FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) HOST CHOICE AND BEHAVIOURAL RESPONSES TO CHANGES IN FLOW REGIME

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The endangered freshwater pearl mussel (*Margaritifera margaritifera*), one of the longest-lived invertebrates, are threatened globally. Scotland, UK, remains a stronghold, however even here the population is declining due to factors such as habitat degradation, pollution and pearl fishing. The study comprised two parts: field surveys of glochidia infection of host salmonid fish, and a novel laboratory flume based study of the mussel's behavioral responses to changes in flow regime. The intricate life cycle of *M.margaritifera* includes a parasitic stage as glochidia attached to gills of salmonids. The preferred host in Scotland is thought to be *Salmo salar* and *Salmo trutta* in the absence of *S.salar*. This has not, however, been empirically tested in the field. Eight rivers in NW Scotland were surveyed using standard electrofishing techniques and encysted glochidia counted. Results suggest *S.trutta* is the primary host fish for glochidia attachment in the rivers surveyed, which contradicts current accepted knowledge about host specificity of *M.margaritifera.* Mussel populations are often found in regulated rivers, however little data exists on response to changes in flow regime. The mussel's behavioral response to changes in flow were investigated in an experimental flume. Mussels buried significantly deeper in conditions of gradually increasing water velocity compared with rapid increases or where water velocity was constant. 68% of individual mussels washed out when the water velocity was rapidly increased. The findings are novel, provide initial recommendations for targeted management actions for the conservation of *M.margaritifera* both in Scotland and internationally, and highlight more research is required.

1 INTRODUCTION

Margaritifera margaritifera is thought to be one of the longest-lived invertebrates in the world, there are records of animals with a life span of 210 years [1]. The species is widely distributed and can be found in Europe, Fennoscandia and north-eastern North America [2]. Evidence has shown that this species is threatened across its range, and while Scotland has been highlighted as one of the species' strongholds, populations within this area are showing evidence of decline [3].

The life cycle of *M.margaritifera* includes a parasitic stage where glochidia released into the water are inhaled by salmonids and attach to the gills. Glochidia are extremely specialised parasites and become encysted on the highly oxygenated gill filaments of salmonids for up to 11 months. At this point it is thought that only 5-10% of the initially attached glochidia metamorphose and excyst as juvenile mussels [4]. Current literature based on research completed in a selection of Scottish rivers clearly defines *S.salar* and *S.trutta* as hosts for glochidia. General consensus seems to be *S.salar* is the main host but in rivers where salmon are not present, *S.trutta* may be the sub-optimal host. Scottish laboratory and field studies have demonstrated huge losses of glochidia during the early stages of *M.margaritifera* development [5, 6]. It is estimated [5] that 95% of glochidia developing on fish do not survive to the juvenile mussel stage and in turn 95% of those that do survive are lost between leaving the host fish and establishing in suitable substrate.

Whilst the understanding of *M.margaritifera*'s life cycle in Scotland has increased [5,6], knowledge gaps remain. Current studies report on field observations were that are based on one small west coast burn and one significantly larger east coast river. In addition, the fish used for laboratory experiments were sourced from 'commercial suppliers' and were not therefore necessarily from the same source water as the glochidia.

Therefore inherited genetic immunity or acquired immunity through previous exposure could not be ascertained. It was recognised [4] that very little was known about individual rivers and relationships between host stock size and reproductive success in a given river. Hastie & Young's (2001) study looked at salmonids from six rivers in northern Scotland two hatcheries. Results illustrated that glochidosis was less prevalent in *S.trutta* than in *S.salar*.

It has been noted therefore [2] that the relative importance of *S.salar* and *S.trutta* in Scottish rivers as *M.margaritifera* host fish has not been well studied. Skinner et al. (2003) hypothesised that as 0+ *S.salar* are often more abundant than 0+ *S.trutta*, *S.trutta* are therefore less likely to be the most important host. Rivers carry varying population ratios of *S.trutta* and *S.salar*, however a number of mussel populations in small streams in Scotland have no (or very few) *S.salar*, and these must be considered to be largely trout-dependent as a *M.margaritifera* host [2]. Unless preferred host is established any conservation measures based on glochidial stage of the life cycle could be in vain. Thus there is an imperative to investigate this more thoroughly.

