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INFECTION AND SUCCESSFUL REINFECTION OF BROWN TROUT [*SALMO TRUTTA* (L.)] WITH GLOCHIDIA OF *MARGARITIFERA MARGARITIFERA* (L.)

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ABSTRACT

Brown trout [*Salmo trutta* (L.)] were successfully reinfected with glochidia of *Margaritifera margaritifera* (L.) in the season following an initial infection. Fingerling trout were exposed to glochidia in September 1982 and, although there was great variation in the numbers on each fish, there was no definite evidence of subsequent decline in glochidial numbers. The glochidia metamorphosed and in May 1983 left the fish, which were retained and reinfected in September 1983. The initial number of glochidia attaching in 1983 was higher than in 1982 but the number present declined to a level similar to that observed the first year. Previous studies have noted that *M. margaritifera* glochidia infect mainly small fish and have suggested that reinfection may be deterred by an immune response, however, our study suggests that, at least in the laboratory, older fish can be successfully reinfected with glochidia.

The freshwater pearl mussel, *Margaritifera margaritifera* (L.), has a holarctic distribution. It lives characteristically in fast running streams but with a glochidial larval stage as an obligate parasite on the gills of salmonid fish (Hendelberg, 1961). In Scotland glochidia are released in a limited period between late July and early September (depending on location) and, if inhaled by a suitable host, attach immediately to the gills. This process is highly inefficient. Once attached to their host, glochidia become encysted, grow slowly until the following May, then drop off as fully metamorphosed free living mussels (Young and Williams, 1984a).

Young and Williams (1984a), studying a largely undisturbed mussel population in northwest Scotland, observed a considerable loss of encysted glochidia from wild brown trout, *Salmo trutta* (L.), between December 1979 and May 1980 and between September 1980 and May 1981, such that only about 5% of the glochidia survived. A similar loss was also noted under laboratory conditions for both brown trout and salmon, *Salmo salar* (L.) (Young and Williams, 1984b). In these cases most fish shed all their glochidia, with those remaining being concentrated on a minority of the hosts, although even these lost some. A similar situation has been observed by other workers (Fustish and Millemann, 1978; Bauer, pers. comm.).

In the wild most small, young fish were infected, whereas most large old fish were not (Young and Williams, 1984a), and other workers, such as Awakura (1968), who studied *Margaritifera laevis* (Haas), have also observed the greater incidence of infection of small fish, but usually under laboratory conditions. Several explanations for this and for the loss of encysted glochidia from their hosts are possible. Only small fish can be near the mussels at the time of glochidial release, or their behaviour patterns can predispose them to infection. Alternatively, larger fish can be less susceptible because of some factor which changes with age (such as epidermal thickness), or possibly because of an immune response which develops after infections in previous years.

In this study brown trout were infected with glochidia in the first year and then retained for reinfection the succeeding year. Progress of each year's infections was monitored.

MATERIALS AND METHODS

Juvenile brown trout with a mean length of 8.7 cm were obtained in September 1982 from a commercial stock, provided by Cantray Fish Farm, Croy, Nairn, Scotland, and were free from obvious signs of disease. Water for the fish farm is obtained from the River Nairn, which is believed not to

harbour freshwater mussels; nevertheless, 12 fish were examined and found to be free of glochidia. Throughout the experimental period the fish were kept outside in University aquaria at ambient temperature, were fed a full commercial diet, and had a generous throughflow of water derived from the River Dee. The water was passed through a slow sand filter before supply to remove any possible glochidia. Some fish were kept as uninfected controls under identical conditions.

The fish had grown to an average of 20.0 cm by September 1983, at the time of the second infection. Abnormally large or small fish were discarded.

Several large mussels were obtained from the River Dee in August 1982, kept in aerated river water at ambient temperature and observed daily. When the first glochidia were found in the water, they were examined to confirm that they were unencysted and active. The mussels were then moved into new water and the aeration was stopped, resulting in the mass release of glochidia. These were collected and kept in gently aerated water until used for infections. The same procedure was followed in 1983, except that the mussels came from the nearby River Spey.

