The Role of Freshwater Mussels in River Bed Dynamics and Sediment Flux

Andrea Leng

Doctor of Philosophy

July 2023

Acknowledgements

First and foremost, I would like to give a huge thanks to my supervisors, Prof. K. Sian Davies-Vollum and Dr. Andrew Ramsey for your positivity, patience, support and encouragement. I would like to thank the Environmental Sciences team, the PGR student office and my fellow students at the University of Derby for your help, support and guidance. A big thank you to Dr. Mark Bulling for your help with the statistical analysis, and to Petra Parmová for assisting me with the sediment analysis. I would also like to thank the science technical team for your assistance with the practical aspects of this project. Special thanks to Dr. Charitos Zapitis for your friendship and support, and for passing on your knowledge of aquaculture, which helped me enormously during this PhD.

I am grateful to the University of Derby for providing the funding for the PhD, and to the Environment Agency for providing me with data for freshwater mussel populations. I also wish to thank Leicestershire County Council, and the Earl of Harrington's Angling Club, for granting me access to field sites.

Many thanks to my friends for helping me to stay sane, and most of all, I want to thank my family for your love, support and understanding during difficult times for all of us.

This thesis is dedicated to my father,

Roy Major Leng

02/03/50 - 12/04/23

Abstract

Freshwater mussels have been found capable of improving water quality in river environments by filtering particles from the water column and depositing them on the river bed as pseudofaecal pellets. Growing recognition of the ecosystem services that freshwater mussels provide in river habitats has led to their inclusion in several river restoration projects. The focus of many of these projects in Europe has been on the freshwater pearl mussel, *Margaritifera margaritifera* with attempts to repopulate rivers with this critically endangered species. However, the very specific habitat requirements of *M. margaritifera* mean that many European rivers are not suitable environments for this species. In comparison to *M. margaritifera*, very little conservation effort has been directed at some of our more common, yet declining freshwater mussel species, and little is known about how these species influence river sediments and habitat conditions. Some of the more common European freshwater mussel species, such as *Anodonta anatina* and *A. cygnea* are capable of living in a much broader range of habitat conditions compared with *M. margaritifera*, meaning they could potentially be of benefit in river remediation projects not suitable for *M. margaritifera*.

To improve understanding of how *Anodonta* species may influence river environments through bioturbation, filtration, and biodeposition, their influence on river sediment characteristics, sediment dynamics, and habitat conditions was investigated in two lowland English rivers and a laboratory-based flume environment. *Anodonta anatina* and *A. cygnea* from Markeaton Brook, Derbyshire and the River Sence, Leicestershire were translocated from mussel-dense reaches to locations within each river where mussels were absent. In both rivers, quadrats where mussels had been removed were compared with control quadrats where mussels were present, whilst quadrats at the sites where mussels had been introduced were compared with control quadrats where mussels were absent.

At sites in both rivers where mussels had been removed from the river bed, significant decreases in hyporheic oxygen saturation were found in all quadrats, compared with the control quadrats that contained mussels. Half of all quadrats where mussels had been introduced to river sites showed significantly increased hyporheic oxygen saturation, with the remaining quadrats showing non-significant increases compared with the control quadrats without mussels. Grain-size distribution patterns of sediment cores taken from the stream bed indicated that the introduction of mussels to the River Sence increased the textural heterogeneity of the river bed sediment, whereas removal of mussels reduced textural heterogeneity of the river bed sediment. No significant differences in river bed textural heterogeneity were found in Markeaton Brook. Mean percentages of organic matter, inorganic carbon, and fine-grained sediment were not significantly affected by the presence of mussels in the majority of quadrats. Significant reductions in water turbidity were found in Markeaton Brook, where mussels were introduced, but no significant differences in turbidity were found in the River Sence. Significantly higher BMWP scores were found where mussels were present in the River Sence but differences in Markeaton Brook were non-significant. The variation in results between the river sites suggest that the extent to which freshwater mussels influence river bed conditions and water quality may be mussel density-dependent and site-specific.

A recirculating flume-based study using fifty *A. anatina* investigated the impact of this species on substrate characteristics, hydrological conditions, and particle flux of a polymodal substrate. River seston was added to the flume at weekly intervals to provide food for the mussels, and water and substrate conditions were monitored for the eight-week duration of the study. A control experiment was also set up with mussels absent from the flume. It was found that the presence of *A. anatina* led to reduced near-bed, 0.6 depth and 0.4 depth velocities, and reduced suspended and dissolved solids in the water column. *Anodonta anatina* reduced the entrainment of fine and organic material but increased the entrainment of sand and gravel compared with the control study. Although water velocities were reduced with mussels in the flume, calculations based on the

grain-sizes entrained into the flume's sediment trap indicated that critical boundary shear stresses were significantly increased with mussels in the flume. Additionally, sediment grain-size distribution patterns and topographical measurements of the substrate surface indicated that the mussels increased the heterogeneity of the substrate.

The results of the research described in this thesis indicate that bioturbation, filtration and biodeposition by *Anodonta* species may positively influence hyporheic oxygen saturation levels, water quality and habitat heterogeneity in river environments. Increased mixing and mobilisation of river bed sediment, and the transferral of material from the water column to the substrate by mussels implies they constitute a critical element in the sediment and nutrient dynamics of fluvial systems.

Contents

Figures and tables

Chapter 1: Introduction

1.1 Background and rationale

Freshwater mussels (Order Unionida (Gray 1854)) are semi-infaunal filter-feeders of the Class Bivalvia (Linnaeus 1758), that can form dense aggregations of multi-species assemblages in fluvial environments (Hornbach and Deneka, 1996; Nichols and Garling, 2000; Vaughn and Spooner, 2006; Atkinson and Forshay, 2022). Unionids may constitute up to 90% of the benthic biomass of a river (Mann, 1964; Negus, 1966), filtering suspended particles from the water and depositing them as pseudofaeces on the river bed (Killeen, Aldridge and Oliver, 2004, p. 3; Atkinson *et al*., 2011; Hoellein *et al*., 2017). Filter-feeding and subsequent biodeposition by unionids serves to improve water clarity in aquatic environments, whilst providing nutrients to benthic species (Vaughn and Spooner, 2006; Strayer, 2014; Lummer, Auerswald and Geist, 2016).

The critical role that unionids have in benthic-pelagic food webs (Beckett *et al.*, 1996; Silverman *et al.*, 1997; Howard and Cuffey, 2006; Spooner and Vaughn, 2006; Aldridge, Fayle and Jackson, 2007; Bontes *et al.*, 2007; Vaughn, Nichols and Spooner, 2008; Burlakova, Karatayev and Karatayev, 2012; Black, Chimenti and Just, 2017) has led to general recognition of their status as "keystone species" and "ecosystem engineers" (Jones, Lawton and Shachak, 1994, 1997; Gutierrez *et al.*, 2003; Moore, 2006; Aldridge, Fayle and Jackson, 2007). However, freshwater mussels have experienced significant declines in recent years, attributed primarily to pearl fishing and habitat degradation (discussed in Section 1.2.5), and as such are now considered globally threatened (Bogan, 1993; Strayer *et al*., 1999; Vaughn, Gido and Spooner, 2004; Brainwood, Burgin and Byrne, 2008; Vaughn, Nichols and Spooner, 2008; Ford, Gullett and May, 2009; Hoke, 2011; Gillis *et al*., 2017).

The role of unionids in influencing the characteristics and dynamics of river sediment is presently understudied. Given the ecosystem services that unionids provide (Lummer, Auerswald and Geist, 2016; Vaughn, 2017), it is important to fully understand how they influence fluvial

environments in order to assess the implications of their continuing demise, and how that demise might be mitigated.

The primary aims of this thesis are to investigate the extent to which freshwater mussels influence:

A) The characteristics and flux of river sediment.
B) The eco-hydrological conditions within rivers.

The term "eco-hydrological" in this context refers to the interactions between hydrological conditions and river biota (Nuttle, 2002).

Specifically, this thesis examines the degree to which freshwater mussels impact river sediment grain-size distribution patterns, organic and inorganic carbon dynamics, sediment transport, river bed topography and heterogeneity, hyporheic oxygen levels, water quality, and habitat conditions. Although the role of mussel shells in improving habitat heterogeneity has previously been considered (Gutierrez *et al.*, 2003), to the author's knowledge, the impact of unionids on the textural heterogeneity of river bed sediment has not previously been investigated.

The wider implications of the conclusions drawn from this research are assessed in the context of the rapid decline in freshwater mussel abundance in Britain and Europe. Improved understanding of the ecosystem services provided by unionids, and their role in river habitat modification can help inform river restoration and management strategies, and would add further justification to the importance of conserving this group of animals.

1.2 Freshwater mussel biology, ecology and conservation

1.2.1 Unionid evolution and taxonomy

Freshwater bivalves first appeared in the Middle to Late Devonian Period; a time when terrestrial genera underwent considerable diversification (Chamberlain, 2007). The Unionida order of freshwater bivalves likely evolved after the End-Permian extinction event (Chamberlain, 2007),

and today they exhibit world-wide distribution, occupying a diverse range of freshwater environments (Williams *et al*., 1993; Killeen, Aldridge and Oliver, 2004, p. 1).

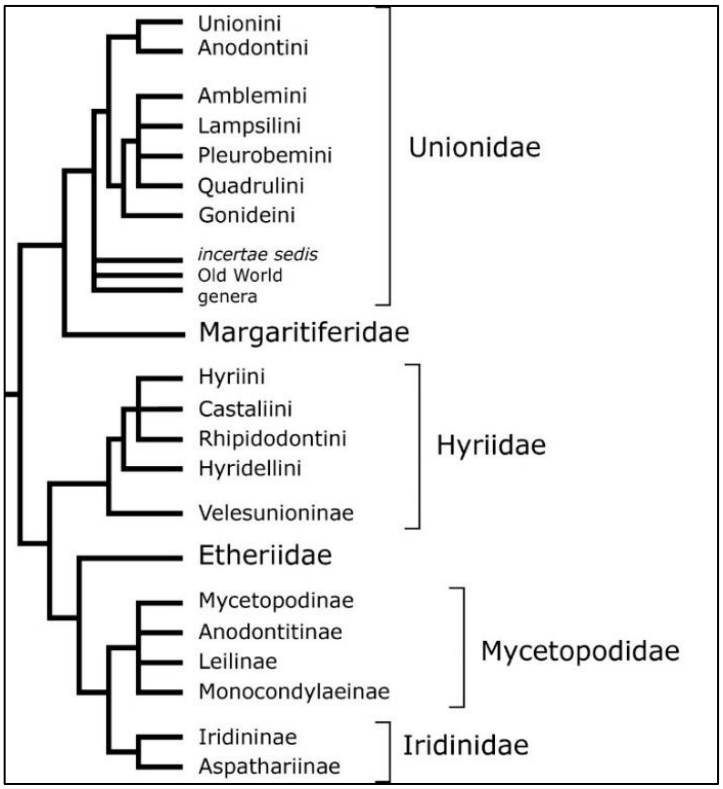


Figure 1.1: Cladogram of Freshwater Mussel Family-Group Level Taxa. Sourced from Graf and Cummings (2007).

Present-day Unionida families include the river mussels (Unionidae (Fleming 1828)) and the pearl mussels (Margaritiferidae (Haas 1940)) (Graf and Cummings, 2007). Margaritiferidae and the unionid subfamilies Unioninae and Anodontinae (Figure 1.1) are native to, and extant in the British Isles, specifically *Margaritifera margaritifera* (Linnaeus 1758), *Unio tumidus* (Philipsson 1788), *Unio pictorum* (Linnaeus 1758), *Pseudanodonta complanata* (Rossmässler 1835), *Anodonta anatina* (Linnaeus 1758) and *Anodonta cygnea* (Linnaeus 1758) (Killeen, Aldridge and Oliver, 2004, p. 6).

1.2.2 Biology and life cycle

Depending on the species, the lifespan of freshwater mussels can range from 10 to 200 years (Ziuganov *et al.*, 2000) with adults reaching 4 to 30 cm in length (Williams *et al.*, 1993). The large variation in longevity is thought to reflect the conditions in which they live, with the colder and more nutrient poor streams inhabited by Margaritiferids necessitating slower metabolic and growth rates, and therefore longer life spans. The warmer and relatively nutrient-rich waters occupied by unionid species allow for greater metabolic rates, faster growth rates and shorter life spans (Bauer and Hochwald, 1991).

Unionid morphology is depicted in Figure 1.2. Like other bivalves, they have their soft body parts enclosed between a pair of calcareous valves which are secreted by the underlying mantle. The valves are mainly calcium carbonate with an inner, often iridescent nacreous layer and an outer proteinaceous layer called the periostracum, which can partially erode away in older individuals. The two valves are joined at the hinge by the ligament on the dorsal side of the animal. As juveniles, the first part of the valve to grow is the pointed tip of the umbo, which is called the beak. Successive sub-concentric layers of shell are then secreted on the outer margin of each valve during seasons of growth, separated by growth lines, which represent periods of winter dormancy (Aldridge, 1999).

The valves are held closed by the adductor muscles providing protection from predators and adverse environmental conditions, whilst the action of the adductors is opposed by the elastic ligament. Thus, when the ambient conditions are conducive to activity, the adductor muscles relax and the valves are allowed to open (Killeen, Aldridge and Oliver, 2004, p. 2).

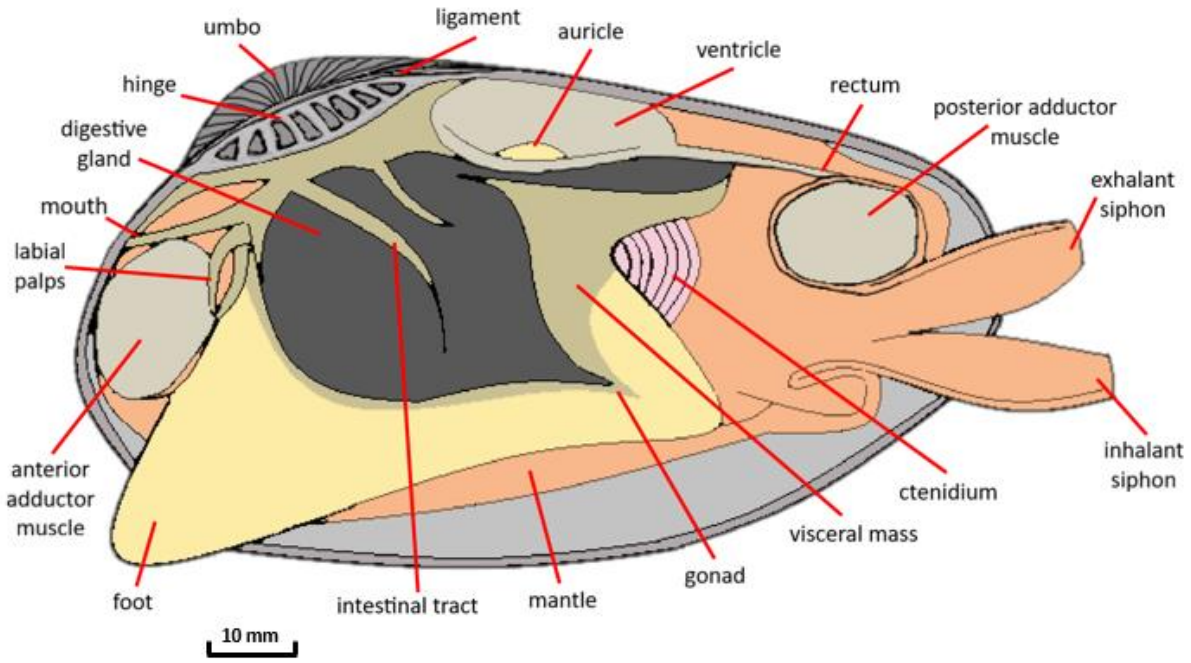


Figure 1.2: Internal soft tissue of a bivalve mollusc.
Re-drawn and modified from Cesari and Pellizzato (1990). © Andrea Leng.

Movement of the muscular foot at the ventral-anterior of the animal enables lateral and vertical movement on and within the substrate (Schwalb and Pusch, 2007; Zieritz, Geist and Gum, 2014; Monaco, Famiani and Iacona, 2016; Zapitis *et al.*, 2021), as well as pedal feeding within the sediment, particularly during their juvenile stage (Cosgrove *et al.*, 2000; Lavictoire *et al.*, 2015). The siphons extend through the posterior margin of the shell, with water drawn in via the inhalant siphon and released via the exhalent siphon. This allows filter feeding and respiration to take place in the ctenidium (gills), which comprise an inner and outer demibranch (Killeen, Aldridge and Oliver, 2004, p. 3). The flow of water is brought about through the movement of cilia and cirri (fused cilia) present on the inner surface of the mantle, demibranchs, and visceral mass. These structures sweep suspended particles from the water toward the mouth via the labial palps (Vaughn, Nichols and Spooner, 2008; Lavictoire *et al.*, 2018).

The demibranchs and palps sort particles so that the digestible constituents pass through the intestinal tract for absorption or egestion as faeces, whilst inedible material is bound in mucus and

deposited as pseudofaecal pellets (Vaughn and Hakenkamp, 2001). Both these biodeposits are ejected via the exhalent siphon (Killeen, Aldridge and Oliver, 2004, p. 3). The rate of filtration can be affected by water temperature, turbidity, turbulence levels and the availability of food (Aldridge, Payne and Miller, 1987; Reeders and Bij de Vaate, 1990; McIvor, 2004; Vaughn, Nichols and Spooner, 2008; Zapitis, Huck and Ramsey, 2021). The outer demibranchs of females also serve as brooding chambers for developing eggs and larvae (glochidia), and this has been shown to reduce the filtration rate of gravid females (Tankersley and Dimock Jr., 1993).

The valves of glochidia are held together at the hinge, and by a single adductor muscle. When mature, the glochidia are released on mucus threads and attach themselves to the gills or skin of fish with the use of hooks, or by grasping with their valves (Barnhart, Haag and Roston, 2008). Freshwater mussels are unique among bivalves in being obligate parasites (Haag and Stoeckel, 2015). This adaptation provides a survival advantage in lotic environments as it enables upstream recruitment, and also in lentic environments where limited water currents may otherwise hinder dispersal (Aldridge and McIvor, 2003; Daraio, Weber and Newton, 2010).

The timing of glochidial release can vary significantly between different populations of the same species depending on the availability of host fish species in the various drainage basins (Aldridge, 1999; Barnhart, Haag and Roston, 2008; Benito-Reyes, Alvarez-Codesal and Sweeting, 2015). Some species of unionid are generalists capable of utilising a variety of host fish species, whereas others are specialists using only a few closely related species (Haag and Stoeckel, 2015). The pearl mussel *(Margaritifera margaritifera)* is a specialist limited to salmonid hosts such as Atlantic salmon (*Salmo salar* (Linnaeus 1758)), brown trout (*Salmo trutta* (Linnaeus 1758)) and Arctic char (*Salvelinus alpinus* (Linnaeus 1758)) (Bauer and Hochwald, 1991; Cosgrove *et al.*, 2000; Benito-Reyes, Alvarez-Codesal and Sweeting, 2015; Clements, 2015; da Silva *et al.*, 2022; Taskinen and Salonen, 2022). A study by Taskinen and Salonen (2022) indicated that *M. margaritifera* may even have regional differences in their preferred salmonid host fish, for example *Salmo trutta* or *S. salar*, with regard to glochidial infection success rates. Indeed, certain

local *M. margaritifera* populations even showed improved glochidial infection success with local strains of their preferred salmonid host species compared to non-local strains (Taskinen and Salonen, 2022). Anodontids, however use a broader range of host fishes including brown trout *(S. trutta),* bullhead (*Cottus gobio* (Linnaeus 1758)), perch (*Perca fluviatilis* (Linnaeus 1758)), minnow (*Phoxinus phoxinus* (Linnaeus 1758)) and chub (*Squalius cephalus* (Linnaeus 1758)) (Bauer and Hochwald, 1991; Huber and Geist, 2017).

The epidermal and epithelial cells of the host fish respond to the presence of an attached glochidium by encapsulating it to form a cyst (Kat, 1984; Bauer and Hochwald, 1991; Rogers-Lowery and Dimock, 2006), and the juveniles generally excyst after three or four weeks of encapsulation (Bauer and Hochwald, 1991; Aldridge and McIvor, 2003). Barnhart *et al.* (2008) indicated that glochidia of less than 100 µm in length can double in size during their parasitic stage, and hypothesised that this would be a necessary trait in river environments to allow the juveniles to deposit themselves on the bed of the river. The excysted juveniles would be subjected to varying degrees of transport whilst suspended in the water and could be resuspended or transported with bedload after settling (Daraio, Weber and Newton, 2010). Therefore, glochidia have little control over where they settle, with only those landing in favourable habitat conditions likely to survive to adulthood (Layzer and Madison, 1995; Daraio, Weber and Newton, 2010).

1.2.3 Ecology

Freshwater mussels can feed across various trophic levels from both filter-feeding from the ambient water, and pedal-feeding from the underlying sediment (Cosgrove *et al.*, 2000; Howard and Cuffey, 2006; Lavictoire *et al.*, 2015; Fogelman *et al.*, 2022). They are thought capable of filtering a wide range of suspended solids, including phytoplankton, zooplankton, detritus, bacteria, inorganic sediment, and dissolved organic matter (Vaughn, Nichols and Spooner, 2008; Shah *et al.*, 2022). Analysis of the gut contents and biodeposits of unionid species from both lakes

and rivers demonstrate that freshwater mussels are able to selectively feed on green algae and diatoms from the water column, which provide them with many essential nutrients (Miura and Yamashiro, 1990; Nichols and Garling, 2000). However, studies indicate that bacteria constitute their primary source of dietary carbon and that they are capable of filtering particles as small as 1 µm in diameter (Jørgensen *et al.*, 1984; Way *et al.*, 1990; Silverman *et al.*, 1997; Lummer, Auerswald and Geist, 2016).

The removal of suspended solids from the water and its subsequent biodeposition in the sediment is thought to have a significant impact on river environments, improving water clarity and providing nutrients to other organisms (Beekey, Mccabe and Marsden, 2004; Vaughn and Spooner, 2006; Strayer, 2014). The ability of unionids to link multiple trophic levels through this benthic-pelagic coupling make them key components of food webs with significant impacts on nutrient cycling, the extent of which depends on their abundance, species composition, and environmental conditions (Howard and Cuffey, 2006; Vaughn, Nichols and Spooner, 2008). Freshwater mussels provide food to a variety of vertebrate species such as wild boar (*Sus scrofa* (Linnaeus, 1758)) (Sousa *et al.*, 2018), otters (*Lutra lutra* (Linnaeus 1758) and *Lontra canadensis* (Schreber, 1777)) (Reid *et al.*, 2013; Zajac, 2014), Muskrat (*Ondatra zibethicus* (Linnaeus 1766)) (Tyrrell and Hornbach, 2010), birds, fish, and after death, to freshwater invertebrates (Williams *et al.*, 1993). Their shells also create habitat for epizoic organisms such as filamentous algae (Vaughn and Hakenkamp, 2001), macrophytes (Podostemaceae) (Vaughn, Spooner and Hoagland, 2002), and caddisflies (Hydropsychidae) (Beckett *et al.*, 1996).

1.2.4 Distribution and habitat

In the British Isles, the freshwater pearl mussel *Margaritifera margaritifera* is mainly confined to the more mountainous areas of Scotland, Northern Ireland, Wales and the north of England (Figure 1.3), where they prefer oligotrophic coarse sand, fine gravel and cobble rivers and streams

(Skinner, Young and Hastie, 2003; Lopes-Lima *et al.*, 2017). The species shows a preference for relatively stable river bed localities, with a mix of sand and fine gravel substrate for burrowing, and cobbles or boulders to provide stability and a reduced shear stress during flood events (Quinlan *et al.*, 2015). Clements (2015) investigated the impact of different flow regimes on the burrowing behaviour of *M. margaritifera* in a series of flume experiments. The three flow regimes compared in the study were a constant velocity of 0.2 m/s, a gradual increase in from 0.2 to 0.7 m/s and a rapid increase in velocity from 0.2 to 0.7 m/s. *Margaritifera margaritifera* were found to bury deeper in the sediment as water velocity increases, however very rapid increases in velocity led to them being washed away. Mussels with lower body mass were shown to bury deeper in the sediment as the velocity increased compared with heavier mussels (Clements, 2015).

In Britain, the painter's mussel *Unio pictorum*, the swollen river mussel *Unio tumidus* and the depressed river mussel *Pseudanodonta complanata* are distributed mainly in the more lowland areas of central, south and east England, and the eastern edge of Wales (Figure 1.3 and 1.4). They have a preference for calcareous rivers, lakes and canals with firm, muddy substrates, or occasionally in sand, gravel and soft mud (Killeen, Aldridge and Oliver, 2004, p. 56).

The duck mussel *Anodonta anatina* and the swan mussel *Anodonta cygnea* are more widespread than the other British unionids, with their range extending over most of England and Wales, southern Scotland and Northern Ireland (Figure 1.4). Whilst *Anodonta cygnea* prefers slower-moving rivers, canals, lakes and ponds with clay and silt substrates, *Anodonta anatina* can be found in the most diverse array of habitats of any freshwater mussel species, including streams, rivers, lakes, ponds, reservoirs and canals with either clay, silt, sand or gravel substrates (Killeen, Aldridge and Oliver, 2004, p. 49; Lopes-Lima *et al.*, 2017).

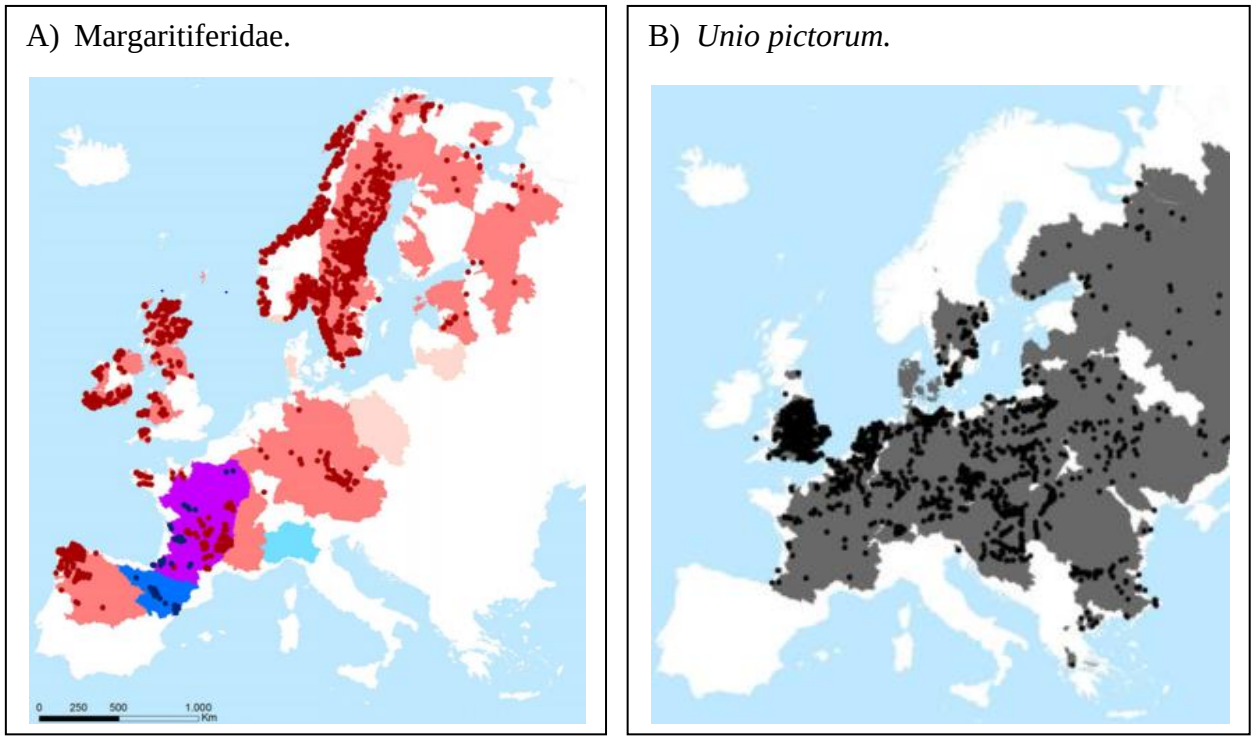


Figure 1.3: A: Distribution of Margaritiferidae in Europe. Known populations of *Margaritifera margaritifera* are shown as red dots, with their post-1992 distributional range shown as a lighter red. Historical (pre-1992) distributions are shown in pale pink. *Margaritifera auricularia* range of distribution is shown in blue and the purple colour indicates the presence of both species. B: *Unio pictorum* distribution in Europe. The black dots show present populations. Grey areas show their distributional range in river basins across Europe. Maps sourced from Lopes-Lima *et al*. (2017).

Previous studies have attempted to link mussel species distribution to certain types of substrate, with inferences made about the impact the sediment will have on ease of burrowing, water clarity, oxygen availability and hyporheic exchange (Brim Box, Dorazio and Liddell, 2002; Brainwood, Burgin and Byrne, 2008; Harriger, Moerke and Badra, 2009; Klos, Rosenberry and Nelson, 2014; Cushway *et al.*, 2022). However, this has proven problematical, with factors such as velocity, shear stress and bed stability appearing to take precedence over the bed sediment characteristics (Huehner, 1987; Layzer and Madison, 1995; Strayer, 1999; Hardison and Layzer, 2001; Brim Box, Dorazio and Liddell, 2002; Hoke, 2011; Lopez and Vaughn, 2021).

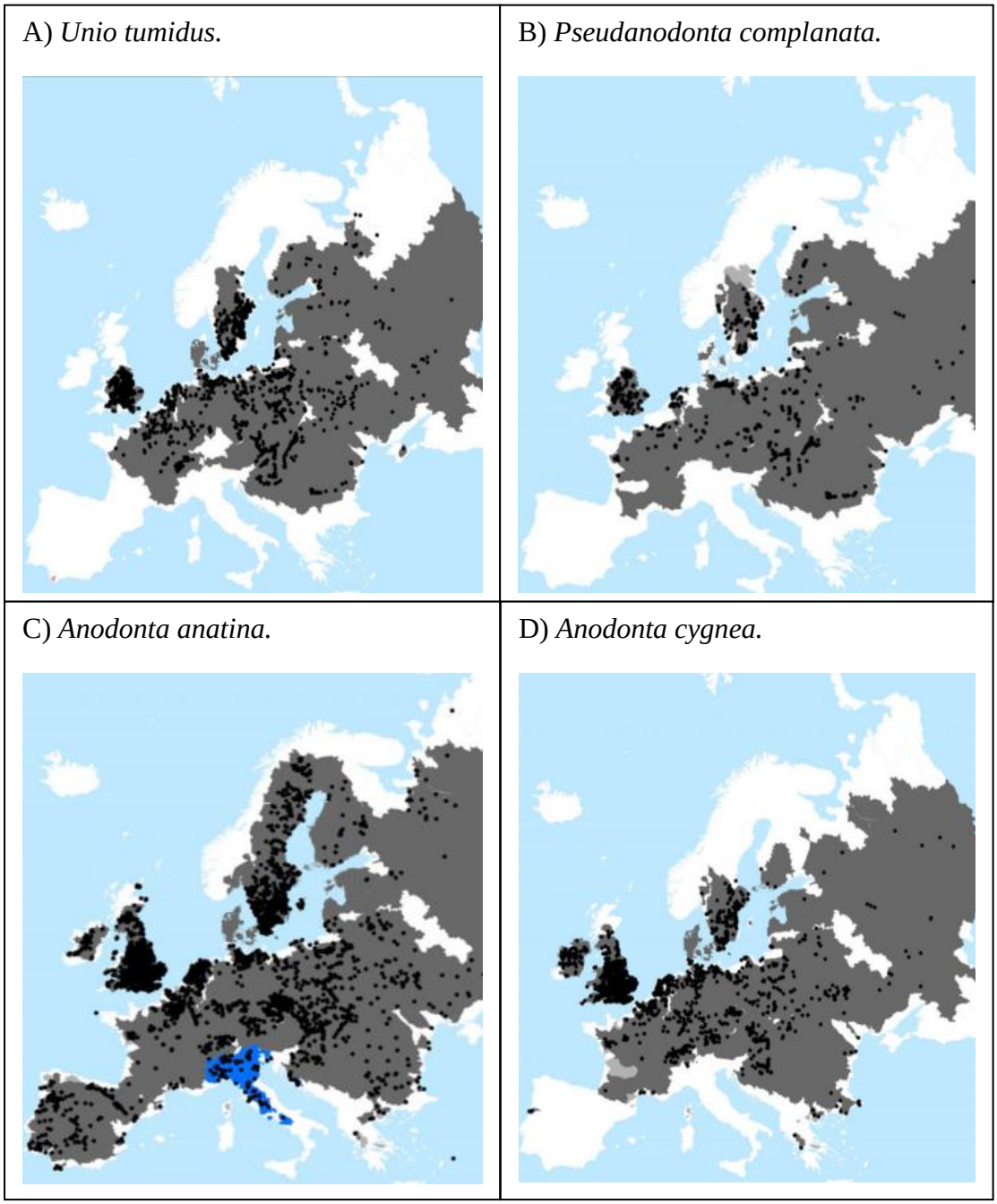


Figure 1.4: Distribution of A: *Unio tumidus,* B: *Pseudanodonta complanata*, C: *Anodonta anatina and Anodonta sp.* (blue), and D: *Anodonta cygnea.* The black dots show present, known populations and the dark grey areas show their post-1992 range. Historical populations (pre-1992) are shown as a lighter grey. Maps sourced from Lopes-Lima *et al*. (2017).

The requirement of unionids for stable areas of river with low shear stress likely accounts for their tendency to form patchy aggregations as opposed to more even distributions (Strayer, 1999; Daraio, 2010), although it has been suggested that clustering may facilitate reproduction (Schwalb and Pusch, 2007). Other factors thought to influence distribution include the availability of

perennially flowing waters, host fish abundance, turbidity, water chemistry, oxygen availability and anthropogenic factors such as pollutants and impoundments (Williams *et al.*, 1993; Štambuk *et al.*, 2009; Hoke, 2011; Johnson, Krstolic and Ostby, 2014; da Silva *et al.*, 2022). Given the longevity of many unionid species, it is likely that variability in environmental conditions operating over extended time scales will impact freshwater mussel distribution. This could be changes to hydrological regimes and drainage basin patterns; for example, periods of drought, changes in sediment influx and the frequency and magnitude of flood events (di Maio and Corkum, 1995).

1.2.5 Conservation

Freshwater mussels are considered indicator species as they are sensitive to degradation of their environment, and their diversity and abundance are often indicative of the overall health of freshwater ecosystems (Williams *et al.*, 1993; Aldridge, Fayle and Jackson, 2007; Štambuk *et al.*, 2009). A number of studies have correlated unionid presence and abundance with higher BMWP (Biological Monitoring Working Party) scores, higher invertebrate taxon richness, and higher invertebrate biomass compared with areas in which they are absent (Beckett *et al.*, 1996; Bially and MacIsaac, 2000; Beekey, Mccabe and Marsden, 2004; Vaughn and Spooner, 2006; Aldridge, Fayle and Jackson, 2007; Zaiko, Daunys and Olenin, 2009; Burlakova, Karatayev and Karatayev, 2012).

Freshwater mussels may reduce fine sediment infiltration into gravel river beds, which can be detrimental to spawning salmonids due to reduction in interstitial flow and oxygen availability (Beschta and Jackson, 1979; Wood and Armitage, 1997; Evans and Wilcox, 2014). Additionally, unionids may play an important role in removing pathogens from the water that could be harmful to fish (Burge *et al.*, 2016). Recent research shows that *Anodonta anatina* can significantly reduce the rate at which rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) are infected by the eye

fluke parasite *Diplostomum pseudospathaceum* (Niewiadomska, 1984) by feeding on the parasite larvae (Gopko *et al.*, 2017). Salmonids play an essential role in the reproductive cycle of unionids (Bauer and Hochwald, 1991; Clements, 2015), thus the loss or decline of salmonid hosts due to habitat loss, water degradation, or the impacts of climate change, would negatively impact unionid populations (da Silva *et al.*, 2022). If freshwater mussels improve the habitat conditions for the host fish, then loss of either mussel or host fish has the potential to initiate a feedback cycle towards eventual extinction.

Unionids as a whole are considered globally threatened (Bogan, 1993; Vaughn and Hakenkamp, 2001), and the freshwater pearl mussel, *Margaritifera margaritifera* and depressed river mussel, *Pseudanodonta complanata* are on the IUCN Red List of threatened species, listed as Endangered and Vulnerable respectively (IUCN, 2022). *Margaritifera margaritifera* numbers in Europe have declined by 87% since the early 20th Century (Moorkens *et al*., 2018), and pearl fishing is thought to be primarily responsible for the obliteration of populations in Britain and Ireland (Killeen, Aldridge and Oliver, 2004, p. 7).

The river Ehen in Cumbria holds England's largest population of *M. margaritifera* with over 100,000 individuals, and is the only river in England with active juvenile recruitment in evidence (Natural England, 2014b). The remaining English rivers with *M. margaritifera* comprise dwindling and aging populations, with no evidence of recruitment in the past 25 years (JNCC, 2019). In the past 20 years, the river Dee in Scotland has lost 99% of its *M. margaritifera* population, thought to be primarily a result of major flood events washing mussels downstream, and possibly out to sea (A.D. Ramsey 2023, pers. comms., 21 July).

The harvesting of pearl mussels is now illegal in the UK, and *M. margaritifera* is now fully protected under the Wildlife and Countryside Act 1981 (Cosgrove *et al.*, 2000). However, the demise of freshwater mussel populations has been further exacerbated by widespread river habitat deterioration (JNCC, 2019). Dams, weirs, and other man-made barriers restrict the movement of

host fish and impede successful reproduction and recruitment (Brim Box and Mossa, 1999; Killeen, Aldridge and Oliver, 2004, p. 8). Changes in catchment land use such as conversion of grassland and woodland to arable land, removal of riparian vegetation, forestry operations, and soil erosion from farm tracks and livestock activity, can increase river bed siltation and suspended sediment load (JNCC, 2019). River bed dredging, and discharge of storm water and sewage into rivers can also increase fine sediment load and reduce the quality of the water and substrate (Cosgrove *et al*., 2000; JNCC, 2019).

Large quantities of fine sediment influx into rivers are considered problematic for freshwater mussels as it can lead reduced dissolved oxygen concentrations in the river bed and ambient water, which may result in recruitment failure (Quinlan *et al*., 2015; Lavictoire *et al*., 2020). Siltation can also reduce the survival of salmon host fish embryos and promote the growth of macrophytes (Chapman, 1988). Large concentrations of suspended fine sediments in the water column can lead to reduced filtration rates in freshwater mussels due to clogging of feeding mechanisms (Tuttle-Raycraft, Morris and Ackerman, 2017).

Margaritifera margaritifera juveniles are particularly vulnerable to low substrate dissolved oxygen levels arising from fine sediment infiltration (Lavictoire *et al*., 2015, 2020). High levels of dissolved oxygen levels found in coarse sand and gravel substrates with high interstitial water flow, have been strongly associated with survival rates of juveniles (Lavictoire *et al*., 2020). This may hold true for all unionid species, however, Lummer, Auerswald and Geist (2016) demonstrated that filter feeding in *Unio pictorum* was not impaired by fine sediment pollution, but rather the mussels play a valuable ecosystem service by removing significant quantities of it from the water column.

River engineering works such as flood defences, channelisation and water abstraction alter hydraulic conditions and rates of sediment transport (Quinlan, 2014). Prolonged periods of low flow resulting from abstraction or drought can cause stress to mussels and host fish due to

increased water temperatures, low oxygen levels, increased concentration of pollutants, and increased deposition of fine sediment (JNCC, 2019).

Nutrient enrichment from fertiliser runoff, animal excrement, and sewage overflow can lead to eutrophication, whilst contaminants in the form of pesticides, heavy metals, pharmaceuticals and industrial chemicals from surface runoff and wastewater present impediments to survival (Bogan, 1993; Brim Box and Mossa, 1999; Brainwood, Burgin and Byrne, 2008; Hoke, 2011; Taskinen *et al.*, 2011; Gillis *et al.*, 2017; Lopes-Lima *et al.*, 2017; Rzodkiewicz, Annis and Woolnough, 2022).

The introduction of invasive species such as the zebra mussel, *Dreissena polymorpha* (Pallas 1771), quagga mussel, *Dreissena rostriformis bugensis* (Andrusov 1897), and Asiatic clam, *Corbicula fluminea* (Muller 1774) reduce the food, space and oxygen available to native mussels due to competition for resources (Williams *et al.*, 1993; Turner, 2010; Atkinson *et al.*, 2011; Hoke, 2011; Majdi, Bardon and Gilbert, 2014; Lopes-Lima *et al.*, 2017; Dobler, Hoos and Geist, 2022). Dense stands of invasive plants such as Himalayan balsam, *Impatiens glandulifera* (Royle) and Japanese knotweed, *Reynoutria japonica* (Houtt) along riparian zones, can leave the banks bare of vegetation when they die down in the winter, exposing the soil to erosion, whilst decay of plant matter in the river may reduce water oxygen levels (JNCC, 2019).

Growing recognition of the ecosystem services provided by unionids, and awareness of their rapid decline has resulted in a variety of Government and NGO (Non-Governmental Organisation) mussel reintroduction and habitat restoration initiatives with varying degrees of success (Lavictoire *et al.*, 2015; Strayer *et al.*, 2019). Many of these initiatives have focussed on the endangered *M. margaritifera* (Table 1.1).

Ex-situ strategies for propagating *M. margaritifera* include captive breeding of mussels collected from rivers in order to artificially infect captive salmonid host fish with glochidia (Thomas, Taylor and de Leaniz, 2010; Strayer *et al.*, 2019). The infected host fish are then released back into the

wild, or alternatively, the excysted juveniles are collected and reared in captivity before being released into rivers (Thomas, Taylor and de Leaniz, 2010; Sime *et al.*, 2017).

In England, the captive breeding of *M. margaritifera* has been led primarily by the Freshwater Biological Association, who have been breeding juvenile mussels since 2007 (FBA, 2023). At the FBA Ark captive breeding facility in Cumbria, juveniles from several English rivers have been bred using salmonid host fish, and then raised in tanks and flumes until they are large and resilient enough to be released into rivers (Lavictoire *et al*., 2015; Gibson, 2018; FBA, 2023). The first juvenile mussels from The Ark were released in 2017, and ongoing monitoring of reintroduced juveniles indicates promising rates of survival (FBA, 2023).

In-situ conservation methods include the creation of protected areas, bankside artificial encystment of host fish with glochidia and the use of mussel cages to rear juveniles in rivers (Thomas, Taylor and de Leaniz, 2010; Horton *et al.*, 2015; Sime *et al.*, 2017; Strayer *et al.*, 2019). Other in-situ conservation strategies include the translocation of mussels from rivers with healthy populations to other rivers, and river catchment restoration projects, involving participation of stakeholders and riparian landowners to improve water quality and optimise habitat conditions (Thomas, Taylor and de Leaniz, 2010; Horton *et al*., 2015; Sime *et al*., 2017; Watt, Hastie and Cosgrove, 2018; Strayer *et al*., 2019).

Ongoing Government and NGO river restoration efforts aimed at maintaining *M. margaritifera* populations involve managing water abstraction; control of invasive species; reducing point source and diffuse pollution from commercial, residential, and agricultural activities; preventing livestock from accessing river banks; and restoration of the natural river morphology and riparian vegetation (Hirst, 2009; Sime *et al*., 2017; JNCC, 2019; North York Moors, 2019; Devon Wildlife Trust, 2021; West Cumbria Rivers Trust, 2021a; Natural England, 2022; Kelly, 2023). Table 1.1 provides a summary of past and present freshwater mussel projects:

Table 1.1 Freshwater mussel projects.

Project	Date	Project Summary	Reference
FBA Ark, Windermere, Cumbria, England	2007-present	Captive breeding facility with juvenile *Margaritifera* from seven different UK river catchments. First captive bred juvenile reintroduced 2017	Lavictoire *et al*. 2015; Gibson, 2018; FBA, 2023
Devon Wildlife Trust, Freshwater Pearl Mussel Project, River Torridge, Devon, England	2019-present	River restoration and reintroduction juvenile *Margaritifera* by temporarily translocating brooding females from the FBA Ark to allow glochidial release into the river	Devon Wildlife Trust, 2021
Yorkshire Water and North York Moors National Park Authority, The Esk Pearl Mussel and Salmon Recovery Project, River Esk, North Yorkshire, England	2009-present	River habitat restoration and reintroduction of juvenile *Margaritifera* from FBA Ark	Hirst, 2009; North York Moors, 2019
West Cumbria Rivers Trust and United Utilities, Irt Freshwater Mussel Project, River Irt, Cumbria, England	2018-present	Reintroduction of juvenile *Margaritifera* from the FBA Ark hatchery to restore river habitat and inform best practice for mussel reintroduction	West Cumbria Rivers Trust, 2021a
West Cumbria Rivers Trust and Scottish Natural Heritage Pearls in Peril Project, River Ehen, Cumbria, England	2012-2021	Bankside artificial encystment of electrofished salmon and trout with glochidia extracted from *Margaritifera*, which were temporarily removed from the river. Restoration of riparian and instream conditions through removal of hard river bank protection works and replacement with soft engineering techniques, including woven willow spiling	Natural England, 2014b; Sime *et al*., 2017; West Cumbria Rivers Trust, 2021b
South Cumbria Rivers Trust and Natural England Life R4ever Project, River Kent, Cumbria, England	2021-2026	Improving habitat conditions for *Margaritifera* by restoring riparian vegetation and natural geomorphology of the catchment. Installing bankside fencing and removal of invasive species. Promote sustainable catchment management through agri-environment schemes. Reintroduction of *Margaritifera* from FBA Ark hatchery	South Cumbria Rivers Trust, 2021; Natural England, 2022

Table 1.1 continued on next page…

Table 1.1 (continued). Freshwater mussel projects.

Project	Date	Project Summary	Reference
Shropshire Hills AONB Partnership, River Clun Recovery Project, River Clun, Shropshire, England	2014-2027	Reducing siltation and improving habitat conditions for *Margaritifera* by working with landowners and restoring riparian habitats. Preventing livestock accessing river banks, runoff mitigation works, and planting 2200 riparian trees. Translocation of *Margaritifera* from degraded areas of the catchment to restored sections of the river.	Natural England, 2014a; Kelly, 2023
Natural Resources Wales and Scottish Natural Heritage Pearls in Peril Project, Afon Eden, Gwynedd, Wales	2012-2016	Restoration of 2.4 km of riverbed habitat, targeting reaches that could provide spawning areas for *Salmo trutta*, the preferred host fish for the local population of *Margaritifera*. Included putting locally sourced clean gravels in tributaries and large boulders with woody debris in the main river. Restoring riparian wetlands by blocking draining ditches and creation of settlement ponds to trap sediment	Sime *et al*., 2017
Scottish Natural Heritage and Cairngorms National Park Authority, tributaries in Deeside and Speyside, Scotland	2005-2015	*Margaritifera* reintroduced to secret locations in the Cairngorms. Subsequent monitoring of host fish showed active encystment in two of the three rivers by 2015	Cairngorms National Park, 2005; Watt, Hastie and Cosgrove, 2018
Scottish Natural Heritage Pearls in Peril Project, River South Esk, Angus, and River Dee, Grampian, Scotland	2015-2017	Removal of 1 km of boulder protection from river banks and restoration of riparian woodlands along more than 80 km of river to provide shade, reduce erosion and restore riparian vegetation communities. Widening of river channel in certain areas to increase habitat available to juvenile *Margaritifera* and spawning salmonid host fish. Boulders dispersed in main channel to recreate diverse riverbed conditions favoured by *Margaritifera*	Sime *et al*., 2017
Ballinderry Rivers Trust, FPM Project, Ballinderry River, County Tyrone, Northern Ireland	1999-present	Captive breeding of *Margaritifera* from the river and release of glochidia into the river	Horton *et al*., 2015

Table 1.1 continued on next page…

Table 1.1 (continued). Freshwater mussel projects.

Project	Date	Project Summary	Reference
The Pearl Mussel Project Ltd., Kenmare, County Kerry, Ireland	2018-present	Providing training, assistance and financial incentives to farmers in Counties Kerry and Mayo in order to improve the quality of watercourses for the benefit of *Margaritifera*	Pearl Mussel Project, 2020
University of Milan and Milano-Nosedo Wastewater Treatment Plant, Milan, Italy	2013-2014	40,000 Zebra mussels collected from Lake Maggiore and Lake Lugano, Italy moved to water treatment facility in Milan for the purpose of removing heavy metals, pharmaceuticals and recreational drugs from wastewater	Binelli *et al*., 2014; Magni *et al*., 2015
Natural Resources Institute of Finland, Laukaa Fish Farm, Laukaa, Finland	2016-present	Freshwater mussels *(Anodonta)* being trialled as part of a recirculating aquaculture system involving *Daphnia* and microalgae	Stevcic, Pulkkinen and Pirhonen, 2018
WWF Sweden, LIFE Project, unnamed river catchment, Sweden	2004-2009	1000 *Margaritifera* collected from a river and reintroduced to a stream within the same catchment as part of a river restoration project	Reform Rivers, 2011
NINA, Trondheim, Brook Hammerbekken, and River Ogna, Central Norway	2008-2010	Brown trout artificially encysted with *Margaritifera* glochidia and released into streams	Larsen, 2013
Austevoll Freshwater Pearl Mussel Breeding Station, Western Norway	2011-present	Captive breeding and reintroduction of *Margaritifera* to 40-50 rivers	Larsen, 2013
Nature Conservation Agency of the Czech Republic, Seminatural Breeding of the Freshwater Pearl Mussel, Aš Region, Czech Republic	2015-2016	*Margaritifera* glochidia reared using captive brown trout and juvenile mussels reintroduced to three rivers	Ochrana Prirody, 2022
Partnership for the Delaware Estuary, Freshwater Mussel Recovery Program, Delaware, New Jersey and Pennsylvania, USA	2013-present	Juvenile freshwater mussels from hatchery in Philadelphia being reintroduced to tributaries of the estuary to restore water quality and reduce cost of water treatment	Partnership for the Delaware Estuary, 2013

Due to the vulnerability of *M. margaritifera* and *P. complanata* populations in Britain, these species will be excluded from the experimental aspects of this research project. The focus of the studies within this project will therefore be on the *Unio* and *Anodonta* species extant in Britain.

1.3 River bed dynamics and the influence of fauna

1.3.1 Sediment transport processes

Sediment grain-sizes transportable by fluvial processes include boulders (>256 mm), cobbles (64-256 mm), gravel (2-64 mm), sand (63-2000 μm), silt (4-63 μm) and clay sized particles (1-4 μm), with the finest fractions more likely to be transported in suspension (Engelund and Hansen, 1967; Roseberry, Schmeeckle and Furbish, 2012; Greenbaum *et al.*, 2020). River sediment can be transported by bedload traction (rolling and sliding), by saltation (a ballistic-like trajectory) or by suspension, whereby particles are supported in the water column by upwardly turbulent forces (Nelson *et al.*, 1995; Church, 2006; Bhattacharyya, Ojha and Mazumder, 2013; Dey, 2014).

Bedload constitutes a small percentage of overall sediment transport in rivers, and flood events capable of mobilising bedload are infrequent in many gravel bed streams (Reid, Frostick and Layman, 1985). Coarser bedload such as gravel, cobbles (64-256 mm) and boulders (>256 mm) require generally higher velocities for entrainment, and can be deposited at higher velocities than for fine sediment deposition (Church, 2006). However, water velocity often fails to accurately predict the grain-size distribution of sediment transported in natural river systems, due to sediment supply often being limited by other variables (Friend, 1993; Rice and Church, 1998). These include the binding of particles by vegetation and biota, flocculation and diagenetic cementation, as well as limitations and variations in sediment supply. Hence, natural rivers are frequently found to be transporting less sediment than they are theoretically capable of (Friend, 1993).

A river's competence is defined as "the ability of a stream flow to mobilise sediment of a given size" (Church, 2006, p. 329), and is represented by the Shields parameter τ^*, derived through Equation 1, which constitutes a measure of dimensionless shear stress (Church 2006). As water flows over the surface of a grain, the velocity difference between the top and the bottom of the grain creates a vertical pressure gradient, generating lift (Gordon, McMahon and Finlayson, 1992).

(1)

$$\tau^* = \frac{\tau}{(\rho_s - \rho) g D_{50}}$$

$\tau*$ = Dimensionless shear stress (N m^{-2}). τ = boundary shear stress (N m^{-2}). ρs = sediment density (kg m^{-3}). ρ = fluid density (kg m^{-3}). g = acceleration of gravity (m s^{-2}). D_{50} = median grain-size (m).

Critical shear stress τ_c (Equation 2) is the amount of shear stress (N m^{-2}) required for a grain of a given size to be set in motion, and represents the point at which the downstream and upward forces acting on a grain overcome the forces holding it in position on the bed (Miller, McCave and Komar, 1977; Wiberg and Smith, 1987).

(2)

$$\tau_c = \tau^* (\rho s - \rho) g D_{50}$$

τ_c = Critical shear stress (N m^{-2}). $\tau*$ = Dimensionless shear stress (N m^{-2}). ρs = sediment density (kg m^{-3}). ρ = fluid density (kg m^{-3}). g = acceleration of gravity (m s^{-2}). D_{50} = median grain-size (m).

The shear stress required for grain entrainment is determined not only by the parameters in Equation 2, but also by the relative size and position of adjacent grains (Wiberg and Smith, 1987; Dey, 2014). Larger grains often protrude from the river bed, thereby reducing the boundary shear stress on the surrounding smaller grains, whilst the larger grains are subjected to increased boundary shear stress due to them being more exposed to the force of the flow (Wiberg and Smith, 1987). Once the larger particles are set in motion, the smaller grains are no longer protected, thus a wide range of sediment sizes tend to be entrained within a narrower range of τ than would be predicted based on their size and density alone (Church, 2006).

Equation 2 becomes inaccurate for fine-grained floccular sediments due to the added complexity of electrostatic forces that generate cohesion (Wiberg and Smith, 1987; Dey, 2014). The smoother

profile of fine-grained sediments also produces less turbulence, making erosion more difficult (Gordon, McMahon and Finlayson, 1992). Additionally, grain packing, grain imbrication and slope angle can influence the shear stress required for mobilisation, hence, determining the threshold of grain motion can be problematic (Rosgen, 1994; Church, 2006).

The critical shear stress required to mobilise grains of a given size will also vary according to hydrological regime and antecedent weather conditions (Reid, Frostick and Layman, 1985). Long periods of time between flood events allow for physical, biological and chemical consolidation of sediment, therefore, isolated flood events may require a period of grain destabilisation before significant bedload transport can commence (Reid, Frostick and Layman, 1985). In gravel streams bedload may occur predominantly on the recessional limb of the first flood of the season, whereas with subsequent floods the destabilised river bed may allow bedload to be generated on the rising limb of the storm hydrograph (Reid, Frostick and Layman, 1985).

Fine material forming the matrix between gravel stream beds can derive from both the bedload and suspended load (Frostick, Lucas and Reid, 1984). Gravel stream beds frequently display stratification with depth due to the scour of finer material from the gravel, which exposes the coarser material on the surface, or by kinematic sieving when the bed sediment is in motion (Evans and Wilcox, 2014). Finer material in suspension can also become trapped between the gravel clasts through downwelling currents to form a matrix. This produces an "armour layer" on the surface, which shields the finer matrix from erosion (Frostick, Lucas and Reid, 1984; Reid and Laronne, 1995; Almedeij and Diplas, 2005; Evans and Wilcox, 2014; Yager, Kenworthy and Monsalve, 2015). Breaching of the armour layer during powerful flood events can expose the finer sub-armour sediment to the flow, resulting in significant mobilisation of the river bed (Reid, Frostick and Layman, 1985; Reid and Laronne, 1995).

1.3.2 River channel morphology and dynamics

The morphology and bed dynamics of any given river result from a complex interplay of physical and human factors, such as local climate, antecedent weather events, geology, topography, soil type, flora, fauna and land use within the drainage basin (Church, 2006). Sediment can be supplied to a river via tributary streams, overland flow, bank erosion, mass movement from the valley sides, bar erosion and bed erosion. Depending on the hydrological and geomorphological conditions at any given time, this influx of sediment may either be deposited on the bed, bars or floodplain, or may be removed through sediment flux downstream (Hooke, 2003). Channel gradient, sediment grain-size, and the quantity of sediment supplied to the river can influence channel morphology, stability, flow characteristics, and the propensity for erosion, deposition and lateral migration (Hooke, 2003; Church, 2006; Yager, Kenworthy and Monsalve, 2015; Nanson and Huang, 2017). Steeper channel gradients and more resistant bedrock are often present in the upper reaches of rivers, meaning the carrying capacity of the river can frequently exceed its sediment supply, leading to net erosion of the river bed (Dey, 2014). In the lower reaches the decreased topographic gradients often reduce the transport capacity of the river, resulting in net deposition and aggradation of the river bed (Dey, 2014).

Spatial and temporal variations in any of the sources and sinks of sediment supplied to a river result in morphological changes within that river (Friend, 1993; Bunte and Abt, 2001). The river's gradient, morphology, velocity and capacity to transport sediment adjust to maintain equilibrium with the prevailing conditions and achieve maximal efficiency and stability (Yager, Kenworthy and Monsalve, 2015; Nanson and Huang, 2017). When the supply of sediment to a section of river is increased beyond the river's capacity, the river responds by depositing sediment, and thus increasing the local elevation and therefore gradient of the bed immediately downstream of the sediment supply point. The increase in gradient leads to an increased reach average shear stress on that section of river, and thus the river's capacity to transport the sediment will also increase. When sediment supply is reduced to below the river's capacity at any given point of the river, the

local elevation and gradient of the bed is reduced through erosion, which in turn slows the flow, reducing the rate of transport and deposition along that stretch of river (Yager, Kenworthy and Monsalve, 2015).

The highest velocity flow of water in a river will tend to follow the thalweg, which is the deepest part of the river channel with the least friction. The flow will switch back and forth diagonally across the channel away from shallower bars and obstructions, which is thought to trigger meander development (Madej, 1999; Bunte and Abt, 2001). Helicoidal flow is generated in the meanders which can lead to undercutting of the outer bank and deposition of material on the inner bank where velocities are lower (Tanner, 1960; Bunte and Abt, 2001; Lai *et al.*, 2017). Lateral expansion of meanders is thought to be driven by a combination of erosion of the concave bank (bank-pull), predominantly during floods, and lateral progradation of inclined heterolithic strata deposited on the convex point bar (bar-push) (Nanson, 1980; Miall, 1985; van de Lageweg *et al.*, 2014).

In gravel and cobble rivers the shallower sections generally display coarser bed sediment, and form riffles, where flow tends to be more turbulent due to the increased roughness, whereas the deeper pools often show reduced levels of turbulence, allowing deposition of finer sediment (Rosgen, 1994; Lisle and Hilton, 1999; Madej, 1999). During low flow conditions, riffles often have higher flow velocities than pools. This scours finer material off the riffles and deposits it in the pools (Lisle and Hilton, 1999). During flood events, the velocity, and therefore shear stress can sometimes be higher in the pools than the riffles, particularly when a recirculating vortex develops. This can scour coarser material out of the pool and deposit it on the riffle (Bunte and Abt, 2001).

Field evidence and hydrological modelling suggest that the grain-size of sediment generally decreases downstream, and sediment will become more rounded (Gasparini, Tucker and Bras, 2004). The downstream reduction in median bed sediment size and angularity is partly as a result

of abrasion of grains occurring when they collide with other sediment during transport (Gasparini, Tucker and Bras, 2004). Additionally, coarser grains such as cobbles and gravel have a reduced likelihood of being transported compared with finer grains due to the greater shear stresses required for entrainment, thus the finer grains are often selectively transported further downstream (Wiberg and Smith, 1987). Textural changes in river bed sediment downstream are often in reality more complex, due to local changes in geology and gradient, and the lateral introduction of sediment from multiple sources along the river's route (Rice and Church, 1998; Bunte and Abt, 2001). The nature of the underlying material being incised by the river can dictate the river bed sediment characteristics. For example, many lowland English streams have muddy banks and bed sediment comprising gravel-sized clasts, resulting from erosion of Mesozoic mudstone bedrock with a cover of Pleistocene glacial and fluvioglacial material (Friend, 1993). These rivers generally only transport the silt and clay-sized fractions outside of significant flood events, which fills interstices between the gravel clasts, yet in rivers of comparable discharge where the Pleistocene cover is sand, the river may continuously transport and deposit this sand, resulting in a contrasting fluvial morphology (Friend, 1993).

1.3.3 River sedimentary structures

Sediment grain-size, water velocity, channel depth and topography determine the nature of sedimentary structures present on the river bed (Ashley *et al*., 1990). Asymmetrical ripples are typical of lower flow regime conditions transporting grain-sizes ranging from silt to coarse sand, with low to moderate velocities (Ashley *et al*., 1990). With increasing velocities the mobilisation of the coarser fractions is associated with the development of dunes, which may be superimposed with ripples (Ashley *et al*., 1990). In silt and sand bed river channels, straight-crested, sinuous and linguoid ripples, dunes and bars migrate downstream as sediment is eroded from the stoss (upstream) side of the bedform and deposited on the lee (downstream) side, forming planar

(tabular), and trough cross-stratification in the vertical sediment profile (Kleinhans, 2002; Best, 2005).

Typical sedimentary structures found in gravel and cobble river beds include pebble imbrication and particle clusters (Figure 1.5), which can form from the stacking-up of pebbles against an obstruction as they are transported along the river bed (Bunte and Abt, 2001; Piedra, Haynes and Hoey, 2012). Larger pebbles can have a stabilising effect on smaller grains in a particle cluster by increasing bed roughness and reducing shear stress, which can give rise to the formation of wake deposits (Figure 1.5) on the lee side of the particle cluster (Bunte and Abt, 2001; Piedra, Haynes and Hoey, 2012). However, flow disturbance generated by larger pebbles can also create localised flow vortices that scour sediment from the upstream side of the obstruction and deposit it on the lee side to form a horseshoe vortex scour structure (Figure 1.6) (Bunte and Abt, 2001). Cross-stratification may form in gravel river beds due to the migration of bars of sediment downstream (Bunte and Abt, 2001).

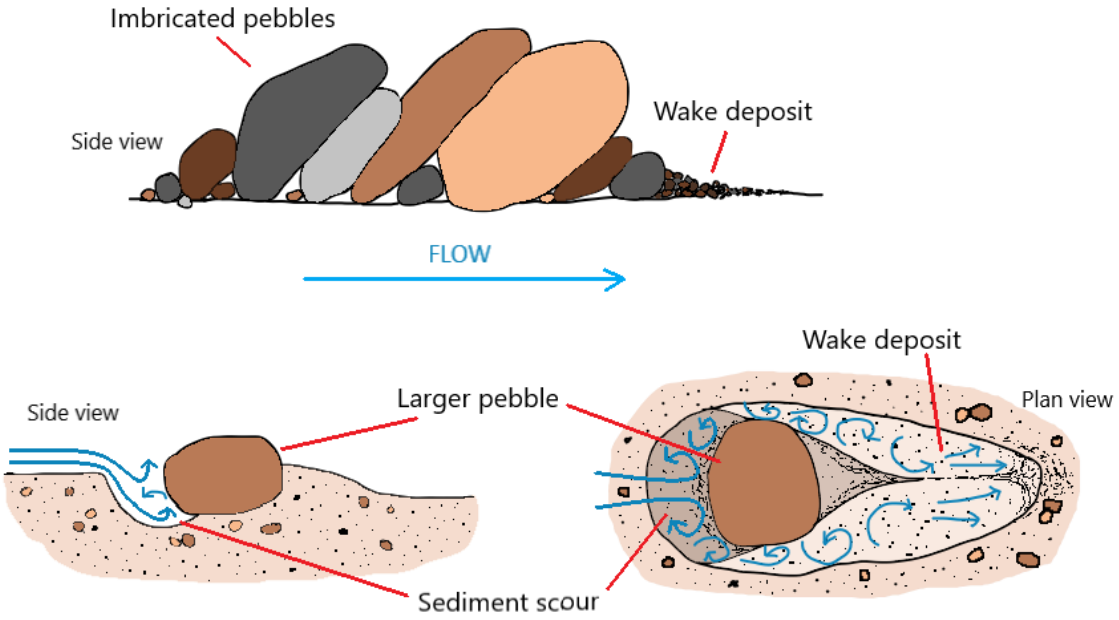


Figure 1.5: Imbricated pebbles in a particle cluster with a finer wake deposit (top) and diagrams to demonstrate horseshoe vortex scour formation around a pebble in a sandy matrix (bottom). Redrawn and modified from Bunte and Abt (2001). © Andrea Leng.

1.3.4 Zoological impacts on river bed characteristics

Jones, Lawton and Shachak (1994, p. 373) coined the term "ecosystem engineers" in reference to organisms that "directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic material." They categorised ecosystem engineers into "autogenic engineers," which modify their environment with their own physical structure, such as corals and trees, and "allogenic engineers," which modify the environment by changing and providing resources to other organisms, for example beavers and burrowing animals (Jones, Lawton and Shachak, 1994). Ecosystem engineers can modify physical resources within a habitat such as availability of light, water, sediment, shelter and strength of current (Crooks, 2002). Ecosystem engineers are capable of facilitating ecosystem stability over long periods of time by altering abundance and distribution of organisms (Jones, Lawton and Shachak, 1997; Corenblit *et al*., 2007). The extent to which ecosystem engineers in rivers and streams are able to impact their environment is determined by their behaviour, size and population density; factors which in turn will be limited by the biotic and abiotic factors prevalent in the river system. If the habitat modification by the ecosystem engineer results in an increased fitness of their population, there may be significant positive feedback effects associated with the increase in abundance (Moore, 2006).

A concept related to ecosystem engineering is that of zoogeomorphology, which specifically addresses the role of animals as geomorphic agents (Corenblit *et al*., 2007). Geomorphological processes can impact fluvial ecosystem functioning, however biotic components within that ecosystem can have collective impacts on fluvial geomorphology. Assemblages of zoogeomorphic species have the potential to profoundly affect fluvial dynamics by influencing erosion, transportation, deposition and mixing of sediment (Corenblit *et al*., 2007; Rice *et al*., 2012), therefore river systems can be considered to have co-evolved with their resident biota (Reinhardt *et al*., 2010). Understanding the complex ecological and geomorphological

interactions in freshwater ecosystems would facilitate the management of the natural capital and ecosystem services they provide (Viles *et al*., 2008).

Various species of fish have been shown to have zoogeomorphological impacts on river sediments (Gottesfeld, 1998; Barber, Nairn and Huntingford, 2001; Statzner, Sagnes and Champagne, 2003; Pledger, Rice and Millett, 2017; Rice *et al*., 2019; Smith, 2019). Whether fish behaviour results in an increase or decrease in river bed shear stresses and sediment mobility is likely dependent on a range of factors, including the degree of structural disturbance and particle sorting by the fish (Pledger, Rice and Millett, 2017).

Male sticklebacks (Gasterosteidae) cement grains of sand and gravel together with a glue-like protein to construct ornamental nests, which reduces grain entrainment and influences river bed topography (Barber, Nairn and Huntingford, 2001). Sockeye salmon (*Oncorhynchus nerka* (Walbaum 1792)) can spawn at high densities, and dig nests (redds) that are over twenty centimetres deep and over two metres wide (Moore, Schindler and Scheuerell, 2004). Sockeye salmon preferentially spawn in shallower freshwater habitats, such as riffles and bars, and redd construction selectively mobilises fine sediment from the spawning areas (Gottesfeld, 1998; Moore, Schindler and Scheuerell, 2004; Hassan *et al.*, 2008). The reworking of gravel by salmon, increases river bed permeability and hyporheic oxygen levels in redd areas, which improves egg to fry survival rates (Gottesfeld, 1998; Han *et al*., 2019). Salmon bioturbation also removes any vertical sorting of sediment and river bed armouring that may have formed during high flows throughout the year, which reduces overall bed sediment transport thresholds (Gottesfeld *et al.*, 2004; Hassan *et al.*, 2008). The fine sediment displaced from riffles and bars during redd construction, accumulates in the undisturbed areas of river bed, filling the pools and widening the channel (Gottesfeld, 1998; Hassan *et al.*, 2008). The resulting habitat modification significantly affects algal biomass, invertebrate densities and community organisation (Moore, Schindler and Scheuerell, 2004).

A series of studies by Gottesfeld (1998), Gottesfeld *et al*. (2004), and Hassan *et al*. (2008) involved monitoring the movement of magnetically-labelled gravel in in the upper reaches of the Fraser River in British Columbia. Movement of the labelled gravel over four years indicated sockeye salmon spawning activity accounted for 11% of annual bedload transport in the upper reaches of the Fraser River (Gottesfeld, 1998). The impact of salmon spawning was found to be more pronounced in the lower gradient, downstream sections of stream, where salmon were thought responsible for 48% of annual bedload transport, compared with 4% on the upstream, higher gradient stretches (Gottesfeld, 1998). Freeze-core samples of the river bed indicated that the amount of vertical mixing of sediment by salmon was often equivalent to that caused by flood events (Gottesfeld *et al*., 2004; Hassan *et al*., 2008). Changes in river bed surface elevation derived from sediment trap data and topographic profiles, indicated that salmon redd construction created a distinct hummocky channel morphology that defined river bed topography for most of the year (Gottesfeld *et al*., 2004; Hassan *et al*., 2008).

Foraging benthic fish can also cause significant disturbance to river bed sediments, influencing grain mobility and sediment flux (Pledger, 2014; Pledger, Rice and Millett, 2017; Rice *et al.*, 2019). Fish foraging can disturb grain fabrics and sedimentary structures such as imbrication, which can reduce sediment stability and critical entrainment stresses (Rice *et al.*, 2019). Conversely, increased topographic roughness and grain protrusion from fish foraging can increase drag, resulting in lower near-bed velocities and reduced grain mobility (Rice *et al.*, 2019).

Fish size and feeding style can determine the depth of sediment disturbed by benthic foraging, with some fish bulldozing sediment, pushing or rotating clasts, or gulping in and spitting out grains in a new location (Rice *et al.*, 2019) determine the depth of sediment disturbed by benthic foraging, with some fish bulldozing sediment, pushing or rotating clasts, or gulping in and spitting out grains in a new location (Rice *et al.*, 2019). In lotic environments, fish tend to orientate themselves in an upstream direction when feeding, which means larger grains are often pushed

upstream, whilst finer grains may be mobilised downstream by the current (Pledger, Rice and Millett, 2017; Rice *et al.*, 2019).

European barbel (*Barbus barbus* (Linnaeus 1758)) and gudgeon (*Gobio gobio* (Linnaeus 1758)) have been found to modify surface characteristics and reduce the critical shear stress of sand and gravels in experimental streams by bioturbating the river sediments (Statzner, Sagnes and Champagne, 2003). An ex-situ experiment with European barbel investigated the impact of their feeding on the structure and composition of gravel-bed river sediments (Pledger, 2014). Within four hours, approximately thirty seven percent of the substrate was modified, increasing the microtopographic roughness and reducing particle imbrication, whilst bedload flux increased by sixty percent during the same period (Pledger, 2014). A similar in-situ experiment demonstrated that European barbel and chub (*Squalius cephalus* (Linnaeus 1758)) modified seventy percent of a gravel substrate in a river during a twelve hour period, increasing microtopographic roughness, coarsening the substrate and increasing mobility of river sediments (Pledger, 2014).

Pledger, Rice and Millett (2017) used in-situ and ex-situ studies to assess the impact of European barbel and chub foraging on gravel river bed characteristics and entrainment in the River Idle, Nottinghamshire, England. Over a 12 hr period, 75% of sediment surface within experimental trays on the river bed were modified by foraging fish, and resulted in changes in bed surface elevation ranging from -98 mm to +92 mm (Pledger, Rice and Millett, 2017). Foraging by barbel and chub led to increases in microtopographic roughness, particle sorting, and grain-size in foraged areas, with larger clasts pushed upstream and finer sediments displaced downstream (Pledger, Rice and Millett, 2017). The impact of foraging fish was found to be widespread, yet spatially heterogeneous across riffles, contributing to overall habitat heterogeneity (Pledger, Rice and Millett, 2017). Subsequent flume entrainment experiments found that foraged sediments were less mobile than non-foraged sediments, with significantly reduced sediment flux under entrainment conditions (Pledger, Rice and Millett, 2017), which is contradictory to earlier flume experiments by Pledger (2014). Reductions in grain entrainment were thought to result from the

large reductions in bed surface elevation caused by fish foraging, as well as the overall coarsening of the sediment resulting from loss of fines (Pledger, Rice and Millett, 2017). Reductions in total bedload flux were also associated with increased flux of coarse sediment (Pledger, Rice and Millett, 2017). The results of the flume experiments likely provide an incomplete picture of the impact of barbel and chub on sediment flux, as the finer sediments ejected from the trays prior to the flume studies would be relatively more mobile than the coarser lag left behind (Pledger, Rice and Millett, 2017). Measured over a wider area of the bed to include areas of fine sediment deposition, the net impact of fish feeding may have provided different results for sediment flux (Pledger, Rice and Millett, 2017).

A series of mesocosm experiments explored how benthic foraging by common bream (*Abramis brama* (Linnaeus 1758)) affects sediment dynamics (Smith, 2019). The results of the mesocosm experiments showed that bream foraging significantly increased water turbidity, and that turbidity levels were positively correlated with fish size, fish number, and food availability (Smith, 2019). Additional mesocosm experiments showed that interspecific competition by common roach (*Rutilus rutilus* (Linnaeus 1758)) further increased turbidity relative to foraging by bream alone, with the same number of fish present (Smith, 2019). The feeding efficiency of roach was also found to be impaired by the high turbidity levels induced by bream foraging (Smith, 2019). Morphological and velocity parameters of bream feeding pits in English lowland streams were scaled and modelled in a recirculating flume to assess their impact on lotic hydraulics (Smith, 2019). Streamwise and vertical velocities increased in the presence of feeding pits, whereas turbulent kinetic energy immediately above the bed was reduced, indicating that bream are significant zoogeomorphic agents in fluvial environments (Smith, 2019).

Rice *et al.* (2019) attempted to evaluate the zoogeomorphic impact of benthic fish foraging across the entire River Trent catchment, England. The spatial extent and abundance of 30 benthivorous fish species across 176 sites were used to create a scoring system to assess their impact, accounting for body size and feeding behaviour (Rice *et al.*, 2019). Benthic feeding was found to

have zoogeomorphic effects at more than 90% of sites, with the magnitude of disturbance dependent on the community composition, and the abundance of key benthivores (Rice *et al.*, 2019).

Invertebrates are also capable of significantly modifying river bed sediments, with their relatively smaller sizes compensated for by high abundances and a diverse range of behaviours (Mason *et al.*, 2019). The foraging behaviour of stoneflies (*Megarcys signata* (Hagen 1874)) has been shown to increase the mobility of fine sediment from the river bed by dislodging it from interstitial spaces (Zanetell and Peckarsky, 1996). However, blackfly (Simuliidae (Newman 1834)) larvae are suspension feeders capable of capturing fine material from the water column and depositing it as faecal pellets on the river bed. The faecal pellets of stonefly can be subsequently transported and deposited downstream, providing food for benthic microbial and invertebrate communities (Wotton *et al.*, 1998).

Silk-spinning caddisfly larvae (Hydropsychidae (Curtis 1835)) construct silk nets and retreats that consolidate sediment by binding grains together, thereby increasing critical shear stress for entrainment (Johnson *et al.*, 2009; Statzner, 2012). Case-building caddisfly larvae such as the Limnephilidae (Kolenati 1848), Glossosomatidae (Wallengren 1891) and Hydroptilidae (Stephens 1836) construct mobile tubular cases out of sand and fine gravel (Mason *et al.*, 2019). The preferential use of specific grain-sizes by caddisflies, and the transportation of the agglutinated grains across the river bed can alter the spatial distribution of sediment and grain mobility (Mason *et al.*, 2019). The grain-sizes utilised, and the shear stresses required to mobilise the cases varies with species, larval stage, and the local availability of sediment (Mason *et al.*, 2019).

