via

מאת: "I P Bodner" <ipbodner@gmail.com>
אל: <vaiem@netvision.net.il>
נשלח: יום שני 22 פברואר 2010 05:21
נושא: herring

his is a copy of an email from J.D. Smith, one of the biggest mechabrim and researchers of nisakis. I underlined the sentence of what he says about herring.

ear Shlomo,
y wife Ros and I returned on Sunday from a wonderful 3-week driving tour of the USA. We visited Memphis, ew Orleans, the Kennedy Space Center, Epcot Center, Savannah, Charleston, etc, etc! We even managed camp for 6 nights in the Deep South!!
ow to "business". I retired some 15 years ago but do science as an (unpaid) hobby. I think my friend ichael Burt mentioned that I'm currently writing a monograph on the nematode parasites of Canadian fishes a labour of love!
any given geographical area all flounder, pollock, herring and salmon might harbour at least one larval nisakis in the flesh -- but the problem is when there are many worms in a given fish. The reasons for great undance in some sea areas rather than others are complex but include the presence of infected final hosts mall and large whales) shedding worm eggs into the sea. As I recall, the European Union and FAO permit a rtain number of worms per unit weight of fish flesh destined for human consumption. If the flesh is deep ozen to at least minus 20 degrees C for 24 hours the worms are killed and the problem is purely cosmetic id not public health.
far as I'm aware, hatched larvae do not grow in the sea; they can survive for 3 to 4 weeks at 13-18 grees C, and for 6-7 weeks at 5-7 degrees C before being ingested by a krill or another crustacean host. ey are about 0.22 to 0.29 mm long without the sheath, and about 0.33 to 0.37 mm long with the sheath.
ive below some old and newer references to Anisakis and related worms in the hope you have access to a ecial library.
ope this information will be useful to you.
intrigued: you are the second person in recent days to have contacted me re Anisakis. Is there perhaps a rrent worry in Europe (or elsewhere)?
ith Best Wishes
hn

via

מאת: "I P Bodner" <ipbodner@gmail.com>
אל: <vaiem@netvision.net.il>
נשלח: יום ראשון 21 פברואר 2010 22:24
צרף: 306-1-618_Final_Report_S14008_Anisakis.pdf
נושא: herring

See page 71. This study is herring from Scotland. Aprox 36% had at worms in the flesh.
PB

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).

? מקור

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.

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.

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Capelin (*Mallotus villosus*) and herring (*Clupea harengus*) as paratenic hosts of *Anisakis simplex*, a parasite of beluga (*Delphinapterus leucas*) in the St. Lawrence estuary

Réjean Hays, Lena N. Measures, and Jean Huot

Abstract: Capelin (*Mallotus villosus*) ($N = 760$) and herring (*Clupea harengus*) ($N = 165$) were collected in the St. Lawrence estuary during the summer of 1994 and 1995 to examine the importance of pelagic fish in transmission of *Anisakis simplex* to cetaceans. Larval *A. simplex* were removed from fish by means of a pepsin-digest solution or by dissection. Prevalence of *A. simplex* in dissected capelin was 5%, with a mean intensity of 1.2. Prevalences of *A. simplex* in herring were 95 and 99%, with mean intensities of 6.2 and 6.8 for pepsin digestion and dissection, respectively. Third-stage larval *A. simplex* found in capelin and herring were compared with third-stage larvae found in cuphausiids and belugas (*Delphinapterus leucas*) from the St. Lawrence estuary and no differences in size or morphology of larvae from these four hosts were observed. Euphausiids, which harboured moulting second-stage and third-stage larvae, are intermediate hosts of *A. simplex*. As there was no apparent development of larvae in herring or capelin, these fish are considered to be paratenic hosts of *A. simplex* in the St. Lawrence estuary.

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Résumé : Afin d'examiner l'importance des poissons pélagiques dans la transmission d'*Anisakis simplex* aux cétacés, 760 capelans (*Mallotus villosus*) et 165 harengs (*Clupea harengus*) ont été récoltés dans l'estuaire du Saint-Laurent pendant les étés 1994 et 1995. Les larves d'*A. simplex* ont été retirées des poissons par digestion à l'aide d'une solution de pepsine, ou par dissection. La prévalence d'*A. simplex* chez les capelans disséqués a été évaluée à 5% et l'intensité moyenne à 1.2. Chez les harengs, la prévalence d'*A. simplex* était de 95% dans les échantillons digérés, et de 99% dans les échantillons disséqués, alors que l'intensité a été évaluée à 6.2 dans le premier cas et à 6.8 dans l'autre. Les larves de troisième stade d'*A. simplex* provenant des capelans et des harengs ont été comparées aux larves du même stade trouvées chez des euphausiacés et chez des bélugas (*Delphinapterus leucas*) de l'estuaire du Saint-Laurent; la taille et la morphologie des larves étaient les mêmes chez les quatres hôtes. Les euphausiacés, qui abritent des larves de deuxième stade en mue et des larves de troisième stade, sont des hôtes intermédiaires d'*A. simplex*. Comme les larves du parasite ne semblent pas subir de développement chez les harengs ou les capelans, ces poissons sont considérés comme des hôtes paraténiques d'*A. simplex* dans l'estuaire du Saint-Laurent.

