

OBSERVATIONAL AND EXPERIMENTAL STUDIES ON THE ACQUISITION OF *ANISAKIS* SP. LARVAE (NEMATODA: ASCARIDIDA) BY TROUT IN FRESH WATER

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Abstract—WOOTTEN R. and SMITH J. W. 1975. Observational and experimental studies on the acquisition of *Anisakis* sp. larvae (Nematoda: Ascaridida) by trout in fresh water. *International Journal for Parasitology* 5: 373–378. In the enclosed fresh-water environment of Hanningfield Reservoir, Essex, England, *Anisakis* sp. larvae (parasites of marine fish) were found in 55 per cent of 40 brown trout and in 26.2 per cent of 61 rainbow trout. Experimental infection by intubating larvae into the stomach was more successful in brown trout (50.6 per cent recovery rate) than in rainbow trout (27 per cent recovery rate). Some larvae reached the body-cavity as early as 2 h after infection. They penetrated the region between the oesophagus and intestine immediately posterior to the caecal openings. Fewer larvae successfully penetrated the gut wall of brown trout within 24 h at 8°C than at $15 \pm 1^\circ\text{C}$. It appears that the reservoir trout acquired *Anisakis* by being fed as juveniles on untreated marine fish offal containing live larvae.

INDEX KEY WORDS: *Anisakis* sp. larvae; larval transfer; fresh water; trout; United Kingdom.

INTRODUCTION

THIRD-STAGE larvae of *Anisakis* sp. are common parasites of marine fish with a world-wide distribution; they have been studied intensively because of their significance as a cause of human anisakiasis (Ruitenbergh, 1970; Oshima, 1972). In British waters *Anisakis* sp. larvae are abundant in important food fishes, e.g. herring (Khalil, 1969; Davey, 1972) and cod (Young, 1972).

The life-cycle of *Anisakis* sp. is wholly marine, with euphausiids (and possibly other invertebrates) serving as first intermediate hosts, a wide variety of marine fish as second intermediate hosts, and marine mammals as final hosts (Oshima, 1972). *Anisakis* sp. larvae have been recorded from migratory salmonids which spend part of their adult life in the sea and presumably acquire the larvae during this time (Bikhovskaya-Pavlovskaya, Gusev, Dubinina, Iziumova, Smirnova, Sokolovskaya, Shtein, Shulman & Epshtein, 1964; Hoffman, 1967).

There are few reports of *Anisakis* sp. larvae from wholly fresh-water fish. Kane (1966) found larvae in brown trout, *Salmo trutta* L. from Lough Sheelin and the River Boyne, Eire. During a general study in 1968 of the metazoan parasite fauna of fish from Hanningfield Reservoir, Essex, England, *Anisakis* sp. larvae were found commonly in brown trout and in rainbow trout, *S. gairdneri* Richardson, 1836 (Wootten, 1973). Bassleer, Puylaert & Van Beneden (1973) have since reported larval *Anisakis* (and *Contracaecum aduncum*) in trout from ponds in the

Belgian Ardennes. The unexpected presence of a marine parasite in fish from enclosed freshwater environments prompted an experimental investigation of a possible means by which the trout became infected. The results of these experiments are given below together with additional information on the occurrence of *Anisakis* sp. larvae in Hanningfield Reservoir trout.

MATERIALS AND METHODS

Some physical and biotic features of Hanningfield Reservoir were described by Wootten (1973). The brown and rainbow trout populations in the reservoir are maintained by annual stocking with hatchery-reared fish, usually of age 2+. Some of the trout are reared in raceways adjacent to the reservoir where they are fed only on dried pelleted food. Most of the trout for stocking are imported from various other fish farms.

Brown and rainbow trout from the reservoir and rainbow trout from the raceways were examined at monthly intervals throughout 1968. Totals of 56 brown trout and 89 rainbow trout from the reservoir and 125 rainbow trout from the raceways were examined. Trout from the reservoir were caught by traps and gill nets. Most fish were killed on capture and immediately deep frozen for later examination but some were examined fresh.

