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The life cycle of *Paraquimperia tenerrima*: a parasite of the European eel *Anguilla anguilla*

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Abstract

Previous studies on the life history of the nematode eel specialist *Paraquimperia tenerrima* (Nematoda: Quimperiidae) have failed to determine whether an intermediate host is required in the life cycle. In the laboratory, eggs failed to hatch below 10 °C, hatching occurring only at temperatures between 11 and 30 °C. Survival of the free-living second stage larvae (L2) was also temperature dependent, with maximal survival between 10 and 20 °C. Total survival of the free-living stages (eggs and L2) is unlikely to exceed a month at normal summer water temperatures, confirming that parasite could not survive the 6 month gap between shedding of eggs in spring and infection of eels in early winter outside of a host. Eels could not be infected directly with L2, nor could a range of common freshwater invertebrate species. Third stage larvae (L3) resembling *P. tenerrima* were found frequently and abundantly in the swimbladder of minnows *Phoxinus phoxinus* from several localities throughout the year and were able to survive in this host in the laboratory for at least 6 months. Third stage larvae identical to these larvae were recovered from minnows experimentally fed L2 of *P. tenerrima*, and eels infected experimentally with naturally and experimentally infected minnows were found to harbour fourth stage larvae (L4) and juvenile *P. tenerrima* in their intestines. Finally, the whole life cycle from eggs to adult was completed in the laboratory, confirming that minnows are an obligate intermediate host for *P. tenerrima.*

Introduction

The quimperiid nematode *Paraquimperia tenerrima* is a specific freshwater parasite of the European eel *Anguilla anguilla.* Its distribution appears to be congruent with that of its definitive host (Moravec, 1994) and in some localities it can be common enough to dominate the intestinal component communities (Kennedy, 1990). Its prevalence and abundance exhibit clear seasonality, as infection of eels takes place in late winter to early spring, the parasite produces eggs in summer and then infection levels decline to reach their lowest levels in autumn and early winter (Chubb, 1982; Nie & Kennedy, 1991). Despite its being so common, the life cycle of this, and indeed of any other species, of *Paraquimperia* is still unknown.

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Moravec (1966a) reported the parasite from the Czech Republic for the first time and later (Moravec, 1974) described the development of its early stages. Mature eggs obtained from adult worms and maintained in water at 20–25 °C hatched to release first stage larvae (L1) after 5–6 days. These moulted to second stage larvae (L2) 3–4 days later, but all had died 6 days after this moult. All attempts to infect invertebrates with L2 failed. An attempt to infect eels directly with L2 in the laboratory gave an ambiguous result: one larva was reported from an eel, but Moravec (1974) himself considered this may have come from a natural infection of the eel prior to its being brought into the laboratory. Based on these findings, Moravec (1994) believed that an intermediate host, an invertebrate or fish, was required in the life cycle of *P. tenerrima* and this belief has been shared by other workers (Jeacock, 1969; Conneely & McCarthy, 1986). Nie (1990) was also unable to find any *P. tenerrima* larvae in a range of invertebrate species taken from a river in which the adult stage was common in eels, nor was he able to

infect any of these species experimentally although some workers (Chubb, 1982; Williams & Boulton, 1985; Lacy, 1986) still considered the possibility of the L2 being eaten by eels or penetrating their skin.

A direct life cycle, whilst a possibility, seems unlikely, and not just because most nematodes parasitic in fish require an intermediate host. Many studies have been carried out on the parasites of eels, but none have reported nematode third stage larvae (L3) that resemble those of a *Paraquimperia* species. The seasonality of the cycle of *P. tenerrima* in eels also poses a problem. Adults become sexually mature in summer after being recruited into eels in winter and early spring (Chubb, 1982; Nie & Kennedy, 1991). Eggs released in summer in turn release L1 which then moult to L2, but this whole process takes only *c*. 16 days at summer water temperatures (Moravec, 1974). There thus remains a gap of around 3 months before recruitment into eels commences in November, during which period the location of the larvae, especially L3, is unknown. It is possible that some eggs or the L2 may remain quiescent or dormant in the river, but it appears rather more likely that the larvae spend this period in an as yet unidentified intermediate host.

