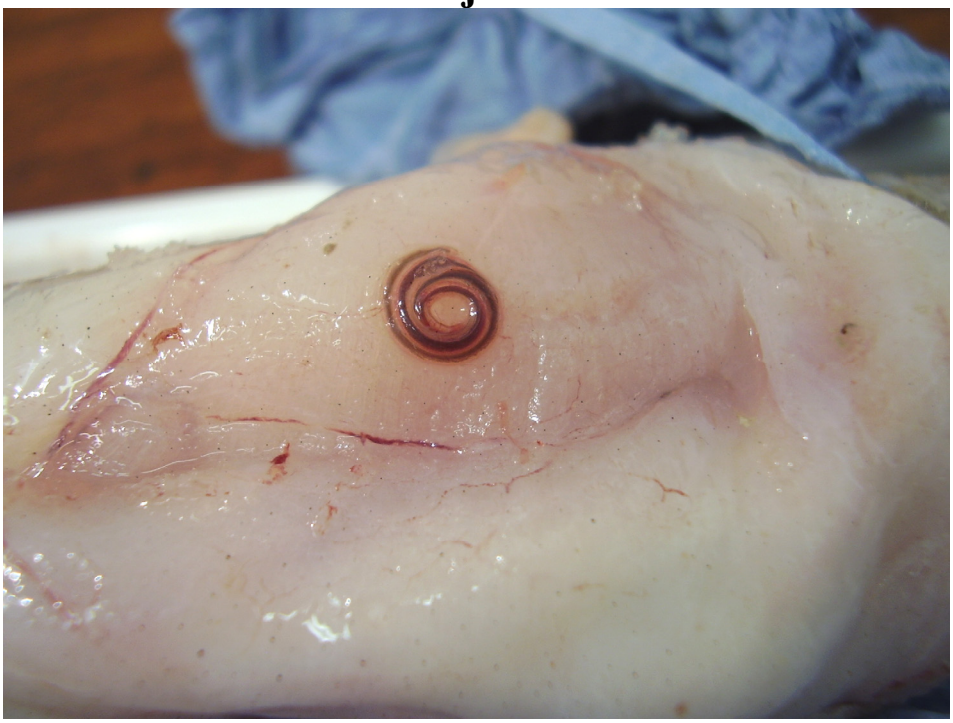
A Survey of *Anisakis* and *Pseudoterranova* in Scottish fisheries and the efficacy of current detection methods

FSAS Project S14008



Allan Petrie[1], Rod Wootten[1], David Bruno[2], Ken MacKenzie[3] and James Bron[1]

[1]Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA,
[2]Fisheries Research Services, 375 Victoria Road, Aberdeen, AB11 9DB
[3]University of Aberdeen, Aberdeen, AB24 3FX

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Executive Summary

The aim of the project was to determine the abundance of larval anisakid nematodes in the flesh of commercially important species of fish in Scottish waters and to assess the efficacy of methods of detection of these parasites.

Monkfish (n = 1075), cod (n = 372), herring (n = 784) and mackerel (n = 358) caught from the N. Sea and waters to the W. and N. of Scotland were examined for the presence of larval anisakids in the flesh. Fish were examined by a variety of methods including, candling and slicing, digestion and pressing.

Two species of nematode, *Anisakis simplex* and *Pseudoterranova decipiens* were found in the flesh of monkfish and cod, but only *A. simplex* occurred in the flesh of herring and mackerel.

The overall prevalence (percentage of total fish sampled found to be infected by worms) and abundance (mean number of worms per fish including both infected and uninfected fish) of *A. simplex* and *P. decipiens* in monkfish were 26.7% and 0.5, and 36% and 0.7, respectively, and in cod 50.3% and 3.3, and 37.9% and 2.5, respectively. In herring the overall prevalence and abundance of *A. simplex* were 36% and 0.7 and in mackerel 22.9% and 0.9, respectively.

P. decipiens was most abundant in monkfish and cod from Scottish coastal waters whilst *A. simplex* tended to be more abundant in offshore waters of the northern N. Sea, around Shetland, and to the N. of Scotland.

In all fish species numbers of both *A. simplex* and *P. decipiens* increased with length of the host, although there was evidence for a levelling off in abundance in larger fish.

In all species of fish examined, except mackerel, there were significantly greater numbers of larval anisakids in flesh from the left side of the fish than the right. Significantly more *A. simplex* were found in the belly flaps than in the fillets in all fish species. *P. decipiens* was significantly more abundant in the fillets in monkfish but there was no difference between numbers of this parasite in belly flaps and fillets in cod.

A comparison of the efficacy of detection methods showed that visual inspection and candling of fillets of monkfish and cod detected only ca. 50% of the numbers of parasites detected by candling and destructive slicing. In monkfish candling was only effective in fillets of up to 2.5 cm in thickness, which equates to a fish of 37cm in length. Visual inspection and candling were more effective in detecting worms in belly flaps recovering at least 75% of the numbers found by candling and slicing. In herring and mackerel candling is not effective but there was no significant difference in the numbers of *A. simplex* recovered by digestion and pressing.

A questionnaire survey of fish processors in Scotland showed that very few carried out any systematic examination of fish for larval nematodes.

Sample numbers of fish required to detect single infected fish from populations with different prevalences of infection have been calculated, assuming detection methods of different sensitivity. The numbers of fish which need to be sampled decreases with increasing prevalence of infection.

1. Introduction

Anisakiasis, a potentially fatal condition associated with the accidental ingestion by humans of larval nematodes in infected fish or squid, affects over 2000 people globally per annum, the incidence of infection increasing with the growing trend in consumption of raw or uncooked seafood (Rosales *et al*. 1999). Europe accounts for 3.5% of the global incidence of infection, with most cases observed in Holland, Germany and France (*ibid.*) and this has led to concerns about anisakids in fish and rejections of fish consignments within the EU. In particular, consignments of monkfish from Scotland have been rejected at European frontiers. Most anisakiasis is associated with ingestion of the nematode *Anisakis* spp with the remainder principally associated with the related *Pseudoterranova* spp. Nematodes, particularly *Pseudoterranova* are also macroscopically visible, leading to infected fish being rendered unaesthetic in appearance, and they are thus a source of concern to the fishing and food industries. Whilst candling of fillets has been employed to detect and remove nematodes before sale of the fish, this method often proves ineffective (McClelland 2002). Despite this, no other practical solutions for the detection of nematodes have been adopted by the industry. Cases of "anisakiasis" are recorded particularly from Japan where raw and lightly cooked fish are commonly eaten and in certain areas of Europe where lightly salted or pickled fish are consumed (e.g. Rosales *et al*. 1999). Although man is an "accidental" host, ingested larvae may nevertheless attempt to penetrate the gastro-intestinal wall and cause acute abdominal symptoms including nausea, fever, abdominal pain, and a range of gastrointestinal disorders and lesions of the stomach and intestine, which can be fatal. A number of authors (e.g. Audicana *et al.* 2002) have also reported a range of allergic reactions in humans exposed to anisakine antigens in seafood. This is of particular concern since food which has been frozen or cooked to kill worms will still retain antigens capable of eliciting an allergic response.

Until recently the nematode species found in the flesh of fish from Scottish waters were thought to be *Anisakis simplex* and *Pseudoterranova decipiens*. Recent research has established that each of these nominal worm species 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 (Pazzi *et al*. 1991). Differences in the biology, distribution and abundance of these different species are not clear, but it is most likely that their general biology will be very similar as outlined below.

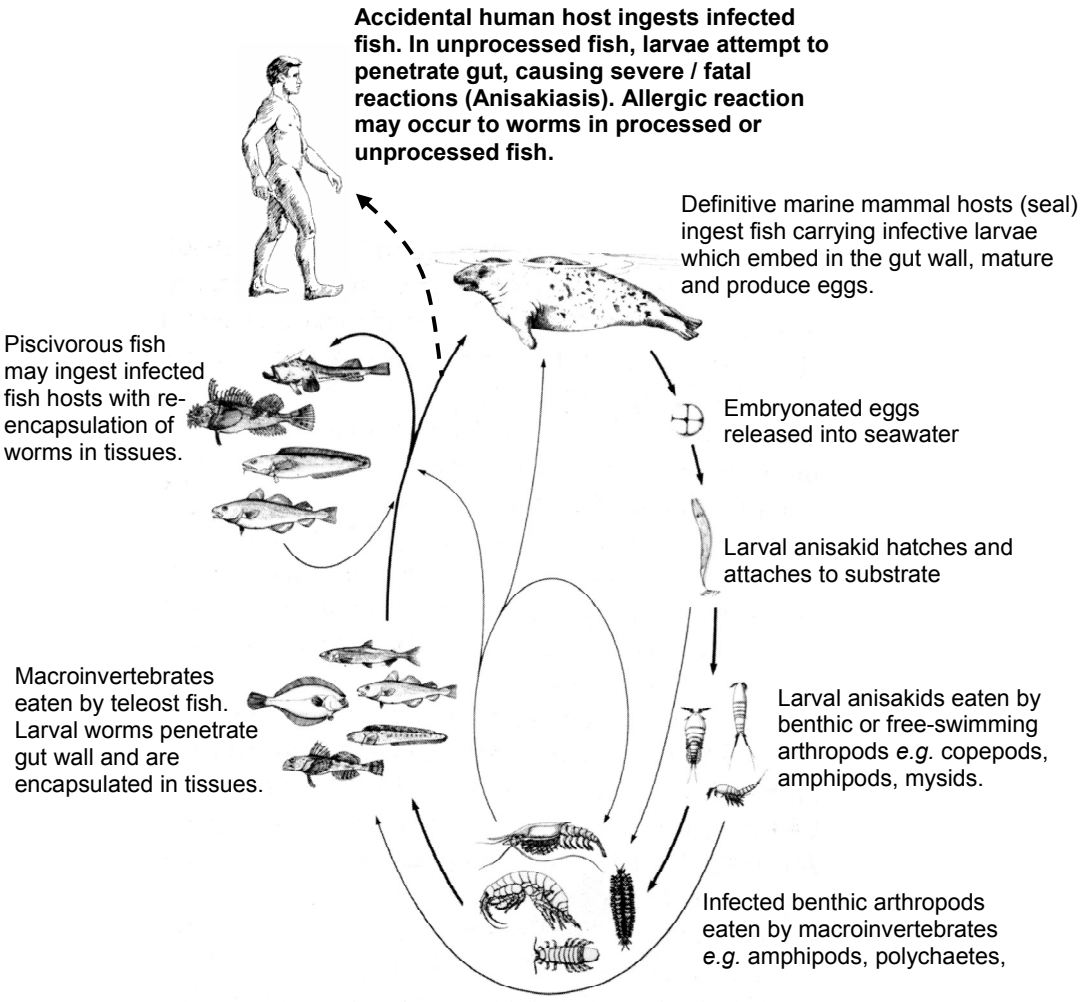


Figure 1 Diagrammatic representation of life-cycle of the sealworm *Pseudoterranova decipiens*. In *Anisakis simplex* the definitive hosts are cetaceans and the invertebrate hosts are often euphausids (Figure adapted from McClelland *et al.*, 1990).

The general life cycle of marine ascaridoid nematodes (Figure 1.) involves both free-living and parasitic stages (Smith and Wootten 1978) and comprises four larval and one adult stage. The life cycles of *A. simplex* and *P. decipiens*, are indirect, using intermediate hosts in their transmission. Eggs develop in the uterus of mature female worms within the digestive tracts of aquatic mammal hosts and are released in faeces (Berland 1991). Eggs of marine anisakids all undergo an incubation period in sea water, with second or third stage larvae hatching as the free living stage e.g. *Anisakis* (Smith 1983), *P. decipiens* (McClelland 2002). Free-living larvae of both nematode species are ingested by intermediate invertebrate hosts, which for Anisakis appear to be principally planktonic malacostracan crustaceans e.g. euphausids (Smith 1983, Smith & Wootten 1978). The first intermediate host of *Pseudoterranova* is a benthic crustacean, and various species of

benthic, epibenthic and natant copepods may become infected (McClelland 2002). The second transport hosts are teleost fish, which become infected by eating invertebrate hosts. Parasites released from infected prey during digestion, penetrate the digestive tract and migrate to various organs within the body cavity of the fish or to the musculature where they are generally encapsulated (Smith and Wootten 1978). Larvae of *A. simplex* occur commonly in pelagic species of fish, presumably as a result of feeding on pelagic invertebrates. *P. decipiens* is thought to predominate in demersal fish species, which feed on infected benthic invertebrate hosts; and infections of *P. decipiens* are common in fish from inshore waters (McClelland 2002), which these authors, among others, related to the distribution of the seal final hosts. Infection of piscivorous fish such as monkfish (*Lophius piscatorius*) and cod (*Gadus morhua*) may also occur by ingestion of infected fish hosts (Scott 1954; Smith 1974; Burt *et al*. 1990a). Larvae may remain within fish for long periods. Third stage larvae ingested in prey by suitable mammalian final hosts are released during digestion, remain in the alimentary tract and moult twice to the fourth stage (pre-adult) and then to the adult stage. *A. simplex* develops to the adult stage in the stomachs and intestines of a wide variety of marine mammals, predominantly cetaceans of various species (Smith and Wootten 1978). There have been many studies on nematode infection in marine fish but in Scottish waters most attention has been paid to cod as it has long been known to be commonly parasitized by *Pseudoterranova* (Rae 1958, 1972). Other authors (Young 1972, Wootten & Waddell 1977) have shown that *Pseudoterranova* was more abundant in cod from coastal waters, but that *Anisakis* was the dominant species in off shore areas. Wootten and Waddell (1977) suggested that this pattern reflected the distribution of the final mammalian hosts, i.e. seals for *Pseudoterranova* and cetaceans for *Anisakis*. These same authors also suggested that an apparent increase in overall infection of cod in the 1960s and 1970s was due to an increase in *Anisakis* numbers. Variations in anisakid numbers are probably related to a wide variety of host and other factors. For example, a recent study of *Anisakis* infections in Baltic herring found host length, condition, sex and gonad development, as well as year, season and sea area to be significant in determining prevalence (Podolska and Horbowy 2003).

Other fish species from Scottish waters have not been so comprehensively studied as cod. There are no published data on monkfish. Mackerel are known to be widely infected with *Anisakis*, reflecting their pelagic habit. Levsen (2007) showed that mackerel from the northern North Sea had a mean number of up to 3.5 *Anisakis* in the flesh, depending on fish size. Large scale surveys of herring from British waters were carried out by Khahl (1969) and Davy (1972) who found the abundance of *Anisakis* was highest in the northern North Sea and around Shetland with much lower levels in fish from the West of Scotland and the southern North Sea. Smith and Wootten (1975) examined a limited number of herring samples from Scottish waters and found infection levels generally comparable with earlier studies, although up to 20% of the total burden of *Anisakis* were in the flesh. A recent study on herring to the West of the British Isles found infection prevalences of up to 98% with abundance levels between 5 and 16 (Cross *et al* 2007).

The objectives of the present study were:

1. To study the prevalence and intensity of anisakine nematode infections of monkfish, cod, herring and mackerel from Scottish waters.

2. To determine the factors affecting the variability of infection on a temporal (seasonal/historical), spatial (different fisheries), intra-population (size, age, sex) and carcass (location in flesh) basis.

3. To determine the efficacy and limitations of current techniques for detecting the presence of nematodes in fish flesh.

2. Experimental Procedures

2.2 Questionnaire

To gauge the current situation with respect to problems experienced and those perceived by fish processors with regards to nematode infections, a questionnaire was drafted and sent to 99 processors within Aberdeen city and Aberdeenshire. A copy of the questionnaire is attached in Appendix 2.

2.3 Fish Collection

In conjunction with FRS Aberdeen, fish were caught by participating on research cruises in which it was expected to catch species required for the project. Briefly, these cruises consisted of monkfish catch cruises, monkfish surveys, ICES (International Council for the Exploration of the Sea) surveys and a herring acoustic survey. A small number of cod (n=36) were caught by a commercial fishing boat to east of Shetland. Each survey is described below in more detail with their corresponding cruise number.

1505S: Monkfish Catch Cruise
Sampling was conducted in waters 58° to 60° north and 1° to 5° west. Samples caught consisted of monkfish of both U.K. species, *Lophius piscatorius* and *Lophius budegassa,* (n=476) and cod (n=4).

1605S: Monkfish Survey
Sampling was conducted in waters 58° to 62° north and 4° east to 2° west. Samples consisted of monkfish of both species (n=288) and cod (n=171).

1705S: ICES Demersal Trawl Survey
Sampling was conducted in waters from 50° to 58° north and 11° to 3° west. Samples caught consisted of monkfish of both species (n=93) and cod (n=45).

1106S: Herring Acoustic Survey
Only herring were caught on this cruise (n=452).

1606S: Monkfish Catch Cruise
Sampling was conducted from 59° to 60° north and 1° to 4° west. Samples consisted of monkfish of both species (n=107) and cod (n=61).

1706S: Monkfish Survey
Sampling was conducted at 57° north and 12° west. Samples consisted of *L. piscatorius* only (n=31).

1806S: ICES Demersal Trawl Survey
Sampling was conducted in waters from 53° to 59° north and 3° to 11° west. Samples consisted of monkfish of both species (n=80), cod (n=55), herring (n=332) and mackerel (n=358).

The ICES squares in which fish from any of the 4 species were caught are illustrated in Figure 2. For the purposes of interpretation the ICES squares have been grouped into larger areas (A-F) which share common features in terms of terrain or biological habitat (Figure 3.). The sample areas have been designated as follows: A = W of Scotland and NW Ireland, B = Inner Hebrides and Irish Sea, C = North coast of Scotland, D = Shetland, E = Northern North Sea, F = Rockall.

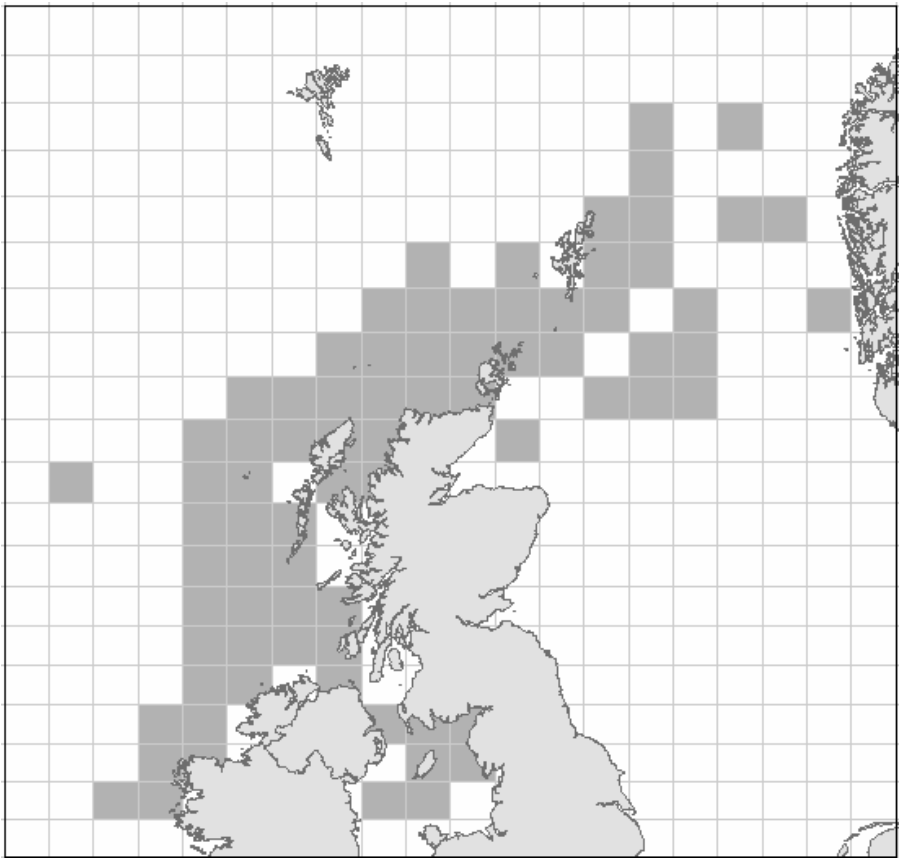


Figure 2: Plot of ICES squares with shaded squares indicating those where fish from any of the 4 species sampled were caught

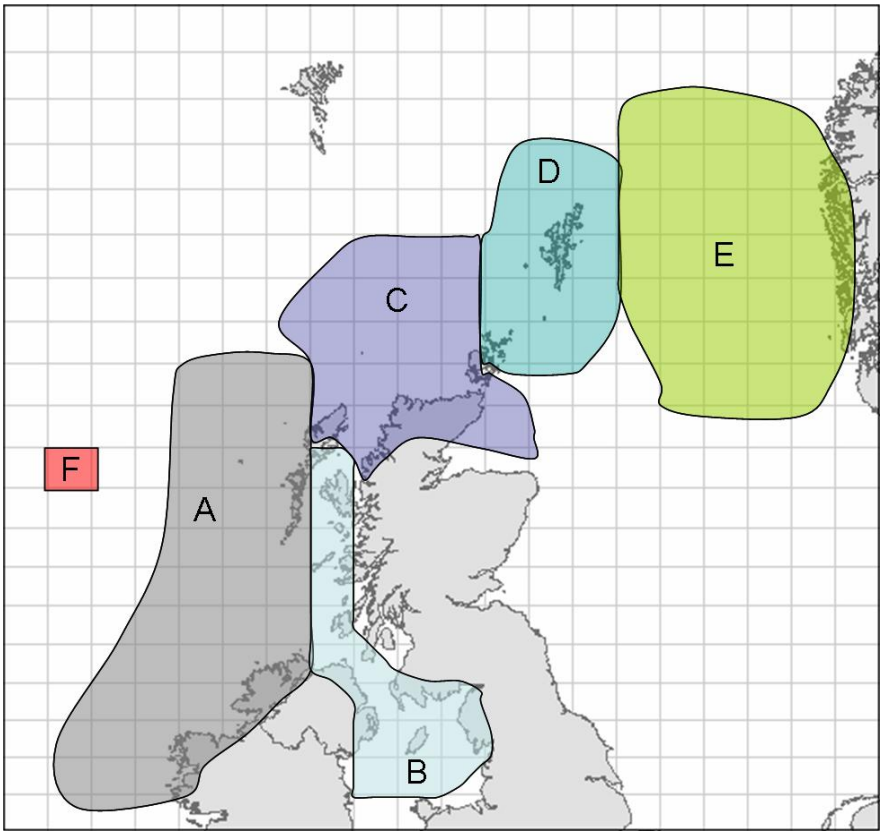


Figure 3: Plot of areas that were defined for splitting catches into geographical capture locations*.

2.4 Sample processing

Preliminary processing of cod and monkfish samples was conducted at sea on-board the research vessel FRV Scotia. This consisted of recording the length of the fish, the whole and gutted weight, and the sex and maturity. The species of monkfish was determined by examining the peritoneal lining of the fish, with a black lining identifying the species as *L. budegassa*. For monkfish, the tails including the belly flaps were separated from the remaining carcass and individually identified and wrapped before being stored at -20°C. For cod, the gutted fish were also individually identified and wrapped before being stored at -20°C. Individual identification codes were created which identified the fish by cruise number, trawl number and fish number.

Herring and mackerel were frozen whole on board ship at -20ºC in groups of 10 fish caught during the same trawl. Fish groups were identified by both cruise number and trawl number. On shore, all samples were stored at -20°C until further processing occurred.

* A = W of Scotland and NW Ireland, B = Inner Hebrides and Irish Sea, C = North coast of Scotland, D = Shetland, E = Northern North Sea, F = Rockall

To detect *Anisakis* and *Pseudoterranova*, fish species were examined by a number of different methods, depending on the species under investigation. These are described below. All nematodes found were identified on the basis of their morphology. The exact location of worms within the flesh of monkfish and cod was recorded on a "fish map" (Appendix 1). This was not possible for herring and mackerel due to the detection methods used.

2.5 Detection Methods

The detection methods employed comprised visual examination (by eye), candling, *i.e.* slicing of the flesh into thin strips on the candling table to aid light transmission (slicing), pepsin digestion or a press method. All nematodes detected, except those from pressing were retained in glass vials containing 95% ethanol, which were identified with the appropriate cruise, trawl and fish number.

Visual examination (Eye) and Candling
In visual examination the flesh was examined by naked eye under ambient fluorescent lighting. In candling the flap or fillet of fish was placed onto a light box, roughly comparable to an industrial candling table. This consisted of a box with a white, translucent plastic sheet overlying a cool white light source, normally comprising 2 or more fluorescent tubes. The light output from the candling table used was a minimum of 20,000 lux. The candling table shines light through fish flesh and reveals nematodes by the shadows they cast.

Slicing
Following candling, flesh was sliced into thin strips (5-10mm) on the candling table and the strips then examined on the candling table as previously described for the presence of nematodes.

Digestion Method
A modification of the digestion method described by Jackson *et al* (1981) was employed. A 0.85% sodium chloride solution was prepared and pepsin added to a concentration of 10mg/l. Depending on the size of the fish either the whole flesh (gutted fish, head removed) or a fillet was placed in an appropriately labelled 1000ml conical flask and either 500ml or 750ml of pepsin solution was added. The pH of the solution was then adjusted with concentrated hydrochloric acid to pH 2 and the solution incubated overnight at 37°C. The following day the solution was sieved (1.5x1mm mesh) and the contents examined for nematodes.

Press system
This method was a modification of that described by Levsen *et al* (2003). In this method fillets of fish were placed into labelled plastic bags and then flattened to 3-5mm using a commercial roll-type printing press (Figure 4.). These flattened fillets were then frozen and subsequently examined under UV-A light conditions where the *Anisakis* fluoresce.

Figure 4. Roll-type printing press employed to flatten fillets of herring and mackerel for worm visualisation.

2.5.1 Monkfish

Monkfish tails were first skinned. Any nematodes that remained attached to the skin and their approximate location in relation to the flesh were noted. After skinning, the flesh was separated into left and right sides (as viewed dorsally) and also into flaps and fillets, which were examined visually under ambient light conditions for the presence of nematodes. Flaps and fillets were then transferred to a candling table for further examination and the number of worms in each recorded. The flaps were then sliced to aid light penetration and examined on the candling table. Fillets were then placed onto the candling table and cut into 5-10mm thick slices, perpendicular to the backbone, and examined. Nematodes were recorded in relation to the body location (left, right, flaps & fillet) and also by the detection method (eye, candle & slice). To compare the efficacy of detection methods, a number of monkfish were also digested following nematode detection by eye, candle and slicing.

2.5.2 Cod

Cod were filleted and skinned and the belly flaps removed. The flaps and fillets were then subsequently examined in the same sequence as described for monkfish except that the flesh was sliced longitudinally. A small number of cod (n=31) from cruise 1606S were

examined for nematodes by the pressing method as described above. As with the monkfish, to compare the efficacy of detection methods, a number cod were digested following nematode detection by eye, candle and slicing.

2.5.3 Herring

Herring were filleted and subsequently examined by digestion or pressing. A total of 394 were digested, giving results on the total loading of *Anisakis*, but no information on location in the flesh. A further 390 were examined by the pressing method which gave information on location. A number of herring (20, 2.5% of total) examined by the press method were subsequently digested to determine the efficacy of this pressing.

2.5.4 Mackerel

All mackerel were examined using the press method as previously described. Of the total number of mackerel samples examined by the press method, 10% (n=36) were subsequently digested to determine the efficacy of this pressing.

2.6 Data Analysis

For the purposes of analysis cod and monkfish samples were divided into different geographical areas A-F (Figure 3.). These reflect different offshore and inshore habitats which will contain distinct stocks of fish, but are also known from previous studies to show different levels of nematode infection due to differences in abundance of crustacean, fish and mammalian species.

The infection of hosts by parasites often follows, as is the case here, a right-skewed and overdispersed distribution (*i.e.* most data lie to the left of a host infection frequency plot, with a few highly infected individuals providing a long right-leading tail to the distribution). For this reason much of the preliminary / univariate data analysis has been carried out using non-parametric techniques. Non-parametric analysis employed the Mann-Whitney U-test for determination of differences between 2 groups or Kruskal-Wallis for determination of differences between multiple groups. For naturally paired samples *e.g.* left and right fillets of the same fish, a Wilcoxon Matched Pairs Test was employed for comparison of two samples. In some instances it will be noted that there are discrepancies between the results provided by the univariate and the multivariate analyses in terms of a particular variable. Whilst in univariate analysis the differences between samples are ascribed wholly to the analysed variable of interest, in multivariate analysis total sample variability is partitioned more appropriately to *all* of the responsible variables and, at the same time, this more accurate partition means that the variability ascribed to random error is thus often reduced. As a result, the significance of a given variable may change with respect to the univariate case. Where this occurs, the results from the multivariate analysis are more likely to be reliable as they derive from a fuller model of the sources of sample variability.

The decision to use plots of means with nominal 95% confidence intervals for most univariate infection data throughout this report reflects the fact that the worm infection data are universally zero inflated (*i.e.* have excessive numbers of uninfected fish). If plotted using a median boxplot, such data would give near identical boxes with no lower bounds and the median centred on the $y = 0$ axis and therefore all samples would appear near identical. Use of the mean and 95% CI provides the reader with a better appreciation of difference between samples, although the two summary values are not reflective of a normal distribution and should not be considered representative of such.

For multivariate analysis a number of techniques were employed including General Linear Modelling (GLM), Generalized Linear Modelling (GLZ) and Generalized Additive Modelling (GAM). Variables were transformed and outliers removed as necessary. The most successful multivariate models, as presented in this report, are Generalized Additive Models (GAMs) carried out according to the principles outlined by Wood (2006). Briefly, this technique allows a response variable (*e.g. Anisakis* numbers) to be modelled in terms of the contribution of a number of continuous (*e.g.* length and depth) and nominal (*e.g.* location, sex and year) explanatory variables. The power of this technique is that it allows both linear and non-linear relationships to be modelled and in particular relies on the fitting of "smoothers" to continuous variables that may better model their contribution. Models were constructed using a reverse stepwise procedure with the initial model incorporating fixed terms, interaction between the fixed terms, and additional individual non-linear length effects for each sex and location where appropriate. Model fit was assessed using AIC scores and by observing model residuals. A parsimonious modelling strategy was employed whereby additional terms, which provided minimal improvement to the model, were excluded rather than included. Although they represented the most successful modelling technique, the models suffered from a lack of data in largest and smallest fish size categories (due to the nature of the fisheries in question) and from the arbitrary (in the sense of being non-systematic) depths of capture employed during most cruises. More structured datasets with larger fish numbers would doubtless assist model performance but are not possible without dedicated (and therefore prohibitively expensive) trawl surveys. With respect to capture location, all comparisons in multivariate models are made with respect to the Shetland capture area (area D) which is a key region for capture of all the species studied in this project (see Appendix 3). Full model specifications for all multivariate analyses are provided in Appendix 4.

A number of standard parasitology statistics relating to infection of hosts are also employed in this study. The term "prevalence" relates to the total percentage of sampled hosts that carry an infection. The term "mean intensity" is the mean number of parasites per fish calculated for the infected fish only. The term "abundance" is the mean number of worms per fish including both infected and uninfected fish.

The majority of statistical analyses presented in this report were conducted using the Statistica 7.1 statistics package, with GAMs constructed using R 2.4.1 and Brodgar 2.5.3. For all analyses, statistical significance was taken as $p<0.05$.

3. Results

3.1 Questionnaire

Of the 99 questionnaires that were sent out, 19 were returned by processors. Of these, 2 processors only deal with fish that have already been filleted *i.e.* are secondary processors. The remaining 17 were aware of the potential presence of nematodes in flesh and all but 2 examine fish for nematodes during processing. However, only 1 processor indicated that they use a candling table for nematode detection and suggested that it was of limited use for monkfish, stating: '*monkfish is very difficult because of the thick skin and flesh*'. One other processor noted that although candling had been previously used it was not now conducted as it was '*not cost effective*'. The remaining processors use only visual inspection for nematode detection. Three processors had experienced rejections of fish, 2 of which were confirmed as being monkfish, with both rejections occurring during import into Italy.

3.2 Number of fish and site of capture

The numbers of fish captured from each cruise are summarised in Table 1. No herring or mackerel were caught within the first year of the project and only cruise 1806S resulted in the collection of all species required for the project.

Table 1: Numbers of fish from each species caught during individual cruises

	Monkfish	Cod	Herring	Mackerel
1505S	476	4	0	0
1605S	288	171	0	0
1705S	93	45	0	0
1106S	0	0	452	0
1606S	107	61	0	0
1706S	31	0	0	0
1806S	80	55	332	358
Totals	1075	336 (+36[†])	784	358

Fish were captured at a variety of sites in Scottish fishing waters. The furthest point west where samples were taken was 12.9°W at Rockall (Area F) consisting only of monkfish. The northern extreme of waters fished was 61°N, the southern extreme occurred at 53°N and the furthest point east was 4°E.

The sites of capture for each of the fish species studied and an indication to the numbers caught per ICES square are illustrated in Figures 5-8. It can be seen that the majority of the samples were caught in waters west of 0°. Sites of capture were dependent upon the scientific requirements of the cruise. The areas where fish were captured were divided into 6 areas as described previously (Figure 3.).

[†] A small number of cod (n=36) were caught by a commercial fishing boat to the east of Shetland.

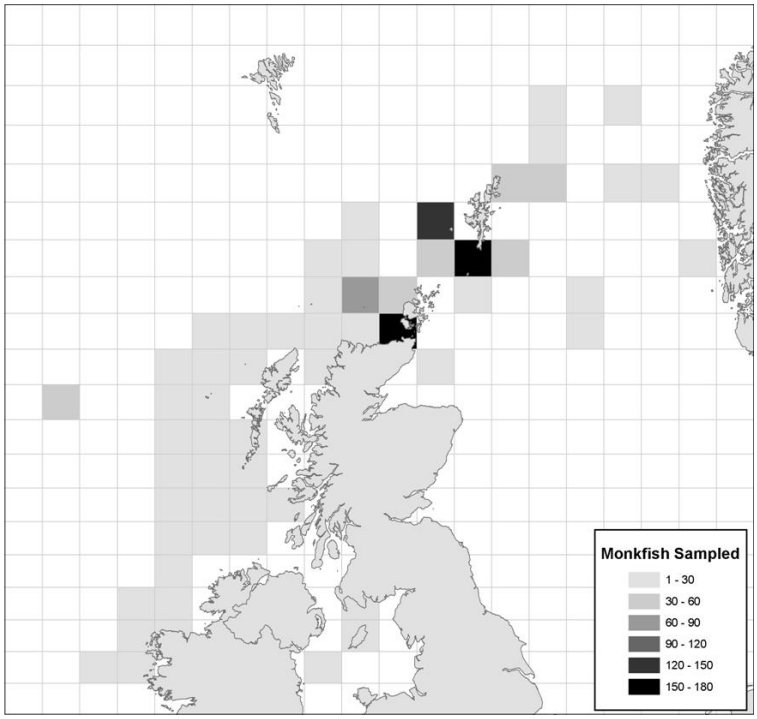


Figure 5: Plot highlighting ICES squares where monkfish were sampled with degree of shading indicating number of monkfish sampled from that ICES square

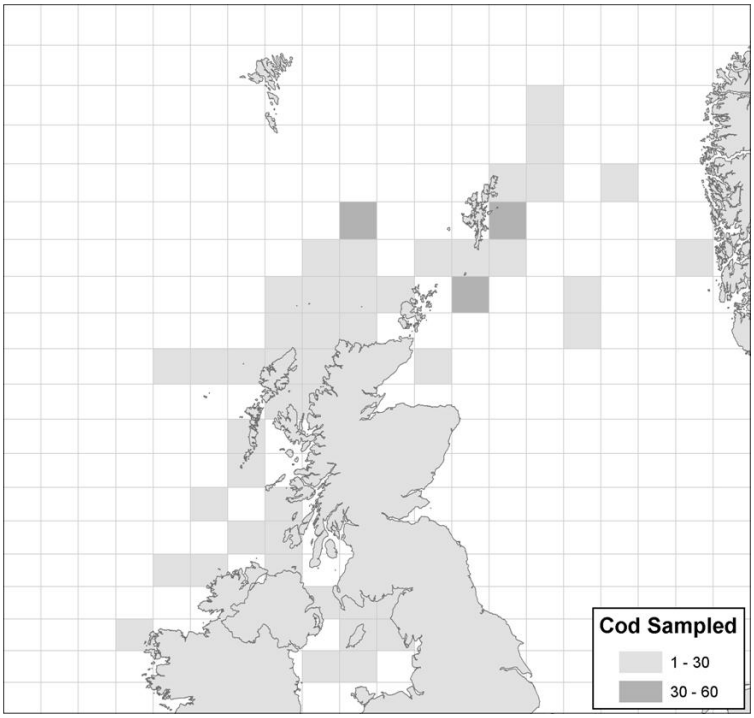


Figure 6: Plot highlighting ICES squares where cod were sampled with degree of shading indicating number of cod sampled from that ICES square

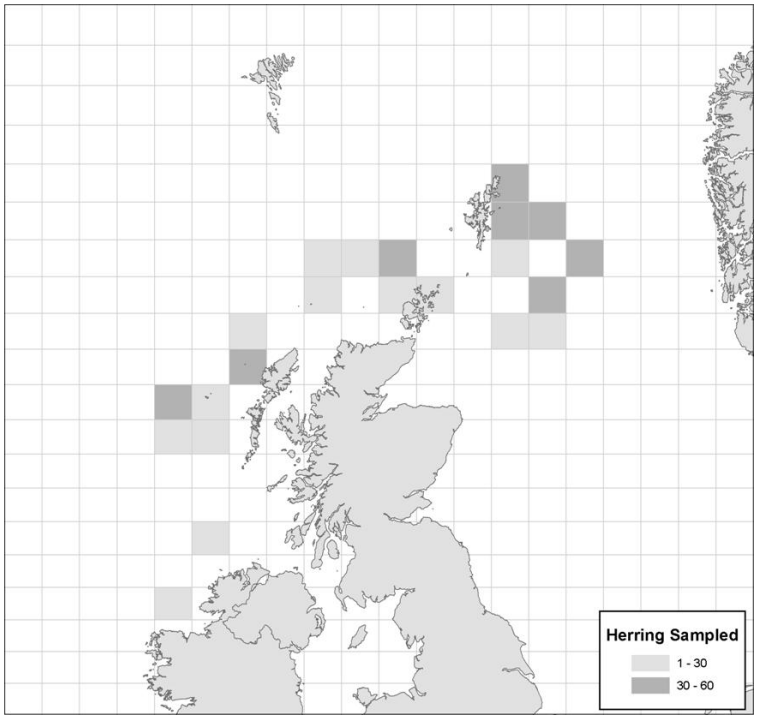


Figure 7: Plot highlighting ICES squares where herring were sampled with degree of shading indicating number of herring sampled from that ICES square

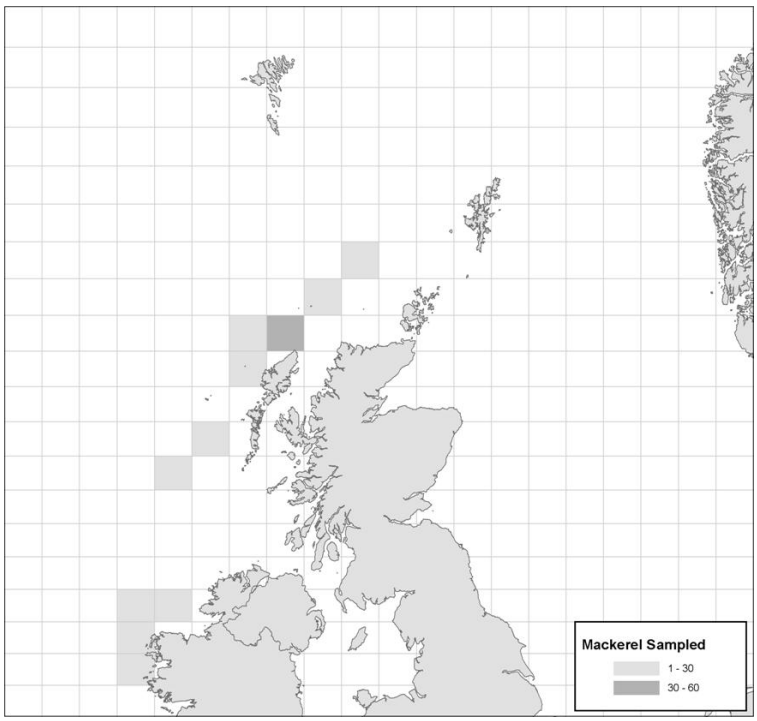


Figure 8: Plot highlighting ICES squares where mackerel were sampled with degree of shading indicating number of mackerel sampled from that ICES square

3.3 Effect of Storage

Following storage of the samples at -20°C or less the quality of the flesh was examined prior to nematode detection. For monkfish, herring and mackerel there was no discernible degradation of the flesh quality so as to affect the detection of the nematodes. However, with cod there were a number of belly flaps where the flesh had degraded to such an extent that it was not possible to determine numbers or location of nematodes and these were therefore not counted.

In the following results, which are presented by species, it should be remembered that statistics concerning worm numbers are associated only with worms recovered from the flesh. Whilst further worms are undoubtedly associated with other areas, such as the gut, these are not considered relevant to questions of human fish consumption.

3.4 Monkfish

A total of 1075 monkfish were examined, of which 40 were found to be *Lophius budegassa* or black-bellied monkfish. The remainder were identified as *L. piscatorius,* the more commonly captured species in northern waters. Because of the low number of fish caught, *L. budegassa* data were omitted from the statistical analysis. However, the *L. budegassa* data were analysed to determine the prevalence of *L. budegassa* captured at each geographical location. These data are presented in Table 2., which indicates that the highest prevalence of *L budegassa* was obtained in area A, with decreasing prevalence moving through area C, to areas D and E

The prevalence of infection of *L. budegassa* with *Anisakis* and *Pseudoterranova* was 25% and 50%, respectively. The infection abundance was 0.6 and 0.95 for *Anisakis* and *Pseudoterranova* respectively and the mean intensity of infection was 2.4 and 1.9.

Table 2: Prevalence of *L. budegassa* as a percentage of total monkfish captured at each of the sampling locations.

Location	$n_{budegassa}$	n_{total}	% Prevalence by location
A	11	121	9.1
B	0	7	0
C	15	337	4.5
D	11	457	2.4
E	3	122	2.5
F	0	31	0

All other statistical analysis and results refer to those obtained for *L. piscatorius* and any further reference to monkfish relates to this species. The median lengths of monkfish (*L. piscatorius*) in relation to the capture location are plotted in Figure 9.

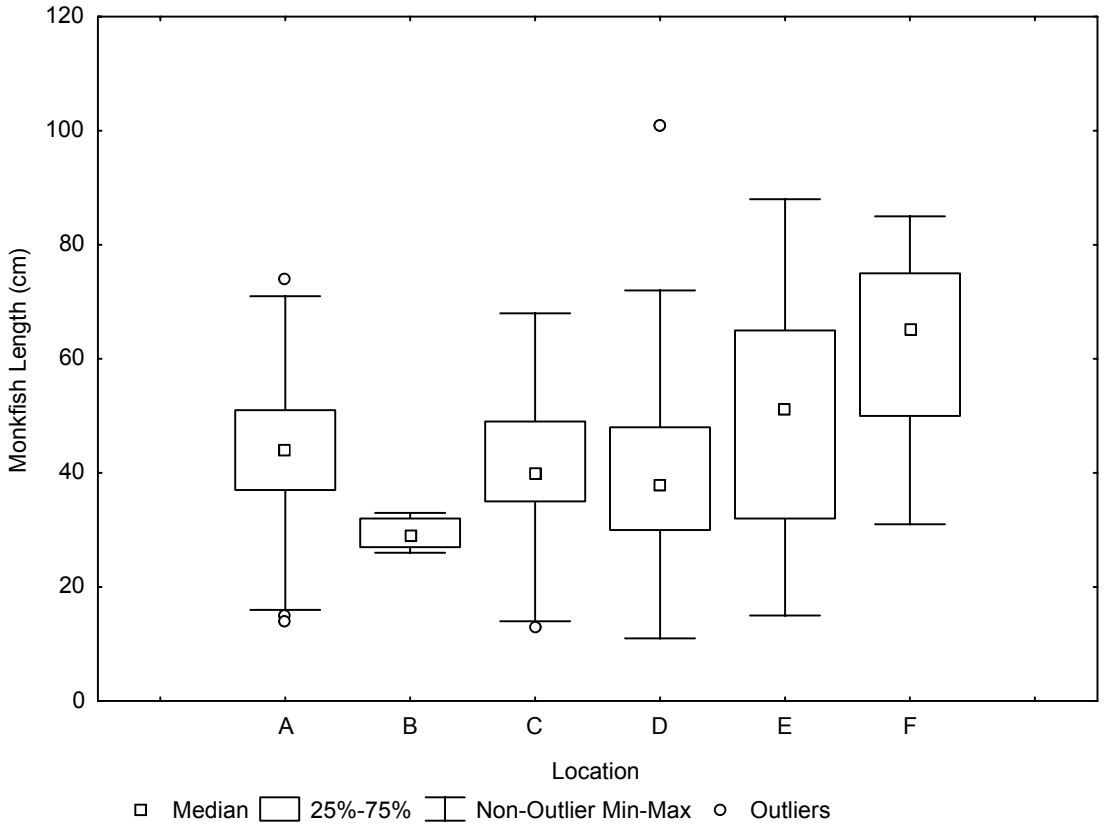


Figure 9: Boxplot of median monkfish length (cm) in relation to the 6 different sample locations.

