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Hydrogeomorphic stressing and the response of endangered freshwater pearl mussels, *Margaritifera margaritifera*

A trait-based approach to inform conservation management

By

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The freshwater pearl mussel, *Margaritifera margaritifera*, is considered vital to conserving the ecological integrity of freshwater systems, fulfilling the criteria of a keystone, flagship, indicator and umbrella species. Yet populations of this rare, long lived freshwater bivalve have witnessed substantial declines across the species' Holarctic range. River systems in the Scottish Highlands continue to support large reproductively viable populations; many of which inhabit regulated rivers, managed for hydroelectric energy production. However, there has been limited study concerning the response of *M. margaritifera* to alterations in habitat characteristics resulting from dam operation. Utilising a combination of field and laboratory experiments, this study aimed to address knowledge gaps regarding interactions between *M. margaritifera* and the hydrogeomorphological processes occurring within their habitat, providing urgently needed empirical evidence to drive future conservation strategies implemented by government (NatureScot, SEPA) and utilised by the hydroelectric industry (SSE).

Initial lab-based work sought to derive methods to non-invasively quantify mussel stress. Here, variation in the expression of particular behavioural metrics was examined in accordance with measurements of oxygen consumption, across environmental stressors, and between two freshwater mussel species. Results from this study revealed an increase in the presence of behavioural traits associated with valve activity in response to stress exposure, in accordance with substantial deviations in metabolic functioning of corresponding individuals. This study corroborates previous work highlighting the potential of bivalve filter feeding organisms as indicator species for alterations in habitat conditions. Furthermore, results exhibit the applicability of these techniques to non-invasively quantify physiological stress in Unionid mussels, towards understanding thresholds in response to environmental stressors across individuals, populations and species.

To understand the response of *M. margaritifera* to alterations in flow regime, resulting from drought conditions and dam operation, flume experiments examined the responses *M.margaritifera* from two different populations, to three different rates of drawdown, using two different spatial arrangements. Results demonstrated a propensity of *M. margaritifera* to detect alterations in flow depth, utilising vertical and horizontal movements to avoid prolonged aerial exposure. Data from this study foregrounded intraspecific variation between populations, indicating potential variation in behavioural phenotypic traits. Results from a field trial in a regulated system, using a subset of the corresponding *M. margaritifera* population, endorsed findings from the flume study. Evidence presented in this study advocates for controlled drawdowns in regulated rivers to assist in reducing mortalities associated with receding water levels, during periods of drought.

Finally, this study developed and tested novel smart-sensors housed within mussel shells to provide an affordable, accurate and accessible tool to record near-bed flow dynamics in aquatic systems. The resulting instrumented shells were found to accurately detect, and potentially predict entrainment events in *M. margaritifera*. Entrainment risk was dependent on the flowrate, shell orientation and size; consequently, highlighting the importance of vertical movements in mitigating flow forcing, and the vulnerability of smaller mussels to high flow events. This tool could assist in identifying suitable habitat for *M. margaritifera*, guiding reintroduction and translocation efforts, and identifying at risk populations to surges in flow discharge.

Recommendations are made towards future conservation management of *M. margaritifera* in Scotland, with a focus on adopting a context-driven approach at the population level. Work in this thesis has provided a foundation for the development of future monitoring techniques to improve understanding of *M. margaritifera* habitat requirements, in consideration of hydrogeomorphological processes.

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AUTHOR DECLARATION

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

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Edward Curley

24.03.2021

CHAPTER 1

1 ECOLOGY OF THE FRESHWATER PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*)

1.1 Life cycle

1.1.1 Overview

The freshwater pearl mussel, *Margaritifera margaritifera,* is a species of freshwater bivalve in the order Unionida. This is a large Unionid species, up to 160mm in length with a heavy (>100g) black oval-shaped shell, often eroded at the umbone (apex) (Moorkens 1999; Ziuganov et al. 1994). Literature concerning the physiology of *M. margaritifera* frequently refers to the longevity of its life span, with adult mussels occasionally found to exceed 200 years in age; thus, establishing the freshwater pearl mussel as one of the longest-lived invertebrates in the world (Ziuganov et al., 1994). The life cycle of this species is complicated: comprising an initial phase of development in the female mussel, subsequently followed by an obligate parasitic stage on host salmonid fish, in addition to a prolonged period of post parasitic development in the aquatic environment (Taeubert et al., 2010). Individual maturation usually occurs between 10 and 15 years of age – when the individual's size exceeds 65mm in length (Skinner et al., 2003). Adult mussels usually remain sexually reproductive throughout their lives, with the proportion of individuals able to produce offspring remaining relatively high in most populations (30-60%), even in relatively sparse aggregations (Bauer 1988; Eybe et al., 2015; Hastie & Young, 2003; Young & Williams, 1984).

1.1.2 Pre-parasitic phase

In the early summer months, male mussels will ejaculate the spermatozoa into the water, which females subsequently inhale through their siphons (Fig 1.2). The spermatozoa then migrate into the demi branch where the female's eggs are deposited (Fig 1.1); it is here where fertilisation occurs (Bauer, 1987; Ziuganov et al., 1994). The fertilised eggs develop for several weeks in the marsupia (Skinner et al., 2003) and are eventually released as tiny larvae, known as glochidia (Young & Williams, 1984). The release of the glochidia occurs in a sudden and highly synchronised event across the pearl mussel population (Fig 1.2). Previous studies suggest a threshold in certain environmental cues, such as temperature, may induce this process (Hastie & Young, 2003). Each female releases between 1-4 million glochidia (Hastie, Young, et al., 2000). During this phase of the life cycle, glochidia resemble small-scale adult mussels, with their shells held apart (Bauer, 1987).

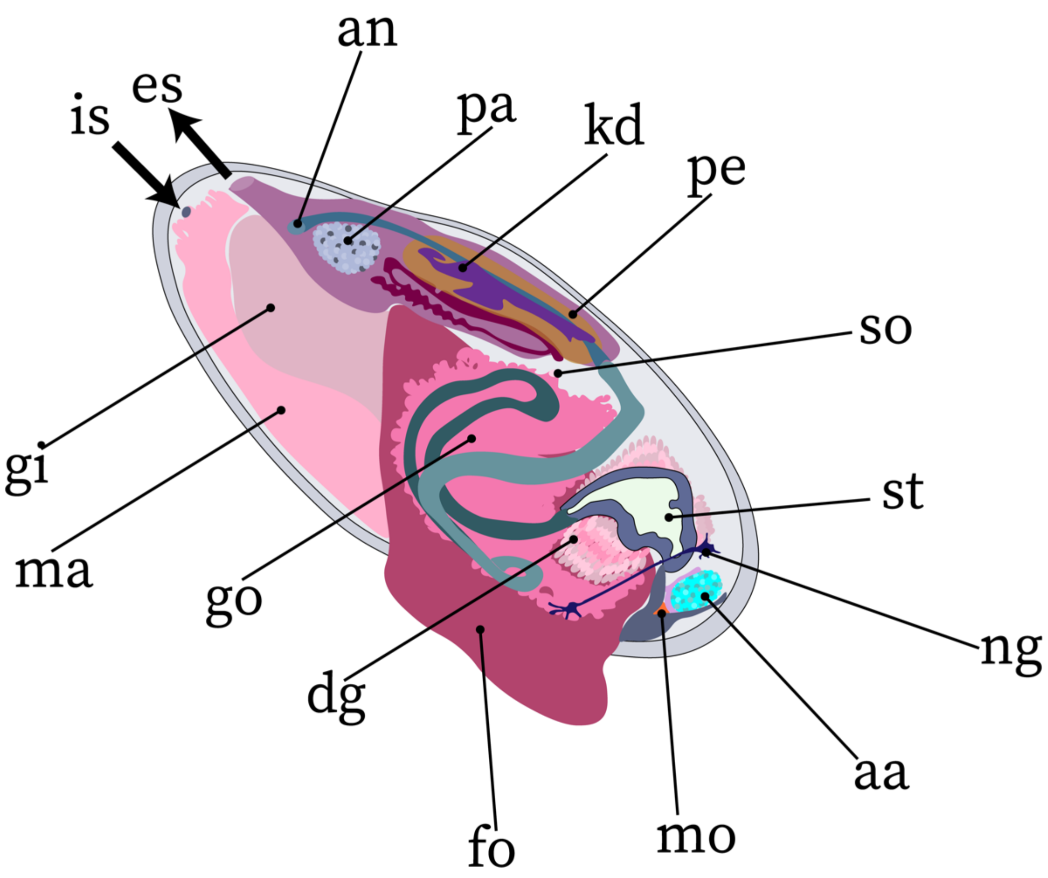

Figure 1.1 The anatomy of a freshwater pearl mussel Margaritifera margaritifera (adapted from Ziuganov et al. (1994)) aa - anterior adductor muscle; an - anus; dg – digestive gland; es – exhalant siphon; fo – foot; gi – gills; go – gonads; is – inhalant siphon; kd – kidney duct; ma – mantle; mo – mouth opening; ng – nervous ganglia; pa – posterior adductor muscle; pe – pericardium; so – sexual opening; st - stomach

1.1.3 Host parasite phase

On release from the gravid females (Fig 1.2), glochidia remain viable for up to six days, during which time they must reach the gill tissue of the host fish (Ziuganov et al., 1994). Those that successfully reach their target hosts, usually do so within hours of release (Young & Williams, 1984). Water currents in conjunction with the natural ventilation systems of the host fish enable the passive transport of glochidia to the gills (Skinner et al., 2003). After inhalation into the host fish (Fig 1.2), the glochidia will proceed to clasp to the epithelial tissues of the gill filaments (Nezlin et al., 1994).Once attached, the glochidia will become rapidly encysted by the surrounding epithelial cells of the host (Nezlin et al., 1994; Taeubert et al., 2013). During this phase of maturation, glochidia grow roughly 6-10 fold in size, and successively metamorphose into juvenile mussels (Ziuganov et al., 1994). As the distributive stage in the freshwater pearl mussel life cycle, it is evident that the glochidia are highly adapted to their role, with modifications to facilitate effective attachment to the hosts. For example, the sharp flange along the ventral border of the shell enables the cutting of the host epithelial tissue during attachment; thus, fixing the glochidia to the gills of the host (Nezlin et al., 1994). Estimates from previous studies suggest that less than 10 glochidia in every million will successfully elicit host infection(Bauer, 1988; Young & Williams, 1984), yet the level of juvenile mortality evoked during this phase of the life-stage is not uncommon, and can be observed even in the most abundant populations (Buddensiek, 1995). The low glochidial success observed in *M. margaritifera* populations is, in theory, compensated for by the low adult mortality and longevity of lifespan and reproductive viability (Skinner et al., 2003).

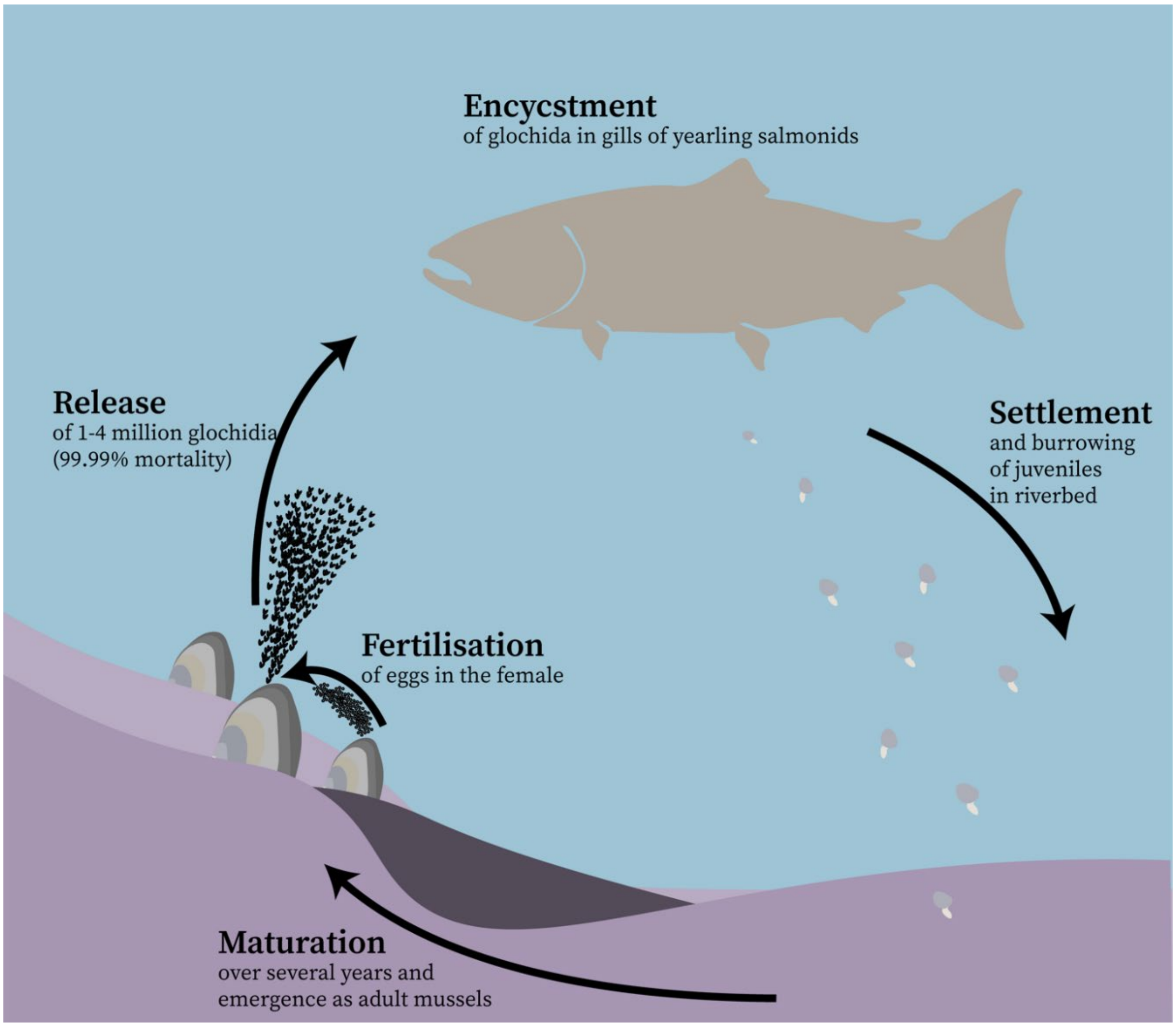

Figure 1.2 Illustration of the lifecycle of the Freshwater pearl mussel (M.margaritifera)

1.1.4 Post-parasitic phase

At 240µm, the juvenile mussel is already fully formed, possessing all the organs characteristic of an adult mussel (Ziuganov et al., 1994). Once this stage of development has been reached, the metamorphosed juveniles will proceed to rupture the surrounding epithelial encystment and become free-living (Ziuganov et al., 1994). The duration of the parasitic stage is highly variable (Ziuganov et al., 1994); however, most juveniles will undergo excystment (Fig 1.2) the following spring (Eybe et al., 2015). During the initial post-parasitic period, juvenile mussels will live completely buried in the substratum of the riverbed’s interstitial zone (Eybe et al., 2015). After several years, the juvenile mussels migrate to the surface (Fig 1.2) of the river bed and begin filter feeding (Taeubert & Geist, 2013; Young & Williams, 1984). This early stage in the juvenile's life is critical for successful survival, and is highly dependent on the physico-chemical characteristics of the surrounding environment (Buddensiek, 1995; Hastie, Boon & Young, 2000; Skinner et al., 2003).

1.2 The Role of Pearl Mussels Within Their Aquatic Habitat

In many of the systems where they are found, freshwater mussels represent the dominant benthic invertebrate species, accounting for 50-90% of the total benthic biomass (Allen & Vaughn, 2010; Strayer & Ralley, 1993). Consequently, freshwater pearl mussels are believed to exert a significant influence over the structure and composition of the aquatic systems they inhabit. According to Geist (2010), *M. margaritifera* fulfil the criteria of an indicator, keystone, flagship and umbrella species (Geist, 2010). Given their complex lifecycle, large size, and sensitivity to environmental alterations, *M. margaritifera* have often been suggested as useful indicators of ecological integrity (Premalatha et al., 2020; Santos et al., 2015). As a keystone species, *M. margaritifera*, directly and indirectly impacts the wider ecologial, hydrological and physical landscapes, with the presence of freshwater pearl mussels believed to significantly enrich aquatic biodiversity and ecosystem functioning (Howard & Cuffey, 2006; Vaughn, 2018). Freshwater pearl mussels have often been recognised for their important role in ecosystems, and as a result have become a central tenet of conservation campaigns; thus, presenting them as a flagship species (Simberloff, 1998). Considerations towards freshwater pearl mussels in conservation schemes, requires a holistic approach, encompassing management at the micro and macro-habitat scales (Strayer et al., 2004). The restoration of river reaches in addition to the wider catchment for the purpose of successfully conserving freshwater pearl mussel populations, is likely to benefit a plethora of aquatic species inhabiting the same ecosystem; hence, highlighting the role of *M. margaritifera* as an umbrella species (Geist, 2010; Simberloff, 1998). With this in mind, the following section will look to examine the mechanisms by which pearl mussels may influence the surrounding environment, with a focus on their filter-feeding function and movement.

Within freshwater ecological communities, *M. margaritifera* principally functions as a filter-feeding organism, accessing planktonic and benthic food supplies (Nichols et al., 2005). The purpose of this filtering activity is to service the mussel's metabolic functioning through gaseous exchange and the absorption of food particles present in the water column (Moorkens, 1999). Food acquisition is facilitated by cilia positioned on the inner mantle surface, demi-branches and visceral mass. The cilia's synchronous movement stimulates water currents both inside and outside of the shell. This creates a continuous supply of freshwater water with replenished oxygen and food, whilst removing waste products (Silverman et al., 1997; Vaughn et al., 2008). As the water enters into the mantle cavity (Fig.1.1) via an exposed inhalant siphon (Skinner et al., 2003), food particles are initially coated in mucus, and then transferred to the digestive grooves for processing (Moorkens, 1999). The water is subsequently passed over a series of gills, where gaseous exchange occurs (Fig.1.1). A set of adductor mussels (Fig.1.1) regulate valve activity (Moorkens, 1999). The adductor mussel comprises a smooth section, facilitating prolonged valve closure during exposure to unfavourable environmental conditions, and a striated section, responsible for enabling rapid valve closure to allow waste ejection from the pallial cavity, and in response to predator attack (Barillé et al., 2000). The size of this muscle has been hypothesised to alter in relation to habitat conditions, with an increasing size shown in response to predator pressure, and prolonged exposure to high turbidity conditions (Dutertre et al., 2009; Theisen, 1982). Actively, individual mussels are able to filter a considerable amount of water in a 24-hour period, with filtration rates ranging between 0.5 - 4.5 L h^{-1} across Unionid species (Atkinson et al., 2013; McIvor, 2004; Pusch et al., 2001; Vaughn et al., 2008).

The substantial biofiltering capacity of this species acts to enhance the aquatic environment for other species; thus, providing an important ecosystem service and demonstrating the role of *M. margaritifera* as a keystone species (Vaughn, 2018). For example, the removal of particles from the water column by filter feeding can substantially reduce phytoplankton and suspended matter, thereby improving the water quality (Lummer et al., 2016; Reid et al., 2013). The filtering of suspended matter, and subsequent biodeposition of the high-quality pelagic resources, elicits a link between the benthic and pelagic environments via the transferal of energy and nutrients from the water column to the sediment (Chowdhury et al., 2016; Vaughn, 2010; Vaughn & Hakenkamp, 2001). The impact of the energy and nutrient input by freshwater mussels is thought to cascade through food webs, with up to 74% of nitrogen within aquatic food webs attributable to mussel excretion processes (Atkinson et al., 2013). This stimulates algal and macroinvertebrate production (Allen et al., 2012; Black et al., 2017; Howard & Cuffey, 2006; Vaughn et al., 2008), and alters the species composition of these communities (Chowdhury et al., 2016; Vaughn & Hakenkamp, 2001). The biodeposition of organic carbon may also facilitate primary and secondary production (Strayer, 2014). Thus, freshwater mussels play an important role in providing regenerated nutrients, particularly in nutrient-limited systems, by enhancing the heterogenous distribution of nutrients across spatial and temporal scales to create "hot spots and hot moments" in production and biogeochemical cycling (McClain et al., 2003; McIntyre et al., 2008; Strayer, 2014).

Freshwater mussel species are often deemed to be sedentary organisms. In rivers, mussels are frequently found to reside within dense assemblages, termed 'mussels beds' (Fig. 1.3), which are patchily distributed and interspersed with regions where mussel occurrence is substantially more sparse (Atkinson & Vaughn, 2015; Strayer, 2008). These self-organised structures are thought to enhance near-bed turbulence, generating greater hydrodynamic habitat complexity (Sansom et al., 2020; Sansom, Atkinson, et al., 2018), in addition to aiding the resuspension of microphytobenthos; thus, facilitating the provision of food (Widdows et al., 2009). Despite studies suggesting mussel beds are static and may persist for decades (Haag, 2012), there is a growing body of evidence to suggest these beds may be more dynamic (Sansom, Bennett, et al., 2018).

Despite their perceived sedentary existence, there is plenty of evidence to suggest that mussels do move and that movement can have a substantial impact on the aquatic environment (Amyot & Downing, 1997; Bartsch et al., 2010; Block et al., 2013; Gough et al., 2012; Johnson & Brown, 2000; Lymbery et al., 2020; Newton et al., 2015). Studies have shown that *M. margaritifera* and similar Unionid mussel species are capable of navigating through the environment by pumping haemolymph through their foot. This enables movement along the riverbed (horizontal movement) and burrow (vertical movement) into the substrate (Clements, 2015; Gough et al., 2012; Lymbery et al., 2020; Newton et al., 2015; Skinner et al., 2003).

Burrowing behaviour has received particular interest within the literature, with adult mussels often observed to burrow into the sediment with two-thirds of their shell submerged in the riverbed substratum, filtering water up to 3cm above the riverbed (Moorkens & Killeen, 2014). Burrowing is understood to be influenced by substrate grain size (Cândido & Romero, 2007; Hyvärinen et al., 2021), and alters with season (Amyot & Downing, 1997; Watters et al., 2001), flow (Gough et al., 2012), disturbance (Lewis & Riebel, 1984), reproductive cycle (Amyot & Downing, 1997), and species composition (Allen & Vaughn, 2009). By burrowing into the substrate mussels are able to anchor themselves and thus avoid potential entrainment due to high flow regimes (Allen & Vaughn, 2009; Sansom, 2018; Thompson et al., 2016). Burrowing may also act as a response to increasing water temperature (Block et al., 2013), and instances where individuals are exposed to air (Lymbery et al., 2020).

The protrusion of the shell provides substrate for epibiont attachment and contributes to habitat complexity, whilst interstices between shells may provide refuge for benthic organisms (Allen & Vaughn, 2009; Gutiérrez et al., 2003; Hopper et al., 2019). The movement of mussels, both horizontally and vertically, assists in sediment mixing, increasing the sediment water content and facilitates oxygen penetration into the riverbed substratum (McCall et al., 1995). The presence of a more oxygen-rich environment within the riverbed substratum is anticipated to alter the microbial communities, with a heightened presence of obligate aerobe and facultative aerobe bacteria (Boeker et al., 2016). Furthermore, the vertical orientation of mussels may stabilise fine-grained sediments, and hence increase the habitat suitability for other organisms (Strayer et al., 2004; Strayer & Ralley, 1993; Vaughn & Hakenkamp, 2001).

Differences in shell morphology, rates of biodeposition and behaviour have been suggested to influence the extent to which freshwater mussels may modify the biogenic structure and hydrogeomorphological characteristics of the habitat (Daniel & Brown, 2014; Vaughn et al., 2008). Substantial variation in the size and sculpture of mussels has been documented between species (Spooner & Vaughn, 2006; Watters, 1994). Preston *et al.,* (2010) also documented variation at the intraspecific level: *M. margaritifera* from two different populations displayed significantly different shell shapes. Differences in shell morphology are understood to be driven by hydrologic conditions, with species adapting their morphology to cope with turbulent flow dynamics by developing shell characteristics that aid in the inhibition of displacement, or the rapid re-positioning to a favourable orientation after dislodgement (Bartsch et al., 2010; Hornbach et al., 2010; Randklev et al., 2019; Watters, 1994). Variation in size and shape would likely alter the extent to which mussels stabilise sediments, and could also be indicative of differences in behavioural traits. Recent studies have highlighted significant differences in behavioural responses of Unionid species to the same hydrological stressors (Gough et al., 2012; Lymbery et al., 2020; Meysman et al., 2006; Saarinen & Taskinen, 2003). Despite this, the extent to which *M. margaritifera* may deviate from the preconceived sessile lifestyle is currently unknown; thus, their overall impact on the surrounding ecology is not yet fully understood.

In addition to uncertainty concerning the potential pathways through which *M. margaritifera* may influence the surrounding ecology in their habitat, there remains a lack of evidence pertaining to the density of mussels required within a system to evoke habitat alterations redolent of an ecosystem engineer, and keystone species. Despite the acknowledgement that, universally, *M. margaritifera* promote biodiversity within inhabited aquatic systems (Geist 2010), at smaller scales the magnitude and direction of their effects are likely to vary depending on an amalgam of variables related to their trophic relationships within the ecosystem (Phillips et al., 2021; Harley & O'Riley, 2011; de Moura Queirós et al., 2011; Bell & Cuddington, 2019), the phenotypic characteristics of the population (Vozzo et al., 2021), and the environmental conditions (Vozzo et al., 2021; Commito et al., 2005; de Moura Queirós et al., 2011). For example, the riverbed structure and composition will determine the availability of suitable habitat for mussel settlement and propagation: in armoured riverbeds, a lack of fine gravels will prevent burrowing by mussels, and thus impede population propagation in the reach due to an inhibition of juvenile settlement (Wegscheider et al., 2019). In such environments, dense mussel beds are unlikely to form; hence, limiting the impact *M. margaritifera* may exert on the near-bed hydrodynamic habitat, and consequently reducing their role in governing habitat suitability for associated benthic macroinvertebrates and fish species (Sansom et al., 2020). The relationship between mussel density and their impact on the surrounding ecology is likely to be non-linear, with their importance as ecosystem engineers potentially emerging above a certain threshold in abundance; a relationship often observed in other keystone ecosystem engineer species (Harley & O'Riley, 2011; Kimbro & Grosholz, 2006; Baxter & Getz, 2005). It is, therefore, important to consider how *M. margaritifera* respond to alterations in their environment, and how variation in habitat conditions cascades to influence the role of *M. margaritifera* in associated communities, towards identifying when and where positive species interactions will be greatest.

1.3 Habitat Requirements

Previous studies have often debated the suitability of habitat and its influence on the dynamics of the associated freshwater communities. In the past, researchers have attempted to elucidate the crucial factors governing the spatial variation and abundance of Unionid populations. In order to deduce these environmental parameters, previous studies have often looked for positive associations between the environmental characteristics of habitat and the condition (e.g. presence, abundance, recruitment) of *M. margaritifera* populations (Tarr, 2008). The freshwater environment is, however, a complex system comprising an intricate web of interconnected variables. As such, previous research has often struggled to identify the particular characteristics that largely define the community dynamics. Here, the ecological parameters have been divided into two components: hydrogeomorphological characteristics, and the biological environment. These environmental parameters are not mutually exclusive in shaping the surrounding freshwater ecology, with evidence to suggest substantial overlap in their effects. However, for the purpose of providing clarity, the following sections will attempt to focus on each as an individual entity.

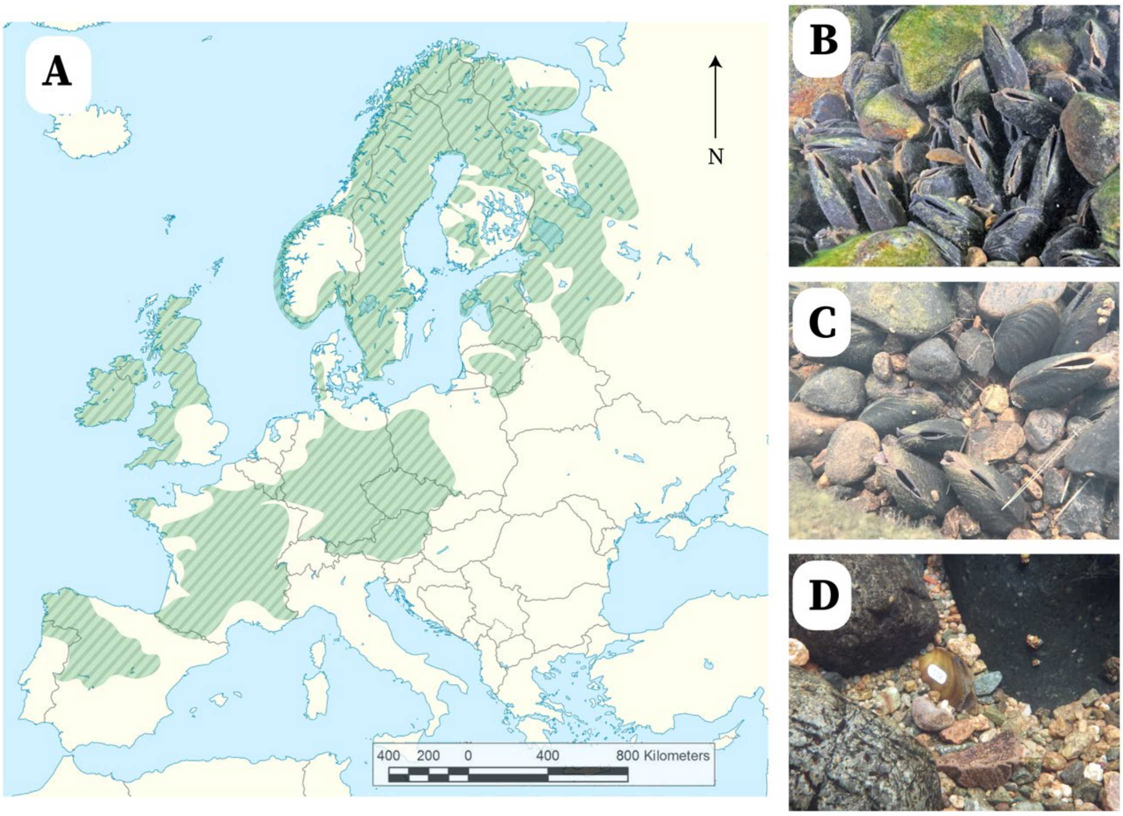

Figure 1.3 The European distribution range of Margaritifera margaritifera. (A) Map of Europe, with the green hatching illustrates the approximate distribution range of the species, adapted from Lopes-Lima et al., (2017). Images illustrating different extents of mussel colonisation of riverbeds, ranging from a high-density mussel bed (B), and medium-density bed (C), to a single mussel inhabiting a patch of gravel sheltered amongst larger boulders (D). *Images of mussel beds from following sources: (B) https://nerc.ukri.org/planetearth/stories/1283/; (C) https://www.pearlmusselproject.ie/freshwater-pearl-mussel.html; (D) Chris West, Cumbria Wildlife Trust.*

1.3.1 Hydrogeomorphological Characteristics

Attention towards *M. margaritifera* in the literature has often focused on its ecological contribution and the importance of conservation efforts in maintaining the presence of this species, for the purpose of upholding the ecological integrity of corresponding river systems. However, the role of hydrogeomorphic processes in governing habitat suitability, for the persistence of functional pearl mussel populations, is still to be fully understood. When accounting for the influence of these processes on the persistence of freshwater pearl mussel populations, previous studies have focused on flow regime, bed stability and connectivity, levels of suspended sediment and the concentration of certain chemicals as key environmental parameters. Yet these characteristics are often examined independently, with few studies examining the interactions between physical and hydrologic characteristics.

Previous research examining the presence of mussel beds and the associated microhabitat characteristics, suggests mussels persist in areas of the riverbed that experience relatively lower boundary shear stresses at relatively high flow rates (Gangloff & Feminella, 2007; Hastie, Boon, et al., 2000; Layzer & Madison, 1995; Quinlan et al., 2015; Scheder et al., 2015; Strayer, 2016). Despite these findings, there is currently no absolute consensus regarding the role of depth and velocity parameters on the condition of freshwater pearl mussel populations.

Very few studies concerning *M. margaritifera* have analysed the near-bed flow regime, arguably the most ecologically relevant data for benthic biota, and often shown to be crucial in determining their spatial distribution (Blanckaert et al., 2013; Long et al., 2011; Oldmeadow et al., 2010; Robson et al., 1999). Hastie et al., (2000) measured velocities at 60% water depth at low, medium and high discharge within a regulated system in Scotland. The results of this study suggested optimum conditions for the species are present at water depths between 0.3-0.4m and flow velocities of 0.25-0.75m s^{-1}. However, this work did not account for near-bed flow dynamics and ultimately neglected the flow conditions most applicable to a benthic dwelling species. Further study by Moorkens & Killeen, (2014) sought to rectify this knowledge gap, conducting research that examined the near-bed flow velocities across mussel beds with varying population densities. Results from this study suggested near-bed flow velocities were higher across mussel beds with greater densities, with optimum habitat and bed densities associated with near-bed flow rates ranging from 0.27-0.31m s^{-1}. These were attributed to mussel beds in good condition. Furthermore, their findings suggest that water height may not be a crucial factor in determining habitat suitability, provided that mussels are covered by a reasonable depth of water (>0.15m). However, it is difficult to discern if the recorded high flow conditions are not a consequence of the enhanced near-bed turbulence caused by alterations in hydrodynamic forcing and skimming flow over the mussel beds (Sansom et al., 2020; Widdows et al., 2009). Moreover, the propellor-based current meter and single point measurement method, may inhibit the accuracy and precision required to fully understand the complex flow dynamics within these regions of the river.

Despite the need for further data relating to the near-bed flow dynamics, inferences can be made towards the types of flow regime believed to solicit more favourable habitat. At lower discharges, organic and inorganic particles previously suspended in the water column, are likely to be deposited on the riverbed. This process of sedimentation may facilitate the formation of algal mats and the subsequent suffocation of mussels (Matthaei et al., 2003). Furthermore, sedimentation reduces the mixing between the interstitial zone and the water column: a critical parameter in aiding the survival of juvenile mussels (Lavictoire et al., 2020). Additionally, flow velocities below a certain threshold will substantially lower the dissolved oxygen levels, whilst imposing greater heat stress on benthic organisms (Skinner et al., 2003). Therefore, moderate flow regimes are important in facilitating the cleaning of silts and algal accumulation from the interstitial spaces, thus ensuring the removal of potentially harmful fine material and the maintenance of channel geomorphic integrity (Quinlan et al., 2015).

At high flow velocities there is a risk of dislodgement, resulting in mortality (Hardison & Layzer, 2001; Hastie, Young, et al., 2000; Hauer, 2015) or substantial energetic expenditure: in response to high velocities mussels will often display defensive behavioural responses such as aggregation and burrowing (Tošenovský & Kobak, 2016). Moreover, high flow velocities may cause modifications to the physical habitat: the erosion and entrainment of bed and bank substrate during scouring events leads to the loss of suitable habitat (Skinner et al., 2003; Thompson et al., 2015). Morales et al. (2006) attempted to examine the effects of hydrodynamic conditions on the habitat suitability of Unionid mussel beds, and found that the magnitude and duration of flow elicited long-term effects on the mussel populations. The results of this work, and similar research, suggest that high flow regimes limit the availability of suitable habitat for juvenile mussels to settle, consequently hindering the functional capacity of populations to reproduce effectively (Allen & Vaughn, 2010; French & Ackerman, 2014; Gangloff & Feminella, 2007; Morales et al., 2006; Randklev et al., 2019).

Examination of the physical environment towards understanding the parameters governing the quality of freshwater pearl mussel habitat have often associated with the theory that the relative stability of riverbeds over extended periods of time (i.e. decades) evokes successful mussel persistence (Geist & Auerswald, 2007; Goodding et al., 2019; Hastie, Young, et al., 2000; Johnson & Brown, 2000; Scheder et al., 2015; Skinner et al., 2003; Zigler et al., 2008). Geist & Auerswald (2007) attempted to understand the physical parameters governing the quality of freshwater pearl mussel habitat using the recruitment of *M. margaritifera* as a biological indicator. The results of this study identified the substrate composition and structure in riverbeds as a critical determinant of condition for the associated ecological communities. Substratum parameters were shown to be powerful predictors of functional capacity in freshwater pearl mussel populations, with concurrence shown in similar studies (Skinner et al., 2003). Adult and juvenile mussels have often been found to occupy analogous regions of the riverbed, displaying relatively similar preferences to substratum characteristics (Hastie et al., 2000). Additionally, substratum parameters are thought to be less prone to short-term fluctuations (Geist & Auerswald, 2007). As such, factors associated with the composition and structure of the substratum are believed to offer suitable modelling tools to assess habitat quality.

Attempts to define the parameters responsible for ensuring substrate stability, require consideration of the substratum composition and structure, in addition to the wider channel structure of the freshwater environment. Previous studies have often considered characteristics associated with channel structure, such as depth and gradient, as potential determinants of substratum stability (Degerman et al., 2009; Gangloff & Feminella, 2007; Quinlan et al., 2015; Skinner et al., 2003). Quinlan et al. (2015) predicted that, at a uniform flow, depth and gradient are likely to govern the shear stress exerted at a given point: areas experiencing greater shear stress are often found in deeper regions of the waterbody; consequently, these regions are unlikely to provide the necessary stability required for *M. margaritifera* colonisation (Quinlan et al., 2015). With regards to substrate structure and composition, Geist & Auerswald, (2007) suggested small patches of well-sorted silt, stabilised by a surrounding bed of larger pebbles and boulders, fulfil the need for both quality and stability; a view commonly shared by other authors (Addy et al., 2012; Jung et al., 2013; Skinner et al., 2003; Varandas et al., 2013). Here, larger substrata consolidate fine sediment, in a mechanistic process known as the ‘hiding effect’, by reducing the hydraulic forces experienced within adjacent patches of substratum (Addy et al., 2012; Knighton, 1998; Quinlan et al., 2015). This substratum structure enables *M. margaritifera* aggregations to colonise regions of the riverbed exposed to high flow regimes, which would otherwise present substantial risks of entrainment (Strayer, 2016). The hydraulic nature of these environments prevent sediment compaction, whilst ensuring the maintenance of high exchange rates at the hyporheic zone; hence, preserving channel geomorphic integrity (Addy et al., 2012; Quinlan et al., 2015).

The paradigm of sediment stability in mussel conservation, advocating that significant areas of the riverbed remain immobile for such expansive periods of time, juxtaposes with fundamental theory in the field of riverbed sediment transport. A key principle in fluvial geomorphology pertains to dominant or channel-forming flow discharge, whereby a natural river channel may reach bankfull flow conditions and the channel morphology is governed by the erosional potential of the flow event; thus, the river is self-formed (Williams, 1978). These self-formed channels do exist, characterised by prolonged stability in the channel bed composition and structure, and displaying partial bed mobility with very slow morphologic change. Yet such channels are relatively small in scale and often restricted to areas displaying high-gradients (Church, 2006). Contrastingly, labile and transitional channel beds, which often experience bed mobility, are more common and have frequently been shown to provide habitat for freshwater mussel species globally (Geist, 2010; Haag, 2012; Quinlan et al., 2015; Strayer, 2008).

The methods used by freshwater ecologists to determine substrate stability may also be insufficient for determining the risk of substrate entrainment. The standard and frequently employed method for the identification of incipient motion flow conditions pertains to Shield's critical shear stress criterion. However, a review by Buffington & Montgomery (1997) illustrated the limitations of such methods, with threshold of motion results exceeding an order of magnitude in some field and laboratory trials. Additionally, the inherent subjectivity in calculations regarding a threshold for mobilisation of sediment grains (Papanicolaou et al., 2002), further limits the propensity of this deterministic treatment of turbulent flow processes to accurately describe incipient flow dynamics (Valyrakis et al., 2011).

Despite concerns regarding the applicability of substrate stability as a determinant of habitat suitability, it is clear that freshwater mussels are reliant on the quality, composition, structure and transport of substratum within the freshwater environment. For burrowing to occur, the mussels (Hastie et al., 2000) require a suitable medium; with the abundance of fine interstitial sediments in the habitat shown to be particularly important for juveniles (Geist & Auerswald, 2007; Hastie et al., 2000). Studies have suggested that the substrate type can alter the behaviour of juvenile *M. margaritifera* (Hyvärinen et al., 2021). However, based on this single parameter, previous studies have often failed to present a positive relationship between the presence of functional populations of *M. margaritifera* and high percentage cover of fine sediment (Hastie et al., 2003). Habitats dominated by fine sediments alone are unsuitable for mussels: they are unlikely to provide adequate protection against entrainment from high flow regimes (Geist & Auerswald, 2007; Hastie et al., 2000). Additionally, in the absence of high flows, these habitats are susceptible to sedimentation and subsequent clogging of the macropore system (Morales et al., 2006; Quinlan et al., 2015; Tarr, 2008), with evidence to suggest the presence of excess fine sediments causes stress in juvenile mussels, which respond by surfacing; consequently, exposing them to risks from predation and entrainment (Hyvärinen et al., 2021).

The early-post settlement period for juvenile *M. margaritifera* is particularly sensitive to physical characteristics of habitat (Hastie, Young, et al., 2000; Lavictoire et al., 2020). During this phase of development, juveniles require a structure that enables exchange between the water body and the interstitial zone (Jung et al., 2013; Skinner et al., 2003). This physical connectivity of free-flowing water and the interstitial zone is often cited as a critical parameter in defining the quality of habitat (Geist & Auerswald, 2007). Areas of the riverbed, where water is able to penetrate the bed substratum, constitute the hyporheic zone. It is within this zone that a large proportion of the total respiration for a freshwater community occurs (Degerman et al., 2009; Geist & Auerswald, 2007).

Insufficient exchange between the two components of the hyporheic zone will generate anoxic conditions within the river substratum, which could be detrimental to the associated aquatic communities (Geist & Auerswald, 2007). The composition and structure of the river bed substratum is, therefore, largely responsible for facilitating the establishment of a rich fauna (Degerman et al., 2009). Structures in the water, such as pebbles and wood, govern the extent of percolation and thus the rate of exchange occurring within the hyporheic zone. This is most prominent when pebbles and gravel dominate the riverbed (Degerman et al., 2009; Varandas et al., 2013; Wilson, Roberts & Reid, 2011). Substratum comprising of pebbles and gravel is particularly crucial in providing an interface between the riverbed and hyporheic interstices, necessary for successful juvenile pearl mussel development (Cosgrove et al., 2016; Jung et al., 2013). Contrastingly, habitat dominated by clay soils with high levels of sedimentation, are often restricted in exchange due to a substantially inhibited macropore system (Geist & Auerswald, 2007). Additionally, saturated clay particles may act as pollutants when contaminated surface waters leach into the ground water (Wilson et al., 2011). As such, it is no surprise that very few authors have reported functional populations of *M. margaritifera* existing in habitat dominated by fine sediment or peat (Cosgrove & Harvey, 2004; Geist & Auerswald, 2007). However, the precise association between physical connectivity and mussel presence and abundance requires greater clarity, particularly when considering the levels of deposited fine sediment necessary to surpass the tolerance levels of pearl mussels.

The transportation of fine particulate matter is a principal component of freshwater systems and constitutes a naturally occurring phenomenon (Kemp et al., 2011); however, high suspended sediment loads may lead to habitat degradation and can significantly impact populations of benthic filter-feeders (Österling, Arvidsson & Greenberg, 2010; Österling & Högberg, 2013). High levels of suspended sediment can subject benthic invertebrates to scouring and abrasion, as well as potentially damaging exposed feeding structures and respiratory organs (Bilotta & Brazier, 2008). Currently, levels of fine sediment, associated with the persistence of functional freshwater pearl mussel populations, have not been adequately categorised.

Previous studies examining habitat degradation due to high levels of suspended fine sediment, have often relied on measurements of water turbidity (Gosselin, 2015; Österling, 2014; Österling et al., 2010). Yet turbidity is a convoluted measure of water quality, masked by the physical and optical characteristics of the fine sediment particles (e.g. the size, shape and composition of particles) in addition to the degree of dissolved colour (e.g. organic substances) (Cavanagh, Hogsden & Harding, 2014; Henley et al., 2000). Moreover, these properties may exhibit significant variance within and across freshwater systems and over time (Bilotta & Brazier, 2008). As such, experimental analysis may often fail to replicate the natural complexity of fine sediment transport in freshwater systems. Despite observing significant variation in the categorisation of 'optimal levels' of suspended fine particulate matter in the literature, there is a definitive consensus towards the negative effects of increased sediment load and dissolved colour on the condition of Unionid mussels (Österling et al., 2010; Quinlan et al., 2015). The benthic, post-parasitic juvenile stage appears to be the most susceptible to high sediment loads in the water column. Österling et al. (2010) advocated the process of sedimentation as a key mechanism, complicit in eliciting the negative effects of high sediment loads on juvenile mussels. Here, Österling et al. (2010) suggested that sedimentation of high volumes of fine particulate matter could inhibit the flow of water in riverbed interstices through colmation, resulting in diminished levels of oxygen and pH, as well as elevated levels of ammonia.

In light of the aforementioned, oligotrophic waters with flow regime that provide adequate near-bed velocities to inhibit sedimentation, whilst also remaining below critical shear stress values that induce substantial riverbed mobility, appear to provide optimal habitat conditions for freshwater pearl mussels. When defining the preferred physical environment for *M. margaritifera* populations, it is apparent that one must take into account the quality of the substrate and the structural nature of the riverbed. Freshwater pearl mussels require substratum which permits physical connectivity at the hyporheic zone (Geist & Auerswald, 2007), in addition to suitable hydraulic refugia that prevents dislodgement from favourable environments (Gangloff & Feminella, 2007). Nevertheless, given the coarse and sometimes unsubstantiated nature of research on this subject, it is hard to provide exact measures of hydrogeomorphological characteristics with which to generate accurate guidelines for mapping suitable habitat for *M. margaritifera* populations. Further work is required to understand the near-bed flow characteristics that provide suitable habitat, with a greater focus on mussel behavioural responses to alterations in the hydrologic and geomorphic environment.

1.3.2 Biological Interactions

The reasons for observed variation in *M. margaritifera* population abundance, due to compositional differences in the associated ecological communities, are often difficult to differentiate from alterations in the physico-chemical nature of the corresponding environment. Nevertheless, when deciphering the biological processes governing the habitat suitability for pearl mussel colonisation, previous studies have often focused on the role of fish host populations and the composition of flora in the habitat.

At the meta-habitat scale, the composition of vegetation largely determines the role and effectiveness of the ecological buffer zone neighbouring a freshwater system. This zone constitutes the littoral environment, as well as the surrounding dry land, which influences the surface water (Degerman et al., 2009). The size of a buffer zone will vary between freshwater systems displaying differences in topography, hydrology and geomorphology (Degerman et al., 2009). When considering the role of the buffer zone, in regulating the condition of the freshwater habitat for pearl mussels, previous literature has predominantly focussed on its influence over the following parameters: sediment and nutrient inputs, macrophyte growth, and the hydrological regime (Hastie et al., 2003; Morales et al., 2004; Skinner, Young and Hastie 2003; Tarr, 2008).

A positive association between the presence of certain vegetation and the abundance of *M. margaritifera* has been cited in previous research: Hastie et al. (2003) described a strong positive association between aquatic mosses, liverworts and lichens with functional populations of freshwater pearl mussels. However, these species of vegetation are unlikely to have a direct association with mussel beds; instead, they are a product of the corresponding environment and indicative of its physico-chemical nature. Nevertheless, riparian tree species, such as alder (*Alnus glutinosa*), located on the riverbanks may directly alter the characteristics of the freshwater pearl mussel habitat (Degerman et al., 2009; Hastie et al., 2003; Wilson, Roberts & Reid 2011). The presence of trees at the riverbank may aid in the stabilisation of the substrate; thus, minimising the amount of sediment entering the waterbody due to erosion. Additionally, the shade offered by trees could prevent significant temperature fluctuations in the water column (Hastie et al., 2004) and inhibit the growth of aquatic plants and filamentous algae in the river channel (Degerman et al., 2009). The extent of shade required to exert this influence has been estimated to be between 60-100% (Degerman et al., 2009), and may alter with respect to nutrient levels, air temperature and flow regime (Skinner et al., 2003).