The aim of this present investigation was to elucidate the life cycle of *P. tenerrima* by experimentally investigating all these possibilities and especially the requirement for, and identity of, any intermediate host. Survival of eggs and larvae in freshwater was determined over a range of temperatures, and the ability of second stage larvae to infect a variety of invertebrates, eels and other fish directly was investigated. Eventually an attempt was successfully made to complete the whole life cycle of the parasite under experimental conditions in the laboratory.

Material and methods

Hatching and survival of larvae

Infected eels were caught in local rivers (Rivers Clyst and Otter, Devon, UK) by electro-fishing, brought back to the laboratory and killed using ethyl-p-aminobenzoate terminal anaesthesia. Intestines were removed, opened and searched for adult female *P. tenerrima.* Eggs were obtained by teasing open these adults. Eggs from different parents were mixed in water at 20 °C. Batches of 50 eggs were transferred to small dishes containing filtered pond water and were kept under a 12 h:12 h light:dark regime at constant temperatures over the range of 0–30 °C to develop. These eggs were examined daily and the number of eggs and larvae counted. Larvae were judged to be alive when they responded to light and stimuli by movement.

Potential for direct infection of eels by second stage larvae

Uninfected eels for experimental infections were obtained from a fish farm, having been fed exclusively on pellets. The absence of infections was confirmed by examination of a control sample. Eels were maintained in experimental groups of ten in tanks in the laboratory at 16–18°C and fed on pellets. One group served as a control, 100 L2 at 6 days post-hatching were introduced into a tank containing the second group, whereas eels from the third group were infected individually with ten L2 by stomach tube whilst under anaesthetic. All eels were killed after four weeks post-infection, when all organs and samples of all tissues were examined for nematode larvae by searching and following digestion.

Potential invertebrate intermediate hosts

Potential candidates for intermediate hosts were selected as being species that were as common and widespread as eels, lived in close proximity to eels or were common eel dietary items and were to be found in rivers at least from late winter to early summer. Samples of a number of species fulfilling these criteria were collected by kick sampling and hand netting from the rivers Clyst and Otter in June and September. The samples were brought back to the laboratory alive, killed and examined immediately for the presence of nematode larvae. On a later occasion, samples of a similar variety of invertebrates were obtained and brought back to the laboratory for experimental infections. Individual invertebrates were kept in small dishes of water at 18–20 °C and exposed to 5–10 L2. They were examined daily over a period of 21 days or for the life span of the larvae, after which they were killed and examined for the presence of parasites. All dead invertebrates were also examined.

Potential fish intermediate hosts

On the same occasions, samples of minnows *Phoxinus phoxinus* were obtained from the same rivers by DC electro-fishing. They were brought back to the laboratory alive and then killed and examined as soon as possible thereafter. Fish were examined by standard techniques for the presence of larval nematodes, supplemented by examination of organ squashes, tissue smears and digestion of tissue. Later in the programme, minnows were sampled from each river monthly over 12 months, brought back to the laboratory and subjected to a similar standard examination. All nematode larvae found were killed and fixed. For purposes of experimental infection, minnows were caught by a similar method from the River Culm, where levels of *P. tenerrima* in eels were known to be very low (no neighbouring locality was found to be completely free of this parasite, but out of a preliminary sample of 100 minnows none were found to be infected). Minnows were maintained at 18 °C in the laboratory and fed flaked food. Larvae for infection were obtained from eggs hatched in the laboratory and were L2 at 6 days post-hatch. Larvae were introduced into tanks of minnows, which were allowed to feed on them naturally. Fish were exposed to increasing densities of larvae; details of the protocol and numbers are shown in table 6. Attempts were also made to infect 14 roach *Rutilus rutilus* obtained from Slapton Ley with 70–140 L2 per fish. All fish were kept at 18 °C and fed daily and were then killed and examined for parasites after 4 weeks. Any nematode larvae were killed and measured. A further batch of 100 minnows from the River Otter was caught in October 1995. These were brought back to the laboratory alive, maintained in a large

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tank at 12 °C and fed daily on flakes. Samples of 30 were killed in October, January and March and examined for parasites as described above. Any nematodes found were removed, counted, killed and measured.

