FOLIA PARASITOLOGICA 46: 111-116, 1999

Post-cyclic transmission in *Pomphorhynchus laevis* (Acanthocephala)

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Key words: transmission, post-cyclic, *Pomphorhynchus laevis*

Abstract. A series of laboratory experiments was conducted to investigate the possibility of post-cyclic transmission in *Pomphorhynchus laevis* (Müller, 1776). Rainbow trout *Oncorhynchus mykiss* (Walbaum) were exposed to *P. laevis* in naturally infected *Cottus gobio* Linnaeus, *Noemacheilus barbatulus* (Linnaeus), *Phoxinus phoxinus* (Linnaeus) and *Leuciscus cephalus* (Linnaeus) and sacrificed one month after infection. Post-cyclic transmission was possible from all four species even though they came from three families and differed in respect of their status and suitability as hosts of *P. laevis*. There was no selection for or against either sex of *P. laevis*, parasites grew in the rainbows and they occupied the same, normal site in the intestine of rainbows irrespective of source host. Post-cyclic transmission of gravid parasites could occur from *C. gobio* but not from *L. cephalus*. It is believed that this failure to transmit larger parasites of either sex reflects the age and so development of the proboscis bulb of *P. laevis* and the extent of the host encapsulation response rather than size or stage of maturity per se. Post-cyclic transmission has the potential to be important in nature.

The phenomenon of post-cyclic transmission has been most clearly described by Nickol (1985) as "When ingested as adults within their definitive hosts some acanthocephalans survive and parasitise the predator". Nickol further commented that little is known regarding post-cyclic transmission in acanthocephalans, although he listed five species from which it had been reported. Uglem and Beck (1972) achieved post-cyclic transmission of *Neoechinorhynchus cristatus* Lynch, 1936 experimentally by force feeding of several species of fish, but showed that only sub-adults established in the predator host and even these had all disappeared seven days post infection (dpi). They were completely unable to achieve transmission in the closely related species *N. crassus* Van Cleeve, 1919. Lassiere and Crompton (1988) demonstrated that post-cyclic transmission could occur in *N. rutili* (Müller, 1780) when rainbow trout *Oncorhynchus mykiss* (Walbaum) were allowed to feed on naturally infected *Gasterosteus aculeatus* Linnaeus in the laboratory. This parasite survived in the predator host for up to 10 dpi. Several authors have suggested that this form of transmission may be important in nature (Hnath 1969, DeMont and Corkum 1982, Nickol 1985, Lassiere and Crompton 1988, Valtonen and Crompton 1990), but they have provided no evidence in support of this. Indeed, until more is known about a phenomenon that has been reported from a few species only and that is not a property of all species or all stages of a species, it is difficult to obtain any idea of its potential significance in natural systems. If parasites transmitted in this manner do not survive long in the predator (Uglem and Beck 1972), post-cyclic transmission may be of little or no importance in nature at all.

Post-cyclic transmission has not hitherto been reported from any species of the genus *Pomphorhynchus*, though Kennedy (1996) drew attention to the possibility of its occurring in *P. laevis* (Müller, 1776) between *Cottus gobio* Linnaeus and *Salmo trutta* Linnaeus in the River Otter. It might in fact be considered unlikely that it would be exhibited by this species in view of the particularly firm attachment of *P. laevis* its host's intestine. This involves a host response such that the bulb of the parasite, and indeed often the whole praesoma of adults, is enclosed in a nodule of host fibrous tissue that forms a tough capsule (Hine and Kennedy 1974, Wanstall et al. 1986, 1988).

Nevertheless, when a preliminary experiment indicated that post-cyclic transmission of *P. laevis* from *C. gobio* to *O. mykiss* could occur in the laboratory, the system was investigated further to provide wider and more detailed information on the phenomenon. The particular aims of the investigation were to provide information on the range of naturally infected fish species that could be sources of parasites for such transmission, to determine whether all stages and both sexes of the parasite could transmit with equal facility, to see whether the parasite could survive for longer than 7 dpi and grow and, if so, to find out whether it located in the same region of the predator's intestine as it does when infection is achieved by ingestion of an infected gammarid intermediate host. The results of these experiments, which achieved the aims, are reported and discussed in this paper.