In both years pairs of brown trout were infected in buckets of aerated river water which contained approximately 500,000 glochidia/5 *l*. Each fish was exposed to glochidia for 3 minutes before being transferred to a large holding tank. Subsamples of the infective glochidia were taken regularly and glochidia were added, as necessary, to maintain the initial density. Approximately 250 fish were infected in 1982 and 100 in 1983. (This infection was carried out by M.R. Young under British Home Office licence No. SHR 1191).

On the day following infection, and at various intervals thereafter samples of 10 fish in 1982/83 and 5 fish in 1983/84 were selected randomly and killed. Fish were weighed, measured and their gills excised and examined immediately. The small sample sizes were necessary so that sufficient fish remained throughout the projected 2-year experimental period. All attached glochidia were counted and between 30 and 50 removed from the cysts and measured, before they and the gills were fixed in aqueous Bouin's fluid. Note was made of any abnormal or dead glochidia.

All trout surviving the September 1982-May 1983 infection were used for reinfection in September 1983. A small number of these may have lost all glochidia in the course of the first infection (by analogy to fish sampled then), but all received an initial load. Only 18 fish (out of 250) died during the first infection period.

On 15 October 1983 many of the fish were found to have a fungal infection. Moribund fish were removed and the remainder were treated with a 2 mg/*l* malachite green solution for 1 hour. This treatment was repeated twice the following week. Subsequent inspection, including the sample taken on 25 November 1983, revealed no apparent sign that encysted glochidia had been affected by this treatment, in that all appeared live and had grown.

On 28 November 1983 chlorine residues contaminated the water supply and killed all fish being held in the University aquaria, including the infected fish, so terminating the experiment.

RESULTS

Glochidia attached successfully to the gills of the brown trout in both years and the initial numbers of glochidia on the fish are indicated by the day 1 samples (Table 1). As can be seen, significantly more glochidia became encysted in 1983 (range 8789-17751) than 1982 (range 637-2737) (Mann Whitney U-test: $p < 0.001$); however, in both years

Table 1. Glochidia present on brown trout during the two infection periods.

	September 1982-June 1983 Days post infection							September 1983-November 1983 Days post infection		
	1	23	50	134	150	190	237	1	20	65
No. of glochidia on each trout	637	59	180	0	1	64		8789	0	0
	868	99	280	0	7	326		10290	0	0
	1060	186	424	0	16	685		14831	0	2915
	1292	204	529	1	28	1354		15475	1823	3325
	1331	271	1435	19	30	1417		17751	3673	3601
	1648	372	1436	25	285	1605	920			
	1846	403	1478	366	302	1779				
	2388	1443	1505	575	497	1934				
	2659	1482	1700	1823	1369	2046				
	2737	1497	2709	2022	2219	2122				
Mean (& median) no. of glochidia/fish	1646.6 (1687.0)	601.6 (778.0)	1167.6 (1444.5)	483.1 (1011.0)	475.4 (1110.0)	1333.2 (1093.0)	— —	13427.2	1099.2	1968.2
Mean (& median) no. of glochidia/infected fish	1646.6 (1687.0)	601.6 (778.0)	1167.6 (1444.5)	690.1 (1011.5)	475.4 (1110.0)	1333.2 (1093.0)	—	13427.2	2748.0	3280.3
Mean longest axis of glochidia - mm	0.07	0.13	0.19	0.20	0.26	0.29	0.35	0.07	0.10	0.18
Mean fish length - cm	8.7							20.0		

the infestation rates were highly variable. The mean number of glochidia per fish fluctuated widely for each subsequent sampling date in the first year's infection, and there was no consistent trend in numbers. In contrast, after infection of fish in September 1983, there was an apparent sharp decline by day 20, and this was substantiated by the sample at day 65. The low sample numbers and high variance of results preclude statistical testing, but the magnitude of the change is readily apparent. In both years, later samples contained some fish with no glochidia, whereas others were heavily infected.

