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An Investigation of Salmonid Host Utilisation by the Endangered Freshwater Pearl Mussel *(Margaritifera margaritifera)*in north-west Scotland

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ABSTRACT

1. The complex life cycle of the globally threatened *M.margaritifera* includes a parasitic stage, where glochidia attach to the gills of fishes of the *Salmo* genus. However the species utilised appears to vary across its range. In previous literature, the reported primary host in Scotland, home to a high proportion of the world's remaining *M.margaritifera* populations, is the Atlantic salmon *Salmo salar* and in its absence, the brown trout *Salmo trutta*.
2. In this study, the prevalence of infection in putative *Salmo* hosts in eight rivers in NW Scotland was determined. At a selected site on each river, where both *S. trutta* and *S. salar* were collected in abundance, *S. trutta* was the preferred host.
3. However at sites where *S. salar* were abundant but *S. trutta* were at low density, *S. salar* showed a high prevalence of infection (with the exception of one river where neither *S.salar* or *S.trutta* were infected). Thus the primary host appears to be very site specific in the rivers sampled.
4. We speculate that this may be because *M. margaritifera* have population specific responses to cues for attachment to a host. Alternatively it may be that host population specific immune responses mediate infections by glochidia. Additionally, larger fish were less likely to be infected than smaller fish and gills 1 and 5 were less infected than gills 2 to 4.
5. One consequence of this finding for both national and international conservation management of this globally endangered species, is that any current or future

management activity must take into account local population host preferences, otherwise conservation efforts may be in vain.

Key words: Atlantic salmon, brown trout, freshwater pearl mussel, glochidia, host fish, *Margaritifera margaritifera*, parasite, Scotland

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1. INTRODUCTION

The Freshwater Pearl Mussel, *Margaritifera margaritifera,* is a very long lived Unionid Bivalve that is endangered and, as a result, highly protected across its range (IUCN, 2017; Machordom, Araujo, Erpenbeck & Ramos, 2003; Ziuganov et al., 2000). Although Scotland is a stronghold for this species, populations here as elsewhere, are showing evidence of decline (Cosgrove et al., 2016; Skinner, Young & Hastie, 2003). A number of causative factors have been suggested, including habitat degradation, pollution, and pearl fishing. However the abundance of fish in rivers that also support *M.margaritifera* may also be a factor in their decline (Hastie & Cosgrove 2001; Langan et al., 2007; Sime 2015). For a very short but critical period in its early life cycle, *M.margaritifera* is parasitic. Mature females release glochidia into the stream flow in summer; these are then carried in water currents and taken into the gill chamber of host fish where they attach by clamping their valves on to epithelial tissue of the gills (Meyers & Millemann, 1977).

This parasitic stage of the life cycle is not well understood. The host fish for *M.margaritifera* are fish of the genus *Salmo*, however the species utilised appears to vary across its range. Atlantic salmon (*Salmo salar)* is reported as the principal host of *M.margaritifera* in Nova Scotia and Russia (Bauer, 1987). In contrast, brown trout (*Salmo trutta)* appears to be the main host species in Germany and central Europe (Bauer, 1987). There are only two *Salmo* species in Scotland, *S. salar* and *S.trutta* (Maitland & Campbell 1992). It has been reported that *S. salar* is the primary host but that *S. trutta* might act as a sub-optimal host where the former species is absent for Scottish populations of *M.margaritifera* (Hastie & Young 2001; Young & Williams 1984a; 1984b).The relative importance of these two species as a host for *M.margaritifera,* is of considerable management importance. The two species occupy different stream habitat types and there is thus considerable variation in the relative abundance of each species at different rivers and different sites within the same river (Klemetsen et al., 2003). This thus affects the probability of contact between glochidia and a suitable host.

This study addressed four questions related to host utilisation by *M.margaritifera*:

1) Which of the two salmonid species is the preferred host for the parasitic stage of the life cycle of the freshwater pearl mussels in selected rivers in north-west Scotland?
2) Is host specificity consistent between *M. margaritifera* populations?
3) Is there a relationship between salmonid size and levels of glochidia encystment?
4) What component parts of the gill structure are parasitized?