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MATERIALS AND METHODS

The great majority of wild fish infected with *P. laevis* were obtained from the River Culm, a tributary of the River Exe, by electro-fishing. One sample of *C. gobio* was obtained from the River Otter by the same method. Samples of as many fish species as possible from the R. Culm were killed and examined by standard methods (Hine and Kennedy 1974), to determine the prevalence, abundance, weight (filter paper dry), sex and state of maturity of *P. laevis* in potential source fish. Sample sizes and summary data on infection levels are shown in Table 1.

Samples of fish to be used as parasite sources in experimental infections were obtained from the same localities on or close to the same date. These fish were brought back to the laboratory and maintained in aquaria for up to a week before infection. Rainbow trout were used as predator fish to receive transmitted parasites, as this species is readily maintained in the laboratory and is a suitable wild host for *P. laevis* in that the parasite can reach adult size and attain full sexual maturity in it (Kennedy et al. 1978, Bates and Kennedy 1990, 1991). The rainbow trout were of similar size (most between 250 and 300 g) and were obtained from a local fish farm known to be free of infection with *P. laevis*. After acclimatisation for up to three weeks in the aquarium at 12°C, rainbow trout were isolated and anaesthetised. Each fish was force fed as appropriate: two headless *C. gobio* from the R. Otter, or one headless *C. gobio*, *Noemacheilus barbatulus* (Linnaeus) or *Phoxinus phoxinus* (Linnaeus) from the R. Culm or pieces of intestine of *Leuciscus cephalus* (Linnaeus). Transmission experiments were restricted to these four species of fish (Table 1) which differed in respect of their suitability as hosts for *P. laevis*, and numbers of rainbows used were dependent upon availability and numbers of wild fish. Rainbows were allowed to recover, were fed regularly on trout pellets and were then sacrificed at 31 dpi from the R. Culm and at 7 dpi from the R. Otter. They were then examined for parasites by standard methods. All *P. laevis* were counted and the position of each along the alimentary tract of rainbows was recorded as a percentage of the gut length from the stomach-intestine junction to the anus. All parasites were sexed and females assigned to one of three categories: F1, immature, with a single discrete ovary or ovarian balls only; F2, maturing, where the ovary had broken up into ovarian balls and maturing acanthors were present; and F3, gravid, where fully mature shelled acanthors were present. All parasites were individually weighed (filter paper dry).

RESULTS

Infection levels of *P. laevis* were high in the R. Culm and the parasite was found in every species of fish examined (Table 1). As judged by the proportion of females reaching the F3 stage and by the size attained by adult males and females, *L. cephalus* is the preferred host of *P. laevis* in this locality. The parasite also matured in *Leuciscus leuciscus* (Linnaeus) in the R. Culm, and in *C. gobio* and *S. trutta* in the R. Otter (Kennedy 1996) but not in the R. Culm. The source species of fish used in the transmission experiments thus differed not only in their suitability as hosts for *P. laevis* but also in the strength of their response to the parasite. Both *L. cephalus* and *N. barbatulus* responded strongly to *P. laevis* by fibrosis and encapsulation ensuring that parasites were firmly attached to the intestine wall. The attachment to *C. gobio* was less firm and to *P. phoxinus* very poor.

Nevertheless, and despite these differences, all attempts at post-cyclic transmission of *P. laevis* succeeded. Three families, Cottidae, Cobitidae and Cyprinidae, were represented by the source fish and as their predator fish was a Salmonidae all transmission was between fish of different families and so para post-cyclic. Transmission was demonstrated from species in which *P. laevis* never matures (*P. phoxinus*), sometimes (Rumpus 1975, Kennedy 1996) matures (*C. gobio* and *N. barbatulus*) and normally (Hine and Kennedy 1974) matures (*L. cephalus*). The proportion of rainbow trout infected ranged from 44.4% to 68%. Abundance levels in source fish and rainbows were similar for *C. gobio* and *N. barbatulus*, increased from *P. phoxinus* to rainbows and decreased from *L. cephalus* to rainbows. An estimate of transmission rate, of 55.5%, could be obtained only in respect of *C. gobio* from the R. Otter.