Each fish was measured and its age determined from epidermal scale readings. The brown trout were from 1+ to 6+ years old and from 193 to 552 mm in length. The rainbow trout from the reservoir were from 1+ to 5+ years old and measured from 264 to 544 mm; those from

TABLE 1—LOCATION OF *Anisakis* sp. LARVAE IN BROWN AND RAINBOW TROUT FROM HANNINGFIELD RESERVOIR

	on Stomach	on Pyloric caeca	No. (%) of larvae found		
			on Intestine	on Mesenteries	in Muscle
Brown trout	5 (15.2)	20 (60.6)	3 (9.1)	3 (9.1)	2 (6.1)
Rainbow trout	10 (38.5)	7 (26.9)	5 (19.2)	4 (15.4)	0 (0)

the raceways were 0+ and 1+ years old and from 72 to 260 mm in length. The viscera of each fish was examined for larval nematodes. The worms were counted and their location in the host noted in most cases. The musculature of the fish was not examined in detail.

The brown and rainbow trout used in infection experiments were obtained from two commercial fish farms in Scotland where they had been fed only on dried pelleted food. The fish used were of age 1+ and from 100 to 232 mm in length. Control batches of 10 brown trout and 10 rainbow trout from each farm were digested using a pepsin-HCl mixture (Smith & Wootten, 1975); no larval nematodes were recovered.

Anisakis sp. larvae were removed from commercially caught herring and stored in Tyrode's solution at $4 \pm 1^\circ\text{C}$ for up to 14 days before use. Trout were anaesthetized with tricaine methane sulphonate (MS222, Sandoz Products Ltd., London, England) at the rate of 0.1 g per l. Five *Anisakis* sp. larvae were introduced into the stomach of each fish using a polythene tube fitted with a pipette. The trout were allowed to recover from anaesthesia in fresh water and observed for 10 min thereafter, during which time there was no evidence of regurgitation of larvae. Brown trout were then maintained in aquarium tanks at 8°C or $15 \pm 1^\circ\text{C}$ and killed at intervals of from 2 h to 6 days after infection; rainbow trout were kept at the latter temperature only and killed at intervals of from 24 h to 24 days after infection. The viscera and musculature of each trout were dissected and examined for *Anisakis* sp. larvae. The location of any nematodes found was noted.

RESULTS

Identity of Anisakis sp. larvae

All larvae found in trout from Hanningfield Reservoir and all those used in experimental infections morphologically resembled the third-stage larvae designated *Anisakis* sp. larva (1) by Berland (1961). Such larvae were reared *in vitro* to the adult stage by Van Banning (1971) and identified as *A. simplex* (of which *A. marina* is a synonym according to Davey, 1971).

Occurrence of larvae in trout from Hanningfield Reservoir

Prevalence of larvae. *Anisakis* sp. larvae were found in brown trout from Hanningfield Reservoir from May to November, 1968, and in rainbow trout from May to December. Twenty-two (55 per cent) of the 40 brown trout and 16 (26.2 per cent) of the 61 rainbow trout caught in these periods were infected. The mean intensity of infection was only 1.7 and 1.6 larvae per infected fish in brown and

rainbow trout respectively. The maximum number of larvae in an individual fish of either trout species was 4, and in over half the infections only a single nematode was present.

Twenty-one (95.5 per cent) of the infected brown trout and 14 (87.5 per cent) of the infected rainbow trout were newly-introduced 2+ fish under 40 cm in length. One 6+ brown trout and two rainbow trout aged 1+ and 3+ were also infected. None of the juvenile rainbow trout from the raceways was infected. Coarse fish species present in the reservoir were also uninfected (Wootten, 1973).

Site of infection. Most *Anisakis* sp. larvae found in both trout species occurred singly in capsules in a variety of locations within the body-cavity of the fish (Table 1). In both brown and rainbow trout over 80 per cent of the larvae found in the body-cavity were encapsulated on the gut. In brown trout the largest number was found amongst the pyloric caeca, although other larvae were located on the wall of the stomach and intestine. In rainbow trout the largest number of larvae was found on the stomach but others occurred amongst the pyloric caeca and on the intestinal wall. Two larvae in brown trout were found unencapsulated with the anterior end embedded in the hypaxial musculature.

