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The reproductive biology of the freshwater pearl mussel
Margaritifera margaritifera (Linn.) in Scotland
II. Laboratory studies

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With 2 figures and 6 tables in the text

Abstract

Laboratory studies of fresh glochidia show that their basal "snapping" rate increases in the presence of fish mucus, blood, gill tissue or fin tissue and that closure follows direct touch on the inter-valve membrane. The glochidia are found only on the gills of their host fish where their distribution does not correspond to either an even pattern or one proportionate to gill area. Difficulties of attachment on the more exposed gill areas, coupled with passive carriage to the gills, might account for the observed distribution. Minnows and Rainbow Trout were unsuitable as hosts, but even on Brown Trout and Salmon only a small proportion (5—12 %) of glochidia survived until release as young mussels. In Scotland glochidia grow a little in the autumn and then complete their growth the following spring.

Introduction

This study examines the biology of the glochidial stage of the Freshwater Pearl Mussel, *Margaritifera margaritifera* (Linn.) under laboratory conditions. It was carried out to amplify and substantiate observations made in parallel in the wild and contains four sections: glochidial behaviour in relation to environmental stimuli; attachment of glochidia to various possible host fish species; growth of glochidia on their hosts; and the fate of glochidia during their development.

These observations, and those made previously (Young & Williams, in prep.) on glochidia in the field, are designed to elucidate the reproductive strategy of *M. maragaritifera*. This species is unusual in several respects. It is Britain's most massive bivalve and yet is restricted to waters low in calcium (Boycott, 1936); it is extremely long-lived reaching at least 70—80 years in Britain (Young & Williams, 1983) and 100 years on the continent (Hendelberg, 1960); and, most relevant here, it has retained its glochidial stage in spite of being found only in lotic habitats. Because of this it needs a migratory glochidial host, a fish, to maintain the upstream populations.

Our field results (Young & Williams, in prep). showed very high losses between glochidial release and attachment on the host fish (99.99 % loss) and

between release from the fish and establishment of young mussels in the substrate (95 % loss), but also equally high losses whilst on the host fish (95 % loss) and this last loss is investigated further here. Clearly the very inefficient retention of glochidia in a lotic environment is balanced by the long reproductive life of the mussel and the production of vast numbers of glochidia each year.

In this study glochidia were collected from mussels from the River Dee, Aberdeenshire, and were used in behavioural experiments and to infect various host fish species. The growth and development of the glochidia were monitored up till the time of liberation from the fish.

Materials and Methods

a) Collection of glochidia

Mussels were examined periodically in the River Dee ,Aberdeenshire, until glochidia were observed which were unencapsulated and "snapping". Tongs were used in this examination and these do not harm the mussels (YOUNG & WILLIAMS, 1983). The gravid mussels were then taken back to the laboratory in river water and the glochidia were collected as they were released. All glochidia used experimentally were collected on the day of use and were kept exclusively in aerated river water.

b) Observations of glochidial behaviour

The rate of "snapping" of glochidia and the proportion closed were measured in unstimulated, newly-released glochidia and in the presence of either the mucous, blood, gill tissue or fin tissue of Brown Trout (*Salmo trutta*). The observations were made on large samples of glochidia transferred to watch glasses in river water immediately before the experiments so as to reduce oxygen stress and overheating. Even so temperatures of about 20°C prevailed, compared with usual River Dee temperatures of 15–18°C at the time of glochidial release. Oxygen concentrations were not measured but the water was aerated until immediately before use. For each trial the basal "snapping" rate was measured for each of the first three minutes, then the stimulus was applied and a further three minutes observations were made. Control runs were made where "snapping" rates were measured over six minutes with no stimulus being applied. Usually three replicates were used for each trial.