Parasites from all four species of source fish survived for at least one month in rainbows and over that period they were *able to grow* as evidenced by the increases in weight. The sex ratio was similar in source fish and rainbows, and there appeared to be no selection for or against either sex (Table 2). It does appear, however, as if post-cyclic transmission of *P. laevis* is only possible with small individuals. It is easy to distinguish juvenile and adult males and females of *P. laevis* by weight in *L. cephalus* and each piece of intestine fed to the rainbows contained both adult and immature parasites. However, no adult males or F3 females were recovered from rainbows, but only juvenile males and F1 and F2 females. The largest parasite recovered from a rainbow was an F2 of 13.84 mg and that from a *L. cephalus* source, and the largest male was 12.31 mg, from a *P. phoxinus* source. Sexual maturity per se does not appear to be a barrier to transmission as F3 individuals were recovered from rainbows a week after infection with *C. gobio* from the R. Otter (Table 1). The decrease in abundance levels in rainbows fed *P. laevis* from a *L. cephalus* source (Table 1) may thus reflect the inability of the larger (above 14 mg) adult parasites in the source fish (61% of all females were F3) to transmit post-cyclically. The immature parasites that were transmitted appeared to have grown and developed perfectly normally.

Regardless of the identity of their source fish, individuals of *P. laevis* occupied remarkably similar regions of the intestine in rainbows (Table 3). All occurred from 40% to 99% and there were no significant differences in the mean position between parasites from the four different species of source fish (Student "*t*" test: all *p* values NS).

Table 1. Infection levels, weight and reproductive characteristics of *Pomphorhynchus laevis* in species of wild, source fish fed to rainbow trout.

Source	Fish species	n	%	× ± SD	%F3	Parasite sex	Wet weight (mg) × ± SD	n
R. Otter	*Cottus gobio*	32	50.0	1.8 ± 2.4	23.5	F3	13.5 ± 6.7	12*
R. Culm	*Cottus gobio*	8	62.5	2.0 ± 2.6	0	M	1.87 ± 0.7	8
						F1	1.98 ± 1.9	8
						M:F ratio	1 : 1	
R. Culm	*Noemacheilus barbatulus*	30	27.5	0.8 ± 2.5	0	M	5.64 ± 3.1	7
						F1	5.14 ± 1.6	7
						M:F ratio	1 : 1	
R. Culm	*Phoxinus phoxinus*	33	9.1	0.1 ± 0.3	0	M	1.63	1
						F1	5.1	2
						M:F ratio	1 : 2	
R. Culm	*Leuciscus cephalus*	11	100.0	68.1 ± 134	61.0	Mj	6.3 ± 3.63	100
						Ma	32.39 ± 0.4	35
						F1	6.49 ± 2.55	56
						F2	10.64 ±4.64	42
						F3	42.65 ±15.9	81
						M:F ratio	1 : 1.32	
R. Culm	*Leuciscus leuciscus*	8	87.5	1.37 ± 1.4	30.0	No infection attempted		
R. Culm	*Gobio gobio*	3	33.3	0.33	0	No infection attempted		
R. Culm	*Anguilla anguilla*	32	62.5	10.9 ± 19.1	0	No infection attempted		
R. Culm	*Salmo trutta*	2	50.0	0.5	0	No infection attempted		

n – number, % – prevalence, × – abundance, %F3 – percentage of females with shelled acanthors, Mj – juvenile males, Ma – adult males. * Data from Kennedy (1996).

Table 2. Infection levels, weight and maturity of *Pomphorhynchus laevis* following post-cyclic transmission to rainbow trout.

Source fish species	n	%	× ± SD	%F3	Parasite sex and wet weight (mg) × ± SD		Maximum weight	n
Noemacheilus barbatulus	9	55.5	0.67 ± 0.7	0	M	0	0	0
					F1	8.67 ± 3.1	12.8	6
					M:F	0 : 6		
Phoxinus phoxinus	10	50.0	1.5 ± 2.1	0	M	6.2 ± 2.7	12.3	8
					F1	9.73 ± 2.4	11.1	7
					M:F	1.14 : 1		
Cottus gobio (R. Culm)	25	68.0	2.24 ± 3.5	0	M	3.81 ± 1.6	6.8	27
					F1	4.13 ± 1.7	8.4	30
					M:F	1 : 1.11		
Leuciscus cephalus	27	44.4	0.55 ± 0.9	0	Mj	7.86 ± 2.5	11.6	8
					Ma	0	0	0
					F1	8.22 ± 2.71	13.8	6
					F2	13.84	13.84	1
					F3	0	0	0
					M:F	1.14 : 1		
Cottus gobio (R. Otter)	10	60.0	2.0 ± 2.7	64.0	Data not available		Data not available	20

n – number, % – prevalence, × – abundance, %F3 – percentage of females with shelled acanthors, Mj – juvenile males, Ma – adult males.