The activity of crayfish (Astacoidea (Latreille, 1802)) in rivers and streams can significantly impact sand and gravel flux, increasing bedform roughness and preventing consolidation of particles (Statzner *et al.*, 2000; Statzner, 2012). This in turn reduces critical shear stress which promotes downstream sediment flux, increases water turbidity, and reduces the height of dunes on

the river bed (Statzner, 2012; Harvey *et al.*, 2014). Crayfish behaviour has also been found to decrease the amount of sand in the interstices of gravel on riffles, reduce the amount of filamentous algae on riffles, and reduce the biofilm cover on sand dunes in pools, impacting benthic community structure (Statzner *et al.*, 2000; Statzner, Peltret and Tomanova, 2003).

Flume experiments were used to model the surface and sub-surface flow around the characteristic pit and mound structures constructed by crayfish in gravel river environments (Han *et al.*, 2019). Analysis of near-bed and hyporheic flow showed downwelling and upwelling regions across the pit, and that water is forced into the bed upstream of the mound, re-emerging at the top of the mound (Han *et al.*, 2019). Calculations of shear stress showed a reduced stability of sediment on the upstream edge of the pit and on the leeside of the mound, with implications for sediment dynamics and flux (Han *et al.*, 2019).

Regarding freshwater mussels, parallels can be drawn from extensive studies carried out on marine bivalves. The presence of the sea scallop *Placopecten magellanicus* (Gmelin 1791) on benthic substrates can result in the formation of horseshoe vortex scour structures when subjected to tidal currents. The obstruction caused by such animals on the substrate is thought to influence the resuspension and flux of material and increases the boundary skin friction of the sediment-water interface, depositing grains immediately down-current from the obstacle (Eckman and Nowell, 1984).

Marine mussels (Mytilidae (Linnaeus 1758)) can exist at high densities of more than 600 individuals per m^2 (Fuentes *et al.*, 2000; Green, 2007), which significantly impact characteristics of sediments in their habitat through the removal of suspended algae and particulates from the water column and the subsequent biodeposition on the sea bed. This has the effect of increasing the amount of organic matter, phosphate, nitrogen, ammonia and chlorophyll-a in the sediment and reducing ambient water turbidity and dissolved oxygen concentrations in the environment (Baudinet *et al.*, 1990; Dame *et al.*, 1991; Mirto *et al.*, 2000; Giles and Pilditch, 2006). Marine

mussels form compact bysally attached beds that have been shown to increase bed roughness and thus reduce water velocities, which increases levels of fine-sediment deposition (van Leeuwen *et al.*, 2010). These zoogeomorphic characteristics have been linked to significant alteration of microbial, meiofaunal and macrofaunal benthic communities, increasing the abundance of some taxa and reducing the abundance of others; changes which likely have wider implications for marine and coastal food webs (Dittmann, 1990; Grenz *et al*., 1990; Beukema and Cadee, 1996; Ragnarsson and Raffaelli, 1999; Mirto *et al*., 2000; Walker *et al*., 2014).

The occurrence of freshwater mussels (Unionida (Gray 1854)) in lentic and lotic environments has been associated with increased abundance of predators, herbivores and detritivores such as mayflies, caddisflies, midge larvae, worms, crustaceans, leeches and dragonflies, as well as inducing changes in bacterial and algal communities (Howard and Cuffey, 2006; Vaughn and Spooner, 2006; Burlakova, Karatayev and Karatayev, 2012; Black, Chimenti and Just, 2017). A number of explanations have been proposed to explain such observations, which serve to justify their status as ecosystem engineers. The shells of unionids provide biogenic structure that can be colonised by other species, offer shelter to invertebrates, and introduce calcium carbonate to the river sediment (Vaughn and Hakenkamp, 2001; Gutierrez *et al.*, 2003; Beekey, Mccabe and Marsden, 2004; Spooner and Vaughn, 2006; Vaughn and Spooner, 2006; Zaiko, Daunys and Olenin, 2009; Burlakova, Karatayev and Karatayev, 2012). Ammonia, nitrate, phosphate, phosphorous, organic matter concentrations and benthic community respiration, have all been positively correlated with freshwater mussel biomass, the effects of which could influence multiple trophic levels (Roditi, Strayer and Findlay, 1997; Hakenkamp and Palmer, 1999; Lavrentyev, Gardner and Yang, 2000; Vaughn and Hakenkamp, 2001; Vaughn, Gido and Spooner, 2004; Turner, 2010). A microcosm study by Boeker *et al*. (2016) found that bioturbation activity by duck mussels *Anodonta anatina* (Linnaeus 1758) increased interstitial oxygen concentration and altered nutrient fluxes along with the composition of bacterial communities in the hyporheic zone. Bioturbation and biodeposition from Chinese pond mussels *Sinanodonta*

woodiana (Lea 1834) has also been shown to significantly influence benthic metabolic processes and nutrient mobilisation (Benelli *et al.*, 2017).

Filter feeding by freshwater mussels transfers large quantities of suspended material from the water column to the sediment through deposition of faeces and pseudofaeces. This improves water clarity for the benefit of producers and therefore grazers, whilst the biodeposits are an easily assimilated, nutrient-rich food source for detritivores. Increased abundance of these groups will in turn promote an increased abundance and diversity of predators (Hakenkamp and Palmer, 1999; Vaughn and Hakenkamp, 2001; Beekey, Mccabe and Marsden, 2004; Vaughn, Nichols and Spooner, 2008). Pseudofaeces is bound in mucus which is thought to have a stabilising and consolidating effect on the sediment, reducing the likelihood of resuspension , although one study found that zebra mussel biodeposits were resuspended at fifty percent lower shear stress than that required to resuspend sediments that have been passively deposited (Roditi, Strayer and Findlay, 1997). Burrowing and pedal feeding by freshwater mussels can disturb sediment and facilitate entrainment of particles, however when sessile, their protrusion from the substrate appears to stabilise the sediment by altering boundary layer shear stress (Vaughn and Hakenkamp, 2001; Gutierrez *et al.*, 2003; Spooner and Vaughn, 2006; Zimmerman and de Szalay, 2007).

A laboratory-based experiment in a model river channel investigated turbulent flow around a live mussel *Amblema plicata* (Say 1817), using particle image velocimetry (PIV), with natural sediments as tracer particles (Kumar *et al.*, 2019). The flow data identified a distinct low velocity region in the wake of the mussel when orientated with the posterior of the mussel facing upstream, whereas the flow quickly recovered in the wake of the mussel with the posterior facing downstream. The PIV measurements also showed concentrated vorticity around the mussel's siphons when filter-feeding in a downstream orientation (Kumar *et al.*, 2019). A simulation to characterise flow and turbulence around freshwater mussels was carried out byWu, Constantinescu and Zeng (2020)to clarify the near-bed hydrodynamics around an isolated partially burrowed fatmucket clam *Lampsilis siliquoidea* (Barnes 1823). The capacity of current around

the mussel to form scour, wake and horseshoe vortex structures, and entrain the mussel in the flow were also investigated. The simulation indicated that the level of erosion in the wake of the mussel was larger than comparative objects in equivalent flow conditions due to strong downwelling currents within the horizontal shear layers, as well as counterrotating vortices that induce upwash behind the mussel. Additionally, the simulation showed increased flow disturbance and drag generated by the exhalent siphon when filter feeding, suggesting that filter feeding does not increase the stability of isolated mussels subject to increasing flow velocities. However, the authors note that this may not apply to non-isolated mussels in mussel beds with multiple individuals (Wu, Constantinescu and Zeng, 2020). A study by Sansom *et al*. (2020), using model mussels in an experimental channel, investigated how freshwater mussels impact near-bed turbulent flow. The results of the study showed that at densities of 25 mussels m^{-2} or greater, the maximum Reynolds shear stress is displaced from the sediment-water interface to the height of the mussel canopy. The reduced near-bed flow velocities at densities of 25 mussels m^{-2} or greater resulted in a 64% reduction in the turbulent shear stress acting on individual mussels, indicating that long-term mussel bed stability may be density-dependent (Sansom *et al*., 2020).

1.4 Research objectives and key questions

This thesis aims to address areas of fluvial sedimentology and freshwater ecology in which knowledge and understanding is lacking, with particular focus on the influence that *Anodonta* species have on the sedimentology of fluvial environments. It also considers the implications of any modification of sediment by mussels on the river ecosystem as a whole. The thesis integrates broad areas of research that include freshwater mussel biology and behavioural ecology, river sediment transport, river bed morphology and the influence of fauna on fluvial systems. It is hoped that an improved understanding of the interactions that freshwater mussels have with their environment will better inform subsequent conservation strategies with regard to this group of

animals and other interacting species. The conclusions drawn should also aid in the understanding of local and catchment-wide factors affecting river sediment flux and bedform development, which would be relevant to hydrological modelling and the management of drainage basins.

The research for this thesis involved observation of mussels and the surrounding sedimentary environment of their natural river habitat, and assessed the impact of their addition to, and removal from the river bed over a period of time. The research also included the observation of mussels in a controlled laboratory-based flume setting to investigate their influence on substrate conditions and sediment transport.

The Research Objectives for this project were as follows:

1) Ascertain if the addition or removal of freshwater mussels to/from the beds of two rivers can significantly impact the sedimentological and eco-hydrological characteristics of the river environments, and examine the precise nature of any observed impacts.
2) Determine if freshwater mussels significantly impact sediment dynamics, substrate topography, and hydrological conditions in a laboratory-based mesocosm fluvial environment, and if so, what these impacts are.

Key Questions addressed in this thesis are:

1) To what extent do freshwater mussels impact river bed grain-size distribution patterns?
2) To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?
3) To what extent do freshwater mussels impact sediment transport in rivers?
4) To what extent do freshwater mussels influence river bed topography?
5) To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?
6) To what extent do freshwater mussels influence water quality and habitat conditions in rivers?

Chapter 2 details preliminary research carried out prior to the investigations described in Chapters 3 and 4, and includes a description of all methods used during the project.

Chapter 3 describes a river investigation addressing Research Objective 1, and Key Questions 1, 2, 5 and 6.

Chapter 4 describes a flume-based investigation addressing Research Objective 2, and Key Questions 1, 2, 3, 4, 5 and 6.

Chapter 5 discusses the results of the two investigations and assesses the wider implications and conclusions drawn from the research, including the potential for further areas of study.

Chapter 2: Preliminary Research and Description of Methods

2.1 Introduction

This chapter details the methods used in the field-based and laboratory-based investigations described in this thesis. The process of selecting suitable study sites, and the decisions regarding the choice of methods, equipment and sample sizes for the research is described and discussed.

Section 2.2 details a series of river surveys carried out in Derbyshire, Nottinghamshire and Leicestershire, in the East Midlands of England. The objectives of the river surveys were to establish the location of suitable sites for the river-based investigation detailed in Chapter 3, and to identify populations of unionids that could be used in the flume-based investigation detailed in Chapter 4.

The river-based investigation involved the translocation of mussels from one section of river to another site within the same river in order to determine how the removal of mussels from, and the introduction of mussels to an area would affect river conditions. It was therefore not only important to identify where mussels were abundant, but also to identify where they were absent. It was also essential that the two sites were within the same river system so that the translocation of mussels would not introduce invasive species or pathogens to another river system. Additionally, in order to establish the reproducibility and reliability of the investigation, two identical concurrent studies were carried out in two separate river systems.

Section 2.3 describes the selection of appropriate techniques and equipment for the river-based investigation, once the location of study sites had been established. Section 2.3 also details the preliminary studies carried out to characterise the hydrological and sedimentological conditions of the mussels' habitat so that these conditions could be more accurately replicated in the flume-based study.

Section 2.4 describes methods specific to the flume-based investigation detailed in Chapter 4 of this thesis.

Regarding the selection of target mussel species for this project, much of the focus of freshwater mussel research and conservation in Britain and Europe has been on the endangered freshwater pearl mussel (*Margaritifera margaritifera* (Linnaeus 1758)), as detailed in section 1.2.5 of this thesis. *Margaritifera margaritifera* is not currently found in the East Midlands of England but populations of the depressed river mussel (*Pseudanodonta complanata* (Rossmässler 1835) have been documented (Lopes-Lima *et al.*, 2017; National Biodiversity Network, 2021). As *M. margaritifera* and *P. complanata* are on the IUCN Red List of threatened species (IUCN, 2022), populations of these species were not selected for use in either investigation as the translocation of mussels from one site to another, or into a flume environment would have increased the risk of mortality (Killeen and Moorkens, 2016; Bolden and Brown, 2002). According to Killeen and Moorkens (2016, Forward, para. 3):

> "Translocation [of mussels] is an effective conservation tool but its use either on its own or in conjunction with other conservation solutions needs rigorous justification and is seen as a recourse of last resort."

Translocation of mussels to river sites previously absent of mussels would likely pose an even greater risk of mortality as the absence of mussels may be indicative of unsuitable habitat conditions (Bolden and Brown, 2002). The unionid species targeted in the river surveys for this project were the swollen river mussel (*Unio tumidus* (Philipsson 1788)), the painter's mussel (*Unio pictorum* (Linnaeus 1758)), the duck mussel (*Anodonta anatina* (Linnaeus 1758)) and the swan mussel (*A. cygnea* (Linnaeus 1758)), all of which have been found within the East Midlands (National Biodiversity Network, 2021). These species have a conservation status of "Least Concern" (IUCN 2022), and are abundant and widespread in England (National Biodiversity Network, 2021), thus the mortality risk associated with their translocation is less critical compared

with the more endangered species. The *Unio* and *Anodonta* species could, however, provide a useful analogue to the more endangered species in gaining a greater understanding of their impact on river bed environments, and help guide future freshwater mussel conservation and river restoration efforts.

2.2 Site selection

2.2.1 River survey methodology

Surveys to locate unionid populations in potential sites for the river investigation described in Chapter 3 were carried out between the 10th May and 19th August 2016. The search for suitable sites was based on pre-determined criteria:

1) It was important that the sites were relatively accessible to allow for regular monitoring of the sites over several months. It was therefore preferable that the chosen sites were in proximity to the University of Derby in the East Midlands of England, where the research was based. Rivers within Derbyshire were given priority, followed by those in the adjacent counties of Nottinghamshire and Leicestershire.
2) It was necessary that any potential sites would allow safe entry and movement through the water for the purpose of gathering data. The river therefore needed to be safely wadeable under base flow conditions, so a base flow water depth of no greater than knee height was considered desirable.
3) The river bed ideally needed to be free from underwater, man-made or natural structures and obstacles that could present a hazard when entering the water.
4) For the investigation to more accurately reflect natural flow and river bed conditions, river sites free from channelisation or other overt forms of engineering were preferred.
5) Sites with invasive mussel species such as the zebra mussel (*Dreissena polymorpha* (Pallas 1771)) were avoided in order to reduce the biosecurity risk.

Potentially suitable river reaches were identified initially by using records of the target unionid species on the National Biodiversity Network database (National Biodiversity Network, 2016), and by using information obtainable through Ordnance Survey© maps and satellite imagery. To locate potential populations of mussels for the flume-based study, some lentic environments were also surveyed in order to establish the presence or absence of mussels. Landowners and local authorities were contacted to obtain access to potentially suitable sites that lacked public access.

Locating suitable sites that m*et al*l the aforementioned criteria proved more problematic than originally envisaged. As the *Unio* and *Anodonta* species in Britain have been less well studied compared with *M. margaritifera*, documented records of their estimated abundance and precise locations within British rivers are sparse, with limited detail. An assemblage of *A. anatina* and *A. cygnea* was known to exist in Markeaton Brook in north-west Derby prior to the survey period, with the presence of juveniles indicating active recruitment was taking place. However, its location within a public park put it at risk of disturbance by members of the public so finding alternative locations was preferable. More effective targeting of study sites was enabled by data provided by the Environment Agency in July 2016, which listed recent kick-sample records of *Anodonta* and *Unio* species within the East Midlands. Many of the locations with records of these species were found to be in canals or in rivers that did not meet the pre-determined criteria for the investigation, however, a number of stream and river reaches were listed that were potentially suitable.

As the primary focus of the river-based study was to investigate the impact of freshwater mussels on river bed sediment, it was necessary to search for mussels without excessively disturbing the river bed of any potential study sites. For this reason the use of dredging equipment and kick sampling methods to survey potential river sites was avoided, and a Nuova Rade jointed aquascope (Figure 2.1) was used to view underneath the water to search for mussels. Use of the aquascope required that the water in the river be of a low enough turbidity and depth that specimens could be observed through the water. A location that provided good visibility through

the water ensured that the characteristics of the river bed sediment could also be observed more easily. Visual searches for unionid shells and shell fragments along the banks of surveyed river reaches were also undertaken. The aquascope surveys were only attempted on days when water conditions were favourable enough to view the river bed, as high flow and turbid water would have limited its effectiveness.

The survey methodology for stream and river reaches entailed a continuous transect (conditions allowing) that followed a zig-zag pattern upstream moving at a 45° angle across the channel from bank to bank, such as in the method described by Young *et al*. (2001) and Harriger, Moerke and Badra (2009). Accessible reaches of the chosen rivers were surveyed with this method and the locations of any mussels or shell debris were recorded using a handheld Garmin Dakota 20 GPS device. This method was selected to span a wide range of depths and microhabitats within each stretch of river and therefore maximise the chance of encountering mussel aggregations, should they be present. Along each river reach surveyed, the dominant bed sediment type was recorded, along with any other information about the river pertinent to its potential use as a study site.

Figure 2.1: Nuova Rade jointed aquascope used for viewing beneath the surface of the water. Image from Nuova Rade (2019).

2.2.2 River survey results

Each river reach and lentic location surveyed was designated a separate site number, with 46 sites surveyed in total. Overview maps of the numbered river reaches and lentic sites surveyed within the East Midlands of England are shown are shown in Figures 2.2 – 2.6. The full survey results are described in Table 2.1, which also includes the site number and grid references for river reach and lentic site surveyed, a brief description of each site, and the freshwater mussel species found. The grid references provided for each river site mark the downstream and upstream limits of each stretch of river surveyed.

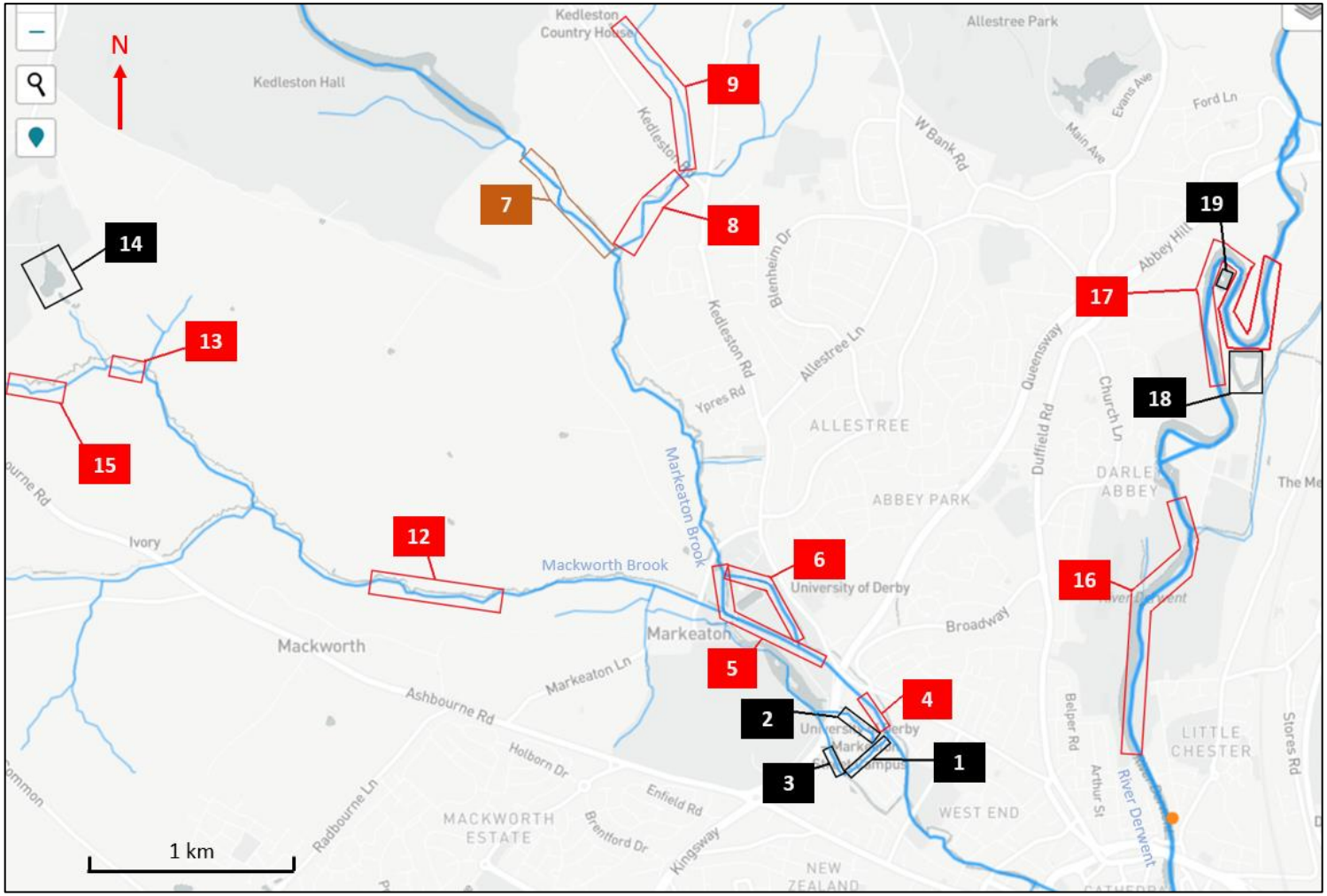


Figure 2.2: Numbered river reaches and lentic sites surveyed to the north and north-west of Derby, Derbyshire. Freshwater mussels were found in the river reaches and lentic sites outlined and numbered in black. Freshwater mussel shell debris was found in the river reach outlined and numbered in brown. No mussels were found in the river reaches outlined and numbered in red. Details of each numbered site are described in Table 2.1. The orange dot on the map is a river gauging station on the River Derwent. Base map sourced from National River Flow Archive, UK CEH (2023). © UKCEH 2023 Open Government Licence v3.0.

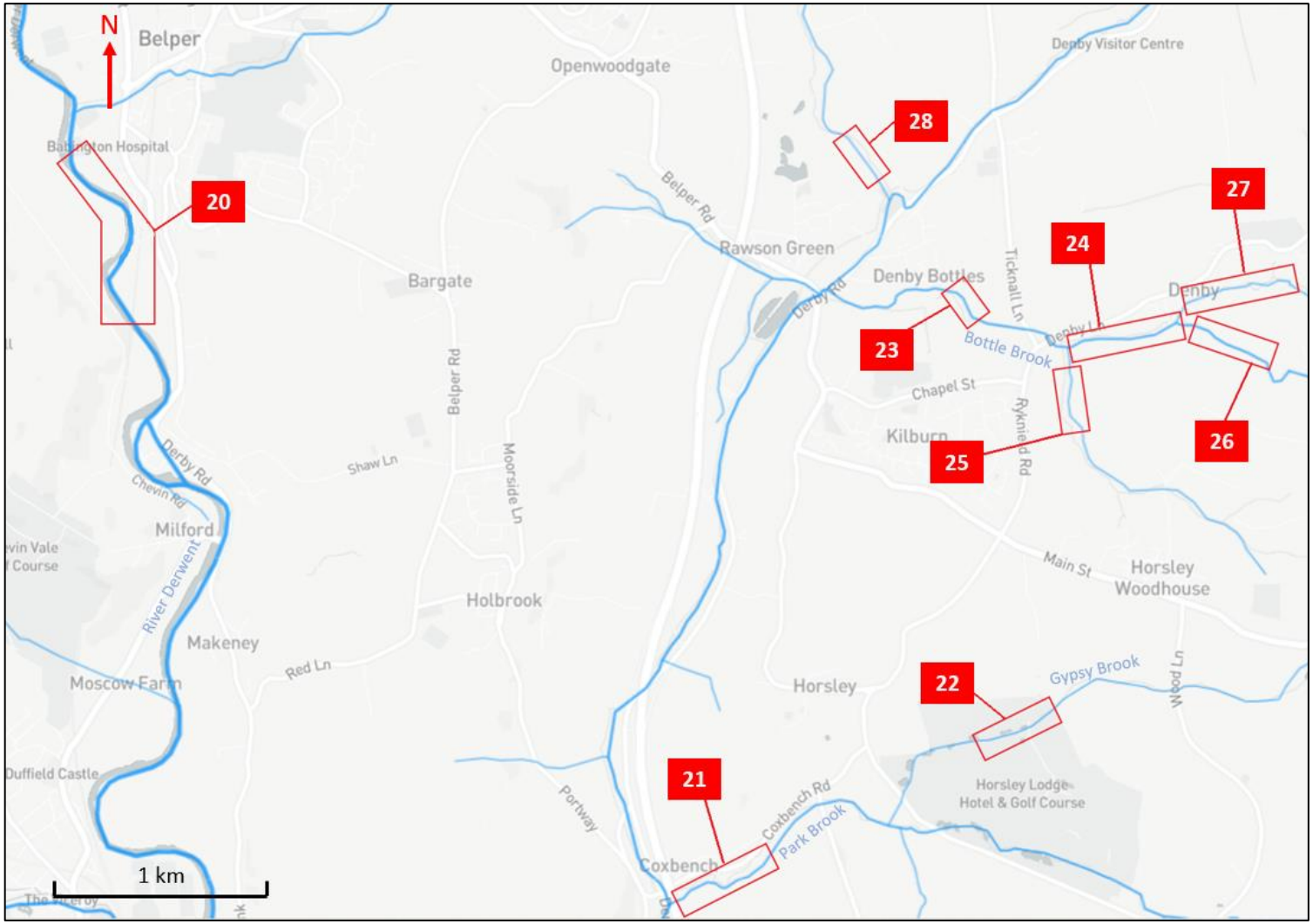


Figure 2.3: Numbered river reaches surveyed to the south and south-east of Belper, Derbyshire. No mussels were found in the river reaches outlined and numbered in red. Details of each numbered site are described in Table 2.1. Base map sourced from National River Flow Archive, UK CEH (2023). © UKCEH 2023 Open Government Licence v3.0.

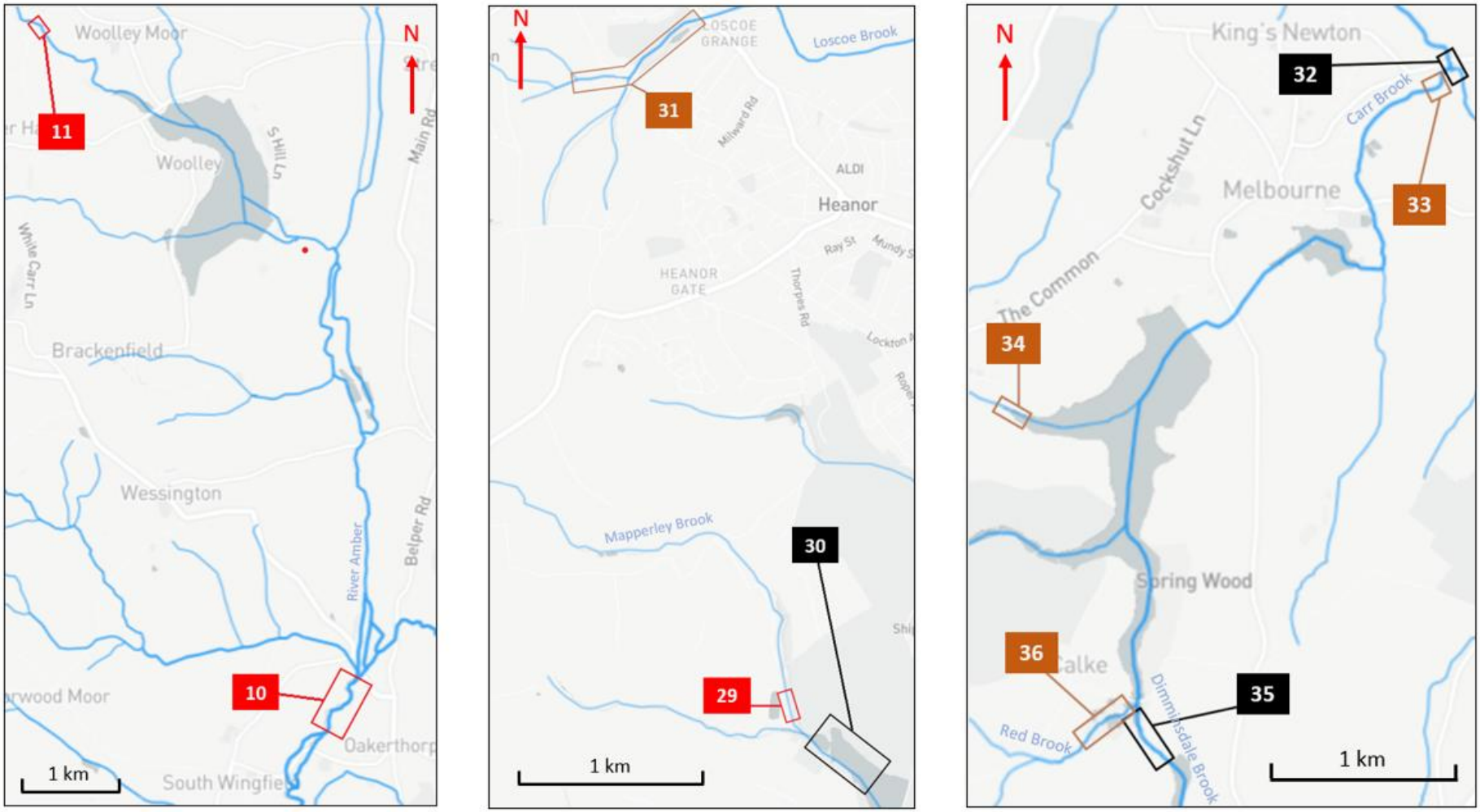


Figure 2.4: Numbered river reaches and lentic sites surveyed to the north of Belper, Derbyshire (left), north-west and south of Heanor, Derbyshire (middle), and to the north-east and south-west of Melbourne, Derbyshire (right). Freshwater mussels were found in the river reaches and lentic sites outlined and numbered in black. Freshwater mussel shell debris was found in the river reaches outlined and numbered in brown. No mussels were found in the river reaches outlined and numbered in red. Details of each numbered site are described in Table 2.1. Base maps sourced from National River Flow Archive, UK CEH (2023).

Figure 2.5: Numbered river reaches surveyed to the south of Trowell, Nottinghamshire (left), east and south of Heather, Leicestershire (middle), and to the west of Thrumpton, Nottinghamshire (right). Freshwater mussels were found in the river reaches outlined and numbered in black. Freshwater mussel shell debris was found in the river reaches outlined and numbered in brown. No mussels were found in the river reaches outlined and numbered in red. Details of each numbered site are described in Table 2.1. Base maps sourced from National River Flow Archive, UK CEH (2023). © UKCEH 2023 Open Government Licence v3.0.

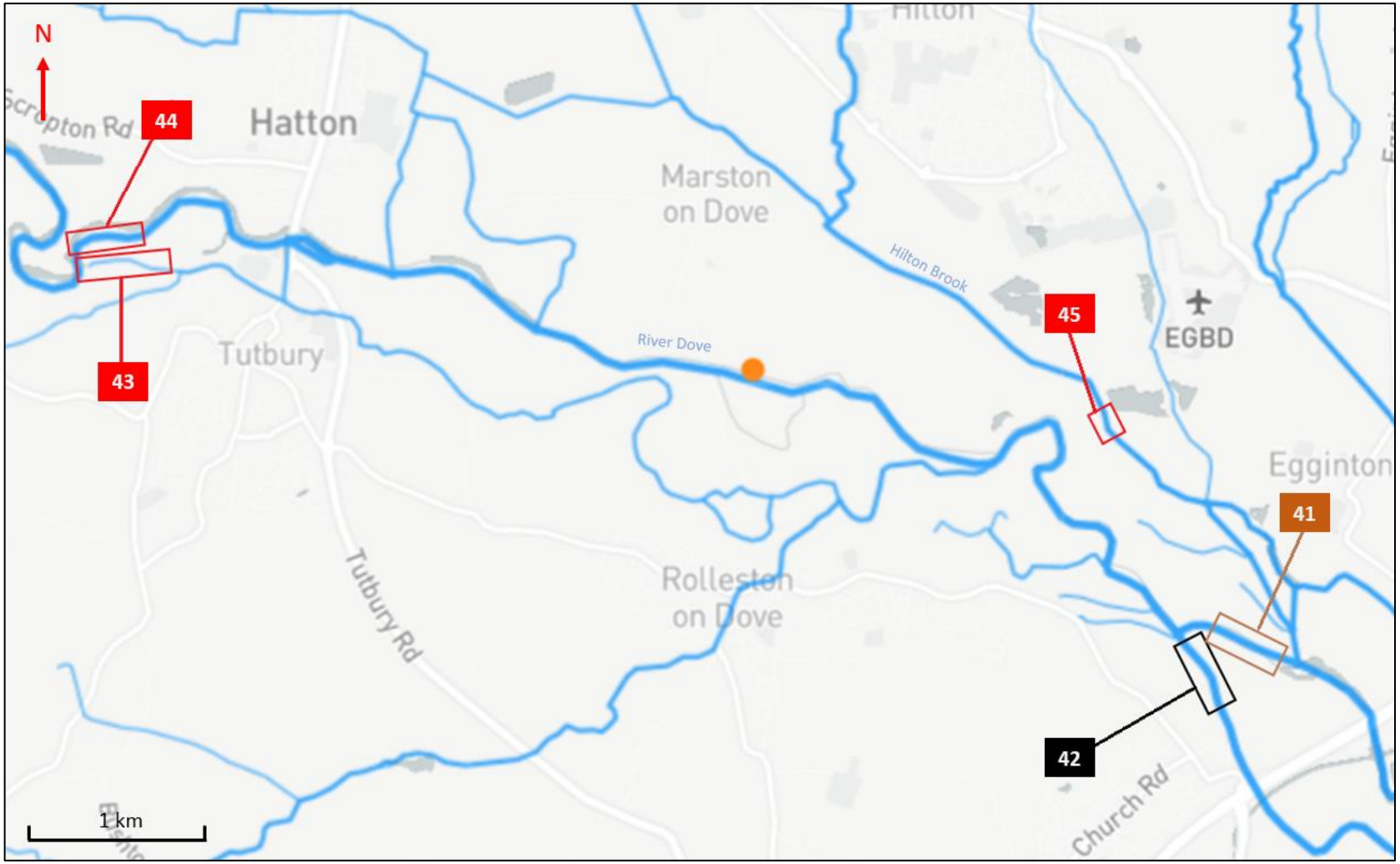


Figure 2.6: Numbered river reaches surveyed to the west of Egginton, Derbyshire. Freshwater mussels were found in the river reaches and outlined and numbered in black. Freshwater mussel shell debris was found in the river reaches outlined and numbered in brown. No mussels were found in the river reaches outlined and numbered in red. Details of each numbered site are described in Table 2.1. The orange dot on the map is a river gauging station on the River Dove. Base map sourced from National River Flow Archive, UK CEH (2023). © UKCEH 2023 Open Government Licence v3.0.

Table 2.1: Results of the river surveys showing the location and description of the river reaches and lentic sites surveyed, and the mussel species of mussel found.

Site	Survey date	River reach/ lentic site designation	UK grid reference	Site characteristics	Occurrence of mussels
1	10/05/16	Markeaton Brook 1	SK 34062,37257 to SK 33907,37112	Sand, gravel and cobble river bed with muddy banks next to public footpath.	*Anodonta anatina* and *A. cygnea* present.
2	10/05/16	Markeaton Brook 2	SK 34007,37187 to SK 33857,37387	Silt, sand, gravel, cobble and exposed bedrock. Adjacent to public park.	Adult and juvenile *Anodonta* and *A. cygnea.*
3	10/05/16	Markeaton Brook 3	SK 33847,37112 to SK 33787,37222	Fishing lake with stream inflow and outflow. Too deep to wade into.	*Anodonta cygnea* present.
4	17/05/16	Markeaton Brook 4	SK 34067,37262 to SK 33947,37457	Sand, gravel and cobble bed sediment with muddy banks. Adjacent to public park.	No mussels found.
5	17/05/16	Markeaton Brook 5	SK 33727,37662 to SK 33282,38062	Markeaton Park. Sand and gravel bed sediment. Weirs and channelisation present.	No mussels found.
6	17/05/16	Markeaton Brook 6	SK 33652,37702 to SK 33397,37912	Small tributary. Silt/clay bed sediment.	No mussels found.
7	21/05/16	Markeaton Brook 7	SK 32722,39547 to SK 32347,40012	Clear water, gravel bed sediment. Adjacent to farmland.	Found mussel shell fragments but no live mussels.
8	21/05/16	Markeaton Brook 8	SK 32782,39522 to SK 33067,39867	Small tributary. Discharge too low to be suitable.	No mussels found.
9	21/05/16	Markeaton Brook 9	SK 33072,40157 to SK 32757,40627	Highly vegetated small stream. Silt/clay substrate.	No mussels found.

10	24/05/16	River Amber 1	SK 38566,56127 to SK 38376,55862	Mostly channelised with poor access. One good access point that is regularly disturbed by people and dogs.	No mussels found.
11	24/05/16	River Amber 2	SK 35946,61572 to SK 36066,61497	Shallow and biodiverse sand and gravel stream with clear water and crayfish.	No mussels found.
12	01/06/16	Mackworth Brook 1	SK 32357,37937 to SK 31642,37972	Difficult to access river. Mixed bed sediment. Appropriate depth and discharge.	No mussels found.
13	16/06/16	Mackworth Brook 2	SK 30582,38912 to SK 30422,38967	Narrow in places. Unlikely suitable due to low flow.	No mussels found.
14	16/06/16	Mackworth Brook 3	SK 30132,39342	Meynell Langley lake	*Anodonta cygnea* present.
15	16/06/16	Flagshaw Brook	SK 30187,38882 to SK 29917,38897	Narrow stream with mixed substrate and exposed bedrock	No mussels found.
16	27/06/16	River Derwent 1	SK 35192,37382 to SK 35347,38442	Sand and mud substrate. Gravel in places. Too deep to access the middle of the channel.	No mussels found.
17	01/07/16	River Derwent 2	SK 35582,38987 to SK 35832,39282	Silt/clay, sand and gravel. Too deep to access middle of channel.	No mussels found.
18	01/07/16	Haslams Lake	SK 35752,39047	Private fishing lake with muddy bottom.	Abundant *A. cygnea.*
19	01/07/16	Woodrow's Pond	SK 35632,39437	Private fishing lake with muddy bottom.	Abundant *A. cygnea.*
20	05/07/16	River Derwent 3	SK 34502,46762 to SK 34197,47587	Sandy substrate adjacent to farmland and public footpath. Deep in places.	No mussels found.

21	06/07/16	Park Brook	SK 37292,43567 to SK 37667,43762	Highly eutrophic water conditions and areas of stagnant water. Gravel and silt/clay bed sediment.	No mussels found.
22	06/07/16	Gypsy Brook	SK 38567,44212 to SK 38862,44317	Narrow stream, silt/clay bed sediment. Very low flow.	No mussels found.
23	07/07/16	Bottle Brook 1	SK 38492,46242 to SK 38587,46162	Lots of litter and fly-tipped material. Gravel and cobble substrate.	No mussels found
24	07/07/16	Bottle Brook 2	SK 38957,46047 to SK39607,46262	Channelised in places. Pebbles and cobble substrate. Grazed land.	No mussels found.
25	09/07/16	Bottle Brook 3	SK 38957,46022 to SK 38977,45732	Grazed land. Shallow vegetated channel.	No mussels found.
26	09/07/16	Bottle Brook 4	SK 39532,46107 to SK 39767,46037	Difficult access in places. Vegetated, silt/clay substrate.	No mussels found.
27	09/07/16	Bottle Brook 5	SK 39662,46312 to SK 39917,46352	Good water quality but low flow. Silt/clay and sand substrate.	No mussels found.
28	26/07/16	Bottle Brook 6	SK 38077,46837 to SK 38002,46902	Adjacent to old coal workings so possibly too acidic and polluted.	No mussels found.
29	27/07/06	Mapperley Brook	SK 42932,44002 to SK 4297,43892	Difficult to access due to vegetation and boggy ground. Small stream.	No mussels found.
30	27/07/16	Mapperley Reservoir	SK 43262,43712	Silt/clay substrate. Reservoir with public footpath.	Numerous *Unio pictorum and Anodonta anatina.*

31	29/07/16	Loscoe Brook	SK 42307,47407 to SK 41512,47167	In public park. Silt/clay substrate. Shallow, small stream with slow turbid water.	Broken *Anodonta* shells found but no living mussels observed.
32	01/08/16	Ramsley Brook	SK 39813,25867 to SK 39803,25967	Clear water but silt/clay substrate generates turbidity when moving around. On farmland.	*Anodonta anatina* present.
33	01/08/16	Carr Brook	SK 39708,25972 to SK 39783,25982	Difficult to access. Silt/clay substrate made viewing difficult.	Shell material found but no individuals found.
34	01/08/16	Scots Brook	SK 36593,23867 to SK 36668,23847	Small, silt/clay bottom stream.	Shell material only.
35	04/08/16	Dimminsdale Brook	SK 37833,21577 to SK 37728,21742	Clear water with sand, gravel and silt/clay, which generates turbidity when accessing water.	Numerous *A. anatina* and *A. cygnea* but presence of *Dreissena polymorpha* shells pose a biosecurity risk.
36	04/08/16	Red Brook	SK 37453,21682 to SK 37633,21832	Small stream with mixed substrate next to major footpath and regularly disturbed.	Shell material found but no live mussels.
37	05/08/16	River Erewash	SK 48312,38997 to SK 48357,38862	Gravel and silt/clay substrate. Wadeable in places but generally deeper than ideal.	*Anodonta cygnea* found plus some shell material.
38	08/08/16	River Sence 1	SK 39514,10987 to SK 39484,1077	Gravel, grit, sand and silt/clay bed sediment. Lots of algal growth but clear water.	Dense mussel bed of mainly *A. anatina* and occasional *A. cygnea.*
39	08/08/16	River Sence 2	SK 394589,10607 to SK 39424,10562	Mixed bed sediment stream close to water treatment works.	Occasional *A. anatina* present.

40	08/08/16	River Sence 3	SK 38074,08747 to SK 38149,08772	Coarser bed sediment than previous sites with abundant algal growth and clear water.	No mussels found.
41	11/08/16	River Dove 1	SK 26248,27477 to 25968,27592	Deep and fast water in places. Mixed river-bed sediment.	Large piles of *Anodonta* shells on banks but no live specimens found.
42	11/08/16	River Dove 2	SK 26098,27177 to SK 25898,27487	Channelised section with cobble and silt/clay bed sediment.	Occasional *A. anatina* present.
43	15/08/16	River Dove 3	SK 20712,29347 to SK 20542,29367	Adjacent to public park. Silt/clay and gravel bed sediment.	No mussels found.
44	15/08/16	River Dove 4	SK 20687,29502 to SK 20482,29487	High velocity flow. Mixed substrate with clear water.	No mussels found.
45	15/08/16	Hilton Brook	SK 25648,28462 to SK 25543,28567	Wadeable biodiverse gravel stream of suitable depth and velocity.	No mussels found.
46	19/08/16	River Soar	SK 49177,30637 to SK 49287,30857	River too deep and turbid to view in the middle so searches confined to the shallower areas near the banks.	*Anodonta* shells found but no living mussels observed.

2.2.3 Conclusions of river survey

Anodonta anatina were found at 8 of the 46 sites surveyed, 7 of which were river reaches and one of which was a reservoir site. *Anodonta cygnea* were found at 9 sites, 5 of which were river reaches and 4 of which were within lakes. *Unio pictorum* were found at only one site, which was in a reservoir. At 7 of the 46 sites surveyed only unionid shell material was found, but no live mussels. No mussels or shell material were found at 26 of the locations surveyed.

Although 13 of the 46 survey sites were found to contain freshwater mussels, many of these were not suitable for the planned river study as they did not meet the predetermined criteria. Some locations were in lakes and reservoirs, and although unsuitable for the river study they could potentially have provided a source of mussels for the ex-situ flume study. Some river reaches were found to have too deep or too turbid water for the study, whereas others were in channelised sections of river or contained only very low densities of mussels.

A potentially suitable assemblage of *Anodonta anatina* and *A. cygnea* was found living in Dimminsdale Brook (Site 35, Figure 2.4 and Table 2.1) in Dimminsdale Nature Reserve, which is managed by the Leicestershire and Rutland Wildlife Trust. However the soft silt-clay bottom made it difficult to access the stream without causing significant disturbance to the bed sediment. Furthermore, numerous *Dreissena polymorpha* shells were found in the stream, which would have presented a risk to biosecurity as they are an invasive species in the UK.

The River Sence in Leicestershire was found to have dense aggregations of *Anodonta anatina* and *A. cygnea* at Site 38 (Figure 2.5 and Table 2.1) and no mussels present at Site 40 (Figure 2.5 and Table 2.1), which lies approximately 3 km downstream of Site 38. Both these sites met the predetermined criteria and were therefore selected for the river study. Markeaton Brook in Derbyshire contained a suitable assemblage of *A. anatina* and *A. cygnea* at Sites 1 and 2 (Figure 2.2 and Table 2.1) and an absence of mussels at Site 4 (Figure 2.2 and Table 2.1). The location of these sites next to a public park put them at risk of being disturbed by members of the public,

however, as no other suitable sites were found within the timeframe available, Sites 2 and 4 were selected for the river study. Due to the close proximity of Markeaton Brook to the University of Derby, mussels from Site 1 and Site 2 were also used in the flume-based investigation.

2.3 Preliminary river studies and description of methods

2.3.1 Review of river bed sediment sampling methods

A number of techniques for sampling river bed sediments were evaluated in order to determine the most appropriate for the planned investigation. As described in section 1.3.1, the grain-size distribution of the surface layer of sediment in a gravel river is often much coarser than that of the sub-surface layer due to the formation of an armour layer (Mosley and Tindale, 1985; Reid, Frostick and Layman, 1985; Diplas and Sutherland, 1988). Due to textural differences between the armour and sub-armour layers, and the difficulty in obtaining a volumetric sample of the surface layer, the armour and sub-armour layers are often sampled separately (Mosley and Tindale, 1985; Diplas and Sutherland, 1988; Petts *et al.*, 1989; Thoms, 1992).

The sampling method detailed by Wolman (1954) for determining the grain-size of surficial sediments on a gravel river bed involves measurement of the B-axis of 100 pebbles using a grid system; the B-axis being the intermediate-length axis of the pebble (Figure 2.7). Measurement of the B-axis allows comparison with sediments that have been classified through sieving, which separates grains by their B-axis length. Other researchers have subsequently found that sample sizes of between 30 and 70 pebbles are sufficient for defining grain-size parameters of a gravel-bed river (Brush and Nolan, 1961; Hey and Thorne, 1983; Mosley and Tindale, 1985). According to Wolman (1954), pebbles from the surface of the river bed can be randomly or systematically selected according to their position within a sampling grid, or by pacing and selecting the pebble at the tip of the operator's boot. The size of each selected pebble is then measured using the Krumbein phi (φ) scale (Krumbein, 1934), categorised using the Wentworth scale (Wentworth,

1922), and then frequency curves are plotted to show the grain-size distribution pattern and facilitate the calculation of median grain-diameter (D_{50}), grain-size percentiles, and grain-size sorting values (Wolman, 1954).

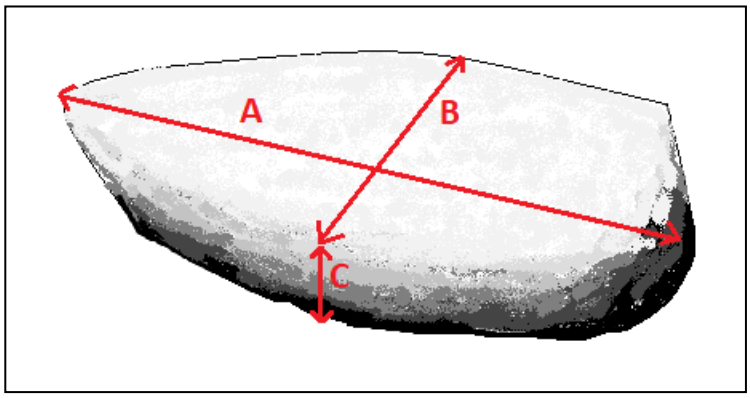


Figure 2.7: Diagram to show pebble axes, with "A" being the longest axis, "B" being the intermediate axis and "C" being the shortest axis of the pebble. © Andrea Leng.

The Wolman method has the advantage of being able to provide a representative sample of coarse sediments across a reach of a stream, for example, a pool, riffle or bar, but it is limited to measuring only the surface layer of sediments, and to pebbles with a B-axis diameter of greater than 2-4 mm (Wolman, 1954). The Wolman method can cause disturbance to stream habitats and is time-consuming over large areas, which could prove problematic when repeated surveys are required (Pearson *et al.*, 2017). There is also potential for operator error due to differences between the selection and measurement of pebbles between operators (Hey and Thorne, 1983). A study on gravel bed material by Hey and Thorne (1983) showed that with the Wolman method, as sample size increases the difference between operators becomes statistically significant, with sample sizes of 30, 60 and 100 pebbles showing no significant differences in the B-axis values, but sample sizes of 120, 180 and 300 showing significant differences between operators. Depending on the sample size, size of the sampling grid, and the variability of the river bed sampled, sampling error can also be introduced, resulting in differences between the sample mean and the population mean (Hey and Thorne, 1983).

An alternative method of sampling river bed sediments is to obtain an areal sample of the sediment surface layer through photography or the application of a wax or adhesive to remove the upper layer of sediment (Diplas and Sutherland, 1988; Diplas and Fripp, 1992). Areal sampling methods are more easily applied to dry sediments with little or no pebble imbrication, and it is widely accepted that areal samples are biased towards the coarser fractions compared with volumetric samples of the same material (Diplas and Sutherland, 1988; Diplas and Fripp, 1992). The presence of voids in the sediment can also complicate analysis due to grains from the sub-surface layer appearing through voids in the surface layer (Diplas and Sutherland, 1988). It is possible to convert grain-size distributions obtained through areal methods to the volumetric equivalent with the use of mathematical conversion models, for example with the modified cube model (Diplas and Sutherland, 1988), which accounts for sediment porosity. However, the high degree of variability in fluvial grain-size distribution curves and differences in porosity can result in varying degrees of bias (Diplas and Fripp, 1992).

More recent developments in the quantification of the grain-size of surface sediments in gravel rivers involve the use of optical granulometry or digital "photo-sieving" techniques to characterise the planimetric dimensions of grains using photographic images of the river bed (Carbonneau, Bergeron and Lane, 2005; Langhammer *et al.*, 2017; Pearson *et al.*, 2017; Woodget, Fyffe and Carbonneau, 2018). Optical granulometry enables analysis of calibrated digital photographs from an underwater camera or Unmanned Aerial Vehicle (UAV) imagery, and has the advantage of allowing rapid, non-invasive sampling of fluvial sediments (Langhammer *et al.*, 2017). Although data collection using photo-sieving methods can be rapid, the post-processing of images can be laborious, and may require manual identification of grain boundaries (Heritage and Milan, 2009). Furthermore, detail of sediments may be partially obscured by surface reflections, and the long exposure times required to capture images in limited light levels can result in motion blur (Langhammer *et al.*, 2017; Woodget and Austrums, 2017). The development of automated image processing software has improved the efficiency of optical granulometry analytical methods

(Carbonneau, Bergeron and Lane, 2005; Heritage and Milan, 2009). Further technological advances have involved the use of survey methods such as Airborne Laser Scanning (ALS) and Terrestrial Laser Scanning (TLS) to produce high resolution Digital Elevation Models (DEMs) utilising topographic data to characterise grain-size distributions, grain roughness, and changes in river bed elevation (Milan, Heritage and Hetherington, 2007; Heritage and Milan, 2009; Milan *et al.*, 2011; Pearson *et al.*, 2017; Woodget, Fyffe and Carbonneau, 2018).

Depending on the resolution of the imagery, analysis of grain-size data from photography and laser scanners is generally limited to the coarser sand and gravel fractions, and to clearly visible sediments, such as in dry, exposed parts of the channel, or clear, shallow-water areas, free from vegetation (Milan, Heritage and Hetherington, 2007; Heritage and Milan, 2009; Langhammer *et al.*, 2017; Pearson *et al.*, 2017; Woodget, Fyffe and Carbonneau, 2018).

Characterisation of the sub-surface and finer fractions of fluvial sediments can be achieved using a range of volumetric sampling methods. Volumetric methods often involve the extraction of a bulk sample of the river bed using various shovels, grab samplers and coring devices (Mosley and Tindale, 1985; Petts *et al.*, 1989; Young *et al.*, 1991; Thoms, 1992; Milan, 1994, 1996; Schuett-Hames *et al.*, 1996; Méndez *et al.*, 2003; Somerfield and Warwick, 2013). Depending on characteristics of the river bed, the extraction of a representative bulk sample can be physically challenging, and the presence of tightly-packed cobbles and boulders can impede the penetration of sampling equipment into the river bed (Thoms, 1992). Removal of the armour layer to enable sampling of the finer sub-surface sediments can result in loss of fines (silt and clay fractions), and has the disadvantage of excluding the layer of sediments most relevant to stream hydraulics from volumetric analysis (Petts *et al.*, 1989). Additionally, withdrawal of the extracted bulk sample through the water column can result in significant loss of fines, particularly when a shovel or grab-sampler is used (Petts *et al.*, 1989; Thoms, 1992; Schuett-Hames *et al.*, 1996).

Loss of fines during extraction of bulk samples can be partially mitigated with the use of a stilling well (such as a bucket without a bottom) to reduce the effect of the current on the sample being collected (Young *et al.*, 1991; Schuett-Hames *et al.*, 1996). Alternatively, loss of fines can be minimised with the use of a McNeil core sampler, which consists of a coring tube driven into the stream bed, and a sediment collection cylinder extending to the water surface (Schuett-Hames *et al.*, 1996). Once the river bed sediment has been removed from the core of the McNeil sampler into the collection cylinder, the base of the cylinder is capped to prevent loss of sediment whilst it is lifted from the water (Schuett-Hames *et al.*, 1996). The McNeil sampler has been shown to provide relatively accurate and representative sampling of overall substrate composition (Young *et al.*, 1991; Schuett-Hames *et al.*, 1996). Disadvantages of the McNeil sampler include difficulty inserting it into coarse or compacted sediment, and also that samples can be heavy and cumbersome to carry due to the amount of water collected (Schuett-Hames *et al.*, 1996).

Another method of collecting sediment samples is with the use of a hand-held piston corer, which employs suction to retain sediment in the coring tube whilst it is withdrawn, and reduces loss of fines and vertical mixing of the sedimentary layers compared with grab-samplers and shovels (Méndez *et al.*, 2003; Somerfield and Warwick, 2013). Freeze-corers use a cryogenic medium such as liquid carbon dioxide or liquid nitrogen to freeze sediment to the walls of the extraction tube (Petts *et al.*, 1989; Young *et al.*, 1991; Thoms, 1992; Dück *et al.*, 2019). By freezing the sediment prior to extraction, loss of fines and disturbance to the vertical sedimentary profile is reduced (Petts *et al.*, 1989; Young *et al.*, 1991; Schuett-Hames *et al.*, 1996). The maximum grain-size sampled by freeze-corers is limited by the diameter of the collection tube, and depending on the model used, they can be prone to undersampling or oversampling certain grain-sizes (Petts *et al.*, 1989; Young *et al.*, 1991; Schuett-Hames *et al.*, 1996).

Whichever bulk-sampling method is used, the removal of bulk samples is inherently destructive, and due to the high degree of lateral variability in fluvial environments, the quantity of sediment required to provide a representative sample can be significant (Mosley and Tindale, 1985; Thoms,

1992). A study by Mosley and Tindale (1985) found that 200 bulk samples, each weighing 100 kg was necessary to estimate mean grain-size of a gravel river bed to within ±10 %. However, due to the impracticability of sampling such large volumes it was concluded that ensuring that the largest grain is less than 5% of the total sample volume resulted in no significant bias (Mosley and Tindale, 1985).

The grain-size distribution of extracted bulk samples can be characterised through a different methods depending on the range of grain-sizes present. Sieve analysis can separate sand and gravel-sized sediments according to their Wentworth class (Wentworth, 1922), and the silt and clay fractions can be characterised based on their sedimentation rate in water with the application of Stokes' Law (the sieve-pipette method), or alternatively, using laser diffraction particle-size analysis (Beuselinck *et al.*, 1998; Akhurst *et al.*, 2011; Dinis and Castilho, 2012).

2.3.2 Preliminary river studies

Preliminary investigations for the river-based study described in Chapter 3 of this thesis were carried out in Markeaton Brook between the 16th March and the 2nd May 2017. The section of Markeaton Brook investigated was what is referred to as Site 1 (Figure 2.2, Table 2.1) described in Section 2.2.2. Site 1 was selected as it was immediately downstream of Site 2, which was chosen for the river-based study. This meant that the collection of sediment samples would not disturb the area of river bed chosen for the main investigation, yet it contained similar densities of *Anodonta anatina* and *A. cygnea* to Site 2, and appeared to have similar hydrological conditions. Mussels from this site were also used in the flume-based study described in Chapter 4.

Decisions regarding the most appropriate sampling techniques and sample sizes were guided by the local river bed conditions at the sites used, and the resident mussel populations. The river study described in Chapter 3 required the translocation of mussels from mussel-dense locations to locations where mussels were absent. The risks associated with translocating large quantities of

mussels to sites that lack resident mussel populations were discussed in Section 2.1. A study by McIvor (2004) involving the translocation of 6000 freshwater mussels to new freshwater sites resulted in survival rates of 47% for *A. anatina* and only 5% for *P. complanata* within five months of them being moved. The results of the river surveys described in Section 2.2.2 indicated that the mussel assemblages present in Markeaton Brook and the River Sence were dominated by *A. anatina*, and restricted to short, isolated reaches of each river. If the planned investigation led to the high mortality rates observed in the McIvor study, this could threaten the future viability of the resident mussel populations, and have implications for the wider river ecosystem. It was also considered that if the population density of mussels in the study was too low, the mussels would be unlikely to have a significant impact on the river bed conditions.

The mussel density at the River Sence site was relatively high, with approximately 50 mussels/m^2, whereas at Markeaton Brook, mussel density was relatively low with 1-2 mussels/ m^2 up to approximately 20 mussels/ m^2 in the most dense part of the mussel bed. To maintain mussel densities approximately equivalent to the high-density reaches without having to risk moving and potentially killing large quantities of mussels, it was determined that the study area within each of the four sites in the planned river investigation would be limited to 4 x 1 m^2 quadrats at each site.

For the reasons discussed in Section 2.3.1 the armour layer and the subsurface sediment were sampled separately using different methods. The restricted quadrat area used in the river study and the presence of mussels within the quadrats influenced the sediment sampling techniques chosen. As the primary focus of the river investigation was to understand the impact of freshwater mussels on river sediment, it was important that the method of sampling caused minimal disturbance to the river bed sediment. Sediment sampling methods such as shovels, grab-samplers and McNeil samplers would have been too destructive to the river bed, and repeated samples within each quadrat would have quickly obliterated any sedimentary changes arising from the presence of mussels. Even the use of commonly-used coring devices such as freeze-corers and

piston corers would have caused significant disturbance to the river bed sediment within each 1 m^2 quadrat if repeated samples were taken over the duration of the study.

The surficial sediments in both the Markeaton Brook and River Sence sites were dominated by a clast-supported armour layer of tightly-packed gravel, cobbles and boulders, whereas the sub-surface sediments comprised a greater proportion of sand, silt and clay with abundant gravel and cobble clasts. Exploratory attempts were made at driving various metal and plastic coring tubes of different diameters into the river bed. However, the presence of frequent coarse bedload at depth meant that the use of coring equipment was extremely difficult and often impossible, even after the coarser armour layer had been removed. To address the problems associated with using the larger coring devices, a 20 ml plastic Luer-Lok syringe was modified by sawing off the tip to form a 90 mm long and 18 mm diameter piston-corer, similar to that described by Somerfield and Warwick (2013) for sampling sediments. To extract a sediment sample from the finer river bed sediment, a small area of the armour layer just large enough to push the syringe through was carefully parted, or if necessary, removed. The plunger of the corer was withdrawn from the barrel of the syringe to allow a 50 mm depth of sediment to enter the corer as it was pressed into the sediment. The plunger was then withdrawn to the full 90 mm in order to generate a negative pressure that held the sediment inside the syringe as it was removed from the river. Although the small diameter and length of the modified syringe limited the depth of sediment and the size of grains that could be extracted from the river bed, it allowed easier penetration into the finer matrix between the gravel clasts, whilst causing minimal loss of fines or disturbance to the river bed. The upper 50 mm of river bed sediment sampled by the syringe is within the zone most likely impacted by freshwater mussel activity (Buddensiek *et al.*, 1990; Quinlan, Malcolm and Gibbins, 2014). Furthermore, it was intended that the grain-size distributions of sediment samples from the river and flume investigations would be characterised through laser diffraction particle-size analysis, which requires only very small volumes of sediment for analysis. Laser diffraction

particle-size analysis enables more precise differentiation of the finer silt and clay fractions affected by mussel filter-feeding and biodeposition compared with sieve analysis.

For sampling of the armour layer, the use of photographic or laser-scanner methods of quantifying grain-size distributions would have avoided excessive disturbance to the river bed. A laser-scanner was not available for use in the investigation but the use of optical granulometry was considered. However, due to the turbidity of the water at the river sites and reflections on the water surface, capturing images of the river bed from above the water, clear enough to differentiate grain boundaries proved difficult. Additionally, the shallow depth of water meant that use of an underwater camera was not feasible as it couldn't be held at a sufficient height above the river bed to capture the images. For the reasons discussed in Section 2.3.1, photogrammetric methods also have an increased potential for error when estimating the B-axis diameter of pebbles in fluvial environments compared with the Wolman method. It was therefore decided that the Wolman method of quantifying the grain-size distribution of the armour layer was preferable. The small study area for the planned investigation also justified the use of the more laborious Wolman method, whereas if the study area had comprised an entire river reach, it may have been more desirable to choose a more time-efficient sampling method. To minimise disturbance to the river bed resulting from the Wolman method of sampling, the armour layer was only sampled at the beginning and the end of the river investigation (detailed in Chapter 3). The main focus of the investigation was on the finer sedimentary fractions collected with the modified syringe as it was thought that mussel filtration and biodeposition would have a greater impact on these grain-sizes, therefore cores of the finer sediment were collected throughout the study.

Although various researchers have previously attempted to establish optimal sample sizes for characterising gravel river grain-size distributions (described in Section 2.3.1), much of this work has been carried out on larger sections of stream with a greater complexity of topographic features compared with the planned river investigation (Wolman, 1954; Brush and Nolan, 1961; Hey and Thorne, 1983; Mosley and Tindale, 1985). Due to the restricted area of river bed used in the

planned study, it was considered probable that lateral variation in grain-size distribution patterns would be considerably less than in if a larger stretch of the river had been studied. As the investigation required minimising disturbance to the river bed during the sampling process, it was considered best practice to carry out pilot studies to establish optimal sample sizes specific to the area of river bed being studied.

To determine the number of sediment and water samples necessary to form reliable and representative data for the river investigation, multiple sediment and water samples were taken, and the running mean and standard error of the mean (SEM) were calculated for each variable measured. Stabilisation of the running mean and the SEM after continual sampling of each variable were considered indicative that the sample mean was representative of the true mean. The data collected in the pilot studies were also used to characterise the habitat conditions of the resident freshwater mussel population to inform the design of the flume-based study described in Chapter 4.

Sediment samples:

Sediment samples for the preliminary study were collected from Markeaton Brook Site 1 (Figure 2.2, Table 2.1, Section 2.2.2) on the 16th March 2017. Sediment cores were extracted with the modified Luer-Lok syringe using the method described previously in Section 2.3.2. The locations of 60 point samples were determined using two randomly-generated numbers that dictated the number of heel to toe paces taken both across the river and upstream along the river to each sample point. The likelihood that each successive sample would be influenced by prior disturbance of the sediment immediately upstream was minimised by starting at the downstream end of the site and working in an upstream direction.

After extraction of each sediment core, the plunger of the modified syringe was depressed to allow the sediment to be extracted into screw-top sample containers, and the pH of the wet sediment samples was measured using a Hanna Checker pH probe. The laser particle size analyser used in

Chapters 3 and 4 of this thesis was not available during the pilot studies, therefore grain-size distributions were characterised through sieve analysis. To do this, the wet sediment samples were poured into funnels with filter papers to remove excess water and then the samples were dried overnight in an oven set to 105°C. A pestle and mortar was used to break apart unconsolidated particles that had aggregated during the drying process. The samples were then sieved through a standard sieve stack, with mesh sizes ranging from 63-8000 µm, for 5 minutes on a sieve shaker to separate into the various grain-size fractions. The mass of each fraction was weighed using a balance with a 0.001 g level of precision.

The coarse sediment of the "armour layer" was sampled on the 2nd May 2017. As each study site in the river investigation comprised a 2 m^2 area (divided into 1 m^2 quadrats), a 2 m^2 area was used for the pilot study to determine the number of samples required, as that would account for any sediment variability across the whole area. To avoid excessive disturbance to the river bed at the planned study site, an area of river bed directly adjacent to the river study site with equivalent conditions was used for the pilot study. Eighty pebbles were selected from randomly-generated positions within the quadrat area and the B-axis was measured to a tenth of a milimeter using Vernier callipers before returning the pebble to its original position.

Water depth and near-bed velocity:

To characterise mussel habitat conditions for the construction of the flume (described in Section 2.4), eighty measurements of water depth and near-bed velocity were taken from Markeaton Brook Site 1 (Figure 2.2, Table 2.1), during base flow conditions on the 17th April 2017. The same random sampling technique described for the sediment samples was used to generate sample locations within the river channel. A meter stick was used to measure the depth of the water, which was recorded in metres. The mean near-bed water velocity at each sample location was then measured over a 30 second period at 0.9 of the depth from the water surface using a Valeport BFM002 flow meter (Figure 2.8). This model was chosen over the 001 model because although it

has a greater margin of error in the reading (±0.01 m/s compared with ±0.004 m/s for the 001 model), the smaller (25 mm radius) impellor size of the 002 enabled operation closer to the river bed, and in the shallow water areas inhabited by the mussels (Valeport Ltd., 2013).

Figure 2.8: Valeport BFM flow meter and wading set showing the 002 impellor above with a diameter of 50 mm and the 001 impellor below with a diameter of 125 mm. Image from Valeport Ltd. (2013).

Velocity at 0.6 depth:

Although useful for characterising near-bed velocities in Markeaton Brook for use in the flume-based study described in Chapter 4, measurement at 0.9 depth proved to be difficult as obstructions from river bed sediment sometimes restricted the movement of the impellor. Furthermore, as the radius of the impellor was 25 mm, it was not possible to measure at 0.9 depth if the river depth was shallower than 0.25 m. It was therefore decided that for assessing the impact of freshwater mussels on water velocities during the river investigation (described in Chapter 3), 0.6 depth would be more suitable. This depth is widely used for the measurement of

river velocity as it is considered the depth most representative of the mean velocity within the channel (Gordon, McMahon and Finlayson, 1992; Moorkens and Killeen, 2014). Water velocity at 0.6 depth was sampled on the 27th April 2017 from a 2 m^2 area of river bed at Markeaton Brook Site 1 (Figure 2.2, Table 2.1) to determine optimal sample sizes for the river-based investigation. Velocity at 0.6 depth was measured using the Valeport BFM002 flow meter at 50 randomly-generated positions within the 2 m^2 quadrat area, as it was considered probable that stabilisation of the running mean and standard error of the mean (SEM) would occur within 50 samples. If the running mean and SEM failed to stabilise after 50 samples, more samples would have been taken.

Turbidity and total dissolved solids:

Water turbidity and total dissolved solids (TDS) were sampled on the 27th April 2017 from the same 2 m^2 area quadrat as the 0.6 depth velocity measurements. Eighty water samples were collected from a depth at the mid-point between the surface of the river bed and the surface of the water from randomly-generated locations within the quadrat area into a sample cell for testing. Turbidity was measured in Formazin Nephelometric Units (FNU) using a Hach 2100Q*is* portable turbidimeter, which was calibrated after every set of 10 samples. TDS in ppm was measured using a Hanna Primo TDS probe calibrated immediately before the samples were taken.

Oxygen Saturation:

Water and hyporheic oxygen saturation were sampled on the 2nd May 2017 from 80 randomly-generated positions within the 2 m^2 area pilot study quadrat in Markeaton Brook Site 1. A YSI ProODO meter was used to measure water and hyporheic oxygen saturation, which was calibrated immediately before the samples were taken. In order for the oxygen readings to reflect those experienced by mussels in their semi-infaunal mode of life, the probe was placed immediately on-top of the substrate surface for the water oxygen readings, and at a depth of 50 mm below the substrate surface for the hyporheic oxygen readings. For each measurement the probe was held in position for 30 seconds to allow the reading to stabilise before the value was recorded.

To enable penetration of the probe into the substrate without damage to the sensor, the steel guard of the probe was covered with a geotextile membrane that allowed the free movement of water and dissolved constituents, but formed a barrier to the movement of insoluble particles. To insert the probe into the finer sub-surface sediment, the armour layer gravel was carefully parted prior to pushing the probe vertically downwards into the substrate. If coarse sediment beneath the surface prevented the probe from being inserted, that sample was rejected and an alternative randomly-generated location would be used.

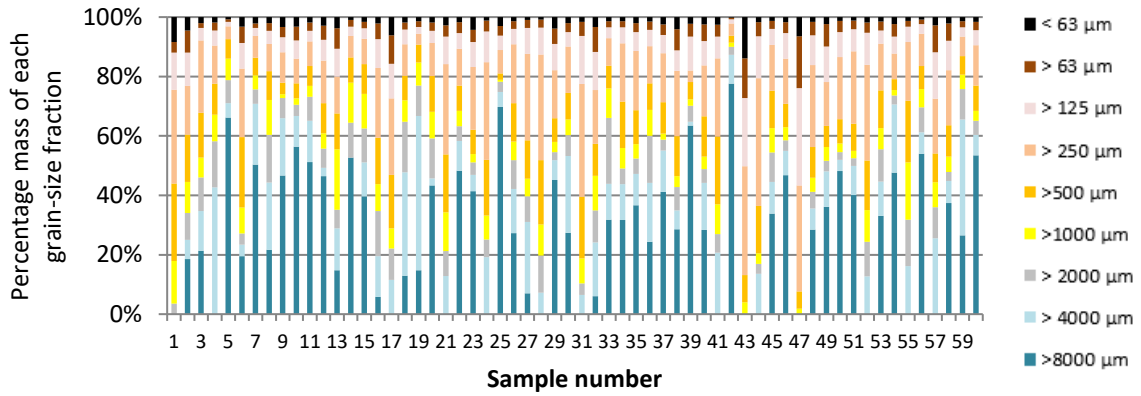
2.3.3 Results of the preliminary studies

Sediment samples:

The breakdown of grain-size fractions by the percentage dry mass for each sample, and the mean percentage mass of each fraction is shown in Figure 2.9. The mean percentage mass of each grain-size fraction found in the sampled stretch of Markeaton Brook was used to inform the grain-size distribution of the flume substrate used in Chapter 4 of this thesis.

Graphs of the running mean and the standard error of the mean (SEM) used to determine optimal sample sizes for the river investigation can be found in Appendix 1 (Figures A-L). The running mean and the SEM were calculated for the < 63 µm (silt and clay), 63-125 µm (very fine sand) and 125-250 µm (fine sand) grain-size fractions as it was considered that freshwater mussels would be more likely to influence the finer sediment grain-sizes as opposed to the coarser fractions. The running means of each of the selected grain-size fractions began to stabilise after 5th sample. The SEM for the selected grain-size fractions stabilise between the 10th and 15th sample. The running mean and SEM for sediment pH level off after the 10th sample. Stabilisation of the running mean and the SEM indicate that the sample mean is representative of the true mean. The results therefore indicated a minimum sample size of 10-15 as optimal for each visit to the river during the investigation detailed in Chapter 3. For the armour layer B-axis measurements

recorded within a 2 m^2 area of Markeaton Brook, the running mean had stabilised by the 20th sample and the SEM stabilised around the 50th sample, thus a sample size of 50 pebbles per quadrat was chosen for the river investigation.



Grain-size fraction (µm)	>8000	4000-8000	2000-4000	1000-2000	500-1000	250-500	125-250	63-125	< 63
Wentworth class	Medium-coarse gravel	Fine gravel	Very fine gravel	Very coarse sand	Coarse sand	Medium sand	Fine sand	Very fine sand	Silt and clay
Mean % mass	28.4	13.2	7.7	6.8	11.3	18.0	7.8	4.2	2.5

Figure 2.9: Grain-size distribution of 60 sediment samples taken from Markeaton Brook Site 1 (detailed in Figure 2.2, Table 2.1), by percentage dry mass of each fraction derived through sieve analysis. Separation of grain-sizes less than 63 µm was not carried out as this was the smallest mesh-size available.

Velocity at 0.6 depth:

The running mean and the SEM for water velocity at 0.6 depth within a 2 m^2 area of Markeaton Brook were used to determine an appropriate minimum sample size for the river investigation detailed in Chapter 3. The running mean of the velocity measurements at 0.6 depth levelled off after the 5th sample and the SEM remained between 0 and 0.01 after the 3rd sample. The flow meter used to measure velocity has a margin of error of 0.01 ms^{-1} so it would have been unrealistic to achieve a SEM below 0.01. The SEM fell below 0.01 after the 4th sample; therefore these

results indicated that 5 measurements of velocity at 0.6 depth per 1 m^2 quadrat were sufficient for each visit to the river site during the planned investigation.

Turbidity and total dissolved solids:

The running means and the SEM for water turbidity and total dissolved solids (TDS) within a 2 m^2 area of Markeaton Brook were calculated to determine optimal sample sizes for the river investigation. The running mean for water turbidity stabilised after the 10^{th} sample. The Hach 2100Q*is* portable turbidimeter used to take the turbidity measurements had an accuracy of 2 % of the reading of the instrument. The mean turbidity in the 2 m^2 quadrat area was 10.5 FNU. As 2 % of 10.5 is 0.21 and the SEM remained less than 0.15, and less than 0.09 at 5 samples, this shows very little fluctuation in the turbidity readings. Any observed variation in readings could be attributed to the error margin of the instrument. TDS readings also showed very little variation during the preliminary study. The Hannah TDS probe used to measure TDS had a 2 % margin of error. Mean TDS was 270 and 2 % of 270 is 5.4. SEM remained at less than 5.5 and after 5 samples it was below 2.1. Thus a sample size of 5 readings per 1 m^2 quadrat was deemed sufficient for the measurement of turbidity and TDS in the river investigation.

Oxygen saturation:

The running means and SEM for sediment and water oxygen saturation within a 2 m^2 area of Markeaton Brook were calculated. The YSI ProODO oxygen meter used to measure sediment and water oxygen saturation had an accuracy of 1 % of the reading of the instrument. Mean hyporheic oxygen saturation in Markeaton Brook was 101.5 %, which means the readings would have a margin of error of 1.015 %. The SEM for hyporheic oxygen saturation remained less than 0.3 and was less than 0.2 at 5 samples. Mean water oxygen saturation was 104.3 %, which meant a margin of error of 1.043 %. The SEM for water oxygen saturation remained less than 0.4 and was less than 0.2 at 5 samples, thus 5 samples per 1 m^2 quadrat was considered sufficient.

2.3.4 Conclusions of the preliminary studies

The results for water turbidity, velocity, total dissolved solids, oxygen saturation and hyporheic oxygen saturation all indicated that a minimum of five samples of each were sufficient to show representative data for a 2 m^2 quadrat area. The river investigation, as described in Chapter 3, involved four study sites, each comprising 4 x 1 m^2 quadrats, making 16 quadrats in total. Taking five measurements from each 1 m^2 quadrat increased confidence in the reliability of the data as there would likely be lower variability in a 1 m^2 area than in a 2 m^2 area of river bed. Furthermore as the river investigation was replicated in two different rivers and was sampled multiple times over a period four months, this further improved confidence in the data due to the multiple data-points involved.