Results of experimental infections

Anisakis sp. larvae successfully penetrated into the body-cavity of both brown and rainbow trout. A considerably higher percentage of the introduced larvae was recovered from brown trout than from rainbow trout. Forty-three (50.6 per cent) of 85 introduced larvae were recovered from the body-cavity of 17 brown trout maintained at $15 \pm 1^\circ\text{C}$ for 24 h or more after infection, compared with 27 (27 per cent) of 100 larvae from 20 rainbow trout kept under similar conditions.

Fifteen *Anisakis* sp. larvae recovered from the body-cavity of brown trout 7 days after initial infection were introduced into 3 other brown trout maintained at $15 \pm 1^\circ\text{C}$. When the latter fish were killed 7 days later, 6 (40 per cent) of the larvae were found in the body-cavity. These 6 larvae were then introduced into another brown trout but failed to penetrate into the body-cavity. In a similar experiment using rainbow trout, 4 (26.7 per cent) of 15 larvae were recovered from the 3 fish infected. These larvae also failed to infect another rainbow trout.

The rate of migration of *Anisakis* sp. larvae from the gut lumen into the body-cavity of brown trout

TABLE 2.—SITE OF PENETRATION OF THE GUT WALL BY *Anisakis* sp. LARVAE IN BROWN TROUT AT $15 \pm 1^\circ\text{C}$ AT DIFFERENT TIME INTERVALS AFTER INFECTION

Time after infection (h)	No. of larvae penetrating			
	Stomach	Pyloric caeca	Caecal intestine	Post-caecal intestine
2	2	0	0	0
4	1	1	0	0
6	4	0	0	1
9/10	2	0	1	2
12	0	0	0	1
24	0	0	0	1

at $15 \pm 1^\circ\text{C}$ is shown in Fig. 1. A number of larvae had penetrated successfully into the body-cavity of the trout only 2 h after infection; most larvae that were able to penetrate apparently did so within 24 h.

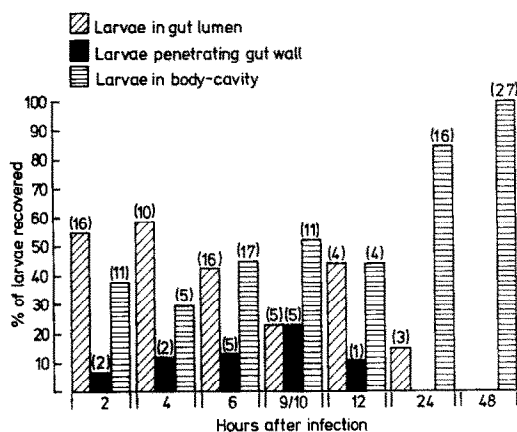


FIG. 1. The rate of migration of *Anisakis* sp. larvae from the gut lumen into the body-cavity of brown trout at $15 \pm 1^\circ\text{C}$. Numbers of larvae are shown in parentheses.

At 8°C larvae were unable to penetrate with the same degree of success into the body-cavity of brown trout (Fig. 2). A much higher proportion of the larvae remained in the gut lumen at all time intervals. However, some larvae did succeed in penetrating into the body-cavity of the trout within 2 h. Some *Anisakis* sp. larvae were observed in the process of penetrating the gut wall of both brown and rainbow trout. In brown trout at $15 \pm 1^\circ\text{C}$, 9 larvae were observed penetrating at the junction of the oesophagus and stomach or through the cardiac limb of the stomach. One larva was observed penetrating a pyloric caecum, whilst 5 larvae were observed penetrating the intestine immediately posterior to the caecal openings. Larvae in brown trout maintained at 8°C and in rainbow trout maintained at $15 \pm 1^\circ\text{C}$ were also observed penetrating the same regions of the gut wall. Larvae were never seen to penetrate the posterior part of the intestine.

The sites at which penetration was observed at different time intervals after infection in brown trout at $15 \pm 1^\circ\text{C}$ are shown in Table 2. From 2 to 4 h after infection penetrating larvae were observed in the region of the stomach and caeca. After 6–10 h penetration also occurred to the post-caecal intestine and, at 12 and 24 h after infection, penetration was observed only in this part of the gut.