DISCUSSION

The experiments reported here have demonstrated conclusively that post-cyclic transmission is possible in *P. laevis*, thus bringing the number of species from which it has been reported to seven (see Nickol 1985, and Lassiere and Crompton 1988, for the identity of the other species). The ability of *P. laevis* to survive, though not necessarily grow, in virtually every species of freshwater fish (Hine and Kennedy 1974, Kennedy et al. 1978) may be related to its facility for post-cyclic transmission. Parasites could be transmitted from fish species belonging to three different families and irrespective of the suitability of the source species as a host. In this respect *P. laevis* is similar to some other species of fish acanthocephalans. Although Hnath (1969) transmitted *Echinorhynchus salmonis* Müller, 1784 from *Oncorhynchus kisutch* (Walbaum) to *Salvelinus fontinalis* (Mitchill) i.e. only within one host family, Lassiere and Crompton (1988) transmitted *Neoechinorhynchus rutili* from *Gasterosteus aculeatus* to rainbow trout and Uglem and Beck (1972) transmitted *N. cristatus* to a number of species of freshwater fish from different families and genera.

Rainbow trout are a suitable host for *P. laevis* in that individuals can grow and attain full sexual maturity in this species (Kennedy et al. 1978, Bates and Kennedy 1990, 1991). Rainbows are easily infected in the laboratory by ingestion of *Gammarus* spp. containing cystacanths and parasites can reach the F3 stage and attain a weight of between 20 and 40 mg by 112 dpi (Bates and Kennedy 1990). These adult parasites locate between 40% and 100% along the alimentary tract, with a mean position between 65% (Bates and Kennedy 1990) and 73% (Bates and Kennedy 1991). Mature parasites also provoke a strong host response, and the praesoma becomes invested by a fibrous capsule of host tissue (Wanstall et al. 1986). The individuals of *P. laevis* transmitted post-cyclically to rainbow trout in the current experiments appeared to behave perfectly normally. They grew in the rainbows, sometimes attaining a greater size than in their source host after only 31 days in rainbows e.g. individuals from *N. barbatulus*, and the maximum sizes of individuals recovered from rainbows are consistent with the size to be expected if rainbows were infected with cystacanths and recovered after one month. The post-cyclically transmitted *P. laevis* did not remain long enough in rainbows to reach full sexual maturity (F3) or provoke the full host inflammation response, but all parasites attached in their normal, preferred site in the intestines of rainbows at around 70%. By contrast, Uglem and Beck (1972) found post-cyclically transmitted *N. cristatus* at the extreme posterior of their normal range in the intestine, but as these parasites survived transmission no longer than 7 days they may in fact have been on their way out of the intestine in any case. Unfortunately, it was generally impossible to obtain a reliable estimate of numbers of parasites fed to rainbows for a combination of reasons, including samples of wild fish being very small, parasites being very over-dispersed (high variance : means) and variation between individual rainbows and batches in their readiness to ingest whole fish or intestines. The unreliability of estimates is evidenced by *C. gobio* and *P. phoxinus* from the R. Culm when more parasites were apparently recovered from rainbows than were administered to them (abundances higher in rainbows than source fish). In the one case where it was possible to estimate transmission rate, the estimate of 55.5% accords well with the high rates reported by Lassiere and Crompton (1988).

Laboratory experiments of this nature can only demonstrate that post-cyclic transmission can occur: they cannot shed any light on the extent to which it may occur in nature or on its importance as a natural phenomenon. In the case of *N. cristatus*, which survived no longer than a week in its predator host following experimental post-cyclic transmission (Uglem and Beck 1972), it would seem likely that this form of transmission would be of little importance in nature. By contrast, Hnath (1969) found that *E. salmonis* transmitted post-cyclically survived for up to 12 weeks. In this species, and in species such as *P. laevis* where parasites attach normally, survive and grow after post-cyclic transmission, the phenomenon could be potentially important in nature. If it does occur to any extent in nature it could explain why *P. laevis* has been found in large, piscivorous *S. trutta* and *Esox lucius* Linnaeus (Hine and Kennedy 1974). Several authors have invoked post-cyclic transmission to explain peculiarities in acanthocephalan transmission and life cycles and have also argued that it will aid dispersal and accentuate overdispersion of parasites in the definitive host (De Mont and Corkum 1982, Nickol 1985, Lassiere and Crompton 1988, Valtonen and Crompton 1990, Kennedy 1996), but even though their arguments are convincing direct proof of its importance in nature is still lacking.