Definitive eel hosts

Eels for infection needed to be large enough to feed naturally on fish and also, if possible, parasite-free. It proved impossible to obtain eels of this size from the eel farm, so they had to be obtained from localities where they were known to harbour very low infections of *P. tenerrima*: Slapton Ley and the River Culm, Devon, UK. Samples of 30 eels from each locality were caught by electro-fishing in March and April and kept in the laboratory until October/November so that any natural infections present at sampling may have been lost. They were maintained at 12 °C on a 12:12 h light:dark regime in groups of ten in tanks with refuges and fed on uninfected fingerling trout. Eels that did not feed on trout served as controls. Those that did feed became experimental animals. For experimental infections eels were transferred individually to experimental tanks. Of 15 eels from each locality, groups of five were exposed individually to: (i) minnows from the River Culm infected in January with *P. tenerrima* larvae at a dose of 200–400 larvae per ten fish and thereafter fed flaked food; (ii) minnows from the River Otter naturally infected with *P. tenerrima* larvae; and (iii) minnows from the River Otter without swimbladders. All minnows were killed before being fed to eels. Eels were fed on fingerling trout after minnows, then killed after 4 weeks. A second group of eels was infected in the same manner as (i) and (ii) and then killed after 8 weeks (it was impossible to obtain enough to repeat (iii) and the controls). All eels killed were examined for *P. tenerrima* and all parasites found were killed and measured.

Results

Hatching and survival

The relationship between hatching rate of *P. tenerrima* eggs and water temperature is shown in fig. 1. No development or cleavage was evident below 10 °C, eggs were clearly starting to decompose after 3 weeks and they disintegrated after 4 weeks. At higher temperatures, the hatching rate and duration of the hatching period was directly related to water temperature. Between 10.1 and 15 °C hatching commenced at 9 days and continued until day 14, whereas between 25.1 and 30 °C all eggs hatched on the third day. Only 38% of the eggs hatched at 10.1–15 °C, 70% at 15.1–20 °C and 60% at 20.1–25 °C, i.e. the proportion of eggs that hatched peaked between 15.1 and 20 °C and declined at both lower and higher temperatures. Larval survivorship was also temperature dependent, being greatest between 10.1 and 20 °C (50% survived to 15 or 16 days) and lowest at 25.1–30 °C (50% survived to 6 days) (fig. 2). Maximal larval survival at the lowest temperatures was only 19 days and maximum egg survival over the same temperature range was only 21 days. This gives a maximum life span for the free-living stages at winter water temperatures of 40 days: the great majority of eggs and larvae would survive for less than a month.

Direct infection of eels by second stage larvae

Free-living L2 were unable to infect eels directly, either by penetrating the skin or following ingestion (table 1).

Infection of potential intermediate hosts

None of the species of invertebrate examined for natural infections with nematode larvae harboured any larval stages of *P. tenerrima* (table 2) even though they were obtained from rivers in which adults of *P. tenerrima*

