HOST CHOICE AND BEHAVIOURAL RESPONSES TO FLOW CHANGE IN THE FRESHWATER PEARL MUSSEL, *MARGARITIFERA MARGARITIFERA*, IN SCOTLAND

Elizabeth Anne Clements

B.Sc. Hons Zoology

Submitted in fulfilment of the requirements for the Degree of Master of Science (R) Ecology and Environmental Studies, MSc.

Institute of Biodiversity, Animal Health and Comparative Medicine
College of Medical, Veterinary and Life Sciences
University of Glasgow

ABSTRACT

Margaritifera margaritifera are one of the longest-lived invertebrates in the world. They are threatened across their range but Scotland remains a stronghold for this species. Even so, in Scotland the population is showing evidence of decline. This study comprised of two parts:

The intricate life cycle of *M. margaritifera* includes a parasitic stage as glochidia attached to the gills of salmonids. The preferred salmonid host in Scotland is thought to be *Salmo salar* and *Salmo trutta* in the absence of *S. salar*. This has not been empirically tested in the field. Eight rivers in the North West of Scotland were surveyed using standard electro fishing techniques. Glochidia encysted on the gills of fish were counted immediately prior to drop off. Results of the study suggest that *S. trutta* is used as the primary host fish for glochidia attachment in the rivers surveyed.

The second part of the study looked at behavioural responses, horizontal and vertical movement, to changes in flow regime. 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. Sixty-eight per cent of individual mussels washed out when the water velocity was rapidly increased. No differences in horizontal distance travelled were observed.

In conclusion the 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 standard habitat description for each discreet population of *M. margaritifera* before management actions can be sufficiently targeted to prevent the continued decline of this species.

Front-page image: *Margaritifera margaritifera* in Scotland. Copy right Elizabeth A Clements

CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

The work for this thesis was funded by IBIS, project 2859 supported by the European Union's INTERREG IVA programme managed by the Special EU Programmes Body (www.seupb.eu)
My thanks go to the IBIS project sponsors.

My thanks go to all my fellow IBIS students based at SCENE and in particular to my flat mates and friends, Nina O' Hanlon, Milly Piggott, and Jo Clarke for their patience, humour and help at every stage of my project. Also to Jessica Fordyce who honed her expert electro fishing, and pie consumption skills on some of the field work for this study and to Hannele Honkanen for entertaining and educating me in the office.

I would like to thank my supervisor Prof. Colin Adams for his guidance during this research project, and Dr Rhian Thomas for her unwavering encouragement throughout. I would also like to thank the staff at SCENE and most of all Rona Brennan, you're a legend!

My sincere thanks go to Bruce and 'Ruby' without whom my fieldwork would have been more challenging and less enjoyable. In the face of whirlwinds, sweltering temperatures and harsh wintery conditions you kept me company and focussed on the job in hand. Without complaint you lifted, shifted and fished, thank you!

Last but by no means least, special thanks must go to my family and in particular my parents for their steadfast support and encouragement in every new challenge I accept, this one has been memorable.

'The freshwater pearl mussel is widely regarded as an indicator, flagship, umbrella and keystone species' (Geist, 2010)

1 GENERAL INTRODUCTION

Bivalvia: Unionoida, freshwater bivalves, are an extraordinarily successful order found on all continents excluding Antarctica, with approximately 900 species worldwide all exclusively restricted to freshwater rivers, streams and lakes and characterised by larvae which have to pass through a parasitic stage on a host fish. Freshwater pearl mussels have a unique lifecycle which includes both parental care and brooding of larvae followed by parasitism of the larvae on freshwater fishes (Graf & Cummings 2007;Bogan & Roe 2008). The adaptions of Unionoida for larval parasitism on fish hosts have influenced mussel biology in many ways including, morphology, behaviour, fecundity, reproductive seasonality, adult habitat specialisation and geographic distribution (Barnhart, Haag & Roston 2008).

1.1 Exploitation and Conservation now and in the future

The exploitation, management and currently the conservation of *M. margaritifera* has been documented since pre-roman times (Skinner, Young & Hastie 2003). The Holarctic distribution of this species is now threatened with extinction or is highly vulnerable in every part of its range (JNCC 2013). Most or all of *M. margaritifera* populations are declining (IUCN, 2011). In Europe, for example, there are populations that have shown no functional recruitment (at least one juvenile found regardless of the overall numbers of adults present) for over 30 years. Given the length of time this animal takes to sexually mature (12-13 years), this has led to the species being defined as 'Critically Endangered' in Europe and globally endangered, (International Union for the Conservation of Nature, IUCN 2011.)

Pearls collected from Scottish *M. margaritifera* were traded in Europe, as early as the 12th Century and by the 16th Century there was significant trade across Britain and Ireland. However, by the 19th Century the level of exploitation reached was unsustainable and the fishery declined to a constant, but small scale level maintained traditionally by travelling people (Skinner *et al.* 2003). In 1991 *M. margaritifera* were added to Schedule 5 of the Wildlife and Countryside Act 1981 but this did not afford the species full protection as, non-destructive fishing was still possible and it remained legal to 'take' *M. margaritifera* and inspect it for pearls before returning it 'unharmed' to the water course, (Young 1991). In 1998 freshwater pearl mussels were afforded full protection, and *M. margaritifera* is

currently listed on Annexes II and V of the EC Habitats Directive and is fully protected under the Wildlife and Countryside Act 1981. In addition to this, *M. margaritifera* is also a UKBAP (UK Biodiversity Action Plan) Priority Species and is included on the Scottish Biodiversity List. In 2011 *M. margaritifera* joined the list of the 365 most endangered species in the world.

In Scotland, of all the rivers historically known to hold populations of *M. margaritifera*, 65% no longer harbour functional populations (Cosgrove *et al.* 2000). Of these functional populations, the relative abundance of 81% of the transects surveyed were found to be either rare (1-50 live *M. margaritifera*) or scarce 51-499 live *M. margaritifera*). Furthermore, at each river containing a population of *M. margaritifera* identifiable threats were reordered, pearl fishing, pollution and river engineering. No population was in its natural state.

1.2 Ecology and life history of *Margaritifera margaritifera*

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 (Ziuganov *et al.* 2000). The species is widely distributed and can be found in Europe, Fennoscandia (the area encompassing Norway, Sweden and Finland), and north-eastern North America (Skinner *et al.* 2003). 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 (Hastie & Cosgrove 2001).

1.3 *Margaritifera margaritifera* life cycle

M. margaritifera become sexually mature from the age of 12-13yr which approximates to lengths of 6.5-7.7cm (Young & Williams 1984a). Mature animals are dioecious, and fertilisation occurs through females ingesting spermatozoa that are released into the water column by males. Eggs, which are deposited in the female demi branch, are then fertilised. Fertilised eggs are maintained in the marsupia where they develop into glochidia (larvae). Five to seven weeks after fertilisation the glochidia, resembling miniature mussels with shells held apart, are ready for release (Bauer 1987). Females produce on average three to four million glochidia over a period of one to four weeks between July and September, each year from maturity to an age of approximately 60 years (Skinner *et al.* 2003).

The glochidia of *M. margaritifera* are highly modified with a number of special features that adapt them for attachment to host fish. When glochidia encounter a suitable host they clasp the gill epithelial tissue between their valves. Gill epithelial cells adjacent to the glochidium become rounded and the glochidium becomes encapsulated by epithelial cells. This cyst formation is very rapid and within two hours of successful attachment adjoining epithelial cells change their shape and structure (Nezlin *et al.* 1994). The glochidia remain encysted on the gills of host fish and grow until they are mature and drop off, in the following spring. The post parasitic stage of *M. margaritifera,* the juvenile mussel, spends several years in the interstitial zone of the river bed restricted to areas where there are high levels of exchange between free water and the interstitial water (Buddensiek 1995).

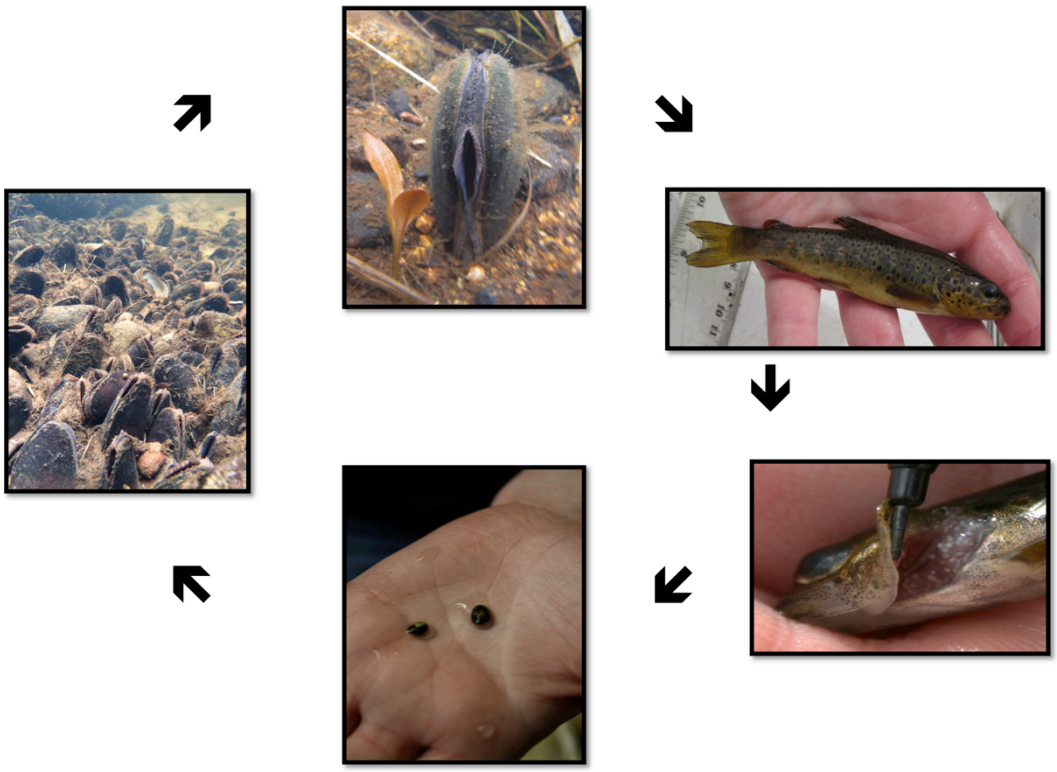

Figure 1-1: Life cycle of Margaritifera margaritifera. *Glochidia are released into the water column to be inhaled by salmonids. Glochidia encyst on the gills of the fish until environmental cues trigger the release of juvenile mussels. Juvenile mussels bury into suitable substrate where they grow to maturity*

1.4 Glochidia and host fish interaction

Young & Williams (1984b) hypothesised that the period of glochidia release was governed by water temperature and that glochidia are emitted from the adult female mussel when the

water temperature is at its highest thus giving the glochidia the greatest chance at being inhaled by the host fish which will be most active when water temperatures are elevated. It has been shown that, more glochidia are released during the day (between 10am and 5pm), when the water temperature is warmer than at any other time of the day (Young & Williams 1984a). Glochidia have no propulsive action and are passively carried to the gills in the water through water currents and natural ventilation of the fish host. Initial attachment success depends on various factors including the prevailing water currents (Young & Williams 1984b).

All host fish for *M. margaritifera* are salmonids and the specific host fish species available to *M. margaritifera* varies throughout its range. Investigations of the glochidia or parasitic stage of *M. margaritifera* were pioneered in North America, by Meyers & Millemann (1977). The authors investigated the susceptibility of salmonid fish to *M. margaritifera* glochidial infection using known numbers of parasites in the laboratory. Comparisons of the susceptibility and infection rates on Coho salmon (*Oncorhynchus kisutch*), Chinook salmon (*Oncorhynchus tshawystcha*), Steelhead trout (*Oncorhynchus mykiss* (sea run Rainbow trout)), Atlantic salmon (*Salmo salar*) and Kokanee salmon (*Oncorhynchus nerka* (land locked Sockeye salmon)) revealed species differences in infection rates. Several factors were suggested to explain the differences in infection rate but no obvious link was found between the position in the cage, gill morphology, ventilating rate or behaviour. As most of the species examined are anadromous, the authors hypothesised that physiological changes associated with parr-smolt metamorphosis may be involved in infection susceptibility through a lower resistance of salmonids to glochidiosis (Karna *et al.*1978). Differences in chemical composition of gill mucus or blood of fish have also been suggested as an explanation in addition to natural and acquired resistance (Karna *et al. 1978).*

In summary evidence to date reports that *M. margaritifera* have a short parasitic larval phase as glochidia attached to the gills of a suitable salmonid host. Across the range of *M. margaritifera* the salmonid utilised varies, further north *S. salar* becomes increasingly more important and is known to be the main host in Nova Scotia and Russia (Bauer 1987). *Salmo trutta* is the main host species in Germany and central Europe (Bauer 1987). The small number of rivers looked at in Scotland suggest that there is some host overlap, with *S. salar* being the main host in some rivers and where they are absent *S. trutta* are the only available host species (Skinner *et al.* 2003).