Both *Anisakis* and *Pseudoterranova* were recovered from sampled monkfish tails and belly flaps. As Figures 10. and 11 illustrate, the latter species may be large and clearly visible on the surface of the tail flesh, presenting an obvious justification for rejection of fish consignments.

Figure 10. Encapsulated *Pseudoterranova* on monkfish tails. The worms are apparent as round red circles on the flesh.

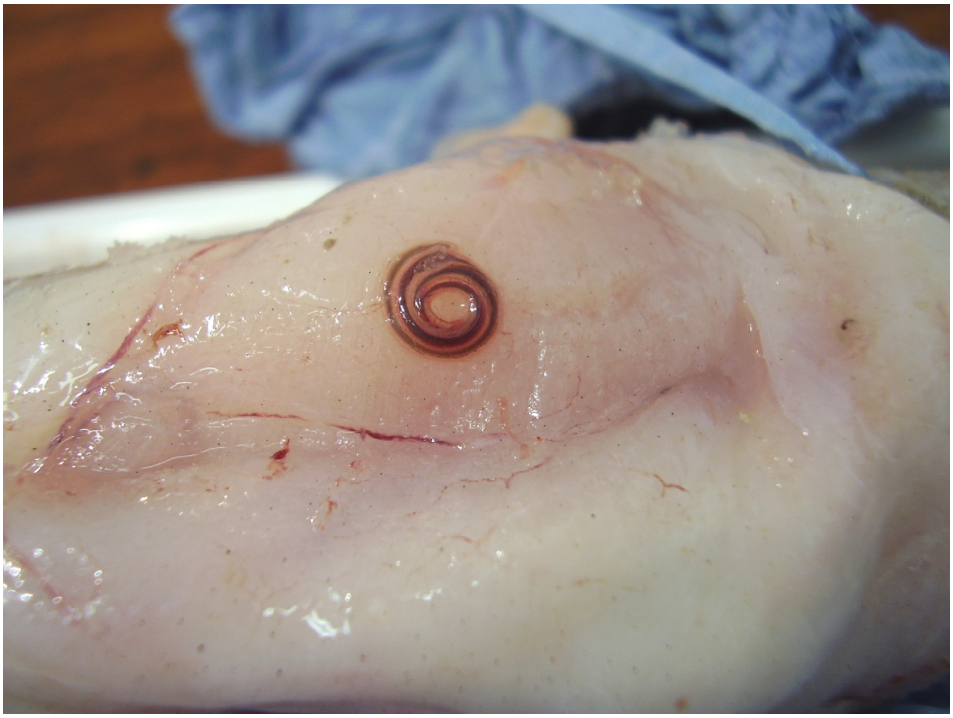


Figure 11. Encapsulated *Pseudoterranova* on monkfish tail.

Figures 12 and 13 show the frequency of monkfish with different levels of *Anisakis* and *Pseudoterranova* infection captured from the six sampling locations. It is clear that for both species, fish carrying no worms are in the majority and that most fish carry few worms (<5). It should also be noted that there is some variation in worm number recovered with respect to site of capture.

The calculated prevalence, mean intensity and abundance for *Anisakis* in monkfish were 26.96, 1.70 and 0.46, respectively. The prevalence, mean intensity and abundance for *Pseudoterranova* in monkfish were 36.04%, 1.92 and 0.69, respectively.

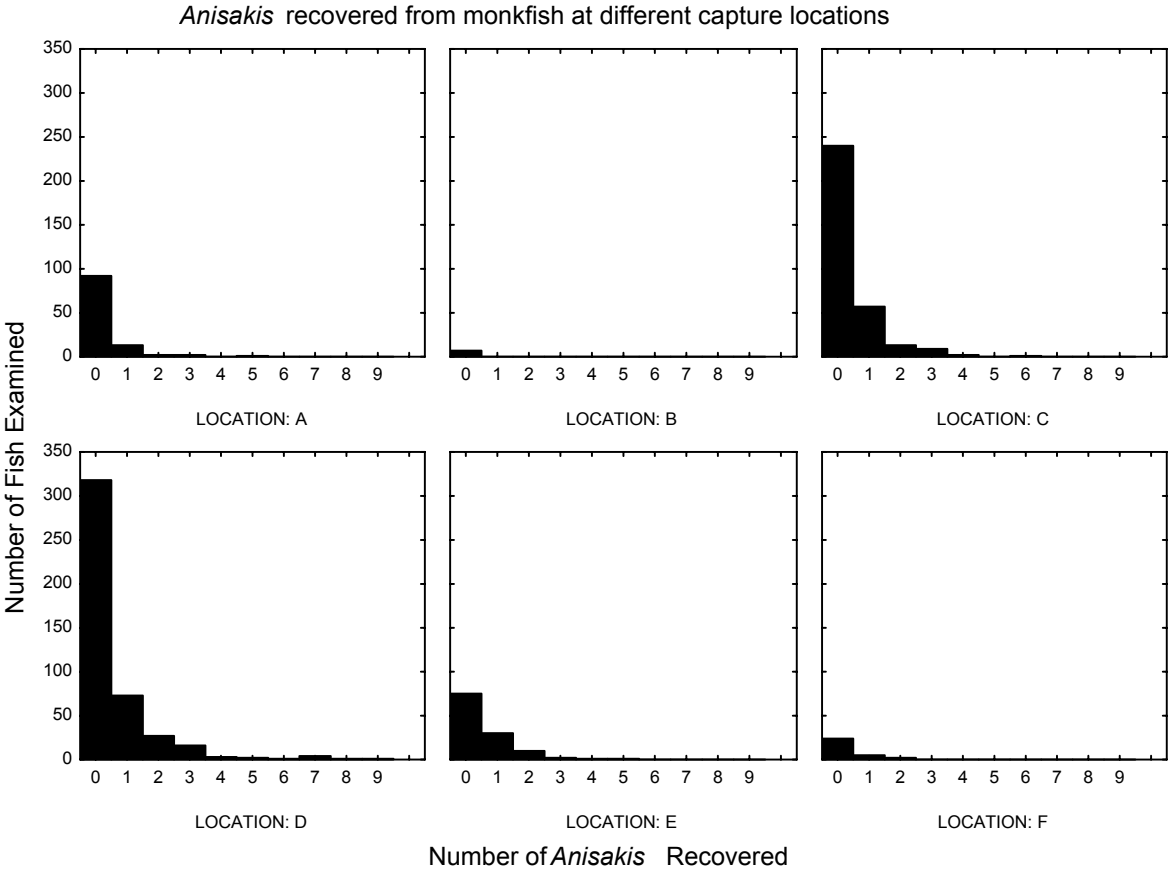


Figure 12. Histogram showing frequency of different infection loads for *Anisakis* in sampled monkfish.

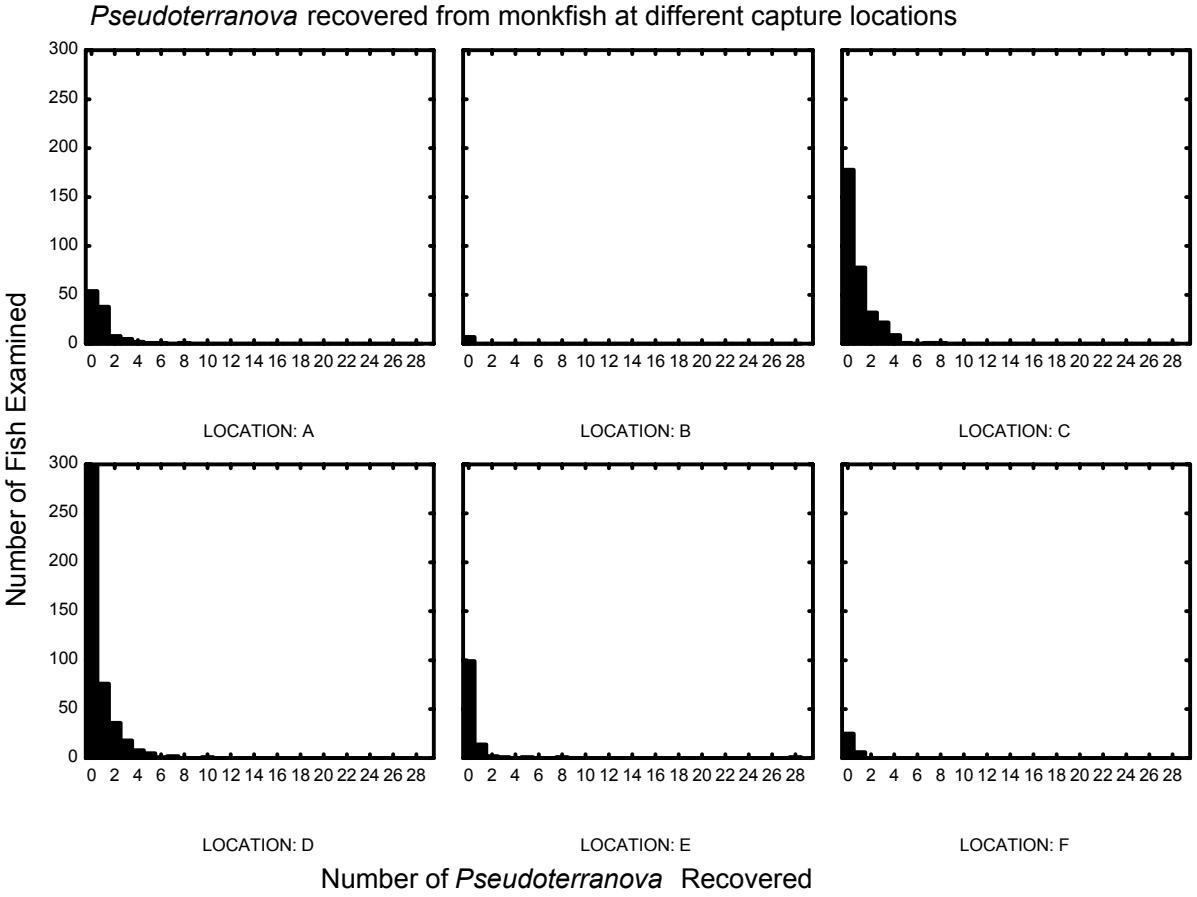


Figure 13. Histogram showing frequency of different infection loads for *Pseudoterranova* in sampled monkfish.

3.4.1 Single factor statistics

The statistical significance of a number of measured factors, on *Anisakis* and *Pseudoterranova* numbers was assessed by Mann-Whitney analysis as follows:

Sex

Anisakis: A plot of mean numbers of *Anisakis* per fish suggested a lower parasite load for females than males (Figure 14.). There was a significant difference ($p_{(adjusted)} = 0.03$) in *Anisakis* numbers between the sexes.

Pseudoterranova: There was no significant difference ($p_{(adjusted)} = 0.9$) between the host sexes for *Pseudoterranova* (Figure 15.).

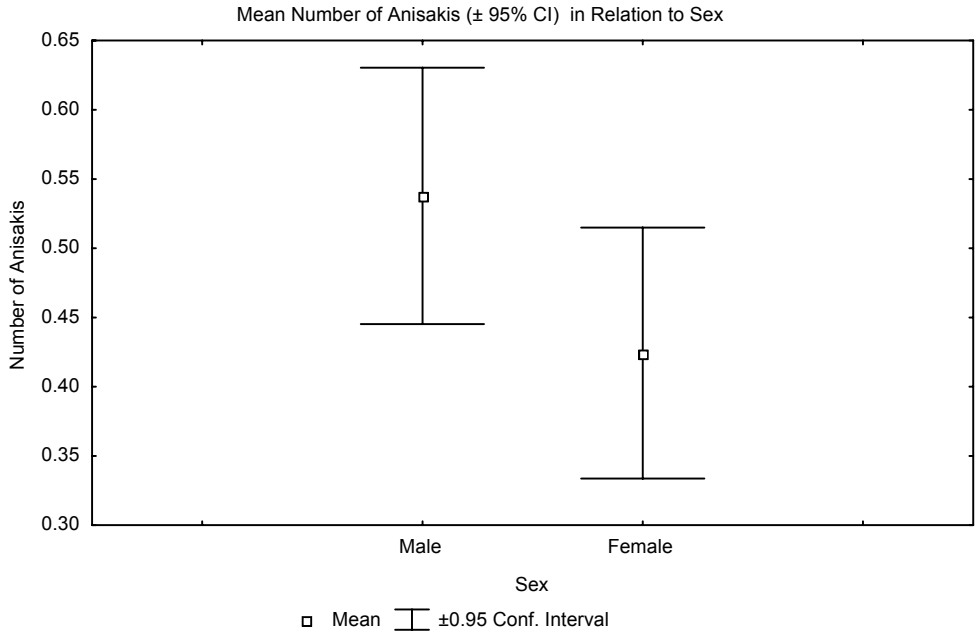


Figure 14: Mean number ± 95% CI of *Anisakis* in monkfish relative to sex

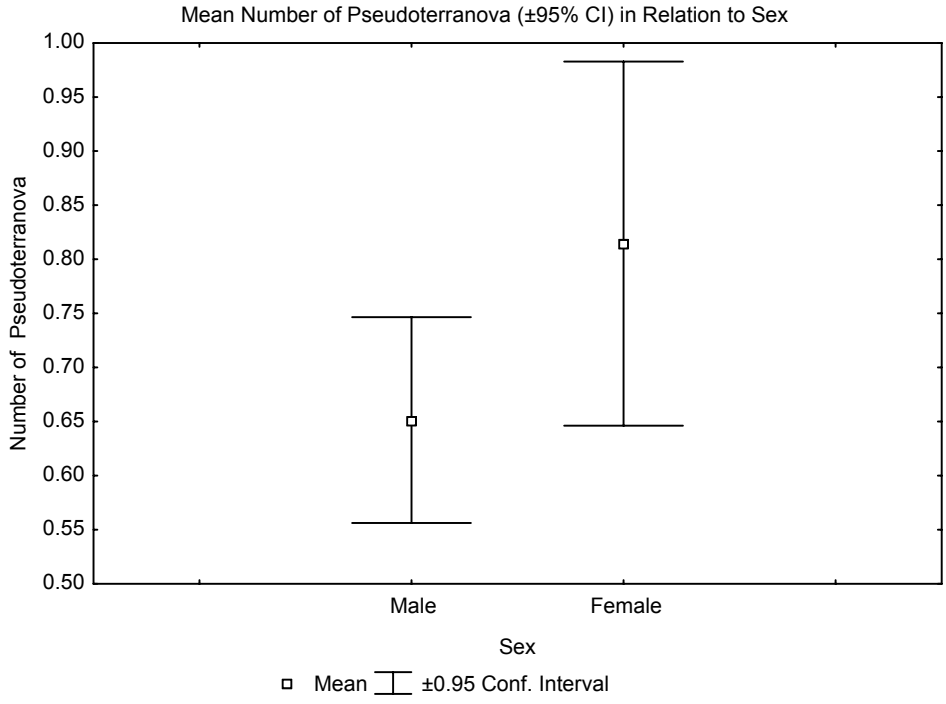


Figure 15: Mean number ± 95% CI of *Pseudoterranova* in monkfish relative to sex

Temporal variation

For analysis of differences between years, only fish that were sampled from similar areas in both years were analysed.

Anisakis: There was a significant difference ($p_{(adjusted)} < 0.001$) between the two sampling years, with a lower number recovered in 2006 (Figure 16.).

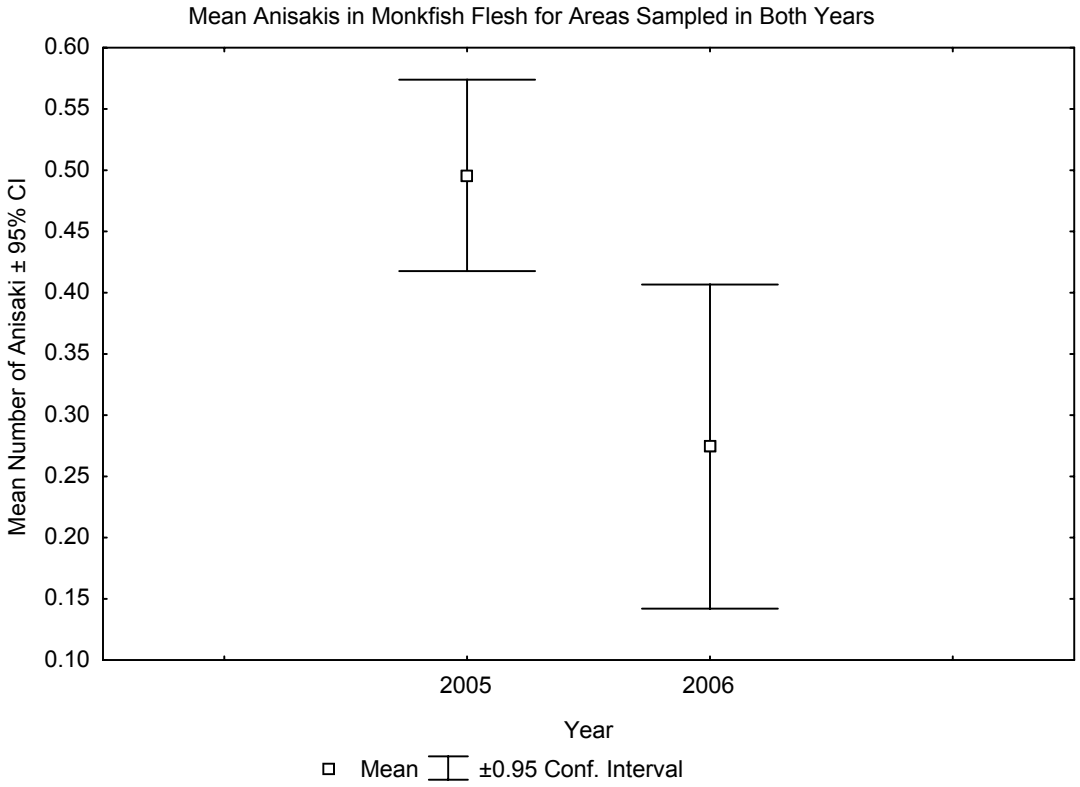


Figure 16: Mean number of *Anisakis* ± 95% CI in monkfish in relation to year of sampling

Pseudoterranova: There were significant differences ($p_{(adjusted)} = 0.04$) in *Pseudoterranova* levels between sampling years with lower numbers recovered in 2006 (Figure 17).

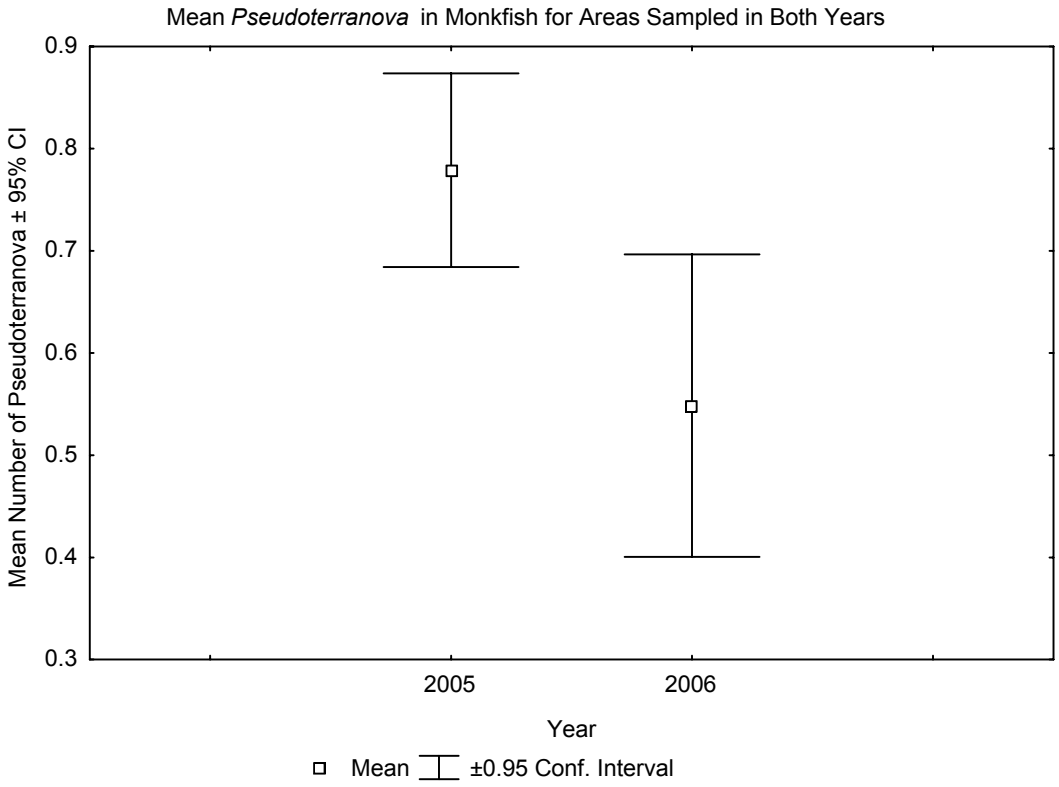


Figure 17: Mean number of *Pseudoterranova* ± 95% CI in monkfish in relation to year of sampling

3.4.2 Analysis of multiple factors

3.4.2.1 Total *Anisakis* numbers in monkfish

The best model for *Anisakis* numbers in monkfish explained only 11.3% of the deviance of the data (a measure of the total variability in numbers between fish samples). The following represent the principal findings of the model:

- Up to a length of ~40-50cm, monkfish showed a significant (p=1.4e-9) increasing probability of having more *Anisakis*, which does not occur in larger fish (see Figure 18.).
- Fish caught in 2006 had significantly (p = 1.90e-05) lower numbers of *Anisakis*, although this may be due to a difference in sampling sites for each year rather than to inter-annual variation at the same sites.
- Female monkfish showed significantly (p=0.031) lower *Anisakis* than male monkfish in the samples studied.
- There were significant (p=1.47e-06) differences in *Anisakis* numbers between locations. If location D (which lies at the centre of Scotland's monkfish captures

and is situated around Shetland) is taken as the baseline, then all other sites showed significantly ($p<0.05$) lower numbers of *Anisakis* save for site F (Rockall).

- There was a significant ($p=0.001$) effect of depth on *Anisakis* numbers, with a largely increasing trend in parasite numbers in fish sampled from depths of ~100m and deeper. There is also an apparent initial fall in numbers from the shallowest depths sampled up to ~100m.

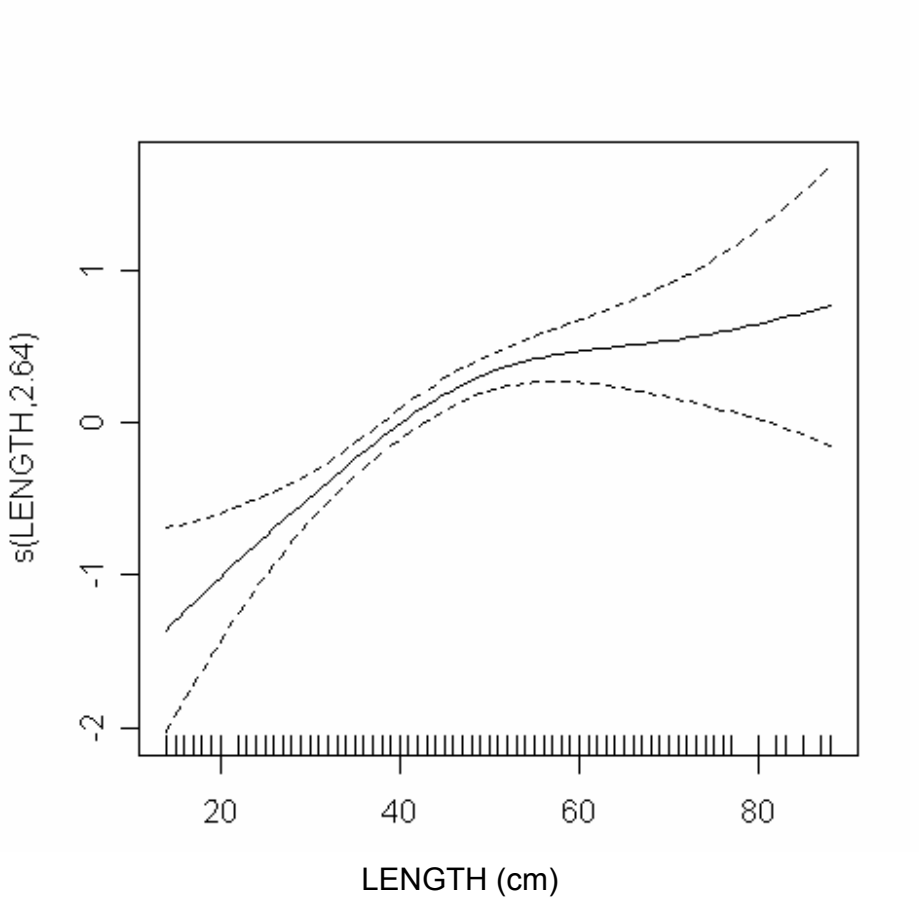


Fig.18. Partial fit of length for model of *Anisakis* in monkfish. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.4.2.2 Total *Pseudoterranova* numbers in monkfish

Similarly to the data for *Anisakis*, the explained deviance for the best *Pseudoterranova* model was only 12.4%. Nevertheless, the following conclusions could be drawn from the model:

- Length was a significant factor ($p=9.86e-11$). Up to a length of ~30-40cm, monkfish showed an increasing probability of having more *Pseudoterranova*, with this

probability levelling off in larger fish, although in fish greater than 70cm it may again begin to rise. However, the latter tendency was obscured by wide confidence limits for larger fish, probably due to small numbers of large monkfish in captured samples (see Figure 19.).

- In 2006 there were significantly less parasites (p=8.73e-06) than in 2005 as for *Anisakis*.
- There was a significant overall location effect (p=1.47e-06), with significantly lower numbers of *Pseudoterranova* at sites E and A, with respect to the Shetland baseline site (D).
- There were significantly (p=0.0012) lower numbers of *Pseudoterranova* with increasing depth.
- There was a significant (p=0.012) effect of sex overall with higher numbers of *Pseudoterranova* in female monkfish. The interaction of sex and location was also significant (p=0.22).

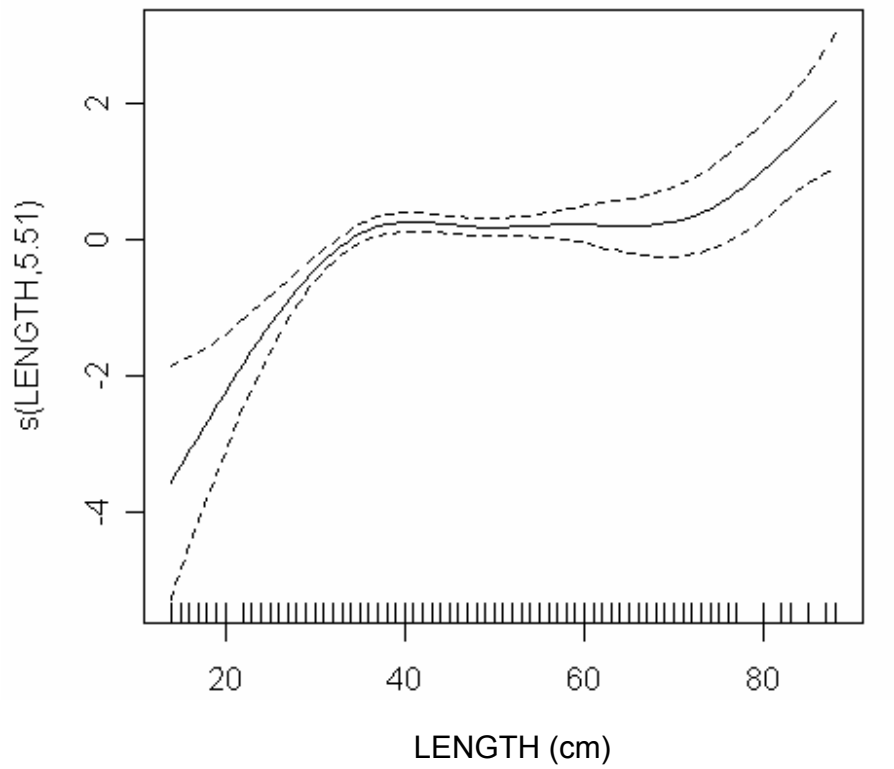


Fig.19. Partial fit of length for model of *Pseudoterranova* in monkfish. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.4.2.3 Presence / Absence data for all worms in monkfish

The presence / absence of worms was modelled using a binomial distribution (*i.e.* a distribution with two outcomes "present" and "absent"). Whilst the explained deviance of the model was only 6.85% (perhaps because the two worm species are, in effect, cancelling one another out in terms of factors having opposite effects on the two species), the model estimates indicate that:

- Fish with lengths up to 40cm had a significantly (p=3.1e-09) increasing probability of having parasites. Fish larger than 40 centimetres have similar probabilities, with a possible slight further rise in probability after 60cm (Figure 20).
- In 2006 the probability of having a parasite was significantly (p=2.64e-06) smaller than in year 2005.
- The probability of the monkfish having a parasite depended significantly (p=0.016) upon location of capture, with significantly lower probability of infection at site E than the reference site D in particular.

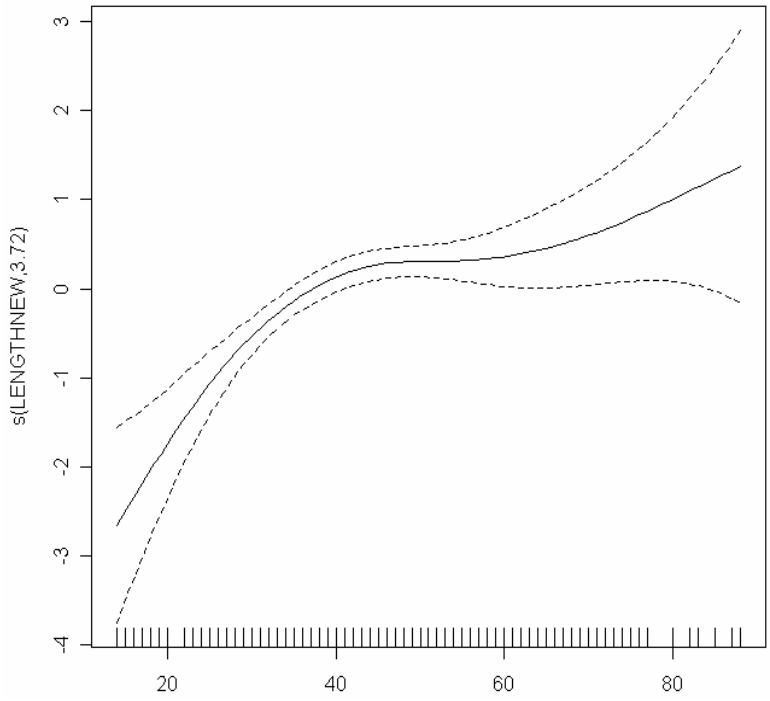


Fig.20. Partial fit of length for model of worm presence / absence data for monkfish. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

The results for the monkfish models overall, showing main terms only, are given in Table 3.

Table 3. Summary table of significant explanatory variables affecting worm numbers in monkfish in best models. X indicates a significant ($p<0.05$) effect. The significance of year may be an artefact of there being some different sample sites in the different years.

Response Variable	Host Length	Depth	Sex	Location	Year
Anisakis numbers	X	X	X	X	X
Pseudoterranova numbers	X	X	X	X	X
Worm Presence / Absence	X			X	X

3.4.3 Prevalence, mean intensity and abundance of worms in monkfish

Data were categorised as follows:

- Male=1, Female=2.
- Stage of ovary or testis maturity, categorised from 1-5 using FRS Marine Laboratory criteria for monkfish with Stage 1 being immature and Stage 5 being fully mature.
- Length class, categorised as 10-29 cm, 30-49 cm, 50-69 cm and 70-89 cm.
- Weight class, categorised as 1-1000g, 1001-2000g, 2001-3000g, 3001-4000g, 40001-5000g, 5001-6000g, 6001-7000g and 7001-8000g.
- Depth class, categorised as 1-100m, 101-200m, 201-300m.

The prevalence data for both *Anisakis* and *Pseudoterranova* infection in relation to sex, maturity stage, length class, weight class, depth class and location are illustrated in Figures 21 - 23.

Table 4 displays the mean intensity and abundance values for *Anisakis* and *Pseudoterranova* for the explanatory variables sex, maturity stage, length class, weight class, depth and location.

Figures 21 - 23 show that there was little difference in the prevalence of both *Anisakis* and *Pseudoterranova* in relation to sex. With regards to *Anisakis* infection and maturity stage, between stages 1 and 3, there was an increase in prevalence, and then a decrease to stages 4 and 5. For *Pseudoterranova*, prevalence increased from stage 1 to 2 and then decreased and levelled off for stages 3 to 5. Considering length class, both *Anisakis* and *Pseudoterranova* had a trend of increasing prevalence with an increase in length, this being more evident for *Anisakis*. This trend of increasing prevalence was not as noticeable when weight class was examined. When the relationship between depth class and *Anisakis* and *Pseudoterranova* prevalence was examined, there was a trend of increasing prevalence of *Anisakis* with an increase in depth and a converse relationship for *Pseudoterranova*. A similar picture emerges in relation to the location data. For *Anisakis*, there was an increase in prevalence from area A to area E, however, the prevalence in area F (Rockall) was similar to that of

area A. In contrast, *Pseudoterranova* prevalence increased from area A to area E, although prevalence in area F was similar to that of E. Note that there were a small number of monkfish sampled from area B, but nematodes were not detected.

Table 4: Abundance and Mean intensity data for *Anisakis* and *Pseudoterranova* in relation to sex, maturity, length class, weight class, depth class and location in monkfish

		Anisakis Abundance	*Pseudoterranova* Abundance	*Anisakis* Mean Intensity	*Pseudoterranova* Mean Intensity
Sex	M	0.3	0.7	1.7	1.7
	F	0.3	0.8	1.7	2.2
Maturity Class	1	0.2	0.6	1.6	1.9
	2	0.4	0.9	1.7	2.1
	3	0.5	0.3	1.6	1.3
	4	0.3	0.5	4.0	1.8
	5	0.3	0.2	2.7	1.0
Length (cm)	10-29	0.1	0.2	1.8	1.6
	30-49	0.3	0.8	1.6	1.9
	50-69	0.4	0.8	1.9	1.9
	70-89	0.4	1.3	1.7	4.7
Weight (g)	1-1000	0.2	0.6	1.5	1.9
	1001-2000	0.3	0.8	1.8	1.8
	2001-3000	0.4	0.5	2.2	1.6
	3001-4000	0.6	1.0	1.4	1.6
	4001-5000	0.4	0.3	1.8	1.0
	5001-6000	0.3	0.2	1.5	1.0
	6001-7000	0.3	6.0	2.5	12.0
	7001-8000	1.0	0.3	1.3	1.0
Depth (m)	1-100	0.2	0.9	1.5	2.0
	101-200	0.3	0.6	1.8	1.9
	201-300	0.4	0.3	1.5	1.2
Location	A	0.2	0.9	1.6	1.7
	B	0.0	0.0	0.0	0.0
	C	0.3	0.8	1.5	1.8
	D	0.3	0.6	1.9	2.0
	E	0.4	0.5	1.5	3.1
	F	0.2	0.2	1.3	1.0

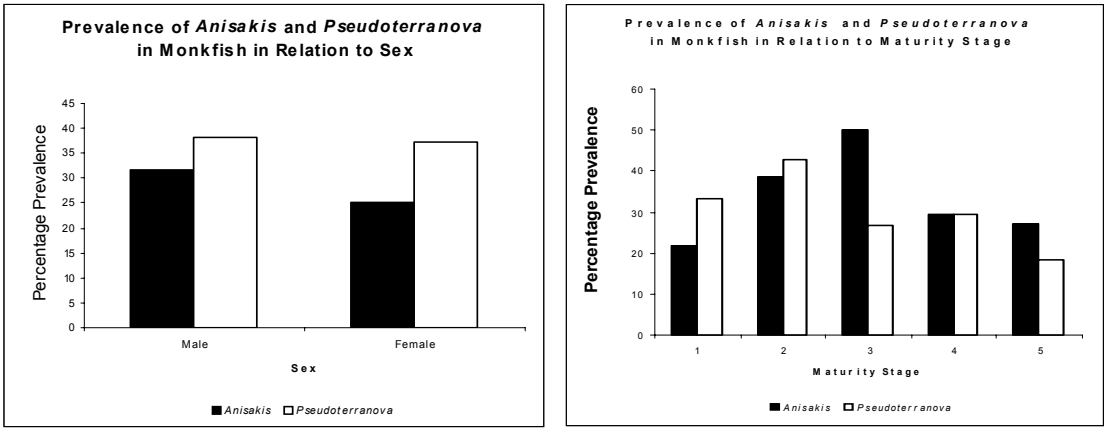


Figure 21: Prevalence of *Anisakis* and *Pseudoterranova* in monkfish in relation to sex and maturity stage.

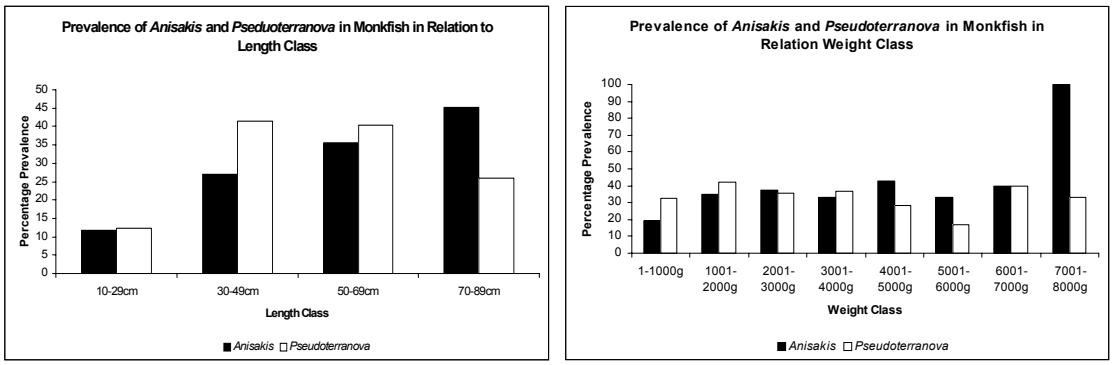


Figure 22: Prevalence of *Anisakis* and *Pseudoterranova* in monkfish in relation to length and weight class.

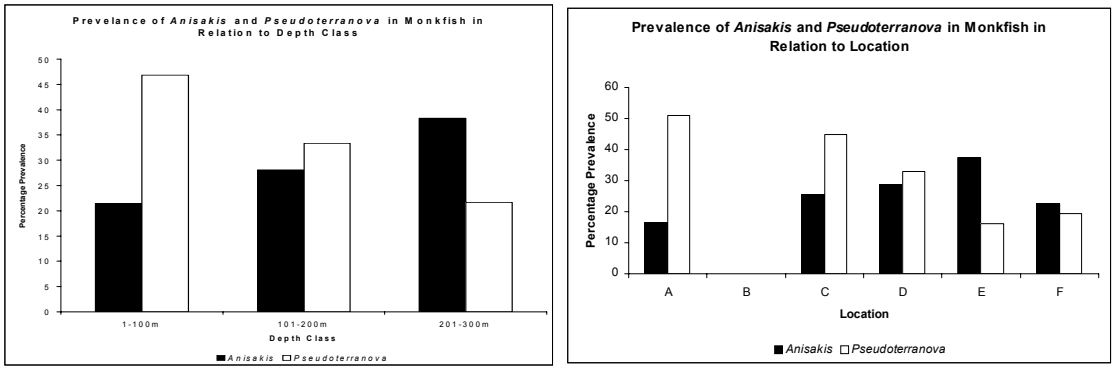


Figure 23: Prevalence of *Anisakis* and *Pseudoterranova* in monkfish in relation to depth class and location.

The abundance and mean intensity of monkfish infection, with respect to the principal variables measured, are shown in Figures 24-26. With respect to depth, *Anisakis* abundance increased with depth whilst *Pseudoterranova* abundance decreased. Whilst *Anisakis* mean intensity appeared to fall with increasing depth, *Pseudoterranova* intensity appeared relatively stable with a slight increase in the middle depth class (Figure 24).

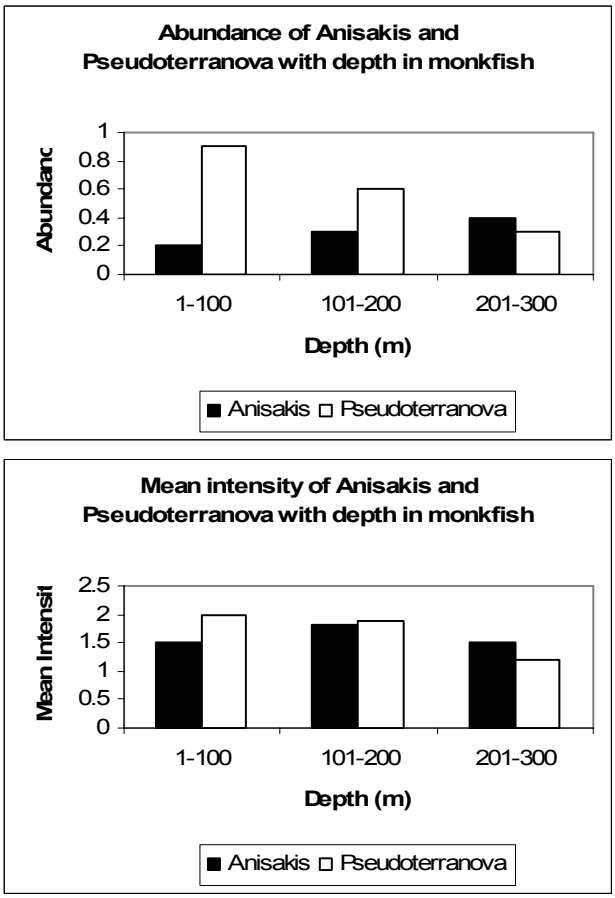


Figure 24: Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in monkfish in relation to depth class.

The abundance of *Anisakis* increased between fish captured at locations A and E (excepting location B where no worms were recovered) but was low in fish captured at location F. *Pseudoterranova* abundance, however, was lower in those fish caught from location A compared to those from location F (similarly excepting location B). The mean intensity of *Anisakis* and *Pseudoterranova* varied according to sampling location, with highest *Anisakis* abundance at location D and highest *Pseudoterranova* abundance at location E (Figure 25).

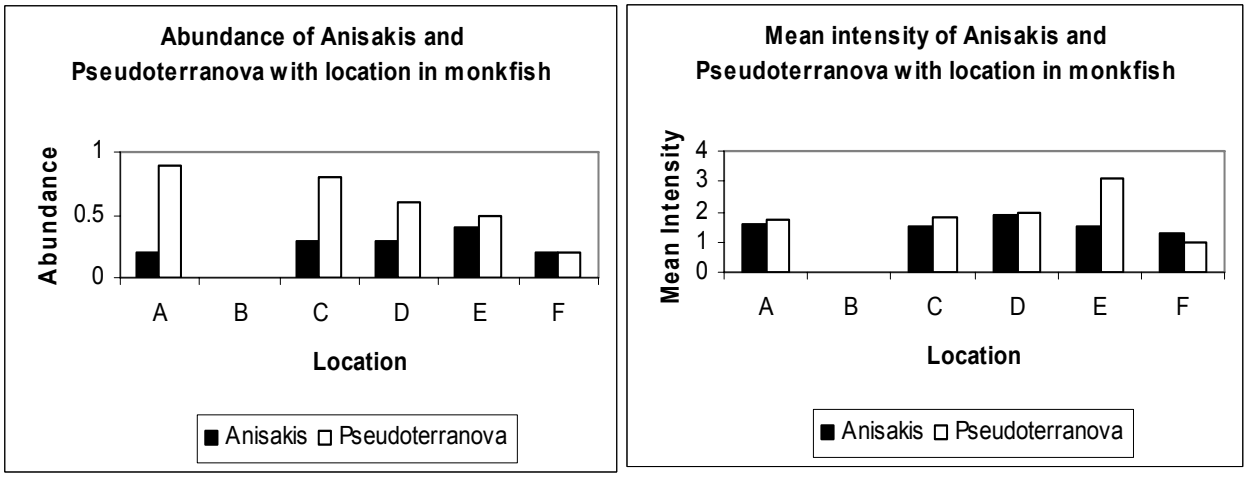


Figure 25: Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in monkfish in relation to location

Anisakis showed greatest abundance in maturity class 3. The abundance of *Pseudoterranova* was greatest for maturity class 2, with lowest numbers in maturity class 5. Mean intensity of *Anisakis* was highest in maturity classes 4 and 5, whilst that of *Pseudoterranova* had lowest numbers in maturity classes 3 and 5 (Figure 26).