The stimulants were added in small amounts directly into the watch glasses, in such a way that most of the glochidia were not in direct contact with them. Two further stimuli used were agitation of the water, to mimic water currents caused by the fish, and direct touch of a fine needle on the membrane between the glochidial valves, to simulate direct touch by fish.

c) Infecting fish with glochidia

Freshly released, infective glochidia were kept in buckets of aerated river water and diluted so that the required concentrations were obtained. In all later experiments 400,000 glochidia in 3 litres of water was the concentration used. Subsamples were

ets containing glochidia for a time consistent with their size, for example Brown Trout and Salmon parr 5–7cm length were infected for 3 minutes. The variations in glochidial concentrations and times used are given in the results. After infection fish were kept in large outdoor tanks of aerated water which comes after only filtration from the River Dee via Aberdeen city supply.

Samples of fish were taken at regular intervals and were killed and examined. No glochidia were found except for those on the gills and these were counted and measured on freshly excised gills and then preserved later in Bouin's fluid.

Brown Trout *Salmo trutta* LINN., Rainbow Trout *S. gairdneri* RICHARDSON and Salmon *S. salar* LINN. were obtained from commercial suppliers and Minnows *Phoxinus phoxinus* (LINN.) were caught in the River Dee. In all cases a preliminary examination of a sample of fish showed no glochidia to be present.

Results

a) Glochidial behaviour

It was observed that unstimulated glochidia have a characteristic "snapping" rate but do not close (up to at least 3 hours after release) unless stressed or stimulated in some way. Table 1 notes the results of this basal situation in comparison with "snapping" rates and closure after stimulation with each of 6 stimuli. These stimuli are:

1. Water agitation — designed to mimic water disturbance by swimming fish;
2. Direct touch — as if by fish;
3. Blood from Brown Trout;
4. Mucus from Brown Trout;
5. Gill tissue from Brown Trout; and
6. Fin tissue from Brown Trout.

From Table 1 it is clear that water movement has no effect, touch causes immediate closure, the presence of blood a small, but significant, increase in snapping rate and mucus, gill and fin a very large snapping rate increase. In addition the latter two induce closure, even when not in direct contact with the glochidia.

The variations in basal rates of "snapping" between batches of glochidia are in most cases not significant but some batches do seem to show a real difference and so comparisons between treatments are not valid. The basal rate variation may be due to age differences between the batches, as unsubstantiated observations showed a decline in rate after a period of hours.

b) Glochidial attachment to host fish

In 1980 four species of fish were exposed to active, mature glochidia and the success and development of the infections were observed until the release

Margaritifera margaritifera (LINN.)

Table 2. The success of glochidial infection of four species of fish in 1980.

Fish species used	Lengths–cms	No. of fish used and dose of glochidia/3 litres	Numbers of glochidia observed on gills mean (+ranges)		
			After 0–5 hrs	After 24 hrs	After 48 hrs
Minnows – *Phoxinus phoxinus*	4.1–6.8 cms	4 at 200,000/5 mins; 10 at 400,000/3 mins	50 (45–55); All >100	0; 5 (0–26)	0; 0
Rainbow trout *Salmo gairdneri*	7.7–8.6 cms	8 at 200,000/2 mins	680 (500–850)	4 (0–79)	0
Salmon *Salmo salar*	4.8–5.9 cms	10 at 400,000/3 mins	Not scored	340 (220–260)	Infection cont See Table 6
Brown Trout *Salmo trutta*	4.8–6.4 cms	10 at 400,000/3 mins	No scored	610 (230–950)	Infection cont See Table 6