Introduction

Anisakis simplex (Rudolphi, 1809, det. Krabbe, 1878), or the "whaleworm," is parasitic in marine pelagic fishes as a larva and uses cetaceans as final hosts. It has been reported from 40 different families of fish worldwide, 16 of which occur in Atlantic Canadian waters (Simard 1997). Kagei (1974) noted that 164 species of fish harbour larval *Anisakis* sp. in Japanese waters. In fish from the Pacific Ocean, infections of larval *Anisakis* sp. have been found in walleye pollock (*Theragra chalcogramma*), cod (*Gadus morhua macrocephalus*), chub mackerel (*Scomber japonicus*), common mackerel (*Pneumatophorus japonicus*), horse mackerel (*Trachurus japonicus*), salmon (*Oncorhynchus* spp.), sardine (*Sardinops melanosticus*), and Pacific herring (*Clupea pallasi*) (see Oshima 1972; Smith and Wootten 1978; Nagasawa 1990). Studies of *A. simplex* in fish from the Atlantic Ocean have focused mainly on Atlantic herring (*Clupea harengus*) (see Khalil 1969; Parsons and Hodder 1971; Davey 1972; Grabda 1974; Smith and Wootten 1975; Beverley-Burton and Pippy 1977; Banning and Becker 1978; Grabda 1983; McGladdery and Burt 1985; McGladdery 1986). These studies show that Atlantic herring can harbour considerable numbers of larval *A. simplex*. But, after 30 years of research on this parasite, it is still uncertain whether fish are obligate intermediate hosts. Kagei (1969) believed that the second moult occurred in fish, which would make them intermediate hosts for *Anisakis* sp. Alternatively, Smith (1971), Oshima (1972), and Shimazu (1974) believed that euphausiids were important as intermediate hosts. Smith (1983) described small larvae in euphausiids that he interpreted as second-stage larvae in the process of moulting to the third stage.

In the St. Lawrence estuary, large whales such as the blue, fin, and minke whale are seen regularly during summer (Michaud 1993). Beluga, which have been identified as final

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R. Hays and J. Huot. Département de biologie et Centre d'Études Nordiques, Université Laval, Ste.-Foy, QC G1K 7P6, Canada.
L.N. Measures.[1] Fisheries and Oceans Canada, Maurice Lamontagne Institute, P.O. Box 1000, Mont-Joli, QC G5H 3Z4, Canada.

[1]Author to whom all correspondence should be addressed (e-mail: measuresL@dfo-mpo.gc.ca).

Table 1. Prevalences and mean intensities of larval *Anisakis simplex* in capelin and herring from the St. Lawrence estuary.

Host species	*N*	Site of collection	Age (years) Mean	Age (years) Range	Prevalence (%)	Intensity Mean	Intensity Range	Method of examination
Capelin	100	Ile Verte[a]	3[b]	2–3	5	1.20	1–2	Dissection (frozen)
	99	Grandes Bergeronnes[a]	—[c]	—	—	—[d]		Pepsin digest (fresh)
	561	Grandes Bergeronnes	—[e]	—	0	0		Pepsin digest (fresh)
Herring	100	Ile Verte	8	4–11	99[f]	6.83[f]	1–38	Dissection (frozen)
	65	Métis-sur-Mer	7	4–10	95[f]	6.19[f]	1–36	Pepsin digest (fresh)

[a]There was no significant difference in numbers of larvae between these two sites.
[b]Capelin were 13.5 (12.0–15.1) cm long. Only six larvae were found.
[c]Capelin were 12.5 (9.9–15.3) cm long.
[d]Prevalence and mean intensity were not determined, as all fish were digested together. Only two larvae were found.
[e]Capelin were 7.8 (5.9–10.6) cm long.
[f]There were no significant differences in prevalence or mean intensity between groups of herring.

hosts of *A. simplex* (see Vladykov 1944; Measures et al. 1995), reside in the St. Lawrence estuary all year. The only available information on the diet of belugas in the St. Lawrence estuary was gathered by Vladykov (1944). His study of summer-hunted belugas showed that the capelin (*Mallotus villosus*) was an important pelagic fish in their diet, particularly during the first months of summer, when belugas eat this fish almost exclusively. He believed that Atlantic herring could be an important food for the St. Lawrence beluga in spring and summer, when herring and capelin spawn in shallow waters of the estuary. Both fish feed on planktonic crustaceans such as euphausiids (Scott and Scott 1988), which have been recently shown to be intermediate hosts of *A. simplex* in the St. Lawrence estuary (Hays et al. 1998).