The preliminary study for the grain-size of the sediment cores indicated a sample size of 5-10 as sufficient. This preliminary study was carried out with random sampling along a longer, wider stretch of river (spanning from bank to bank) than the quadrat area used in the river investigation, which was positioned in the middle of the channel. The extended area of the preliminary study likely had a much higher degree of variability than each of the 1 m^2 area quadrats used in the river investigation. As the planned river study ran over several months it was important to minimise disturbance to the river bed caused by the extraction of sediment samples, as this would influence the conclusions of the study. It was therefore considered that for each time it was sampled, five sediment samples per 1 m^2 quadrat would be most appropriate for the river investigation.

The preliminary study for bed sediment B-axis size indicated a sample size of 50 pebbles per 2 m^2 area quadrat would be appropriate, which is consistent with previous research on gravel-bed rivers (Brush and Nolan, 1961; Hey and Thorne, 1983; Mosley and Tindale, 1985). To further improve confidence in the data a sample size of 50 pebbles per 1 m^2 quadrat was chosen for the river investigation.

The preliminary studies to characterise the habitat conditions of the mussels used in the flume-based investigation indicated the near-bed velocity within the flume should range from 0.00-0.202 ms^{-1} with a mean of 0.106 ms^{-1}. The indicated depth of the water in the flume was 0.23 m. The grain-size distribution of the flume substrate was based on the percentage mass of the grain-size categories found in Markeaton Brook, detailed in Figure 2.9.

2.4 Preliminary flume studies and description of methods

2.4.1 Flume design and construction

A recirculating flume with a novel design was constructed for the purpose of investigating the influence of unionids on fluvial hydrological and sedimentological conditions in a more controlled environment than a natural river setting. The flume was designed using SketchUp Make (2017) CAD software (Figure 2.10). The glass exterior of the flume was constructed by Midland Aquatics (www.midlandaquatics.gbr.cc) and the acrylic insert was constructed by Atlas Plastics and Fabrication Ltd (www.atlasplastics.co.uk). Both the glass exterior and the acrylic insert were fully transparent to allow clear viewing of the contents of the flume. The acrylic insert comprised two straight sections of channel and two curved sections, the dimensions of which are shown in Figure 2.10.

A wider flume channel would have been preferred to more accurately replicate the channel shape and conditions of the mussel's habitat. However, due to spatial constraints within the laboratory, the channel width was restricted to 200 mm. The 200 mm wide channel allowed the mussels to freely move past each other but it constrained their motion to primarily upstream and downstream migration, thus restricting the extent to which they could move in a cross-channel direction. It should be taken into consideration that their natural burrowing and feeding behaviour may have been affected by the narrow width. The narrow channel width may also have impacted the nature of bedforms such as ripples, dunes and cross-stratification that could form in the channel by

restricting lateral sediment migration. Limiting the spatial extent available for natural phenomena to occur in a model environment can result in problems with scaling and boundary effects that can arise when conditions are scaled down from those present in natural systems (Kirkegaard *et al.*, 2011; Gorrick and Rodríguez, 2014). Scaling down of conditions in a laboratory setting, with model boundaries and constraints not present in nature can impact the processes being simulated, giving rise to inaccurate or misleading data (Kirkegaard *et al*., 2011).

The central section of the acrylic insert of the flume housed the filter system, which was necessary to remove excess waste products from the water such as ammonia and nitrite which can become toxic to unionids over time (Augspurger *et al.*, 2003). The filter system comprised layers of reticulated polyether aquatic filter foam, polymer filter wool and ceramic media, the purpose of which was to provide substrate for colonisation by the nitrifying bacteria and archaea that remove the nitrogenous waste (Sauder *et al.*, 2011).

Water pumps with adjustable velocity directed the filtered water from the central chamber to the outer channel, generating flow in a clockwise direction. As the filtered water was pumped out of the central chamber it was replaced by water from the channel recirculating through the filter system inlet. The water in the flume tank was tap water dechlorinated with Sera Aquatan water conditioner. The water was kept at ambient room temperature, maintaining a temperature of 21 to 23 °C. This was in range of the water temperatures measured in the mussel's habitat at that time of year.

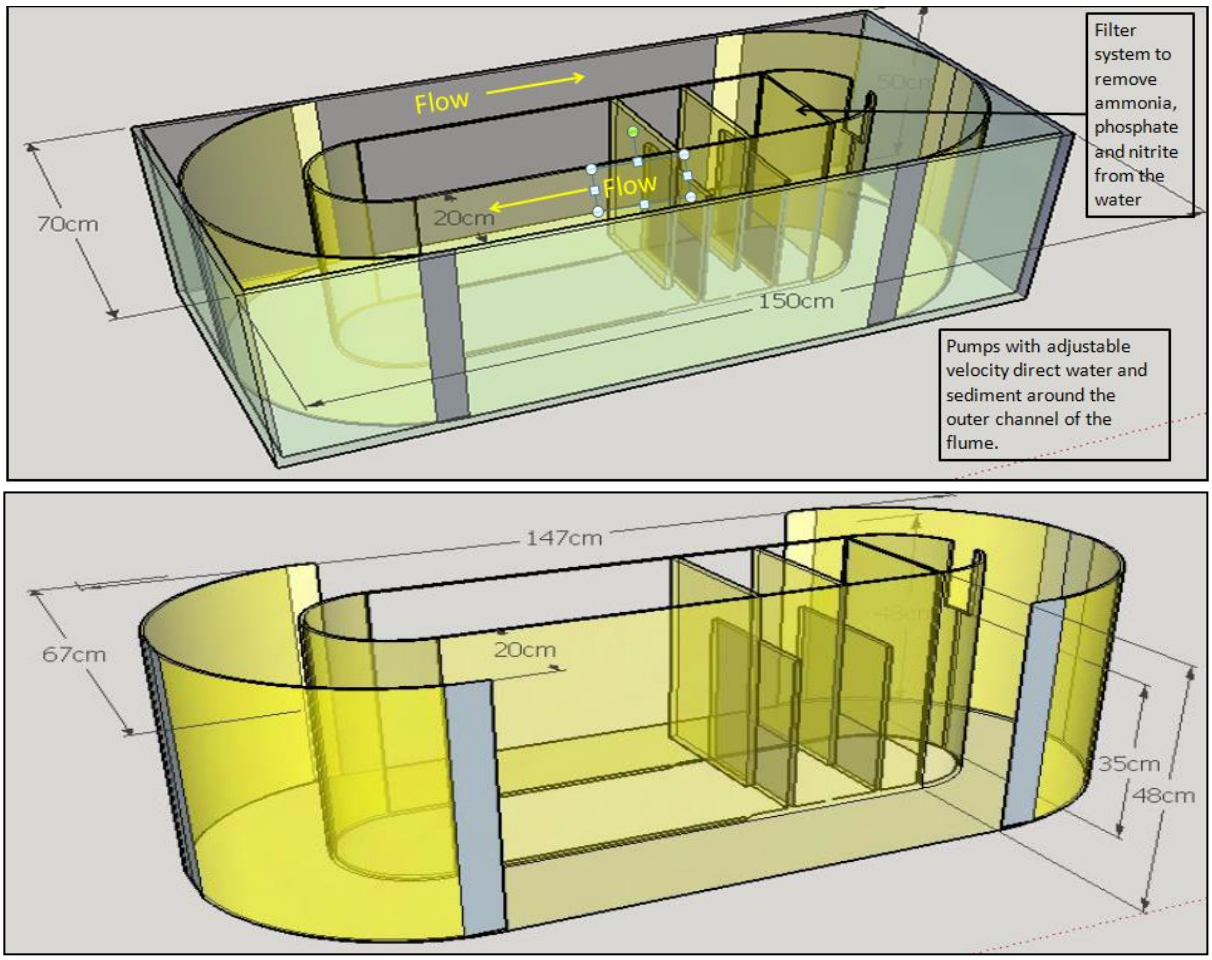


Figure 2.10: The dimensions of the flume tank (top) and the acrylic insert (bottom) designed using SketchUp Make (2017) CAD software. © Andrea Leng.

2.4.2 Flume channel substrate

The grain-size distribution of the flume substrate was based the results of the preliminary study in Markeaton Brook (Figure 2.9). In order to approximate the grain-size profile of the polymodal river bed sediment, a selection of aquarium sands and gravels were sieved into grain-size fractions and the required mass of each fraction were combined together to produce the substrate mix detailed in Table 2.2. Armour layer sediment was not included in the flume substrate, as the cobble and boulder-sized sediment present in Markeaton Brook may have caused damage to the glass of the flume. It was also thought that the mussels would be less likely to affect the transport of these grain-sizes. It should be noted, however, that the lack of armour layer in the flume likely

influenced the mobility of other grain-sizes, and may have affected mussel behaviour during the investigation.

Grain-size of aquarium sand/gravel (Wentworth class)	Grain-size of aquarium sand/gravel (µm)	Mass of grain-size fraction (kg)	Mass of grain-size fraction (%)
Fine-medium sand	63 to 500	49.5	33
Coarse sand	>500	16.5	11
Very coarse sand	>1000	10.5	7
Very fine gravel	>2000	12.0	8
Fine gravel	>4000	19.5	13
Medium gravel	>8000-16000	42.0	28
Total		150.0	100

Table 2.2: The proportion of each grain-size fraction present in the initial substrate mix of the flume.

Although present in small quantities (2.5%) in the sediment samples from the pilot study, grain-sizes of less than 63 µm (silt and clay) were excluded from the initial substrate but were added incrementally throughout the study as explained in Chapter 4 of this thesis. This was to avoid the problem of large quantities of non-cohesive, fine sediment remaining in suspension in the water, which would reduce visibility and potentially cause problems for the mussels. Excessive quantities of fine sediment in suspension is thought to impact freshwater mussel growth and survival due to the clogging of filter-feeding mechanisms (di Maio and Corkum, 1995; Brim Box and Mossa, 1999; Gascho Landis, Haag and Stoeckel, 2013). The sand and gravel substrate mix was rinsed several times in tap water and fully homogenised before being added to the flume tank to ensure the various grain-sizes were evenly distributed, both laterally and vertically in the flume's channel. A total of 150 kg of sand and gravel was added, which came to a depth of 0.13

cm in the channel. A 250 ml glass beaker was inserted into the sediment to form a sediment trap at the downstream end of one of the straight sections of channel in the flume, mid-way across the channel. It was covered with a net to prevent mussels from falling into the trap, but with a wide-enough mesh that the coarsest sediment in the flume could easily fall into the trap.

2.4.3 Water depth and initial velocity

The depth of water and the mean water velocity in the flume was based on the preliminary studies in Markeaton Brook described in Section 2.3. The mean water depth in Markeaton Brook was 0.23 metres, however, this water depth proved problematic in the flume, as the channel depth was too shallow to allow adequate water flow from the channel into the filter inlet shown in Figure 2.9. At that depth the volume of water able to pass through the inlet was of an insufficient volume to replace the water being removed by the pumps, leading to big differences in the height and pressure of the water on either side of the inlet, causing the walls of the central filter section to collapse inwards. To avoid compromising the structural integrity of the flume, it was found that a water depth of 0.27 metres was necessary. This depth was still within range of the depths observed in Markeaton Brook during the preliminary study described in Section 2.3, which showed depths ranging from 0.06 to 0.45 m. The level of water was marked on the glass of the flume and this level was maintained as evaporation occurred by the addition of RO (reverse osmosis) water to the central filter section so as to avoid disturbing the sediment in the flume's channel.

In order to choose an appropriate water velocity for the study it was considered that the near-bed (0.9 depth) velocity would be of most importance with regard to the preferences of the mussels, as this is the section of water column that they inhabit. Based on the results of the preliminary study described in Section 2.3, the range of near-bed velocity in the mussel's habitat was found to be between 0 and 0.2 ms^{-1} with a mean near-bed velocity of 0.106 ms^{-1}. Measurements of near-bed

velocity were taken across the full length and width of the flume channel using a Valeport BFM002 flow meter (described in Section 2.3.2) and the flow from the water pumps was adjusted until the water velocities all locations within the flume at 0.9 depth fell within the range of velocities observed within the mussels habitat. Photographs of the flume set-up are shown in Figures 2.11-2.13.

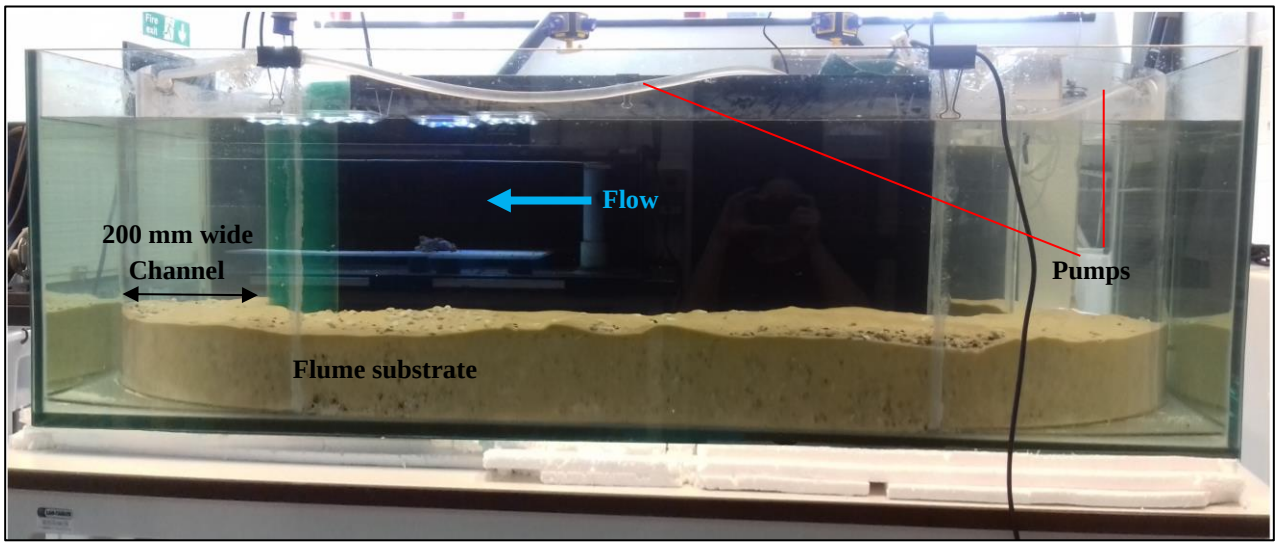


Figure 2.11: Side-view of the flume set-up showing the substrate mix and water level. Pumps remove water from the central filter section and direct it into the channel to generate directional flow of water. © Andrea Leng.

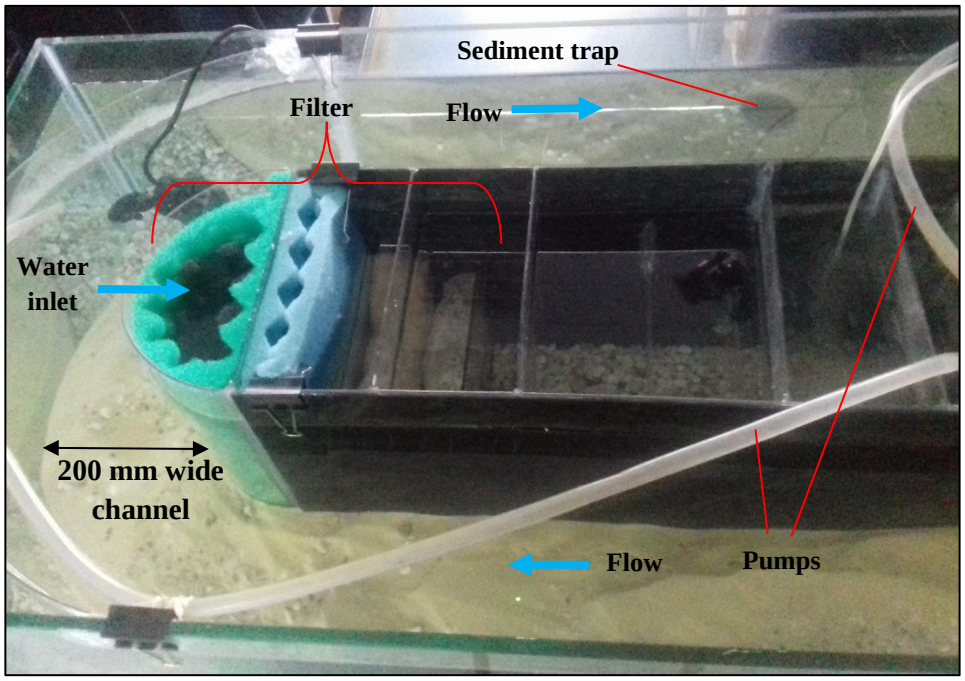


Figure 2.12: View of the flume from above showing where water is drawn into the central filter section from the channel. © Andrea Leng.

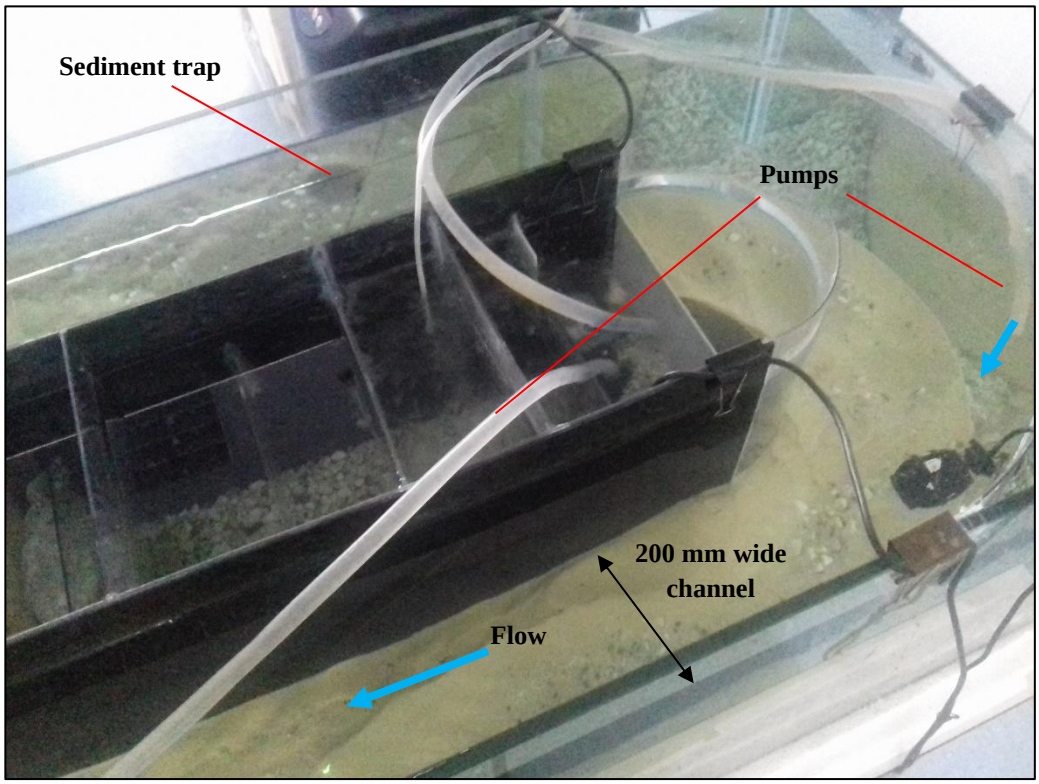


Figure 2.13: View of the flume from above showing where water is pumped out of the central filter section into the channel. © Andrea Leng.

2.4.4 Preliminary flume studies

Preliminary studies for the flume-based investigation were carried out on the 15th May 2017 to determine optimal sample sizes for the flume-based investigation described in Chapter 4. Sediment and water were introduced to the flume as described in Section 2.4.2 and 2.4.3 on the 13th May 2017 to allow time for the sediment to adjust to the flume's flow regime. Fifty measurements of water turbidity, water oxygen saturation and substrate oxygen saturation were taken from randomly-generated positions within the flume's channel using the equipment and methods described in Section 2.3.2.

Sediment samples were not collected during the preliminary studies to avoid disturbance to the substrate mix prior to the main investigation. Removal of sediment cores prior to the flume-based investigation may have resulted in preferential removal of certain grain-sizes depending on how they had been distributed within the flume during sediment transport. Successive sampling of the

sediment during the flume investigation (described in Chapter 4) would eventually lead to substantial losses of sediment from the flume. The removal of sediment cores may also have obscured any effects that the mussels may have had on the sediment. It was therefore important to keep the number of sediment samples collected from the flume to a minimum. The river environment of Markeaton Brook, from which the grain-size distribution of the flume substrate mix was derived, likely displayed a much higher degree of sediment variability than the homogenised sediment within the confines of the flume. Preliminary studies in Markeaton Brook, indicated an optimal sample size of five for the collection of sediment cores, therefore five sediment cores were also taken from the flume each time it was sampled, using the method described in Section 2.3.2.

2.4.5 Results of the preliminary flume studies

The running mean and the standard error of the mean (SEM) were calculated for each set of data to determine optimal sample sizes for the flume investigation. The results are displayed in Appendix 1, Figures M-O. The results for turbidity show that the running mean stabilised after the 5th sample and SEM remained less than 0.02 after the 3rd sample and was less than 0.01 by the 10th sample. The Hach 2100Q*is* turbidimeter had an accuracy of 2% of the reading of the instrument. Mean turbidity was 0.54 and 2% of 0.54 is 0.01. It was therefore considered that 10 samples would be sufficient to characterise the conditions within the flume, as the level of consistency in readings was acceptable given the accuracy of the instrument.

The running means water and substrate oxygen saturation had stabilised by the 5th reading. The SEM for water oxygen saturation remained less than 0.06, and was 0.04 at 5 samples and 0.03 at 10 samples. The SEM for sediment and water oxygen saturation was 0.04 at 5 samples and 0.03 at 10 samples. The YSI ProODO oxygen meter had an accuracy of 1% of the reading of the instrument. Mean water oxygen saturation was 99.0%, which gives an instrument error of

0.990%. Mean substrate oxygen saturation was 98.7%, which means an instrument error of 0.987%. 10 samples of each was therefore deemed sufficient to represent the conditions present in the flume.

2.4.6 Conclusions of the preliminary flume studies

The results for the water quality variables described in Section 2.4.5 show a high level of consistency around the flume, which likely reflects the relatively small volume of water and sediment contained within the flume compared with a river environment. The running means and SEM calculations show that five readings of each variable would have been sufficient to gather reliable and representative data. However, as the sampling of water in the flume involved minimal disturbance to the substrate conditions, ten readings were taken each sampling period in order to further improve reliability.

2.5 Additional methods used in the river and flume investigations

2.5.1 Macroinvertebrate sampling methodology

During the river investigation described in Chapter 3, sampling of macroinvertebrates was carried out using the UK-standard three minute kick sample method, followed by a one minute hand search (Murray-Bligh, 1999; O'Hare *et al.*, 2007) using a 1 mm mesh-diameter hand net. Invertebrates collected in the net were transferred to a tray of water and identified to the lowest practicable taxonomic level. As this method requires significant disturbance to the river bed sediment to the extent that invertebrates are swept downstream into the net, it would likely have dislodged both mussels and large amounts of sediment from the quadrat area. To avoid disruption to the river bed sediment, of main importance to the investigation, kick samples were carried out immediately downstream of each of the 16 river study quadrats, with one kick sample per quadrat.

The downstream location of the kick samples relative to the quadrats may not have been fully representative of the faunal assemblages present in the immediate vicinity of the mussels due to the lack of mussel bioturbation and the increased distance from the mussels. However, the fluvial transportation of sediment, water and pseudofaeces from the quadrats would likely have had some level of impact on the river bed immediately downstream of them, and was therefore of considered relevant to this study. All equipment used in the investigation was thoroughly cleaned and dried after each use to minimise the likelihood of invasive species being transferred from one river to the other.

2.5.2 Sediment grain-size analysis

A Beckman Coulter LS 13 320 laser particle size analyser was used for the characterisation of sediment grain-size distributions from the river sediment samples. For this purpose it was felt that analysis of the wet sediment would more accurately maintain the textural characteristics of the sediment compared with if it had been dried. Oven drying of sediment can cause aggregation of fine particles (Mason, 2016) and could conceivably impact the integrity of mussel pseudofaecal pellets of relevance to this study.

Before laser particle size analysis takes place, it is common practice for samples to be pre-treated with hydrogen peroxide and a dispersing agent in order to remove organic matter and disperse aggregations of grains (Allen and Thornley, 2004; Gray, Pasternack and Watson, 2010; Schulte *et al.*, 2016). However, for studies of a biological nature it is considered best practice to avoid pre-treatment, as the organic detritus, organo-mineral complexes and biodeposits are considered integral components of the sediment (Mason, 2016; Schulte *et al.*, 2016).

Excess water was carefully removed from the sample containers with the use of a Pasteur pipette so as not to disturb the fine sediment that had settled out of suspension. The remaining sediment "paste" was then transferred to a bowl, where it was thoroughly mixed with a spatula to

homogenise it. A spatula was used to remove a sub-sample, which was washed with de-ionised water through a 1000 µm mesh-size wire sieve into a stainless steel tray to remove the coarser fraction. The particle size analyser is capable of measuring grains up to 2000 µm in diameter (coarse sand size); however a sieve of this mesh-size could have allowed passage of grains with a long axis greater that 2000 µm if they fitted through the mesh on their intermediate axis. The threshold was therefore set at 1000 µm to avoid errors in the laser analysis. The grain-size distribution of the coarser fraction was determined through sieve analysis, which is described later in this section.

The finer fraction from the stainless steel sieve pan was washed through a funnel into a glass beaker using de-ionised water. A magnetic stirrer was used to keep the contents of the beaker in suspension as it was transferred to the particle size analyser using a Pasteur pipette. The size of aliquot used in the analysis is determined by the degree of obscuration caused by each sample in the particle size analyser and will vary depending on the grain-size distribution of each sample. Finer-grained samples will contain a greater number of particles for a given volume, and therefore a smaller aliquot will be required in order to get a representative sample for analysis compared with coarser-grained samples (Beckman Coulter, 2011). The required quantity of each sample was analysed three times, averaged and visualised graphically by the LS 13 320 software.

The remainder of each homogenised sample that was not used in the laser particle size analysis was oven dried at 105°C for 24 hrs, weighed and sieved into grain-size fractions of >8000, >4000, >2000, >1000, >500 and $\leq$500 µm using a nested stainless steel sieve stack. The mass of each fraction with a grain diameter of more than 500 µm was recorded to three decimal places and the percentage of each fraction was calculated for each sample. The percentage >500 -1000 µm fraction of each sample was calculated by both the laser particle size analyser and through sieve analysis. This grain-size category could therefore be used to merge and normalise the data from both methodologies to determine the percentage of each grain-size category in each sample. Research by Dinis and Castilho (2012) suggested a threshold grain-size of 1000 µm was indeed

appropriate for the integration of sieving and laser data, and thus supports my choice of methodology.

2.5.3 Sediment organic and inorganic content

As the size of the sediment samples taken from each quadrat were relatively small, to avoid significant disruption to the river bed conditions, it was necessary to re-use the sediment from the sieve-analysis (described in Section 2.5.2) in the determination of sediment organic and inorganic carbon content. Therefore for each sample, the sieved fractions were recombined; a pestle and mortar were used to break up any large fragments of shell and organic matter and the samples were transferred to weighed porcelain crucibles. The mass of each crucible and sample was recorded to four decimal places and the samples were placed in a muffle furnace at 475°C for 12 hours in order to determine organic carbon content by the percentage loss on ignition. They were then re-weighed and returned to the muffle furnace for 12 hours at 800°C before being weighed again to determine the percentage inorganic carbon (carbonate) content of each sample. This methodology has been shown to be the optimal loss on ignition procedure for determining the organic and inorganic carbon content of stream sediments (Wang, Li and Wang, 2011).

2.5.4 Flume topographic profiles

The use of Digital Elevation Models to record changes in river bed topography was described in Section 2.3.1. As the resources required to produce DEMs were not available during the study period, the degree to which the mussels altered the topographic profile, bed roughness and wetted perimeter in the flume was investigated with a series of topographic transects taken across the length and width of the flume channel substrate at the beginning and end of the study. The length of wetted perimeter across the channel, and the topographic profile length along the length of the

channel, was used as an indication of bed roughness of the flume substrate, as an increased bed roughness would produce a longer topographic profile.

Six lateral transects were positioned across the straight sections of the flume channel, with three on each straight section, equidistant apart. Along each transect, the sediment depth of the substrate was measured every 50 mm across the width channel from bank to bank. A longitudinal transect recorded the mid-channel depth every 50 mm along the length of the channel. To measure the depth at each sampling point a soil pin was inserted vertically into the sediment until it touched the base of the tank. The level of the sediment surface was marked on the pin and then measured with a ruler once the pin had been removed. Trigonometry was then applied by using the sediment depth at each sampling point and the horizontal distance between each sampling point to calculate the hypotenuse length between each point on the substrate surface that the substrate depth was measured. The hypotenuse lengths of each transect with mussels in the flume were statistically compared to those in the control study. The sum of all the hypotenuse lengths for each transect was taken as a measure of the wetted perimeter/ longitudinal topographic profile length of that transect.

This method was used to minimise disturbance to the substrate surface, as although the insertion of the soil pin caused some disturbance to grains, the more conventional use of a metal chain to measure the distance along the substrate surface was found to cause more disturbance to substrate. Additionally, by measuring the individual distances and depths between each sampling point it allowed the topographic profiles to be visualised graphically. Photographs were also taken of the sediment surface, its lateral profile and bedform features at the beginning and end of the study to compare visible differences in topographic features with mussels present and absent from the flume.

2.5.5 Flume water velocity measurements

During the flume investigation, water velocity was measured using a Valeport BFM002 flow meter (described in Section 2.3.2) at four depth intervals above 18 point-locations on the surface of the bed spanning the length and width of the straight sections of channel, making 72 measurements in total. The selected depth intervals were 0.1 depth (near-surface), 0.4 depth, 0.6 depth and near-bed, which was 3.5 cm above the substrate surface due to the limitations of the flow meter. These depths were chosen to provide a measurement of how mussels impacted water velocities along the full length, width and depth of the channel, and were based on widely used techniques in the measurement of river velocities (Moorkens and Killeen, 2014). The mean velocity over a 30 second period was recorded for each of the 72 locations within the flume.

2.5.6 Flume sediment flux

In order to establish how the mussels influenced sediment flux over time, the quantity and grain-size distribution of material entering the sediment trap (described in Section 2.4.2) was examined. At the end of the study the sediment trap was removed and the excess water was carefully decanted using a Pasteur pipette so as not to disturb the sediment in the beaker. The vertical changes in grain-size were graphically logged by viewing the vertical accretion of sediment at the upstream, downstream and both lateral edges of the glass beaker to produce four graphic logs for experimental run. The height of each sedimentary layer was recorded in millimetres using a ruler and the changes in grain-size were recorded in µm with the use of a grain-size card and hand lens.

The total vertical height in millimetres of each graphic log of the sediment trap infill was divided by 10 to determine the sediment depth measurement interval. Starting at 0 mm (the base of each log), median grain-size (D_{50}) in metres was recorded at every 10^{th} of the height of each log to produce 11 grain-size samples for each graphic log, generating 44 samples for each of the two experimental runs. Equation 2 described in Chapter 1, Section 1.3.1 was used to compare critical

shear stress τ_c over the course of each experimental run using the D_{50} values from the graphic logs. For the other parameters within Equation 2, a τ^* value of 0.047 N m^{-2} was based on estimates for poorly sorted sediments in gravel bed rivers (Miller, McCave and Komar, 1977). ρs, the sediment density was based on the density of quartz (2650 kg m^{-3}). ρ, fluid density was based on the water density of 1000 kg m^{-3}. g was the acceleration of gravity (9.81 m s^{-2}).

The sediment from the sediment trap was dried at 105°C for 24 hrs and then the total dry mass (g) was recorded. The sediment was homogenised and divided into two using the cone and quarter method described by (Schumacher *et al.*, 1990). One half of this was further coned and quartered into eight sub-samples. These were used to measure the percentage of organic and inorganic carbon in the sediment trap material using the loss on ignition method described in Section 2.5.3.

The other half of the sediment from the trap was sieved into grain-size fractions of >8000, >4000, >2000, >1000, >500 and $\leq$500 µm and the mass of each fraction was recorded to three decimal places. The fractions with a grain-size $\leq$1000 µm were recombined, homogenised and divided into a further eight sub-samples using the cone and quarter method. Grain-size distributions of the sub-samples were characterised using a Beckman Coulter LS 13 320 laser particle size analyser, and then merged and normalised with the sieve data using the method described in Section 2.5.2.

2.5.7 Statistical methodology

When deciding on a suitable method for the statistical analysis of the river data, consideration had to be given to the dynamic and unpredictable nature of the fluvial environment. It is impossible in a river to control all the variables to ensure that the control quadrats have an identical set of conditions to the experimental quadrats. Variations in river discharge and sediment supply over the course of the study, and natural fluvial processes such as erosion, transport and deposition could result in increasing or decreasing velocity, shear stress, grain-size or other abiotic or biotic

variables in certain quadrats compared with others over time. Furthermore, there may already have been statistically significant sedimentological and hydrological differences between the various quadrats before the removal or introduction of mussels to the area. The use of statistical methods that compared sets of data measured at the end of the study, without accounting for differences between quadrats at the start of the study, would have been inappropriate in this investigation. It was therefore important to choose a statistical method that compared how the data in each experimental quadrat changed relative to its respective control quadrat in terms of the rate of increase or decrease in each variable over time.

For each of the water quality and sedimentological parameters measured in this investigation, R software was used to create linear models for the change in each individual measurement over the duration of the study. In order to establish if the change in each variable over time was significantly different to the control quadrats, the mean beta coefficients derived from the linear models were compared using a Welch two sample t-test, as described by McDonald (2014). By comparing the rate of change of variables in each experimental quadrat relative to the variables in each respective control quadrat, it meant that it was not necessary to measure river discharge at each site throughout the duration of the study, as both experimental and control quadrats would be subject to the same variations in flow discharge and sediment supply.

For the reporting of statistical results, highly significant p values of less than 0.001 are reported as "< 0.001," non-significant p values of greater than 0.05 are reported as "> 0.05," and for p values of 0.001 to 0.05 the exact p value is provided to indicate the significancy of the results and level of confidence in the data.

Chapter 3: The Influence of Freshwater Mussels on the Sedimentological and Eco-hydrological Conditions of Two Lowland English Rivers

3.1 Introduction

Multiple previous studies have investigated the extent to which the sedimentological and hydrological conditions within river environments influence freshwater mussel behaviour and survival (Lewis and Riebel, 1984; Huehner, 1987; Libois and Hallet-Libois, 1987; di Maio and Corkum, 1995; Layzer and Madison, 1995; Hamilton, Brim Box and Dorazio, 1997; Strayer, 1999; Hardison and Layzer, 2001; Bolden and Brown, 2002; Brim Box, Dorazio and Liddell, 2002; Hastie *et al.*, 2003; Geist and Auerswald, 2007; Schwalb and Pusch, 2007; Brainwood, Burgin and Byrne, 2008; Harriger, Moerke and Badra, 2009; Daraio, Weber and Newton, 2010; Mueller *et al.*, 2011; Johnson, Krstolic and Ostby, 2014; Klos, Rosenberry and Nelson, 2014; Moorkens and Killeen, 2014; Scheder *et al.*, 2015). This prior research has served to improve our understanding of the habitat requirements of freshwater mussels, whilst facilitating and informing conservation and reintroduction strategies (Cope and Waller, 1995; Hamilton, Brim Box and Dorazio, 1997; Skinner, Young and Hastie, 2003; Luzier and Miller, 2009; Gum, Lange and Geist, 2011; Gascho Landis, Haag and Stoeckel, 2013; Lavictoire *et al.*, 2015; Quinlan, C. Gibbins, *et al.*, 2015; Lopes-Lima *et al.*, 2018). In contrast, our understanding of the degree to which freshwater mussels influence river sediment and fluvial hydrology is limited.

The potential positive impacts of freshwater mussels on fluvial environments were discussed in Sections 1.2.5 and 1.3.4. Growing recognition of the capacity of freshwater mussels to engineer their environment and improve water quality has led to their inclusion in several river restoration projects, as detailed in Section 1.2.5. However, previous studies have suggested both positive and negative effects of freshwater mussels in river habitats; for example, studies have indicated that freshwater mussels may reduce substrate dissolved oxygen concentrations, and increase fine and organic matter in the river bed sediment (Hakenkamp and Palmer, 1999; Beekey, Mccabe and

Marsden, 2004; Howard and Cuffey, 2006; Spooner and Vaughn, 2006; Turner, 2010). Increases in organic benthic matter from freshwater mussel biodeposition have been shown to have positive effects on some benthic macroinvertebrate communities (Howard and Cuffey, 2006; Spooner and Vaughn, 2006). Conversely, fine sediment infiltration into river bed sediment and reduced hyporheic oxygen levels can be detrimental to a number of freshwater species, including salmonids, who require clean, well-oxygenated gravels for spawning (Chapman, 1988; Wood and Armitage, 1997). A study by Boeker *et al.* (2016) drew contradictory conclusions, indicating that the freshwater mussel *Anodonta anatina* (Linnaeus 1758) led to significant increases in hyporheic oxygen saturation levels, attributed to mussel bioturbation. Bioturbation may also result in displacement of fine and organic sediment from the substrate surface to deeper within the hyporheic zone (Gerino, 1990; Majdi, Bardon and Gilbert, 2014), which would have implications for carbon cycling and sediment flux downstream. Alternatively, bioturbation may resuspend buried sediment into the water column, thus reducing the fine and organic content of the substrate (Zimmerman and de Szalay, 2007).

Given the uncertainties that still persist regarding the impact of unionids on river bed sediments, it is important to address these so that river restoration and mussel reintroduction projects are better informed, with an improved chance of success. An increased understanding of how unionids affect river sediments and resident fauna would also ensure that mussel reintroduction projects are better targeted, depending on the specific conservation objectives within the river in question. The investigation described in this chapter aimed to address areas of uncertainty with regard to the impact of mussels on river bed sediment, and better understand their impact on the eco-hydrological conditions in two English rivers. Translocation of freshwater mussels from mussel-dense reaches to mussel-free sites within each river enabled examination of the resultant impacts on the water, sediment and macro-invertebrate communities.

This field study addresses Research Objective 1 described in Section 1.4:

> *"Ascertain if the addition or removal of freshwater mussels to/from the beds of two rivers can significantly impact the sedimentological and eco-hydrological characteristics of the river environments, and examine the precise nature of any observed impacts."*

The field study aimed to address the following Key Questions described in Section 1.4:

1) *"To what extent do freshwater mussels impact river bed grain-size distribution patterns?"*
2) *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?"*
5) *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"*
6) *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"*

3.2 Catchment characteristics

3.2.1 Markeaton Brook

Markeaton Brook lies to the north-west of Derby, Derbyshire, UK, and has a length of 17 km from its source to its confluence with the river Derwent in Derby (Ordnance Survey, 2015). The 64 km^2 catchment is primarily comprised of improved grassland, agricultural and horticultural land, with the exception of its lower reaches, which are largely urbanised (NERC (CEH), 2017). The nearest weather station in Watnall, Nottinghamshire, records a mean annual precipitation of 709.4 mm (Met Office, 2019). The source of Markeaton Brook is a spring rising from interbedded limestones and mudstones of the Lower Carboniferous, at an altitude of 160 m (UK grid reference SK 263,448) to the south-east of Hulland Ward village, where it is initially named Mercaston Brook (British Geological Survey, 2019). Immediately downstream of the source it cuts through

Early Triassic siltstone and sandstone before incising back down into Lower Carboniferous limestones and mudstones just south of the village of Mercaston.

On its south-eastward course, Markeaton Brook it is joined by a series of tributaries, which include Black Brook, Hungerhill Brook, Wildpark Brook and Greenlane Brook, before its name changes to Cutler Brook just south of the village of Weston Underwood (Ordnance Survey 2015). The channel has been heavily modified with weirs and landscaping within the grounds of Kedleston Hall to form a series of lakes, downstream of which it takes the name of Markeaton Brook (Holdich *et al.*, 2006; National Trust, 2019). Downstream of Kedleston Hall and for the remainder of the river's course, Markeaton Brook flows over Early to Middle Triassic siltstone and mudstone, as well as superficial deposits of clay, silt, sand and gravel (British Geological Survey, 2019). At Markeaton Park in the north-west of Derby, the river merges with Mackworth Brook, and from here onwards has been subjected to a high degree of modification through the construction of lakes, diversionary channels, mills, weirs and flood-relief culverts (Holden-Brown and Tolley, 2011). From Markeaton Park the river flows mainly underground beneath Derby through two main culverts, one of which joins the river Derwent in Darley Park in the northern suburbs of the city, and the other joining the Derwent in the east of the city centre (Holden-Brown and Tolley, 2011). The stretch of Markeaton Brook between Markeaton Park and its confluence with The Derwent has been designated a Main River by the Environment Agency, in reference to the flood risk and requirement for active flood mitigation strategies (Environment Agency, 2019b). The Markeaton Brook catchment is shown in Figure 3.1.

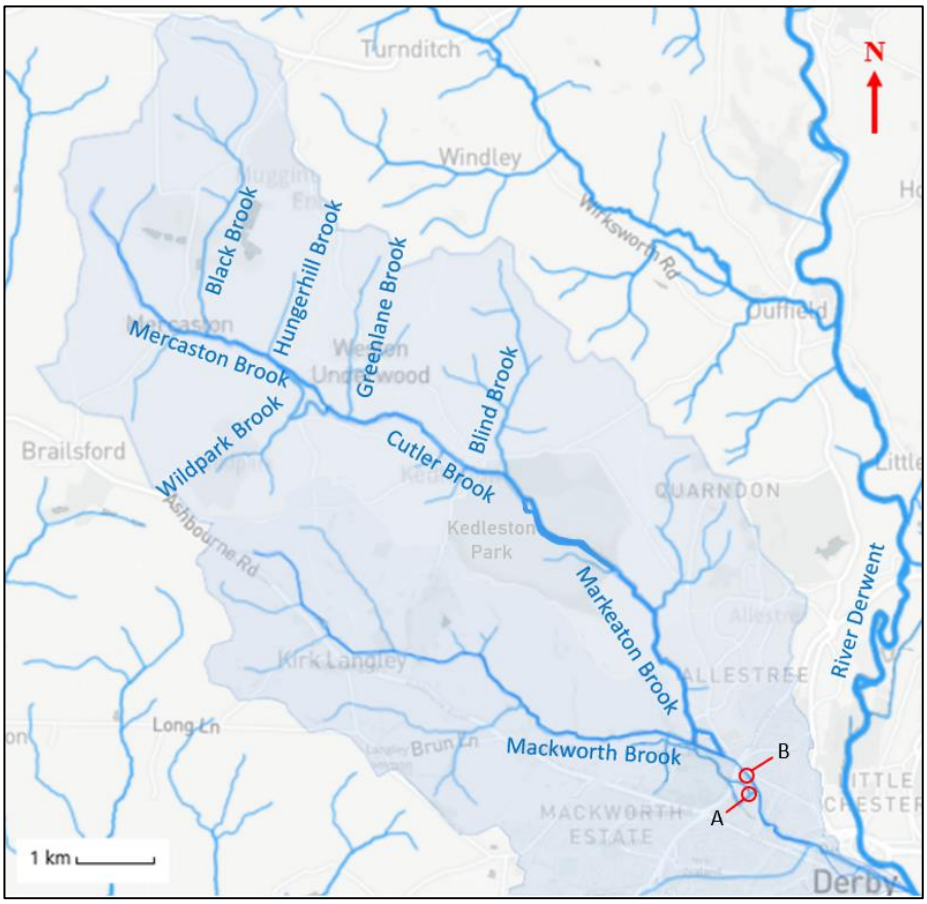


Figure 3.1: The Markeaton Brook catchment (shaded in pale blue) and the location of the two study sites (marked "A" and "B"). Base map sourced from DEFRA (2021). © Crown Copyright 2021 Open Government Licence v3.0.

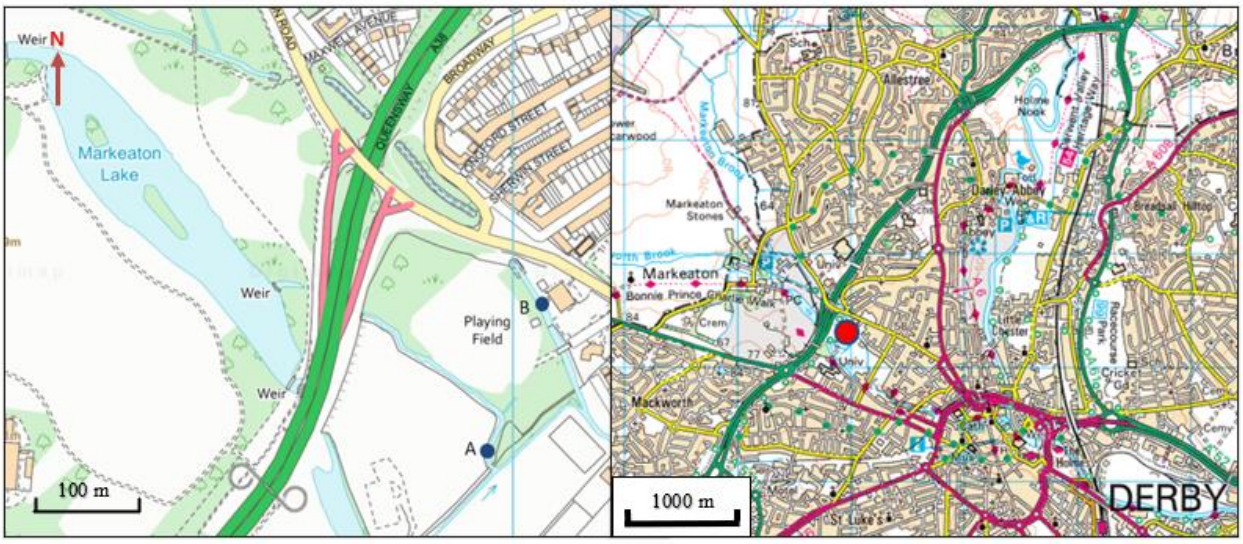


Figure 3.2: Location of the two Markeaton Brook study sites. Site A lies to the south of a recreation ground and site B lies to the east of the recreation ground as shown in the map on the left. Site A is flowing from NNE to SSW and Site B is flowing from NNW to SSE. The location of the recreation ground in the north-west of Derby is shown with a red dot on the map on the right. Base maps sourced from Ordnance Survey (2019). © Crown Copyright 2019 Ordnance Survey (AC0000851941) Open Government Licence v3.0.

The two study sites in Markeaton Brook are located adjacent to a recreation ground (Figure 3.2), immediately downstream of Markeaton Park at an altitude of 54 m. Site A (UK grid reference SK 3397,3725), which contains freshwater mussels, is located to the south of the recreation ground. It is located on the river reach designated as site number 2 (Figure 2.2, Table 2.1) described in Section 2.2.2. Site B (UK grid reference SK 3404,3738) is to the east of the recreation ground, and has an absence of freshwater mussels. It is located on the river reach designated as site number 4 (Figure 2.2, Table 2.1) described in Section 2.2.2. Both sites are under the cover of trees with heavily vegetated banks (Figures 3.3 and 3.4), and have a river bed consisting of clay, silt, sand, gravel and cobbles. Under typical flow conditions observed during this study, the channel width of Site A was 2.5-3.0 m and Site B was 3.4-4.5 m. The typical water depth at both sites ranged from 0.10-0.28 m.

White-clawed crayfish (*Austropotamobius pallipes* (Lereboullet, 1858)), signal crayfish (*Pacifastacus leniusculus* (Dana 1952)), duck mussel (*Anodonta* anatina (Linnaeus 1758)) and swan mussel (*Anodonta cygnea* (Linnaeus 1758)) have been recorded in Markeaton Brook, as well as 17 fish species that include brown trout (*Salmo trutta* (Linnaeus 1758)), pike (*Esox lucius* (Linnaeus 1758)), chub (*Squalius cephalus* (Linnaeus 1758)), perch (*Perca fluviatilis* (Linnaeus 1758)), bullhead (*Cottus gobio* (Linnaeus 1758)) and brook lamprey (*Lampetra planeri* (Bloch, 1784)) (Holdich *et al.*, 2006; National Biodiversity Network, 2021). The environmental quality of the section of Markeaton Brook relevant to this study has been classified as “Moderate” by the Environment Agency (Environment Agency, 2019a), with the majority of ecological and chemical analysis indicating water quality as “good” (Environment Agency, 2019a).

Figure 3.3: Markeaton Brook site A viewed looking to the north in an upstream direction. The channel is 2.5 - 3.0 m wide. © Andrea Leng.

Figure 3.4: Markeaton Brook site B viewed looking east in a downstream direction. The channel is 3.4 - 4.5 m wide. Two sets of adjacent 1 m^2 quadrats used in the investigation are shown in the photograph. There are approximately four metres distance between the two sets of quadrats. © Andrea Leng.

3.2.2 River Sence

The Sence rises on Bardon Hill in the Charnwood Forest area of Leicestershire, UK (Ordnance Survey, 2016b). It emerges from the boundary between Middle Triassic mudstones and overlying Pleistocene deposits at an altitude of 198 m (British Geological Survey, 2019). The Sence has a length of 28 km from its source to its confluence with the river Anker on the Leicestershire-Warwickshire border, north-east of Atherstone, Warwickshire (Ordnance Survey, 2016a).

The main tributaries of the Sence lie within the country of Leicestershire, and include Ibstock Brook, Carlton Brook and the Tweed (Ordnance Survey, 2016a, 2016b). Ibstock Brook drains the area south of Ibstock, joining the Sence near Shackerstone, and Carlton Brook rises near Market Bosworth and meets the Sence near Congerstone (Ordnance Survey, 2016b). The source of the Tweed is near Barwell and it enters the Sence at Ratcliffe Culey, just north of Atherstone (Ordnance Survey, 2016a). The entire drainage basin covers an area of 176.3 km^2 (Environment Agency, 2019a). The geology of the catchment is dominated by Early-Middle Triassic mudstones, siltstones and sandstones, with Upper Carboniferous coal measures exposed at the former opencast colliery of Sence Valley Forest Park, near Ibstock (British Geological Survey, 2019). Mean annual precipitation recorded at Sutton Bonington, Nottinghamshire, the nearest weather station to the catchment, is 620.2 mm (Met Office, 2019). The main land uses within the catchment are agricultural and horticultural, woodland, urban, amenity and industrial (NERC (CEH), 2017). The upper Sence catchment, of relevance to the field study, and the location of the two field sites are shown in Figure 3.5.

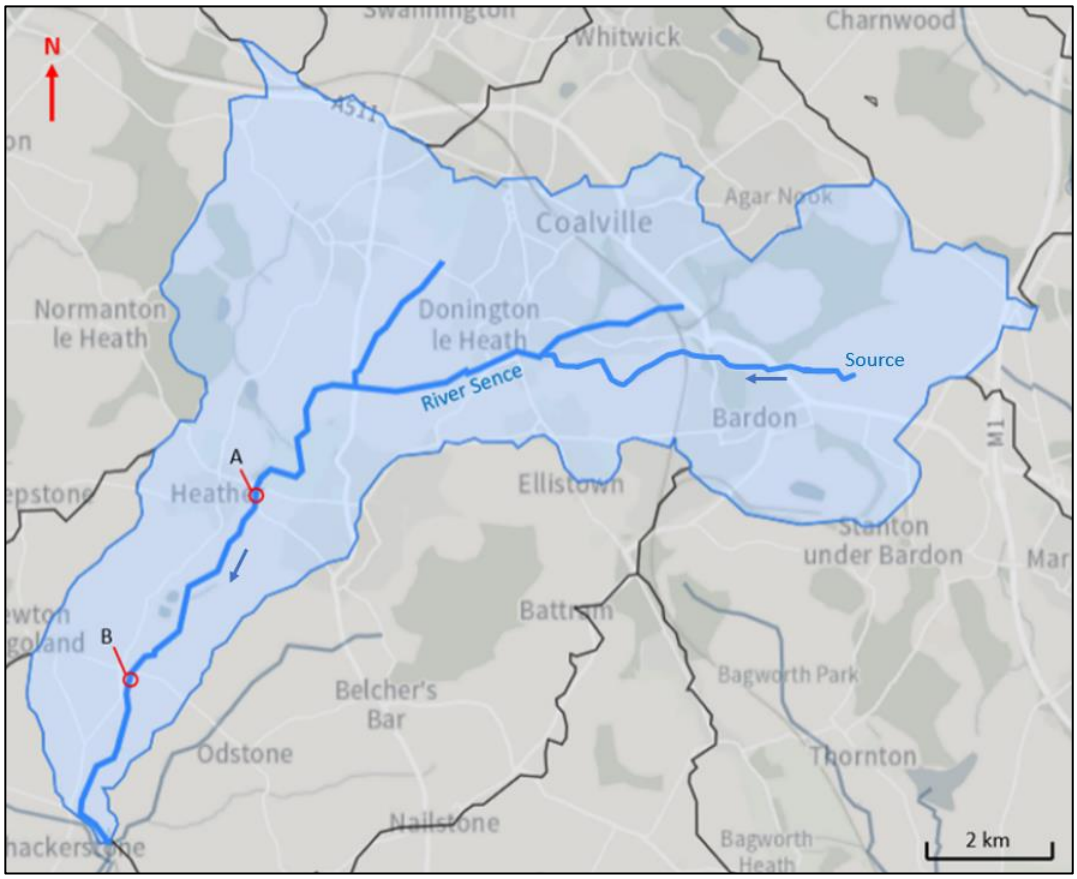


Figure 3.5: The upper Sence catchment (shaded in pale blue) from its source to its confluence with Ibstock Brook near Shackerstone, Leicestershire. The direction of river flow is indicated with blue arrows, and the two study sites are labelled "A" and "B". Base map sourced from DEFRA (2021). © Crown Copyright 2021 Open Government Licence v3.0.

Site A (UK grid reference SK 3948,1077), which contains abundant freshwater mussels, is located to the south of Sence Valley Forest Park, off Pisca Lane between Heather and Ibstock (Figure 3.6). It is located on the river reach designated as site number 38 (Figure 2.2, Table 2.1) described in Section 2.2.2. Site A lies at an altitude of 114 m within an area of pastoral farm land, with a hedgerow and trees alongside the right hand bank (Figure 3.7). At the time of the study the channel width was 4.8 - 5.0 m, with a water depth of 0.06 - 0.25 m. The river bed sediment consisted of clay, silt, gravel and cobbles with abundant bivalve shell material.

Site B (UK grid reference SK 3807,0875) has an absence of freshwater mussels and is located just south of Newton Lane, between Newton Burgoland and Odstone, south-west of Ibstock, at an

altitude of 101 m. It is located on the river reach designated as site number 40 (Figure 2.2, Table 2.1) described in Section 2.2.2. Site B is approximately 2 km downstream of a sewage treatment works, with arable farm land on either side of the densely vegetated banks (Figure 3.8). At the time of the study the channel width ranged from 4.4 - 4.6 m, with a water depth ranging from 0.10 - 0.19 m. The bed sediment comprised mainly sand, gravel, cobbles and boulders, with a relatively dense cover of filamentous algae and macrophytes.

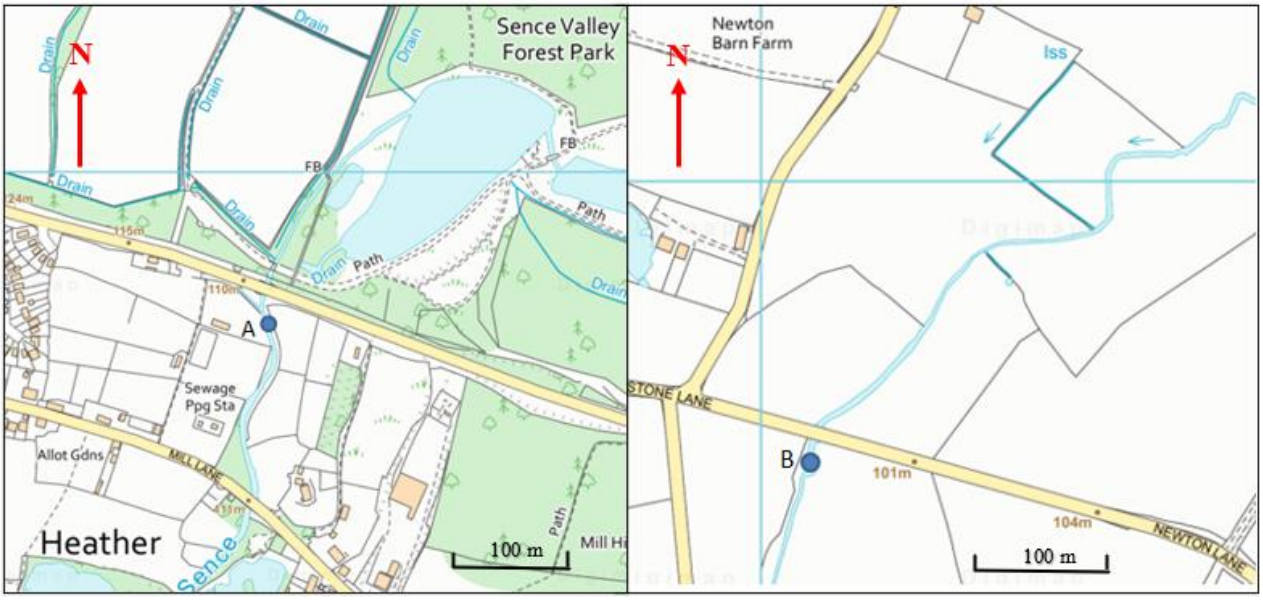


Figure 3.6: River Sence site's A (left) and B (right). The Sence is flowing from NE to SW. Site A lies to the east of the village of Heather, Leicestershire and downstream of Sence Valley Forest Park. Site B is located downstream of, and to the south of site A, and is surrounded by arable land. Base maps sourced from Ordnance Survey (2019). © Crown Copyright 2019 Ordnance Survey (AC0000851941) Open Government Licence v3.0.

Figure 3.7: River Sence site A viewed looking south in a downstream direction. The channel is 4.8 - 5.0 m wide. © Andrea Leng.

Figure 3.8: River Sence site B viewed looking SW in a downstream direction. The channel is 4.4 - 4.6 m wide. © Andrea Leng.

The environmental condition of the stretch of the Sence that the two sites are located within has been classified as "Poor" by the Environment Agency (Environment Agency, 2019a). Reasons

given for the poor rating include high phosphate levels arising from runoff from farming and discharge of sewage, as well as the presence of physical barriers to the migration of fish (Environment Agency, 2019a).

159 species of bird, 27 mammal species and 18 fishes have been recorded in the Sence Valley, including the marsh harrier (*Circus aeruginosus* (Linnaeus 1758)), Montagu's harrier (*Circus pygargus* (Linnaeus 1759), osprey (*Pandion haliaetus* (Linnaeus 1758)), otter (*Lutra lutra* (Linnaeus 1758)), Daubenton's bat (*Myotis daubentonii* (Kuhl 1817)), noctule bat (*Nyctalus noctula* (Schreber 1774)), roach (*Rutilus rutilus* (Linnaeus 1758)), chub (*Squalius cephalus* (Linnaeus 1758)), perch (*Perca fluviatilis* (Linnaeus 1758)) and brown trout (*Salmo trutta* (Linnaeus 1758)) (National Biodiversity Network, 2021).

3.3 Data collection and processing

3.3.1 Experimental design

The selection of sites within Markeaton Brook and the River Sence was based on survey work described in Section 2.2 of this thesis. Both Markeaton Brook and the River Sence had sections of river where mussels were abundant, as well as sections of river where mussels were absent. The river sites forming the basis of this investigation are referred to as follows:

- Markeaton Brook A (MBA): Mussels present.
- Markeaton Brook B (MBB): Mussels absent.
- River Sence A (RSA): Mussels present.
- River Sence B (RSB): Mussels absent.

The investigation ran for three months from May to August 2017. It was initially planned for the investigation to run over several months, but heavy rain in July 2017 washed a large proportion of the mussels from MBB, resulting in the Markeaton Brook study being terminated after 8 weeks.

Further loss of mussels at RSB in August resulted in the River Sence study being terminated after 14 weeks. The loss of mussels at MBB and RSB validated the decision to limit the number of mussels being translocated (as discussed in Section 2.3.2), and provides and explanation as to why mussels were not present at these sites prior to the study.

All equipment used in this investigation was thoroughly cleaned and dried after each use to minimise the likelihood of invasive species being transferred from one river to the other. At each of the four river sites, two pairs of adjacent 1 m^2 quadrats were set up using string, making 16 quadrats in total. The quadrats were held in position above the water by suspending the string across the channel and attaching it to each bank using tent pegs as shown in Figure 3.9. The novel quadrat design was used because the metal pegs holding the quadrats could be left in position on the banks, ensuring that the quadrats would be in the same position each time the river was sampled.

Figure 3.9: Two adjacent 1 m^2 quadrats positioned above the surface of the water. The quadrats were made using string which was attached to the river banks using tent pegs. © Andrea Leng.

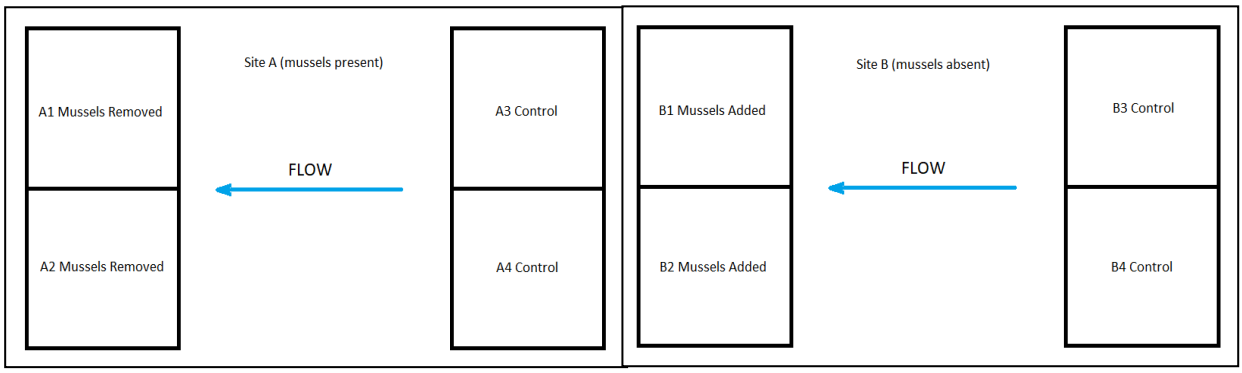


Figure 3.10: Diagram to show the quadrats present in each of the two rivers. Each river had two sites – Site A (left) with mussels originally present and Site B (right) with mussels originally absent. Each of these sites contained four quadrats, two of which were control quadrats positioned upstream of the quadrats where mussels had been either removed or added.

At each site, the two adjacent upstream quadrats served as control quadrats. Therefore at MBA and RSA there were mussels present in these quadrats and at MBB and RSB mussels were absent from these quadrats. Mussels were removed from the two adjacent downstream quadrats at MBA and RSA and mussels were introduced to the two adjacent downstream quadrats at MBB and RSB (Figure 3.10). These quadrats were positioned downstream of the control quadrats so that the addition or removal of mussels to/from these quadrats would not impact the control quadrats.

Before any mussels were removed from the river bed, the initial set of water and sediment samples were taken so that the sediment disturbance caused by the removal of mussels would not interfere with any of the equipment readings. The collection of the water and sediment samples is described in sections 3.3.2 and 3.3.3. At MBA and RSA a Nuova Rade jointed aquascope (as described in Section 2.2.1) was used to locate the mussels present in the four quadrats. All located mussels were then removed from the river bed by hand and placed on the river bank, as shown in Figure 3.11, where they were counted and labelled by scratching a number onto their shell using a pair of compasses. This method of numbering was utilised as it was only visible on close inspection, so was less likely to alert passers-by or predators to their presence compared with other methods of identification.

Figure 3.11: Freshwater mussels laid on the bank before being counted, identified and labelled. Mussel assemblages in both rivers comprised a mix of *Anodonta anatina* and *A. cygnea*. © Andrea Leng.

The dimensions of each mussel were recorded using Vernier callipers to measure their length and diameter, and this data was used to aid in identifying the species present (Appendix 4). The mussel assemblage found in the MBA quadrats was primarily *Anodonta anatina* with a smaller proportion of individuals more closely resembling *A. cygnea.* The length of mussel shells in MBA ranged from 55 to 94 mm, with a mean length of 69.6 mm and a median length of 70.0 mm. In RSA, *A. cygnea* was the most abundant species, with *A. anatina* also present. Shell lengths at RSA ranged from 75 to 123 mm, with a mean length of 99.4 mm and a median length of 97.0 mm.

At RSA, 217 mussels were found in the four quadrats. This number was divided in to four, which gave 54 mussels with one left over. The smallest mussel was returned to the river outside of the quadrats and 54 mussels were placed in each of the two control quadrats at RSA. The mussels

were positioned equidistant-apart, with their anterior-ventral edge pressed partially into the sediment and their posteriors pointing downstream. The remaining mussels were transported in buckets and 54 were placed in each of the two downstream quadrats at RSB. Care was taken to ensure the mussels were distributed as equally as possible in terms of the number of each species, and the size of each individual mussel in the four quadrats. MBA had a lower abundance of mussels compared with RSA, with 83 mussels found in and around the immediate area of the four quadrats. Seventeen additional mussels were gathered from the river bed downstream of the quadrats to provide a total of 100 mussels for the study. Twenty-five mussels were placed in each control quadrat at MBA and 25 were placed in each of the downstream quadrats at MBB using the same method as for the River Sence.

As the quadrats were only marked with string above the surface of the water, there was no physical barrier preventing mussels from moving in or out of the quadrats. Putting the mussels in cages or putting a fence or wall around them would have made it easier to control the number of mussels in each quadrat; however it would have impacted the flow of water and sediment through the sampling area. As the main focus of this investigation was to see how the mussels impacted the river hydrology and bed sediment characteristics, the decision was made to keep the quadrats open. Each river site was visited every two weeks in order to collect data. Upon arrival at the sites the river bed upstream, downstream and either side of the quadrats was thoroughly searched using the aquascope. Any labelled mussels that had moved out of the quadrats were noted and returned to their original position. Any mussels moving from the surrounding area of river bed into the quadrats at MBA and RSA were removed and placed outside of the quadrats. The number of mussels present in each quadrat and the number that had moved out of or into each quadrat were recorded at each visit to the sites.

3.3.2 Hydrological conditions

Using the methods and equipment described in 2.3.2, samples (n=5) of water turbidity, total dissolved solids (TDS), water oxygen saturation and mean water velocity at 0.6 depth were taken from each of the 16 quadrats at the beginning of the study, then every two weeks for the duration of the investigation. Samples were collected from randomly-generated locations within each quadrat. Sample sizes were determined by the preliminary study detailed in Section 2.3.3.

Although river discharge is one of the primary factors influencing sediment transport, bedforms, and macroinvertebrate assemblages in rivers (Dunbar *et al.*, 2010; Gong *et al.*, 2014; Nicholas *et al.*, 2016), discharge was not measured during this investigation. This was because the comparative quadrat approach examined how hydrological and sedimentological conditions changed in the control quadrats relative to the experimental quadrats over the duration of the study, whilst subject to the same variability in river discharge. Additionally, as each quadrat only constituted a small area of the channel as opposed to the entire channel width, it was considered more relevant to record changes in river velocity within each quadrat to explore how mussels impact hydrological conditions within their immediate area.

It was anticipated that increased flow resistance resulting from the protrusion of mussel shells into the water column would locally reduce near-bed velocities. It was also considered possible that increased bed roughness from mussel bioturbation would increase flow resistance, leading to reduced water velocities where mussels were present. Testing how mussels impact water velocity with the comparative quadrat approach therefore enabled inferences to be made about how a more extensive mussel bed across an entire river reach may influence hydrological conditions. For reference, river discharge, river level, and rainfall data for the study period, recorded at the nearest river gauging stations and rain gauges to the study sites, are provided in Appendix 3.

3.3.3 River bed conditions

Details of the preliminary research to determine the most appropriate methodologies for sampling the river bed sediment are described in Section 2.3. The armour layer was sampled at the beginning and at the end of the investigation, using the method described in 2.3.2. After measurements were taken, each pebble was carefully returned to its original position. Samples of the finer sediment beneath the armour layer (n=5) were taken at the beginning of the study, and then every two weeks for the duration of the investigation from randomly-generated locations within each quadrat. Hyporheic oxygen saturation was measured, and sediment cores were collected using the methods and equipment described in 2.3.2. The sediment core samples were stored vertically in the screw-top sample containers at 4 °C before they were further processed. Descriptions of the methods used to process and analyse the sediment core samples to determine sediment grain-size distributions are described in Section 2.5.2. Methods to determine the organic and inorganic carbon content of the sediment core samples are described in Section 2.5.3.

3.3.4 Macroinvertebrate surveys

To assess the impact of the presence or absence of mussels on benthic macroinvertebrate populations, kick samples were undertaken every four weeks at each river site using the method described in Section 2.5.1. BMWP (Biological Monitoring Working Party) scores were calculated from the kick sample data to assess the mussels' impact on water quality. The BMWP score system is an assessment of water quality based on the relative abundance of pollution-sensitive and pollution-tolerant invertebrate taxa (Walley and Hawkes, 1997). Details of the method used in calculating the BMWP scores are provided in Appendix 2.

3.3.5 Statistical analysis

Quadrats with mussels either introduced or removed were compared with control quadrats using the statistical methodology described in Section 2.5.7. In each river, quadrats A1 and A2 had mussels removed from them and A3 and A4 were the control quadrats with mussels present. Therefore A1 was statistically compared with A3, and A2 was statistically compared with A4. Quadrats B1 and B2 in each river had mussels introduced to them, so they were compared to the control quadrats with mussels absent; B3 and B4 respectively. By comparing each quadrat with its control quadrat independently in two different rivers, the repeatability and reliability of any observed results could be more conclusively demonstrated.

3.4 Results

3.4.1 Hydrology and water quality

Turbidity

Mean water turbidity levels at MBA fluctuated between 3.01 and 8.90 Formazin Nephelometric Units (FNU) over the 8 weeks of the study (Figure 3.12). Turbidity was generally higher in the quadrats where mussels had been removed, but not significantly so.

The mean beta coefficient of the change in turbidity over time in A1, where mussels were removed (M=-0.44, SD=0.19) was not significantly different to A3, where mussels were present (M=-0.54, SD=0.29); $t(6.91)=-0.67$, $p > 0.05$.

The mean beta coefficient of the change in turbidity over time in A2, where mussels were removed (M=-0.47, SD=0.29) was not significantly different to A4, where mussels were present (M=-0.81, SD=0.53); $t(6.17)=-1.28$, $p > 0.05$.

Overall, the removal of mussels from quadrats at MBA did not result in significantly different turbidity levels compared with the quadrats where they were present.

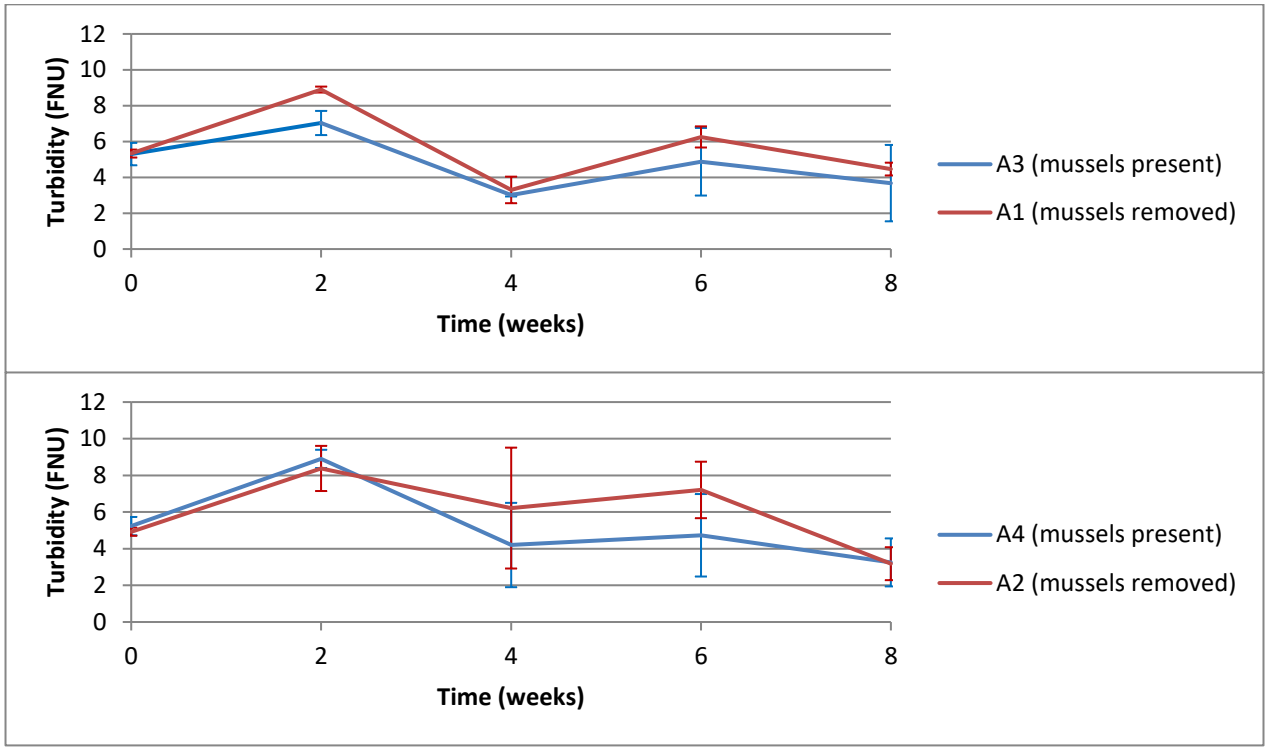


Figure 3.12. Mean (± 1 SD) water turbidity in Formazin Nephelometric Units (FNU) at MBA over the duration of the study.

At MBB the introduction of mussels to B1 and B2 resulted in higher turbidity levels compared with the control quadrats by week 2. By week 4 turbidity levels in B1 and B2 fell below those in B3 and B4, and remained lower for the duration of the study (Figure 3.13).

The mean beta coefficient of the change in turbidity over time in B1, where mussels were added (M=-0.45, SD=0.13) was significantly more negative than B3, where mussels were absent (M=0.47, SD=0.19); $t(7.14)=8.98$, $p < 0.001$.

The mean beta coefficient of the change in turbidity over time in B2, where mussels were added (M=-0.30, SD=0.40) was significantly more negative than B4, where mussels were absent (M=0.59, SD=0.23); $t(6.37)=4.39$, $p = 0.004$.

The introduction of mussels to quadrats at MBB resulted in significantly reduced turbidity levels compared with the quadrats where they were absent.

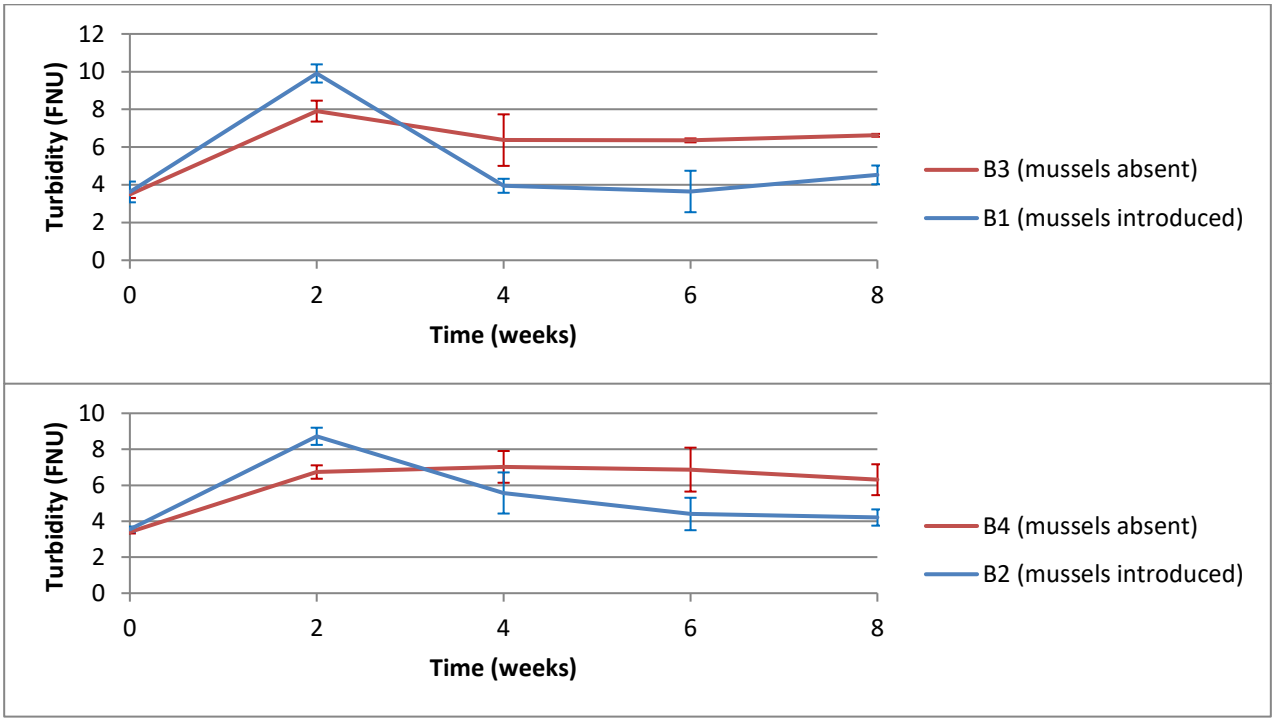


Figure 3.13. Mean (± 1 SD) water turbidity in Formazin Nephelometric Units (FNU) at MBB over the duration of the study.