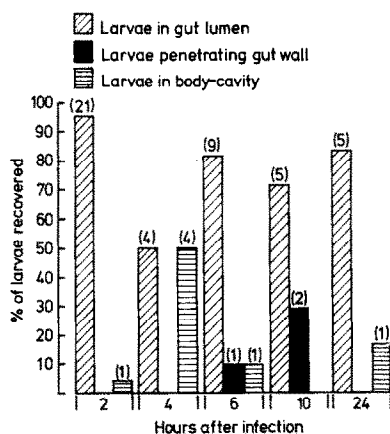


FIG. 2. The rate of migration of *Anisakis* sp. larvae from the gut lumen into the body-cavity of brown trout at 8°C . Numbers of larvae are shown in parentheses.

The location of fully penetrated *Anisakis* sp. larvae in the body-cavity of brown and rainbow trout maintained at $15 \pm 1^\circ\text{C}$ is shown in Table 3. The highest number of larvae in both host species was found amongst the pyloric caeca. Over 60 per cent of the larvae from brown trout and over 85 per cent from rainbow trout were located on the anterior part of the alimentary canal. Larvae found in the hypaxial musculature had, with one exception, only the anterior end embedded. The exceptional worm, in a brown trout, was completely buried within the musculature. Three larvae in brown trout and one larva in a rainbow trout were found in the swim-bladder. None of the larvae recovered from either trout species was encapsulated.

TABLE 3—LOCATION OF *Anisakis* sp. LARVAE IN EXPERIMENTALLY INFECTED BROWN AND RAINBOW TROUT AT $15 \pm 1^\circ\text{C}$

	No. (%) of larvae found on/in					
	Stomach	Pyloric caeca	Intestine	Mesenteries	Swim-bladder	Muscle
Brown trout	14 (15.4)	36 (39.6)	3 (3.3)	24 (26.4)	3 (3.3)	11 (12.1)
Rainbow trout	5 (18.5)	14 (51.9)	4 (14.8)	1 (3.7)	1 (3.7)	2 (7.4)

DISCUSSION

The results of the experiments described above demonstrate that *Anisakis* sp. larvae can infect trout in a fresh-water environment if ingested by the fish. The transfer of *Anisakis* sp. larvae between marine fish was achieved by Smith (1974) who infected haddock and whiting with larvae taken from Atlantic salmon and herring respectively. Scott (1954) transferred larvae of the related nematode *Porrocaecum* (= *Phocanema* = *Terranova*) *decepiens* from smelt to cod. Wild trout in fresh-water environments are extremely unlikely to come into contact with *Anisakis* sp. larvae, but juvenile fish reared in trout farms may become infected if fed untreated marine fish offal containing live larvae. The practice of feeding such offal to farmed fish is quite common in Europe as it provides a cheap supply of food. Bassleer *et al.* (1973) suggested that the trout they investigated may have become infected in this way. As most of the trout in Hanningfield Reservoir had been reared in various other fish farms it seems certain that they acquired their infections with *Anisakis* sp. larvae as juveniles by feeding on untreated offal. It is probable that the larvae of *Anisakis* sp. are able to survive for a considerable time in the fish host, so that larvae acquired by juvenile trout would still be present when the fish were introduced into the reservoir. The longevity of *Anisakis* sp. larvae may account for the observed infections of a 3+ and a 6+ trout but it is possible that the latter fish, which was over 60 cm long, may have acquired the larvae by cannibalism of newly-introduced trout. The present experiments show that *Anisakis* sp. larvae are capable of infecting a second fish if ingested. As relatively few trout in Hanningfield Reservoir survive to a large enough size to become cannibalistic it is unlikely that there is any widespread circulation of larvae within the reservoir. Trout are not preyed upon by other fish species in the reservoir so the latter do not become infected.

Brown trout were more heavily infected than rainbow trout in both natural and experimental circumstances. The reasons for this difference in susceptibility of the two trout species are not clear. No dead larvae were found in the body-cavity of either naturally or experimentally infected trout, and it seems probable that the lower number of parasites in rainbow trout is due to the inability of larvae to penetrate the gut wall so readily as in brown trout.