It is widely accepted that anthropogenic changes in river flows are a key component of freshwater habitat and species decline. Habitat alteration can impact both directly and indirectly on aquatic organisms including effects on mortality, disruption of reproductive cues, reduced migration and food web disruptions [7]. Our current understanding of flows that are ecologically relevant to the maintenance of *M.margaritifera* beds in Scotland is poor, as is information about how changes in flow conditions may subsequently affect mussels. Changing flow conditions affect river habitats and it is generally understood that higher peak flows can destabilise river beds through mobilisation of larger clast sizes and of mussels themselves. In turn, lower base flows can result in decreases in the velocity of water over adult mussels and through river bed interstices which can have a detrimental impact as smaller fine sediment particles can fall out of suspension and accumulate in the river bed [8]. Moorkens & Killeen (2014) highlight the importance of maintaining ecologically appropriate velocities over mussel beds as an important aspect of their management and conservation.

This study aims to firstly, investigate relative importance of *S.salar* and *S.trutta* as host fish species for *M.margaritifera* in a selection of Scottish rivers. Secondly, the behavioural responses of adult *M.margaritifera* to three contrasting flow regimes will be investigated. The three flow regimes were defined as; 1) constant velocity, 2) rapidly increasing velocity, and 3) gradually increasing velocity.

2 SALMONID HOST PREFERENCE OF PARASITIC GLOCHIDIA IN *MARGARITIFERA MARGARITIFERA*

2.1 Methods

The study was carried out between May and June 2013. Eight sites on 8 rivers were chosen through discussion with Scottish Natural Heritage (SNH). Site selection was based on the presence of *M.margaritifera* and both *S.trutta* and *S.salar*. All 8 watercourses were in NW Scotland, and in an attempt to protect locations, sites are not named here and will be referred to as sites a-h. Salmonid fish were collected using a standard 500W DC backpack electrofishing gear. Care was taken to avoid trampling on visible *M.margaritifera* beds. Collected fish were anaesthetised identified, measured, (fork length (mm)) and the number of encysted glochidia counted. Glochidia were large enough to count by eye, the fish were held in the hand on their dorsal side, by gently pressing below the head, the operculum opened and the gills and gill filaments were visible. Using a wool needle to gently part the gills it was possible to count individually encysted glochidia on the anterior and posterior surfaces separately of all 5 gills on both left and right sides of the fish. All fish were returned to the watercourse within the section they were taken after a period of recovery. R statistical software v.3.0.2 provided the platform for all data analysis [9]. Total number of glochidia counted per fish was investigated as the response variable with fork length and site as explanatory variables in a general linear model. In addition, a general linear model was used to investigate glochidia count per gill with the anterior /posterior side of gill, gill number and left and right side of fish as explanatory variables. An interaction between side of gill and gill number was included. Each explanatory variable in the model was assessed in sequence using significance testing between models (ANOVA; likelihood ratio tests [LRT]). An analysis of deviance was used to test the significance of the interaction within the model.

2.2 Results

Three of the eight rivers examined were excluded from analysis for the following reasons; River c only one *S.trutta* was caught, river d only *S.salar* were caught therefore no comparison between species could be made

and a third, river e only *S.salar* were caught and none of the 94 fish caught were infected with glochidia (Table 2.1).

Site	Total number of fish sampled:	Number of infected *S. trutta*:	Number of uninfected *S. trutta*:	Number of infected *S. salar*:	Number of uninfected *S. salar:*	Mean fork length *S. trutta*(mm):	St dev *S. trutta*:	Mean fork length *S. salar* (mm):	St dev *S. salar:*
A	42	22	18	0	2	106.3	24.5	139.5	9.5
B	255	15	8	0	232	90.7	31.4	74.3	15.1
C	56	0	1	34	21	-	-	69.2	8.6
D	46	0	0	14	32	-	-	81.2	26.6
E	90	0	0	0	90	-	-	83.7	15.2
F	143	4	17	0	122	101.6	25.4	76.7	15.0
G	117	29	84	0	4	114.3	6.1	114.3	6.1
H	81	4	32	0	45	98.3	28.1	88.2	13.6