As *M. margaritifera* are declining across its range there have been many studies attempting to ascertain the cause of the decline. Arvidsson *et al.* (2012) found that *S. trutta* density along with other biotic factors of mussel population size and density can affect recruitment. Their study showed that increasing mussel density and *S. trutta* density had positive effects on juvenile recruitment. A high density of adult mussels would result in a large number of glochidia being released and if there is a high density of host fish then the chances of glochidia successfully encountering and infecting a fish are greater. Their results also showed that mussel density was more important than *S. trutta* density. This may be because the release of glochidia over time will be relatively stable but the density of *S. trutta* can vary greatly between years (Österling, Arvidsson & Greenberg 2010).

1.5 *Margaritifera margaritifera* habitat requirements

The next stage of the life cycle of *M. margaritifera* is completed with the excystment of juvenile mussels from the host fish and the settlement of the juveniles on suitable substrate. One determinant of the success of this stage is where the host fish is at the time of excystment and if the host fish is in the vicinity of appropriate substrate for the juvenile mussel to bury. Mussel assemblages are constrained by the distribution and abundance of fish hosts, dispersal by fish hosts is an important limiting factor to mussel communities. Mussel and fish assemblages both respond to the environment, and mussels are found to occupy a range of habitats due to the release from fish hosts in different locations, (Vaughn & Taylor 2000). In addition to the success of the initial colonisation by *M. margaritifera*, distribution of mussel beds in a river may be the result of an even layer of juvenile mussels being released from fish hosts across the riverbed but where mussels encounter suboptimal habitat the juveniles die (Morales *et al.* 2006) .

In 2006 Morales *et al*. looked at the application of a numerical model to analyse the effects of substrate and hydrodynamic conditions on the suitability and formation of unionid mussel beds. A model that would predict the location of mussel beds was thought to be a valuable tool for the management of these species. The results from the simulation on the Upper Mississippi River demonstrated that juvenile unionid mussels travelled downstream up to 2km before settling when in the high velocity of the main stem of the river, and less when in the lower velocity areas. Morales *et al.* (2006) found that high flows were the limiting condition and substrate stability was the determining factor for mussel beds in medium to high flows. As flows increased the number of suitable areas decreased. Juvenile

mussels are small particles (in the order of 0.2mm), so they are unlikely to be able to settle in areas where particles that are equal to or greater than 0.025mm are actively transported with the flow. This may be cause for concern if increasing velocities preclude recruitment of young individuals and therefore hinder the long-term survival of otherwise healthy mussel beds.

Juvenile *M. margaritifera*, once established in the substrate, require high quality stream substratum and high quality water for completion of their life cycle (Geist & Auerswald 2007). Specific substrate requirements differ from other species and in 2000 Hastie *et al*. recognised a need to more clearly describe the habitat requirements of *M. margaritifera* in all its life stages in order to tailor conservation measures more appropriately to this rapidly declining species. At the time they highlighted the lack of knowledge in relation to stream hydrological processes on the microhabitat and spatial structures of mussel populations and especially the recruitment of juveniles (Hastie *et al*. 2000). These authors recommended that a standard habitat description was required for every river containing *M. margaritifera,* this has not yet been done.

Moving on in the life cycle of *M. margaritifera* from the settlement and establishment of juvenile mussels to adult mussels in a self sustaining mussel bed, adult mussels are found primarily at bed level, partially buried and partly projecting into the water column. The distribution of adult mussels in a river reflects the morphological conditions and processes across a range of spatial scales (Quinlan *et al.* 2014). Quinlan *et al.* (2014) effectively outline the limitations of the existing literature and knowledge on depth and velocity at which adult *M. margaritifera* are found. In particular the authors highlight that it remains unclear whether mussel abundance-water velocity relationships are governed by how forces influence bed stability and thus the scour of mussels or by bioenergetics the independent movement of individuals. An additional point of debate is centred on local habitat suitability governing spatial variation and abundance of organisms, this relies on organisms being mobile and having the ability to search out and occupy optimal conditions. A number of studies have attempted to model this and the factors governing the abundance and distribution of freshwater mussel populations (Hardison & Layzer 2001, Allen & Vaughn 2010). Similarities can be drawn from studies when hydraulic variables and mussel density were considered. In summary it was found that mussel density was lowest where high shear stress was experienced in spates (Gangloff & Feminella 2007). In addition to this Morales *et al.* 2006 found that the distribution of mussel beds coincided

with stable areas of substrate at mean maximum annual discharge. Quinlan *et al.* (2014) outline 4 questions that highlight the current gaps in knowledge of the hydraulic requirements of *M. margaritifera*, they are: i. Which parameters and approaches most usefully describe hydraulic habitat as scales relevant to adult and juvenile mussels? ii. What are the causal mechanisms underpinning observed relationships between hydraulic parameters and mussel distributions? iii. Do mussels engineer hydraulic habitat conditions? iv. How do hydraulic conditions influence the settlement and distribution of mussels immediately after they leave their salmonid hosts? Quinlan *et al.* (2014) emphasize that to answer question ii there is a requirement for the use of new technologies and extensive field studies.

1.6 Aims

Thus there are two areas in which our knowledge of the ecology of this vulnerable species is poor and hampering effective conservation actions:

1) Host choice of glochidia; In Scotland it is assumed but not empirically proven that *S. salar* are the preferred salmonid host for the parasitic stage of the life cycle of *M. margaritifera* (Skinner *et al.* 2003). There is no definitive survey of which rivers in the North West of Scotland are dependent on *S. salar*, resident *S. trutta* or Sea Trout. There is concern that if the sea trout populations have crashed and there is a decline in *S. salar* then only the watercourses where *M. margaritifera* main host is *S. trutta* will be unaffected. Therefore a major aim of the study presented here was to establish host preference through field studies immediately prior to excystment.
2) Behavioural responses to changes in flow velocity; little is known about how adult *M. margaritifera* respond to changes in flow velocity in natural and regulated watercourses. Optimal velocity conditions exist in the literature but these are anecdotal and investigation into changes in flow are required to provide evidence to base practical management on. In addition to this a better understanding of appropriate management of existing regulated watercourses that contain *M. margaritifera* beds and actions to actively manage catchments to enhance habitats for *M. margaritifera* would fill a gap in our understanding of how to manage Scotland's fragile but important *M. margaritifera* populations.

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

2.1 Introduction

As *M. margaritifera* are thought to live a mostly sedentary life any expansion of range can only occur through transportation of glochidia on a host fish and it is thought that this parasitic relationship between mussels and fish began as phoresy as juveniles obtain a selective advantage from upstream dispersal (Barnhart *et al.* 2008). The evolutionary origin of this relationship is not well understood but it is assumed that the beneficial phoretic relationships through frequent contact with fish provided an opportunity for natural selection (Barnhart *et al.* 2008). It was suggested by Barnhart *et al.* (2008) that evolution of adaptations to facilitate mechanical attachment to fish may have developed from the production of larval threads entangling and adhering to fish and therefore facilitating upstream transportation.

Host specificity is a critical feature of the evolutionary diversification and conservation biology of Unionoida and *M. margaritifera*. The intricate relationships between mussels and fish are easily disrupted (Barnhart *et al.* 2008) Specifically in the life cycle of *M. margaritifera* in Scotland, during the parasitic stage glochidia are released into the water and are inhaled by salmonids and attach to the gills (Figure 1.1). The gills provide a highly oxygenated, large surface area for attachment in soft tissue with a minimal mucus layer and the glochidia remain there 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 (Hastie & Young 2001). Current literature based on research completed in a selection of Scottish rivers clearly defines *S. salar* and *S. trutta* as hosts for glochidia (Young & Williams 1984a, Young & Williams 1984b, Hastie & Young 2001). The general consensus is that *S. salar* is the main host but in rivers where salmon are not present, then *S. trutta* may be the sub optimal host (Skinner *et al.* 2003).

In Scotland the only native stream dwelling salmonids are *S. trutta*, and *S. salar*. While the focus of both Meyers and Millemann (1977) and Karna *et al.* (1978) studies was *M. margaritifera* infection, from the group of salmonids they investigated only *S. salar* is present in Scotland and their evidence demonstrated that *S. salar*, showed a wide range of susceptibility and resistance to glochidia infection. In Scotland laboratory and field studies looking at these fish as hosts have demonstrated huge losses of glochidia during the early

stages of *M. margaritifera* development (Young & Williams 1984b, Young & Williams 1984a). Young and Williams (1984b) estimated 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 becoming establishing in suitable substrate. It is evident from this that the successful completion of the glochidial stage of the life cycle is essential. An inability to complete this part of the life cycle would result in recruitment failure a bottleneck in the population and potentially a whole year class of *M. margaritifera* could be lost from the system.

Although the papers by Young and Williams in the 80's (Young & Williams 1984b, Young & Williams 1984a), greatly increased our understanding of the life cycle of the freshwater pearl mussel in Scotland the scope of the studies left some gaps in our knowledge . Their field observations were based on one small west coast 'burn' and one significantly larger east coast river. In addition to this, the fish used for the 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. Nearly 20 years after Young and Williams (1984a, 1984b) initial studies of freshwater pearl mussels in Scotland, Hastie and Young collaborated to add to our knowledge of *M. margaritifera* glochidosis (Hastie & Young 2001). They recognised that at the time very little was known about individual rivers and relationships between host stock size and reproductive success in a given river. Their 2001 study looked at salmonids from six rivers in northern Scotland all known to contain large numbers of *M. margaritifera* and, two hatcheries where the water supply was taken from rivers known to contain *M. margaritifera*. The results of their work showed that less encysted glochidia were found on individual *S. trutta* than on *S. salar.* Two of the six rivers studied, the Kerry and the South Esk, showed median infection loads on 0+ *S. trutta* to be significantly lower than those on 0+ *S. salar*. One river, the Spey showed no significant difference between mean infection loads of 1+ *S. trutta* and *S. salar*.

The release of artificially infected host fish into rivers was recognised by Hastie & Young (2003) to be a feasible conservation option in Scotland to augment declining *M. margaritifera* populations. The authors highlighted that extensive mussel cultivation trials at a number of sites where *M. margaritifera* populations have been wiped out by pearl fishing and still contained suitable habitat conditions could be suitable for this work.

In Scotland the locations of *M. margaritifera* populations vary from large east coast rivers like the River Dee (136km long, 17 major tributaries and draining a catchment of 2100km^2) all the way through to smaller west coast rivers of only a few kilometres in length. It is not currently known if the salmonid host utilised by *M. margaritifera* populations found in this variety of locations is the same in each and every river. Unless this 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. Skinner *et al.* (2003) noted that the relative importance of *S. salar* and *S. trutta* in Scottish rivers as *M. margaritifera* host fish has not been well studied. It was hypothesised that as 0+ *S. salar* are often more abundant than 0+ *S. trutta* therefore *S. trutta* are less likely to be the most important host of *M. margaritifera* (Skinner *et al.* 2003). 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 (Skinner *et al*. 2003). Geist *et al.* found that a poor status of host fish can only explain the lack of juvenile reproduction of *M. margaritifera* in a very limited number of streams. The long life span and long reproductive life of a freshwater pearl mussel reduces the number of host fish required to maintain a population. Low densities of host fish can be buffered by low mortality rates among juvenile mussels at the post parasitic stage, (Geist *et al*. 2006). Leading on from this it can be hypothesised that in areas with poor substrate quality, a higher density of host fish species is required to compensate. But density of host fish should not be looked at in isolation there also needs to be some consideration of varied susceptibilities and immune responses from different strains of *S. trutta*.

The primary aim of this study is to investigate the relative importance of *S. salar* and *S. trutta* as host fish species for *M. margaritifera* in the field in a selection of rivers in Scotland by looking at the number of encysted glochidia on individual fish immediately prior to excystment. *M. margaritifera* glochidia can and do infect both *S. trutta* and *S. salar* in Scotland. In line with current understanding in Scotland, in rivers where *S. trutta* and *S. salar* are both present it is assumed that *M. margaritifera* glochidia attach to the gill filaments of *S. salar* in greater numbers than *S. trutta.*

Secondary, to the relative importance of host salmonid to *M. margaritifera,* is the number and position of encysted glochidia on the gill filaments of the infected fish. In previous laboratory studies where 400 000 glochidia in 3 litres of water with 2 fish for 3 mins

showed that *M. margaritifera* glochidia, with no free swimming ability, are inhaled by salmonids and attach to middle sections and ventral zones 4 weeks after infection (Young & Williams 1984b). Young and Williams (1984b) also noted that 95% of glochidia that initially attach to the host fish are shed before excystment as juvenile *M. margaritifera*. It can be assumed that in the wild immediately prior to excystment the greatest number of glochidia will be recorded on the central gill filaments. This study will record the number and position of glochidia on the gill filaments of fish in the field immediately prior to excystment.