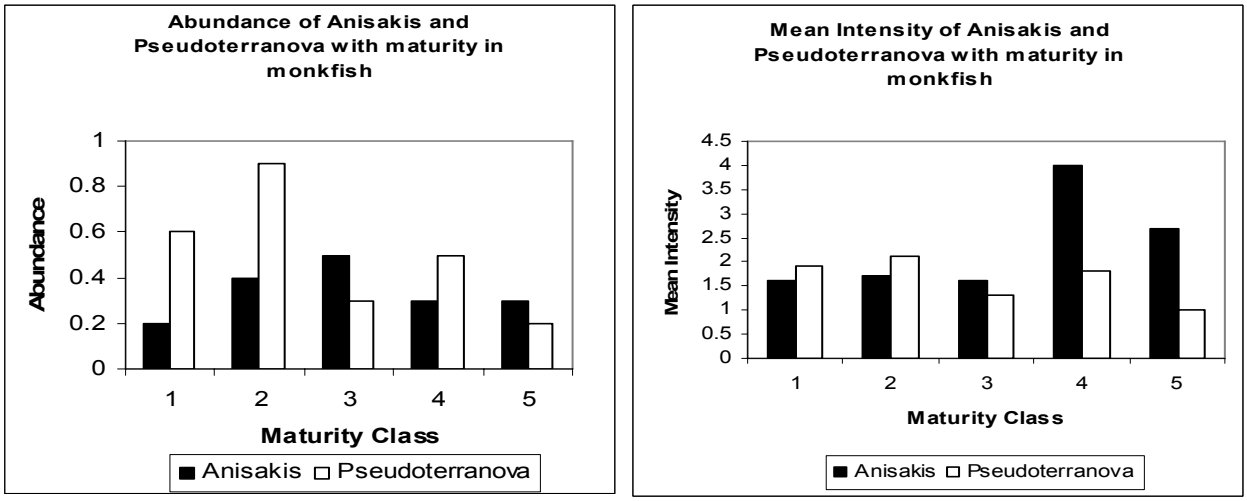


Figure 26: Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in monkfish in relation to maturity class

The abundance of both *Anisakis* and *Pseudoterranova* increased with increasing fish length, whilst the mean intensity of infection for both species was relatively stable save for a large increase for *Pseudoterranova* in length class 4 (Figure 27).

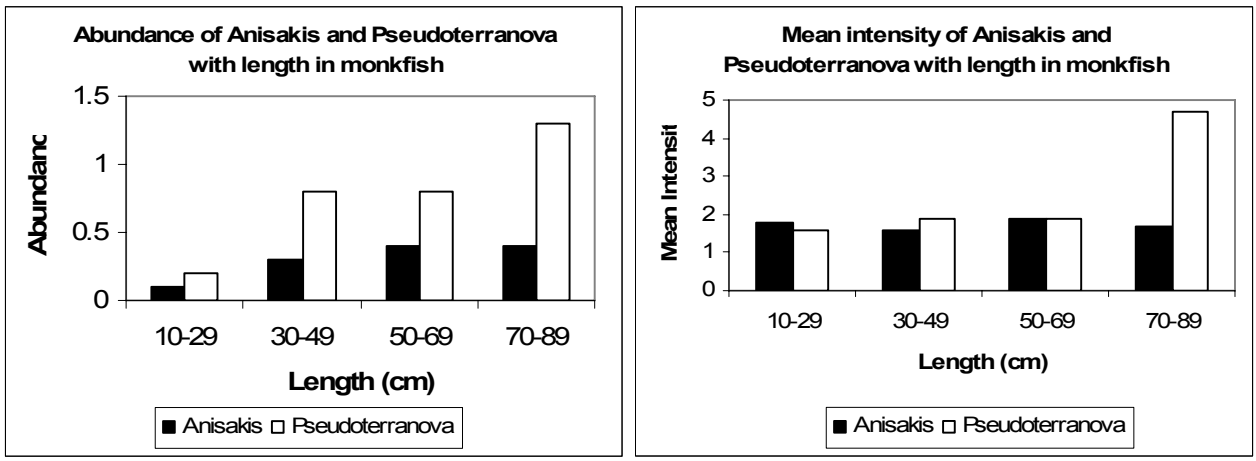


Figure 27: Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in monkfish in relation to length class

3.4.4 Body Distribution and Detection Methods

The distribution of *Anisakis* and *Pseudoterranova* within the flesh of monkfish was determined by visual inspection, candling and slicing. The distribution of nematodes within the flesh was divided into those obtained from left and right sides and also those derived from flaps and fillets. The mean numbers of *Anisakis* and *Pseudoterranova* in relation to body distribution are illustrated in Figures 28 - 31. Wilcoxon Matched Pairs Tests highlighted significant differences in the number of *Anisakis* between both the belly flap and the fillet ($p=0.0002$) and also between the left and right sides ($p<0.000001$). Similarly, significant differences for *Pseudoterranova* were detected between the belly flap and the fillet ($p<0.000001$) and the left and right sides ($p=0.000759$). With regards to *Anisakis* infection, a significantly greater number were detected within the belly flap compared to the flesh ($p=0.0002$). This however was reversed for *Pseudoterranova* with more being detected within the fillet ($p=0.000001$).

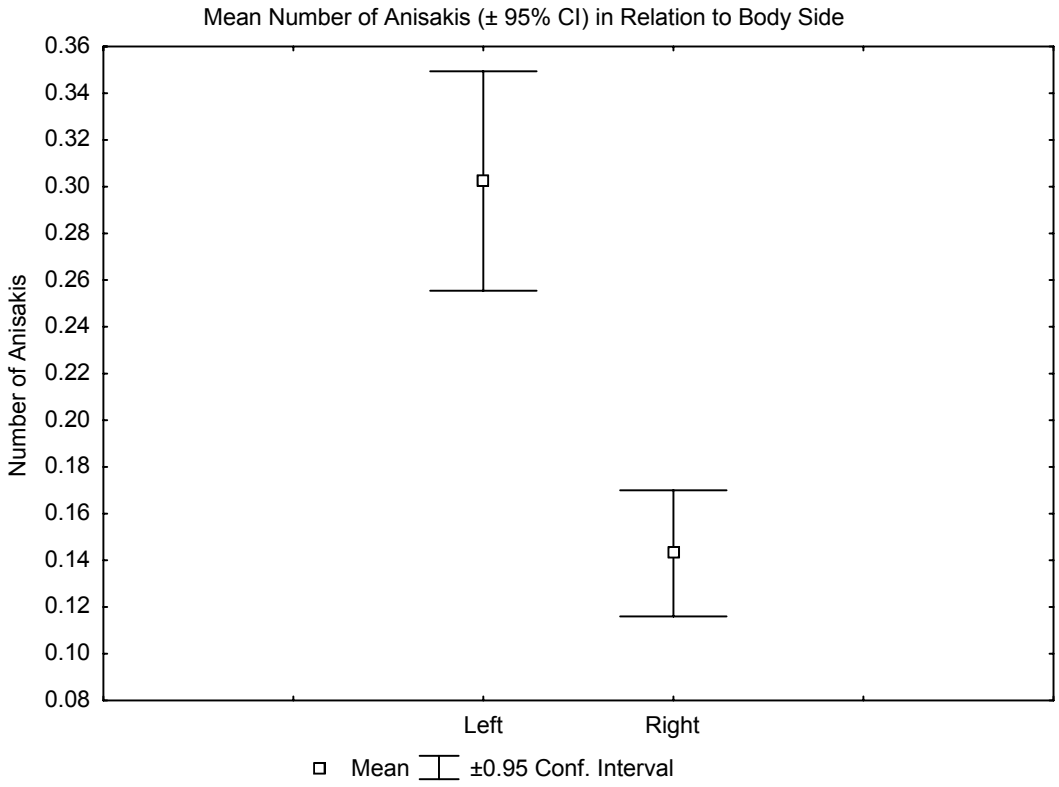


Figure 28: Mean number of *Anisakis* in monkfish in left and right sides of fish.

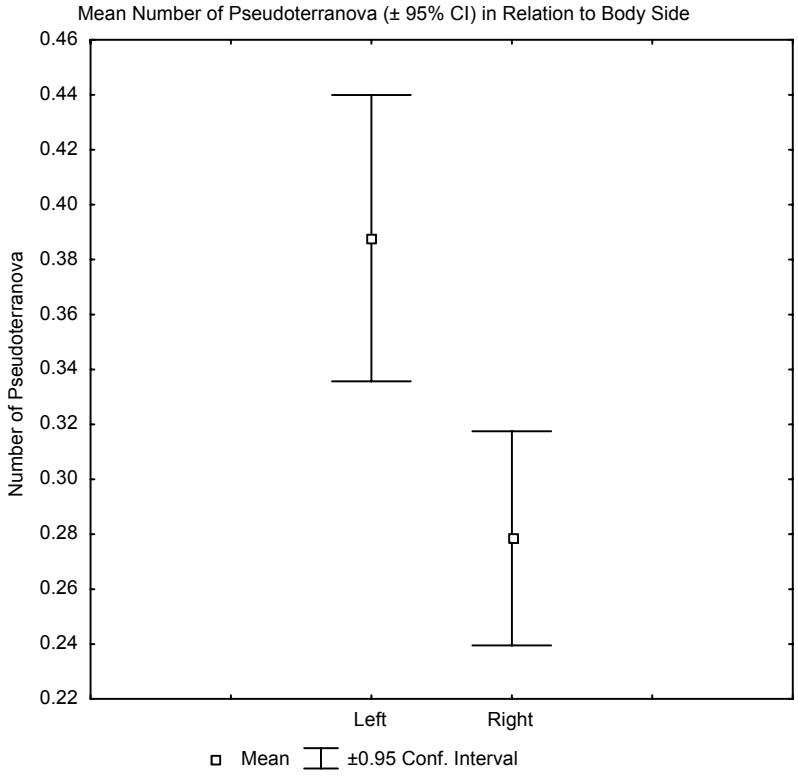


Figure 29. Mean number of *Pseudoterranova* in monkfish in left and right sides of fish.

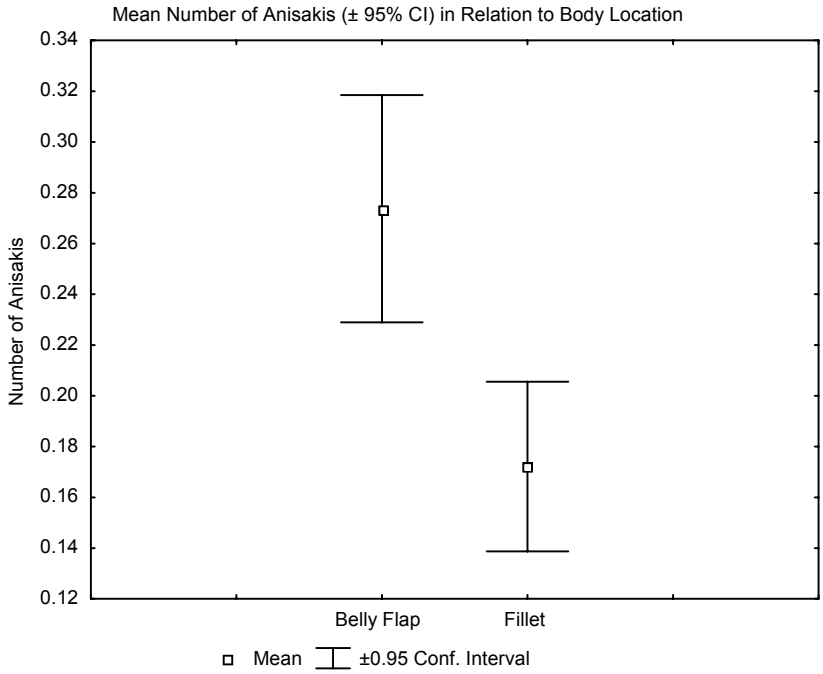


Figure 30. Mean number of *Anisakis* in monkfish in flaps and fillets

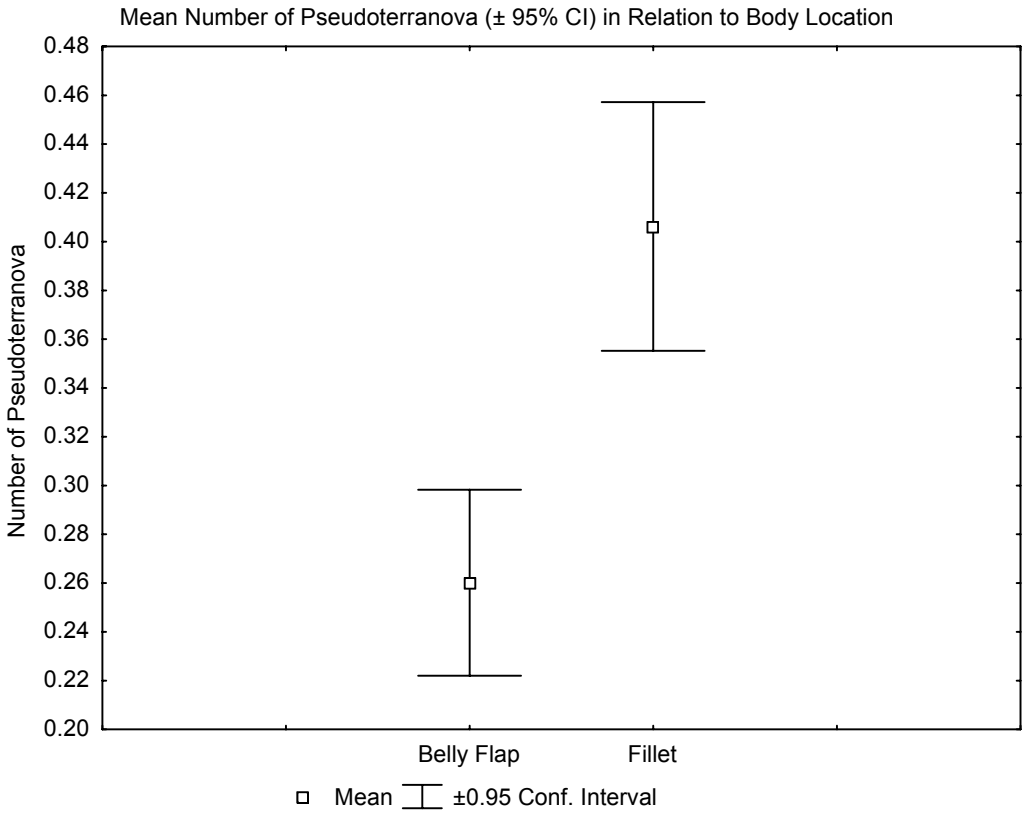


Figure 31. Mean number of *Pseudoterranova* in monkfish in flaps and fillets

When comparing the distribution of the nematodes in the sides of the fish using Wilcoxon's Matched Pairs Test, both *Anisakis* and *Pseudoterranova* were found in significantly higher numbers on the left side of the fish ($p<0.000001$ and $p=0.000759$ respectively). Figures 32 and 33 illustrate the overall prevalence, mean intensity and abundance of *Anisakis* and *Pseudoterranova* infection in monkfish in relation to side and belly flap and fillets.

When considering detection methods, three methods were investigated. The results are presented with the assumption that candling would detect those worms already detected by direct visual inspection (E) as well as those detected by candling alone (termed E + C, with the C representing candling) and that slicing (termed E + C + S, with the S representing slicing) would detect all the worms previously detected by both visual inspection and candling. The means for the three methods employed to detect *Anisakis* and *Pseudoterranova* in belly flaps and fillets are illustrated in Figures 34 to 37. Statistical analysis, using a Friedman ANOVA, highlighted significant differences ($p<0.001$) between the 3 detection methods when considering either *Anisakis or Pseudoterranova* in the belly flap and fillet. The numbers of *Anisakis* and *Pseudoterranova* and the percentage of the total worms detected by these 3 methods are provided in Table 5.

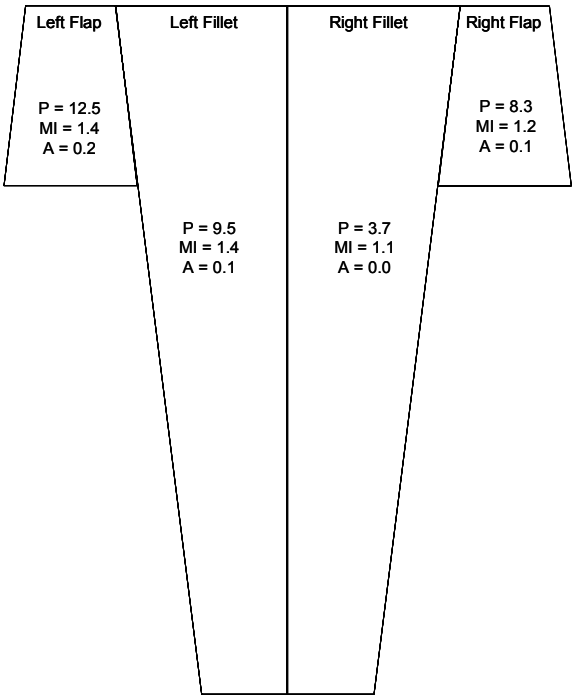


Figure 32. Overall prevalence (P), mean intensity (MI) and abundance (A) for *Anisakis* in monkfish flesh in relation to left, right, flaps and fillets

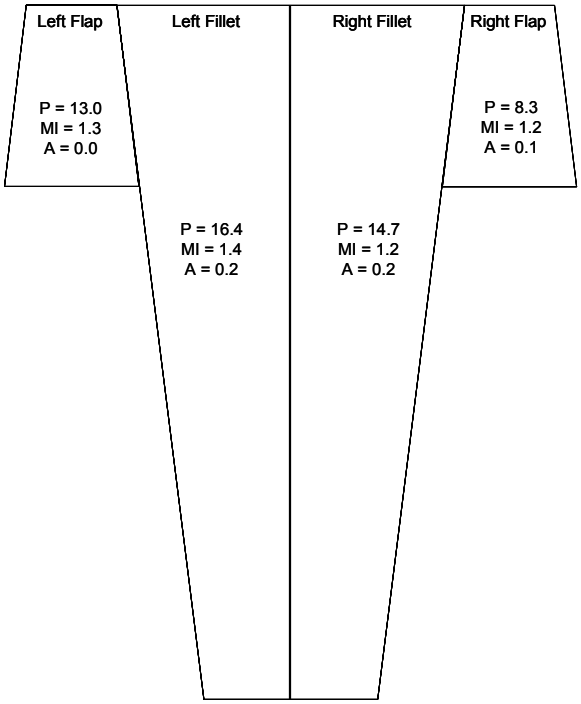


Figure 33. Overall prevalence (P), mean intensity (MI) and abundance (A) for *Pseudoterranova* in monkfish flesh in relation to left, right, flaps and fillets

Table 5: Numbers of and percentage of total *Anisakis* and *Pseudoterranova* detected by the 3 detection methods in belly flap and fillets in monkfish.

		Anisakis		*Pseudoterranova*	
		N	%	N	%
Flaps	Eye	34	11.6	221	72.9
	E + C	278	94.9	293	96.7
	E + C + S	293	100	303	100
Fillets	Eye	26	14	121	26.9
	E + C	31	16.8	143	31.8
	E + C + S	185	100	450	100

From the results it can be seen that within the flaps, very few (11.6%) of *Anisakis* are detected by visual examination, with the majority (94.9%) detected by candling. For *Pseudoterranova* present in the flaps, the majority are detected by visual examination (72.9%) and/or candling (96.7%). When considering the fillets, visual examination and

candling only account for 16.8% of *Anisakis* that are present. For *Pseudoterranova*, visual examination and candling only detect 31.8% of the worms present.

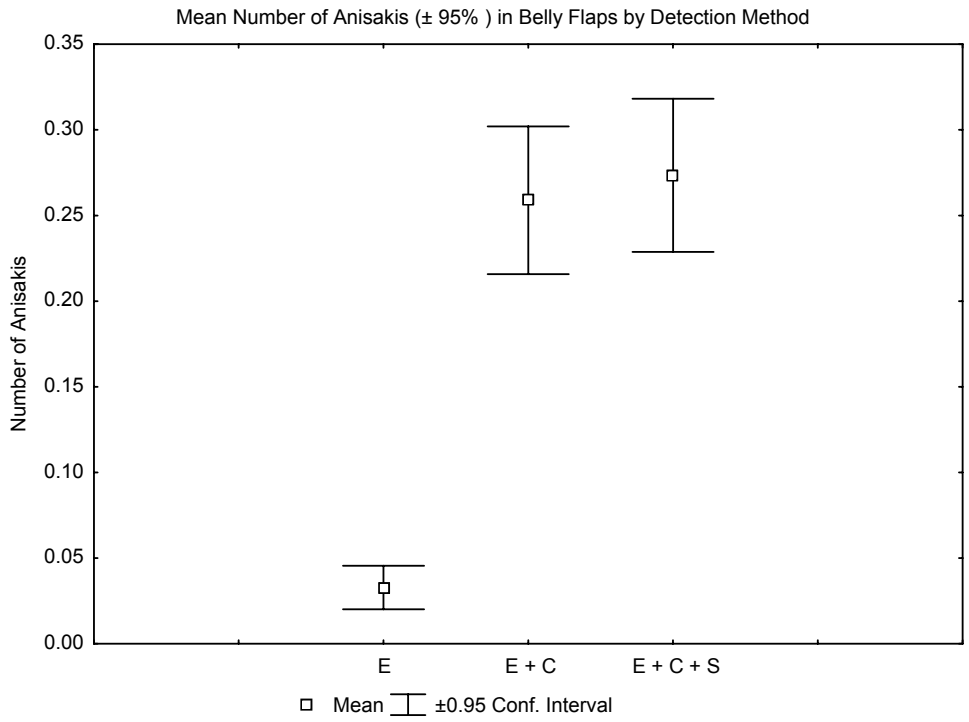


Figure 34. Mean number of *Anisakis* in monkfish belly flaps detected by each of the three detection methods.

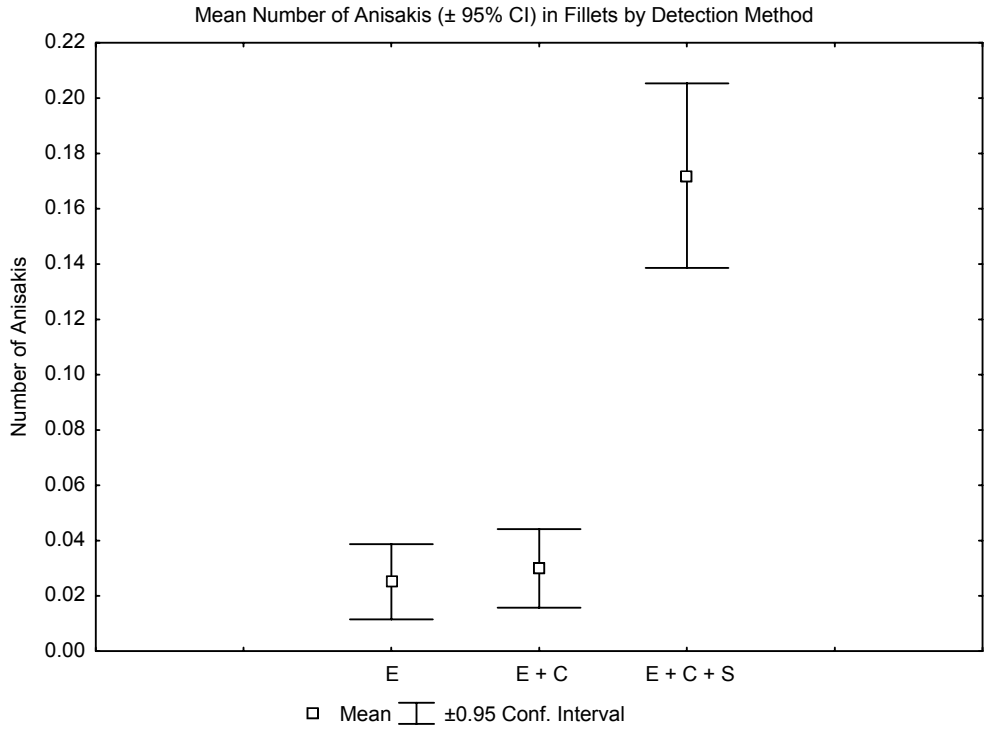


Figure 35. Mean number of *Anisakis* in monkfish fillets detected by each of the three detection methods.

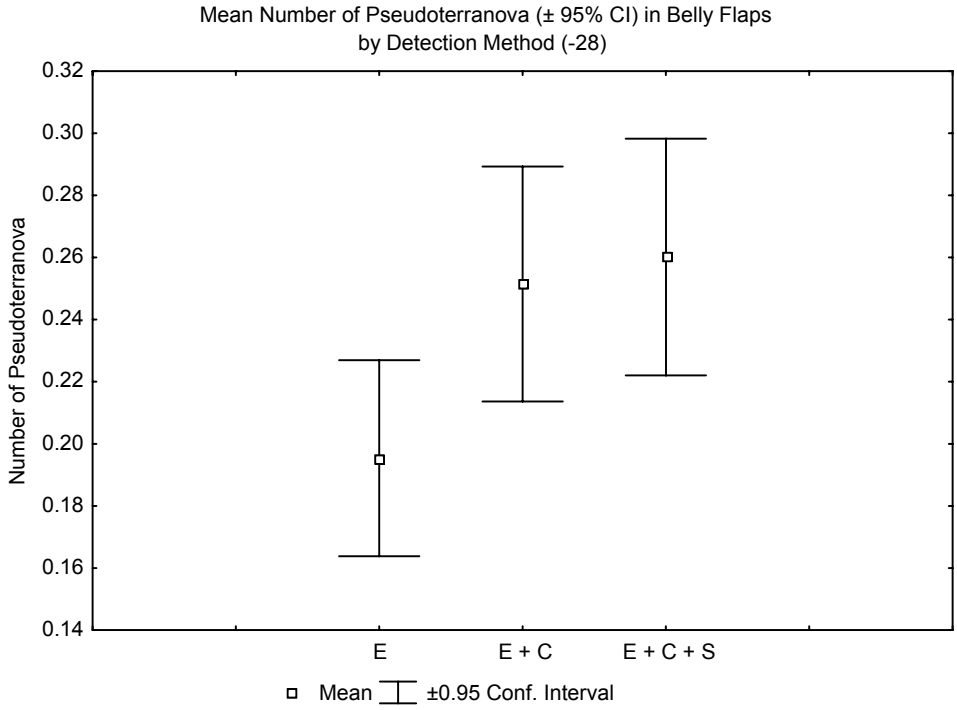


Figure 36. Mean number of *Pseudoterranova* in monkfish belly flaps detected by each of the three detection methods

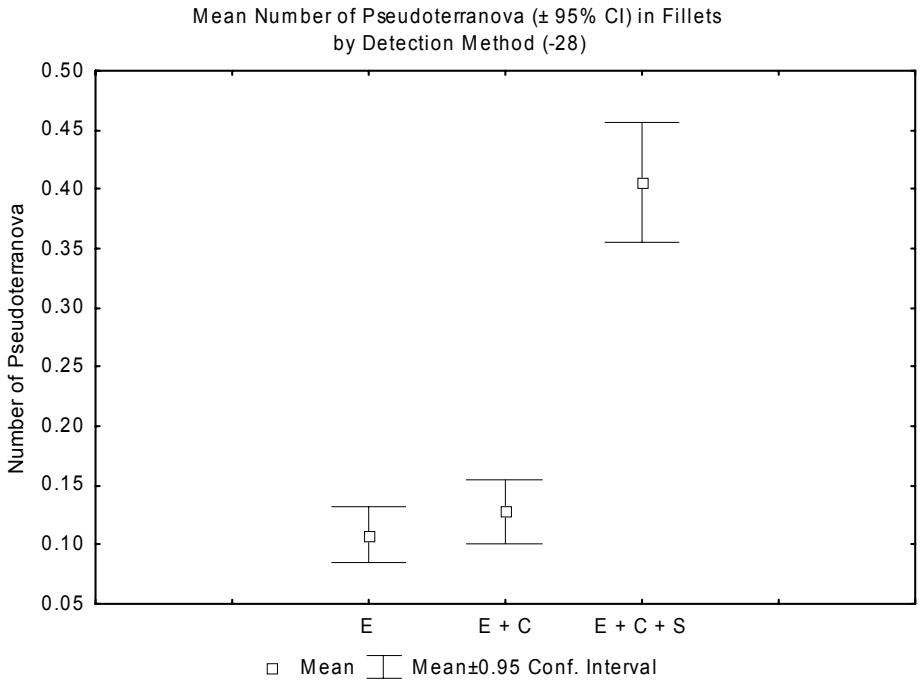


Figure 37. Mean number of *Pseudoterranova* in monkfish fillets detected by each of the three detection methods

Experiments carried out to determine the maximum depth of fillet for which candling is still effective for detecting worms, indicated that worms could not be detected in fillets more than 2.5 cm thick. Figure 38., which shows the flesh thickness plotted against fish length, shows that the maximum thickness of flesh for candling (~2.5cm) occurs at a fish length of ~37cm. This means that, to all intents and purposes, candling alone can not be used to detect worms in monkfish of a commercial size.

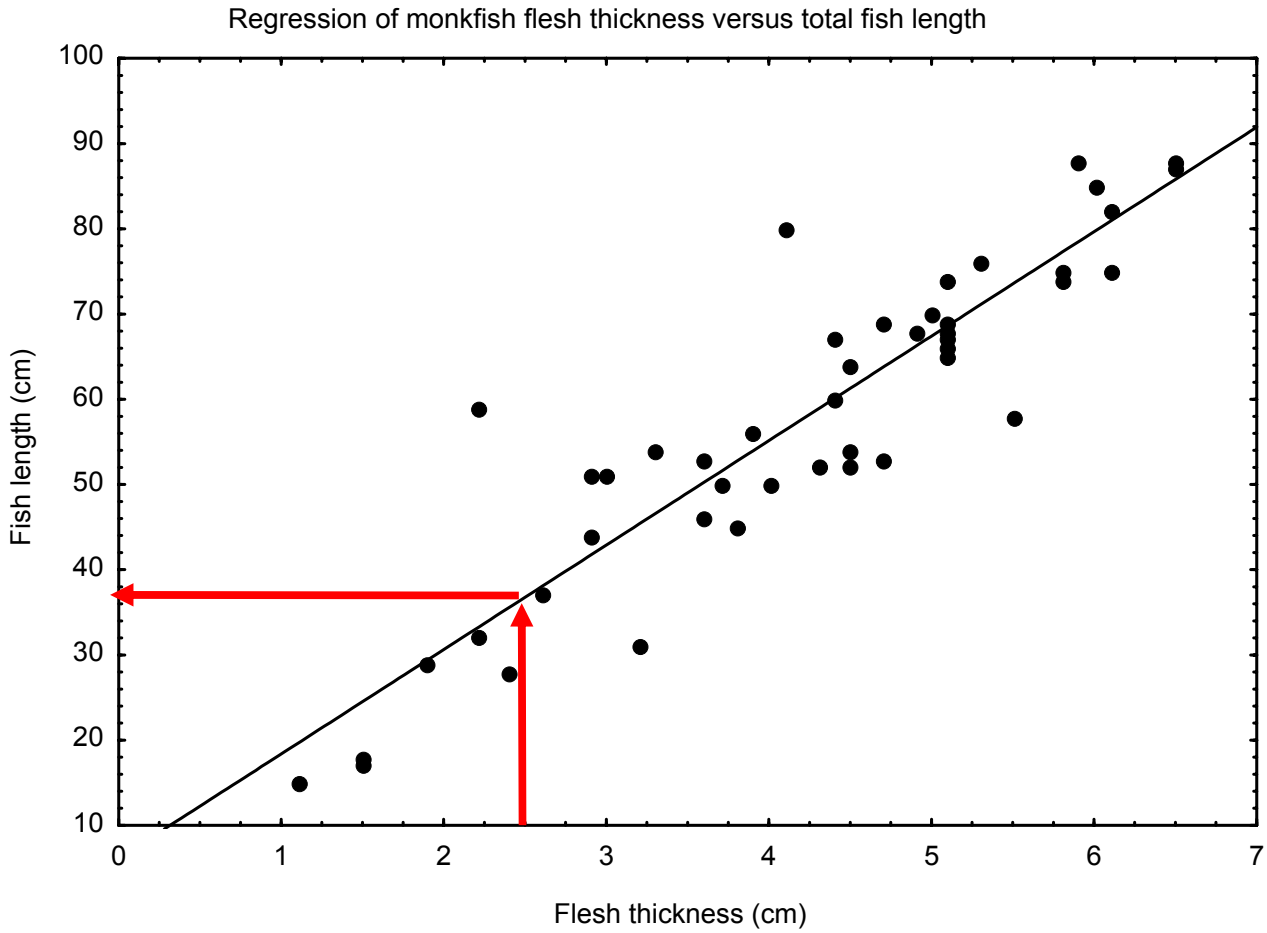


Figure 38. Regression of flesh thickness against fish length. Candling allows worm detection to a depth of ~2.5cm equating to a fish length of circa 37cm (see arrows).

3.5 Cod

A total of 372 cod were examined for the presence of *Anisakis* and *Pseudoterranova*. Figure 39. shows a single cod fillet examined using a UV trans-illuminator with encapsulated *Anisakis* larvae apparent as bright fluorescent spots in the flesh.

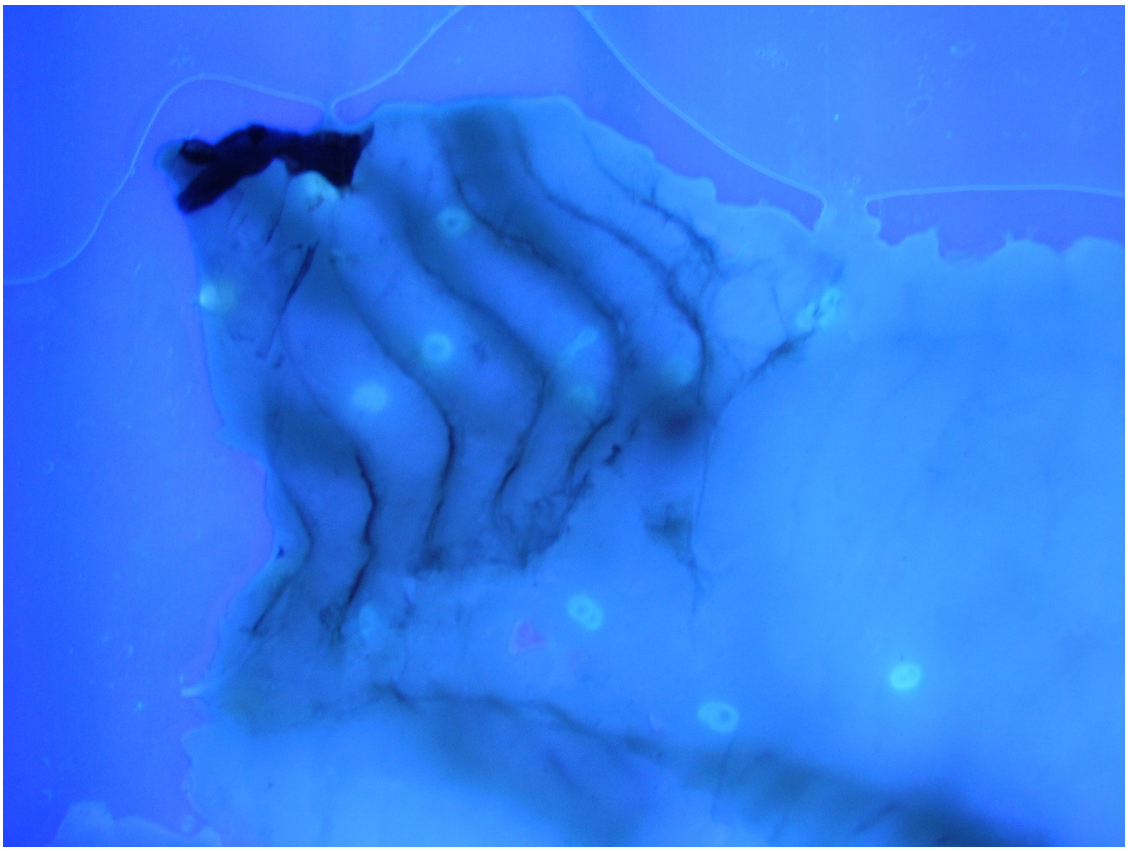


Figure 39. Encapsulated *Anisakis* larvae apparent as bright fluorescent spots in flesh of cod illuminated by UV light.

The calculated prevalence, mean intensity and abundance for *Anisakis* in cod were 50.3%, 6.49 and 3.26, respectively. The prevalence, mean intensity and abundance for *Pseudoterranova* in cod were 37.9%, 6.65 and 2.52, respectively.

Figures 40 and 41 show the frequency of cod with different levels of *Anisakis* and *Pseudoterranova* infection captured from the six sampling locations. For both species of worm, fish carrying no worms are in the majority and most fish carry few worms (although generally more than monkfish). It should also be noted that there is considerable variation in worm number recovered with respect to site of capture.

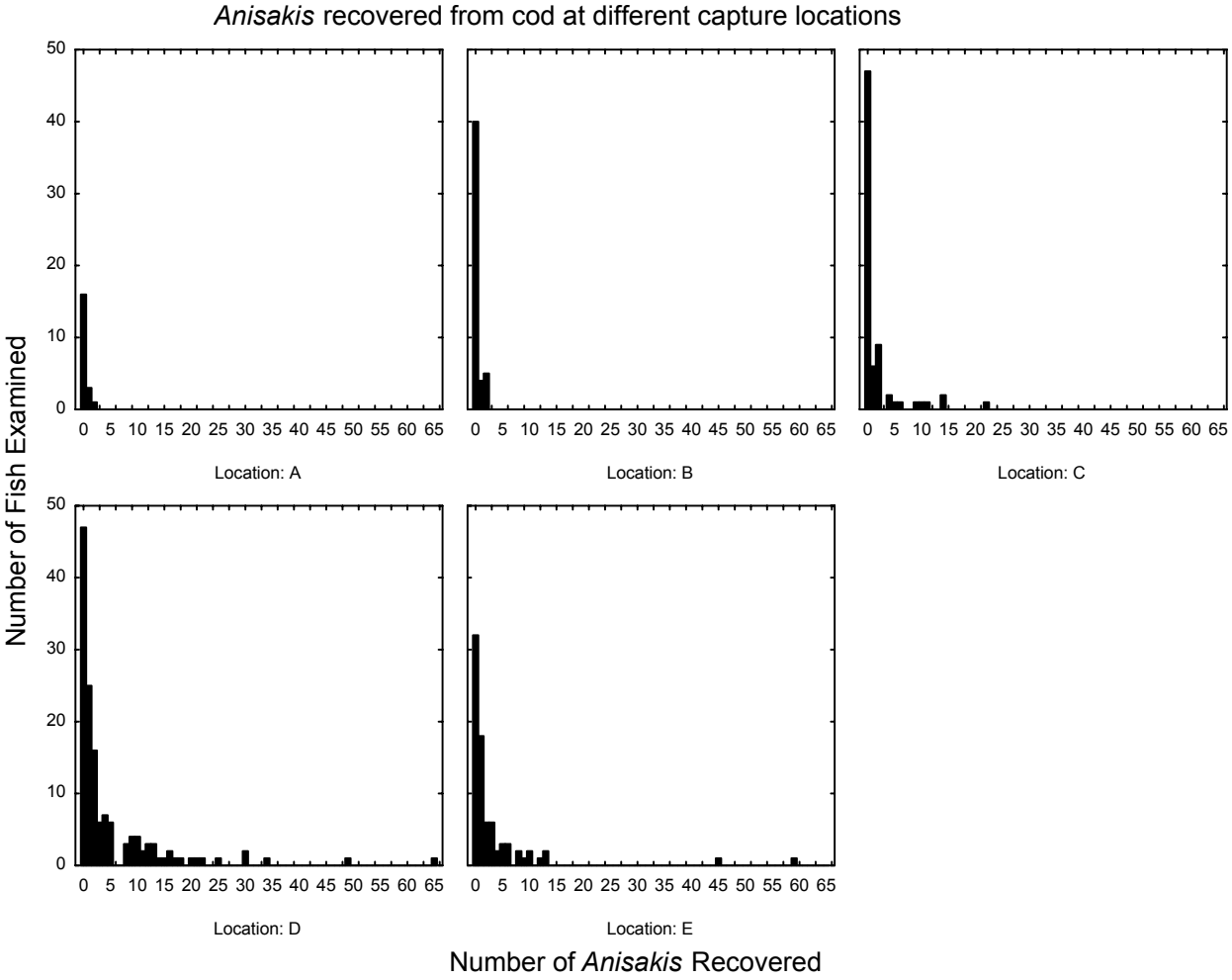


Figure 40. Histogram showing frequency of different infection loads for *Anisakis* in sampled cod.

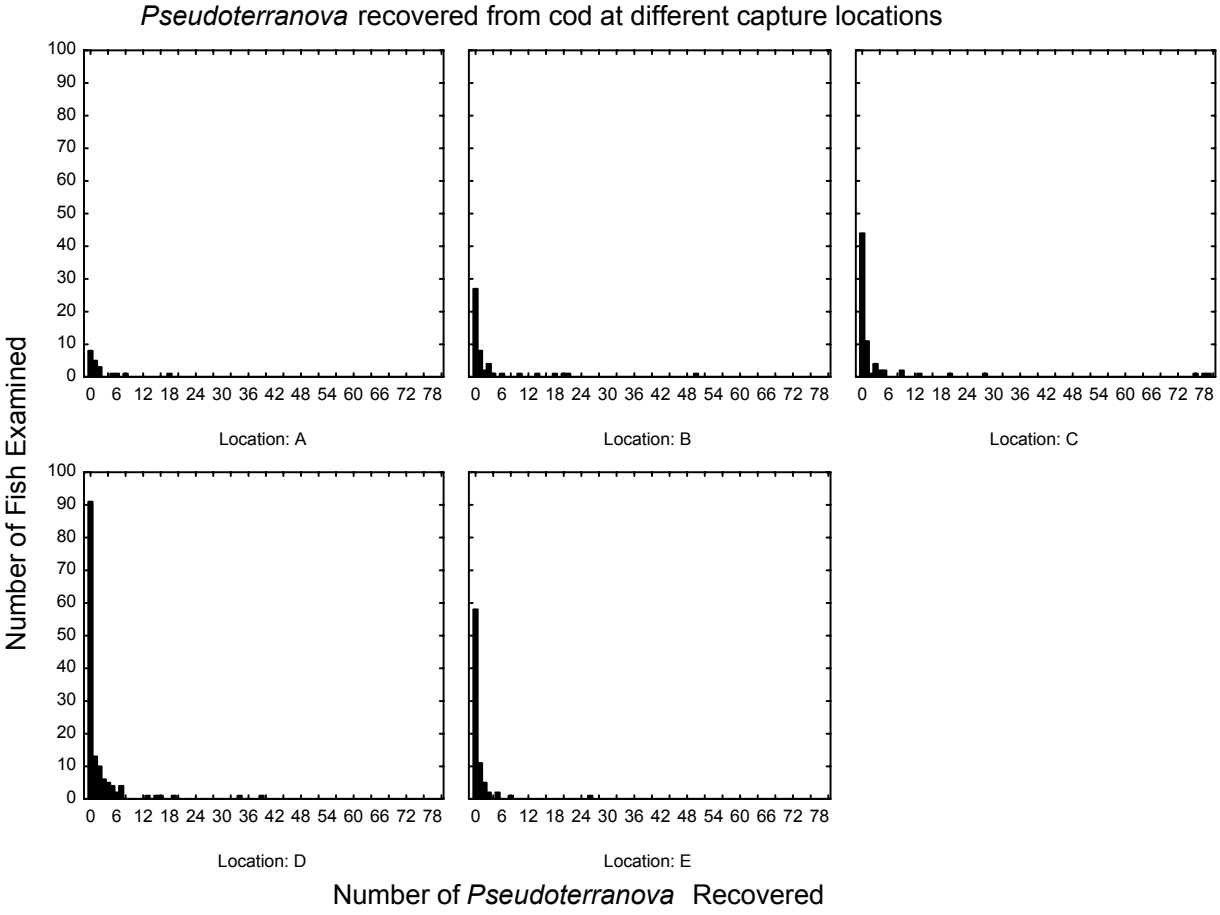


Figure 41. Histogram showing frequency of different infection loads for *Pseudoterranova* in sampled cod.