2. METHODS

Potential fish hosts were collected from sites on eight rivers in north-west Scotland by electrofishing between the 7th May 2013 and 20th June 2013. Osterling (2011) using a similar method to that used here, indicated that June, the period immediately before glochidia are shed (Hastie & Young, 2001) is the most appropriate period for visual counting of glochidia. Similarly, Reid et al., (2013) conducted visual counting of glochidia on salmonids in June. To protect *M.margaritifera* locations from potential illegal pearl-harvesting, rivers are not named here but referred to only as rivers A-H. A suitable site on each river was selected for its suitability as salmon and trout habitat and which were located downstream of, and in close proximity (<30m) to *M.margaritifera* beds. Salmonid fish were collected using a standard 500W DC backpack electro-fisher with one operator and an assistant. Electrofishing has previously been shown to not adversely affect the short-term survival of *M.margaritifera* (Hastie & Boon, 2001).Collected fish were anaesthetised, identified, measured (fork length in mm) and the number of encysted glochidia counted. At this time (immediately prior to excystment), glochidia were large enough to count by eye. The fish were held in the hand on their dorsal surface and the operculum gently lifted to make the gill filaments visible. Using a blunt needle to part the gills it was possible to count individually encysted glochidia on the anterior and posterior surfaces of the gill filaments separately for all five gills (from gill 1, to gill 5 (numbered from anterior to posterior)on both left and right sides of the fish. Two people replicated counts on a random sample of fish, to ensure accuracy and consistency of this visual count.

All fish were returned to the site on each river from which they were taken after a period of recovery.

Glochidia frequencies were analysed using chi-squared analysis to test for difference between host species and rivers. The total number of glochidia counted per fish and the number on each gill arch was modelled in a general linear model (GLM) using fork lengthand gill arch number as explanatory variables in R (Crawley, 2007).The relevance of inclusion of each explanatory variable in the model was assessed in sequence using significance testing between models (ANOVA; likelihood ratio tests [LRT]).

3. RESULTS

Across the eight rivers, a total of 830 fish (combined *S. salar* and *S. trutta*) were examined for the glochidia of *M.margaritifera* (Table 1).The combined-river mean fork length (mm) for *S.trutta* was 102.2 mm (+/- 23.1 S.D.) and *S.salar* 90.9mm (+/- 13.7).The overall mean prevalence of infection in both species across the eight rivers was 14.7% (122 fish). No infected fish of either species were detected in River E. If River E is excluded from analysis, of the 740 remaining fish,16.5% were infected and 83.5% uninfected.

Across all seven rivers where infection was detected, the combined prevalence of infection for *S.trutta* (31.6% of 234 examined) was higher than that for *S.salar* (9.5% of 506 examined)(χ^2=435;df=1; P<0.0001).

To examine the question of host specificity, data from five rivers are informative. At rivers B, F and H both *S. trutta* and *S. salar* were collected in numbers large enough to test for host use differences. At the sampled site within each of these rivers the prevalence of infection of *S. trutta* (ranging from 11-65%) was statistically significantly higher than for *S. salar* (0% for all three sites) (Table 1). In contrast, two additional rivers show a high prevalence of infection by *S. salar*. The sampled site at rivers C and D showed 62% and 30% infection prevalence in *S. salar* respectively but there was only one *S. trutta* collected (at river C) (Table 1), indicating that a very high prevalence of *S. salar* infection by glochidia is possible at least when *S. trutta* are not available. At one further river (G) infection prevalence of *S. trutta* was high (26%) but only four *S. salar* were collected so there were no significant differences between species.

For *S.trutta* and *S.salar* combined, the number of glochidia detected on fish with any infection, was significantly negatively related to fish fork length (p<0.001). Thus smaller fish had significantly heavier glochidia load compared with fish with longer fork lengths (Figure 1).

The GLM investigated the number of encysted glochidia on all five gills, and revealed a significant two-way interaction between the side of the gill filament that was infected (anterior or posterior) and the gill number (one to five)(Table2), but left/right side of the fish was not significant. A *post hoc* Tukey test revealed there to be significantly more encysted glochidia on gills two, three and four (which did not differ from each other) than on gills one and five. In addition, *post hoc* testing also showed that there were significantly more encysted glochidia on the anterior surface of gills two, three and four ($p < 0.001$, $p < 0.001$ and $p < 0.01$) compared with the posterior side (Table 2).