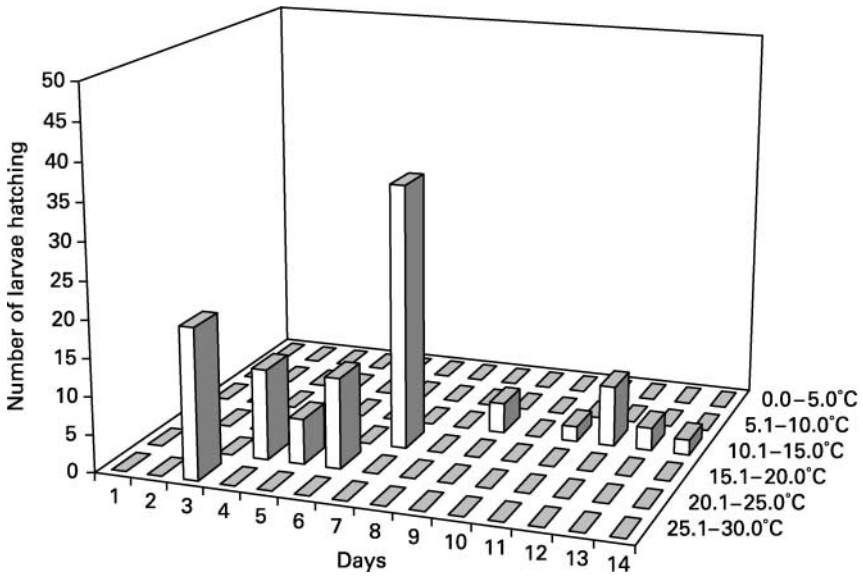

Fig. 1. Hatching rates of *Paraquimperia tenerrima* at temperatures between 0 and 30°C.

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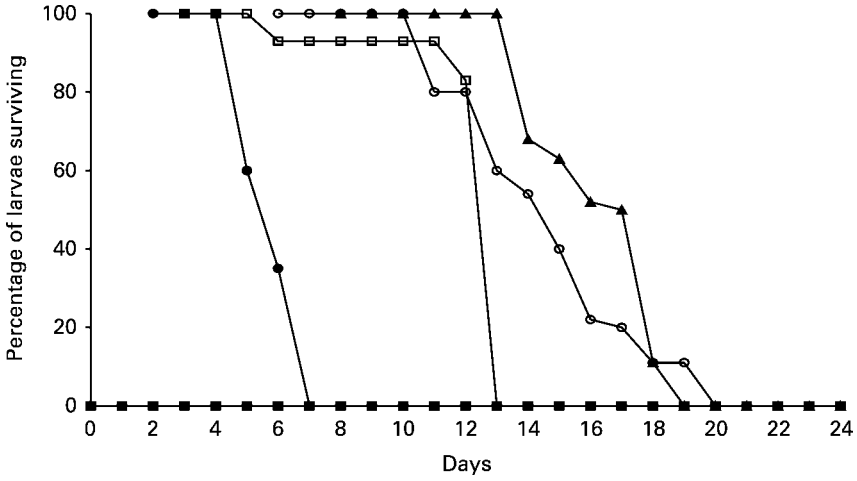

Fig. 2. Percentage survival of larvae of *Paraquimperia tenerrima* at: 5.1–10.0°C (■), 10.1–15.0°C (▲), 15.1–20.0°C (○), 20.1–25.0°C (□), 25.1–30.0°C (●)

were present in eels. Similarly, none of the species of invertebrate experimentally exposed to L2 of *P. tenerrima* became infected with larvae. It was evident that chironomid larvae ingested the L2 as larvae of *P. tenerrima* were visible in the intestines of some of the experimental animals, but the larvae passed through the gut and were excreted.

Examination of several species of fish revealed the presence of small, colourless nematode larvae in the swimbladder wall of minnows and, at a much lower level, dace (table 3). Further investigations therefore concentrated upon minnows since prevalence levels of these larvae attained 38% in the River Clyst in October, and 91% in the River Otter in the same month. The larvae were

Table 1. Experimental infections of *Anguilla anguilla* with second stage larvae of *Paraquimperia tenerrima* administered directly at a dose of ten per eel.

Procedure	N	Eel length (mm) range and (median)	Eel weight (g) range and (median)	Parasites recovered
Controls	10	97–190 (142)	0.9–7.7 (4.0)	0
Exposed to larvae	10	97–179 (157)	0.9–6.6 (4.3)	0
Stomach tube	10	108–190 (155)	1.2–7.7 (4.5)	0

N, number examined.