Table 2.1 Data from all 8 rivers surveyed

The combined *S.trutta* and *S.salar* mean rate of infection with glochidia across all sites (7 in total, River e excluded as no infected fish were caught) was 8.71 (sd 12.00) or, 14.70% of all fish caught. Across all sites (7, River e excluded) mean incidence of infection of *S.trutta* was 10.57 (sd 11.54), 8.92% and *S.salar* 6.85 (sd 13.06), 5.78%. However the incidence of infection varied between sites from site G where 26.5% of fish caught were infected to site F where only 2.8% of fish caught were infected. A Chi squared analysis of the combined infection rate of all fish in watercourses where both *S.trutta* and *S.salar* were infected with glochidia showed infection rates between rivers differed significantly; $X2 = 111.5$, $df=4$, $N=632$, $p<0.005$.

In five of the remaining rivers (A, B, F, G, H) surveyed that contained both *S.salar* and *S.trutta* the only species found to have encysted glochidia visible by eye on their gills was *S.trutta*. In river D no *S.trutta* were caught and only *S.salar* were infected (Table 2.1). (In river C only one *S.trutta* was caught and found to be uninfected.) When comparing the watercourses it can be seen that the numbers of *S.trutta* and *S.salar* caught varied. The catch from river B was 91% *S.salar*. This pattern was similarly repeated in river F where *S.trutta* accounted for only 15% of the total catch. In comparison, river H had a more even split between species *S.salar* 55%, *S.trutta* 45%, but still *S.trutta* was the species found to be infected with glochidia.

To test for differences in the occurrence of glochidia between *S.trutta* and *S.salar* a chi squared test compared the relative frequency of glochidia infection on each. Under conditions where less than 6% of all fish caught were infected (site B, F and H), there is a significant difference between the infection rate observed in *S.trutta* and *S.salar* and the expected infection. Expected values for rate of infection were calculated for each individual river and were based on the percentage of the catch that were found to be infected and the assumption that both *S.trutta* and *S.salar* could be infected at the same rate (Table 2.2). In summary infection rates for sites B, F and H were significantly higher for *S.trutta* than *S.salar*.

Site	Rate of infection (% of all fish)	X^2	p value
A	52	2.31	<0.21
B	5.9	160.76	<0.0001
F	2.8	24	<0.001
G	26.5	1.50	>0.30 <0.20
H	4.9	5.2	<0.05

Table 2.2. Chi squared analysis of the frequency of occurrence of glochidia on *S.trutta* and *S.salar* for each of the five sites where they were found to occur in the same watercourse

Further analysis focused on fork length of fish and numbers of encysted glochidia. Analysis revealed that the total number of glochidia found on fish was significantly negatively related to fish fork length with smaller fish having significantly heavier loads of glochidia compared with fish with longer fork length ($p<0.001$). A generalised linear model (GLM) revealed significant site-specific differences and a significant effect of an interaction between site and fork length of total number of glochidia on fish. Predicted values of glochidia encystment abundance were given by the model and calculated using the formula; glochidial loading = fork.length * x + site; where x= measured fork length of fish. This relationship between mean fork length and glochidia encystment abundance across all sites equates to an average decrease in glochidia loading of 1 glochidia per 10mm of fork length of fish over all sites surveyed.

The minimum model investigating the number of encysted glochidia across all five gills revealed a significant two-way interaction between side of gill (anterior or posterior) and the gill number (one to five).In addition to this the side of gill(anterior or posterior) and gill number (one to five) was found to be significant in determining the number of encysted glochidia but left or right side of fish was not significant. A post hoc Tukey test revealed there to be significantly more encysted glochidia on gills two, three and four with fewer encysted glochidia on gills one and five. There was no significant difference in the total number of glochidia on gills two, three, and four. The post hoc Tukey test also revealed that there were significantly more encysted glochidia on the anterior side of the gills two, three and four ($p <0.001$, $p<0.001$ and $p<0.01$) compared with the posterior side.