The first objective of this study was to assess the importance of capelin and herring in transmission of larval *A. simplex* to belugas. The second objective was to determine what role fish play in the life cycle of *A. simplex* in the St. Lawrence estuary, i.e., are they intermediate or paratenic hosts? In an intermediate host there is morphological development, moulting and usually growth of a parasite. In contrast, in a paratenic host there is generally little or no development or growth of the parasite and the paratenic host serves to transport the parasite from one host (usually the intermediate) to another (usually the final) host (see Anderson 1992).

Materials and methods

Collection of fish

Herring and capelin were collected in the St. Lawrence estuary. On May 7, 1994, capelin (N = 100) and on May 19, 1994, herring (N = 100) were collected at Ile Verte (48°02′N, 69°26′W) using a weir (mesh size 20 mm). All fish were frozen immediately after capture. On June 30, 1994, capelin (N = 660) were collected near Grandes Bergeronnes (48°14′N, 69°30′W) using a Marinovich pelagic trawl net (mesh size 0.5 mm) set at a depth of 5–10 m and towed for 15 min at 3 kn by the *Calanus II*, a Fisheries and Oceans Canada research vessel. On May 30, 1995, herring (N = 65) were collected at Métis-sur-Mer (48°40′N, 68°00′W) using a weir (mesh size 30 mm). Fish were kept refrigerated at 4°C until digested artificially within 4–48 h. Weir samples were collected at low tide, day or night.

Examination of fish by the digestion technique

Capelin were measured (total length), the body cavity was opened, and whole specimens were placed in modified Baermann apparatuses. Each apparatus contained 50 capelin in 2.5 L of pepsin solution (1 L H_2O, 8.5 g NaCl, 7 mL HCl, and 6 g pepsin). Herring were weighed, measured (total length), and identified by sex, and otoliths were removed for age determination. Age was determined by counting annuli on otoliths treated with dichloroethane. Internal organs and both fillets of each fish were separated and placed in two different Baermann apparatuses containing 2.5 L of pepsin solution (Table 1). After 24, 48 and 72 h at room temperature (18–21°C), 200 mL of solution from capelin and herring samples was withdrawn from the bottom of each Baermann apparatus and examined with a dissecting microscope.

Examination of fish by dissection technique

Fish from the May 7 and May 19, 1994, samples were defrosted, weighed, measured (total length), and identified by sex. Otoliths were removed for age determination. Body cavities were examined and flushed with saline (0.65%). Each body organ was pressed between glass plates and examined using a dissecting microscope (40×) for nematode larvae. Fillets were skinned, thin-sliced, and examined with the aid of a light source.

The terms prevalence, mean intensity, and abundance were used to define levels of *A. simplex* infection in fish (see Margolis et al. 1982).

Examination of nematodes

Larvae were fixed in hot glycerin–alcohol (9 parts 70% alcohol : 1 part glycerin) and cleared by allowing the alcohol to evaporate. Worms mounted in glycerin on glass slides were studied and measured using a compound microscope (Leitz Diaplan) equipped with a drawing tube interfaced with a digitizer and computer. Identification of larval *Anisakis* sp. as *A. simplex* was made using Pippy and van Banning (1975) and Beverley-Burton et al. (1977). Third-stage larval *A. simplex* from the stomach of a stranded beluga from the St. Lawrence estuary (see Measures et al. 1995) and third-stage larval *A. simplex* from euphausiids collected in the St. Lawrence estuary (Hays et al. 1998) were also studied and measured. Voucher specimens were deposited in the Canadian Museum of Nature, P.O. Box 3443, Station D, Ottawa, ON K1A 0M8 (CMNPA 1997–0030 to 0034).

Results

Abundance of *Anisakis simplex* in capelin

Seven hundred and sixty capelin were collected in the St. Lawrence estuary from May 1994 to May 1995 (Table 1). Small capelin (N = 561) taken near Grandes Bergeronnes were free of larval *A. simplex* (Table 1). Abundance of larval *A. simplex* was low in large capelin from Ile Verte and

Grandes Bergeronnes, with a total of eight larvae found in 199 capelin (Table 1). There was no significant difference in numbers of larval *A. simplex* in large capelin from Ile Verte and from Grandes Bergeronnes (Poisson distribution, $p = 0.1445$). The smallest infected capelin were 13.7 cm long and contained only one larva. The highest intensity was observed in a capelin measuring 15.1 cm in length that contained two larval *A. simplex*. Overall abundance was 0.01 for all sampled capelin ($N = 760$).