1. The initial fate of glochidial infections on the four species of fish are shown in Table 2. The variation in the size of dose used does not detract from the obvious result that both Minnows and Rainbow Trout lose all glochidia within 48 hrs of infection. Moreover the initial number attaching to Minnows seems lower than for the other species and observations showed that glochidia did not even encyst on Minnows, whereas they did on the others. Rainbow Trout 48 hours after infection showed many small "scars" on the gills which we assume to have become associated with glochidia.
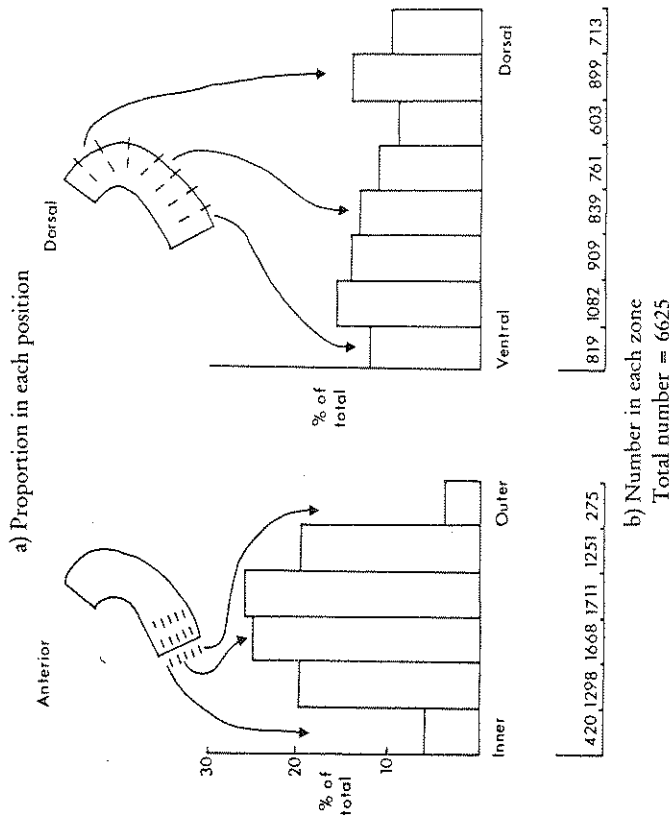
Brown Trout and Salmon both became infected succesfully but showed wide variation in the numbers of glochidia which they harboured. The cause of this variation may be attributed partly to the variations in the size of the fish used and it precludes comparison of the infection rate between the species.

2. The position of glochidia on the gills

An analysis was made of the distribution of glochidia on the gills using the gills from five randomly selected Brown Trout. The gills were arbitrarily divided into 6 anterior/posterior sections and 8 dorso-ventral zones as shown in Table 3. As far as possible the section and zone areas were made equal but the glochidia were not distributed equally. As Table 3 shows there were sig-nificantly more glochidia on the middle sections rather than the...

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Table 1. The responses of glochidia to the presence of various fish related stimuli.

* Figures are means from 3 replicates. *** Highly significant P = <0.001. NS Not significant.

Stimulus tested	No. of snaps/100 gloch's/min						% closed		χ² value	Summary of response
	Initial snapping rate 1	2	3 min	4	5	6 min (Rate after stimulus)	0–3 min	After 6 min		
...ol*	19	17	8	21	15	19	0	0	0.39 NS	No change
agitation (...cates)	10 min immediately / 8 min before			7 min immediately / 15 min after			0	0	0.004 NS	No effect
tactile ...us	–			Immediate closure				–	–	Immediate closure
blood ...ce of Brown	27	24	29	62	75	73	0	0	19.26 ***	Small increase No closure
mucus ...ce of Brown	38	37	33	216	252	299	0	0	165.12 ***	Large increase No closure
gill ...ce of Brown	26	32	33	287	10	–	0	100 (all closed)	206.31 ***	Large increase All close
fin ...ce of Brown	12	9	15	243	273	170	0	95	195.41 ***	Large increase Most close

Table 3. The number and proportion of glochidia on each of various zones on the gills of 5 different Brown Trout 4 weeks after infection.

a) Proportion in each position

b) Number in each zone
Total number = 6625

| 420 | 1298 | 1668 | 1711 | 1251 | 275 |

| 819 | 1082 | 909 | 839 | 761 | 603 | 899 | 713 |

Difference between observed distribution and even distribution:

Inner/Outer　　= *** χ^2 = 1721.8　　　d.f. = 5
Ventral/Dorsal = *** χ^2 = 174.7　　　 d.f. = 7

and 3 and avoidance of dorsal zones 6 and 8. For this analysis the glochidia from similar sections and zones from all gills were summed.

A further analysis was made of the number of glochidia from each gill pair and the results are shown in Table 4.