3 BEHAVIOURAL RESPONSES TO FLOW CHANGE OF *MARGARITIFERA MARGARITIFERA*

3.1 Methods

150 *M.margaritifera* were collected in November 2013 from a river in NE Scotland. The conchological parameters of each mussel were systematically collected, photographs taken and identification number painted on the shell. A flume at the Scottish Centre for Ecology and the Natural Environment (SCENE) (Lat: 560 07'43. 73" N; Long: 0040 36'43. 20"W) was used for all the experiments. The water supplied for the flume was sourced directly from Loch Lomond with no recirculation. Water temperature was ambient from the loch. One tonne of washed gravel (20mm - 40mm) was used to fill the flume to a depth of 25cm, providing enough depth for the largest mussel collected to bury completely. The water depth above the gravel was maintained at 30cm and was circulated at a speed of $0.25ms^{-1}$ for 14 days to allow the gravel to settle and biofilms to develop.

A quadrat was placed directly above the flume to mark the experimental arena for all experiments. The quadrat was 60cm x 90cm in size and each square cell in the quadrat was $5cm^2$. Mussels were placed flat on the gravel with the widest part of the mussel in the centre of the cell. For all experiments the mussels were put in the flume for an acclimation period of 15hrs, over night, during which the velocity was constant at 0.25 ms^{-1}. Immediately prior to the experiment starting, the location of each mussel in the quadrat was recorded along with the burial position (vertical movement), which was a score on a four-point continuum (Burial index 1: flat on bed or propped up against rock. No visible active use of foot or anchoring. Burial index 2: flat on bed with foot protruding. Burial index 3: anchored on substrate with hinge parallel to bed or partially buried with hinge visible. Burial index 4: completely buried, no valve visible).

Observations of each individual *M.margaritifera* were made at 7 recording points during the duration of each experiment. Horizontal movement (cm) was estimated using changes in grid position by an individual between sequential observations and distances between midpoints of cells within the grid. Total horizontal movement was calculated as a sum of the changes in grid position.

The movement of individual *M.margaritifera* was investigated under three different flow regimes; constant flow, fast increase in velocity and gradual increase in velocity. Constant: the flow of water through the flume was maintained at a mean velocity of $0.231ms^{-1}$ (sd 0.04). After an initial acclimation period of 15 hours the position and burial of each mussel was recorded every 90 minutes (60 minutes elapsed then 30 minutes recording period) for 7 recording periods or 540 minutes. Fast increase: following the 15-hour acclimation period, the flow of water through the flume was increased rapidly and maintained at an average velocity of 0.697 ms^{-1}. The position and burial of each mussel was recorded every 90 minutes (60 minutes then 30 minutes recording period). Gradual increase: following the 15 hour acclimation period the flow of water through the flume was increased incrementally every 30 minutes followed by a 30 minute recording period for the first 270 minutes. At this point the maximum flow obtainable in the flume was achieved and this flow was maintained for a further 180 minutes with recordings of the position and burial of the mussels taken every 60 minutes until the mussels had been in the flume for a period of 450 minutes.

Four response variables: burial (vertical movement or depth of burial), speed of burial, distance travelled (horizontal movement), and washout (when the mussel becomes entrained in the flow and is removed

from the experimental arena) were analysed using linear mixed effect models. For each response variable, the minimum adequate model was found using the simplification method [9]. Each explanatory variable in the model was assessed and non-significant terms removed from the model in sequence. This was completed by significance testing between models (ANOVA; likelihood ratio tests [LRT]) and sequential backward elimination of terms of no significance. Post hoc pairwise comparisons were performed by Tukey's honest significant difference (HSD) test. Each model included the effect of time, experimental days, as a random effect. "Experimental days" was the total number of days elapsed since the mussels had been removed from the watercourse and been maintained in the trough prior to the trial commencing. The explanatory variables included were; weight of mussel, velocity change, mean velocity at siphon level experienced by mussel over the whole experiment, an interaction between weight of mussel and velocity change and an interaction between weight of mussel and mean velocity at siphon level. Recording period (two - seven, recording period one was the initial position in the flume,) and corresponding burial of a mussel at that recording period was used as an indication of speed of burial. Differences in washout out frequency among the three different flow regimes investigated were analysed using a chi-square test.

