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 Deposited on: 09 February 2018
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<th>Aquatic Conservation: Marine and Freshwater Ecosystems</th>
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<td>Manuscript ID</td>
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</tr>
<tr>
<td>Wiley - Manuscript type:</td>
<td>Short Communication</td>
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<td>Date Submitted by the Author:</td>
<td>n/a</td>
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<td>Complete List of Authors:</td>
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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 encystment), 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 length and 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 90.9 mm (+/- 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) ($\chi^2=435; df=1; P<0.0001$).

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
test 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 glochidia 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.

5. ACKNOWLEDGEMENTS

Funding was provided by the European Union INTERREG IVA Programme (project 2859 ‘IBIS’) managed by the Special EU Programmes Body and the Scottish Government project SP004. We thank Scottish Natural Heritage, District Salmon Fisheries Boards,
proprietors and landowners for access, and Bruce Wallace and Jennifer Dodd for assistance with data collection.

6. REFERENCES


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Atlantic salmon (Salmo salar) and brown trout (Salmo trutta) differ in their suitability as hosts for the endangered freshwater pearl mussel (Margaritifera margaritifera) in northern Fennoscandian rivers. *Freshwater Biology*, 62, 8, 1346-1358.


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

<table>
<thead>
<tr>
<th>River</th>
<th>S. trutta N</th>
<th>S. trutta %</th>
<th>S. salar N</th>
<th>S. salar %</th>
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<tr>
<td>A</td>
<td>40</td>
<td>22</td>
<td>55</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>23</td>
<td>15</td>
<td>65</td>
<td>232</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>55</td>
</tr>
<tr>
<td>D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>46</td>
</tr>
<tr>
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<tr>
<td>F</td>
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<td>19</td>
<td>122</td>
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<tr>
<td>G</td>
<td>113</td>
<td>29</td>
<td>26</td>
<td>4</td>
</tr>
<tr>
<td>H</td>
<td>36</td>
<td>4</td>
<td>11</td>
<td>45</td>
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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*.

<table>
<thead>
<tr>
<th>Gill Number</th>
<th>Mean glochidia count</th>
<th>Standard deviation</th>
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<tr>
<td></td>
<td>Anterior</td>
<td>Posterior</td>
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<tr>
<td>1</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>11.3</td>
<td>3.7</td>
</tr>
<tr>
<td>3</td>
<td>10.8</td>
<td>5.0</td>
</tr>
<tr>
<td>4</td>
<td>9.8</td>
<td>5.2</td>
</tr>
<tr>
<td>5</td>
<td>6.4</td>
<td>3.5</td>
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</table>
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)
Figure 1. Relationship between the fork length (mm) of individual fish caught and the total glochidia counted
\( (F=96.43, \text{df}=1, r^2=0.06, p=<0.001) \).

107x79mm (300 x 300 DPI)