As interesting as an investigation of the conditions under which post-cyclic transmission can be achieved is an analysis of the conditions under which it fails to occur. Uglem and Beck (1972) could only demonstrate this form of transmission in sub-adult *N. cristatus*: neither adult *N. cristatus* nor any stage of *N. crassus* could be transmitted in this manner. In the case of *P. laevis*, post-cyclic transmission could not be achieved for large adults of either sex. It seems very likely that this failure is related to the size of the parasite rather than to its stage of development or the suitability of the source fish as a host of the parasite. Some individuals of *P. laevis* can and do reach sexual maturity in *C. gobio* and *N. barbatulus*, though the proportion doing so

Table 3. Position of *Pomphorhynchus laevis* in the intestine of rainbow trout following experimental post-cyclic transmission.

Source fish	Mean (%) position * ± SE	Range	n
Noemacheilus barbatulus	70.3 ± 7.1	40.0 - 94.1	6
Phoxinus phoxinus	72.8 ± 3.7	40.5 - 97.1	15
Cottus gobio	68.7 ± 1.7	46.7 - 99.2	56
Leuciscus cephalus	71.5 ± 4.5	43.8 - 92.1	15

* percentage of the gut length from stomach-intestine junction to the anus

appears to vary from locality to locality. In the R. Avon, only 0.5% of female *P. laevis* reached the F3 stage in *C. gobio* and only 2% in *N. barbatulus* (Rumpus 1975): in the R. Otter 23.5% became gravid in *C. gobio* but none reached the F3 stage in *N. barbatulus* (Kennedy 1996), whereas in the Rivers Culm (Table 1) and Severn (Brown 1989) no gravid specimens were found in either host species. Gravid *P. laevis* from *C. gobio* in the R. Otter attain a mean size of 13.5 mg (Kennedy 1996) and it proved possible to transmit some of these gravid parasites to rainbows post-cyclically. Parasites of both sexes and of weights up to 11.34 mg (from *N. barbatulus*) could be transmitted to rainbow trout, as could F2 and F3 individuals of similar weight. What proved impossible was to transmit large parasites from *L. cephalus*, even though they comprise the greater proportion of the population in the naturally infected source fish.

It is suggested that this may relate to the attachment of the parasite and not to its size or maturity per se. Large, adult *P. laevis* of both sexes in *L. cephalus* have well developed bulbs and provoke encapsulation of the praesoma with fibrous tissue of host origin (Hine and Kennedy 1974). This capsule is exceptionally tough and difficult to break up mechanically and is very slow to digest (unpublished data). It would seem probable that such a large parasite in which the attachment structures were surrounded by tough fibrous tissue would have great difficulty in re-attaching itself to the intestine wall of any other individual fish following post-cyclic transfer and so would be passed straight out of the alimentary tract without ever attaching. The bulb and host response are also well developed in *N. barbatulus* towards sexually mature *P. laevis* if the praesoma penetrates the intestinal wall (Wanstall et al. 1988), but all the individuals of *P. laevis* from this host used in the current experiments came from the R. Culm and were immature although the attachment was firm. These transferred successfully, as did parasites from *C. gobio* where the bulb is less well developed and the host response weaker (Rumpus 1975) and from *P. phoxinus* in which parasites are very loosely attached. It is therefore suggested that it is the state of development of the bulb and the extent of the host response, both of which are cross-correlated with age, that limit the extent of post-cyclic transmission in *P. laevis* rather than age, or size or state of maturity per se.

Acknowledgements. I am very grateful to Mr P. and Mrs J. Shears for technical assistance in the field and laboratory, to Mr D. Butterton for permission to use some unpublished data and to an unknown referee who first suggested that post-cyclic transmission might occur from *Cottus gobio*.

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Received 28 September 1998 Accepted 7 January 1999