2.2 Materials and Methods

2.2.1 Fieldwork – Electro fishing

The study was carried out between 7th May 2013 and 20th June 2013. Young and Williams (1984a) found that in Scotland juvenile *M. margaritifera* were released from their hosts between late June and early July therefore electro fishing was planned for the period of time immediately prior to excystment in May and June. Eight sites on 8 rivers were chosen through discussion with Scottish Natural Heritage (SNH), and based on a report by Sinclair (2011). Site selection was based on the presence of *M. margaritifera* and both *S. trutta* and *S. salar*. All of the watercourses investigated were in northwest Scotland, due to the sensitive nature of the work and in an attempt to protect *M. margaritifera* locations, sites are not named here and will be referred to as sites a-h. At each site a visit was made prior to electro fishing to ascertain the presence of suitable habitat for young salmonids and locate *M. margaritifera* beds.

Salmonid fish were collected using a standard 500W DC backpack electro fishing gear by an operator with one additional person wading to catch stunned fish. In each watercourse the starting point for the survey was a safe access point downstream of the first suitable juvenile salmonid habitat located. Electro fishing continued upstream from this point with particular sampling attention paid to suitable juvenile salmonid habitat. Care was taken to avoid trampling on visible *M. margaritifera* beds. Fishing was effective in all streams between 400v and 500v and continued at each site until the battery pack failed at approximately 40 min. All fish collected were anaesthetised, identified, measured, (fork length (mm)) and the number of encysted glochidia counted. At this time 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. Two people, repeated counts on a random sample of fish, to ascertain the accuracy of this visual count, accuracy (or error) between observers never exceeded one.

Figure 2-1: Encysted M. margaritifera *glochidia on* S. trutta. White dots on gill filaments are glochidia

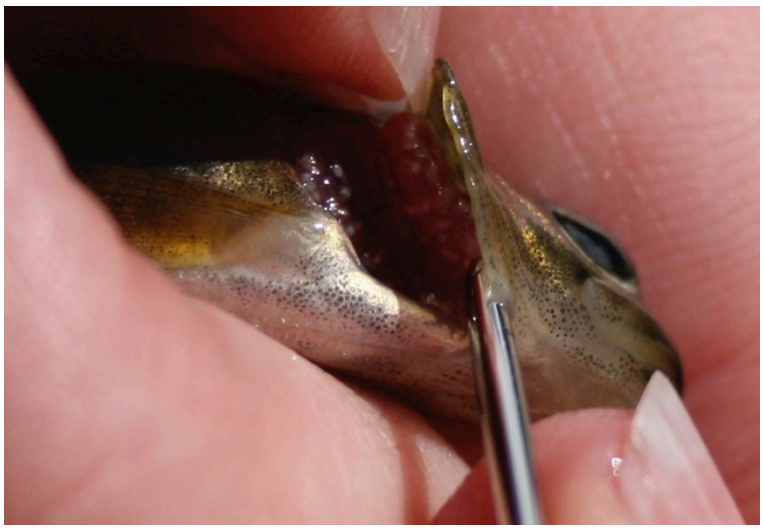

Figure 2-2: Encysted M. margaritifera *glochidia on* S. trutta. White dots on gill filaments are glochidia

A sample of the scales of the fish was also taken from a random selection of fish. All fish were returned to the watercourse within the section they were taken after a period of recovery. After approximately 40 minutes the battery packs were replaced and electro fishing continued in this manner until a sufficient sample size,of more that 40 individual fish had been recorded. No attempt was made to undertake a quantitative evaluation of fish density as it is already known that that successful recruitment is positively related to both host fish density and mussels density (Österling, Greenberg & Arvidsson 2008).

2.2.2 Statistical analysis

R statistical software v.3.0.2, and associated packages provided the platform for all data analysis (Crawley 2007).

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. This was to test the assumption that *S. salar* is the primary host for *M. margaritifera* glochidia in watercourses that contain both *S. salar* and *S. trutta*. In addition to this a Chi squared analysis was used to investigate whether the rate of infection by glochidia for all fish, *S. trutta* and *S. salar* combined, differed between sites. The combined infection rate of fish at each site gives some indication as to the differences in rate of infection at each site regardless of salmonid species. Additional Chi squared analyses were used to ascertain if *S. trutta* and *S. salar* were infected at an equal rate with glochidia, this provides indication of salmonid preference in each watercourse.

Following on from the analysis of salmonid infection 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. This analysis was to test the assumption based on laboratory studies, (Young & Williams 1984b), that glochidia are located in middle and ventral zones of host fish gills.

2.3 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).

Table 2-1: Data from all 8 rivers surveyed

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):	Standard deviation *S. trutta*:	Mean fork length *S. salar* (mm):	Standard deviation *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

The combined *S. trutta* and *S. salar* mean rate of infection with *M. margaritifera* 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 of *M. margaritifera* showed infection rates between rivers differed significantly; $X^2 = 111.5$, df=4,N=632, $p<0.005$.

In all eight rivers were surveyed, one river (E) was excluded as no infected fish were caught. 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

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 they were found to occur in the same watercourse.*

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

To analyse any 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 salmonid in each watercourse with expected infection rate based on the observed rate of infection of all fish caught and the assumption that *S. trutta* and *S. salar* have an equal chance of being infected. 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 (Table 2.2). In summary infection rates for sites B, F and H were significantly higher for *S. trutta* than *S. salar*.

Following on from this, further analysis focussed on the fork length of fish and numbers of encysted glochidia. The analysis revealed that the total number of glochidia found on fish was significantly negatively related to fish fork length (Figure 2.3) 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, for example river A = 5.76 + fork length * -0.01. This relationship between mean fork length and glochidia encystment abundance across all sites equates to an average decrease in glochidia loading of 1 glochidium per 10mm of fork length of fish over all sites surveyed.

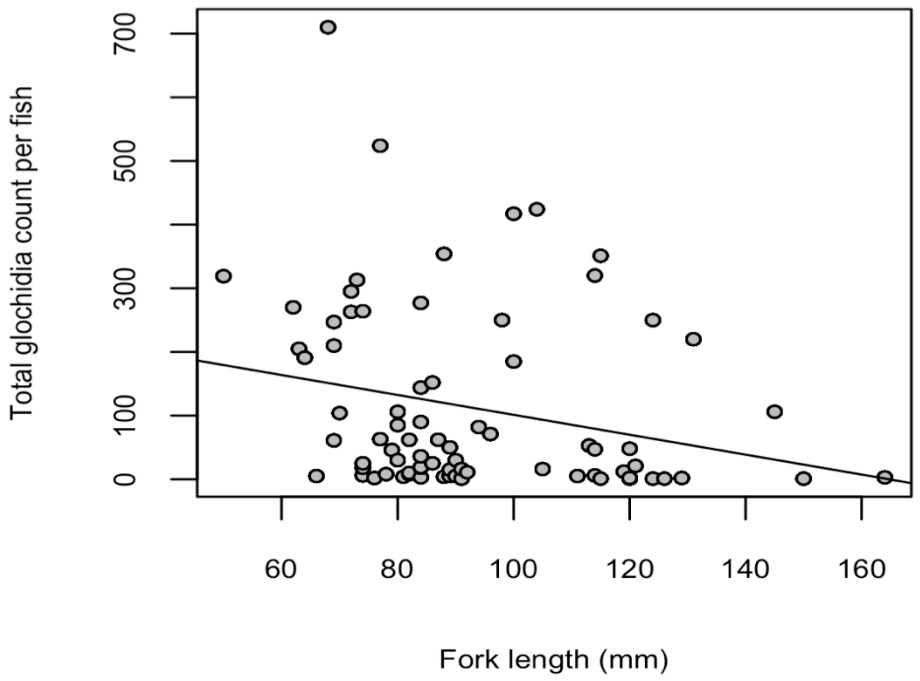

Figure 2-3: Linear regression showing an inverse relationship between the fork length of seventy four infected S. trutta *caught and the total glochidia counted on each individual fish (F=96.43, df =1, r^2 = 0.06, p = <0.001).*

Table 2-3: Results of GLM analysing the effect of fork length and site on the total number of glochidia per infected S. trutta

	Estimate	Std. Error	z value	p value
Intercept	6.22	0.02	275.20	<0.001
Fork length: River G	0.02	0.00	61.05	<0.001
Fork length: River H	-0.00	0.00	-3.57	<0.001
Fork length: River F	-0.03	0.00	-16.67	<0.001
Fork length: River B	0.00	0.00	12.119	<0.001
Fork length	-0.02	0.00	-67.86	<0.001
River G	-2.49	0.04	-69.74	<0.001
River H	-0.81	0.10	-8.72	<0.001
River F	1.08	0.13	7.98	<0.001
River B	0.15	0.03	5.43	<0.001

In addition to this, an analysis of deviance to test the significance of the interaction between site and fork length in the model revealed that an interaction between site and fork length and both the explanatory variables of site and the fork length and were significant in determining the total number of glochidia encysted on fish (Table 2.4).

*Table 2-4: Analysis of deviance testing the significance of the interaction between fork length of fish and site in determining the number of encysted glochidia on fish (*S. trutta*.)*

	Degrees of freedom	Deviance	Residual degrees of freedom	Residual deviance	Pr(>Chi)
Null			1539	249569	
Fork Length	1	18182	1538	231386	<0.001
Site	4	34054	1534	197333	<0.001
Fork length: site	4	4666	1530	192667	<0.001

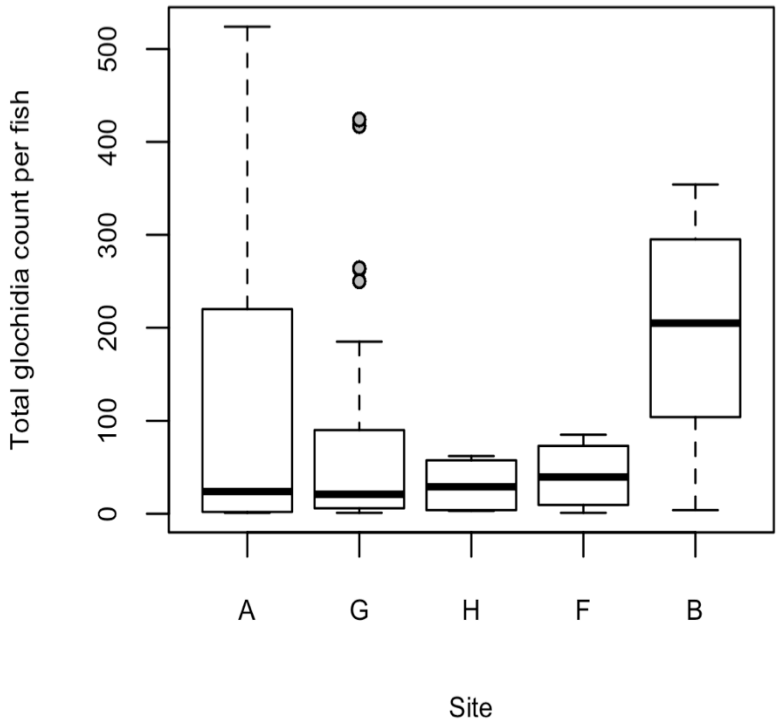

Figure 2-4: Total counts of glochidia. Bold line highlights the median count for each river, box shows the 25th and 75th percentile and whiskers minimum and maximum counts of glochidia on each river

Table 2-5: Summary of the total number of encysted glochidia counted per fish at each site

Site	Mean glochidia count per fish	Standard deviation of glochidia count per fish
A	106.3	146.1
G	80.4	117.9
H	30.8	27.1
F	41.3	33.6
B	222.7	164.2

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), (Table 2.6). 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.

Table 2-6: Results of ANOVA to investigate abundance and position of M. margaritifera *glochidia on gills of* S. trutta*. A significant interaction between anterior/posterior side of gill and gill number was found*

	Degrees of freedom	**Sum Sq**	**Mean Sq**	**F value**	**p value**
Side of gill: Gill number	4	4.8	1.198	4.975	<0.0001
Side of gill	1	16.3	16.254	67.496	<0.0001
Gill Number	4	73.0	18.248	75.779	<0.0001
Side of fish	1	0.0	0.014	0.059	0.8
Residuals	1529	368.2	0.241		

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 (Table 2.7, Figure 2.5).