Prevalence and intensity of *A. simplex* in capelin and herring

Prevalence of *A. simplex* in herring was almost 100%. No significant differences in prevalence or mean intensity were seen between herring captured at Ile Verte and examined by dissection and those captured at Métis-sur-Mer and examined by pepsin digestion (prevalence: $Z = 1.48$, $p > 0.05$; mean intensity: Student's t test, $t = 0.69$, $p > 0.05$) (Table 1). Because there were no significant differences, the two herring samples were grouped together and then compared with the capelin sample. The grouped herring sample ($N = 165$) had a mean intensity of 6.58, significantly higher than 1.20, the mean intensity for capelin from Ile Verte (Student's t test, $t = -10.94$, $p < 0.05$).

Prevalences of *A. simplex* in herring according to age were as follows: age 4, $N = 2$, 50%; age 5, $N = 12$, 100%; age 6, $N = 34$, 91%; age 7, $N = 38$, 100%; age 8, $N = 28$, 100%; age 9, $N = 20$, 100%; age 10, $N = 21$, 100%; age 11, $N = 9$, 100%. Only 4 of 164 herring were uninfected with *A. simplex* larvae. These uninfected herring were amongst the youngest of the sample, one was 4 years old and three were 6 years old. Intensity of infection with larval *A. simplex* increased with age of herring as demonstrated by linear regression analysis (Fig. 7).

Location of *Anisakis simplex* in herring

Most larval *A. simplex* found in herring were located in the abdominal cavity. In 100 dissected previously frozen herring from Ile Verte, 638 (94.4%) larvae were found in the abdominal cavity and 38 (5.6%) were in the muscle. In 65 digested fresh herring from Métis-sur-Mer, 349 (90.9%) larvae were found in the abdominal cavity and 35 (9.1%) were in the muscle. There were no significant differences between collection sites in numbers of larvae in the abdominal cavity and muscle (χ^2, $p < 0.05$).

Morphology and morphometrics of larval *Anisakis simplex* from different hosts

Larval *A. simplex* removed from capelin and herring from the St. Lawrence estuary were morphologically identical. They possessed a boring tooth (Fig. 3), an excretory pore at the base of the lips, a divided oesophagus (preventriculus and cylindrical ventriculus with no appendages), no intestinal caecum, an oblique ventricular-intestinal junction, anal glands at the base of the rectum, and a mucron (tail spine) at the tip of a rounded tail (Fig. 4). All larvae bore only one cuticle and were presumed to be in the third stage. The morphology of third-stage larval *A. simplex* from four different hosts (euphausiid, capelin, herring, and beluga) was identical for diagnostic characters given above (see Figs. 1–6). However, increasing development of the lip pulp beneath the cuticle in the lip region at the anterior extremity of larval *A. simplex* from different hosts was observed (Figs. 1, 3, and 5). For example, the lip pulp was wider and higher in larvae from fish than in those from euphausiids and also in larvae from belugas than in those from fish or euphausiids.

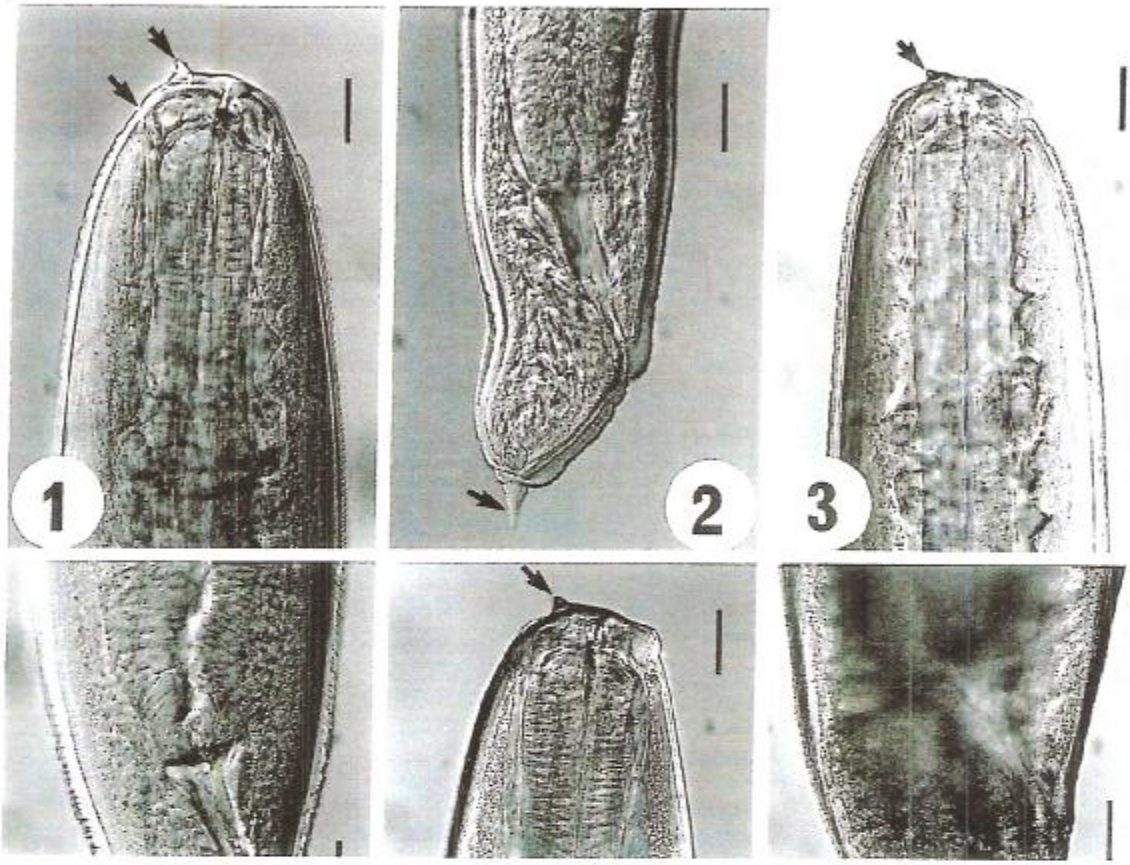
Larval *A. simplex* from frozen herring were not statistically longer than those from fresh herring (Student's t test, $p > 0.05$) or those from euphausiids (Table 2) (Tukey's HSD test, $p > 0.05$). Larval *A. simplex* from capelin and belugas also showed no significant differences (Tukey's HSD test, $p > 0.05$) in total length (Table 2). All other comparisons between total length of larval *A. simplex* from different hosts showed significant differences (ANOVA, $p < 0.0001$) (Table 2). For example, larvae from capelin and belugas were significantly shorter than those from herring and euphausiids.