Penetration of the gut wall of brown trout was apparently restricted to the region from the oesophagus to the intestine immediately posterior to the caecal openings. There was some evidence that larvae which penetrated in the first few hours after infection did so mainly in the stomach region but that penetration of more posterior regions occurred subsequently as the worms passed down the gut. *Anisakis* sp. larvae were observed penetrating the stomach wall and pyloric caeca of haddock and whiting by Smith (1974), and Scott (1954) suggested that the larvae of *Porrocaecum decepiens* penetrated the stomach wall of cod. The 4 *Anisakis* sp. larvae found in the lumen of the swim-bladder in experimentally infected trout may have migrated there through the pneumatic duct.

Anisakis sp. larvae were able to penetrate the gut wall of brown trout very rapidly at both 8°C and $15 \pm 1^\circ\text{C}$ so that some larvae were found in the body-cavity only 2 h after infection. Larvae which failed to penetrate the gut wall within the first 24 h at $15 \pm 1^\circ\text{C}$ were apparently eliminated with the host's faeces. No larvae were found in the gut lumen of any of the fish kept at $15 \pm 1^\circ\text{C}$ and examined more than 24 h after infection.

Fewer larvae successfully penetrated the gut wall of brown trout within 24 h at 8°C than at $15 \pm 1^\circ\text{C}$. This is possibly due to the inhibitory effect on larval activity of the lower temperature. After 24 h a large proportion of introduced larvae remained within the gut lumen of brown trout kept at 8°C and it may be that at least some of these larvae would have penetrated the gut wall if given a longer time period. Smith (1974) found that *Anisakis* sp. larvae were able to penetrate the gut wall of haddock within 24 h at $9\text{--}10^\circ\text{C}$, but penetrated larvae were not found in whiting until 70 h after infection.

The mechanism by which an *Anisakis* sp. larva penetrates the gut wall of host fish is unknown but possibly involves the release of histolytic enzymes from the excretory gland through the excretory pore, which opens close to the boring tooth at the anterior end of the larva. Mueller (1927) described secretory granules in the excretory gland of adult *Anisakis simplex* and suggested that secretions from the gland may be used to destroy host tissue. More recently, Lee, Chen & Lin (1973) found that the excretory gland of larval *Anisakis* contains large amounts of granules with a crystalline appearance which they suggested may contain histolytic enzymes. Ruitenbergh & Loenderloot (1971) demonstrated the presence of enzymes, particularly non-specific

esterase, in the excretory gland of *Anisakis* sp. larvae. The action of histolytic enzymes secreted through the excretory pore onto the host's gut wall, possibly combined with the action of the boring tooth, seems the most likely method by which *Anisakis* sp. larvae are able to penetrate rapidly through the gut wall.

In both naturally and experimentally infected trout most *Anisakis* sp. larvae recovered from the body-cavity were located along the length of the gut, especially amongst the fat and mesenteries surrounding the stomach and pyloric caeca. Most of the remaining larvae were found in the anterior half of the body-cavity. The distribution of the larvae in trout reflects the site of penetration through the anterior region of the gut.

An appreciable proportion of the total numbers of *Anisakis* sp. larvae recovered from experimental trout was found partially or totally embedded in the hypaxial musculature of the fish. A few larvae were found partially embedded in the hypaxial musculature of brown trout from Hanningfield Reservoir; since the musculature of these fish was not searched completely, any totally embedded larvae were not detected.

Anisakis sp. larvae occur in considerable numbers in the musculature of herring (Smith & Wootten, 1975), cod (Young, 1972) and whiting (MacKenzie & Wootten, 1973) with most of the larvae situated in the hypaxial musculature. The larvae of *Anisakis* sp. found in marine fish are usually encapsulated in a flat spiral (Khalil, 1969); the capsule was found by Prusevich (1964) to be of host origin. Larvae in trout from Hanningfield Reservoir were usually coiled loosely within a thin host capsule. Larvae from experimental trout were not encapsulated although it is probable that insufficient time had elapsed for a capsule to form. On the other hand, Smith (1974) found that a delicate capsule was present around some larvae in the body-cavity of haddock only 34 h after infection.

The feeding of untreated marine fish offal to trout in fish farms may lead to infection with *Anisakis* sp. larvae. However, if marine fish offal is deep frozen to -20°C for 24 h before being fed to trout any *Anisakis* sp. larvae present are killed (Gustafson, 1953).

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