Table 2-7: Summary of the total numbers of encysted glochidia counted on anterior and posterior gill filaments on each of the five gills of infected S. trutta

	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

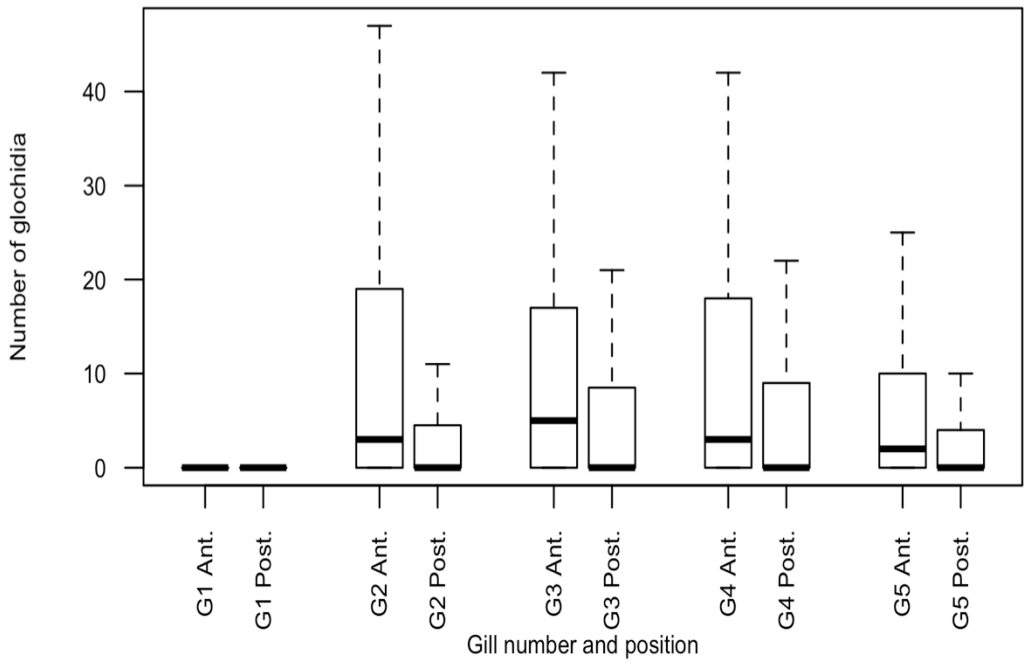

Figure 2-5: Numbers of encysted glochidia on each gill 1-5 and anterior and posterior sides of the gill filaments. Figure shows median in bold, box represents 25th and 75th percentile, box shows the minimum and maximum counts

2.4 Discussion

The primary aim of this study was to investigate the relative importance of two wild salmonid populations as host fish for *M. margaritifera* in Scotland. Previous studies in Scotland have looked at encystment in laboratory settings and recorded numbers of glochidia immediately after encystment. This study was carried out wholly in the field and examined *S. salar* and *S. trutta* for glochidia in the spring immediately prior to excystment when individual glochidia could be counted by eye.

Throughout the range of *M. margaritifera,* glochidia are known to encyst on salmonid fish as the host for the parasitic stage of the life cycle. There is a general understanding in the literature that the salmonid host varies but in Scotland it is currently understood to primarily be *S. salar* (Hastie & Young 2001,Skinner *et al*. 2003). In rivers where *S. salar* are not present then *S. trutta* are the host. A number of smaller rivers in Scotland known to hold *M. margaritifera* beds have no *S. salar* and here the host must be *S. trutta*.

Interestingly and perhaps most significant for future management of *M. margaritifera* in Scotland are that the results from this study do not correspond with current understanding. In two of the rivers surveyed *S. salar* was the dominant species recorded but not the primary host for *M. margaritifera* glochidia, this strongly indicates a preference for *S. trutta* at these sites.

In 2000 Riusech *et al*. looked at host suitability and utilisation in two Unionoida *Venustaconcha* species found North America. Their investigation specifically looked at the transformation success of encysted glochidia and highlighted possible differences in compatibility within species of mussels and hosts particularly where habitat preferences are likely to vary genetically among populations, (Riusech & Barnhart 2000). A recommendation from the study by Riusech *et al.* (2000) was that when studying compatibility or otherwise of host fish species the locality and origin of fish should be recorded and that a single test is not necessarily representative of compatibility throughout the range. Following Riesech *et al.* (2000), Rogers *et al.* (2001) looked at the endangered *Epioblasma florentina walkeri* also found in North America. They found that there was a higher transformation rate of glochidia on fish from watercourses where the mussels were found than on fish from catchments that had no mussels. The hypothesis drawn from these findings was that host fish suitability is mediated by varying immune response and that coadaptation of sympatric host fish and mussel populations seemingly enhances

compatability (Rogers, Watson & Neves 2001). Evidence from other Unionoida show that mussels can adapt simultaneously to distantly related hosts but can also be sensitive to slight genetic differences among related species or populations of a single species (Barnhart *et al.* 2008).

In Central Europe *S. trutta* are known to be the primary host of *M. margaritifera* and in 2010 Taeubert *et al.* looked at the suitability of different salmonid strains as hosts, (Taeubert *et al.* 2010). They looked at three strains of *S. trutta* and one of *S. salar* and demonstrated that all became infected with glochidia but that the susceptibility to infection varied between the *S. salar* and the *S. trutta*, infection rate on the *S. salar* was much lower. The most suitable hosts were *S. trutta* from within the natural distribution of *M. margaritifera*. Taeubert *et al.* (2010) suggested that there could be a possible host specific adaptation of *M. margaritifera* larvae immunological rejection reaction that could be more effective in fish from rivers with no *M. margaritifera*.

It can be assumed that glochidia of *M. margaritifera* are adapted to survive the innate defensive response of both *S. salar* and *S. trutta* in Scotland. Fish hosts can acquire immunity via adaptive immune responses but this develops slowly and would affect glochidia after multiple infections (Barnhart *et al.* 2008) which could explain why *M. margaritifera* are principally found on 0+ fish. In addition to this adaptation to a host fish species by natural selection requires glochidia to make contact with the host. *M. margaritifera* has a non targeted release of glochidia and it could be considered that they have become specialists with one host species if that species is over whelmingly abundant in the watercourse (Barnhart *et al.* 2008). This was not the case in my study (Table 2.1), rivers B and F were *S. salar* dominated but *S. trutta* was the host fish, river G was *S. trutta* dominated and *S. trutta* was the host, river H had similar numbers of *S. salar* and *S. trutta* and *S. trutta* was the host fish.

In Scotland the stocking of *S. salar* and *S. trutta* has been used to augment fish populations for many years, and as our knowledge and understanding of fisheries management has increased the importance of maintaining genetically distinct populations has been recognised. The Focussing Atlantic Salmon Management on Populations (FASMOP) project looked specifically at population structuring within *S. salar* stocks in Scotland (240+ sites and 12000+ fish). The sites where my surveys were conducted fell into three fisheries districts taking part in the FASMOP project (Cauwelier *et al.* 2013, Stradmeyer *et*

al. 2013, Coulson *et al*. 2013). Two of the districts exhibited significant but weak genetic differences between samples taken at difference locations within the catchment illustrating genetic structuring in *S. salar* in the watercourses surveyed. The weak genetic differences could be a result of historic stocking and mixing of fish between watercourses. One of the districts showed moderate to strong genetic differences, they showed differences over time and distinct differences that can be directly associated with aquaculture escapees.

It is widely accepted that fish from different rivers exhibit different traits, different run timing, smolting age and sea age maturity and that these behaviours have a genetic factor (Taylor 1991, Primmer 2011). It is possible that the intricate relationship between sessile *M. margaritifera* and changing genetic structuring of the more mobile salmonids, through anthropogenic means in addition to natural dispersal, could in part be responsible for the decline in *M. margaritifera*. The salmonids in the watercourses now could have a better immunological rejection reaction (Taeubert *et al.* 2010) to the glochidia of *M. margaritifera* than the fish that maintained the *M. margaritifera* populations previously. Any advances in the FASMOP or other projects using a set of genetic markers to differentiate between wild and farmed salmon allowing for widespread sample and screening to identify individuals of pure ancestry and explore possible levels of introgression between wild and farmed individuals could shine a light on a genetic element of the *S. salar* host interaction with *M. margaritifera* glochidia.

Prior to the FASMOP project, Marine Scotland Science (2011) reported on genetic analysis of brown trout and sea trout in the north west of Scotland. Initial analysis showed that each river had its own unique breeding population. In addition to this multiple genetic groups were found in the samples of sea trout taken in the estuarine locations. Further studies are required to fully understand the genetic structuring of *S. trutta* but it is indication that perhaps in a similar way to *M. margaritifera* in Germany (Taeubert *et al.* 2010) investigation into suitable *S. salar* and *S. trutta* strains may inform *M. margaritifera* management actions in the future.

In Norway it has been established that *M. margaritifera* are either *S. trutta* of *S. salar* dependent (Karlsson, Larsen & Hindar 2013). Karlsson *et al*. (2013) study looked at nineteen rivers in 4 distinct geographical areas and took genetic samples from *M. margaritifera* known to be either *S. trutta* or *S. salar* dependent. In populations utilising *S. salar* there was found to be more genetic variation within populations and lower genetic

variation among populations compared with *M. margaritifera* with *S. trutta* as a host fish. Host affiliation explained more genetic differentiation among *M. margaritifera* populations than geographical range. There was also found to be strong reproductive isolation between *M. margaritifera* populations. The study did find that in *M. margaritifera* populations within the same river using different hosts species showed large genetic differences but populations using the same host species showed small non significant genetic differences. It was not possible in their study to determine functional local adaptation and/or distinct evolutionary lineages but the genetic isolation indicates a functional divergence between the two groups.

In addition to changes in host fish population it has long been known that pearl fishers moved *M. margaritifera* from catchment to catchment and further afield in Britain in attempts to increase the number and size of harvestable *M. margaritifera* beds (Goodwin 1985, Skinner *et al.* 2003). Detailed knowledge on preferred hosts in each watercourse or even within an individual watercourse has never been available in Scotland therefore *M. margaritifera* may have been reintroduced or introduced into rivers where they do not match the available salmonid host. In line with similar recommendations for *M. margaritifera* in Norway made by Karlsson *et al.* (2014) any management actions in relation to salmonids or *M. margaritifera* in Scotland needs to take into account specific host fish utilization, how genetically isolated and potentially locally adapted *M. margaritifera* and host fish are.

In addition to defining significant differences in host utilisation my results also showed there was a significant effect of site on the total number of glochidia counted on fish. This could possibly be explained by the size and distribution of *M. margaritifera* beds. To quantify the effect of site it would be necessary to complete *M. margaritifera* surveys along with electro fishing and this is perhaps something that could be done in the future.

With a fragile population that is in decline more investigation into genetic structuring of host fish and *M. margaritifera* populations appears to be essential to inform the management and conservation of this species.

A secondary aim of the study was to ascertain where on the gill arches and in what numbers encysted glochidia are found immediately prior to excystment. Due to the declining numbers of *M. margaritifera* and the protection afforded to salmonids in

Scotland my methodology to assess glochidial encystment on fish was partly driven by the requirement for the method to be non-lethal. In addition to this, previous work on *M. margaritifera* encystment has focussed on numbers of glochidia attached immediately post infection and prior to large numbers of non-viable glochidia being shed in the autumn months. It is detrimental to an ever declining species to use lethal survey methods that essentially remove viable glochidia from the population, therefore a method was developed ensuring that surveys could be completed quickly, in the field, and with minimum equipment requirements. The results from my study correspond with previous findings of glochidial attachment (Paling 1968, Young & Williams 1984b). The majority of encysted glochidia are located on the anterior sides of gills two, three and four. This location is unsurprising as it corresponds with the areas with the greatest respiratory current flows, and the glochidia therefore are located in highly oxygenated areas with the greatest circulation. The first and fifth pairs of gill slits together only carry about one sixth of the total respiratory current (Paling 1968) and this was reflected in the results. No glochidia were recorded on gill one and only an average 6.4 on gill five (Table 2.7). This non-lethal method is only possible immediately prior to excystment when glochidia are visible with the naked eye. An alternative non destructive photo method has been developed, a photograph is taken of the glochidia larvae on the first gill arch by placing a steel spatula between the first and second gill arch (Österling 2011). This method has the advantage of being possible when the glochidia are not easily visible by the naked eye. The development of non-destructive methods for surveying glochidia of *M. margaritifera* and other endangered unionids is essential as long as there is a requirement to survey every life stage accurately to inform conservation and management decisions.