Table 2. The occurrence of *Paraquimperia tenerrima* larvae in naturally occurring invertebrates in the rivers Clyst and Otter, Devon, UK in 1994 and in experimentally infected (5–10 L2 per individual) invertebrates.

	River Clyst				River Otter						
	June		Sept.		June		Sept.		Experimental		
Taxon	N	Ni	N	Ni	N	Ni	N	Ni	Nx	Nr	Ni
Daphnia	50	0	50	0	15	0	17	0	20	5	0
Cyclops	50	0	50	0	0	0	15	0	20	5	0
Ostracoda	50	0	50	0	32	0	12	0	0	0	0
Asellus	50	0	50	0	15	0	17	0	10	5	0
Gammarus	50	0	50	0	15	0	17	0	10	5	0
Caenis	37	0	24	0	14	0	2	0	5	4	0
Agrion	30	0	17	0	10	0	14	0	5	4	0
Chironomidae	50	0	50	0	17	0	27	0	10	5	0
Limnephilidae	50	0	27	0	15	0	24	0	5	4	0
Hydropsyche	46	0	21	0	15	0	16	0	0	0	0
Sialis	50	0	34	0	15	0	17	0	5	4	0
Potamopyrgus	50	0	50	0	16	0	17	0	10	5	0
Lymnaea	43	0	39	0	0	0	0	0	10	4	0
Planorbis	39	0	35	0	0	0	0	0	0	0	0

N, number examined; Ni, number infected; Nx, number exposed; Nr, number of replicates.

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Table 3. The occurrence of *Paraquimperia tenerrima* in wild fish in the rivers Clyst and Otter, Devon, UK in 1994.

		River Clyst			River Otter		
Fish species	Month	N	Ni	%	N	Ni	%
Cottus gobio	Sept.	33	0	0	20	0	0
Noemacheilus barbatulus	Sept.	20	0	0	20	0	0
Gasterosteus aculeatus	Sept.	50	0	0	4	0	0
Phoxinus phoxinus	Sept.	32	5	15.6	38	32	84.2
	Oct.	21	8	38.1	23	21	91.3
Leuciscus leuciscus	Oct.	0	0	0	3	1	33.3

N, number examined; Ni, number infected; %, prevalence.

coiled within the wall of the swimbladder: they were not encysted and nor did they cause any inflammation. They were viable and moved freely if disturbed with a needle. The larvae were longer (mean 0.77 mm ± SD 0.06, $n = 15$) than L2 of *P. tenerrima* (0.54 mm ± 0.03, $n = 17$). Stage 3 larvae of *P. tenerrima* are unknown and thus no comparison with the larvae from minnows is possible, but the larvae from minnows are similar in appearance to L2 and of a length that might be anticipated for the L3. The larvae were not an accidental or occasional infection in minnows, but were present in both rivers throughout the year (table 4).

Larvae were more prevalent and abundant in the River Otter but there was no clear evidence of seasonality in either parameter in either river and the larvae were available for infecting a definitive host all the year round. The abundance of parasites was directly proportional to fish length:

$$\text{River Otter } y \text{ (abundance)} = 1.4 \times (\text{fishlength mm}) - 4.6 \; (R = 0.5, \; P > 0.01, \; n = 248$$

$$\text{River Clyst } y = 0.4 \times -1.1 \; (R = 0.49, \; P > 0.01, \; n = 237)$$

and infections first appeared in minnows at 30 mm long. Larvae survived in minnows for at least 6 months (table 5), over which period prevalence changed little but abundance and intensity declined. The larvae grew from 0.77 mm (± 0.07, $n = 25$) in October to 0.84 mm (± 0.03, $n = 17$) in January and 0.87 mm (± 0.04, $n = 25$) in March.