3.5.1 Single factor statistics

The statistical significance of a number of measured factors, upon *Anisakis* and *Pseudoterranova* numbers in cod, was assessed by Mann-Whitney analysis and the results were as follows:

Sex
There were no significant differences between the sexes, with values of $p_{(adjusted)}=0.69$ for *Anisakis* and $p_{(adjusted)}=0.348$ for *Pseudoterranova*. (Figure 42-43)

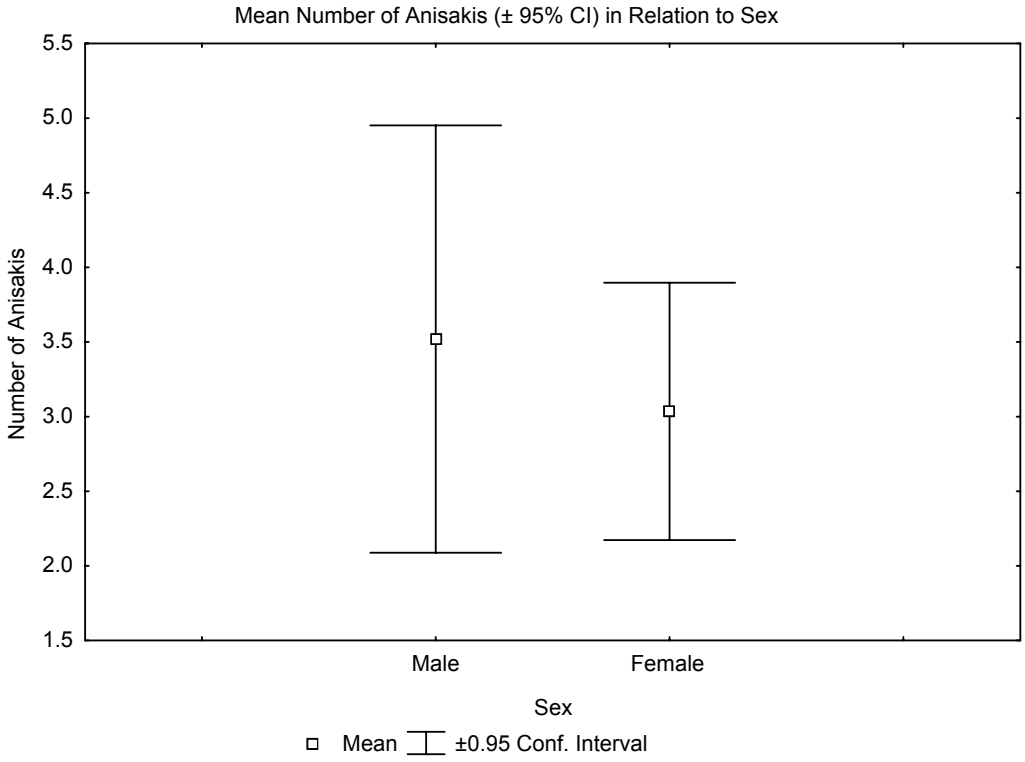


Figure 42. Mean Number of *Anisakis* in cod in relation to sex

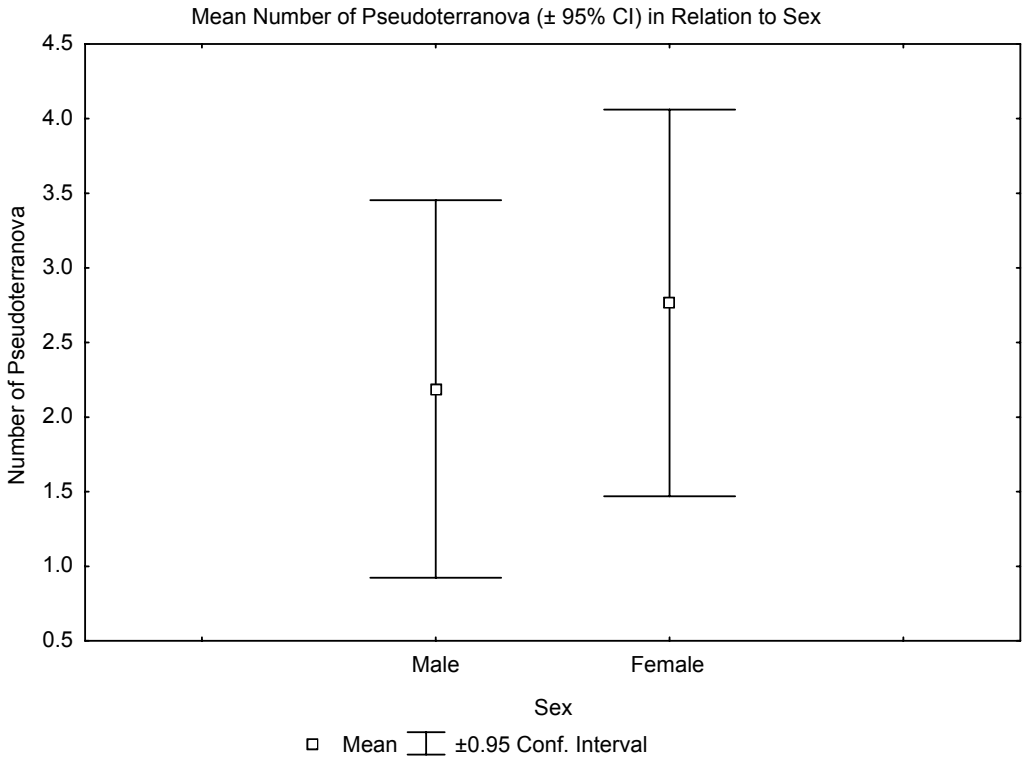


Figure 43. Mean Number of *Pseudoterranova* in cod in relation to sex

Temporal variation

Anisakis: Statistical analysis highlighted a significant difference between the 2 years, $p_{(adjusted)} < 0.001$, with lower numbers obtained in 2006 (Figure 44).

Pseudoterranova: Statistical analysis showed that there was no significant difference ($p_{(adjusted)} = 0.083$) between years for this species.

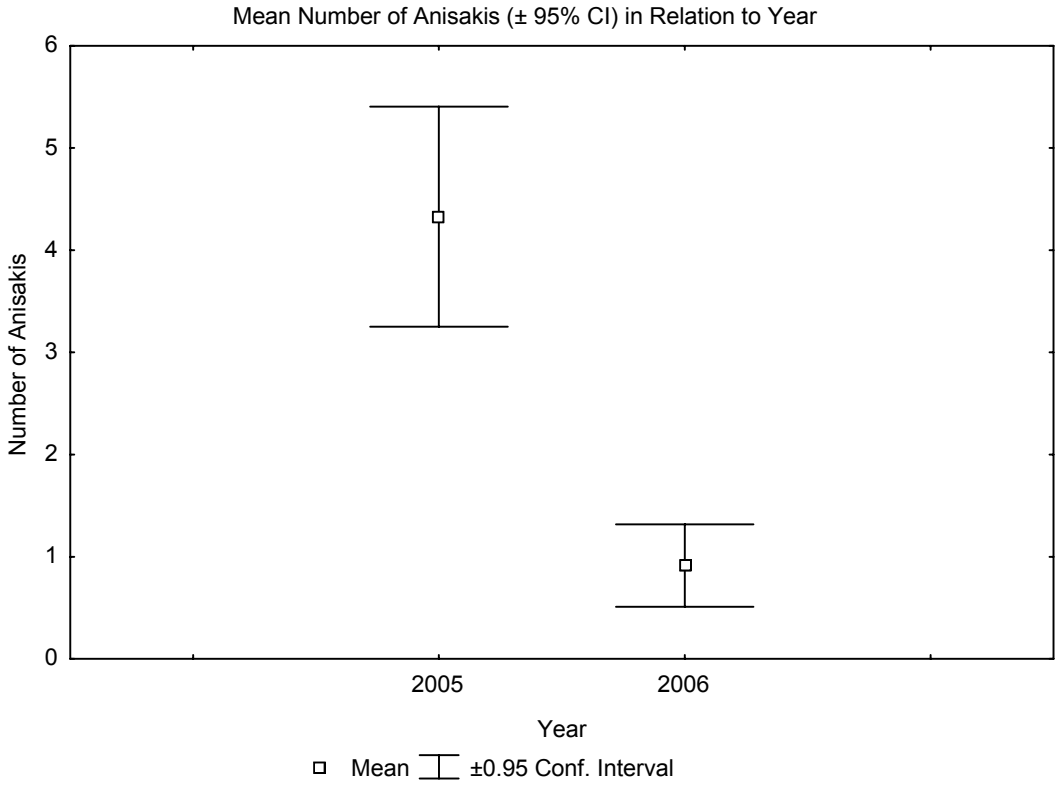


Figure 44: Mean Number of *Anisakis* in cod in relation to year

3.5.2 Analysis of multiple factors

3.5.2.1 Total *Anisakis* numbers in cod

The best model for *Anisakis* numbers in cod explained 38% of the "deviance", a measure of the total variability in numbers between fish samples. The following represent the principal findings of the model:

- Length had a significant ($p<1.11e-9$) effect on *Anisakis* numbers in cod, with a steeply increasing probability of more parasites from ~40cm-~60cm. At 60cm and above, there was a lesser effect of length on *Anisakis* numbers, although they still

appear to increase with length. However, there are fewer sampled fish in this size category, hence the confidence limits are broader (Figure 45).

- Location had a significant (p=1.82e-7) effect on *Anisakis* numbers in cod, with locations B, C and A having significantly lower probabilities of higher numbers of *Anisakis* than location D (Shetland).

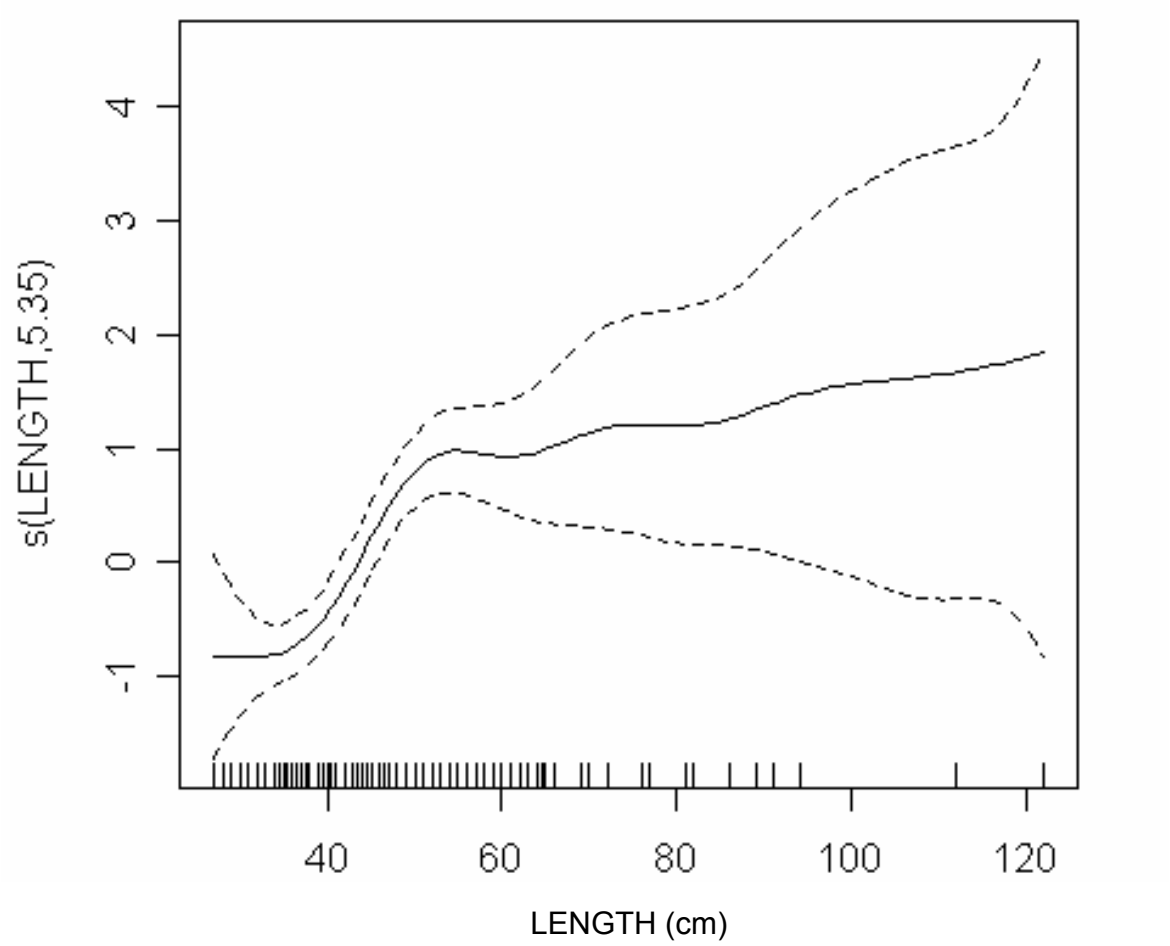


Figure 45. Partial fit of length for model of *Anisakis* in cod. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.5.2.2 Total *Pseudoterranova* numbers in cod

The best model for *Pseudoterranova* numbers in cod explained 38% of the deviance (a measure of the total variability in numbers between fish samples). The following represent the principal findings of the model:

- Length had a highly significant (p=5.76e-9) effect on *Pseudoterranova* numbers with steeply increasing numbers occurring from ~30cm-~60cm. Above 60cm the rate of increase flattens off, with more stability in numbers occurring above 80cm. Above 80cm it should, however, be noted that there are relatively large confidence limits associated with the estimate (Figure 46).
- Depth was a significant (p=0.0151) factor in determining *Pseudoterranova* numbers in cod, this effect displaying a modal distribution with relatively little risk up to ~80m, peak levels at ~150m and decreasing levels thereafter. It should be noted that confidence limits at the lowest and highest depths are relatively wide making the tails of the prediction more uncertain.
- There was a significant effect of location (p=0.000427) on *Pseudoterranova* numbers, with sites B and C having significantly higher probabilities of increased numbers than the baseline site D (with site A almost significant p=0.071).
- Sex was a significant (p=0.039) determinant of numbers, with an increased probability of higher numbers associated with female fish.

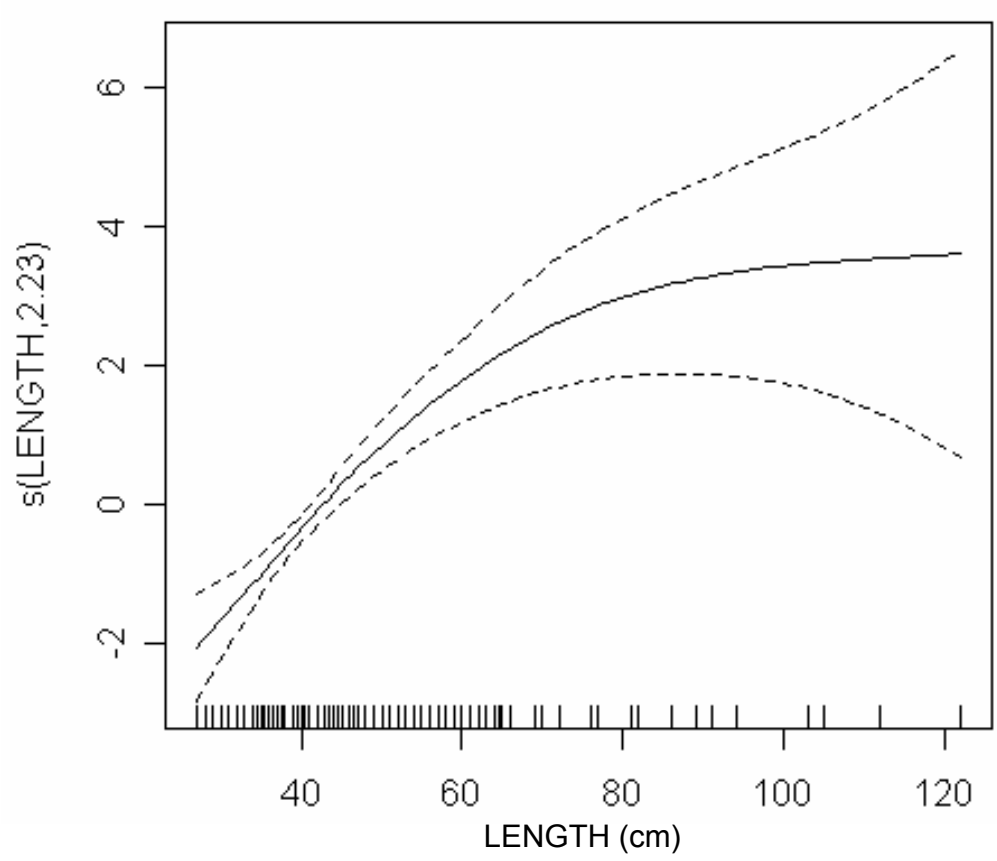


Figure 46. Partial fit of length for model of *Pseudoterranova* in cod. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.5.2.3 Presence / Absence data for all worms in cod

The presence / absence of worms in cod was modelled using a binomial distribution (*i.e.* a distribution with two outcomes "present" and "absent").The best binomial model explained 10.6% of the deviance, a measure of the total variability in numbers between fish samples, in the worm presence / absence data, with the following effects predominating:

- Overall, there was a significant (5.02e-06) positive effect of length on the presence of worms in cod.
- Depth had a significant (p=0.023) effect on worm presence in cod. The wide confidence limits at the highest and lowest depths sampled, however, make interpretation of the true relationship difficult, although there appears to be a peak of infection at ~120m with lower probabilities at lesser and greater depths.

Table 6. provides a summary of the findings for models of multiple factors affecting worm numbers in cod.

Table 6. Summary table of significant explanatory variables affecting worm numbers in cod in best models. X indicates a significant ($p<0.05$) effect. The significance of year may be an artefact of different sample sites in different years.

Variable	Host Length	Depth	Sex	Location	Year
Anisakis numbers	X			X	
Pseudoterranova numbers	X	X		X	
Worm Presence / Absence	X	X			

3.5.3 Prevalence, mean intensity and abundance of worms in cod

The following data are categorised as follows:

- Male=1, Female=2.
- Stage of ovary or testis maturity, categorised from 1-4 using FRS Marine Laboratory criteria for cod with Stage 1 being immature.
- .Length class, categorised as 10-29 cm, 30-49 cm, 50-69 cm and 70-89 cm, 90-109 cm and 110-129 cm.
- Weight class, categorised as 1-500g to 8001-8500g in 500g intervals.
- Depth class, categorised as 1-100m, 101-200m, 201-300m.

The relationship of prevalence in both *Anisakis* and *Pseudoterranova* infections to the variables sex, maturity stage, length class, weight class, depth class and location are shown in Figures 47 to 49. Table 7 displays the mean intensity and abundance values for sex, maturity stage, length class, weight class, depth class and location. The results indicate that there were no apparent differences between prevalence of either *Anisakis* or *Pseudoterranova* in cod with respect to sex. With an increase in maturity stage there was seen to be a trend of increasing prevalence of both *Anisakis* and *Pseudoterranova* in cod.

Likewise, a similar trend was observed for increases in length class. A slightly more complicated picture was observed for weight class. In this case, a trend of increasing prevalence up to approximately 2500g was observed followed by a slight fall and then an increase up to 100% prevalence for both species in cod over 4000g.

Table 7. Mean intensity and abundance for *Anisakis* and *Pseudoterranova* for cod in relation to sex, maturity, length class, weight class, depth class and location.

		Anisakis Abundance	*Pseudoterranova* Abundance	*Anisakis* Mean Intensity	*Pseudoterranova* Mean Intensity
Sex	M	3.52	2.19	6.95	5.27
	F	3.04	2.77	5.78	8.01
Maturity Class	1	1.50	0.69	3.32	2.82
	2	3.43	2.96	6.51	7.17
	3	8.74	5.65	11.82	10
	4	10.07	11.07	15.67	12.92
Length (cm)	10-29	0.58	0.11	1.57	1
	30-49	2.20	0.80	4.97	3.2
	50-69	4.90	5.52	14.26	16.55
	70-89	3.91	16.18	4.78	22.25
	90-109	32.25	4.25	32.25	4.25
	110-129	12.00	2.50	12.00	2.5
Weight (g)	1-500	0.96	0.63	2.42	3.74
	501-1000	2.00	0.71	4.8	2.93
	1001-1500	6.75	2.42	9.48	4.09
	1501-2000	4.15	6.65	6.71	9.83
	2001-2500	3.79	2.93	5.89	5.86
	2501-3000	2.00	16.88	3.2	19.29
	3001-3500	4.00	22.40	6.67	28
	3501-4000	0.50	9	1	18
	4001-4500	11.00	5	11	5
	4501-5000	3.00	25.5	3	25.5
	5001-5500	0	1	0	1
	5501-6000	6.00	0	6	0
	6001-6500	2.00	4.5	2	4.5
	6501-7000	15.00	3	15	3
	7001-7500	45	5	45	5
	7501-8000	59	1	59	1
	8001-8500	16	2	16	2
Depth (m)	1-100	1.69	1.08	5.38	3.75
	101-200	2.98	2.83	5.74	9.04
	201-300	11.17	1.83	16.75	2.75
Location	A	0.25	2.4	1.25	4
	B	0.29	3.41	1.56	7.59
	C	1.76	4.93	4.88	12.68
	D	5.33	1.91	7.83	5.4
	E	3.58	0.89	5.72	3.23

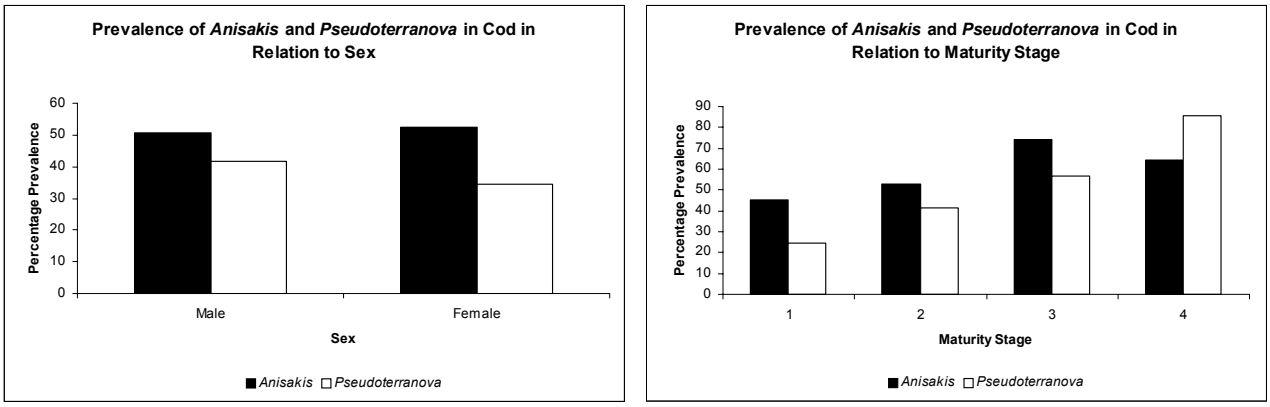


Figure 47. Prevalence of *Anisakis* and *Pseudoterranova* in cod in relation to sex and maturity stage

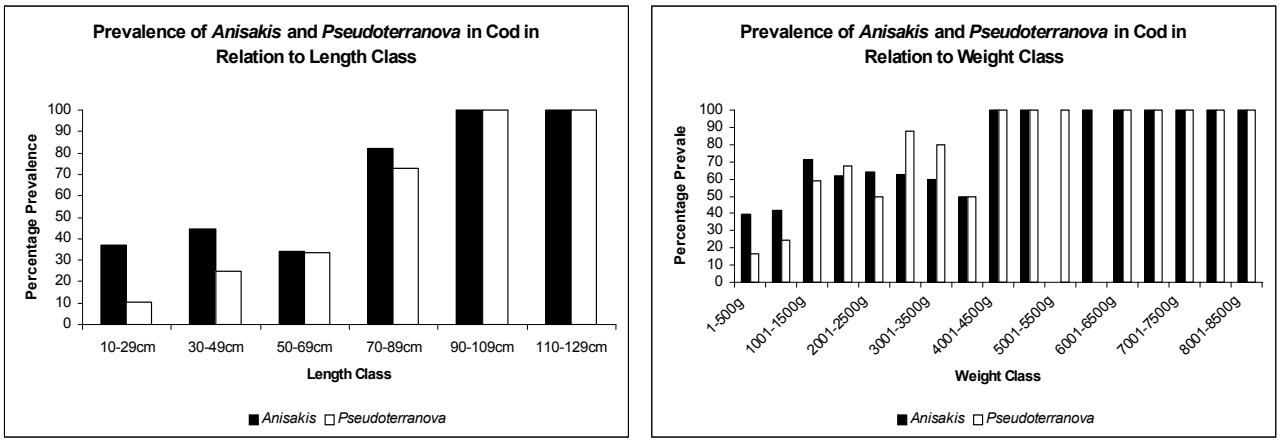


Figure 48. Prevalence of *Anisakis* and *Pseudoterranova* in cod in relation to length and weight class

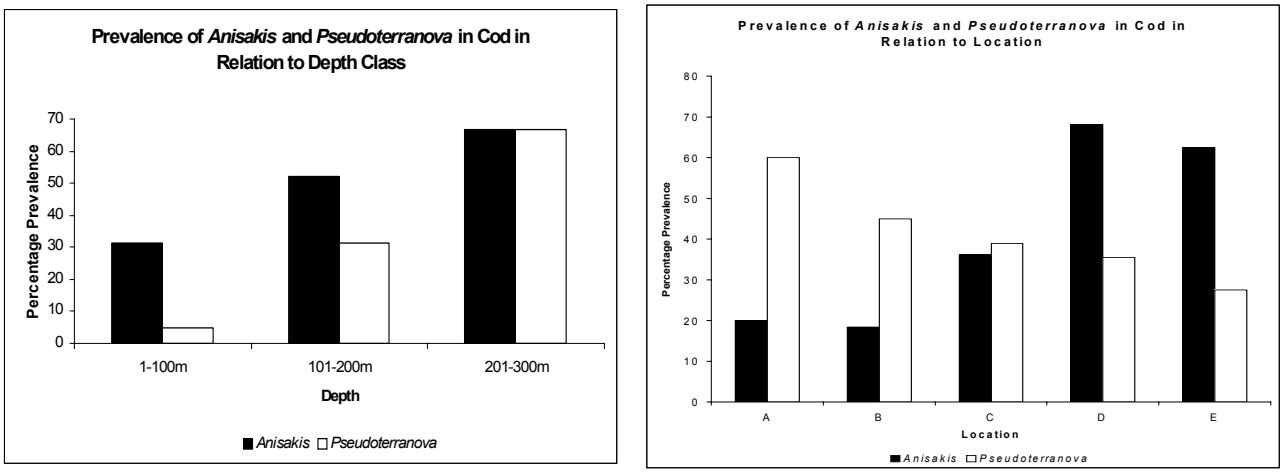


Figure 49. Prevalence of *Anisakis* and *Pseudoterranova* in cod in relation to depth class and location

When considering depth class, an increase in prevalence of both *Anisakis* and *Pseudoterranova* was seen with an increase in depth. An increase in *Anisakis* prevalence and a decrease in *Pseudoterranova* prevalence were observed when comparing locations A through E.

The abundance and mean intensity data for *Anisakis* and *Pseudoterranova* in cod are plotted in Figures 50-51

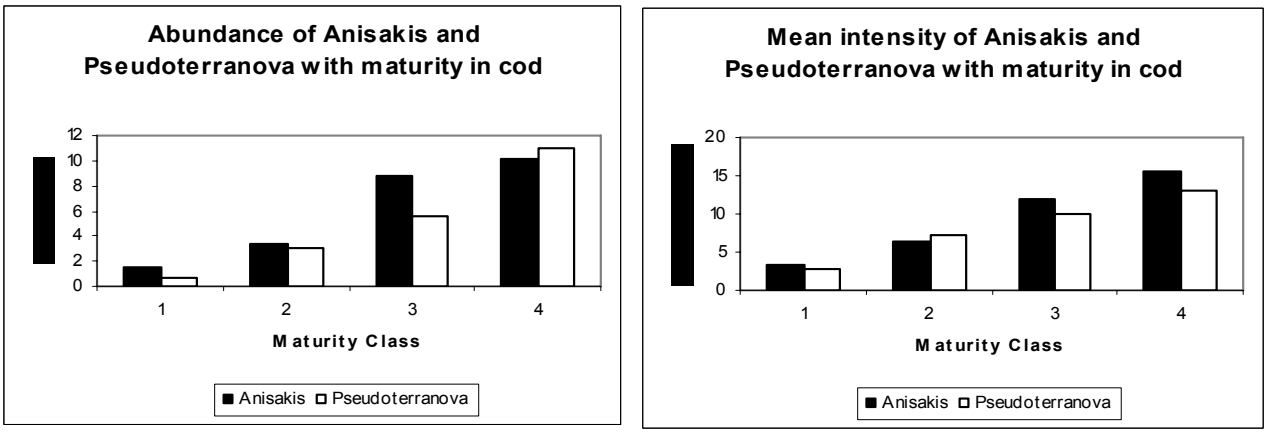


Figure 50. Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in cod in relation to maturity class

Mean intensity of *Anisakis* infection increased from length class 1 (10-29cm) to 3 (50-69cm) with a peak intensity at length class 5 (90-109cm), although there was an apparently anomalously low value in length class 4 (70-89cm). *Pseudoterranova* mean intensity increased up to length class 4 with a substantial fall thereafter (Figure 51).

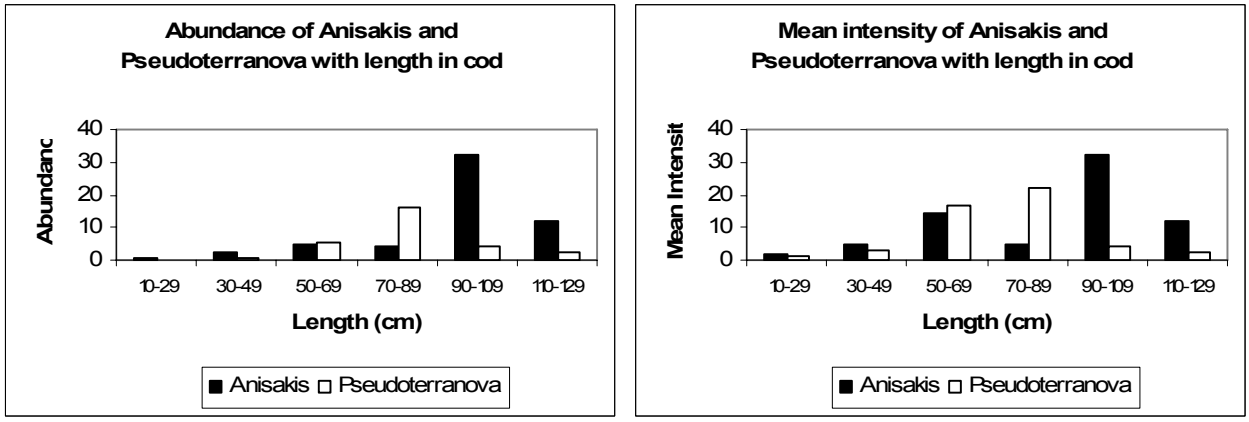


Figure 51. Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in cod in relation to length class.

The abundance of *Anisakis* in cod increased with depth, whilst that of *Pseudoterranova* was highest at depth class 2. Mean intensity of *Anisakis* was greatest at depth class 3 whilst that of *Pseudoterranova* was greatest at depth class 2 (Figure 52).

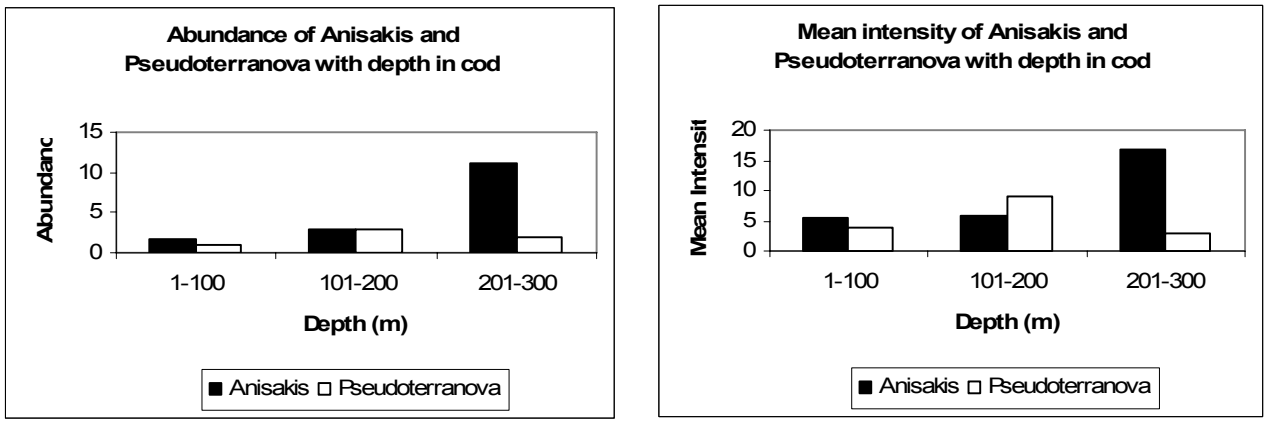


Figure 52. Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in cod in relation to depth class.

There was substantial variability in *Anisakis* abundance with regard to sampling location with low abundance at locations A and B and highest abundance at locations D and E. Abundance of *Pseudoterranova* rose from locations A to C and fell through locations D and E. Mean intensity of *Anisakis* infection increased from locations A through D with a slight fall at location E. *Pseudoterranova* mean intensity rose through sites A to C then fell through sites D and E (Figure 53).

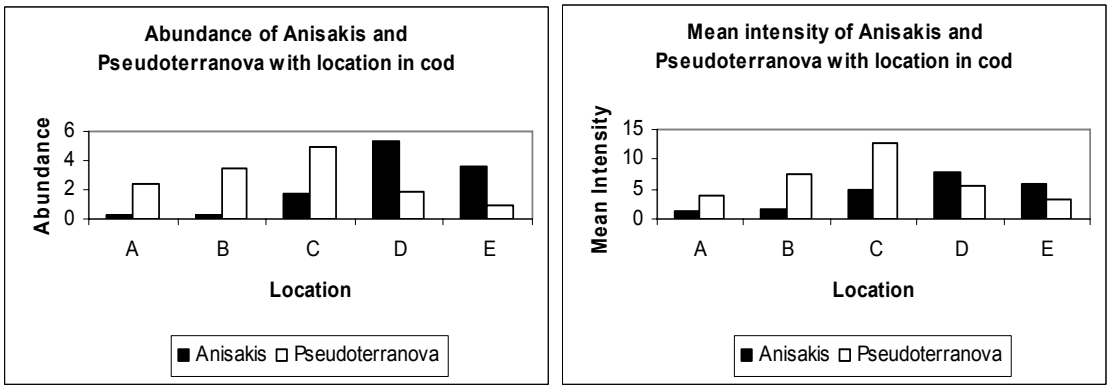


Figure 53. Abundance and mean intensity of *Anisakis* and *Pseudoterranova* in cod in relation to location

3.5.4 Body distribution and detection methods

The distribution of *Anisakis* and *Pseudoterranova* within the flesh of cod was determined by direct visual inspection, candling and slicing. Similarly to monkfish, the flesh was divided into left and right, belly flap and fillets. The mean numbers of *Anisakis* and *Pseudoterranova* in relation to body distribution are shown in Figures 54 to 57. Statistical analysis was conducted using the Wilcoxon Matched Pairs test.

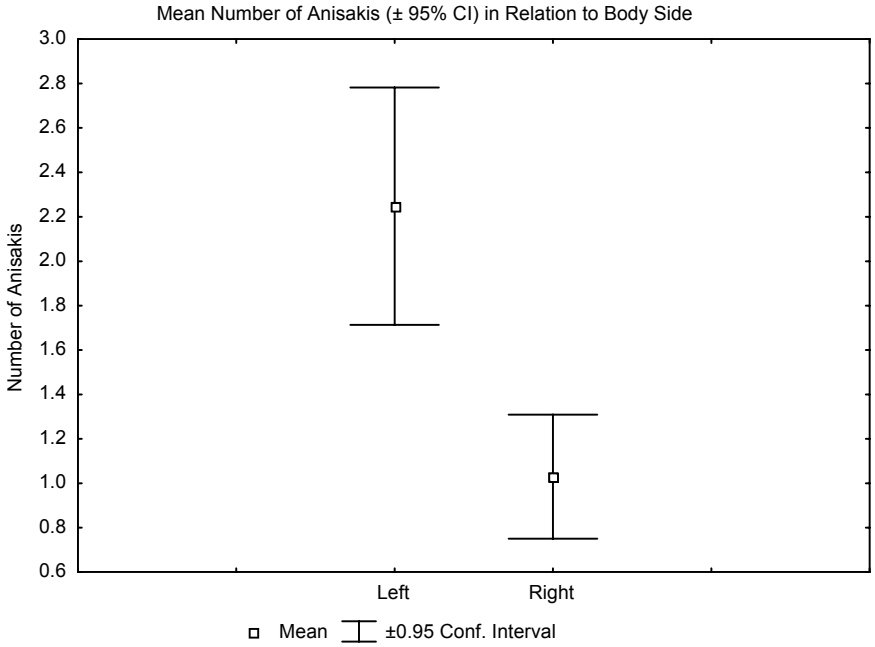


Figure 54. Mean number of *Anisakis* in cod in relation to body side

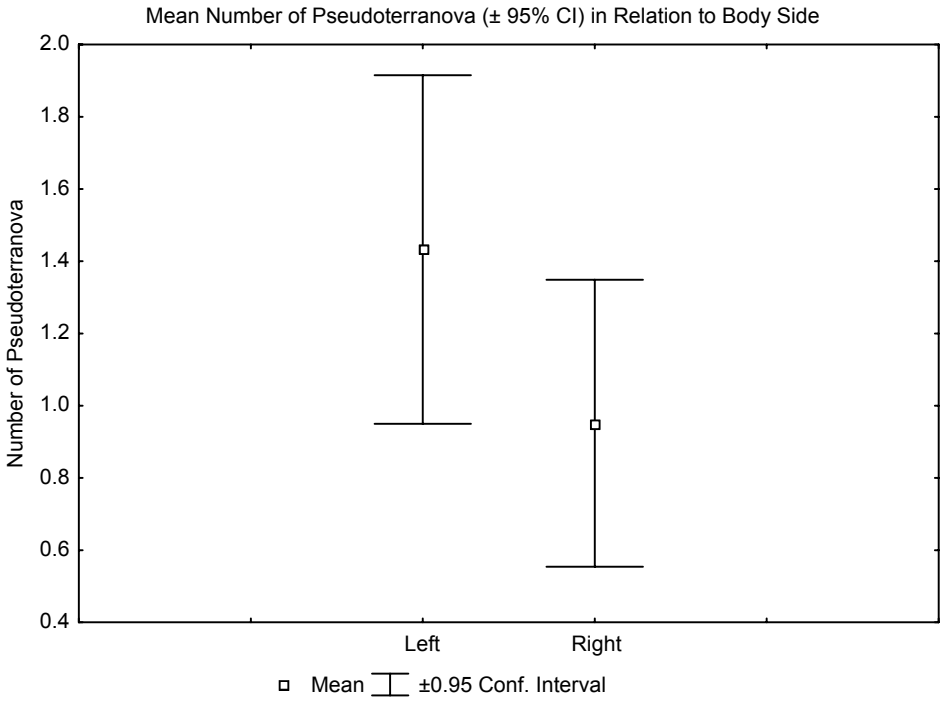


Figure 55. Mean number of *Pseudoterranova* in cod in relation to body side

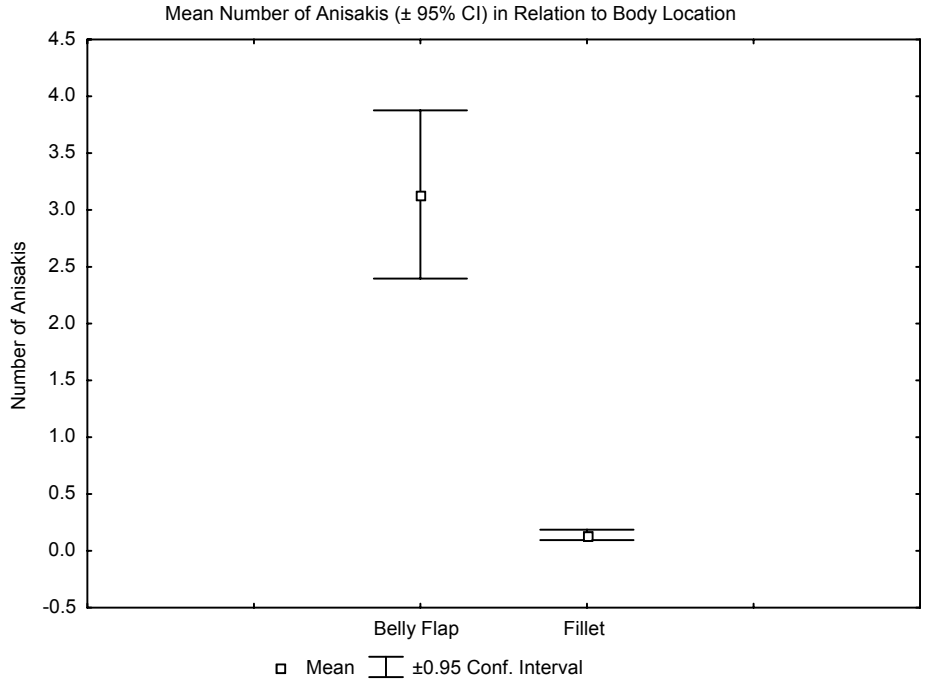


Figure 56. Mean number of *Anisakis* in cod in belly flaps and fillets

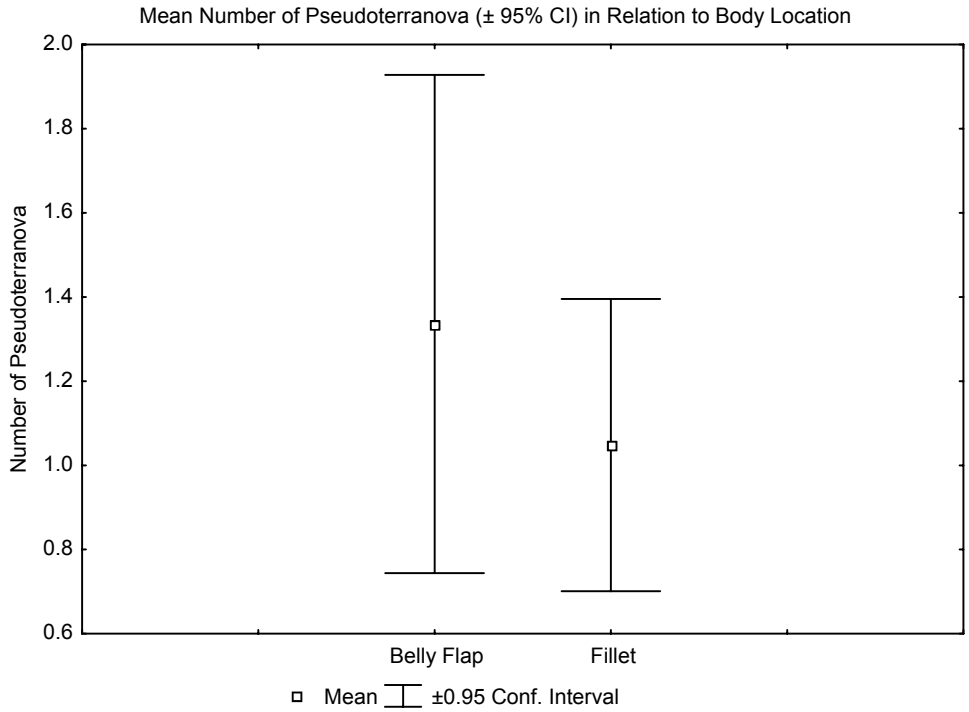


Figure 57. Mean number of *Pseudoterranova* in cod in belly flaps and fillets

Comparing *Anisakis* distribution between the left and right sides of the fish highlights significantly more *Anisakis* ($p < 0.000001$) in the left than the right side. Likewise significantly more ($p < 0.000001$) *Anisakis* were detected in the belly flaps compared to the fillets. For *Pseudoterranova,* there were significantly more worms detected in the left side than the right side ($p < 0.000001$), however there was no significant difference between the distributions in belly flaps and fillets ($p=0.396$).