3 BEHAVIOURAL RESPONSES TO FLOW CHANGE OF *MARGARITIFERA MARGARITIFERA*

3.1 Introduction

It is widely accepted that anthropogenic changes in river flows at an ecologically relevant level are a key component of freshwater habitat and species decline (Bunn & Arthington 2002). 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 (Poff *et al.* 1997). Our current understanding of flows that are ecologically relevant to the maintenance of *M. margaritifera* beds in Scotland is poor. In 2003 Skinner *et al.* published an account of the ecology of *M. margaritifera* as part of the 'Life In UK Rivers' project to develop methods for 'conserving the wildlife and habitats of rivers within the Natura 2000 network of European protected sites'. Within this report Skinner *et al*. (2003) summarise the depth and velocity requirements of freshwater mussels as being within the range of 0.1-2 m and 0.1-2 ms $^{-1}$. Hastie *et al*. (2000) describe the optimum conditions for *M. margaritifera* as 0.3-0.4m water depth and 0.25-0.75ms^{-1} velocity. Information about how changes in flow conditions may subsequently affect mussels is data deficient. Changing flow conditions affect river habitat and it is generally understood that higher peak flows increase the severity of flood events and 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, (Moorkens & Killeen 2014). Moorkens and Killeen (2014) highlight the importance of maintaining ecologically appropriate velocities over mussel beds as an important aspect of their management and conservation.

Poff *et al.* (1997) highlighted that ecological impacts of dams depend on the degree of flow modification relative to conditions pre impoundment. The River Kerry in Scotland is a regulated watercourse managed by Scottish and Southern Electricity and is known to hold a large and functioning population of *M. margaritifera*. In 2012 Thomas and Hoey studied historical records and aerial photographs to show the river to be relatively stable with respect to channel morphology and position of channel banks and concluded that the dam on the river acts as a sediment trap consequently the lack of fine sediment appears to be beneficial to juvenile mussels. The presence of mussels in the River Kerry was found to be positively associated with cobble-boulder substrates interspersed with pockets of fine

sediment. This study suggests that a modified flow regime has produced a habitat capable of sustaining a functional population of *M. margaritifera*. Given that the River Kerry maintains a functioning population of *M. margaritifera* when other populations in Northwest Scotland and further afield are declining, this suggests that favourable conditions can be created and maintained by the regulated flow at this location.

In 2011 Galbraith and Vaughan looked at three species of unionid *Quadrula* species and found that dam operation can have effects on a variety of mussel life history strategies, including lower mussel densities, higher parasitism and hermaphroditism, and reduced body condition. Most importantly the authors highlighted that population disturbances were not as severe in watercourses where the dam operation mimicked natural flows.

It is evident from a review of the literature that a more detailed knowledge of hydrological factors governing the maintenance of *M. margaritifera* in the wild and in regulated rivers is required in order to manage these fragile populations for the future. Quinlan *et al*. (2014) have highlighted field studies that could be a valuable addition to our knowledge and Thomas and Hoey (2012) outline a valuable case study of a regulated river in Scotland. Although field studies provide valuable insights into flow requirements of *M. margaritifera,* controlled experimental manipulation study has the power to yield more precise data on the flow needs of this important species.

For the second part of my study I am going to look at the behavioural responses of adult *M. margaritifera* to three contrasting flow regimes, two substrate complexities and two groupings of animals. In addition to the behavioural response of *M. margaritifera* to velocity changes the behavioural responses of *M. margaritifera* under two different substrate complexities will be investigated as the watercourses in which *M. margaritifera* are found in are not the same, some are dominated by cobble-boulder substrate interspersed with small amounts of smaller gravel for mussels to bury into, others have more uniform smaller sized gravels. Third and finally I will investigate the effect of association of individuals by grouping and not grouping animals together to ascertain if there is a group effect modifying the behaviour of the individuals. All of these experiments will be carried out using an artificial river or experimental flume and the starting point will be our current knowledge of the range of velocities *M. margaritifera* are found in in Scotland.

This study tests three hypotheses: 1) Adult *M. margaritifera* move a greater vertical distance and bury horizontally when the water velocity is increased in order to prevent entrainment. 2) In more complex substrates adult *M. margaritifera* actively search out variations in flow to utilise areas of lower flow as means for protection. 3) Mussels in close proximity to each other do not move horizontally or vertically as a means of protection from damaging flows.

3.2 Materials and Methods

3.2.1 Collection of M. margaritifera

One hundred and fifty *M. margaritifera* were collected (SNH licence; 17698, 27703,) in November 2013 from a river in the North East of Scotland. Adult mussels were removed by hand and stored in aerated cool boxes, lined with substrate from the watercourse to allow the mussels to bury during transport. The area from which the mussels were removed was a disused mill lade where the water velocity at collection was 0.01ms^{-1}, the bed level and the substrate uniform. Although the lade is no longer in use, it remains hydrologically connected to the main channel but is protected from significant changes in flow velocity except in full flood conditions. The water depth at time of collection was 30cm, this was 'normal' water levels for the lade (pers. com. Steve Hawkins, River Bailiff).

3.2.2 Maintenance of M. margaritifera

Gravel and sand collected from the watercourse at the time of mussel collection was put into a shallow trough approximately 25cm deep and filled with water directly sourced from Loch Lomond. The gravel and water depth was maintained at levels that allowed the mussels to move and bury in the trough unhindered. There was no notable flow of water through the trough but a constant trickle inflow and outflow was maintained to prevent stagnation of the water. The conchological parameters (length and width of shell, depth of animal at widest part) of each mussel were systematically collected, photographs taken and identification number painted on the shell.

3.2.3 Experimental set up

A flume (Figure 3.1) at the Scottish Centre for Ecology and the Natural Environment (SCENE) (Lat: 56^0 07'43. 73" N; Long: 004^0 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 water temperature from the loch (range 6.20^0 c to 9.90^{0}c; mean ± SE for the duration of the experiment), ensuring that the temperature throughout the experiment reflected natural temperatures experienced by *M. margaritifera* during this period of their life cycle. The experiment was conducted on a photoperiod of 9L: 15D cycle.

Consistent velocity across the experimental arena was desirable; therefore only one straight side of the flume was used (Figure 3.1). 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 bio films to develop.

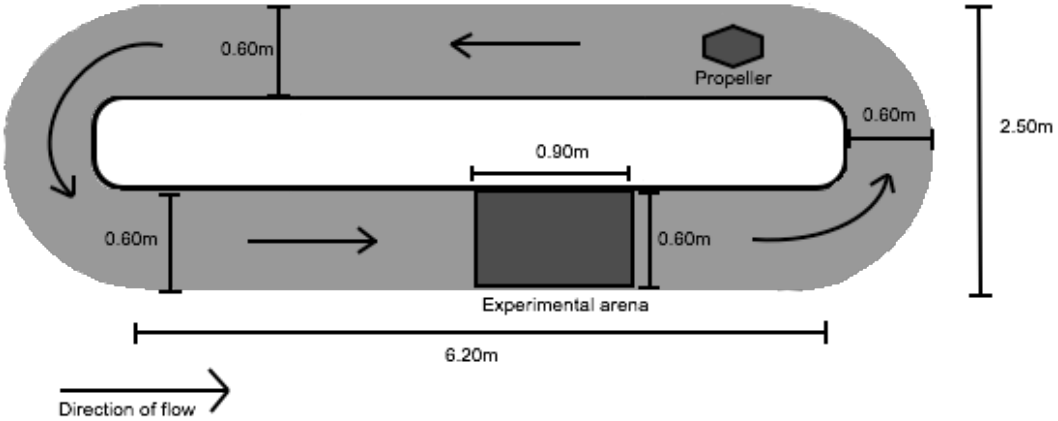

Figure 3-1: Drawing of flume from above showing dimensions, position of propeller, position of quadrat used to define experimental arena and direction of flow of water denoted by arrows

A quadrat was placed directly above the flume to mark the experimental arena (Figure 3.1, Figure 3.2), for all experiments. The positions of mussels were marked in the quadrat using canes and a coordinate system: 1 to 12 right to left and 1 to 18 downstream to upstream. The quadrat was 60cm x 90cm in size and each square cell in the quadrat was 5cm^2.

Figure 3-2: Image shows canes marking positions to place mussels in prior to trial starting

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 15hr, over night, during which the velocity was constant at 0.25 ms^{-1}. In the morning immediately prior to the experiment starting the location of the each mussel in the quadrat was recorded along with the burial position (vertical movement), which was a score on a

four-point continuum (Table 3.1). For the purposes of this experiment the scale 1-4 was considered to be continuous.

Table 3-1: Semi quantitative index of burial. Scores ranged 1-4 with increasing depth buried. The score provides a functional index that takes account of variable animal size

Score	**Description of burial**
1	Flat on bed or propped up against rock. No visible active use of foot or anchoring
2	Flat on bed with foot protruding
3	Anchored on the substrate with hinge parallel to the bed or partially buried with hinge visible
4	Completely buried, no valve visible

Observations of each individual *M. margaritifera* were made at 7 recording points during each experiment (Table 3.2, Table 3.3). 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 (Allen & Vaughn 2009). Total horizontal movement was calculated as a sum of the changes in grid position.

Three experimental conditions were manipulated in this experiment: flow regime, mussel distribution and substrate.

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 $0.231 ms^{-1}$ (sd 0.04). After an initial acclimation period of 15 hours the position in the flume 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 (Table 3.2).

Fast increase: following the 15-hour acclimation period the flow of the 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) for 540 minutes the same recording period as for constant flows (Table 3.3).

Table 3-2: Recording period and corresponding minutes in flume for constant and rapid increase in flow velocity trials

Recording Period	Minutes in Trial
2	90
3	180
4	270
5	330
6	450
7	540

Table 3-3: Recording period and corresponding minutes in flume for gradual increase in flow trials

Recording Period	Minutes in Trial
2	90
3	180
4	270
5	330
6	390
7	450

Gradual increase: following the 15 hour acclimation period the flow of the 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.

The starting distribution of mussels within the experimental arena was defined as clustered or dispersed.

Clustered: individual mussels were placed in the centre of the arena in two rows of three at grid points 7:11, 6:11, 5:11, 7:9, 6:9, 5:9 (Figure 3.3).

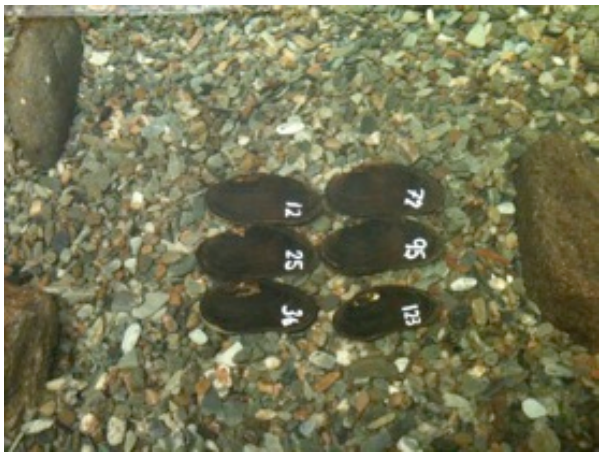

Figure 3-3: Distribution of individual mussels within the experimental arena. Clustered grouping of mussels in a complex substrate

Figure 3-4: Distribution of individual mussels within the experimental arena. Dispersed grouping of mussels in a simple substrate

Dispersed: Individual mussels were placed across the arena in a uniform formation at grid points 2:8, 4:11, 4:5, 6:9, 9:11, 9:5 to ensure that replication was possible (Figure 3.4).

The third and final variable investigated was substrate complexity and its effect on movement and burial of mussels. All experiments had a substrate of 25cm deep gravel.

Simple: Only the gravel substrate was placed in the flume (Figure 3.4).

Complex: the linear flow of water was broken up by placing three rocks into the flume

within the experimental arena creating some heterogeneity in the flow of water (Figure 3.3).

In the trials of fast increase in velocity and gradual increase in velocity all *M. margaritifera* used in the experiments were naïve to the flume and were randomly chosen from the maintenance trough. For each experiment (Table 3.4) six mussels were chosen randomly from the 150 numbered mussels in the holding trough. In trials where the velocity remained constant, individual mussels were used twice. The same group of mussels for the simple substrate, constant velocity trials (clu.sim.con and dis.sim.con (Table 3.4)) and then a different group of mussels for the complex substrate constant velocity trials (clu.com.con and dis.sim.con (Table 3.4)). The constant velocity trials were timed such that the mussels were left in the maintenance trough undisturbed for 24 days between periods in the flume. Observations showed mussels in all trials to be ventilating following the 15 hour over night acclimation period. This was assumed to be indication that the mussels were alive and functioning normally. Within an hour of being removed from their existing position and being placed flat on the gravel, ventilation was observed. Constant flow velocity with no increase in velocity during the trial was considered to be the control for the experiment and as such was not replicated. All other trial conditions were replicated three times.