4. DISCUSSION

In Scotland, literature indicates that *S. salar* is the primary host for *M. margaritifera* glochidia, but where this species is not present, then *S. trutta* might be a sub-optimal host (Hastie & Young, 2001; Young & Williams, 1984b). Data from the study presented here shows that, not only is *S. trutta* a suitable host for the glochidia stage of the life cycle but that, at least at some sampled sites within rivers, *S. trutta* is the preferred host even when there are potential *S. salar* hosts present. Although only seven rivers with glochidia infection were examined here, at two of these *S. salar* was the main host infected. At both of these however, S. *trutta* density was too low to determine if *S. trutta* would have been the principal host species if present and thus if *S. salar* was the optimal or a sub-optimal host. Thus one conclusion of the study presented here is that there appears to be one dominant host species for the parasitic phase of the *M. margaritifera* life-cycle, but that the primary host used varies between each river. Thus at any one river, *salar* or *S. trutta* will be the primary host and carry the bulk of the infections. Similar findings have been reported from Scandinavia (Karlsson, Larsen, & Hindar, 2014; Larsen, Hårsaker, Bakken & Barstad (2000); Salonen, Luhta, Moilanen, Oulasvirta, Turunen & Taskinen, 2017). Whilst effort was made to ensure that selected sites on each river had both salmon and trout juvenile habitat, it is acknowledged that the relative abundance of each species at each site is likely to reflect local habitat type. However for five rivers sufficient numbers of both species were collected to make meaningful between species comparison. The level of glochidia encystment at any site is also likely to be affected by the proximity of *M.margaritifera* beds to suitable juvenile fish habitat. Previous work showed the closer mussel beds and good juvenile fish habitat were, the higher the levels of encystment (Cosgrove & Hastie, 2001; Hastie, Watt & Cosgrove, 2011). In order to minimise the capture of fish that were not infected efforts were thus made to electrofish within 30m or less downstream of mussel beds to capture as high glochidia encystment as possible. The timing of glochidia counts may also have an effect. It is possible that some glochidia may have already dropped of the fish by June, however previous studies indicate that this period is the time when such counts may be made most successfully (Osterling, 2011). These caveats to this study may well have affected the absolute count number on fish presented here but they are highly unlikely to affect the between species differences in infection unless it is argued that the timing of excystment differs between species.

The mechanism through which glochidia may infect only, or almost only, one *Salmo* species when two are present is uncertain. The infection process is thought to be largely passive, in that glochidia are taken into the gill cavity and exposed to the gill epithelium through the normal respiration process in the host fish (Meyers & Millemann 1977). However, there are at least two possible routes through which the observed selectivity may occur. The glochidia may fail to attach to the exposed epithelium at the appropriate time, if exposed to the "wrong" *Salmo* host. Alternatively the glochidia may attach to its host but the host may initiate an immune response causing the shedding of attached glochidia.

There is some circumstantial evidence in support of the first of these possibilities. Karlsson and colleagues (Karlsson et al. 2014) examined the population genetics of mussels from rivers where the prevalence of either *S. trutta* infection or *S. salar* infection dominated. They concluded that *M.margaritifera* from *S. trutta* dominated host populations were genetically different from populations which were *S. salar* host dominated. One potential inference from this study is that there are significant genetic differences between populations of *M. margaritifera* and that populations respond differently to each of the putative *Salmo* hosts. In the UK a study (Cauwelier, Verspoor, Tarr, Thomson, & Young, 2009) has shown that freshwater pearl mussel populations show a major evolutionary split into northern and southern phylogenetic groups. They also found that mussels from different river systems belonged to separate breeding populations and the levels of genetic diversity within breeding populations varied significantly and were higher in Scotland, compared to England and Wales.

There is also similar circumstantial support for the second of these two possibilities. *S. salar* is known to exhibit significant between-population variation in genes of the Major Histocompatibility Complex, which has consequences for immune-competence. This is assumed to be a response to the differential exposure of populations to parasites (Dionne, Miller, Dodson, Caron & Bernatchez, 2007). It is a reasonable proposition to suggest that discrete *Salmo* populations may similarly show different response capabilities on exposure to *M. margaritifera* glochidia as a result of their separate evolutionary past. In addition, the study presented here showed that larger and thus older fish had lower infection levels than smaller and younger fish. Although speculative, one possibility is that older fish acquire some immunity as a result of previous exposure that offers some protection from subsequent glochida infection, as has been shown in a previous study (Thomas, Taylor & Garcia de Leaniz, 2014).

Data presented here also shows that *M. margaritifera* infection does not affect all gills equally. The outer gill arch and the inner gill arch had lower levels of infection than the middle three arches. It is likely that the inner arch is partly protected by its relative position behind the four others and that the first gill arch might be less infected as a result of the abrasion effect of the opercular bone on glochidia.