As is clear in Table 4 the number of glochidia on the gill pairs differed significantly both from the numbers expected if the distribution was even and also from the numbers expected if glochidia were distributed in proportion to the gill area of each pair. However the χ^2 value for the latter comparison is much lower than for the former indicating that relative gill area may have some effect. The differences are due to a slight preponderance of glochidia on gill pairs 1 and 4 and a corresponding lack on pairs 2 and 3.

3. Growth of glochidia on their fish hosts

Glochidia were measured regularly from the time of attachment to their hosts until they had completed development. Table 5 lists the results and

Table 4. The number and proportion of glochidia on each pair of gills summed for 5 different Brown Trout and the proportionate areas of each pair of gills.

	Gill pair				Total no. of glochidia
	Anterior		Posterior		
	1	2	3	4	
No. of glochidia on each gill pair	2114	1890	1533	1135	6672
Proportion of total no. of glochidia	31.6 %	28.3 %	22.9 %	17.0 %	
Porportionate gill area for each pair	29.4 %	29.9 %	24.1 %	16.7 %	
Expected no's if distribution was: even	1668	1668	1668	1668	
: proportionate to gill area	1960	1993	1606	1112	

Difference between observed distribution and:

even　　　　　　　　　　　= *** χ^2 = 330.1　　　d.f. = 3
:proportionate to gill area = *** χ^2 = 21.2　　　 d.f. = 3

Fig. 1 illustrates them. Measurements in mm were made of the greatest dimension (i.e. the length) on each occasion and each sample exceeded 50 (and usually exceeded 100).

Small initial growth in the autumn is followed by a cessation of growth over the winter and then a rapid growth from March to May with full size being reached in June. There is some evidence of a decrease in size in November (although the high variability of the results make analysis difficult) and this may have been caused either by sample variation or by a real loss from the fish of larger glochidia. (At approximately this time of year some glochidia are lost from their host fish, see section [b]4 below.) In Table 5 the mode is used rather than the mean. This avoids bias in the presentation of the results due to dead (and hence non-growing) glochidia remaining on the fish and being measured.

4. The fate of glochidial infections on their fish hosts

200 Brown Trout and 200 Salmon were infected with the same dose of glochidia in August 1980. Regular samples were then taken during the period of glochidial development and these demonstrated a loss of glochidia from both species. These results are summarised in Table 6 and Fig. 2. The regular samples were of ten randomly chosen fish. Young mussels developed successfully on both host species and samples were timed so that the last (at

Table 5. The growth of glochidia on Brown Trout from October 1979 to June 1980.

Date of infection – 18 September 1979
Infection rate – 200,000 glochidia/3 litres – fish exposed to glochidia for 2½ minutes (all fish 6–8 cm long)
Size of glochidia prior to infection – 0.07 mm long

Date of sample	Days after infection	Modal size mm (+no at mode)	Size range mm	N
16 Oct 1979	28	0.11 (60)	0.09–0.12	111
29 Oct	41	0.15 (54)	0.06–0.21	110
12 Nov	55	0.15 (46)	0.09–0.24	95
30 Nov	73	0.15 (143)	0.09–0.24	319
10 Dec	83	0.21 (52)	0.09–0.27	141
20 Dec	93	0.12 (86)	0.09–0.18	150
9 Jan 1980	113	0.12 (143)	0.09–0.27	345
21 Jan	125	0.18 (52)	0.12–0.24	127
4 Feb	139	0.18 (46)	0.09–0.24	132
18 Feb	153	0.21 (59)	0.09–0.30	196
4 Mar	167	0.21 (47)	0.15–0.30	144
18 Mar	181	0.21 (66)	0.12–0.27	156
15 Apr	209	0.21 (30)	0.18–0.30	86
16 May	241	0.27 (23)	0.21–0.39	81
12 June	268	0.39 (45)	0.27–0.42	96