In RSA the mean water turbidity ranged from 3.23 to 11.08 FNU of the course of the study (Figure 3.14).

The mean beta coefficient of the change in turbidity over time in A1, where mussels were removed (M=0.04, SD=0.40) was not significantly different to A3, where mussels were present (M=-0.45, SD=0.53); t (7.44)=-1.67, $p > 0.05$.

The mean beta coefficient of the change in turbidity over time in A2, where mussels were removed (M=-0.09, SD=0.38) was not significantly different to A4, where mussels were present (M=-0.50, SD=0.23); t (6.68)=-2.06, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different turbidity levels compared with quadrats where they were present.

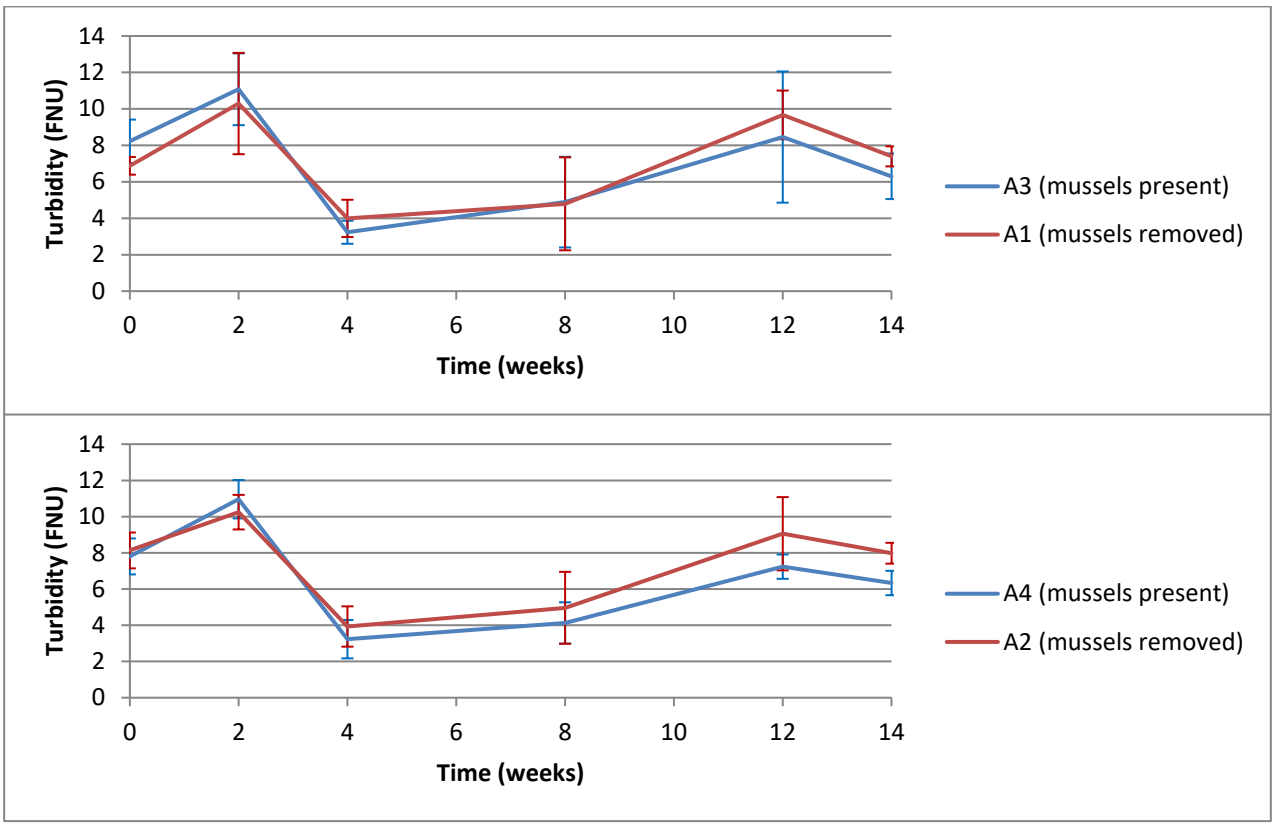


Figure 3.14. Mean (± 1 SD) water turbidity in Formazin Nephelometric Units (FNU) at RSA over the duration of the study.

At RSB mean turbidity ranged from 3.31 to 14.18 FNU over the 14 weeks of the study. Turbidity levels in the quadrats where mussels had been introduced dropped below those in the control quadrats by week 4, where they remained for the end of the investigation (Figure 3.15).

The mean beta coefficient of the change in turbidity over time in B1, where mussels were added (M=-1.61, SD=0.73) was not significantly different to B3, where mussels were absent (M=-1.39, SD=0.36); t (5.88)=0.62, $p > 0.05$.

The mean beta coefficient of the change in turbidity over time in B2, where mussels were added (M=-1.40, SD=0.62) was not significantly different to B4, where mussels were absent (M=-0.89, SD=0.39); t (6.73)=1.54, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in significantly different turbidity levels compared with quadrats where they were absent.

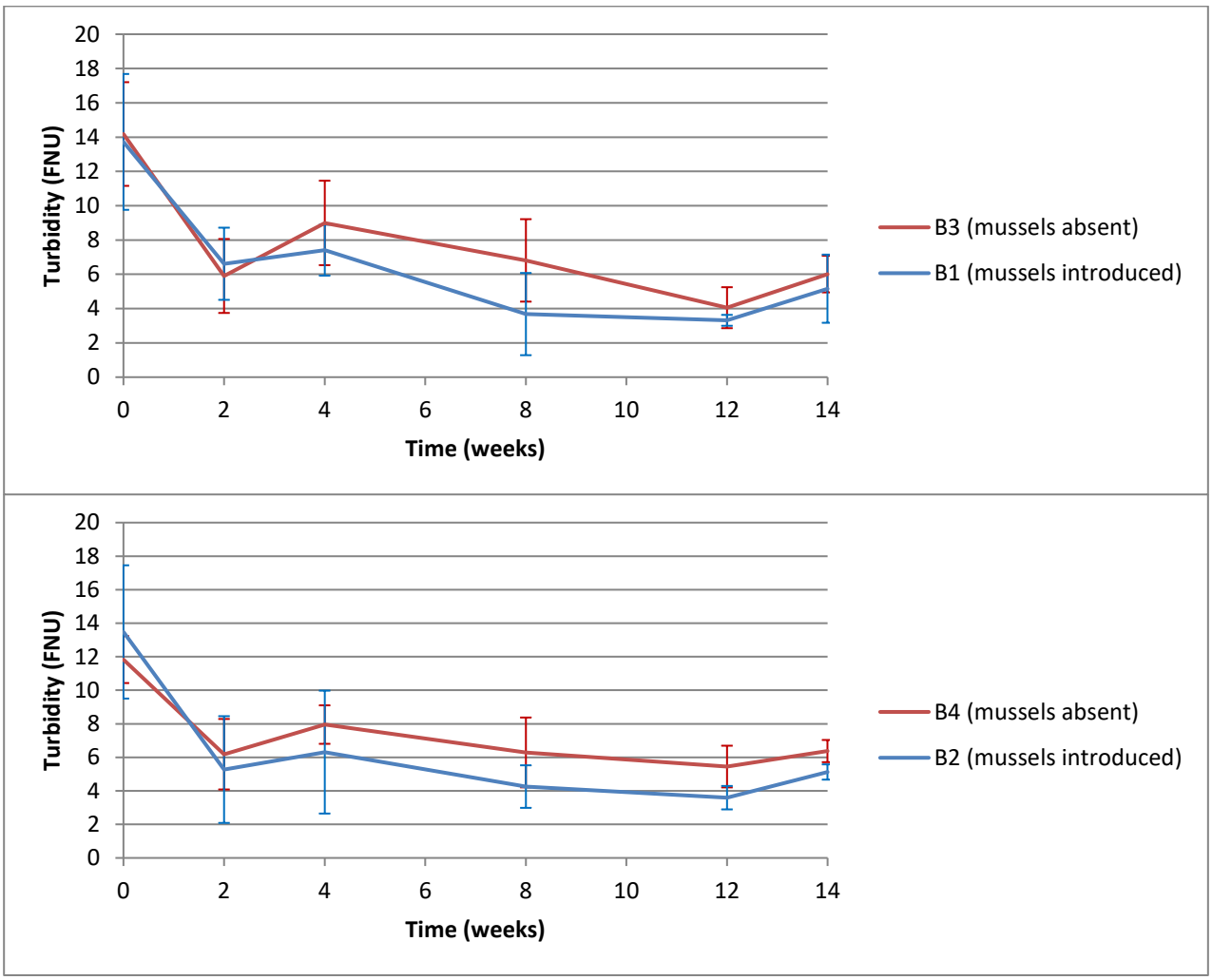


Figure 3.15. Mean (± 1 SD) water turbidity in Formazin Nephelometric Units (FNU) at RSB over the duration of the study.

Total dissolved solids

Mean total dissolved solids (TDS) in the water at MBA rose from 279 ppm at the start of the investigation to 284-285 ppm by the end of the study (Figure 3.16).

The mean beta coefficient of the change in TDS over time in A1, where mussels were removed (M=1.34, SD=0.61) was not significantly different to A3, where mussels were present (M=1.30, SD=0.60); t (7.80)=-0.10, $p > 0.05$.

The mean beta coefficient of the change in TDS over time in A2, where mussels were removed (M=1.32, SD=0.23) was not significantly different to A4, where mussels were present (M=1.20, SD=0.44); t (5.99)=-0.54, p > 0.05.

The removal of mussels from quadrats at MBA did not result in significantly different levels of TDS compared with quadrats where they were present.

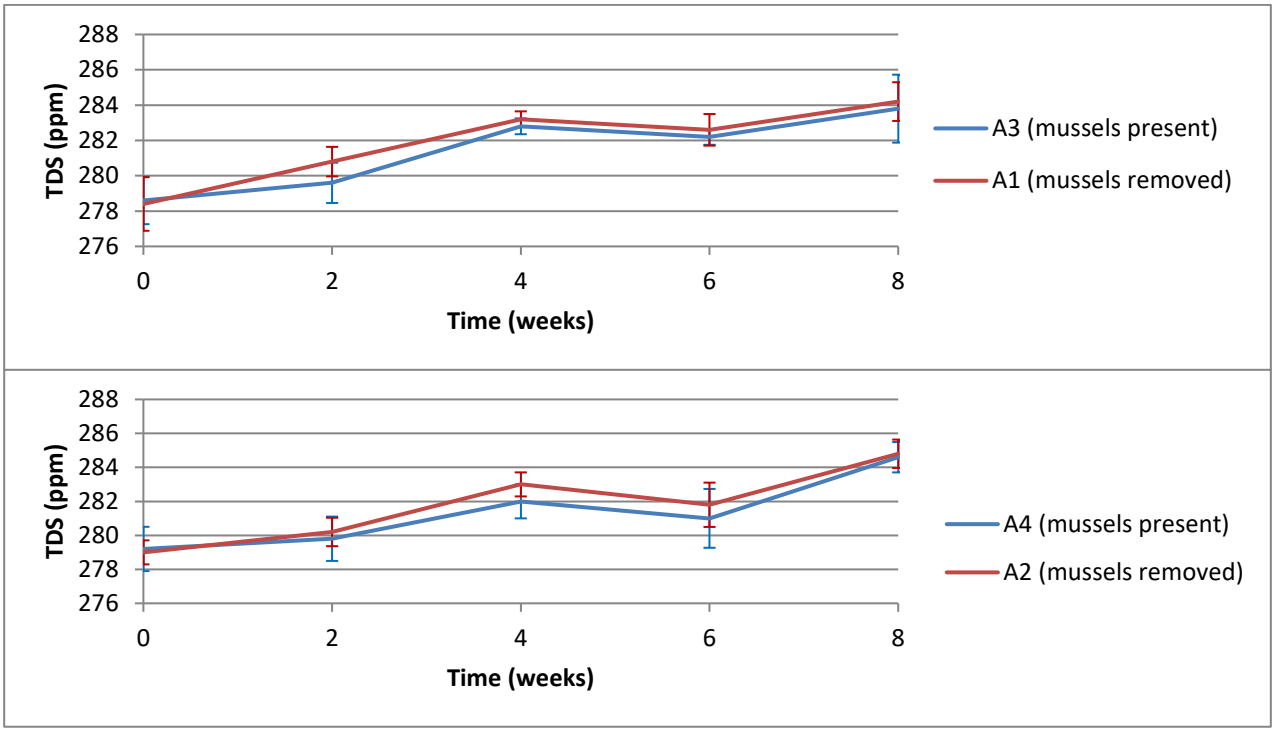


Figure 3.16. Mean (± 1 SD) total dissolved solids (TDS) at MBA over the duration of the study.

Mean TDS at MBB reduced from 277-278 ppm at the beginning of the study to 256-259 ppm by weeks 4-6, before increasing to 271-272 ppm by week 8 (Figure 3.17).

The mean beta coefficient of the change in TDS over time in B1, where mussels were added (M=-2.30, SD=0.37) was not significantly different to B3, where mussels were absent (M=-2.54, SD=0.38); t (7.99)=-1.02, p > 0.05.

The mean beta coefficient of the change in TDS over time in B2, where mussels were added (M=-2.42, SD=1.01) was not significantly different to B4, where mussels were absent (M=-2.68, SD=0.67); t (6.93)=-0.48, p > 0.05.

The introduction of mussels to quadrats at MBB did not result in significantly different levels of TDS compared with quadrats where they were absent.

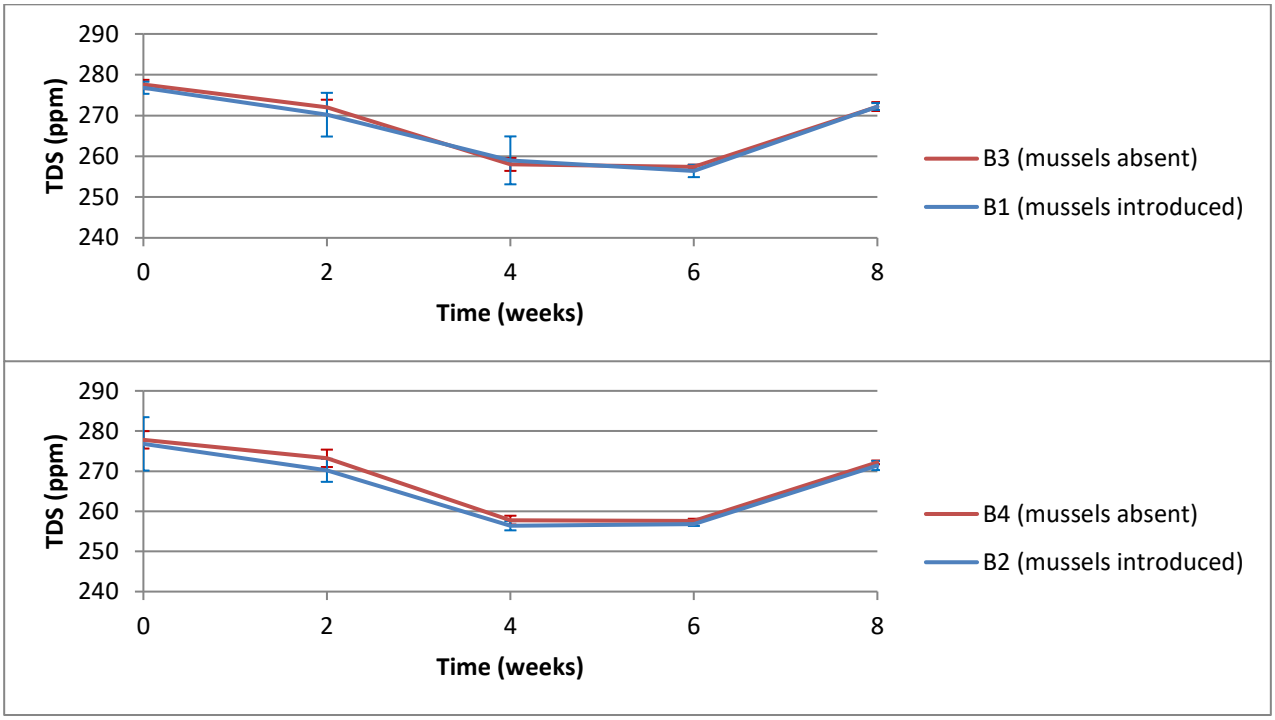


Figure 3.17. Mean (± 1 SD) total dissolved solids (TDS) at MBB over the duration of the study.

Mean TDS at RSA ranged from 290 to 348 ppm for the majority of the study until week 14, where it dropped to 251-253 ppm in the four quadrats (Figure 3.18).

The mean beta coefficient of the change in TDS over time in A1, where mussels were removed (M=-1.28, SD=0.68) was not significantly different to A3, where mussels were present (M=1.56, SD=0.08); t (4.10)=-0.91, $p > 0.05$.

The mean beta coefficient of the change in TDS over time in A2, where mussels were removed (M=-1.44, SD=0.10) was not significantly different to A4, where mussels were present (M=1.53, SD=0.13); t (7.49)=-1.29, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different levels of TDS compared with quadrats where they were present.

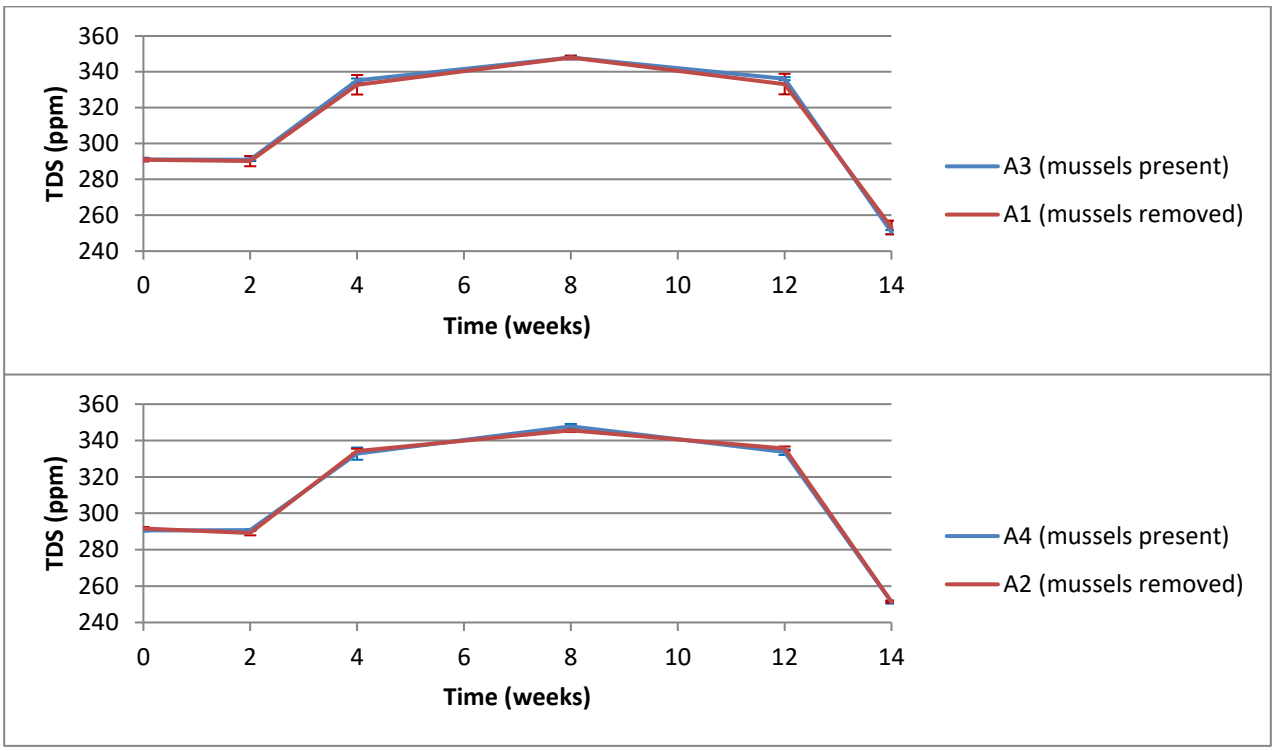


Figure 3.18. Mean (± 1 SD) total dissolved solids (TDS) at RSA over the duration of the study.

Mean TDS levels at RSB followed a similar pattern to RSA, ranging from 260 to 356 ppm (Figure 3.19).

The mean beta coefficient of the change in TDS over time in B1, where mussels were added (M=-7.74, SD=0.15) was not significantly different to B3, where mussels were absent (M=-7.92, SD=0.11); t (7.21)=-2.16, $p > 0.05$.

The mean beta coefficient of the change in TDS over time in B2, where mussels were added (M=-7.82, SD=0.33) was not significantly different to B4, where mussels were absent (M=-7.83, SD=0.25); t (7.45)=-0.09, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in significantly different levels of TDS compared with quadrats where they were absent.

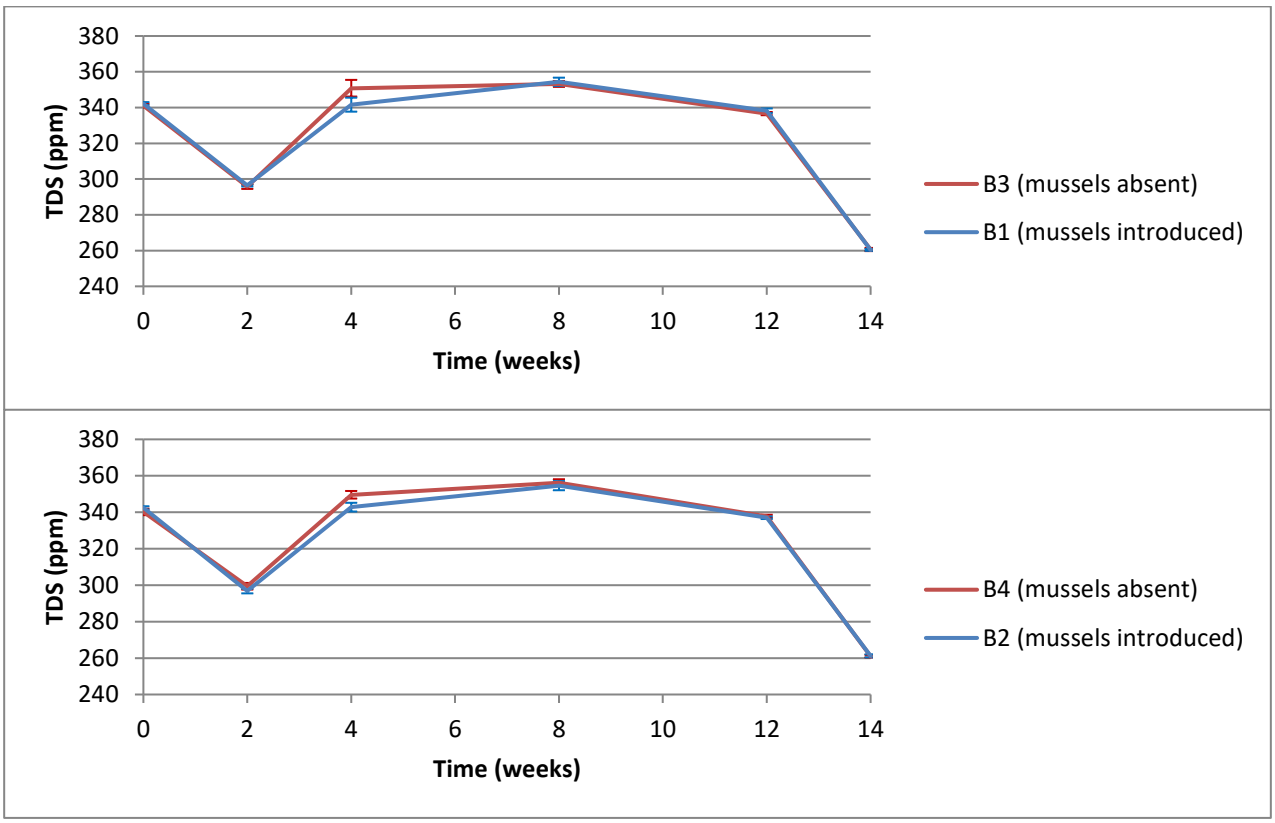


Figure 3.19. Mean (± 1 SD) total dissolved solids (TDS) at RSB over the duration of the study.

Water oxygen saturation

Mean water percentage oxygen saturation in all four quadrats at MBA steadily dropped from 104-105 % at the beginning of the study to 32-35 % by week 6 before rising again to 56-62 % (Figure 3.20).

The mean beta coefficient of the change in water oxygen saturation over time in A1, where mussels were removed (M=-14.16, SD=0.47) was not significantly different to A3, where mussels were present (M=-13.45, SD=0.77); t (6.58)=1.75, $p > 0.05$.

The mean beta coefficient of the change in water oxygen saturation over time in A2, where mussels were removed (M=-14.53, SD=1.25) was not significantly different to A4, where mussels were present (M=-14.31, SD= 0.91); t (7.31)=0.32, $p > 0.05$. The removal of mussels from quadrats at MBA did not significantly alter oxygen saturation levels compared with quadrats where they were present.

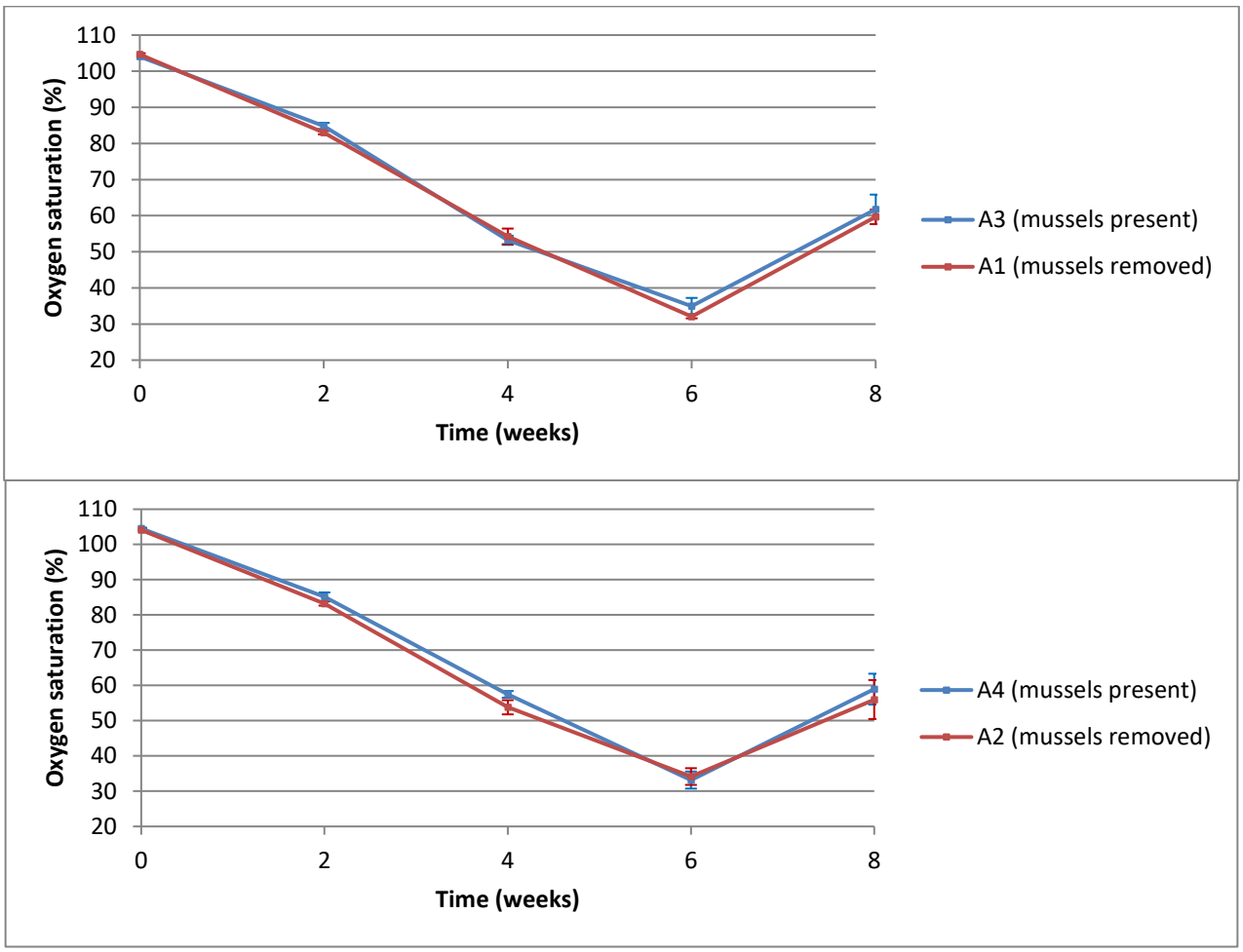


Figure 3.20. Mean (± 1 SD) percentage oxygen saturation at MBA over the duration of the study.

The addition of mussels to MBB did not significantly alter water oxygen saturation levels, with mean percentage oxygen saturation falling from 108 % at the start of the study to 85-86 % by week 4 before steadily increasing to 91-92 % in a ll quadrats by week 8 (Figure 3.21).

The mean beta coefficient of the change in water oxygen saturation over time in B1, where mussels were added (M=-3.69, SD=0.17) was not significantly different to B3, where mussels were absent (M=-3.74, SD=0.19); t (7.92)=-0.42, $p > 0.05$.

The mean beta coefficient of the change in water oxygen saturation over time in B2, where mussels were added (M=-3.70, SD=0.13) was not significantly different to B4, where mussels were absent (M=-3.75, SD=0.08); t (6.82)=-0.75, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not significantly alter oxygen saturation levels compared with quadrats where they were absent.

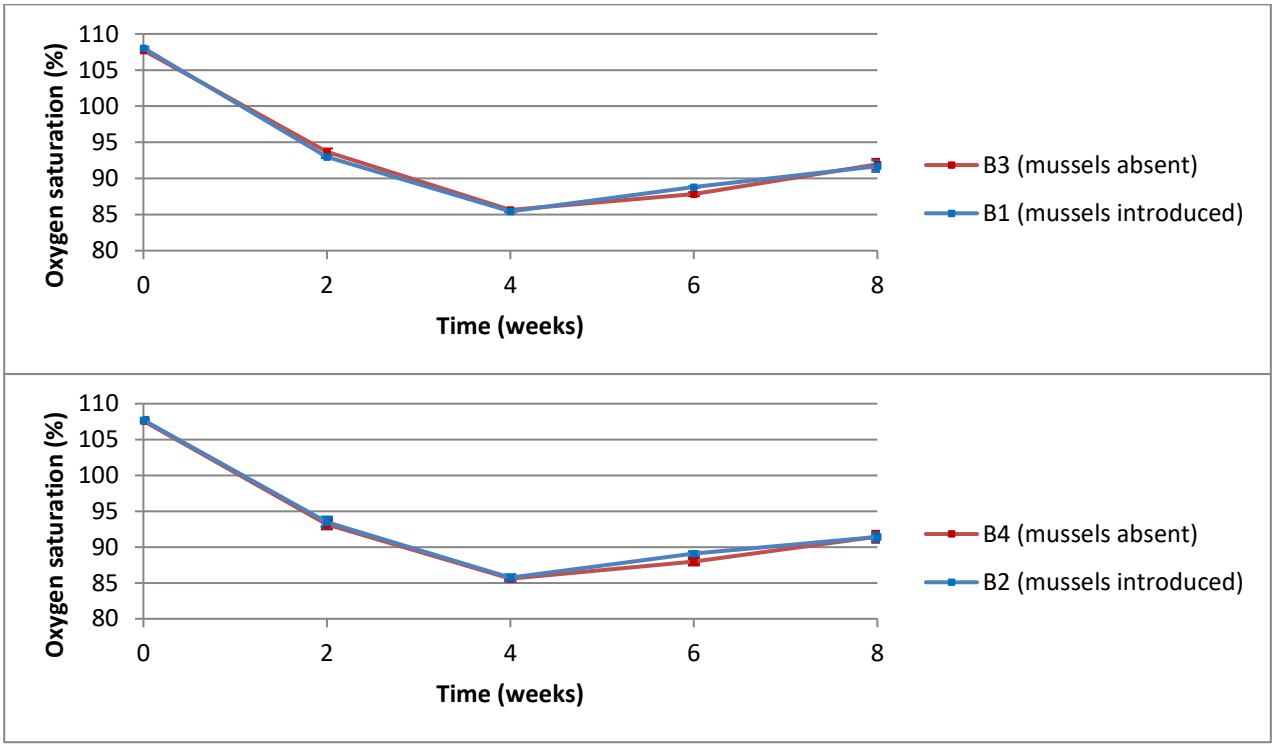


Figure 3.21. Mean (± 1 SD) percentage oxygen saturation at MBB over the duration of the study.

At RSA mean percentage water oxygen saturation fell from 150-156 % at the beginning of the study to 115-120 % by week 8, rising to 130-132 % by week 14 in the four quadrats (Figure 3.22).

The mean beta coefficient of the change in water oxygen saturation over time in A1, where mussels were removed (M=-3.63, SD=0.47) was not significantly different to A3, where mussels were present (M=-4.34, SD=0.59); t (7.61)=-2.11, $p > 0.05$.

The mean beta coefficient of the change in water oxygen saturation over time in A2, where mussels were removed (M=-3.90, SD=0.24) was not significantly different to A4, where mussels were present (M=-3.70, SD=0.44); t (6.19)=0.90, $p > 0.05$.

The removal of mussels from quadrats at RSA did not significantly alter oxygen saturation levels compared with quadrats where they were present.

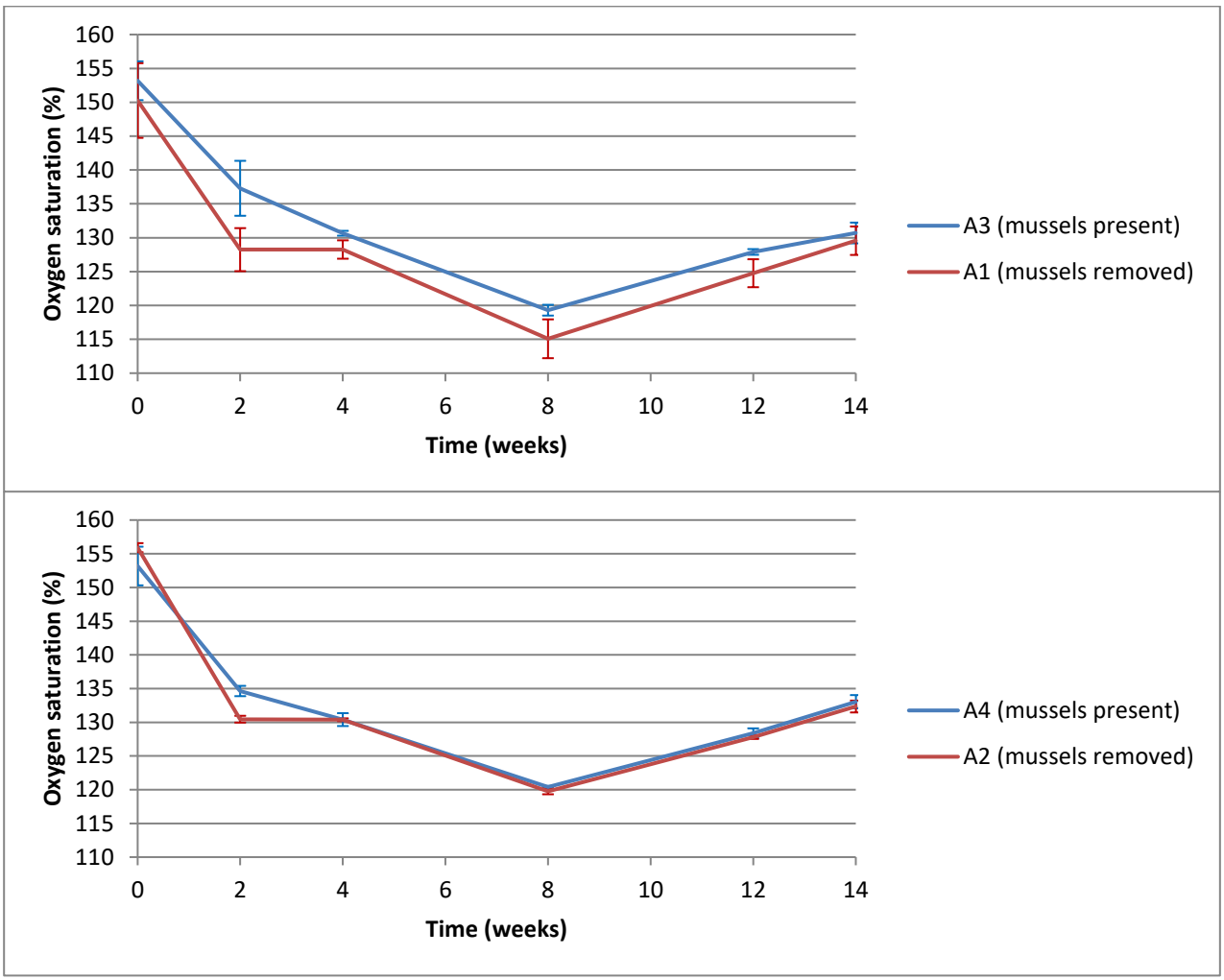


Figure 3.22. Mean (± 1 SD) percentage oxygen saturation at RSA over the duration of the study.

Mean percentage water oxygen saturation levels at RSB were highest at the start of the study at 102-103 % in all four quadrats. By week 4 oxygen saturation had dropped to 83-85 %, fluctuating between 84 and 91 % for the remainder of the study (Figure 3.23).

The mean beta coefficient of the change in water oxygen saturation over time in B1, where mussels were added (M=-2.38, SD=0.19) was not significantly different to B3, where mussels were absent (M=-2.20, SD=0.13); $t(7.14)=1.78$, $p > 0.05$.

The mean beta coefficient of the change in water oxygen saturation over time in B2, where mussels were added (M=-2.25, SD=0.07) was not significantly different to B4, where mussels were absent (M=-2.21, SD=0.04); $t(5.89)=0.92$, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not significantly alter oxygen saturation levels compared with quadrats where they were absent.

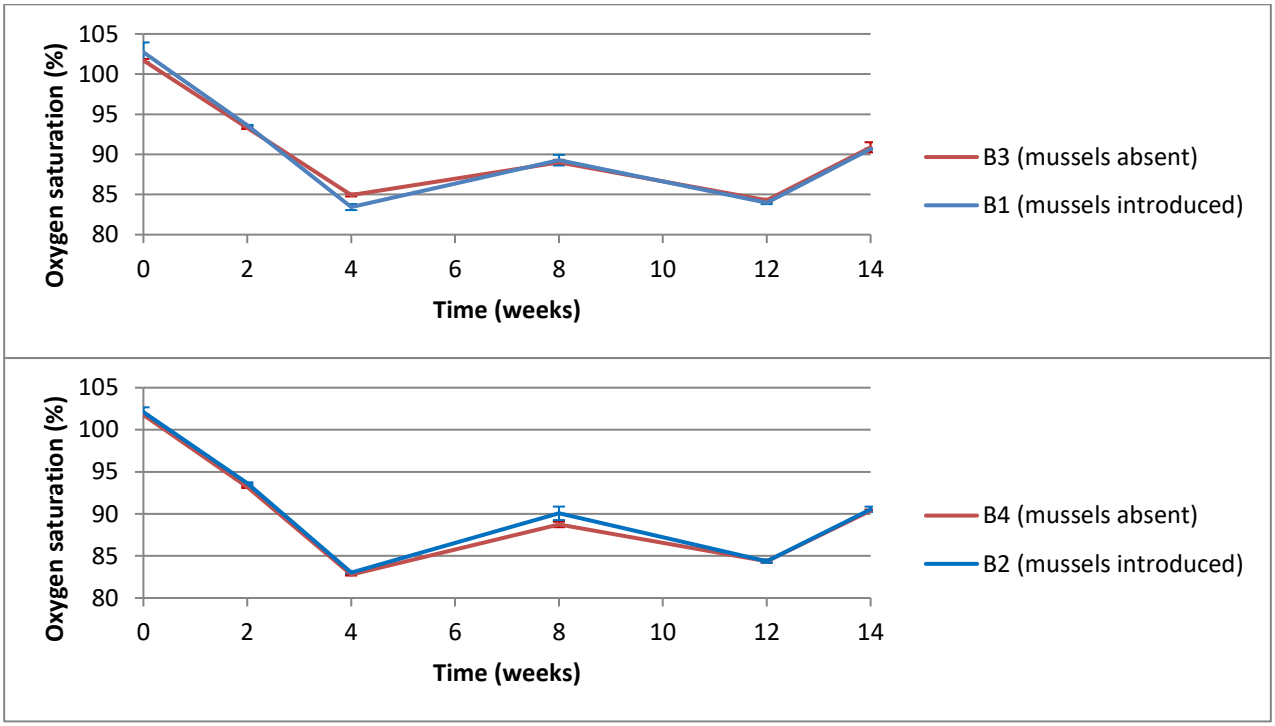


Figure 3.23. Mean (± 1 SD) percentage oxygen saturation at RSB over the duration of the study.

Water velocity

Mean water velocity at 0.6 depth ranged from 0.14 to 0.19 ms^{-1} in the four MBA quadrats for the first two weeks of the study (Figure 3.24). From week 4 to 8 of the investigation water velocity at MBA was below the threshold required to move the impeller of the flow meter. The minimum velocity required to move the Valeport Model 002 flow meter is 0.046 ms^{-1} (Valeport Ltd., 2013).

The mean beta coefficient of the change in river velocity over time in A1, where mussels were removed (M=-0.02, SD=0.04) was not significantly different to A3, where mussels were present (M=-0.05, SD=0.01); t (4.06)=-1.60, $p > 0.05$.

The mean beta coefficient of the change in river velocity over time in A2, where mussels were removed (M=-0.05, SD=0.01) was not significantly different to A4, where mussels were present (M=-0.06, SD=0.01); t (6.34)=-2.07, $p > 0.05$.

The removal of mussels from quadrats at MBA did not significantly alter the velocity of the water compared with quadrats where they were present.

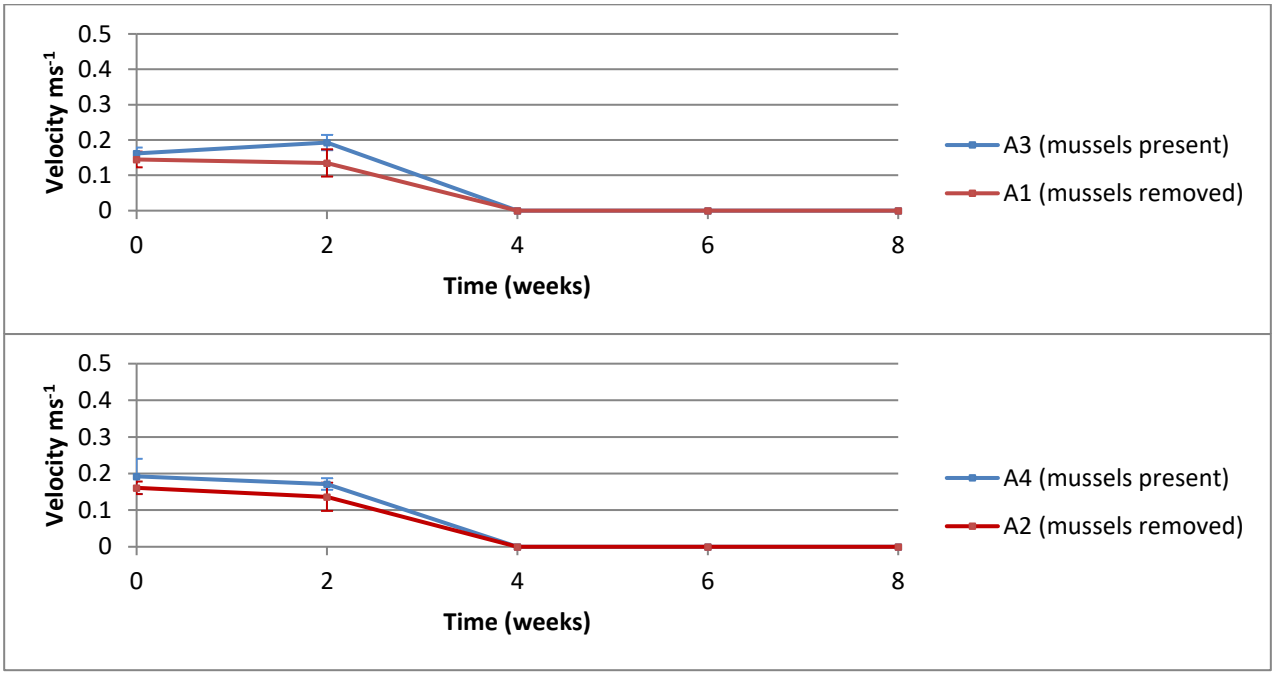


Figure 3.24. Mean (± 1 SD) water velocity at MBA over the duration of the study.

At MBB mean water velocity at 0.6 depth ranged from 0.16 to 0.48 ms^{-1} in the four quadrats, with the peak discharge occurring in week 2 of the study (Figure 3.25). In B1 where mussels were introduced, the velocity was initially higher than B3, where they were absent. However from week 2 until the end of the investigation the velocity in B1 dropped below that of the control quadrat, but not significantly so.

The mean beta coefficient of the change in river velocity over time in B1, where mussels were added (M=-0.04, SD=0.004) was not significantly different to B3, where mussels were absent (M=-0.03, SD=0.01); t (4.84)=1.99, $p > 0.05$.

The mean beta coefficient of the change in river velocity over time in B2, where mussels were added (M=-0.04, SD=0.008) was not significantly different to B4, where mussels were absent (M=-0.05, SD=0.01); t (6.17)=-1.54, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not significantly alter the velocity of the water compared with quadrats where they were absent.

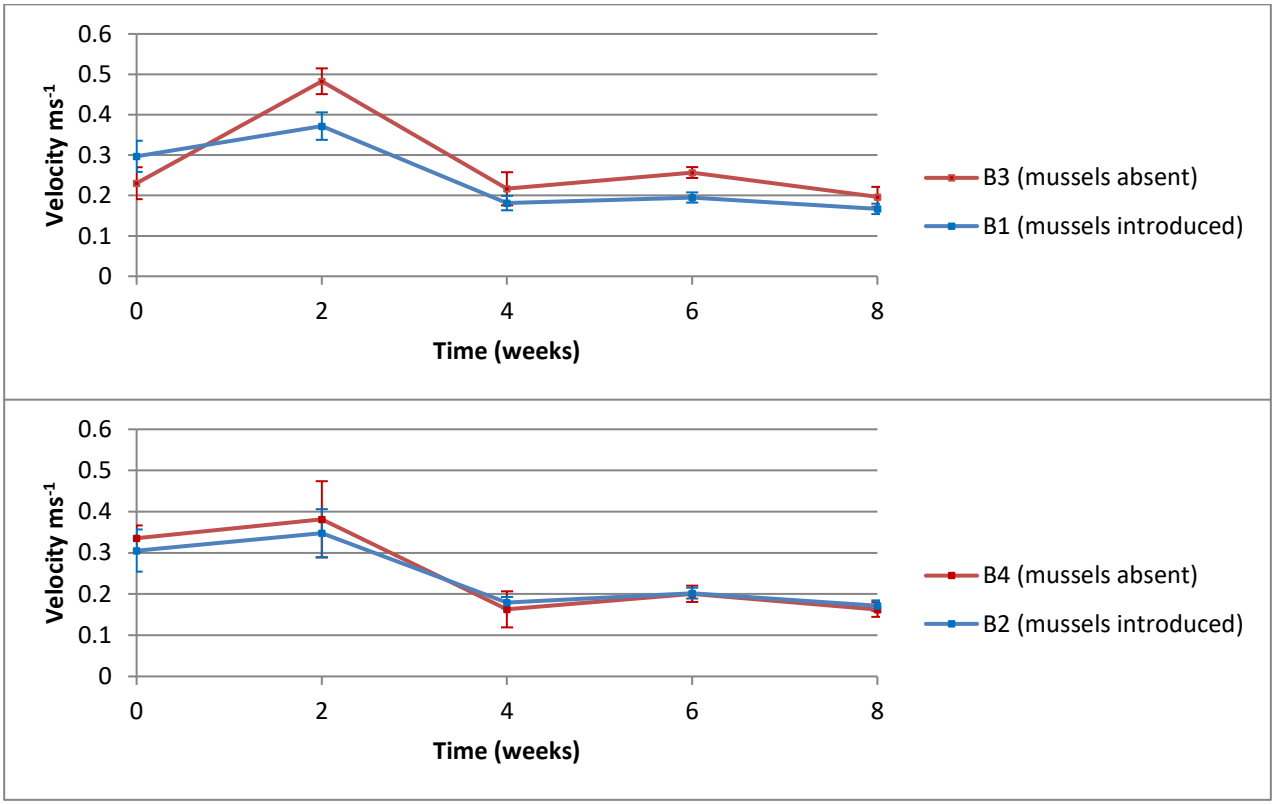


Figure 3.25. Mean (± 1 SD) water velocity at MBB over the duration of the study.

At RSA mean water velocities at 0.6 depth fluctuated between 0.10 to 0.50 ms^{-1} over the 14 weeks of the investigation (Figure 3.26). From week 10 to 14, the velocity in quadrats A1 and A2, from which mussels had been removed had increased above that of the quadrats that contained mussels.

The mean beta coefficient of the change in river velocity over time in A1, where mussels were removed (M=0.01, SD=0.01) was significantly greater than A3, where mussels were present (M=-0.02, SD=0.002); t (4.29)=-3.88, p = 0.0157.

The mean beta coefficient of the change in river velocity over time in A2, where mussels were removed (M=0.03, SD=0.01) was significantly greater than A4, where mussels were present (M=-0.01, SD=0.02); t (6.82)=-3.06, p = 0.0189.

The removal of mussels from quadrats at RSA resulted in significantly increased velocities at 0.6 depth compared with quadrats where they were present.

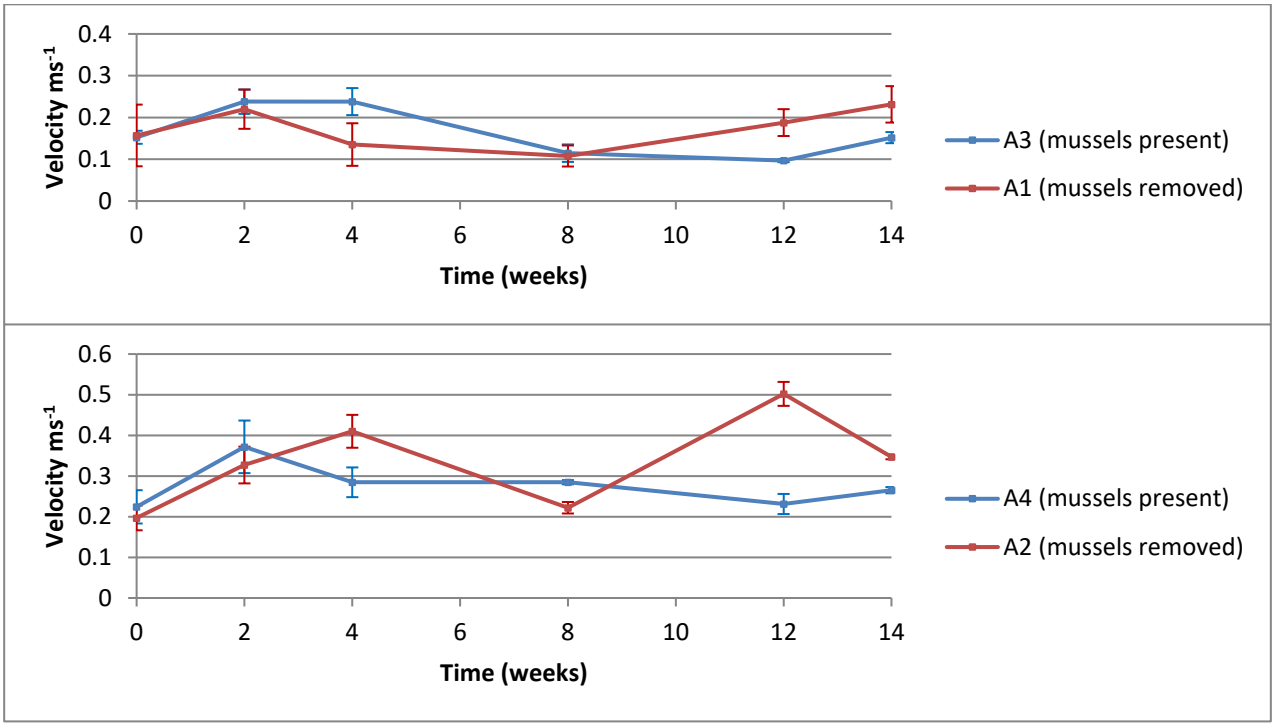


Figure 3.26. Mean (± 1 SD) water velocity at RSA over the duration of the study.

Mean water velocities at 0.6 depth at RSB remained relatively consistent throughout the study (Figure 3.27). B1, B2 and B3 had velocities ranging from 0.13 to 0.33 ms^{-1}, whereas the mean velocity in B4 ranged from 0.39 to 0.58 ms^{-1}.

The mean beta coefficient of the change in river velocity over time in B1, where mussels were added (M=0.00, SD=0.01) was not significantly different to B3, where mussels were absent (M=-0.01, SD=0.03); t (4.56)=-0.61, $p > 0.05$.

The mean beta coefficient of the change in river velocity over time in B2, where mussels were added (M=0.01, SD=0.01) was not significantly different to B4, where mussels were absent (M=0.01, SD=0.01); t (7.80)=0.04, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not significantly alter the velocity of the water compared with quadrats where they were absent.

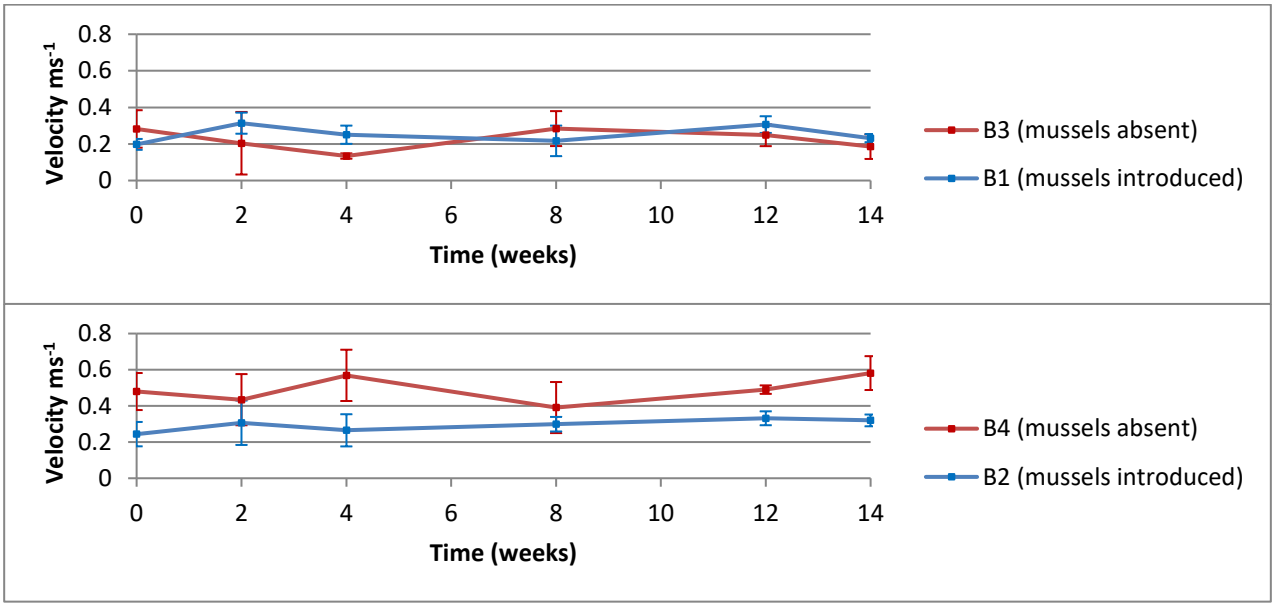


Figure 3.27. Mean (± 1 SD) water velocity at RSB over the duration of the study.

A summary of the hydrology and water quality results is shown in Table 3.1, showing the impact of freshwater mussels on the measured hydrological variables at each river site.

Table 3.1. Summary of results for the four river sites, showing whether the introduction or removal of freshwater mussels led to a significant increase or decrease in each measured hydrological variable compared with the control quadrats.

River site / Variable	MBA	MBB	RSA	RSB
Water Turbidity (FNU)	No significant difference	Significantly decreased where mussels were introduced	No significant difference	No significant difference
Total dissolved solids (ppm)	No significant difference	No significant difference	No significant difference	No significant difference
Water oxygen saturation (%)	No significant difference	No significant difference	No significant difference	No significant difference
Water velocity at 0.6 depth (ms^{-1})	No significant difference	No significant difference	Significantly increased where mussels were removed	No significant difference

3.4.2 River bed characteristics

Substrate oxygen saturation

At the beginning of the study the mean percentage hyporheic oxygen saturation at MBA was similar in all four quadrats, with values ranging from 99 to 102 %. After this point until the end of the study the mean hyporheic oxygen saturation levels in the quadrats with mussels removed began to decrease below that of the quadrats containing mussels (Figure 3.28). By the end of the study mean percentage hyporheic oxygen saturation in A1 and A2 had dropped to 44 % and 46 % respectively, whereas the control quadrats containing mussels had oxygen saturation levels of 58-59 %.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in A1, where mussels were removed (M=-14.45, SD=1.44) was significantly more negative than A3, where mussels were present (M=-9.71, SD=0.50); t (4.93)=6.94, p = 0.0010.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in A2, where mussels were removed (M=-13.35, SD=1.74) was significantly more negative than A4, where mussels were present (M=-10.50, SD=0.41); t (4.45)=3.57, p = 0.0195.

The removal of mussels from quadrats at MBA resulted in significantly reduced hyporheic oxygen saturation levels compared with quadrats where they were present.

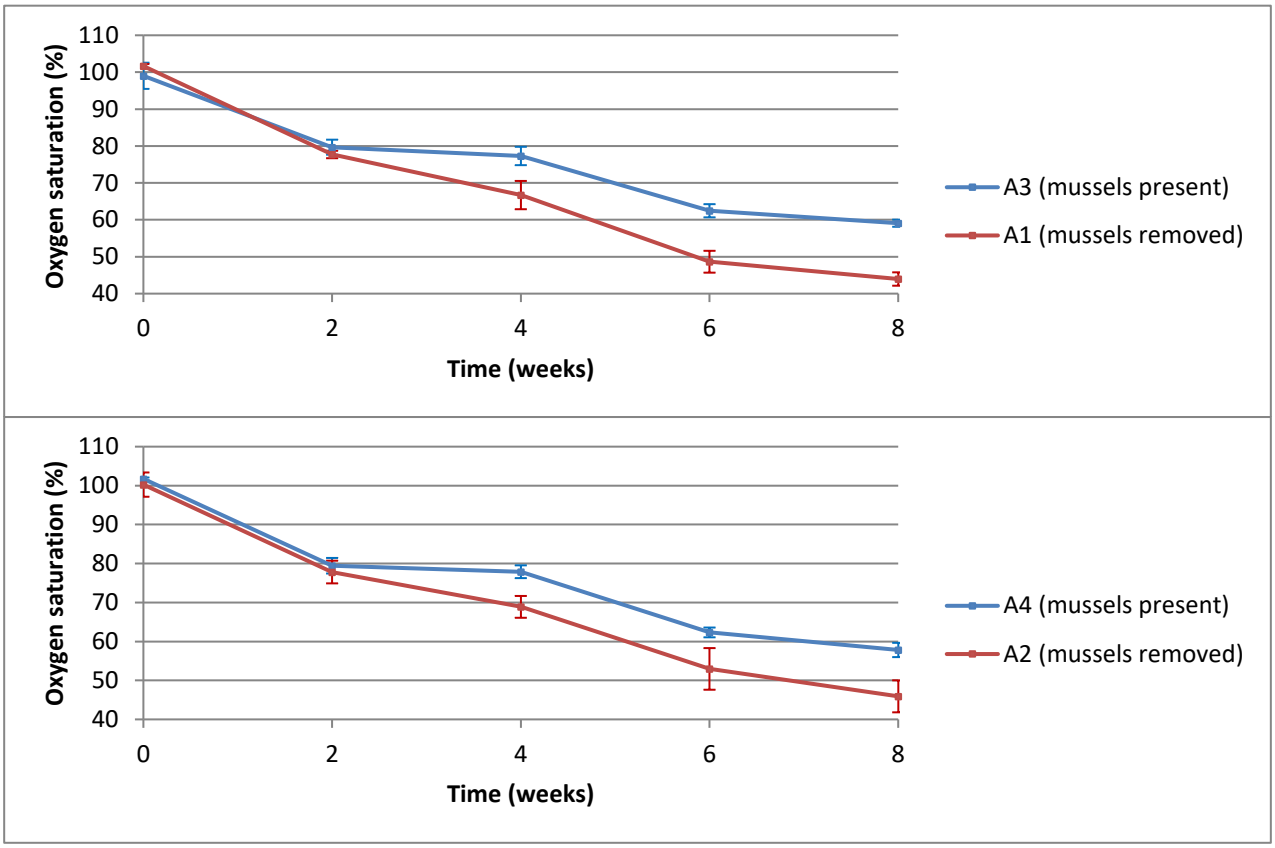


Figure 3.28. Mean (± 1 SD) percentage hyporheic oxygen saturation at MBA over the duration of the study.

At MBB mean hyporheic oxygen saturation was at similar levels in all four quadrats until week 4 when oxygen saturation in the quadrats containing mussels began to increase above the levels in the control quadrats (Figure 3.29). By week 8 the mean hyporheic oxygen saturation was 7 % higher in B1 than in B3, and in B2 the hyporheic oxygen saturation was 4 % higher than in B4.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in B1, where mussels were added (M=-1.42, SD=0.78) was significantly less negative than B3, where mussels were absent (M=-3.29, SD=0.76); t (7.99)=-3.87, p = 0.0047.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in A2, where mussels were added (M=-1.47, SD=0.93) was not significantly different to A4, where mussels were absent (M=-2.34, SD=0.93); t (4.38)=-2.34, p > 0.05.

The introduction of mussels to MBB resulted in significantly increased hyporheic oxygen saturation levels in quadrat B1 compared with where they were absent in quadrat B3. The introduction of mussels to quadrat B2 did not result in significantly different hyporheic oxygen saturation levels to quadrat B4, where they were absent.

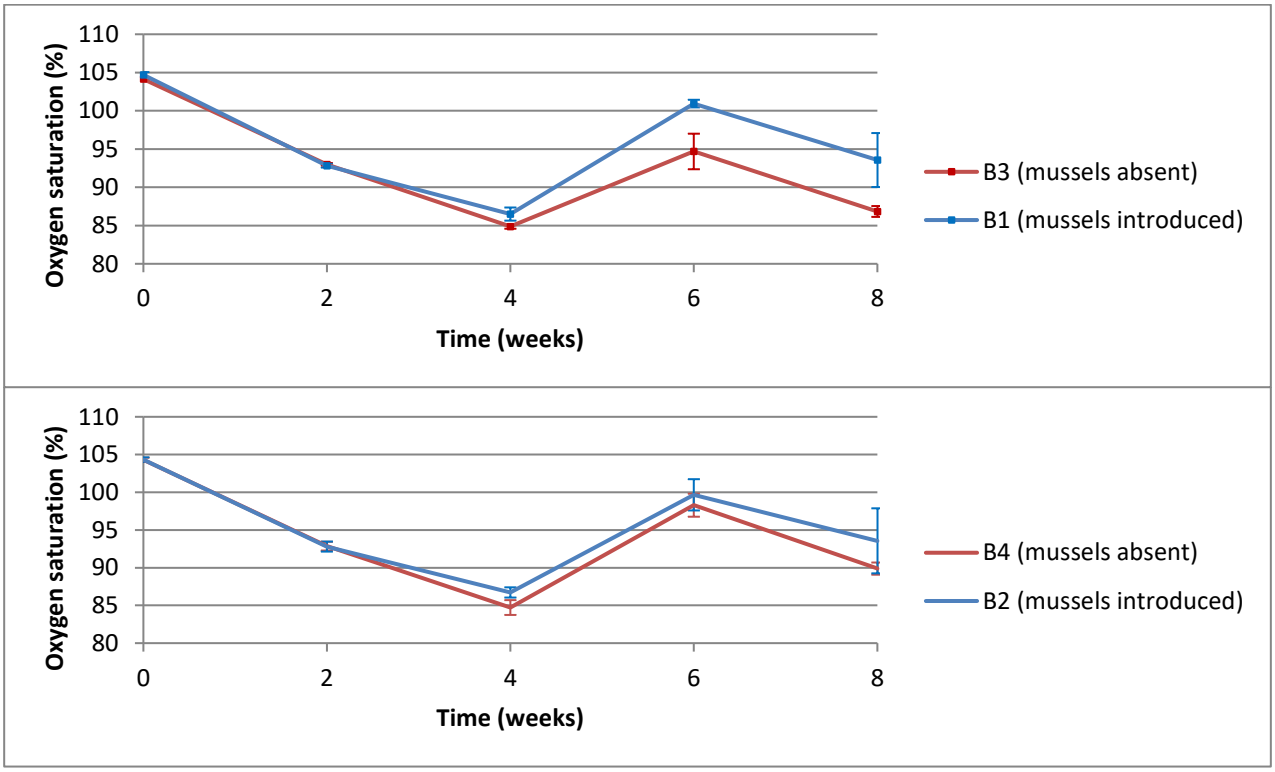


Figure 3.29. Mean (± 1 SD) percentage hyporheic oxygen saturation at MBB over the duration of the study.

At RSA mean percentage hyporheic oxygen saturation levels were of similar levels in the four quadrats until week 6 when levels in the quadrats without mussels dropped below those present in the quadrats with mussels (Figure 3.30). Between week 8 and 14 the mean percentage hyporheic

oxygen saturation in the quadrats with mussels removed was 4-9 % lower than in the quadrats with mussels present.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in A1, where mussels were removed (M=-6.08, SD=1.34) was significantly more negative than A3, where mussels were present (M=-3.36, SD=0.44); $t(4.86)=4.30$, $p = 0.0083$.

The mean beta coefficient of the change in hyporheic oxygen saturation over time in A2, where mussels were removed (M=-5.04, SD=0.50) was significantly more negative than A4, where mussels were present (M=-3.47, SD=0.32); $t(6.83)=5.93$, $p < 0.001$.

The removal of mussels from quadrats at RSA resulted in significantly reduced hyporheic oxygen saturation levels in compared with quadrats where they were present.

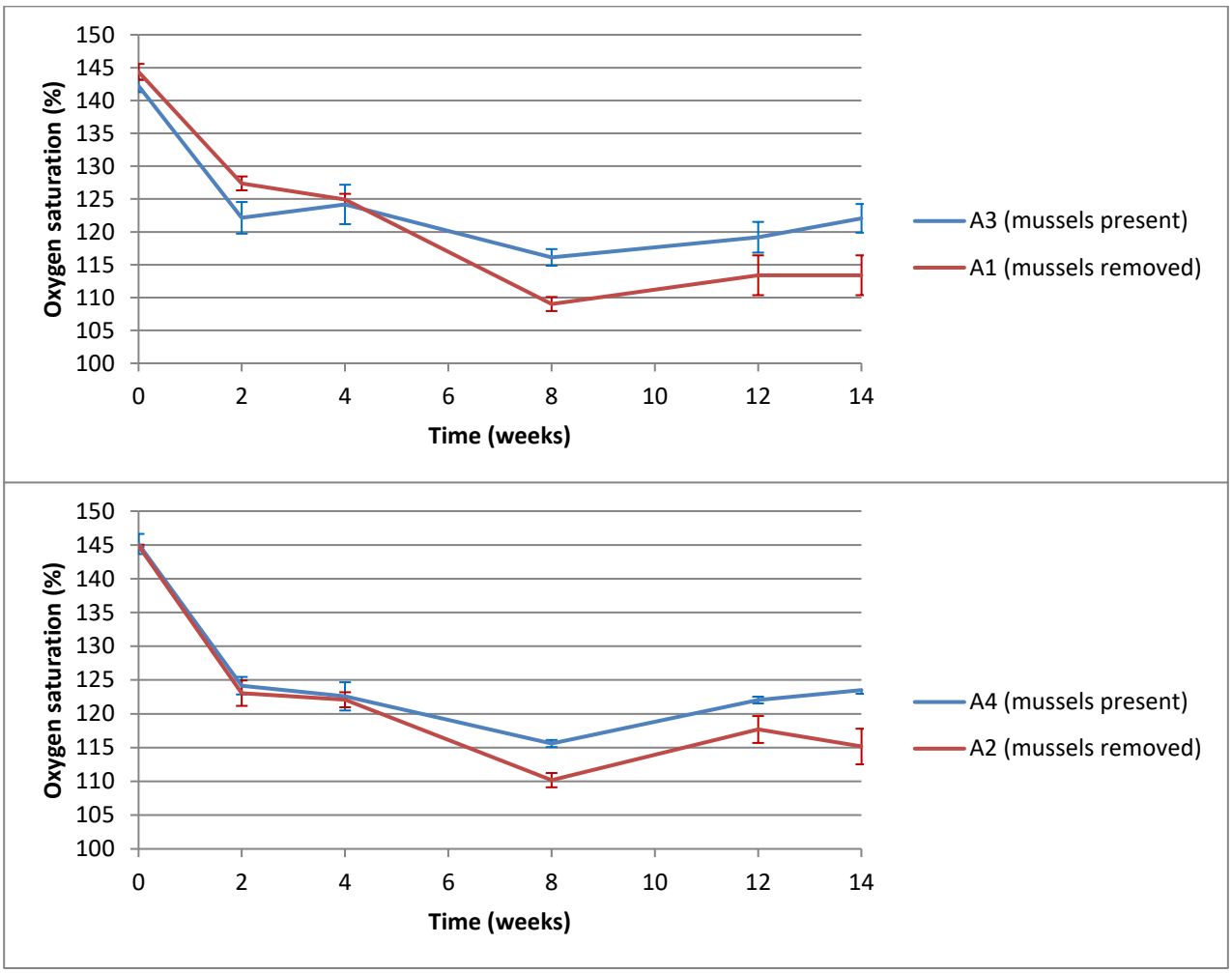


Figure 3.30. Mean (± 1 SD) percentage hyporheic saturation at RSA over the duration of the study.

At the beginning of the study the mean percentage hyporheic oxygen saturation at RSB was similar in all four quadrats, with mean values ranging from 99-100 % (Figure 3.31). From the second week until the end of the study the mean percentage hyporheic oxygen saturation in the quadrats with mussels introduced was greater than in the quadrats with mussels absent. By week 14 quadrat B1 had a hyporheic oxygen saturation of 97 % compared with B3, which was at 94 % oxygen saturation. B2 had hyporheic oxygen saturation levels of 99 %, compared with 96 % for the B4 control quadrat.

The mean beta coefficient of the change in water oxygen saturation over time in B1, where mussels were added (M=0.46, SD=0.32) was significantly greater than B3, where mussels were absent (M=-0.02, SD=0.31); t (8.00)=-2.39, p = 0.0440.

The mean beta coefficient of the change in water oxygen saturation over time in B2, where mussels were added (M=0.47, SD=0.55) was not significantly different to B4, where mussels were absent (M=0.17, SD=0.58); t (7.97)=-0.84, p > 0.05.

The introduction of mussels to RSB resulted in significantly increased hyporheic oxygen saturation levels in quadrat B1 compared with where they were absent in quadrat B3. The introduction of mussels to quadrat B2 did not result in significantly different hyporheic oxygen saturation levels to quadrat B4, where they were absent.

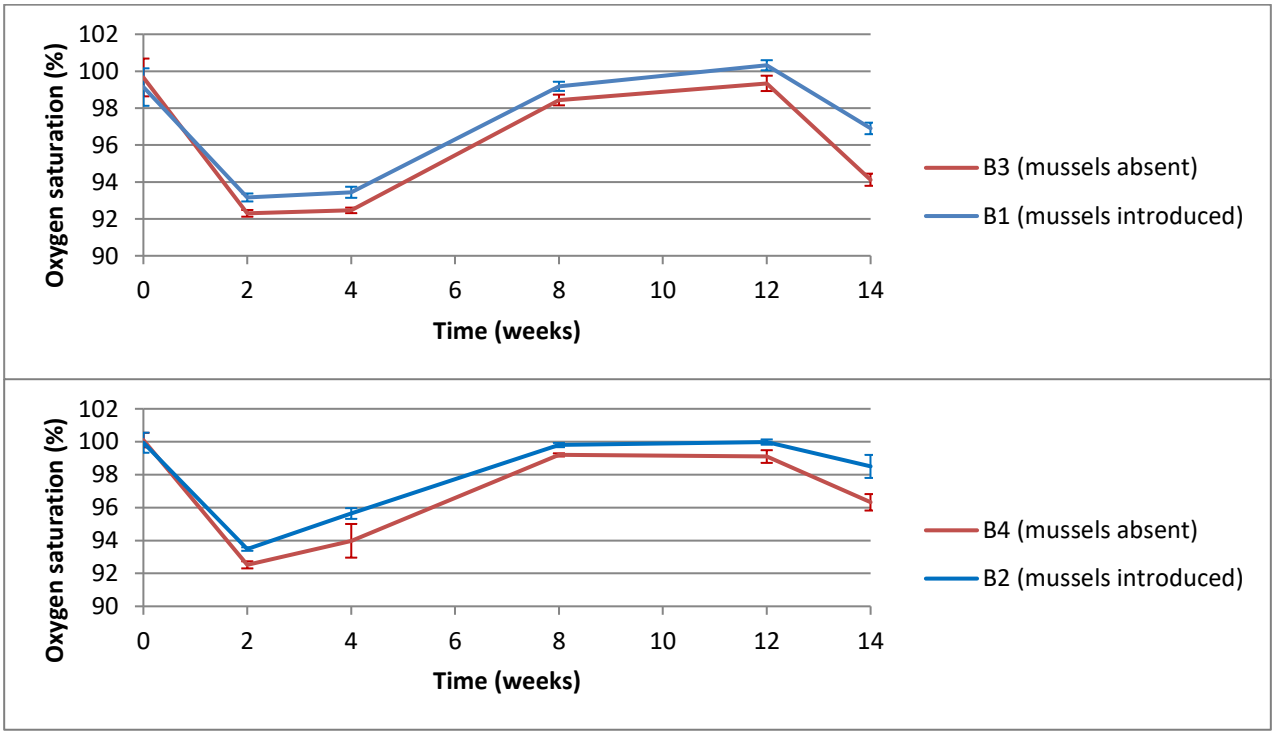


Figure 3.31. Mean (± 1 SD) percentage hyporheic oxygen saturation at RSB over the duration of the study.

Substrate grain-size

The B-axis length of sediment in the "armour layer" at MBA ranged from 5.6-200 mm at the beginning of the study, with mean values ranging from 17.3-44.1 mm. Median B-axis length in A1, with mussels removed was 44.7 mm at the beginning of the study and 31.7 mm at the end of the study. In A3, with mussels present, median B-axis length was 30.8 mm at the start of the study and 29.5 mm at the end (Figure 3.32).