Table 3-4: List of experimental conditions. Three elements of trial varied: Arrangement of mussels within the experimental arena, complexity of substrate and velocity change

Experiment	Arrangement	Substrate	Velocity change
clu.sim.con	Clustered	Simple	Constant
dis.sim.con	Dispersed	Simple	Constant
clu.com.con	Clustered	Complex	Constant
dis.com.con	Dispersed	Complex	Constant
clu.sim.grad	Clustered	Simple	Gradual
dis.sim.grad	Dispersed	Simple	Gradual
clu.sim.fast	Clustered	Simple	Fast
dis.sim.fast	Dispersed	Simple	Fast
clu.com.grad	Clustered	Complex	Gradual
dis.com.fast	Dispersed	Complex	Fast

3.2.4 Statistical analysis

Four response variables: burial (burial index 1-4), speed of burial, distance travelled (cm), 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 detailed by Crawley (2007). 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 (R package: multcomp, function: glht [Tukey]). 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.

3.2.4.1 Burial- Vertical movement

All mussels were analysed and their burial state at last observation was used as a measure of burial state under the prevailing experimental conditions. Last observation was that either immediately prior to washout or at the conclusion of the trial. The explanatory variables included in the mixed effect model were; weight of mussel, substrate complexity, arena distribution, 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. An interaction between individual weight of a mussel and velocity change was included to investigate the effect size on behavioural response to flow conditions.

3.2.4.2 Speed of burial

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. For instance if a mussel buried to level four at recording period two, then that is indication of rapid burial. If by recording period seven a mussel is only buried to level two than that is indication of slow burial. Burial positions were recorded at the end of the experimental period or the recording period immediately prior to washout. As mussels under rapid increase in flow conditions were recorded at slightly different periods of times the time corresponding to recording period two-seven for constant and gradual trials was used for comparison.

3.2.4.3 Distance travelled – Horizontal movement

The explanatory variables examined in the mixed effect model for total distance travelled were velocity change (constant, fast, gradual), arena distribution and substrate complexity. For this analysis only mussels that remained within the experimental arena to the conclusion of the trial were used.

3.2.4.4 Washout

Differences in washout out frequency among the three different flow regimes investigated were analysed using a Chi-square test. Individuals from each regime were combined to obtain expected frequencies.

3.3 Results

The experiments focussed on the behaviour of mussels it did not look in detail at the conchological parameters of individual mussels. A significant positive relationship was found between length of shell and weight of mussel, weight was chosen as the single parameter to represent size of the mussel (r^2=0.87,t (70 =22.0, p<0.001).

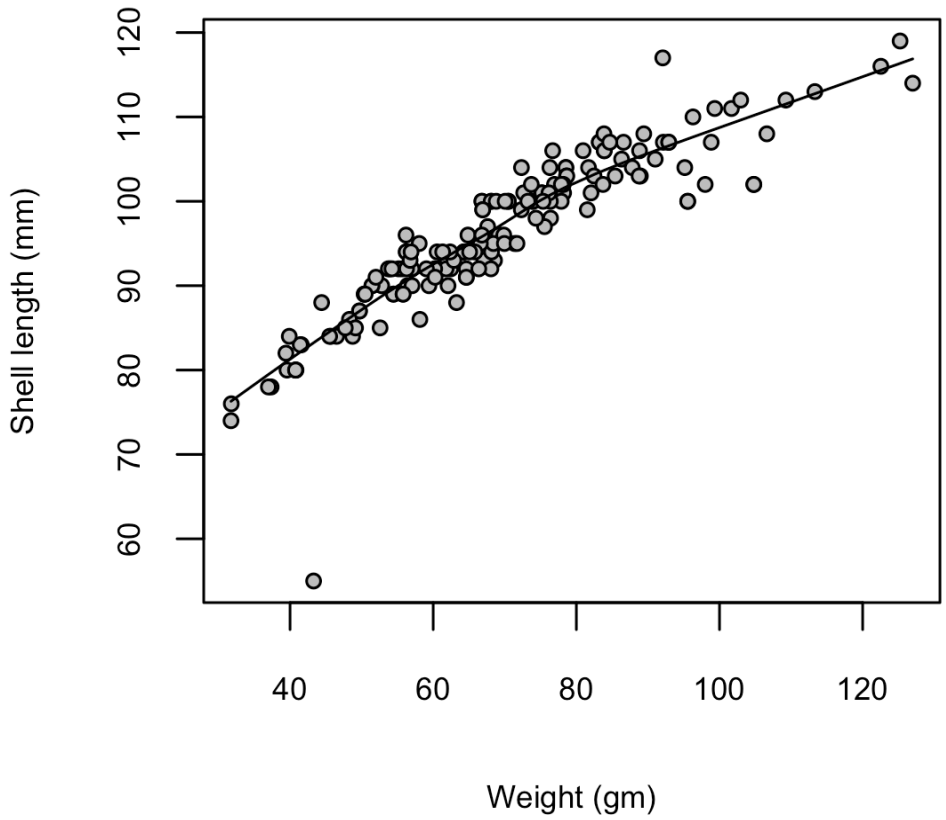

Figure 3-5: Size of mussel. As weight of individual mussel increases so does shell length

3.3.1.1 Burial – Vertical Movement

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, by the end of the trial lighter mussels were observed to have buried deeper.

Table 3-5: Results of mixed effect model to explain variation in depth of burial in individual mussels under 10 different trial conditions

	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

In total 42% (n=70) of *M. margaritifera* across all experimental conditions buried, 12% (n=20) of the individuals buried completely (level 4, Table 3.1), during the experiment, 11 of these were in a gradual increase in velocity trial, five in fast increase in velocity trials and four in a 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. Suggesting detection of an increase in velocity.

Mean velocity (ms^{-1}) experienced by an individual mussel over the course of a trial significantly influenced the degree to which an individual buried. Where individuals experienced greater mean velocities over the period of the trial they were found to bury deeper (Figure 3.6). 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 $0.691ms^{-1}$).

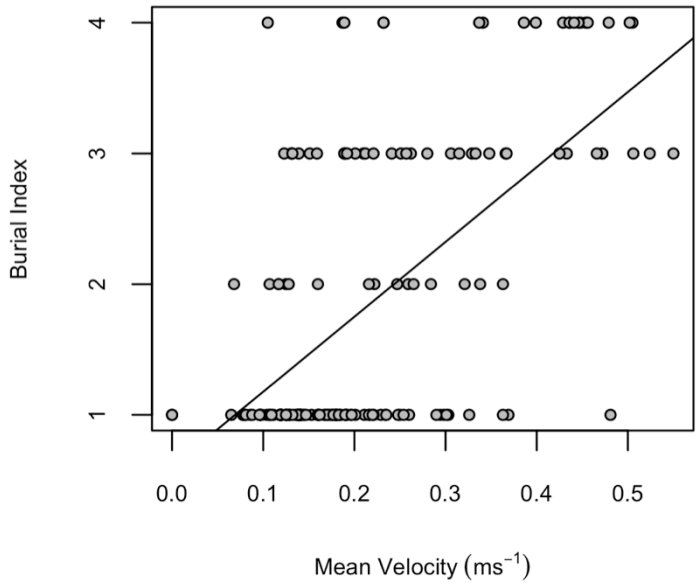

Figure 3-6: Higher mean velocities experienced by individual mussels during a trial increased the depth of burial

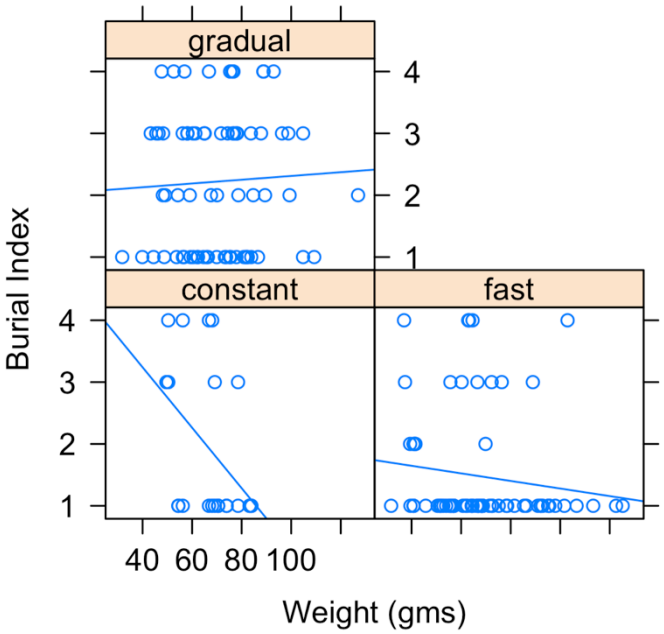

Figure 3-7: An interaction between weight of mussel and velocity regime influences the final burial position of mussels

3.3.1.2 Speed of Burial

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.

Table 3-6: 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

	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

Mussels under constant velocity conditions buried to a mean depth of two on the burial index during the trials (Table 3.1). Mussels under gradual increase in velocity buried more gradually compared with burial under fast increases in velocity (Table 3.6). 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.

Similarly to the results from the analysis of depth of burial under the three velocity regimes 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 trials. In addition to this, 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 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 (Table 3.1).

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.

Table 3-7: Numbers of individual mussels washed out of the experimental arena at recording periods one - six. Data for recording period seven is the number of mussels remaining at the end of the trial

		Recording Period					
Velocity change		2	3	4	5	6	7
Constant	Mean burial depth	0	0	0	0	0	2
	Standard Deviation	0	0	0	0	0	1
	Number of mussels	0	0	0	0	0	**24**
Fast	Mean burial depth	1	1.7	3	2	1	4
	Standard Deviation	0	1.2	0	1	0	1
	Number of mussels	56	3	1	2	1	**9**
Gradual	Mean burial depth	2	1.5	2	2	2	3
	Standard Deviation	1	0.8	1	1	0	1
	Number of mussels	2	6	18	2	2	**42**

3.3.1.3 Washout

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 (Table 3.7) this coincided with a mean burial depth of two, the foot exposed to the flow.

Washout of mussels differed significantly among velocity changes (x^2= 65.3213, d.f =2, p <0.001). In total 93 mussels, 55% of all mussels washed out during the trials. In fast trials only nine 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.

3.3.1.4 Total distance travelled – Horizontal Movement

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.

3.3.1.5 Substrate and arena distribution

Neither the complexity of substrate during trials or the distribution and grouping of indiviudals within the experimental arena had a significant affect on any of the trials.

3.4 Discussion

The aim of this study was to ascertain how *M. margaritifera* respond to changes in flow regime by analysing horizontal and vertical (burial) movements as behaviour utilised to search for suitable habitat or as a means of protection from damaging flows.

The flume used in this study allowed for flow regimes to be altered within the depth and velocity parameters described by Hastie *et al.* (2000), as optimal conditions for *M. margaritifera* following their study on the River Kerry '…a typical medium sized upland river'.

Analysis of the results showed that rate of change; how quickly flow changes and the velocity: the speed of water, impacted most on the responses of *M. margaritifera*. The investigation into burial revealed that in gradually and rapidly increasing velocity conditions *M. margaritifera* were found to bury deeper and faster when compared with flow regimes that remained constant over the same period of time. Depth of burial was not significantly different in individuals that remained to the end of the trials in rapidly increasing velocities compared with gradually increasing velocities. The depth that individuals buried to in rapidly and gradually increasing velocities was significantly deeper than that of individuals held at constant velocity conditions.

Mean velocity at siphon level of mussels that remained to the end of the trial, a total of 450 minutes, was found to be a significant influence on the depth of burial. As mean velocity at siphon level increased so did the depth of burial. The mean velocity at siphon level of *M. margaritifera* that remained to the end of the trial under rapid and gradual increases in velocity were 0.399 ms^{-1} (sd 0.105 ms^{-1}) and 0.307ms^{-1} (sd 0.138ms^{-1}) respectively.

Perhaps one of the most significant findings from this study when considering the maintenance of *M. margaritifera* beds is the number of mussels washed out during trials. Under constant flow conditions the velocity was maintained at a mean of 0.231ms^{-1} this is just under the optimal conditions for *M. margaritifera* as described in the literature (Hastie *et al.* 2000; Skinner *et al.* 2003). Under these conditions no mussels washed out. However, under rapid increases in velocity (to a mean velocity of 0.703ms^{-1}, sd 0.031 ms^{-1}), more than half (68%) of the mussels in these trials were washed out of the experimental arena. Of the mussels washed out, 78% were washed out as soon as the velocity was increased. In contrast less than half (32%) of mussels in trials where the velocity of the water was

increased gradually over time were washed out of the experimental arena. Most individuals were washed out at recording period four and observations suggested that these were mostly mussels that were anchored to the substrate by their foot but had not buried. Mussels were orientated parallel to the flow and film footage recorded during the experiment shows washout occurring when the foot could no longer remain tight to the substrate; the foot is retracted and the mussel washed way.