Young mussels released from fish – 26 June – 10 July 1980
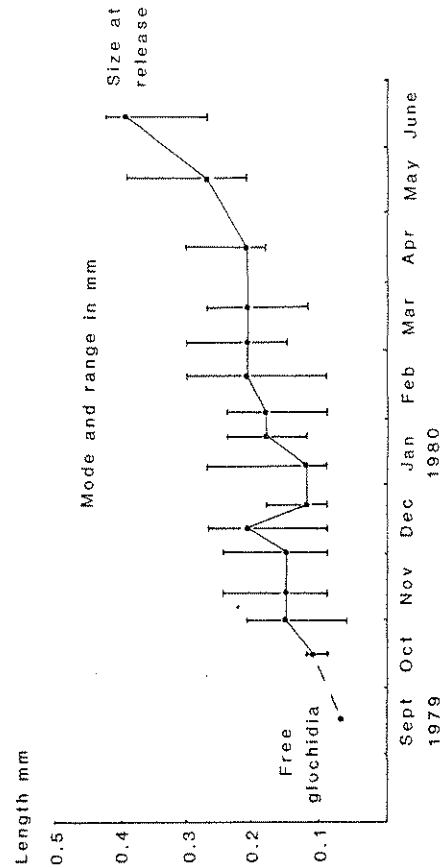Length mode = 0.39 mm (16)
Range = 0.33–0.48 mm
N = 50

Table 6. The number of glochidia remaining on their host fish at different intervals after infection. (Dose used: 400,000 glochidia/3 litres for 3 mins.)

Fish species and initial size-length (cm)	No. of glochidia/fish*					Eventual size of fish-length (cm)
	Days after infection					
	1	40	113	288	293	
Brown Trout *Salmo trutty*	230	0	0	0	0	
	353	0	0	0	0	
	362	0	0	0	0	
4.8–6.4 cms	554	0	0	0	0	10.4–15.3 cms
	598	0	0	0	0	
x̄=6.0	680	0	0	0	0	x̄=13.2
	746	0	0	0	0	
	773	1	0	0	0	
	863	6	20	0	59	
	950	751	443	136	166	
Mean no./fish	610	76	46	14	23	
Salmon *Salmo salar*	220	19	13	No	0	
	270	22	15	sample	0	
	281	24	15		0	
4.8–5.9 cms	295	46	48		12	6.1–8.3 cms
	301	62	62		13	
x̄=5.4	312	97	67		22	x̄=6.6
	350	165	188		33	
	376	179	252		34	
	386	228	285		73	
	620	296	285		234	
Mean no./fish	340	114	123		42	

* Each sample is of 10 different fish

Difference between initial fish sizes P<0.01** Student's t-test
Difference between initial glochidial no's on:
Salmon and Brown Trout: P<0.001***
Difference between glochidial no's on Salmon: } Mann Whitney U test
Day 1/Day 40 P<0.01**
Day 40/Day 113 P>0.05 N.S.

293 days/post-infection) immediately preceded the release of these young mussels. (In fact a very small proportion could have been released by day 293, though none were found free in the fish tanks.)

Table 6 reveals that the same dose produced a significantly smaller initial glochidial load on the Salmon than on the Brown Trout. However the salmon used were slightly, but significantly, smaller than the Trout and these differences might be associated.