There are a number of important management consequences that stem from these findings. Firstly because the host species may not be the same for each population, then any assessment of the density of hosts as one possible stressor on a population of *M. margaritifera* has to take account of the *Salmo* species that is the relevant host for that specific *M. margaritifera* population. It cannot be assumed that one host species is a simple suitable replacement for the other. Secondly, the very clear host specificity for one or the other of the two *Salmo* species reported here and elsewhere (see Bauer, 1987) suggests that if *M. margaritifera* glochidia are forced to use the less preferred host species at any river (because density of the preferred host is very low) then there may be some consequences for individual survival of animals in the longer term.

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Table 1. The prevalence of infection and sample size in two Salmo species in eight rivers in north-west Scotland.

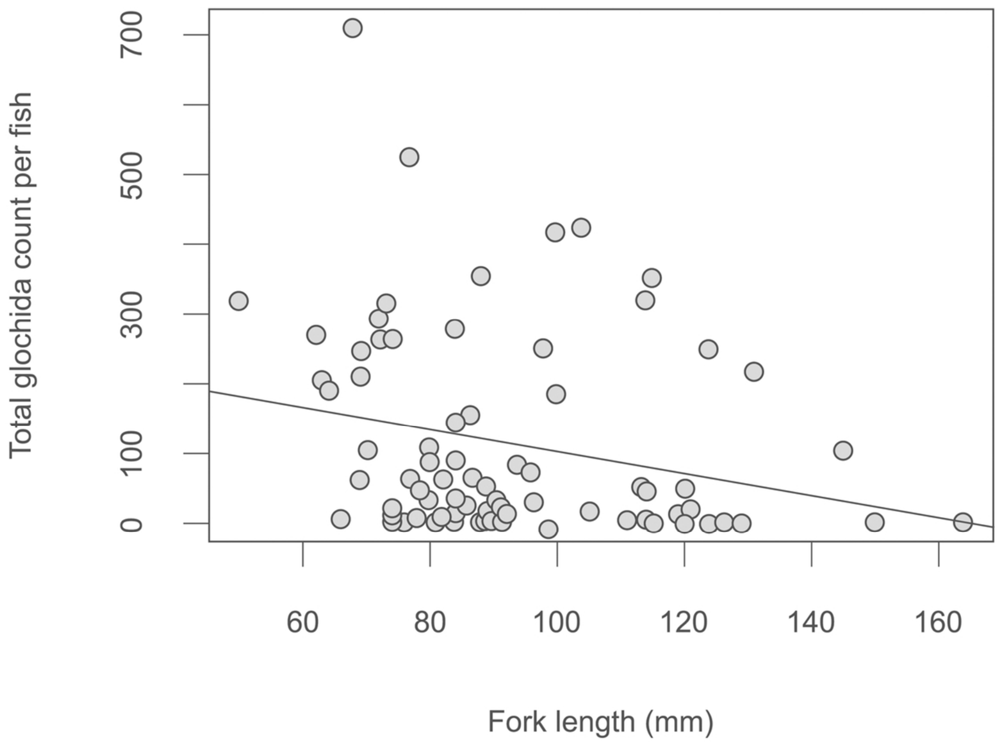
River	*S. trutta* N	*S. trutta* N Infected	*S. trutta* % Infected	*S. salar* N	*S. salar* N Infected	*S. salar* % Infected
A	40	22	55	2	0	0
B	23	15	65	232	0	0
C	1	0	0	55	34	62
D	0	0	0	46	14	30
E	0	0	0	90	0	0
F	21	4	19	122	0	0
G	113	29	26	4	0	0
H	36	4	11	45	0	0

Table 2. Summary of the total numbers of encysted glochidia counted on anterior and posterior sides of the gill filaments on each of the five gills of infected *S.trutta and S. salar.*

	Mean glochidia count		**Standard deviation**	
Gill Number	**Anterior**	**Posterior**	**Anterior**	**Posterior**
1	0.0	0.0	0.0	0.0
2	11.3	3.7	15.1	6.9
3	10.8	5.0	14.5	8.4
4	9.8	5.2	13.1	7.9
5	6.4	3.5	8.7	6.4

Figure 1. Relationship between the fork length (mm) of individual fish caught and the total glochidia counted ($F=96.43$, $df=1$, $r^2 = 0.06$, $p =<0.001$).

(Separate jpeg file)



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107x79mm (300 x 300 DPI)