Figures 58 and 59 illustrate the prevalence, mean intensity and abundance of infection within cod flesh for all samples of *Anisakis* and *Pseudoterranova* respectively

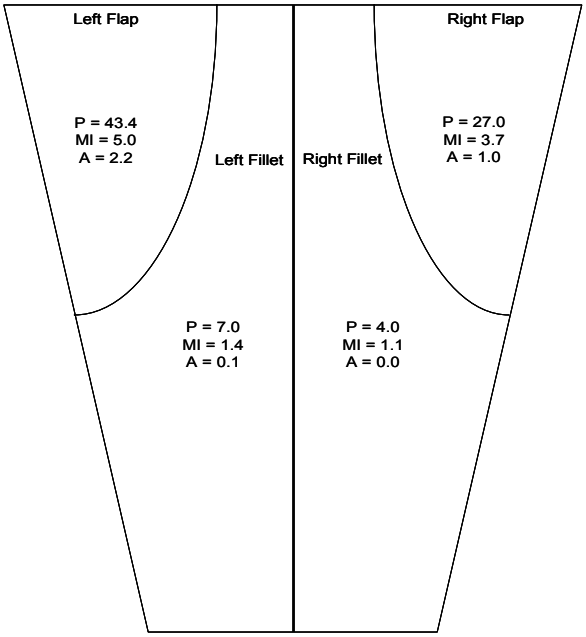


Figure 58. Prevalence (P), mean intensity (MI) and abundance (A) data for Anisakis in cod flesh

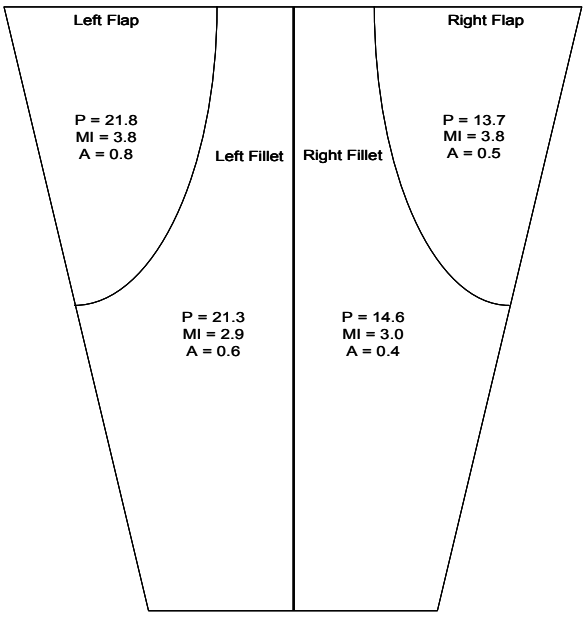


Figure 59. Prevalence (P), mean intensity (MI) and abundance (A) data for *Pseudoterranova* in cod flesh

When consideration was given to the detection methods used, the same classification for the detection methods used for monkfish was utilised.

The mean number of *Anisakis* and *Pseudoterranova* detected in the belly flaps and fillets of cod are illustrated in Figures 60 to 63. By visual examination the detection of *Anisakis* in the belly flaps was low, but detection was increased dramatically by candling, with only a slight increase in numbers detected by slicing. In the flesh, slicing detected significantly more worms than both visual inspection and candling. Few *Pseudoterranova* were detected by visual examination alone, with a slight increase in the detection rate following candling, again followed by a slight increase with slicing. Very few *Pseudoterranova* were detected in the flesh by eye or candling, but there was a large increase in detection following slicing.

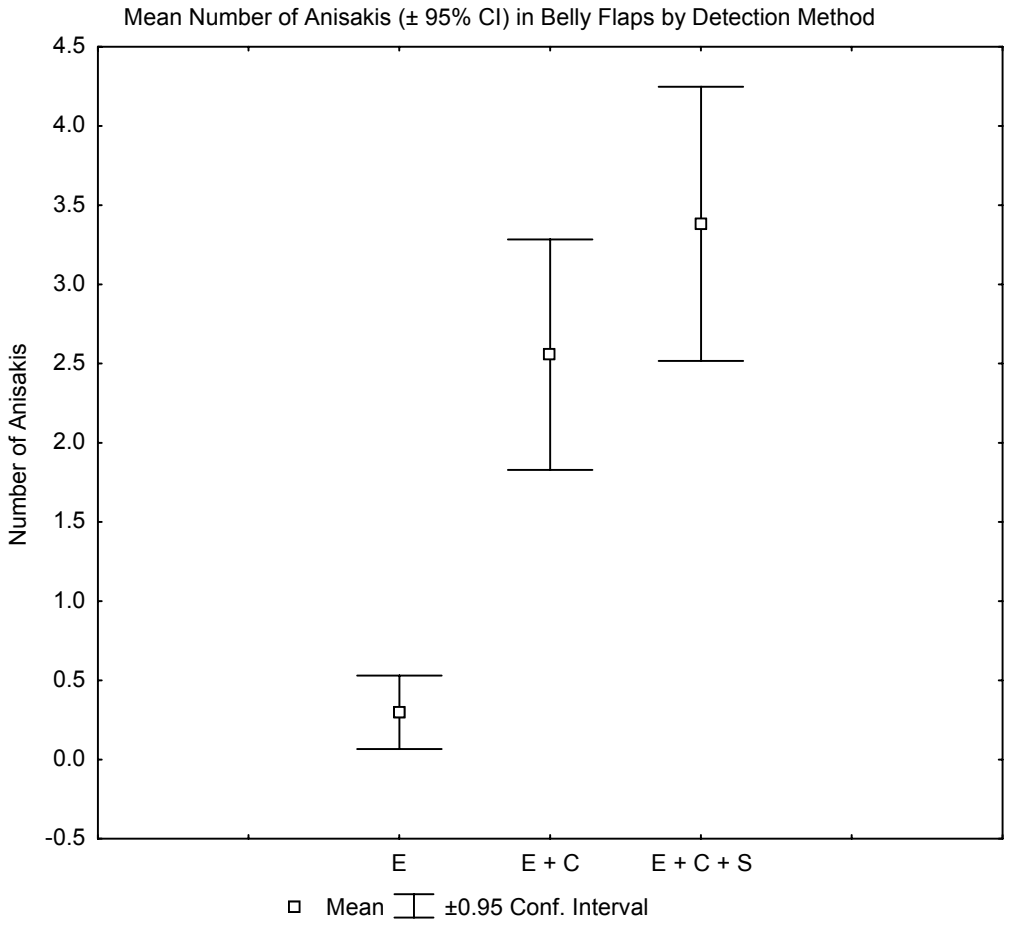


Figure 60. Mean number of *Anisakis* in cod belly flaps detected by each of the three detection methods

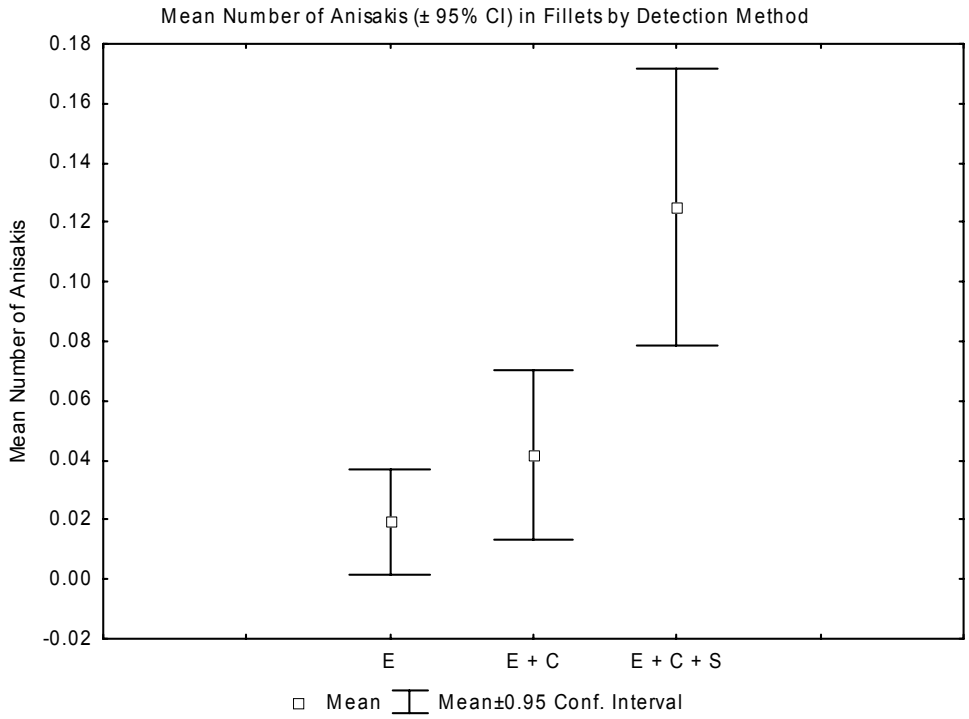


Figure 61. Mean number of *Anisakis* in cod fillets detected by each of the three detection methods

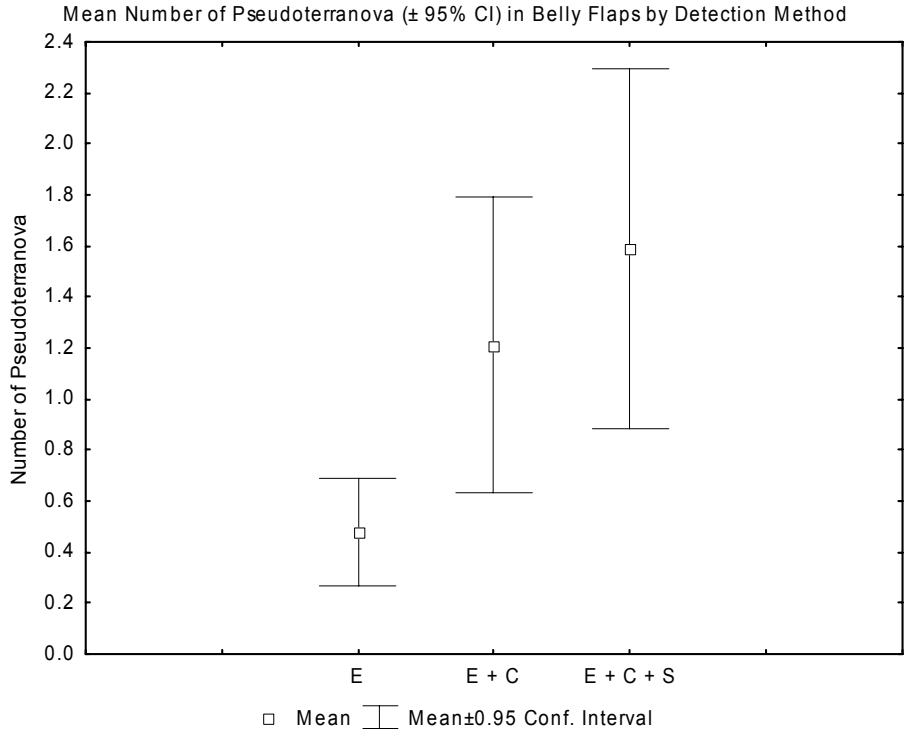


Figure 62. Mean number of *Pseudoterranova* in cod belly flaps detected by each of the three detection methods

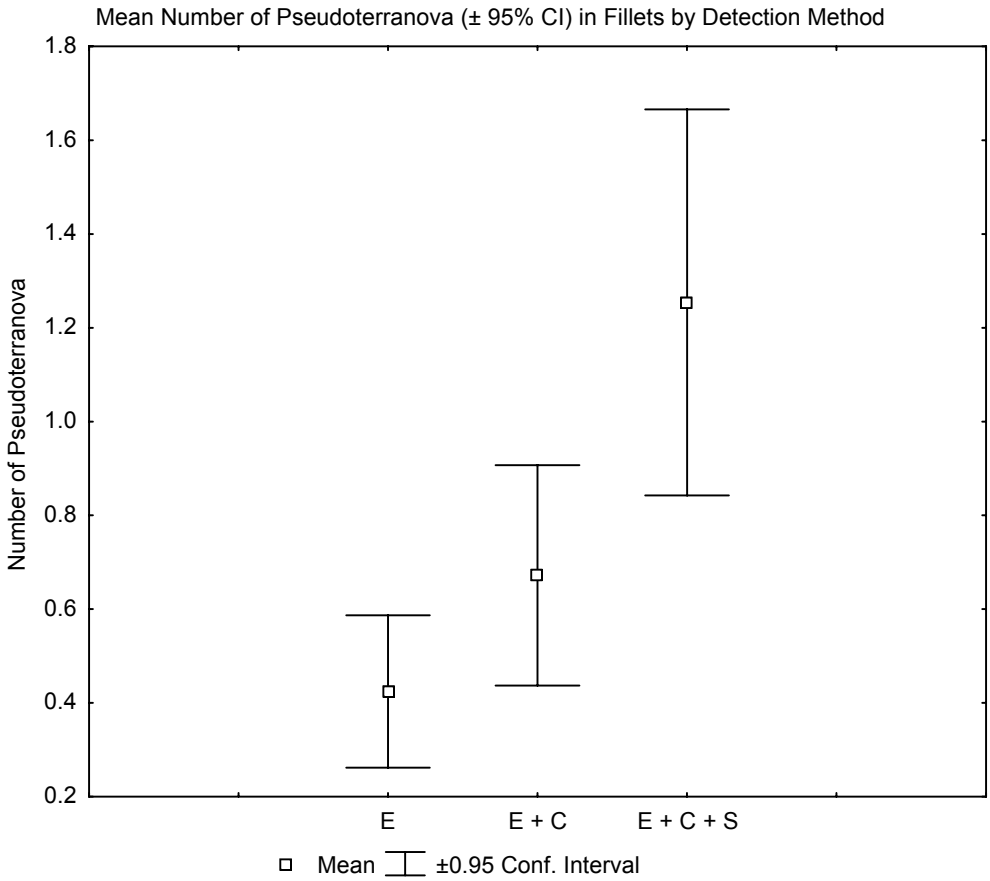


Figure 63. Mean number of *Pseudoterranova* in cod fillets detected by each of the three detection methods.

The numbers of *Anisakis* and *Pseudoterranova* detected and the percentage of the total worms detected by the 3 methods are illustrated in Table 8. Only 8.8% of *Anisakis* in the belly flaps were detected by eye, rising to 75.5% following candling. In the flesh, only 33.3% were detected by eye and candling. For *Pseudoterranova* in the belly flaps, candling detected 76.2% of the total load, whereas in the flesh candling only accounted for approximately half (53.6%) the worm load.

Table 8. Numbers of and percentages of total *Anisakis* and *Pseudoterranova* detected by the 3 detection methods in belly flap and fillets in cod

		Anisakis		*Pseudoterranova*	
		N	%	n	%
Flaps	Eye	93	8.8	149	30.1
	E + C	795	75.5	377	76.2
	E + C + S	1052	100	495	100
Fillets	Eye	6	15.4	132	33.8
	E + C	13	33.3	209	53.6
	E + C + S	39	100	390	100

3.6 Mackerel

A total of 358 mackerel were examined for the presence of *Anisakis* and *Pseudoterranova*. Eighty-two fish were infected with *Anisakis* and none were infected with *Pseudoterranova.* A total count of 328 *Anisakis* was recovered.

The calculated prevalence, mean intensity and abundance for *Anisakis* in mackerel were 22.9%, 4 and 0.92, respectively.

As with cod and monkfish, Figure 64 demonstrates that the majority of fish carried no worms with the remainder mostly carrying less than 10 worms each.

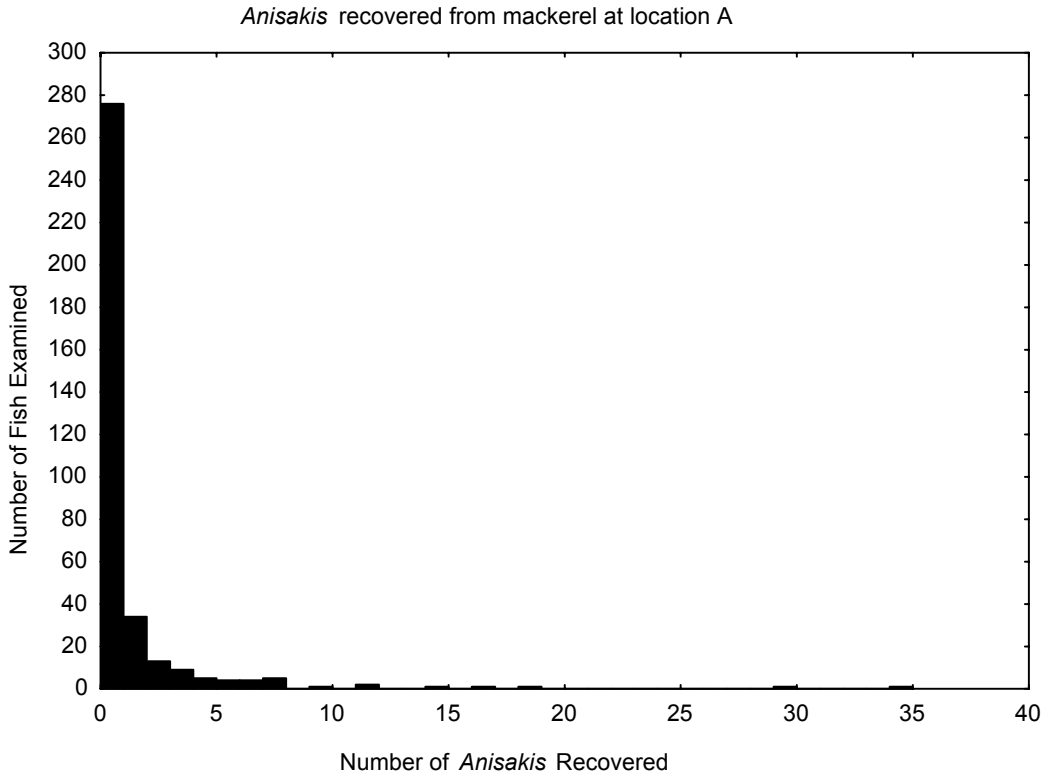


Figure 64. Histogram showing frequency of different infection loads for *Anisakis* in sampled mackerel.

3.6.1 Single factor statistics

There were fewer measured factors to explain worm numbers in mackerel since the analysed samples were taken in a single year from a single location and therefore represent a single stock of fish. Thus, only the effect of sex was analysed.

Figure 65. shows the mean number of *Anisakis* associated with the two sexes. Statistical analysis for this effect indicated it to be non-significant. (p(adj.)=0.82).

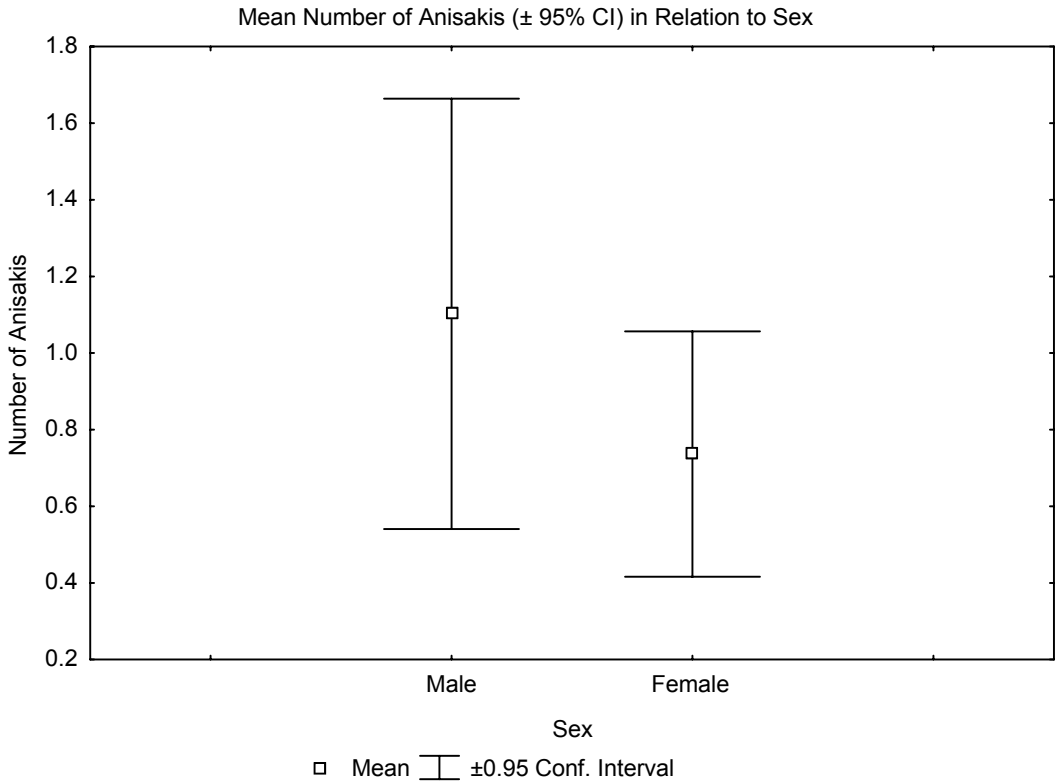


Figure 65. Mean Number of *Anisakis* in mackerel in relation to sex

3.6.2 Analysis of multiple factors

3.6.2.1 Total *Anisakis* numbers in mackerel

The best model for *Anisakis* numbers in mackerel explained 28.4% of the deviance (a measure of the total variability in numbers between fish samples). The following represent the principal findings of the model:

- Length was the major variable having a significant (p=9.6e -06) effect on the probability of *Anisakis* infection in mackerel, with increasing numbers of *Anisakis* found up to ~25 cm after which numbers were more stable, with a small increasing probability (Figure 66).

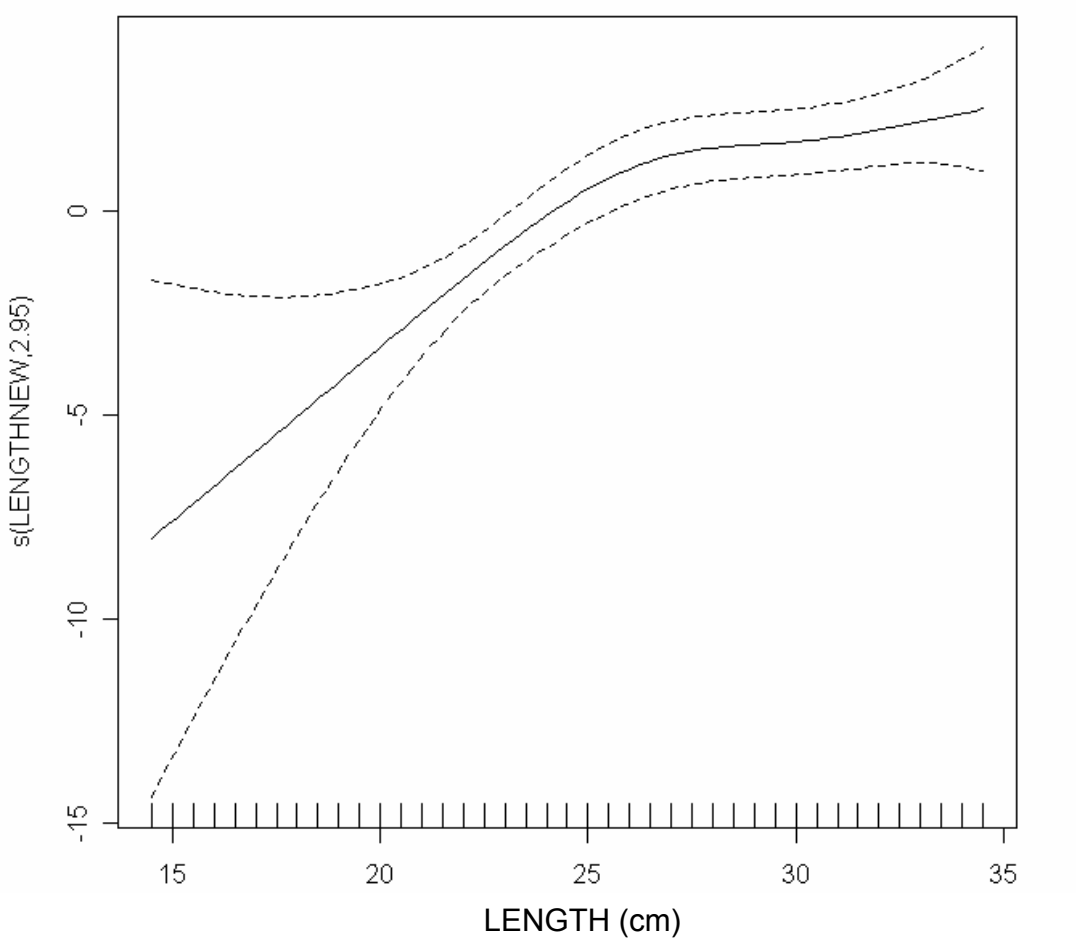


Figure 66. Partial fit of length for model of *Anisakis* in mackerel. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.6.2.2 Presence / Absence data for *Anisakis* in mackerel

The presence / absence of worms in mackerel was modelled using a binomial distribution (*i.e.* a distribution with two outcomes "present" and "absent").The best binomial model explained only 15.5% of the deviance, a measure of the total variability in numbers between fish samples, in the worm presence / absence data, with the following effects predominating:

- Length was the only variable having a significant (p=0.00065) effect on the probability of worm infection in mackerel, with an increasing likelihood of obtaining worms found up to ~25 cm after which numbers were more stable, with a minor increasing tendency (Figure 67).

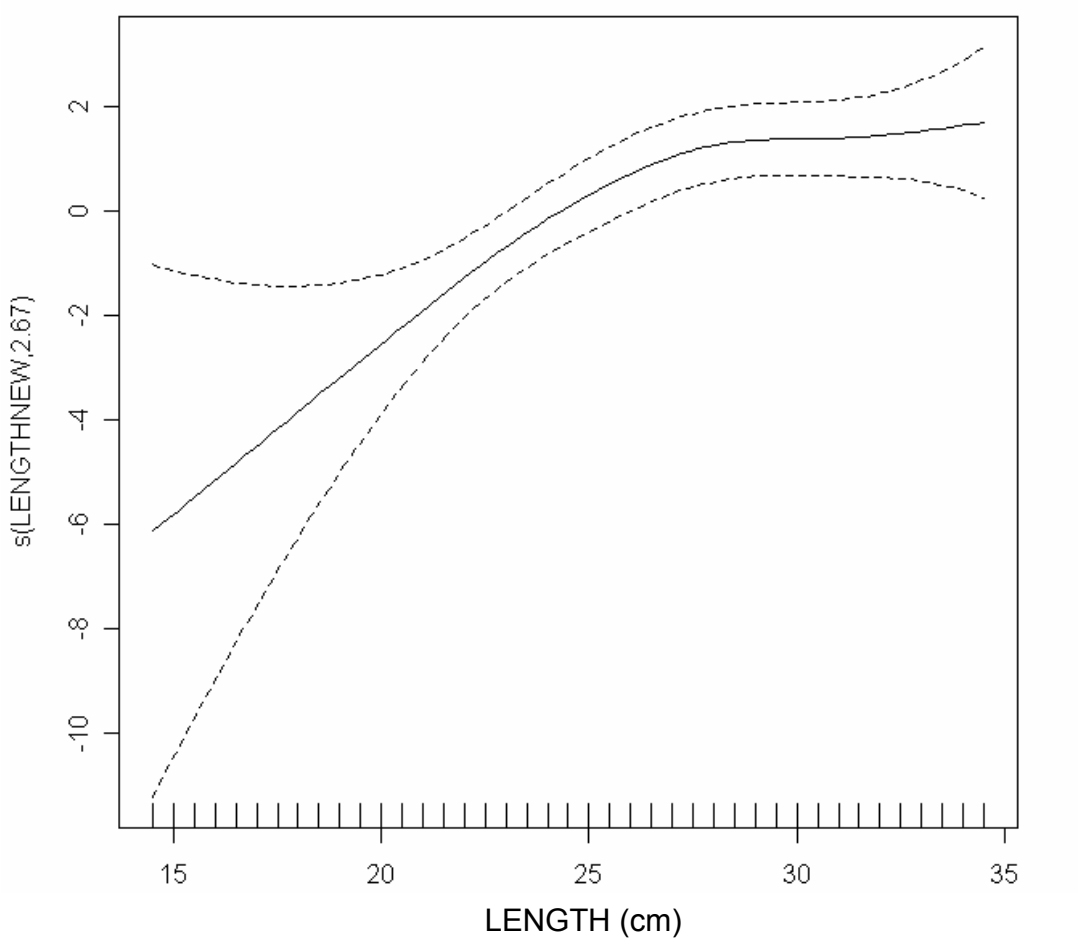


Figure 67. Partial fit of length for model of presence / absence of *Anisakis* in mackerel. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

Table 9. provides a summary of the significant factors identified by the analysis of multiple factors.

Table 9. Summary table of significant explanatory variables affecting worm numbers in mackerel in best models. X indicates a significant ($p<0.05$) effect. Mackerel were only sampled in one year at a single location hence missing factors (NA).

Variable	**Host Length**	**Depth**	**Sex**	**Location**	**Year**
Anisakis	X			NA	NA
Presence / Absence of Worms	X			NA	NA

3.6.3 Body distribution and detection methods

The distribution of *Anisakis* within the flesh of mackerel was determined by visual inspection of pressed fillets. The distribution of nematodes within the flesh was divided into those recovered from the left and right sides and also those recovered from flaps and fillets. The mean numbers of *Anisakis* in relation to body distribution are illustrated in Figures 68-70. Wilcoxon matched pairs tests highlighted significant differences in mean number of *Anisakis* between both the belly flap and the fillet ($p < 0.000001$) and also between the left and right sides ($p = 0.337$). There were also significant differences between numbers recovered from right flaps and fillets ($p<0.000001$) and left flaps and fillets ($p<0.000001$).

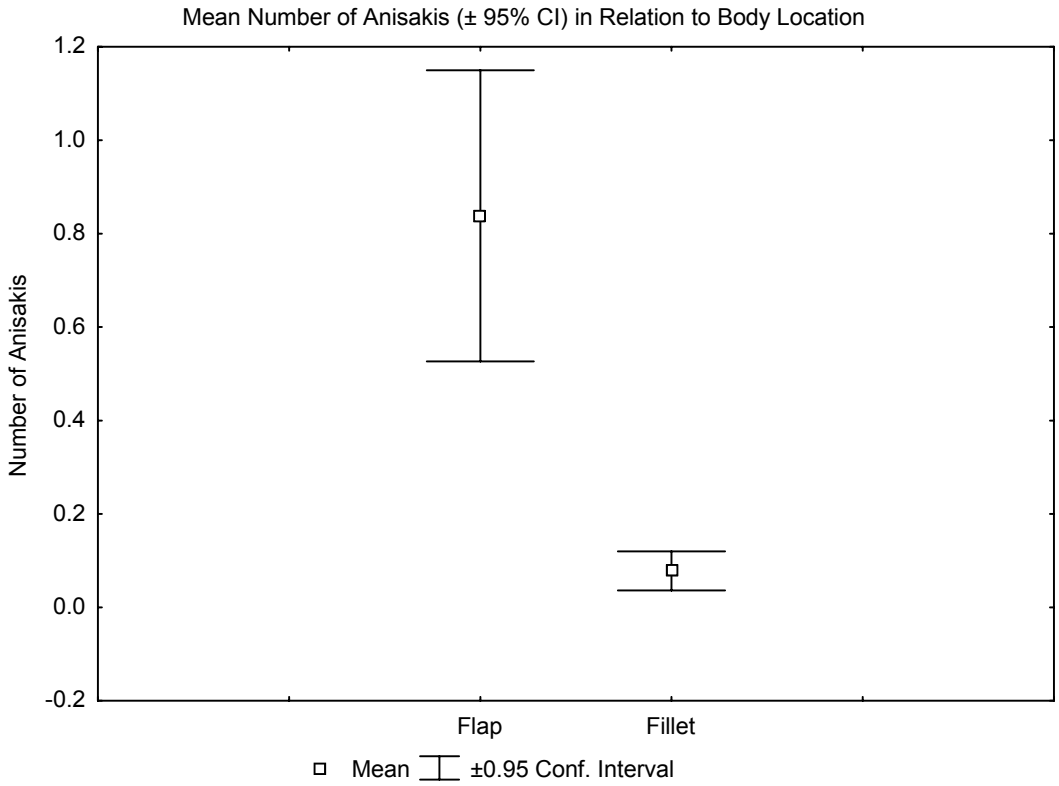


Figure 68. Mean number of *Anisakis* in mackerel belly flaps and fillets

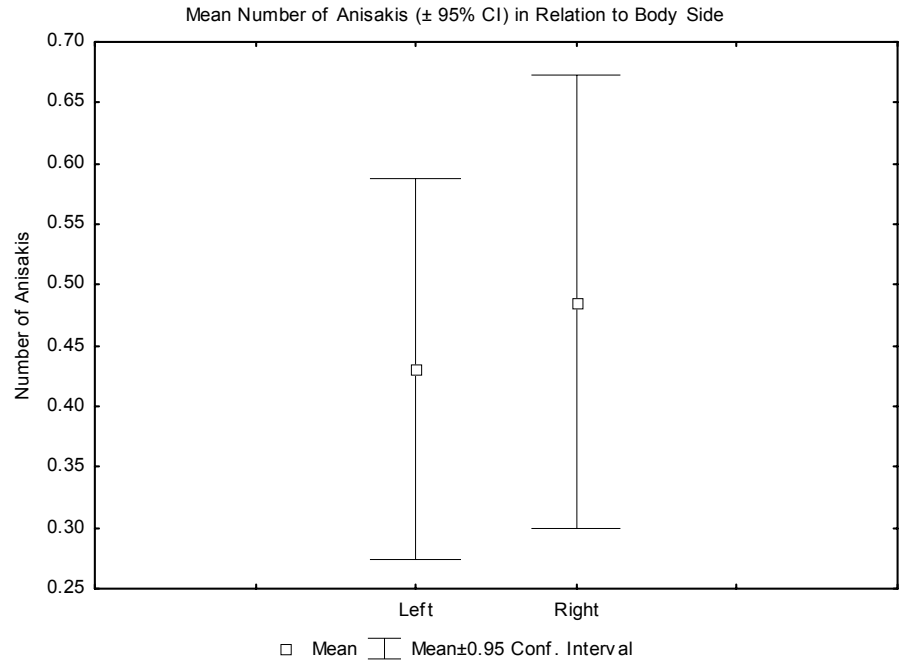


Figure 69. Mean number of *Anisakis* in mackerel in left and right sides of fish

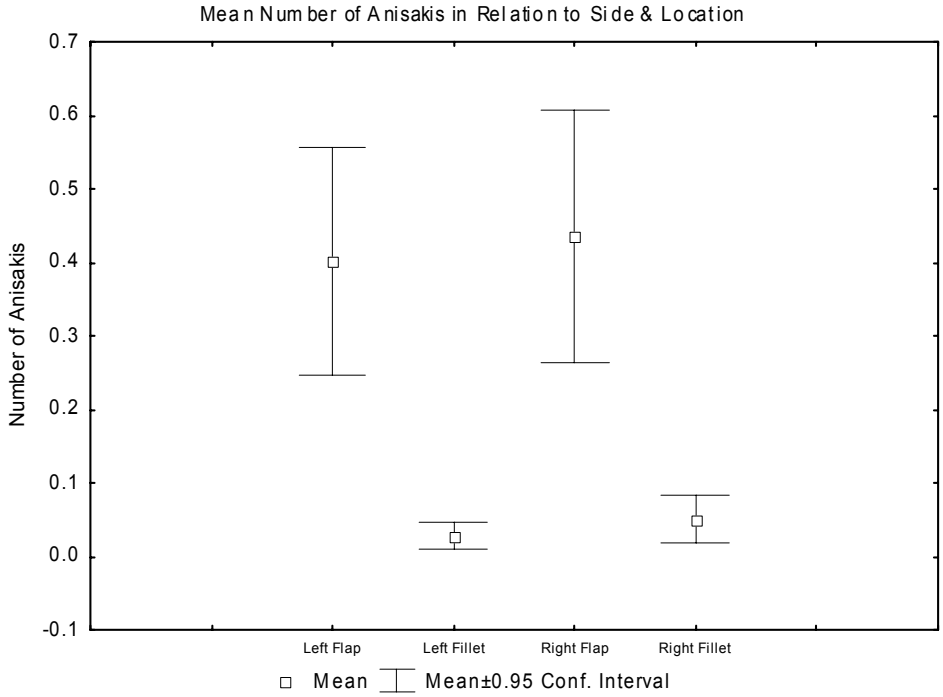


Figure 70. Mean number of *Anisakis* in mackerel in left and right fillets and flaps.

3.7 Herring

A total of 784 herring were examined for the presence of *Anisakis* and *Pseudoterranova*. Of the 784 herring examined, 285 were infected with *Anisakis* and none were infected with *Pseudoterranova,* with a total count of 526 *Anisakis*.

The calculated prevalence, mean intensity and abundance for *Anisakis* in herring were 36%, 1.85 and 0.67, respectively. Figure 71. illustrates a single *Anisakis* detected by UV illumination of pressed fillets.

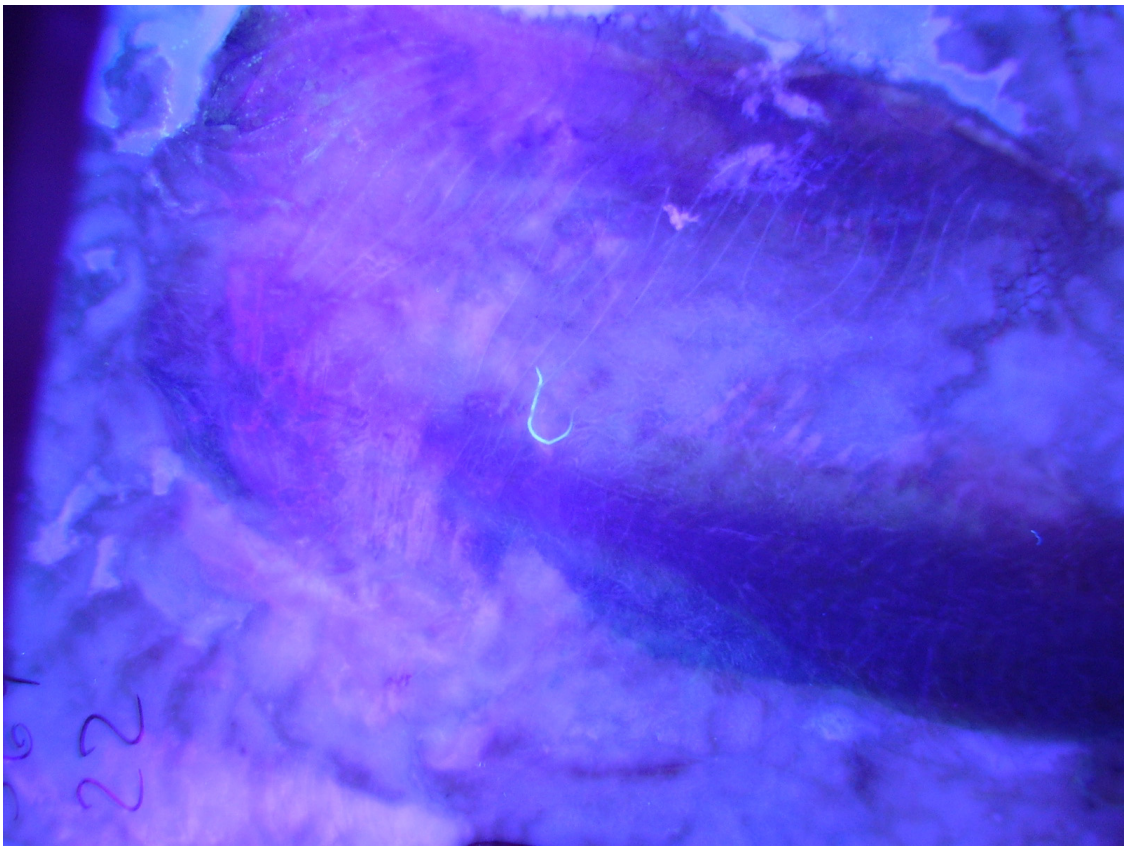


Figure 71. Single *Anisakis* larva fluorescing when exposed to UV following pressing of herring fillet.

Very few worms were recovered from site A and at site B the majority of fish sampled carried no worms, with those that were infected mostly carrying <3 worms (Figure 72).

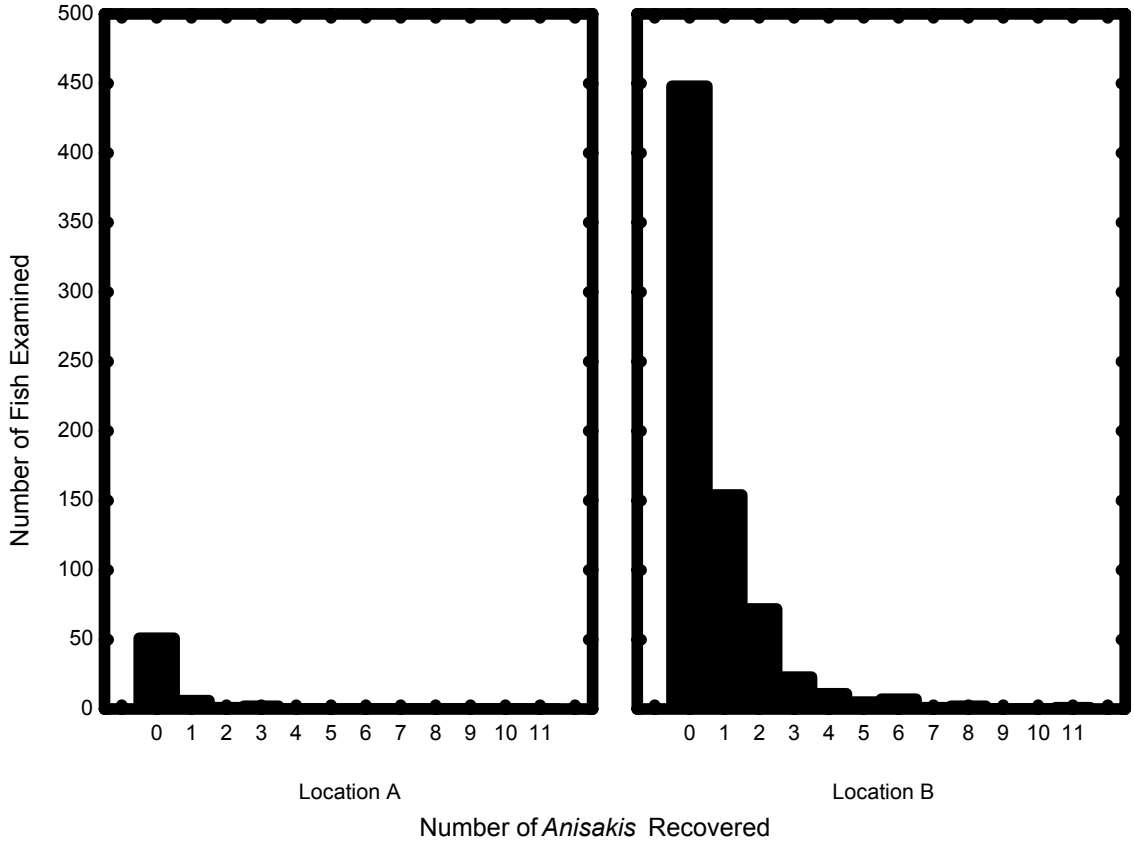


Figure 72. Histogram showing frequency of different infection loads for *Anisakis* in sampled herring.