By day 40 most Trout had lost their glochidia, although some fish retained large numbers, and this pattern then continued so that at day 293 most
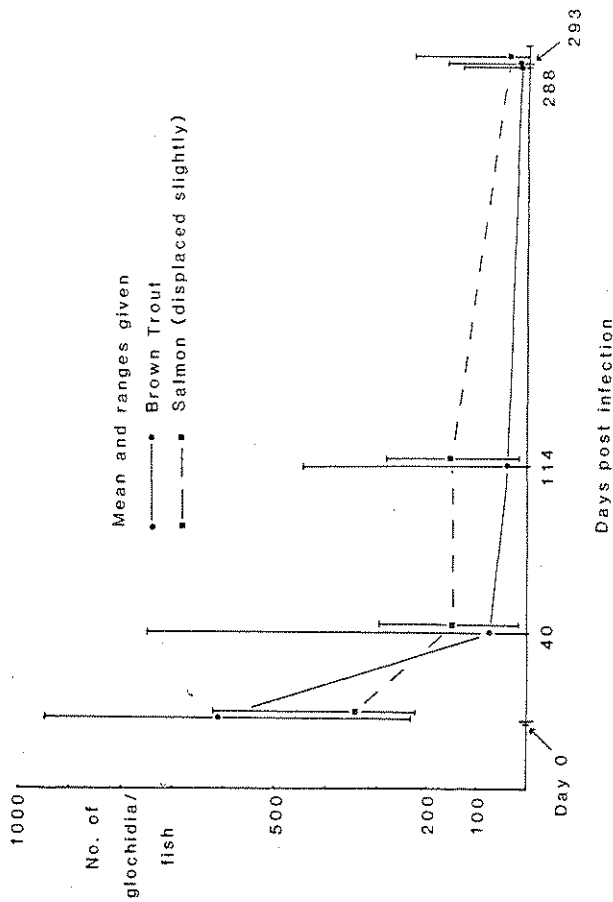

Fig. 1. The growth of glochidia on Brown Trout from Oct 1979 to June 1980.

Water agitation, designed to simulate water currents caused by fish movement, had no effect but touching the glochidia on the membrane between the valves elicited immediate closure of the valves. In contrast to the observations of MEYERS, MILLEMANN & FUSTISH we never observed reopening of glochidia, even though they had closed on nothing. It seems appropriate that water currents should not induce a response as *M. margaritifera* usually lives in fairly turbulent streams.

When Brown Trout blood, mucus, gill tissue or fin tissue are added to the water containing the glochidia there is a rapid, significance increase in the "snapping" rate indicating the ability of the glochidia to detect appropriate water-borne chemicals. The response to blood was less than to the other stimuli and it may be that mucus, which would be present on the fish gills and fins, contains or is the active substance. WOOD (1974) found a similar activation of the glochidia of *Anodonta cygnea* LINN. by fish mucus and even managed to make an initial analysis of the active components. It is easy to imagine that an increased "snapping" rate would enhance the chances of glochidia attaching successfully to trout gills and presumably the usual behavioural sequence is for glochidia to detect the presence of trout at close range, perhaps only when inhaled, to increase their snapping rate and then to clamp shut immediately the inter-value membrane is touched.

2. Glochidial attachment to their host fish

It is clear that different fish species differ in their susceptibility to *M. margaritifera* glochidia (Table 2). Minnows are common fish in many British mussel rivers and have been suggested as possible hosts (e.g. by MURPHY, 1942; HARMS, 1907; BJORK, 1962) however glochidia do not even become encysted on their gills and are lost within a few hours. Rainbow Trout are usually regarded as rather unsuitable hosts (KARNA & MILLEMANN, 1978; BAUER, pers. comm.) and our results confirm this. Although the glochidia apparently became encysted they were then shed within 24 hours leaving visible hyperplastic scars on the gills. This reaction is more extreme than that observed by KARNA & MILLEMANN for fish infected with American *M. margaritifera*, where a few glochidia did persist, but is similar to that reported by AWAKURA (1968) who found no successful glochidial attachment by *M. laevis* on *S. gairdneri*. Brown Trout and Salmon proved suitable as hosts and the further development of their infections is discussed below.

Other studies have noted a variety of host fish; WELLMAN (1943) quotes *Esox lucius* LINN. and *Perca fluviatilis* LINN. as possible hosts, MULLER (1957) notes *Thymallus thymallus* (LINN.) and ELLIS (1978) includes *Cottus gobio* LINN., however most recent authors (such as BAUER, 1979) seem to agree that Salmonidae are the usual hosts. MEYERS & MILLEMANN (1977) infected several American salmonid species with glochidia from American *M. margaritifera* and found varying susceptibilities which they and others attribute to varying host immune response (FUSTISH & MILLEMANN, 1978; KARNA & MILLEMANN, 1978; MEYERS, MILLEMANN & FUSTISH, 1980). They observed well developed hyperplasia in some species as we did occasionally after our experimental fish

No. of glochidia/fish

1000
500
200
100

Mean and ranges given
——— Brown Trout
– – – Salmon (displaced slightly)

Day 0   40   114   288 293

Days post infection

Fig. 2. The no. of glochidia on samples of 10 different fish at various days post infection.

trout were without glochidia. The numbers retained by the few infected fish seem lower than the numbers attaching initially but this is not testable with the small sample sizes used.