Glochidia resulting from the infection in September 1982 grew quickly before winter, reaching 0.19 mm (mean longest axis) by day 50. Growth resumed in early spring and metamorphosis to free-living mussels occurred in late May 1983. Glochidia which attached in September 1983 grew to 0.18 mm (mean longest axis) by day 65, similar to the growth rate recorded in 1982 and they appeared clear and "healthy", in spite of the treatment for a fungal infection earlier in their development.

No fish died at the time of either infection and all subsequently grew at a rate similar to the uninfected control fish.

DISCUSSION

There are strong similarities between these results and those of other workers. The marked decline in attached glochidia after the initial infestation in September 1983 is similar to that recorded by Fustish and Millemann (1978) [on chinook salmon *Oncorhyncus tshawytscha* (Walbaum) and coho salmon *O. kisutch* (Walbaum)] and Young and Williams (1984a, b). However R. Dettmer (pers. comm.) did not find this with a German population of *Margaritifera margaritifera* on brown trout, where there was no decline from initial levels of 100-200 glochidia on 10 cm fish.

In other studies different species and sizes of host fish have been used, as well as other species of *Margaritifera*. However, the eventual numbers of glochidia per infected fish recorded here are close to the ranges previously reported. Karna and Millemann (1978) reported *Margaritifera* glochidial infections of less than 100 to more than 1000 on 4-7 cm chinook salmon and Fustish and Millemann (1978), working with fish of 4-6 cm, noticed declines from initial mean glochidial loads of 1547 on coho salmon, and 938 on chinook salmon. Young and Williams (1984a) reported wild brown trout with mean natural infections of 923 glochidia per fish in 1979 and 458 per fish in 1980; in both cases a significant reduction followed. The levels of 2750-3300 per 20 cm fish in September 1983 are higher than previously reported, but the fish, at 20 cm, were larger and no fish died at the time of infection. In contrast, Murphy (1942) reported the deaths of 7 cm brown trout infected with 100-295 glochidia of Californian *Margaritifera*, and Meyers and Millemann (1977) also reported fish mortality in various species of experimentally infected fish, some of which proved unsuitable as hosts. The much greater initial loads of glochidia in September 1983 than September 1982 could have been due to a larger available gill area on the larger fish, to a greater volume of water respired by the larger fish, or to increased stress sufferd by larger fish in the buckets (due to lowered oxygen levels and more contact with the other fish), resulting in a higher gill ventilation rate.

Unfortunately it was necessary to use glochidia from mussels from different rivers in 1982 and 1983, although the rivers are in proximity. Different "strains" of *Margaritifera margaritifera* can occur in these two rivers, but Purser (1985) did not detect differences between them using electrophoresis. However Kat (1983) did find differences between nearby *Elliptio* populations in the United States and it is possible that the slightly different infection patterns in 1982 and 1983 reported here were due to differences between the glochidia.

Previous studies have noted that young host fish were both more heavily infected than older fish and that a higher proportion of them were infected (Awakura, 1968; Karna and Millemann, 1978; and Young and Williams, 1984a) and this has been tentatively ascribed to three possible factors. Glochidial release in late summer can occur when only the younger host fish are near the mussel beds. This is feasible in Scotland where adult brown trout tend to live mainly in lochs, returning to streams in winter to spawn after the period of glochidial release (Young and Williams, 1984a). Alternatively, older fish can be inherently less suitable hosts than younger fish due to a thicker mucus layer, epithelium, or other physical feature. Lastly, observations showing hyperplasia and other histological effects associated with glochidiosis suggest that an immune response can be involved (Meyers, *et al.*, 1980). Our results clearly show successful reinfection of 20 cm fish and so suggest that if an immune response is induced by glochidia, then it is weak or transitory. Furthermore there is no physical reason which prevents infection of older fish.

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