3.7.1 Single factor statistics

The effects of the following factors were examined:

Sex

Figure 73. provides an overview of mean numbers of *Anisakis* with respect to sex in herring. Sex had no apparent significant effect on *Anisakis* numbers in herring (p(adj.)=0.756).

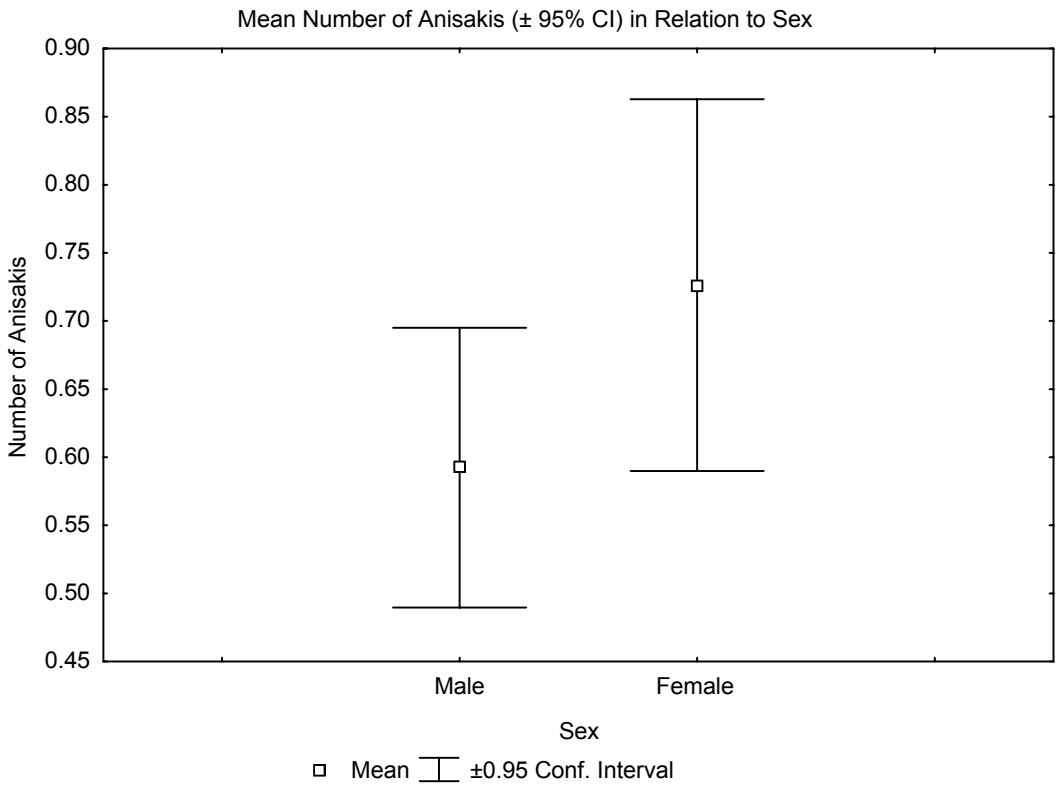


Figure 73. Mean Number of *Anisakis* in herring in relation to sex

Location

Figure 74. shows *Anisakis* numbers for the two locations examined. A single factor test suggests that location had a significant effect on *Anisakis* numbers in herring (p(adj.)=0.000371), with fish collected from area B having a higher *Anisakis* infection levels.

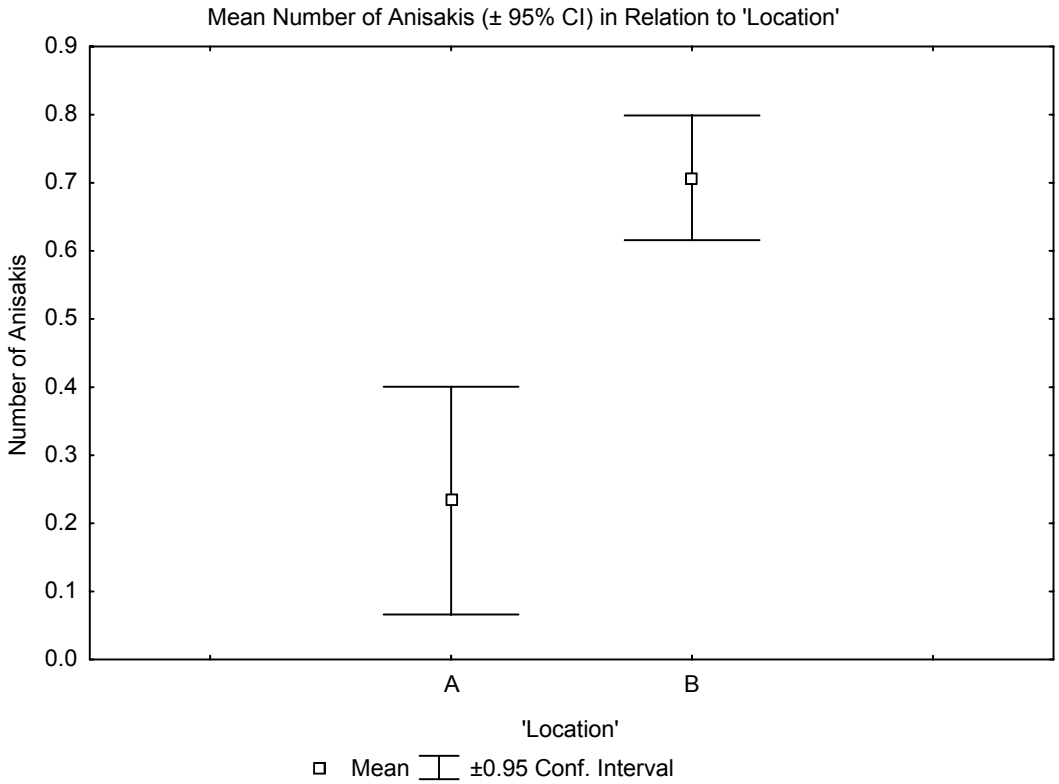


Figure 74: Mean Number of *Anisakis* in herring in relation to location

3.7.2 Analysis of multiple factors

3.7.2.1 Total *Anisakis* numbers in herring

The best model for *Anisakis* numbers in herring explained only 12.2% of the deviance. The following represent the principal findings of the model:

- Length had a significant (p=0.000279) effect on *Anisakis* number in herring, with numbers increasing up to ~ 25cm and then levelling off with a possible increase from >28cm, although the latter is uncertain due to broad confidence limits. (Figure 75).
- Depth also showed a significant (1.67e-06) effect on the probability of *Anisakis* infection, with an apparent fall in probability down to 80m with levelling off thereafter (however, confidence bounds are similarly relatively wide for this variable).
- There is a significant (5.55e-05) effect of year on *Anisakis* numbers in herring, which may suggest inter-annual variability.
- Sex was a significant (p=0.00116), explanatory variable for *Anisakis* in herring with females having significantly higher numbers than males.

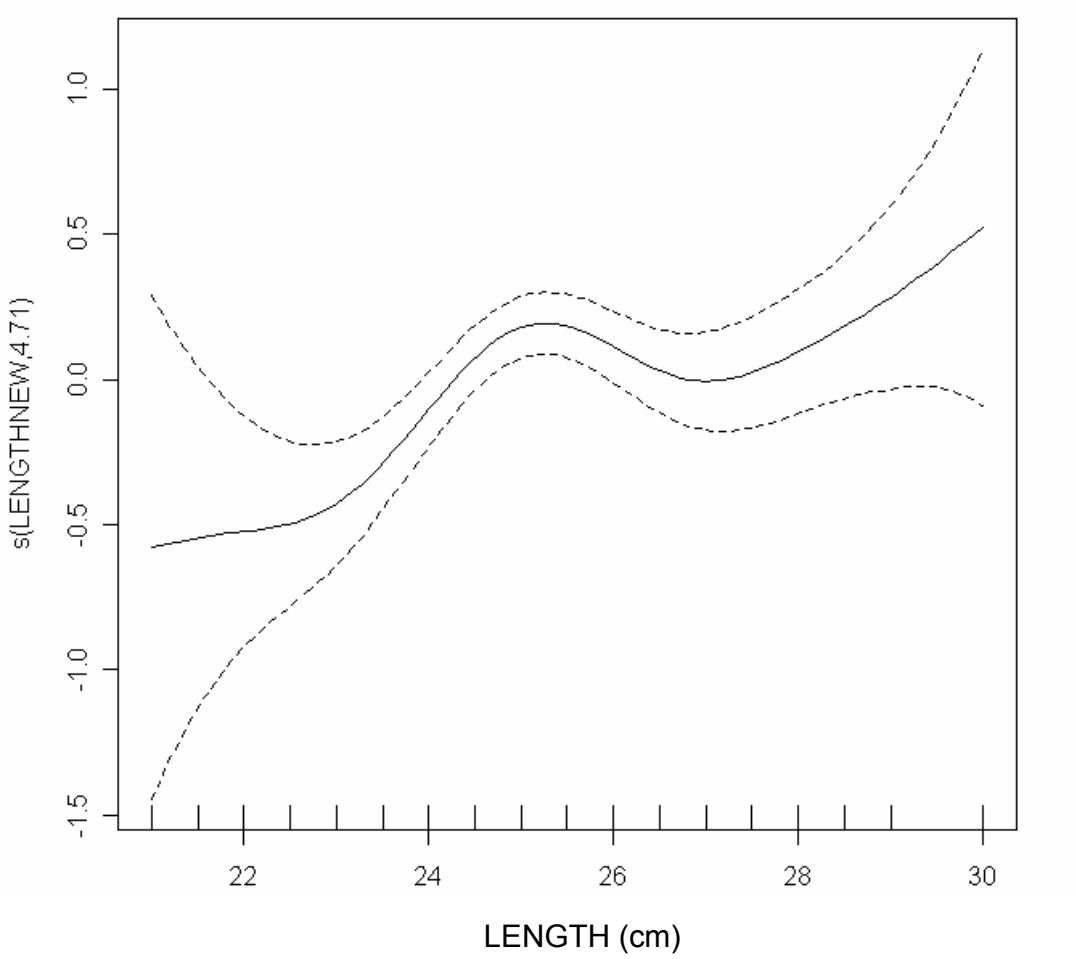


Figure 75. Partial fit of length for model of *Anisakis* numbers in herring. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

3.7.2.2 Presence / Absence data for *Anisakis* in herring

The presence / absence of worms in herring was modelled using a binomial distribution (*i.e.* a distribution with two outcomes "present" and "absent").The best binomial model explained only 3.22% of the deviance, a measure of the total variability in numbers between fish samples, in the worm presence / absence data, with the following effects predominating:

- The interaction of length and sex (*i.e.* through probable sexual dimorphism) was significantly (p=0.043) associated with the increased probability of infection, with infection increasing with increasing size above ~23cm. An apparent drop in the probable number of fish infected between 21-23cm may be an artifact of wide confidence limits (Figure 76.).

- The variable year was significantly (p=8.96e-06) associated with worm infection, with prevalence being higher in 2006.

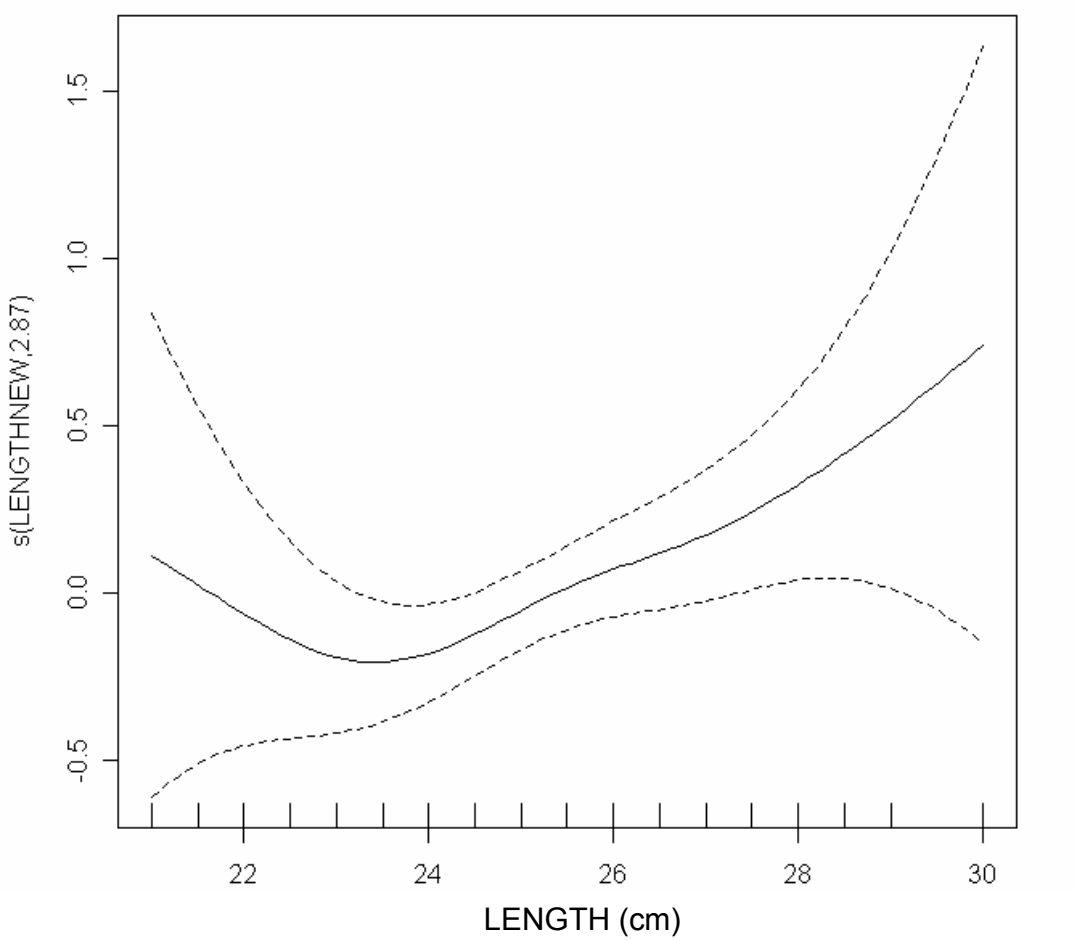


Figure 76. Partial fit of length for model of presence / absence of *Anisakis* in herring. The *x*-axis shows the fish length in cm and the *y*-axis shows the contribution of the smoothing function used to associate the number of worms recovered with the length of the fish they were recovered from. Higher numbers on the *y*-axis denote an increased probability of higher worm numbers. Internal ticks on the *x*-axis mark the samples obtained.

A summary of the significant factors identified through the analysis of multiple factors is provided in Table 10.

Table 10. Summary table of significant explanatory variables affecting worm numbers in herring in best models. X indicates a significant ($p<0.05$) effect. Worms were only recovered from herring sampled in one location hence missing factor (NA).

Variable	**Host Length**	**Depth**	**Sex**	**Location**	**Year**
Anisakis	X	X	X	NA	X
Presence / Absence of Worms	X			NA	X

3.7.3 Body distribution and detection methods

Figure 77. shows the mean number of *Anisakis* recovered by the two detection methods. There was no significant difference in the number of worms recovered by the two detection methods employed – digestion and physical pressing (Mann-Whitney, p=0.318).

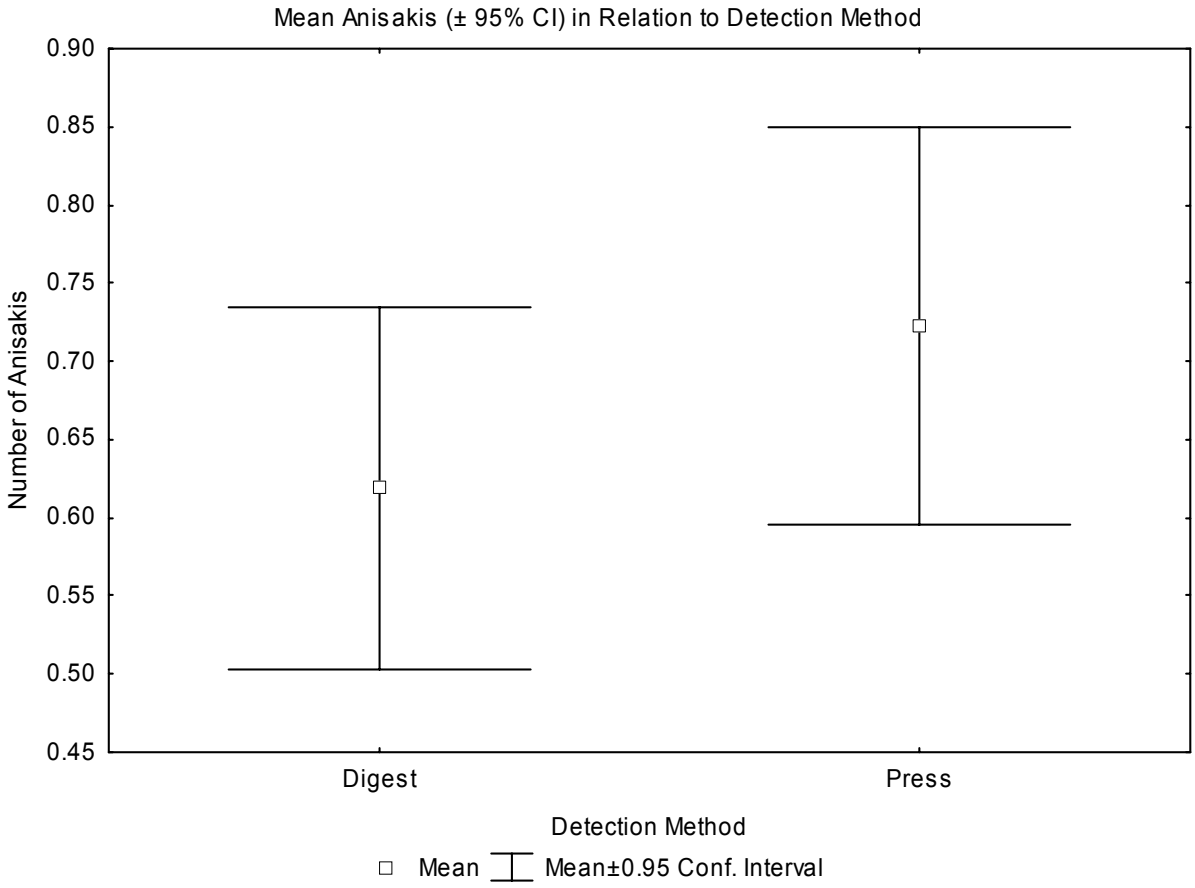


Figure 77. Mean number of *Anisakis* recovered from herring using two alternative methods – digestion and pressing.

The distribution of *Anisakis* within the flesh of herring was determined by visual inspection of pressed fillets. The distribution of nematodes within the flesh was divided between those recovered from left and right sides of the fish and between those recovered from flaps and fillets. The mean numbers of *Anisakis* in relation to body distribution are illustrated in Figures 78 and 79. Wilcoxon Matched Pairs tests highlighted significant differences in mean number of *Anisakis* between both the belly flap and the fillet ($p < 0.000001$) and also between the left and right sides ($p = 0.027$). There were also significant differences between numbers recovered from right flaps and fillets ($p<0.000001$) and left flaps and fillets ($p<0.000001$).

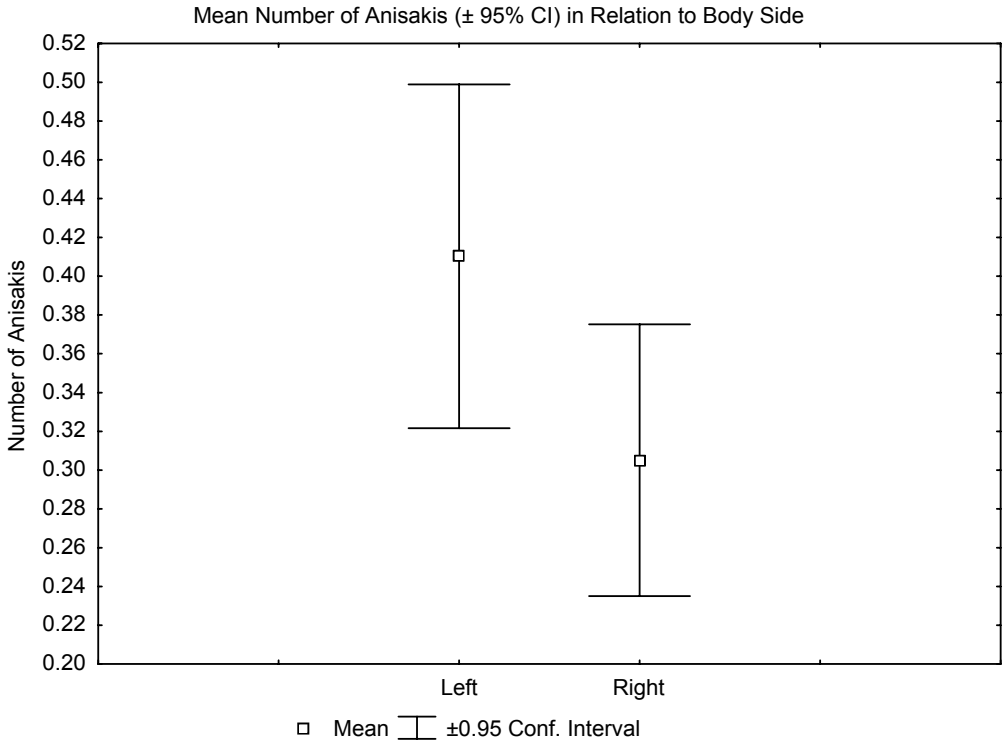


Figure 78. Mean number of *Anisakis* recovered from right and left sides of herring.

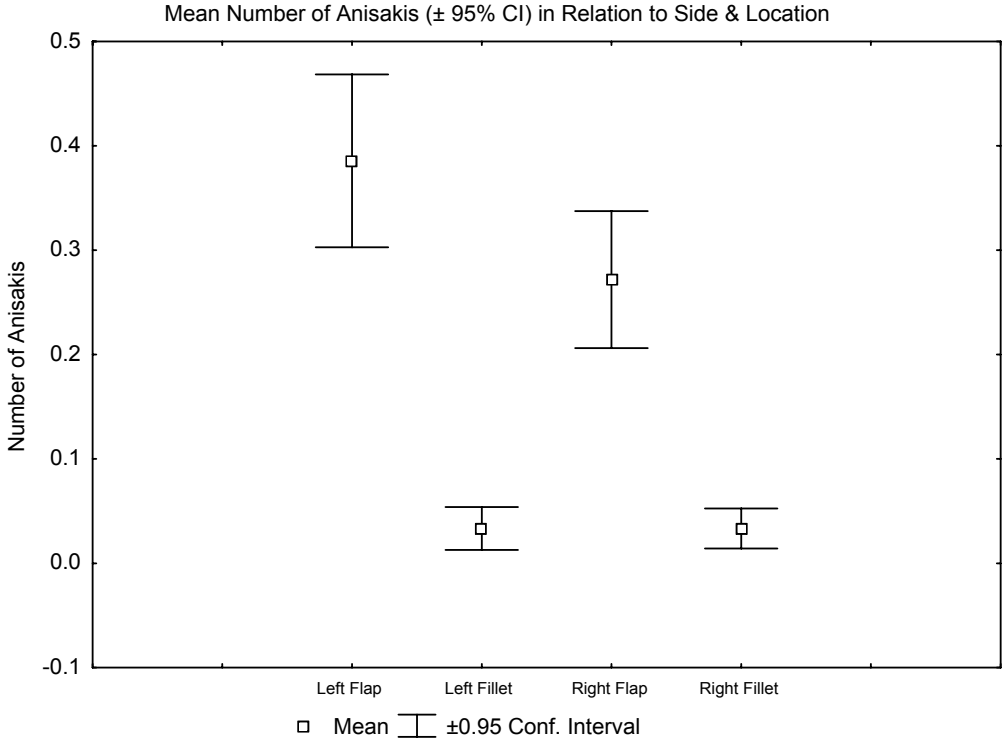


Figure 79. Mean number of *Anisakis* recovered from left and right flaps and fillets of herring.

3.8 Detection of anisakids for food security

3.8.1 Sampling requirements

From the current study and from earlier work it is clear that between 20->50% of captured fish of all the sampled species are likely to contain *Anisakis* or *Pseudoterranova* in the flesh. The number of hosts to sample for worms therefore depends entirely upon the stated intention of the sampling process. If the objective of sampling is to detect the presence / absence of worms, then Figure 80. shows the number of fish that need to be sampled in order to find a single fish carrying worms in flesh, given different prevalences of worms in the population to be sampled and different sensitivities of the protocol used to detect the fish. For instance, digestion / slicing will find ~100% of *Pseudoterranova* so that smaller numbers of fish need to be sampled to find an infected fish than would be the case if one was trying to detect *Anisakis* by visual inspection alone (detection efficiency 10%). At a population prevalence of 20% it would be necessary to sample 15 or 150 fish for *Pseudoterranova* or *Anisakis* respectively, with these detection efficiencies to obtain a 95% chance of successfully detecting a single infected fish.

The same information may also be obtained from Figures 81-83, which highlight specific detection efficiencies. These figures assume that *Pseudoterranova* is the principal target of the search, since this large red worm is the most likely to be spotted by customs officials or consumers. Clearly, more fish need to be sampled if detection is by eye rather than by digestion / slicing. As a rule of thumb, a sample of 30 fish is often taken, which would detect the presence of worms in a population down to a prevalence of 10% using a detection method with 100% efficiency (see Figure 83). It is, however, difficult to imagine how this sampling approach might be used to reduce consumer exposure or customs rejection of consignments.

An alternative sampling strategy, illustrated in Figure 84, assumes that there is some prevalence above which it will not be possible to sell the fish or above which consumer exposure might be considered to be too high. In this case the number of fish to sample is selected as the number required to determine a given prevalence within certain limits of accuracy. For instance, 100 fish would be needed to estimate a population prevalence of 50% to an accuracy of ±10% using a detection efficiency of 100% and 64 fish would be needed to determine a prevalence of 20%.

In practice, the optimal screening strategy may be, particularly for monkfish, to visually screen the surface of all flesh for the presence of the large red *Pseudoterranova* and to remove the worm where encountered.

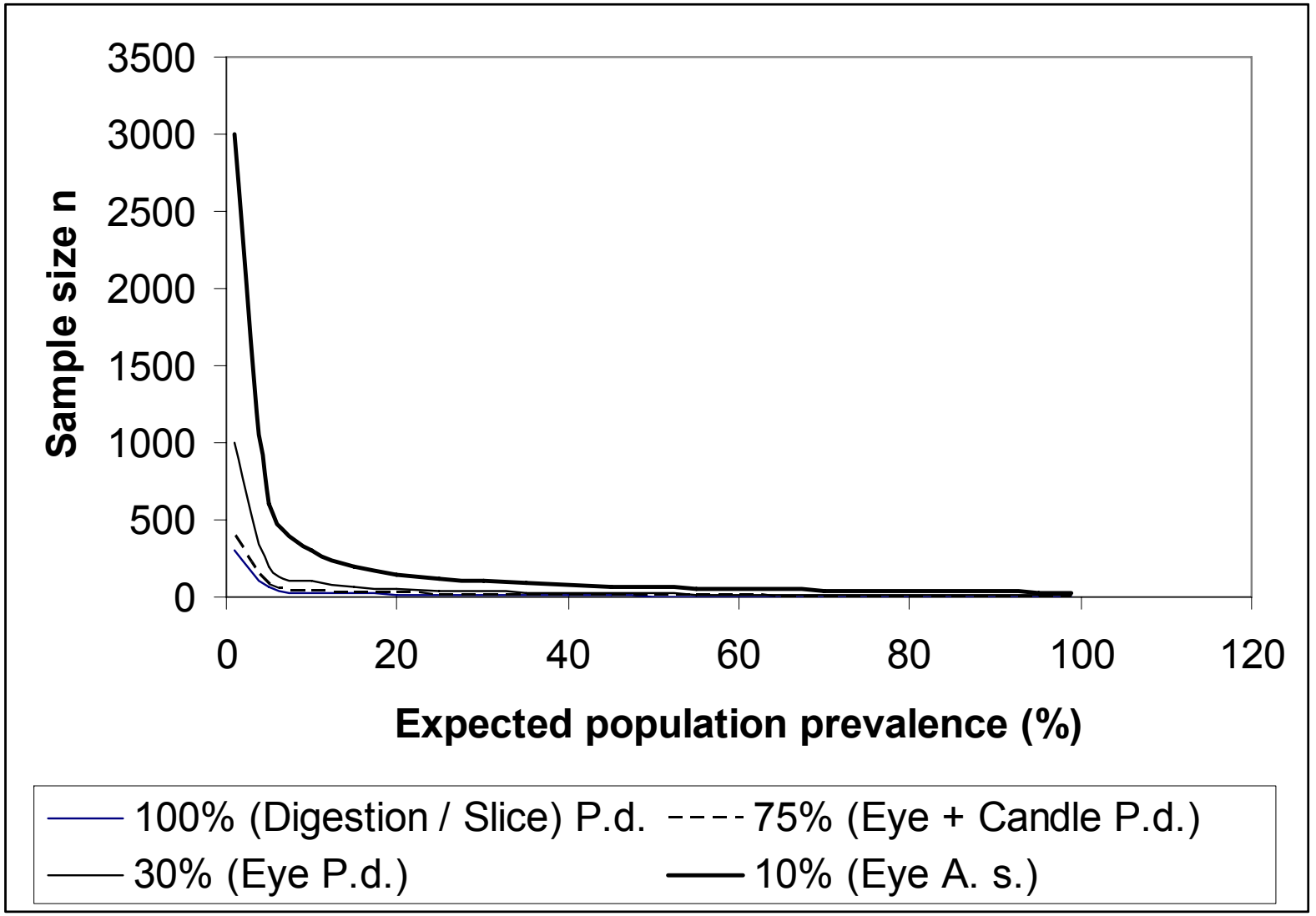


Figure 80. Sample number required to detect a single infected fish from a population with an expected target prevalence given different detection sensitivities (calculated according to des Clers, 1994).

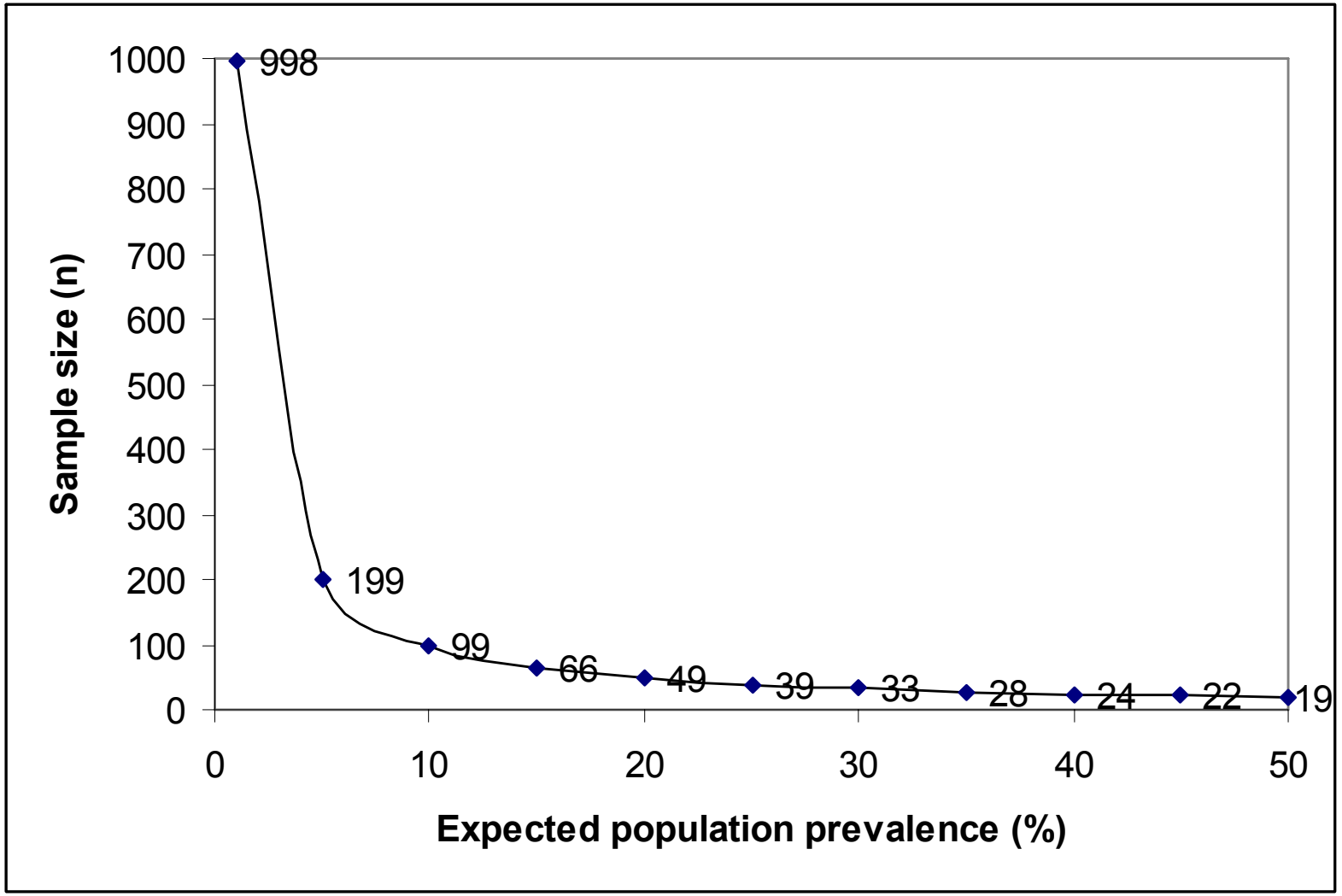


Figure 81. Sample number required to detect a single fish infected with *P. decipiens* by eye only (30% detection efficiency) from a population with a stated target prevalence (calculated according to des Clers, 1994).

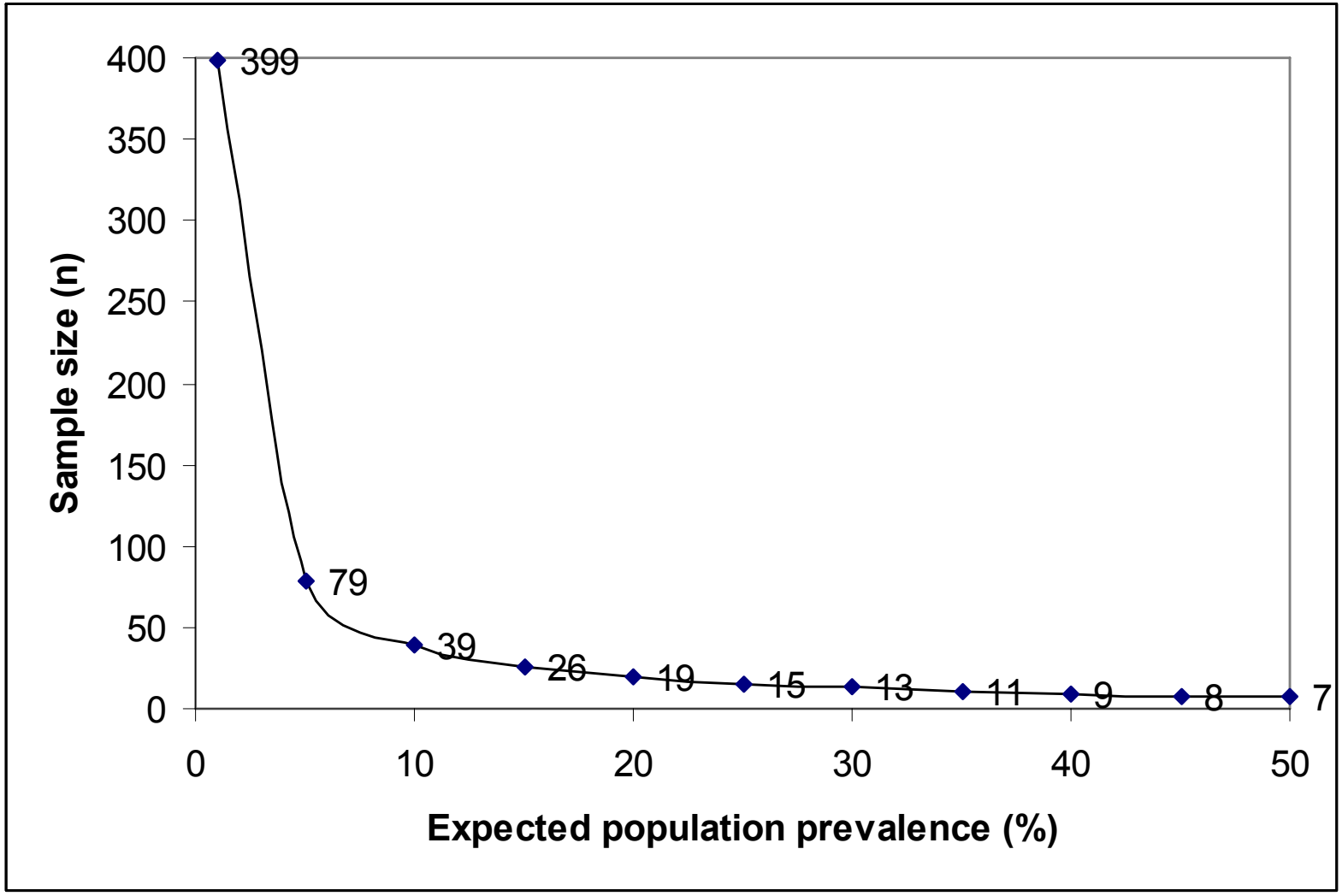


Figure 82. Sample number required to detect a single fish infected with *P. decipiens* by eye and candling only (75% detection efficiency) from a population with an expected target prevalence (calculated according to des Clers, 1994).

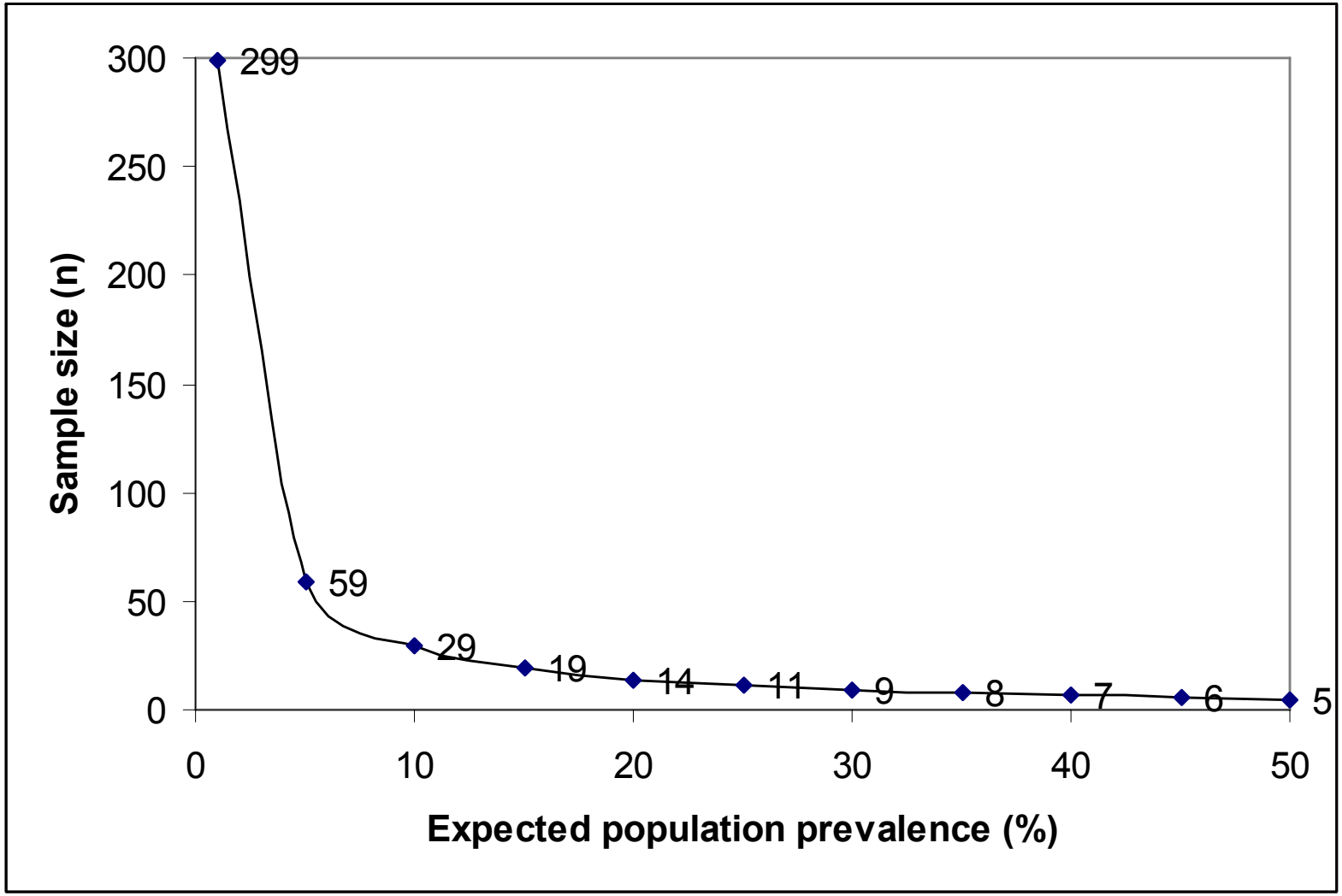


Figure 83. Sample number required to detect a single fish infected with *P. decipiens* by digestion or slicing and candling (~100% detection efficiency) from a population with an expected target prevalence (calculated according to des Clers, 1994).

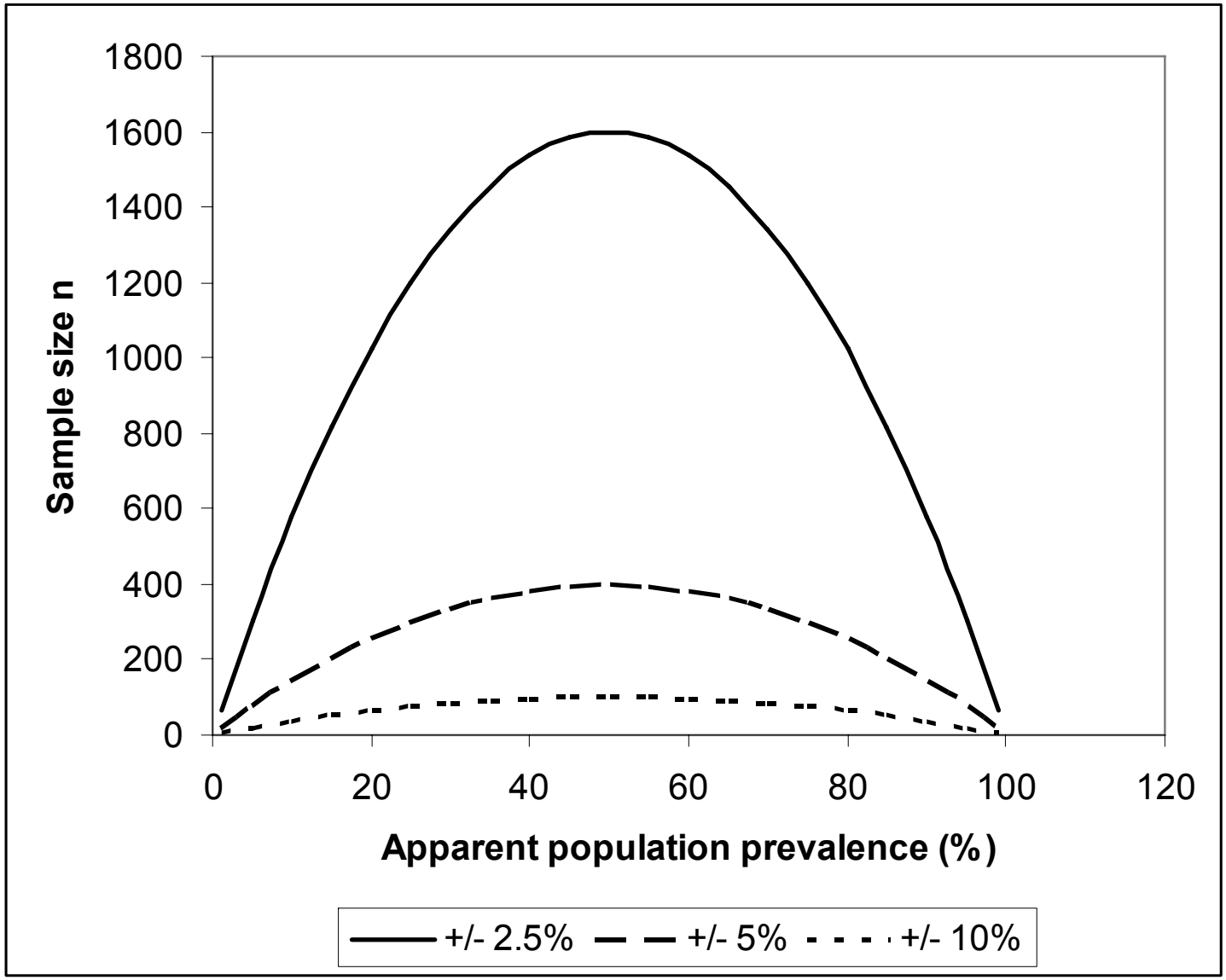


Figure 84. Sample number required to detect a given target prevalence to within a given precision (calculated according to des Clers, 1994).