In minnows experimentally infected with *P. tenerrima* L2, both prevalence and abundance of nematodes in the swimbladder increased with the experimental dose (table 6), with prevalence increasing from 70% through 75% to 90% and abundance from 1.2 to 14.1. Only one control fish was infected and that with a single larva and it seems likely that this was the result of a natural infection as it could never be guaranteed that experimental fish were initially free of infection. By contrast with the success of the infections in minnow, only a single roach was infected. Larvae recovered from these experimentally infected minnows were 0.56 ± 0.6 mm in length, smaller than larvae obtained from naturally infected minnows and of a length similar to L2. In every respect, including appearance and site in the host, the experimentally infected L3 of *P. tenerrima* resembled the larvae found naturally in the swimbladder of minnows.

Infection of eels

Four weeks after experimental infections of eels with *P. tenerrima* larvae in both naturally and experimentally infected minnows, larvae, presumably fourth stage (L4), were found in the stomach of eels (table 7). They showed a preference for the diverticulum and were free, viable and active. No larvae of any species were recovered from eels fed minnows from which the swimbladders had been removed, indicating clearly that the source of the

Table 4. Quarterly changes in the levels of infection of swimbladders of minnows in the rivers Otter and Clyst, Devon, UK in 1995 with the third stage larvae of *Paraquimperia tenerrima*.

	Month			
	January	April	July	October
River Otter				
Number of fish examined	30	20	22	40
Number of fish infected	27	9	15	39
Prevalence	90.0	45.0	68.2	97.5
Mean abundance (SD)	5.0 (4.2)	0.6 (0.7)	2.0 (3.1)	3.9 (3.6)
Mean intensity (SD)	5.6 (4.1)	1.2 (4.4)	3.0 (3.5)	3.9 (3.5)
River Clyst				
Number of fish examined	19	37	24	34
Number of fish infected	9	11	8	8
Prevalence	47.3	29.7	33.3	33.3
Mean abundance (SD)	0.7 (1.1)	0.6 (1.4)	0.5 (1.0)	0.5 (2.3)
Mean intensity (SD)	1.6 (1.0)	2.0 (1.9)	1.6 (1.1)	1.6 (1.5)

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Table 5. Survival of *Paraquimperia tenerrima* in *Phoxinus phoxinus* collected from the River Otter, Devon, UK in October 1995 and maintained in the laboratory.

Date	N	Ni	Np	%	Abundance (SD)
Oct. 1995	31	27	158	87.1	5.1 (4.3)
Jan. 1996	31	25	111	80.1	3.6 (3.6)
Mar. 1966	26	25	85	84.6	3.3 (3.0)

N, number of fish; Ni, number infected; Np, total number of parasites.

nematodes in the eel stomachs was the larvae in the swimblader of minnows. At 8 weeks post-infection some larvae were still present in the stomach, but many were now to be found in the small intestine. Recovery rates were higher from experimentally infected minnows, probably because experimental infection levels were higher than in naturally infected minnows. Changes in parasite length throughout the life cycle of *P. tenerrima* are summarized in fig. 3.

Discussion

Although both the adult stage of *P. tenerrima* in eels (Moravec, 1966a,b, 1994; Nie & Kennedy, 1991) and eggs and development up to the L2 stage (Moravec, 1974) have been studied in some detail, the complete life cycle is unknown and L3 and L4 have not been identified as such. Despite the suggestions of some authors (Chubb, 1982; Lacy, 1986; Williams & Boulton, 1985) that *P. tenerrima* has a direct life cycle, field data on the seasonal occurrence of *P. tenerrima* in adult eels tend to favour the hypothesis that the parasite has an indirect cycle and requires an intermediate host. The failure of both Moravec (1974) and Nie (1990) to infect eels directly with L2 supports this view. Furthermore, Moravec's (1966a,b, 1974) studies on the development of *P. tenerrima* to L2 and subsequent larval survival indicated that the free-living stages were short lived. It was thus very difficult to explain the 6 month gap, identified from field studies, between maturation of adults in eels in summer and re-infection of eels with larvae the following spring (Nie & Kennedy, 1991) on the basis of the parasites overwintering as free-living stages. Nevertheless, data on larval survival required confirmation, as did the failure of attempts to infect invertebrates and eels directly. Ultimately, the only convincing evidence for an indirect life cycle must be established by the completion of the life cycle in the laboratory under experimental conditions.