When assessing the relationship between the abundance of unionid mussels and the presence of aquatic vegetation, previous studies have often found a negative association; with functional populations of *M. margaritifera* rarely observed in areas of the riverbed displaying dense macrophyte stands (Burlakova & Karatayev, 2007; Schultz & Dibble, 2012; Tarr, 2008). Reasons for this observed association are often unclear and unsubstantiated with experimental validation. It is likely that the impact of macrophytes on freshwater mussels is mediated by the size and density of the macrophyte beds, in addition to broader habitat characteristics such as flow regime, and natural seasonal and diurnal variations, which may elicit both positive and negative effects on associated unionid communities, depending on the circumstances (Moore, Collier & Duggan, 2018).

The profuse branching that occurs within compact macrophyte stands generates structural complexity in the waterbody, and restricts water movement (Burlakova & Karatayev, 2007). As a result, dense macrophyte beds may provide shelter from flow forcing, with macrophyte structures shielding near downstream regions from high shear stresses (Preiner et al., 2020) that may elicit mussel entrainment. However, alterations to the flow regime within dense macrophyte stands subsequently affect the processes that govern the nature of the physical environment, such as the erosion and re-suspension of sediments. For example, the trapping of fine sediments within dense macrophyte stands (Cronin et al., 2006) may lead to the clogging of interstitial pores within the riverbed substratum, thus degrading habitat conditions for adult and juvenile *M. margaritifera*, and potentially causing suffocation, as observed in the River Spey following the introduction of *Ranunculus* species (Sime, 2014). This issue is further exacerbated when considering that dense root masses of the macrophyte beds may trap mussels in the riverbed, preventing movement away from the unfavourable conditions (Moore, Collier & Duggan, 2018). Furthermore, dense root systems prevent the burrowing of juvenile mussels into the sediment; thus, prohibiting future mussel colonisation in sections of riverbed inhabited by dense macrophyte beds (Burlakova & Karatayev, 2007).

The presence of macrophytes has often been attributed to an increase in water quality with macrophytes shown to assimilate nitrogen and phosphorus from the water column (through their foliage) and the river sediments (through their roots) (Preiner et al., 2020; Gyosheva et al., 2020). Consequently, macrophytes may provide a valuable ecosystem service by dampening the effects of nitrogen and phosphorous loading into riverine systems due to catchment activities, and hence mitigate potential eutrophication within lotic habitat, which is potentially lethal to freshwater mussels (Racchetti et al., 2017). However, dense macrophyte stands may produce environmental micro-gradients within the water column (e.g. light, pH, temperature and dissolved oxygen); eliciting modifications to the habitat, and the composition of associated flora and fauna (Schultz & Dibble, 2012 ;Burlakova & Karatayev, 2007; Van Donk & Van de Bund, 2002). For example, macrophytes have been shown to alter the abundance of food resources for freshwater mussels, such as phytoplankton, cyanobacteria and algae, through competition for resources (e.g. nitrogen, phosphorous, light), as well as directly supressing their growth through the secretion of allelopathic chemicals (Bakker et al., 2010; Erhard & Gross, 2006). Additionally, the stratification of dissolved oxygen in the water column may cause a reduction in dissolved oxygen near the bed surface, impacting the survival of juvenile and adult mussels (Wright et al., 2012); work by Geist and Auerswald (2007) denoted the importance of redox potential in determining habitat suitability for *M. margaritifera*. The stagnation of river flow in vegetated regions is also likely to limit the ability of adult mussels to filter feed effectively (Kumar et al., 2019), with lower flow velocities reducing nutrient turnover, which will impact individual metabolic processes, with implications towards reproductive capabilities and long-term persistence .

The mass senescence of macrophytes at the end of summer, constitutes another mechanism by which macrophytes adversely impact freshwater mussel populations (Moore, Collier & Duggan, 2018). This mass die-off of macrophytes at the end of the summer can cause an accumulation of dead organic matter in aquatic systems, culminating in a reduction in redox potential and dissolved oxygen concentrations (Lopes-Lima et al., 2016). An example of this process was documented within a population of swan mussels (*Anodonta cygnea*), where high mortality was observed across three populations located in the Iberian Peninsula following the mass senescence of water hyacinth, Eichhornia crassipes (Lopes-Lima et al., 2016).

Macrophytes may also impact freshwater mussels via interactions with host salmonid fishes. Host fish may gain benefits from the presence of macrophytes, which support large invertebrate communities and thus offer a potentially important source of food (Monahan & Caffrey, 1996). Yet the response of fish species to differing macrophyte densities is species specific, with some preferring dense vegetation, whilst others thrive in sparser macrophyte aggregations (Johnson et al., 1988). Regardless of species, a threshold in macrophyte density exists, beyond which the foraging efficiency of fish decreases with increasing macrophyte density (Schultz & Dibble, 2012). The presence of dense macrophyte beds may generate large sand deposits downstream, which are likely to clog interstitial spaces required by host salmonid eggs, alevins and fry (Laughton et al., 2008). Furthermore, root systems within these vegetated regions are likely to consolidate sediments, preventing female salmon from creating redds during spawning (Laughton et al., 2008).

In addition to the indirect impacts of macrophytes on fish ecology, predominately fostered via modifications to the hydrogeomorphic processes within aquatic habitat, macrophyte species may also directly impact fish condition. For example, some invasive macrophyte species secrete allelopathic chemicals, which are known to directly impact fish growth and survival, with individuals shown to avoid areas of the river reach where dense stands of associated macrophytes reside (Linden & Lehtiniemi, 2005). As a result, the presence of such macrophytes may encumber encounters between mussels and their host fish, and reduce the availability of habitat in the river reach where juveniles may colonise following excystment (Schultz & Dibble, 2012).

When considering species of fauna that may exert an influence over the condition of *M. margaritifera* habitat, previous studies have often alluded to the importance of host fish populations (Degerman et al., 2009; Skinner, Young & Hastie 2003). The success of reproduction within populations of *M. margaritifera* ultimately depends on the availability of suitable host fish. These hosts become progressively resistant to the glochidial infection with increasing age. Consequently, fish in the first three year-classes constitute the majority of the available host populations (Eybe et al., 2015; Skinner et al., 2003). The immune system of host fish is, therefore, essential towards the successful glochidial metamorphosis into free living-juveniles (Taeubert et al., 2010; Haag, 2012; Taeubert & Geist, 2017). Studies have shown that the duration of the parasitic phase influences the size and post-parasitic fitness of juvenile mussels, with juvenile mussels displaying longer parasitic phases shown to develop size, growth rate, and survival advantages over those with shorter parasitic phases (Marwaha et al., 2017).

It was assumed that glochidial infestation of host fish was undertaken without depletion of host condition; however, recent studies have highlighted that *M. margaritifera* encystment can increase fish recovery times following exposure to stressors (Thomas et al., 2014), evoke an immune response (Thomas et al., 2014), diminish foraging success (Österling et al., 2014), reduce competitive ability (Filipsson et al., 2016) and hinder swimming capacity (Taeubert & Geist, 2013). Research by Filipson et al. (2017) postulated that the observed increase in metabolism and haematocrit within infested fish may suggest that glochidia encystment has a physiological effect on host fish, possibly acting to compensate for the respiratory stress caused by glochidia (Filipsson et al., 2017). However, work by Castrillo et al. (2021) noted that Atlantic Salmon (*Salmo salar*) fry infested with approximately 22 larvae per fish displayed no clinical signs of stress during the trial, with gills recovering to their normal morphology almost completely in a short time, suggesting a minimal impact on fish health after glochidiosis. Consequently, the extent to which glochidial infection impacts host fish physiology is dependent on the extent of parasitism.

Historically, the extent to which successful encystment occurs has largely been controlled by host fish abundance in *M. margaritifera* habitat. The minimum density of hosts necessary to ensure populations of *M. margaritifera* remain functional in the long-term can, in theory, be calculated using a variety of factors, such as the probability of glochidia reaching the host; the fecundity of female mussels; and the number of generations (Skinner et al., 2003). Previous studies have often placed estimates for the necessary host density in the range of 0.05-0.3 fish m^{-2} (Bauer, 1991; Ziuganov et al., 1994). However, given the complex nature of the mussel's lifecycle, and the high mortality rates often observed during this process, such calculations are often plagued with broad assumptions and must, therefore, be treated with great caution (Skinner et al., 2003).

An association between the success of glochidia and the numbers of host fish in a river system is probable: a higher density of fish would likely improve the probability of glochidia encountering and infecting a host (Degerman et al., 2009). In systems where a depleted host population is the primary cause of stagnation in the corresponding *M. margaritifera* population, anthropogenic augmentation of host fish populations is necessary, but dependent on the availability of suitable habitat (Skinner et al., 2003). Additionally, one must account for the particular species of salmonid necessary to evoke improved glochidia survival. Previous studies have suggested that the Atlantic salmon constitutes the primary host for *M. margaritifera* in the northern areas of the mussel's distribution, whereas brown trout (*Salmo trutta*) dominate the southern regions (Bauer, 1987). In Scotland, this distinction is less apparent, with greater overlap between the two species (Hastie & Young, 2003). Nevertheless, it appears that *M. margaritifera* populations are often affiliated with one of the fish species, but not both; suggesting a genetic differentiation between mussel populations, resulting from strong reproductive isolation (Karlsson et al., 2014). This notion is often overlooked in restoration and management plans and, as such, previous attempts at promoting greater reproductive success in *M. margaritifera*, through the enhancement of host fish populations, have experienced minimal success (Degerman et al., 2009; Karlsson, Larsen & Hindar, 2014).

1.4 Current state of populations

The natural range of the freshwater pearl mussel extends from western Russian, across Europe, to the north-eastern seaboard of North America (Eybe et al., 2015). The freshwater pearl mussel was once abundant across its Holarctic range (Skinner et al., 2003); however, literature concerning the condition of current populations, has documented substantial declines in *M. margaritifera* abundance (Arvidsson et al., 2012; Bauer & Wachtler, 2001; Cosgrove et al., 2016; Österling et al., 2010). A review of the species' global populations by Young et al. (2001) suggested only 100 reproductively viable ('Functional') populations may still exist in the world; whilst estimates by Bauer (1988) suggest that populations of *M. margaritifera*, located across central and southern Europe, have witnessed a reduction of 95-100% in abundance over the last century (Fig. 1.3). Consequently, the International Union for the Conservation of Nature (IUCN) heightened the classification of this species to 'critically endangered' across Europe; emphasising the vulnerability of the Unionid to extinction (Cuttelod, Seddon & Neubert, 2011; Lopes-Lima et al., 2016). Additionally, the European Union Habitats Directive, which promotes the conservation of rare, threatened and endemic species, places the freshwater pearl mussel under Annex II (core areas of the species habitat are designated as sites of community importance; managed in accordance with the species ecological requirements) and Annex V (species subjected to taking in the wild and exploitation, must be managed appropriately).

Despite efforts to protect this species, population declines across Europe persist (Geist, 2010), with freshwater pearl mussels believed to be extinct in Poland and Lithuania (Moorkens, 2011). Populations in central and southern Europe remain, but at greatly reduced levels with substantial fragmentation in population distributions. Additionally, many of the remaining populations are classified as "functionally extinct", given the lack of recent recruitment and low genetic diversity (Geist, 2010; Lois et al., 2014; Stoeckle et al., 2017). Despite this, surveys in northern latitudes have identified notable exceptions in Scotland, Ireland, Fenno-Scandinavia and Russia (Lopes-Lima et al., 2016), where large populations with high genetic diversity have been found (Geist et al., 2010, 2018).

In accordance with trends exhibited across mainland Europe, *M. margaritifera* in the UK has experienced widespread extinction across many regions of England, Wales and Northern Ireland, with the remaining functional populations predominantly restricted to areas of Scotland and northern England (Fig. 1.3) (Chesney & Oliver, 1998; Young & Williams, 1984). Concerning these findings, Cosgrove et al., (2000) undertook an extensive survey of 150 major waterways located across Scotland from 1996-1999, to determine the state of the remaining populations. Approximately two-thirds of the freshwater systems, previously occupied by freshwater pearl mussels, were classed as 'extinct' (mussels absent) or 'functionally extinct' (no evidence of recruitment in the populations over the last 15 years) (Cosgrove et al., 2000). Further study was conducted between 2013 and 2015; the findings of which depicted recent recruitment in almost two-thirds of the extant populations (Cosgrove et al., 2016). Many of these functional sites were located in the north and west of Scotland, suggesting that, despite extinction in many of the Lowland regions, river systems in the Scottish Highlands still hold some of the largest remaining functional *M. margaritifera* populations (Cosgrove et al., 2016). The results of the study undertaken by Cosgrove et al. (2016) also recorded a reduced rate of extinction since the turn of the century. However, given the high-profile nature of the conservation efforts currently in place, sustained losses to the one of the last remaining global hotspots of *M. margaritifera* represent a significant cause for concern.

Whilst there is encouragement to be taken from the fact that Scotland remains a stronghold for *M. margaritifera* populations, a trend of continued population decline persists. Furthermore, the strength of population recruitment in those remaining functional populations remains unknown, and may mask the true extent of vulnerability evoked by this species (Cosgrove et al., 2016). In light of this, conservation efforts must strive to identify and combat the causes provoking this continued decline, in order to ensure the sustainability of *M. margaritifera* populations in Scotland.

CHAPTER 2

2 CONSERVATION MANAGEMENT OF THE FRESHWATER PEARL MUSSEL

The freshwater pearl mussel has been subjected to substantial declines in abundance across its Holarctic range (Arvidsson et al., 2012; Bauer & Wachtler, 2001; Cosgrove et al., 2016). Addressing the causes of this are, however, locally different and often confounded by the interconnectedness of the associated hydrogeomorphological, physico-chemical and ecological parameters. To examine the reasons for decline, one must first account for the habitat characteristics necessary for successful *M. margaritifera* proliferation (see 1.3). One must also recognise the lifecycle of this species (see 1.1): *M. margaritifera* is a particularly slow growing mussel that displays an exceedingly low reproductive rate; thus, necessitating years to recover from a significant disturbance event. With consideration towards the aforementioned, the following section will highlight several of the most commonly cited threats to *M. margaritifera*, detailing historical drivers of decline as well as current and emerging threats to populations, encompassing both periodic disturbance events that result in abrupt changes to the system, with the possibility of recovery, and more chronic stressors, which impede system recovery and may cause more permanent shifts in the ecosystem state (Borics et al., 2013). Following this, an overview of the current conservation management practises will be provided, highlighting areas in which current approaches have been limited by a lack of data, and culminating in a summary of the aims and hypotheses that will dictate the direction of study in this thesis.

2.1 Factors Evoking Population Decline

2.1.1 Illegal Pearl Fishing

The exploitation of *M. margaritifera* has been prevalent over the past two millennia (Young & Williams, 1984), with evidence to suggest Scottish pearls were widely traded across Europe during the 12th Century (Skinner et al., 2003). Commercial exploitation of the species, at a much larger scale, was undertaken in the 16th century across the United Kingdom, with the introduction of bailiffs, employed to manage watercourses and ensure the most valuable pearls were registered to the monarchy (Skinner et al., 2003). However, the practise of pearl mussel fishing is often destructive; pearl fishers often resorted to the prolific killing of the specimens they obtained, in order to increase efficiency (Tarr, 2008). Consequently, by the 19th century, many of the populations of *M. margaritifera* had been exploited beyond sustainable levels and a substantial decline in the species abundance was becoming evident across Britain. Pearl mussel fishing practises subsequently transcended into small-scale fisheries during the 20th century, until complete legal protection was afforded to the species in 1998 (Skinner et al., 2003). Nevertheless, the extent of pearl mussel fishing witnessed over the 19th and 20th centuries remains evident to this day, with almost every known population of *M. margaritifera* displaying hallmarks of substantial historical exploitation (Cosgrove et al., 2000). Thus, previous studies have often highlighted the role of unsustainable pearl fishing practises as a crucial driver of population extinction in many freshwater systems (Addy et al., 2012; Hastie, 2006; Lopes-Lima et al., 2016). In Scotland, Cosgrove et al. (2000) attributed pearl fishing as one of the principal causes of decline in 153 (99%) populations during the first national survey undertaken between 1996 and 1999.

Although the species is now comprehensively protected by law, the policing of watercourses has thus-far failed to eradicate the threat posed by illegal fishing practises (Cosgrove et al., 2000). Despite this, many of the large, protected salmon rivers located in the North East of Scotland have recorded a 90% reduction in mussel destruction, emanating from fishing practices since the ban was implemented (Hastie, 2006; Tarr, 2008); reports of small-scale casual pearl fishing in some of the more remote and less protected watercourses, have persisted (Hastie, 2006). This trend towards illegal fishing practises in remote freshwater systems has gathered momentum within recent decades, owing to the improved infrastructure in the Scottish Highlands, which has improved accessibility to the watercourses (Cosgrove et al., 2000). Of particular concern is the vulnerability of the populations of *M. margaritifera* in these smaller, more remote river systems. Here, the species is likely to be less abundant; thus, heightening the population's vulnerability to destructive fishing practises (Langan et al., 2007). Furthermore, Hastie (2006) highlights the destructive, opportunistic nature of the recent illegal pearl fishing practises, citing the substantial destruction of young, non-pearl bearing mussels as particularly worrying.

Despite the early causes of decline in *M. margaritifera* undoubtedly emanating from commercial fishing practises, some authors have questioned whether persistent illegal exploitation remains a primary cause of concern for the conservation management of this species (Bauer, 1988; Lopes-Lima et al., 2016; Moorkens, 1999; Skinner et al., 2003; Wilson et al., 2011). Nevertheless, given the scarcity of functional populations, it is crucial that those still displaying signs of active recruitment are conserved. Illegal fishing practises still solicit substantial damage to some *M. margaritifera* populations, despite being construed as negligible at the global scale. Additionally, when accounting for the difficulties in recording and identifying illegal fishing incidents, it is likely that the occurrence and devastation of this practise is widely underestimated (Cosgrove et al., 2016). It is therefore important for conservation workers to remain vigilant and actively enforce the prohibition of illegal fishing practises (Cosgrove et al., 2016; Hastie, 2006).

2.1.2 Pollution

The adverse effects of pollution and poor water quality on populations of *M. margaritifera*, have often been documented in literature concerning the species' conservation (Bauer, 1988; Degerman et al., 2009; Moorkens, 1999). However, despite the vast array of contaminants that could potentially elicit adverse effects on populations of *M. margaritifera*, very few studies have explicitly addressed the issue (Boon et al., 2019; Lopes-Lima et al., 2016). A review by Boon et al. (2019) highlighted the need for information concerning a suite of chemical attributes required to assess the water quality in rivers, some of which are believed to be directly toxic or "promote harmful conditions", whilst others are thought to indicate damaging activities.

Within Europe, the decline of pearl mussel populations largely coincided with the industrial growth of the 20th Century (Frank & Gerstmann, 2007). The largest population losses were often observed in areas displaying extensive industrialisation, characterised by the widespread use of fossil fuels and agricultural chemicals; hence, implicating the role of diffuse chemicals as a crucial driver of *M. margaritifera* decline (Frank & Gerstmann, 2007). Earlier work by Bauer (1988) supports this notion with observations to suggest a positive association between the concentration of nitrate in the waterbody and mortality observed within adult mussels. Survivorship curves, produced by this study, depict drastic reductions in the lifespan of mussels in rivers polluted by high levels of nitrate: 0.5ppm, 1.5ppm and 3ppm in unpolluted, moderately polluted and heavily polluted rivers respectively. Nutrient loading into the waterbody may elicit negative impacts on the corresponding ecological communities through indirect pathways. For example, heightened nutrient concentrations are often indicative of eutrophication in the waterbody (Moorkens, 1999; Skinner, Young & Hastie 2003). High levels of eutrophication will often stimulate primary production in the waterbody, subsequently leading to increased detrital loads (Bauer, 1988). Large amounts of detritus on the riverbed can inhibit the exchange at the hyporheic zone; thus reducing the quality of habitat for juveniles to settle (Bauer, 1988; Skinner, Young & Hastie 2003). Even in low levels, eutrophication can evoke substantial limitations to the survival of *M. margaritifera* populations (Skinner et al., 2003).

Within Scottish populations, Cosgrove et al. (2016) found evidence to suggest that 24.6% of the 126 sampled populations were affected by pollution between 1999 and 2015. Caution must be taken when analysing these results, with instances of pollution often highly localised and, therefore unlikely to solicit large-scale population declines (Cosgrove et al., 2016). Despite this, point-source contamination within a freshwater system can be detrimental to aggregations of Unionid mussels (Cosgrove et al., 2000; Lopes-Lima et al., 2016). Populations of *M. margaritifera* inhabiting rural areas are likely to suffer from point-source pollution emanating from agricultural activity, such as the leakage of sheep dip and slurry into local water sources (Tarr, 2008). Virtue and Clayton (1997) found that, within a single river catchment in Scotland, 47 sheep dips were discharged directly into the freshwater system, often as a result of poor operational methods. The introduction of excess quantities of easily degradable organic matter has been attributed as a common cause of reduced levels of dissolved oxygen in open water (Studer et al., 2017). This has implications for the acquisition of sufficient oxygen for respiration in adult mussels, resulting in heightened individual stress and a subsequent loss of condition (Moorkens, 1999).

The ecological ramifications of point-source pollution from agricultural and industrial activities can be highly varied. For example, the presence of particular heavy metals and organic pollutants in the waterbody are known to induce calcium deficiency within freshwater mussels (Degerman et al., 2009; Lopes-Lima et al., 2016; Naimo, 1995). Compounds present in these pollutants such as organochlorine insecticide DDT and its metabolite DDE may disrupt the ion channels associated with calcium metabolism, with evidence to suggest a similar consequence of exposure to elevated concentrations of heavy metals such as cadmium (Frank & Gerstmann, 2007; Varandas et al., 2013). Frank and Gerstmann (2007) found that *M. margaritifera* populations exhibiting higher concentrations of these pollutants, often displayed a significantly lowered calcium pool. Mussel species will often dedicate a large fraction of their metabolic energy budget towards calcium uptake (Mackie & Flippance, 1983). As a result, exposure to these chemicals may constitute a substantial energetic stress that could impede the long-term survival of an individual (Frank & Gerstmann, 2007). Alterations in the acid-base chemistry of the water may also impact shell growth and mussel condition. Acidification has become a particular issue within Scandinavia, with intervention often required to counteract this process (Henrikson & Brodin, 1995). Severe acidification can instigate the gradual degradation of the calcareous shell and interfere with the regulation of acid-base mantle fluid (Fromm, 1980). Additional ecological impacts of point source pollution, often highlighted in previous research, include the loss of salmonid hosts; and the localised extinction of *M. margaritifera*, resulting from toxic poisoning (Moorkens, 1999).

Previous studies have often disregarded point-source pollution as an implicit factor driving large-scale population declines; instead, authors advocate consideration towards diffuse sources of chronic low-level pollution, resulting from land use practises in the catchment area, as crucial determinants of *M. margaritifera* survival throughout the species range (Cosgrove et al., 2016; Lopes-Lima et al., 2016). Land designated to urban development, forestry and agriculture may enhance the nutrient and sediment loads transported to the watercourse via surface run-off. These diffuse pollutants enter the watercourse along large parts of the system and are consequently difficult to manage (Beggel & Geist, 2015; Lopes-Lima et al., 2016). For example, increased loads of Sodium Chloride, a common de-icing salt, has been shown to represent a significant contaminant to freshwater systems (Kaushal et al., 2005). Up to 60% of the de-icing salt applied to roads ultimately runs off into surface water bodies(Perera et al., 2013), with research showing the presence of these diffuse contaminants impacts the filtration behaviour of freshwater mussels (Hartmann et al., 2016) and reduces the successful attachment of glochidia to host fish (Beggel & Geist, 2015).

Conservation management concerning land use practises in the catchment area has often focused on tackling increased nutrient and sediment loads entering the watercourse, with evidence to suggest that this may be one of the most significant drivers of *M. margaritifera* decline (Degerman et al., 2009). Nitrogen and phosphorous concentrations are increasing in many regions across Europe (Douda, 2010; Galloway et al., 2008; Grizzetti et al., 2011), although phosphorous levels in pearl mussel rivers in northern and western Europe are lower than those recorded in systems located in central and southern Europe (Degerman et al., 2013; Gosselin, 2015; Lois, 2015; Moorkens, 2006). Nevertheless, a single occurrence of heightened phosphorous concentrations can evoke an episode of elevated agal productivity, culminating in a reduction in oxygen in the riverbed gravels, with a risk of potential loss to several years of juvenile recruitment (Ní Chatháin et al., 2012)

The presence of high concentrations of fine sediment may evoke increased levels of turbidity and deposition (Jones et al., 2012). Fine sediment deposition leads to the clogging of interstitial spaces, limiting oxygen supply to these regions (Denic et al., 2015). When coupled with increased oxygen demands, fostered by eutrophication due to heightened levels of phosphorous and nitrogen (resulting in algal and macrophyte growth and decay) this process could have significant ramifications for the survival of juvenile mussels (Geist & Auerswald, 2007). The tolerance of different life-stages to certain sediment loads is currently understudied. However, work by Altmuller and Dettmer (2006) suggest that, when efforts to reduce sediment entering the river are made, mussel habitat suitability is drastically improved. Furthermore, research by Lavictoire et al. (2020) sought to quantify the grain sizes attributable to successful juvenile propagation. Findings from this study revealed juvenile survival was heightened when individuals presided within substratum dominated by grain sizes of 1-2 mm, compared to 0.25-1 mm, suggesting juveniles benefit from larger pore sizes which enable higher flow into interstitial spaces, facilitating greater dissolved oxygen delivery.

Nutrient and sediment loading from land-use practises are likely to be further exacerbated due to disturbance to the ecological buffer zone surrounding a freshwater system. Practises such as forest plantations, overgrazing and urban development, will likely yield substantial alterations to the water cycle and groundwater runoff, in addition to modifications to processes governing sedimentation and water acidification (Degerman et al., 2009; Wilson, Roberts & Reid 2011). Tarr (2008) proposed the implementation of riparian buffers and sediment traps as useful methods to counteract the increased sedimentation caused by agriculture and forestry, a suggestion shared by others (Degerman et al., 2009; Henley et al., 2000).

Despite the perceived threat of point-source and diffuse pollution to the maintenance of functional *M. margaritifera* populations, environmental quality standards committed to ensuring legally 'safe' limits of diffuse pollutants, known to diminish the ecological integrity of nearby freshwater systems, have only recently been outlined (Boon et al., 2019; Lopes-Lima et al., 2016). As such, their efficacy towards identifying and mitigating pollution in river systems remains to be seen. Of particular concern to conservation efforts, is the enhanced susceptibility of freshwater pearl mussels to the presence of these diffuse xenochemicals, when compared to other freshwater species; primarily due their longevity, feeding behaviour (i.e. filter-feeding), and habitat (Beggel & Geist, 2015; Lopes-Lima et al., 2016). A report by Young, (2005) collated information from the literature concerning water quality parameters and associated responses of freshwater mussels. However, few studies have been conducted since to test the anecdotal evidence presented, in addition to examining whether the response of other Unionid species are synonymous with those likely to be exhibited by *M. margaritifera*.

2.1.3 Deterioration of host fish populations

Freshwater ecosystems across Europe have experienced the disappearance of many native fish species over recent decades (Lopes-Lima et al., 2016). Many Unionid mussel species are reliant on fish to provide the host-parasite interaction necessary for juvenile development. This potential limitation in the availability of hosts can be particularly damaging to the reproductive viability of mussels with restricted host-fish spectra, such as the freshwater pearl mussel, which relies on two salmonid species: the brown trout (*Salmo trutta*) and the Atlantic salmon (*Salmo salar*) (Bauer, 1988; Lopes-Lima et al., 2016).

Brown trout and Atlantic salmon populations have experienced substantial declines throughout Europe in recent years (Hastie & Cosgrove, 2001). Within the northwest of Scotland, a region often recognised as a stronghold for *M. margaritifera* populations, salmon catches saw a marked deterioration over the 1990s, reaching historically low-levels by the turn of the century (Hastie & Cosgrove, 2001; Skinner, Young & Hastie 2003). Additional declines were experienced within sea trout stocks across many Scottish river systems (Cosgrove et al., 2016). In a survey conducted by Cosgrove et al. (2016), 28 watercourses located in the West Highlands (48.9%) exhibited *M. margaritifera* populations at risk to low host fish density, a percentage the authors considered conservative in their estimations. Numerous factors have been suggested as the cause of the host-fish population collapse, including over-fishing; climate change; the degradation of habitat; and the introduction of novel parasites, such as sea lice (Skinner et al., 2003).

Growing demand for seafood over the past three decades has engendered a rapid expansion of aquaculture operations, with marine net-pen salmon farming representing a key industry in countries such as Scotland and Norway (Medcalf et al., 2021). Of particular concern is the introgression and transmission of pathogens and parasites from farmed salmon to wild populations, attributed to the spatial over-lap of aquaculture operations and salmon migration routes (Krkošek, 2017). Specifically, the heightened transmission of the salmon louse, *Lepeophtheirus salmonisis,* within wild salmon stocks has been shown to influence growth and survival by compromising immune and osmoregulatory function (Pike & Wadsworth, 1999; Wagner et al., 2003; Finstad et al., 2010) whilst also reducing food-conversion efficiency (Costello, 2009; Abolofiaet al., 2017). With climate change, and the expected increase in water temperatures, these issues are likely to be compounded further: higher temperatures accelerate sea-louse development (Hamre et al., 2019); salmonid hearts perform less effectively at high temperature (e.g.dysrhythmia and reduced maximum stroke volume and cardiac output; Eliason et al., 2013). Thus, stressors associated with ocean warming and coastal salmon aquaculture can compromise wild salmon fitness through the impairment of vital organs (Medcalf et al., 2021). It is likely that multiple stressors acting on host fish may exacerbate the physiological effects of glochidial parasitism, with potential implications towards the condition of resulting juvenile mussels (Defo et al., 2019). Despite this, research concerning the consequences of these effects on the persistence of salmonid populations, and their viability as host fish for *M. margaritifera* is sparse. Therefore, the consequences to *M. margaritifera* conservation remain unclear.

The deterioration of salmonid stocks is frequently affiliated with recruitment failure in corresponding *M. margaritifera* populations (Young, Cosgrove & Hastie, 2001; Ziuganov et al., 1994). Consequently, previous studies often emphasise the role of host-fish abundance in governing the successful reproduction of freshwater pearl mussels (Cosgrove et al., 2016; Cuttelod, Seddon & Neubert, 2011). Nevertheless, the threshold at which a decline in host fish populations begins to substantially impede mussel recruitment remains largely unknown. Studies concerning the calculation of appropriate host population densities (Bauer, 1988) have often neglected the importance of suitable hosts, instead focusing on entire population densities, rather than the presence of fish in the first three year-classes: the predominant hosts for *M. margaritifera* (Degerman et al., 2009). However, there remains a lack of sampling data with which to conclusively attribute the fall in catch of adult salmonids with declines in the density of juvenile fish (Langan et al., 2007; Skinner et al., 2003). Furthermore, attempts by conservation management to counter the host fish declines, by stocking salmonid populations, often overlooks localised host specificity (Jung et al., 2013). Pearl mussel populations display apparent preferences to certain salmonid hosts (Young & Williams, 1984). In the absence of this knowledge, a species introduction would be unlikely to stimulate enhanced recruitment in *M. margaritifera*, and may even negatively influence mussel reproductive success: the introduced species may out-compete the remaining suitable hosts (Degerman et al., 2009).

Assessment of the results emanating from previous baseline studies highlights the need for further research into the relationship between host density, host specificity and mussel recruitment. The persistence of freshwater pearl mussels ultimately depends on the presence of host fish species (Cosgrove & Hastie, 2001; Jung et al., 2013). With consideration towards this close relationship between salmonids and pearl mussels, and the paralleled deterioration of the corresponding populations, an integrated conservation management plan would likely prove an effective approach (Hastie & Cosgrove, 2001).

2.1.4 Introduction of non-native species

Ecosystems across the globe have witnessed an exponential growth in non-native species invasions over the past century; often attributed to the rise of international trade and travel, presenting pathways for introduction (Hulme, 2009). The presence of fauna and flora species in non-native environments has been accredited with significant modifications to the associated ecology (Molnar et al., 2008; Postnote, 2008; Tangley, 2003). Within freshwater systems, macrophytes and non-insect invertebrate species are often recognised as the most prolific invaders (Roy et al., 2012), with previous studies often citing their role as “ecosystem engineers”, highlighting the propensity of these species to enact fundamental alterations to the physico-chemical nature of the associated environment (Heegaard et al., 2001; Schultz & Dibble, 2012). Despite the introduction of non-native species gaining widespread interest from ecologists and conservationists alike, this topic is rarely alluded to in literature concerning the freshwater pearl mussel (Tarr, 2008). Nevertheless, alterations to the community dynamics following the introduction of these non-native species could threaten the persistence of *M. margaritifera* populations, and should not be neglected.

When considering the impact of non-native invertebrate species introductions across the freshwater systems of Europe, previous studies have often highlighted the formidable nature of the invasive zebra mussel, *Dreissena polymorpha* (Cosgrove et al., 2016; Miller & Watzin, 2007; Ricciardi, Neves & Rasmussen, 1998; Tarr, 2008). Observations from invaded waterbodies have frequently described high density zebra mussel beds, developing over several years following initial introduction (Ricciardi, 2003). The rapid colonisation of freshwater systems by *D. polymorpha* is often accompanied by precipitous depletions within native Unionid populations (Ricciardi et al., 1998). These epifaunal bivalves are rarely hindered by substratum type (provided the material is hard), and have frequently been found attached to Unionid mussel shells: the bysall threads of zebra mussels facilitate their attachment to the valves of native bivalves (Byrne et al., 1995). The fouling from *D. polymorpha* is believed to impinge on the activity and locomotion of the native bivalves, with heavily infested Unionid mussels often exhibiting signs of metabolic stress and starvation (Baker & Hornbach, 1997; Byrne et al., 1995). Additionally, *D. polymorpha* may also inhibit Unionid survival indirectly, via competition for food and nutrients (Baker & Hornbach, 1997): the filtering activity of this invasive species, after expansive population growth, can lead to mass depletion of seston (Ricciardi et al., 1998; Strayer & Malcom, 2007).

Another invasive invertebrate species, the signal crayfish (*Pacifastacus leniusculus*), has also received substantial attention in literature: often highlighted as a threat to the ecological integrity of freshwater communities (Degerman et al., 2009; Johnson, Rice & Reid 2011; Lopes-Lima et al., 2016; Rice et al., 2014). When assessing the impact of *P. leniusculus* on populations of freshwater pearl mussel, previous studies have often highlighted the potential predatory nature of this invader (Degerman et al., 2009; Josefsson & Andersson, 2001; Lopes-Lima et al., 2016). Signal crayfish often prefer smaller prey and are, therefore, unlikely to predate on adult pearl mussels (Degerman et al., 2009). However, juvenile *M. margaritifera* may fall prey to invading crayfish (Degerman et al., 2009); consequently weakening population recruitment within the corresponding mussel aggregations. Furthermore, a growing body of evidence in the literature suggests this invasive species represents an important zoogeomorphic agent within invaded waterbodies (Johnson et al., 2011; Rice et al., 2014). Signal crayfish burrow into river substratum; dismantling the sedimentary structures that ensure stability, whilst increasing rates of entrainment and bedload transport (Rice et al., 2014; Statzner et al., 2003). Thus, the activity of *P. leniusculus* may have broad implications for catchment-scale sediment dynamics (Harvey et al., 2014); subsequently reducing the quality of habitat for *M. margaritifera.*

Presently these invasive alien invertebrate species remain largely absent from the freshwater systems inhabited by Scottish *M. margaritifera* populations. Despite this, sampling data held by the Spey Fishery Board (2014) has identified the presence of known breeding populations of *P. leniusculus* on the River Dee and Clyde; catchments adjacent to globally important *M. margaritifera* strongholds (Cosgrove et al., 2016). Additionally, sparse populations of *D. polymorpha* have been recorded in Scotland; although, it remains to be seen whether the zebra mussel can replicate the expansive nature of its invasion, depicted in the more southerly regions of Britain (Aldridge et al., 2004).

Assessment of current threats to *M. margaritifera* from invasive species, highlight macrophytes such as *Ranunculus* and Elodea spp., which have already colonised numerous freshwater systems in Scotland, as potentially the greatest threat to freshwater pearl mussel populations. Invasive macrophyte species frequently demonstrate a competitive advantage over native counterparts; epitomised through their utilisation of previously uninhabited regions of the freshwater habitat and the production of dense stands within colonised beds (Grudnik et al., 2014; Jimenez-Valverde et al., 2011; Kunii, 1981; Minchin & Boelens, 2008; Vernon & Hamilton, 2011). The successful colonisation of a river bed by macrophytes is often associated with changes to the flow regime, and alterations to the processes governing sediment transport (Declerck et al., 2011; Scheffer et al., 1993; Schultz & Dibble, 2012; Van Donk & Van de Bund, 2002). Within these dense macrophyte stands, hydrochemical and temperature depth gradients can often form, which may lead to shifts in the associated invertebrate and macrophyte community dynamics (Rørslett et al., 1986). Invasive non-native macrophytes have also been shown to directly suppress the growth of invertebrate species, by emitting allelopathic chemicals; subsequently inhibiting the survival of nearby invertebrate taxa (Erhard & Gross, 2006; Hilt & Gross, 2008; Kelly et al., 2015; Schultz & Dibble, 2012; Van Donk & Van de Bund, 2002). Furthermore, the decay of plant material during the autumn months could produce a substantial quantity of organic matter and, in environments subjected to low water flow velocity, this matter may become entrained in the riverbed; risking potential suffocation of sessile organisms present in benthic regions (Moorkens & Killeen, 2014). Nevertheless, very few papers have attempted to decipher whether these ecological changes also apply to the introduction of invasive macrophytes in *M. margaritifera* habitat (Laughton et al., 2008).

2.1.5 Hydrological Management Schemes

The construction of hydroelectric dams in freshwater systems has often been attributed to the decline in *M. margaritifera* populations, and the degradation of aquatic communities more broadly. Many river systems in Europe have experienced physical alterations from human activity (Lopes-Lima et al., 2016). Currently, more than 7000 large (>15m high) dams exist in Europe (Limburg & Waldman, 2009). However, with a drive towards renewable sources of energy, in the face of a global climate crisis and diminishing fossil fuel reserves, pressure is mounting for the establishment of new dams across the continent. The broad impacts of hydrological management schemes, on existing ecological communities in the freshwater environment, have received substantial attention from numerous authors. Impoundments are known to alter flow and sediment regimes, substrate composition and nutrient flow, in addition to inducing habitat fragmentation and loss of connectivity (Gangloff & Feminella, 2007; Grill et al., 2019; Lu et al., 2018; Marteau et al., 2018; Nilsson & Berggren, 2000; Poff et al., 2007; Rosenberg et al., 2000). These alterations to the abiotic nature of regulated freshwater ecosystems are responsible for eliciting modifications to the aquatic biota through the loss of functional diversity, impediment of migration, increased homogenisation of communities, changes to food webs and distortion of reproductive cues (Gallardo et al., 2008; Lytle & Poff, 2004; Moran et al., 2018; Rolls & Arthington, 2014; Ziv et al., 2012). Furthermore, an increase in demand for renewable energy sources has seen a shift towards increasing support for small hydro-power plants, often located in upstream areas of river basins, which have remained largely understudied to-date. With regards to the aforementioned, several authors have suggested that dam construction may constitute one of the greatest threats to freshwater systems in the coming decades (Geist, 2011). The following section will look to evaluate this information further, with a specific focus on the impact of dams on the conservation of *M. margaritifera* populations.

Alterations to natural hydrogeomorphology

When examining the effects of hydrological management schemes in freshwater systems, conservation management will often allude to the changes in flow regime as a key factor driving observed ecological changes (Addy et al., 2012; Zigler et al., 2008). For example, the water depth will often exceed natural levels in the river reach situated upstream of a dam construction; subsequently reducing the flow and altering the temperature regime within the waterbody (Addy et al., 2012). As these lotic habitats become progressively more lentic in their characteristics, the waterbody is likely to become more stratified, with gradients in water temperature developing. This thermal stratification is often accompanied by alterations in dissolved oxygen, acidity and carbon-dioxide content (Layzer & Scott, 2006; Vaughn & Taylor, 1999). The channel border and backwater habitats may also offer depositional sites for fine-grained sediments and associated contaminants, such as cadmium and other heavy metals (Frank & Gerstmann, 2007; Naimo, Atchison & Holland-Bartels, 1992), potentially exposing mussel beds to highly toxic chemicals. Juvenile mussels are particularly susceptible to these silty conditions, with the infiltration of interstices by fine sediment degrading habitat (Addy et al., 2012), and associated compaction inhibiting development by limiting exchange between free and interstitial water (Geist & Auerswald, 2007). Despite these perceived risks to associated upstream *M. margaritifera* aggregations, areas downstream of impoundments remain the focus of literature concerning the ecological ramifications of dam construction. When considering areas of the watercourse downstream of impoundments, authors have often drawn attention to modified flow regimes as particular areas of concern for the condition of freshwater mussels (Addy et al., 2012).

Hydropeaking, the raising and lowering of water levels in the river system, has been attributed to significant declines in freshwater mussel populations within regulated systems. At high flow velocity, discharged water has been shown to displace mussels and prevent the settlement of juveniles in nearby regions of the riverbed (Lopes-Lima et al., 2016; Tarr, 2008); thus, impairing population recruitment. Additionally, high flow regimes modify the sediment distribution in the watercourse through scouring and erosional processes (Addy et al., 2012). If sustained by hydrological management schemes, high flow regimes ultimately hinder the establishment of sediment stability: a critical parameter governing *M. margaritifera* habitat suitability (Vaughn & Taylor, 1999). When coupled with the trapping of sediments by impoundments, which disrupts the continuity of sediment transport through the system and inhibits substrate replenishment, dams may prevent the realisation of habitat complexity by further amplifying these erosional processes (Mathias Kondolf et al., 2014). This loss of fine sediment from the substratum, and subsequent development of coarse armoured surface layers downstream of the impoundment (Addy et al., 2012; Nilsson & Berggren, 2000), may incur a substantial reduction in suitable habitat for freshwater mussels due to a lack of suitable medium in which to bury (Wegscheider et al., 2019). The resulting alterations in sediment cycling may have far-reaching consequences concerning the availability of suitable habitat for *M. margaritifera:* a 13km extinction of mussel species was reported downstream of an impoundment in the Caney Fork River, USA; largely attributed to the loss of fine sediments (Layzer et al., 1993). Similar studies have also reported extinction gradients for freshwater mussel species, encompassing kilometres of downstream habitat (Vaughn & Taylor, 1999).

A reduction in the frequency of competent flows due to flow regulations can cause the narrowing of the channel width. Here, modified flow regimes are unable to sustain geomorphic features of the channel and, when coupled with the stabilisation of water levels, facilitates the encroachment of vegetation onto previously active bars (Addy et al., 2012; Casado et al., 2016; Kondolf et al., 2002; Petts & Gurnell, 2005). This contraction of the channel width can impact the availability of mussel habitat (Jones & Byrne, 2010), with pearl mussels often found to inhabit regions of the riverbed close to the channel banks, which offer greater stability, and can be particularly susceptible to adjustments in channel widths (Addy et al., 2012; Hastie, Young, et al., 2000; Morales et al., 2004; Varandas et al., 2013). Extended periods of low flow induced by hydrological management schemes, may also leave mussels stranded outside of the waterbody (Lopes-Lima et al., 2016). Mussels are slow moving organisms and are thought to be unlikely to respond to a swift recession in the water levels (Vaughn & Taylor, 1999). Although, a growing body of evidence in the literature suggests some freshwater mussel species are able to detect alterations in the water depth and track reductions in flow height to avoid prolonged aerial exposure (Gough et al., 2012; Newton et al., 2015). Nevertheless, in circumstances where exposure to the terrestrial environment is only brief, thermal extremes and desiccation may impose substantial physiological stress on individuals; potentially diminishing an individual's reproductive or immunological competence (McMahon, 1991; Vaughn & Taylor, 1999). Additionally, prolonged emersion will ultimately lead to the desiccation and death of freshwater mussels (Martel et al., 2010). The length of time required for this to occur may vary though, with recent studies having provided evidence to suggest tolerance to emersion varies depending on the species of freshwater mussel, with some species shown to survive up to 8.5 days out of water (Nakano, 2018).

In addition to alterations in flow regime, the river reach situated downstream of an impoundment will also experience fluctuations in the temperature regime, as a consequence of thermal stratification in the reservoir upstream of the impoundment (Ellis, 1942; Gallardo et al., 2008; Grill et al., 2019). The release of unseasonal cold or warm water can alter ecological responses to modifications in flow regime, with hypolimnetic releases shown to reduce water temperatures by up to 15°C for hundreds of kilometres downstream (Olden & Naiman, 2010; Rolls & Bond, 2017). Previous studies have provided evidence to suggest the discharge of cold hypolimnetic water is associated with significantly diminished pearl mussel populations downstream (Addy et al., 2012). Alterations to temperature regimes possibly impact temporal behavioural mechanisms. For example, temperature has been shown to influence the search efficiency of mussels, with water temperatures governing the occurrence and extent of horizontal and vertical movements (Amyot & Downing, 1997; Block et al., 2013; Wegscheider et al., 2019). Furthermore, the timing of oviposition and glochidia release events appear to be temperature dependant, with cold temperatures potentially suppressing the timing of these events by weeks (Hastie & Young, 2003), with potential implications for the rates of successful attachment to the host fish. Cold temperatures suppress the metabolic rate of organisms and may, therefore, drive this inhibition of reproductive activity (Vaughn & Taylor, 1999). Cooler water temperatures in the summer months are potentially damaging to existing adult and juvenile *M. margaritifera* as well, when high metabolic rates are required to facilitate growth in individuals (Layzer et al., 1993). Moreover, abnormal temperature regimes might indirectly influence mussel species via changes to the surrounding ecological community. For example, fluctuations in the thermal regime can disrupt the reproductive nature of host salmonids, whilst providing a competitive advantage to more temperature-tolerant species; consequently, engendering a change in the fish communities, which may impose colonisation barriers to *M. margaritifera* (Burr et al., 1996; Layzer et al., 1993).

Habitat Fragmentation

In addition to the modification of physico-chemical characteristics of the habitat, hydrological management schemes can substantially alter the ecological nature of the freshwater environment, with direct implications for the corresponding Unionid populations. With regards to *M. margaritifera* populations, a key concern lies with the persistence of host fish populations. Episodes of glochidia release and attachment constitute the most susceptible stage in the development of *M. margaritifera*, as failure to reach a viable host results in mortality (Brainwood et al., 2008). Minimum densities of host fish necessary for successful glochidia infection were originally thought to be between 10 and 20 fish per 100m^{-2} (Bauer, 1991; Ziuganov et al., 1994). However, data from a study by Österling, Arvidsson and Greenberg, (2010) indicated this could be even lower, with a suggested density between 5-10 fish per 100m^{-2}. In many cases, the density of host fish may not be the key driver of population decline (Geist et al., 2006); however, reductions in the abundance of host fish populations should be monitored, with their sustained presence necessary to ensure successful conservation of *M. margaritifera*. Insufficient host fish densities, are evoked by the presence of impoundments in a river system through two key pathways (Addy et al., 2012): first, by generating barriers to prevent migration of fish upstream; second, by degrading host fish habitat.

With regards to the degradation of host fish habitat, a review by Poff and Zimmerman, (2010) found that alterations in flow, emanating from dam operation, have a significant negative impact on fish populations, with associated reductions in abundance, diversity and demographic numbers. The armouring of bed layers may also impact the host fish populations within corresponding systems by degrading spawning habitat (Kondolf, 1997). The extent of spawning habitat significantly influences the recruitment of juvenile salmon, with substrate composition and structure forming a first order control on site suitability (Addy et al., 2012; Gilvear et al., 2002; Kondolf & Wolman, 1993). For salmonid species, optimal spawning habitat comprises riverbeds dominated by gravel substrates with a median diameter up to 10% of an individual's body length (Armstrong et al., 2003; Kondolf & Wolman, 1993). Yet in regulated systems, gravel dominated beds may be lost due to a combination of erosional processes and a lack of substrate replenishment (Kondolf et al., 2014).