4. Discussion

4.1 Survey

The results of the questionnaire survey suggested that the fish processing industry are aware of the presence of larval nematodes but that inspection of products is apparently almost entirely confined to visual examination. That this may not be carried out very thoroughly is indicated by the rejection of monkfish consignments to Italy where *Pseudoterranova* on the tails have been very obvious.

4.2 Fish Infection Levels

4.2.1 Monkfish

Larval anisakid nematodes were common in the flesh of monkfish, with *Pseudoterranova* more abundant than *Anisakis*. Indeed, *Psuedoterranova* levels were similar to those of cod, which has long been considered the fish species most heavily infected with this parasite in Scottish waters (Rae 1972, Wootten & Waddell 1977). There is no historical data which allows inferences on whether there has been any increase in infection of either nematode species in monkfish in recent years. The common infection of monkfish with *Anisakis* and *Pseudoterranova* undoubtedly reflects their piscivorous habit, with infections being acquired through the ingestion of already infected prey fish. The abundance of *Pseudoterranova* in monkfish is probably due to its benthic habitat, where it is more likely to come into contact with prey that have already acquired infections through feeding on infected invertebrates or other fish.

Monkfish showed a rise in numbers of *Pseutoterranova* with increase in length up to about 40 cm, after which it levelled off. There may have been a further increase in fish over 70 cm but the numbers of such fish examined were small. *Anisakis* showed a similar increase in numbers in fish up to 50 cm in length and then also levelled off. Larval anisakids are long lived and it might therefore be expected that number would rise throughout the life of the fish host. That this does not seem to occur in monkfish might be due to a failure to acquire new infections, which seems unlikely, or that invading worms fail to reach the muscle. Possible reasons for the latter might be the greater distance involved in such a migration in larger fish, or a greater host response which encapsulates worms within the viscera.

For both *Anisakis* and *Pseudoterranova* there were significantly higher numbers of parasites in female monkfish. The reasons for this are unknown, but may reflect a larger size of female fish at a comparable age.

Monkfish showed significant differences in infection with both *Anisakis* and *Pseudoterranova* according to their location of capture. Fish from the offshore northern North Sea and from West of the Hebrides had significantly less *Pseudoterranova* than those from around Shetland, whilst, with the exception of Rockall, monkfish from all other areas had significantly less *Anisakis* than fish from Shetland. The geographic distribution of anisakids seems to depend to a large extent on the abundance of the final mammalian host. Thus, it is not surprising that *Pseudoterranova* was less abundant in offshore waters where grey seals are scarce (Young 1972, Wootten & Waddell 1977). Other studies (Smith & Wootten 1978, Wootten & Waddell 1977) have shown that *Anisakis* is more abundant in the northern North Sea than in coastal waters, probably because of the distribution of cetacean final hosts

and euphausid crustacean hosts. Although it is thus not unexpected that higher numbers of *Anisakis* were found in monkfish from around Shetland it is surprising that the parasite was less abundant in fish from the central northern North Sea. The pattern of final host distribution may also explain the effect of depth of capture on parasite numbers, with a decrease in *Pseudoterranova* with increasing depth and a reverse situation with *Anisakis*.

In monkfish, as in cod and mackerel, analysis suggested significant year to year variation in parasite numbers. Although it would be surprising if such variation did not occur, no conclusions can be drawn from the present study because of the non-uniformity of samples between years.

4.2.2 Cod

Cod were commonly infected with both *Anisakis* and *Pseudoterranova,* but showed a higher prevalence, mean intensity and abundance of *Anisakis* than monkfish. Although the prevalence of *Pseudoterranova* was similar to that of monkfish, mean intensity and abundance were higher. As in the latter species, the levels of infection reflect the piscivorous diet of cod and, in the case of *Pseudoterranova*, the consumption of benthic invertebrate hosts.

Both *Anisakis* and *Pseudoterranova* numbers increased with length of cod. This was very marked for fish up to 60 cm in length and appeared to continue to a lesser extent in larger fish, although the numbers of such fish examined were low. Other authors have found similar relationships (Wootten & Waddell 1977, des Clers 1992). This increase, as in monkfish, reflects the longevity of the parasites and increased consumption of infected fish hosts.

Sampling area had a major influence on parasite numbers in cod. *Anisakis* was most abundant in fish from around Shetland and significantly less so in cod from waters to the West and North of mainland Scotland. Conversely *Pseudoterranova* was much more abundant in the latter areas. As with monkfish this distribution is likely to be largely driven by final host distribution and it corresponds to previous findings (Young 1972, Wootten & Waddell 1977). The finding that peak levels of *Pseudoterranova* occurred at depths of ca 150m also reflects the predominantly coastal distribution of the parasite.

It is difficult to compare infection levels of cod found in the present study with earlier reports because differences in some sampling areas and sizes of fish. However, it would certainly seem that there has been no reduction in numbers of either parasite species and in some areas there has been a substantial increase (Wootten & Waddell 1977). This is particularly true for *Pseudoterranova* from the North coast of Scotland and, surprisingly, the offshore Northern North Sea and also for *Anisakis* in fish from the North and West mainland coasts, as well as the offshore North Sea. There is no comparable data for Shetland. Increases in *Pseudoterranova* numbers might be driven by changes in size of grey seal populations but for *Anisakis* the reasons are unknown.

4.2.3 Mackerel

The mackerel examined in this study were from a single location sampled at one time and therefore it was not possible to explore the effect of a range of variables on infection levels.

Only *Anisakis* was found in mackerel which is certainly because of the pelagic habit of the fish and its planktonic invertebrate diet. Mackerel thus have no opportunity to come into contact with *Pseudoterranova.*

The prevalence, intensity and abundance of *Anisakis* in the flesh of mackerel was not high compared with the finding of a previous study on the same stock of fish (Levsen 2007) and this is probably due to the relatively small size of fish samples. There was a significant association of *Anisakis* numbers with increasing length of mackerel, again reflecting the longevity of the parasite and continued consumption of infected prey by the fish. Interestingly, Levsen (2007) showed that in mackerel above 450g in weight there was a significantly lower abundance of *Anisakis* than in smaller fish. Unfortunately fish of this larger size were not examined in the present study.

Smith (1984) described a migration of *Anisakis* from the viscera into the flesh of mackerel after death of the fish. Such a migration cannot be ruled out in the present study since fish were not gutted before freezing.

4.2.4 Herring

Two samples of herring were examined, one of which was from the West of Scotland. This sample population was found to be uninfected with either *Anisakis* or *Pseudoterranova*. Conversely, the sample from the North Sea had a relatively high prevalence, intensity and abundance of infection, although only *Anisakis* was found which, as in mackerel, reflects the pelagic habitat and planktonic diet of herring.

The difference in infection levels between the samples from the North Sea and the West of Scotland is in agreement with previous studies (Khalil 1969, Davy 1972, Cross *et al.* 2007), where fish from the West of Scotland were generally found to be more lightly infected with *Anisakis.* West coast herring represent different stock(s) from those in the North Sea and the populations do not mix.

Given that only one sample of herring was examined from the North Sea it is not possible to make definite comparisons of the worm burdens recorded here with the results of earlier studies. However, the numbers found are not dissimilar to those recorded by Smith & Wootten (1975).

There was generally a positive association between length of herring and members of *Anisakis*, probably due to the same factors as discussed for mackerel.

As with mackerel, some authors have suggested that there is a migration of *Anisakis* from the viscera into the flesh after the death of the host (e.g. Smith & Wootten 1975), others have found no evidence of a migration (e.g. Roepstorff *et al.* 1993). Since in this study herring were frozen ungutted it was not possible to determine if any migration had occurred.

4.3 Location in host

In all the fish species examined there were significant differences in the location of *Anisakis* and *Pseudoterranova* in various parts of the musculature.

The great majority of *Anisakis* were recovered from the flaps or hypaxial muscles surrounding the body cavity in all species of fish. Only small numbers of worms had penetrated as far as the fillets or epaxial muscles. This general pattern of distribution of *Anisakis* has been observed previously by a number of authors in a variety of fish species, including cod (e.g. see Wootten & Waddell 1977, Brattey & Bishop 1992). *Anisakis* larvae penetrate through the stomach wall of fish (Wootten & Smith 1975) and from there will reach the flaps first. Most apparently are encapsulated there rather than penetrating further to reach the fillets.

The significance of this distribution of *Anisakis* within the flesh is that the majority of parasites will be removed if the flaps are trimmed off and discarded during processing, as in fact occurs in many products.

With *Pseudoterranova* the situation is rather different. In monkfish the majority (60%) were found within the fillet or tail and in cod, although the largest proportion of worms were found in the flaps, ca 45% occurred in the fillets. Other authors have found that relatively large numbers of *Pseudoterranova* can be found in the fillets of cod, although not to the extent found here (Wootten & Waddell 1977). The latter authors found that the distribution of *Pseudoterranova* was age-related, with an increasing proportion of worms found in the flaps in older and larger fish. It is not clear why a larger proportion of *Pseudoterranova* reach the fillets. It may be a function of their larger size which allows a longer migration. As with *Anisakis,* removal of the flaps during processing will remove a large proportion of the *Pseudoterranova* present, although substantial numbers may remain in the fillet.

In all fish species examined, with the exception of mackerel, significantly more parasites of both species were found in the musculature of the left side of the fish. This may be because of the disposition of organs within the body cavity which might to some extent obstruct the path of migrating worms into the right side musculature. Why this effect was not seen in mackerel is unclear.

4.4 Detection methods

For all fish species analysed it is clear that only destructive methods, *ie* slicing, digestion or pressing can recover the majority of worms from fillets, particularly in larger fish. Thus to quantify worms in a given catch it will be necessary to destructively screen a sub-sample of the fish (see below).

4.4.1 Monkfish

The results of this study indicate that that unaided observation by eye is a poor method for detecting either species of worm in the fillets of monkfish as the small white *Anisakis* are easily missed and the large red *Pseudoterranova* can only be seen if lying close to the surface. Whilst detection of *Pseudoterranova* in flaps is easier using visual examination (since the tissue is much thinner), this remains a poor way to detect and quantify worms in the edible portions of the fish. Whilst *Anisakis* can be recovered from flaps by candling, this method picks up very few worms in fillets and slicing is necessary for good recovery. Candling and candling with slicing are highly effective for picking up *Pseudoterranova* in flaps but again, only slicing allows them to be recovered from fillets in numbers. These observations support previous reports of poor detection performance by candling alone (Levsen & Lunestad, 2005). Overall this indicates that only destructive methods will pick up the majority of

worms in fillets, and, with the observation that candling of whole fillets is only effective for monkfish of <37 cm in length, the latter is clearly not a practical way to screen whole catches. Destructive methods could, however, be used for sub-samples to establish whether or not catches from a given stock or locations are highly infected. Visual examination of the skinned tail, for the more obvious *Pseudoterranova*, is, however, useful for screening samples in a more general way and could realistically be used for screening whole catches prior to sale or export. This follows from the fact that monkfish tails are manually processed prior to export, making visual screening straightforward and from the fact that the large red *Pseudoterranova* are easily seen when handling the fish. Moreover, it is likely that these surface worms are the worms which trigger rejection of consignments.

4.4.2 Cod

Results for cod, in terms of detection of worms, were very similar to those for monkfish, with the unaided eye performing poorly for flaps and fillets, particularly for *Anisakis*. Again, only candling and slicing together allowed the majority of worms of both species to be recovered from fillets indicating that only destructive methods can truly quantify worms in these fish. As with monkfish visual examination and candling of cod fillets, particularly if skinned, could be a realistic way of screening fish during processing for the more obvious species of *Pseudoterranova*. This would, however, be an inefficient method for detecting *Anisakis* and *Pseudoterranova* in thick fillets.

4.4.3 Mackerel and herring

Because both of these species are captured in large numbers, either sold in the round (whole and ungutted) or filleted automatically, and because mackerel in particular has dark flesh, visual observation of these fish for worms using eye, candling or slicing is not practical. Two destructive techniques, digestion and pressing were both employed to detect worms in this study and were both found to be equally successful in detecting *Anisakis*, the only worm present in these species. Since pressing is the more practical of these techniques, it is clear that this method should be the method of choice for screening sub-samples of these species.

5. Summary and Conclusions

This study has shown that fish from Scottish waters are commonly parasitized by *Anisakis simplex* and / or *Pseudoterranova decipiens*. The latter is most significant from an aesthetic or fish rejection point of view because of its large size and colouration. On the other hand, *Anisakis* is perhaps more significant from a human health aspect because of its tendency to infect fish which may be eaten uncooked or lightly cured, such as herring, and because of difficulties of detection due to its small size and lack of colour.

Monkfish and cod were found to be heavily infected by both *Anisakis* and *Pseudoterranova* with respect to historical records and overall worm infection was quite high in all areas sampled, suggesting that targeting different fishing grounds would not be very effective in terms of lowering worm numbers. There may be more variation in *Anisakis* numbers in herring with respect to geographical location but further work would be required on this. Mackerel stocks and northern N. Sea herring would appear likely to be widely affected.

In all fish species examined there was a tendency for infection to increase with length, but even the smallest commercial sizes are infected.

Given that there seems little opportunity for targeted fishing to prevent worms being present in fish for human consumption, processing techniques would appear to represent the best chance of removing worms before sale.

In most cases the majority of worms in the flesh are present in the belly flaps. Therefore, if these are trimmed off during processing, as indeed does occur for most whitefish, the numbers of worms in fish products reaching the consumer will be greatly reduced, although a sizeable proportion will remain.

Detection of worms by available means is inefficient but even simple visual inspection should lead to the removal of the most obvious parasites. This would be particularly true for *Pseudoterranova* and, if carried out, might have prevented some of the recent cases of rejection of monkfish shipments. Candling of fillets could also remove a further proportion of worms, again particularly *Pseudoterranova.* This technique would be quite effective with small fish, but its efficiency decreases rapidly in thicker fillets. Visual inspection and candling are not effective for dark fleshed fish such as herring and mackerel and for such species it is essential that current freezing regulations are adhered to for fish that are to be consumed raw or almost raw.

The study has shown that it is possible to accurately detect the numbers of worms in the flesh of fish using destructive methods of examination. Thus, for whitefish, a combination of candling and slicing of the flesh is efficient. For small fish, and those with dark flesh such as herring and mackerel, digestion techniques or pressing of the flesh followed by examination under UV light are very effective. These techniques are not likely to be of routine interest to processors, except where they may need information on the numbers of worms in particular consignments. However, they could be of value to authorities who require an assessment of worm numbers.

A further complication in such sub-sampling is the question of how many fish need to be sampled to detect a given level of infection. The study has provided examples of the numbers involved to detect a

given infection using detection methods of varying efficiencies. At low levels of infection the numbers of fish involved might be very large and are probably impractical, at least for high value species. If infection levels are high, or the acceptable likelihood of detecting infected fish is low then numbers become more realistic. Such sub-sampling methods are likely to be most useful for low value, small species such as herring or mackerel.

Recommendations

- Filleted and skinned monkfish and cod should always be inspected, at least by eye and preferably by candling, during processing or before sale to remove obvious worms.

- The belly flaps should be removed from monkfish and cod during processing or before sale for fillet use to remove a large proportion of worms present.

- To obtain an estimate of the prevalence of infection in any batch of fish a sample should be subjected to a destructive test such as digestion, or in the case of small fish such as herring or mackerel, pressing under UV light. The number of fish to be tested will depend upon the acceptable level of prevalence but should not be less than 30.

APPENDIX 1 - Example of fish map

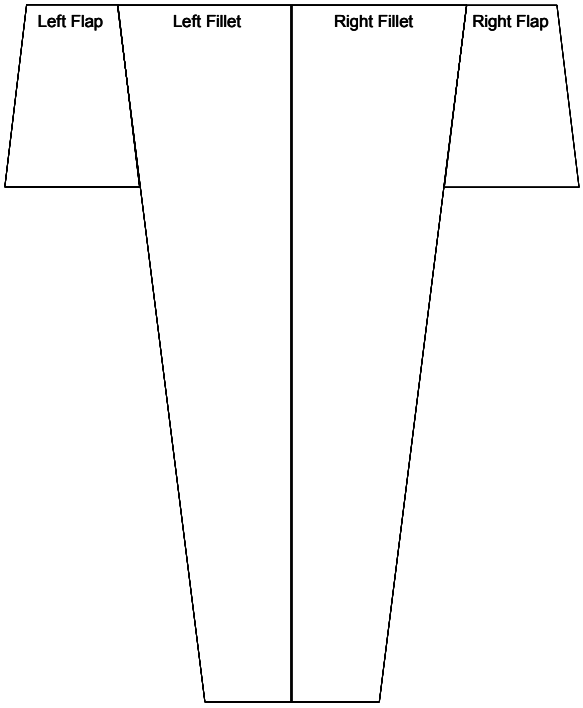


Figure Ia Example of flesh location map for monkfish.

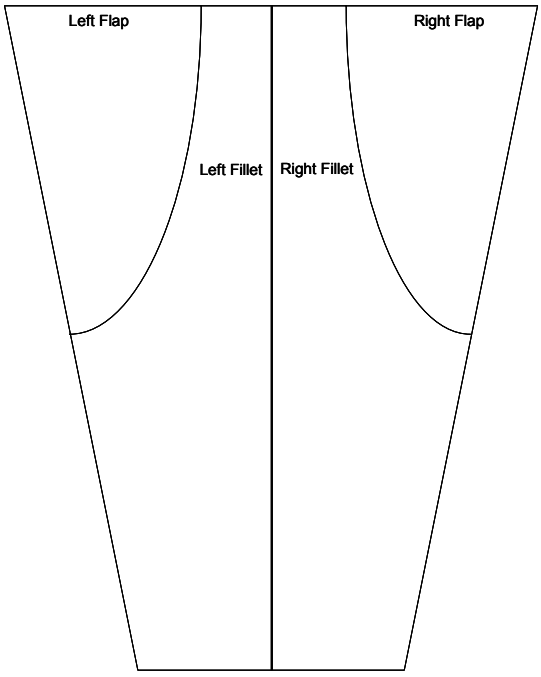


Figure IIa. Example of flesh location map for cod, mackerel and herring.

APPENDIX 2 - Questionnaire

WHITEFISH PROCESSORS QUESTIONNAIRE FOR NEMATODE WORMS 2005

All information received will be assured complete confidentiality

COMPANY DETAILS	
Name:	
Company Name:	
Address:	
Phone Number:	
Email Address:	

Please indicate if you process the following species and from which location (if known);	
Monkfish	
Cod	
Whiting	
Others	

Are your staff familiar with worms in fish, especially those in the attached photographs?	
Is it your current practise to examine fish for these worms?	
If so, what methods do you use for detecting worms in fish?	
Please provide details of equipment/facilities used for these procedures:	
In what species of fish are worms most often detected?	
Are fish ever examined by your Environmental Health Officer? If yes, how often?	
Have you ever experienced rejections of exported consignments due to worms? If so, which fish, from which country and when?	
Would you find recommendations for best practice with regard to detection/removal of worms useful?	
Thank you very much for your time. We will keep you informed of any future developments.	
If you require further information contact Allan Petrie on 01224 295523 or email allan.petrie@stir.ac.uk	

APPENDIX 3 - Scottish Catch Data

Maps of Scottish fish captures in 2005 according to compiled Scottish Executive figures. Maps obtained from: http://www.scotland.gov.uk/Topics/Fisheries/Sea-fisheries/marketing/maps on 28-08-07.

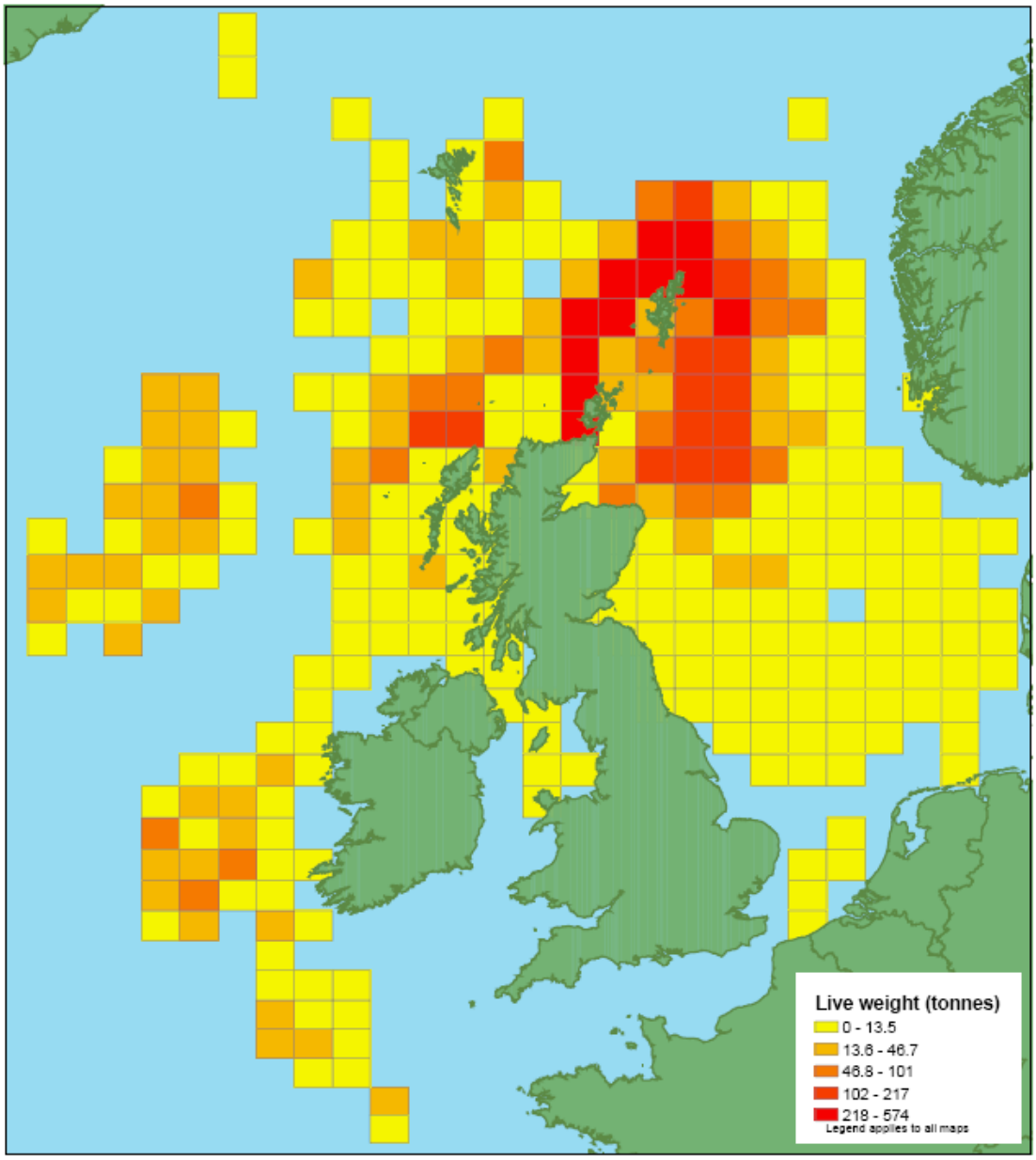


Figure IIIa. Monkfish Captures by Scottish vessels in 2005

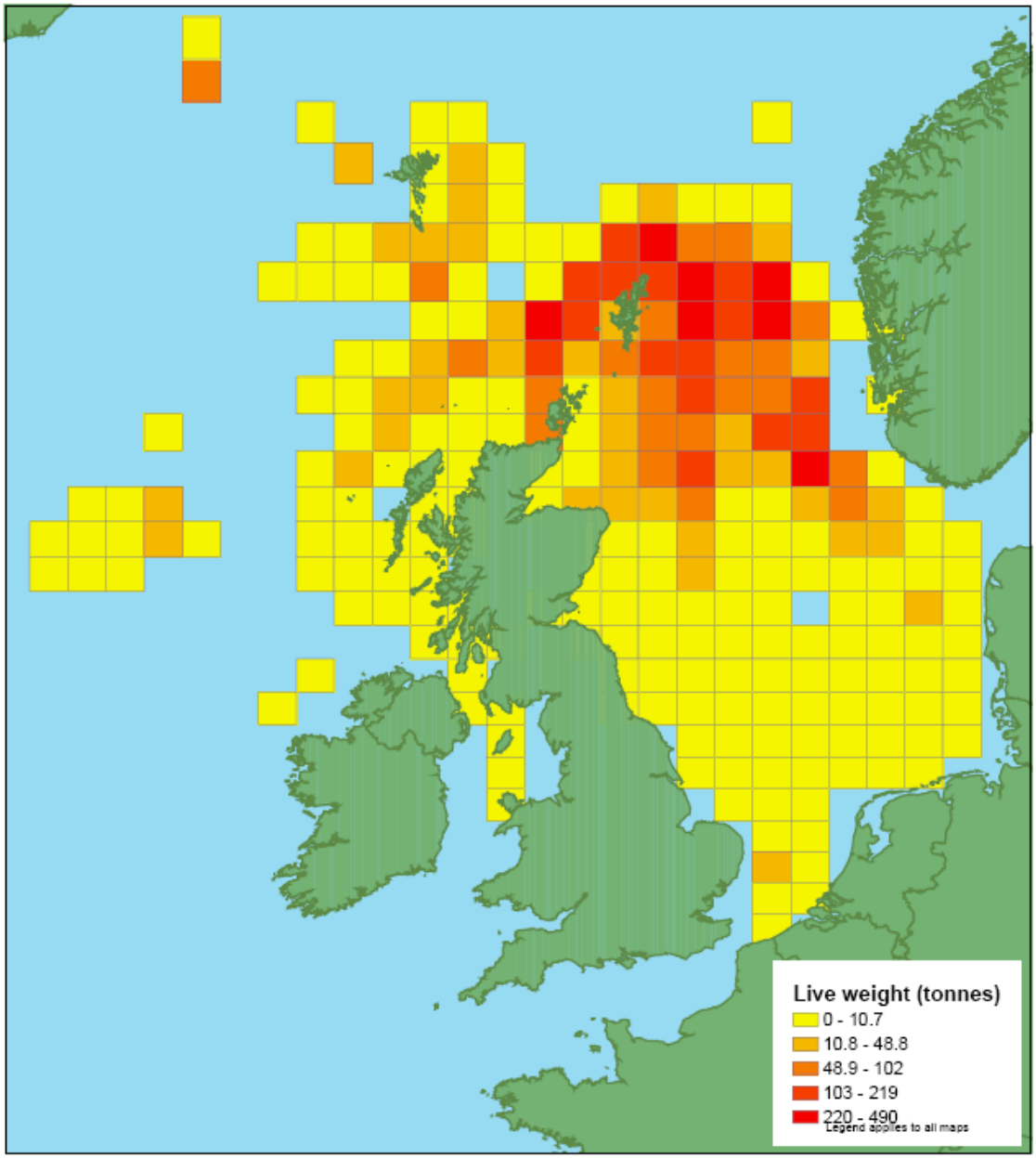


Figure IIIb. Cod captures by Scottish vessels in 2005.

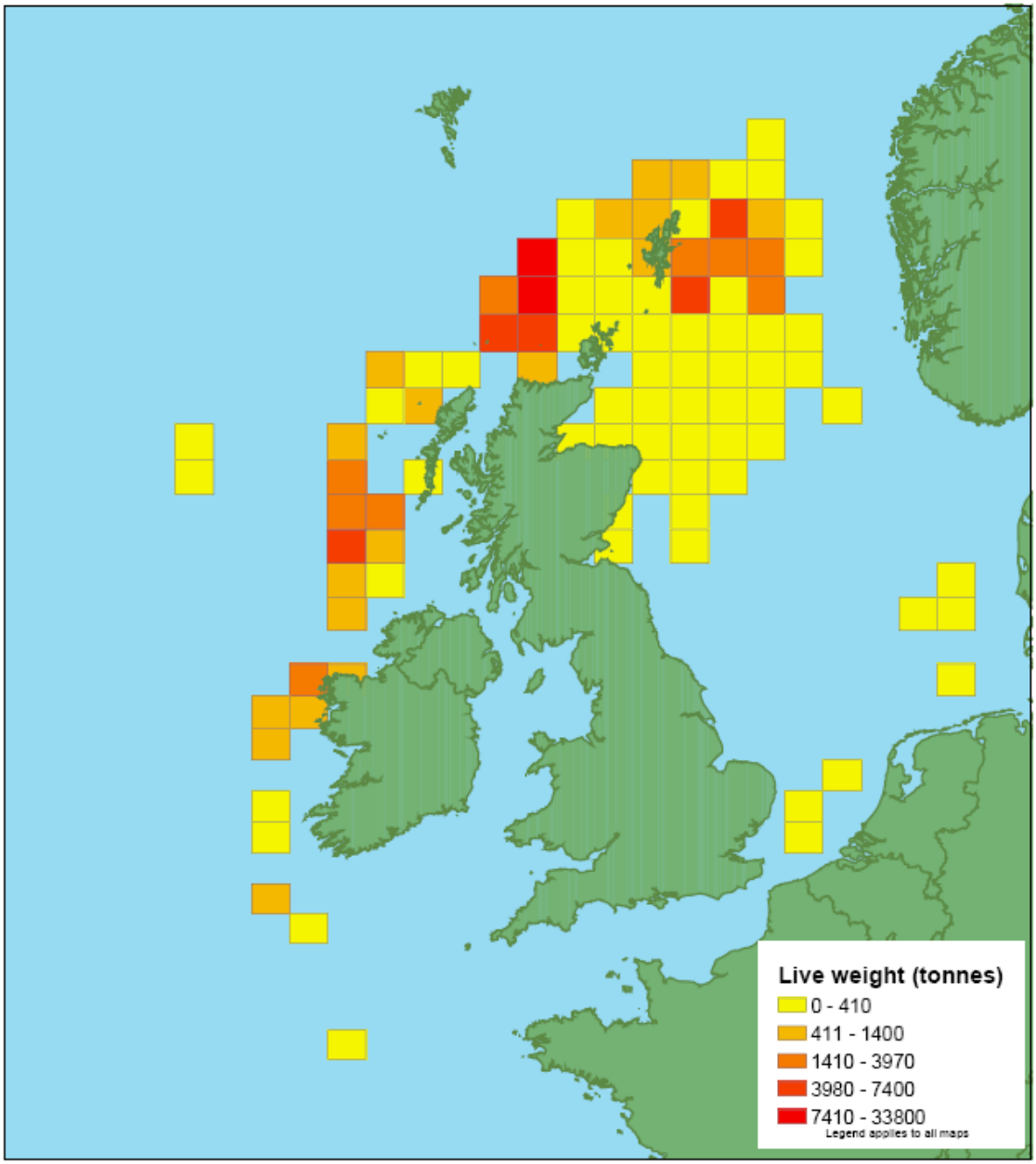


Figure IIIc. Mackerel captures by Scottish vessels in 2005.

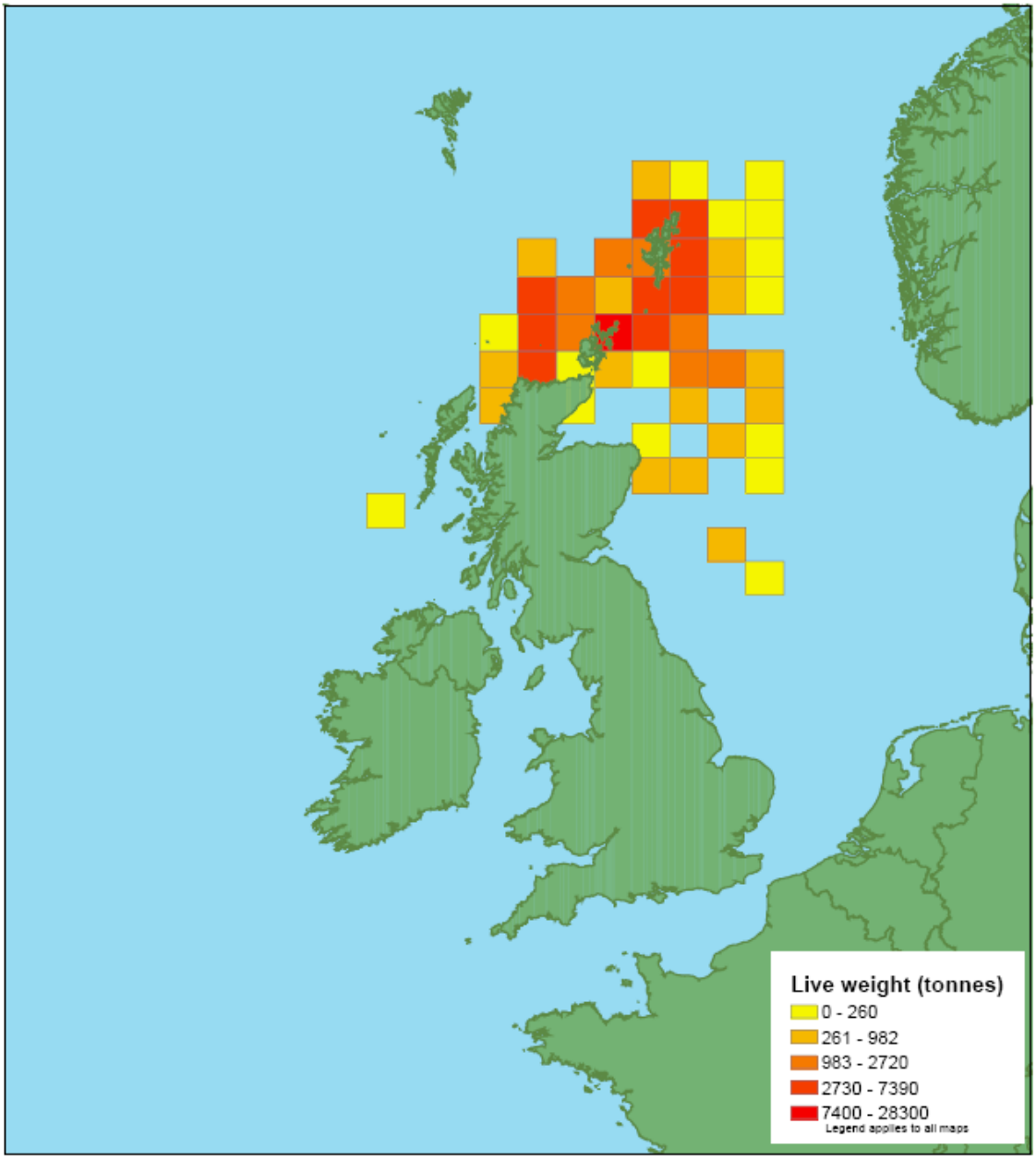


Figure IIId. Herring captures by Scottish vessels in 2005.

APPENDIX 4 – Model Specifications

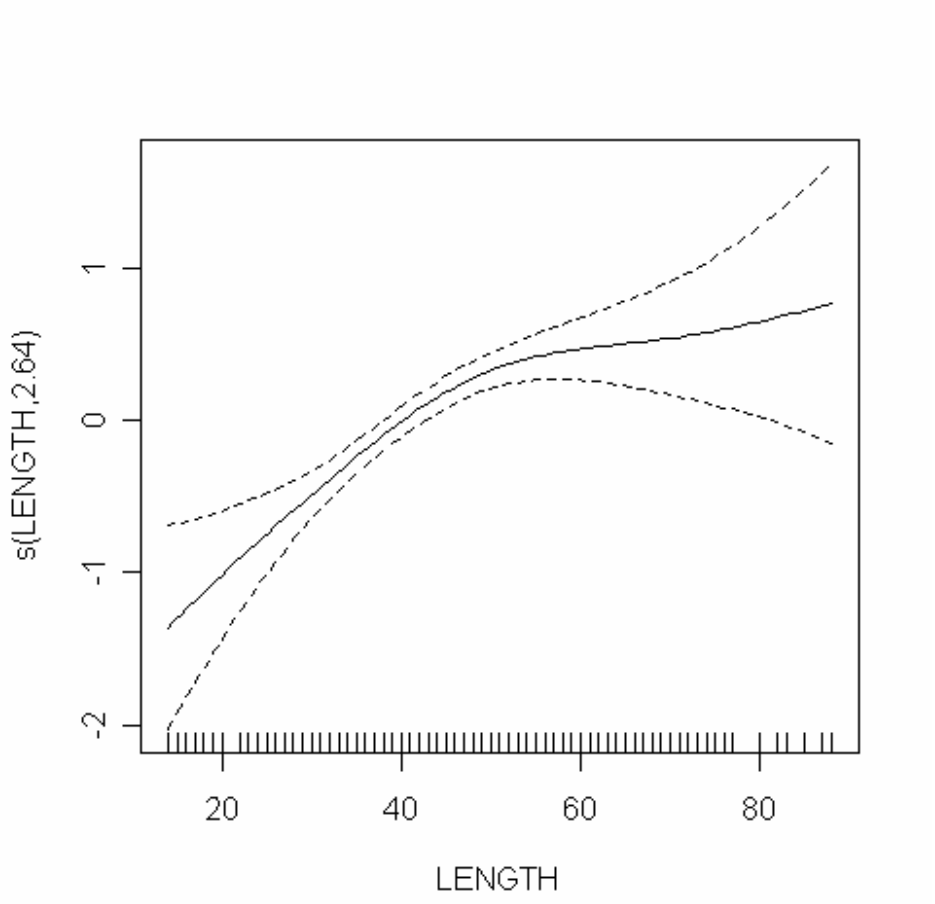
The following models are Generalized Additive Models (GAMs) carried out according to the principles outlined by Wood (2006) and applied using the mgcv library for R. Models were constructed using a reverse stepwise procedure with the initial model incorporating fixed terms, interaction between the fixed terms, and additional individual non-linear length effects for each sex and location where appropriate. Poisson, semi-Poisson and negative binomial distributions were tested according to the requirements of the distribution of the data, with a binomial model used for presence / absence data. Model fit was assessed using AIC scores (using formula -2log(Likelihood) + 2*df) and by observing model residuals. A parsimonious modelling strategy was employed whereby additional terms, which provided minimal improvement to the model, were excluded rather than included. Although they represented the most successful modelling technique, the models suffered from a lack of data in largest and smallest fish size categories (due to the nature of the fisheries in question) and from the arbitrary (in the sense of being non-systematic) depths of capture employed during most cruises. Outliers have been removed where necessary for modelling as have individuals with missing data. Modelling or worm presence / absence in particular was very poor since the two species show diametrically opposite infection behaviours for a number of the key factors measured.