The present experiments on egg and larval survival have confirmed that free-living stages of *P. tenerrima* (eggs and L2) could survive in rivers for only a short time;

Table 6. Experimental infections of minnow *Phoxinus phoxinus* from the River Culm, Devon, UK exposed to second stage larvae of *Paraquimperia tenerrima* and examined at 4 weeks post-infection.

Treatment	N	Length range of fish (mm)	Prevalence	Abundance (SD)	Maximum
50–100 larvae	20	2.3–3.5	70.0	1.2 (1.1)	3
Control	20	2.3–3.8	0	0	0
101–200 larvae	20	2.5–4.2	75.0	1.7 (1.7)	7
Control	20	2.1–4.0	0	0	0
201—500 larvae	10	3.0–4.0	90.0	14.1 (10.6)	30
Control	10	2.5–3.8	10.0	0.1 (0.3)	1

Table 7. Experimental infections of eels *Anguilla anguilla* with minnows *Phoxinus phoxinus* infected with larvae of *Paraquimperia tenerrima*.

					Parasite recovery					
					Stomach			Intestine		
Treatment	N	Source of eel	Time	Eel size (mm)	%	x (SD)	Mx	%	x (SD)	Mx
Lab. inf.	5	R. Otter	4 wpi	353–385	80.0	11.6 (7.5)	19	0	0	0
Lab. inf.	5	R. Otter	8 wpi	376–450	100.0	6.8 (2.3)	10	100.0	5.6 (3.4)	9
Nat. inf.	5	R. Otter	4 wpi	380–495	100.0	6.2 (3.6)	10	0	0	0
Nat. inf.	5	R. Otter	8 wpi	363–470	80.0	4.4 (2.5)	8	80.0	3.0 (2.4)	6
NSB	5	R. Otter	4 wpi	340–440	0	0	0	0	0	0
Control	5	R. Otter	4 wpi	349–426	0	0	0	0	0	0
Lab. inf.	5	Slapton	4 wpi	359–426	100.0	15.0 (7.9)	27	0	0	0
Lab. inf.	5	Slapton	8 wpi	380–502	80.0	5.2 (3.8)	10	80.0	6.0 (4.6)	12
Nat. inf.	5	Slapton	4 wpi	375–469	100.0	5.4 (3.2)	8	0	0	0
Nat. inf.	5	Slapton	8 wpi	338–400	80.0	2.6 (1.7)	4	80.0	3.2 (2.8)	7
NSB	5	Slapton	4 wpi	316–405	0	0	0	0	0	0
Control	5	Slapton	4 wpi	394–527	0	0	0	0	0	0

Lab. inf., laboratory infected; Nat. inf., naturally infected; NSB, wild minnows infected naturally but with the swim bladder removed; Control, uninfected eels; wpi, weeks post infection; %, prevalence; x, abundance; SD, standard deviation; Mx, maximum number.

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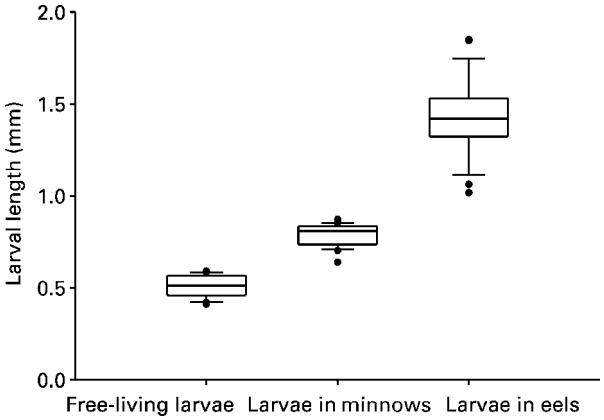

Fig. 3. Box plot depicting data from all developmental stages of *Paraquimperia tenerrima* (n = 31 for each stage). Median value displayed as a bar, the interquartile range as a box, and the range as the whiskers. Outliers are also shown as individual points.