3.2 Results

A significant positive relationship was found between length of shell and weight of mussel (Figure 3.1), weight was chosen as the single parameter to represent size of the mussel (r^2=0.87,t (70 =22.0, p<0.001). Vertical movement of *M.margaritifera* or depth of burial of individuals into the substrate did vary significantly between different flow regimes, and mean velocities experienced by individuals over the course of the trial. The minimum model also revealed a significant two-way interaction between mussel weight and change in velocity. Lighter mussels were found to bury deeper in constant velocity conditions. This significant interaction was also found to influence the final burial position of individuals. Under constant velocity conditions lighter mussels had buried more by the end of the trial. This was also the case in conditions where the velocity was increased rapidly.

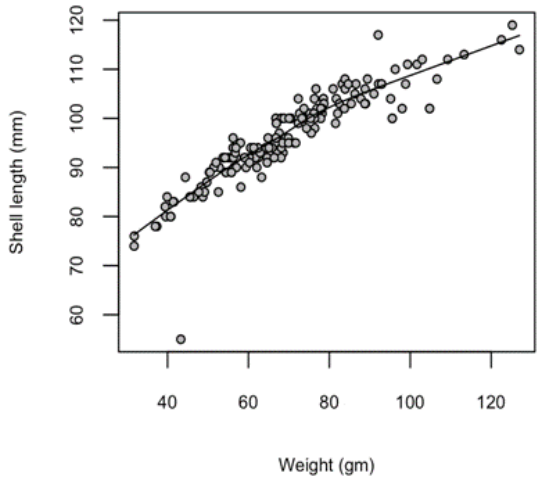

Figure 3.1. Relationship between mussel length and weight.

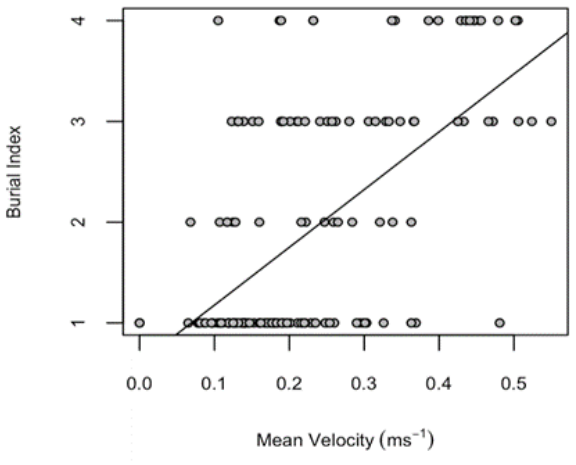

Figure 3.2. Higher mean velocities experienced by individual mussels increased burial depth.

In total 42% (n=70) of *M.margaritifera* across all experimental conditions buried, 12% (n=20) of the individuals buried completely during the experiment, 11 of these were in a gradual increase in velocity trial, 5 in fast increase in velocity trials and 4 in constant velocity. Mussels were found to bury significantly deeper in conditions of gradually increasing water velocity compared with fast increases in water velocity or where water velocity was kept constant throughout the experiment.

Mean velocity experienced by an individual over the course of a trial significantly influenced the degree to which they buried. Where individuals experienced greater mean velocities over the period they were found to bury deeper (Figure 3.2). Tukey HSD test revealed a significant difference in mean burial depth between fast increases in water velocity and constant water velocities (z =-2.93, p = 0.0087) and between gradual increases in water velocity and constant water velocities (z = -3.045, p = 0.0060). There was no significant difference between gradual and fast increases in water velocity. The mean water velocity at siphon level experienced by an

individual in gradually increasing velocities at the last recording period was 0.441ms^{-1}, (min 0.047ms^{-1} max 0.853ms^{-1}) and in rapidly increasing velocities was 0.503ms^{-1}, (min 0.105ms^{-1}max 0.691ms^{-1}).

	Value	Standard Error	Degrees of freedom	t value	p value
Intercept	4.10	1.03	137	3.98	p<0.001
Weight: Fast velocity change	0.04	0.02	137	2.49	0.01
Weight: Gradual velocity change	0.04	0.02	137	2.80	0.01
Fast velocity change	-3.14	1.08	24	-2.91	0.01
Gradual velocity change	-3.39	1.10	24	-3.07	0.01
Mean velocity	5.88	0.64	137	9.15	p<0.001

Table 3.1. Results of mixed effect model to explain variation in depth of burial in individual mussels under 10 different trial conditions

The minimum model revealed a significant effect of velocity change and time spent in the trial on the final burial position of individuals. Burial position varied significantly at each recording period under the three different flow velocities. In all trials mussels that remained to the end of the experiment were buried deeper than at the first recording period of the trial. Mussels in the constant flow conditions all remained to the end of the trial and were buried to a depth of two. In fast and gradually increasing flows mussels that remained to the end of the trials were buried to level three and above.