Studies concerning the impact of dams on fish species have frequently highlighted the impediment of migratory fish passage upstream of impoundments (Brainwood et al., 2008; Sutherland et al., 2020; Watters, 1999; Winemiller et al., 2016), with fish abundance upstream of dams reduced by 60% in some cases (Moran et al., 2018). Obstacles to fish migration prevent access to stretches of the river reach and may result in juvenile and adult salmonid fish co-inhabiting stretches of the watercourse. In this instance, adult fish are likely to monopolise the available resources, subsequently inhibiting the survival of juvenile fish (Degerman et al., 2009). Attachment of glochidia is more prolific in younger fish; consequently, the fragmentation of a freshwater system may severely deplete recruitment within *M. margaritifera* populations, due to a diminished host availability reducing the chances of infection (Arvidsson et al., 2012). A study of fish host populations in Swedish watercourses found the median density of brown trout under-yearlings to be 8.2 per 100m^2 in migratory populations, compared to 0.6 in resident populations (Söderberg et al., 2008); therefore, highlighting the importance of migratory fish populations in facilitating effective recruitment in pearl mussel populations. At the meta-habitat scale, the stagnation of fish migration may inhibit gene flow between mussel aggregations, causing disruption to the natural structure of the species population within the corresponding freshwater system (Geist, 2011; Geist & Kuehn, 2005). This isolation and fragmentation of *M. margaritifera* is particularly concerning when considering the long-term persistence of the species in some watercourses: recruitment may be insufficient to accommodate substantial population recovery after a disturbance event (Lopes-Lima et al., 2016).

The rise of small hydropower plants

Despite a wealth of literature concerning the impact of large hydropower schemes on the ecological integrity of the corresponding river systems, very few studies have examined the environmental impacts of smaller hydropower schemes (Sutherland et al., 2020). Globally there are estimated to be more than 80,000 small hydropower dams located on upland streams, with 11 small dams to every large dam (Couto & Olden, 2018). With an increase in demand for renewable energy sources resulting in a shift towards increasing support for small hydro-power plants, it is crucial future research and corresponding conservation management strategies examine the effects of small hydropower schemes on freshwater mussel populations.

Small dams are chiefly designed and built for hydropower generation, and often constructed anywhere in the catchment that provides appropriate physical characteristics (Deitch et al., 2013; Lu et al., 2018). Regulations and guidance imposed on large hydropower projects, towards decreasing the negative impacts of operations on the ecology of associated river systems, are rarely reciprocated with smaller schemes (Sutherland et al., 2020; Winemiller et al., 2016). Yet small hydropower dams exert the same characteristic alterations to the river system that one would expect from a larger scheme, with known alterations to the natural flow regimes and sediment characteristics (Moran et al., 2018). Although their individual impact on the prevailing environmental conditions may be perceived as less substantial than larger schemes, the cumulative impact of many small dams on a single river reach may be severe (Deitch et al., 2013; Lu et al., 2018). Moreover, research by Kibler and Tullos (2013) suggests the biophysical impacts per megawatt of energy produced by small schemes may even exceed those of larger dams.

A key concern for conservation management with regards to the impact of small hydropower dams on *M. margaritifera* populations pertains to the location of such schemes: often built on upland streams that have historically experienced limited anthropogenic activity (Vörösmarty et al., 2010), where functional freshwater pearl mussel populations have often been found to reside (Hastie, Boon, et al., 2000). These systems are regarded as important habitats for maintaining hydrological connectivity, crucial to the conservation of aquatic biodiversity (Lange et al., 2018). As with larger schemes, there are likely to be direct and indirect effects of these small hydropower schemes on *M. margaritifera* populations and their condition.

Examination of the direct effects of small hydropower schemes on freshwater pearl mussels have comprised correlative studies, assessing abundance and distribution of mussel beds within regulated systems (Sousa et al., 2020; Watters, 1996). Results of these studies found that adult and juvenile pearl mussels were significantly more abundant upstream of dams compared to downstream (97.4% more) and within reservoirs (98.5% more), with such patterns largely explained by variance in sediment and water chemistry characteristics (Sousa et al., 2020). Of particular note was the substantial loss of individuals from areas that had become reservoirs following dam construction, attributed to the accumulation of fine sediments and organic matter, culminating in low dissolved oxygen levels (Sousa et al., 2020). In river reaches compounded by successive small hydropower schemes, these effects could be detrimental to the survival of *M. margaritifera* populations. Approximately three to four times more reservoir area can develop upstream of successive small impoundments compared to a single large dam (Rosenberg et al., 2000), potentially culminating in vast swathes of the river becoming progressively more lentic in form.

Assessment of the indirect effects of small hydropower schemes, can be largely attributed to potential alterations in host fish populations. Due to historically limited regulations concerning the construction requirements of small dams, many lack fish ladders or locks; thus, providing potential barriers to fish migration (Watters, 1996). Furthermore, river reaches regulated by multiple small hydropower schemes often experience shifts in the community structure and composition of fish species, with a reduction in overall species richness, accompanied by higher proportions of eurytopic species: a common response of fish communities to impoundments (Jumani et al., 2018). Eurytopic species are better adapted to the presence of unnaturally high or low flow regimes caused by dam operation, and represent a lotic to lentic shift in community dominance patterns (Carlisle et al., 2011).Research by Benejam et al. (2016) specifically highlighted a loss in brown trout as a result of the cumulative effects of small hydropower plants on fish migration and habitat quality. Consequently, there is cause for concern regarding the sustainability of host fish populations within such systems, and the resulting viability of functional populations upstream of successive small dam structures.

Hydropower within Scotland

With a temperate maritime climate, providing an average (2010-2020) precipitation of 1752.3mm per annum and modest evaporative demands, Scotland's hydroclimate is analogous to that of north Atlantic countries. Consequently, the country has over 6000 rivers and a total stream length exceeding 100,000km (Mackay et al., 1998). Thus, Scotland holds an abundance of water resources, presenting an exploitable resource equivalent to 16,000m^3 per person per annum, compared to 2090m^3 for the UK as a whole (Scottish Office, 2000). With this is mind, it is perhaps no surprise that Scottish rivers have been subjected to a long history of river flow management and regulation, culminating in a cultural identity as a "hydro nation" (Scottish Government, 2020).

Scotland's first hydropower scheme was constructed in 1885 in Greenock, Renfrewshire, which was shortly followed by a 18kW hydropower dam at St Benedict's Abbey, Fort Augustus (Payne, 1988). A national scale development of hydropower followed in the 20th century, with the most significant expansion occurring between 1950 and 1965, under the guidance of the North of Scotland Hydro-Electricity Board, during which 74 hydropower dams were constructed, totalling a capacity in excess of 950MW (DECC, 2020; Payne, 1988). Since the mid-20th century development, Scotland has witnessed further expansion of the hydropower sector, and an increased profile as a source of renewable energy complimenting the country's climate policy (Nelson, 2013). Despite recently appearing in the top ten hydropower countries worldwide, concerning hydropower's contribution to total national electricity generation (IEA, 2011), Scotland has shifted towards greater reliance on wind energy as its principal source of renewable infrastructure (DECC, 2020).

Despite a deep vein of history in river management and dam construction, examination of the impacts of river regulation on the corresponding ecology occurred later on, growing concurrently with the expansion of scientific investigation and frameworks to disseminate relevant data using quantitative methodologies (Petts & Gurnell, 2005). As a result, pre-impoundment data pertaining to channel geomorphological alterations and modifications to natural flow regimes in Scotland are limited (Carling, 1988; Nelson, 2013). Prior to the 1950s hydro expansion, early understanding regarding the environmental and ecological impact of hydroelectric dam operations predominately comprised an awareness of potential channel degradation and the inhibition of salmonid migration (Petts & Gurnell, 2005). Following an increase in the rate of large dam construction in the 1950s and 60s, scientists and practitioners developed a greater appreciation of the wider temporal and spatial-scale effects of hydrological management practises, resulting in theories on which the central tenets of modern fluvial geomorphological research are founded (Brandt, 2000; Petts & Gurnell, 2005).

From these early postulations, a wealth of literature has been formulated to discern the impacts of hydroelectric operations on the corresponding Scottish river systems, with greater emphasis being placed on mitigating the loss of ecological integrity (Addy et al., 2012; Geris et al., 2015; Gilvear et al., 2002; Gilvear, 2004; Gowans et al., 2003; Langan et al., 2007; Moir et al., 2002; Winterbottom, 2000). Governmental bodies such as the Scottish Environmental Protection Agency (SEPA) and NatureScot oversee the management of river systems, with legislature such as the Water Environment Regulations (SEPA, 2018), applying regulatory controls on the environmental flow practises to minimise the potential impact of impoundments on the ecology of associated ecosystems. These regulations are largely driven by empirical data collected by the aforementioned organisations, yet there remains a dearth in research concerning direct examination of non-fish species', such as *M. margaritifera,* responses to alterations in flow.

Despite regulatory controls on river systems, a recent drive towards renewable energy within Scotland has increased pressure to maximise production from the available resources. This has included the use of traditional storage hydroelectric schemes, in addition to renewed interest in large and small-scale hydroelectric dam development to bolster renewable energy infrastructure (Forrest & Wallace, 2009). As a result, the last three years have witnessed a significant change in water management, with storage reservoirs being drawn down significantly lower than before to maximise energy production, and to prevent uncontrolled spill when hydroeletric generation is halted to avoid overloading the energy grid (A Stephen 2021, personal communication, 10 March). However, significantly lower storage levels may inhibit the ability to maintain agreed statutory minimum (compensation) flows during drought conditions. These compensation flows are often higher than the full range of annual flows expected under natural conditions. Unnaturally high minimum flows consequently create permanent wetted widths, with a bank-to-bank flow retained throughout the year, prompting substantial colonisation by freshwater pearl mussels. Thus, dense *M. margaritifera* beds are often found in regions of regulated rivers which present shallow gradients and water depths, unlikley to be inhabited under natural flow conditions. Yet, to avoid complete dessication of the river system during epsiodes of drought, a reduction in flow below compensation levels may be neccessary, resulting in extended emersion of mussel beds and subsequent mass mortality (A Stephen 2021, personal communication, 10 March). Therefore, empirical evidence is urgently required to drive future conservation strategies with regards to river flow management, whilst also accounting for expected alterations in environmental conditions due to climate change (Addy et al., 2012).

2.1.6 Climate Change

Climate change represents one of the greatest threats to freshwater biodiversity (IPCC, 2014; Thomas et al., 2004; Woodward et al., 2010). The impact of associated alterations in climatic conditions are likely to vary across species and ecosystems depending on the nature and magnitude of modifications, in addition to the challenges to conservation management these alterations incur (Cosgrove et al., 2012; Thomas et al., 2004). Within freshwater systems the most prevalent impacts of climate change are anticipated to take the form of modifications to thermal and hydrological regimes (Sundt-Hansen et al., 2018).

Global mean surface air temperatures have risen by 0.81°C over the last decade, with a further increase of 1.4-3.1°C predicted by the end of this century (IPCC, 2014): a trend likely to be replicated in freshwater systems (Payton et al., 2016). Significant alterations to the thermal regimes in the water body could potentially induce drastic changes to the dynamics of freshwater communities. Temperature is known to govern the physiology and metabolic rates of organisms, in addition to influencing fecundity, recruitment, mortality and species distributions (Carr et al., 2019). Thus, alterations in freshwater communities from modified thermal regimes are anticipated to develop through species-specific differences in physiological tolerance, adaptive response to stress and life history characteristics; culminating in a scenario of ‘winners’ versus ‘losers’ (Payton et al., 2016).

When researching the effects of climate change on species of bivalve, in relation to individual response towards thermal stress, previous studies have often focussed on comparisons across and within species distributed along latitudinal gradients (Compton et al., 2007; Spooner & Vaughn, 2008; Thorp et al., 1998). Research conducted by Payton, Johnson, and Jenny (2016) revealed species-specific responses to similar temperature regimes between closely related Unionid species, with apparent differences in biochemical, physiological and molecular responses; suggesting response mechanisms are unique to Unionid species, and may be non-comparable.

With regards to *M. margaritifera,* evidence suggests moderate elevations in water temperature may augment juvenile recruitment, which could act to increase population recruitment (Hastie et al., 2003; Hruška, 1992). However, thermal effects can drive potential shifts in pearl mussel reproduction by several months. This may decouple the timing of reproduction with heightened abundances of host salmonid fry in the corresponding river systems (Cosgrove et al., 2012). More drastic increases in water temperature could potentially cause faster growth in adult mussels and subsequently reduce their life-span: lowering the number of reproductive episodes (Ziuganov et al., 1994). At the southern edge of its distribution, in the Iberian Peninsula, increases in thermal regime could potentially incur the loss of population recruitment due to an exceedance of the mussels' physiological capacity (Sousa et al., 2015). Despite these concerns, freshwater mussels have been shown to be more thermally tolerant than their corresponding host fish species, with salmonid growth, survival and demographic characteristics strongly influenced by temperature (Pandolfo et al., 2012; Sundt-Hansen et al., 2018). Therefore, alterations in thermal regime may evoke more substantial indirect effects on *M. margaritifera* populations. Within Scotland, predicted alterations to temperature regime suggest a constriction in the availability of suitable habitat for salmonid species (Jackson et al., 2018). With cold-water oligotrophic streams and rivers anticipated to experience the most drastic alterations in water temperature (Bolotov et al., 2018), there is concern that many populations of *M. margaritifera* in Scotland could consequently experience a drastic decline in the availability of host fish.

In consideration of the hydrological modifications resulting from future climate change, previous studies have often drawn attention towards increased inter-annual variation in levels of precipitation and the consequential effects on flow regimes (Lopes-Lima et al., 2016). In the 1980s, the hydrological behaviour of Scottish rivers significantly altered, with records displaying new maximum flood thresholds, increased occurrence of high flow regimes and elevated annual runoffs (Hastie, Ellis & Gaywood 2003). These extreme climatic events (e.g. drought and flood events) are predicted to occur in greater frequency and magnitude, in accordance with future climate change (Orr et al., 2015).

Within Scotland the magnitude and frequency of flood events is expected to significantly increase, with 1 in 200 year flood events potentially reducing to 1 in 18 year occurrences, under the "H-Wet" scenario of UKCIP02 (Cameron, 2006). Periodic, low-intensity flooding is likely to provide beneficial habitat characteristics to associated mussel populations via low-impact scouring events: fine sediments and pollutants are flushed away without risking mussel displacement (Hastie et al., 2003; Nobles & Zhang, 2004). However, high magnitude flood events have been shown to adversely affect mussels, with considerable population die-offs frequently witnessed after flood events (Hastie et al., 2001; Sousa et al., 2012). Research by Hastie et al. (2001) highlighted the impact high flow events can have on *M. margaritifera* populations: reporting a 4-8% mortality (fifty thousand individuals) after a 1 in 100 year flood event, with juvenile mussels (<10 years old) disproportionately affected. Further study, regarding the role of high flows in shaping freshwater mussel populations, revealed an inhibition of juvenile settlement and a loss of stable substrates to facilitate burrowing (French & Ackerman, 2014; Randklev et al., 2019). Of the 71 extant populations in Scotland, 63 inhabit small-moderate, shallow watercourses, which are anticipated to be highly susceptible to flooding (Cosgrove et al., 2012). However, 89% of the river systems inhabited by extant populations contain "sizeable" lakes in the catchment, which may act to buffer populations by reducing the magnitude of extreme flow events through the accumulation and release of water over greater periods of time; consequently, ameliorating flood and spate events (Cosgrove et al., 2012).

Perceptions of Scotland as a predominately wet country have potentially limited research into climate future scenarios presenting greater water scarcity. Despite this, evidence from recent studies suggests more frequent periods of low summer discharge due to extended episodes of low precipitation, in addition to lower levels of ground water, with extreme drought events expected to increase from 1 in 20 years to 1 in 3 years (Kirkpatrick et al., 2021). A report conducted by Natures cot, found that a recent drought in 2018 caused substantial damage to *M. margaritifera* population condition in six rivers across Scotland, although the extent of mortality is unclear due to a lack of survey data within the respective systems before and after the drought occurrence (Cosgrove et al., 2021). Nevertheless, other examples outside of the UK provide clear evidence of the extent to which prolonged drought conditions can impair population condition in Unionid species. For example, a 93% loss of total mussel abundance was observed in some areas of the watercourse following a drought in Georgia between 1999 and 2001 (Golladay et al., 2004). In Portugal, a large die-off was observed within a population of *M. margaritifera* following a severe drought in 2017 (Sousa et al., 2018). Here, mussel deaths were attributed to a rapid decrease in flow conditions, culminating in the marooning of mussels on the exposed river bed, with mortality attributed to desiccation and predation by wild boar. This predation of emersed individual mussels by terrestrial species during periods of drought is well documented in the literature, and believed to contribute substantially to the observed mortalities (Morales et al., 2011; Walters & Ford, 2013). The resistance and resilience of *M. margaritifera* to alterations in flow is thought to be relatively low, with the species believed to be sedentary (Sousa et al., 2018). Despite this, recent studies concerning the response of freshwater mussel species inhabiting drought-prone systems have demonstrated the utilisation of behavioural strategies to avoid or mitigate the effects of emersion: individuals tracked receding water levels (horizontal movement) or burrowed (vertical movement) further into the riverbed substratum (Gough et al., 2012; Lymbery et al., 2020). The presence of such behaviours in pearl mussels are yet to be studied but necessitate investigation to solicit a better understanding of the risks associated with water level reduction.

Although it is clear that climate change presents significant risks to pearl mussel populations, there remains significant variation in the predicted climate scenarios likely to be experienced (Cameron, 2006); thus, confounding efforts to assimilate a coherent conservation management plan for river systems inhabited by *M. margaritifera*. Attempts to comprehend particular outcomes of climate change must account for the complexity of the systems they are impacting, and adopt a multifaceted approach to quantify the uncertainties of climate change impact studies (Prudhomme et al., 2003). For example, assumptions of a species response to climate change, solely based on the analysis of thermal tolerance, neglects the complex amalgam of long that accompany this environmental phenomenon (Grabowski & Gurnell, 2016; Woodward, Perkins & Brown 2010). Furthermore, the synergy of a multitude of stressors and disturbance events may act to intensify the effects of climate change: summer droughts may lead to the amplification of eutrophication and other forms of pollution, due to elevated concentrations of associated pollutants; in addition to expected increases in temperature and habitat fragmentation (Woodward et al., 2010).

2.2 Current Conservation Strategies

Despite the considerable population declines observed across the species' Holarctic range, the prospects for recovery are higher than for many other endangered species, due to the longevity of *M. margaritifera* and their high reproductive potential (Bauer, 1992; Geist, 2010). To mitigate declines observed across *M. margaritifera* populations, early conservation management focused predominately on the examination of abiotic habitat characteristics and interspecies dynamics, towards identifying habitat requirements (Geist, 2010). Yet these early approaches often failed to attend to the persistence of genetic and phenotypic diversity within and across populations (Moritz, 2002; Preston et al., 2010), often prescribing a one-size-fits-all approach. More recent conservation management schemes have placed greater emphasis on the conservation of biodiversity between and within species, consequently preserving evolutionary capabilities to adapt to future alterations in the environment (Geist, 2010). These efforts have focused on the restoration of degraded habitats and propagation of populations *ex-situ* towards subsequent reintroduction and translocation efforts. Such schemes are buffered with regulations and legislation at a national and international level, with the creation of protected areas (Ziuganov et al., 1994). Furthermore, the development of defined monitoring techniques to track the condition of populations as well as identify phenotypic and genetic variation (Geist et al., 2018; Tuttle-Raycraft & Ackerman, 2020; Zieritz et al., 2010), has assisted in providing context-driven management techniques, to target limited resources more effectively.

2.2.1 Restoration of degraded habitat

Mussels are intimately associated with their habitat and current drivers of decline are largely attributed to habitat degradation (Downing et al., 2010). Therefore, in the absence of suitable habitat, conservation measures such as reintroductions and translocations are destined to fail (McMurray & Roe, 2019; Thomas et al., 2010). Attempts to restore river systems with the aim of re-establishing a more suitable environment for aquatic species are often long and costly processes, with expenditure on stream restoration exceeding $1 billion per annum in the USA alone (Renwick & Rakovan, 2010). Irrespective of variation in their size and scale, most restoration projects within lotic systems have prescribed to the theory that increasing habitat heterogeneity results in improved environmental conditions, and an associated heightening of biodiversity (Guzelj et al., 2020).

River restoration projects have been undertaken across Europe, but despite Europe Union directives it appears there are disparities in restoration trends, attributed to cultural differences (Guzelj et al., 2020). For example, restoration of the Skjern river, Denmark, comprised the excavation of the riverbed with the aim of reverting back to a historical flow path. This necessitated substantial capital investment, with the system requiring a long time to recover to full ecological functionality after the completion of construction work (Zingraff-Hamed et al., 2017). Contrastingly, in France, a successful, large-scale, process-based approach was adopted whereby sections of the river were designated specific corridors in which self-forming processes govern the channel formation; thus, anthropogenic mitigation was rarely necessitated (Piégay et al., 2005). Other restoration approaches utilised by conservation management comprise the inclusion of fish passages to allow migration past dams (Benson et al., 2018), and defragmentation through dam removal (Lejon et al., 2009). However, previous fish passage designs have been ineffective and can be used to justify harmful dams (Brown et al., 2013), whilst the removal of dams can evoke a substantial release of fine sediment and pollutants into the watercourse that may significantly impede freshwater mussel populations downstream (Ferreira-Rodríguez et al., 2019; Österling et al., 2010). Holistic restoration schemes which encompass the entirety of river catchments are anticipated to be the most successful in evoking *M. margaritifera* population proliferation (Geist, 2010). Nevertheless, there remains a distinct lack of long-term monitoring following restoration schemes to evaluate their potential success, with even fewer projects reporting their failures (Gardeström et al., 2013).

2.2.2 Propagation of populations

To overcome habitat deficiencies at the population level, conservation management has frequently utilised artificial culturing and breeding techniques (Barnhart, 2006). These techniques are often viewed as a last resort to retain the evolutionary potential of priority populations residing within heavily disturbed habitats, which require extensive restoration (Eybe et al., 2015). Here, adult mussels are collected from wild populations and brought into captivity, establishing living gene banks, where recovery is aided in the hope of achieving self-sustaining populations (Thomas et al., 2010). This *ex-situ* conservation of *M. margaritifera* populations involves some or all of the following (Hastie & Young, 2003): fertilisation of females in captivity (Preston et al., 2007); infection and encystment of glochidia in suitable fish hosts (Taeubert et al., 2013); stocking of infected host fish into historical mussel rivers (Hruška, 2001); harvesting and rearing of excysted larvae (Gum et al., 2011); release of captive-reared juvenile mussels (Kyle et al., 2017).

A significant benefit of this approach corresponds to the control over environmental parameters, allowing practitioners to provide optimal mussel habitat, and subsequently increasing the survival of juvenile mussels (Lavictoire et al., 2016). Despite the optimisation of near-perfect techniques to culture certain freshwater mussel species *ex-situ* (Lopes-Lima et al., 2014), captive rearing techniques for *M. margaritifera* have proven more difficult (Lavictoire et al., 2016). A variety of methods to elicit the successful propagation of *M. margaritifera* have been trialled in Europe (Eybe et al., 2013; Gum et al., 2011; Hastie & Young, 2003; Lange & Selheim, 2011; Scriven et al., 2011), yet data concerning juvenile growth and survival is often absent, likely due to high mortality rates. Research is continuing to be undertaken to refine methods (Lavictoire et al., 2020; Wilson et al., 2012), with recent data concerning the use of mussel silos offering new, and potentially more successful pathways to reduce the high observed mortalities during juvenile reintroductions into natural river systems (Kyle et al., 2017). Nevertheless, further investigation into habitat suitability (Gray & Kreeger, 2014) in addition to the role of phenotypic (Preston et al., 2010) and genetic (Ferreira-Rodríguez et al., 2019; Geist, 2010) drivers of variation across individuals and populations, is required to tease apart the reasons for these high mortality rates.

2.2.3 Translocations

A less resource intensive method for protecting freshwater mussel populations from degraded habitat conditions, known to impede proliferation, pertains to the translocation of adult populations within and between rivers (Killeen & Moorkens, 2016). Here, individuals are moved in an attempt to establish, re-establish or augment populations (Cope & Waller, 1995; Haag & Williams, 2014). Despite frequent use of translocations, dating back hundreds of years (Killeen & Moorkens, 2016), empirical data concerning the viability of these techniques is surprisingly limited (Cope & Waller, 1995), leading some to question the efficacy of such approaches (Haag & Williams, 2014). A report by Killeen and Moorkens, (2016) attempted to collate and summarise data from 25 examples of translocation in *M. margaritifera* populations across Europe and North America. Results from this study suggest a mean population loss of 62% at the receptor sites. Minimal success in previous translocation efforts could be ascribed to biased mark-recapture techniques (Meador et al., 2011), improper handling and transport of mussels (Yusufzai et al., 2010) and unsuitable habitat at the receptor sites (Cope et al., 2003). Moreover, translocation attempts appear to be more successful when conducted within the same system, with Valovirta (1998) reporting 90% short-term survival for an intra-river translocation, compared to 50% survival for an inter-river translocation. The reason for this could be grounded in phenotypic differences between populations, with significant differences in shell morphology between populations of *M. margaritifera* thought to reflect ecophenotypic variations, governed by the hydrologic conditions within respective habitats (Hastie, Young, et al., 2000; Preston et al., 2010). However, genotypic differences within and across populations may also drive these observations (Geist, 2010). A recent study concerning translocation efforts in other Unionid species has provided evidence to suggest methods could be improved to achieve greater success, with habitat quality in receptor sites a key factor in realising the sustainability of the relocated population (Tsakiris et al., 2017). Thus, there is a need to study *M. margaritifera* responses to translocations for the purpose of developing and enhancing this conservation strategy.

2.2.4 Legislation and regulation

Within the European Union, populations of freshwater pearl mussels are protected by the European Habitats Directive, which elicits the safeguarding of the species via designated special areas of conservation (Council of the European Communities, 1992). However, the Habitats Directive was published in 1992, with assumptions largely denoted from data collected in the 1980s. Therefore, knowledge concerning the condition of ecosystems across Europe may be inaccurate, with potential implications for conservation management of systems inhabited by *M. margaritifera* (Ferreira-Rodríguez et al., 2019; Modesto et al., 2018). As such, it is crucial that appropriate spatial scales for conservation units are defined to ensure effective management practises (Abell et al., 2007).

Conservation units take two forms: evolutionary significant units (ESU), which constitute a population (or group of populations) that is ecologically and genetically distinct from other units; and management units (MU), defined as populations that are demographically distinct from one another (Funk et al., 2012). Maintenance of ESUs is anticipated to raise the evolutionary potential of populations, consequently enhancing their potential resilience to future environmental alterations, whilst the preservation of multiple MUs is expected to aid the conservation of genetic diversity. Despite recent advances in the dissemination of genetic diversity across populations of *M. margaritifera* (Geist et al., 2018), further research is required to identify relevant MUs, with implications towards the success of stocking and breeding activities with freshwater mussels (Geist, 2010).

2.2.5 Monitoring techniques

A consistent approach to monitor pearl mussel populations had remained underdefined until 2017, when the European Committee for Standardization (CEN) released the standard for pearl mussel monitoring (British Standards Institution, 2017), representing the collaborative efforts of 40 specialists in pearl mussel biology, across 10 countries. The aim of this work was to aid conservation of *M. margaritifera* by outlining recommendations for the monitoring of populations and their habitat, with the hope of ascertaining comparable data across temporal and spatial scales to enable the early identification of population declines, and potential threats, in addition to offering guidance towards low impact monitoring techniques (Boon et al., 2019). Despite this progress, efforts to refine these techniques remain ongoing.

Research by Killeen and Moorkens (2020) sought to develop and refine current habitat mapping techniques to determine the extent and condition of suitable habitat for juvenile mussels, whilst work by Dobler et al. (2019) demonstrated the use of ecological niche models to investigate whether current conservation efforts are sufficient in protecting populations of *M. margaritifera*. Yet there remains a distinct lack of depth regarding assessments of hydrogeomorphic characteristics in mussel habitats, with standard monitoring techniques focused on the visual assessment of substrate structure and composition in association with examination of river discharge (Boon et al., 2019). Information and guidance regarding the detailed analysis of near-bed flow hydrodynamics is limited, despite often being considered the most ecologically relevant data for benthic biota; crucial in determining their spatial distribution (Blanckaert et al., 2013; Long et al., 2011; Oldmeadow et al., 2010; Robson et al., 1999). Thus, there remains significant gaps in current monitoring techniques, preventing a thorough understanding of the species' habitat requirements.

A key concern for many practitioners when undertaking habitat and population assessments of *M. margaritifera* pertains to the invasive and potentially destructive nature of the corresponding techniques (Killeen & Moorkens, 2020). Additional constraints concerning current quantitative and qualitative monitoring methods include their time consuming nature; possible underestimation of the abundance and composition of populations; limited application when presented with unfavourable climatic conditions on the day; and requirement for skilled taxonomic specialists (Sansom & Sassoubre, 2017). To overcome this, studies have assessed the utility of non-destructive, non-invasive monitoring tools such as environmental (e)DNA. Environmental DNA is a relatively novel approach that tests for the presence or absence of aquatic species through the examination of water samples (Stoeckle et al., 2016). Studies have demonstrated the effectiveness of eDNA, with evidence to suggest it could offer greater sensitivity than existing monitoring methods (Wilcox et al., 2016). However, the use of these techniques has largely been confined to the examination of fish and amphibians (Ficetola et al., 2008; Goldberg et al., 2011; Jerde et al., 2011; Takahara et al., 2012), with studies analysing the efficacy of eDNA to detect freshwater mussel populations only recently being explored.

Early research concerning the adoption of eDNA techniques to monitor freshwater mussel populations suggest reliable detection rates, with extensive downstream transport enabling the detection of populations located several kilometres upstream (Carlsson et al., 2017; Stoeckle et al., 2016; Wacker et al., 2019). Despite this, detection rates for systems inhabited by sparsely populated aggregations are often low, whilst seasonal variation can be significant: up to 20-fold increases in eDNA concentrations were observed when comparing results across spring and summer months (Wacker et al., 2019). Downstream transport may also act to inhibit the applicability of eDNA techniques to infer local abundance of mussel populations (Wacker et al., 2019). Further study to understand eDNA transport and decay is required to address these constraints, with the creation of models to account for these processes potentially offering reliable approaches to discern local presence and abundance of mussel populations (Sansom & Sassoubre, 2017). However, such techniques are unlikely to provide an indication of the population condition.

To date, research concerning the monitoring of population condition, has often relied on a correlative approach, identifying associations between environmental characteristics of habitat and the condition (e.g. presence, abundance, recruitment) of *M. margaritifera* populations (Morales et al., 2006; Strayer

& Ralley, 1993; Tarr, 2008). However, predictive models are often limited in their application across larger temporal and geographic scales due to their over-specificity of context-dependent parameters (Campbell & Hilderbrand, 2017; Strayer, 2008), in addition to an apparent lack of experimental evidence concerning interactions between the environment and the life-history traits of freshwater mussels, to provide a strong scientific basis for model assumptions (Clements, 2015; French & Ackerman, 2014).

To bridge this gap, recent attempts to accurately quantify the health of freshwater mussels have focused on the examination of biological processes in response to alterations in the environment (Fritts, Peterson, Hazelton, et al., 2015; Gagné et al., 2002). These techniques may assist in detecting early warning signs before the occurrence of mortality (Handy & Depledge, 1999), provide a method to study the effects of sublethal stressors (Hartmann et al., 2016), and aid the evaluation of population condition in response to translocation and restoration efforts (Gray & Kreeger, 2014; Roznere et al., 2017; Salerno et al., 2018). However, no known studies have illustrated the propensity of these methods towards the assessment of *M. margaritifera* condition.

2.3 Limitations to Future Conservation Efforts

A cross-examination of the current knowledge concerning habitat suitability, the drivers of population decline, and associated conservation management for *M. margaritifera* populations reveals a substantial lack of information concerning hydrogeomorphological processes and associated responses of freshwater pearl mussels.

In 1994, Statzner and Borchardt (1994) suggested the incorporation of fluid dynamics into studies of freshwater ecology would accelerate ecological theory beyond past and current research approaches. Yet progress has been slow, with knowledge of how organisms have evolved and adapated in response to flow conditions in their habiat remaining largely absent (Statzner, 2008). This notion is clearly reflected in the examination of techniques used to assess the hydrologic characteristics of freshwater pearl mussel habitat, which have rarely extended beyond point measurements at 60% flow depth, with only one known study having examined near-bed flow regimes in pearl mussel habitat (Moorkens & Killeen, 2014). With regards to examinations of riverbed structure and composition, conservation management has often focused on surveys to examine riverbed stability, which rely on descriptive analyses. However, these approaches risk substantial user-bias (Johnson & Brown, 2000; Schwendel et al., 2010) and are grounded in theory that contradicts fundamental understanding of fluvial substrate mechanics (Sansom, 2018).

In addition to the coarse nature of methods to define hydrogeomorphological processes, no known studies have examined the responses of *M. margaritifera* to alterations in flow or habitat structure, due to assumptions of a perceived sedentary existence. However, this notion would appear to contradict findings observed in other Unionid species, with a growing body of evidence to suggest that mussels do respond to alterations in environmental characteristics: seasonal movement is attributed to reproductive purposes (Amyot & Downing, 1997); movement has also been observed in response to changes in the hydrologic environment (Bartsch et al., 2010; Block et al., 2013; Clements, 2015; French & Ackerman, 2014; Hamstead et al., 2019; Johnson & Brown, 2000); with burrowing also found to assist in the prevention of zebra mussel infestation (Burlakova & Karatayev, 2007). Thus, there is a need to define the life-history traits of *M. margaritifera* in response to alterations in environmental conditions, with a focus on metrics associated with hydrogeomorphological process. This research is urgently required to inform conservation management.

In Scotland empirical evidence of *M. margaritifera* responses to alterations in hydrogeomorphology is particularly warranted when considering the immense pressures derived from river management practises and future climate change. There remains a lack of knowledge regarding the interaction between hydropower development and climate change. Yet synergistic amd antagonistic interactions between hydropower plants and climate change are likely to arise; altering the prevailing habitat conditions, with modifications to patterns in discharge and temperature regime anticipated to significantly affect life-histories of aquatic species, in addition to broader ecosystem functions. Thus, an understanding of the response of freshwater pearl mussels to alterations in discharge and associated environmental parameters may assist conservation, through the provision of data to define risk in populations and identify the form and function of potential intervention management practises.

2.4 Aims and Objectives of Current Study

In recognition of the need to derive empirical evidence towards understanding the interactions between *M. margaritifera* and hydrogeomorphological processes, this project seeks to investigate the response of freshwater pearl mussels to a variety of environmental stressors. Specifically, a multi-disciplinary approach will be adopted to tackle gaps in the knowledge concerning mussel responses to alterations in flow, whilst also examining novel methods to improve current monitoring of populations to inform conservation management. Research regarding the analysis of mussel responses will focus on the examination of differences across individuals and populations, to assess the need for context-driven conservation management techniques. It is hoped the results of this work will provide data to better inform river management of this highly endangered species in Scotland, with findings that are applicable to broader conservation management efforts across global freshwater systems.

Research conducted as part of this thesis is presented in three empirical chapters (3-5). Much of the material presented in these chapters has been either published or accepted for publication. The following paragraphs will summarise the key aims and objectives for each chapter.

Chapter Three presents work that aimed to derive methods to non-invasively quantify stress in freshwater mussels. To do so, the study examined stress responses in freshwater mussels, through the analysis of their behavioural traits, in conjunction with aerobic metabolic rate. To assess the consistency of metabolic and behavioural responses to stress across unionid species, the responses of two unionid species were compared: the freshwater pearl mussel, *M. margaritifera*; and the duck mussel, *A. anatina*. To provide context to the expression of certain behavioural and physiological responses, this study looked to compare the response of the two species across two stressors: emersion; and heightened total suspended solids. This work tested the hypothesis that behavioural responses of *M. margaritifera* serve as a biomarker of physiological stress, unique to the individual, population and environmental stressor.

Chapter Four aimed to address knowledge gaps concerning the response of *M. margaritifera* to alterations in flow regime, important to informing river management towards conserving populations during significant disturbance events such as drought. To do so, this study examined the response of *M. margaritifera* to controlled water level drawdowns in a lab and field setting. To account for potential adaptation to conditions within their natural habitat, two different populations (one from a regulated system, and one from a semi-natural system) of *M. margaritifera* were compared. To determine whether a threshold in response exists, mussels responses were compared across different rates of drawdown. To examine the role of proximity to conspecifics, two different density treatments were compared: elevated density; low density. This work tested the hypothesis that freshwater pearl mussels display a propensity to detect alterations in flow depth that risk emersion, utilising behavioural responses that assist in the mitigation of mortality resulting from prolonged aerial exposure, which differ in occurrence and extent across individuals and populations.

Chapter Five provides an overview of research that aimed to provide a direct, non-intrusive, low-cost and accessible tool to evaluate near-bed incipient flow conditions and predict entrainment risk in unionid mussels. To achieve this, the study sought to design, for the first time, an instrumented freshwater mussel which comprises embedded inertial microelectromechanical sensors (MEMS) within freshwater mussel shells. To ensure biological relevance, the sensors were housed in different sized shells and calibrated, before experiencing simple functionality tests in a lab environment. Dedicated flume experiments were then undertaken to assess instrumented shell movement metrics, for a range of flow conditions and initial orientations (fully exposed and partially buried). Experimentally obtained results were analysed towards identifying metrics that offer distinct criterion for assessing the risk to entrainment. Finally, the utility of this tool was validated under identified metrics for indirectly assessing flow conditions that result in greater stressing of the mussels. This research tested the following hypotheses: (1) behavioural responses of *M. margaritifera* determine the extent of stress incurred by increased flow forcing, with greater shell exposure resulting in a heightened risk of entrainment; (2) examination of ecologically relevent near-bed flow conditions can be ascertained using data from inertial microelectromechanical sensors (MEMS) embedded within freshwater mussel shells to provide a direct, non-intrusive, low-cost and accessible tool for freshwater habitat assessment.

CHAPTER 3

3 BEHAVIOURAL AND METABOLIC RESPONSES OF UNIONIDA MUSSELS TO STRESS

Material presented in this chapter are included in the manuscript Curley E.A.M., Thomas R., Adams, C.E. & Stephen A. (in press, July 2021), Behavioural and metabolic responses of Unionida mussels to stress, Aquatic Conservation: Marine and Freshwater Ecosystems

ABSTRACT

1. The aim of this study was to assess the extent to which the behavioural traits of freshwater mussels provide suitable indicators of stress in individuals, towards the advancement of non-invasive, remote monitoring techniques to examine population condition.
2. Variation in the expression of particular behavioural metrics was examined in accordance with measurements of oxygen consumption, across environmental stressors (aerial exposure and high concentrations of suspended inorganic matter), and between two freshwater mussel species (*M. margaritifera* and *Anodonta anatina*)
3. Aerobic metabolic rate was quantified using intermittent respirometry, and behaviour was observed using time-lapse footage. Comparisons of metabolic response and the occurrence of behavioural traits, across the two stressors, focused on differences between the twenty-four-hour pre-exposure period (pre-exposure); the first three hours of post-exposure (immediate post-exposure); and the time following the initial three hours of post-exposure until the end of the experimental run (extended post-exposure).
4. The results of this study demonstrated a relationship between the frequency of occurrence of behavioural responses to stress exposure, associated with valve activity, and significant changes in the metabolic functioning of *A. anatina* and *M. margaritifera* mussels. Findings from the study also highlighted substantial intraspecific variation across species and stressors.
5. Data from this research could assist in the development of novel bio-sensors that track mussel valve activity remotely, in their natural environment. When coupled with real-time data examining alterations in environmental metrics, this technology could assist in the monitoring of population condition and aid conservation management.

Keywords: behaviour, physiology, remote sensing, hydropower, invertebrates, pollution, river

3.1 Introduction

Freshwater bivalve mussels of the order Unionida are one of the fastest diminishing taxa globally (Geist, 2011; Graf & Cummings, 2007; Lydeard et al., 2004), and considered to be vital to the health of the wider freshwater ecosystems (Vaughn, 2018): functioning as biomonitors of adverse habitat conditions (Lummer et al., 2016; Scheder et al., 2015; Vaughn, 2010); enhancing nutrient cycling and trophic interactions in freshwater communities (Allen et al., 2012; Boeker et al., 2016; Vaughn, 2010); whilst also contributing to habitat diversity in benthic environments (Boeker et al., 2016; Spooner & Vaughn, 2008). Despite this, conservation efforts have often been constrained by a limited understanding of their biology and insufficient identification of conservation units (Ferreira-Rodríguez et al., 2019; Fraser & Bernatches, 2001; Lopes-Lima et al., 2020)

One area of study that has witnessed increasing interest as a means of addressing these gaps in knowledge concerns the use of biomonitoring tools, or the tracking of specific biological processes, and how these processes respond to alterations in the environment (Blaise & Gagné, 2009; Farcy et al., 2013; Fritts et al., 2015; Gagné et al., 2002; Galloway & Depledge, 2001). The study of biological responses may assist in detecting early warning signs before the occurrence of mortality (Handy & Depledge, 1999), provide a method to study the effects of sublethal stressors (Hartmann et al., 2016), and aid the evaluation of population condition in response to translocation and restoration efforts (Gray & Kreeger, 2014; Roznere et al., 2017; Salerno et al., 2018).

Behaviours reflect an individual's response to a combination of environmental and physiological factors, and therefore have the capacity to provide sensitive, non-invasive indicators of stress in individuals (Hartmann et al., 2016; Hasenbein et al., 2015; Robson et al., 2009). Examples within the literature of non-lethal techniques for examining stress in freshwater and marine mussels often focus on two behavioural responses. Firstly, movement: specifically how a mussel may use its foot to move along the riverbed, or to burrow into substrate (Bartsch et al., 2010; Block et al., 2013; Clements, 2015; French & Ackerman, 2014; Johnson & Brown, 2000). Secondly, filtration: the active movement of water through the mantle, which facilitates respiratory and reproductive processes; previous studies suggest both valve activity and clearance rates mirror individual responses to environmental change (Nagai et al., 2006; Robson et al., 2012; Salerno et al., 2018; Tuttle-Raycraft et al., 2017; Wilson et al., 2005). Both behaviours have the potential to provide an easy and cost-effective biomarker of stress (Hartmann et al., 2016; Kádár et al., 2001; Liao et al., 2009; Lummer et al., 2016; Newton & Cope, 2006; Robson et al., 2009), which could be scaled up to populations and species.

Despite interest regarding the use of behavioural traits as potential non-invasive indicators of stress in freshwater mussels, few studies have researched the physiological mechanisms which may drive their expression during stress exposure (Archambault et al., 2014; Farcy et al., 2013). Research examining oxygen consumption rates ($\dot{M}O_2$)(a measure of aerobic metabolic rate) in other aquatic species, has received significant attention as a method for testing hypotheses that relate variation in physiological traits with intraspecific variation in behaviour and life history traits (Biro & Stamps, 2010; Burton et al., 2011; Rosewarne et al., 2016), with recent research demonstrating the efficacy of these techniques to evaluate stress in unionid mussels (Gibson, 2019; Haney et al., 2020). In the study reported here, we examined stress responses in freshwater mussels, through the analysis of their behavioural traits, in conjunction with aerobic metabolic rate.

Very few studies, linking the expression of behavioural traits with physiological condition in freshwater mussel responses to stress, have examined individual variability (Hartmann et al., 2016). During stress exposure, animals may prioritise specific physiological functions and behaviours (Killen et al., 2013); however, the expression of a trait may not be consistent across species and individuals (Biro & Stamps, 2010; Burton et al., 2011; Dingemanse et al., 2009; Jolles et al., 2017). Specifically, the extent to which an individual prioritises the expression of particular behavioural and physiological traits during stress exposure is thought to vary between conspecifics (Dingemanse et al., 2009). Therefore, to assess the consistency of metabolic and behavioural responses to stress across unionid species, we examined the responses of two unionid species: the freshwater pearl mussel, *Margaritifera margaritifera;* and the duck mussel, *Anodonta anatina*.

Previous research has almost solely focussed on identifying the response of behavioural traits to chemical pollutant stressors, with little consideration towards other environmental stressors. The prevailing ecological conditions within an animal's habitat are known to accentuate the importance of particular traits; thus, different stressors may evoke different responses and, therefore, highlight otherwise subtle differences between populations and species (Cook et al., 2011; Killen et al., 2013). To provide context to the expression of certain traits, individual responses should be observed across several environmental parameters, representative of common stressors a population experiences in the natural habitat, and presented at a magnitude necessary to evoke a response; thus, determining whether the response is linear or has a threshold effect. Consequently, this study looked to compare the response of the two species across two stressors.

There are two stressors to unionid mussels commonly identified in the literature that might be expected to induce stress. First, a reduced river discharge resulting in the aerial exposure of benthic living mussels (Bradley et al., 2012; Environment Agency, 2013; Lopes-Lima et al., 2016; SEPA, 2014). Second, high concentrations of fine particulate matter, resulting from erosional and depositional processes (Addy et al., 2012; Frank & Gerstmann, 2007; Naimo et al., 1992; Tuttle-Raycraft et al., 2017).

The purpose of this study was to investigate mussel behaviour as a biomarker for stress in unionid mussel species towards the creation of new techniques to assist in their conservation. To do so, this study tested the following three hypothesises. First, the physiology of mussels, measured as oxygen consumption, shows a quantitative response to stressors. Second, the expression of certain behavioural traits, measured as frequency of occurrence, shows a quantitative response to stressors. Finally, behaviour can be used as a non-invasive, non-destructive biomarker of underlying physiology in freshwater mussel species.

3.2 Methods

3.2.1 Mussel collection

A sample of *A. anatina*, were collected in December 2016 from Ryat Linn reservoir, East Renfrewshire, Scotland, during reservoir maintenance work when water depth was reduced, permitting access to deep, silty areas of the bed.

A sample of *M. margaritifera* mussels were collected, under licence, in the summer of 2017 from a mill lade, hydrologically connected to the main channel of the South Esk River, Scotland. The lade substratum largely consisted of uniform beds containing fine silt and gravel, interspersed with larger boulders and wood.

Adult mussels were removed by hand and stored in aerated cool boxes, lined with substrate and filled with water from the corresponding system. Collected individuals were held in two tanks (one for each species) at the Scottish Centre for Ecology and the Natural Environment (SCENE). Each tank contained washed gravel (0.1-25mm) to a depth of 100mm, fed with water from Loch Lomond at ambient temperature, 14°C ± 4.2 (annual mean temperature ± SD), to a depth of 200mm and a flow rate to mimic conditions of the respective habitats. Each individual mussel was marked with a unique number for identification on the shell using correction fluid, weighed, and measured for shell length, width and height (Table 3.1).

Table 3.1 Summary of the morphological characteristics found in the experimental sample populations of *Anodonta anatina* and *Margaritifera margaritifera*; presenting the number of individuals (n), and the mean measurement and the standard deviation (mean ± SD) for shell length, width, height and total weight.

Species	n	Length (mm)	Width (mm)	Height (mm)	Total weight (g)
Anodonta anatina	20	90.45±3.03	49.14±2.16	27.75±2.20	81.98±7.35
Margaritifera margaritifera	20	93.33±8.18	41.70±3.57	25.41±2.34	62.80±13.74

3.2.2 Experimental set-up

The experimental setup (Fig.3.1) consisted of four metabolic chambers (1.25L). Each chamber was placed within a separate holding tank (70L) and submerged in water. Washed gravel (0.1-25mm) was placed in each chamber to a depth of 50mm. Air stones were placed within each holding tank to maintain maximum dissolved oxygen levels. Underwater digital cameras (GoPro Hero 8 Black, Gopro, Inc. San Mateo CA, USA) were positioned inside the holding tanks, facing the metabolic chamber and programmed to capture time-lapse footage (one image min^{-1}). A wooden plank was placed across on length of the holding tank, to accommodate individuals during the aerial exposure experiments, with camera mounts adhered to the plank in locations to enable continuous capture of behavioural responses. Adequate water circulation within the holding tanks was maintained using two Eheim pumps (1046 Universal). The experimental apparatus was located within a Constant Temperature Unit at SCENE; ensuring water temperatures were maintained at 15 ± 0.5°C throughout an experimental run.

3.2.3 Experimental overview

The experiment was designed to compare the physiological response (metabolic rate) and behavioural responses of the same individuals of two different mussel species to two different stressors (air exposure and suspended sediment; Fig.3.1). Forty mussels (20 from each species) were randomly selected for the experiment using a random number generator based on the unique identification numbers given to each indivual. Each individual was exposed to both stressors or control conditions. There were four treatments for each of the aerial exposure and turbidity exposure experiments, including one control condition (see Table 3.2 for details of magnitude). Before, during and after exposure to each stressor condition, the behaviour of each individual was quantified, with oxygen consumption rates recorded before and after exposure.