From the washout statistics it can be seen that the parameters within which flow regime can be varied before *M. margaritifera* are scoured from their existing position anchored on or buried in the substrate are narrow. It is well understood that if there is an increase in the variation of the magnitude and frequency of a high velocity regime, riverine species are more susceptible to being washed out or stranded (Poff *et al.* 1997). The results here support this, 78% of mussels in rapid increases in velocity were washed out of the experimental arena compared with less than 32% in gradually increasing velocity condition.

Previous studies have shown that *Elliptio complanata,* a freshwater lake dwelling bivalve, is endobenthic during the winter months, moving very little and only emerging in the spring and summer months (Amyot & Downing 1997). Amyot and Downing (1997) also found that horizontal movements were correlated with the spring and summer months, coinciding with spawning. In addition to this Schwalb and Pusch (2007) reported that in the field, 90% of the mussels that they were studying moved up to 25cm horizontally in a week. This distance was significantly related to temperature with increases in mean distance moved between May and October, the warmer months of the year (Schwalb & Pusch 2007). My trials on *M. margaritifera* were carried out over a four-month period between November and February when water temperatures were cooler (range 6.20^0C to 9.90^0C).

In the experiments, 82% of individuals did move horizontally from the original position they were placed in the experimental arena but this was not predicted by any of the flow variables that were investigated. This horizontal movement was in turn a significant predictor of burial or speed of burial. As *M. margaritifera* spawn during the spring and summer months it is possible that similar to *E.complanata, M. margaritifera* are less mobile during the winter months and the horizontal movement observed was a result of disturbance.

When the *M. margaritifera* were collected for the experiment they were removed from a bed of *M. margaritifera* by hand. No individuals were entirely buried, and no animals were dug from the substrate but all were buried within the substrate and in close proximity to each other in most cases touching at least one other individual. The *M. margaritifera* were collected from the watercourse and maintained in a trough for several days. During this time they moved horizontally around the trough, buried and were observed to be ventilating. Some individuals buried completely into the substrate. Before each trial *M. margaritifera* were removed from the trough and placed into the flume for a period of 15hours. All individuals were seen to be ventilating but not all had returned to an upright position within the 15-hour settling period. It is possible for *M. margaritifera* to ventilate without being in an upright position but as in most bivalves it can be assumed that they are better adapted to feed, respire, reproduce and excrete when orientated in the substrate and flow (Waller, Gutreuter & Rach 1999). Burial within the substrate to some extent provides stability and protection. It was shown by Waller *et al.* (1999) that water temperature affected the 'righting' mechanism of four freshwater bivalves studied, increased water temperature reduced the amount of time to right (Waller *et al.* 1999). If my study had been carried out in the spring or summer when the water temperature was higher and the need to be correctly orientated in the watercourse to aid spawning was essential then the results may have been different. In addition to this, the disturbance caused by handling and moving the *M. margaritifera* from the watercourse to the trough and then from the trough to the flume could have negatively impacted on the burial and horizontal movement of the mussels. Waller *et al*. (1999) highlighted a need to model disturbance rate and frequency of establishment to advise in surveys, relocation and harvesting of bivalves. This could have implications for *M. margaritifera* conservation management actions if re locations are to be considered.

Another point of interest when investigating burial of *M. margaritifera* is that although all the *M. margaritifera* were adults and were collected from a single population, with no attempt made to grade the animals into size or age classes (shell length from 55mm to 119mm with a mean shell length of 89.12mm and weight range of 19.75gms to 126.9gms mean weight 68.91gms), lighter mussels were found to bury deeper than heavier mussels into the substrate in conditions where the velocity was constant or increased rapidly. Schwalb and Pusch (2007) also looked at horizontal and vertical movements of three species of bivalve (*Unio tumidus, Unio pictorum, Anadonta anatina)*, and found there to be a significant difference in burial depth of different sized mussels with smaller mussels

burying deeper. There can be no direct comparison with the flume studies presented here and those carried out by Schwalb and Pusch (2007) as they used length of shell as a proxy for size and age class. But it is indication that the size, weight or length could be affecting the depth to which mussels bury.

Maio and Corkum (1997) looked at burrowing and orientation of unionids in a 'stable river'; a river with little variation in flow over time, and an 'event river'; a river with greater variation in flow over time. They found that individuals oriented themselves more parallel to the flow in the event river than in the stable river. The authors also found that mussels in the event river were larger than in the stable river. Their study suggests that differences in burrowing behaviour may be attributes that enhance adaptation to specific conditions experienced by unionids in different rivers. The results and hypothesis of Schwalb and Pusch (2007) also support the work of Maio & Corkum (1997) suggesting that unionid bivalves circumvent dislodgement in extreme flows by burrowing, and that flow velocity may be the dominant driving factor affecting burrowing activity. The *M. margaritifera* collected for my trials were taken from a slow moving redundant mill lade, they were not exposed to regular high flow events and due to the location were buffered to some extent from spates and low flow events. It could be that *M. margaritifera* studied here were simply not adapted to the flow regimes tested. To test this, it would be necessary to repeat these trials with *M. margaritifera* from a selection of rivers with different flow regimes. In addition to this, detailed field observations as outlined previously by Quinlan *et al.* (2014) would be highly beneficial to understanding how *M. margaritifera* are adapted to different flow conditions. If the results from my study are representative of *M. margaritifera* then the optimal flow conditions for this species may be even narrower than previously thought.

Unio crassus is another freshwater bivalve that inhabits small or medium sized rivers with gravel, sandy to muddy bottoms and clean water. Due to its significant and continuing decline it is now, like *M. margaritifera*, protected under EU laws. *U.crassus* is threatened by many similar pressures faced by *M. margaritifera,*(Zajac & Zajac 2011). Zajac and Zajac (2011) investigated how *U.crassus* moved after dislodgement. Their study showed that the distance travelled by *U.crassus* differed considerably between riffle and pool experiments, mussels moved randomly upstream and downstream to escape shallow water. They hypothesized that in the riffle habitat there are higher flow velocities and increased risk of dislodgement. The larger particle size of substrate in the riffle structures impedes

movement and therefore the riffle is thought to promote burial. In my experiment, along with changes in velocity, two different substrate complexities were tested. Essentially, the simple substrate provided silt free gravel deep enough for the largest *M. margaritifera* to completely bury into. The more complex habitat included three boulders placed within the experimental arena. This formation essentially broke up the linear flow of the water and provided areas of flow variation within the experimental arena. Analysis of the results did not provide any evidence to suggest that habitat complexity changed how the *M. margaritifera* moved horizontally or vertically. Although not statistically significant it should be noted that on more than one occasion, individuals were seen to be moved by the increase in velocity and were then stopped from being washed out of the experimental arena by a boulder. Over the remaining time in the trial individual *M. margaritifera* would proceed to extend the pedal foot to search the gravel, anchor and then bury into the substrate. The design of the experiment was such that accurate velocity measurements could be taken but casual observations suggest that more investigation to how habitat complexity affects the stability of *M. margaritifera* beds would be useful.

Hydrological variability creates and maintains a temporal and spatial mosaic of habitats available to be exploited by a wide range of aquatic species. The ability of a species to adapt to the environmental dynamism will in turn predict the success of species in an environment where the habitat is periodically destroyed and recreated over a range of time scales, (Poff *et al.* 1997). The two-substrate complexities investigated for this study did not look at a range of variability in habitats to which *M. margaritifera* could be exposed. In any river where unionids are found their existence is patchy across the riverbed. Strayer (1999) looked at the use of flow refuges of four North American freshwater bivalves and found that mussels were found in well defined patches but that water depth, velocity, and grain size did not relate to the location of patches. There was some correlation between the location of mussel beds and areas of low flow refuges, greater numbers of mussels were found in flow refuges than outside. Occupying areas of low flow was found to partially explain how these long-lived species are able to persist in rivers where substrates are regularly mobilised (Strayer 1999). Interestingly for *M. margaritifera* in Scotland, Stayer (1999) found that even where mussel densities and micro habitat variables were correlated within a site they were not between sites, for example mussels could be found in gravel in one watercourse and mud in another.

The experiments completed for my study do not encapsulate the full complexity of behaviours that could be exhibited by *M. margaritifera* in rivers and mussel beds across Scotland in the wide variety of habitats, sizes and locations they are found. Other studies on unionids illustrate the potential to collect similar information on how *M. margaritifera* respond to different flow regimes and Quinlan *et al*. (2014) suggest how field studies could add to our knowledge. In addition to this, flume studies similar to this one could provide a valuable insight into behavioural mechanisms used by *M. margaritifera* in watercourses.

The principal limitation of my study was that only adult mussels were used and therefore the aggregations were probably not representative of how mussel beds are structured. Only two substrate complexities were studied, neither was found to be significant in the determining horizontal or vertical movements of mussels but other studies suggest habitat could be significant. The *M. margaritifera* used were from a relatively slow flowing stable watercourse and as such could be adapted to this habitat and therefore not representative of *M. margaritifera* from watercourses with higher flows or of a more varied nature. No literature exists to suggest an appropriate period of time for *M. margaritifera* to 'right' themselves following disturbance and this may have affected washout of individuals that were ventilating but not buried at the beginning of trials. In addition to this the trials were completed during winter months when mussels are known to be less active.

Critical components of flow regime that regulate geomorphic and ecological processes in river ecosystems are clearly understood. Specific hydrological phenomena can be used to describe, floods and low flow events and thus the integrity of a water course, (Poff *et al.* 1997). The impact on aquatic systems of change in any one component is well documented and summarised by Poff *et al*.(1997). In turn it is these factors along with other associated variables of water quality, quantity, geology, topography, to name but a few that influence the distribution and abundance of riverine species and regulate the ecological integrity of a watercourse. Hastie *et al*. (2000) recommended that a standard habitat description was required for every river containing *M. margaritifera,* this is still to be completed. There remains a gap in in our knowledge about how *M. margaritifera* in Scotland are adapted to the rivers in which they are found and the specific geomorphic and ecological processes that determine habitat suitability. In Scotland *M. margaritifera* are found in east coast and west coast rivers of various size, substrate and character. Each river containing *M. margaritifera* will vary in climate, geology, topography, vegetation cover, and land use practices all of which impact on flow regimes. The human influence on flow regimes,

changing the hydrological variability of watercourses and predictable patterns may be impacting on the success or otherwise of *M. margaritifera* in Scotland.

4 GENERAL CONCLUSION

The principal aim of the first part of my study was to ascertain empirically in the field the preferred salmonid host species utilised by *M. margaritifera* in a selection of Scottish rivers known to contain both *S. salar* and *S. trutta*. This was identified as a gap in our current understanding of the life cycle of *M. margaritifera,* a declining species globally but with an important population found in Scotland. My study looked at encysted glochidia immediately prior to excystment in an attempt to ascertain which host fish species hold glochidia throughout this phase of the life cycle right up to when viable juvenile *M. margaritifera* drop off the gills of the fish. Contrary to the existing literature which suggests that glochidosis is less prevalent in *S. trutta* when compared with *S. salar* (Young & Williams 1984b, Young & Williams 1984a), (Hastie & Young 2001) my study has shown that in five of the rivers surveyed that contained both *S. trutta* and *S. salar* only *S. trutta* were infected with the glochidia of *M. margaritifera*. 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 utilised. In addition to this the infection rate or number of glochidia 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 my study and the conservation of *M. margaritifera* in Scotland my first recommendation would be to establish host preference for each distinct mussel population. Geist *et al.* (2006) investigated *S. trutta* as host fish in watercourses in Germany, Czech Republic, France and Finland. They found that stocking with *S. trutta* had no positive effect on streams investigated. Geist *et al.* (2006) reported that a lack of juvenile *M. margaritifera* and lack of suitable host fish was only rarely observed. Functional *M. margaritifera* populations with relatively high numbers of juveniles had significantly lower densities and biomass of host fish than pearl mussels without recent recruitment. Therefore, it appears that based on the work of Geist *et al*. (2006), increasing numbers of *S. trutta* in watercourses where this species is the primary host species would not improve *M. margaritifera* populations.