The mean beta coefficient of the change in bed sediment B-axis length over time in A1, where mussels were removed (M=-12.37, SD=25.13) was significantly more negative than A3, where mussels were present (M=0.41, SD=25.67); t (97.96)=2.51, p = 0.0135.

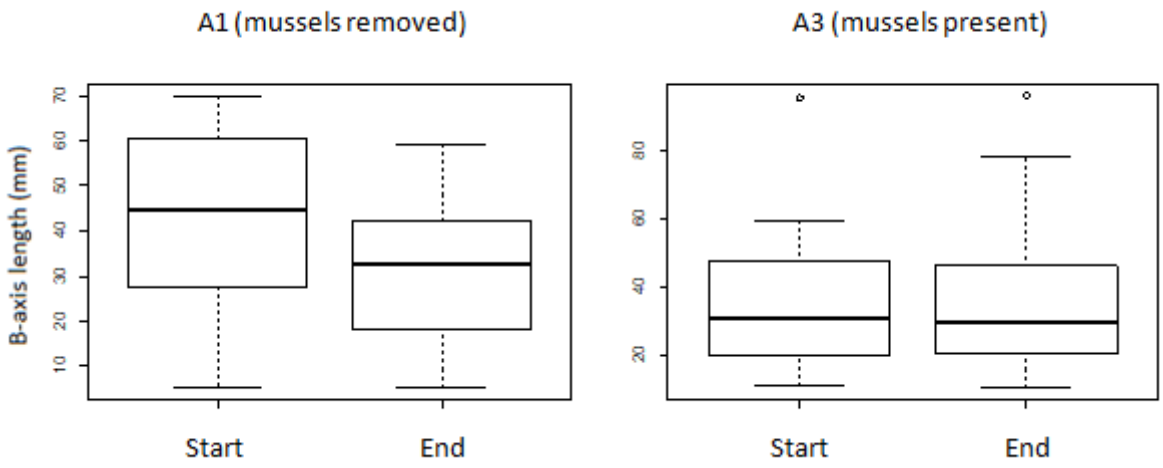


Figure 3.32. Boxplots showing the change pebble B-axis length in quadrat MBA1, with mussels removed compared with MBA3, with mussels present, at the beginning and end of the study.

In quadrat A2, with mussels removed, median B-axis was 37.2 mm at the start of the study and 33.8 mm at the end. Median B-axis length in the A4 control quadrat was 16.1 mm at the start of the study and 19.2 mm at the end (Figure 3.33).

The mean beta coefficient of the change in bed sediment B-axis length over time in A2, where mussels were removed (M=15.19, SD=16.08) was significantly lower than A4, where mussels were present (M=25.871, SD=28.88); t (76.71)=2.281, p = 0.0252.

The removal of mussels from quadrats at MBA resulted in significantly reduced pebble B-axis lengths compared with quadrats where they were present.

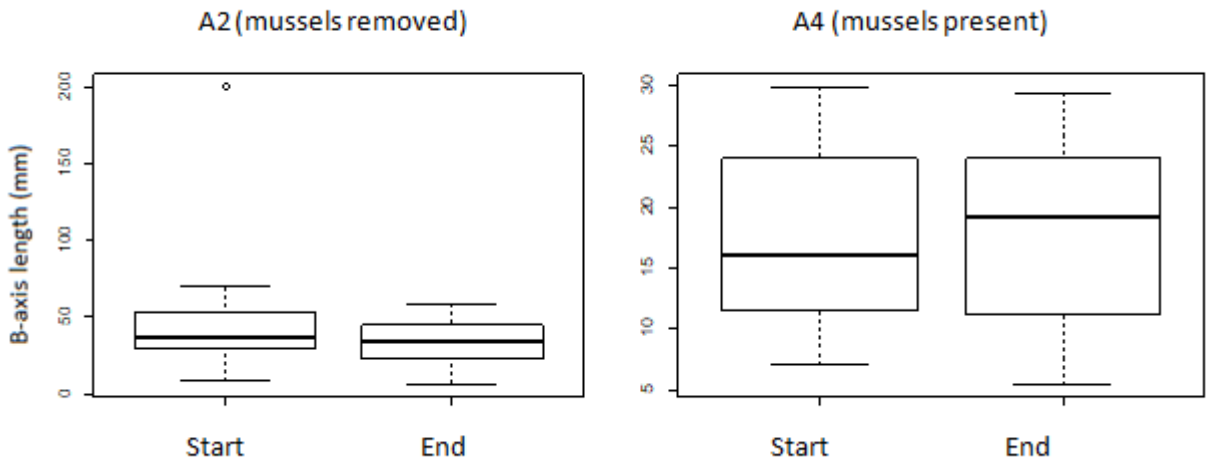


Figure 3.33. Boxplots showing change in pebble B-axis length in quadrat MBA2, with mussels removed compared with MBA4, with mussels present, at the beginning and end of the study.

At MBB pebble B-axis length ranged from 5.5-145 mm at the beginning of the study, with mean B-axis in the four quadrats ranging from 37.1-48.0 mm. Median B-axis length in B1, with mussels introduced was 40.2 mm at the beginning of the study and 30.4 mm at the end of the study. In B3, with mussels absent, median B-axis length was 32.4 mm at the start of the study and 28.6 mm at the end (Figure 3.34).

The mean beta coefficient of the change in bed sediment B-axis length over time in B1, where mussels were added (M=-0.99, SD=22.97) was not significantly different to B3, where mussels were absent (M=-2.28, SD=-27.90); t (94.51)=-0.25, p > 0.05.

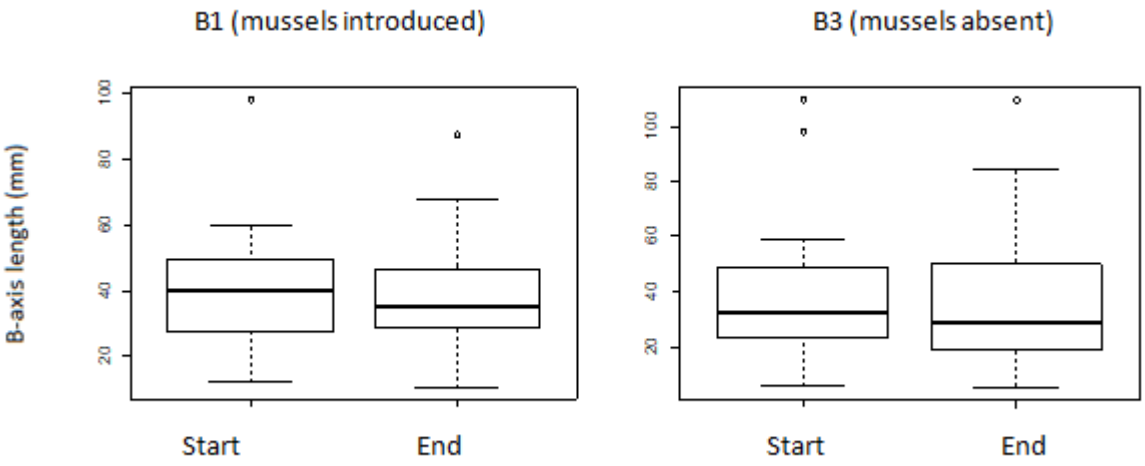


Figure 3.34. Boxplots showing change in pebble B-axis length in quadrat MBB1, with mussels introduced compared with MBB3, with mussels absent, at the beginning and end of the study.

In quadrat B2, with mussels introduced, median B-axis was 41.6 mm at the start of the study and 41.0 mm at the end. Median B-axis length in the B4 control quadrat was 47.4 mm at the start of the study and 50.6 mm at the end (Figure 3.35).

The mean beta coefficient of the change in bed sediment B-axis length over time in B2, where mussels were added (M=11.04, SD=34.16) was not significantly different to B4, where mussels were absent (M=1.61, SD=33.80); t (97.99)=-1.39, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not result in significantly different pebble B-axis lengths compared with quadrats where they were absent.

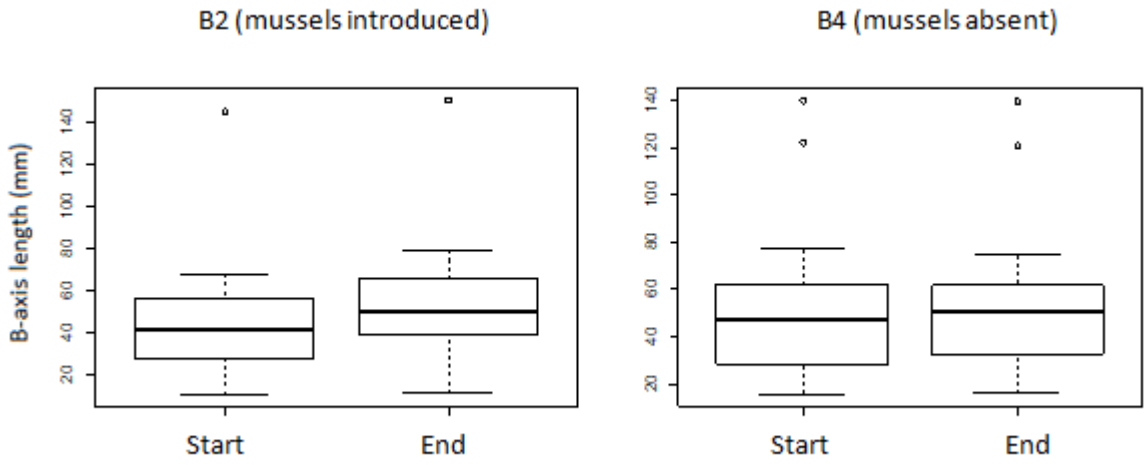


Figure 3.35. Boxplots showing change in pebble B-axis length in quadrat MBB2, with mussels introduced compared with MBB4, with mussels absent, at the beginning and end of the study.

The pebble B-axis length of river bed sediment at RSA ranged from 5.2-139.7 mm at the beginning of the study, with mean values ranging from 29.1-45.7 mm. Median B-axis length in A1, with mussels removed was 29.0 mm at the beginning of the study and 37.3 mm at the end of the study. In A3, with mussels present, median B-axis length was 43.2 mm at the start of the study and 46.6 mm at the end (Figure 3.36).

The mean beta coefficient of the change in bed sediment B-axis length over time in A1, where mussels were removed (M=8.58, SD=16.13) was not significantly different to A3, where mussels were present (M=2.84, SD=33.11); $t(71.01)=-1.10$, $p > 0.05$.

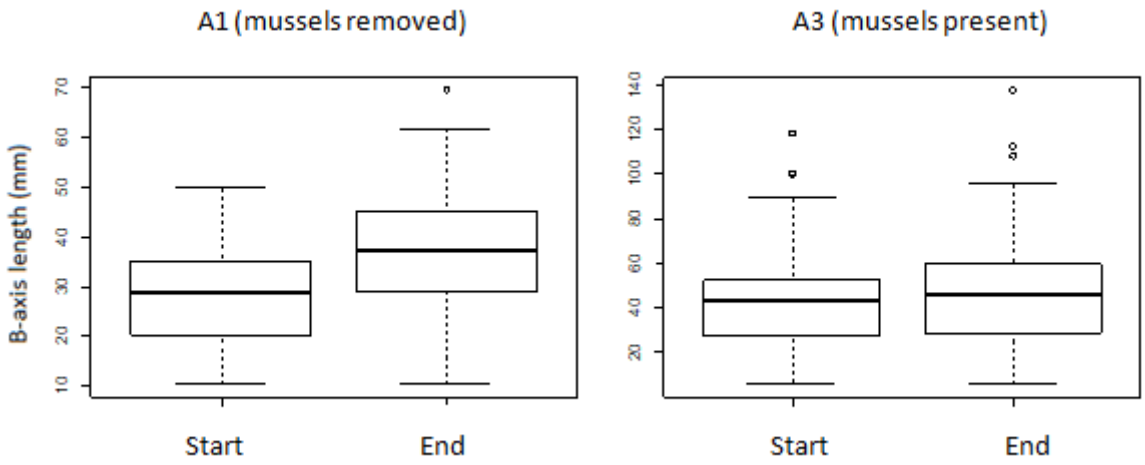


Figure 3.36. Boxplots showing change in pebble B-axis length in quadrat RSA1, with mussels removed compared with RSA3, with mussels present, at the beginning and end of the study.

In quadrat A2, with mussels removed, median B-axis was 37.3 mm at the start of the study and 48.0 mm at the end. Median B-axis length in the A4 control quadrat was 40.6 mm at the start of the study and 34.0 mm at the end (Figure 3.37).

The mean beta coefficient of the change in bed sediment B-axis length over time in A2, where mussels were removed (M=8.56, SD=23.23) was not significantly different to A3, where mussels were present (M=-4.99, SD= 43.50); t (74.86)=-1.94, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different pebble B-axis lengths compared with quadrats where they were present.

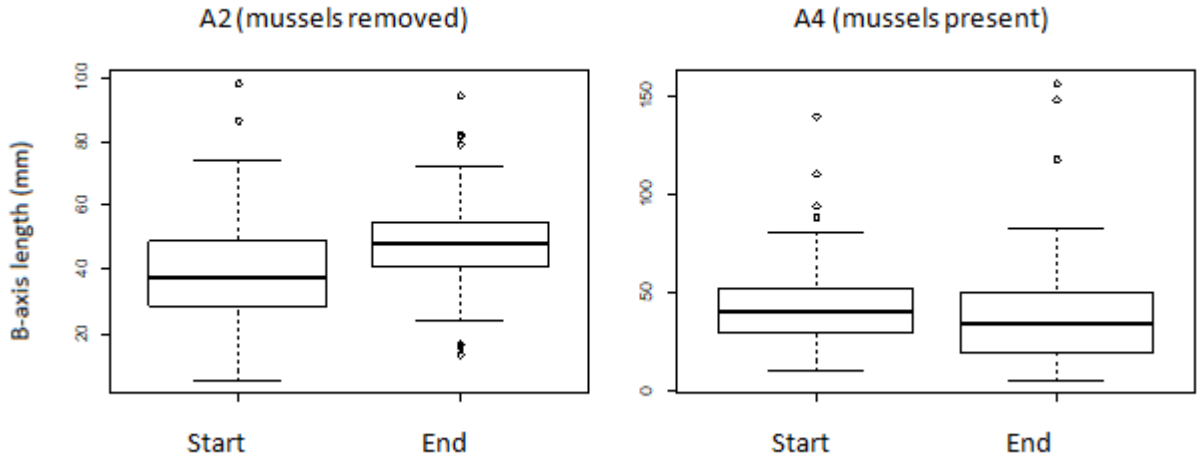


Figure 3.37. Boxplots showing change in pebble B-axis length in quadrat RSA2, with mussels removed compared with RSA4, with mussels present, at the beginning and end of the study.

At RSB pebble B-axis length ranged from 5.1-450.5 mm at the beginning of the study, with mean B-axis in the four quadrats ranging from 46.7-62.5 mm. Median B-axis length in B1, with mussels introduced was 43.5 mm at the beginning of the study and 45.6 mm at the end of the study. In B3, with mussels absent, median B-axis length was 34.7 mm at the start of the study and 34.8 mm at the end (Figure 3.38).

The mean beta coefficient of the change in bed sediment B-axis length over time in B1, where mussels were added (M=2.29, SD=85.56) was not significantly different to B3, where mussels were absent (M=-1.34, SD=75.35); $t(96.46)=-0.22$, $p > 0.05$.

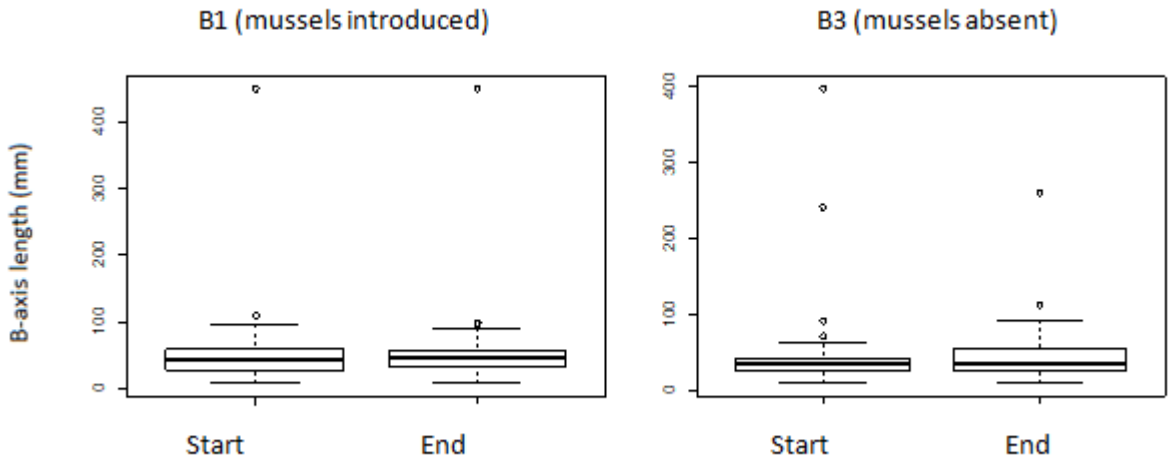


Figure 3.38. Boxplots showing change in pebble B-axis length in quadrat RSB1, with mussels introduced compared with RSB3, with mussels absent, at the beginning and end of the study.

In quadrat B2, with mussels introduced, median B-axis was 36.8 mm at the start of the study and 45.7 mm at the end. Median B-axis length in the B4 control quadrat was 53.7 mm at the start of the study and 45.7 mm at the end (Figure 3.39).

The mean beta coefficient of the change in bed sediment B-axis length over time in B2, where mussels were added (M=8.92, SD=60.54) was not significantly different to B4, where mussels were absent (M=-12.22, SD=61.25); t (97.99)=-1.74, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in significantly different pebble B-axis lengths compared with quadrats where they were absent.

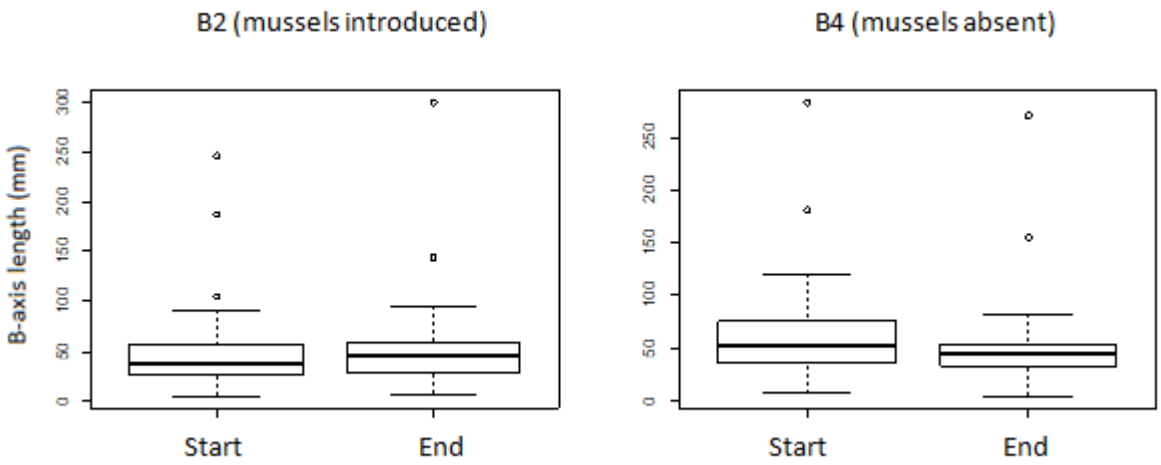


Figure 3.39. Boxplots showing change in pebble B-axis length in quadrat RSB2, with mussels introduced compared with RSB4, with mussels absent, at the beginning and end of the study.

Combined data from sieve analysis and laser particle size analysis of sediment core samples taken from MBA showed that the mean grain-size present within A1 and A2, where mussels were removed, was initially finer than the control quadrats A3 and A4 (Figure 3.40). Mean grain-size of the samples from A1 and A2 was 2022 and 1621 µm respectively at the start of the study, whereas mean grain-size in A3 and A4 was 4051 and 4382 µm. Mean grain-size in all four quadrats increased throughout the 8 weeks of the study, with mean values ranging from 2834-5314 µm at the end of the investigation.

The mean beta coefficient of the change in mean grain size over time in A1, where mussels were removed (M=1341.34, SD=2533.21) was not significantly different to A3, where mussels were present (M=631.48, SD=-4000.25); t (7.33)=-0.51, $p > 0.05$.

The mean beta coefficient of the change in mean grain-size over time in A2, where mussels were removed (M=606.47, SD=1042.07) was not significantly different to A4, where mussels were present (M=414.09, SD=2187.54); t (5.73)=-0.18, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in significantly different mean sediment grain-size compared with quadrats where they were present.

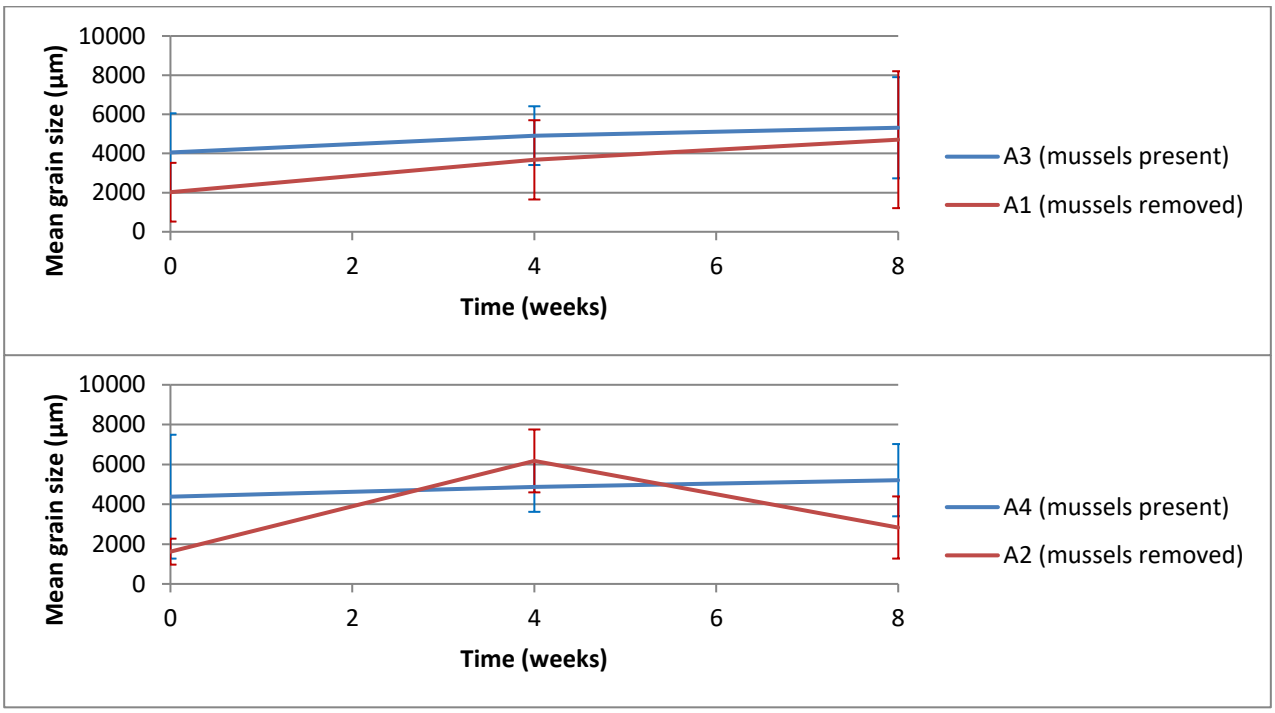


Figure 3.40. Mean (± 1 SD) grain-size of sediment core samples taken from MBA over the duration of the study.

Mean grain-size at MBB decreased in all four quadrats over the duration of the study from 4441-5857 µm at the beginning to 2593-4641 µm at the end of the study (Figure 3.41).

The mean beta coefficient of the change in mean grain-size over time in B1, where mussels were added (M=-1086.10, SD=1078.93) was not significantly different to B3, where mussels were absent (M=-786.66, SD=1797.98); t (6.55)=0.32, $p > 0.05$.

The mean beta coefficient of the change in mean grain-size over time when in B2, where mussels were added (M=-1631.74, SD=1561.62) was not significantly different to B4, where mussels were absent (M=100.34, SD=1887.92); t (7.73)=1.58, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not result in significantly different mean sediment grain-size compared with quadrats where they were absent.

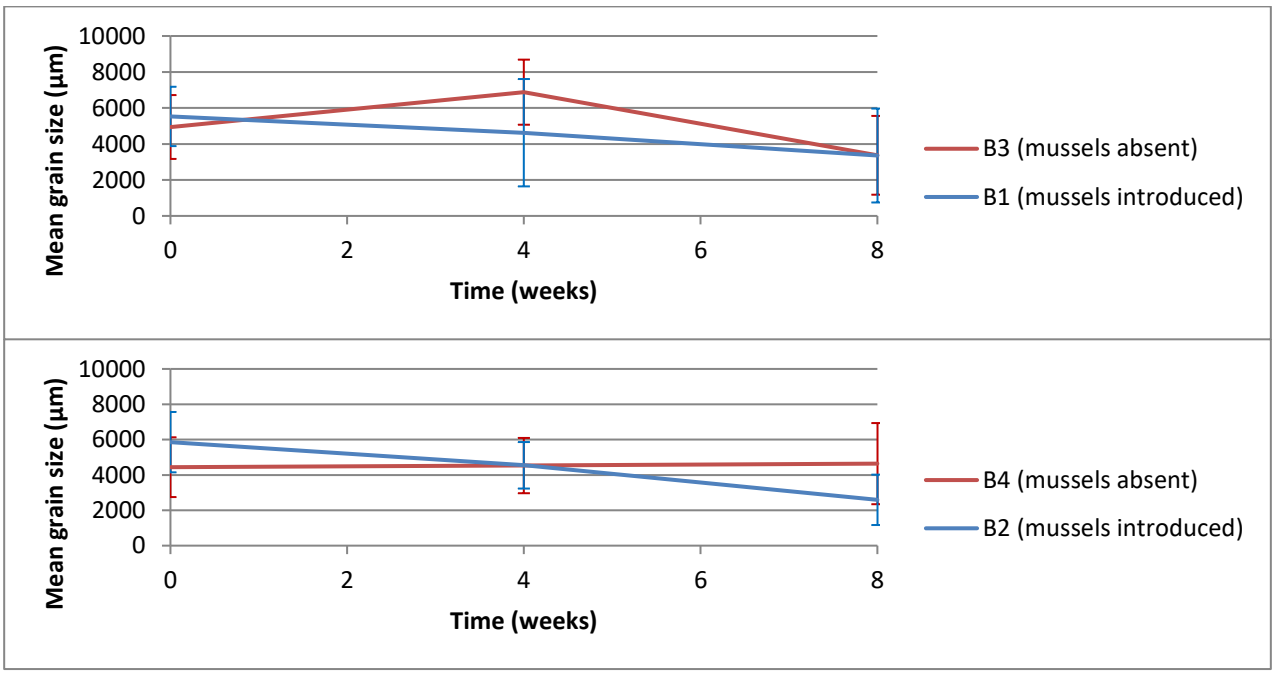


Figure 3.41. Mean (± 1 SD) grain-size of sediment core samples taken from MBB over the duration of the study.

Mean sediment grain-size at RSA ranged from 4657-7546 µm at the beginning of the study and 3815-6031 µm at the end of the study (Figure 3.42).

The mean beta coefficient of the change in mean grain-size over time in A1, where mussels were removed (M=272.74, SD=727.14) was not significantly different to A3, where mussels were present (M=-365.08, SD=969.05); t (7.42)=-1.18, $p > 0.05$.

The mean beta coefficient of the change in mean grain-size over time in A2, where mussels were removed (M=-485.70, SD=730.86) was not significantly different to A4, where mussels were present (M=-730.48, SD=744.03); t (8.00)=-0.52, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different mean sediment grain-size to quadrats where they were present.

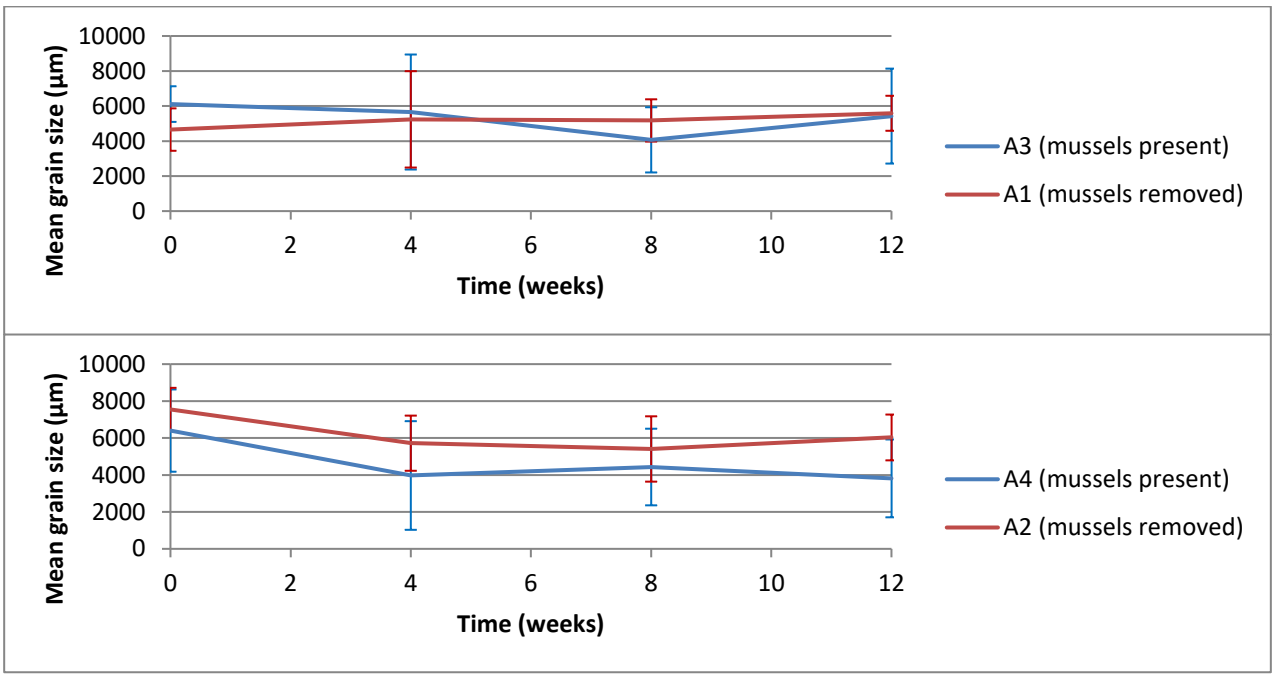


Figure 3.42. Mean (± 1 SD) grain-size of sediment core samples taken from RSA over the duration of the study.

Mean sediment grain-size at RSB decreased in all four quadrats over the duration of the study, from 4902-6711 µm at the beginning of the study to 4158-5271 µm at the end of the study (Figure 3.43).

The mean beta coefficient of the change in mean grain-size over time in B1, where mussels were added (M=-745.42, SD=1552.73) was not significantly different to B3, where mussels were absent (M=-615.32, SD=375.49); t (4.47)=0.18, $p > 0.05$.

The mean beta coefficient of the change in mean grain-size over time in B2, where mussels were added (M=-481.90, SD=1419.17) was not significantly different to B4, where mussels were absent (M=-444.90, SD=828.67); t (6.44)=0.05, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in significantly different mean sediment grain-size compared with quadrats where they were absent.

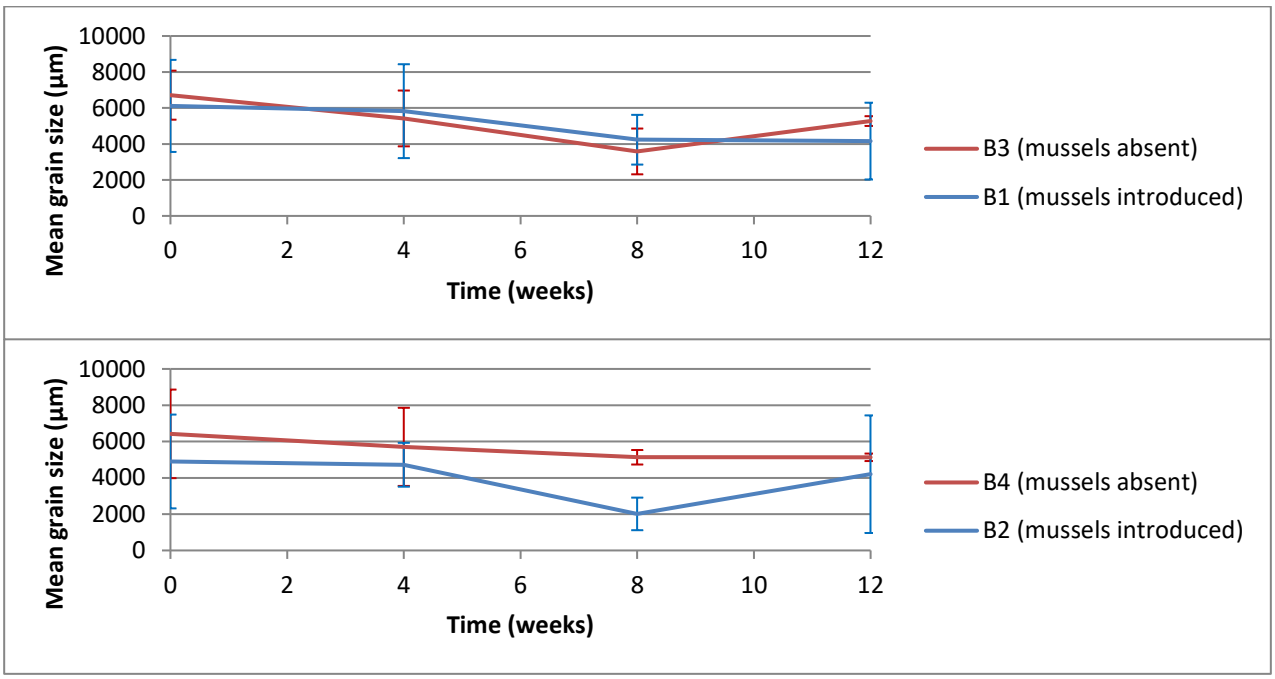


Figure 3.43. Mean (± 1 SD) grain-size of sediment core samples taken from RSB over the duration of the study.

The mean percentage of fines (<63 µm) in sediment core samples taken from MBA at the beginning of the study was initially higher in A1 and A2 at 22.2 % and 14.2 % respectively, compared with A3 and A4 at 7.9 % and 7.8 % respectively. By week 4 the mean percentage of fines was similar in all four quadrats, ranging from 3.8-7.2 % (Figure 3.44). By week 8 the mean percentage of fines ranged from 5.1-9.1 % in the four quadrats.

The mean beta coefficient of the change in the mean percentage of fines over time in A1, where mussels were removed (M=-7.58, SD=8.18) was not significantly different to A3, where mussels were present (M=0.24, SD=4.16); t (5.94)=1.91, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage of fines over time in A2, where mussels were removed (M=-2.59, SD=5.18) was not significantly different to A4, where mussels were present (M=-1.32, SD=1.67); t (4.82)=0.52, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in a significantly different percentage of fines compared with quadrats where they were present.

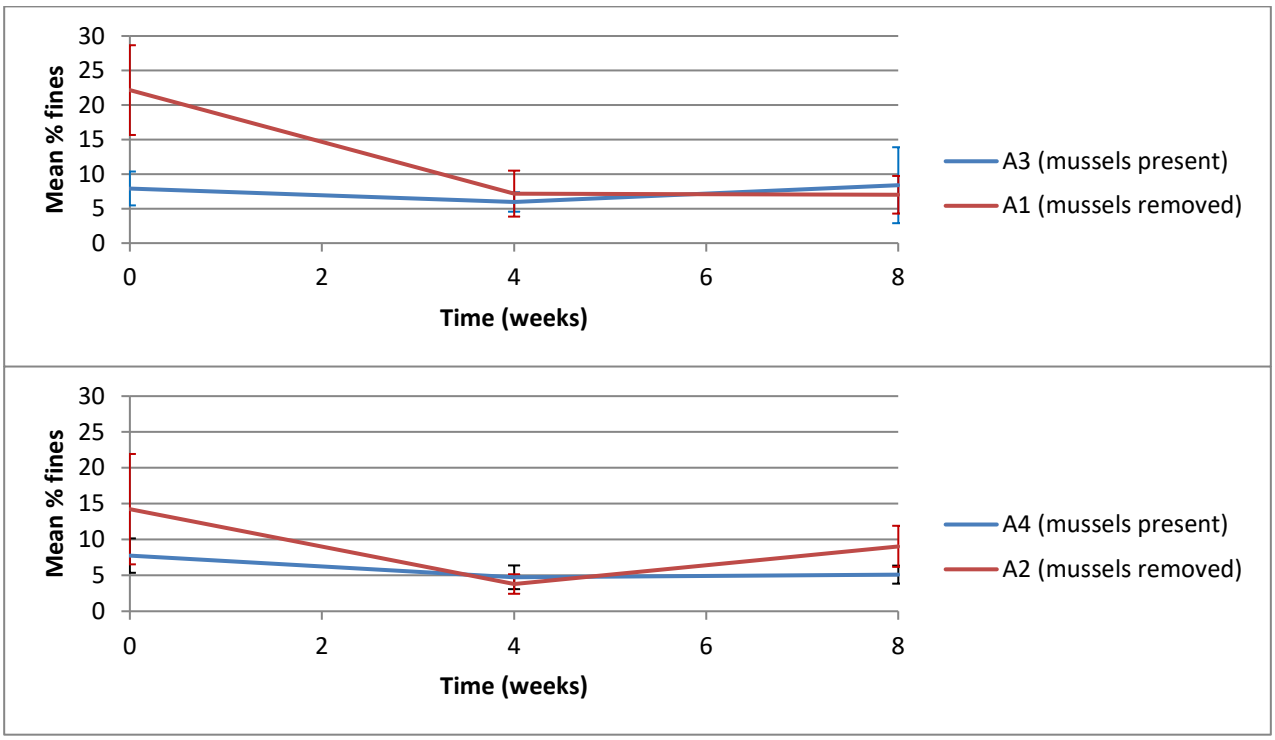


Figure 3.44. Mean (± 1 SD) percentage of fines (<63 µm) in sediment core samples taken from MBA over the duration of the study.

The mean percentage of fines in the four quadrats at MBB ranged from 16.9-20.36 % at the beginning of the study, reducing to 9.6-14.1 % by week 4 (Figure 3.45). By week 8 the mean percentage of fines in B1, B2 and B3 had increased to 24.5-30.8 %, whereas in B4 it had only increased to 16.2 %.

The mean beta coefficient of the change in the mean percentage of fines over time in B1, where mussels were added (M=-2.85, SD=5.27) was not significantly different to B3, where mussels were absent (M=3.77, SD=11.32); t (5.65)=0.16, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage of fines over time in B2, where mussels were added (M=6.97, SD=12.70) was not significantly different to B4, where mussels were absent (M=-2.08, SD=8.83); t (7.13)=-1.31, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not result in a significantly different percentage of fines compared with quadrats where they were absent.

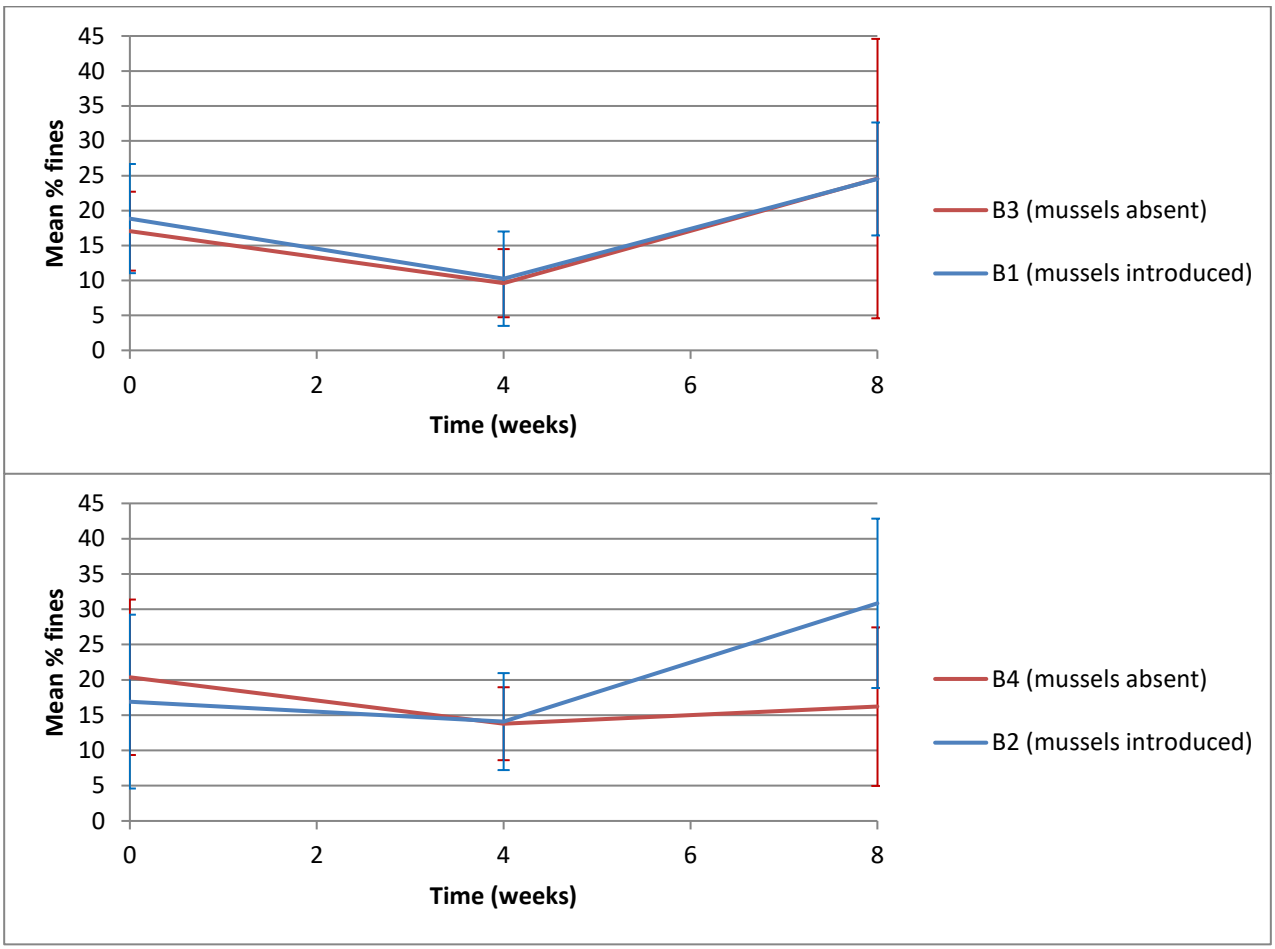


Figure 3.45. Mean (± 1 SD) percentage of fines (<63 µm) in sediment core samples taken from MBB over the duration of the study.

At RSA the mean percentage of fines in A1, where mussels had been removed was initially higher (14.7 %) than the A3 control quadrat (8.0 %), which contained mussels. After week 4 the percentage of fines in A1 reduced below that of the control quadrat, and by week 12 the mean percentage of fines in A1 was 11.2 %, whereas the A3 quadrat containing mussels was at 13.9 % fines (Figure 3.46).

The A2 and A4 quadrats had a mean % fines of 7.5% and 9.3 % respectively. As was the case with the A1 and A3 quadrats, the mean % fines in quadrat with mussels removed reduced throughout the study to 5.9 %, whereas the A4 quadrat with mussels showed an increased percentage of fines (24.4 %) by week 12.

The mean beta coefficient of the change in the mean percentage of fines over time in A1, where mussels were removed (M=-1.39, SD=2.49) was significantly more negative than A3, where mussels were present (M=2.46, SD=1.49); t (6.55)=2.96, p = 0.0228.

The mean beta coefficient of the change in the mean percentage of fines over time in A2, where mussels were removed (M=-0.24, SD=0.66) was not significantly different to A4, where mussels were present (M=3.96, SD=6.08); t (4.09)=1.54, p > 0.05.

The removal of mussels from quadrat A1 at RSA resulted in a significantly reduced percentage of fines compared with quadrat A3, where they were present. The removal of mussels from quadrat A2 at RSA did not result in a significantly different percentage of fines to quadrat A4, where they were present.

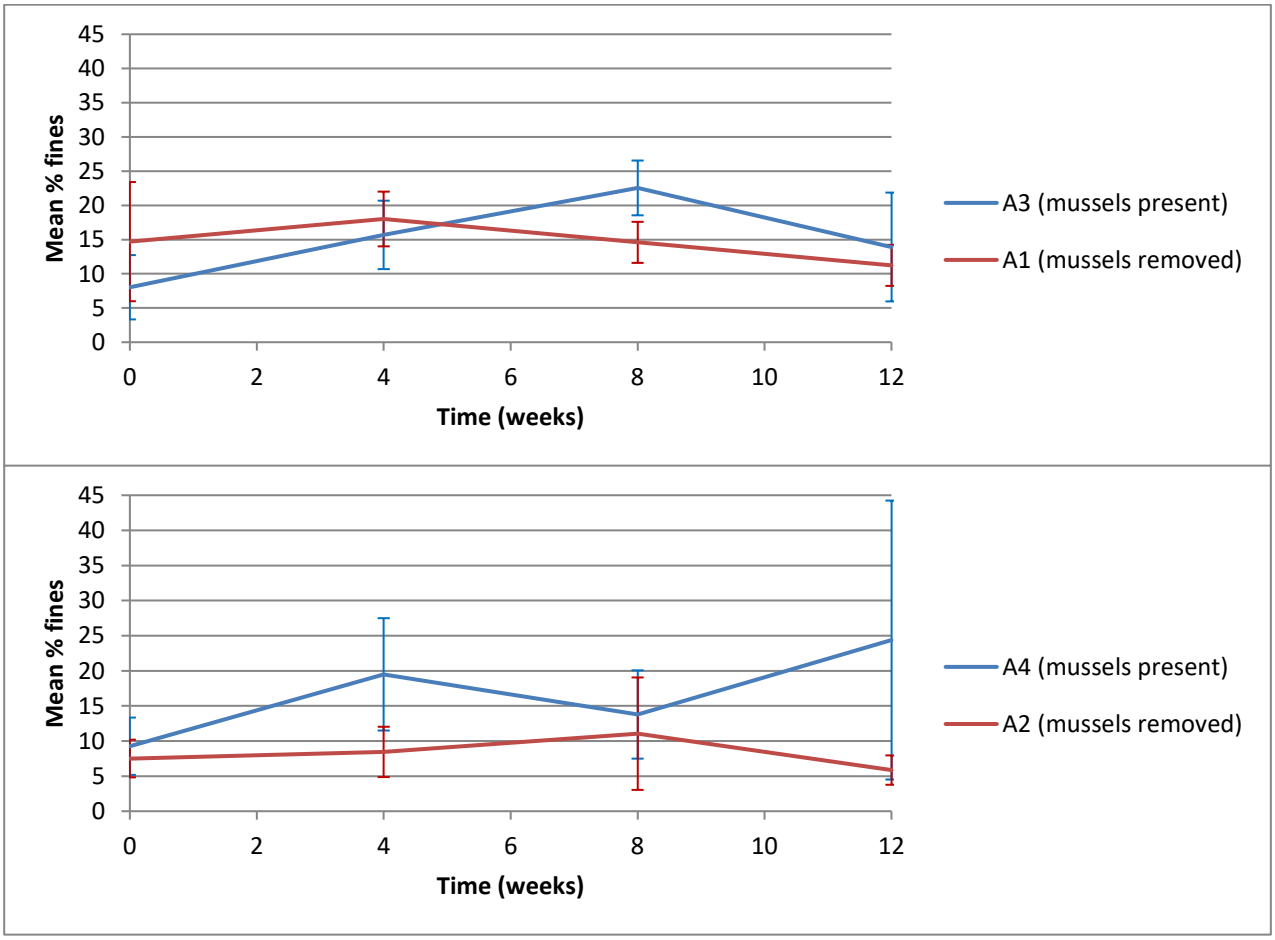


Figure: 3.46. Mean (± 1 SD) percentage of fines (<63 µm) in sediment core samples taken from RSA over the duration of the study.

At RSB The mean percentage of fines ranged from 3.8-14.6 % over the duration of the study (Figure 3.47).

The mean beta coefficient of the change in the mean percentage of fines over time in B1, where mussels were added (M=0.49, SD=2.85) was not significantly different to B3, where mussels were absent (M=0.51, SD=1.03); t (5.03)=0.02, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage of fines over time in B2, where mussels were added (M=-0.64, SD=4.85) was not significantly different to B4, where mussels were absent (M=0.27, SD=1.89); t (5.19)=0.39, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in a significantly different percentage of fines compared with quadrats where they were absent.

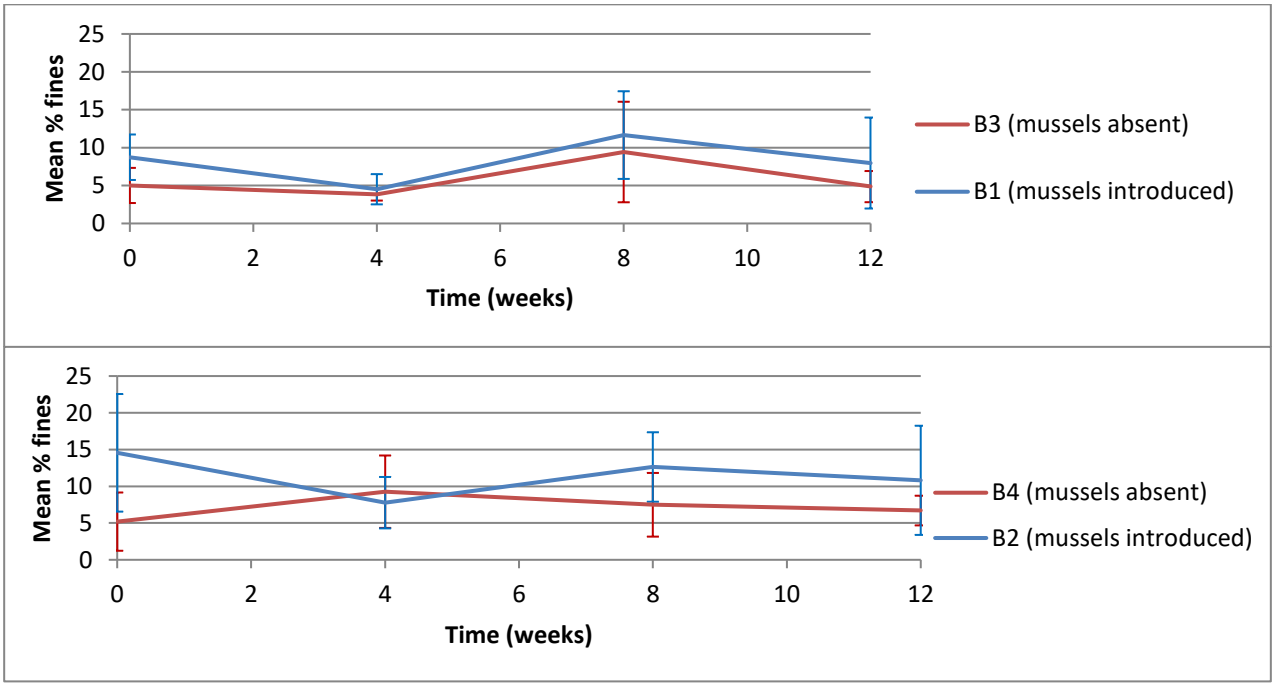


Figure 3.47. Mean (± 1 SD) percentage of fines (<63 µm) in sediment core samples taken from RSB over the duration of the study.

Grain-size distributions graphs of the homogenised sediment core samples taken from Markeaton Brook and the River Sence are shown in Figures 3.48 - 3.55. The grain-size distribution graphs show the mean percentage volume of each grain-size fraction (differential volume) of the sediment

core sample taken at the beginning and the end of the investigation. The mean percentage volume of each fraction was calculated from aliquots taken from each of the homogenised sediment cores as described in section 3.3.3. The legends (M.A1.1a - M.B4.3e and S.A1.1a - S.B4.4e) on each of the graphs in Figures 3.48 - 3.55 show the unique identifier designated to individual sediment core samples taken from randomly generated locations within each quadrat.

At the beginning of the study the samples from MBA show peaks in the grain-size abundance of the 8-16 µm, 125-500 µm and 4000-8000 µm fractions (Figures 3.48 and 3.49). Grain-size distributions in quadrats A2, A3 and A4 remained relatively unchanged throughout the study. Quadrat A1, from which mussels had been removed, showed a decreased abundance of clay and fine silt (0.2-16 µm) by the end of the study.

The sediment samples from MBB initially contained higher proportions of silt (4-63 µm) and lower proportions of fine-medium sand (125-500 µm) compared with MBA, whilst the proportion of gravel (>2000 µm) at both sites was comparable. By the end of the study samples from all four quadrats displayed an increased proportion of silt and sand compared with the gravel fraction, with no noticeable differences between the quadrats with mussels and the control quadrats (Figures 3.50 and 3.51).

Sediment samples from RSA showed peaks in the abundance of the 8-31 µm, 250-500 µm and 2000-8000 µm grain-size categories at the beginning of the study (Figures 3.52 and 3.53). The removal of mussels from A1 and A2 resulted in loss of fines (0.1-31 µm) compared with A3 and A4 by the end of the study. The A1 and A2 sediment samples from week 12 display a relatively consistent grain-size distribution pattern compared with the more varied grain-size distribution patterns found within the A3 and A4 quadrats by the end of the study. The more uniform grain-size distribution patterns found in the A1 and A2 quadrats by week 12 indicates a decline in substrate heterogeneity after mussels were removed.

Sediment samples from RSB show peaks in the 8-16 µm, 250-500 µm and 4000-8000 µm at the start of the investigation (Figures 3.54 and 3.55). Each sample from B3 and B4 shows a relatively consistent grain-size distribution pattern by week 12 of the study, whereas the relative proportion of clay, silt, sand and gravel in the B1 and B2 samples shows a much higher degree of variability. The more varied grain-size distribution patterns observed in the mussel-containing quadrats compared with the control quadrats by week 12 indicates an increased substrate heterogeneity in the presence of mussels.

In summary, the removal of mussels from quadrats at MBA and RSA resulted in a reduced proportion of clay and fine silt in the sediment compared with where mussels were present. No noticeable differences in grain-size distributions were found in quadrats at MBB where mussels were introduced compared with where they were absent. Increased variability in grain-size distribution patterns were found in quadrats at RSA and RSB where mussels were present, compared with where they were absent, indicating that mussels increased the heterogeneity of the substrate at the River Sence sites.

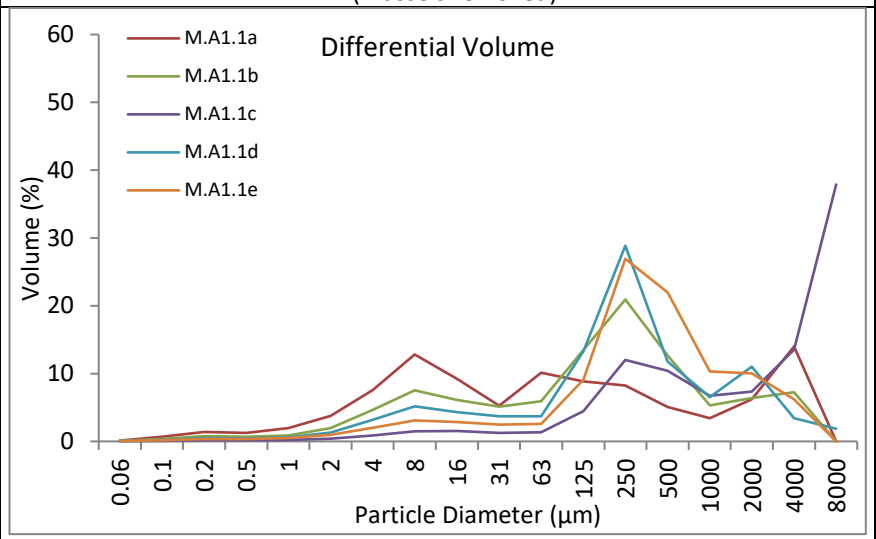
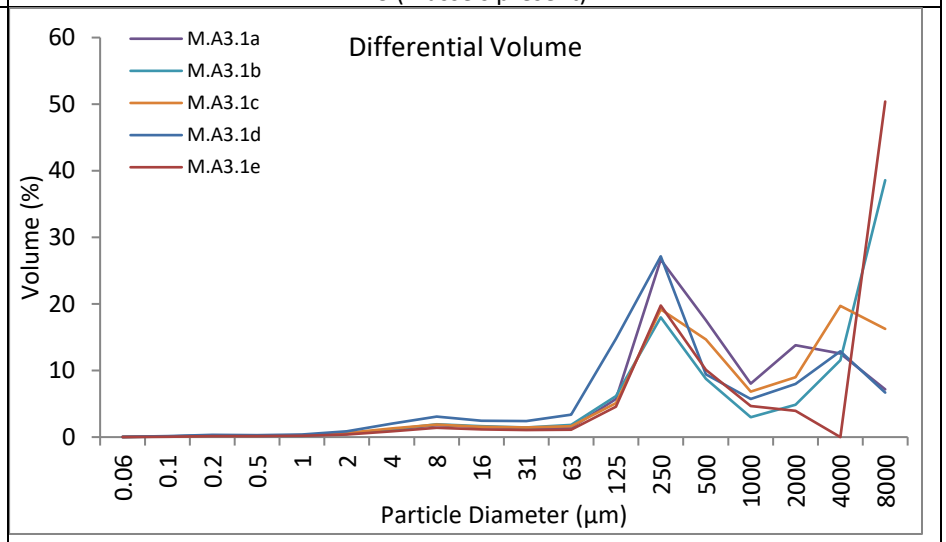
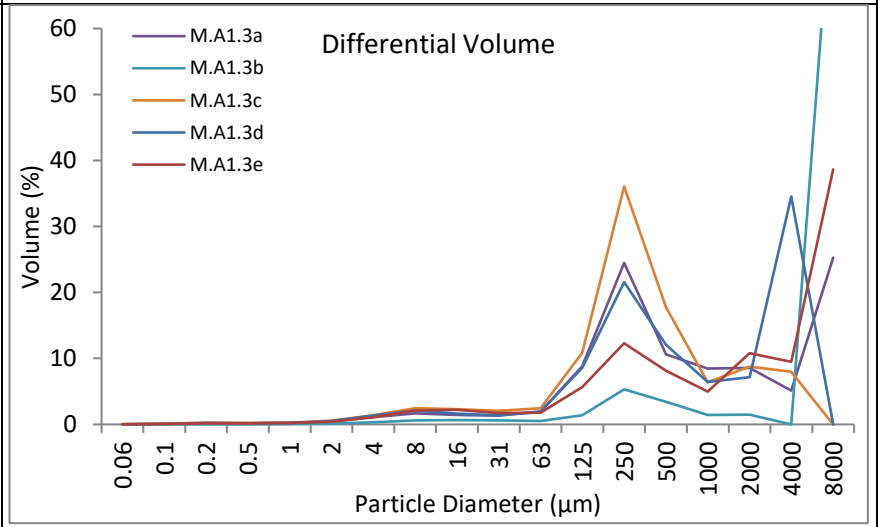
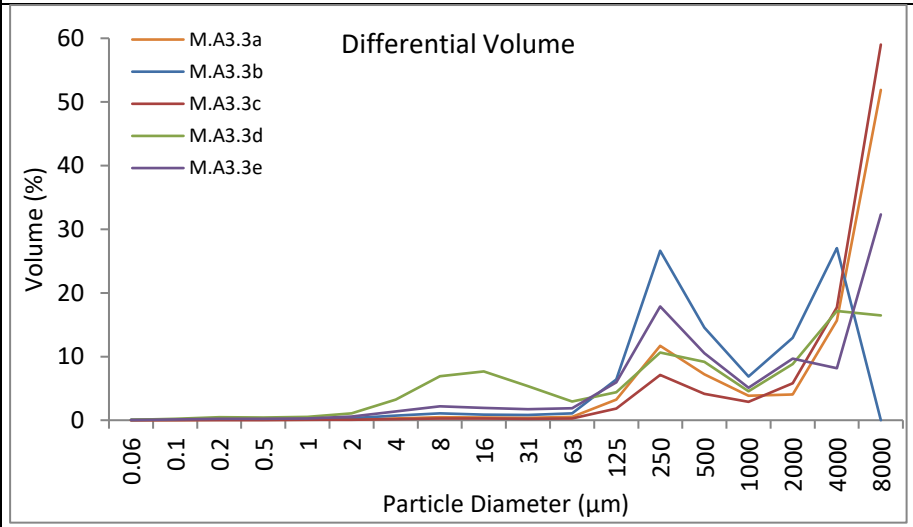
Time (weeks)	Sediment grain-size distribution A1 (mussels removed)	Sediment grain-size distribution A3 (mussels present)
0		
8		

Figure 3.48. Grain-size distribution of sediment samples in A1 (mussels removed) and A3 (mussels present) from MBA at the beginning and end of the study.

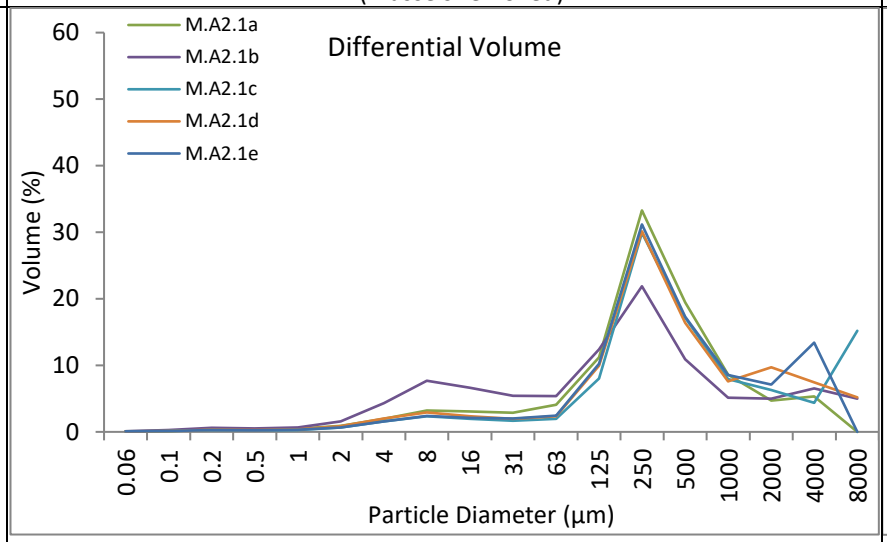
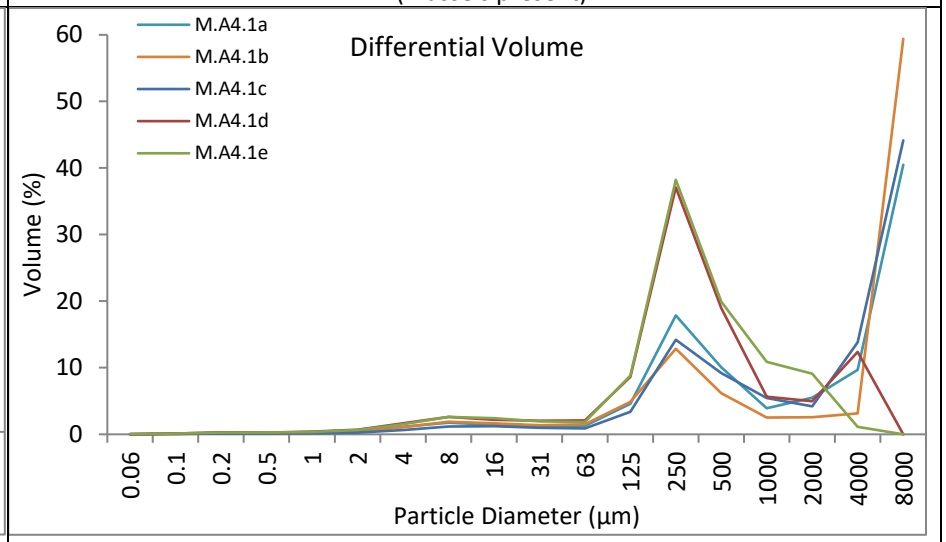
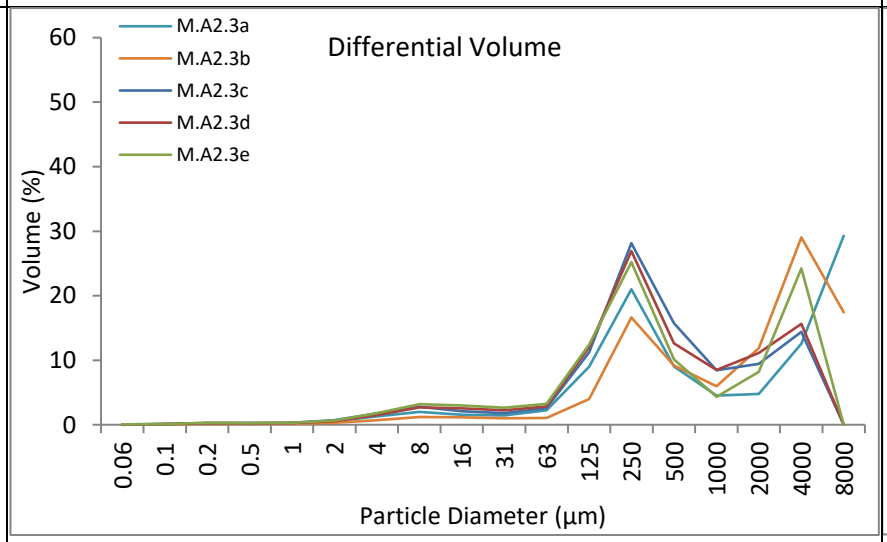
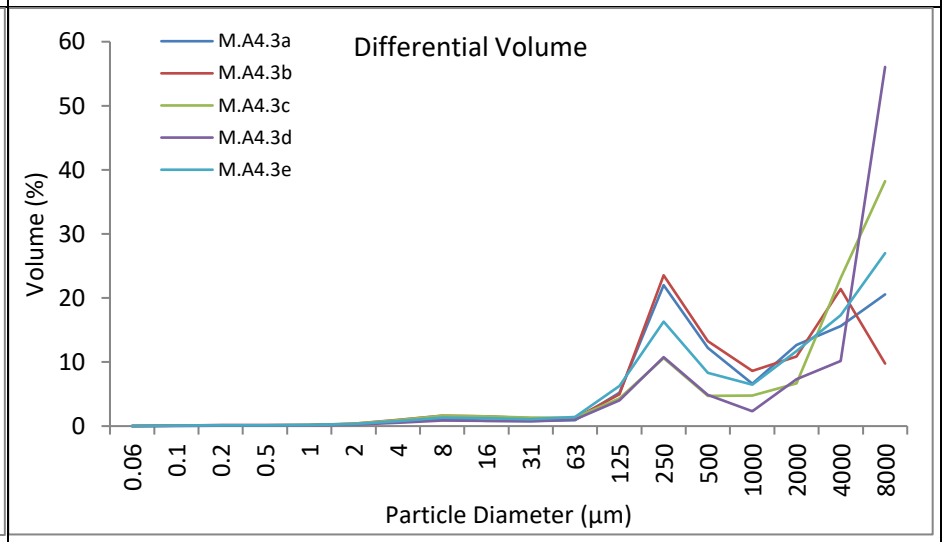
Time (weeks)	Sediment grain-size distribution A2 (mussels removed)	Sediment grain-size distribution A4 (mussels present)
0		
8		

Figure 3.49. Grain-size distribution of sediment samples in A2 (mussels removed) and A4 (mussels present) from MBA at the beginning and end of the study.

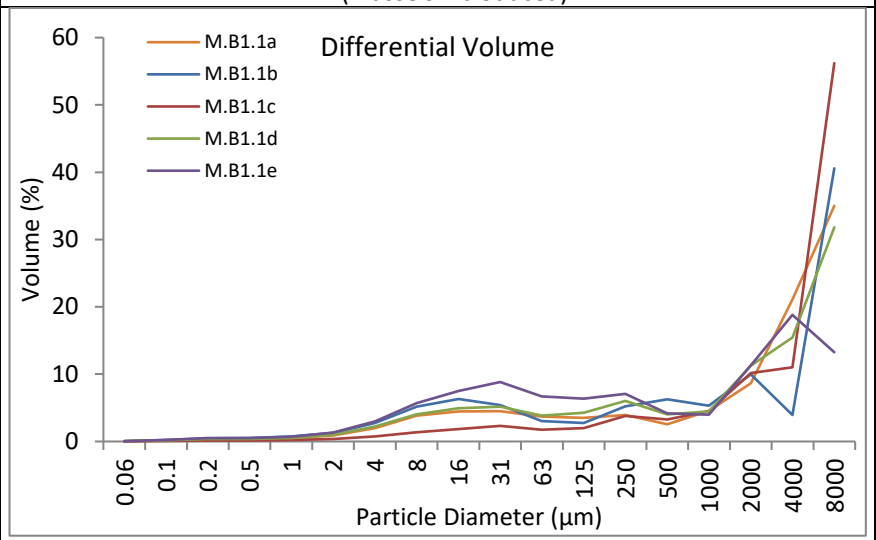
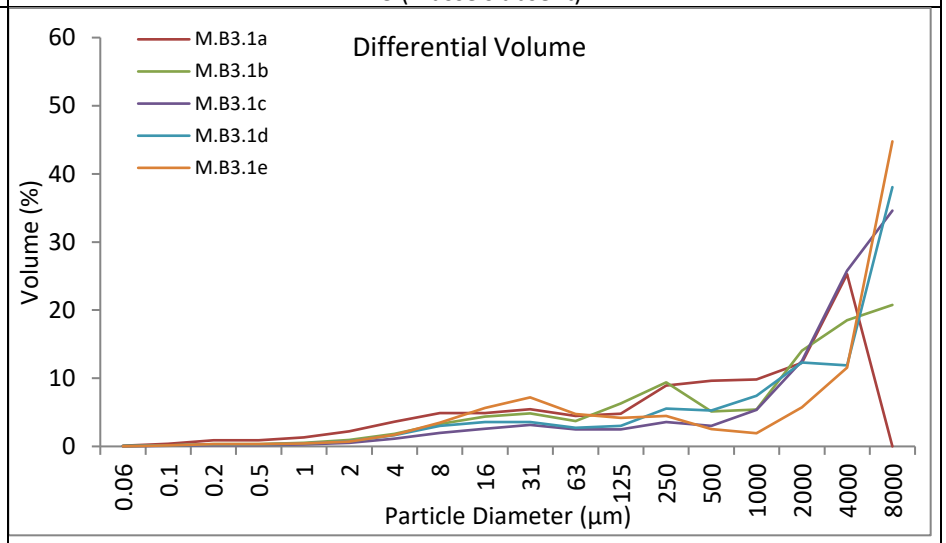
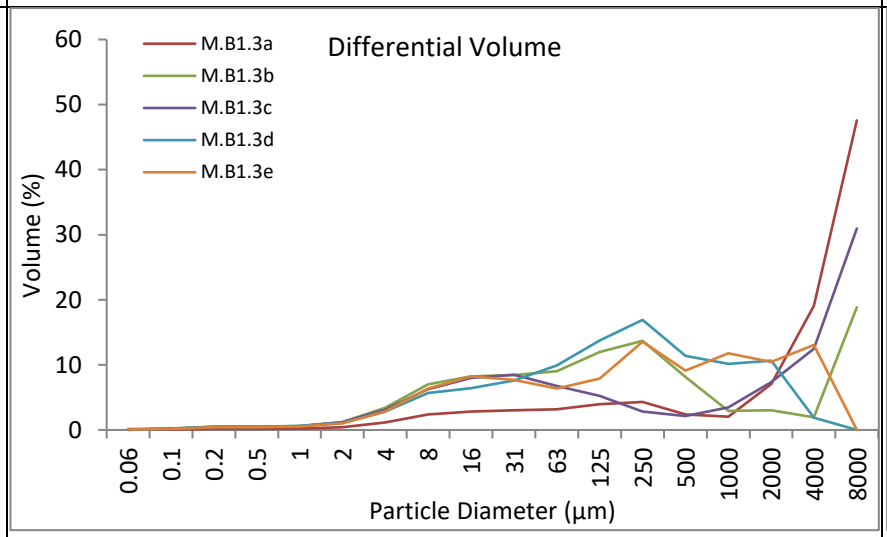
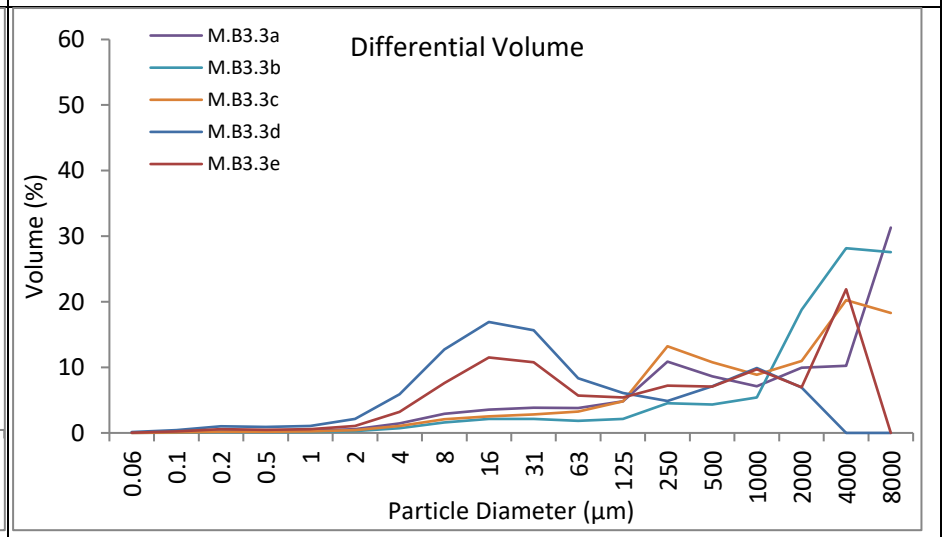
Time (weeks)	Sediment grain-size distribution B1 (mussels introduced)	Sediment grain-size distribution B3 (mussels absent)
0		
8		

Figure 3.50. Grain-size distribution of sediment samples in B1 (mussels introduced) and B3 (mussels absent) from MBB at the beginning and end of the study.