Each trial was conducted on four mussels simultaneously, each experiencing one of four treatment conditions (Low, Medium and High stress magnitude and a control group) and consisted of six sequential steps: (1) a two-day acclimation period (2) a two hour background check (3) a 24 hour pre-exposure period (4) a stress exposure (Table 3.2) (5) a minimum 18 hour post-exposure period (6) two hour background check. An individual experienced two trials, one with each of the two stressors. A period of six weeks rest was given to each mussel between the two trials, with marginal differences in individual SMR suggesting this was adequate for recovery (Table 3.3).

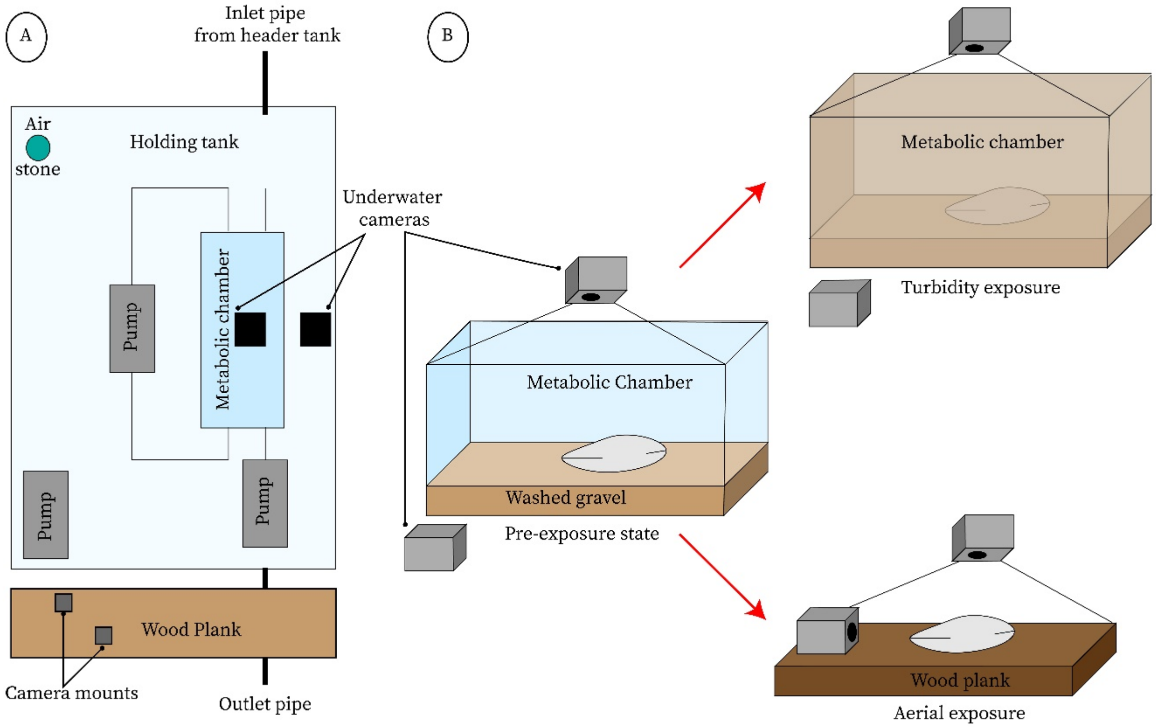

Figure 3.1 Schematic of the (A) experiment set-up observed within each of the four holding tanks, and (B) the conditions a mussel experienced during pre-exposure and two stressor exposures: aerial exposure and turbidity exposure.

Before the experimental stress exposure commenced, all mussels were acclimated to an experimental temperature, 15 ± 0.5°C, for two days in a, 30L, tank with untreated fresh water pumped from Loch Lomond and natural algal concentrations.

Two background checks, undertaken in the absence of mussels, recorded oxygen reduction in the metabolic chambers for two hours, before and after a trial, to obtain measures of microbial respiration (Svendsen et al., 2016). Oxygen concentration in the metabolic chambers during this period was regressed on time in both background check periods to quantify changes in background respiration over the course of a trial. Approximated background respiration was subsequently subtracted from measurements of mussel oxygen consumption.

After the background check, individuals were placed in the corresponding metabolic chamber for a pre-exposure period. Here, mussels remained for 24 hours undisturbed to record potential diurnal fluctuations in metabolic rate, and provide sufficient acclimation time (Gibson, 2019). Following this, mussels were exposed to the relevant stressor (Table 3.2). After stress exposure, mussels were left undisturbed in the metabolic chambers for a minimum of 18 hours before removal from the experimental set-up and the final background check commenced.

Comparisons of metabolic responses across the two stressors focused on differences in oxygen consumption between the twenty-four-hour pre-exposure period (pre-exposure); the first three hours of post-exposure (immediate post-exposure); and the time following the initial three hours of post-exposure until the end of the experimental run (extended post-exposure).

Table 3.2 An overview of the stressors used to elicit a physiological and behavioural response in Anodonta anatina and Margaritifera margaritifera mussels.

Stressor	**Implementation of exposure**	**Control Conditions**	**Low stress conditions**	**Medium stress conditions**	**High stress conditions**
Aerial exposure	Removal from metabolic chamber and placed in terrestrial conditions	Remain in metabolic chamber throughout experimental run	Placed in terrestrial environment for 1h30 and then placed back into metabolic chamber	Placed in terrestrial environment for 3h00 and then placed back into metabolic chamber	Placed in terrestrial environment for 4h30 and then placed back into metabolic chamber
Turbidity	Addition of ‘Polsperse 10’ kaolin to the water of holding tank until a pre-set turbidity measurement (Nephelometric Turbidity Unit; NTU) reached	0 NTU for 3h30	2 NTU (1 mg L^{-1}) for 3h30 then flushed out and replaced with 0 NTU water	20 NTU (153 mg L^{-1}) for 3h30 then flushed out and replaced with 0 NTU water	40 NTU (320 mg L^{-1}) for 3h30 then flushed out and replaced with 0 NTU water

3.2.4 Oxygen consumption

Oxygen consumption ($\dot{M}O_2$; mg O_2 h^{-1}) was measured using intermittent respirometry, using a computer-controlled setup that recorded oxygen partial pressure and temperature (sampling rate, 10 sec). Water oxygen content in the metabolic chambers was measured using optodes (Firesting 4-Channel oxygen meters, Pyroscience; www.pyro-science.com).

Intermittent respirometry was conducted according to the technique described by Svendsen et al., (2016). One complete measurement cycle (“loop”) comprised a five minute “open-system flush period” (flush state) and a 30 minute “closed-system, metabolism determination cycle” (closed state). This loop repeated consecutively until the end of an experimental run.

Individual $\dot{M}O_2$ was recorded during the “closed state” by measuring oxygen reduction in the metaboic chamber - calculated using linear least squares regression. The first ten minutes and the final two minutes of the closed-state-readings were excluded to ensure the linear component of oxygen reduction was captured in the absence of "noise" (Svendsen et al., 2016); resulting from pump operation.

3.2.5 Metabolic rate analysis

To determine an individual's metabolic rate as a proportion of metabolic tissue (mg O_2 $h^{-1}Kg^{-1}$), required metabolic tissue weights.

To obtain *A. anatina* metabolic tissue weights, mussels were sacrificed at the end of the experiment. Harvested tissues were dried at 70°C for two days to provide final dry tissue weights. Individual *M. margaritifera* were not sacrificed to obtain dry tissue weights, due to their endangered status. Instead, wet tissue weights were estimated. Empty shells were collected from the sampled population. To estimate live shell weight, the relationship between shell length, width and height and dry weight was calculated using a linear regression constructed from dead shells. Estimated live shell weight was then estimated from shell linear dimensions and subtracted from the total wet weight of live individuals, to estimate wet tissue weight for live *M. margaritifera* .

Mass independent metabolic rates (MIMR) were calculated to standardise metabolic rates and reduce the intraspecific variation (up to three fold differences in SMR observed between conspecifics), using residuals from a regression analysis between standard metabolic rate (SMR) and tissue weight ($P<0.001$) (Auer et al., 2015). Body mass and metabolic rates were $\log_{10}$-transformed prior to analyses to normalize and linearize the data.

Individual standard metabolic rates (SMR; mg O_2 h^{-1} Kg^{-1}) were calculated using oxygen consumption measures in the final 10 hours of pre-exposure. Readings taken during this period, within one standard deviation of the mean, were averaged to generate a final estimated SMR for the corresponding individual (see Table 3.3 for summary of calculations).

*Table 3.3 A summary of the calculations for standard metabolic rate (SMR; mg O_2 h^{-1} kg^{-1}) in Margaritifera margaritifera and Anodonta anatina sample populations. The percentage difference between individual's SMR readings for each of the two stressor experiments was calculated ((SMR Aerial/ SMR Turbidity)*100), with average individual variation for each species shown.*

Species	n	Mean (±SD)	Range	Avg. Individual Variation
Margaritifera margaritifera	20	12.86 ± 4.99	6.11 - 23.49	6.13
Anodonta anatina	20	176.29 ± 55.36	89.52 - 262.16	2.93

3.2.6 Behavioural analysis

Behavioural analyses were conducted only on mussels experiencing high and medium stress magnitude. Underwater digital cameras captured time-lapse footage from two hours before stress exposure to four hours after stress exposure.

Behaviour was quantified from video film only during 30-minute closed phases of intermittent respirometry cycles. Three behavioural metrics were quantified. First, "transition frequency", defined as the number of observations where the width of a mussel's shell aperture changed between successive images. Second, "avoidance behaviour", defined as the number of observations where the mussel's shell was closed. Finally, "foot extension", which recorded the number of observations where the foot of the mussel protruded from the shell and was clearly visible: distinctly different to the mussel's undisturbed resting behaviour, during which the foot is anchored into the substrate and isn't visible.

Comparisons of observed behaviour comprised the mean time or frequency of each behaviour during each of three time periods (pre-exposure, stress exposure and post-exposure).

3.3 Statistical analysis

Data were investigated using mixed effects models executed in R version 3.5.3 (R Core Team, 2020).

3.3.1 Physiological response

Three statistical approaches were used to analyse alterations in individual metabolic rate over time and in response to varied levels of stress magnitude.

First, analysis of individual MIMR measures, recorded at each 30 minute "closed state", were used as the only response variable in a mixed effects model, with Time (pre-exposure, immediate post-exposure and extended post-exposure), Stress magnitude (High, Medium, Low or Control) as covariates and Individual as a random variable.

Second, analysis of individual metabolic differential, calculated by taking the difference in mean MIMR between pre-exposure and immediate post exposure; irrespective of the direction of change (i.e. positive or negative). The metabolic differential was entered as the primary response variable in a mixed effects model, with Stress magnitude as a covariate and Individual as a random variable.

Finally, analysis of metabolic variability, calculated using confidence intervals (CI) of the mean MIMR for pre-exposure, immediate post-exposure and extended post-exposure. CI was entered into a mixed effects model as the primary response variable, with Stress magnitude and Time as covariates and Individual as a random variable.

3.3.2 Behavioural response

Analysis of each behavioural response (Transition frequency, Avoidance behaviour and Foot extension) was undertaken separately using two statistical approaches.

First, analysing the occurrence of the behavioural metric across an experimental run, using the average length of time the behavioural metric was observed during 30-minute closed states as the primary response variable in a mixed effects model, with Stress magnitude (High or Medium) and Time (pre-exposure, stress exposure and post-exposure) entered as covariates, and Individual as a random variable.

Second, a two-step approach, to assess the relationship between the occurrence of a behaviour and an individual's metabolic rate, using a mixed effects model designed to handle zero-inflated data. The presence or absence of the behavioural metric during the closed state was entered as the primary response variable into an initial mode, with Time (pre-exposure, immediate post-exposure and extended post-exposure), Stress magnitude (High or Medium) and MIMR entered as covariates, and Individual and Time (minutes) as random variables. A second linear mixed effects model was then created to determine which covariates affect the occurrence of the behavioural metric in the positive non-zero data, with Individual and Time (minutes) as random variables.

3.4 Results

3.4.1 Metabolic rate

Analysis of MIMR revealed differences in metabolic response between the two species (Fig.3.2).

There was a significant effect of stressor magnitude on MIMR, during aerial exposure experiments, in both species. In *M. margaritifera* a significant difference between the control group and the high stress magnitude group ($P<0.05$) was shown. For *A. anatina,* a significant difference between high magnitude aerial exposure and the control ($P<0.05$) was shown (Fig.3.2).

For turbidity exposure experiments, marginal differences in *M. margaritifera* MIMR between the control and low ($P=0.08$) magnitude grouping were found, in addition to marginal differences between control and high ($P=0.08$) magnitude grouping. No significant differences between the control group and the stressor magnitude groups were found in the turbidity exposure experiments for *A. anatina*.

Analysis of MIMR over time, during aerial exposure experiments, found MIMR was significantly lower during extended post-stress exposure ($P<0.001$) compared to pre-stress exposure in the high stress magnitude grouping for *M. margaritifera*. The same analysis for *A. anatina* found significantly lower MIMR during immediate post-exposure compared to pre-exposure ($P<0.01$) in the medium magnitude group. Contrastingly, MIMR significantly increased during immediate post-exposure compared to pre-exposure in *A. anatina*'s high stress magnitude group ($P<0.001$).

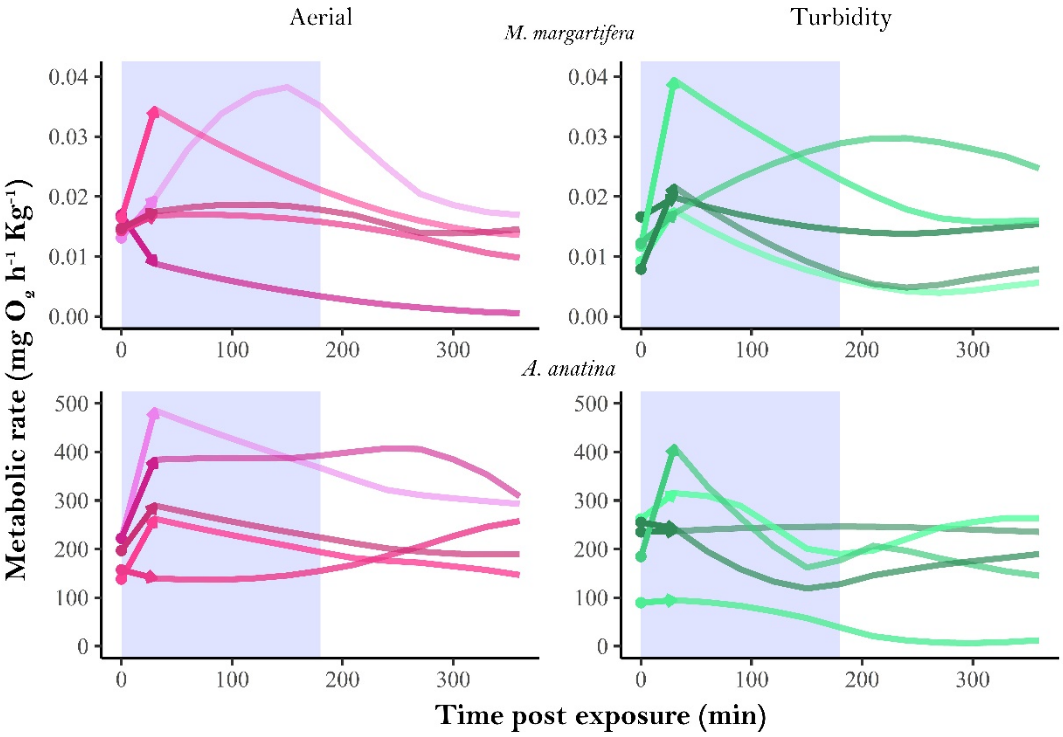

Figure 3.2 Variation in metabolic rate of individual mussels in response to high magnitude stress exposure. A comparison of changes in metabolic rate of M. margaritifera and A. anatina following aerial exposure and turbidity exposure. Each curve represents data for one individual and has undergone LOESS smoothing in R. Each line begins with a dot at the point of Standard metabolic rate (SMR) for the corresponding individual. An arrow from the point of SMR to the first metabolic reading at 30 minutes displays the initial direction of change in metabolic rate. Shaded area represents immediate post-exposure period, with extended post-exposure present thereafter

No further significant differences in MIMR over time were identified for aerial or turbidity exposure experiments in *M. margaritifera*. However, in *A. anatina*, the MIMR significantly increased during immediate post-exposure when compared to pre-exposure ($P<0.01$) across all stress magnitude groups, during turbidity experiments.

3.4.2 Metabolic differential

Assessment of the metabolic differential revealed similarities between the two stressors and between *M. margaritifera* and *A. anatina* (Fig.3.3).

A significant effect of stressor magnitude on the size of the metabolic differential during aerial exposure experiments was shown in both species. For *M. margaritifera*, the differential was significantly higher in the low ($P<0.05$) and high ($P<0.05$) stressor magnitude groups, compared to the control group. Similarly, the metabolic differential was significantly higher in the low stressor magnitude groups compared to the control ($P<0.05$) in *A. anatina*.

For turbidity exposure experiments, no significant differences between stress magnitude groups and the control were found in *M. margaritifera* and *A. anatina*.

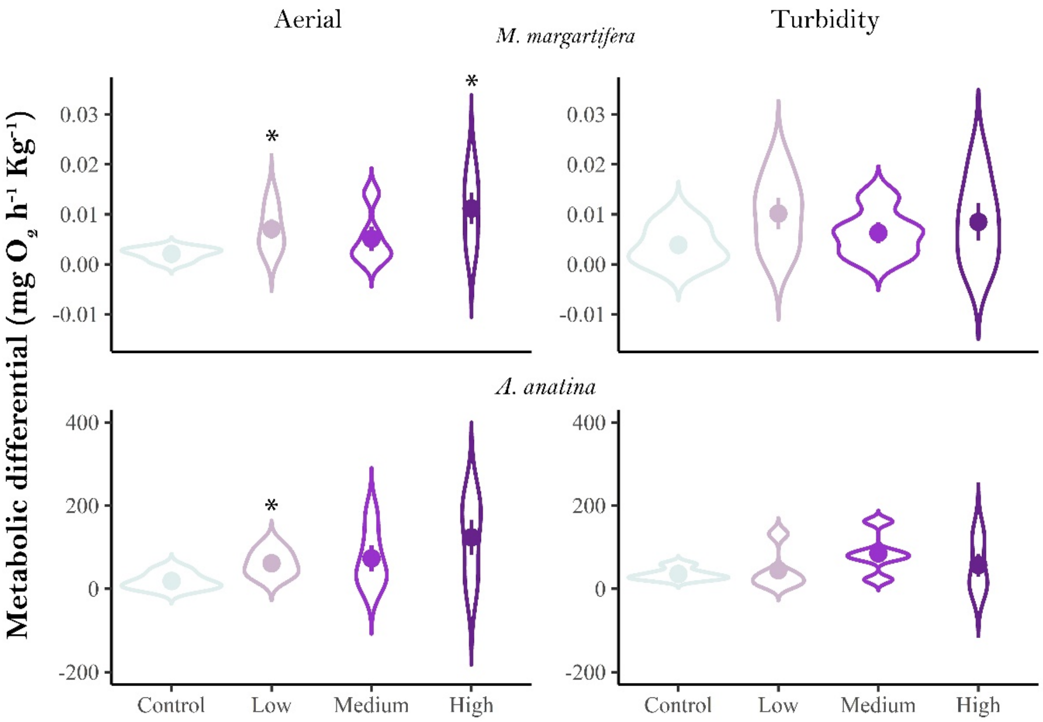

Figure 3.3 The effect of stress magnitude on the extent of change in metabolic rate in response to stress exposure. A comparison of metabolic differential in M. margaritifera and A. anatina following aerial exposure and turbidity exposure. The violin plot shows the mean and standard error for the metabolic differential of corresponding individuals across the stress magnitude groups. The significant differences highlighted in the output of Kruskal-Wallis tests, for comparisons of mean metabolic differential between stress magnitude groups and the control, are displayed with an asterisk (= P<0.05;** = P<0.01; *** = P<0.001).*

3.4.3 Metabolic variability

Examination of metabolic variability (CI) for *M. margaritifera* and *A. anatina* revealed some similarities in responses (Fig.3.4).

A significant effect of stress magnitude on metabolic variability in both turbidity ($P<0.05$) and aerial exposure experiments ($P<0.05$) was found in both species. Examination of aerial exposure showed a significant increase ($P<0.05$) in CI for all three stress magnitude groups when compared to the control, in both species, with no significant differences between stress magnitude groups shown in either species. Analysis of turbidity exposure found a significant increase ($P<0.05$) in CI for all three stress magnitude groups when compared to the control in *M. margaritifera*. Contrastingly, this was only true for the medium ($P<0.05$) and high ($P<0.05$) magnitude groups for *A. anatina*.

Examination of CI over time revealed similarities between the two stressors and the two species. During aerial and turbidity exposure experiments, *M. margaritifera* within each of the low ($P<0.01$), medium ($P<0.001$) and high ($P<0.001$) stress magnitude groups displayed a significant increase in CI during immediate post-exposure compared to pre-exposure. During aerial and turbidity exposure experiments with *A. anatina,* the control group ($P<0.05$), as well as each of the low ($P<0.01$), medium

($P<0.001$) and high ($P<0.001$) stress magnitude groups, displayed a significant increase in CI during immediate post-exposure compared to pre-exposure. During aerial exposure experiments, *M. margaritifera*'s extended post-exposure CI remained significantly higher than pre-exposure in all three stress magnitude groups ($P<0.05$). For *A. anatina,* the extended post-exposure CI remained significantly higher than the pre-exposure only in the medium stress magnitude group ($P<0.05$). For turbidity exposure experiments, no significant differences between pre-exposure and extended post-exposure CI readings were shown for either species.

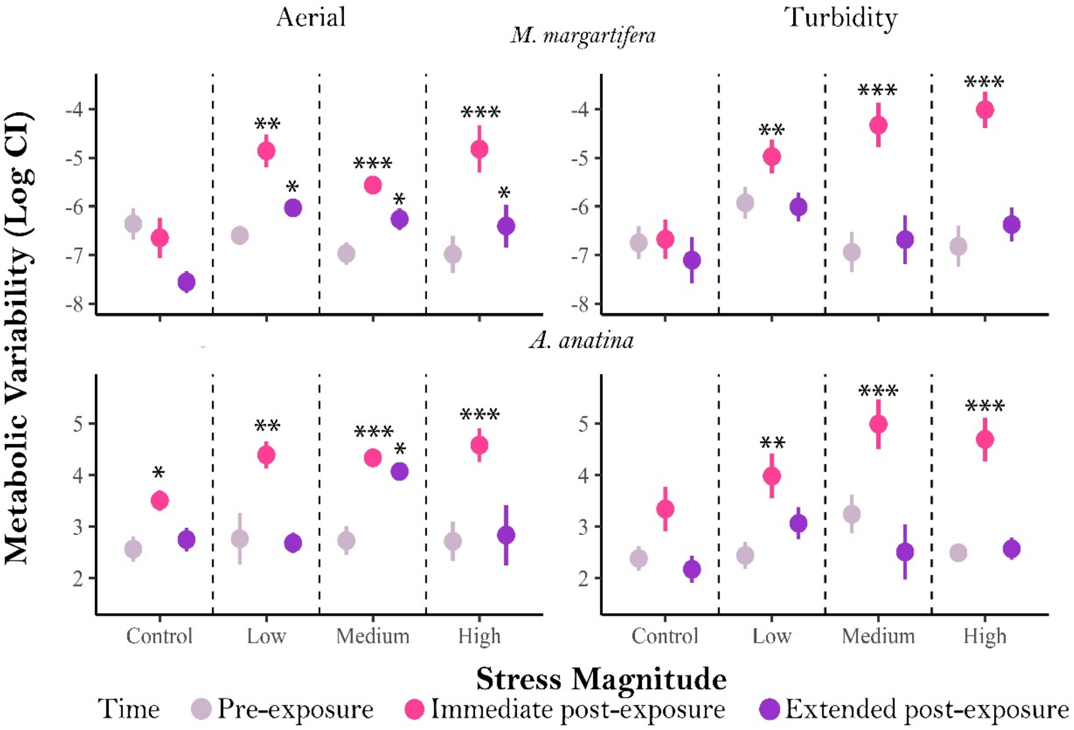

Figure 3.4 The effect of stress magnitude on variability in metabolic rate of individual mussels over time. A comparison of metabolic variability (confidence interval of mean metabolic rate of individual during corresponding time period) in M. margaritifera and A. anatina following aerial exposure and turbidity exposure. Each point corresponds to the mean confidence interval (± standard error) for the relative stressor, stress magnitude, species and time. The significant differences highlighted in the output of the Kruskal-Wallis tests, for comparisons of the mean confidence intervals within stress magnitude groups and between the pre-exposure time period and the two post-exposure time periods, are marked with an asterisk (= $P<0.05$;** = $P<0.01$; *** = $P<0.001$).*

3.4.4 Transition frequency

Examination of the transition frequency (alterations to a mussel's shell aperture width) as a behavioural response revealed some similarities at the interspecific level (Fig.3.5).

Stress magnitude did not significantly affect the occurrence of transition frequency during aerial exposure experiments in *M. margaritifera*; yet, a significant effect was found for *A. anatina* ($P<0.05$). During turbidity experiments, stress magnitude was shown to have a significant effect on the occurrence of transition frequency in *M. margaritifera* ($P<0.01$); contrastingly, no significant effect was found in *A. anatina*.

For *M. margaritifera* during aerial exposure, time had a significant effect on the occurrence of transition frequency, with transition frequency shown to be significantly higher during stress exposure (*P*<0.001) and post-exposure(*P*<0.001) compared to pre-exposure conditions. The same was true for *A. anatina*. During turbidity experiments, a significant effect of time on the occurrence of transition frequency in *M. margaritifera* was shown; this was limited to a significant difference between stress exposure and pre-exposure (*P*<0.001). A significant effect of time was also shown for *A. anatina* during turbidity experiments with both stress exposure and post exposure conditions shown to be significantly higher than pre-exposure (*P*<0.001).

Mixed effects models examining transition frequency with respect to metabolic activity found a significant association between the frequency of transition frequency and the metabolic rate (MIMR for *M. margaritifera*) in aerial exposure experiments: higher levels of transition frequency were related to higher readings of metabolic rate (*P*<0.001 for *M. margaritifera*; *P*<0.05 for *A. anatina*). Time was also a significant predictor of transition frequency in aerial experiments, with higher transition frequency during immediate post-exposure compared to pre-exposure (*P*<0.01) in both species. No significant predictors of transition frequency were found in turbidity exposure experiments.

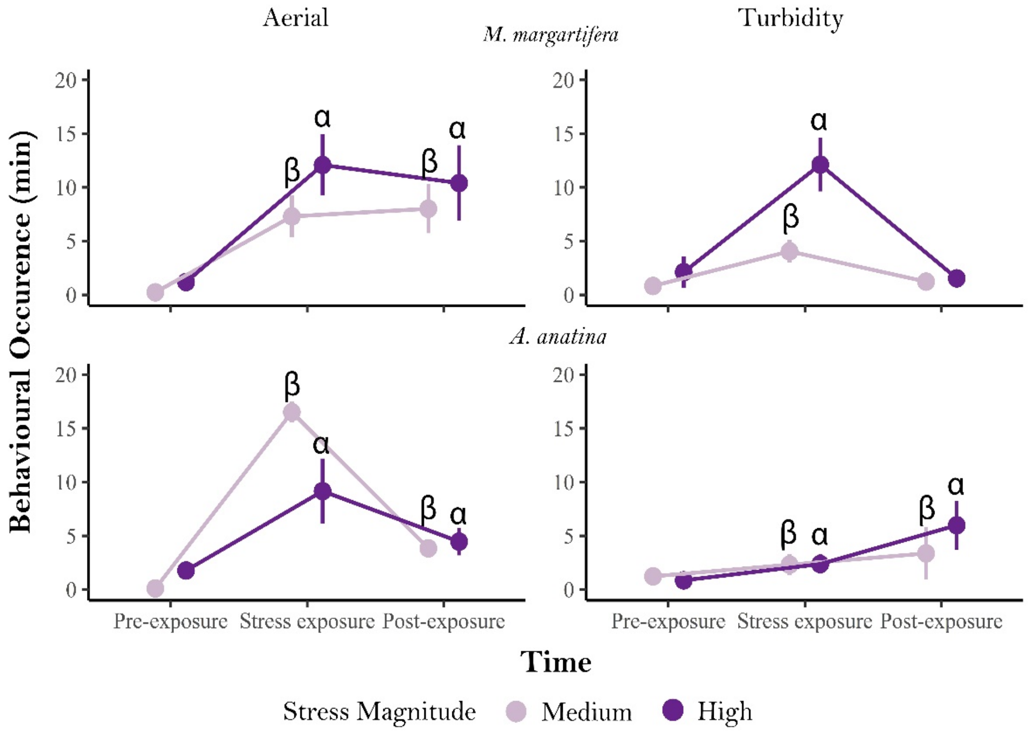

Figure 3.5 The effect of stress magnitude on the occurrence of transition frequency over time. A comparison of the mean (± standard error) proportion of time transition frequency occurs in M. margaritifera and A. anatina following aerial exposure and turbidity exposure, across the medium and high stress magnitude groups. Comparisons between the pre-exposure time period and the two subsequent time periods (Stress exposure; Post-exposure) were undertaken using Kruskal-Wallis tests. Significant differences, in the occurrence of transition frequency between the pre-exposure time period and the two subsequent time periods, for the high and medium stress magnitude groupings are marked with alpha (α) and beta (β) respectively. Lines have been added to connect data points and aid visualisation and are not representative of a continuous trend in the data set.

3.4.5 Avoidance behaviour

Examination of Avoidance (observations where the mussel's shell was closed) as a behavioural response revealed some similarities at the interspecific level.

There was no significant effect of stress magnitude during aerial exposure and turbidity experiments.

For *M. margaritifera* in aerial exposure experiments, time had a significant effect on the occurrence of avoidance behaviour, with avoidance behaviour significantly higher during stress exposure ($P<0.001$) and post-exposure($P<0.05$) compared to pre-exposure conditions. For *A. anatina* in aerial exposure experiments, there was a significant effect of time, but this was limited to a significant difference between stress exposure and pre-exposure ($P<0.001$). There was no significant effect of time on the occurrence of avoidance behaviour in either species, during turbidity experiments.

Mixed effects models examining avoidance behaviour in *M. margaritifera* and *A. anatina* with respect to metabolic activity revealed no significant effects of the predictor variables on the presence of this behaviour across the two stressors.

3.4.6 Foot extension

Assessment of foot extension as a behavioural response revealed some differences between the two species.

Stress magnitude significantly affected the occurrence of foot extension in *M. margaritifera* during aerial exposure experiments ($P<0.001$). No further significant effects of stress magnitude were found in either species, across both stressors.

For *M. margaritifera* in aerial exposure experiments, time had a significant effect on the occurrence of foot extension, with foot extension significantly higher during stress exposure ($P<0.05$) compared to pre-exposure conditions. No further significant effects of time were found for the occurrence of foot extension in either species, and across both stressors.

Mixed effects models examining foot extension in *M. margaritifera* and *A. anatina* with respect to metabolic activity revealed no significant effects of the predictor variables on the presence of this behaviour across the two stressors.

3.5 Discussion

The results of this study re-affirmed the notion that the behavioural response of unionid mussels to stress exposure provides a useful biomarker for examining the effects of environmental parameters on individual condition. Previous studies have established filtration and evasive behavioural strategies as biomonitoring tools to investigate tolerance to set concentrations of specific pollutants and between periods of rest and exposure (Haney et al., 2020; Hartmann et al., 2016; Liao et al., 2009; Premalatha et al., 2020; Tran et al., 2003). Nevertheless, this is the first known study that has attempted to identify common behavioural responses in freshwater mussel species across multiple environmental stressors, and associate these with measures of physiological stress. The results of this study provide evidence of behavioural responses to stress exposure that can be linked to physiological condition, specifically to metabolic rate, in *A. anatina* and *M. margaritifera* mussels. The study also revealed substantial intraspecific variation; highlighting the importance of individual variability when examining stress response across populations.

3.5.1 Metabolic response

A key component of this study was to examine whether physiology, measured as oxygen consumption, displayed a quantitative response to stressors. Initial findings showed significant differences in individual metabolic functioning, across both species, with mussels found to exhibit idiosyncratic metabolic responses to stress exposure: some mussels appeared to heighten their metabolic rates, whilst others displayed a metabolic depression immediately after stress exposure. However, significant individual variation was already present before exposure to the stressors, with a three-fold and four-fold difference between the maximum and minimum values for SMR in *A. anatina* and *M. margaritifera* respectively; a common finding in many other aquatic species (Burton et al., 2011; Kristín & Gvoždík, 2012; Leeuwen et al., 2012; Metcalfe et al., 2015). Therefore, quantification of physiological responses to stress required analysis that sought trends amongst the noise of individual variation.

Deviation from normal metabolic functioning, for extended periods of time following stress exposure, was found to be common across species and stressors; thus, presenting metabolic variability as a potential method for quantifying response to stressors. This observed increase in metabolic variability and frequent failure to return to normal metabolic functioning within the experimental time limit, following stress exposure in this study, is well documented in the literature: studies concerning metabolic response of bivalves to stress exposure, provide evidence to suggest individuals will sometimes require days to return to pre-exposure levels (Lopes-Lima et al., 2016; Newton & Cope, 2006; Payton et al., 2016; Ridgway et al., 2014; Robson et al., 2012).

It is likely that both stressors used in this study would impact the physiological functioning of freshwater mussels. Aerial exposure removes the appropriate medium for the mussel's specialised respiratory structures; consequently, preventing filtration activity from fulfilling an individual's metabolic requirements. The subsequent establishment of an energy deficit may force a substantial reduction in energy dissipation to prevent fatal thermodynamic imbalance and cell death (Thomsen & Melzner, 2010; Widdows & Shick, 1985). Contrastingly, high concentrations of inorganic suspended sediments are thought to increase the energetic demand of particle processing, with the active excretion of undesired compounds in pseudofaeces incurring an energetic cost to individuals (Lummer et al., 2016; Tuttle-Raycraft, 2018; Vaughn et al., 2008). For both stressors, the perceived deviation from the standard metabolic rate following intense physiological activity is perhaps, therefore, reflective of individual's continued attempts to adjust their filtration rates to compensate for disturbance to osmoregulation (Hartmann et al., 2016), nutrient turnover (Lorenz & Pusch, 2013) and respiratory processes (Shick et al., 1986).

Attempts to discern if a threshold in species response to the two stressors existed, revealed no substantial differences between stress magnitude groups, only between mussels that experienced stress, and those that didn't. Alexander, Thorp & Fell (1994) discovered a similar response in metabolic rate to increasing turbidity with *Dreissenia polymorpha*: acute exposure to suspended solids evoked a depressed metabolic rate; however, oxygen consumption did not cease or continue to decline at higher turbidity levels. Therefore, the results concerning physiological response to stress exposure suggest a binary response to the presence or absence of stress; contrary to a positive linear relationship between heightened response and greater levels of stress initially imagined.

3.5.2 Behavioural response

In addition to examining individual physiology, this study also assessed whether behavioural responses to stress could be quantified. For both species and stressors, transition frequency increased in occurrence in response to stress exposure.

Exposure to terrestrial conditions and suspended fine particulate matter would likely have constrained the capacity of mussels to function as filter feeders (Alexander et al., 1994; Shick et al., 1986; Tuttle-Raycraft et al., 2017; Widdows & Shick, 1985). To endure aerial exposure, the adoption of brief periods of air breathing may have assisted in the removal of metabolic bi-products through aerial diffusion, such as anaerobically produced CO_2; thus, permitting the conservation of energy stores and consequently preventing early fatigue (Shick et al., 1986). To cope with increased suspended fine particulate matter during turbidity experiments, a consistent alteration of valve activity would assist in modulating an individual's exposure to fine particulate matter: exposure may incur damage to the filter-feeding apparatus with inorganic solids overloading the gut and gills; interfering with filter-feeding functions and efficient gaseous exchange (Alexander et al., 1994). Despite providing a potential coping mechanism, brief periods of aerobic respiration during exposure to either stressor may not relinquish the reliance on anaerobic pathways (De Zwaan & Wijsman, 1976), with periods of closure interspersing phases of aerobic respiration to prevent physiological damage (Liao et al., 2009).

The implementation of anaerobic pathways to compensate the energetic requirements of an individual during stress exposure would necessitate a recovery period after the removal of the stressor, dependent on aerobic metabolism (Burton et al., 2011; Haney et al., 2020; Richards et al., 2002; Robson et al., 2012). To assist recovery, a constant movement of the shell aperture may have acted to facilitate an augmented filtration rate, by pumping the water over the gills; thus, providing a pathway for reducing the incurred oxygen deficit and removing potentially harmful substances (Robson et al., 2012;

Widdows & Shick, 1985). It would, therefore, appear that the increased occurrence of transition frequency during and after stress exposure, reflects a propensity of mussels to utilise behavioural traits to cope with stressors; however, this application appears to be specific to the stressor, the species and the metabolic scope of an individual.

Individuals, displaying heightened transition frequency in response to stress exposure, may be more likely to recover faster and, therefore, display a prompt return to normal activities after stress exposure (Marras et al., 2010). Although, for transition frequency to occur, an individual is required to generate frequent shell movement, which necessitates the use of adductor mussels and is, therefore, likely to be energetically demanding (Shick et al., 1986). Individuals with a higher metabolic rate or aerobic scope are more likely to cope with the energetic requirements of transition frequency, and thus, utilise this behavioural trait more often. Furthermore, individuals of the same species were collected from the same study site, suggesting environmental conditions in the habitat were unlikely to shape the observable phenotypic variation in behavioural and physiological traits, provided that heritability of an individual's physiological profile is low (Burton et al., 2011).

Avoidance behaviour and foot extension were observed less frequently in mussels after stress exposure and varied between the two species; thus, generating large zero-inflated data sets. Both avoidance behaviour and movement have been documented as responses to alterations in the environment (Allen & Vaughn, 2009; Archambault et al., 2013; Block et al., 2013; Gough et al., 2012; Hartmann et al., 2016). The low frequency of occurrence for these two behaviours could be due to the type of stressors used, with the expression of certain behavioural traits occurring more often in response to particular stressors. Additionally, due to limitations in the experimental design, oxygen consumption rates could not be obtained during stress exposure periods. It is during these periods that these behavioural metrics were often observed; thus, attempts to compare physiological change with avoidance behaviour may have suffered from a lack of data. Further tests, with an improved experimental approach that allows for continuous respiratory readings with larger mussel groupings, and using different stressors may, therefore, be required to test the link between the presence of such behaviours and the physiological mechanisms underlying their occurrence.

3.5.3 Variation in species and stressors

Results from this study suggest species specific responses to the stressors, often perceived to reflect differences in physiology (Ganser et al., 2013; Gough et al., 2012; Haney et al., 2020). A key driver of these differences could also be the environmental conditions the populations experienced in their natural habitats. The sample populations used in this study were collected from ecosystems displaying very different habitat characteristics. The lentic system *A. anatina* were collected from was subjected to frequent water abstractions and displayed poor water quality, suggesting a potential tolerance to prolonged stress exposure and previous experience with both stressors. Contrastingly, *M. margaritifera* were taken from a mill lade, hydrologically connected to the main channel of the river, which presented relatively consistent depth and flow conditions in addition to good water quality. This suggests there were perhaps differences in the sensitivity to the stressor (Hart et al., 2019), with the *M. margaritifera* population less adapted to the presence of the experimental stressors for extended durations (Johnson et al., 2018; Lummer et al., 2016). Due to the significant differences in habitat the species samples were acquired from, and the potential for this to be a significant driver of individual responses, this study is limited in its propensity to tease apart species differences.

In addition to interspecific differences in response, this study also highlighted differences in response to the two stressors, perhaps reflective of differences in the magnitude of stress caused by each stressor. However, there were differences in how these stressors were induced for this study: mussels were handled during the aerial exposure stressor experiments, but were not handled during the turbidity exposure study. Handling mussels could have heightened the extent of stress individuals experienced during the aerial exposure experiments. Without handling the control mussels, it is difficult to quantify the extent to which this evoked stress within mussels. Nevertheless, evidence from the literature regarding the impact of short-term handling on individual suggests this could be negligible (Gray & Kreeger, 2014; Miller et al., 1995; Ohlman & Pegg, 2020)

It is likely that, for both species, suspended sediment presents a more commonly encountered environmental condition, compared to aerial exposure. Furthermore, a suitable respiratory medium still exists in these circumstances. The lower magnitude of stress, caused by exposure to suspended fine particulate inorganic matter, would likely have permitted a faster recovery; hence, why both metabolic and behavioural metrics were often similar in the pre-exposure and post-exposure conditions during turbidity experiments. Despite differences in the magnitude of stress caused, the data from this study would suggest both stressors have a significant impact on the physiology of both species and cause stress to some degree. For both stressors, it is likely that prolonged exposure would cause significant loss of individual condition, eventually culminating in mortality.

3.5.4 Implications for behaviour as a biomarker

Results from this study suggest that exposure to an environmental stressor can be detected by measuring transition frequency in unionid mussels. This study demonstrates a clear distinction in the presence of this behaviour between periods prior to stress exposure and following exposure, which can be linked to alterations in metabolic functioning. Measurements of transition frequency could, therefore, form the basis for a biomonitoring tool to detect the onset of stress in populations.

Recording the frequency of occurrence of this behaviour over time could assist practitioners in identifying when individuals are experiencing prolonged stress, and requiring conservation intervention. This biomonitoring tool may also be deployed to aid relocation and restoration efforts, towards the conservation of populations, with research concerning the use bioindicators of unionid fitness already having demonstrated the applicability of such approaches (Gray & Kreeger, 2014; Roznere et al., 2017). For example, studies acting as prerequisites to a translocation scheme could deploy a small subset of a population into a habitat of interest, and subsequently conduct monitoring of transition frequency to assist practitioners in gauging habitat suitability.

To quantify the extent of stress caused, research must account for individual variability. To do so, laboratory-based experiments could identify the most responsive individuals within a sample of the population to act as indicators for overall population condition. The thresholds for individual stress response could be identified by focusing on the presence of transitions frequency across a variety of stressors in these indicator individuals; thus, accounting for population-specific variation in response.

This study focused exclusively on adult mussels, and hence did not account for variation in response across life-stages. Research would suggest that juvenile mussels are perhaps more susceptible to environmental stressors such as heightened fine particulate matter (Geist, 2010; Geist & Auerswald, 2007; Tuttle-Raycraft et al., 2017). Although, given their size, studies such as this may be difficult to replicate with a sample of juvenile mussels. Therefore, biomonitoring techniques reliant on the monitoring of transition frequency may be limited to adult mussels, yet could be used as a proxy to infer juvenile population condition.

To identify the onset and frequency of occurrence of behavioural metrics, this study relied on direct observation using high resolution camera technology. This method provided a useful method of unrestricted categorisation of behavioural traits, but required extensive analysis of the image data, and would be difficult to undertake in a field setting. The use of animal-attached remote sensing technologies such as Hall sensors, circumvents such issues, and allows measurements of mussel valve movement (valvometry) to be acquired at high resolutions and in real-time (Nagai et al., 2006; Robson et al., 2012). Previous studies have provided evidence to suggest both avoidance behaviour and transition frequency could be analysed using bio-sensor technology (Hartmann et al., 2016; Lorenz & Pusch, 2013; Lummer et al., 2016). However, this technology is currently limited to lab-based experiments and yet to be tested in the field as a remote sensing technique.

3.6 Conclusion

To ascertain information that is specific to certain species across variable, spatial, and temporal scales, in addition to being predictive, prescriptive and scalable, ecologists must move away from “long tail” scientific methods (Hampton et al., 2013). The adoption of a context-driven approach to ecology, which examines the physical attributes of the ecological landscape in addition to how the animals respond to changes in their habitat, is likely to provide appropriate data for enacting successful conservation management. Using remote sensing to detect the occurrence of transition frequency in indicator individuals, may assist such an approach: data to suggest how a population is responding to alterations in environmental conditions before, during and after conservation management (e.g. river restoration and re-introduction schemes), could assist the quantification of project success; providing population-specific thresholds to identify when particular environmental variables begin to negatively affect the condition of individuals. This paper highlights the potential of this approach, for eliciting successful conservation of the endangered freshwater unionid mussels.

CHAPTER 4

4 ADAPTIVE RESPONSES OF FRESHWATER PEARL MUSSELS, MARGARITIFERA MARGARITIFERA, TO MANAGED DRAWDOWNS

Material presented in this chapter are included in the manuscript Curley E.A.M., Thomas R., Adams, C.E. & Stephen A. (accepted July 2021), Adaptive responses of freshwater pearl mussels, Margaritifera margaritifera, to managed drawdowns, Aquatic Conservation: Marine and Freshwater Ecosystems.

ABSTRACT

1. Growing demand to maximise energy production from renewable sources has led to significant alterations in water management practises in regulated river systems, which threaten to exacerbate anticipated future water shortages caused by severe drought episodes, brought on by climate change.
2. Across Scotland, many highly managed systems are inhabited by some of the last remaining reproductively viable populations of freshwater pearl mussel *Margaritifera margaritifera*. However, due to a lack of empirical evidence on the response of *M. margaritifera* to alterations in flow regime, it is unclear how best to mitigate the potentially lethal effects of prolonged episodes of low flow.
3. This study addressed this knowledge gap by examining the response of *M.margaritifera* to controlled water level drawdowns in laboratory and field settings. Using a flume set-up, the responses of 50 *M.margaritifera* from two different populations (25 from a regulated system; 25 from a semi-natural system) were compared, across three different rates of drawdown (50 mm h^{-1}; 30 mm h^{1} ; 15 mm h^{-1}), using two different spatial arrangements (low density; elevated density). A field trial examined the responses of 18 *M. margaritifera* in a regulated system, with a hydroelectric dam facilitating a controlled drawdown of 30 mm h^{1}.
4. The study showed that *M. margaritifera* can detect alterations in flow depth, which culminate in the emersion of mussel beds, and respond by undertaking vertical and horizontal movements to mitigate the risk of prolonged aerial exposure. Moreover, significant differences between populations were observed, with mussels from the regulated system more successful in tracking water levels to avoid emersion, indicating between population variation in behavioural phenotypic traits. Results from the field trial corroborated findings from the flume experiments, with 80% of mussels shown to successfully avoid emersion by tracking receding water levels.

5. Findings from this study advocate for the potential role of controlled drawdowns in regulated rivers to assist in reducing mortalities associated with receding water levels, during periods of drought. Between population differences in response highlight a need to adopt a context-dependent approach to conservation efforts.

Keywords: behaviour, movement, freshwater mussels, dams, drought, conservation

4.1 Introduction

The freshwater pearl mussel, *Margaritifera margaritifera*, is one of the most threatened (Arvidsson et al., 2012; Bauer & Wachtler, 2001; Cosgrove et al., 2016), and widely researched unionid species, with evidence to suggest it fulfils the criteria for an indicator, flagship, umbrella and keystone species (Geist, 2010). Studies examining the ecology of *M. margaritifera* are likely important to the conservation of oligotrophic stream ecosystems (Boon et al., 2019), and applicable to wider conservation efforts concerning unionid species.

In the European Union, populations of this rare, long lived freshwater bivalve (Geist, 2010; Ziuganov et al., 1994) are protected by the European Habitats Directive, which provides for safeguarding of the species via designated Special Areas of Conservation (Council of the European Communities, 1992), yet declines across populations persist (Cosgrove et al., 2016; Geist, 2010; Lopes-Lima et al., 2016).

Scotland remains one of the last strongholds for the species, with river systems in the Scottish Highlands continuing to support large reproductively viable populations (Cosgrove et al., 2016); defined by their ability to self-sustain without requiring the addition of new genetic material, originating from outside the system (British Standards Institution, 2017). Many of these important populations inhabit regulated rivers, managed for hydroelectric energy production. Thus, attempts to address the causes of decline, and implement long term conservation strategies in Scotland, have frequently highlighted the importance of hydrological management schemes (Araujo & Álvarez-Cobelas, 2016; Gosselin, 2015; Layzer & Madison, 1995).

The regulation of rivers for hydroelectricity has been attributed to substantial losses to populations of Unionid mussels globally (Campbell & Hilderbrand, 2017; von Proschwitz & Wengström, 2020; Wegscheider et al., 2019), with studies often referring to the fragmentation of populations due to the inhibition of migration of their host (during the parasitic phase of the life cycle), together with altered river flow, sediment and temperature regimes as key threats to freshwater mussels emanating from the presence of impoundments (Araujo et al., 2018; Ferreira-Rodríguez et al., 2019; Modesto et al., 2018; Winemiller et al., 2016).