By day 40 the Salmon had lost a significant number of glochidia, with the loss being more evenly spread between the fish than in the Brown Trout. The glochidia present at day 40 were retained until day 113 but there was then a further decline between day 113 and day 293. Although considered very unlikely, since no young mussels were found in the fish tanks, this latter decline may have been due to the release of young mussels from the fish.

During the course of infection the Brown Trout grew from a mean length of 6.0 cms to one of 13.0 cms but the Salmon only 5.4 cms to 6.6 cms.

Discussion

1. Glochidial behaviour

Infective, free glochidia have a characteristic basal "snapping" rate of about 15—20 snaps/100 glochidia/minute but casual observation suggests that this rate declines as the glochidia age and glochidia more than 24 hours after release generally appear lifeless. Our experiments were carried out only with glochidia which had been released the same day. These basic observations agree with those of MEYERS, MILLEMANN & FUSTISH (1980), who also observed a basal "winking" rate. Our results show dramatic changes from this situation when glochidia are exposed to certain fish-related stimuli.

3. Growth of glochidia on their host fish

Glochidia in Scotland grow after attachment to their host fish until October/November and then complete their growth after March of the following year. There was an apparent, but statistically insignificant, peak size in early December, followed by a slight fall, but it is felt that this may be due merely to random sample variation. There is no rational reason for such a fall in mean size unless it is due to the preferential loss from the fish of the larger glochidia. Certainly some glochidia are lost from the fish in autumn, although most are lost before November, but these can be recovered from the holding tanks and are only partially developed and dead and cannot be measured accurately.

BAUER (1979) notes that in parts of Bavaria development of glochidia proceeds quickly enough for full development to be achieved by October when the young mussels leave the fish. He showed by transfer experiments to more northern streams that this trait was genetically determined rather than being due to immediate environmental factors (such as stream temperature) and we believe that all Scottish populations defer full development until the spring, as is true for most populations of M. margaritifera.

4. The fate of glochidial infestations on their fish hosts

Table 6 and Fig. 2 show a remarkable loss of glochidia from both Salmon and Brown Trout by 40 days after infection. The Brown Trout results show wide variation with most fish losing all their glochidia, whilst a few retain up to half of their initial load through to full glochidial development. Salmon are similar although more fish retain some glochidia throughout. After day 40 there was no further significant loss from either fish species. Unfortunately no samples were taken between day 1 and day 40 and so the actual time of the loss remains unknown.

The initial infection levels differed between the fish species used, but so did their sizes, and the larger Brown Trout harboured more glochidia, presumably because of their greater gill area and their greater ventilation rate. The concentration of glochidia in the infection chambers and the length of exposure was the same for both fish species.

FUSTISH & MILLEMANN (1978) observed a rather similar loss, after only 5 days, in both Coho and Chinook salmon, with the former losing most of their glochidia whilst the latter kept over 75% until development was complete. They associated hyperplasia and other evidence of an immune response with this loss and, in a variety of tests, they and their coworkers noted that Chinook salmon, the supposed natural host in Oregon where they worked, showed a lesser response than a range of related fish species (MEYERS & MILLEMANN, 1977; KARNA & MILLEMANN, 1978; MEYERS, MILLEMANN & FUSTISH,

had lost their glochidia. The American workers cited above used some salmonid species not found in Europe and it is possible, if not likely, that different host fish of M. margaritifera are adapted to different host fish.