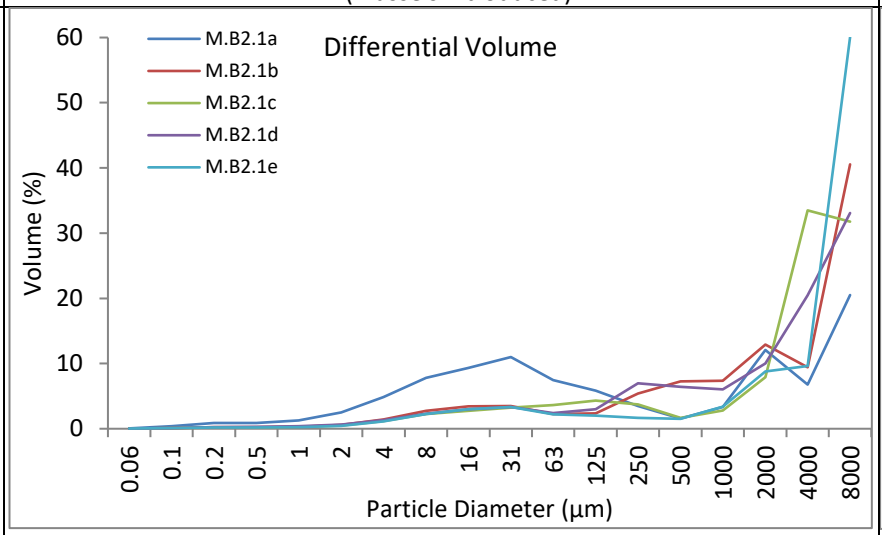
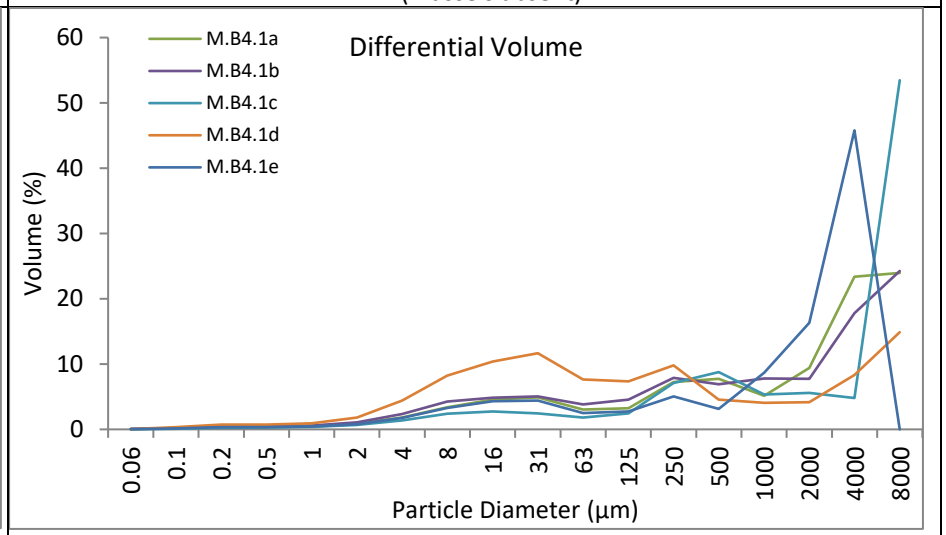
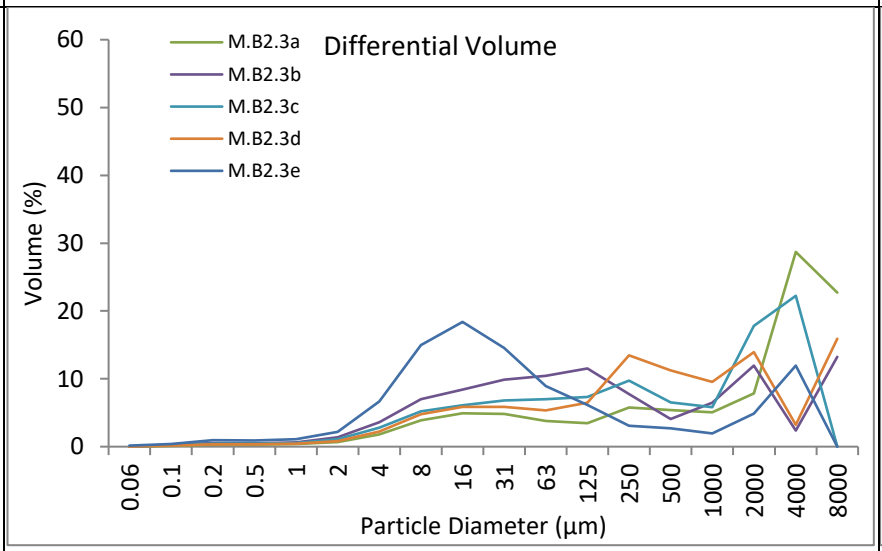
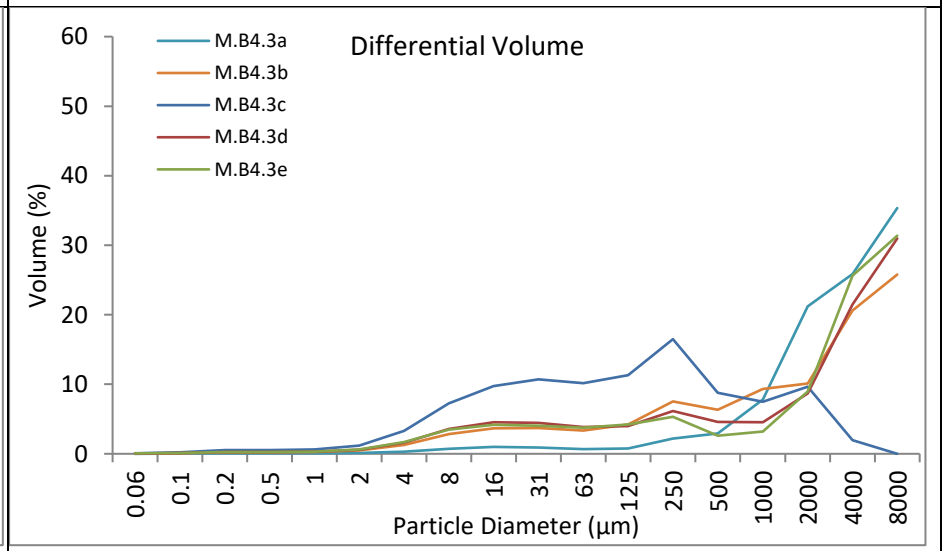
Time (weeks)	Sediment grain-size distribution B2 (mussels introduced)	Sediment grain-size distribution B4 (mussels absent)
0		
8		

Figure 3.51. Grain-size distribution of sediment samples in B2 (mussels introduced) and B4 (mussels absent) from MBB at the beginning and end of the study.

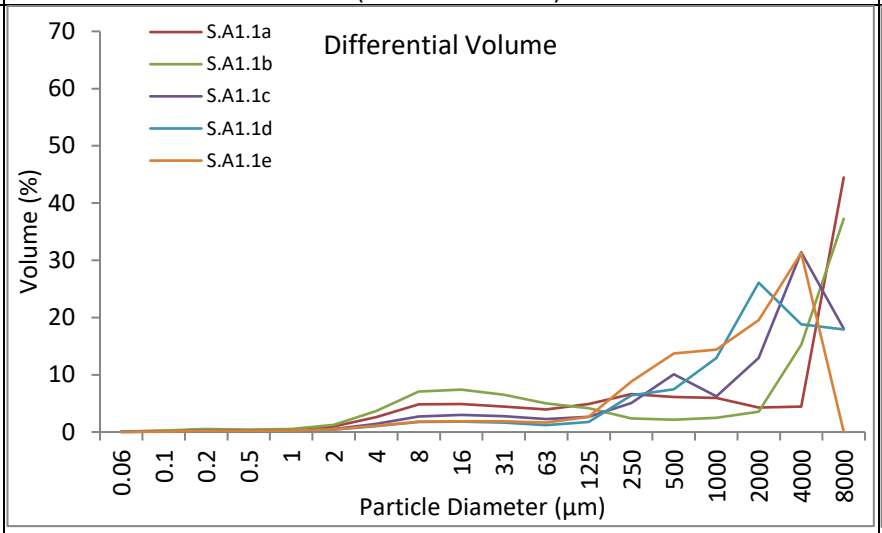
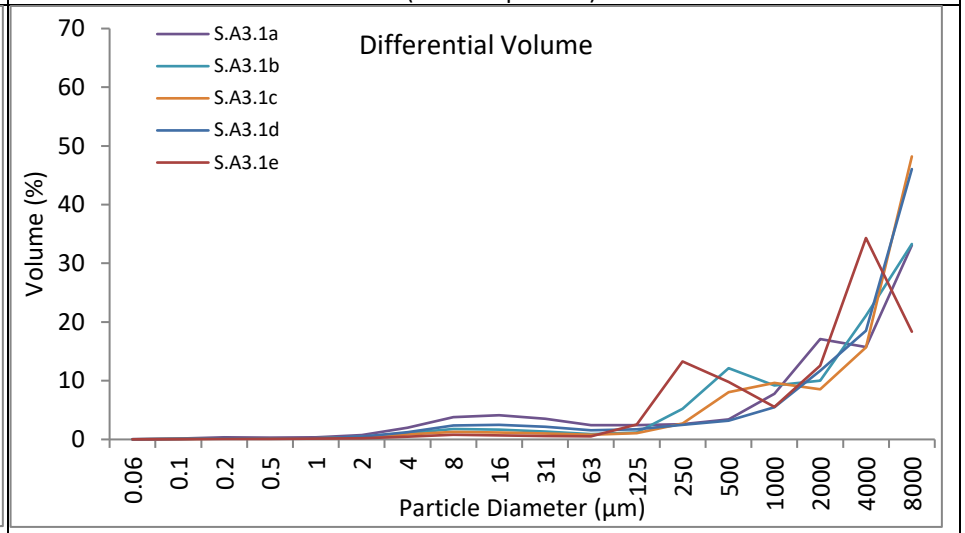
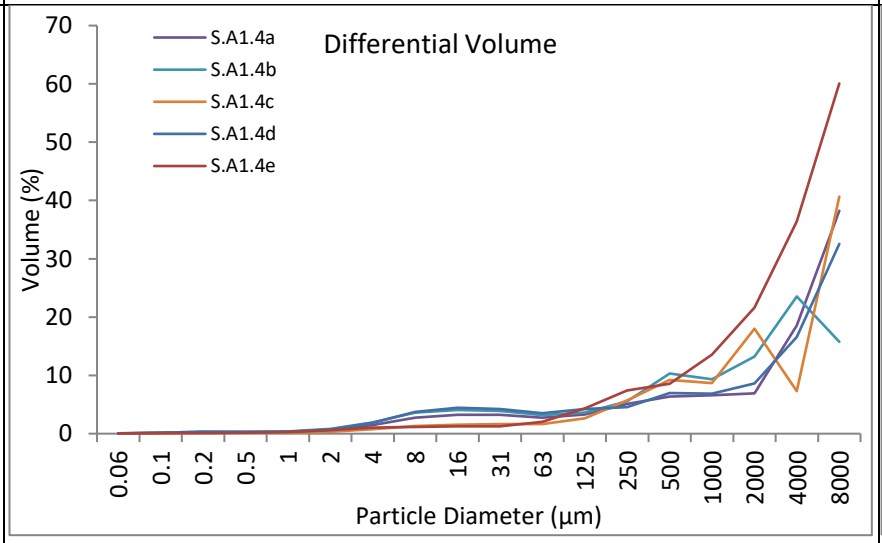
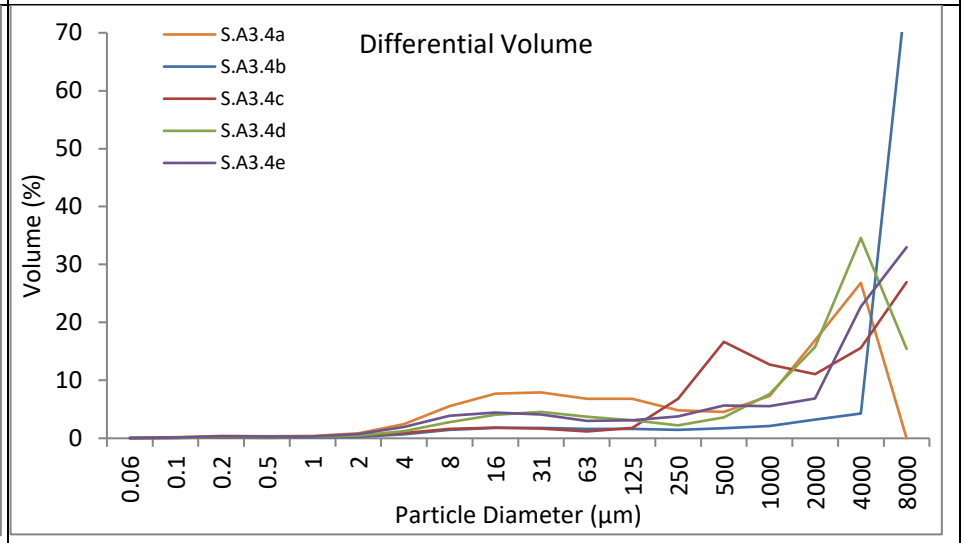
Time (weeks)	Sediment grain-size distribution A1 (mussels removed)	Sediment grain-size distribution A3 (mussels present)
0		
12		

Figure 3.52. Grain-size distribution of sediment samples in A1 (mussels removed) and A3 (mussels present) from RSA at the beginning and end of the study.

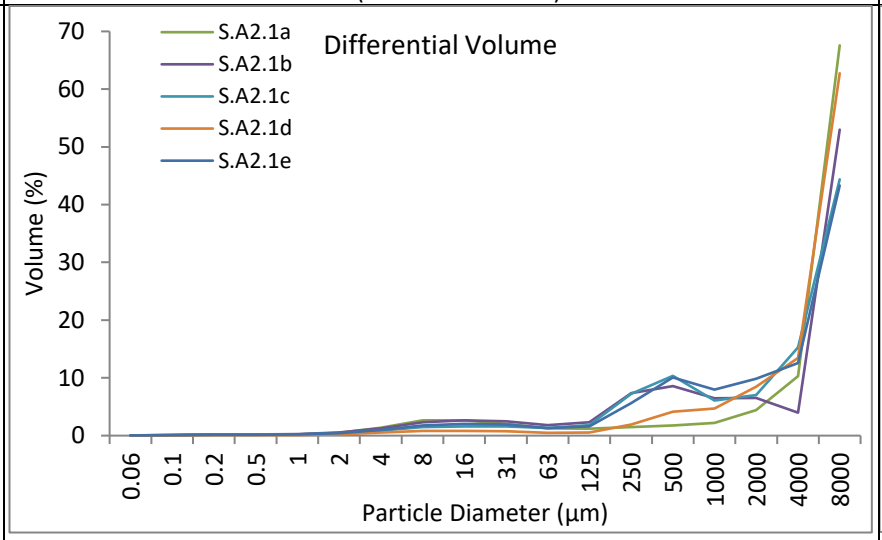
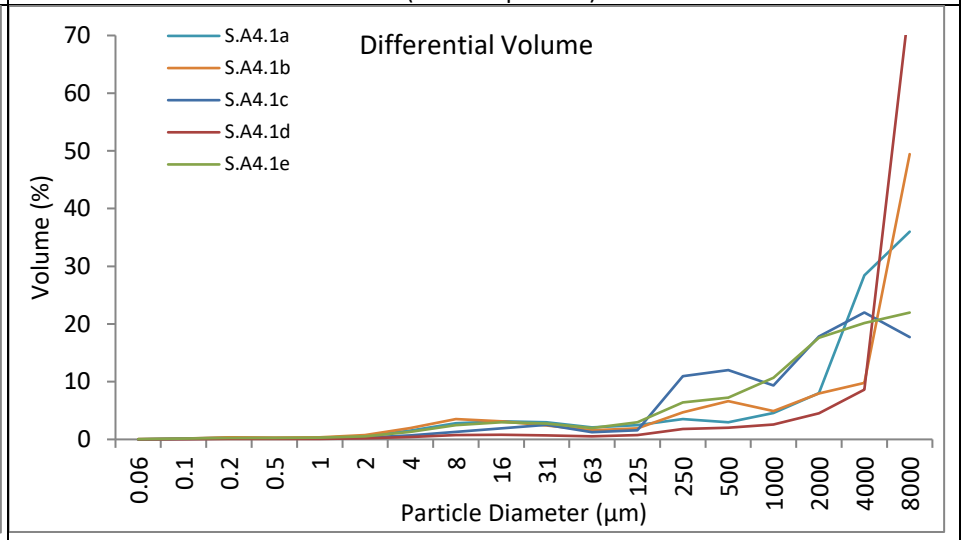
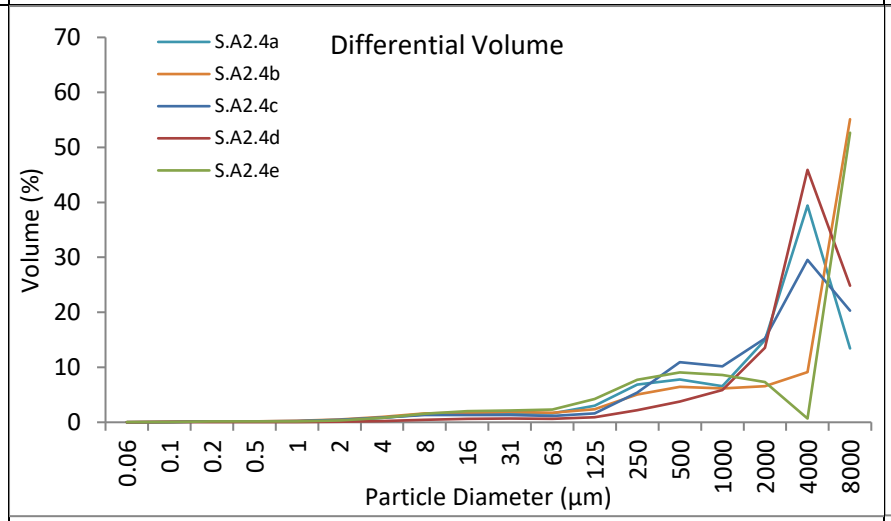
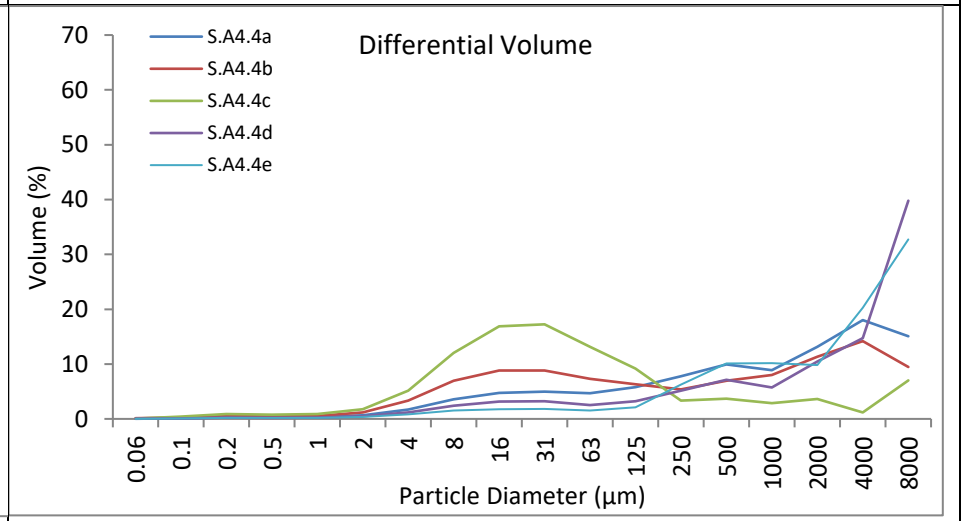
Time (weeks)	Sediment grain-size distribution A2 (mussels removed)	Sediment grain-size distribution A4 (mussels present)
0		
12		

Figure 3.53. Grain-size distribution of sediment samples in A2 (mussels removed) and A4 (mussels present) from RSA at the beginning and end of the study.

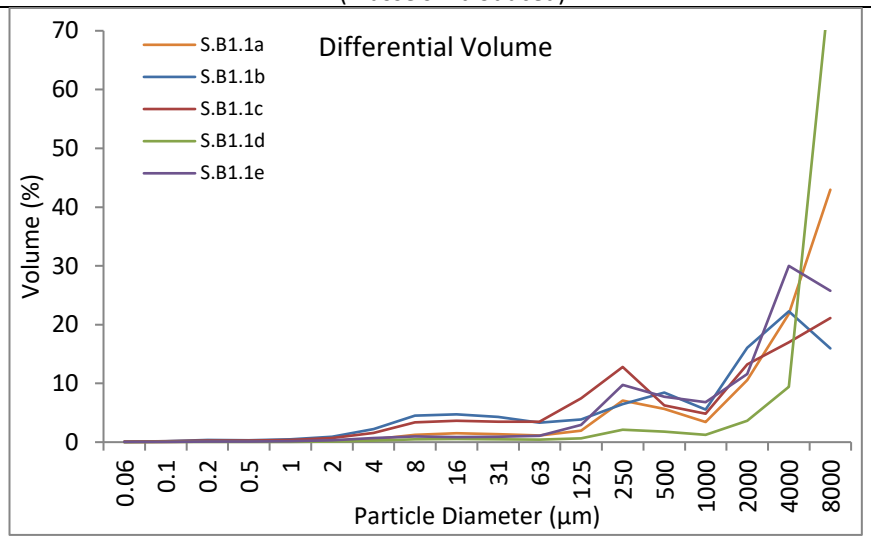
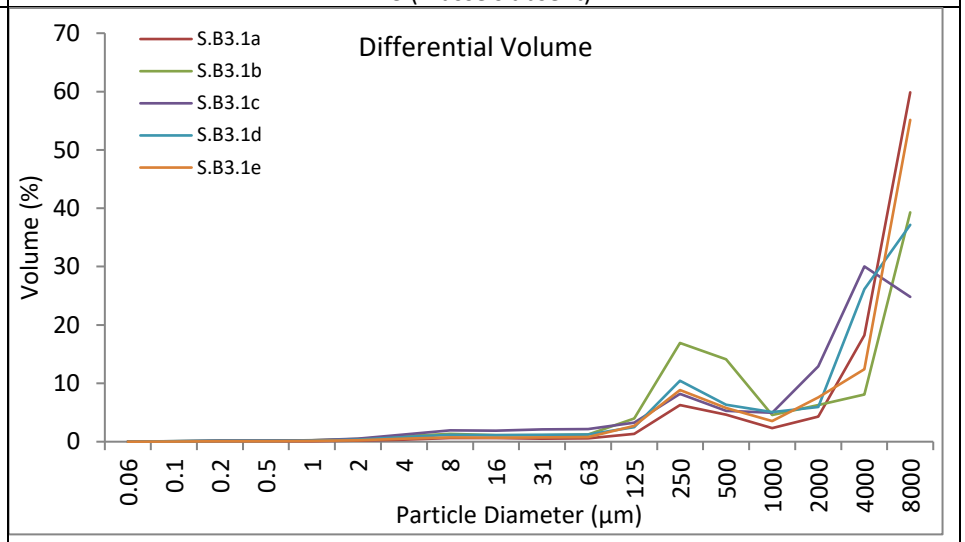
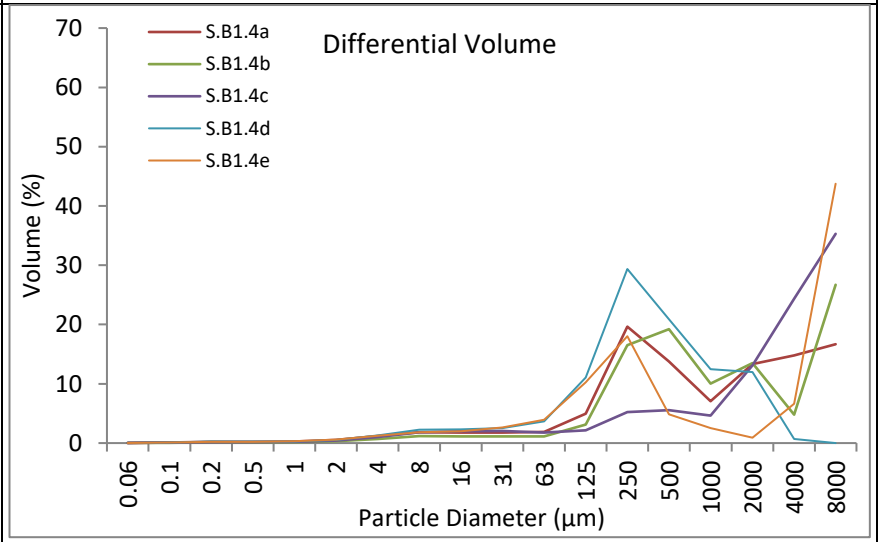
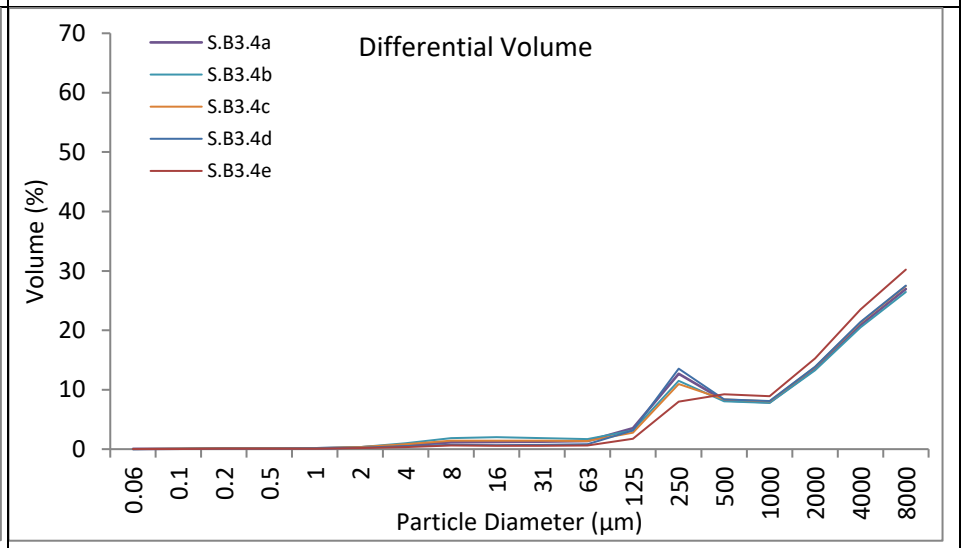
Time (weeks)	Sediment grain-size distribution B1 (mussels introduced)	Sediment grain-size distribution B3 (mussels absent)
0		
12		

Figure 3.54. Grain-size distribution of sediment samples in B1 (mussels introduced) and B3 (mussels absent) from RSB at the beginning and end of the study.

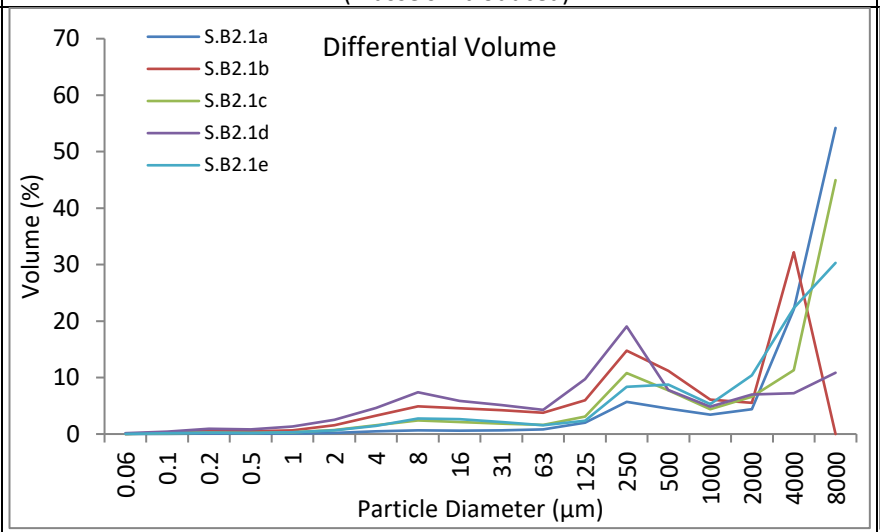
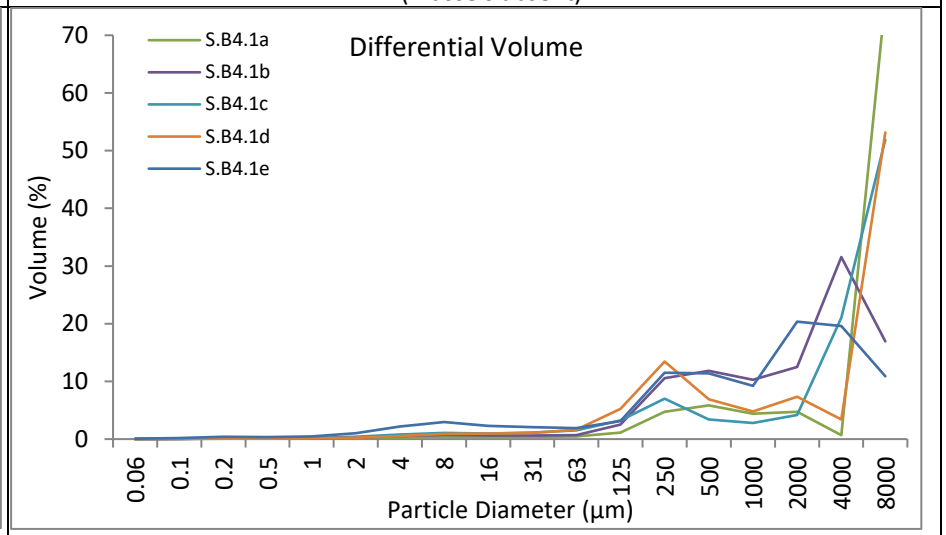
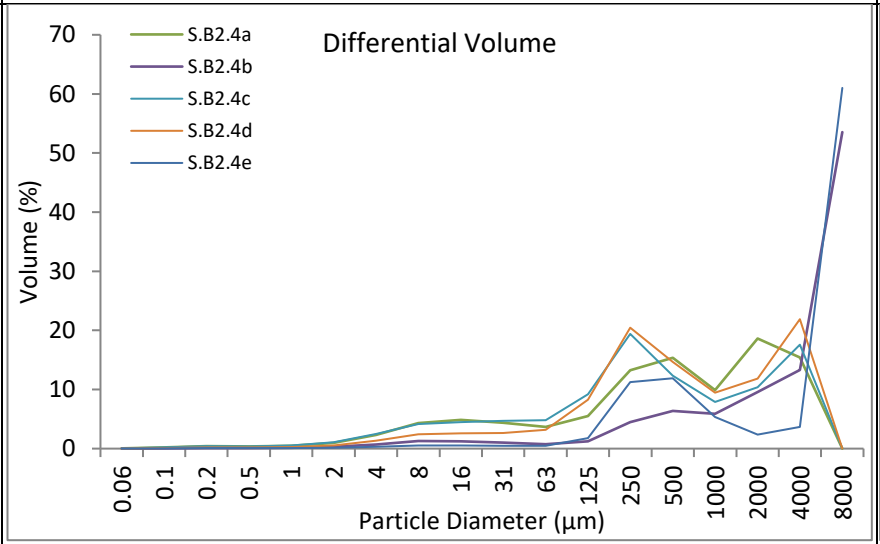
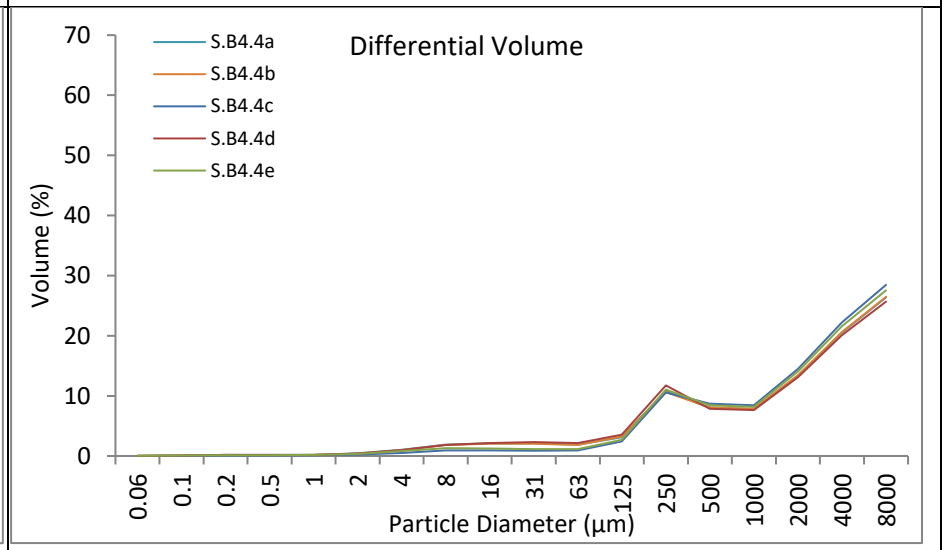
Time (weeks)	Sediment grain-size distribution B2 (mussels introduced)	Sediment grain-size distribution B4 (mussels absent)
0		
12		

Figure 3.55. Grain-size distribution of sediment samples in B2 (mussels introduced) and B4 (mussels absent) from RSB at the beginning and end of the study.

Substrate organic and inorganic carbon content

The mean percentage of organic carbon determined by loss on ignition (LOI) in the sediment samples from MBA decreased from 1.77-2.25 % in A1 and A3 at the beginning of the study to 1.19-1.60 by the end of the study. The mean percentage of organic carbon in A1, with mussels removed, had a similar rate of decrease to the A3 control quadrat throughout the investigation (Figure 3.56).

The removal of mussels from A2 resulted in the mean LOI reducing from 3.64 % at the beginning of the study to 1.79 % by week 4. The mean percentage LOI of A2 remained relatively unchanged by week 8 at 1.86 %. The A4 control quadrat maintained mean LOI values of 1.25-1.48 % throughout the study.

The mean beta coefficient of the change in the mean percentage LOI over time in A1, where mussels were removed ($M=-0.33$, $SD=0.76$) was not significantly different to A3, where mussels were present ($M=-0.29$, $SD=0.16$); $t(4.37)=0.09$, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage LOI over time in A2, where mussels were removed ($M=-0.89$, $SD=1.44$) was not significantly different to A4, where mussels were present ($M=-0.06$, $SD=0.42$); $t(4.69)=1.24$, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in a significantly different percentage of substrate organic carbon compared with quadrats where they were present.

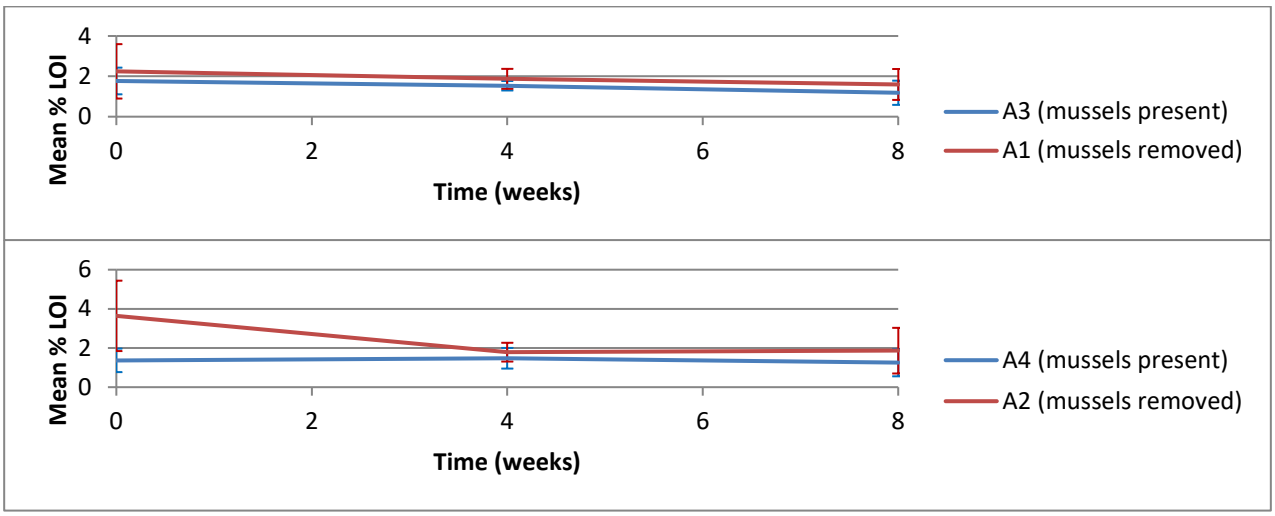


Figure 3.56. Mean (± 1 SD) percentage of organic carbon by loss on ignition (LOI) in sediment core samples taken from MBA over the duration of the study.

At MBB the percentage LOI of the sediment samples was similar in all four quadrats at the beginning of the study with mean values of 1.28-1.60 %. By week 4 the percentage LOI of had risen to 1.66-2.05 % in the four quadrats (Figure 3.57). By week 8 the percentage LOI in the B2 and B3 quadrats had risen to 3.81 and 3.33 % respectively, whereas the B1 and B4 quadrats remained relatively unchanged at 1.82-1.94 % LOI.

The mean beta coefficient of the change in the mean percentage LOI over time in B1, where mussels were added (M=0.33, SD=0.27) was not significantly different to B3, where mussels were absent (M=0.94, SD=0.81); t (4.85)=1.62, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage LOI over time in B2, where mussels were added (M=1.10, SD=1.41) was not significantly different to B4, where mussels were absent (M=0.19, SD=0.76); t (6.13)=-1.28, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not result in a significantly different percentage of substrate organic carbon compared with quadrats where they were absent.

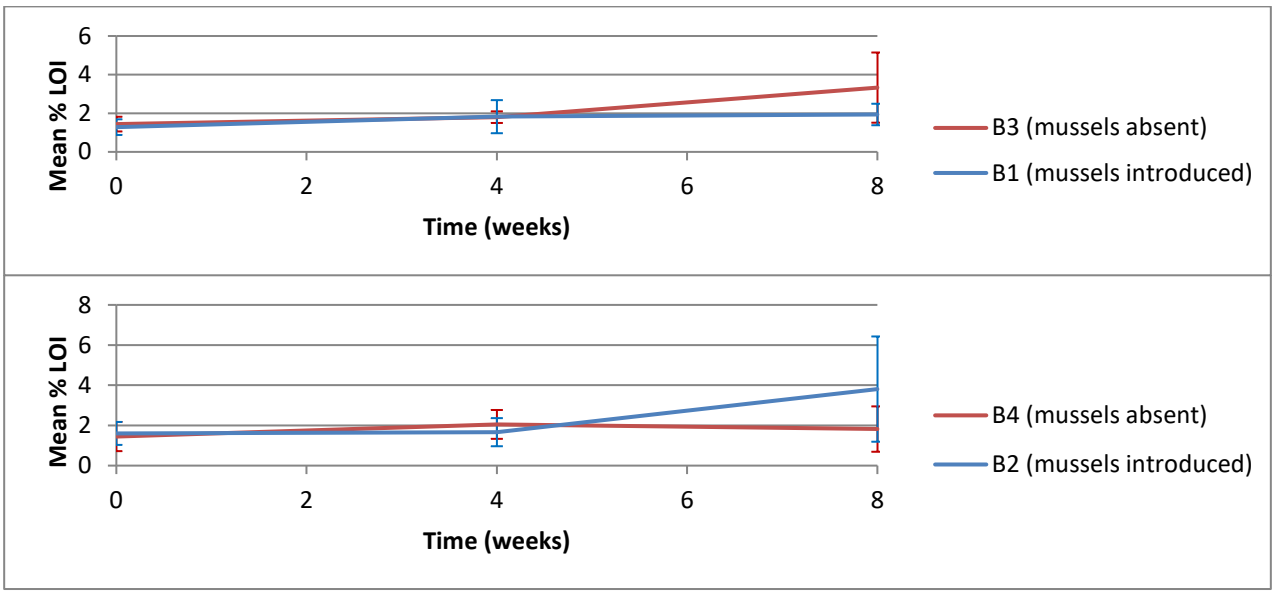


Figure 3.57. Mean (± 1 SD) percentage of organic carbon by loss on ignition (LOI) in sediment core samples taken from MBB over the duration of the study.

At RSA the mean percentage LOI decreased in A1, with mussels removed relative to the A3 control quadrat yet increased in A2 relative to the A4 control quadrat over the course of the study (Figure 3.58). The changes in percentage LOI observed in the quadrats with mussels removed were not significantly different to the control quadrats.

The mean beta coefficient of the change in the mean percentage LOI over time in A1, where mussels were removed (M=-0.06, SD=0.43) was not significantly different to A3, where mussels were present (M=0.45, SD=0.86); $t(5.87)=0.91$, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage LOI over time in A2, where mussels were removed (M=0.84, SD=0.86) was not significantly different to A4, where mussels were present (M=0.10, SD=0.77); $t(7.91)=-1.43$, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in a significantly different percentage of substrate organic carbon compared with quadrats where they were present.

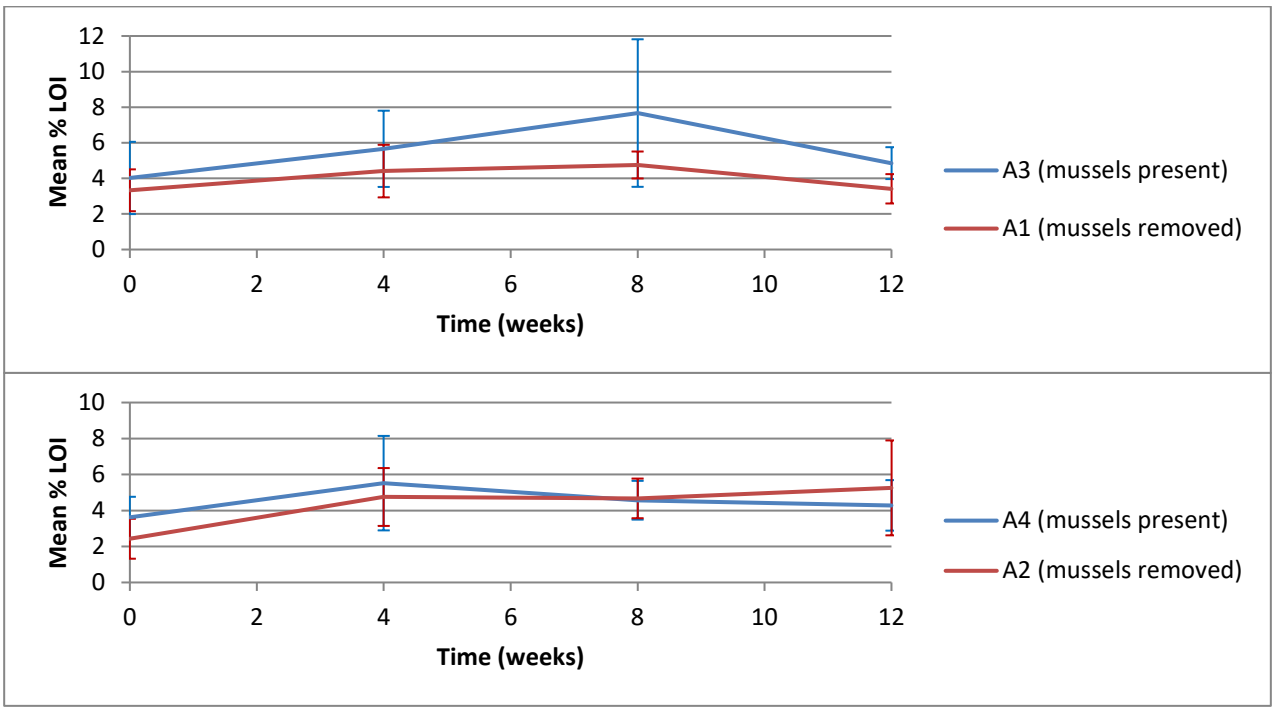


Figure 3.58. Mean (± 1 SD) percentage of organic carbon by loss on ignition (LOI) in sediment core samples taken from RSA over the duration of the study.

At RSB the mean percentage LOI of samples from B1, B2 and B3 ranged from 1.38-1.58 % at the beginning of the study. B4 initially had a higher mean percentage LOI of 2.33 % (Figure 3.59). The mean percentage LOI increased in B1 and B2 by week 12, with mussels introduced to 2.37 % and 2.74 % respectively. The mean percentage LOI in the B3 control quadrat remained relatively unchanged throughout the study, whereas the B4 control quadrat showed a reduced organic content of 1.63 % by the end of the study.

The mean beta coefficient of the change in the mean percentage LOI over time in B1, where mussels were added (M=0.24, SD=0.48) was not significantly different to B3, where mussels were absent (M=0.04, SD=0.11); t (4.41)=-0.89, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage LOI over time in B2, where mussels were added (M=0.50, SD=0.34) was significantly greater than B4, where mussels were absent (M=-0.17, SD=0.23); t (6.95)=-3.68, $p = 0.008$.

The introduction of mussels to quadrat B1 at RSB did not result in a significantly different percentage of substrate organic carbon compared with B3, where they were absent. The introduction of mussels to quadrat B2 at RSB resulted in a significantly increased percentage of substrate organic carbon compared with B4, where they were absent.

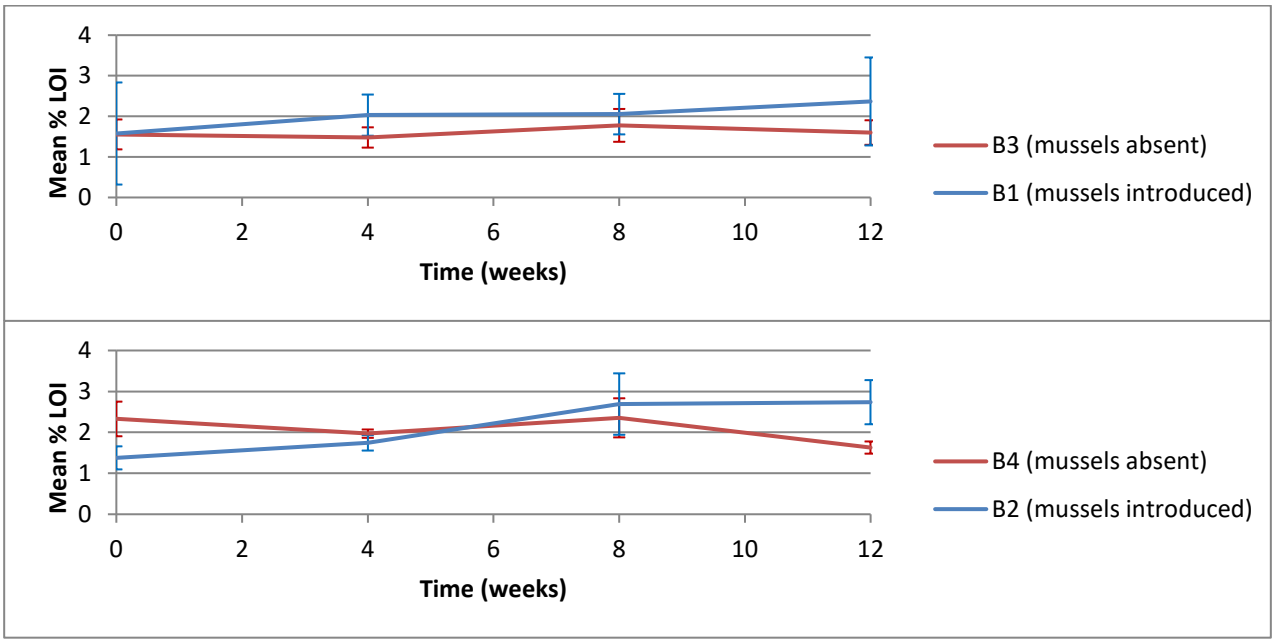


Figure 3.59. Mean (± 1 SD) percentage of organic carbon by loss on ignition (LOI) in sediment core samples taken from RSB over the duration of the study.

The mean percentage inorganic carbon (IC) in each of the quadrats at MBA remained relatively unchanged throughout the study with mean values ranging from 1.96-5.26 % (Figure 3.60).

The mean beta coefficient of the change in the mean percentage IC over time in A1, where mussels were removed (M=0.17, SD=1.75) was not significantly different to A3, where mussels were present (M=-0.09, SD=0.38); t (4.38)=-0.33, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage IC over time in A2, where mussels were removed (M=-0.87, SD=1.29) was not significantly different to A4, where mussels were present (M=-0.35, SD=1.03); t (7.63)=0.70, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in a significantly different percentage of substrate inorganic carbon compared with quadrats where they were present.

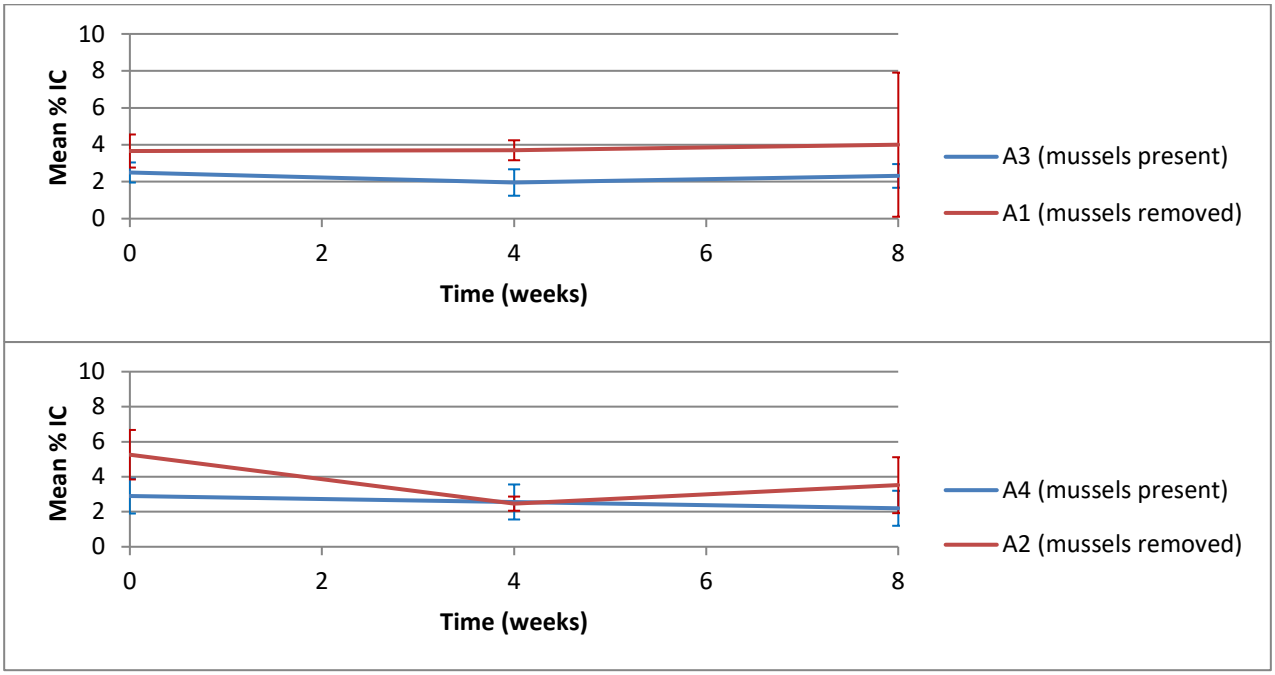


Figure 3.60. Mean (± 1 SD) percentage of inorganic carbon (IC) in sediment core samples taken from MBA over the duration of the study.

The mean percentage IC at MBB ranged from 3.50-5.33 % in the four quadrats at the beginning of the study (Figure 3.61). The mean percentage IC remained relatively unchanged in B1 and B3 throughout the study. The percentage IC increased in B2, with mussels introduced relative to the B4 control quadrat by week 4. By week 8, the mean percentage IC in B2 significantly reduced below that of B4.

The mean beta coefficient of the change in the mean percentage IC over time in B1, where mussels were added (M=0.31, SD=0.60) was not significantly different to B3, where mussels were absent (M=0.73, SD=2.29); t (4.55)=0.39, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage IC over time in B2, where mussels were added (M=0.04, SD=1.59) was significantly less than when B4, where mussels were absent (M=0.04, SD=1.27); t (7.63)=2.75, $p = 0.0261$.

The introduction of mussels to quadrat B1 at MBB did not result in a significantly different percentage of substrate inorganic carbon compared with B3, where they were absent. The introduction of mussels to quadrat B2 at MBB resulted in a significantly reduced percentage of substrate inorganic carbon compared with where they were absent.

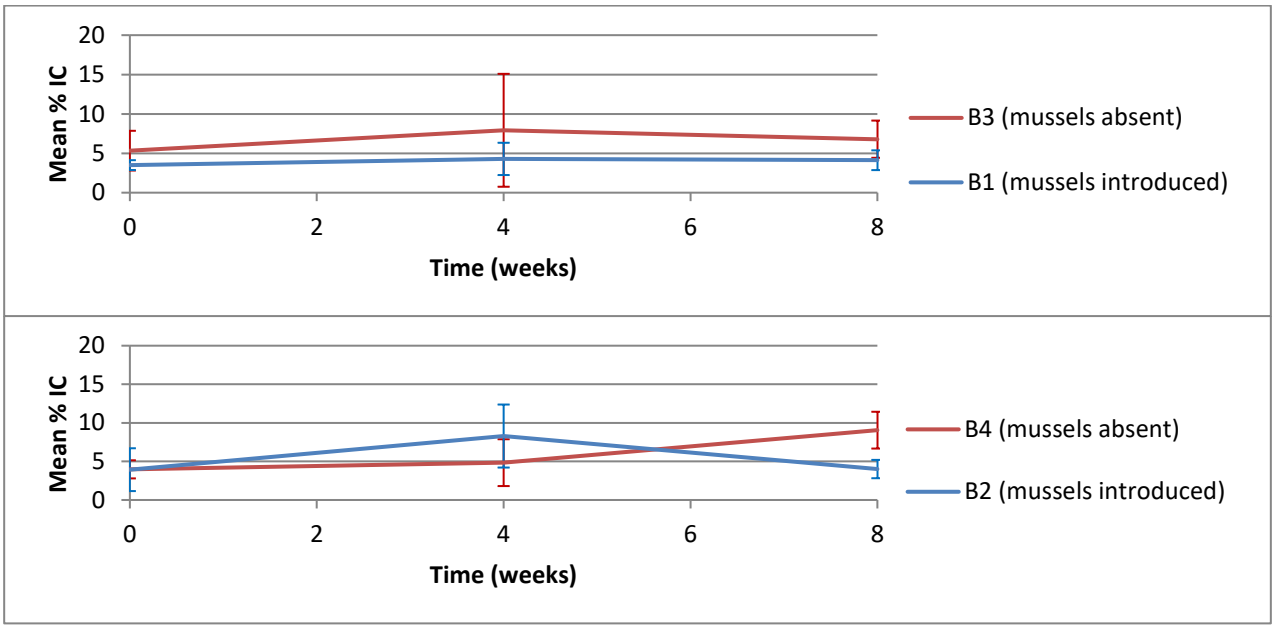


Figure 3.61. Mean (± 1 SD) percentage of inorganic carbon (IC) in sediment core samples taken from MBB over the duration of the study.

At RSA, the mean percentage IC in the A3 control quadrat increased relative to the A1 quadrat, with mussels removed by week 4 of the study, but remained relatively unchanged from week 8-12, with mean values ranging from 3.74-5.77 %. The mean percentage IC in the A2 and A4 quadrats remained within 3.38-4.12 for the duration of the study (Figure 3.62).

The mean beta coefficient of the change in the mean percentage IC over time in A1, where mussels were removed (M=-0.30, SD=1.74) was not significantly different to A3, where mussels were present (M=0.28, SD=0.83); $t(5.73)=0.68$, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage IC over time in A2, where mussels were removed (M=0.33, SD=0.95) was not significantly different to A4, where mussels were present (M=0.11, SD=0.41); $t(5.45)=-0.46$, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in a significantly different percentage of substrate inorganic carbon compared with quadrats where they were present.

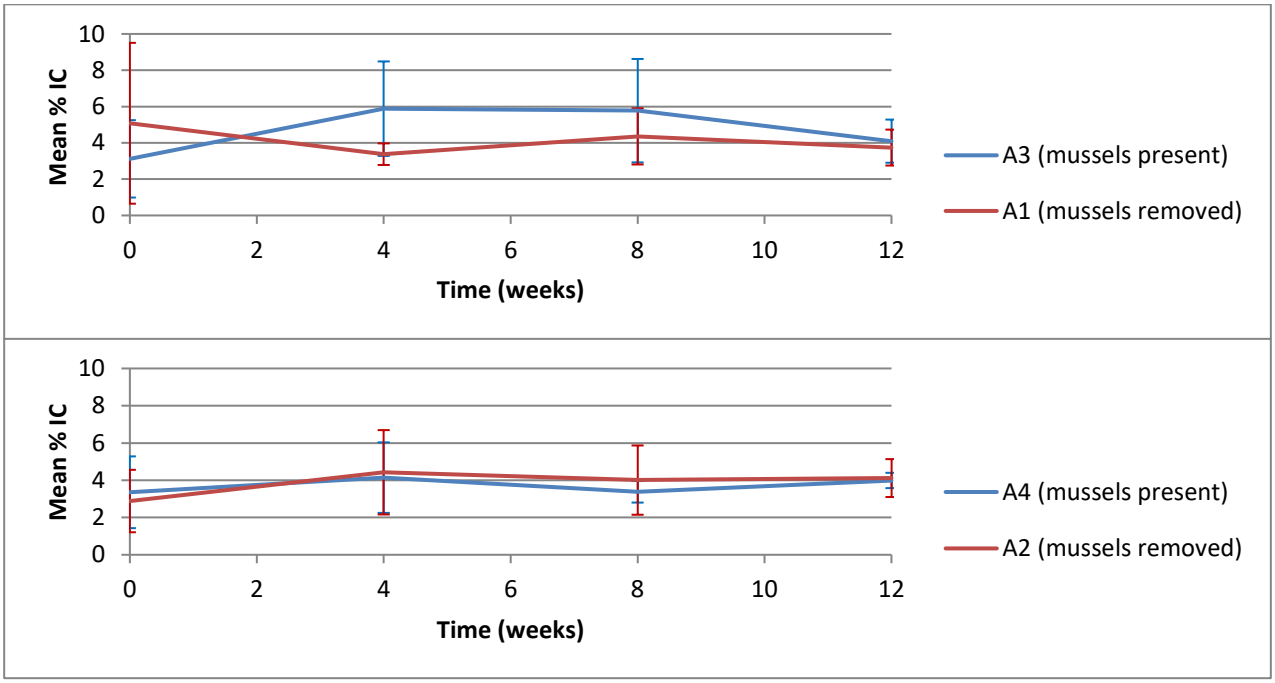


Figure 3.62. Mean (± 1 SD) percentage of inorganic carbon (IC) in sediment core samples taken from RSA over the duration of the study.

At RSB the mean percentage IC was initially higher in B1 and B3 compared with B2 and B4 at 1.33-1.80 % and 0.64-0.69 % respectively. From weeks 4-12 the mean percentage IC in B1 and B3 dropped to levels comparable with B2 and B4, with mean values in all four quadrats remaining at 0.41-0.65 % (Figure 3.63).

The mean beta coefficient of the change in the mean percentage IC over time in B1, where mussels were added (M=-0.37, SD=0.57) was not significantly different to B3, where mussels were absent (M=-0.21, SD=0.61); t (7.96)=0.42, $p > 0.05$.

The mean beta coefficient of the change in the mean percentage IC over time B2, where mussels were added (M=-0.00, SD=0.15) was not significantly different to B4, where mussels were absent (M=-0.01, SD=0.11); t (7.29)=-0.06, $p > 0.05$.

The introduction of mussels to quadrats at RSB did not result in a significantly different percentage of substrate inorganic carbon compared with quadrats where they were absent.

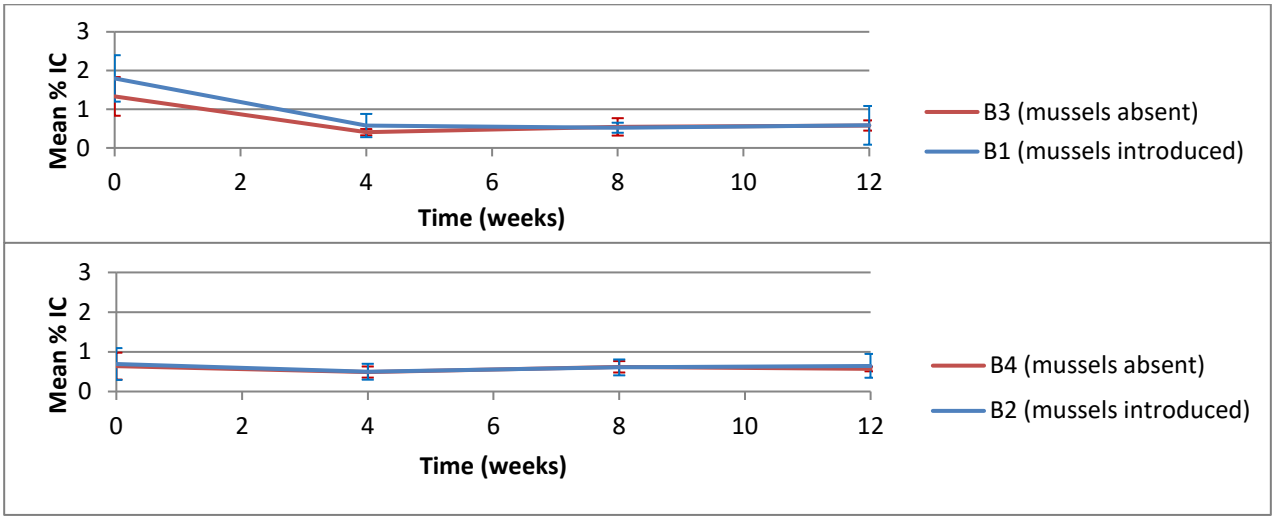


Figure 3.63. Mean (± 1 SD) percentage of inorganic carbon (IC) in sediment core samples taken from RSB over the duration of the study.

A summary of results showing how the presence of freshwater mussels impacted the substrate conditions at the four river sites is shown in Table 3.2:

Table 3.2. Summary of results for the substrate conditions at the four river sites, showing whether the introduction or removal of freshwater mussels led to a significant increase or decrease in each measured variable compared with the control quadrats.

River site / Variable	MBA	MBB	RSA	RSB
Substrate oxygen saturation (%)	Significantly decreased where mussels were removed	Significantly increased in B1 where mussels were introduced. No significant difference in B2	Significantly decreased where mussels were removed	Significantly increased in B1 where mussels were introduced. No significant difference in B2
Armour layer pebble B-axis length (mm)	Significantly decreased where mussels were removed	No significant difference	No significant difference	No significant difference
Mean sediment grain-size (µm)	No significant difference	No significant difference	No significant difference	No significant difference
Fines (<63 µm) (%)	No significant difference	No significant difference	Significantly decreased in A1 where mussels were removed. No significant difference in A2	No significant difference
Substrate heterogeneity	No significant difference	No significant difference	Reduced heterogeneity where mussels were removed	Increased heterogeneity where mussels were introduced
Organic carbon (%)	No significant difference	No significant difference	No significant difference	No significant difference in B1. Significantly increased in B2 where mussels were introduced
Inorganic carbon (%)	No significant difference	No significant difference in B1. Significantly reduced in B2 where mussels were introduced	No significant difference	No significant difference

3.4.3 Macroinvertebrates and BMWP scores

As only one kick sample per quadrat was carried out at each visit to the river sites, the kick sample data from the two replicate quadrats and two control quadrats at each site have been analysed together in order to establish mean beta coefficients for each pair of quadrats.

At MBA the mean BMWP scores for A1 and A2 were initially similar to those for A3 and A4 at 33.9 and 36.2 respectively. By week 4 the mean scores for A1 and A2, where mussels had been removed had dropped to 20.3 compared with 30.5 for A3 and A4. By week 8 mean BMWP scores in the A3 and A4 quadrats with mussels had increased to 45.6, whereas the A1 and A2 quadrats maintained relatively low BMWP scores of 20.8 (Figure 3.64).

The mean beta coefficient of the change in BMWP scores over time in A1 and A2, where mussels were removed (M=-6.55, SD=7.28) was not significantly different to A3 and A4, where mussels were present (M=4.70, SD=1.34); t (1.07)=2.15, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in significantly different BMWP scores compared with quadrats where they were present.

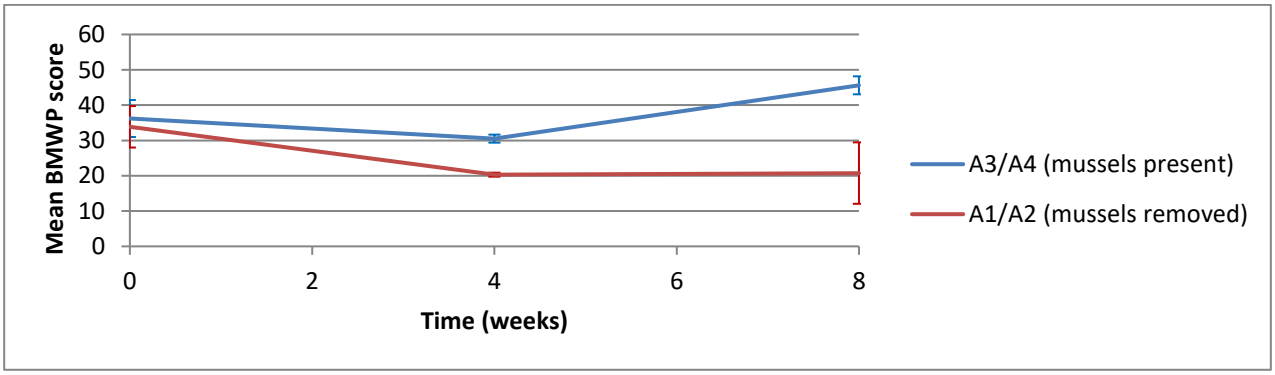


Figure 3.64. Mean (± 1 SD) BMWP scores at MBA over the duration of the study.

At MBB both pairs of quadrats had mean BMWP scores of 27.8-28.2, which are lower than the scores for MBA. By the end of the study mean BMWP scores for B1 and B2, with mussels introduced had increased to 47.7 compared with 45.3 for B3 and B4 (Figure 3.65).

The mean beta coefficient of the change in BMWP scores over time in B1 and B2, where mussels were added (M=9.93, SD=10.08) was not significantly different to B3 and B4, where mussels were absent (M=8.53, SD=9.51); t (1.99)=-0.14, p > 0.05.

The introduction of mussels to quadrats at MBB did not result in significantly different BMWP scores compared with quadrats where they were absent.

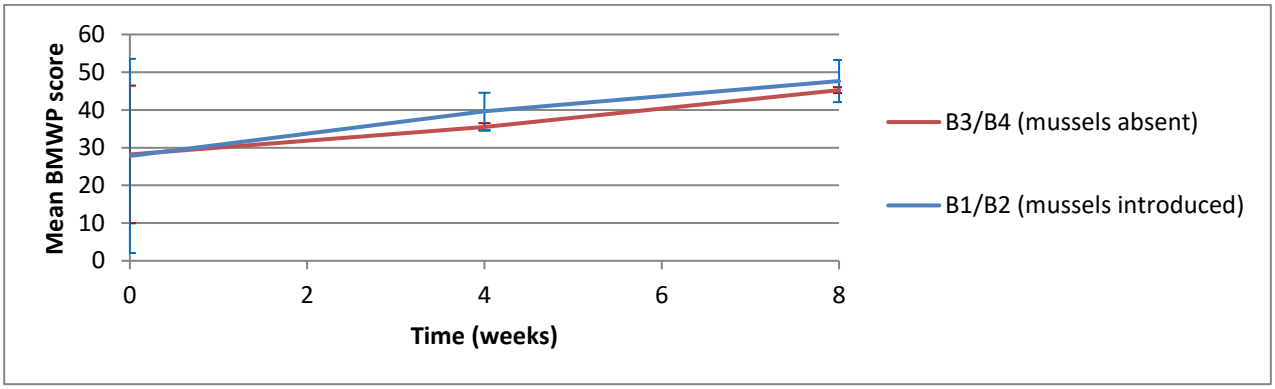


Figure 3.65. Mean (± 1 SD) BMWP scores at MBB over the duration of the study.

At the beginning of the study mean BMWP scores at RSA ranged from 50.8-54.7 (Figure 3.66). By week 4 mean BMWP scores in A1 and A2, with mussels removed had decreased to 24.9 compared with 32.8 for A3 and A4. By week 12 mean BMWP scores in A3 and A4 were significantly higher than A1 and A2 at 67.6 and 37.5 respectively.

The mean beta coefficient of the change in BMWP scores over time in A1 and A3, where mussels were removed (M=-4.79, SD=0.81) was significantly more negative than A3 and A4, where mussels were present (M=4.71, SD=0.67); t (1.93)=12.72, p = 0.007.

The removal of mussels from quadrats at RSA resulted in significantly reduced BMWP scores compared with quadrats where they were present.

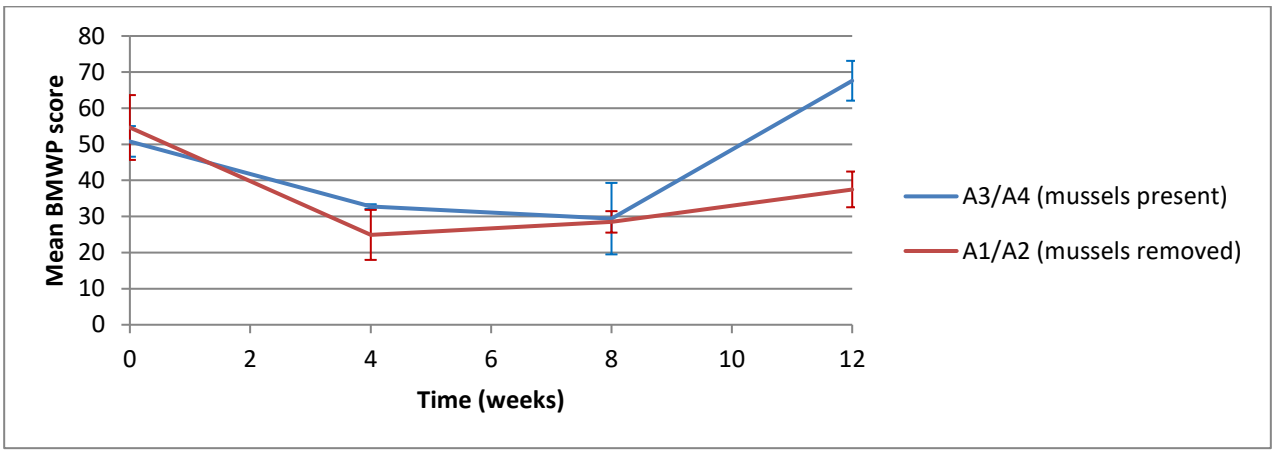


Figure 3.66. Mean (± 1 SD) BMWP scores at RSA over the duration of the study.

At RSB, mean BMWP scores for B1 and B2, with mussels introduced increased from 43.9 to 61.0 by week 8 before dropping slightly to 59.4 by week 12 (Figure 3.67). The B3 and B4 control quadrats maintained BMWP scores of 35.0-43.9 throughout the investigation.

The mean beta coefficient of the change in BMWP scores over time in B1 and B2, where mussels were added (M=5.23, SD=0.25) was significantly more positive than B3 and B4, where mussels were absent (M=-2.78, SD=0.16); t (1.70)=-37.48, p = 0.0018.

The introduction of mussels to quadrats at RSB resulted in significantly increased BMWP scores compared with quadrats where they were absent.

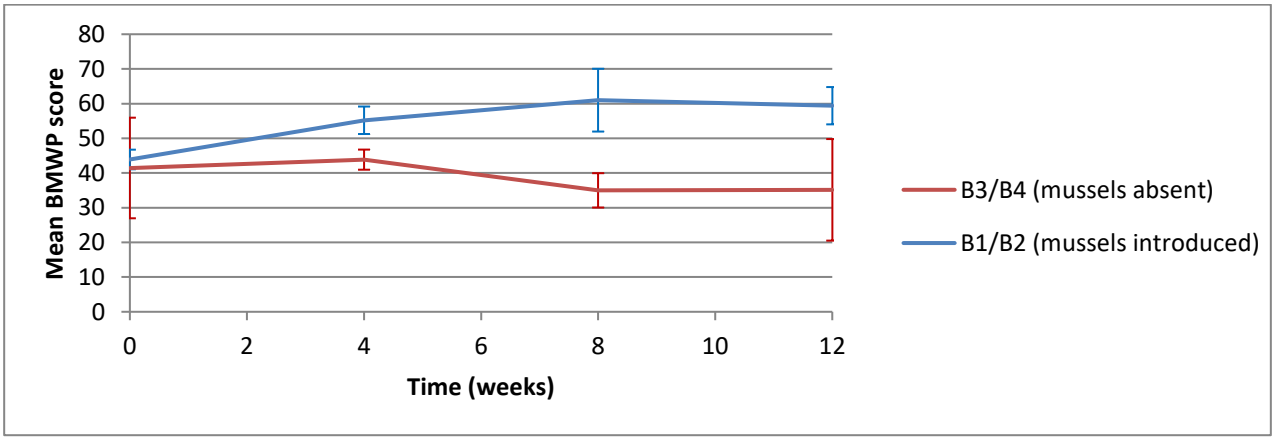


Figure 3.67. Mean (± 1 SD) BMWP scores at RSB over the duration of the study.

As Ephemeroptera (mayflies) and Trichoptera (caddisflies) are considered biological indicators of water quality (Stoyanova *et al.*, 2014), and due to the relatively high abundance of Ephemeroptera and Trichoptera species in the kick sample data, additional analysis was carried out for these invertebrate orders. At MBA the mean abundance of Ephemeroptera in A1 and A2, with mussels removed was initially higher than in the A3 and A4 control quadrats (Figure 3.68). By week 4 the number of Ephemeroptera in all four quadrats significantly decreased to between 0 and 4 individuals per quadrat, with the number in A3 and A4 marginally above the numbers in A1 and A2 for the remainder of the study.

The mean beta coefficient of the change in abundance of Ephemeroptera over time in A1 and A2, where mussels were removed (M=-13.25, SD=3.18) was not significantly different to A3 and A4, where mussels were present (M=-7.00, SD=6.36); t (1.47)=1.24, $p > 0.05$.

The removal of mussels from quadrats at MBA did not result in significantly different Ephemeroptera abundance compared with quadrats where they were present.

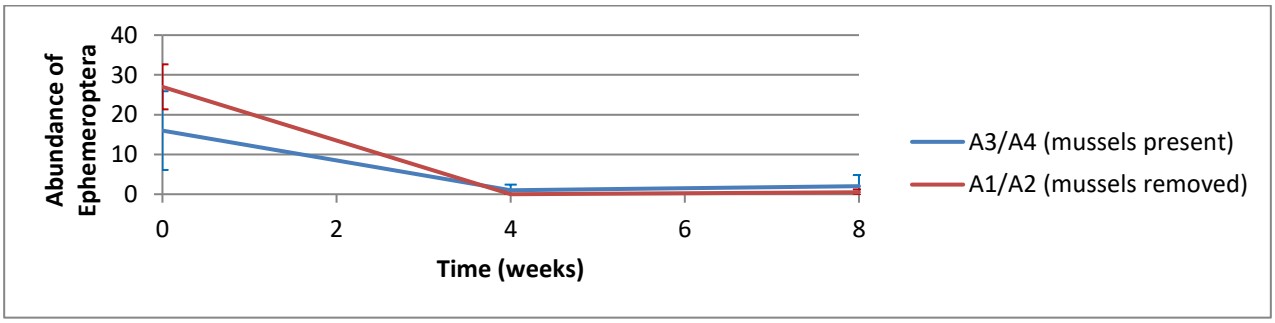


Figure 3.68. Mean (± 1 SD) abundance of Ephemeroptera (mayflies) at MBA over the duration of the study.

At MBB mean Ephemeroptera abundance decreased from 4.5-7 at the beginning of the study to 2-4.5 by week 8 (Figure 3.69). The mean beta coefficient of the change in abundance of Ephemeroptera over time in B1 and B2, where mussels were added (M=-1.25, SD=4.60) was not significantly different to B3 and B4, where mussels were absent (M=-1.25, SD=3.88); t (1.95)=0.00, $p > 0.05$.

The introduction of mussels to quadrats at MBB did not result in significantly different Ephemeroptera abundance compared with quadrats where they were absent.

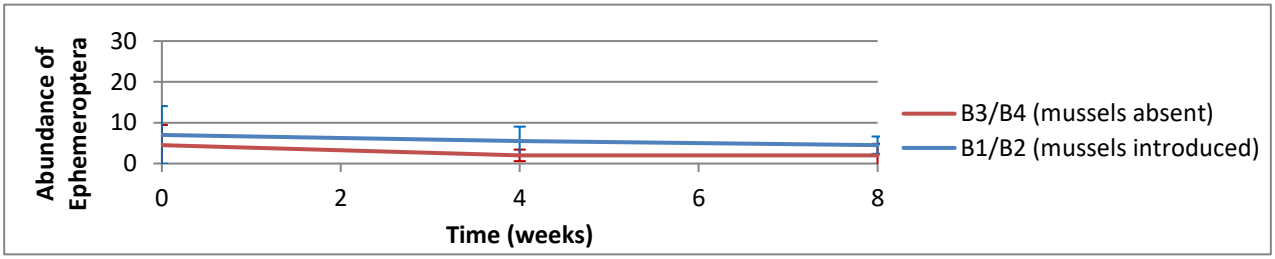


Figure 3.69. Mean (± 1 SD) abundance of Ephemeroptera (mayflies) at MBB over the duration of the study.