In Scotland, Water Environment Regulations (SEPA, 2018) apply regulatory controls on the environmental flow practices in regulated rivers to minimise the potential impact of impoundments on the ecology of associated ecosystems. These regulations define a constant minimum discharge value, termed compensation flow, for impoundments, with operations often exceeding such values but never allowed to be lower. However, recent drought conditions have led to negotiation between hydroelectric dam operators and governmental agencies around greater flexibility in reducing the statutory minimum compensation flow during extended low runoff conditions. Despite this, there are concerns this may not be sufficient to mitigate the impacts of more severe droughts anticipated in future climate scenarios (Dr Alastair Stephen 2021, personal communication, 23 February). A key component of the desire to uphold the minimum discharge values concerns the potential aerial exposure of *M. margaritifera*, and subsequent population mortality under drought conditions.

From a conservation perspective, it is important to understand how species such as *M. margaritifera* respond to more extreme flow events. Desiccation, resulting from low flow discharge, represents a clear threat to efforts to maintain and improve population health of mussel species such as *M. margaritifera* (Hardison & Layzer, 2001; Hastie et al., 2000; Hauer, 2015; Lymbery et al., 2020; Randklev et al., 2018; Sousa et al., 2018). Within Scotland, more frequent periods of low summer discharge are anticipated due to extended episodes of low precipitation, in addition to lower levels of ground water, with extreme drought events expected to increase from 1:20 years to 1:3 years (Cosgrove et al., 2021; Kirkpatrick et al., 2021). Regulated systems are not exempt from the threats of extreme flow events, with management needing to ensure that the operation of dams assists in providing ecological resilience to the increased incidence of floods and droughts resulting from climate change, whilst also meeting operational requirements (Schneider et al., 2013; Sundt-Hansen et al., 2018). However, without the empirical evidence to inform how *M. margaritifera* respond to alterations in managed flows, the effects of changes in dam operation are unknown.

Despite the frequency to which impoundments are attributed as potential inhibitors of successful *M. margaritifera* conservation in the literature, few studies have examined the effects of dams on freshwater mussels (Sousa et al., 2020). Those that have, undertook observational studies, utilising correlative approaches to discern the potential factors governing the spatial variation and abundance of populations in relation to the presence and proximity of impoundments operation (Addy et al., 2012; Sousa et al., 2020), with no direct examination of *M. margaritifera* responses to alterations in flow characteristics resulting from dam operation.

Current conservation management of *M. margaritifera* is grounded in the principal that individuals are sedentary, and hence unable to utilise the surrounding environment to adapt to changes in flow. Yet there is a growing body of evidence to suggest that mussels do respond to alterations in environmental characteristics: mussels exhibit movement seasonally for reproductive purposes (Amyot & Downing, 1997); and in response to alterations in the hydrologic environment (Bartsch et al., 2010; Block et al., 2013; Clements, 2015; French & Ackerman, 2014; Hamstead et al., 2019; Johnson & Brown, 2000); with suggestions that burrowing may also assist in the avoidance of zebra mussel (*Dreissena polymorpha*) infestation (Burlakova & Karatayev, 2007). There is, therefore, a need to investigate whether such responses are also displayed by *M. margaritifera*, with potential repercussions for conservation management strategies.

Recent studies, observing the response of other freshwater mussel species inhabiting drought-prone systems, have provided evidence to suggest individuals utilise behavioural strategies to avoid or mitigate the effects of emersion by tracking receding water levels (horizontal movement) or burrowing (vertical movement) further into the riverbed substratum (Gough et al., 2012; Lymbery et al., 2020). Similar research has also been conducted within regulated systems to examine the response of two freshwater mussel species (*Amblema plicata* and *Lampsilis cardium*) to controlled drawdowns (Newton et al., 2015), revealing similar findings. However, such studies are yet to determine whether these responses are population specific. The extent to which individuals prioritise the expression of particular behavioural traits is thought to vary between conspecifics and across populations, presenting potential variation in response depending on the physiology of the individual and the environment they inhabit (Allen & Vaughn, 2009; Daniel & Brown, 2014; Dingemanse et al., 2009; Gough et al., 2012). The magnitude of a stressor is also likely to influence the response of individuals, with Newton *et al.,* (2015) postulating that larger movements by freshwater mussels are prompted by greater extents of stress. However, no known study has asssessed whether the rate at which emersion occurs affects the response of freshwater mussels. Thus, any researh concerning the response of freshwater mussels to alterations in flow regime, must account for variation across individuals and populations, as well as variation in the rate of drawdown.

The purpose of this study was to investigate the responses of *M. margaritifera* to alterations in flow depth that risk emersion, with the aim of providing empirical evidence to drive the effective mitigation of negative climate change effects on populations in regulated systems. To do so, this study utilised field and lab approaches to test the following four hypotheses. First, *M. margaritifera* show a behavioural response to receding water levels (drawdown), in the form of horizontal and vertical movements. Second, the movement of mussels shows a quantitative response to different rates of drawdown. Third, the movement of mussels in response to drawdown varies across populations inhabiting regulated and semi-natural systems. Finally, the movement of mussels in response to drawdown, varies with the proximity to conspecifics.

4.2 Material and methods

4.2.1 Flume study

(i) Mussel collection

M. margaritifera mussels were collected, under licence (100197), in the summer of 2017 from a disused mill lade, hydrologically connected to the main channel of the South Esk River, Scotland. This semi-natural system presented relatively homogenous flow regimes, less turbulent than those experienced in the main channel. However, regular alterations in flow depth were expected, and desiccation of the remaining population occurred in the summer of 2019.

A group of *M. margaritifera* mussels were collected, under licence (141417), in the summer of 2019 from the River Lyon, Scotland; a regulated system controlled by two hydroelectric dams, situated approximately 6km apart (Fig.4.2). The collections were from habitat located approximately 19 km downstream from the nearest impoundment, in the main channel of the river, which experienced heterogenous turbulent flow regimes. The River Lyon is a 391km^2 headwater catchment of the River Tay, the largest river in Scotland, and comprises one of the most intensely regulated catchments in the UK (Geris et al., 2015).

Adult mussels were removed by hand and stored in aerated cool boxes, lined with substrate and filled with water from the corresponding system. Collected individuals were held in two tanks (one for each population) at the Scottish Centre for Ecology and the Natural Environment (SCENE). Each tank contained washed gravel (0.1-25mm) to a depth of 100mm, fed with water from Loch Lomond at ambient temperature, 14°C ± 4.2 (annual mean temperature ± SD), to a depth of 200mm and a flow rate to mimic conditions of the respective habitats. Each individual mussel was marked on the shell using correction fluid and given a unique identification number, weighed and measured for shell length, width and height.

(ii) Experimental set-up

Experiments were conducted in a controlled, recirculating flume system, with a viewing chamber (Fig.4.1a), located at SCENE. The 0.6m wide channel can support flows up to 0.4m in depth, at a maximum flow rate of 0.2 m s-1. Flow is controlled by an adjustable electrical propeller.

The system was fed with untreated water from Loch Lomond. Flow velocity was kept constant (0.1 m s^{-1}). Water input rate was maintained at 0.001 m^{3}s^{-1}, using a pre-defined turbine operational capacity, with water draining back into the loch via an out-flow pipe (Fig.4.1a). Water temperature reflected ambient levels observed in the loch.

In a straight section of the flume, cement blocks were layered to form a step-gradient foundation, perpendicular to the flow direction (Fig.4.1b). To gain adequate hydraulic roughness, an artificial bed surface comprised layers of water-worked uniformly sized fine gravel (median d_{50} = 15–25 mm) covering the cement blocks, to a minimum depth of 100mm. Fine gravel was chosen to replicate the substratum conditions often highlighted as favourable for burrowing by Unionid mussels (Geist & Auerswald, 2007; Hastie, Boon, et al., 2000). The resulting structure (0.6 x 0.27 x 6.2m) comprised two sections of flat riverbed (0.08 x 6.2m), defined as the upper and lower plateau, separated by a 33° slope (0.44 x 6.2m).

Flow profiles were recorded across the artificial riverbed using a Acoustic Doppler Velocimeter probe (ADV Vectrino II, Nortek AS, Rud, Norway), at an operational propellor frequency known to produce average flow velocities of $0.1 ms^{-1}$ in the flume channel, and a flow depth (h_t; distance from base of flume bed to waters' edge; see Fig.4.1a) of 390mm. Using this data, a test section (1.4m x 0.6m) was located 4.6m downstream from the inception of the artificial bed, where hydraulically rough, turbulent flow was fully developed at the point of shell placement (Fig.4.1a).

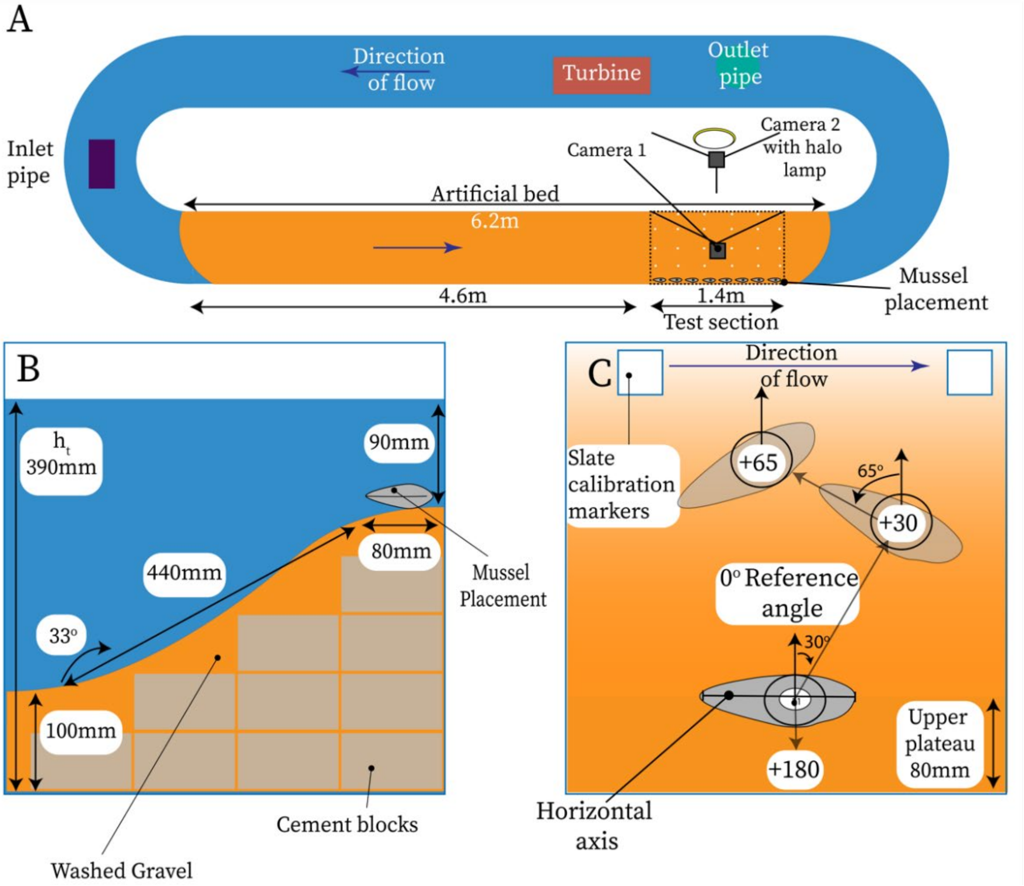

Figure 4.1 Schematic of the (A) experiment flume set-up, highlighting the location of the test section, with an (B) illustration of the cross-section of the artificial bed and an (C) overview of the method used to determine the direction of mussel movement, which defined direction as a positive angular displacement from the reference direction, based on the individual's orientation at the beginning of the trial: perpendicular to the horizontal axis of a mussel, pointing down the gradient of the riverbed

Two high-speed commercial video cameras operating at 60 frames-per-second (GoPro Hero 8 Black, Gopro, Inc. San Mateo CA, USA) were positioned at the centre of the experimental area to capture mussel movement. The first, placed above the flume channel, parallel to the bed, filmed the micro-topography below. The second, positioned inside the viewing chamber, filmed horizontally across the micro-topography. A halo lamp was placed behind the second camera to provide an ultra-bright light source. To calibrate the images taken by the cameras to known distances on the test section, 20mm x 20mm square slate pieces were painted white with correction fluid and placed across the experimental test section (Fig.4.1c).

(iii) Experimental overview

Laboratory based experiments were designed to compare the behavioural responses of individuals from two different populations (regulated and semi-natural) to reductions in flow height, h_t, which incur partial aerial exposure of the gravel bed. Fifty mussels (25 from each population) were randomly selected for the experiment. Each individual was exposed to three different drawdown rates, calculated from analysis of annual (2018) flow heights in a regulated Scottish river, inhabited by a large functional *M. margaritifera* population: 50 mm h^{-1}(Δh_{50}), 30 mm h^{1} (Δh_{30}), 15 mm h^{-1} (Δh_{15}) (mean drawdown of a regulated river (150mm) over three, six and twenty-four hours respectively). Two animal density treatments were tested for each of the three drawdown rates: low density (D1; individuals placed 140mm apart) and elevated density (D2; individuals placed 10mm apart). An individual experienced six trials, one for each drawdown rate and density treatment combination.

Each trial was conducted on eight mussels simultaneously, each experiencing the same drawdown rate and density treatment. At the start of a trial, mussels were placed in a line, perpendicular to the flow direction, across the elevated plateau of the study site in the pre-defined density arrangement (Fig.4.1a). The flow height (h; distance from top of riverbed substratum to waters' edge) at the plateau was 90mm (Fig.4.1b) and circulated at an average velocity of 0.1ms^{-1} (SD 0.03). A trial comprised three sequential experimental periods: first, a pre-drawdown period, comprising a two-hour settleing period with h_t kept constant; second, a drawdown period, with h_t reduced by 150mm at the pre-determined drawdown rate; finally, a post-drawdown period, comprising a 12-hour period with flow height kept constant at the reduced levels. Throughout a trial the flow velocity was kept constant at 0.1ms^{-1} (SD 0.05). Both high-speed commercial video cameras were turned on at the beginning of a trial and recorded time-lapse footage, at 30 second intervals, until the end of the 12-hour post-drawdown period. Mussels experienced a period of one to eight weeks rest between trials, to understand how fatigue may impact response. The extent of rest was determined by randomly assigning individuals to each trial within a pre-determined schedule.

Analysis of mussel responses to drawdown was undertaken using tracking software, examining when mussels moved, how far they moved and which direction they moved (see section 4.2.3 for details).

4.2.2 Field study

(i) Location

The field experiment was conducted at a test site, located on a point bar, on the inside bend of a meander of the River Lyon, approximately 2km upstream of the impoundment at Stronuich and 4 km downstream of the impoundment at Loch Lyon (Fig.4.2). Regulation of the flow regime at the impoundment on Loch Lyon provided adequate control of the hydrodynamics at the test site, in the absence of significant catchment effects. The point bar offered a gentle slope of relatively homogenous gravel (lacking significant obstacles to mussel movement), with a median size of d_{50} = 20–35 mm, which could be completely submerged during high flow regimes, elicited by dam operation at Loch Lyon.

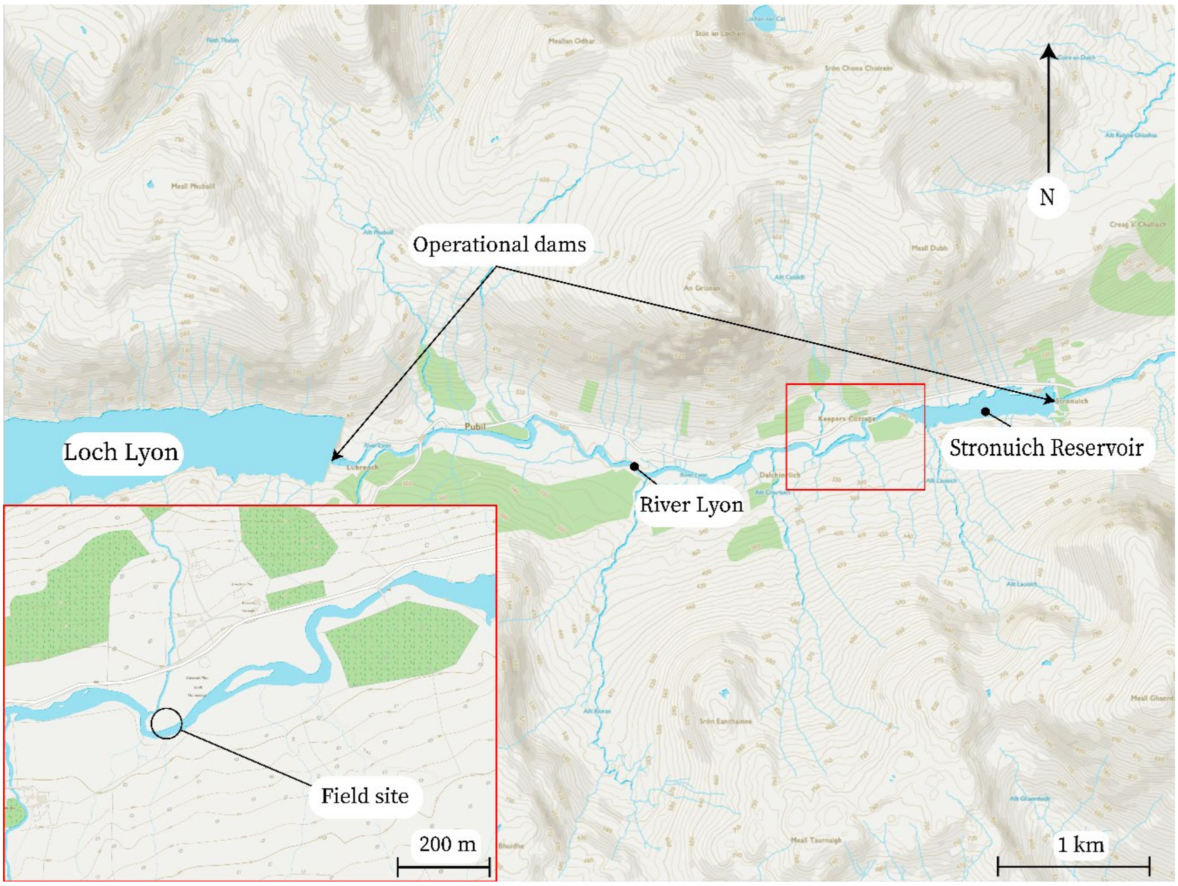

Figure 4.2 A map of the site used in the field study, noting the location of the two operational dams and the location of the field site, where mussel movement was examined in relation to a controlled drawdown.

(ii) Mussel collection

The day before the experiment was conducted, a sample of 18 *M. margaritifera* mussels were collected, under licence, 22 km downstream of the impoundment at Stronuich, from a habitat located in the main channel of the river, experiencing heterogenous turbulent flow regimes. Each individual mussel was marked, using fluorescent pink aquarium-grade spray paint, and given a unique number for identification, weighed, and measured for shell length, width and height. The mussels were placed in mesh plastic crates and positioned in the water at the test site, with bricks placed on the lid to prevent dislodgement.

(iii) Experimental set-up

A scaffold structure was built and placed within the river (see Fig.4.3a for setup) and three selfie sticks attached to hold the following: two high-speed commercial video cameras, operating at 60 frames-per-second (GoPro Hero 8 Black, Gopro, Inc. San Mateo CA, USA), facing either side of the ridge to capture mussel movement; one halo light to illuminate the test section overnight. A measuring stick was fixed to the outer support beam to record alterations in flow height.

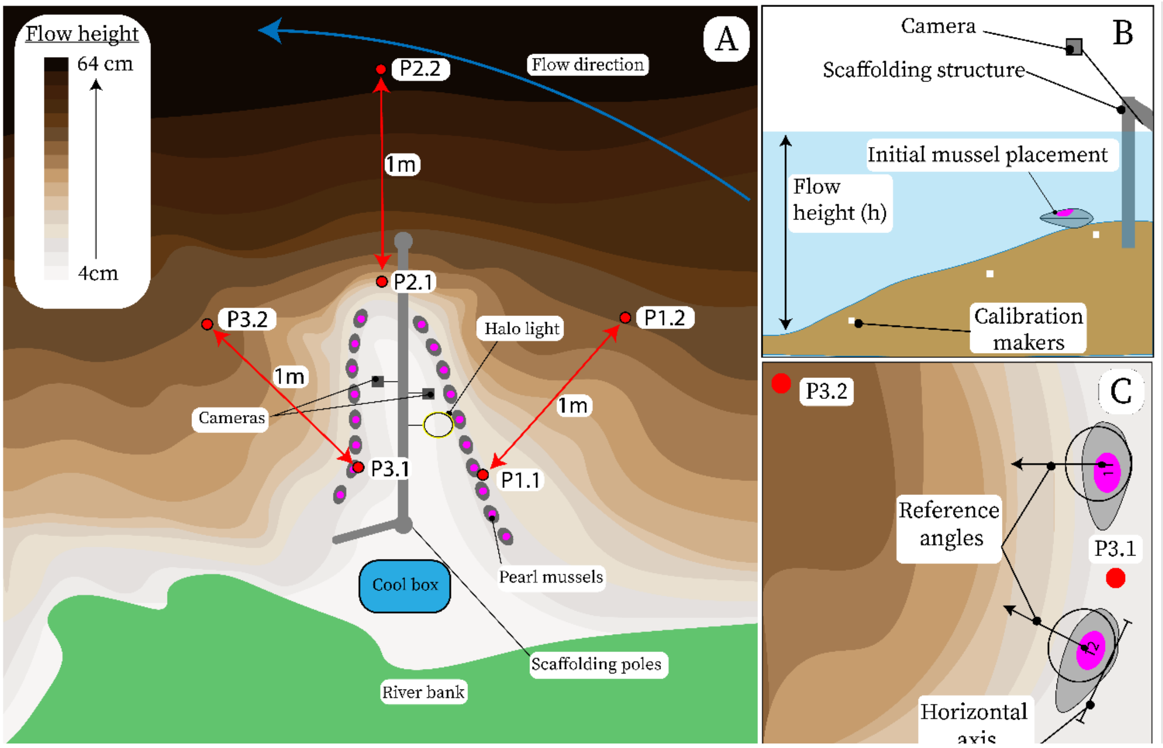

Figure 4.3 Schematic of the (A) experiment field set-up, highlighting the location of the test site, located on a point bar, on the inside bend of a meander with locations of the initial mussel placement, scaffold structure and locations for taking ADV measurements: three locations around scaffold structure (P1.1;P2.1;P3.1) and three corresponding locations 1m downgradient (P1.2;P2.2;P3.2) (B) An illustration of the cross-section of the ridge. (C) Overview of the method used to determine the direction of mussel movement, which defined direction as a positive angular displacement from the reference direction, pointing perpendicular to the mussel's horizontal axis, directly down the riverbed slope to the nearest refuge from emersion.

A coolbox was attached to the inner support beam and contained two 12v batteries running in parallel connected to an invertor, which powered the cameras and light throughout the experiment. To calibrate the images taken by the cameras to known distances on the test site, thin slate squares (20mm x 20mm) were painted, using white aquarium grade spray paint, and placed across the experimental test section (Fig.4.3b).

(iv) Experimental overview

The field experiment was designed to examine whether results from the flume trials could be replicated within a river system. As with the flume component of this study, the experiment sought to compare the behavioural responses of 18 individuals, collected from the corresponding river system, to a reduction in flow height, which incurs partial aerial exposure of the riverbed. The experiment comprised a single trial, using a target drawdown rate of 30 mm h^{-1} (Δh_3) and a low-density arrangement. The experiment was undertaken in early November 2020.

The trial was conducted on 18 mussels simultaneously. At the start of the trial, a target flow of $16m^3s^{-1}$ was delivered from the impoundment at Loch Lyon, with an average water temperature of 7°C. A meter stick was used to record alterations in flow height within a 1m radius of the scaffold structure to ascertain the gradient of the slope. Flow profiles were then recorded at six locations (Fig.4.3a) using a handheld Acoustic Doppler Velocimeter probe (Flowtracker2, Sontek, San Diego, USA) to examine alterations in flow velocity, with respect to changes in water height, across the test site: three locations across the ridge where the scaffold structure was located (P11; P21;P31); three corresponding locations within 1m of each point on the ridge, which presented the largest recorded flow heights (P12;P22;P32).

Following recording of the flow profiles, mussels were placed horizontally on the bed at the top of the ridge, facing down the gradient, at two sites located either side of the scaffold structure: nine at P11, facing the flow traveling into the meander; nine at P31, facing the flow traveling away from the meander (Fig.4.3a). The flow height (h), where mussels were initially positioned, was 120mm. Mussels were left undisturbed, in the absence of significant changes to flow regime for three hours, comprising a pre-drawdown period. Following this, three incremental reductions in flow were conducted over six-hours, as part of the drawdown period: resulting in a 200mm reduction in water height. Flow profiles were recorded at the six aforementioned locations, 30 minutes after each incremental reduction in flow; thus, providing four flow profiles at each location, with data to indicate alterations in mean flow velocity and flow height at each point over the duration of the trial. At the end of the drawdown period, a post-drawdown period began with the final flow regime maintained for a further 8 hours, after which normal operations at the dam on Loch Lyon resumed.

Analysis of mussel responses to drawdown was undertaken using tracking software, examining when mussels moved, how far they moved and which direction they moved (see section 4.2.3 for details).

4.2.3 Behavioural Analysis

After each trial, video footage stored on the camera's micro-SD was downloaded, compiled and edited using software (Adobe Premier Pro, 2020) to remove image distortion, and enhance visibility. The image sequences were subsequently organised into three groups corresponding to the three sequential experimental periods. Images were imported into Fiji (Schindelin et al., 2012) and analysed using the MTrackJ plugin (Meijering & Dzyubackyk, 2012), which allowed for the tracking of individual mussels based on their unique identification number. The following variables were quantified for each individual in a trial: (1) total distance of movement (cm); (2) average direction of movement from the reference angle (°); (3) initial response (see below for description); (4) final resting position.

Direction of movement pertained to the average angular change of an individual's displacement vector (pointing from the previous point to the current point on an individual's track) relative to reference co-ordinate system of the image (with the origin taken as the previous point). Angle values ranged from 180° to 0°, where 0° refers to a movement that runs parallel in the direction of the reference angle, and 180° a movement that runs parallel in the opposite direction of the reference angle (Fig.4.1c). The reference co-ordinate system of an image was based on the individual's orientation at the beginning of the trial: perpendicular to the horizontal axis of a mussel, pointing down the gradient of the riverbed. For flume trials the reference co-ordinate system was the same for all individuals in a given trial due to the homogenous conditions: the reference angle ran perpendicular to the flow direction (Fig.4.1c). However, for field studies this varied with individual positioning, yet the reference angle always constituted the shortest linear direction an individual would need to move to arrive at the deepest part of the riverbed, within 1m of the scaffold structure (Fig.4.3c).

Initial response comprised the categorisation of the extent to which a mussel was exposed to air when their first movement was recorded: fully exposed (<10% water contact); partially exposed (10-90% water contact); fully submerged (>90% shell submerged in water).

Final resting position pertained to the categorisation of the extent to which a mussel was buried in the substrate at the end of a trial, with mussels recorded as having burrowed if they had an angle to the sediment of at least 45° and sediment covering the shell to the umbo or greater (in accordance with Lymbery et al., 2020). The three categories for final resting position were as follows: fully exposed to the flow, horizontal on the bed (100% shell exposed); partially buried (20-80% shell exposed); completely buried (<20% shell exposed).

4.3 Statistical analysis

4.3.1 Flume study

Four statistical approaches were used to examine successful avoidance of aerial exposure by individual mussels in laboratory conditions.

First, a two-step approach, using the presence or absence of successful aerial exposure avoidance by individual mussels as the primary response variable in a binomial mixed effects model. The first step analysed this response with Population (regulated system vs. semi-natural system), Drawdown rate ($\Delta h_{50,}$ Δh_{30},Δh_{15}) and Density (low vs. elevated) entered as co-variates, and Individual as a random variable. Using the optimal model obtained from the first step, the second step added greater complexity by including more predictor variables with Direction of movement (^{o}), Total distance of movement (cm), Initial response (Submerged, Partially exposed, Fully exposed), Morphology (Length * Height; mm), Group Success (% successful avoidance), Last handled (Time since individual was last used in a trial: <1week; 1-2 weeks; 2-4 weeks; >1 month), and Temperature (^{o}C) entered as co-variates, and Individual as a random variable.

Second, the repeatability of individual avoidance success (presence or absence of successful aerial exposure avoidance) across drawdown rates was examined using GLMM-based repeatabilities from a multiplicative model fitted by Penalised-Quasi Likelihood (PQL), with estimated repeatability on the logit-link scale, according to methods outlined by Nakagawa and Schielzeth (2010). Here, the presence or absence of successful aerial exposure avoidance by individual mussels was entered as the primary response variable with Population (regulated system vs. semi-natural system) and Drawdown rate ($\Delta h_{50,}$ Δh_{30},Δh_{15}) entered as co-variates, and Individual as a random variable

Third, a two-step approach, to assess the relationship between the total distance and direction of individual movement and the experimental period, using a hurdle model designed to handle zero-inflated data. The presence or absence of movement was entered as the primary response variable in a binomial mixed effects model, with Experimental period (pre-drawdown, drawdown, post-drawdown), Drawdown rate ($\Delta h_{50,}$ Δh_{30},Δh_{15}), Density (low and elevated), Last handled (<1week; 1-2 weeks; 2-4 weeks; 1-2 months;> 2 months), and Initial Response (Submerged, Partially exposed, Fully exposed) entered as co-variates, and Individual as a random variable. Two mixed effects models were subsequently created to determine which covariates affect the total distance and direction of movement in the positive non-zero data, with Individual as a random variable in both: one model with total distance of movement (cm) as the primary response variable, and Direction of movement (^{o})

entered as a co-variate; one model with Direction of movement (°) as the primary response variable, and Total distance of movement (cm) entered as a co-variate.

Fourth, examination of the extent to which individuals burrow following response to a drawdown, with final resting position (fully exposed, partially buried, completely buried) entered as the response variable in a multinomial logistic regression, with Total Distance (cm), Direction of movement (°), Population (regulated system vs. semi-natural system), Drawdown rate (Δh_{50}, Δh_{30},Δh_{15}) and Density (low vs. elevated) entered as co-variates.

4.3.2 Field study

Three statistical approaches were used to examine successful avoidance of aerial exposure by individual mussels in field conditions.

First, the presence or absence of successful aerial exposure avoidance by individual mussels as the primary response variable, in a binomial logistic regression, with Direction of movement (°), Total distance moved (cm), Initial Response (Submerged, Partially exposed, Fully exposed), Morphology (Length * Height), Flow height differential ($\Delta h_{x,y}$; difference in flow height between starting position and corresponding point located downgradient of the riverbed, for example $\Delta h_{p11,p12}$) and Mean flow Velocity (U; at starting position) entered as co-variates.

Second, a two-step approach, to assess the relationship between the distance and direction of individual movement and the experimental period, using a hurdle model designed to handle zero-inflated data. The presence or absence of movement was entered as the primary response variable in a binomial logistic regression, with Experimental period (pre-drawdown, drawdown, post-drawdown), Initial Response (Submerged, Partially exposed, Fully exposed), Morphology (Length * Height), Flow height differential ($\Delta h_{x,y}$), Mean flow Velocity at starting position (U_1) and Mean flow Velocity at the corresponding point located downgradient of the riverbed (U_2) entered as co-variates. Two linear regression models were subsequently created to determine which covariates affect the distance and direction of movement in the positive non-zero data: one model with Total distance of movement (cm) as the primary response variable, and Direction of movement (°) entered as a co-variate; one model with Direction of movement (°) as the primary response variable, and Total distance moved (cm) entered as a co-variate.

Third, a McNemar test, to examine whether the frequency of successful avoidance seen within the field experiment was similar to the frequency of successful avoidance observed within the laboratory experiments using a drawdown rate of Δh_{30} with individuals from the regulated system, placed in the low-density arrangement.

4.4 Results

Examination of the results from both flume and field trials found that mussels displayed horizontal and vertical movements in response to drawdown events. In all trials, mussel movement occurred more frequently during the drawdown and post-drawdown periods compared to the pre-drawdown settlement period (Fig.4.5). During the flume trials, the use of these movements to successfully avoid emersion varied with respect to the drawdown rate and population. Here, the highest rate of successful emersion avoidance was shown during trials using individuals from the regulated system at a drawdown rate of Δh_{50}: 58% of individuals successfully avoided emersion. Contrastingly, the lowest success rate (20%) was shown during trials with individuals from the semi-natural system at a drawdown rate of Δh_{50} (Table 4.1). Few individuals repeatedly demonstrated successful emersion avoidance across the six flume trials, with only 17% of individuals that successfully avoided aerial exposure in at least one trial, managing to replicate this success across all six trials, compared to 38% for the semi-natural system (Table 4.2). A comparison between flume and field trials found that successful emersion avoidance was higher in the field trial: 72% of individuals successfully tracked receding water levels to avoid prolonged aerial exposure in the field trial, compared to 46% for individuals from the same system, experiencing the same density and drawdown conditions in the flume study (Table 4.3).

4.4.1 Flume Data

(i) Successful avoidance

Initial analysis of the presence or absence of successful aerial exposure avoidance by individual mussels revealed significant differences across populations and drawdown rates. However, no significant effect of Density was revealed.

Examination of differences between populations revealed contrasting responses to the same drawdown rates. Individuals from the regulated system were shown to be more likely to avoid aerial exposure during the Δh_{50} drawdown rate compared to individuals from the semi-natural system ($P<0.05$). No significant differences were shown between populations when comparing trials with Δh_{30} and Δh_{15} drawdown rates.

Examination of differences within populations, revealed variation across the three drawdown rates, specific to the population (Table 4.1). Individuals from the semi-natural system displayed significantly lower avoidance success at Δh_{50} drawdown rate compared to Δh_{15} ($P<0.01$), with no significant difference in avoidance success between the Δh_{15} and Δh_{30} rates of drawdown. Individuals from the regulated system displayed significantly higher avoidance success during trials with a Δh_{50} drawdown rate compared to Δh_{30} ($P<0.05$), with no significant difference between the Δh_{15} and Δh_{30} trials.

The second stage of analysis, expanding on the initial model with more predictor variables, revealed variation in avoidance success in response to the total distance of movement, direction of movement, initial response and temperature. Successful avoidance of aerial exposure significantly increased with increasing distance moved (*P*<0.001)(Fig.4.4). Individuals from the regulated system were shown to move significantly more than those from the semi-natural system (*P*<0.05), but this had a negligible effect on their potential to successfully avoid aerial exposure.

Table 4.1 A comparison of the proportion of individuals that successfully avoided aerial exposure for a given drawdown rate and population.

Population	Drawdown Rate ($mm\ h^{-1}$)	Successful Avoidance (%) No	Yes
Semi-natural	15	57	43
	30	58	42
	50	80	20
Regulated	15	46	54
	30	60	40
	50	42	58

Successful avoidance of aerial exposure significantly increased in response to movements that were directed closer to the reference angle for an individual (*P*<0.01)(Fig.4.4). Individuals that displayed a response whilst partially or fully submerged in water were significantly more likely to avoid aerial exposure (*P*<0.05). Temperature interacted with drawdown rate, with a significant reduction in avoidance success shown for drawdown rates of 50 mm h^{-1} compared to 15 mm h^{-1}, when temperatures were higher. No further significant effects of temperature on avoidance success, across drawdown rates, were found.

(ii) Repeatability of Avoidance

The proportion of total variance accounted for by differences among individuals was found to be relatively low (*R*= 0.221, *P*<0.05), with consistent successful avoidance across the three drawdown rates shown to be rare for individuals in both populations (Table 4.2). However, it appears that consistency in successful avoidance is greater in individuals from the semi-natural system (Table 4.2).

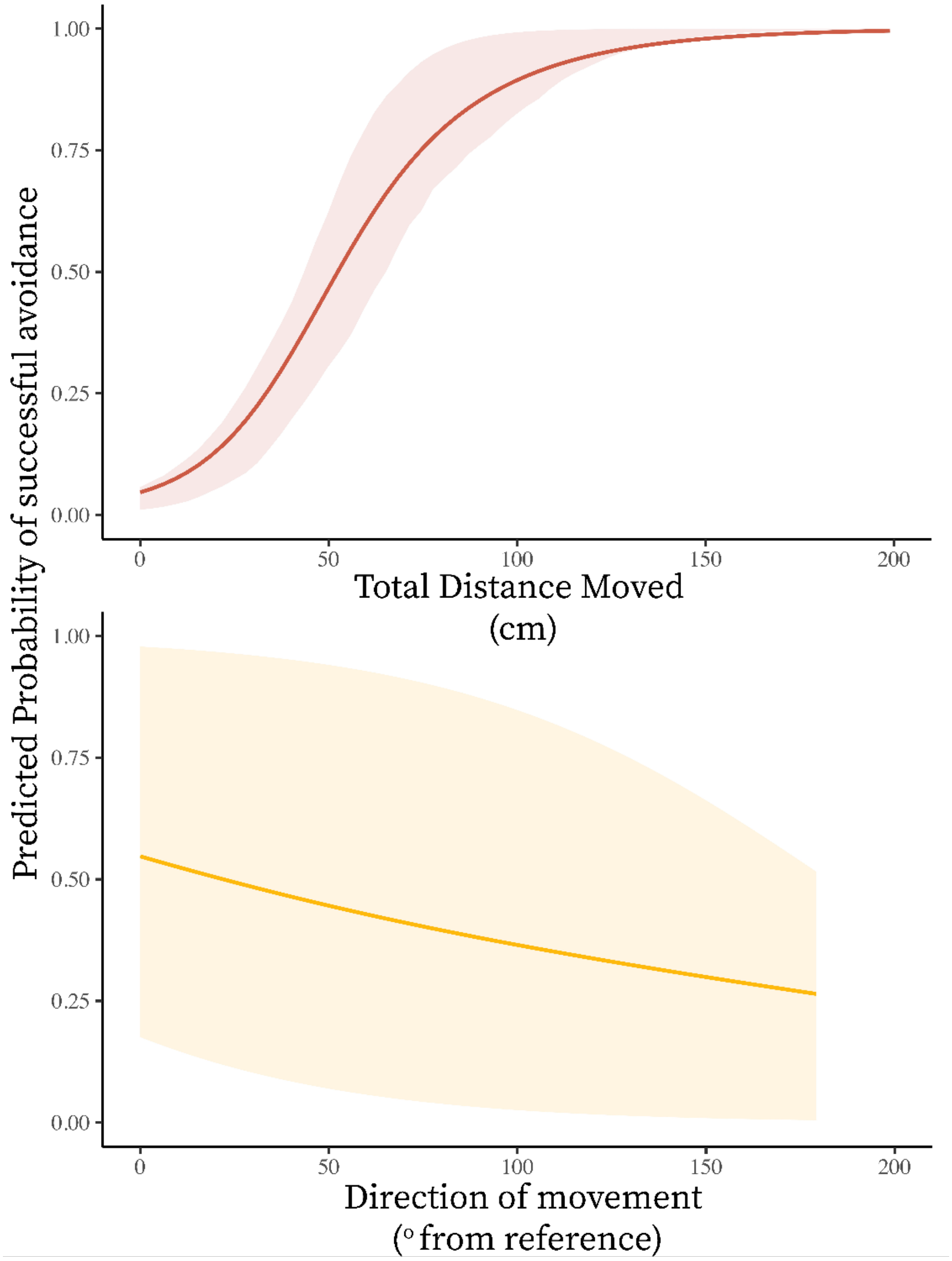

Figure 4.4 The average marginal predicted probability of successful avoidance of emersion associated with alterations in the length and direction of mussel movement, with upper and lower quartile displayed.

Table 4.2. The repeatability of individual mussel avoidance of aerial exposure during lab experiments, with comparisons between drawdown rates, and across populations. The percentage of individuals that avoided aerial exposure in both (or all) corresponding drawdown rates is shown (% Repeated Avoidance). Of those individuals that repeated avoidance, the percentage of those that were sampled from a regulated system or semi-natural system is given (Between populations). Of all the individuals sampled from a population that successfully avoided aerial exposure in at least one experiment with a corresponding drawdown rate, the percentage of those that displayed repeated avoidance is shown (Within populations).

Comparison Drawdown Rates (Δh_A:Δh_B)	% Repeated Avoidance	Between populations % Regulated	Between populations % Semi-natural	Within populations % Regulated	Within populations % Semi -natural
Δh_{50} : Δh_{30}	69	56	44	68	69
Δh_{50} : Δh_{15}	59	70	29	50	79
Δh_{30} : Δh_{15}	64	35	64	68	57
Δh_{15}: Δh_{30}: Δh_{50}	22	40	60	17	38

(iii) Presence of movement

Examination of the presence or absence of individual movement revealed differences across experimental period, population, drawdown rate, density, when individuals were last handled, and initial response.

The occurrence of movement was significantly higher during drawdown ($P<0.001$) and post-drawdown ($P<0.001$) compared to the pre-drawdown period, across all three drawdown rates. Comparisons across populations found that during the post-drawdown period, individuals from the regulated system were marginally more likely to display movement than individuals from the semi-natural system ($P=0.055$). No other significant differences were observed between populations.

Analysis of movement across drawdown rates, found that movement was more frequently observed during trials with Δh_{30} ($P<0.01$) and Δh_{50} ($P<0.01$) compared to those with a drawdown rate of Δh_{15}.

Individuals were more likely to move when they had rested for more than two months, compared to individuals that had rested less than a week between trials ($P<0.05$). No further significant differences in how much rest individuals had received were found.

Movement was significantly more frequent during experiments with mussels placed at an elevated density compared to the low-density treatment ($P<0.01$). During the drawdown period, mussels that displayed an initial response when fully submerged were significantly more likely to move than individuals that displayed an initial response when fully exposed ($P<0.001$). No further significant interactions between initial response, experimental stage and movement were observed.

(iv) Total distance moved

Examination of the factors determining how far individuals moved revealed differences across experimental period, drawdown rate, population, direction of movement and initial response.

The distance moved by an individual significantly increased during drawdown ($P<0.001$) and post-drawdown ($P<0.001$) compared to the pre-drawdown period, across all three drawdown rates (Fig.4.5). Comparisons across populations (Fig.4.5), found that individuals from the regulated system moved marginally further than those from the semi-natural system during the post-drawdown period ($P=0.055$). No further differences in the extent of movement across experimental periods were observed.

The distance moved by individuals was significantly greater during experiments with a drawdown rate of Δh_{50} ($P<0.001$) or Δh_{30} ($P<0.001$) compared to Δh_{15}. No further differences in the extent of movement across drawdown rates periods were observed.

The distances moved by individuals that displayed a response whilst partially ($P<0.05$) or fully submerged ($P<0.001$) were significantly higher during the drawdown period compared to the individuals that initially responded when fully exposed to air (Fig.4.5). No further differences were observed in the extent of movement when comparing the initial response of individuals.

The distance moved by individuals was significantly larger when the direction of movement was closer to the reference angle for the individual ($P<0.001$) (Fig.4.7).

(v) Direction of movement

Examination of the factors determining the direction individuals moved revealed differences across experimental periods, drawdown rates, populations, initial responses and lengths of movement.

The direction of movement was significantly closer to the reference angle for an individual during drawdown ($P<0.001$) and post-drawdown ($P<0.001$) compared to the pre-drawdown period, across all three drawdown rates (Fig.4.6).

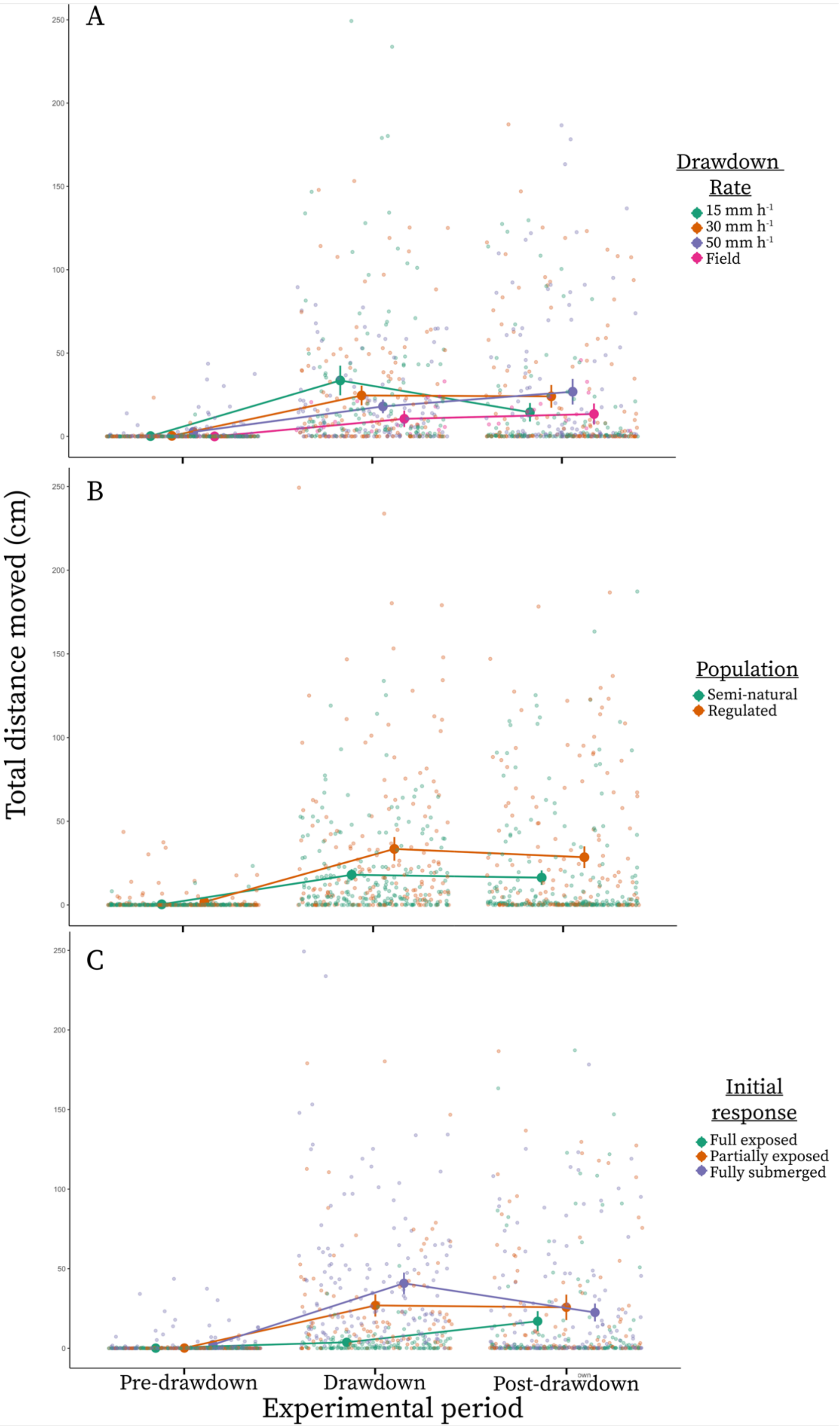

Figure 4.5 Variation in the total distance mussels covered during a trial, accounting for difference across (A) drawdown rate, (B) population, and (C) the extent to which mussels were emersed when they first displayed movement in a trial. Differences in distance covered are presented across the three experimental stages of a trial (pre-drawdown; drawdown; post-drawdown). Raw values for each individual are illustrated as individual points. The mean total distance (± standard error) of all individuals for a given drawdown rate, population or initial response, are presented across the three experimental periods. Lines have been added to connect mean data points and aid visualisation and are not representative of a continuous trend in the data set.

Comparisons across populations found that, at a drawdown rate of Δh_{50}, the direction of movement by individuals from the regulated system was significantly closer to the reference angle ($P<0.05$) compared to individuals from the semi-natural population (Fig.4.6). The direction of movement was significantly closer to the reference angle for individuals from the semi-natural population during the drawdown period, compared to individuals from the regulated system during the same period ($P<0.01$). The direction of movement was significantly closer to the reference angle for individuals displaying an initial response when partially submerged, ($P<0.001$), when they originated from the regulated system compared to the semi-natural system (Fig.4.6). The direction of movement by individuals, displaying an initial response when fully submerged, was marginally closer to the reference angle when they originated from the regulated system compared to the semi-natural system ($P=0.056$). No further differences were observed in the direction of movement when comparing the population, initial response, drawdown rate and experimental period.

Direction of movement was significantly closer to the reference angle for individuals that moved greater distances during a trial ($P<0.001$) (Fig.4.7).

(vi) Extent of burial

Examination of the factors determining the position individuals adopt following a response to drawdown revealed differences across drawdown rates, population, as well as the total distance and direction of movement ($X^2(12) = 177.14$, $P<0.001$, McFadden $R^2=0.23$).