Discussion

The present study permitted a comparison between pelagic fish (capelin and herring) from the St. Lawrence estuary infected with *A. simplex* and published data from the Gulf of St. Lawrence, where most studies of fish parasites in Canadian Atlantic waters are conducted. Capelin from the St. Lawrence estuary were lightly infected with *A. simplex*, prevalence reaching only 5%. Arthur et al. (1995) found 28% of 25 capelin collected in May and June 1994 off Ile Verte in the St. Lawrence estuary to be infected with *A. simplex*; however, their fish were larger and likely older (mean total length 15.6 cm) than those collected in the present study (mean total lengths 7.8, 12.5, and 13.5 cm for the three samples). Arthur et al. (1995) also collected capelin from four other locations in the Gulf of St. Lawrence, but prevalences were lower, ranging from 12 to 24%. Marcogliese (1995) reported prevalences of *A. simplex* ranging from 0 to 19% for capelin (length range 9–17 cm) in the Gulf of St. Lawrence. The relatively small size of the capelin in the present study may account for the prevalence of *A. simplex* observed. Despite this, capelin may be important hosts in the transmission of this parasite to fin and minke whales, which consume tons of capelin annually (Scott and Scott 1988).

Herring were more heavily infected with *A. simplex* than capelin in the St. Lawrence estuary, with a prevalence of almost 100% and a mean intensity close to seven larvae. Three surveys have examined *A. simplex* in herring from the Gulf of St. Lawrence. Parsons and Hodder (1971) reported a prevalence of 25 and 29% for larval nematodes (*Anisakis* sp. and *Contracaecum* sp.) in herring collected in Baie des Chaleurs ($N = 44$) and near the Magdalen Islands ($N = 500$), respectively, but prevalence of larval *A. simplex* alone was probably lower. McGladdery (1986) reported a prevalence of 50% for *A. simplex* in herring ($N = 10$) from near Shediac, New Brunswick. Marcogliese (1995) sampled herring near Anticosti Island and southwest Newfoundland ($N = 167$) and observed prevalences ranging from 6 to 54% (the age of herring collected was not given). Prevalence of *A. simplex* in herring depends on herring age and this is discussed below. Alternatively, high infection rates of *A. simplex* in herring could be attributed to many species of whales in this restricted area during summer, particularly the beluga, which

Figs. 1–6. Third-stage larval *Anisakis simplex* from euphausiids (Figs. 1 and 2), herring (Figs. 3 and 4), and belugas (Figs. 5 and 6). Scale bars = 50 µm. Figs. 1, 3, and 5 show the anterior extremity with a boring tooth (arrow). The excretory pore is also indicated (arrow) in Fig. 1. Figs. 2, 4, and 6 show the posterior extremity, including the tail with the mucron (arrow). The third moult appears to be beginning in the region of the mucron in Fig. 6.



occurs here all year (Michaud 1993) and is a final host (Vladykov 1944; Measures et al. 1995). In addition, herring from the St. Lawrence estuary may represent a different stock than those sampled from the Gulf. The existence of different stocks of herring in Canadian and adjacent waters has been recognized by Iles and Sinclair (1982) (but see McQuinn 1997). Belugas in the St. Lawrence estuary are reported to eat herring (Vladykov 1944), and herring with high intensities of *A. simplex* are likely important in transmission of *A. simplex* to belugas in this region.