Small fish (up to 7 cms long) were used as hosts in this study and YOUNG & WILLIAMS (in prep.) found that it was mainly small fish which were involved in natural infections in the Stac Burn, Wester Ross. KARNA & MILLEMANN (1978) also noted that fingerlings were more susceptible than larger fish but no-one has shown why this should be and since some large fish are found with natural infections it is clearly not an absolute phenomenon. It may be related more to the size of the fish living in the mussel streams at the time of glochidial release than to a different individual response.

Glochidia do not attach uniformly over the gill areas. They predominate on the middle section of each hemibranch (rather than the inner or outer portions), on gill pairs 1 and 2 and to some extent on different dorso-ventral sections (Tables 3 and 4). These distributions differ significantly both from theoretical even distributions and also from those expected if the numbers of glochidia were distributed in proportion to gill area.

PALING (1968) observed a distribution of Anodonta sp. glochidia on Brown Trout gills which he regarded as being in proportion to the volume of water passing over each gill and our results also differ significantly from his ($x^2 = 226.4$, $P = <0.001$). PALING's view is that Anodonta sp. glochidia are carried passively to their gill position and can attach successfully wherever they arrive. Such glochidia have hooks and may be more efficient at attachment than the hookless M. margaritifera, so that the observed lack of glochidia on the outer sections of each gill reported here may be due to an attachment failure. PALING used figures for relative gill area taken from HUGHES (1966), who measured the areas on one large fish. These figures differ significantly from those we obtained, as a mean figure from 5 fish, and its seems probable that relative areas change as fish grow.

Our view is that M. margaritifera glochidia are carried passively to gills in proportion to the volume of water passing over each gill (since they have no propulsive mechanism) but that they show a variable attachment success on different sections depending on factors such as the difficulty of attachment in fast currents. This leads to an eventual distribution which is related directly neither to relative gill area nor to relative volumes of water passing over each section.

All glochidia were found on the gills and this seems to be the usual situation; however KARNA & MILLEMANN (1968) found some on the gill rakers and even the fins of their heavily infected test fish on rare occasions and BAER (1951) notes that they may occur on the fins of cyprinoid fish. In our view this last observation is likely to be due to confusion with other Unionid glochidia.

1980). It seems logical and satisfactory that the natural host species should show only a poorly developed immune response to the glochidia but this situation does not seem to apply in Scotland where Brown Trout, which are indisputably a natural host, nevertheless shows such a dramatic glochidial loss. Similarly Salmon, whose parr are probably also a natural host in some Scottish rivers, also show this loss.

It is possible that different strains of fish may also vary in their susceptibilities to glochidia, and since the fish used in this study were obtained from commercial stocks this might be thought to complicate interpretation of the results. However similar losses were also observed in a natural infection of Brown Trout in the Stac Burn, Wester Ross (YOUNG & WILLIAMS, in prep.) and so such losses are obviously not restricted to captive stock.

Summary

Glochidia of the Freshwater Pearl Mussel show a basal "snapping" rate which increases significantly if Brown Trout blood, mucus, gill tissue or fin tissue are added to the water. Furthermore, the latter two initiate glochidial closure, as does a direct touch on the inter-valve membrane. It is probable that in the wild the "snapping" rate increases in the immediate vicinity of a fish and successful attachment follows closure initiated by the direct touch of the gill filaments.

Glochidia failed to encyst successfully on Minnows (*Phoxinus phoxinus*) and were lost from Rainbow Trout (*Salmo gairdneri*) within 48 hours of encystment. Successful development occurred on Brown Trout (*S. trutta*) and Salmon (*S. salar*).

Glochidia are carried passively to the fish gills but encyst most successfully on the middle and ventral sections. The distribution between gill pairs does not correspond to the proportionate gill areas as there are more than expected on gills 1 and 4. Possibly attachment is only fully successful on more "protected" gill areas.

Glochidia grow slightly between attachment in September and the onset of winter and then complete their growth the following spring.

On both Brown Trout and Salmon the majority of glochidia are lost within 40 days of attachment. The remainder then persist (or perhaps are lost at a very slow rate) until their development is complete after about 290 days; by this time 12 % or less remain.

Acknowledgements

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