Individuals were more likely to completely burrow at drawdown rates of Δh_{30} ($P<0.05$) and Δh_{50} ($P<0.01$) compared to Δh_{15}. Individuals from the regulated system were more likely to completely burrow ($P<0.001$) and marginally more likely to partially burrow ($P=0.064$) than individuals from the semi-natural system. Individuals that moved greater distances were more likely to partially burrow ($P<0.001$) and completely burrow ($P<0.001$) than remain fully exposed to the flow. Individuals that moved in a direction closer to the reference angle were marginally more likely to completely burrow ($P=0.06$).

4.4.2 Field data

(i) Successful avoidance

Analysis revealed variation in avoidance success in response to the length of movement and direction of movement; however, neither variable was found to have a significant effect.

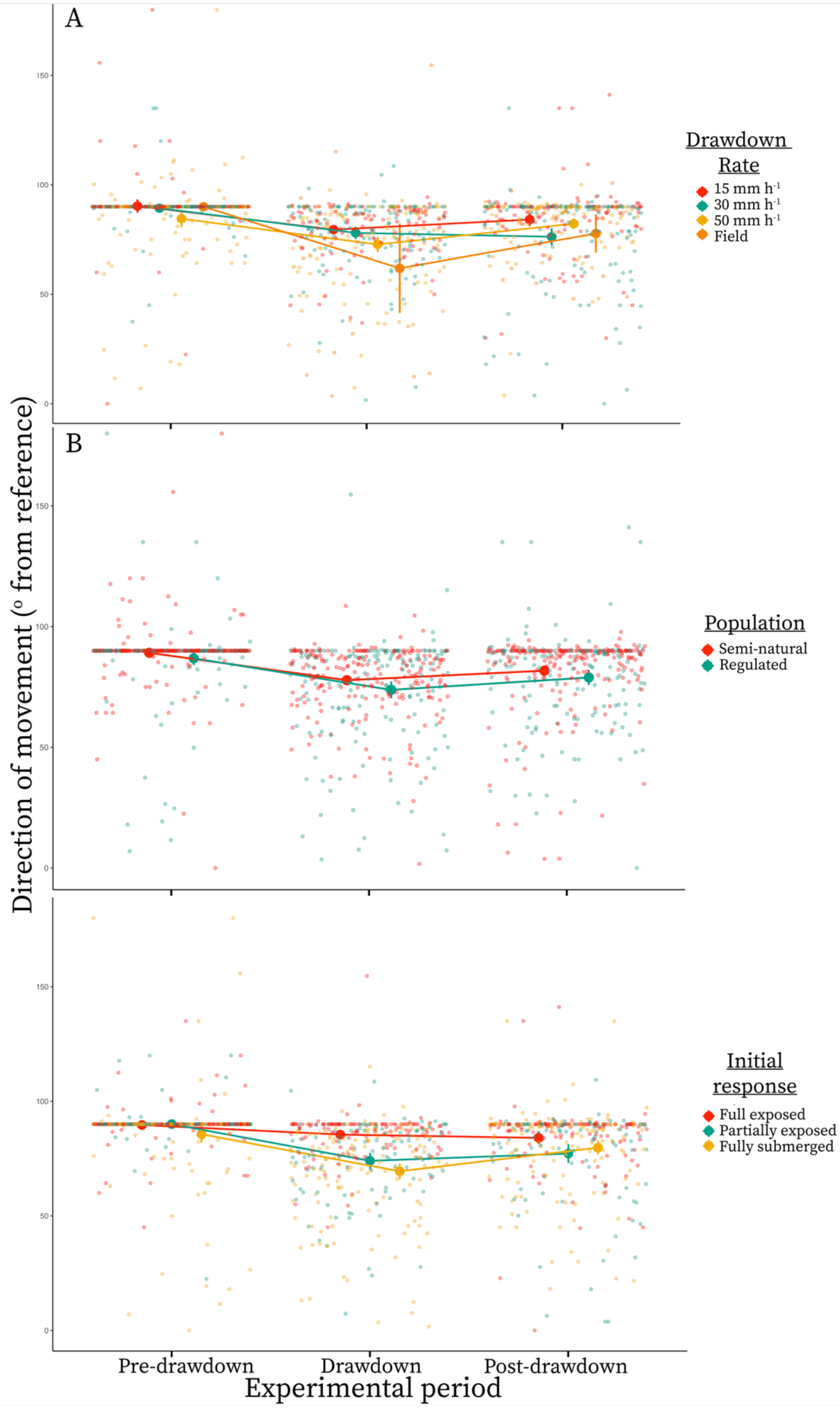

Figure 4.6 Variation in the average direction of movement displayed by individual mussels during a trial, accounting for difference across (A) drawdown rate, (B) population, and (C) the extent to which mussels were emersed when they first displayed movement in a trial. Differences in average direction of movement are presented across the three experimental stages of a trial (pre-drawdown; drawdown; post-drawdown). Raw values for each individual are illustrated as individual points. The mean direction of movement (± standard error) of all individuals for a given drawdown rate, population or initial response, are presented across the three experimental periods. Lines have been added to connect mean data points and aid visualisation and are not representative of a continuous trend in the data set.

(ii) Presence of movement

Examination of the presence or absence of individual movement revealed differences across experimental period and initial response. The frequency of movement did not significantly alter across experimental periods. Individuals that displayed an initial response when fully submerged were marginally (P=0.054) more likely to display movement compared to those that responded whilst partially or fully exposed to air. No further significant predictors of movement were found.

(iii) Total distance moved

Examination of the factors determining how far individuals moved revealed differences across experimental period, morphology and direction of movement.

Individuals moved significantly further during the drawdown ($P<0.05$) and post-drawdown ($P<0.05$) periods compared to the pre-exposure period. No significant difference in total distance moved was observed between the drawdown and post-drawdown period. Individuals that were larger in size moved significantly further ($P<0.05$). Individuals that moved in a direction closer to the reference angle also moved significantly further ($P<0.05$) (Fig.4.7). No further significant predictors of how far individuals moved were found.

(iv) Direction of movement

Examination of the factors determining the direction individuals moved revealed differences across morphology, initial response, flow height differential and total distance moved.

Individuals that displayed an initial response when fully submerged were significantly ($P<0.05$) more likely to display a direction of movement closer to the reference angle compared to those that responded whilst partially or fully exposed to air (Fig.4.6). Individuals that were larger in size were marginally (P=0.061) more likely to display a direction of movement closer to the reference angle compared to those that responded whilst partially or fully exposed to air. Individuals that were placed in an environment with greater differences in flow height were significantly ($P<0.05$) less likely to display a direction of movement closer to the reference angle. Individuals that moved further were significantly ($P<0.05$) more likely to display a direction of movement closer to the reference angle (Fig.4.7). No further significant predictors of the direction individuals moved were found.

(v) Comparisons with flume study

The avoidance success of individuals in the field experiment was significantly higher than the avoidance success of individuals from the same population experiencing the same drawdown rate, with the same density arrangement in lab experiments (X^2=11.077, $P<$ 0.001), see Table 4.3.

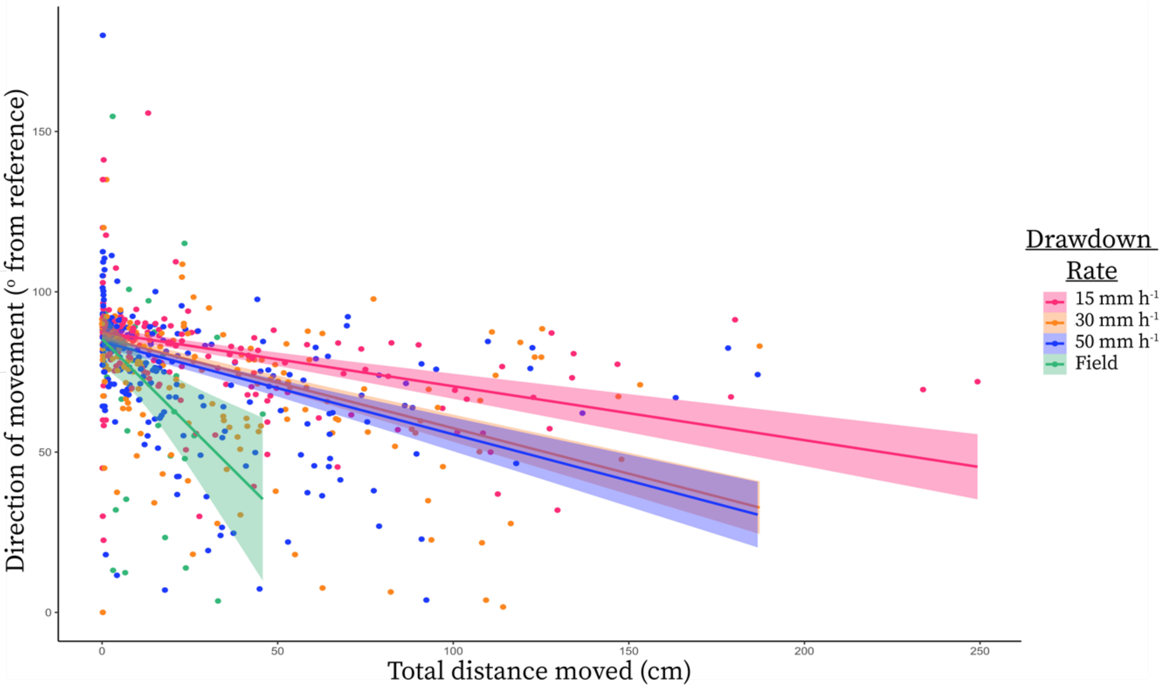

Figure 4.7 Variation in the average direction of movement in association with differences in the total distance covered by individual mussels during a trial, accounting for variation across drawdown rate. Estimates of the conditional mean function, for the three drawdown rates used in the flume studies and the target 3cm h^{-1} drawdown rate utilised in the field trial, are presented with corresponding 95% confidence intervals

Table 4.3 A comparison of the percentage of individuals successfully avoiding emersion between trials using a low-density arrangement, with a 3cm h^{-1} drawdown in a field setting and trials with individuals from the same population, placed in a low-density arrangement, experiencing a 3cm h^{-1} drawdown in a laboratory setting.

Experimental Setting	Successful avoidance (%)	
	Yes	No
Laboratory	46	54
Field	72	28

4.5 Discussion

The data presented in this paper demonstrate, for the first time, the responses of *M. margaritifera* to reductions in water depth that risk emersion, with evidence from both lab and field trials. Previous studies have established the potential for freshwater mussel species to identify alterations in the hydrologic environment and consequently undertake behavioural responses to mitigate the risk of prolonged aerial exposure (Balfour & Smock, 1995; Gough et al., 2012; Lymbery et al., 2020). Yet these have been consigned to species inhabiting intermittent freshwater systems within arid and semi-arid climates, with no consideration towards species within temperate environments that are likely to experience more extreme hydrologic alterations due to future climate change. Overall, the results presented here, illustrate how *M. margaritifera* display similar behavioural responses to fellow unionid species: exhibiting a propensity to detect reductions in flow and move down a gradient in the riverbed to avoid emersion, which alters in response to modifications to the rate of drawdown. The study also revealed substantial intraspecific variation at the population and individual level, with differences in the arrangement of individuals, with respect to conspecifics and the environmental conditions, also determining successful avoidance of prolonged aerial exposure.

4.5.1 Behavioural responses to emersion

The successful avoidance of emersion by *M. margaritifera*, during controlled reductions in water depth, was observed in all trials. However, the proportion of mussels successfully avoiding prolonged aerial exposure during trials varied significantly across different rates of drawdown, with a high of 58% success for a given population and drawdown rate, compared to a low of 20% (Table 4.1). A key component of this study was to, therefore, examine the behavioural traits utilised by *M. margaritifera* in response to reductions in the water depth, which may mitigate potentially lethal prolonged aerial exposure, and examine the potential factors that drive variation in successful avoidance across individuals and trials.

Findings from this study strongly suggest that horizontal movement is an important behaviour utilised by *M. margaritifera* to track receding water levels and consequently avoid prolonged aerial exposure. Traditionally, freshwater mussels have been viewed as a relatively sedentary species; however, results from this study and similar research suggest individuals may move significant distances across the riverbed (Gough et al., 2012; Lymbery et al., 2020; Negishi et al., 2011; Schwalb & Pusch, 2007). In this study, mussels moved significantly more during and after a drawdown event compared to before (Fig.4.5), suggesting receding water levels provided a stimulus for movement. Mussels were shown to move up to 3 metres during the course of a single trial conducted over a 24-hour period, with evidence from the literature to suggest this is not uncommon: over the course of a year *Elliptio complanate* were found to move up to 46.2 metres in a small stream (Balfour & Smock, 1995). The results of this study suggest greater extents of horizontal movement across the riverbed confer survival benefits to the respective individuals, with the probability of successful emersion avoidance increasing with an increase in the total distance individuals covered over the course of a trial.

Further examination of the movement patterns revealed a divergence from previously held beliefs that the direction of movement exhibited by mussels is predominately random (Balfour & Smock, 1995; Schwalb & Pusch, 2007). Instead, results from this study revealed individual movement to predominately occur during and following a drawdown event, with individuals often displaying movement that directed them to lower gradients in the riverbed over the shortest distances. Additionally, individuals that covered greater distances in their movement were also those that moved in a more direct manner to reach deeper water. Thus, it appears successful tracking of receding water levels, by moving down the riverbed gradient in a horizontal motion, following the water's edge, provides a positive stimulus for greater movement. This strong directionality of movement is a result shared by similar studies (Newton et al., 2015).

Results emanating from this study also identified vertical movement as a common behavioural response to drawdown. Previous studies have noted this behaviour in other freshwater mussel species in response to drought, conferring greater survival, but it does not appear to be universal to all (Amyot & Downing, 1997; Negishi et al., 2011). Gough et al. (2012) proposed three different behavioural strategies undertaken by three different freshwater mussel species: species with low physiological tolerance to emersion moved horizontally to track receding water levels; species with high physiological tolerance to emersion displayed minimal horizontal movement, and instead burrowed immediately when emersed; finally, those with a semi-tolerance to emersion tracked water levels and then burrowed. Other studies have revealed results to bolster this hypothesis (Lymbery et al., 2020; Newton et al., 2015). Here, *M. margaritifera* were often shown to mimic the responses of a semi-tolerant species, with vertical movement usually displayed by mussels that tracked receding water levels and subsequently avoided emersion.

The tolerance of *M. margaritifera* to desiccation remains largely unknown. The endangered status of the species has likely prevented the acquisition of licensing to undertake such work. However, results from this study suggest brief exposure of up to 24 hours elicits some degree of stress in individuals. Mussels afforded more than two months rest between trials were more likely to respond to emersion by moving, than those who had less than a week between trials. Thus, it appears mussels experienced some degree of fatigue due to a drawdown event, with full recovery potentially requiring 4-8 weeks. Curley et al., (*in press*) revealed similar findings when inducing aerial exposure in *M. margaritifera*, with observed variation in recovery dependant on the metabolic scope of an individual.

Variation in individual response was prevalent across the trials of this study, epitomised by the significant differences in the initial responses of mussels to drawdown, which appeared to indicate the extent of movement anticipated for an individual in response to receding water levels. Individuals that displayed initial movement whilst fully or partially submerged were far more likely to respond to a drawdown event, and successfully avoid prolonged aerial exposure. The movements displayed by these individuals appeared to amplify in response to faster drawdown rates: implying a heightened behavioural response to increased extents of stress; a common observation in studies concerning behavioural responses to stress in freshwater mussel species (Hartmann et al., 2016; Hasenbein et al., 2015; Robson et al., 2009).

Mussels that refrained from movement whilst fully or partially submerged, often displayed no movement throughout the duration of a trial, and instead remained horizontal on the riverbed with their valves closed. This behavioural response could assist in sealing in any moisture and avoiding substantial water loss (Newton et al., 2015). However, the effectiveness of such strategies would be marginal in circumstances with prolonged aerial exposure, as this necessitates anaerobic respiration and the subsequent build-up of toxic metabolic bi-products (Collas et al., 2014; Robson et al., 2009). The physiology of individuals may drive this variation in response, with some individuals appearing more alert to alterations in their environment. Observations during trials also noted the use of the foot by individuals as a potential means to detect stimuli for movement during the initial response: mussels appeared to extend their foot in search of water, moving in the direction of the water's edge where the foot made contact or closing their shell aperture if no contact was made. Research by Yeager et al. (1994) reported the use of pedal feeding by juvenile Rainbow Mussels, whereby cilia on the foot facilitates particle ingestion; thus, highlighting the use of the foot by freshwater mussels for purposes other than horizontal and vertical movements. Nonetheless, studies concerning the use of the foot as a sensory organ are unknown, and may warrant investigation.

Previous studies have often noted the role of thermal regimes in governing the response of freshwater mussels (Archambault et al., 2013; Bolotov et al., 2018; Denic et al., 2015). Indications from this study suggest temperature is likely to impact the potential for *M. margaritifera* to successfully avoid emersion, with lower temperatures appearing to correlate with higher rates of success in the avoidance of prolonged aerial exposure: the highest success rates were observed in the field with an average water temperature of 7°C. However, due to the study design, it was difficult to discern the true effect of temperature: the lowest temperatures recorded during trials with mussels from the regulated system were at the highest drawdown rate (Δh_{50}); the highest temperatures recorded during trials with mussels from the semi-natural system were at the highest drawdown rate (Δh_{50}). It is, therefore, unclear whether the higher temperatures prompted a reduced success rate in the semi-natural population, indicating differences across populations, or a threshold in mussel response was evoked at significantly higher drawdown rates.

4.5.2 Variation across population

In addition to substantial individual variation in response to receding water levels, this study also revealed significant differences across populations. Findings presented here, represents the first known study of intraspecific variation in the behavioural responses of unionid mussels, originating from different populations, to alterations in the hydrologic environment.

Examination of differences in the behavioural responses to drawdown found that individuals from the regulated systems demonstrated greater horizontal and vertical movements than those from the semi-natural system. Within the flume studies these differences in the extent of movement did not translate into a higher frequency of successful evasions of emersion, except for trials conducted at the highest drawdown rate (Δh_{50}). For all flume trials, the gradient was less than half a metre in length. It is anticipated that reductions in the water level *in-situ* may confer a greater extent of emersion across the riverbed, with mussels required to move more than half a meter to avoid prolonged aerial exposure (Newton et al., 2015). Therefore, the response of individuals from the regulated system may present substantial survival benefits over their semi-natural counterparts in such circumstances.

Further analysis of differences between populations at the highest drawdown rate, revealed a significant deviation in response to the highest rate of drawdown, with individuals from the regulated system more successful at avoiding emersion. The prevailing environmental characteristics within an animal's habitat are known to accentuate the importance of particular traits (Cook et al., 2011; Killen et al., 2013); thus, individuals from the regulated system may be more adept at responding to a recession of the water levels, given that such conditions are more likely to occur in their habitat. The adaptation of life history traits by populations of *M. margaritifera* has been alluded to in previous studies, with Preston et al. (2010) highlighting significant differences in shell morphology across populations, linked to the hydrologic characteristics of the habitats they reside within.

These population specific responses were further evident when examining the repeatability of response across drawdown rates. Despite displaying a higher frequency of successful avoidance overall, individuals from the regulated system were seemingly less consistent in their response, with only 17% of individuals that successfully avoided aerial exposure in at least one trial, managing to replicate this success across all six trials, compared to 38% for the semi-natural system (Table 4.2). Further assessment of the conditions that may have caused this decrease in consistency for mussels from the regulated system, revealed a significant drop in repeatability between trials with a drawdown rate of Δh_{30} compared to Δh_{15}. This correlated with an overall reduction in the proportion of individuals successfully avoiding prolonged emersion when comparing trials with a drawdown rate of Δh_{30} to Δh_{15}, and Δh_{50}. The reasons for this are unclear, and deviate from the negative linear response to increasing drawdown rate observed within the semi-natural population. Further study with a larger subsample of the population may be needed to dissect and understand this perceived trend. Nevertheless, the results from analysis of repeatability reveal the presence of individuals more capable of responding to changes in the hydrologic environment, within both populations.

4.5.3 Findings from the field

Assessment of the results from the field component of this study revealed a significantly higher success rates in the avoidance of emersion by individuals, compared to their counterparts in the flume trials. Given the relatively low sample size, and high proportion of successful avoidance, it was hard to disentangle the main drivers for successful avoidance. However, similar to lab studies, those individuals that displayed an initial response whilst fully or partially submerged, were likely to display more directionality in their movements and move greater distances, resulting in a higher likelihood of success in avoiding emersion.

Despite conveying broad similarities with the flume studies, the field trial discerned two key factors governing mussel response that had not previously been identified. First, the effect of individual morphology, with larger mussels shown to move further and display greater directionality. Second, the effect of riverbed slope, with more pronounced shifts in flow height reducing the directionality of movement.

The perceived variation in mussel response to dewatering, due to morphological differences, appears to contribute further to contrasting accounts in the literature: some studies suggest an absence of a morphological effect on a mussel's response (Lymbery et al., 2020; Schwalb & Pusch, 2007); whilst others have insinuated that smaller individuals are more susceptible to droughts (Sousa et al., 2018). Morphology is expected to impact individual response to alterations in the hydrologic environment, with increasing size found to increase the metabolic scope of individuals (Curley *et al., in press*); thus, enabling them to cope with the energetic requirements of movement. Yet differences in movement attributed to morphological dissimilarities, are likely to be compounded by more significant effects pertaining to variation across populations, species and stressors; hence, the reason morphology was not shown to be a significant driver of response in the flume trials.

Observations of mussel movement with regards to their positioning, showed a greater variation in the movement patterns of mussels when positioned on steeper slopes. Findings by Newton, et al. (2015) suggest that highly sloped surfaces may cue directional movement of mussels, and provide easier access to areas with greater flow depth. Steeper slopes are also likely to present more stable conditions for movement(Lamb et al., 2008). Yet more dynamic near bed flows are expected on steeper slopes (Armanini & Gregoretti, 2005), which may govern the mussel response and cause substantial alterations in the direction of movement over shorter distances. Thus, further study is required to understand the potential hydrogeomorphological processes in the near bed environment that determine successful mussel tracking of receding water levels.

4.5.4 Implications for conservation & river regulation

Within Scotland, extreme drought events are anticipated to increase in frequency in the future, with extended episodes of low summer discharge resulting from reduced precipitation in the spring and summer months (Kirkpatrick et al., 2021). Despite fears that such events may culminate in large-scale mortalities across *M. margaritifera* populations (Cosgrove et al., 2021; Morales et al., 2011; Santos et al., 2015),citing the immobility of such species as a reason for their vulnerability (Sousa et al., 2018), few studies have attempted to examine how individuals respond to alterations in the hydrologic regime.

Results from this study provide evidence to suggest a divergence from the current conservation management strategies, founded upon the belief that *M. margaritifera* are sedentary, and hence unable to respond to alterations in their environment. Here, for the first time, empirical evidence is provided to suggest that *M. margaritifera* can successfully detect alterations in flow depth, which culminate in the emersion of mussel beds, and respond through vertical and horizontal movements to mitigate the risk of prolonged aerial exposure. These results build upon a growing body of literature, revealing the propensity of freshwater mussel species to utilise behavioural strategies to avoid or mitigate the effects of emersion (Amyot & Downing, 1997; Negishi et al., 2011; Gough, Gascho Landis & Stoeckel, 2012; Ganser, Newton & Haro, 2015; Lymbery et al., 2020). Furthermore, results presented in this paper reveal variation in response across individuals and populations, suggesting individual physiology and habitat characteristics shape the extent to which mussels evoke behavioural strategies to successfully avoid emersion. Thus, attempts to discern the risk of populations to recession in the water levels, must adopt a context-dependent approach, with drawdown experiments on a sample population advised to reliably predict population response, and expected mortality.

Results from this study also advocate for greater assimilation of water level management as a tool for the conservation of freshwater mussel species. Data presented here suggests the management of water levels during drought conditions could assist in the preservation of populations, with controlled drawdowns facilitating mussel movement into safer regions of the riverbed. Moreover, the provision of more natural flow may also assist in harbouring greater population resilience to alterations in flow depth; a view echoed in similar studies (Newton et al., 2015; Sousa et al., 2020). With an increase in demand for renewable energy sources resulting in a shift towards increasing support for small hydro-power plants, it is crucial future research and corresponding conservation management strategies further examine the response of freshwater mussels to river regulation. Attempts to bridge the gap between conservation management and river regulation will likely facilitate improved conditions for aquatic species within associated systems, and may also provide a tool to buffer populations against the effects of climate change.

CHAPTER 5

5 SMART SENSORS TO PREDICT ENTRAINMENT OF FRESHWATER MUSSELS: A NEW TOOL IN FRESHWATER HABITAT ASSESSMENT

Material presented in this chapter are included in the manuscript Curley E.A.M., Valyrakis, M., Thomas R., Adams, C.E. & Stephen A. (2021), Smart sensors to predict entrainment of freshwater mussels: a new tool in freshwater habitat assessment. Science of the Total Environment, 787, 147586. doi: https://doi.org/10.1016/j.scitotenv.2021.147586 .

ABSTRACT

1. The identification and protection of suitable habitat for freshwater species, intimately linked to their physical environment, is dependent on the quantification and assessment of associated dynamic hydrogeomorphological processes. However, a consistent approach to accurately record and monitor near-bed flow characteristics, remains largely undefined in freshwater ecology.
2. The purpose of this work was to provide a direct, non-intrusive, low-cost and accessible tool to evaluate near-bed incipient flow conditions and predict entrainment risk in unionid mussels, which comprise some of the most imperilled species in the world and represent a key taxonomic group towards ensuring the health of wider freshwater ecosystems.
3. This study designed, for the first time, an instrumented freshwater mussel, encompassing inertial microelectromechanical sensors (MEMS), housed within *Margaritifera margaritifera* shells.
4. Following initial calibration of the embedded sensors to ensure accurate detection of three-dimensional displacement, dedicated flume experiments were undertaken to assess instrumented shell movement metrics, for a range of flow conditions and shell orientations.
5. Analysis found that data from the sensors' readings could successfully discern, and potentially predict, entrainment events through the examination of variability in recordings of total acceleration, with entrainment risk shown to vary across flowrate, shell orientation and size.
6. Instrumented shells could provide a valuable tool for assisting conservation management of unionid species: aiding the identification of suitable habitat in reintroduction schemes; monitoring habitat stability over time to provide metrics for the evaluation of restoration schemes. Instrumented shells could also assist habitat suitability surveys for a range of freshwater species which inhabit the benthic regions of freshwater ecosystems.

7. Evidence from this study suggests further research into this tool may yield methods for accurately predicting more complex flow metrics associated with hydraulic stress. It is therefore clear that the potential of this tool is still to be fully investigated.

Keywords: inertial sensor; frequency of entrainment; habitat suitability; unionid conservation; instrumented shell; hydraulic stressing

5.1 Introduction

The identification and protection of areas providing suitable habitat for species is crucial in the mitigation of biodiversity loss across ecosystems. Such methods are fundamental to conservation management in terrestrial and marine ecosystems, yet are often underutilised in efforts to conserve freshwater habitat (Decker et al., 2017; Moilanen et al., 2009). Considering that freshwater ecosystems represent some of the most threatened in the world, it is clear that more needs to be done to counteract the heightening extinction rate of species residing therein (Geist, 2011).

Efforts to bridge the gap between limited funding and the need for more extensive research, have led to calls for schemes to focus on keystone species, with the hope that their conservation will assist in inducing improvements to the health of the wider ecosystem (Bolotov et al., 2018). Freshwater unionid mussels (Mollusca: Bivalvia: Unionidae) are often considered important to ecosystem health by functioning as biomonitors of adverse habitat conditions (Lummer et al., 2016; Scheder et al., 2015; Vaughn, 2010) in addition to enhancing nutrient cycling and trophic interactions in freshwater communities (Allen et al., 2012; Boeker et al., 2016; Vaughn, 2010), whilst also contributing to habitat diversity in benthic environments (Boeker et al., 2016; Spooner & Vaughn, 2008), which is particularly noteworthy given the biomass of unionids has been shown to often exceed that of all other benthic organisms in the habitat (Vaughn et al., 2004). Work by Geist (2010) suggested that one such species, the freshwater pearl mussel (*Margaritifera margaritifera*), fulfils the criteria for an indicator, flagship and keystone species. Despite their perceived importance, unionid mussels still represent some of the most imperilled species in the world (Lydeard et al., 2004).

Research concerning the habitat requirements of unionid mussels is necessary to overcome limitations in conservation efforts (Boon et al., 2019; Cope et al., 2003; Geist, 2010), with Quinlan et al. (2015) highlighting the role of hydrogeomorphological processes in governing habitat suitability within freshwater ecosystems as an area requiring further attention. Hydraulic forcing has been shown to shape riverbed structure and composition (Biron et al., 2012; Crowder & Diplas, 2002, 2006; Waddle & Holmquist, 2013), with potential implications towards habitat suitability for freshwater species that are intimately linked to their physical environment, such as *M. margartifera*; however, hydraulic stressing of freshwater mussels may be unique, and the response of mussels to alterations in flow has rarely been studied.

From a conservation perspective, entrainment incidents in populations of freshwater mussel species, resulting from high flow discharge, represent a clear threat to efforts to maintain and improve population health (Hardison & Layzer, 2001; Hastie et al., 2000; Hauer, 2015). Research by Hastie et al. (2001) highlighted the impact high flow events can have on unionid populations: reporting a 4-8% mortality (fifty thousand individuals) after a 1:100 year flood event, with juvenile mussels (<10 years old) disproportionately affected. Further study, regarding the role of high flows in shaping freshwater mussel populations, revealed an inhibition of juvenile settlement and a loss of stable substrates to facilitate burrowing (French & Ackerman, 2014; Randklev et al., 2019). It is likely that these events will occur more frequently and at greater magnitude in the future due to the effects of climate change (Cameron, 2006; Prudhomme et al., 2003; Schneider et al., 2013).

Altered flow regimes associated with impoundments may further confound conservation efforts, with hydrological management schemes often attributed as key threats to the persistence of freshwater mussel populations (Hamstead et al., 2019; Lu et al., 2018; Nilsson & Berggren, 2000; Sousa et al., 2020). Yet few studies have directly assessed the response of freshwater mussel to alterations in the hydrologic environment, resulting from dam operation (Newton et al., 2015). Consequently, there remains a lack of empirical data to inform water management of the best flow operation practises to minimise hydraulic stressing of freshwater mussels. There is, therefore, a need to understand and predict entrainment events for the purposes of detecting the presence of suitable habitat and identifying at-risk populations, in addition to providing empirical data to inform water management.

Studies attempting to link mussels with their physical and hydrodynamic habitat have rarely examined near-bed flow metrics (Moorkens & Killeen, 2014); arguably the most ecologically relevant data for benthic biota, and often shown to be crucial in determining their spatial distribution (Blanckaert et al., 2013; Long et al., 2011; Oldmeadow et al., 2010; Robson et al., 1999). Instead, research has centred on correlative approaches towards determining abundance and distribution with hydraulic variables, or using predictive statistical models and computer simulations, with limited success: often attributed to inadequate flow data (limited range of measurements or a lack of direct measurements) (Gangloff & Feminella, 2007; Hardison & Layzer, 2001; Layzer & Madison, 1995; Morales et al., 2006). The few studies that have quantified interactions between freshwater mussels and hydrodynamics have provided evidence to suggest that dense mussel beds reduce near-bed flow velocity and significantly alter the surrounding flow dynamics (Kumar et al., 2019; Sansom et al., 2020), whilst greater shell exposure is expected to elicit greater hydrodynamic stress to the individual (Sansom et al., 2018). Attempts to directly quantify mussel entrainment values (Thompson et al., 2016) revealed a potential interaction between substrate composition and structure, shell morphology, and individual behaviour; however, the experimental design of such studies limited the applicability of the results, with uncertainty concerning the reliability of flow measurements.

Recent advances have been made, concerning the prediction of turbulent flow conditions that may elicit the entrainment of substrate particles (Diplas et al., 2008; Valyrakis et al., 2010, 2013) yet these have yet to be applied to ecological studies. Instead, there remains a dependency on indirect, time-consuming and often inaccurate methods for examining flow parameters and associated bed morphology: predictive models that attempt to understand the hydrodynamics of a river reach are often based on single point measurements (0.6*flow height), compared across a limited range of flow conditions (Bey & Sullivan, 2015; Morales et al., 2006; Scheder et al., 2015); surveys to examine riverbed stability are founded on descriptive analyses, which risks substantial user-bias (Johnson & Brown, 2000; Schwendel et al., 2010). Attempts have been made to formalise methods for elucidating habitat suitability (Boon et al., 2019), yet accurate methods to record and monitor near-bed hydrogeomorphological characteristics remain undefined.

Technological advancements in the last decade have provided a means of deviating from traditional methods of monitoring surrogate flow metrics, through the use of sensors. Results from studies in the field of riverbed sediment transport suggest the potential of such methods to analyse sediment entrainment directly (Akeila et al., 2010; Gronz et al., 2016; Kularatna & Abeywardana, 2008; Valyrakis & Alexakis, 2016), with work by Al-Obaidi et al. (2020) demonstrating a link between logged readings and sediment entrainment based on derived performance indicators. However, research concerning entrainment risk to freshwater biota must consider the impact of behaviour and morphology, with individuals unlikely to respond in a similar fashion to sediment particles when exposed to turbulent flows (Blanckaert et al., 2013; Kozarek et al., 2010).

The aim of this research was to adapt work with smart sensors from the field of riverbed sediment transport, and examine the suitability of this technology to evaluate the incipient flow conditions at which unionid species are entrained from the riverbed surface; with the hope of providing a direct, non-intrusive, low-cost and accessible tool to assist conservation management in examining near-bed flow dynamics. We hypothesised that the utilization of inertial microelectromechanical sensors (MEMS), housed within mussel shells, could provide an accurate method of identifying and predicting entrainment events. To test this, empty mussel shells, filled with silicone, were used as the instrumented shell to house the sensors. Interpretation and verification of the data emanating from the smart sensors was aided by video capture, which enabled the accurate placement of the shells and subsequent tracking of their movement. To our knowledge, this research would represent the first to employ such sensors for ecological assessment and identification of optimal habitat for a freshwater species. The objectives were:

(i) to design, for the first time, an instrumented freshwater mussel which comprises embedded inertial microelectromechanical sensors (MEMS) within freshwater mussel shells

(ii) to calibrate the instrumented shells, for different sizes, and test their functionality in a laboratory setting

(iii) to conduct dedicated flume experiments to assess instrumented shell movement metrics, for a range of flow conditions and initial orientations (fully exposed and partially buried)

(iv) to analyse the experimentally obtained results towards identifying metrics that offer distinct criterion for assessing the risk to entrainment

(v) to validate the utility of this tool under identified metrics for indirectly assessing flow conditions that result in greater stressing of the mussels

5.2 Defining entrainment of freshwater mussels

The purpose of this study was to assess the extent to which an individual mussel may become more stressed in response to increased flow forcing, depending on its shell morphology and orientation. To achieve this, focus was placed on the examination of the modes of movement observed in freshwater mussels when experiencing hydraulic stress, which result in partial or full entrainment. No known study has previously attempted to define these modes before in unionid mussels. Consequently, initial work sought to define the theoretical modes of movement that would likely be observed for the two orientations that were to be studied: fully exposed, resting horizontally on the bed surface (Fig.5.1); and partially buried, positioned vertically with 50% of the shell area submerged in the substrate (Fig.5.2).

For a fully exposed mussel, the key modes of entrainment were anticipated to be sliding and rolling. However, for the partially buried mussel, the flow forces acting on the shell were likely to be insufficient to remove it from its pocket: because of the shell's asymmetrical shape, with the wider, heavier element anchored below the surface, and the narrower part exposed to the flow (often orientated to streamline with the flow, as observed in live mussel beds), hydrodynamic lift forces were expected to be less effective in displacing the mussel in a saltation mode. Instead, as the hydrodynamic forces increased, the shell was expected to move back and forth or sideways initially, until it repositioned itself into an orientation that enabled full entrainment out of its pocket as the experiment progressed.

A partial or full entrainment event was expected to be dependent on the mode of movement, with the shell potentially displaying a combination of modes during an entrainment event. To quantify their occurrence, the conditions necessary to identify the presence and extent of each mode of movement were articulated. Critical values, which determined the presence or absence of a mode of movement were based upon the minimal assessed displacements captured by high-speed video cameras, operating at 120 frames-per-second, that could be confirmed with a high degree of confidence.

For the fully exposed shell, three modes of movement were defined: a sliding movement (Fig.5.1a), defined as a linear displacement along the x-axis of the shell, greater than or equal to 1mm ($\Delta_x > \Delta_{x,c}$); a change of planar orientation (Fig.5.1b), concerning movement around the z axis, greater than or equal to 2 degrees ($\Delta\theta_z > \Delta\theta_{z,c}$); and a rolling movement (Fig.5.1c), pertaining to an angular displacement of the shell around the y axis, greater than or equal to 2 degrees ($\Delta\theta_y > \Delta\theta_{y,c}$).

For the partially buried shell, three modes of movement were also defined: a change in planar orientation (Fig.5.2a), which concerned movement around the y axis, greater than or equal to 2 degrees ($\Delta\theta_y > \Delta\theta_{y,c}$); a sideways movement relative to the flow (Fig.5.2b), defined by an angular displacement around the x axis, greater than or equal to 2 degrees ($\Delta\theta_x > \Delta\theta_{x,c}$); and a forward or backward movement (Fig.5.2c), categorised as an angular displacement around the z axis, greater than or equal to 2 degrees ($\Delta\theta_z > \Delta\theta_{z,c}$).

By articulating the method for quantifying the presence and magnitude for each mode of movement, output from the sensor could be used to accurately determine entrainment rates, which may serve as a metric to assess stress to freshwater mussels. Furthermore, validation of the sensor data by visual assessment of shell movement was improved, as the positioning of cameras could be optimised to detect the pre-defined movements.

5.3 Considerations to Sensor Design

Having defined the modes of movement that result in entrainment, focus shifted towards outlining the design considerations and specifications that an inertial measurement unit must have to successfully identify these instances of entrainment at threshold flow conditions. The following were outlined as key considerations for design: the size, shape and weight of the sensor; the availability and type of inertial sensor; the format for data transfer and storage; the energy supply and storage; the range of angular velocities and accelerations. Table S1 highlights the design requirements outlined by the research team for the instrumented shell and sensor.

In response to the criteria (Table S1), Invensense MPU-9250 inertial sensors (Invensense Inc., San Jose, CA, USA) were selected for use in this research. This inertial sensor provides measurements concerning three-axis acceleration, three-axis rotational velocity, and three-axis magnetometer, in addition to an internal Digital Motion Processor, which supports up to 16g of measurable acceleration, 2000°/s of measurable rotational velocity at an output frequency in excess of 200Hz. With regards to data transfer and storage, discrete flash integrated circuits were selected for their predictable access, erase and write times, with the S25FL128S (Cypress Semiconductor Corporation, San Jose, CA, USA) specifically used due to its low unit cost, high data logging speed of 1.5MB/s and 16MB storage size. A serial converter to TTL 6-pin was used for charging and data transfer, ensuring a direct connection with the PC. An in-house Python code was developed to retrieve the data stored on the sensor's flash, which increased the speed of downloading the data to a PC substantially, with an hour of data logging requiring three minutes to download to a PC. The data was downloaded in an easy to use .csv format. In consideration of energy storage, rechargeable coin cells (Varta Microbattery produced under CoinPower, Varta Micorbattery GmbH, Ellwangen, Germany) were selected as cost-effective, practical alternatives to lithium batteries, and provided approximately 80 minutes of power from a single charge. For the final design of the sensor (Fig.S1), attention was paid towards ensuring the distribution of the mass across the instrumented shell was biologically relevant: similar to that of live mussels.

5.4 Methodology

5.4.1 Design and creation of instrumented shell

Empty *M. margaritifera* shells from previously deceased individuals were collected from the South Esk River, Scotland, placed in bleach solution for 24 hours and cleaned. Three shells, differing in size and morphology, were selected to function as instrumented shells, to represent a diversity in *M. margaritifera* size class: small; medium; large (For overview of dimensions and what these pertain to see Table S2 and Figure S2b respectively). To easily distinguish the instrumented shell from the surrounding bed, the outer area was painted white with aquarium-grade spray paint (Fig.S2a). For easy visual identification of shell motions, the central x and y axis of the shell were marked on the outer shell using red permanent marker, providing a high contrast colour to the white shell. Shells were filled with aquarium-grade silicone to a weight appropriate for the mussel size, accounting for the weight of the sensor (Fig.S2a). To house the sensor in the instrumented shell, a sensor was placed within a sealed impermeable cellophane wrap and pressed into the silicone before it had set to create an impression mould located on the intersection between the central x and y axis of the shell.

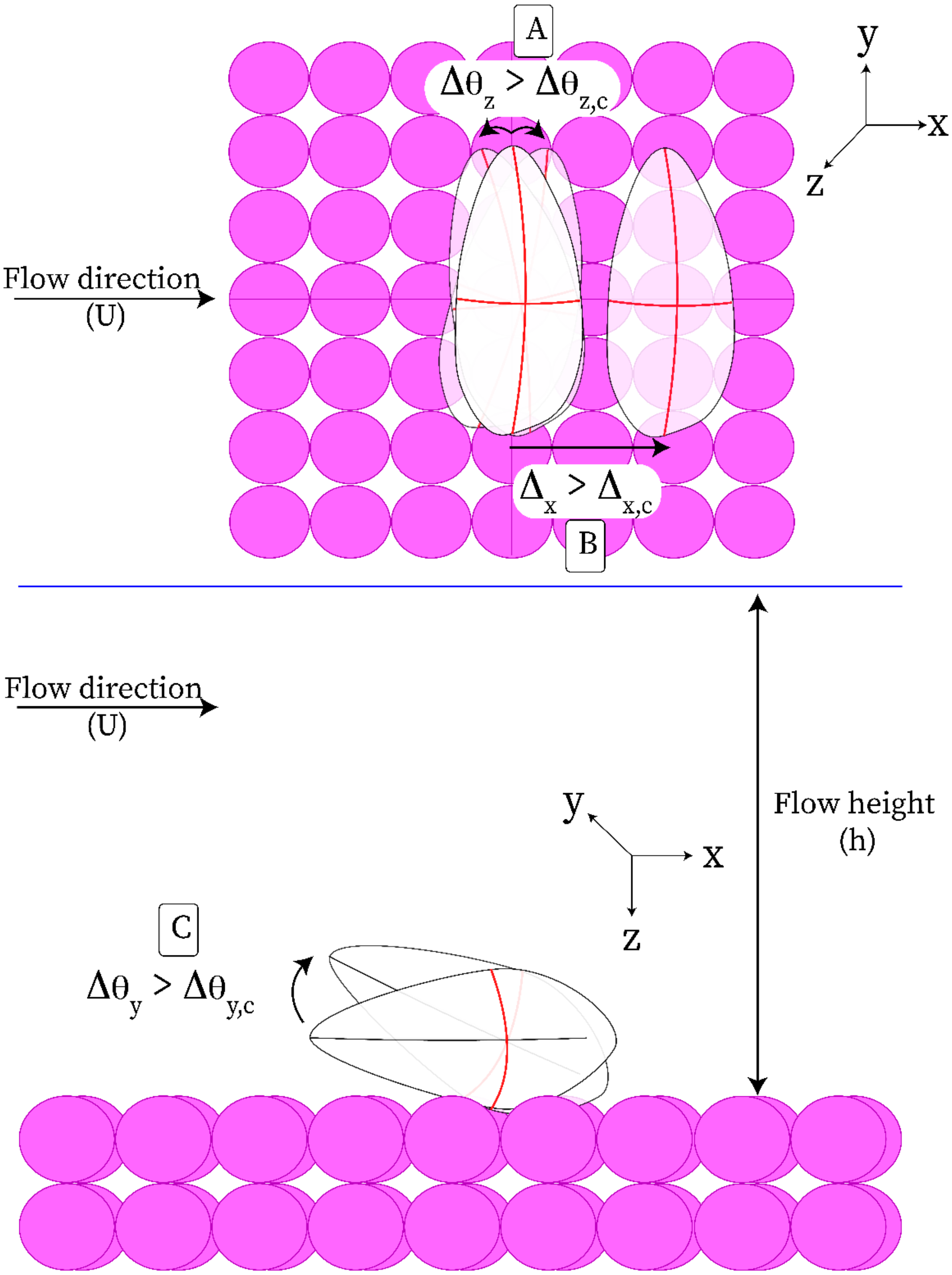

Figure 5.1 The three expected modes of movement to initiate the partial or full entrainment of a fully exposed mussel shell, positioned horizontally on a riverbed (pink circles) with the shell aperture facing the flow. Descriptive formulae are noted next to the corresponding movement. The top diagram depicts a change of planar orientation around the z axis (A; $\Delta\theta_z > \Delta\theta_{z,c}$), in addition to the sliding of the shell along the x-axis in a linear displacement (B; $\Delta_x > \Delta_{x,c}$). The bottom, cross-sectional, diagram shows the rolling of the shell in an angular displacement around the y axis (C; $\Delta\theta_y > \Delta\theta_{y,c}$). For each diagram the position of the pre-defined x, y and z axes for the shell are noted, in addition to the direction of the flow. The bottom, cross-sectional, diagram includes reference to flow height (h), which measured the distance between the water's edge (blue line) and the top of the substrate shells.

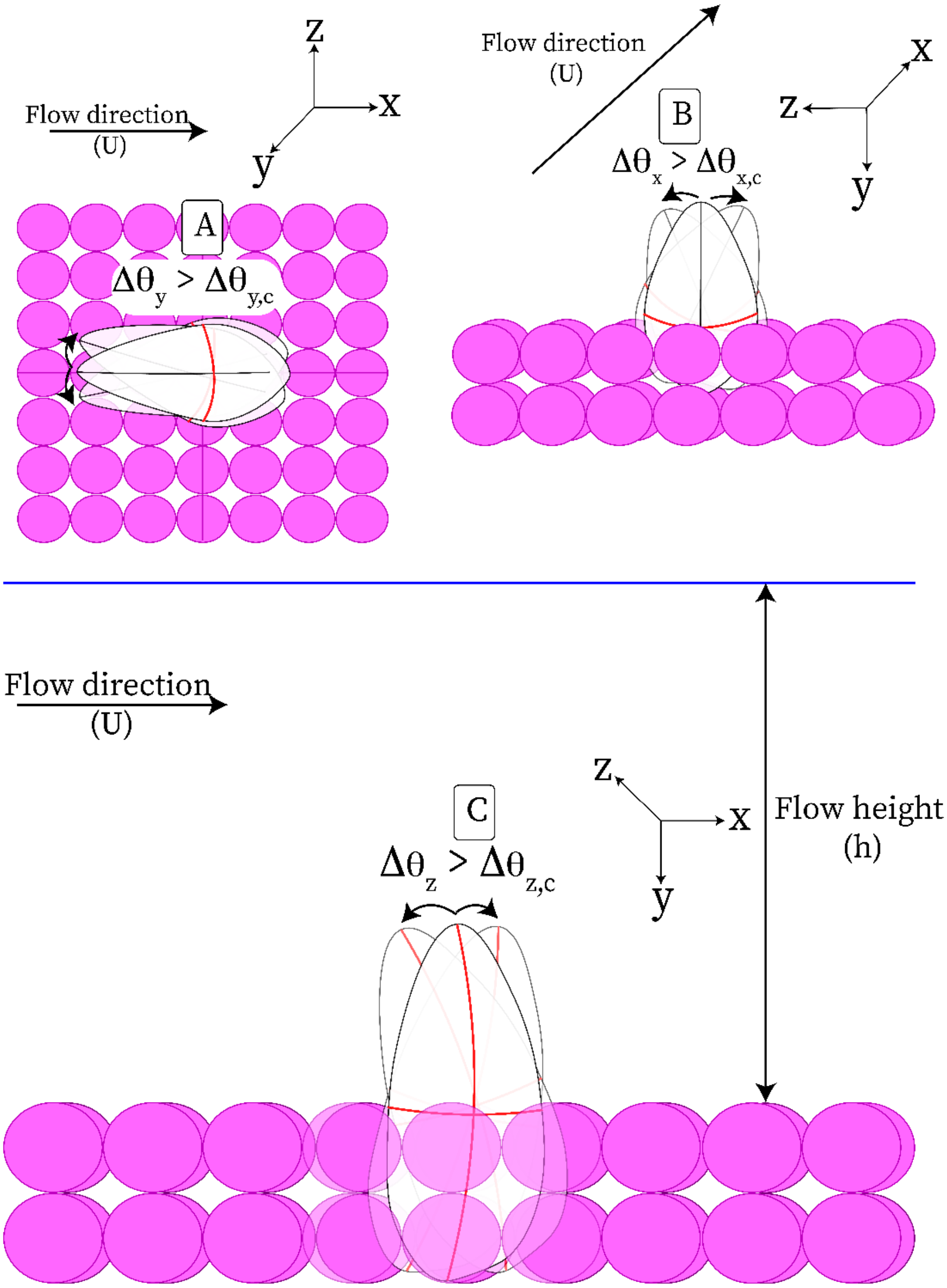

Figure 5.2 An illustration of three expected modes of movement to initiate the partial or full entrainment of a partially buried mussel shell, positioned vertically in the riverbed substrate (pink circles), with 50% of the shell area exposed to the water, and the shell aperture facing the flow: a change in the planar orientation of the shell around the y axis (A;$\Delta\theta_y > \Delta\theta_{y,c}$*); the top of the shell moving sideways relative to the flow (B;*$\Delta\theta_x > \Delta\theta_{x,c}$*); the forward/backward movement of the shell (C;*$\Delta\theta_z > \Delta\theta_{z,c}$*). For each diagram the position of the pre-defined x, y and z axes for the shell are noted, in addition to the direction of the flow. The bottom, cross-sectional, diagram includes reference to flow height (h), which measured the distance between the water's edge (blue line) and the top of the substrate.*

5.4.2 Calibration of instrumented shell

Two calibration tests were undertaken to examine how the data output from the sensor's triaxial gyroscope and accelerometer correlate with known changes in acceleration and rotation, when housed within the instrumented shell. The three shell sizes were used during the two calibration tests, to ensure there was no size dependent effects on the sensitivity of the data. For both calibration tests, the sensor was housed within the corresponding instrumented shell and placed on a non-slip craft mat (Fig.S3). The craft matt was divided into 10mm x 10mm squares. At the centre of the matt a circle (100mm radius) was drawn with a marker pen, with 36 lines drawn from the centre of the circle to the perimeter, representing incremental changes in 10°. For both calibration tests, the instrumented shell was placed in the middle of the circle, with the shell aperture facing the 0° mark. To ensure continuity with the starting position, the instrumented shells were aligned as follows: the central x axis of the shell aligned with the 180° and 0° line of the circle, whilst the central y axis of the shell aligned with the 90° and 270° lines of the circle. Each calibration test for an instrumented shell comprised ten repeats of the same movement, with a repositioning back to the starting position at the end of each repeat.