Prevalence and intensity of *A. simplex* in herring from the St. Lawrence estuary increased with the age of the fish, indicating an accumulation of larvae over time, as observed by Parsons and Hodder (1971), Oshima (1972), Smith and Wootten (1978), McGladdery and Burt (1985), and McGladdery (1986). Contrary to McGladdery and Burt (1985), who examined herring collected in the Gulf of St. Lawrence and elsewhere on the east coast of Canada, intensity of larval *A. simplex* from the St. Lawrence estuary did not decrease in herring over 9 years old (Fig. 7).

Larval *Anisakis* spp. are colourless and tightly coiled in a spiral, making them difficult to find in the flesh of fish. The most efficient and reliable technique for removing nematode larvae from fish is digestion of fresh tissues using a mixture of pepsin and hydrochloric acid (Smith and Wootten 1975). Less than 10% of larval *A. simplex* found in herring from the St. Lawrence estuary were located in the muscle. The difference between the percentage of larval *A. simplex* found in the muscle of digested fresh herring (9.1%) and the percentage found in the muscle of dissected frozen herring (5.6%) may be due to the greater efficiency of the digestion technique in locating larvae. However, the two samples were

Fig. 7. Relationship between intensity of larval *A. simplex* infection and age of herring.

Table 2. Larval measurements (mm) and morphometric ratios of *Anisakis simplex* from euphausiids, capelin, herring, and belugas in the St. Lawrence estuary.

Host	Euphausiids*	Herring (N = 60)	Capelin (N = 5)	Belugas (N = 1)
No. of *A. simplex*	100	60	6	30
TL†	26.74*a*	25.96*a*	22.92*b*	20.69*b*
	(10.00–39.30)	(15.68–31.78)	(20.00–28.94)	(13.30–27.86)
MW	0.47	0.54	0.53	0.45
	(0.19–0.62)	(0.32–0.72)	(0.31–0.68)	(0.33–0.54)
PL	2.07	2.04	2.10	1.87
	(1.09–2.66)	(1.33–2.51)	(1.75–2.43)	(1.38–2.45)
VL	0.93	0.91	0.85	0.75
	(0.46–1.23)	(0.67–1.19)	(0.43–1.21)	(0.46–0.92)
VW	0.21	0.20	0.19	0.20
	(0.10–0.27)	(0.08–0.28)	(0.13–0.24)	(0.13–0.29)
TAL	0.15	0.15	0.15	0.14
	(0.09–0.22)	(0.09–0.21)	(0.10–0.20)	(0.11–0.18)
%MW/TL	1.75	2.11	2.32	2.23
	(1.23–2.36)	(1.29–3.81)	(1.55–2.74)	(1.90–2.87)
%PL/TL	7.87	7.90	9.25	9.15
	(5.95–10.92)	(5.86–10.36)	(7.77–10.71)	(7.49–11.96)
%VL/TL	3.52	3.54	3.72	3.64
	(2.10–4.60)	(2.43–5.09)	(2.03–4.64)	(2.56–4.93)
%VW/TL	0.77	0.79	0.84	0.99
	(0.51–1.04)	(0.36–1.35)	(0.65–1.17)	(0.50–1.41)
%TAL/TL	0.57	0.60	0.67	0.70
	(0.33–0.90)	(0.37–0.96)	(0.46–0.98)	(0.51–1.05)

Note: Values are given as the mean, with the range in parentheses. TL, total length; MW, maximum width; PL, proventriculus length; VL, ventriculus length; VW, ventriculus width; TAL, tail length.

*The number of euphausiids (*Thysanoessa raschii, Meganyctiphanes norvegica*) infected could not be determined, as they were digested together (Hays et al. 1998).

†Values followed by the same letter are not statistically different.

collected from separate sites in the estuary and the difference in prevalence may be site-related. Consumers should be aware that unless they are properly frozen (20°C for 24 h), these fish, if eaten lightly cooked, lightly marinated, or lightly smoked, are a potential health risk.

Japanese workers reported similarities in morphology and size of larval *Anisakis* sp. from euphausiids and fishes (Oshima et al. 1969; Kagei 1974). Shimazu and Oshima (1972) also observed that larval *Anisakis* sp. from euphausiids seemed to be in the same developmental stage as those from marine fish. Larval *A. simplex* from fish are usually identified as third-stage larvae (Shimazu 1974; Grabda 1976; Smith and Wootten 1984; Weerasooriya et al. 1986; Larizza and Vovlas 1995). Beverley-Burton et al. (1977) reported the mean length of third-stage *A. simplex* in herring and Atlantic salmon (*Salmo salar*) to be 19.69 mm

belly flaps?
whiting: related to cod...