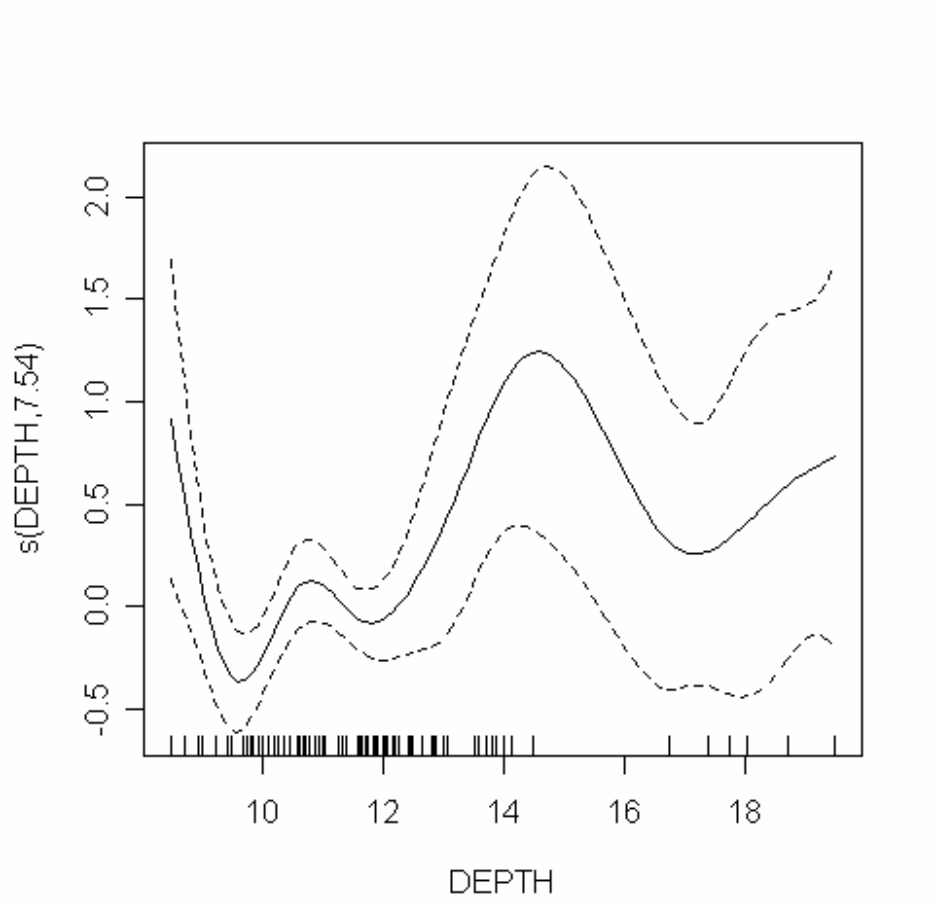
Anisakis in Monkfish

Family: poisson
Link function: log

```
Formula:
Y1 ~ 1 + as.factor(YEAR) + as.factor(LOCATION) + as.factor(SEX) +
    s(DEPTH) + s(LENGTH)

Parametric coefficients:
                    Estimate Std. Error z value Pr(>|z|)
(Intercept)          -0.26941    0.08376  -3.217 0.001297 **
as.factor(YEAR)2     -0.71795    0.16789  -4.276 1.90e-05 ***
as.factor(LOCATION)C -0.39906    0.11441  -3.488 0.000487 ***
as.factor(LOCATION)E -0.58625    0.18794  -3.119 0.001813 **
as.factor(LOCATION)F -0.97824    0.53260  -1.837 0.066251 .
as.factor(LOCATION)A -1.15750    0.25787  -4.489 7.17e-06 ***
as.factor(SEX)2      -0.20505    0.09524  -2.153 0.031325 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
           edf Est.rank Chi.sq  p-value
s(DEPTH)  7.544        9  26.15  0.00193 **
s(LENGTH) 2.644        6  52.52 1.46e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.054   Deviance explained = 11.3%
UBRE score = 0.29462  Scale est. = 1         n = 961

Dispersion parameter                       =  1
Deviance                                   =  1209.76
n           (null degrees of freedom)      =  960
df.residual (residual degrees of freedom)  =  943.81
df          (n-df.residual)                =  16.19

Overdispersion (Deviance/df.residual )     =  1.28

AIC according to formula: -2log(Likelihood) + 2*df   = 1884.86
This AIC formulation is recommended.
```

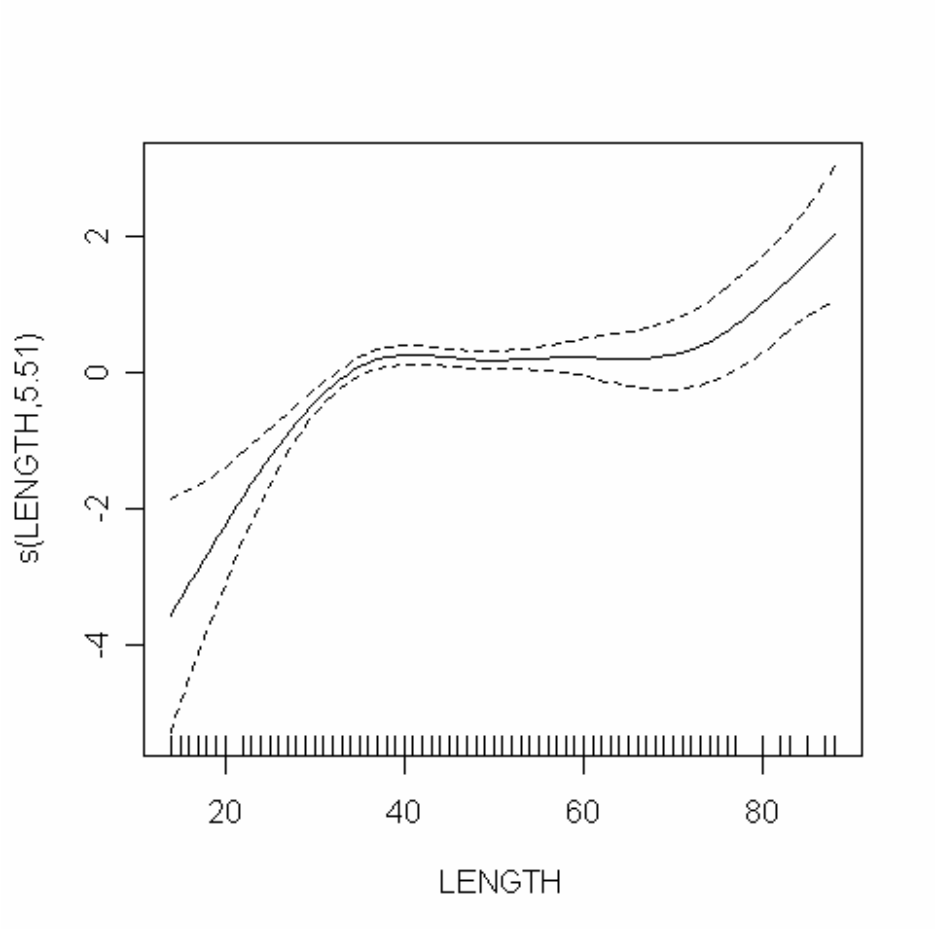
```
Alternative AIC formulation:
AIC according to formula: (Deviance + 2*df  )/n = 1.29


Parametric Terms:
                                 df Chi.sq  p-value
DEPTH                             1 10.476  0.00121
factor(YEAR)                      1 19.771  8.73e-06
factor(SEX)                       1  6.281  0.01221
factor(LOCATION)                  4 32.562  1.47e-06
factor(SEX):factor(LOCATION)      4 11.446  0.02199
```




s(DEPTH,7.54)
-0.5
0.0
0.5
1.0
1.5
2.0
10
12
14
16
18
DEPTH

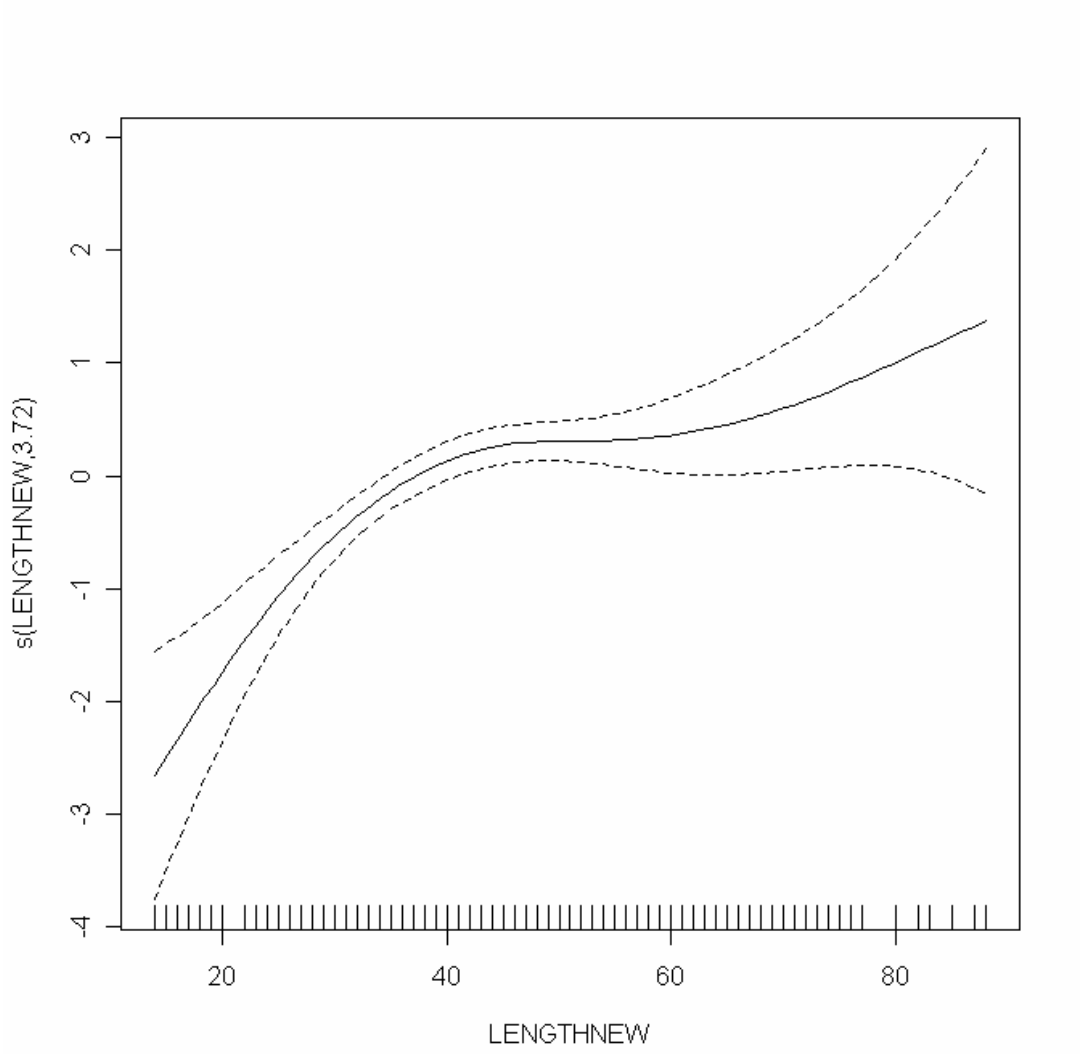
Pseudoterranova in Monkfish

```
Family: poisson
Link function: log

Formula:
Y1 ~ 1 + DEPTH + as.factor(YEAR) + as.factor(LOCATION) + as.factor(SEX)
     + s(LENGTH) + as.factor(SEX):as.factor(LOCATION)

Parametric coefficients:
                                    Estimate Std. Error z value Pr(>|z|)
(Intercept)                          0.89545    0.42401   2.112 0.034696 *
DEPTH                               -0.12136    0.03749  -3.237 0.001209 **
as.factor(YEAR)2                    -0.51482    0.11578  -4.446 8.73e-06 ***
as.factor(LOCATION)3                 0.12338    0.12608   0.979 0.327796
as.factor(LOCATION)5                -1.41011    0.36923  -3.819 0.000134 ***
as.factor(LOCATION)6                 0.22117    0.58146   0.380 0.703675
as.factor(LOCATION)7                 0.63003    0.17099   3.685 0.000229 ***
as.factor(SEX)2                      0.29906    0.11933   2.506 0.012207 *
as.factor(LOCATION)3:as.factor(SEX)2 -0.23884    0.17198  -1.389 0.164901
as.factor(LOCATION)5:as.factor(SEX)2  0.70507    0.43455   1.623 0.104695
as.factor(LOCATION)6:as.factor(SEX)2 -1.45093    0.87731  -1.654 0.098160 .
as.factor(LOCATION)7:as.factor(SEX)2 -0.49517    0.23893  -2.072 0.038229 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
           edf Est.rank Chi.sq  p-value
s(LENGTH) 5.505        9  65.85 9.86e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.0661   Deviance explained = 12.4%
UBRE score = 0.48531  Scale est. = 1         n = 961

Dispersion parameter                      =  1
Deviance                                  =  1392.37
n           (null degrees of freedom)     =  960
df.residual (residual degrees of freedom) =  943.49
df          (n-df.residual)               =  16.51

Overdispersion (Deviance/df.residual )    =  1.48

AIC according to formula: -2log(Likelihood) + 2*df   = 2305.64
This AIC formulation is recommended.
Alternative AIC formulation:
AIC according to formula: (Deviance + 2*df  )/n = 1.48

Parametric Terms:
                                 df Chi.sq  p-value
DEPTH2                            1 10.476  0.00121
factor(YEAR)                      1 19.771  8.73e-06
factor(SEXNEW)                    1  6.281  0.01221
factor(LOCATION)                  4 32.562  1.47e-06
factor(SEXNEW):factor(LOCATION)   4 11.446  0.02199
```


s(LENGTH,5.51)
2
0
-2
-4
20
40
60
80
LENGTH

Presence / Absence of worms in Monkfish

```
Family: binomial
Link function: logit

Formula:
PARA ~ s(LENGTH) + factor(YEAR) + factor(LOCATION)

Parametric coefficients:
                  Estimate Std. Error z value Pr(>|z|)
(Intercept)        0.43550    0.11269   3.865 0.000111 ***
factor(YEAR)2     -0.86405    0.18397  -4.697 2.64e-06 ***
factor(LOCATION)C -0.01928    0.16000  -0.120 0.904110
factor(LOCATION)E -0.67573    0.24267  -2.785 0.005359 **
factor(LOCATION)F -0.68570    0.46797  -1.465 0.142848
factor(LOCATION)A  0.27812    0.23773   1.170 0.242053
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
               edf Est.rank Chi.sq p-value
s(LENGTH) 3.716        8  55.79 3.1e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.0817   Deviance explained = 6.85%
UBRE score = 0.30424   Scale est. = 1   n = 961

AIC 1253.377

Parametric Terms:
                df Chi.sq   p-value
factor(YEAR)     1  22.06   2.64e-06
factor(LOCATION)  4  12.14   0.0163
```

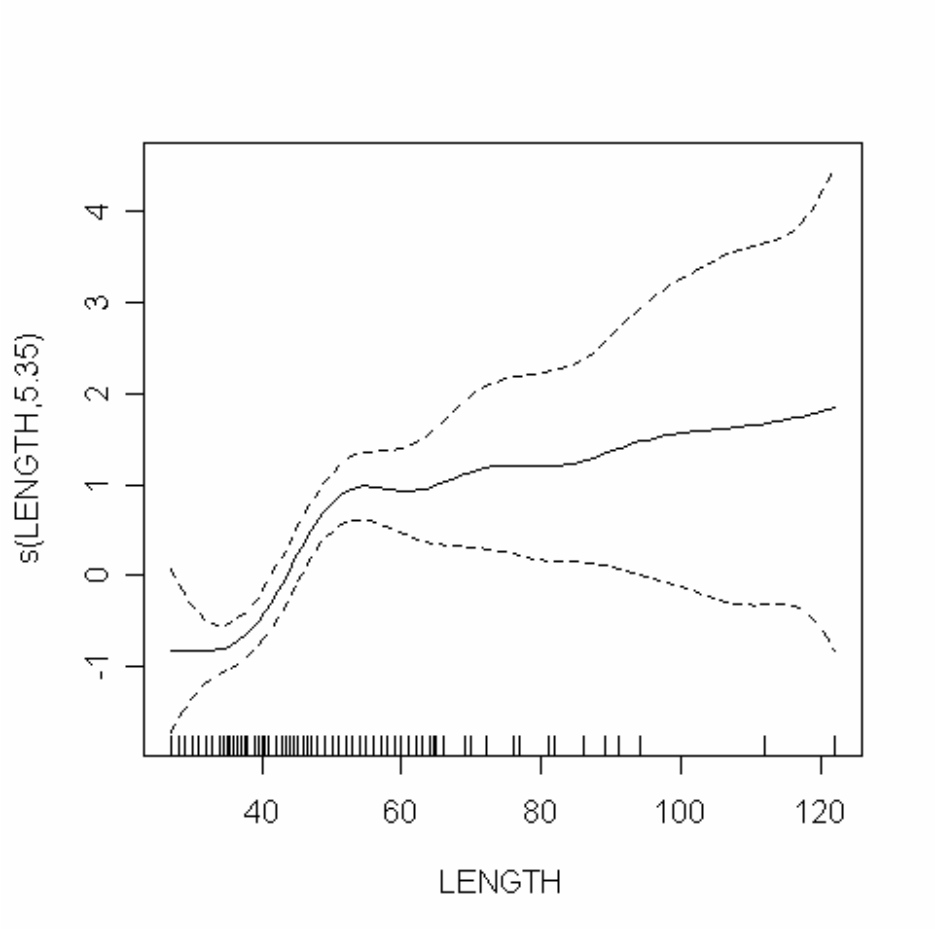

s(LENGTHNEW,3.72)
3
2
1
0
-1
-2
-3
-4
20
40
60
80
LENGTHNEW

Anisakis in Cod

```
Family: Negative Binomial(0.4505)
Link function: log

Formula:
Y1 ~ 1 + as.factor(LOCATION) + as.factor(SEX) + s(LENGTH) + as.factor(SEX):as.factor(LOCATION)

Parametric coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                          1.24687    0.22220   5.612 4.34e-08 ***
as.factor(LOCATION)B                -2.84609    0.54204  -5.251 2.76e-07 ***
as.factor(LOCATION)C                -1.56193    0.47058  -3.319  0.00101 **
as.factor(LOCATION)E                -0.73269    0.38764  -1.890  0.05965 .
as.factor(LOCATION)A                -3.05299    0.99236  -3.076  0.00228 **
as.factor(SEX)2                      0.03126    0.29062   0.108  0.91439
as.factor(LOCATION)B:as.factor(SEX)2 -0.64801    0.85946  -0.754  0.45142
as.factor(LOCATION)C:as.factor(SEX)2  1.09786    0.56671   1.937  0.05359 .
as.factor(LOCATION)E:as.factor(SEX)2 -0.23326    0.49437  -0.472  0.63737
as.factor(LOCATION)A:as.factor(SEX)2 -0.27960    1.28098  -0.218  0.82736
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
            edf Est.rank     F  p-value
s(LENGTH) 5.353        9 7.301 1.11e-09 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.284   Deviance explained =   38%
GCV score = 1.0479   Scale est. = 1         n = 336

Dispersion parameter                      =  1
Deviance                                  =  253.31
n           (null degrees of freedom)     =  335
df.residual (residual degrees of freedom) =  320.65
df          (n-df.residual)               =  14.35

Overdispersion (Deviance/df.residual )    =  0.79

AIC according to formula: -2log(Likelihood) + 2*df   = 1181.04
This AIC formulation is recommended.
Alternative AIC formulation:
AIC according to formula: (Deviance + 2*df  )/n = 0.84
```

Parametric Terms:

	df	F	p-value
factor(SEXNEW)	1	0.012	0.914
factor(LOCATION)	4	9.766	1.82e-07
factor(SEXNEW):factor(LOCATION)	4	1.496	0.203

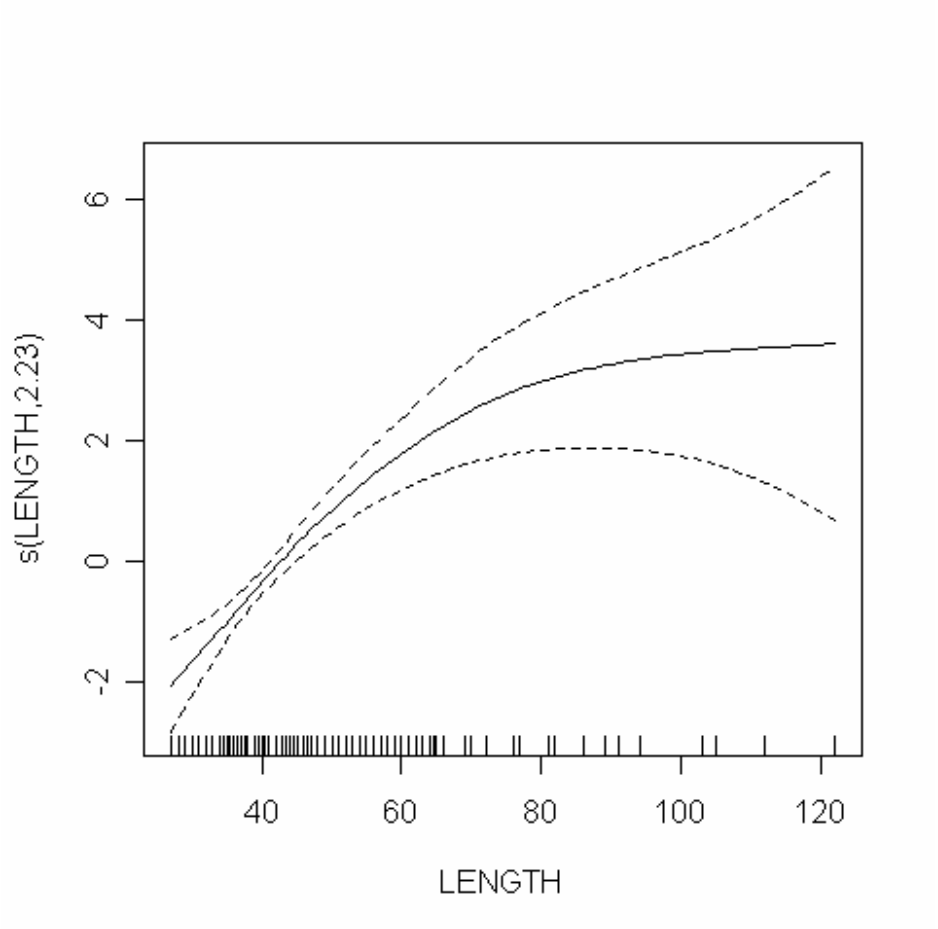

s(LENGTH,5.35)
-1
0
1
2
3
4
40
60
80
100
120
LENGTH

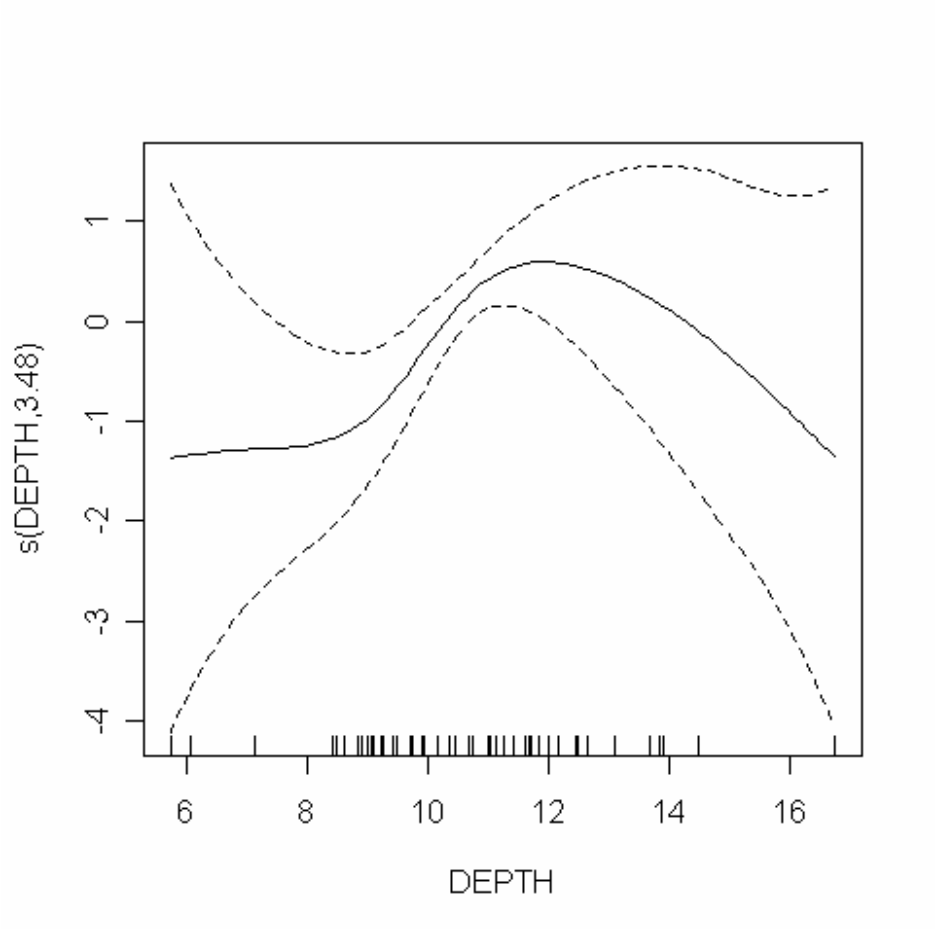
Pseudoterranova in Cod

```
Family: Negative Binomial(0.1902)
Link function: log

Formula:
Y1 ~ 1 + as.factor(LOCATION) + as.factor(SEX) + s(LENGTH) + s(DEPTH) +
    as.factor(SEX):as.factor(LOCATION)

Parametric coefficients:
                                    Estimate Std. Error t value Pr(>|t|)
(Intercept)                          -1.2587     0.4685  -2.687 0.007625 **
as.factor(LOCATION)2                  1.6716     0.8426   1.984 0.048211 *
as.factor(LOCATION)3                  2.3745     0.6837   3.473 0.000592 ***
as.factor(LOCATION)5                 -0.6396     0.7504  -0.852 0.394744
as.factor(LOCATION)7                  1.7718     0.9762   1.815 0.070559 .
as.factor(SEX)2                       1.2184     0.5865   2.077 0.038633 *
as.factor(LOCATION)2:as.factor(SEX)2 -0.8653     0.9608  -0.901 0.368541
as.factor(LOCATION)3:as.factor(SEX)2 -1.1083     0.8707  -1.273 0.204091
as.factor(LOCATION)5:as.factor(SEX)2 -1.4468     0.9196  -1.573 0.116722
as.factor(LOCATION)7:as.factor(SEX)2 -2.7042     1.2978  -2.084 0.038045 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
           edf Est.rank      F  p-value
s(LENGTH) 2.231        5 10.130 5.76e-09 ***
s(DEPTH)  3.480        7  2.537   0.0151 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =     -4   Deviance explained =   38%
GCV score = 1.0534   Scale est. = 1         n = 310

Dispersion parameter                       =  1
Deviance                                   =  151.77
n          (null degrees of freedom)       =  309
df.residual (residual degrees of freedom)  =  294.29
df          (n-df.residual)                =  14.71

Overdispersion (Deviance/df.residual )     =  0.52

AIC according to formula: -2log(Likelihood) + 2*df   = 802.08
This AIC formulation is recommended.
Alternative AIC formulation:
AIC according to formula: (Deviance + 2*df  )/n = 0.59
```

Parametric Terms:

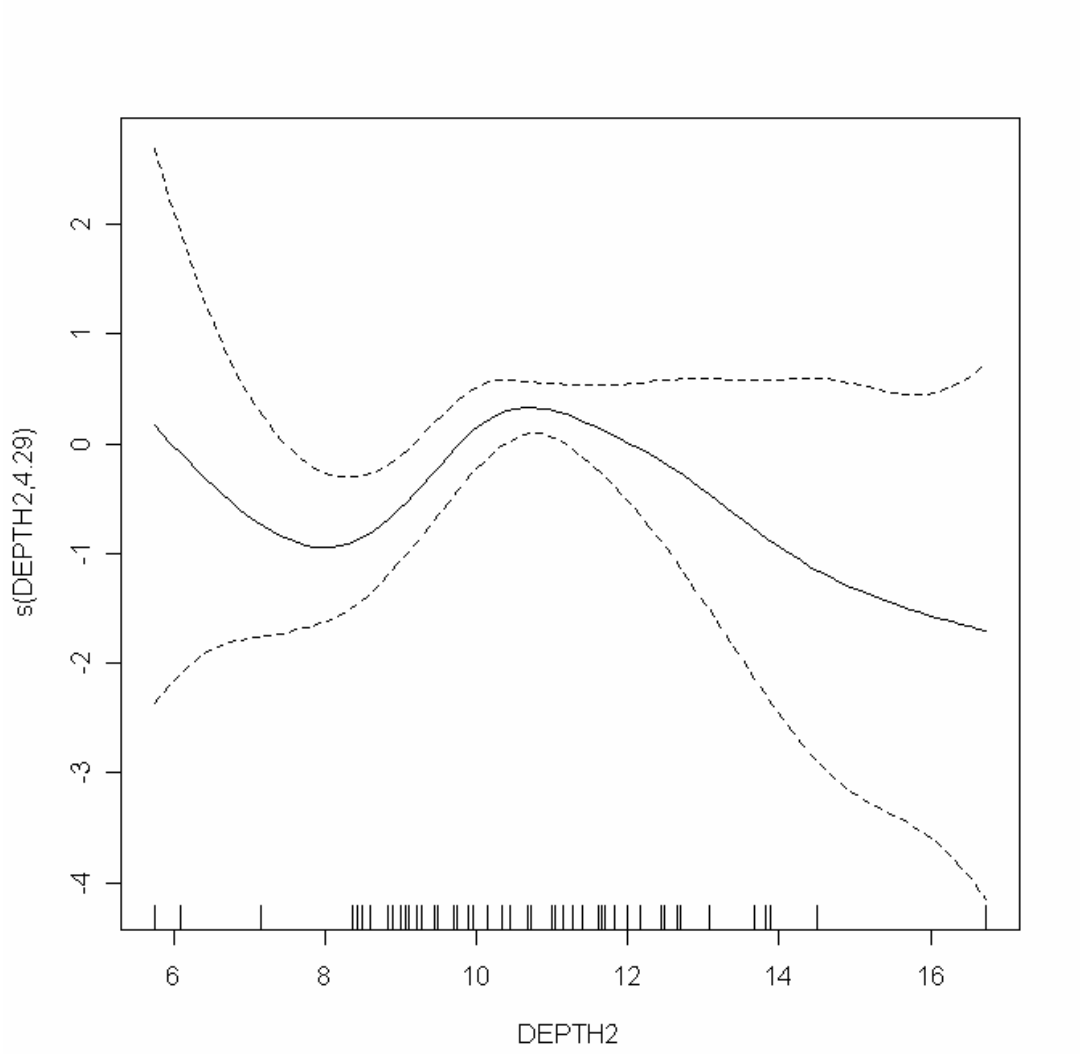
	df	F	p-value
factor(SEXNEW)	1	4.316	0.038633
factor(LOCATION)	4	5.247	0.000427
factor(SEXNEW):factor(LOCATION)	4	1.356	0.249317


s(LENGTH,2.23)
6
4
2
0
-2
40
60
80
100
120
LENGTH


s(DEPTH,3.48)
1
0
-1
-2
-3
-4
6
8
10
12
14
16
DEPTH

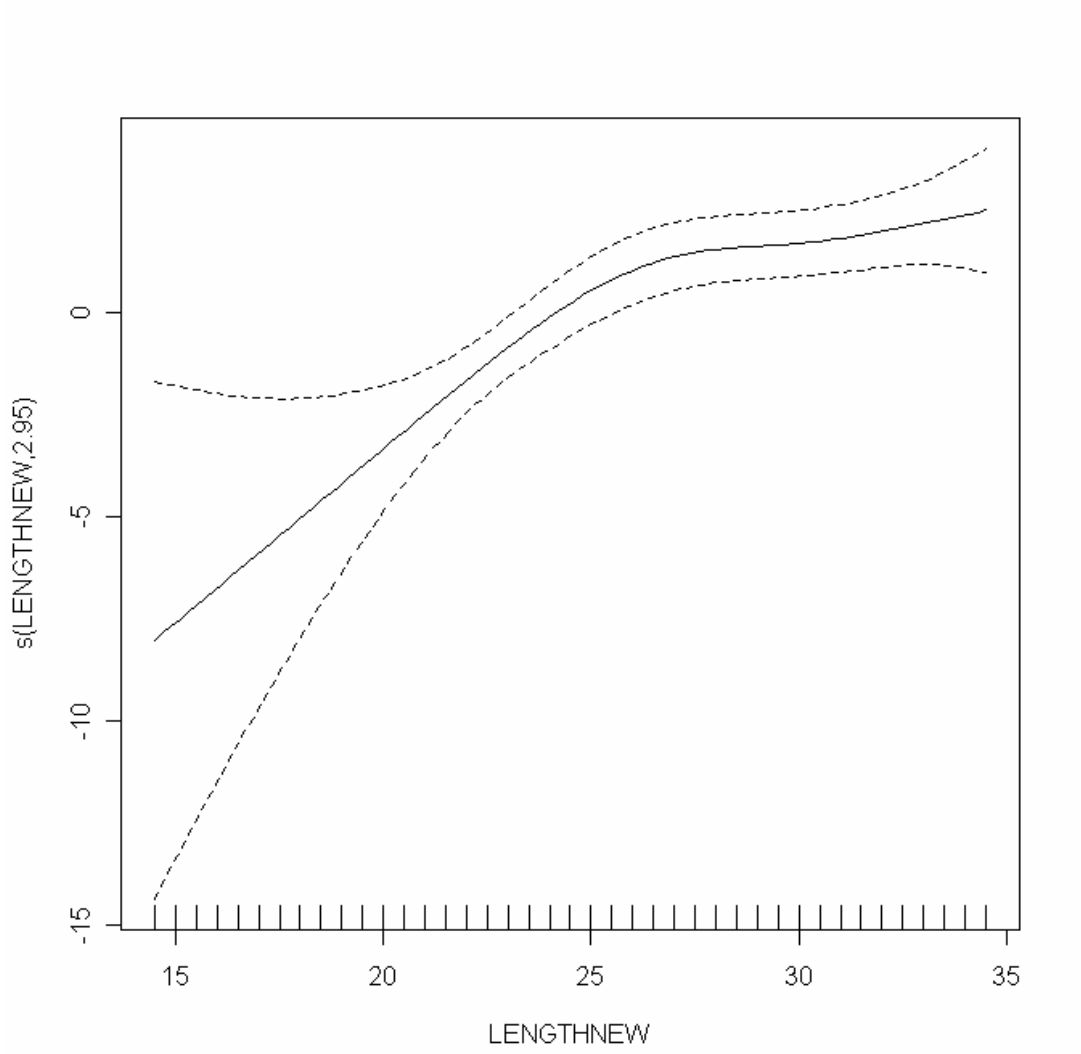
Presence / Absence of worms in Cod

```
Family: binomial
Link function: logit

Formula:
PARA ~ s(DEPTH) + (LENGTHNEW)

Parametric coefficients:
            Estimate  Std. Error  z value  Pr(>|z|)
(Intercept) -2.26845    0.61583  -3.684    0.00023  ***
LENGTH       0.06646    0.01456   4.564    5.02e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
           edf Est.rank Chi.sq p-value
s(DEPTH) 4.293        9  19.25  0.0231 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.111   Deviance explained = 10.6%
UBRE score = 0.21355  Scale est. = 1         n = 317
AIC = 384.6951
```


s(DEPTH2,4.29)
2
1
0
-1
-2
-3
-4
6
8
10
12
14
16
DEPTH2

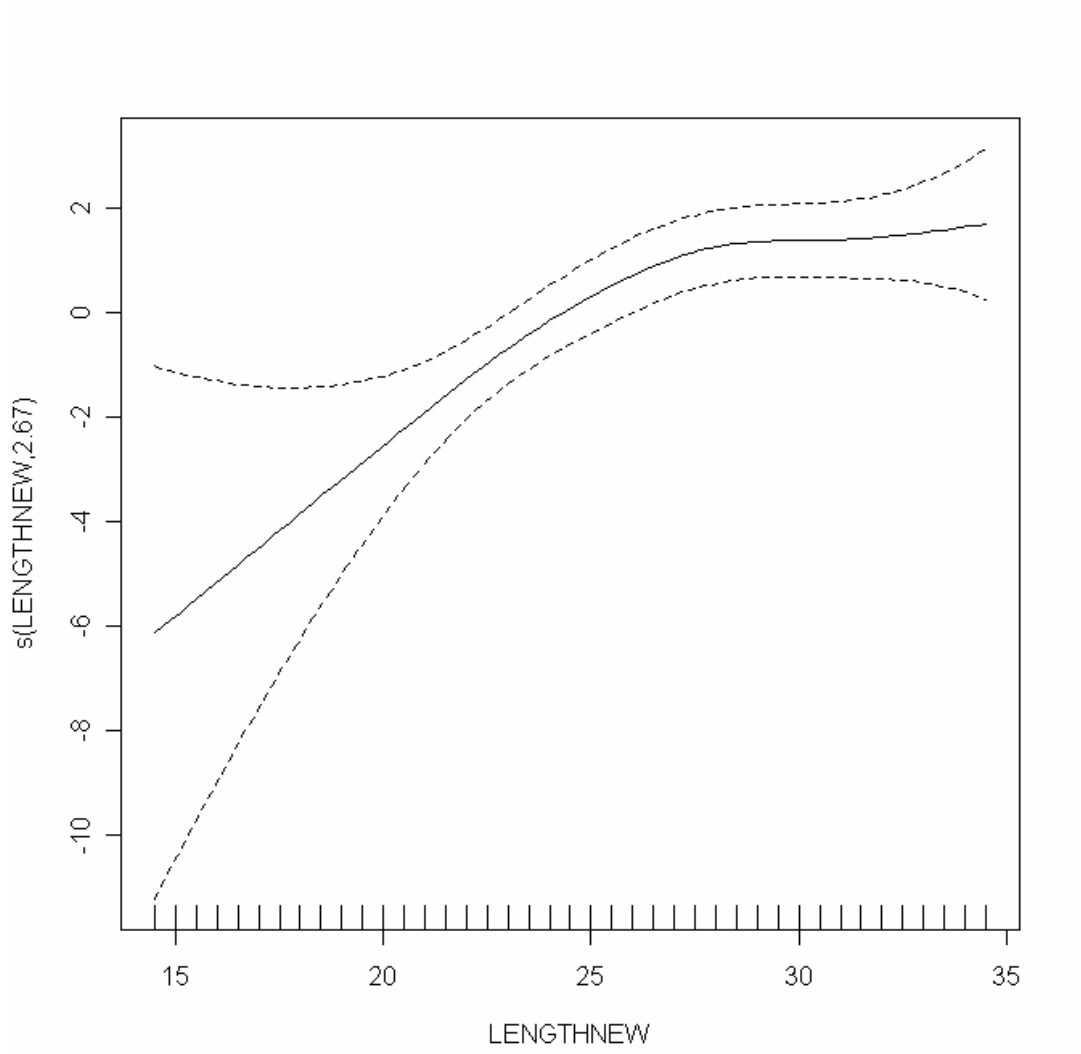
Anisakis in Mackerel

```
Family: Negative Binomial
Link function: log

Formula:
TOTALA ~ s(LENGTHNEW)

Parametric coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  -1.5979     0.3069  -5.206 3.28e-07 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
             edf      Est.rank     F        p-value
s(LENGTH)     2.95        6        5.769     9.6e-06  ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.0653   Deviance explained = 28.4%
GCV score = 1.0113   Scale est. = 1         n = 355
AIC = 631.3303
```


s(LENGTHNEW,2.95)
0
-5
-10
-15
15
20
25
30
35
LENGTHNEW

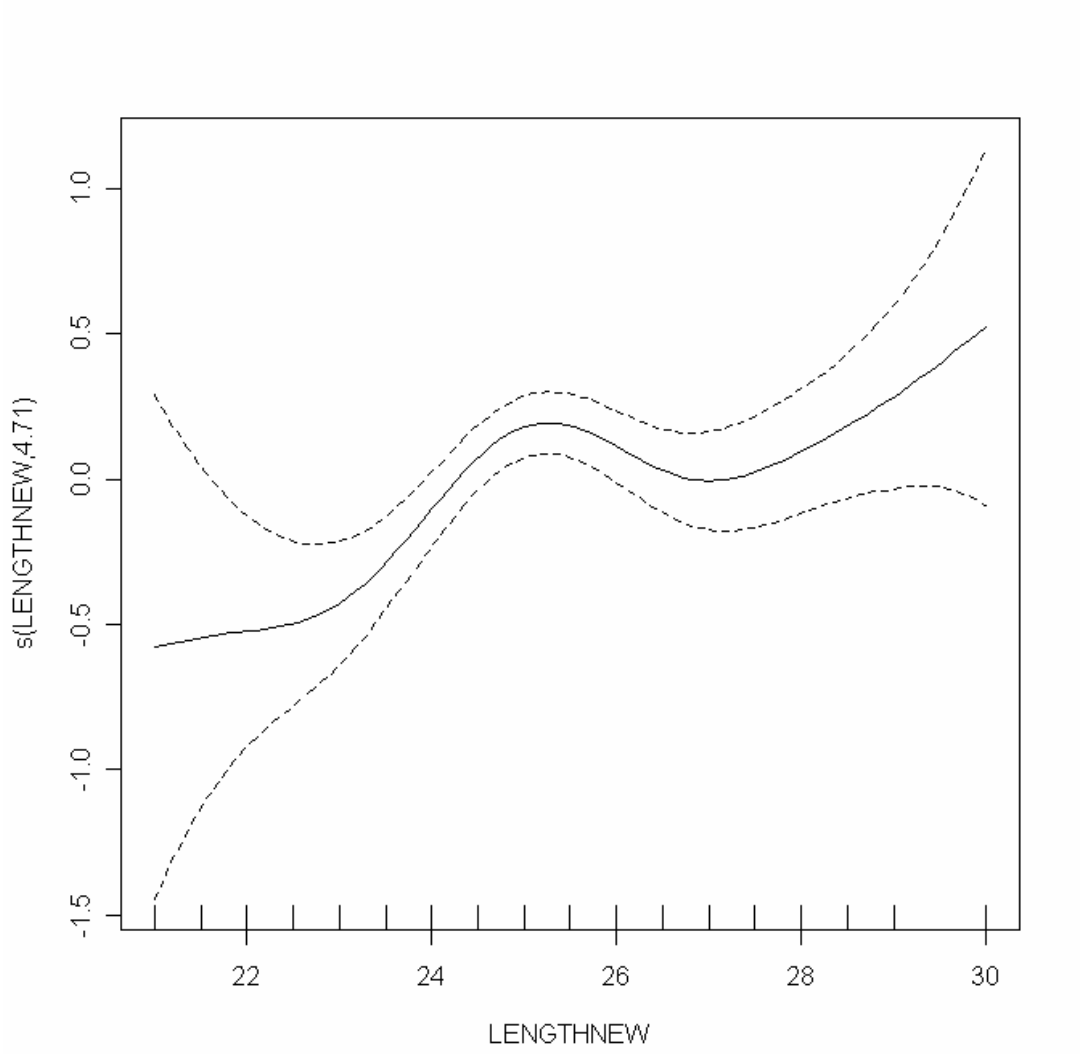
Presence / Absence of *Anisakis* in Mackerel

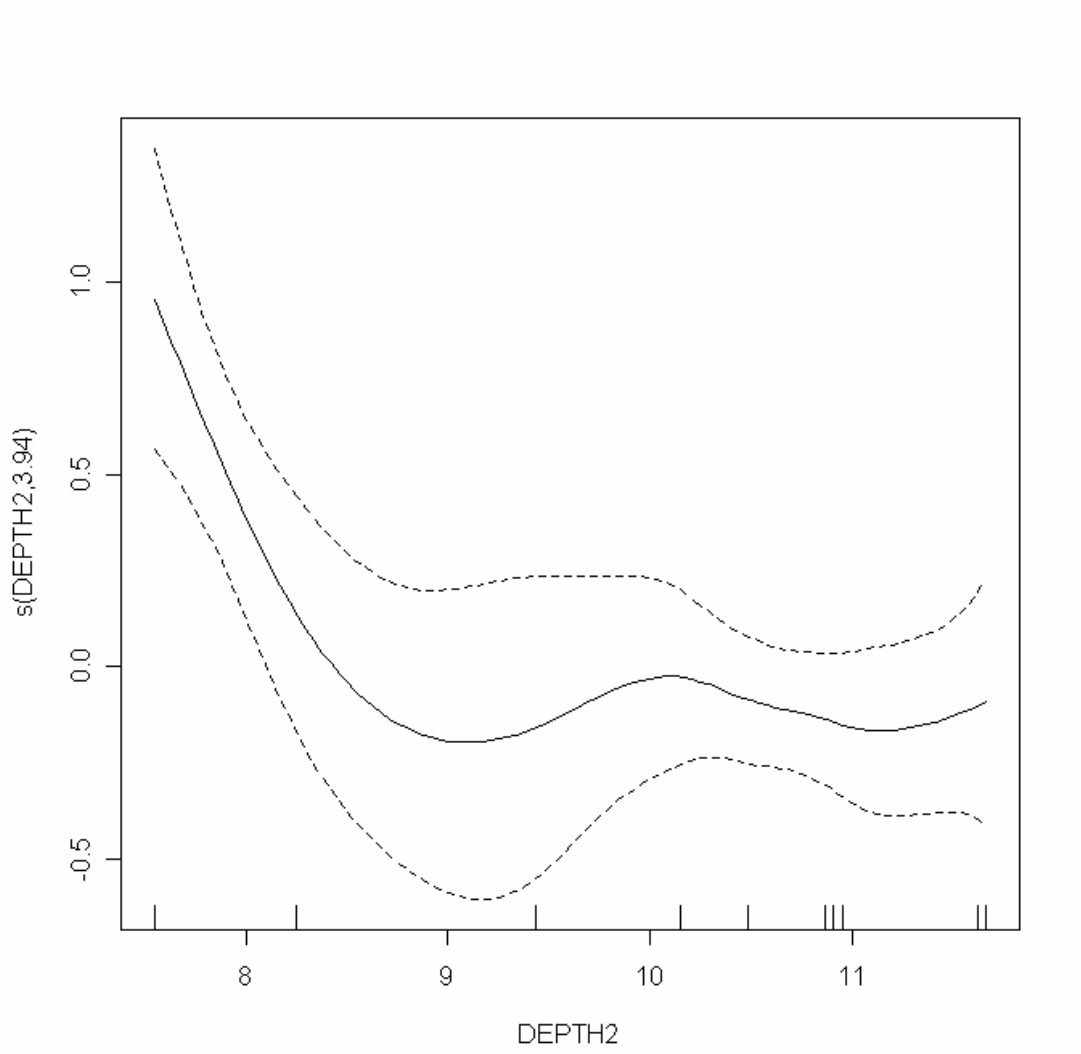
```
Family: binomial
Link function: logit

Formula:
PARA ~ s(LENGTH)

Parametric coefficients:
            Estimate   Std. Error  z value    Pr(>|z|)
(Intercept)  -1.9941     0.2828    -7.051     1.77e-12 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
               edf     Est.rank  Chi.sq    p-value
s(LENGTH)     2.672        6     23.47     0.000654 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.116   Deviance explained = 15.5%
UBRE score = -0.083813  Scale est. = 1         n = 355

AIC = 325.2463
```


s(LENGTHNEW,2.67)
2
0
-2
-4
-6
-8
-10
15
20
25
30
35
LENGTHNEW

Anisakis in Herring

```
Family: poisson
Link function: log

Formula:
TOTALA ~ s(LENGTH, by = as.numeric(SEX)) + s(DEPTH, by = as.numeric(YEAR)) +
factor(YEAR) + factor(SEX)

Parametric coefficients:
                Estimate   Std. Error   z value   Pr(>|z|)
(Intercept)      -0.8831     0.1231    -7.173   7.33e-13 ***
factor(YEAR)2    -1.4345     0.3558    -4.031   5.55e-05 ***
factor(SEX)2      0.4740     0.1459     3.249   0.00116  **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                           edf    Est.rank  Chi.sq  p-value
s(LENGTH):as.numeric(SEX)   4.713     9      31.15   0.000279 ***
s(DEPTH):as.numeric(YEAR)   3.939     8      41.51   1.67e-06 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.0336   Deviance explained = 12.2%
UBRE score = 0.38918  Scale est. = 1         n = 423
AIC = 878.7443
```


s(LENGTHNEW,4.71)
1.0
0.5
0.0
-0.5
-1.0
-1.5
22
24
26
28
30
LENGTHNEW


s(DEPTH2,3.94)
1.0
0.5
0.0
-0.5
8
9
10
11
DEPTH2

Presence / Absence of *Anisakis* in Herring

```
Family: binomial
Link function: logit

Formula:
PARA ~ s(LENGTH, by = as.numeric(SEX)) + factor(YEAR) + factor(SEX)

Parametric coefficients:
                Estimate Std. Error z value  Pr(>|z|)
(Intercept)     -0.73717    0.16600  -4.441  8.96e-06 ***
factor(YEAR)2    0.87829    0.28349   3.098  0.00195 **
factor(SEXNEW)2  0.04501    0.20908   0.215  0.82954
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Approximate significance of smooth terms:
                                edf Est.rank Chi.sq p-value
s(LENGTH):as.numeric(SEX) 2.871        6  13.02  0.0428 *
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

R-sq.(adj) =  0.0307   Deviance explained = 3.22%
UBRE score = 0.29703  Scale est. = 1         n = 423
AIC = 548.6429
```

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