My second recommendation, once salmonid species has been established, would be to investigate the genetic strain of salmonid found to be the host for *M. margaritifera*. Taubert *et al.* (2010) reported that infection of glochidia on the gills of suitable host fish was most successful on *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 (Cauwelier *et al*. 2013, Stradmeyer *et al*. 2013, Coulson *et al*. 2013). 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 with any focus on the genetics of *M. margaritifera* in Scotland. In Norway Karlsson *et al.* (2013) reported 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.

In 2014 Douda *et al.* presented an approach for the evaluation of population-led differences in host compatibility of natural populations of dependent species. Their study looked at *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 focussed. 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 my study suggests that *S. trutta* are the primary host fish for *M. margaritifera* my study rivers were all located in the north west 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 my study. Defining distinct management units using the method

outlined by Douda *et al.* (2014) could assist in the prioritisation of resources in a suite of management actions.

The second aim of my study was to investigate the behavioural responses of adult *M. margaritifera* to changes in flow regime. The results of my study showed that rate of change; how quickly flow changes and the velocity: the speed of water, 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 78% 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 many 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. Hastie *et al.* (2000) recommended that a standard habitat description is required for each river that contains *M. margaritifera,* the results from my study suggest that the optimal flow conditions for this species may be even narrower than previously thought and therefore support this recommendation. The need for detailed field observations has also been highlighted by Quinlan *et al.* (2014), without this an understanding of how *M. margaritifera* are adapted to different flow conditions will remain illusive and effectively managing regulated watercourses that contain *M. margaritifera* will be inaccurate at best.

In conclusion my 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 of *M. margaritifera* before management actions can be sufficiently targeted to prevent the continued decline of this species.

5 REFERENCES

Allen, D.C. & Vaughn, C.C. (2009) Burrowing behavior of freshwater mussels in experimentally manipulated communities. *Journal of the North American Benthological Society*, **28**, 93–100.

Allen, D.C. & Vaughn, C.C. (2010) Complex hydraulic and substrate variables limit freshwater mussel species richness and abundance. *Journal of the North American Benthological Society*, **29**, 383–394.

Amyot, J.-P. & Downing, J. (1997) Seasonal variation in vertical and horizontal movement of the freshwater bivalve Elliptio complanata (Mollusca: Unionidae). *Freshwater Biology*, **37**, 345–354.

Arvidsson, B.L., Karlsson, J. & Österling, M.E. (2012) Recruitment of the threatened mussel Margaritifera margaritifera in relation to mussel population size, mussel density and host density. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **22**, 526–532.

Barnhart, M.C., Haag, W.R. & Roston, W.N. (2008) Adaptations to host infection and larval parasitism in Unionoida. *Journal of the North American Benthological Society*, **27**, 370–394.

Bauer, G. (1987) The parasitic stage of the freshwater pearl mussel (Margaritifera margaritifera L.) 3 Host relationships. *Archiv fur Hydrobiologie*, 413–423.

Bogan, A.E. & Roe, K.J. (2008) Freshwater bivalve (Unioniformes) diversity, systematics, and evolution: status and future directions. *Journal of the North American Benthological Society*, **27**, 349–369.

Buddensiek, V. (1995) The culture of juvenile freshwater pearl mussels Margaritifera margaritifera L . In cages: a contribution to conservation programmes and the knowledge of habitat requirements. *Biological Conservation*, **74**, 33–40.

Bunn, S.E. & Arthington, A.H. (2002) Basic Principles and Ecological Consequences of Altered Flow Regimes for Aquatic Biodiversity. *Environmental Management*, **30**, 492–507.

Cosgrove, P.J., Young, M.R., Hastie, L.C., Gaywood, M. & Boon, P.J. (2000) The status of the freshwater pearl mussel Margaritifera margaritifera Linn . in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **10**, 197–208.

Coulson, M.W., Cunningham, P., Armstrong, A., Cauwelier, E., Stradmeyer, L., Gilbey, J., Sinclair, C. & Verspoor, E. (2013a) *Marine Scotland Science Report 12 / 13 An Overview of Population Genetic Structuring of Atlantic Salmon in Wester Ross Fisheries Trust*.

Coulson, M.W., Webster, L.M.I., Baum, D., Armstrong, A., Cauwelier, E., Stradmeyer, L., Gilbey, J., Sinclair, C. & Verspoor, E. (2013b) *Marine Scotland Science Report 11 / 13 An Overview of Population Genetic Structuring in the Lochaber Fisheries*.

Douda, K., Sell, J., Kubíková-Peláková, L., Horký, P., Kaczmarczyk, A. & Mioduchowska, M. (2014) Host compatibility as a critical factor in management unit recognition: population-level differences in mussel-fish relationships. *Journal of Applied Ecology*, 1–11.

Gangloff, M.M. & Feminella, J.W. (2007) Stream channel geomorphology influences mussel abundance in southern Appalachian streams, U.S.A. *Freshwater Biology*, **52**, 64–74.

Geist, J. & Auerswald, K. (2007) Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (Margaritifera margaritifera). *Freshwater Biology*, **52**, 2299–2316.

Geist, J., Porkka, M. & Kuehn, R. (2006) The status of host fish populations and fish species richness in European freshwater pearl mussel (Margaritifera margaritifera) streams. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **16**, 251–266.

Graf, D.L. & Cummings, K.S. (2007) Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies*, **73**, 291–314.

Hardison, B.S. & Layzer, J.B. (2001) Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers. *Regulated Rivers: Research & Management*, **17**, 77–84.

Hastie, L.C., Boon, P.J., Young, M.R., Station, C.F. & Ab, A. (2000) Physical microhabitat requirements of freshwater pearl mussels , Margaritifera margaritifera (L .). *Hydrobiologia*, 59–71.

Hastie, L.E.E. & Cosgrove, P. (2001) The Decline of Migratory salmonid stocks: A new threat to pearl mussels in Scotland. *Migratory salmonids and pearl mussels*, 85–96.

Hastie, L.C. & Young, M.R. (2001) Freshwater pearl mussel (Margaritifera margaritifera) glochidiosis in wild and farmed salmonid stocks in Scotland. , 109–119.

Hastie, L.C. & Young, M.R. (2003) *Conservation of the Freshwater Pearl Mussel 1: Captive Breeding Techniques. Conserving Ntura 2000 Rivers Conservation Techniques Series No.2. English Nature, Peterborough*.

Karlsson, S., Larsen, B.M. & Hindar, K. (2013) Host-dependent genetic variation in freshwater pearl mussel (Margaritifera margaritifera L.). *Hydrobiologia*, **735**, 179–190.

Karna, D.W., Millemann, R.E. & Karnat, D.W. (1978) Glochidiosis of salmonid fishes. III.Comparative susceptibility to natul infection with Margaritifera margaritifera (L.) (Pelecypoda; Margaritanidae) and associated histopathology. *Journal of Parasitology*, **64**, 528–537.

Maio, J. Di & Corkum, L.D. (1997) Patterns of orientation in unionids as a function of rivers with differing hydrological variability. *Journal of Molluscan Studies*, **63**, 531–539.

Meyers, T.R. & Millemann, R.E. (1977) Glochidiosis of salmonid fishes. I. Comparative susceptibility to experimental infection with Margaritifera margaritifera (L.) (Pelecypoda: Margaritanidae). *The Journal of parasitology*, **63**, 728–33.

Moorkens, E.A. & Killeen, I.J. (2014) Assessing near-bed velocity in a recruiting population of the endangered freshwater pearl mussel (Margaritifera margaritifera) in Ireland. *Aquatic Conservation: Marine and Freshwater Ecosystems*.

Morales, Y., Weber, L.J., Mynett, a. E. & Newton, T.J. (2006) Effects of substrate and hydrodynamic conditions on the formation of mussel beds in a large river. *Journal of the North American Benthological Society*, **25**, 664–676.

Nezlin, L.P., Cunjak, R.A., Zotin, A.A. & Ziuganov, V. V. (1994) Glochidium morphology of the freshwater pearl mussel (Margaritifera margaritifera) and glochidiosis of Atlantic salmon (Salmo salar): a study by scanning electron microscopy. *Canadian Journal of Zoology*, **72**.

Österling, M.E. (2011) Test and application of a non-destructive photo-method investigating the parasitic stage of the threatened mussel Margaritifera margaritifera on its host fish Salmo trutta. , **144**, 2984–2990.

Österling, M.E., Arvidsson, B.L. & Greenberg, L. a. (2010) Habitat degradation and the decline of the threatened mussel Margaritifera margaritifera: influence of turbidity and sedimentation on the mussel and its host. *Journal of Applied Ecology*, **47**, 759–768.

Österling, M.E., Greenberg, L.A. & Arvidsson, B.L. (2008) Relationship of biotic and abiotic factors to recruitment patterns in Margaritifera margaritifera. *Biological Conservation*, **141**, 1365–1370.

Paling, B.Y.J.E. (1968) A method of estimating the relative volumes of water flowing over the different gills of a freshwater fish. *Journal of Experimental Biology*, **48**, 533–544.

Poff, N.L., Allan, J.D., Bain, M.B., Karr, J.R., Prestegaard, K.L., Richter, B.D., Sparks, R.E. & Stromberg, J.C. (1997) The Natural Flow Regime A paradigm for river conservation and restoration. *Bioscience*, **47**, 769–784.

Primmer, C.R. (2011) Genetics of local adaptation in salmonid fishes. *Heredity*, **106**, 401–403.

Quinlan, E., Gibbins, C., Malcolm, I., Batalla, R., Vericat, D. & Hastie, L. (2014) A review of the physical habitat requirements and research priorities needed to underpin conservation of the endangered freshwater pearl mussel Margaritifera margaritifera. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **26**, 107–124.

Riusech, F.A. & Barnhart, M.C. (2000) Host suitability and utilization in Venustaconcha ellipsiformis and Venustaconcha pleasii (Bivalvia : Unionidae) from the Ozark Plateaus . , 83–91.

Rogers, S.O., Watson, B.T. & Neves, R.J. (2001) Life history and population biology of the endangered tan riffleshell (Epioblasma florentina Life history and population biology of the endangered tan riffleshell (Epioblasma florentina walkeri) (Bivalvia : Unionidae). *Journal of North American Benthological Scoiety*, **20**, 582–594.

Schwalb, A.N. & Pusch, M.T. (2007) Horizontal and vertical movements of unionid mussels in a lowland river. *Journal of North American Benthological Scoiety*, **26**, 261–272.

Skinner, A., Young, M. & Hastie, L. (2003) *Ecology of the Freshwater Pearl Mussel. Conserving Natura 2000 Rivers*.

Stradmeyer, L., Gilbey, J., Sinclair, C. & Verspoor, E. (2013) *Marine Scotland Science Report 13 / 13 An Overview of Population Genetic Structuring in West Sutherland Fisheries Trust*.

Strayer, D.L. (1999) Use of Flow Refuges by Unionid Mussels in Rivers Use of flow refuges by unionid mussels in rivers. *Journal of North American Benthological Scoiety*, **18**, 468–476.

Taeubert, J.-E., Denic, M., Gum, B., Lange, M. & Geist, J. (2010) Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (Margaritifera margaritifera L.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, **20**, 728–734.

Taylor, E.B. (1991) A review of local adaptation in Salmonidae with particular reference to Pacific and Atlantic salmon. *Aquaculture*, 185–207.

Vaughn, C.C. & Taylor, C.M. (2000) Macroecology of a host-parasite relationship. *Ecography*, **23**, 11–20.

Waller, D.L., Gutreuter, S. & Rach, J.J. (1999) Behavioural responses to disturbance in freshwater mussels with implications for conservation and management. *Journal of the North American Benthological Society*, **18**, 381–390.

Young, M.R. (1991) Conserving the freshwater pearl mussel (Margaritifera margaritifera L.) in the British Isles and Continental europe. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **1**, 73–77.

Young, M.R. & Williams, J. (1984a) The reproductive biology of the freshwater pearl mussel Margaritifera margaritifera in Scotland 2. Laboratory Studies. *Archiv fur Hydrobiologie*, **100**, 29–43.

Young, M.R. & Williams, J. (1984b) The reproductive biology of the freshwater pearl mussel Margaritifera margaritifera in Scotland. 1. Field Studies. *Archiv fur Hydrobiologie*, **99**, 405–422.

Zajac, K. & Zajac, T. (2011) The role of active individual movement in habitat selection in the endangered freshwater mussel Unio Crassus Philipsson 1788. *Journal of Conchology*, **40**, 446–461.

Ziuganov, V., Miguel, E.S., Neves, R.J., Longa, A., Fernández, C., Amaro, R., Beletsky, V., Popkovitch, E., Kaliuzhin, S. & Johnson, T. (2000) Life Span Variation of the Freshwater Pearl Shell: A Model Species for Testing Longevity Mechanisms in Animals. *AMBIO: A Journal of the Human Environment*, **29**, 102.