Source: Torry Research St GB

Worms in herring & fish

Introduction

Parasitic round worms are frequently found in the guts and in the flesh of fish. Although only a small proportion of fish sold to the public is affected in this way, the worms are unsightly and consumers naturally object to their presence. This note gives a brief account of the nature and occurrence of round worms in fish, and describes means of reducing infestation in fish used as food. The note should also help fish traders and environmental health officers to dispel some of the misunderstanding of the problem when answering complaints from concerned members of the public.

What are parasites?

Animal parasites live in or on other animals from which they obtain at least some of their vital requirements, particularly nourishment. In general each kind of parasite confines itself to one kind of animal or group of animals, known as the host. Some parasites need more than one host at different stages in their development, the adult parasite living off one animal and the young or larval forms living off other animals.

guts = stomach & intestines

Some fish parasites live on the outside of fish, others within the body; most are removed during gutting and washing. The kinds most frequently met with during subsequent handling and distribution are worms. Round worms, or nematodes, in larval form are found in the guts and in the flesh of many fish marketed in the United Kingdom; two kinds predominate, the 'cod worm' and the 'herring worm'.

The 'cod worm', which is often found in cod, is also found in many other species. Its scientific name is Phocanema decipiens; other outdated scientific names are Porrocaecum decipiens or Terranova decipiens. It grows up to 4 cm long in fish, and varies in colour from creamy white to dark brown. It is frequently found in the flesh of fish, particularly in the belly flaps, where it often remains for long periods curled up and encased in a sac-like membrane produced by the fish tissue.

The 'herring worm' is often found in herring, mackerel, whiting and blue whiting, but it also occurs in many other species. Its scientific name is Anisakis simplex. It grows up to 2 cm long in fish, is almost colourless, and is found tightly coiled and encased in the guts and flesh, sometimes in considerable numbers, particularly in the belly flaps. Anisakis can migrate from guts to flesh in fish left ungutted after capture, notably in herring, mackerel and blue whiting. → 2

How do round worms get into fish?

The life history of a parasitic round worm is complex. The adult lives in the stomach of a marine mammal, Phocanema in the grey seal and Anisakis mainly in dolphins, porpoises and whales. Eggs of the parasite pass into the sea with the mammal's excreta, and when the eggs hatch the microscopic larvae must invade a new host in order to develop. The larval worms of Anisakis are eaten by a small shrimplike crustacean, a euphausiid; the first host of Phocanema is a small isopod crustacean that lives on the sea bed.

When crustaceans infested with Anisakis or Phocanema are eaten by a fish the larval worms are released into its stomach. They then bore through the stomach wall and eventually become encased in the guts or in the flesh of the host fish. The life cycle of the parasite is completed when an infested fish is eaten by a suitable marine mammal.

Large fish tend to be more heavily infested by round worms than small fish of the same species. This is because large fish eat more, and therefore ingest greater numbers of parasites, and also because the larval worms, although inactive, can survive for a long time in fish, and therefore their numbers accumulate as the fish grows older.

Are round worms dangerous?

There have been cases of human illness caused by the ingestion of live Phocanema or Anisakis larvae in countries where raw or lightly cured fish is commonly eaten. By 1980, there had been only one reported case of illness in the United Kingdom caused by larval round worms from fish; this is because in the UK fish products are normally cooked before consumption. Phocanema and Anisakis larvae are killed in 1 minute at a temperature of 60°C or over. In practice this means that cooking a fillet 3 cm thick for 10 minutes at 60°C will kill any worms present. The temperature of a cold smoking process, for example kippering, is not high enough to kill parasites, but in a commercial hot smoking

process a high enough temperature is usually maintained for long enough to kill them. Freezing of fish at - 20°C for 60 hours kills all worms

Anisakis larvae are resistant to salting; immersion in 80° brine, 21 per cent salt, for 10 days will kill all larvae, but in brine of lower strength they can survive for much longer. Anisakis is also resistant to marinating. When there is any doubt about whether Anisakis wi survive a process it is safest to use frozen fish. The ability of Phocanema to withstand salting or marinating is not known, but it is probably similar to that of Anisakis.

Can infestation of fish be reduced?

The abundance of Phocanema and Anisakis varies in fish from different areas; Phocanema is usually more abundant in inshore fish, whereas Anisakis occurs in greater numbers in offshore fish, but many species of fish from all areas fished by British vessels are likely to be infested to some degree. Of the two kinds of worm, Anisakis is by far the more abundant and widespread.

The only way to reduce the numbers of parasites reaching the consumer is to inspect the fish and process them in such a way that most parasites are removed. The guts and gut cavity of many fish are often heavily infested; whiting for example often contain large numbers of Anisakis. For this reason it is always advisable to gut fish and clean out the gut cavity before offering them for sale. Most Phocanema and almost all Anisakis in fish flesh are found in the belly flaps; it follows that trimming off and discarding flaps from fillets will remove most of the worms. The greater the area of flap discarded, the greater will be the proportion of worms removed.