Two high-speed video cameras operating at 120 frames-per-second (GoPro Hero 8 Black, Gopro, Inc. San Mateo CA, USA) were positioned to capture the instrumented shells' movement during the tests, to ensure accurate estimation of movement via a visual analysis. Both calibration tests were designed to mimic expected movement of the instrumented shell when experiencing significant hydraulic stress.

Data from the accelerometer and gyroscope were used to determine the total acceleration and total angular velocity (Fig.S1) respectively for the sensor using Equation (5.1):

$$a = \sqrt{a_x^2 + a_y^2 + a_z^2} \tag{5.1}$$

where a_x, a_y and a_z are the x, y and z components of the acceleration, with ω_x, ω_x and ω_x substituting the aforementioned as the x, y and z components of angular velocity when using the equation to calculate total angular velocity (ω; rad/s).

(i) Rotation around central axis

The first calibration test sought to reproduce a change of planar orientation ($\Delta\theta_z > \Delta\theta_{z,c}$) for a fully exposed mussel, (Fig.5.1a), concerning a 30° movement around the z axis. To facilitate this movement, a cylindrical metal probe (Length: 300mm; circumference: 15.71mm) was used to push the edge of the shell backwards, from its starting position, until the y axis aligned with the 120° and 300° markings (Fig.S3b). To ensure rotation around the central axis, and to limit sliding of the shell, a metal pin was placed through the intersection of the shell's x-axis and y-axis, which punctured the craft mat. A marker was placed on each shell to allow for accurate repetition of the point of contact with the metal probe each time. An estimate of the angular velocity for each experimental repeat was made using Equation (5.2):

$$\omega = \frac{\Delta\alpha}{t} \tag{5.2}$$

where ω represents angular velocity (rad/s), $\Delta\alpha$ is the change in angle and t is the time in which the angle change occurs. Total angular velocity was calculated from the sensor's gyroscope data. The mean recorded angular velocity results from the gyroscope, for the ten experimental repeats, are presented in Table S3.

The standard deviation for the ten experimental repeats is relatively large across the three instrumented shells, and likely represents the level of human error in operating the metal probe; facilitating the change in shell orientation. The average error, between the theoretical estimation of total angular velocity and the readings from the sensor, was 5.61% and deemed to be acceptably low considering the measurements were made by hand and the theoretical estimation is based on a smooth and consistent movement with no sliding, which could not be guaranteed.

(ii) Sliding along surface

The second calibration test sought to reproduce the sliding of a fully exposed shell along the x-axis in a linear displacement ($\Delta_x > \Delta_{x,c}$). To enable this movement, the cylindrical metal probe was modified, with a metal plate (135mm x 30mm x 5mm) fixed to one end (Fig.S3c). The metal plate provided a flat surface to push the instrumented shell backwards, whilst minimising substantial changes in the angle of the shell. For each experimental repeat the metal probe pushed the shell backwards by 20mm. An estimate of the acceleration for each experimental repeat was calculated using Equation (5.3):

$$a = \frac{2s}{t^2} \tag{5.3}$$

where a represents acceleration (m s^{-1}), s is displacement (m), and t is time (s).

Furthermore, calibration tests were undertaken using raw data. Total acceleration was calculated from the sensor's accelerometer data. The mean recorded acceleration results from the accelerometer, for the ten experimental repeats, are presented in Table S4.

Similarly, to the calibration tests for angular velocity, the standard deviation for the ten experimental repeats is relatively large across the three instrumented shells: likely to be reflective of human error. The average error, between the theoretical estimation of total acceleration and the readings from the sensor, was 4.86%, and deemed to be acceptably low considering the measurements were made by hand and the theoretical estimation is based on a smooth and consistent movement, which could not be guaranteed.

5.4.3 Examination of sensor operation

Sensing checks were undertaken to ensure the sensor adequately detected alterations in the movement of the instrumented shells, when housed within the shell, and submerged in water. To conduct the sensing checks, a sensor was first placed in each of the three instrumented shells. The sensors were wrapped in cellophane for waterproofing and placed in the impression mould of the relevant shell. and centred at their centre of gravity, with the x-axis and y-axis of the sensor aligned with the central x-axis and y-axis of the shell. The shell was then placed within a glass aquarium tank (600mm x 480mm x 300mm), on a substrate of fine gravel (0.5-4mm) to a depth of 15mm, and then submerged in water to a depth of 300mm. An Eheim Universal 1250 centrifugal pump was placed in the aquarium tank, 250mm away from the instrumented shell's position, and connected to a timer switch, which ensured the pump cycled between 2 minutes of operation at an output of 1200 l/h and 2 minutes of rest. Two sensing checks were undertaken for each of the three instrumented shell sizes: first, partially buried in substrate to 50% shell exposure; second, fully exposed, lying horizontal on surface with shell aperture facing the pump. The sequence for a sensing check was as follows: (1) sensor switched on and placed inside instrumented shell; (2) shell placed in aquarium tank either partially buried or fully exposed; (3) Eheim pump underwent three cycles of operation and rest; (4) pump switched off; (5) instrumented shell removed from the tank, with sensor subsequently extracted and switched off. The resulting motion of the sensor was then checked, with readings from the accelerometer and gyroscope observed separately over the three axes, as shown in Figure 5.3.

From the sensing checks, it was observed that the ranges of 16g and 2000°/s were sufficient for recording the angular velocity and acceleration of the instrumented shells. Additionally, the cellophane wrapping around the sensor was proven to provide effective waterproofing.

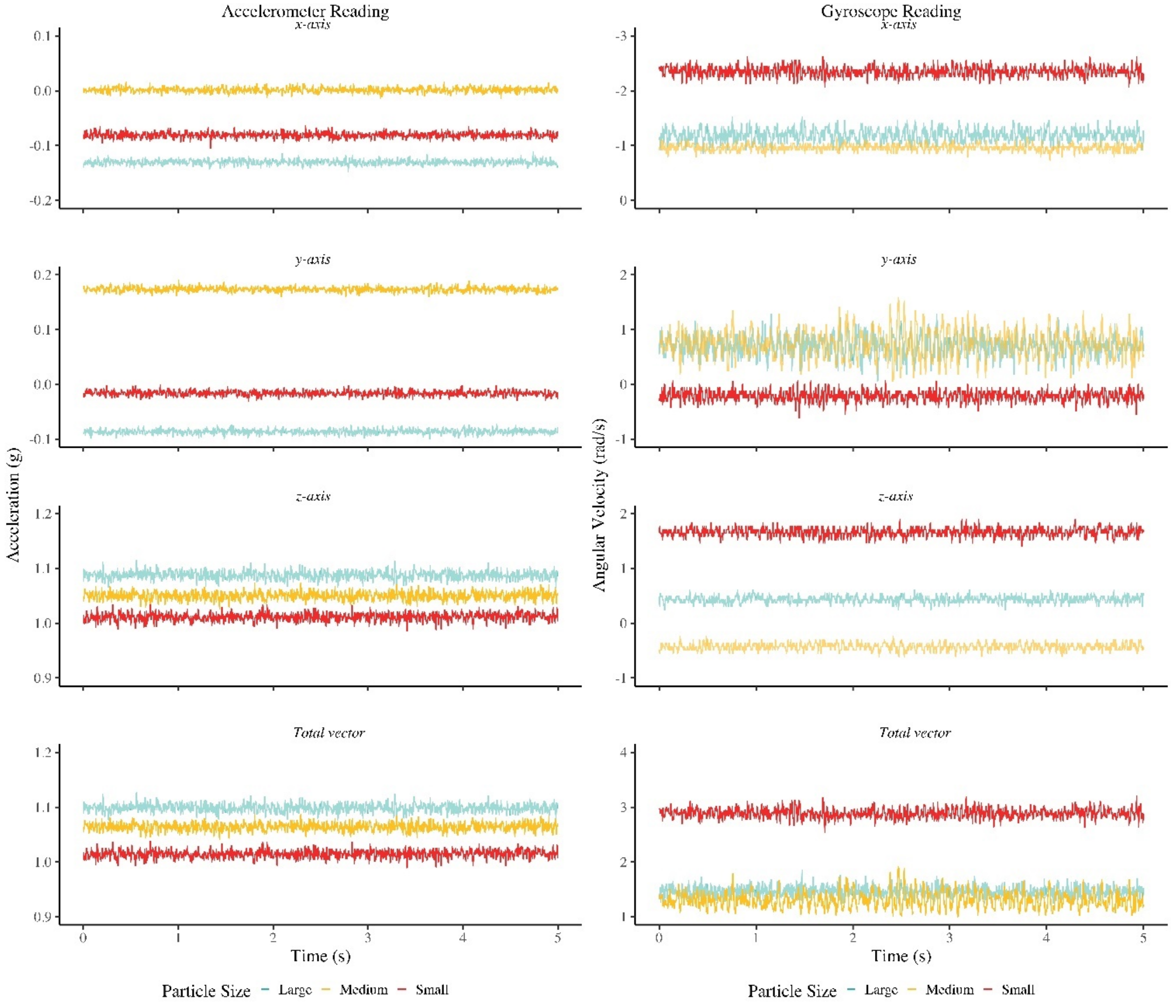

Figure 5.3 Logged readings from sensor's accelerometer and gyroscope during initial sensing check and endurance experiment

5.4.4 Flume set-up and test section

To further examine the application of the instrumented shells, experiments were conducted in a well-controlled 8 m long water recirculating flume, located at the Water Engineering Lab, University of Glasgow (Fig.5.4a). The 0.9m wide open channel can support flows of up to 0.4m in depth, with water provided at a maximum capacity of 0.2m^3/s, controlled by a torque inverter with which operational pump frequency can be adjusted. An adjustable tailgate, located at the outlet, provided a means of artificially attaining intended flow depths at reasonable flow velocities. To gain adequate hydraulic roughness, the bed surface comprised layers of water-worked uniformly sized fine gravel, with a median size of d_{50} = 15–25 mm, to a depth of 120mm. Fine gravel was chosen to replicate the substratum conditions often highlighted as favourable for unionid mussels to burrow into (Geist & Auerswald, 2007; Hastie et al., 2000). A test section (1m x 0.5m) was located 5.7m downstream of the channel inlet, 1.3m from the outlet, to ensure hydraulically rough turbulent flow was fully developed at the point of shell placement. The test section was positioned along the centreline of the flume, 0.2m, from the flume's glass walls.

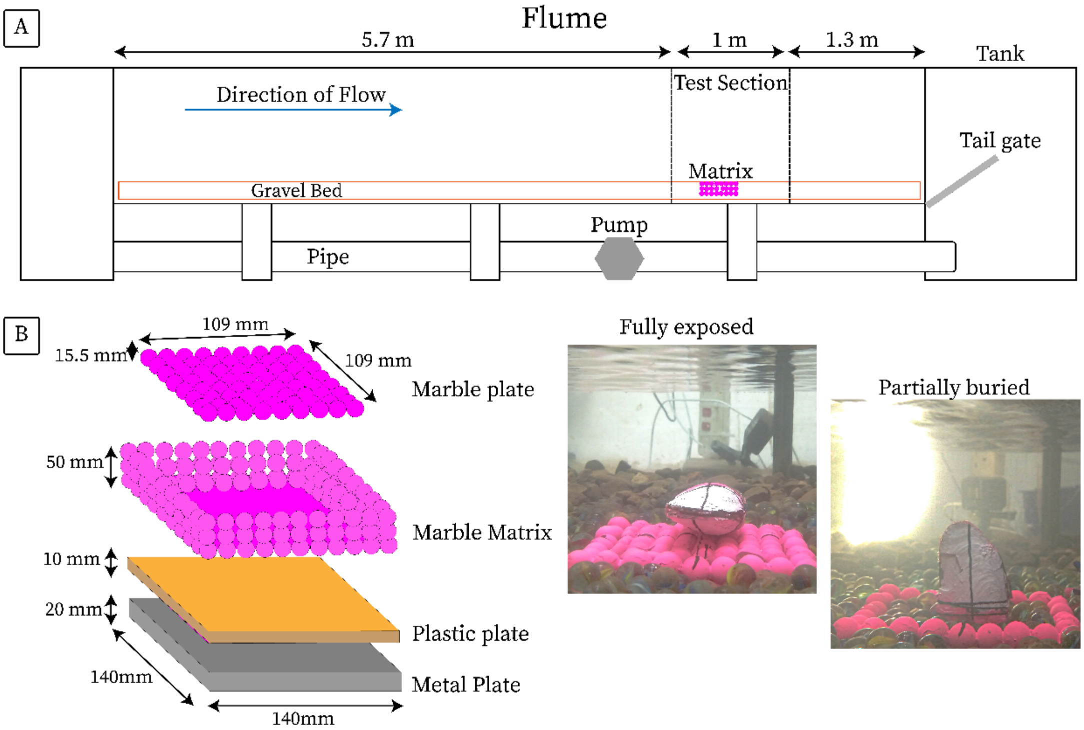

Figure 5.4 Schematic of the (A) experiment flume set-up, highlighting the location of the test section and marble matrix, with an overview of the (B) marble matrix structure, in addition to illustrations of the matrix accommodating the two orientations of instrumented shell

Within the test section, layers of gravel were replaced by a homogenous bed of 15.5mm diameter marble spheres, which surrounded a rigid marble matrix, by a radius of 100mm, from the edge of the matrix to the gravel bed. The introduction of a more homogenous local micro-topography assisted the development of flow near the instrumented particle; thus, reducing the likelihood of significant flux in the near-bed flow dynamics, which may result in anomalies in the sensor reading. The marble matrix (140mm x 140mm x 50mm) was formed by gluing layers of marbles together in a rectilinear well-packed arrangement, with a 3D printed plastic plate (140mm x 140mm x 10mm) as a foundation (Fig.5.4b). The matrix comprised four walls, of two marbles in depth, and three marbles high. A hollow region in the centre of the rectilinear arrangement accommodated loose marbles; enabling the burial of the instrumented shell, whose incipient motions were to be studied. A second rectilinear structure was formed to create a plate (109mm x 109mm x 15.5mm), which could be placed on top of the hollow region of the matrix; thus, forming a rigid bed micro topography to permit the horizontal placement of the instrumented shell. Underneath the marble matrix, resided a metal plate (140mm x 140mm x 20mm) on top of a layer of fine gravel, which ensured the matrix resided at the same depth as the surrounding bed of loose marble spheres, with a gradient to match the surrounding topography. The marble matrix was painted neon pink with an aquarium-grade spray paint, to distinguish it from the surrounding bed, and achieve high contrast with the instrumented shells. The central x and y axis of the marble matrix were marked using black permanent marker, which allowed for consistent alignment of the shells before each experimental repeat, and easier visual identification of shell movement during the experiment by highlighting when a deviation from the axis alignment of shell and matrix had occurred.

Two high-speed commercial video cameras operating at 120 frames-per-second (GoPro Hero 8 Black, Gopro, Inc. San Mateo CA, USA) were positioned to capture the instrumented shell's movement. The first, placed within the flume channel near the flume wall to avoid interference with the flow properties near the instrumented shell, filmed horizontally across the micro-topography. The second, positioned above the flume channel parallel to the bed, filmed the micro-topography below. A halo lamp was placed above the second camera to provide an ultra-bright light source.

5.4.5 Experimental protocol

(i) Preparation of the shell

At the commencement of an experiment, the sensor was switched on and data logging enabled. The sensor was wrapped in layers of cellophane for waterproofing and positioned in the instrumented shell, with the sensor's x axis aligned with that of the shell (Fig.S2a). The shell was subsequently closed with small rubber bands to hold the two shell pieces together. The shell was placed within a glass beaker located in a bucket filled with water to a depth of 450mm and held for two minutes to allow trapped air to escape. The beaker was removed and weighed to record the shell's wet weight. The beaker was lowered into the flume, and the shell placed according to predefined positioning criteria: (i) fully exposed, lying horizontally on the marble matrix with the shell aperture facing the flow, parallel to the bed, with the shell's central x and y axis aligned with that of the microtopography (Fig.5.1); (ii) partially buried, positioned vertically, with 50% of the shell buried in the loose marbles of the matrix, the shell aperture facing the flow, and the shell's y axis perpendicular to that of the microtopography (Fig.5.2).

(ii) Preliminary tests

Preliminary experiments were undertaken to detect the range of flow rates where the instrumented shell started becoming entrained, until the point at which the instrumented shell was difficult to maintain in its orientation during initial placement. Preliminary experiments were conducted for each of the three instrumented shell sizes. After preparation of the shell was complete it was placed according to one of the two predefined positioning criteria. The flume was then run to provide an incremental increase in flowrate until mobilisation of the shell was observed and noted. Preliminary tests were repeated three times for each instrumented shell in a given orientation; thus, undertaking six preliminary tests for each instrumented shell size.

(iii) Experimental tests

The experiment was designed to compare the response of the three instrumented shells across different flowrates, when positioned in two distinctive orientations, and examine whether these responses could be accurately correlated with readings from the accelerometer and gyroscope of the sensors housed therein.

Using data obtained from preliminary testing, three experimental flowrates were identified for each size of instrumented shell: High flowrate provided conditions known to induce frequent entrainment; Low flowrate comprised conditions where no shell movement was observed; Intermediate flowrate provided conditions where shell movement had occasionally been noted.

For each experiment, pump frequency was set and held at the pre-defined rate to provide conditions specific for a given size of instrumented shell and corresponding experimental flowrate (Table 5.1). After pump frequency was set, the flume was left undisturbed for two hours to ensure stable flow conditions were allowed to develop. Following this, flow height (h) was recorded using a digital depth gauge. Measurements of flow height corresponded to the vertical distance from the water's edge to the top of a particle positioned in the experimental matrix (Fig.5.1). To ensure consistency in recordings of flow height, the digital depth gauge was positioned at a designated site, pertaining to a circular mark drawn onto a particle of the experimental matrix, located 1cm upstream of the instrumented shell in the centre of the flume channel (45cm from the flume walls). The digital depth gauge was then removed and an Acoustic Doppler Velocimeter probe (ADV Vectrino II, Nortek AS, Rud, Norway) was then positioned at 0.6*flow height, in its place, and recorded the flow velocity in the flume channel for 8 minutes. The ADV probe was then removed before sensor measurements were taken. See Table 5.1 for an overview of the flow height (h) and mean flow velocity, corresponding to the three experimental flowrates for each instrumented shell size.

The shell was placed according to one of the two predefined positioning criteria. An incubation period of two minutes proceeded initial placement of the shell, before a measurement period of ten minutes, where the shell was left undisturbed. The instrumented shell was allowed to move from its initial positioning downstream, due to sufficiently energetic instantaneous near bed surface flow structures, with full entrainment from the test section permitted within a measurement period. At the end of a measurement period the shell was picked up, moved back and forth along its x axis to provide a distinct fingerprint in the sensor data (indicating the end of an experimental repeat), and placed on the marble matrix, accordingly to the positioning criteria. Four further incubation and measurement periods followed to provide five replicates for one shell size, in one orientation, at a given experimental flowrate. At the end of an experiment, the sensor was removed from the shell, turned off and re-charged before data acquisition.

Table 5.1 The mean flow velocity readings, and corresponding flow heights, associated with the High, Intermediate and Low experimental flowrates assigned to the Large, Medium and Small instrumented shells

	Mean Flow Velocity at 60% Flow Height ($m\ s^{-1}$)			Flow Height (mm)		
Size	High	Intermediate	Low	High	Medium	Low
Large	0.147	0.102	0.053	142.63	135.82	115.07
Medium	0.111	0.055	0.053	141.29	129.67	115.07
Small	0.107	0.055	0.053	140.20	129.67	115.07

5.4.6 Data analysis

After each test, data was inputted into Matlab for further processing. Inverse uncertainty quantification was conducted using parameter calibration and data fusion, for the purposes of creating a filter to undertake data fusion and estimate the final corrected acceleration, angular velocity and orientation. The filter used a nine-axis Kalman filter structure (Kalman, 1960), incorporating inputs of expected accelerometer, gyroscope and magnetometer noise. Data from the calibration tests provided the accelerometer and gyroscope noise as inputs for the inertial sensor fusion filter.

5.5 Results and Discussion

5.5.1 Initial data interpretation

After filtering the data through inertial sensor fusion, the results were used to calculate corrected total acceleration using Equation (5.1). For each experiment, the corrected total acceleration was compared with a visual analysis of the video data from the two cameras. By comparing the two data sources, a threshold in the total acceleration data could be made, pertaining to the occurrence of one, or multiple, modes of movement by the instrumented shell.

Calculating thresholds of entrainment

Initial analysis sought to evaluate whether data from the sensor could accurately and reliably detect modes of movement, which result in partial or full entrainment of the instrumented shell, when exposed to sufficient forcing from turbulent flow conditions. An illustration of this work is shown in Figure 5.5. Here, a fully exposed Large instrumented shell underwent an experimental run with High experimental flow conditions. Total acceleration values exceeding 11.38m s^{-2} indicated a partial entrainment event. Examination of the data presented in Figure 5.5, shows six partial entrainment events, defined by noticeable shifts in shell orientation, without full displacement from the pocket. The shell appears to undergo a change of planar orientation around its z axis. The first partial entrainment event occurred at 0.9 seconds and spanned a period of 1.32 seconds; depicting multiple peaks in the total acceleration, the most prominent of which occurred at 1.38 seconds with a maximum value of 45.109 m s^{-2}.

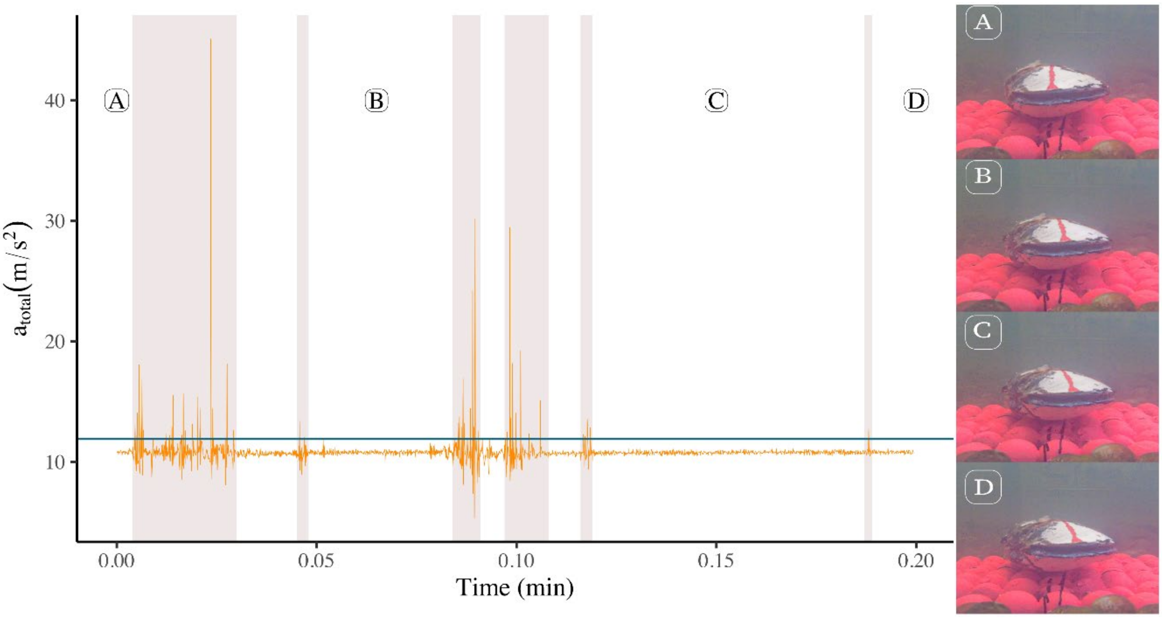

*Figure 5.5 The identification of instrumented shell entrainment events using the fused sensor readings for total acceleration (m s^{-2}). Data presented here was recorded during an experimental run using the Large instrumented shell, in the 'fully exposed' orientation, during the High experimental flow conditions. Highlighted regions represent singular entrainment events in the instrumented shell. The thick blue line represents the threshold in total acceleration readings (average total acceleration ± 6*SD; 10.792 ± 0.708) above which visible movements in the instrumented shell could be detected by the two high-speed video cameras, operating at 120 frames-per-second (Go Pro Hero 8); positioned to capture the instrumented shell's movement during the tests. Corresponding instrumented shell orientation is shown at four (A,B,C,D) periods within the time-series data, demonstrating a gradual change in planar orientation around the shell's z axis. Photos were edited to improve visibility of the instrumented shell.*

For each of the remaining five partial entrainment events shown in Figure 5.5, it is interesting to note that the more significant increases in total acceleration from the sensor's readings, denoted by peaks typically exceeding 20 m s^{-2}, appeared to correlate with significant shifts in orientation; thus, indicating a relationship between the extent of movement in the instrumented shell and the corresponding readings for total acceleration, emanating from the sensor housed within. It therefore appears that the data acquisition rate by the sensor and cameras is sufficient for recording the modes of movement above the pre-defined critical values.

There were instances where energetic events, caused by turbulent flowing water, resulted in noticeable deviations from mean total acceleration readings; however, these were not forceful enough to generate instances where the critical value for a mode of movement was exceeded. These short-lived energetic events, which were insufficient in duration to result in any movement of the instrumented shell, were termed 'twitches' (Valyrakis et al., 2010).

It would appear that substantial shifts in shell movement, highlighted by peaks in total acceleration, often position the shell in an orientation that incurs lesser forcing from the flow. Significant peaks in total acceleration were often greatest during the first entrainment event and rarely matched in magnitude later in an experimental repeat, with movement of the shell mimicking this trend: a substantial shift in orientation occurring initially, followed by smaller movements thereafter. This water-working of the shell, to a potentially more hydrodynamic orientation is similar to the process observed in riverbed substratum, whereby the onset of sediment transport is a continuous transition, from a creeping state to a granular flow (Houssais et al., 2015).

5.5.2 Entrainment Frequency

The procedure highlighted in Figure 5.5, for identifying thresholds in total acceleration to indicate entrainment events, was applied to the results for all three instrumented shell sizes, across the respective High, Intermediate and Low experimental flowrates. Using the results from this analysis, the frequency of entrainment, f_E, was calculated for each experiment by dividing the total number of entrainment events recorded over the five experimental repeats by the total experimental time in seconds. Results emanating from these calculations are displayed in Table 5.2, with the corresponding mean (±SD) total acceleration from sensor's accelerometer readings for the given shell size, orientation and experimental flowrate. Analysis of these results revealed a difference in the frequency of entrainment across experimental flowrate, shell orientation and size.

For mussels positioned horizontally on the bed surface, fully exposed to the flow, a visual assessment of change in f_E in response to altering flowrate and instrumented shell size is presented in Figure 5.6. Here, examination of the frequency of entrainment reveals an increase in f_E with increasing mean flow velocity, across the three instrumented shell sizes; thus, suggesting heightened flow rate induces increased rates of entrainment irrespective of shell size. However, the extent to which f_E increases in response to increasing mean flow velocity is not consistent across the three instrumented shell sizes (Table 5.2).

Interpolation of the data was undertaken by applying a cubic Hermite spline to the f_E calculations (Fig.5.6), to further assess the trends in the frequency of entrainment across the three sizes of instrumented shell. Analysis of these results revealed variation in readings of f_E when comparing the responses of the three instrumented shell sizes to the same flow conditions: at a mean flow velocity of 0.1m s^{-1}, f_E was shown to be 50% higher in the Small instrumented shell compared to the Medium instrumented shell, with no entrainment events recorded for the Large instrumented shell. Therefore, smaller shell sizes appear more likely to be entrained than their larger counterparts, when exposed to the same flow conditions.

Table 5.2 The frequency of entrainment (f_E) events for the three instrumented shell sizes, across the two orientations and three experimental flowrates. Mean (±SD) total acceleration (m s^{-2}) from sensor's accelerometer readings are given for the three instrumented shell sizes, across the two orientations and three experimental flowrates.

Size	Orientation	f_E (Hz)			Mean (±SD) Total Acceleration (m s^{-2}) from Sensor's Accelerometer Readings		
		High	Intermediate	Low	High	Intermediate	Low
Large	Horizontal	0.0002	0	0	10.792 ± 0.118	10.714 ± 0.089	10.786 ±0.075
	Buried	0	0	0	9.534 ± 0.095	9.665 ± 0.069	9.683 ± 0.051
Medium	Horizontal	0.0003	0	0	10.350 ± 0.094	10.329 ± 0.077	10.454 ± 0.0747
	Buried	0	0	0	9.737 ± 0.078	9.634 ± 0.050	9.729 ± 0.046
Small	Horizontal	0.0004	0	0	9.937 ± 0.094	9.929 ± 0.075	9.939 ± 0.073
	Buried	0	0	0	9.546 ± 0.075	9.853 ± 0.049	9.906 ± 0.046

Comparisons of f_E during the High experimental flow rate conditions, for the respective instrumented shell sizes, revealed further variation in their responses: f_E was 33% higher in the Small instrumented shell compared to the Medium instrumented shell, despite a 3% increase in mean flow velocity; f_E was 100% higher in the Small instrumented shell compared to the Large instrumented shell, despite a 27% increase in mean flow velocity; f_E was 50% higher in the Medium instrumented shell compared to the Large instrumented shell, despite a 24% increase in mean flow velocity. Consequently, smaller shell sizes appear to display a more pronounced shift in f_E in response to increasing flowrate, with a larger shell size incurring a more gradual change in f_E in response to increasing flowrate.

Assessment of the effects of orientation on the risk of entrainment to the three instrumented shell sizes, revealed a complete absence of any modes of movement in the shells across the respective experimental flowrates when partially buried in the riverbed. Consequently, it appears that submerging the shell in the riverbed substratum significantly reduces the risk of entrainment to the instrumented shell. Further research, with increased mean flow velocities, to induce greater stress from the flow are required to discern to what extent partial burial inhibits movement, and whether differences across shell sizes exist, as was shown when the shells were fully exposed.

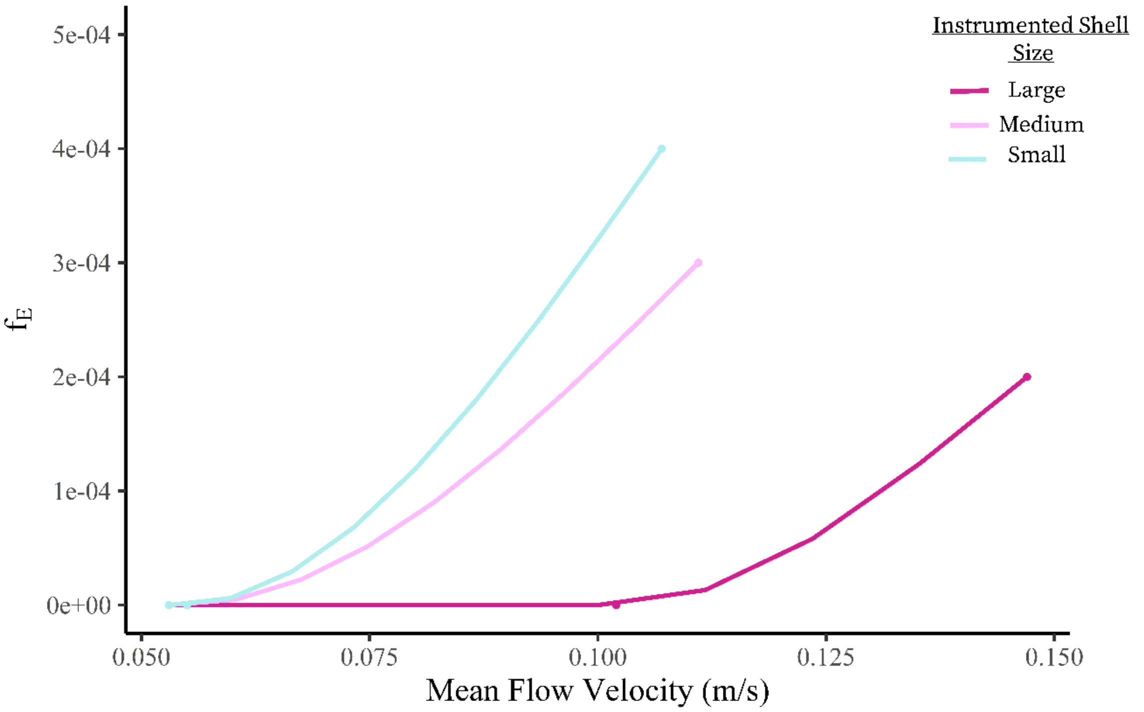

Figure 5.6 Alterations in the frequency of entrainment, f_E, with respected to increasing mean flow velocity (m s^{-1}), across the three sizes of instrumented shell (Large, Medium and Small) positioned horizontally on the bed surface, fully exposed to the flow. Interpolation of the data points was undertaken using cubic Hermite spline to assist visualisation of trends with respect to alterations in f_E for each instrumented shell size

Despite an absence entrainment for the partially buried shells (Table 5.2), analysis of the data would suggest that other metrics could be generated to quantify the stress experienced by instrumented shells when partially buried. For example, increasing variation around mean total acceleration, in response to increasing flowrate, was observed in all three sizes of instrumented shell, across both orientations (Table 5.2). Figure S4 examines this notion further, with the spread of data for each experiment illustrated in violin plots. Here, it is evident that the range of values recorded for total acceleration greatly increases, particularly when comparing the Low and Intermediate experimental flowrates to the High flowrate. The instrumented sensors therefore appear capable of providing evidence of hydraulic stress in the absence of visual examinations of shell movement.

Relevance to practical applications

Partial or full entrainment, twitching and no entrainment were successfully detected using the total acceleration results after defining a threshold value, a result shared in similar studies (Al-Obaidi et al., 2020). For all three instrumented shell sizes, the threshold value for entrainment was set at six standard deviations from the mean total acceleration for the experiment; thus, suggesting a commonality in the extent of variation in total acceleration related to a partial or full entrainment event. Variation in total acceleration readings could, therefore, be utilised to predict the probability of shell entrainment.

Theories concerning the probability of entrainment of individual particles in the riverbed are well established in hydraulic engineering (Valyrakis et al., 2011). These probabilistic approaches suggest that the assessment of the frequency of entrainment of the most exposed particle should be sufficient to identify the risk of onset of scour before an event has occurred. Research by Al-Obaidi et al. (2020) reinforced this notion by demonstrating the use of instrumented particles for predicting the risk of scour initiation to asssess water infrastructure hazards. Despite this development of tools to monitor the occurrence of scour within hydraulic engineering, such practices have rarely been adopted in more ecologically orientated research. Ecological studies concerning habitat suitability from the perspective of substrate stability have often relied on visual categorisations of riverbed composition, correlated with flow metrics such as shear stress to predict sediment stability (Daraio et al., 2010; Morales et al., 2006). However, geomorphological and hydrological hazards, such as river bed destabilisation, often develop at a rate that exceeds the capacity of practitioners to detect and respond with the use of current tools.

Data from this research has shown that the fusion of recordings from the inertial sensors, and the subsequent creation of metrics such as the frequency of entrainment, have the potential to provide performance indicators associated with bed stability and the risk for scour (Al-Obaidi et al., 2020). For this study, the instrumented shells were created using the shells of deceased *M. margaritifera*; consequently, such tools are likely to be well suited to applications in the field concerning the examination of potential entrainment risk in live mussel beds. Given the morphological similarities, instrumented shells could be placed in orientations that mimic the behaviour of live mussels *in-situ* and provide an indication of riverbed stability and the hydraulic stress individuals may be experiencing. However, further work is required to provide evidence to suggest the trends observed for the fully exposed shell in this study are replicable when positioned in other orientations.

The use of instrumented shells is unlikely to be limited to the study of freshwater mussels, but instead could be applied to a wide range of freshwater species, intimately linked to their physical environment: close associations with substratum stability have been shown in many lotic organisms, ranging from algae and aquatic plants (Grabowski & Gurnell, 2016; Matthaei et al., 2003; Smith et al., 2009), to invertebrates (Nakano et al., 2018; Schwendel et al., 2010) and fish (Bey & Sullivan, 2015). The use of the instrumented shells highlighted in this research could, therefore, be useful in the assessment of freshwater habitats; providing a direct, cost effective means of quantitatively examining the stability of the near-bed habitat, which is crucial for the conservation of a plethora of species.

Biological relevance

An evaluation of the biological relevance of these results appears to confirm the long-held understanding that freshwater mussels, such as *M. margaritifera*, may minimise their risk of entrainment by burying into substratum (Allen & Vaughn, 2009; Sansom, 2018). However, the effect of burial on the mitigation of entrainment risk may not be consistent across individuals: observed differences in f_E across the three sizes of instrumented shell when fully exposed, indicates that variation in shell morphology may impact the extent of stress experienced, in response to increasing flow forcing.

Previous studies suggest that interspecific differences in shell morphology may be driven by hydrological conditions, with species adapting their morphology to cope with turbulent flow dynamics, by developing shell characteristics that aid in the inhibition of displacement or the rapid re-positioning to a favourable orientation after dislodgement (Bartsch et al., 2010; Hornbach et al., 2010; Randklev et al., 2019; Watters, 1994). However, variation in shell morphology is also prevalent at the intraspecific level (Preston et al., 2010). Within this study, morphological differences were also shown for individuals from the same population, with measurements of Height, Width and Length not shown to scale linearly with increasing shell size (Table S2). Therefore, phenotypic variation in shell morphology may drive substantial differences in the extent to which individuals and populations can cope with similar levels of hydraulic forcing.

The utilisation of behaviours such as burial to mitigate the risk of entrainment, may be deployed to different extents across individuals, depending on their shell morphology. With regards to the results emanating from this study, it would appear that individuals with similar morphological characteristics to the Small instrumented shell, may be less likely to move in their environment, because movement out of a partially buried position to a more exposed orientation significantly increases their risk of entrainment. Furthermore, such individuals may also bury themselves to a greater extent as it would appear increased exposure presents a heightened risk of entrainment compared to individuals with larger shell sizes. Consequently, the capacity of smaller mussels to utilise movement to respond to alterations in conditions, may be substantially reduced; thus, making such individuals more at risk to unfavourable environmental conditions. This may explain why mussels form dense beds and remain relatively sedentary therein: dense beds (>25 mussels m^{-2}) generate flow environments that decrease the turbulent shear stresses acting to dislodge individuals, and increase food provision through the heightened suspension of microphytobenthos (Sansom et al., 2020; Widdows et al., 2009).

Expanding this study to accommodate greater phenotypic variation in shell morphology, over a broader range of hydraulic conditions with different extents of shell burial, may allow for the disentanglement of these complex interactions. Data from such research could be particularly useful in assisting conservation management in highlighting populations most at risk to entrainment, in addition to identifying whether the morphological characteristics of individuals, designated for reintroduction schemes, are suited to the hydrological environment of the recipient habitats.

5.5.3 Key Findings

The integration of electronic systems in a biologically relevant scale is a complex undertaking, but the potential rewards from such work from an ecological and environmental standpoint are significant. This study is the first known attempt at adapting the use of smart sensors in the assessment of habitat suitability, with results to suggest a broad applicability of such a tool for a multitude of freshwater species. The design features a sensor that is significantly smaller than others seen in comparative research (25mm; Al-Obaidi et al., 2020), and light enough (approx. 6g) to facilitate use at a micro-scale, with a user interface that is easily understood and enables the remote monitoring of riverbed stability and hydraulic stress.

Here, instrumented shells, created by placing inertial sensors within the empty shells of deceased *M.margaritfera*, have enabled the successful direct identification, and potential prediction, of entrainment events through the examination of variability in recordings of total acceleration. With a focus of conservation work in freshwater systems being placed upon restoration and reintroduction schemes, it is clear the instrumented shell could provide a valuable tool for assisting practitioners: through the identification of sites that provide suitable substrate for individual placement, by determining areas at the micro-habitat scale where thresholds for entrainment fall into appropriate levels of safety for newly relocated individuals; and for the monitoring of substrate stability over time to identify the progress of restoration schemes after substantial anthropogenic alterations to the physical environment.

Analysis of variation in total acceleration, beyond generating metrics for predicting entrainment, has provided evidence to suggest further study into this tool could yield methods for accurately predicting more complex flow metrics associated with hydraulic stress: by studying the variation in total acceleration across a broad range of flows, and in association with more detailed analysis of the flow dynamics. Consequently, the instrumented shells could be used to accurately examine near bed flows and provide a means for practitioners to assess complex hydrodynamics remotely, with data that is with easy to access, process and understand. It is therefore clear that the potential of this tool is still to be fully uncovered.

Initial findings presented here are encouraging, with the identification of a tool to predict entrainment risk to freshwater mussels that can assist conservation managers in defining suitable habitat, whilst offering an array of routes for further study.

CHAPTER 6

6 DISCUSSION

This thesis garnered empirical evidence concerning interactions between *M. margaritifera* and the hydrogeomorphological processes that determine habitat suitability and population condition. The purpose of this work was to explore novel methods to inform conservation action regarding this species in Scotland, and across its holarctic range, with findings that can be applied to broader conservation management efforts in freshwater systems across the globe. To achieve this, research centred on three keys areas of investigation. First, the examination of methods to determine the condition of individual mussels, through the analysis of behavioural responses to potentially harmful environmental conditions, coupled with an investigation of physiological indicators of stress. Second, the analysis of *M. margaritifera* responses to reductions in water depth that incur emersion, utilising tracking software to determine how mussels may cope with future episodes of prolonged drought, and the potential role of river management in mitigating the associated impacts. Finally, the assessment of novel methods to determine mussel stress from flow forcing, investigating the role of behaviour in mitigating these stressors, in addition to providing a tool for measuring near bed flow metrics to quantify habitat suitability in benthic regions of lotic systems. Results from this work outlined the importance of habitat characteristics and life history traits in mitigating potential threats to populations of this highly endangered species, with implications for future conservation management practises. Data presented in this study has provided clear paths for future research, with an emphasis on interdisciplinary efforts to provide novel methods to quantify population and habitat condition, which embrace the complexity of environmental characteristics.

6.1 Behavioural Responses of Freshwater Pearl Mussels

Results from this study demonstrated a propensity of *M. margaritifera* to alter their behaviour in response to modifications to environmental characteristics. Principally, *M. margaritifera* evoked responses in the form of alterations to filter functioning, as well as an increase in the presence and extent of horizontal and vertical movements.

Filter functioning facilitates respiratory and reproductive processes in freshwater mussels, and has often been the focus of studies examining behaviour as a biomarker in ecotoxicology research (Gilroy et al., 2017; Hartmann et al., 2016; Lummer et al., 2016; Tuttle-Raycraft et al., 2017) and research investigating the role of freshwater mussel filtering in shaping habitat characteristics (Tran &

Ackerman, 2019; Vanden Byllaardt & Ackerman, 2014; Vaughn et al., 2004). Yet work conducted as part of this thesis represents the first known study to demonstrate variation in filtration activity in response to unfavourable environmental conditions within *M. margaritifera.* Here, behavioural responses to stress exposure were defined in *M. margaritifera*, and another Unionid species, *A. anatina*. Alterations in transition frequency, associated with valve activity, were observed in responses to increases in the concentration of total suspended solids (TSS), as well as emersion. Analogous studies, examining transition frequency in *A. anatina* also demonstrated an increase in the presence and occurrence of this behaviour during periods where mussels were exposed to chemical stressors, in the form of road de-icing salts (Hartmann et al., 2016).

Heightened concentrations of organic and inorganic suspended particles within the water column are known to impact the filter function of freshwater mussels, with studies often highlighting a reduction in clearance rates in response to heightened TSS (Foster-Smith, 1975; Madon et al., 1998; Tuttle-Raycraft et al., 2017). Attempts to determine a threshold in response to increasing concentrations of TSS were unsuccessful in this study, although previous studies have often alluded to a 8 mg L^{-1} threshold at which clearance rates are significantly reduced, compared to controls in which TSS are zero (Foster-Smith, 1975; Gascho Landis et al., 2013; Madon et al., 1998; Tuttle-Raycraft et al., 2017). Studies specifically examining alterations in the filtering capacity of freshwater mussels in response to algal fluxes have reported similar findings across Unionid species, with lowered clearance rates in response to heightened concentrations of algal matter (Ackerman, 1999; Mistry & Ackerman, 2018; Vanden Byllaardt & Ackerman, 2014). When assessing the reasons for this perceived response, authors have often alluded to an increase in particle processing time: mussels have been shown to selectively partition resources obtained through filter functioning, with greater suspended particle concentrations increasing the processing time required within the filtering structures (Mistry & Ackerman, 2018). Additionally, increases in suspended solids are believed to increase the time necessary for individuals to clean the gill surfaces (Foster-Smith, 1975).

Previous studies have demonstrated the role of heightened suspended matter on the filter functioning of freshwater mussels; however, there has been limited study into the associated physiological consequences. Furthermore, there are no known studies that have examined the physiological consequences of stress exposure in *M. margaritifera*. Results presented in this thesis illustrated the correlation between a heightened occurrence of behaviours reflecting filter functioning and significant modifications to the metabolic functioning of individuals. The processes of particle selection, and subsequent rejection of unwanted particles from the inhalant siphon as pseudofaeces, are likely to increase with a heightened concentration of material in the water, causing mussels to eject more particles and feed less (Foster-Smith, 1975; Widdows et al., 1979). This is likely to be energetically demanding and could reduce condition of individuals overtime (Lummer et al., 2016; Tuttle-Raycraft, 2018; Vaughn et al., 2008). Furthermore, reduced clearance rates, resulting from high TSS, could impede reproductive success in populations (Gascho Landis et al., 2013; Gascho Landis & Stoeckel, 2016), due to a reduction in the chances of females encountering sperm during filter feeding, and an increase in pseudofaeces production causing the binding of sperm in mucus and its subsequent ejection before fertilisation (Gascho Landis et al., 2013).

Although no known studies have examined the effect of emersion on *M. margaritifera,* the removal of an appropriate respiratory medium for the mussel's specialised structures prevents filtration activity from fulfilling an individual's metabolic requirements. Therefore, alterations in metabolic functioning are believed to be reflective of attempts to compensate for alterations to osmoregulation, nutrient turnover and respiratory processes (Hartmann et al., 2016; Lorenz & Pusch, 2013; Shick et al., 1986). Nevertheless, the study of freshwater mussel responses to emersion, presented in Chapter Three of this thesis, comprised an experimental design that limited mussel movement; hence, prohibiting the expression of the full suite of behavioural responses potentially available to individuals, for the purpose of mitigating the negative effects of prolonged aerial exposure.