	Value	Standard Error	Degrees of Freedom	t-value	p-value
Intercept	-0.28	0.32	136	-0.86	0.3935
Fast velocity change	1.40	0.31	24	4.46	0.0002
Gradual velocity change	0.88	0.26	24	3.46	0.0021
Recording period 3	0.76	0.34	136	2.26	0.0255
Recording period 4	0.90	0.31	136	2.93	0.0040
Recording period 5	0.84	0.45	136	1.87	0.0641
Recording period 6	0.89	0.53	136	1.68	0.0958
Recording period 7	2.19	0.24	136	9.04	0.0000

Table 3.2. Results of mixed effect model investigating the speed of burial of mussels under different flow conditions over time. Minutes spent in trial was highly significant in determining burial of individual mussels.

Mussels under gradual increase in velocity buried more gradually compared with burial under fast increases in velocity (Table 3.2). Gradually increasing the velocity did not appear to increase the speed of burial of mussels. Between recording periods six and seven the velocity was not increased and was recorded at a mean velocity of 0.441 ms^{-1}, (standard deviation 0.252 ms^{-1}). In gradual increases in velocity, 42 mussels remained to the end of the trials and they had buried to a mean depth of level three, they were actively anchored to the substrate. A post hoc Tukey test revealed a significant difference in the speed of burial between trials where the water velocity was increased rapidly and where it was kept constant ($z = 4.459$, $p = <0.001$).

In trials where the increase in velocity was fast, a total of 56 individuals were washed out of the experimental arena at recording period two which corresponded with the velocity being rapidly increased. At this time the mussels had not buried and were flat on the substrate and with nothing to anchor the mussel to the substrate they became entrained in the flow of water. The remaining mussels in the fast trials buried more rapidly in comparison to gradually increasing and constant velocity trials. Those mussels that remained to the end of the trials in fast increases in velocity buried the deepest of all. In addition, mussels in fast increase in velocity trials buried on average to level three before being washed out at time recording period four. In comparison at the same recording period mussels under gradually increasing velocities only buried to a mean depth of level two. This could be an indication that mussels in the fast increases in velocity condition bury faster. Only nine individual *M.margaritifera* remained to the end of the fast increase in velocity trials. These remaining mussels were buried on average to level four completely buried.

Observations of fast increase in velocity trials showed that if the foot of the mussel was exposed at level two or three and was not actively in the substrate burying the mussel vertically but was instead 'searching' then the foot acted as a sail and the mussel became entrained in the flow and was washed out of the experimental arena.

No mussels were washed out of the experimental arena under constant water velocity conditions. The greatest number of mussels washed out of the experimental arena at recording period four which coincided with a mean burial depth of two, the foot exposed to the flow. Washout of mussels differed significantly among velocity changes ($x2= 65.3213$, d.f =2, $p <0.001$). In total 93 mussels, 55% of all mussels washed out during the trials. In fast trials, 9 of the starting 72 mussels remained to the end of the trial. Conversely 42 mussels (58%) remained to the end of trials where the water velocity was increased gradually. It can be assumed that for a mussel to remain in position within the watercourse it would be necessary for the mussel to anchor or bury.
For analysis of total distance travelled by mussels, only individuals that remained within the experimental arena and were not washed out were used for analysis (n=72, 43%). Distance travelled by mussels on the surface of the substrate around the experimental arena was not predicted by any of the explanatory variables investigated. Total distance travelled by an individual mussel over the course of one experiment varied between 5cm and 105cm. Some individuals (18%) did not move from their original position. Only one mussel travelled 105cm.

4 DISCUSSION

SALMONID HOST PREFERENCE

Contrary to the existing literature which suggests that glochidosis is less prevalent in *S.trutta* when compared with *S.salar* [4,5,6], the study has shown that in five of the rivers surveyed that contained both *S.trutta* and *S.salar*, only *S.trutta* were infected with glochidia. Numbers of each species of fish caught varied from river to river but when *S.salar* was dominant in number, when *S.trutta* was dominant in number, and when there was an equal split in the catch between both species, *S.trutta* was found to be the host fish most utilised. In addition to this, the infection rate encysted on fish was found to be significantly affected by site and size of fish (fish with shorter fork length held more encysted glochidia).