around one month under optimal temperature conditions. At cold (winter below 10 °C) temperatures, eggs could not develop at all and soon disintegrated. As temperatures rose, a greater proportion of eggs developed but survivorship of the L2 declined. These results confirm that eggs could not survive and overwinter in rivers and at summer temperatures eggs and larvae could not survive in the water for the 6 months needed to bridge the infection gap. Confirmation that eels could not be infected by L2 by direct by penetration of the skin or ingestion was also obtained. Together, these findings strongly suggest that an intermediate host is required in the life cycle.

Both invertebrates and vertebrates have been suggested as possible intermediate hosts (Moravec, 1994). However, none of the invertebrate species examined were found to be infected with *P. tenerrima*, and attempts to experimentally infect a variety of invertebrates with *P. tenerrima* larvae were all unsuccessful. This confirms the findings of other workers, including Moravec (1974) and Nie (1990), who were equally unsuccessful in infecting freshwater invertebrates. There is always an element of chance in the selection of species for examination and infection and so negative results can never be entirely conclusive. Nevertheless, the fact that larvae of *P. tenerrima* have never been found in invertebrates does suggest that the intermediate host is not a freshwater invertebrate and this focuses attention upon fish as a possible host.

The finding of nematode larvae in the wall of the swimbladder of minnows was surprising, as no previous studies on the parasites of minnows (e.g. Bibby, 1972), or check lists (e.g. Kennedy, 1974; Holland & Kennedy, 1998) or host–nematode lists (e.g. Moravec, 1994) mention nematode larvae in this site in this host. This may be a matter of chance in that the localities in which minnow parasites have been studied may not have contained eels parasitized by *P. tenerrima*. It may also reflect the fact that the swimbladder wall was not considered as a suitable site for larval nematodes until the appearance of *Anguillicola crassus* in Europe. Indeed, it was only when the swimbladders of minnows were being examined for larvae of *A. crassus* by one of the authors (JAS) in autumn (September and October 1994) that these unknown larvae were found. The larvae could not be identified, but they were considered to be similar in appearance to what might be expected of L3 of *P. tenerrima* as they were of an appropriate size, being slightly larger than L2. Larval prevalence and abundance increased with size of minnows (Shears, unpublished observations), the larvae were present in minnows in both the rivers Otter and Clyst throughout the year (table 4) and individual larvae could survive for at least 6 months (table 5). If these were larvae of *P. tenerrima*, this would explain how and where the parasite was able to survive overwinter and why recruitment to eels took place in winter and early spring. Minnows are known to be a component of the diet of eels (Sinha & Jones, 1975; Tesch, 1977) and so the larvae would be transmitted to eels by ingestion of minnows. Transmission would be likely to occur at a higher level during the winter and early spring when both species of fish live in close proximity along river margins in comparison to the summer when minnows shoal, are more active and are a more difficult prey to catch. Whatever the identity of the larvae, minnows appeared to be a normal and natural host for their growth and development. At this stage in the investigation therefore there appeared to be an *a priori* case on both morphological and biological grounds for considering the larvae to be the larval stages of *P. tenerrima.*

Confirmation of this was obtained from controlled experimental infections. It was established that the L2 of *P. tenerrima* could infect minnows under these conditions. and that they located and survived in the swimbladder wall. Further confirmation that minnows were the intermediate host of the parasite came from the successful infection of eels with larvae initially obtained from eggs in the laboratory, then passaged through the swimbladders of minnows which were in turn fed to eels. The parasites initially located in the stomach diverticulum, probably as L4, but later moved into the intestine, the preferred site of adult *P. tenerrima* in eels. The presence of larvae in dace suggests that other fish may also be able to serve as intermediate hosts, and this can be tested, but by completing the whole life cycle in the laboratory the present investigations have demonstrated conclusively that *P. tenerrima* does require an obligate intermediate host and that the minnow is such a host.

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