Over the past two decades, a growing body of literature has documented the presence and utilisation of horizontal and vertical movements evoked by Unionid mussels to alterations in their environment, with evidence to suggest that mussels display this behaviour for reproductive purposes (Amyot & Downing, 1997); in response to alterations in the hydrologic environment (Bartsch et al., 2010; Block et al., 2013; Clements, 2015; French & Ackerman, 2014; Hamstead et al., 2019; Johnson & Brown, 2000); and to avoid zebra mussel infestation (Burlakova & Karatayev, 2007). Research presented in Chapters Four and Five of this thesis built on this foundational knowledge by examining vertical and horizontal movements in *M. margaritifera*, in response to alterations in the hydrologic environment. Results presented in Chapter Four, demonstrate the utilisation of vertical and horizontal movements within *M. margaritifera* in response to reductions in the water depth, which incur emersion. Here, mussels were shown to track receding water levels and subsequently avoid emersion, with some mussels found to move up to three metres in length over 24-hours. Findings from similar studies, suggest horizontal movements in response to receding water levels are common across Unionid species (Gough et al., 2012; Negishi et al., 2011; Newton et al., 2015; Schwalb & Pusch, 2007). It is unlikely that horizontal movements are solely utilised for the purposes of tracking receding water levels, though no known studies have examined the presence of horizontal movement in *M. margaritifera* with respect to other environment stimuli.

In contrast to the dearth of information related to horizontal movements, the study of burrowing behaviour, facilitated through vertical movements, in unionid species has received substantial attention within the literature (Allen & Vaughn, 2009; Block et al., 2013; French & Ackerman, 2014; Hyvärinen et al., 2021; Lymbery et al., 2020; Saarinen & Taskinen, 2003; Vaughn & Hakenkamp, 2001). Vertical movements by unionid mussels have often been attributed to seasonal variation in conditions. For example, the surfacing of mussels from the substratum has been shown to increase in accordance with increases in temperature and day length, with suggestions that these alterations in locomotion correspond to reproduction and the acquisition of food resources (Amyot & Downing, 1997; Block et al., 2013; Negishi et al., 2011). In Chapter Four of this thesis, vertical movements were often found to be displayed by individuals that successfully tracked receding water levels, which resulted in an avoidance of prolonged aerial exposure. According to criteria outlined by Gough et al. (2012), *M. margaritifera* responses to drawdown are synonymous with those of a semi-tolerant species to drought conditions. Burrowing has been observed in response to emersion in previous studies, in addition to conditions which present shallow water depth subjected to high temperatures, and may therefore function as a mechanism to seek thermal refuge and moisture within sediments (Gagnon et al., 2004; Golladay et al., 2004; Newton et al., 2015). Elevated temperatures are known to alter the metabolic rates of freshwater mussels, culminating in a reduction in available energy for key biological processes such as reproduction and growth (Ganser et al., 2015). However, burrowing behaviour has also been postulated as an adaptation to mitigate flow forcing on the mussels, which may induce entrainment, with flow velocity found to be the dominant factor driving the extent of vertical movement in three unionid species (Schwalb & Pusch, 2007). Results presented in Chapter Five corroborate this theory, with the partial burial (50% surface area) of an *M. margaritifera* shell conferring a significant reduction to entrainment risk, compared to fully exposed shells, across three size classes. Here, evidence suggests vertical movements mitigate entrainment risk; however, further research is required to clarify theories concerning other proximate cues that drive vertical movement behaviours.

In addition to identifying behavioural responses in the form of alterations to filter functioning and locomotion, results presented in this thesis also revealed substantial variation in the presence and extent to which these responses were observed across individuals, populations and species. These perceived differences are likely driven by an amalgamation of phenotypic plasticity, genetic determination and habitat characteristics.

Phenotypic plasticity is thought to be crucial in ensuring an organism's survival when exposed to environmental perturbations (Piersma & Drent, 2003). This notion is particularly relevant to lotic systems, which are inherently dynamic across temporal and spatial scales. Research examining morphological, physiological and behavioural traits in freshwater mussels indicates an inherent phenotypic plasticity that facilitates the occupation of different environmental conditions, with physiology, growth, and shell morphology, in addition to differences in feeding structure, shown to alter with respect to particular environmental factors (Galbraith et al., 2009; Payne et al., 1995; Silverman et al., 1997; Zieritz et al., 2010).

Within marine mussels, differences in clearance rates among conspecifics are attributed to gill structure, siphon positioning and gill to palp size ratios, which are believed to vary depending on the hydrodynamics in the habitat (Dutertre et al., 2009; Troost et al., 2009). Recent studies with freshwater species would indicate a similar pattern, with local adaptations in gill structure representative of differences in hydrodynamic conditions (Vanden Byllaardt & Ackerman, 2014), with similar clearance rates observed in different species inhabiting the same hydrodynamic habitat (Silverman et al., 1997). For example, Tuttle-Raycraft and Ackerman, (2019) demonstrated the presence of denser gill surfaces and larger palp structures within a population of *Lampsilis siliquoidea* from a turbid river, compared to conspecifics from a more pristine environment. These morphological characteristics are believed to be plastic in response to the prevailing environmental conditions (Ouellette-Plante et al., 2017) with increasing size of labial palps believed to elicit more efficient particle sorting and pseudofaeces production (Payne et al., 1995; Widdows et al., 1979). Data presented in Chapter Three of this thesis provides evidence to reaffirm these observations. Here, *M. margaritifera* collected from a more pristine environment experienced greater physiological stress, and displayed reduced filter functioning during exposure to TSS, compared to individuals inhabiting a system dominated by fine sediments, prone to intermittent episodes of high turbidity due to the resuspension of sediments during high flow conditions (Ferré et al., 2005).

Studies examining the extent to which phenotypic plasticity develops in unionid species, and consequently governs behavioural responses and physiological function, found that clearance rates of juveniles of the same species, from the same source population, reared in different conditions, respond differently to the same stressor (Tuttle-Raycraft & Ackerman, 2020). Therefore, differences in mussel filter functioning comprise an element of phenotypic variation. Phenotypic variation has also been shown to drive alterations in shell morphology, with alterations in shell sculpture determined by modifications to temperature and flow regimes (Peyer et al., 2010; Preston et al., 2010; Zieritz et al., 2010). However, no known study has examined phenotypic variation in relation to differences in the locomotion of freshwater mussels. Instead, previous studies have focused on differences across species. For example, Gough et al. (2012) demonstrated significant differences in the behaviour of three different species of freshwater mussels, inhabiting the same system, to reductions in water depth. Differences in the behavioural strategies were aligned with variation in corresponding species tolerance to drought conditions, highlighting three different strategies to cope with drought: burrowing (tolerant), tracking (intolerant) and track then burrow (semi-tolerant). Similar studies have since provided evidence to substantiate this narrative (Lymbery et al., 2020; Newton et al., 2015). Research presented in Chapter Four highlighted significant differences in response at the intraspecific level, with variation in horizontal and vertical movements shown across populations of *M. margaritifera* inhabiting systems governed by markedly different flow regimes. Thus, presenting the case that habitat conditions may drive phenotypic variation in mussel responses to alterations in flow: *M. margaritifera* inhabiting the regulated system experience drawdown events within their habitat and have, therefore, developed adaptative behaviours to cope. However, in the absence of data concerning the genetic variation within and between the corresponding populations, the drivers of this response are difficult to extricate.

Limits to the extent that phenotypic plasticity governs freshwater mussel responses to environmental perturbations are likely to exist, with temporal and spatial alterations in habitat conditions, in addition to genetic variation, influencing the response of organisms.

Despite plasticity in the feeding structures evoking a greater resilience in freshwater mussels to episodes of heightened TSS, an accumulation of fine sediment will ultimately smother juvenile mussels and inhibit their capacity to burrow, eventually leading to mortality and a corresponding lack of recruitment in the population (Quinlan et al., 2015; Tarr, 2008; Tuttle-Raycraft & Ackerman, 2019). Increases in flow velocity may also elicit a reduction in clearance rates via partial or full valve closure caused by interference with the bivalve pump, or instabilities due to lift and drag forces on the shell and siphons (Ackerman, 1999; Jorgensen et al., 1988). Furthermore, the impact of high concentrations of TSS on the filtering capacity of freshwater mussels is likely to vary depending on the composition of suspended matter, with particle quality, from a nutritional standpoint, further modulating filter feeding functioning within freshwater mussels (Tuttle-Raycraft & Ackerman, 2018).

Alterations in habitat conditions are also likely to limit the extent to which horizontal and vertical movements prevent substantial physiological stress from environmental perturbations. For example, the gradient of the riverbed has been suggested as stimulus for horizontal movement (Newton et al., 2015). Therefore, in habitats where the gradient is relatively shallow, this stimulus for movement is likely to be diminished. Consequently, mussels positioned on shallow slopes may be less likely to move in response to a recession in the water level. Additionally, the composition and structure of riverbed substratum is likely to dictate the extent to which mussels can move, either by presenting obstacles to movement, or displaying an absence of suitable medium in which to move: armoured bed layers often lack the fine gravels necessary to permit vertical movements by adult and juvenile freshwater mussels (Addy et al., 2012). Results presented in Chapter Three appear to validate this theory. Here, the directionality of movement patterns in *M. margaritifera* appeared to be impacted by the gradient of the riverbed and associated near-bed flow dynamics, in addition to the rate at which water levels receded.

Recent studies have demonstrated substantial genetic diversity within and across populations of *M. margaritifera* (Geist et al., 2010, 2018; Stoeckle et al., 2017; Zanatta et al., 2018); however, no known studies have sought to investigate the role of genetic variation in mussel responses to alterations in habitat conditions. Differences among individual growth rates at the intraspecific level have been attributed to an underlying genetic variation (Larson et al., 2014; Meyer & Manahan, 2010), with shell size and survival believed to be correlated (Meador et al., 2011; Villella et al., 2004). Size-dependent effects have been demonstrated with regards to mussel clearance rates, with a non-linear effect of size on clearance rates often found within freshwater and marine bivalves (Riisgard, 2001; Vanden Byllaardt & Ackerman, 2014). Results presented in Chapter Three revealed similar findings, with significant differences in metabolic rate associated with the size of individuals. Findings from this study revealed the metabolic scope of individuals is instrumental in determining the extent to which individuals can undertake behavioural responses to mitigate the negative effects incurred during exposure to a stressor. Thus, smaller individuals, with a reduced metabolic scope, are perhaps less capable of responding to environmental perturbations. Data presented in Chapters Four and Five, further highlight the role of shell size in mitigating stress incurred by alterations in flow regime, with larger individuals shown to move further in response to receding water levels, and experience a reduced risk of entrainment in response to greater flow forcing. Despite suggestions that genetics determine unionid growth, reasons for variation with respect to size remain largely understudied, with the ramifications of high variability in growth rarely discussed with respect to species conservation (Haag & Williams, 2014). Although, in instances where intrinsic variation in growth is high, the role of external conditions and genetic variation in determining individual growth and the potential impacts of stressors is hard to disentangle (Larson et al., 2014).

6.2 Conservation Implications

Results presented in this thesis, provide evidence of *M. margaritifera* responses to environmental stressors associated with hydrogeomorphological processes within aquatic systems. Furthermore, new methodologies outlined in this thesis, provide a means to non-invasively determine stress within freshwater mussels and examine near-bed flow metrics. The following section will assess how the findings from this thesis could ultimately affect the conservation management of *M. margaritifera,* and freshwater systems more generally.

Chapter Four of this thesis presented work representing the first known study examining the response of *M. margaritifera* to drawdown events, which incur emersion. Results from this work suggest mussels can detect alterations in the hydrologic environment and respond through horizontal and vertical movements. Empirical evidence from this study will be disseminated to the relevant industrial (SSE) and governmental (NatureScot and SEPA) stakeholders, with the hope of informing new legislation regarding the management of flow regimes in regulated systems across Scotland. It is anticipated the results of this work will be particularly applicable to guiding future management and intervention practises concerning the increasing frequency and magnitude of drought events in Scotland. It is imperative that the dissemination of potential changes to the management of regulated systems transcends beyond corporations involved in large-scale dam projects, and towards private small-scale hydroelectric dam operators; a sector that is seeing a marked expansion, and one that has thus far experienced limited governance concerning the associated ecological impacts.

Within Scotland, the last decade has witnessed a trend of more frequent and severe drought events, prompting river regulators and conservation managers to pay closer attention towards the potential implications of drought scenarios brought on by future climate change. Kirkpatrick et al. (2021) revealed an expected increase in drought events within Scotland as a result of future climate change, with extreme drought events likely to occur every three years, constituting an average increase of 11 extra drought months per decade. Moreover, a report by Cosgrove et al. (2021) revealed evidence to suggest prolonged episodes of high temperatures, low rainfall and subsequent low river discharge present significant threats to the viability of *M. margaritifera* populations across Scotland. The report highlighted the role of a recent drought in 2018, which evoked substantial mortalities within *M. margaritifera* populations across six different systems, a finding echoed in reports of population loss due to severe drought in the Iberian Peninsula (Sousa et al., 2018). However, in the absence of regular systemic monitoring of watercourses, many of the findings in this report remain speculative and may only hint at the overall damage incurred (Cosgrove et al., 2021).

Findings presented in this thesis suggest that, in regulated watercourses, managed drawdowns may assist in mitigating mortalities in *M. margaritifera* populations, resulting from episodes of drought. In accordance with current water management regimes, compensation flows in many of Scotland's regulated rivers are unlikely to prevent complete desiccation of the corresponding river reaches during extreme drought events. There is, therefore, a need to re-assess current management practises towards increasing the resilience of managed river systems to future climate change. Examination of anecdotal evidence provided by NatureScot, concerning the impact of prolonged droughts on regulated systems, revealed that in the past decade, episodes of drought have resulted in some systems being several weeks away from running out of storage water (I Sime 2021, Freshwater & Wetlands Advice Manager, NatureScot, personal communication, 3 March). A reluctance of conservation management to reduce compensatory flows is grounded in the fear that emersion of mussel beds will result in mortality of all exposed individuals. However, findings presented here suggest *M. margaritifera* display responses to drawdown redolent of a semi-tolerant species (Gough et al., 2012). In the field trial of the drawdown study (Chapter Four), a managed drawdown of 30 mm h^{-1} was sufficient to elicit the horizontal movement of mussels and subsequent avoidance of prolonged aerial exposure in 80% of the sample population. However, the proportion of successful cases of emersion avoidance within a population is likely to be context driven, dependent on the habitat conditions and the population.

To examine the risk of mortality associated with managed drawdowns within a river reach, it is recommended that flume-based studies should be undertaken with a subset of the corresponding *M. margaritifera* population. Here, the response of individuals to a recession in the water levels should be tested, with potential variations in riverbed gradient, mussel arrangement and drawdown rate explored to provide greater context. According to results presented in Chapter Four of this thesis, behavioural responses which indicate higher rates of successful emersion avoidance in *M. margaritifera* populations include greater extents of horizontal movement, compared to vertical movement; locomotion activity that is directional (i.e. moving down a gradient rather than laterally across); and a high proportion of mussels that display initial movements whilst fully submerged, compared to those that remain stationary until partially or fully emersed. Using this information, surveys of mussel aggregations could determine whether the habitat conditions are sufficient to facilitate the tracking of receding water levels by individuals. For example, in circumstances where the gradient is too shallow, the slope of the riverbed may be insufficient to provide a stimulus for movement. Furthermore, a shallow gradient may exacerbate successful emersion avoidance by requiring mussels to move distances that exceed their physiological limits. It is suggested that the distance between the existing water's edge and the water's edge following drawdown should not exceed 50cm in a 24-hour period, with smaller incremental changes of less than 20cm likely to evoke more successful responses in mussels. Additionally, risk assessments should consider the presence and extent of obstacles in the riverbed that may impede movement. Movement is likely to be limited in densely packed mussel beds, or circumstances where the riverbed is composed of larger boulders with limited gravel and fine sediment to traverse across. In cases where the habitat conditions are deemed unsuitable to facilitate successful tracking of the water level by horizontal movements, mussels may have to be translocated by hand. Evidence presented in this thesis suggests zero mortality is expected in cases where emersion is negated beyond 24 hours.

Previous studies have frequently highlighted the need to operate regulated rivers in accordance with more natural flow regimes (Addy et al., 2012; Sousa et al., 2020) to assist in the restoration and maintenance of suitable habitat for *M. margaritifera* populations. Alterations in dam operation, which provide flow regimes analogous to the natural variability of the corresponding system, may also encourage greater resilience to severe shifts in the hydrologic regime. Findings presented in this thesis highlighted differences in behaviour across *M. margaritifera* populations to the same stressor, suggesting habitat conditions may drive individual responses. Previous studies have highlighted the role of habitat characteristics in governing phenotypic plasticity, with freshwater mussels shown to alter their morphology in response to modifications to their environment, such as heightened concentrations of TSS (Preston et al., 2010; Tuttle-Raycraft & Ackerman, 2020; Zieritz et al., 2010). Thus, a pristine environment with constant flow conditions may suppress the development of behavioural phenotypic plasticity in response to environmental alterations, ultimately inciting a greater vulnerability in the corresponding population to future perturbations brought on by climate change.

An example of this can be found in the River Kerry, Scotland, where the maintenance of bank-to-bank flows has augmented the *M. margaritifera* population. Here, mussels have colonised areas where they would be unlikely to occur under more natural flow regimes, with dense mussel beds forming in shallow gradients with low water depths. From a conservation perspective, the River Kerry has one of the best mussel populations in Scotland, designated as a Special Area of Conservation (cSAC) under the European Habitats Directive (European Council Directive 92/43/ EEC). As a result, conservation management has defaulted to adopting a laissez-faire approach with regards to current flow regime practises (Birkeland, 2003). However, the population remains vulnerable to significant alterations in flow (Hastie et al., 2001), with current distributional patterns expected to inhibit horizontal movements. A return to flow regimes that encompass the natural variability could assist in evoking alterations to *M. margaritifera* distribution, by providing conditions that necessitate movement down the riverbed to areas that provide a buffer against low flow regimes triggered by drought.

Greater variability in the hydrogeomorphological environment may also assist in evoking resilience in juveniles, with implications for the success of reintroduction schemes. The propagation and subsequent release of hatchery-raised mussels for the purpose of conducting species reintroductions into habitats that have witnessed extirpation, or to augment existing populations, has become a frequently relied upon approach within conservation management (Haag & Williams, 2014; Hoftyzer et al., 2008). Yet captive rearing techniques for *M. margaritifera* success have seen limited success. Variation in rearing conditions for juvenile mussels may determine their morphological and physiological characteristics, and thus define their resilience to certain environmental stressors (Hoftyzer et al., 2008; Tuttle-Raycraft & Ackerman, 2020; Zieritz et al., 2010). Therefore, to enhance the success of reintroductions, rearing conditions should aim to replicate the characteristics of the site where mussels are to be reintroduced, eliciting the development of phenotypic traits that garner greatest resilience to the prevailing environment within the corresponding habitat. The improved success of recent rearing techniques using silos in sites designated for reintroductions is reflective of this notion, with juveniles developing phenotypic traits in accordance with the natural habitat characteristics. For example, juvenile *M. margaritifera* housed in silos have been found to produce significantly more byssus threads, in response to the high-flow regimes in the environment, compared to those raised in artificial flumes *ex-situ* (L Lavictoire 2021, Mussel Reintroductions Research Officer & Interim Head of Science, FBA, personal communication, 17 March). Despite this, it is unclear how long phenotypic traits remain plastic and whether this is developmental or flexible, with adult mussels perhaps displaying a lowered propensity to adapt to alterations in the environment.

Long-term biomonitoring, or the tracking of biological processes, in response to the prevailing habitat conditions may serve to overcome uncertainty regarding the health of *M. margaritifera* populations, and could assist in highlighting when mussels are unable to adapt and cope with particular environmental characteristics. Research by Roznere et al. (2017) highlighted the utility of such techniques to quantify stress in *Amblema plicata* in response to relocation and captivity. The results of the study suggest the analysis of metabolites provides a framework to guide improvements of conditions within hatcheries, whilst also aiding the evaluation of mussel condition following restoration and reintroduction efforts. Findings outlined in Chapter Three of this thesis, represent the first known study to examine the use of biomonitoring tools with *M. margaritifera*. This work highlighted the propensity of remote, non-invasive techniques, which examine valve activity linked to alterations in metabolic functioning, to determine mussel stress in response to exposure to environmental stressors. The methodology formulated from this study could be applied to a subsample of any *M. margaritifera* population to determine the thresholds at which mussels become stressed in response to isolated stressors; thus, permitting a context-driven evaluation of habitat requirements for populations. The development of animal-attached remote sensing technologies such as Hall sensors (Hartmann et al., 2016; Lummer et al., 2016; Nagai et al., 2006; Robson et al., 2012), could facilitate the transition of this tool into a field environment, permitting long-term monitoring of populations to aid in the quantification of stress in mussels following translocation, reintroduction and restoration schemes. These techniques could compliment practises that rely on more invasive, single point metabolomic analyses. Although metabolomic studies have yet to be undertaken with *M. margaritifera,* funding has been acquired to conduct multiple stressors experiments, examining metabolic response of juvenile mussels in captivity to high turbidity and temperature (L Lavictoire 2021, personal communication, 17 March). Thus, it would appear biomonitoring techniques are beginning to gain traction as a potential tool in *M. margaritifera* conservation.

In addition to underlining the utility of biomonitoring techniques to aid the conservation of *M. margaritifera*, research as part of this thesis sought to define new methods to quantify biologically relevant near-bed flow characteristics. The results of this study, presented in Chapter Five, identified a low-cost accessible tool to directly predict the entrainment risk to mussels through the creation of an Instrumented Shell. Previous studies examining near-bed flows relevant to the habitat suitability of *M. margaritifera* have been limited (Moorkens & Killeen, 2014), with no known studies having quantified the interaction between *M. margaritifera* and hydrodynamics in a lab setting. As such, work presented in this thesis represents the first known study to quantify stress resulting from flow forcing in *M. margaritifera*. The instrumented shell design presented in this thesis is adaptable to different shell sizes and morphologies, and can be positioned to mimic the orientation of live mussels. Therefore, data emanating from the housed inertial sensors present biologically relevant information concerning the extent to which individuals experience near-bed flow regimes, which can be applied to a range of bivalve species. Furthermore, the examination of near-bed flow metrics using this tool, provides data that is relevant to other aquatic species, the life-histories of which are intertwined with the physical habitat of river systems. For example, the host salmonid fish for *M. margaritifera*, which require stable gravel substrates for spawning (Addy et al., 2012; Armstrong et al., 2003; Gilvear et al., 2002; Kondolf & Wolman, 1993).

Initial work with the instrumented shells, demonstrated their propensity to successfully detect and predict entrainment events. Thus, instrumented shells provide data relevant to the assessment of suitable habitat for *M. margaritifera,* with consideration towards the stability of the substrate, often highlighted as a crucial determinant towards *M. margaritifera* persistence (Geist & Auerswald, 2007; Goodding et al., 2019; Hastie et al., 2000; Johnson & Brown, 2000; Scheder et al., 2015; Skinner et al., 2003; Zigler et al., 2008). This tool could be used to aid translocation and reintroduction efforts, by identifying patches of riverbed substratum which are at low risk of scouring and, hence, provide suitable habitat for both adult and juvenile mussels. Another application of this tool pertains to the placement of instrumented shells in riverbed substratum next to live mussels, to ascertain the near-bed flow characteristics suitable for the persistence of M. *margaritifera* in a given system.

Instrumented shells may assist in determining the success of restoration schemes following modifications to the structure and composition of the riverbed, with data to indicate the suitability of newly formed habitats to *M. margaritifera*. Research by Pedersen et al. (2014), examining the success of river restoration schemes, highlighted concerns that water managers often risk restoring habitats to conditions that are unsustainable over longer temporal scales, because they neglect the physical laws governing interactions between flow and geomorphology; a sentiment echoed in analogous studies (Palmer et al., 2010; Vaughan et al., 2009). It is envisaged that instrumented shells could bridge this gap in the knowledge, by providing a tool to effectively understand and monitor hydrogeomorphological processes, with quantitative assessments of flow forcing and substrate stability that can easily be interpreted and devoid of user bias. Nevertheless, further study to evaluate the accuracy to which sensor readings quantify more complex flow metrics is required. Additionally, the utility of this tool in a field environment is yet to be studied.

6.3 Limitations of Current Study and Directions for Future Research

The aim of this research was to address knowledge gaps concerning the response of *M. margaritifera* to hydrogeomorphological processes, and provide methods to monitor these interactions towards aiding current conservation efforts. However, in many cases this thesis has provided a foundational knowledge on which to build, with further work required to discern if the results of this research are applicable across different habitats, populations, and species. With this in mind, the following section will outline recommendations for further study.

An examination of the results from the stressor study, presented in Chapter Three, suggest non-invasive biomonitoring of mussel filtration behaviour may provide an effective means to determine stress in unionid species. However, there were areas of the methodology that require refinement. For example, mussel handling should be reduced further, particularly during the stress exposure phase, with modifications to the experimental design required to avoid manual removal of mussels from the metabolic chambers for the aerial exposure stressor. Moreover, the use of Hall sensors is recommended, following an initial trial to gauge the extent to which individuals modify valve activity, to alleviate any user bias in determining Transition Frequency. In the absence of such sensors, the use of fluorescent markers in the shells is recommended to aid in the visual assessment of changes to the valve gape, with the use of open-source tracking software such as MJTrack offering a means to automate the process. With the aforementioned modifications, this methodology is capable of examining various different stressors associated with hydrogeomorphology, but should focus on those associated with filter functioning, with behavioural responses involving locomotion restricted.

Future research, based on work conducted in chapter three, should look to replicate this study with populations from different systems and align this with studies that examine the genetic structure of the respective populations. Furthermore, research should align any laboratory studies with surveys in the natural habitat where sample populations are taken, to understand the environmental conditions that mussels are likely to experience. By combining these two aspects of research, studies can begin to untangle the role of habitat conditions in shaping the tolerance of mussel populations to environmental parameters, whilst also providing data concerning the role of habitat, and the genetic background of populations, in driving the physiological development of mussels. To accompany this work, research should focus on a broader use of stressors that embody a more holistic interpretation of the environmental conditions populations are likely to encounter in their habitat. For example, the addition of road salts, heavy metals, nitrates and phosphates, as well as modifications to temperature regime, flow velocity and the composition of TSS (more organic vs more inorganic), would cover many of the key environmental stressors highlighted in the literature, cited as potential determinants of population condition in *M. margaritifera*. Furthermore, it is recommended that measurements from the field, pertaining to the environmental parameters to be tested, are used to foreground the experimental conditions to be compared across multiple trials, ensuring biological relevance to findings. Data from this work could provide quantitative validation of the thresholds in mussel stress regarding environmental characteristics, unique to populations. This data would provide a more thorough context-dependent framework for the conservation of certain populations and systems to be used by conservation management. Furthermore, the development of technology akin to Hall sensors, which could be operated remotely, may present opportunities to track these behavioural responses in a field environment. If coupled with the recording of environmental parameters such as TSS, phosphorous concentrations and temperature, then biomonitoring of mussel populations could provide detailed information concerning the status of populations over time and in response to alterations in the habitat conditions.

Results from the drawdown study demonstrated differences in the response of individuals to receding water levels across populations of *M. margaritifera*, suggesting a potential role of habitat characteristics in determining mussel response. With regards to refinement of the methodology, it is recommended that future flume studies undertake an extensive analysis of the flow structures across the artificial riverbed. The flume utilised in this study was an inadequate length to allow for a test section larger than 1.4m in length, with the structure of the flume preventing the development of a steady flow, devoid of significant turbulent structures, further upstream. It is likely that the hydrodynamic environment elicits a significant driver of mussel movement, and therefore one must ensure the flow forces experienced by mussels are not significantly different. If this cannot be achieved then variation in the flow experienced at an individual level must be adequately accounted for during data analysis. Here, limitations with test-section size prevented experiments using more than 10 individuals at a time, hindering attempts to undertake a more thorough assessment of the mussel arrangements in determining individual response, which is crucial in providing biologically relevant findings. For example, mussel arrangements were limited to a single line of individuals, perpendicular to the flow, whereas mussels in their natural habitat are usually found in more densely packed spatial arrangements that require mussels to traverse around conspecifics to reach lower regions of the riverbed. Additionally, experiments presented in chapter three are conducted on largely homogenous gravel substrates; thus, further limiting study into the role of obstacles in defining mussel movement.

Previous work examining phenotypic plasticity in unionids, has highlighted how concentrations of TSS can determine variation in the morphology of filter feeding organs within juveniles (Tuttle-Raycraft & Ackerman, 2020). Thus, it would be interesting to replicate these studies with alterations in flow regime to examine if a similar divergence in phenotypic traits develops within juveniles, and whether this is possible to elicit in adult mussels as well. For example, samples of juvenile and adult mussels from the same population, presenting similar genetic histories, could be placed in flume systems that differ in the frequency to which significant alterations in flow depth are enacted. This could be undertaken over several months, with flume studies then conducted to discern if different flow regimes have facilitated a shift in the response of individuals to drawdowns, and whether these differences are more prevalent at different life stages of *M. margaritifera*. Furthermore, an expansion of the drawdown study with populations from different regulated systems, which vary in their operational flow regimes, would assist in defining flow practises that generate greater resilience within populations to alterations in flow. For example, within Scotland, analysis of variation in mussel responses between populations inhabiting the River Kerry, operated with negligible flow alterations and constant wetted widths, and the River Morriston, where wetted widths vary significantly and alterations to flow regime are more frequent, would provide an excellent comparison. The three drawdown rates used in this study appeared adequate to identify differences within and between populations, although drawdown rates greater than 50 mm h^{-1}, and lower than 15 mm h^{-1} may be useful to further examine thresholds in response. Modifications to the riverbed slope would also tease apart any notion concerning its role in providing a stimulus for movement, but this should be accompanied by analysis of the near-bed flow regimes to understand the interactions between flow characteristics and bed structure. Additionally, the drawdown study revealed no significant role of mussel arrangement in determining the successful avoidance of emersion; however, mussels were always arranged in a vertical line. Therefore, studies examining differences in response in circumstances where initial arrangements are closely aligned to conditions found in mussel beds, whereby individuals are impeded in horizontal movement by conspecifics located down-gradient, would provide valuable information concerning the role of mussel arrangements in dictating behavioural responses. Finally, as with the stress exposure study, any research concerning perceived phenotypic variation across populations, should aim to incorporate the examination of genetic variation as well.

The final area of study presented in this thesis pertains to the use of inertial sensors to quantify biologically relevant near-bed flow forcing on *M. margaritifera* shells. Results presented in Chapter Five provide a framework to further test the Instrumented Shell as a potential tool to directly examine near-bed flow metrics and identify suitable habitat for benthic dwelling species, such as *M. margaritifera*. Here, examination of the data highlighted variation in total acceleration as a potential route to determine more complex flow metrics, beyond Frequency of Entrainment, f_E. This work deviates from similar research concerning smart sensors in the study of riverbed transport, by housing the sensors within an asymmetrically shaped particle. In comparison, similar studies have focused on the use of smart sensors housed within spheres (AlObaidi et al., 2020; Maniatis et al., 2020). Placement of the smart sensors within asymmetrical particles presents novel challenges concerning the flow dynamics around the shell: research by Wu, Constantinescu and Zeng (2020) revealed stronger upwelling and downwelling motion, in addition to asymmetric flow patterns around the shell, which increase in intensity with increasing mean flow velocity, compared to circular cylinders of same height and width. To overcome these asymmetrical flow patterns, research presented in this thesis focused on examination of total acceleration along the x, y and z axis. This enabled a consistent and reliable framework to gauge entrainment risk, across different sizes and orientations of the instrumented shell.

Future work should aim to analyse the correlation between recordings in total acceleration, expanding on the foundation provided here, with a more in-depth examination of measurements of near-bed flow, in addition to metrics such as Shear Stress and Froude number; thus, aligning with recent explorations of interactions between unionid species and complex hydrodynamics (Kumar et al., 2019; Mistry & Ackerman, 2018; Sansom et al., 2020). Such work should be undertaken in a flume environment initially, using a wide range of flow regimes, in a set-up analogous to that presented in chapter five. Field testing of the instrumented shell is also required. It is recommended that field testing should be undertaken in a regulated river, permitting controlled modifications in flow regime that can be maintained throughout the duration of trials. To do so, it is suggested that pockets, similar to those created for the lab experiments, are created to provide a known substrate on which to place the instrumented shells; thus, alleviating the potential alterations in bed stability which factor into the shell movement. Transects should be drawn up across the wetted width of the designated river and bathymetry assessments undertaken along the transect lines. At sites along the transect that present markedly different flow depths, the pockets should be placed. Field capable acoustic doppler velocimeters (ADV) should be used to record the flow profiles above the pocket, before the instrumented shells is placed in the pocket and left to record 10-minute intervals, with a minimum of three repeats. Analysis of this work should focus on aligning the ADV recordings and sensor data, along with data from the laboratory experiments, to allow for a cross examination of sensor readings, and a determination of sensor accuracy. If successful, further study into the utility of the inertial sensors when housed in shells from different populations of *M. margaritifera,* representing variation in morphometrics, would facilitate exploration of the role of shell sculpture in determining how mussels experience flow forcing. When combined with a genetic analysis of sample populations, one could determine the extent to which hydrodynamic conditions in the habitat govern the morphological development of mussels. This data would be helpful for rearing techniques, where juveniles may require certain hydrodynamic conditions to be present to ensure they develop shell sculpture and adaptive behaviours that are best suited to the sites they are due to be reintroduced into. If proven to be successful with *M. margaritifera* shells, trials with inertial sensors housed in other bivalve species could be undertaken to provide a global applicability of this tool, whilst further research into the application of inertial sensors could yield methods to instrument live mussels, and enable long-term data longing beyond the one-hour capacity of sensors used here. Field-verified instrumented shells could be used to assist the tracking of changes in the hydrodynamic habitat over time, following river restoration schemes, and allow for practitioners to quantify when habitats become suitable for reintroductions. Finally, instrumented shell could be deployed to map changes in near-bed flow conditions downstream and upstream of impoundments to understand the extent to which river regulation practises impact habitat suitability for benthic-dwelling species.

6.4 Conclusions

Empirical evidence presented in this thesis has addressed knowledge gaps concerning interactions between *M. margaritifera* and hydrogeomorphological processes. Here, justification for a review of current conservation management practises is presented towards the adoption of a context-driven approach to achieving sustainable, functional populations, centred on the response of the organism. In accordance with theory proposed by Geist (2010), this research utilised the role of *M. margaritifera* as an indicator, flagship, keystone and umbrella species to develop approaches which aid the conservation of corresponding populations, in addition to the wider aquatic environment. Here, the role of *M. margaritifera* as an indicator species was explored as a basis to generate biomonitoring techniques, which may detect when conditions become unfavourable in the freshwater environment. Additionally, the role of *M. margaritifera* as a keystone species, interacting with hydrogeomorphological processes to shape the surrounding habitat, gave rise to the development of instrumented shells to improve understanding of complex hydrodynamics in the near-bed environment. Few other species inhabiting benthic environments are large enough to accommodate remote sensing equipment, yet these environments host some of the most important aquatic organisms, governing ecosystem health within freshwater habitat. As a result, current remote sensing technologies are likely to rely on *M. margaritifera*, and other large bivalve species, to offer insights into the condition of benthic environments; thus, reinforcing the narrative of *M. margaritifera* as a flagship and umbrella species. It is therefore clear that conservation efforts concerning *M. margaritifera* provide an opportunity to address the holistic pressures acting on freshwater environments, yet further research is required to tease apart the interactions between the species and corresponding environment. These interactions are likely to be complex in nature, requiring a collaborative, multidisciplinary approach, adopting novel insights and techniques to unravel the inherent interconnectedness present within freshwater systems. This project has presented evidence that such approaches yield valuable insights, providing a basis for future research to build upon.

Table S1 An overview of the desirable sensor design features for the purpose of detecting entrainment risk in freshwater mussels

Feature	Desired Metric	Justification
Height	< 20 mm	Small enough to fit into cavity of shell
Diameter	< 30 mm	
Weight	< 10 g	To avoid distorting weight balance of the shell
Time of operation	>1 h	Enabling sufficient data collection and repetition of experiments. Allowing robust field deployment.
Logging frequency range	>200Hz	Adequately detects micro vibrations experienced within the shell
Range of angular velocities	>1000°/s	Sufficiently captures the mussel's complete rate of angular displacements
Range of angular accelerations	> 8 g	To capture the short-lived, highly dynamic impulsive forces acting on the mussel shell
Data storage	Flash storage. Logging speed >1MB/s	Providing predictable access, erase, write times that can cope with logging frequency of data requirements
Inertial sensor	Triaxial accelerometer and triaxial gyroscope	To detect movement in various forms in three-dimensional space

Table S2 Dimensions of the large, medium and small instrumented shells. Measurements provided in mm.

Shell Size	Length	Height	Width
Large	102	51	35
Medium	86	39	29
Small	69	32	23

Table S3 Calibration results for the total angular velocity across the three instrumented shell sizes. Mean (±SD) total angular velocity (rad/s) from sensor's gyroscope readings are compared against the theorised estimation, with the average error between theoretical and actual readings provided.

Shell Size	Mean (±SD) Theoretical Total Angular Velocity (rad/s) Estimated Using Equation (2)	Mean (±SD) Total Angular Velocity (rad/s) from Sensor's Gyroscope Readings	Mean % Error
Large	1.49 ± 0.55	1.47 ± 0.45	5.71
Medium	1.61 ± 0.59	1.60 ± 0.45	6.22
Small	1.35 ± 0.39	1.30 ± 0.45	5.9

Table S4 Calibration results for the total acceleration across the three instrumented shell sizes. Mean (±SD) total acceleration (m s^{-2}) from sensor's accelerometer readings are compared against the theorised estimation, with the average error between theoretical and actual readings provided.

Shell Size	Mean (±SD) Theoretical Total Acceleration (m s^{-2}) Estimated Using Equation (3)	Mean (±SD) Total Acceleration (m s^{-2}) from Sensor's Accelerometer Readings	Mean % Error
Large	0.051 ± 0.019	0.057 ± 0.024	3.2
Medium	0.052 ± 0.020	0.056 ± 0.020	5.9
Small	0.086 ± 0.024	0.085 ± 0.022	5.5

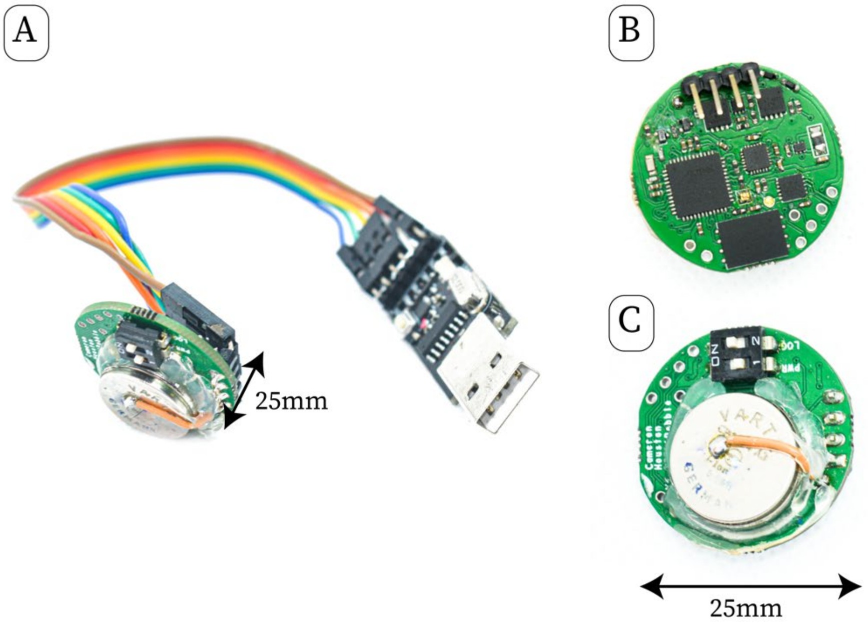

Figure S6.1 The 25mm MEMS sensor. Complete sensor with serial converter to TTL 6-pin attached, which facilitates charging and data transfer when connected via the USB Type-A to a PC (A). The top of the sensor displaying 4 pin connector, inertial sensor and discrete flash (B). The bottom of the sensor, showing the on/off switch and the Varta rechargeable coin cell (C).

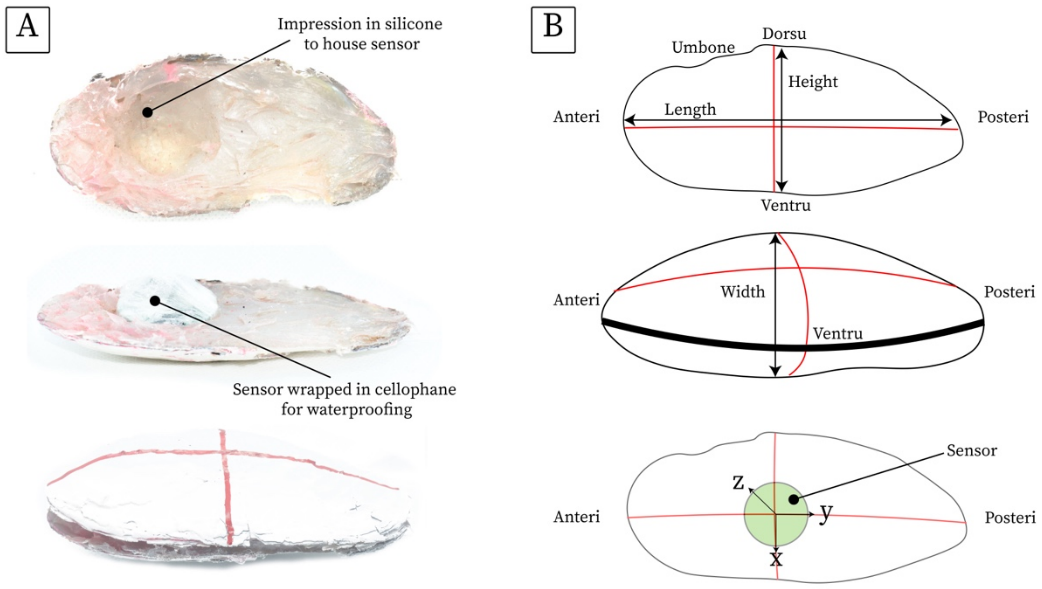

Figure S6.2 Visualisation of the instrumented shell. (A) Instrumented shell design to house sensor (B) Shell Dimensions, with position of shell x,y and z axis aligned with that of the sensor

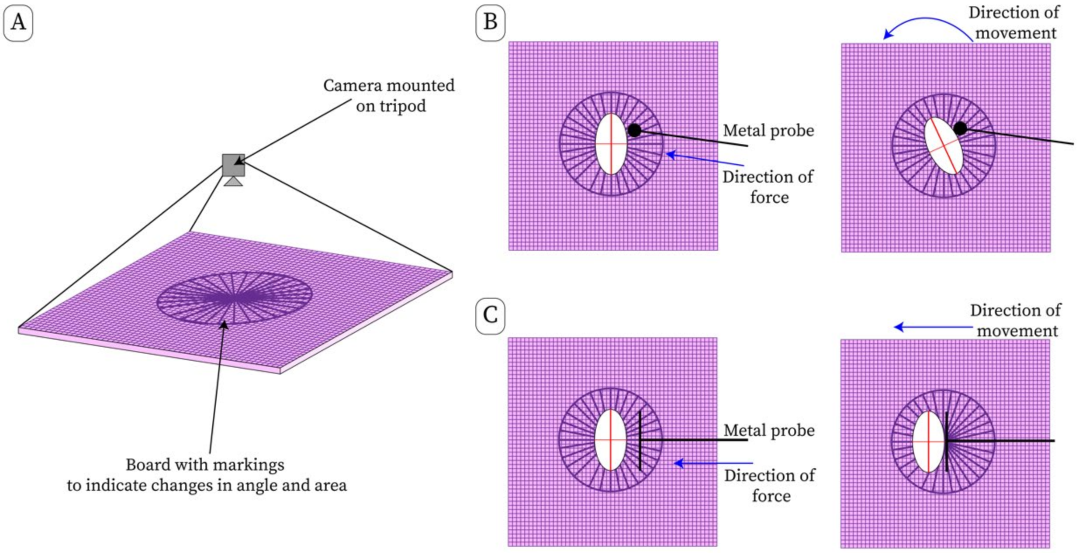

Figure S6.3 Overview of calibration test set-up, (A) displaying the craft mat and camera, (B) the trial facilitating a 30° change along the shell's y axis, and (C) the second trial, initiating an acceleration of the instrumented shell along the x-axis, with the shell moved back 2cm.

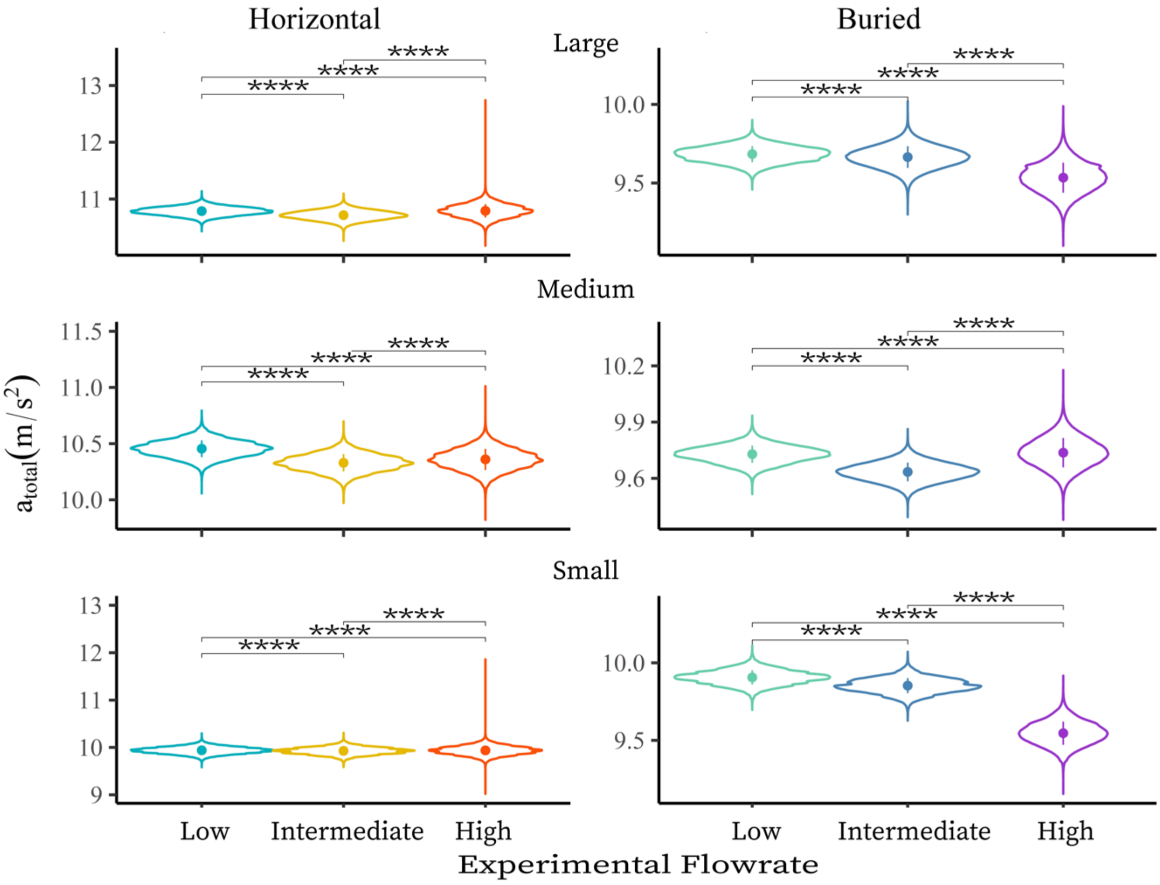

Figure S6.4 Variation in total acceleration (m s^{-2}). Displaying mean and standard deviation for the total acceleration from the sensor's accelerometer readings, across the three shell sizes and experimental flowrates, for the two orientations. Significant differences highlighted in the output of Kruskal-Wallis tests, for comparison of total acceleration across the High, Medium and Low flow velocity treatments for a given instrumented particle size, are displayed with an asterisk (= $p<0.05$;** = $P<0.01$; *** = $p<0.001$).*

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