At RSA the mean abundance of Ephemeroptera was 4.5 in A1 and A2 and 5 in A3 and A4 (Figure 3.70). From week 4 to the remainder of the study, no Ephemeroptera were found in the A1 and A2 quadrats, that had mussels removed. From week 4-8 mean abundance of Ephemeroptera in A3 and A4, with mussels present was 2.5, and by week 12 it had increased to 11.

The mean beta coefficient of the change in abundance of Ephemeroptera over time in A1 and A2, where mussels were removed (M=0.00, SD=2.81) was not significantly different to A3 and A4, where mussels were present (M=1.80, SD=0.42); t (1.00)=6.00, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different Ephemeroptera abundance compared with quadrats where they were present.

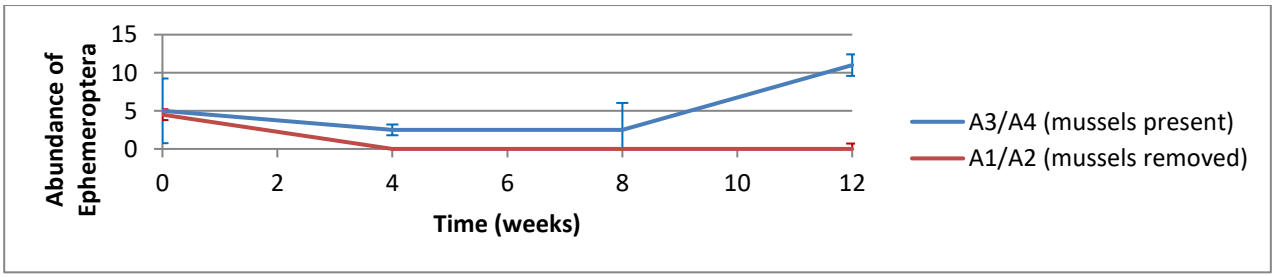


Figure 3.70. Mean (± 1 SD) abundance of Ephemeroptera (mayflies) at RSA over the duration of the study.

Mean abundance of Ephemeroptera at RSB ranged from 2.5-3 individuals at the beginning of the study (Figure 3.71). By week 4 mean Ephemeroptera abundance had increased to 13 in B1 and B2, with mussels introduced, compared with 6 for B3 and B4, with mussels absent. The number of Ephemeroptera in the quadrats with mussels remained higher than the control quadrats for the remainder of the study, with a mean abundance of 9.5 in B1 and B2 by week 12, compared with 3.5 in B3 and B4.

The mean beta coefficient of the change in abundance of Ephemeroptera over time in B1 and B2, where mussels were added (M=1.00, SD=2.55) was not significantly different to B3 and B4, where mussels were absent (M=-0.05, SD=0.35); t (1.04)=-0.58, p > 0.05.

The introduction of mussels to quadrats at RSB did not result in significantly different Ephemeroptera abundance compared with quadrats where they were absent.

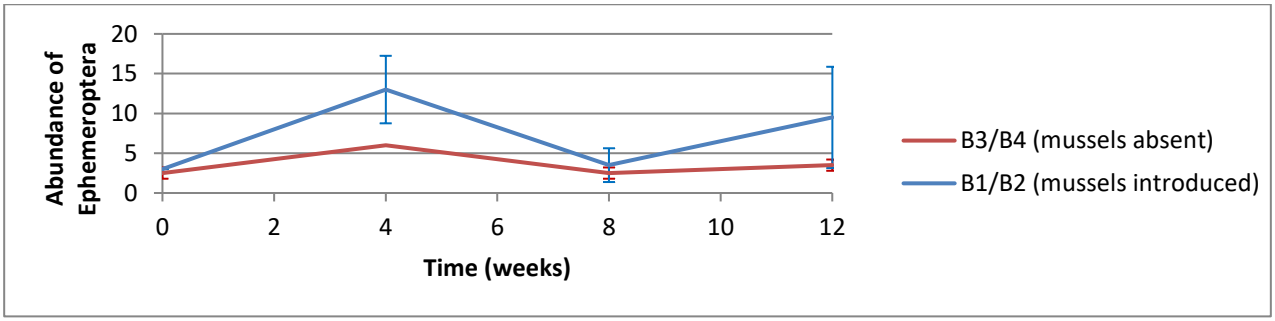


Figure 3.71: Mean (± 1 SD) abundance of Ephemeroptera (mayflies) at RSB over the duration of the study.

At MBA mean abundance of Trichoptera decreased in the A3 and A4 control quadrats from 15.5 at the start of the study to 5 by week 4, increasing to 10 by week 8 (Figure 3.72). In A1 and A2, with mussels removed, mean Trichoptera abundance decreased from 8 to 2 by week 4. On week 8 no Trichoptera were found in A1 or A2.

The mean beta coefficient of the change in abundance of Trichoptera over time in A1 and A2, where mussels were removed (M=-4.00, SD=1.41) was not significantly different to A3 and A4, where mussels were present (M=-2.75, SD=3.89); t (1.26)=0.43, p > 0.05.

The removal of mussels from quadrats at MBA did not result in significantly different Trichoptera abundance compared with where they were present.

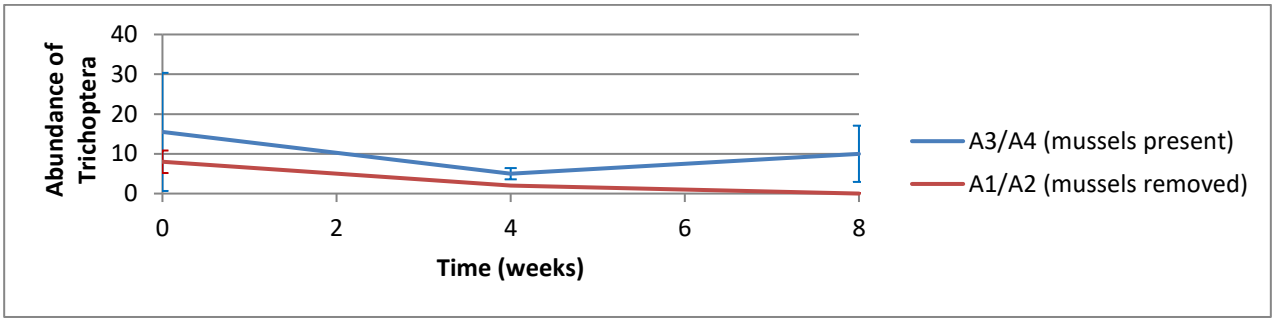


Figure 3.72. Mean (± 1 SD) abundance of Trichoptera (caddisflies) at MBA over the duration of the study.

At MBB mean abundance of Trichoptera initially decreased in the B3 and B4 control quadrats from 6.5 to 2 by week 4, before increasing to 9.5 by week 8 (Figure 3.73). In B1 and B2, with mussels introduced mean Trichoptera abundance increased from 8.5 to 10 by week 4, further increasing to 13.5 by week 8.

The mean beta coefficient of the change in abundance of Trichoptera over time in B1 and B2, where mussels were added (M=2.50, SD=7.07) was not significantly different to B3 and B4, where mussels were absent (M=1.50, SD=3.54); t (1.47)=-0.18, p > 0.05.

The introduction of mussels to quadrats at MBB did not result in significantly different Trichoptera abundance compared with quadrats where they were absent.

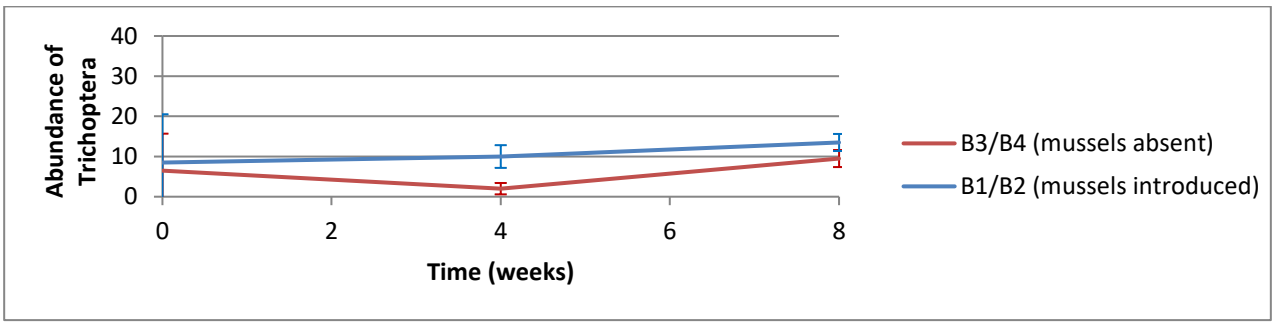


Figure 3.73. Mean (± 1 SD) abundance of Trichoptera (caddisflies) at MBB over the duration of the study.

Mean abundance of Trichoptera was 10.5 to 11.5 at RSA at the beginning of the study. By week 4, mean Trichoptera abundance had decreased to 1 in A1 and A2, with mussels removed and 3 in A3 and A4 with mussels present (Figure 3.74). In A1 and A2 mean Trichoptera abundance remained low at 1-1.5 for the remainder of the study, however, in A3 and A4 it increased to 27-27.5 from week 8-12.

The mean beta coefficient of the change in abundance of Trichoptera over time in A1 and A2, where mussels were removed (M=-2.20, SD=2.83) was not significantly different to A3 and A4, where mussels were present (M=7.40, SD=1.41); t (1.01)=4.79, $p > 0.05$.

The removal of mussels from quadrats at RSA did not result in significantly different Trichoptera abundance compared with quadrats where they were present.

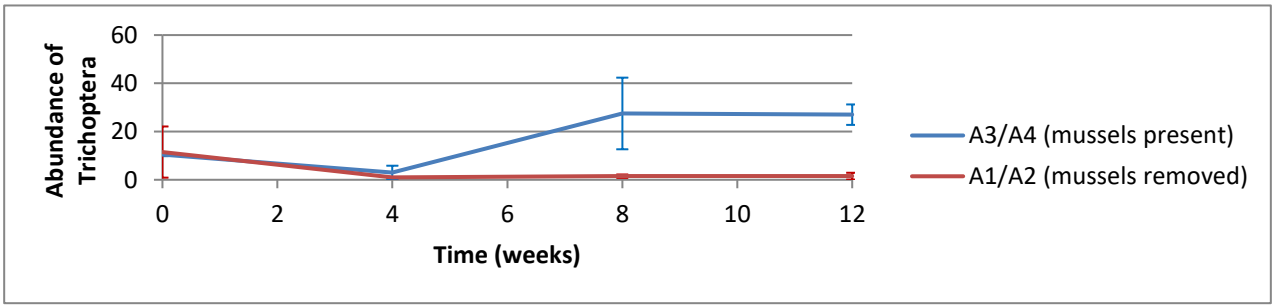


Figure 3.74. Mean (± 1 SD) abundance of Trichoptera (caddisflies) at RSA over the duration of the study.

At RSB the mean abundance of Trichoptera ranged from 2.5-7 up until the 4th week of the study (Figure 3.75). By week 8 mean Trichoptera abundance in B1 and B2, with mussels introduced,

increased to 23 compared with 2.5 for B3 and B4. By week 12 mean Trichoptera abundance was 6.5 in B1 and B2 and 2 in B3 and B4.

The mean beta coefficient of the change in abundance of Trichoptera over time in B1 and B2, where mussels were added (M=1.90, SD=2.26) was not significantly different to B3, B4, where mussels were absent (M=-0.45, SD=0.49); t (1.10)=-1.43, p > 0.05.

The introduction of mussels to quadrats at RSB did not result in significantly different Trichoptera abundance compared with quadrats where they were absent.

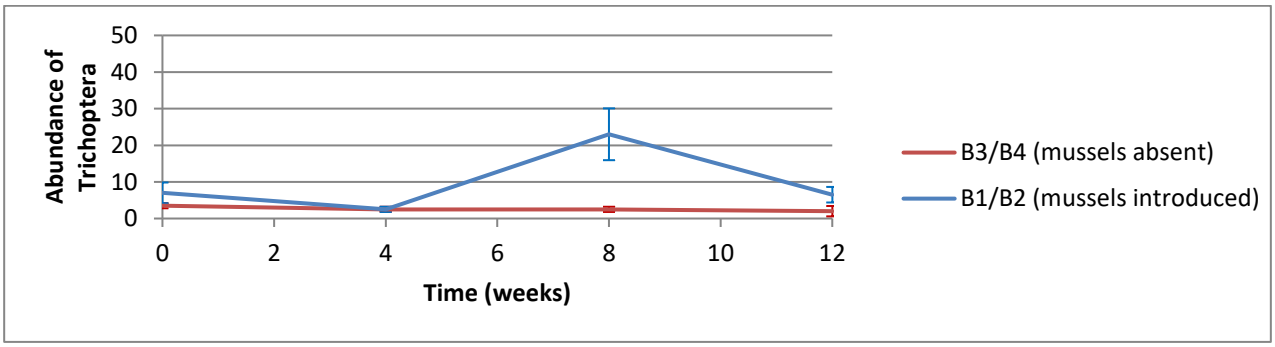


Figure 3.75. Mean (± 1 SD) abundance of Trichoptera (caddisflies) at RSB over the duration of the study.

The results for the macroinvertebrate and BMWP data at each river site are summarised in Table 3.3, which shows how the presence of freshwater significantly affected each of the measured variables compared with when mussels were absent.

Table 3.3. Summary of results for the four river sites, showing whether the introduction or removal of freshwater mussels led to a significant increase or decrease in each measured biotic variable compared with the control quadrats.

River site / Variable	MBA	MBB	RSA	RSB
BMWP scores	No significant difference	No significant difference	Significantly reduced where mussels were removed	Significantly increased where mussels were introduced
Ephemeroptera abundance	No significant difference	No significant difference	No significant difference	No significant difference
Trichoptera abundance	No significant difference	No significant difference	No significant difference	No significant difference

3.5 Discussion

The results of this investigation suggest that presence of freshwater mussels impacted the biotic and abiotic conditions within the four river sites to differing degrees. The variability in the results and the conclusions drawn from each site may be attributable to pre-existing differences in the hydrological, sedimentological and biological conditions prevalent at the various river sites, which may have affected the behaviour of the mussels, or their capacity to engineer their environment.

The influence of the mussels on substrate conditions was more evident in this investigation than their impact on water quality. Where the presence of any given volume of water in each quadrat is only transitory, the flux of sediment in and out of the quadrat is, for the large part, a much slower process. The length of time that mussels are physically interacting with the sediment is therefore greater than is the case with the ambient water.

Addressing Key Question 5 *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"* (described in Section 1.4), six out of eight quadrats containing mussels showed significantly more positive beta coefficients for hyporheic oxygen saturation compared with quadrats where they were absent. A possible explanation for the more positive trend in substrate oxygen levels within mussel-containing quadrats could be that mussel bioturbation may enabling infiltration of more oxygenated water into the hyporheic zone, as has been demonstrated for other invertebrate bioturbators (Boeker *et al.*, 2016; Shrivastava, Stewardson and Arora, 2021). It is also possible that changes in microbial or macroinvertebrate communities resulting from mussel bioturbation and biodeposition may have contributed to higher hyporheic oxygen levels. Higher levels of sediment oxygen saturation would be of benefit to infaunal invertebrate species, as low sediment oxygen levels can restrict the depth at which many macroinvertebrates can burrow and feed (Rasmussen, 1988; Swan and Palmer, 2000; Krieger *et al.*, 2007; Jones *et al.*, 2011). Higher hyporheic oxygen saturation would also provide more favourable spawning conditions for host fish, such as salmonids (Beschta and Jackson, 1979; Chapman, 1988; Malcolm *et al.*, 2009).

The two quadrats that did not show significant differences in hyporheic oxygen saturation were both at sites where mussels had been introduced rather than removed, with one at MBB and the other at RSB. These quadrats showed increased hyporheic oxygen saturation in the presence of mussels compared with the control quadrats but the differences were not enough to be significant. It is possible that as these sites did not previously contain mussels the conditions may not have been optimal for them. The greater mean bed sediment size at MBB and RSB compared with MBA and RSA may have made it more difficult for the mussels to burrow into the sediment and influence the oxygen saturation levels in at these sites.

The results for hyporheic oxygen saturation in this study concur with research by Boeker *et al.* (2016) showing higher hyporheic oxygen levels in the presence of freshwater mussels, and is contradictory to the conclusion of studies by Hakenkamp and Palmer (1999), Beekey, Mccabe and

Marsden (2004) and Turner (2010), indicating that freshwater mussels reduced hyporheic oxygen levels. The studies suggesting that freshwater mussels reduced hyporheic oxygen levels were conducted with mussels that are not native to the UK; specifically, *Dreissena polymorpha* (Pallas 1771) (Beekey, Mccabe and Marsden, 2004; Turner, 2010), *Dreissena rostriformis bugensis* (Andrusov 1897) (Turner, 2010), and *Corbicula fluminea* (Muller 1774) (Hakenkamp and Palmer, 1999), whereas the study by Boeker *et al*. (2016) indicating mussels increase hyporheic oxygen levels was conducted using *Anodonta anatina*. The mussels found in Markeaton Brook and the River Sence were a mixed assemblage of *A. anatina* and *A. cygnea*, which suggests that native *Anodonta* species positively impact hyporheic oxygen levels in rivers, whereas non-native species may have a detrimental impact on hyporheic oxygen levels.

Key Question 1 *"To what extent do freshwater mussels impact river bed grain-size distribution patterns?"* was not fully resolved during this investigation. Mean river bed sediment B-axis size and mean grain-size of the sediment cores were not significantly affected by the presence of mussels, with the exception of MBA, for which the removal of mussels significantly reduced river bed sediment B-axis length in both quadrats. The higher bed sediment B-axis lengths on the surface of the river bed in the presence of mussels at MBA may be explained by kinematic sieving of fine grains through the interstices between coarser bed sediment (Evans and Wilcox, 2014) as a result of mussel bioturbation. The creation of burrows by mussels may have also allowed fine and organic material present on the surface of the river bed to sink down into the burrows and be incorporated into the sub-surface environment rather than remaining on the surface. Alternatively, mobilisation of the river bed sediment by mussel bioturbation may have resulted in scouring of finer material from the surface of the river bed.

Five out of eight quadrats containing mussels showed a trend towards an increased mean percentage of fines (<63 μm) in the sediment cores compared with the quadrats with mussels absent. However, significant results were only found at RSA, where the removal of mussels from

A1 significantly reduced the mean percentage of fines compared with A3. The increased proportion of fine sediment in the presence of mussels is likely due to biodeposition by the mussels. The protrusion of their shells from the river bed may also have disrupted and slowed the flow of water, which may have led to increased rates of fine sediment deposition around the mussels.

The impact of mussels on the grain-size distribution of sediment from the sediment cores was less pronounced in Markeaton Brook compared with the River Sence, which may have been due to the relatively low densities of mussels found in Markeaton Brook. However, the removal of mussels from quadrat A1 at MBA resulted in a reduced proportion of clay and fine silt compared with A3 over the duration of the study. At RSA the proportion of silt and clay reduced over the duration of the study where mussels had been removed, compared to where they were present.

At both RSA and RSB in quadrats where mussels were absent, the sediment core samples show an increased consistency in grain-size distribution patterns by the end of the study. By week 12, all the samples taken from the control quadrats had an almost identical grain-size distribution pattern, whereas the quadrats with mussels present displayed much greater variation in terms of the relative proportions of clay, silt, sand and gravel. These results indicate that in the River Sence, the presence of mussels was associated with greater substrate heterogeneity compared with quadrats without mussels.

The mechanism giving rise to the greater heterogeneity of the substrate may be a combined effect from filter-feeding and biodeposition increasing the amount of fines on the river bed, alongside the impact of mussel bioturbation mobilising sediment both laterally and vertically. Where mussels have been actively burrowing they may locally reduce the threshold for grain entrainment by altering the arrangement of grains and disrupting biofilms (de Smit *et al.*, 2021), resulting in scouring of fines from the surface. Parts of the river bed recently disturbed by mussels may therefore display increased sand and gravel at the sediment-water interface, whereas areas of the

river bed that have not been subject to recent mussel activity would likely collect greater quantities of the finer sediment.

The physical disruption to the flow of water from the mussels' shells protruding from the river bed may have also given rise to some localised variation in grain-size due to the scour and sediment wake effects often observed where there is an obstruction within the flow of a river (Bunte and Abt, 2001). The disruption to the flow and increased bed friction may have generated localised downwelling and upwelling forces and variations in near-bed velocity, resulting in fine sediment deposition in some areas and erosion of fine sediment in others.

The impact of the mussels on substrate heterogeneity observed in this study is an important finding as substrate heterogeneity translates to river bed habitat heterogeneity for resident species. Increased habitat heterogeneity and substrate complexity have been positively correlated with species diversity and abundance in river environments, as it increases the range of available ecological niches (Duan, Wang and Tian, 2008; Luck *et al.*, 2010; Reid, Brierley and Boothroyd, 2010; Platt, 2011; Wyżga *et al.*, 2012; Bellmore and Baxter, 2014; Wohl, 2016). Furthermore, a greater degree of river bed complexity is thought to increase the resilience of river communities to disturbance such as fluctuations in discharge from floods events and droughts, as it increases the range of microhabitats and refuges available to species (Luck *et al.*, 2010; Wyżga *et al.*, 2012; Wohl, 2016). The mussels impact on substrate heterogeneity is therefore not only of relevance to Key Question 1, but also of relevance to Key Question 6: *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"*

Also addressing Key Question 6, significantly higher BMWP scores were found in the presence of mussels at both RSA and RSB by the end of the investigation, which concurs with research by Aldridge, Fayle and Jackson (2007) showing BMWP scores to be strongly correlated with freshwater mussel density. The quadrats containing mussels at MBA and MBB also had higher BMWP scores compared with where they were absent, although the differences were not found to

be significant. This may be due to the lower density of mussels in Markeaton Brook compared with the River Sence, or it may result from the lack of replicates available for analysis, as only one kick sample per quadrat was carried out each time they were sampled. Furthermore, due to the necessity of avoiding disturbance to the river bed sediment within each quadrat, the kick samples were carried out immediately downstream of each quadrat. As the mussels were not directly interacting with the part of the river bed the kick samples were taken from, the extent to which mussels influence macroinvertebrate communities may be greater than the data suggests.

Taxa appearing to respond most positively to the presence of mussels in this study include the Ephemeroptera (mayflies) and the Trichoptera (caddisflies), which are considered to be indicative of good water quality (Stoyanova *et al.*, 2014). Six out of eight sites showed greater abundance of these taxa where mussels were present, although the differences in abundance were not statistically significant. The lack of replicates for the kick sample data likely impacted the statistical significance of these results, as prior research has associated freshwater mussels with an increased abundance of Ephemeroptera, Trichoptera, Oligochaeta (worms), Crustacea (crustaceans) and Anisoptera (dragonflies) (Howard and Cuffey, 2006; Vaughn and Spooner, 2006; Burlakova, Karatayev and Karatayev, 2012).

The degree to which freshwater mussels impact water quality and river velocity in rivers was unclear from this investigation. No significant differences in water oxygen saturation levels or the amount of total dissolved solids in the water were found at any of the four river sites. One river site (MBB) showed significantly reduced turbidity levels in the two quadrats where mussels had been introduced. Although turbidity levels were consistently found to be lower in the presence of mussels, any observed differences in turbidity at the other three sites were not found to be significant. Significantly increased water velocities were found at RSA in the quadrats where mussels had been removed, which may be attributed to reduced bed roughness and flow resistance when mussels were absent from the river bed. However, no significant differences were found for

velocity at the other sites, which means it is not possible to draw conclusions with regard to how mussels impact river velocities.

The ambiguity of results for the water quality parameters may be due to the limited impact that a relatively small quadrat of mussels can have on water that is continually being replenished by the flow of the river. It is likely that filtration by the mussels and their physical presence on the river bed is influencing hydrodynamics and turbidity levels, but 25-54 mussels in each 1 m^2 quadrat was insufficient to significantly affect water that was present in each quadrat for only a matter of seconds. Increasing the scale of this investigation in terms of the area of mussel bed involved would likely demonstrate the impact of mussels with a greater level of confidence. Experiments with *Anodonta anatina* have shown that individual mussels are capable of filtering up to half a litre of water per hour (McIvor, 2004). Therefore, where the impact of each 1 m^2 of mussels may have been minimal, the cumulative impact of a large mussel bed on water quality parameters may be considerable.

Key Question 2 *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?"* could not be conclusively resolved during this investigation. One quadrat at RSB, where mussels had been introduced, showed a significantly increased percentage of organic matter in the sediment by the end of the study, and one quadrat at MBB, where mussels were introduced, showed a significantly reduced percentage of carbonate in the sediment. No significant changes were found in any of the other quadrats with regards to organic and inorganic carbon. It was anticipated that the presence of mussels would lead to significant increases in organic matter due to their filter-feeding and subsequent biodeposition. As this was only observed in one quadrat it may be that the biodeposits are being either re-suspended and transported downstream, or consumed and removed by other organisms. It is also possible that bioturbation by the mussels is vertically dispersing the organic matter within the river bed sediment but the rate

of accumulation was too low to produce significantly increased levels of organics within the time-frame of this experiment.

The reduced carbonate content of the sediment at MBB where mussels had been introduced may indicate that the mussels were incorporating the carbonate into their shells or tissues, however the lack of significant results in the other quadrats makes it difficult to draw conclusions with a sufficient level of confidence. It is widely regarded that freshwater mussels increase the carbonate content of the sediment through the accumulation of shell material (Vaughn and Hakenkamp, 2001; Gutierrez *et al.*, 2003; Beekey, Mccabe and Marsden, 2004; Spooner and Vaughn, 2006; Vaughn and Spooner, 2006; Zaiko, Daunys and Olenin, 2009; Burlakova, Karatayev and Karatayev, 2012). The absence of any increased sediment carbonate in this study is likely due to the limited timescale involved. Accumulation of shell material within the river bed would be primarily due to mussels that have died, and no dead mussels were found in any of the quadrats during the study. If a mussel had died it would require time for physical, chemical and biological processes to break down that shell material and incorporate it into the sediment.

Some carbonate may be introduced to the river bed as fragments break off living mussels due to abrasion from bed sediment, either as the mussels burrow into the sediment or by impacts from bedload in motion. Additionally, if the mussel is set in motion by high velocity flow during flood events there may also be some detachment of shell material due to attrition with the river bed. The limited temporal and spatial scale of this investigation provided insufficient opportunity for these processes to take place.

One of the limitations of this investigation was the small sample sizes involved, which affected the significancy of some of the results. The difficulty in finding river sites with dense mussel beds that could be used in a translocation study restricted the number of mussels that could be placed in each quadrat. As a result, the quadrat size was limited to 1 m^2 to maintain mussel densities at sufficient levels to influence the river bed conditions within each quadrat. However, the small

quadrat area used in the study limited the size and number of sediment samples that could be collected from each quadrat, as repeated sampling would likely have obliterated any effects of mussel activity may have been evident in subsequent sampling of the river bed sediment. A more effective strategy may have been to sample only at the beginning and the end of the study, which would have enabled more samples to be collected on each occasion that could then be statistically compared. That approach was not taken during this study because it was not known how long the translocated mussels would be able to survive in their new habitat. Due to the risk of mussel mortality in potentially sub-optimal conditions, and the risk of being washed away in high discharge events, it was considered a safer approach to carry out regular sampling, and examine the change in variables over time.

Another limitation of this study is the limited time-frame involved, as the loss of mussels from MBB and RSB after periods of high discharge resulted in the investigation being terminated early. The 8-12 week duration of this study may have compromised the degree to which mussels could influence sedimentological and hydrological conditions at the river sites. A potential solution to this problem would be to run the investigation by only examining the effects of removing mussels from an area of the river bed, rather than attempting to translocate mussels to a river site with sub-optimal conditions. Alternatively the experiment could be attempted at a river site with historical mussel populations that have been lost, providing that the original cause of the mussels disappearance from the river has been addressed. In such cases it may be advisable to run a pilot study translocating a small number of mussels to assess their rates of survival over several months before attempting to translocate larger numbers of mussels. If the survival chances of translocated mussels were less uncertain, it may have justified moving larger numbers of mussels to cover a whole river reach, which would have provided much a more informative insight into their impact on hydrological and sedimentological conditions in rivers.

Due to the large number of variables measured at each visit to the river, and the time-consuming nature of some of the data collection techniques such as the kick sampling, measurements of sediment transport were not carried out during this investigation. The interdisciplinary nature of this thesis meant there was a need to balance both biological and sedimentological aspects of the research, and it was considered important that all data were collected on the same day so that the various variables and quadrats could be effectively compared, thus limiting the amount of data that could be collected during each site visit. In retrospect, the lack of kick sample replicates, and the downstream position of the kick samples relative to the quadrats meant that the biotic data was not as useful as anticipated. Quantification of sediment transport rates with the use of tracer particles or sediment traps, together with measurement of river discharge at each site, may have been more informative with regard to the aims of this thesis, and this presents an opportunity for further study.

In summary, the most conclusive results for this investigation were for the hyporheic oxygen saturation levels, with higher levels observed in quadrats with mussels at all four of the river sites. The greater hyporheic oxygen levels in the mussel quadrats may be attributable to the effects of mussel bioturbation, but may also be influenced by changes in microbial or macroinvertebrate communities in those quadrats due to mussel activity.

Significantly greater BMWP scores and substrate heterogeneity were found where mussels were present in the River Sence, indicating an improvement in water quality and habitat conditions in the presence of mussels. The impact on BMWP scores at Markeaton Brook was found to be not significant, and substrate heterogeneity was also unaffected at this site. This may be due to the lower densities of mussels in Markeaton Brook, or may be due to pre-existing differences between the two rivers, such as variations in sediment supply, discharge, river bed conditions or impacts of other biota.

Results for water velocity, water turbidity, substrate organic and inorganic carbon, bed sediment B-axis length, and the percentage of fines in the substrate were inconclusive, with significantly different results found in some quadrats with mussels but not others. The lack of significant results in some quadrats may have been due to the relatively small sample sizes and number of mussels used in each quadrat, as well as the limited size of each quadrat area. The complex and dynamic nature of the river environments used in this study may have also influenced the significancy of some of the results, due to the difficulty in controlling all the interacting variables within each quadrat. This is addressed in Chapter 4, which describes the design of an ex-situ study that provided a more controlled environment, away from the influence of extrinsic factors that may have impacted the results of this river investigation.

Chapter 4: The Impact of Freshwater Mussels on the Water and Sediment Conditions of a Mesocosm Fluvial Environment

4.1 Introduction

The potential for mussels to influence river bed topography, river bed grain-size distributions, and sediment flux in rivers has implications for hydrological modelling, carbon cycling, water quality, and the suitability of the habitat for other freshwater species. Understanding the extent to which freshwater mussels affect habitat conditions and sediment mobility in rivers is therefore of importance when assessing their potential role in habitat remediation and river restoration projects. However, studies on the impact of freshwater mussels on river bed sediment dynamics are lacking.

Studies on marine bivalves such as scallops (Pectinidae (Wilkes 1810)) and mussels (Mytilidae (Linnaeus 1758)) have demonstrated that bivalves can influence the movement of sediment through biodeposition and by causing obstruction to the flow, which increases bed roughness (Eckman and Nowell, 1984; Grant, Emerson and Shumway, 1993; van Leeuwen *et al.*, 2010). An experiment with the unionid species *Actinonaias ligamentina* (Lamarck 1819), *Amblema plicata* (Say 1817) and *Rotundaria pustulosa* (I. Lea 1831) showed that increased species richness was associated with increased erosion of gravel in an artificial stream environment at both high and low mussel densities (Allen and Vaughn, 2011). The orientation of the mussels relative to the flow was found to significantly influence the amount of gravel eroded, with less erosion taking place when mussels were in their typical life-position, aligned parallel to the flow with their posteriors pointing downstream (Allen and Vaughn, 2011).

The river investigation described in Chapter 3 suggested that freshwater mussels may positively influence hyporheic oxygen levels, BMWP scores and heterogeneity of the river bed environment. However, non-significant results for other water quality and sedimentary variables measured in the study meant that a number of the key questions described in Section 1.4 remained unresolved.

The relatively small quadrat size and number of mussels used in the river-based study likely resulted in any impact on water quality variables such as turbidity, TDS and water oxygen levels being undetectable in a flowing river environment. The flume-based study described in this chapter aimed to address this by recirculating water around a flume containing freshwater mussels, in order to simulate the effect of water in a river flowing over a much more extensive mussel bed. The use of a flume mesocosm also enabled more effective control of extrinsic variables such as other resident biota, changes in sediment supply and river flow conditions that may have affected the results of the river study. Furthermore, the flume study enabled investigation of the mussel's impact on sediment transport and river bed topography, which were not quantified during the river study.

The flume-based study described in this chapter aimed to address Research Objective 2 described in Section 1.4:

> *"Determine if freshwater mussels significantly impact sediment dynamics, river bed topography, and hydrological conditions in a laboratory-based mesocosm fluvial environment, and if so, what these impacts are."*

The flume study aimed to address the following Key Questions described in Section 1.4:

1) *"To what extent do freshwater mussels impact river bed grain-size distribution patterns?"*
2) *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?"*
3) *"To what extent do freshwater mussels impact sediment transport in rivers?"*
4) *"To what extent do freshwater mussels influence river bed topography?"*
5) *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"*
6) *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"*

4.2 Data collection and processing

4.2.1 Experimental design

The design and construction of the recirculating flume used in this investigation is described in Sections 2.4.1 - 2.4.3. The investigation took place concurrently with the river-based study between the 20th May and the 27th September 2017. The flume investigation involved a control study with mussels absent, followed by an identical study with mussels present, each with a duration of 8 weeks. Sediment and water was introduced to the flume a week before the beginning of both studies to allow time for the sediment to stabilise in response to the flume's flow regime. At the beginning of each study the first set of water and sediment samples were taken. The collection of water and sediment samples is described in Sections 4.2.2 – 4.2.4.

River seston from a fixed location in Markeaton Brook was collected using a plankton net, strained of water until it ceased dripping, and 25 ml of the wet seston was added to the flume at weekly intervals, immediately after each set of water and sediment samples had been taken. The seston served to provide food for the mussels that most closely approximated their natural diet, and also introduced fine, suspended sediment to the flume which was not present in the initial sand-gravel substrate. Although drying and weighing the seston would have more accurately quantified the amount being added each week, it would have also have likely killed any living components of the seston. As unionids have been shown to be selective feeders (Miura and Yamashiro, 1990; Nichols and Garling, 2000; Atkinson *et al*., 2011) an absence of live seston could potentially have affected their feeding behaviour.

Fifty *Anodonta anatina* (Linnaeus 1758) from Markeaton Brook were introduced to the flume at the beginning of study, after the first set of samples had been taken. The size of the mussels ranged from 66-91 mm in length, with a mean shell length of 76 mm (Appendix 5). The mussels were placed in the flume equidistant-apart in two rows along the channel, and were positioned with their anterior-ventral edge pressed partially into the sediment and their posteriors pointing

down-current (Figure 4.1), which was the life-position most commonly observed in Markeaton Brook when the mussels where collected.

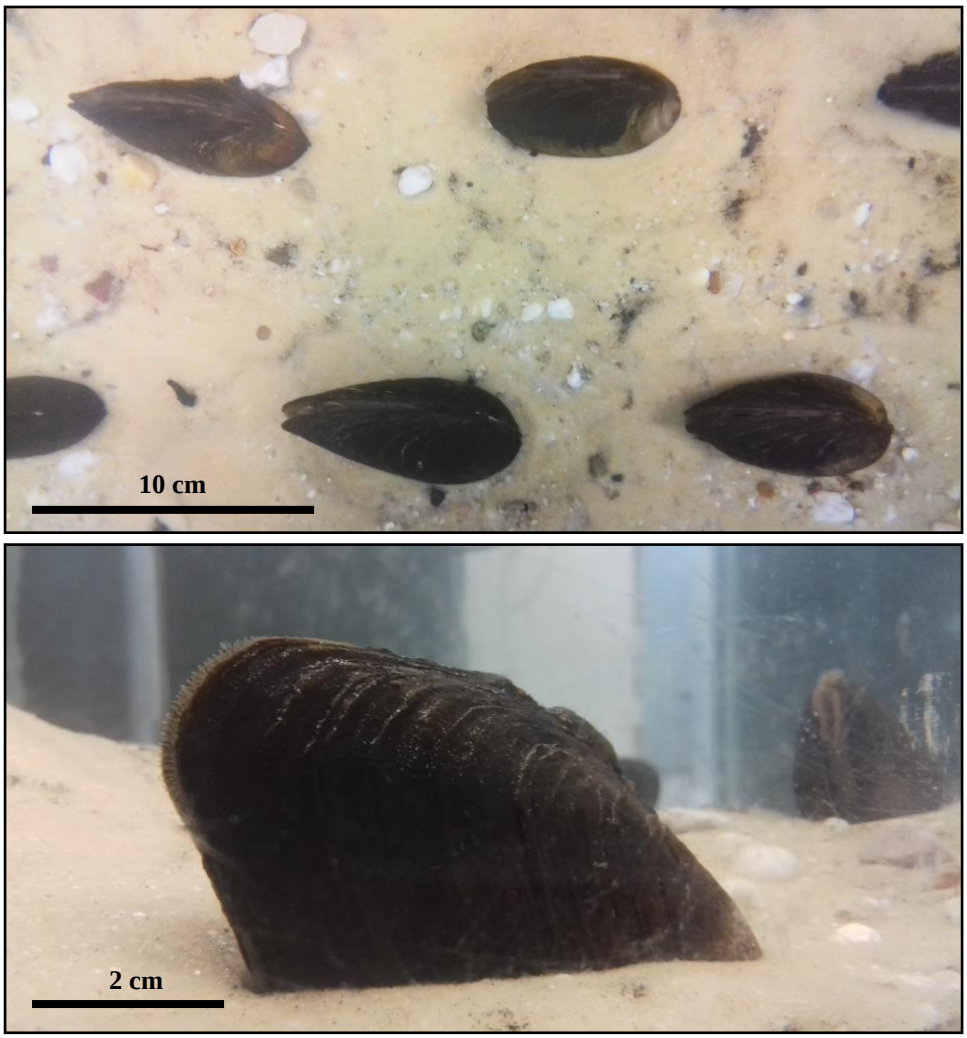


Figure 4.1: *Anodonta anatina* in the flume at the beginning of the study, positioned in two rows with their posteriors pointing down-current and their anterior-ventral edge pressed partially in the sediment. © Andrea Leng.

4.2.2 Measurements of water quality

Water samples (n=10) were taken at the beginning of the study and then every two weeks for the duration of the study from randomly-generated locations within the flume's channel. Water turbidity, total dissolved solids (TDS), and water oxygen saturation were measured using the methods and equipment described in Section 2.3.2. The sample sizes were determined by the preliminary studies described in Sections 2.4.4 – 2.4.6.

Other aspects of water quality typical of standard aquarium maintenance were monitored throughout the duration of the study using weekly spot-checks to ensure conditions in the flume were appropriate for the mussels. This involved the use of an API® Freshwater Master Test Kit, a Hagen Nutrafin® Master Test Kit and eSHa® Aqua Quick Test Strips to monitor water pH, ammonia, nitrite, nitrate, chloride, calcium and carbonate hardness. Water temperature was also monitored throughout the study.

4.2.3 Substrate conditions

Sediment samples (n=5) were taken from randomly-generated locations within the channel at the beginning of the study and then every two weeks thereafter. Hyporheic oxygen saturation was measured and sediment cores were extracted using the methods and equipment described in Section 2.3.2. At the end of the study an additional two sediment samples were taken from both the control experiment and the experiment with mussels in order to increase the size of the dataset for comparison between the two. The analytical methods use in the characterisation of sediment grain-size distributions and organic and inorganic carbon content of the sediment follow the methodology described in Sections 2.5.2 and 2.5.3.

4.2.4 Velocity, topography and sediment flux

Lateral and vertical sampling of water velocity was carried out at the beginning and the end of the study using the method described in Section 2.5.5. To investigate how mussels altered the topographic profile, bed roughness and wetted perimeter in the flume, topographic profiles were taken of the substrate surface at the beginning and the end of the study using the method described in Section 2.5.4. Differences in sediment flux and critical boundary shear stresses with mussels present and absent from the flume were analysed using the methods described in Section 2.5.6.

4.2.5 Statistical analysis

Data from the flume investigation was statistically analysed using the method described in Section 2.5.7, by comparing how each measured variable changed over the 8-week duration of the study with mussels in the flume, relative to the control study. Non-parametric data was statistically analysed with a Mann-Whitney U test to compare data from the flume with mussels present to the control study, with mussels absent. The specific datasets that were analysed using a Mann-Whitney U test have been noted as such in Section 4.4.

4.3 Results

4.3.1 Impact on water quality and suspended load

Turbidity

When mussels were absent from the flume the mean water turbidity steadily increased throughout the 8 weeks of the study from 0.5 Formazin Nephelometric Units (FNU) to 7.28 FNU in response to the seston that was added at weekly intervals. However when mussels were present, there was an initial increase in mean turbidity from 0.49 to 1.42 FNU in the first two weeks, after which it stabilised at levels below 0.77 FNU (Figure 4.2).

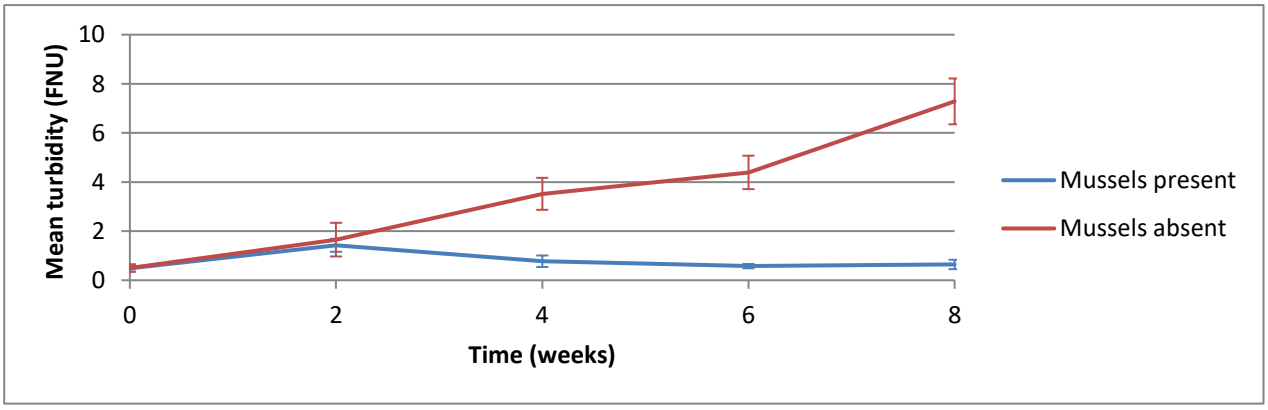


Figure 4.2: Graph to show the change in mean (± 1 SD) water turbidity in Formazin Nephelometric Units (FNU) over the duration of the study with a weekly addition of seston to the flume.

The mean beta coefficient of the change in turbidity over time when mussels were present (M=-0.05, SD=0.05) was significantly lower than when mussels were absent (M=1.63, SD=0.18); t (10.50)=28.56, $p < 0.001$. Mean turbidity was therefore reduced by the presence of mussels.

Total dissolved solids

In the control study when mussels were absent, mean total dissolved solids (TDS) dropped from 359 ppm at the start of the study to 329 ppm at the end. When mussels were present it dropped from 359 ppm to 247 ppm by the end of the study (Figure. 4.3).

The mean beta coefficient of the change in TDS over time when mussels were present (M=-20.68, SD=0.60) was significantly more negative than when mussels were absent (M=-7.22, SD=0.35); t (6.45)=43.49, $p < 0.001$. Therefore, TDS was significantly lower when mussels were in the flume compared with when they were absent.

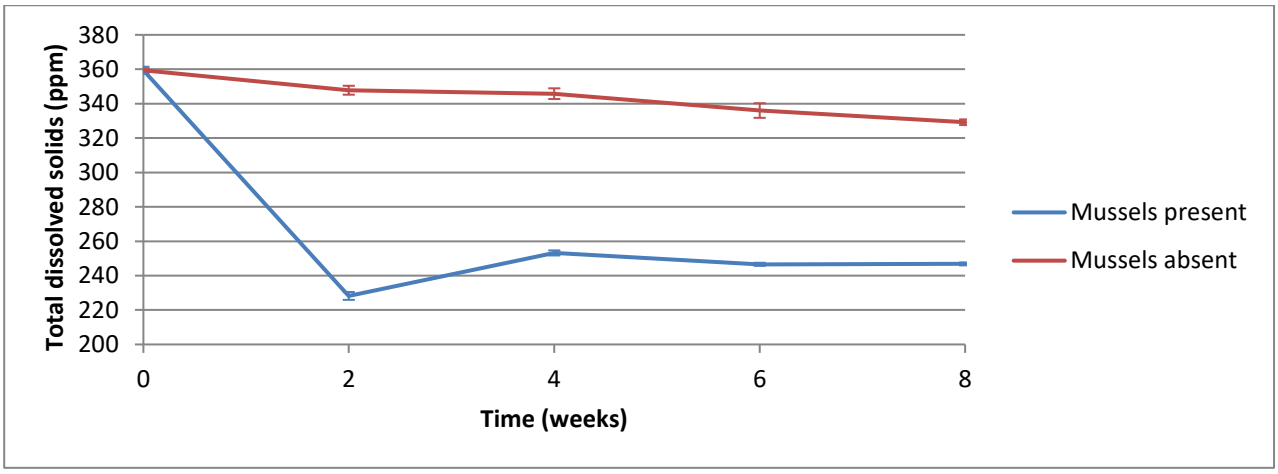


Figure 4.3: Graph to show the change in mean (± 1 SD) total dissolved solids (TDS) over the duration of the study.

Water oxygen saturation

When mussels were absent, the mean percentage water oxygen saturation rose from 99.0 % at the beginning of the study to 100.4 % by the end. With mussels in the flume the mean oxygen

saturation dropped from 99.1 % to 94.1 % by week 2. It then rose to 97.2 % by week 6 before dropping slightly to 96.2 % by the end of the study (Figure 4.4). The mean beta coefficient of the change in water oxygen saturation over time when mussels were present (M=-0.26, SD=0.05) was significantly lower than when mussels were absent (M=0.38, SD=0.05); t (17.64)=28.74, p < 0.001. Therefore, water oxygen saturation was significantly reduced by the presence of mussels.

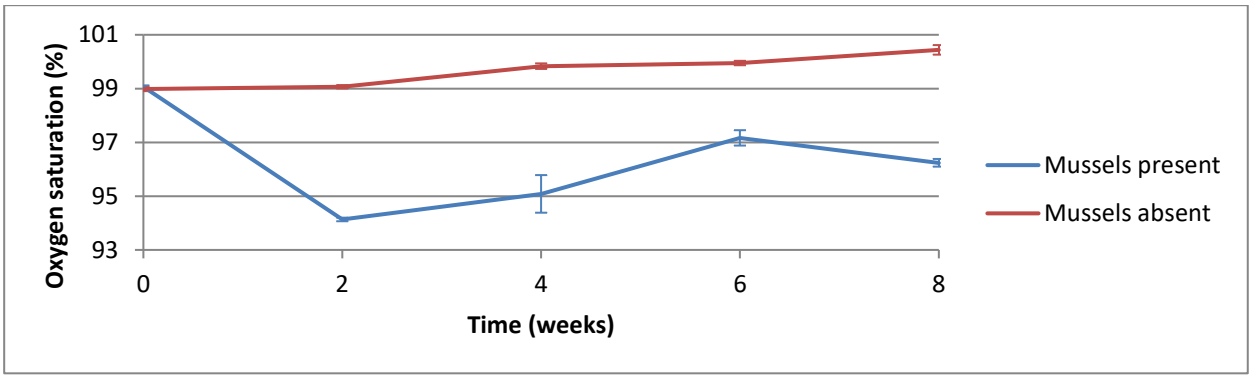


Figure 4.4: Graph to show the change in the mean (± 1 SD) percentage water oxygen saturation over the duration of the study.

4.3.2 Influence on substrate

Substrate oxygen saturation

The mean percentage oxygen saturation of the sediment remained relatively consistent during the control experiment, ranging from 98.6 % at the beginning of the study to 99.3 % at the end. When mussels were present the change in hyporheic oxygen saturation followed a similar pattern to the water oxygen saturation levels, with the percentage saturation initially dropping in the first two weeks from 98.7 % to 94.9 %, before rising steadily to 97.3 % in week 6 before dropping to 96.6% in the final week (Figure 4.5).

The mean beta coefficient of the change in hyporheic oxygen saturation over time when mussels were present (M=-0.19, SD=0.17) was significantly lower than when mussels were absent (M=0.20, SD=0.18); t (17.96)=5.08, p < 0.001. Therefore, hyporheic oxygen saturation was significantly reduced by the presence of mussels.

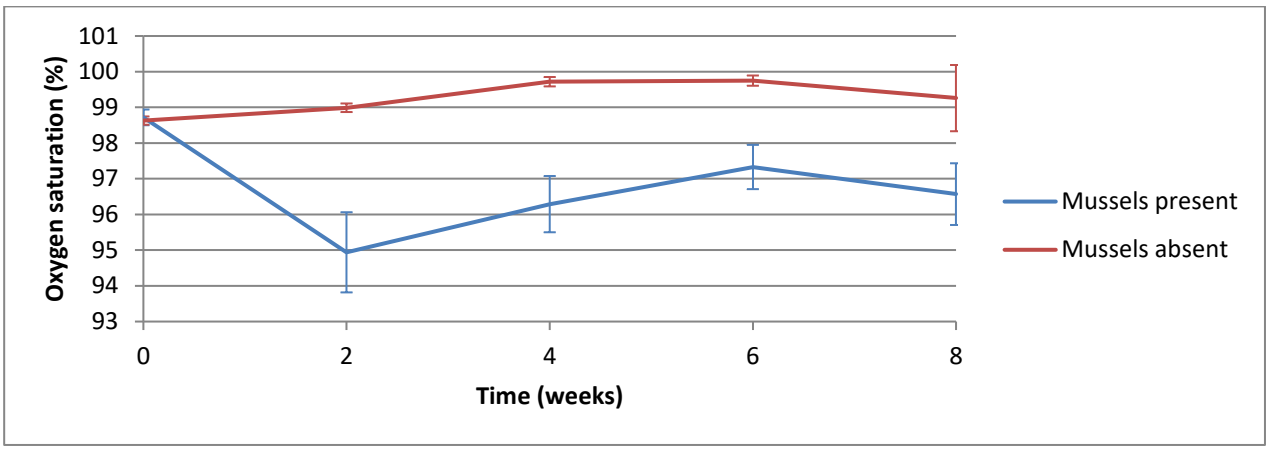


Figure 4.5: Graph to show the change in the mean (± 1 SD) percentage hyporheic oxygen saturation over the duration of the study.

Substrate grain-size

The mean grain-size of the substrate samples taken at the end of the control experiment was 45679 µm whereas at the end of the experiment with mussels the mean grain-size was 3482 µm. However, a Mann-Whitney U test comparing the median values of each of the samples from the control study with the mussel study showed no significant difference between the median grain-size of the substrate samples when mussels were present (*Mdn*=970.0, SD=869.9) compared with when they were absent (*Mdn*=2399, SD=685.3); w=39, $p > 0.05$. The range of the median grain-size values for the substrate samples taken at the end of the study are shown in Figure 4.6.

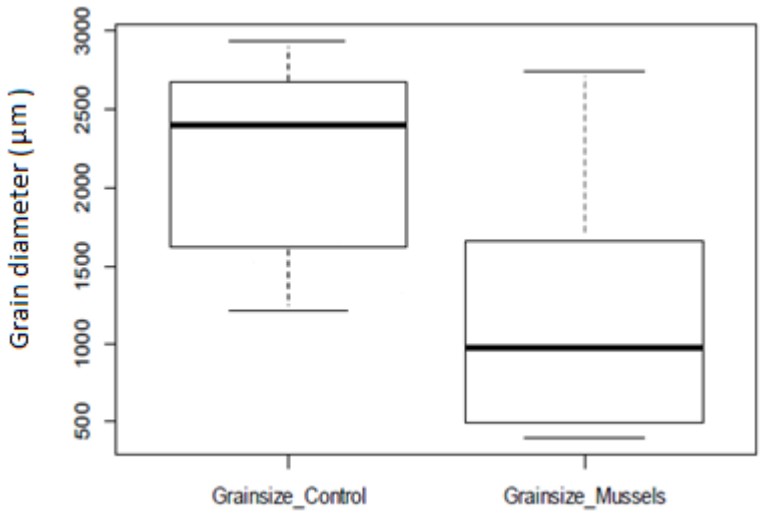


Figure 4.6: Box plot comparing the median grain-size of substrate samples taken at the end of the control study with samples taken at the end of the study with mussels present in the flume. No significant difference was found between the medians of the two samples.

The mean percentage of fines (<63 µm) in the substrate decreased in both the control experiment and with mussels present in the first two weeks of the study (Figure 4.7). From weeks 2 to 8 the percentage of fines remained relatively unchanged in the control study with mean values ranging from 0.6 – 0.7 %. With mussels present, the mean percentage of fines had dropped to 0.6 % by week 4 of the study, but rose to 0.9 % by week 6 and then dropped slightly to 0.8 % by week 8. The mean beta coefficient of the percentage of fines in the substrate when mussels were present (M=0.005, SD=0.04) was significantly greater than when mussels were absent (M=-1.230, SD=0.04); t(8.00)=-4.84, p = 0.001. Therefore, the mean percentage of fines being incorporated into the substrate was significantly greater in the presence of mussels.

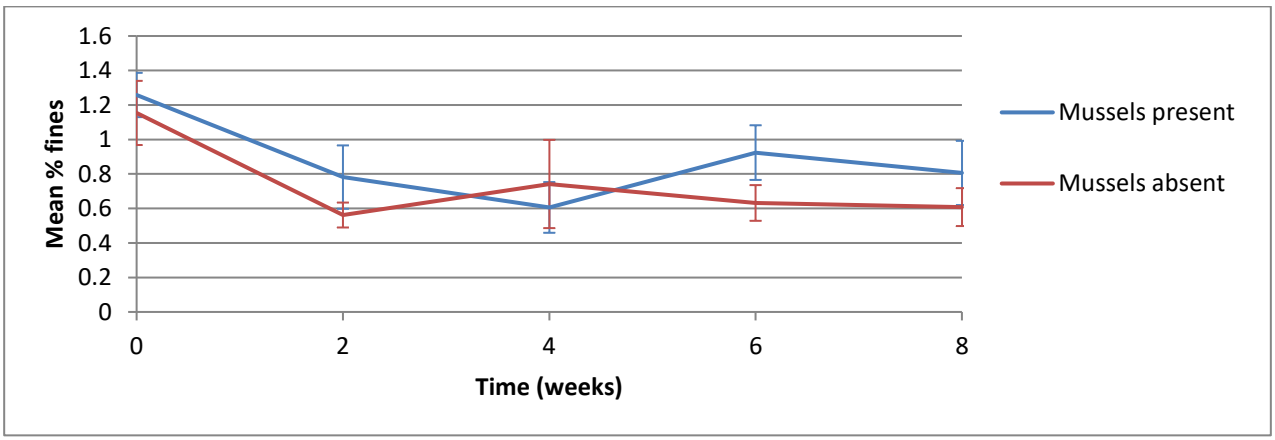


Figure 4.7: Graph to show the change in the mean (± 1 SD) percentage of fines (<63 µm) in the substrate over the duration of the study.

The grain-size distribution of the substrate samples with mussels present and absent from the flume are shown in Figures 4.8 to 4.10. The grain-size distribution graphs show the mean percentage volume of each grain-size fraction (differential volume) of the sediment core sample taken every two weeks throughout the investigation. The mean percentage volume of each fraction was calculated from aliquots taken from each of the homogenised sediment cores as described in Section 2.5.2. The legends (Fa.1 – Fe.7 and F1.1 – F5.7) on each of the graphs in Figures 4.8 to 4.10 show the unique identifier designated to individual sediment core samples taken from randomly generated locations within the flume.

Throughout the eight-week study the substrate grain-size consistently ranged from <0.04 µm to >8000 µm with mussels both present and absent from the flume, with the highest peaks in the fine-medium sand and the very fine-medium gravel Wentworth classes. The grain-size distribution of the control study displays increasing homogeneity of the sampled substrate throughout the eight weeks of the study. By the end of the study very little variation is seen in the grain-size distribution of the various samples taken from around the flume, with each sample showing a relatively consistent pattern (Figures 4.9 and 4.10). In comparison, with mussels present in the flume the grain-size distribution pattern maintains relatively high levels of

heterogeneity throughout the eight weeks of the study, with noticeable differences in the distribution patterns of the various sediment core samples.

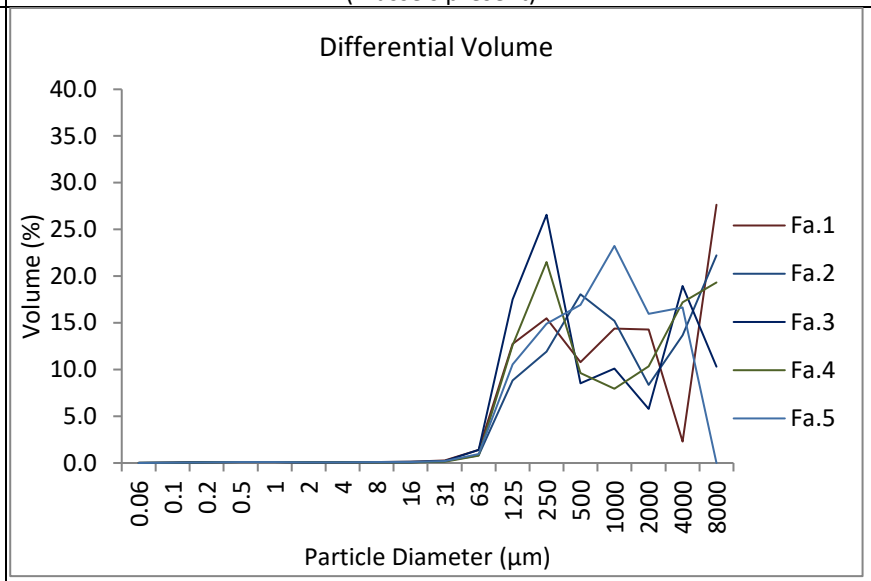
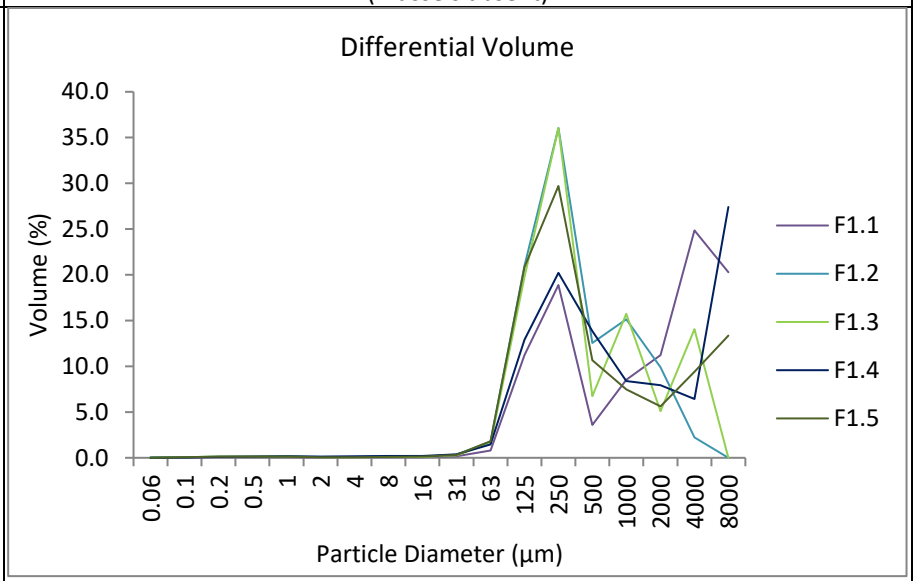
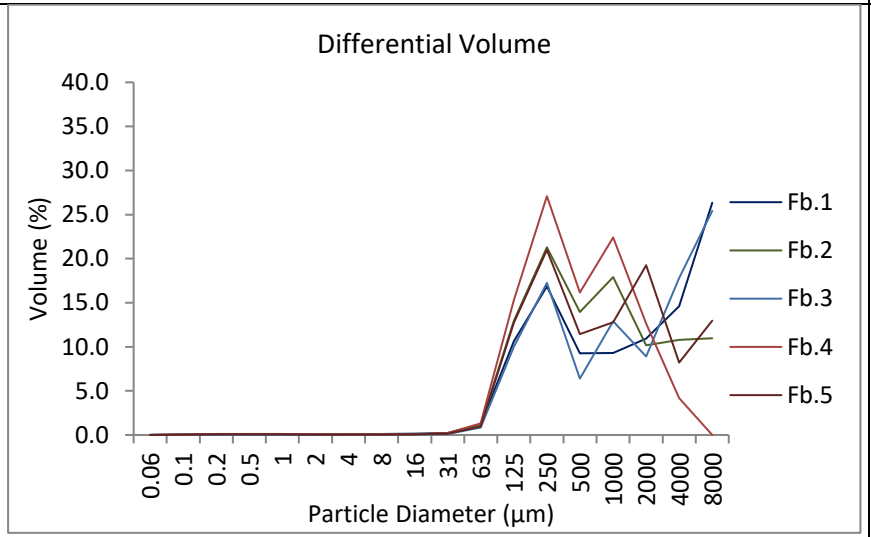
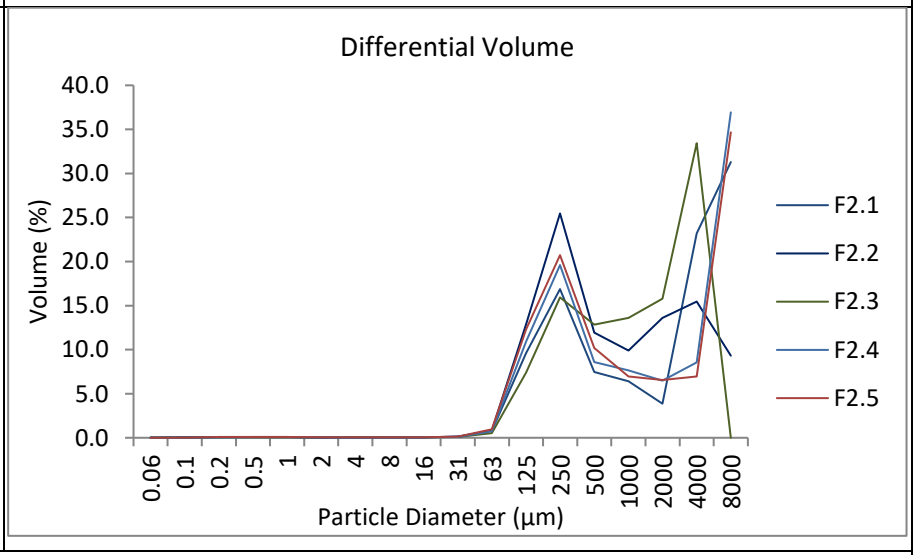
Time (weeks)	Substrate grain-size distribution (mussels present)	Substrate grain-size distribution (mussels absent)
0		
2		

Figure 4.8: Comparison of the grain-size distribution of substrate samples with mussels present and absent from the flume from the start and 2nd week of the study.

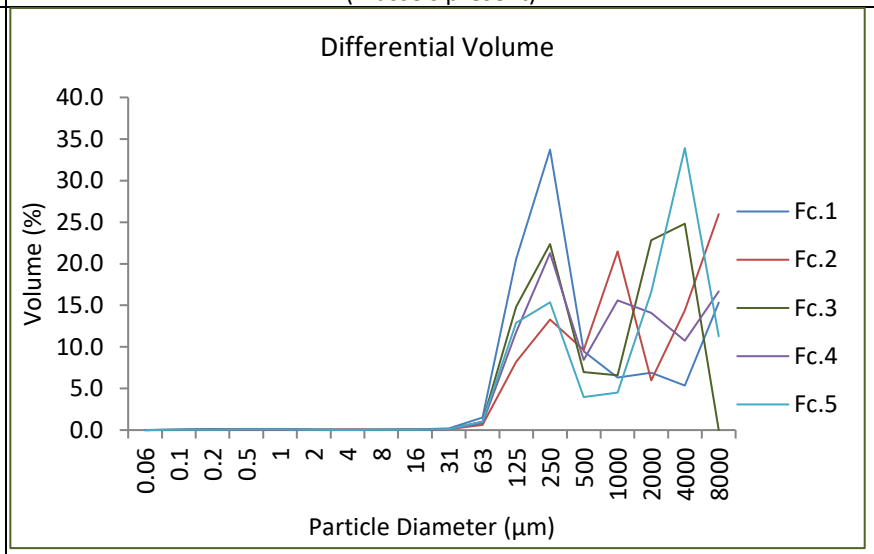
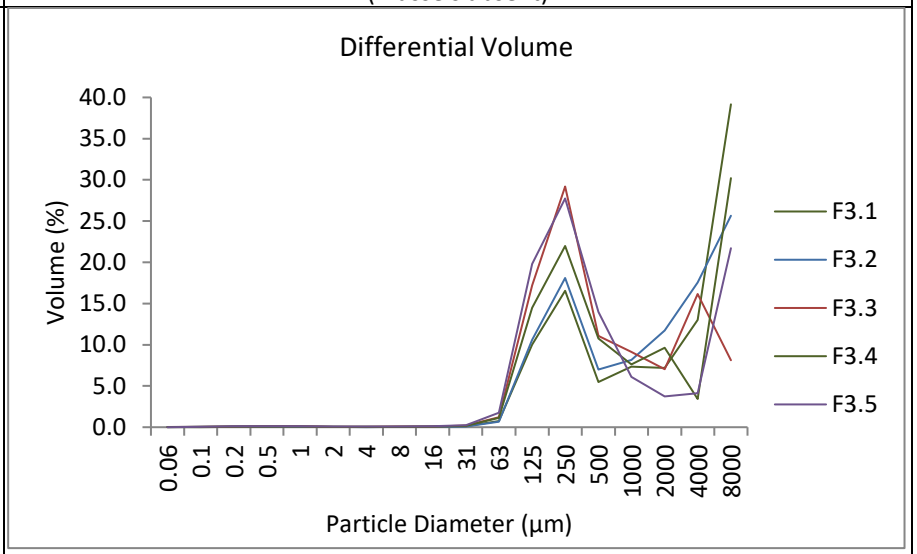
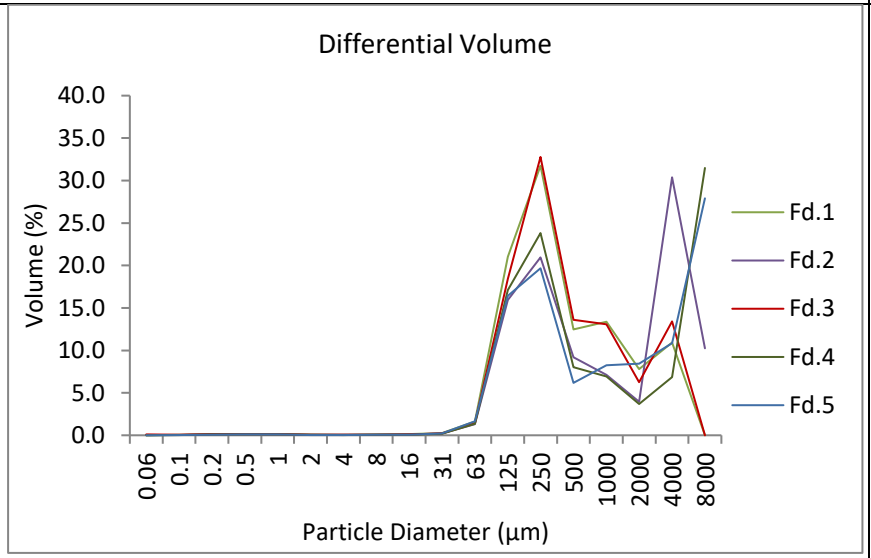
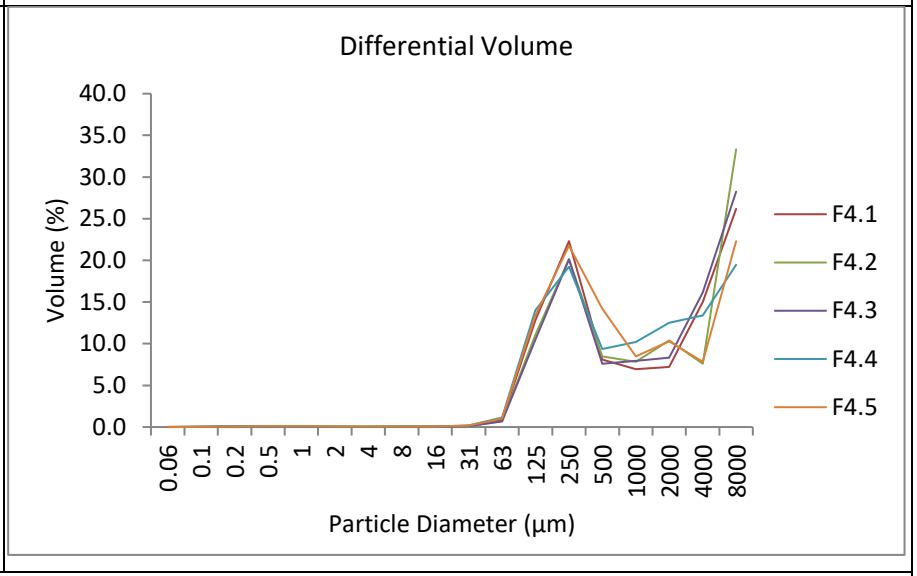
Time (weeks)	Substrate grain-size distribution (mussels present)	Substrate grain-size distribution (mussels absent)
4		
6		

Figure 4.9: Comparison of the grain-size distribution of substrate samples with mussels present and absent from the flume from the 4th and 6th week of the study.

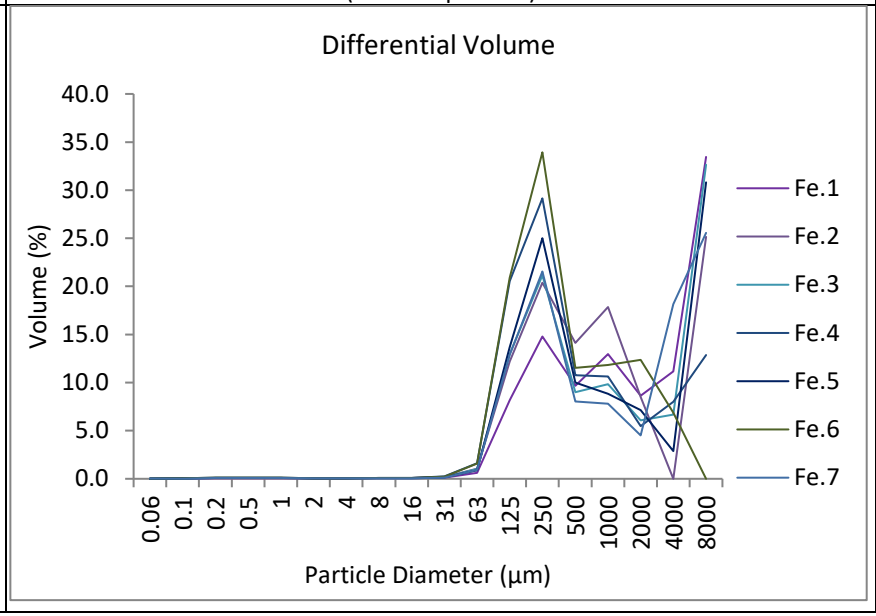
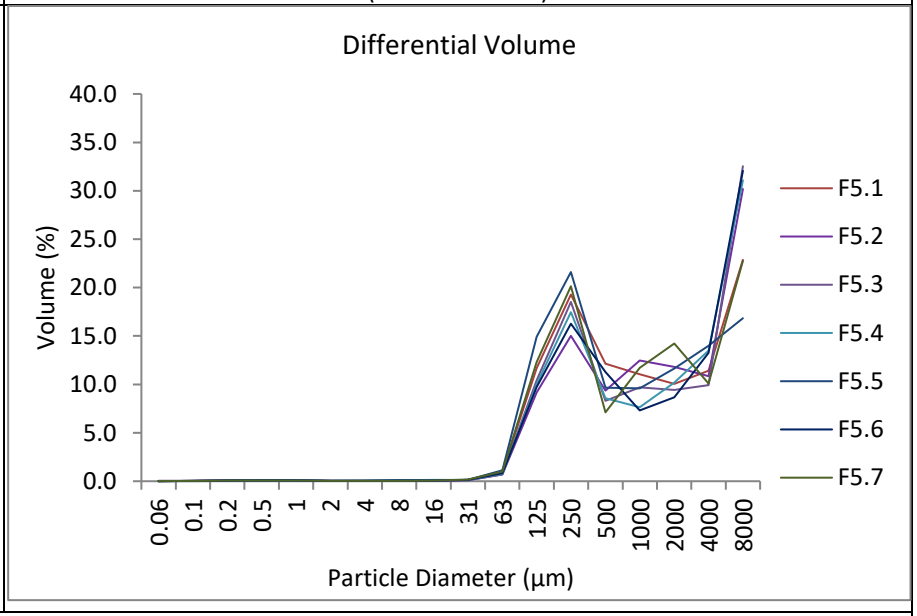
Time (weeks)	Substrate grain-size distribution (mussels present)	Substrate grain-size distribution (mussels absent)
8		

Figure 4.10. Comparison of the grain-size distribution of substrate samples with mussels present and absent from the flume from the 8th week of the study. When mussels were absent (right) the sediment samples display a relatively consistent grain-size distribution around the flume. When mussels were present the grain-size distributions indicate increased heterogeneity of the substrate.

Substrate organic and inorganic carbon content

The mean percentage of organic carbon in the substrate determined by loss on ignition (LOI) is shown in Figure 4.11. Substrate organic carbon increased from 0.14 % at the beginning of the control study to 0.30 % by the second week in response to the addition of seston to the flume. Thereafter, the percentage remained relatively consistent, ranging from 0.26 to 0.32 % for the duration of the study. With mussels present it rose from 0.23 to 0.25 % by the second week. From weeks 4 to 8 it ranged from 0.11 to 0.16 %. The mean beta coefficient of the LOI over time when mussels were absent (M=0.03, SD=0.06) was not significantly different to when mussels were present (M=-0.03, SD=0.01); t (4.45)= 2.18, $p > 0.05$. Therefore, the percentage of organic carbon in the substrate when mussels were present was not significantly different to the control study.

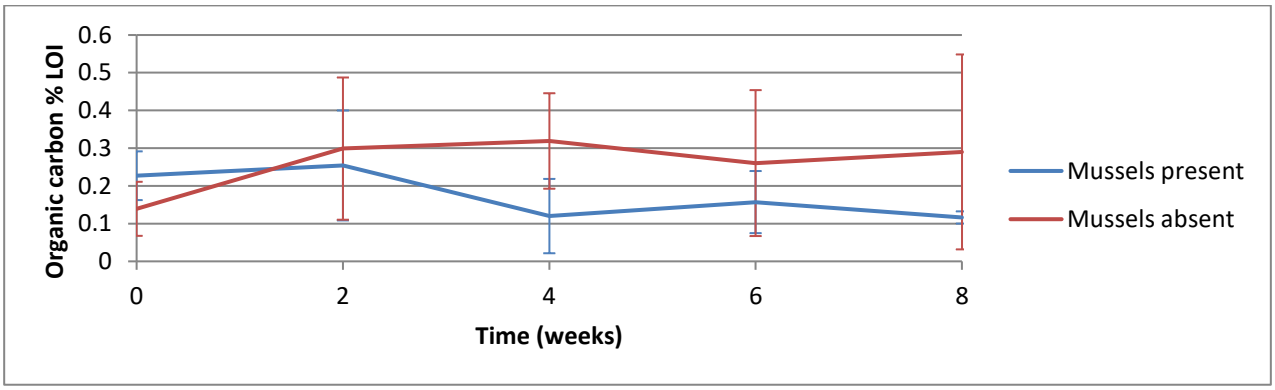


Figure 4.11: Graph to show the change in the mean (± 1 SD) percentage of organic carbon by loss on ignition (LOI) in the substrate over the duration of the study.