Visual inspection of fillets will reveal worms embedded near the surface; these can be removed easily with a knife. Worms embedded deep ir the flesh are not immediately obvious, but some can be detected by candling, that is shining a bright light through the fillet. In commercial practice candling is effective in detecting Phocanema in thin skinless fillets of white fish, particularly cod; the method does not work well on thick fillets with the skin on. Candling is less effective in detecting Anisakis. Time can be saved by candling a sample of fillets from a batch of suspect fish to determine the level of infestation; it can then be decided whether the whole batch needs to be candled, and whether the batch is more suitable for one purpose than another.

Design and use of a candling table

The simplest kind of candling table is a box about 50 cm square with a ground glass or perspex top about 6 mm thick. The inside of the box is white, and is lit by two fluorescent tubes giving a white, not a coloured, light. Electrical wiring should be installed by an electrician who understands the wet conditions in which the box is to be used. The box should be ventilated but splashproof.

To use the box, the fillet is laid down on the illuminated top; worms show up as dark shadows in the flesh, and can be removed with forceps or a knife. Light from above the box should be restricted; the box is useless in bright sunlight for example. An experienced operator can handle up to 300 fillets an hour, but the eyes rapidly become fatigued and efficiency falls during long spells, with the consequent risk of greater numbers of worms passing undetected.

How serious is the problem of worms in fish?

No matter how carefully fish is inspected by processors, caterers and retailers, some worms will occasionally be discovered in fish by the consumer. In reply to complaints it should be pointed out that every reasonable precaution is taken to prevent worms being present in the edible part of a fish. A model purchase specification proposes a maximum of 3 worms in 3·2 kg of fillets of white fish in the United Kingdom, as judged by visual examination. An international standard, Codex Alimentarius, allows a maximum of 5 worms in 1 kg of fish of certain species; only worms of encapsulated diameter of 3 mm, or 1 cm in length, are considered to be of significance. It should therefore be emphasized that the presence of worms in fish offered for sale does not imply carelessness or bad practice on the part of the processor or retailer. It can also be explained that the presence of worms does not reduce the nutritional value of the fish, and that correct cooking or freezing will kill all parasites.

The information in this note has been prepared jointly by the Marine Laboratory of the Department of Agriculture and Fisheries for Scotland and the Torry Research Station of the Ministry of Agriculture, Fisheries and Food.

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.

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).

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RESEARCH THEN AND NOW ON THE ANISAKIDAE NEMATODES[1]

BETTY JUNE MYERS[2]

Southwest Foundation for Research and Education, San Antonio, Texas 78284

MYERS, B. J. 1976. Research then and now on the Anisakidae nematodes. *Trans. Amer. Micros. Soc.*, 95: 137–142. The history of nematodes responsible for human anisakiasis spans almost 500 years. Our knowledge of this group parallels the advancement of research from visual observation—through development and refinement of the microscope and advancement of technology—to newer research tools, such as the electron microscope.

The nematodes of the family Anisakidae were probably first recognized in fish hosts as early as the 13th century, in marine mammals in the early 1700's, as an occasional human disease in 1867, and as a more common human infection in the 1950's and 1960's. Since 1970, eight human cases have been documented from North America.

As early as 1742, ascarid-like nematodes were described from a now extinct sea cow (Stiles & Hassall, 1899). Linnaeus, in the 12th edition of the *Systema Naturae*, described *Gordius marinus* using his simple microscope (Hollfus, 1970). Extensive monographs were prepared during the 1800's. Dujardin (1845) dissected over 2,000 vertebrates and described their parasites. In 1857, studies on cystic worms resulted in description of immature forms of anisakine nematodes (Von Siebold, 1857). On the basis of the internal characteristics these larvae were linked with adult forms occurring in marine mammals and birds. The theory of strayed parasites—those which enter the wrong host and never mature—was postulated. This is what we now call *larva migrans*. As early as 1832, eggs from anisakine nematodes were hatched and attempts were made to grow the larval stages to adults (Stiles & Hassall, 1899).

In 1876, an "ascarid" was vomited by a child in Greenland (Leuckart, 1876). This first observed human case led to an examination of the "ascarids" of seals in 1878 (Krabbe, 1878a,b). Among these "ascarids" was a specimen identified as *Ascaris decipiens*, destined to become *Phocanema decipiens*, the

[1] Based on Past-President's address, delivered at the 91st Annual Meeting of the American Microscopical Society, held in New Orleans, Louisiana, November 1975. I wish to acknowledge the Fisheries Research Board of Canada (under whose auspices the studies on the "cod worm" were carried out while I was at the Institute of Parasitology, McGill University), and the Food and Drug Administration, U.S. Department of Health Education and Welfare, for present support under contract no. 223-74-2140 and 2149. I am grateful to Dr. George J. Jackson for encouragement and review of the manuscript.

[2] Address after 1 May 1976: National Institutes of Health, Bethesda, Maryland 20014.