To further this study with the aim of conservation of *M.margaritifera* in Scotland, a recommendation would be to establish host preference for each distinct mussel population. Once salmonid species has been established, a second recommendation would be to investigate the genetic strain of salmonid found to be the host for *M.margaritifera*. It has been reported [10] that infection of glochidia on the gills of suitable host fish was most successful on a *S.trutta* strain originating from the natural distribution of *M.margaritifera*. Stocking of *S.trutta* and *S.salar* has long been used by fisheries managers to augment fish stocks, and introgression of genetics of fish not originating in Scotland has been documented [e.g. 11]. It is possible that over time the genetics of fish stocks suitable as host fish for *M.margaritifera* glochidia have become diluted.

In addition to investigating the genetics of host fish populations, little work has been carried out on the genetics of *M.margaritifera* in Scotland. In Norway it is reported [12] that host affiliation explains genetic differentiation among populations and there is a strong reproductive isolation between populations of *M.margaritifera*. Historically *M.margaritifera* have been moved around Scotland and Great Britain by pearl fishers to increase the size of existing beds or 'seed' new populations in habitat thought to be appropriate. Augmenting previously exploited mussel beds with individuals from different areas, watercourses or from completely different catchments and geographical locations may not have achieved the goals of increasing dwindling populations as the host fish species may not have been appropriate either in species or genetic strain.

An approach for the evaluation of population-led differences in host compatibility of natural populations of dependent species has been presented [13]. This study looked at the mussel *Unio Crassus* and host fish species in a fragmented river system in Central Europe. They showed that experimental testing of physiological host compatibility could be effectively used for the detection of different management units. Previous to this study it was recognised that there were differences in the ability of U.crassus to infest particular host fish species between nearby and recently isolated populations of mussels. This method of population-level evaluations of host compatibility has benefits in recognising management units where management targets and actions can then be focused. It is possible to identify sources of variability in host fish relationships and therefore direct management actions more closely. This method could be potentially useful in our understanding of *M.margaritifera* in Scotland. Although this study suggests that *S.trutta* are the primary host fish for *M.margaritifera* in the rivers selected, the rivers were all located in the NW of Scotland. *M.margaritifera* are known to be found in larger rivers on the east coast of Scotland and further afield in England and Wales. In these locations the primarily host fish may or may not be *S.trutta*. In addition to this there may be within river variation that was not detected in this study. Defining distinct management units using the method outlined [13] could assist in the prioritisation of resources in a suite of management actions.

BEHAVIOURAL RESPONSES TO FLOW CHANGE

Results showed that rate of flow change and the velocity, impacted most on the responses of *M.margaritifera*. Individuals were found to actively respond to changes in flow by vertical and horizontal movements. Perhaps most significant in the management of watercourses that contain *M.margaritifera* is that 68% of mussels in rapid increases in velocity were washed out of the experimental arena compared with less than 32% in gradually increasing velocity condition. The flume studies had limitations in that the experimental variables were governed by the conditions that could be achieved in the flume available and the time frame within which the work was completed. With this taken into consideration the study provides a valuable starting point for further investigations both in the field and in experimental controlled conditions of an artificial river or flume. It is suggested [14] that a standard habitat description is required for each river that contains *M.margaritifera*, the results from this study support this recommendation. The need for detailed field observations is highlighted, without this an understanding of how *M.margaritifera* are adapted to different flow conditions will remain elusive and effectively managing regulated watercourses that contain *M.margaritifera* will be inaccurate at best.

In conclusion the study has highlighted gaps in our current understanding of the ecology of *M.margaritifera* in Scotland and emphasised the need for more detailed in depth study. *M.margaritifera* have a complex life history strategy, velocity conditions within which mussels beds are maintained are varied and complex, as is the relationship of *M.margaritifera* glochidia and host fish species. There remains a need for a standard habitat description with ecological requirements to be written for each discreet population before management actions can be sufficiently targeted to prevent the continued decline of this species.

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