The change in the mean percentage of inorganic carbon in the substrate over the duration of the study is shown in Figure 4.12. In the control study the amount of inorganic carbon changed very little, ranging from 4.1 to 4.8 %. With mussels present there was more fluctuation in the inorganic carbon content, where it ranged from 3.2 to 6.0 % over the course of the study. The mean beta coefficient of percentage of inorganic carbon over time when mussels were absent (M=-0.15, SD=0.80) was not significantly different to when mussels were present (M=-0.48, SD=0.72); t

(7.92)= 0.70, p > 0.05. Therefore, the percentage of inorganic carbon in the substrate when mussels were present was not significantly different to the control study.

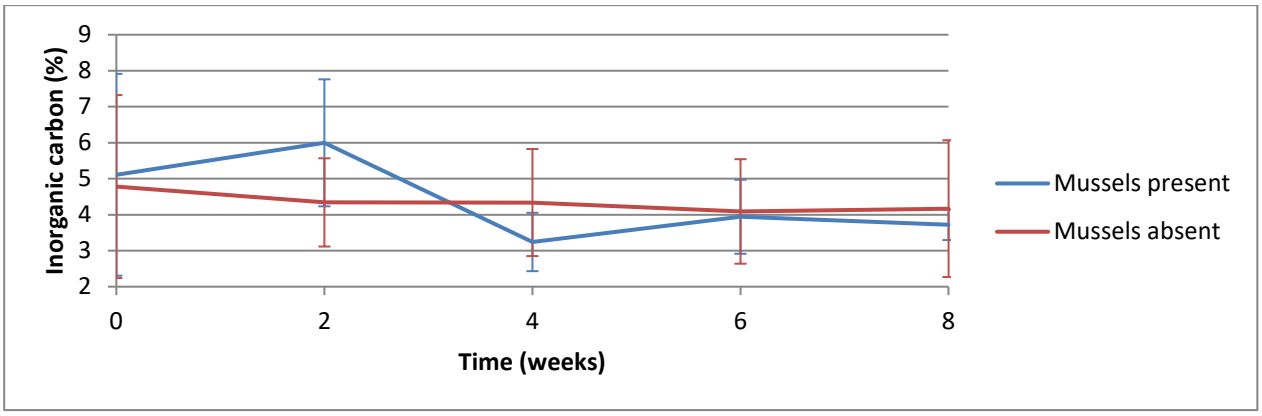


Figure 4.12: Graph to show the change in the mean (± 1 SD) percentage of inorganic carbon in the substrate over the duration of the study.

4.3.3 Velocity, bed topography and sediment dynamics

Water velocity

Velocity profiles based on the mean water velocities at four depths within the flume at the end of the eight-week study are shown in Figure. 4.13. A Welch two sample t-test was used to compare the mean water velocity at each depth with mussels present and absent from the flume. The mean near-bed (0.9 depth) velocity when mussels were present (M=0.05, SD=0.04) was significantly lower than when mussels were absent (M=0.10, SD=0.03); t (31.55)=4.31, p < 0.001. The mean velocity at 0.6 depth when mussels were present (M=0.07, SD=0.03) was significantly lower than when mussels were absent (M=0.12, SD=0.03); t (33.98)=4.50, p < 0.001. The mean velocity at 0.4 depth when mussels were present (M=0.08, SD=0.02) was significantly lower than when mussels were absent (M=0.12, SD=0.04); t (23.64)=3.70, p = 0.001. The mean near-surface velocity (0.1 depth) when mussels were present (M=0.08, SD=0.03) was not significantly different to when mussels were absent (M=0.08, SD=0.02); t (33.86)=0.33, p > 0.05. Therefore, the

presence of mussels led to significantly reduced near-bed (0.9 depth), 0.6 depth and 0.4 depth velocities, but near-surface (0.1 depth) velocities remained unaffected by mussels.

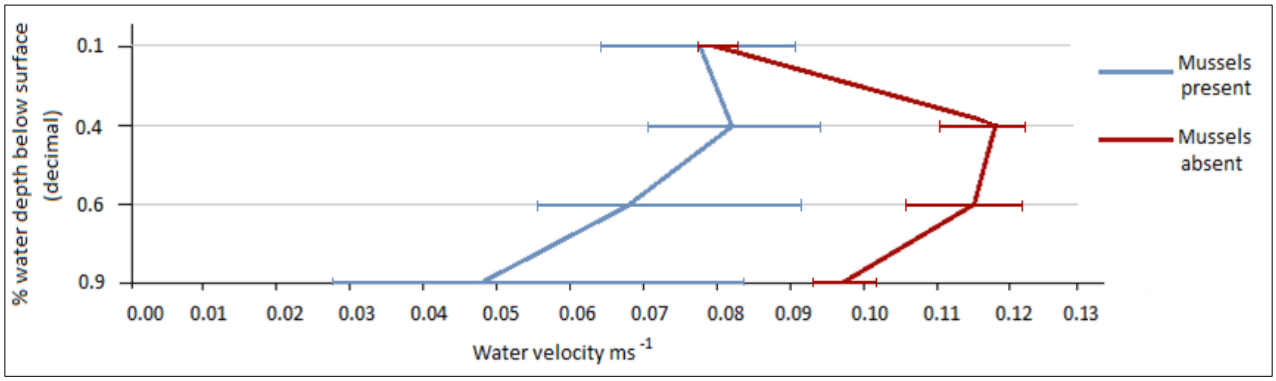


Figure 4.13: Graph to show the mean (± 1 SD) water velocities at four depths within the flume channel after 8 weeks with mussels present and with mussels absent from the flume. The channel had a mean total depth of 0.27 m.

Bed topography

The lateral and longitudinal topographic profiles of the flume substrate can be found in Appendix 6. In the control study, the majority of lateral profiles from the curved sections of the flume channel showed an increased vertical sediment depth on the inner edge compared with the outer edge of the channel. Lateral profiles from the straight sections of flume showed that the vertical thickness of sediment was greater mid-channel compared with the sides of the channel in the control study. With mussels present in the flume, differences in the vertical accretion of sediment on the inside and outside edge of the curved sections of channel was less apparent, indicating that mussels disrupted fluvial bedform development in the flume.

The individual profile lengths between each sampling point along the substrate surface at the end of the control and mussel study were compared using a Mann-Whitney U test. There was no significant difference between the lateral topographic profile lengths when mussels were present (*Mdn* = 0.207, SD=0.004) compared with when they were absent (*Mdn* =0.205, SD=0.016); w=16, $p > 0.05$, indicating that mussels did not significantly increase bed roughness or wetted perimeter

length across the width of the channel. The longitudinal wetted profile lengths when mussels were present (*Mdn*= 0.051, SD=0.0026) were significantly longer than when mussels were absent (*Mdn*=0.050, SD=0.0006); w=62, p = 0.006, indicating that mussels increased bed roughness and topographic profile length along the length of the channel. The differences in the topographic roughness and heterogeneity of the substrate with mussels present and absent from the flume can be observed in Figures 4.14 to 4.16.

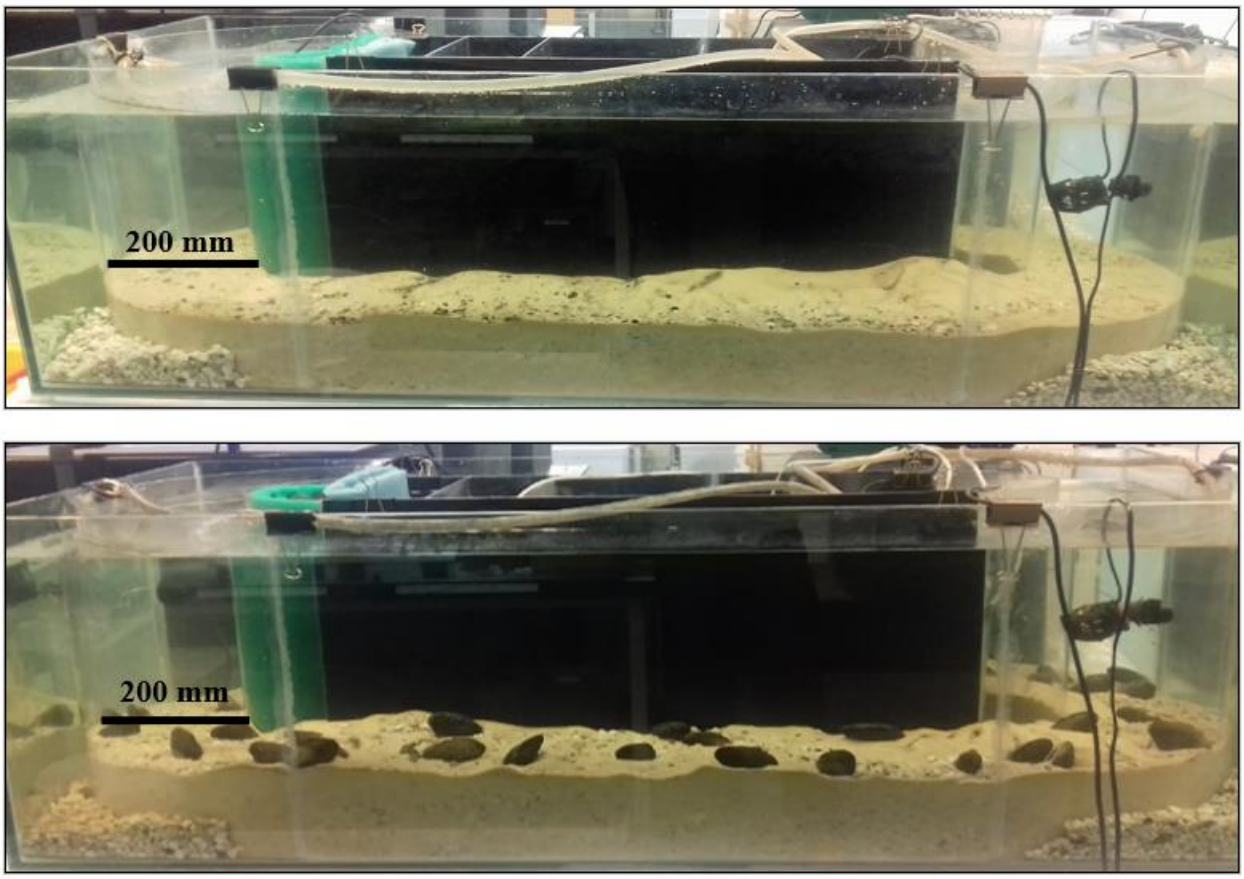


Figure 4.14: Flume substrate after 8 weeks with mussels absent (top) and with mussels present (bottom). The mean depth of sediment in the flume is 130 mm. Note the increased topographic roughness and heterogeneity of the substrate with mussels present. © Andrea Leng.

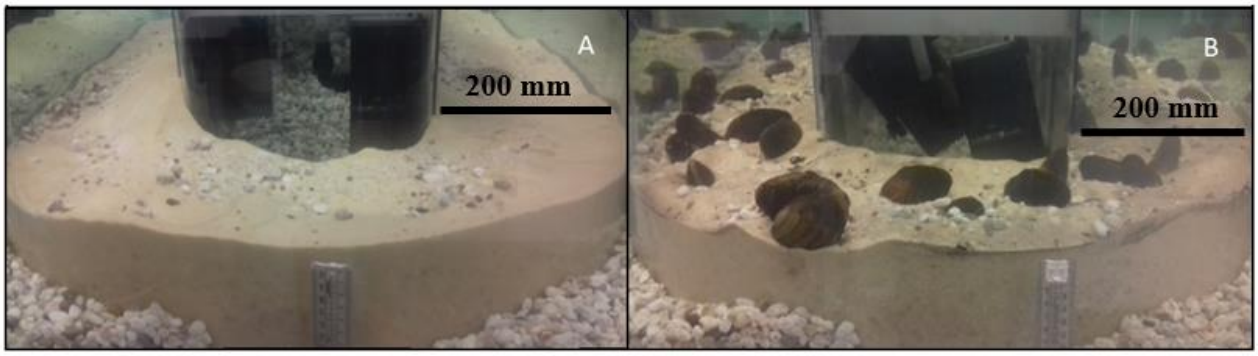


Figure 4.15. Flume substrate after 8 weeks with mussels absent from the flume (A) and with mussels present (B). Note the greater quantity of organic matter in photograph B from mussel biodeposits, and the increased topographic roughness and heterogeneity of the substrate resulting from mussel bioturbation. © Andrea Leng.

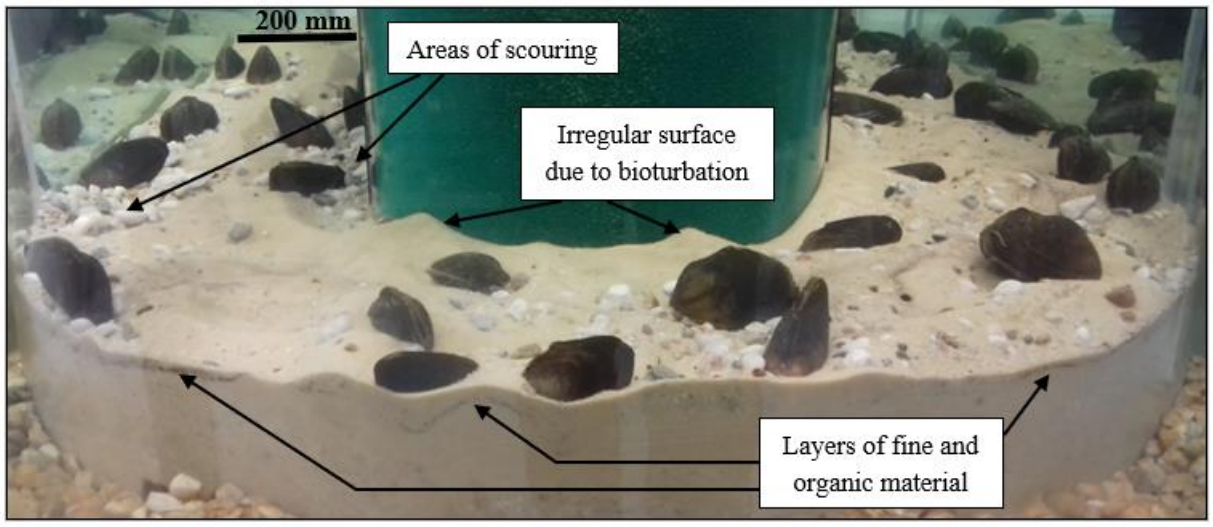


Figure 4.16. Evidence of mussel bioturbation: The lateral and vertical movement of the mussels has led to areas of scouring, where removal of finer grain-sizes has left patches of gravel. Layers of fine sediment and organic material can be observed below the substrate surface, disturbed by mussels extending their foot into the sediment. © Andrea Leng.

Sediment dynamics

Graphic logs of the vertical accretion of sediment in the sediment trap at the end of the 8 weeks are shown in Figure 4.17. The logs were recorded from the upstream, downstream and both lateral edges (left and right) of the glass beaker once it had been removed from the flume at the

end of the study. With mussels absent the initial sediment trap infill was dominated by fine to medium sand, becoming increasingly rich in organic matter and finer material up through the section. The later infill was mainly comprised of organic matter and finer material which had been introduced to the flume through the addition of seston. With mussels present, the sediment trap also contained primarily fine to medium sand with laminations of fine and organic material, however periodic pulses of coarser sand and gravel entering the trap were evident. The later infill was less concentrated in fine and organic material compared with the control study, with a greater quantity of sand being present in the upper part of the four logs.

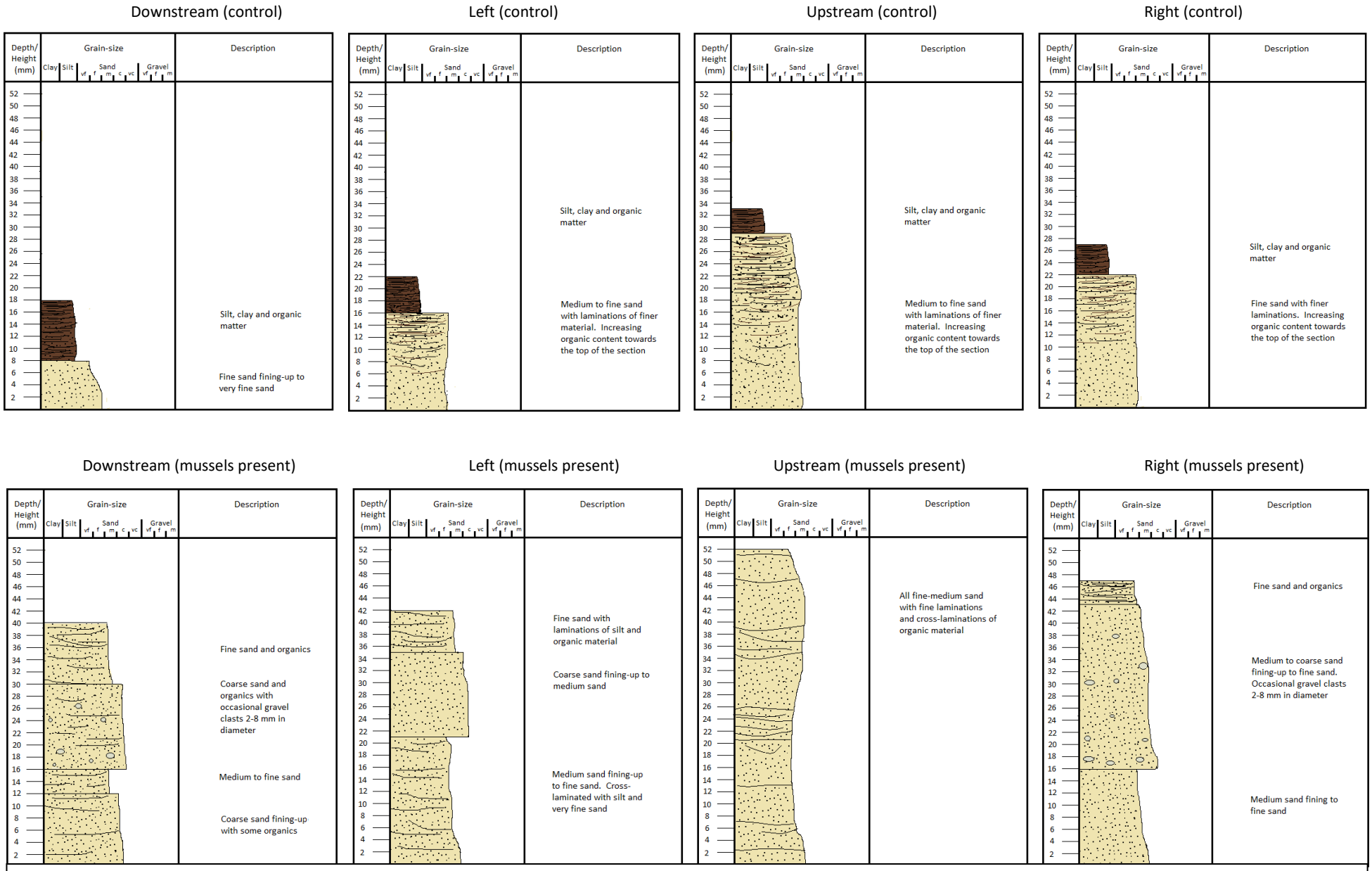


Figure 4.17: Graphic logs from the downstream, left, upstream and right edge of the sediment trap recorded at the end of the study with mussels absent (top) and present (bottom).

A Mann-Whitney U test was used to compare the percentage of organic matter by loss on ignition in the sediment trap when mussels were present and absent (Figure 4.18). When mussels were present the percentage of organic matter (*Mdn*= 0.23, SD=0.06) was significantly less than when mussels were absent (*Mdn*=2.48, SD=0.94); w=64, p < 0.001.

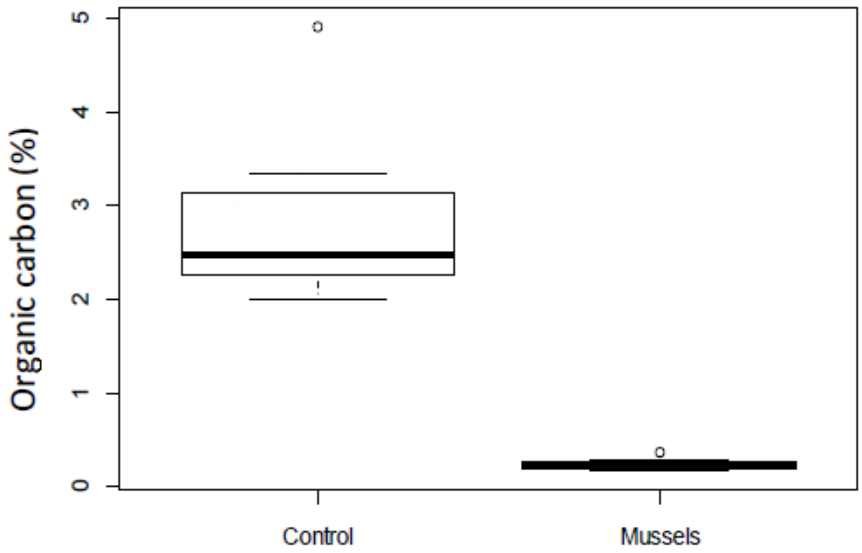


Figure 4.18: Box plot comparing the median percentage organic matter in samples taken from the control study sediment trap and the mussel study sediment trap. The percentage of organic matter in the trap was significantly less when mussels were present compared with when they were absent; *p < 0.001*.

The percentage of inorganic carbon in the sediment trap (Figure 4.19) when mussels were present (*Mdn*= 2.58, SD=0.55) was also significantly less than when mussels were absent (*Mdn*=4.86, SD=1.23); w=62, p < 0.001.

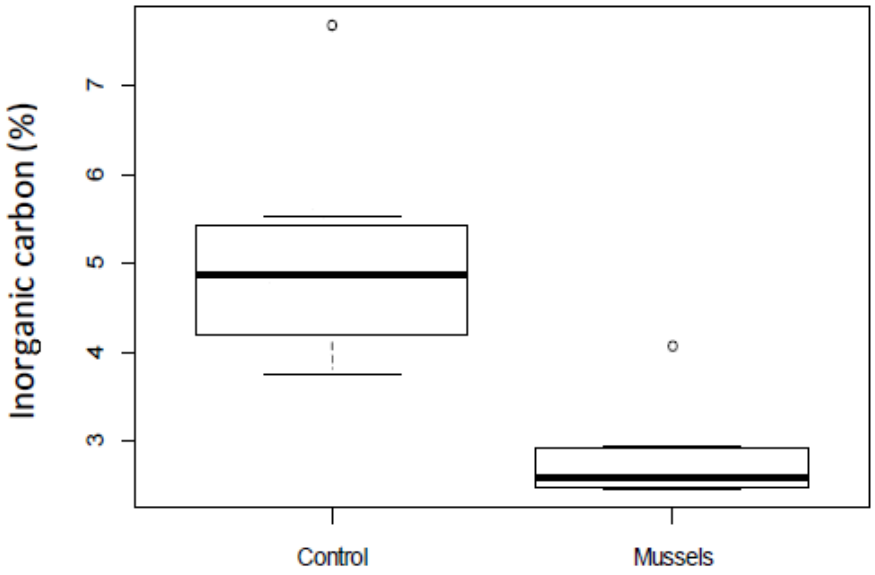


Figure 4.19: Box plot comparing the median percentage inorganic carbon in samples taken from the control study sediment trap and the mussel study sediment trap. The percentage of inorganic carbon in the trap was significantly less when mussels were present compared with when they were absent; $p < 0.001$.

When mussels were absent, a total dry mass of 127.4 g of material was collected in the sediment trap by the end of the study. When mussels were present 226.0 g of material had collected in the sediment trap.

Figure 4.20 shows the grain-size distribution of the contents of the sediment trap at the end of the study.

The mean grain-size of the sediment was 450 µm in the control study, compared with 1329 µm when mussels were present. Median grain-size was similar when mussels were both present and absent at 296 µm and 280 µm respectively. The modal grain-size was 225 µm in both studies. In the presence of mussels a lower percentage (1.2 %) of sediment trap material comprised grains with a diameter of <63 µm, compared with 2.2 % of the sediment when mussels were absent. In the control study 41.2 % of the sediment entering the trap had a grain-size of <250 µm, whereas when mussels were present only 36.2 % of the sediment was <250 µm. A higher percentage (10.1

%) of coarser sediment (>4000 µm) entered the sediment trap when mussels were present compared with 0.3 % when they were absent.

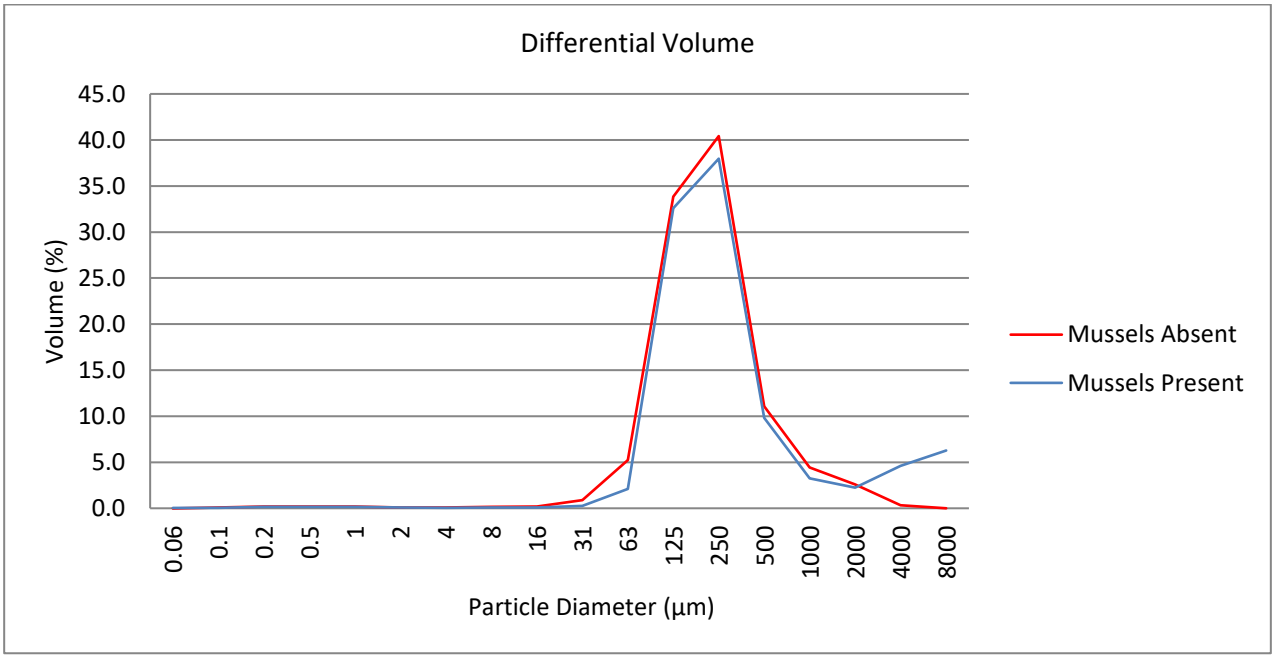


Figure 4.20: Graph to show the grain-size distribution of sediment collected in the sediment trap by the end of the study. An increased percentage of gravel was entrained in the flow in the presence of mussels but a reduced percentage of clays, silts and organics entered the sediment trap.

Changes in the critical boundary shear stress τ_c over the duration of the study calculated using grain-size data from the sediment trap are shown in Figure 4.21. When mussels were absent from the flume, grain-sizes comprising the initial 60 % volume of the sediment trap infill indicated that mean τ_c remained between 0.18 and 0.24 Nm^{-2}. The latter 40 % volume of the sediment trap infill showed a gradual reduction in mean τ_c from 0.20 to 0.03 Nm^{-2} in the latter phase of the study. When mussels were present in the flume, grain-sizes entering the sediment trap indicated increased and more variable τ_c over the duration of the study, compared with when they were absent, with mean τ_c ranging from 0.17 to 0.48 Nm^{-2}.

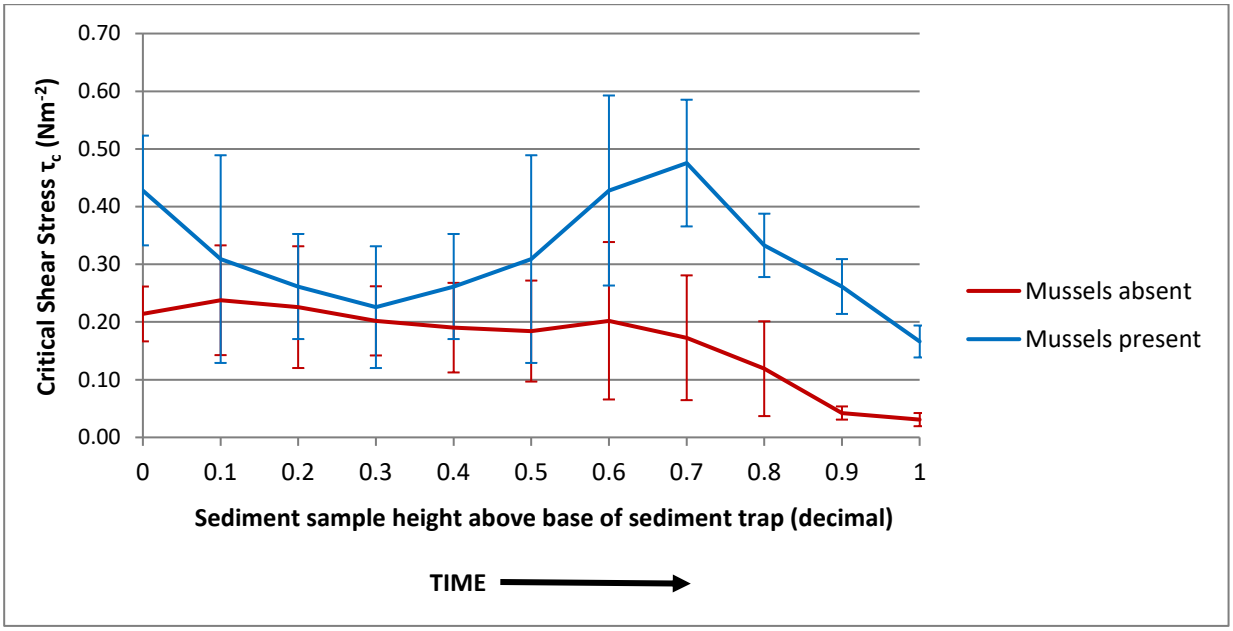


Figure 4.21: Graph to show the mean (± 1 SD) critical boundary shear stress (τ_c) over the duration of the study based on logs of sediment accumulated in the sediment trap. Sediment samples taken at height 0 were from the base of the sediment trap (the initial infill from the beginning of the 8 week study) and samples taken at height 1 were from the top of the sediment trap (latest infill towards the end of the study).

A Mann-Whitney U test was used to compare the theoretical critical boundary shear stress τ_c required to mobilise sediment collected in the sediment trap when mussels were present and absent from the flume (Figure 4.22). The critical shear stress τ_c when mussels were present (*Mdn*= 0.29, SD=0.14) was significantly greater than when mussels were absent (*Mdn*=0.19, SD=0.10); w=396.5, $p < 0.001$.

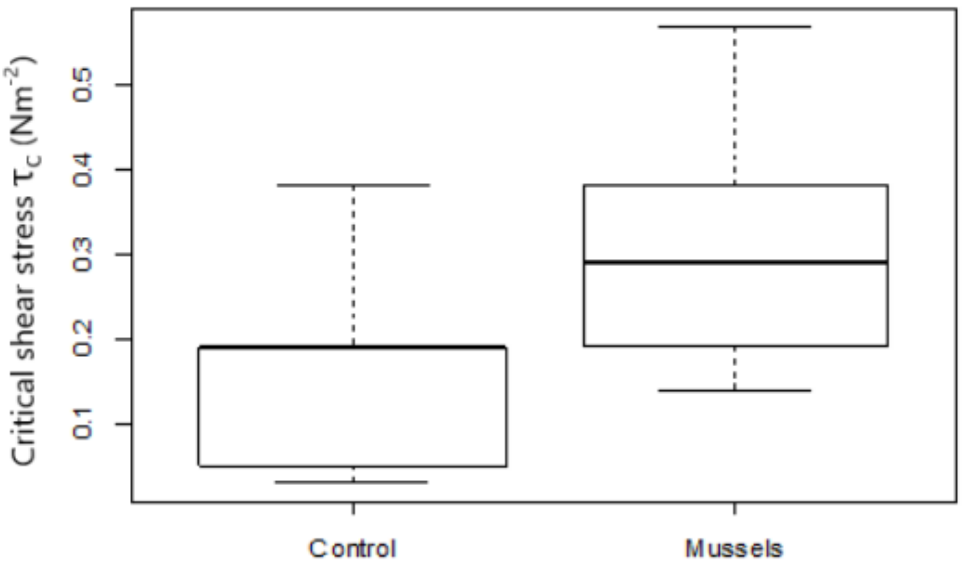


Figure 4.22: Box plot comparing the median critical boundary shear stress τ_c for entrainment of sediment deposited in the sediment trap when mussels were present and absent from the flume. The critical shear stress τ_c when mussels were present was significantly greater than when they were absent; $p < 0.001$.

4.4 Discussion

The results of the flume experiments showed that *Anodonta anatina* significantly reduced turbidity and TDS within the water column, which was likely due to filter-feeding and subsequent biodeposition of pseudofaecal pellets by the mussels. These results relate to Key Question 6 *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"* which was not fully resolved after the river investigation (described in Chapter 3) due to the limited quadrat area and number of mussels used in that study. The results for turbidity and TDS are also relevant to Key Question 3 *"To what extent do freshwater mussels impact sediment transport in rivers?"* as they indicate that freshwater mussels reduce the downstream flux of dissolved and suspended sediment in fluvial environments. Further research is required to determine how mussels affect the concentration of specific dissolved mineral ions in fluvial environments, as it would provide more informative mineral flux data than TDS alone.

The positive impact of *A. anatina* on water clarity observed in this study indicates that this species may provide a valuable ecosystem service in river environments. Reduced water turbidity levels would be of benefit to species who require good water clarity for predation, foraging, locomotion, and evasion of predators in river environments, such as bird, mammal, fish, amphibian, and macroinvertebrate species (Kjelland *et al.*, 2015; Ortega *et al.*, 2020). Improved water clarity would also increase the light available to macrophytes in river environments, which would be of benefit to grazing organisms.

Also addressing Key Questions 3 and 6, *A. anatina* significantly reduced water velocities at all measured depths within the flume with the exception of near-surface velocity. This is most likely due to the increased bed friction and flow disturbance arising from the mussels' shells protruding into the water column, and turbulence from the flow of water through their inhalant and exhalent siphons. Increased bed roughness resulting from mussel bioturbation likely also increased flow resistance, thus reducing the velocity of water in the flume.

Key Question 4 *"To what extent do freshwater mussels influence river bed topography?"* was addressed by investigating how the mussels affected lateral and longitudinal topographic profile lengths of the flume substrate, which were used as a measure of bed roughness. *Anodonta anatina* significantly increased the longitudinal topographic profile length of the channel substrate surface, which indicates an increase in bed roughness due to mussel bioturbation.

Lateral profile (wetted perimeter) lengths across the channel substrate with mussels present in the flume were not significantly different to controls. This may be because fluvial bedform development in the flume channel during the control study resulted in large differences between the depth of sediment on the inside and outside edge of the curved sections of channel, which, like bioturbation, increased the length of the wetted perimeter. In the control study, the lower water velocities on the inside of the curved section of channel would have been more conducive to sediment deposition, whereas the higher water velocities and bed shear stresses on the outside

edge of the channel would have made erosion more likely. Differences between the depth of sediment in the outside and inside edge of the curved sections of channel were much less apparent with mussels present, indicating that mussels disrupted the development of fluvial bedforms in the flume.

The impact of freshwater mussels on river bed topography and near-bed flow velocities could potentially be transformational in terms of the suitability of stream habitats for a variety of freshwater species. In streams that experience frequent flood events, spatial heterogeneity in near-bed flow patterns can provide flow refugia, vital for the long-term persistence of invertebrate populations (Lancaster *et al.*, 2006). Hydrodynamical modelling of marine mussel (*Mytilus edulis* (Linnaeus 1758)) beds demonstrated reduced water velocities over, and in the wake of mussel beds, attributed to the greater bed roughness of the bed compared with the surrounding sea floor (van Leeuwen *et al.*, 2010). Therefore, it is conceivable that dense aggregations of freshwater mussels may provide patches of relatively tranquil water for invertebrates to feed and reproduce, or for macrophytes to take root where they might otherwise get washed downstream.

The use of longitudinal and lateral transects in this study enabled impacts of mussel bioturbation on substrate topography to be quantified, however it did not provide three-dimensional visualisation of the entire substrate surface. The use of three-dimensional imaging techniques such as laser scanners to produce high resolution Digital Elevation Models (DEMs) of the substrate surface would have provided more detailed information about how the mussels impacted substrate topography over the duration of the study.

The mussels significantly increased the percentage of fines in the substrate, which may be due to their ability to capture suspended sediment from the water and deposit in the form of faeces and pseudofaeces. It is also possible that the more irregular bed topography and significantly reduced water velocities resulted in increased deposition of fine sediment with mussels in the flume. In a river setting, this finer sediment would provide important nutrients for micro-organisms,

invertebrates and producers, but could potentially be problematic for species that require gravel with low amounts of fine sediment, such as spawning salmonids (Beschta and Jackson, 1979; Wood and Armitage, 1997; Evans and Wilcox, 2014).

Despite the significant increase in substrate fines in the presence of mussels, there was no significant alteration to the median grain-size of the flume substrate when mussels were present. This may be due to the relatively small volume of fine material in the flume substrate compared with the volume of sand and gravel. Although median grain-size of the substrate was unaffected, the distribution of sediment around the flume was impacted by the presence of mussels, with an increased variability in grain-size observed across, and along the length of the channel. The grain-size distribution of the substrate samples taken from the control study show increasing homogeneity of the substrate throughout the course of the study, whereas the samples from the mussel study show increased variability in grain-size distributions over time.

The substrate grain-size distribution results from this study support the conclusions of the river study, that freshwater mussels improve the heterogeneity of the river bed, and visual observations and photographs (shown in Section 4.3.3) also support this conclusion. The flume substrate was smooth and uniform in appearance around the entirety of the channel by the end of the control study, whereas the bed surface with mussels present displayed an irregular appearance with patches of gravel and areas of finer material. The areas of finer sediment likely result from the filter-feeding and subsequent biodeposition of fines by the mussels, as well as the reduced near-bed velocities. Flow disturbance around the mussels and due to the uneven bed surface would have led to the development of wake deposits and areas of reduced shear stress where the finer sediment could be deposited. The gravel patches could have arisen through the scouring away of sand and finer material that had been mobilised by the mussels, and the increased turbulence generated by the uneven bed profile. They could also have resulted from kinematic sieving of the finer material through the coarser material as it is moved around by bioturbation. These observed affects could be very important in terms of improving habitat heterogeneity, and thus diversity

within river environments, due to the greater availability of different substrates and river bed microhabitats supporting a greater range of species with varying modes of life.

Key Question 3 "*To what extent do freshwater mussels impact sediment transport in rivers?"* was primarily addressed using grain-size analysis and graphic logs of the sediment trap infill. Grain-size analysis of sediment in the sediment trap showed that an increased percentage of gravel was entrained in the flow in the presence of mussels but a reduced percentage of clay, silt and fine sand entered the trap. Graphic logs of the sediment in the trap show that in the control experiment, most of the movement of sediment appeared to happen early in the study as the early infill was primarily sand, whereas the later infill was mainly the organic and fine sediment that had been introduced each week in the form of seston. This would suggest that once the substrate had found equilibrium with the flume's flow regime and the bedforms had become established, there was very little grain movement of the sand and gravel substrate. With mussels in the flume, observations of the mussel behaviour and the progressive infilling of the sediment trap indicated that the mussels continued to mobilise sand and gravel throughout the duration of study.

The water velocities present in the flume with mussels present were lower than in the control study, so the shear stress and ability of the water to erode and transport sediment should also have been reduced. However, with mussels present, critical boundary shear stress (τ_c) calculated using grain-sizes entering the sediment trap, showed significantly greater τ_c compared with when they were absent. With mussels present, the sand and gravel substrate continued to be disturbed and mobilised throughout the study, which led to increased entrainment of the coarser fractions and a greater quantity of sediment entering the trap. Disturbance to grains from mussel bioturbation would have the effect of disrupting grain fabrics and would help overcome the cohesive, frictional, gravitational and inertial forces holding the grains in place, thus allowing the grains to be entrained more readily. The irregular substrate surface resulting from mussel bioturbation and the protrusion of the mussels into the water column may also have increased sediment scouring due to

the formation of localised turbulent eddies and flow vortices, contributing to increased critical boundary shear stresses. Furthermore, wake deposits forming on the lee side of the mussels would become vulnerable to the flow each time a mussel moved, resulting in rapid entrainment of a range of grain-sizes.

During periods when the mussels were stationary, the critical boundary shear stresses within the flume would have been insufficient to maintain the downstream motion of the sand and gravel fractions, as evidenced by laminations and cross-laminations of finer sediment in the sediment trap. Therefore, after the initial disturbance by mussels, the coarse sediment fractions would likely have been rapidly deposited. Nevertheless, the cumulative impact of entire mussel populations on river sediment flux is likely to be considerable over extended periods of time.

Addressing Key Question 2 *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?,"* a reduced percentage of organic and inorganic carbon entered the sediment trap when mussels were present, suggesting they were capturing this material from the water column and preventing its entrainment. Despite the reduced entrainment of organic and inorganic carbon with mussels present, analysis of substrate samples did not find a corresponding increase in organic and inorganic carbon in the substrate. Photographs of the sub-surface sediment through the glass of the flume (Section 4.3.3, Figures 4.15 and 4.16), clearly show significant quantities of organic matter from the introduced seston buried beneath the substrate surface, which was not visible in the control study. This indicates that the increased mixing of the substrate by mussels resulted in burial and vertical dispersal of the organic matter, whereas when mussels were absent, the deposited seston would have remained on the surface of the substrate. As the sediment samples were only taken from the upper 50 mm of substrate, organic and inorganic carbon buried to deeper levels of the substrate by the mussels, may not have been accounted for. An improved coring method that enabled vertical changes in organic and inorganic carbon concentrations to be quantified would further elucidate the mechanisms involved.

The transferral of carbon into the hyporheic environment by mussels may have significant implications with regard to carbon cycling in fluvial systems, due to the mussels' potential role in carbon sequestration. More research is required to establish how much of the buried organic and inorganic carbon remains fixed in the hyporheic environment, and how much is cycled back into the atmosphere through physical, chemical and biological processes. Furthermore, a proportion of the organic and inorganic carbon captured by the mussels would have been incorporated into their own body tissues or lost through respiration. In a river environment the death of unionids would add significant quantities of organic matter and carbonate to the sediment, which was not replicated in this study.

Regarding Key Question 5 *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"* it was anticipated that bioturbation by the mussels would improve hyporheic oxygen levels by allowing more oxygen-rich water to penetrate into the substrate, as observed in the river study. However, in the flume study, the substrate oxygen saturation results appear to mirror the water oxygen saturation results, which likely reflects the relative ease at which the water in the channel could permeate into the flume substrate compared with the river bed sediments.

In the natural river bed habitat, the presence of clay and silt in the substrate would have reduced the permeability of the river bed compared with the sand and gravel substrate of the flume, which would have impeded the flow of oxygenated river water into the hyporheic zone. In river sites where mussels were present, vertical mixing of the substrate by mussels would have transferred more oxygenated water into the river bed sediment compared with sites where they were absent. Additionally, the presence of fine, cohesive sediment in the river substrate may have helped maintain the structural integrity of mussel burrows, enabling greater penetration of more oxygenated water into the hyporheic zone compared with river sites without mussels. The lack of fine, cohesive sediment in the flume substrate would have increased the likelihood that mussel burrows would immediately collapse if a mussel moved to a new location within the flume channel, making the impact of bioturbation on substrate oxygen levels less pronounced.

Therefore, although the river study demonstrated that *A. anatina* can positively impact hyporheic oxygen levels in rivers, the results of the flume study imply that *A. anatina* may not improve hyporheic oxygen levels in river sediments with very low proportions of silt and clay. Further research is required to establish which river bed sediment grain-size distributions respond most positively in terms of hyporheic oxygen levels, when subjected to mussel bioturbation.

A limitation of this investigation was that it was carried out in an artificial flume environment with different abiotic and biotic conditions to the mussels' natural habitat in Markeaton Brook. Although attempts were made to replicate hydrological and sedimentological conditions found in Markeaton Brook, there were several important differences between the two environments that may have impacted the results of the study. One key difference was the absence of cobbles, boulders, and armour layer sediment in the flume, which were present in Markeaton Brook. Although *A. anatina* are frequently found in canals, streams and rivers with clay, silt and sand substrates (Killeen, Aldridge and Oliver, 2004, p. 49; Lopes-Lima *et al*., 2017), the lack of coarse sediment in the flume would inevitably have affected sediment transport rates, critical boundary shear stresses and hydrodynamics, and may have impacted the behaviour of the mussels. The lack of fine, cohesive sediments such as clay, silt and organic matter in the initial substrate mix will also have impacted sediment transport rates, hyporheic oxygen levels and mussel behaviour.

Mussel behaviour and sedimentary processes in the flume may have been affected by the confined and unnatural shape of the flume channel. The lack of variability in flow discharge and sediment supply present in a natural river setting are also likely to have had an impact on mussel behaviour. Additionally, the lack of other stream biota in the flume may have resulted in important interspecific positive or negative feedback effects being unaccounted for.

Another limitation of this investigation was that changes in the rate of sediment flux over time were not represented by quantitative data. The temporal changes in sediment flux could be inferred by the superposition of sedimentary layers of the different grain-sizes measured in the

graphic logs, however the grain-size distribution was only fully characterised from the bulk samples taken from the sediment trap at the end of the study. Sampling of the sediment trap contents at regular intervals throughout the study would have provided more detailed sediment flux data, and would have provided more insight into how changes in mussel behaviour affected sediment transport rates. The beaker forming the sediment trap in these experiments was not removed and analysed during the study as its removal would have caused significant disturbance to the substrate. An improved sediment trap design that enabled regular removal and analysis of the contents of the sediment trap would have improved this investigation. Utilisation of tracer particles and techniques such as particle image velocimetry (PIV) to further quantify and visualise how unionids alter flow dynamics would provide additional insight into their impact on river sediment flux.

The flume investigation could have been improved by running the experiment for longer periods of time, or by manipulating variables such as water velocity, substrate grain-size, mussel density or mussel species to provide more information about freshwater mussel behaviour and their impact on sediment transport. Limited laboratory space where the flume was housed meant that this was unfortunately not possible at the time, as the space was required for other projects. Furthermore, the processing and analysis of sediment samples for both the river and flume investigations proved extremely time-demanding, and constituted a significant proportion of the allocated time for this project, hence, there are still some potential areas of research that would benefit from further study.

The results of this investigation indicate that freshwater mussels have the capacity to significantly influence erosional, transportational and depositional sedimentary processes in fluvial environments. Given that freshwater mussels can exist at very high densities within rivers (Mann, 1964; Negus, 1966; Aldridge, Fayle and Jackson, 2007), increased mixing and mobilisation of river bed sediment, improved habitat heterogeneity and the transferral of fine and organic

sediment from the water to the substrate by mussels implies they constitute a critical element in the sediment and nutrient dynamics of fluvial systems.

Chapter 5: Discussion

5.1 Research approach

The primary aim of this research project was to ascertain the degree to which freshwater mussels impact the sedimentological and eco-hydrological conditions within river environments. To achieve this aim, this project was broken down into two Research Objectives and six Key Questions, described in Section 1.4 of this thesis.

Research Objective 1 *"Ascertain if the addition or removal of freshwater mussels to/from the beds of two rivers can significantly impact the sedimentological and eco-hydrological characteristics of the river environments, and examine the precise nature of any observed impacts"* was primarily addressed in the river-based investigation described in Chapter 3, but also required preliminary river survey work and river studies described in Chapter 2. The river-based investigation allowed the mussels' impact on fluvial conditions to be assessed in their natural habitat, accounting for any biotic or abiotic factors that may have enhanced or negated the mussels' influence on their environment.

Mussels were introduced to quadrats at one river site in Markeaton Brook and one site in the River Sence, where mussels were previously absent. Mussels were removed from quadrats at one site in Markeaton Brook and one site in the River Sence that previously contained mussels. Experimental quadrats (where mussels had either been removed from, or introduced to the river bed) were compared with control quadrats at each of the four river sites. To examine differences in the rate of change of measured variables over time, linear models were constructed for each variable, and the beta coefficients of the linear models were statistically compared. By comparing the rate at which variables in experimental quadrats changed over time relative to variables in the control quadrats, any pre-existing differences between the quadrats could be accounted for. The comparative quadrat method also meant that any natural variations in flow discharge, sediment supply or other hydrological variables throughout the study would impact both experimental and

control quadrats, therefore any observed differences in the beta coefficients of each variable could be more confidently attributed to the presence or absence of mussels.

Key Questions addressed in Chapter 3 were as follows:

1) *"To what extent do freshwater mussels impact river bed grain-size distribution patterns?"*
2) *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?"*
5) *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"*
6) *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"*

Research Objective 2 *"Determine if freshwater mussels significantly impact sediment dynamics, substrate topography, and hydrological conditions in a laboratory-based mesocosm fluvial environment, and if so, what these impacts are"* was primarily addressed in the flume-based investigation described in Chapter 4, but also involved preliminary river-based and flume-based studies detailed in Chapter 2. The flume-based investigation allowed for better control of the complex variables present in the natural river environment, and enabled investigation of the impact that freshwater mussels have on river sediment dynamics and water quality away from extrinsic factors that may have influenced the results of the river study.

The flume-based investigation involved an 8-week control study without mussels in the flume followed by an 8-week study with 50 *Anodonta anatina* (Linnaeus 1758) in the flume. In each study, water was continually recirculated across a polymodal substrate for a period of 8 weeks at water velocities comparable to those found in Markeaton Brook, where the mussels were collected from. The flume contained a sediment trap, enabling quantification of critical boundary shear stresses in the flume. Changes in hydrological and sedimentological variables over the duration of each 8-week study were statistically compared to determine the degree to which freshwater mussels influenced conditions in the mesocosm fluvial environment.

Key Questions addressed in Chapter 4 were as follows:

1) *"To what extent do freshwater mussels impact river bed grain-size distribution patterns?"*
2) *"To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?"*
3) *"To what extent do freshwater mussels impact sediment transport in rivers?"*
4) *"To what extent do freshwater mussels influence river bed topography?"*
5) *"To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?"*
6) *"To what extent do freshwater mussels influence water quality and habitat conditions in rivers?"*

5.2 Key findings

The results of the river and flume investigations indicate that freshwater mussels affect river environments in a variety of important ways, including impacts on hyporheic conditions, sediment grain-size distributions, sediment transport, habitat heterogeneity and water quality. The main findings of this research project and how they address each of the Key Questions set out in Section 1.4 are discussed in this section.

5.2.1 Key Question 1 - To what extent do freshwater mussels impact river bed grain-size distribution patterns?

The flume investigation results indicated that *Anodonta anatina* significantly increased the percentage of fine sediment (< 63 µm) in the substrate. The increase in substrate fines with mussels in the flume corresponded with significantly reduced concentrations of dissolved and suspended solids in the water column, suggesting that mussels were capturing sediment from the water and depositing it in the substrate. However, significant differences in the percentage of

substrate fines were not observed in the river investigation, with the exception one quadrat in the River Sence, which showed a significant decrease in fines (< 63 μm) where mussels had been removed.

The results of the flume study are in agreement with field-based studies on the impact of invasive zebra mussel (*Dreissena polymorpha* (Pallas 1771)) and Asiatic clam (*Corbicula fluminea* (Müller 1774)) populations on water turbidity and fine-sediment deposition (Cohen *et al.*, 1984; Klerks, Fraleigh and Lawniczak, 1996). Photographs (Figures 4.15 and 4.16) taken through the glass walls of the flume showed increased quantities of fine and organic sediment buried beneath the sediment surface with mussels present, compared with the control study when they were absent from the flume. This would suggest that not only were mussels capturing and biodepositing this material, they were also incorporating this material into the hyporheic environment through bioturbation. The transferral of fine-grained sediment into the hyporheic environment may be a direct result of vertical mixing of grains and pseudofaecal pellets as the mussels burrowed laterally and vertically into the substrate. Increased substrate fines may also have resulted from the indirect effect of the mussels' influence on fluvial sedimentary processes, water velocities, grain entrainment thresholds and sediment transport rates.

Although the flume investigation indicated that *A. anatina* increased the percentage of fine sediment in the substrate, the lack of significant results in the river investigation makes it difficult to draw definitive conclusions to this effect. Furthermore, both quadrats in Markeaton Brook, where mussels had been removed, showed significant reductions in armour layer pebble B-axis length compared with the quadrats containing mussels, which may imply that the presence of mussels lead to an overall coarsening of river bed surface material at this site. Statistical differences in the grain-size of the armour layer were not found at any of the other river sites, so this observation remains inconclusive. The lack of armour layer sediment or fine, cohesive sediment in the original flume substrate mix may have influenced mussel behaviour and sediment mobility in the flume investigation. It may also be that in a natural river environment, the

mussels' transferral of fine and organic sediment to the river bed is partially offset by the action of other organisms, as biodeposits would provide a rich source of nutrients to other freshwater biota.

Freshwater mussels were shown to increase the textural heterogeneity of the river bed in both the flume investigation, and in both River Sence quadrats where they were introduced. Additionally, both quadrats in the River Sence where mussels where removed showed a reduction in textural heterogeneity of the river bed compared with the control quadrats. The difference in textural heterogeneity of the substrate in the presence or absence of mussels was demonstrated from the grain-size distribution results of the sediment core samples taken from the river bed and the flume substrate. With mussels present in the flume and the River Sence, the substrate sediment core samples displayed greater lateral variability in grain-size distribution patterns of the river bed and the flume channel substrate, with some areas of the river bed showing an increased proportion of sand and gravel, and others showing higher proportions of silt and clay by the end of each investigation. In comparison, the removal of mussels from the River Sence, and the absence of mussels from the flume resulted in an increasingly homogenous substrate, with all sediment core sample displaying virtually identical grain-size distribution patterns. These results suggest that, although freshwater mussels did not significantly impact the overall mean sediment grain-size of the substrate, they did alter its spatial distribution.

Photographs (Figures 4.14 - 4.16) taken of the flume substrate at the end of the investigation corroborate the grain-size data from the sediment cores, showing a much higher degree of textural variability in substrate surface material, with mussels present in the flume. Mussel bioturbation in the flume resulted in scoured patches of gravel, where finer-grained sediments had been eroded away, and patches of finer sand, silt and organics in parts of the channel not recently occupied by mussels. The mussels' impact on the textural heterogeneity of the river bed was less clear in Markeaton Brook, compared with the River Sence and the flume investigation. This may be because of the lower densities of mussels in Markeaton Brook compared with the River Sence, or

may be due to other biotic or abiotic differences between the two rivers that may have influenced mussel behaviour, or the degree to which they could influence the river bed sediment.

5.2.2 Key Question 2 - To what extent do freshwater mussels influence organic and inorganic carbon dynamics in rivers?

Anodonta anatina significantly reduced the entrainment of organic and inorganic carbon into the sediment trap in the flume investigation. The reduction of organic and inorganic carbon in the sediment trap was likely due to the mussels filter-feeding on the seston that was introduced to the flume each week throughout the investigation. However, the entrainment of seston may also have been impacted by the different flow and substrate conditions with mussels present in the flume, affecting rates of transport, deposition and burial of sediment.

Although mussels significantly reduced the entrainment of carbon into the sediment trap, the percentage of organic and inorganic carbon in the substrate sediment core samples with mussels in the flume, was not significantly different to the control study. Significant differences in substrate organic and inorganic carbon were also not evident in the river investigation. Though not confirmed statistically, Figures 4.15 and 4.16 show distinct layers of fine, organic sediment in the flume substrate with mussels present, that were not apparent in the control study, indicating that mussels were increasing the amount of this material in the substrate. The difference in significance of the results of the sediment trap carbon compared with the substrate carbon may be due the mussels' digestion and metabolism of the captured seston, which would mean that only a proportion of carbon filtered from the water would be egested in the faecal and pseudofaecal pellets (Vaughn and Hakenkamp, 2001). Additionally, as the sediment core samples were only taken from the upper 50 mm of substrate, it is possible that some of the deposited carbon was buried deeper within the hyporheic zone as a result of fluvial processes and mussel bioturbation, and thus not accounted for in the samples of the substrate.

5.2.3 Key Question 3 - To what extent do freshwater mussels impact sediment transport in rivers?

Despite the reduced water velocities when mussels were in the flume, the presence of *A. anatina* resulted in an increased volume of sand and gravel entering the sediment trap compared with the control study, when mussels were absent. The increased entrainment of coarse sediment with mussels in the flume was likely to be a direct result of mussel bioturbation mobilising grains, but could also result from differing flow conditions with mussels in the flume, which may have increased levels of turbulence and erosion.

Based on the grain-sizes entering the sediment trap, theoretical critical boundary shear stresses were significantly higher when mussels were in the flume. The sediment trap data also showed more variability in critical boundary shear stress (τ_c) when mussels were present compared with the control study. During the investigation, there were periods of time when the τ_c with mussels in the flume fell within the range of that recorded in the control study, with values ranging from 0.17 - 0.23 Nm^{-2}, whereas at other times the theoretical τ_c was much higher, increasing to a maximum of 0.48 Nm^{-2}. The fluctuations in τ_c observed when mussels were in the flume could be attributed to variable levels of mussel activity, as when mussels were stationary the reduced near-bed velocities compared with the control study may have resulted in a lower shear stress environment and a reduced likelihood for grain entrainment. When the mussels were actively burrowing in the substrate they would have mobilised sand and gravel by helping to overcome the inertial, frictional, gravitational and cohesive forces holding the grains in place (Miller, McCave and Komar, 1977; Wiberg and Smith, 1987). Mussel bioturbation would also have disturbed grain fabrics and sedimentary structures such as grain imbrication and wake deposits, which would have reduced entrainment thresholds (Rosgen, 1994; Church, 2006; Rice *et al*., 2019). Furthermore, mobilisation of gravel by the mussels would have exposed surrounding smaller grains to increased shear stresses (Wiberg and Smith, 1987; Church, 2006), leading to intermittent pulses of grain entrainment during periods of mussel bioturbation. As the sediment trap was not removed and

analysed until the end of the study, it is not possible to determine from these data how long each period of increased grain entrainment lasted once the mussel or mussels that initiated the destabilisation of grains became inactive again.

The mussels influence on critical shear stresses and grain entrainment thresholds in the flume can be compared to other species that have been shown to increase grain mobility, such as sockeye salmon (*Oncorhynchus nerka* (Walbaum 1792)) (Gottesfeld, 1998; Gottesfeld *et al*., 2004; Hassan *et al*., 2008), European barbel (*Barbus barbus* (Linnaeus 1758)) (Statzner, Sagnes and Champagne, 2003; Pledger, 2014), gudgeon (*Gobio gobio* (Linnaeus 1758)) (Statzner, Sagnes and Champagne, 2003), chub (*Squalius cephalus* (Linnaeus 1758)) (Pledger, 2014), spinycheek crayfish (*Faxonius limosus* (Rafinesque 1817)) (Statzner, 2012) and signal crayfish (*Pacifastacus leniusculus* (Dana 1852)) (Harvey *et al*., 2014), as discussed in Section 1.3.4. Prior research on the impact on sediment stability by freshwater bivalves has drawn variable conclusions, suggestive that stabilisation or destabilisation of sediment by mussels may depend on their density, species and behaviour (Zimmerman and de Szalay, 2007; Allen and Vaughn, 2011). Increased species richness of freshwater bivalves was shown to increase the erosion of gravels in a model stream environment (Allen and Vaughn, 2011). This was attributed to the higher variability of shell morphology and burrowing behaviour increasing stream bed topographic complexity, which could increase turbulence, and therefore erosion (Allen and Vaughn, 2011).

Zimmerman and de Szalay (2007) found that placement of the unionids *Actinonaias ligamentina* (Lamarck 1819) and *Ptychobranchus fasciolaris* (Rafinesque 1820) into a model stream environment initially caused destabilisation of sediments as they positioned and oriented themselves on and into the stream bed. Once positioned, they remained relatively sessile, and caused stabilisation of the river bed sediments by Week 4 of the study (Zimmerman and de Szalay, 2007). In comparison, *A, anatina* individuals in the flume-based investigation, described in Chapter 4 were seen to still be actively moving around the flume channel in Week 8 of the study. However, data from the sediment trap data show a gradual reduction in median grain-size

in the latter part of the sediment trap infill, indicating a reduction in the τ_c towards the end of the study. It is possible that if the investigation ran for longer than 8 weeks, further reductions in sediment flux would have been observed due to the mussels being more acclimatised to the conditions in the flume.

Although *A. anatina* increased the entrainment of sand and gravel in the flume-based study, they reduced the amount of fine sediment entering the flume's sediment trap, suggesting that filtration and biodeposition by the mussels captured this material and reduced its entrainment. This finding corresponds with the increased percentage of fines in the substrate and the reduced levels of water turbidity with mussels in the flume. It is conceivable that over time, the mussels introduction of fine-grained and organic sediment into the substrate through biodeposition could have had a stabilising effect on the coarser-grained sediment as it infills the interstices between grains, which would mitigate some of the destabilising effects of mussel bioturbation. However this could also have implications for hyporheic oxygen levels and the suitability of the environment for other stream biota.

The flume substrate differed from the natural river bed environment in several ways including a lack of armour layer sediment, lack of cobbles and boulders, lack of fine matrix material and lack of other stream biota. The flume environment also lacked variability in flow discharge, sediment supply and other abiotic factors. These differences will have affected grain entrainment thresholds, boundary shear stresses and sediment transport rates. Mussel behaviour may also have been impacted by the differences between the flume and river environments. Testing the mussels impact on sediment transport in a natural river bed environment over longer periods of time, would provide improved understanding of some of these issues.

5.2.4 Key Question 4 - To what extent do freshwater mussels influence river bed topography?

The longitudinal topographic profile length of the substrate was significantly increased when mussels were in the flume compared with when they were absent. The increased topographic profile length indicates that the substrate surface was more uneven and irregular with mussels in the flume, compared with a smoother surface when they were absent. The increased topographic roughness was likely predominantly due to mussel bioturbation, but may also have been influenced by the protrusion of the mussels' shells into the water column, which may have led to localised scouring due to the generation of turbulent eddies and flow vortices, as well as areas of lower shear stress where deposition could occur. Further investigation using particle image velocimetry (PIV) and tracer particles, as described by Kumar *et al*. (2019), would help clarify the mechanisms involved.

The more uneven bed topography with mussels in the flume may have been partly causative in the reduction in water velocities observed when mussels were present, as increased topographic roughness can lead to increased levels of drag (Rice *et al*., 2019). Similar effects on topographic roughness have been seen with foraging European barbel and chub that were shown to increase microtopographic roughness, which was associated with increased sediment mobility (Pledger, 2014; Pledger, Rice and Millett, 2017; Rice *et al*., 2019).

The lateral profile transects taken across the width of the flume channel did not show significant differences in topographic roughness between the study with mussels and the control study. However, differences were apparent with regard to the shape of the channel. In the control study differential erosion and deposition around the curved sections of channel resulted in an increased depth of sediment towards the inside edge of the channel compared with the outer edge, where water velocities and shear stresses would have been greater. The straight sections of channel showed a greater depth of sediment approximately mid-way across the channel in the control study. In comparison, with mussels in the flume, the depth of sediment on either side of the

channel was approximately equal at the end of the study with no clear pattern in the spatial distribution of sediment around the channel. This would suggest that either the physical presence of the mussels disrupting the flow of water, or the impacts of mussel bioturbation, interfered with the development of bedforms within the flume.

It should be taken into account that the mussels' influence on substrate topography observed in the flume study was investigated with a substrate that lacked an armour layer, and comprised approximately equal proportions of sand and gravel. The results of the study may therefore not be directly applicable to gravel-dominated river beds that do display an armour layer. Due to the size and mass of individual freshwater mussels it is likely that they would have limited capacity to mobilise coarser bedload such as cobbles and boulders. However, in rivers with higher proportions of finer sediment, the results of this study suggest that populations of freshwater mussels could significantly alter river bed topography. This could be further explored with the use of laser scanners to produce high resolution Digital Elevation Models (DEMs) of mussel beds in natural river environments (as described in Section 2.3.1) to determine more precisely the extent to which mussels can impact the topography of different river bed sediments.

***5.2.5 Key Question* 5** - To what extent do freshwater mussels impact hyporheic oxygen levels in rivers?

In the river investigation, at sites where mussels had been introduced to the river bed, half of all quadrats showed significantly increased hyporheic oxygen saturation, with the remaining quadrats showing non-significant increases in hyporheic oxygen saturation compared with the control quadrats. Furthermore, at sites where mussels had been removed from the river bed, significant decreases in hyporheic oxygen saturation were found in all quadrats, compared with the control quadrats that contained mussels. These results suggest that the presence of mussels facilitated the transferral of oxygenated water from the pelagic to the hyporheic environment, and are consistent

with research by Boeker *et al*. (2016) and Black, Chimenti and Just (2017), who found increases in the concentration of interstitial oxygen and aerobic bacteria as a result of freshwater mussel bioturbation. The conclusions of the river investigation differ from those of Hakenkamp and Palmer (1999), Beekey, Mccabe and Marsden (2004) and Turner (2010), who found reductions in hyporheic oxygen levels in the presence of mussels, although those studies were conducted on mussels that are not native to the UK.

As one quadrat in the River Sence, and one quadrat in Markeaton Brook showed non-significant increases in hyporheic oxygen saturation where mussels were introduced, it may have been the case that those sites were not ideal conditions for mussels, as they were previously absent from those sites. Additionally the coarser bed sediment size of the armour layer at each of those sites would have made it more difficult for the mussels to burrow into the river bed, which may have been partly the reason for many of the mussels being washed away during a period of high river discharge.

The mussels' impact on hyporheic oxygen saturation was not replicated in the flume, which likely reflects the higher proportion of sand and gravel, and lower proportion of fine, cohesive sediment in the flume substrate. The high porosity and permeability of the flume's sand and gravel substrate may have increased the rate of hyporheic exchange relative to the river bed sediment. The higher permeability of the flume substrate may, therefore, have diminished the level of impact that the mussels could have on hyporheic oxygen saturation in the flume. The low levels of fine, cohesive sediment in the flume could also have reduced the stability of burrow structures, which may have been more liable to collapse. It is conceivable that burrows made by mussels into more cohesive river sediments would maintain their structure for longer periods of time, providing pathways for the transferral of water and nutrients between the pelagic and hyporheic zones. Using natural river sediments rather than a non-cohesive mixture of grain-sizes in the flume would have allowed the mussels' influence on hyporheic oxygen levels to be more accurately tested in a laboratory-based setting.

5.2.6 Key Question 6 - To what extent do freshwater mussels influence water quality and habitat conditions in rivers?

In addition to the abiotic factors examined in Key Questions 1-5, a number of additional abiotic and biotic factors were investigated to assess the influence of freshwater mussels on water quality and habitat conditions in rivers. These included measurements of water turbidity, total dissolved solids (TDS), water oxygen saturation, water velocity, Biological Monitoring Working Party (BMWP) scores, and the abundance of key indicator macroinvertebrate taxa.

Water turbidity and TDS were significantly reduced with *A. anatina* in the flume, compared to the control study when they were absent. High levels of dissolved and suspended solids in the water column can be detrimental to many plant and animal species due to impaired visibility, light levels, and clogging of filter feeding mechanisms (Kjelland *et al*., 2015; Tuttle-Raycraft, Morris and Ackerman, 2017; Ortega *et al*., 2020). The mussels influence on turbidity and TDS would therefore be beneficial to a variety of freshwater organisms. Removal of nutrients and algae from the water column would also be beneficial in mitigation of eutrophication in freshwater habitats (Stevcic, Pulkkinen and Pirhonen, 2018).

The ability of mussels to improve water clarity and remove nutrients from the water is likely to be dependent on the pre-existing water conditions, as highly eutrophic conditions and high levels of suspended solids have been found to detrimentally impact freshwater mussel filter-feeding, recruitment and survival (McIvor, 2004; Gascho Landis, Haag and Stoeckel, 2013; Tuttle-Raycraft, Morris and Ackerman, 2017).

It should be noted that in the river investigation, no significant differences in TDS were observed, and significant decreases in turbidity were found in Markeaton Brook, where mussels were introduced, but not in the River Sence. However, this was likely due to the relatively small size of the quadrat and limited number of mussels in each quadrat, as well as the flowing water conditions. Additionally, based on personal observations of the mussels' behaviour, it is also

probable that they would have closed their shells as a defence mechanism at the time the water samples were taken, meaning they may have not been actively filtering at the time. Even if the mussels were filtering when the samples were taken, the rate at which the mussels in each quadrat would have been able to clear dissolved and suspended solids from the water column would likely be insufficient to be detectable in a flowing water environment. However, based on the results of flume investigation, where water was recirculated across a bed of mussels, the impact of a more extensive mussel bed on water clarity would likely be significant.

Despite the more positive trend in hyporheic oxygen levels observed in the mussel-containing quadrats in the river investigation, water oxygen saturation levels were not significantly different to controls. The lack of significant results for water oxygen levels was likely for the same reasons as the lack of significant TDS and turbidity results in the river investigation, and represents a limitation of the experimental design.

In the flume-based investigation, *A. anatina* were found to significantly reduce water oxygen saturation compared with the control study. However, it should be noted that the flume contained very few photosynthetic organisms that would serve to replenish dissolved oxygen levels in a natural river environment, such as macrophytes and algae. The seston, introduced to the flume each week would likely have contained variable quantities of phytoplankton, that may have released some oxygen into the water. However, the results for water turbidity demonstrate that the mussels were very effective at filtering the seston from the water column, and mussels are capable of selectively feeding on microalgae, diatoms and cyanobacteria (Miura and Yamashiro, 1990; Nichols and Garling, 2000). It is therefore not surprising that 50 actively respiring mussels in a recirculating flume led to significantly reduced water oxygen saturations levels.

Due to the relatively large biomass of individual mussels compared with many other freshwater invertebrates, the mussels' demand for dissolved oxygen may potentially be problematic in environments with large numbers of mussels, where oxygen saturation is a limiting factor. It is

also possible that decomposition of mussel biodeposits may lead to localised reductions in water oxygen saturation levels. However, the mussels' removal of dissolved and suspended solids from the water column may help mitigate this potentially negative impact, as high turbidity levels reduce the amount of light available to photosynthetic organisms, and high algal abundance, and high concentrations of dissolved nutrient levels can contribute to eutrophication and resultant hypoxia. It is therefore more appropriate to assess the mussels net influence on water oxygen saturation in a natural aquatic environment, where other biotic interactions affecting oxygen balance can be accounted for.

In the flume investigation, mussels were found to significantly reduce water velocities at all measured depths, with the exception of near-surface velocity. This may have been due to the increased surface drag and disruption to the flow of water from the protrusion of their shells into the water column, as well as the more uneven bed topography. These effects are similar to those described by van Leeuwen *et al*. (2010) for marine mussels (*Mytilus edulis* (Linnaeus 1758)), which were found to increase bed roughness, reduce water velocities, and increase levels of fine-sediment deposition.

Reduced water velocities in the proximity of freshwater mussel beds may provide zones of more tranquil water, which would benefit flow-sensitive macroinvertebrate species (Lancaster *et al*., 2006). Mussels could also contribute to the availability of flow refugia during flood events, which are essential for the long-term survival of invertebrate populations in stream and river habitats (Lancaster *et al*., 2006).

Reductions in water velocities in mussel-containing quadrats in the river investigation were found to be non-significant, with the exception of River Sence Site A, which showed significantly increased velocities in all quadrats where mussels had been removed from the river bed. The small size of the quadrats in the river study would have lessened the degree of impact that the mussels could have on river velocities compared with a more extensive mussel bed. Furthermore,

Markeaton Brook Site A experienced extremely low flow conditions during the last three weeks of the study, which meant that mean water velocities of 0 ms^{-1} were recorded in both mussel-containing quadrats and control quadrats. This meant that any differences in velocity were undetectable with the model of flow meter used. It is possible that if a more precise flow meter was used, with a smaller impellor capable of measuring closer to the river bed, such as an electromagnetic flow meter, then more significant differences in velocity may have been detected in the river study.

Significant differences in BMWP scores were found in the River Sence in mussel-containing quadrats compared with quadrats where they were absent. At River Sence Site A, BMWP scores were significantly reduced where mussels were removed, and at Site B, they were significantly increased were mussels were introduced to the river bed. The BMWP score system is a measure of water quality based on the relative abundance of pollution-sensitive and pollution-tolerant invertebrate taxa (Walley and Hawkes, 1997), so these results suggest that mussels improved habitat conditions for pollution-sensitive species in the River Sence.

The BMWP results from the River Sence are in agreement with previous work by Aldridge, Fayle and Jackson (2007), who found BMWP scores to be strongly associated with freshwater mussel density. However, the results from the Sence suggest that freshwater mussels may be causative in the increased BMWP scores rather than merely associated with them. However, the lack of significant results for BMWP scores in Markeaton Brook make it difficult to state this conclusively, and more research is required to confirm this. The lower mussel densities in Markeaton Brook compared with the River Sence may have limited the extent to which mussels could modify habitat conditions in that river.

In the River Sence, the abundance of Ephemeroptera (mayflies) and Trichoptera (caddisflies) also increased in mussel-containing quadrats compared with quadrats without mussels, although not significantly so. Ephemeroptera and Trichoptera are invertebrate taxa that are key indicators of

good water quality (Stoyanova *et al*., 2014). In Markeaton Brook, the abundance of these taxa declined significantly in the Site A quadrats during the period of very low flow conditions in the latter part of the study. The decline in abundance may reflect a deterioration of habitat conditions at Site A during this period of the study, and may provide an explanation for the non-significant results for the abundance of these taxa.

The mussel's impact on textural heterogeneity of the river bed discussed in Section 5.2.1 could also positively influence habitat conditions for many freshwater species, as substrate heterogeneity has been positively associated with diversity and abundance of freshwater species (Duan, Wang and Tian, 2008; Luck *et al*., 2010; Reid, Brierley and Boothroyd, 2010; Platt, 2011; Wyżga *et al*., 2012; Bellmore and Baxter, 2014; Wohl, 2016). This is because a more complex and varied river bed increases the variety of microhabitats and ecological niches available to species, and provides refugia during fluctuations in river discharge (Duan, Wang and Tian, 2008; Luck *et al*., 2010; Reid, Brierley and Boothroyd, 2010; Platt, 2011; Wyżga *et al*., 2012; Bellmore and Baxter, 2014; Wohl, 2016). Other freshwater species found to have similar effects on substrate heterogeneity include European barbel and chub, which have been found to increase habitat heterogeneity across riffles in river environments as a result of spatially heterogeneous foraging behaviour (Pledger, Rice and Millett, 2017).

5.3 Wider implications

5.3.1 Improved hyporheic oxygen levels

The results from the river study indicate that the presence of freshwater mussels was associated with higher oxygen saturation levels in the hyporheic zone, which is the subsurface layer of the river bed where groundwater and surficial waters interact (Boulton and Hancock, 2006). Higher hyporheic oxygen levels may be attributed to the effects of mussel bioturbation of the river bed

sediment, although these effects were not replicated in the flume substrate, which contained much lower quantities of silt and clay.

As well as increasing hyporheic oxygen levels through the downward flux of surface waters into the river bed, the improved hyporheic exchange from bioturbation may facilitate the upwelling of nutrient-rich groundwaters to the river environment (Boulton and Hancock, 2006). Increased mixing of groundwater and surface water can promote microbial diversity, which facilitates a range of metabolic processes, such as denitrification, oxidation of minerals, precipitation of metal oxides, and bioremediation of organic compounds (Grimm and Fisher, 1984; Jones and Holmes, 1996; Conant, Cherry and Gillham, 2004; Landmeyer *et al.*, 2010; Nogaro *et al.*, 2013; Boano *et al.*, 2014; Wu, Wang and He, 2017). The resultant biochemical reactions associated with improved hyporheic exchange can strongly influence benthic macroinvertebrate communities and maintain the health of freshwater food webs (Boulton and Hancock, 2006; Boano *et al.*, 2014).

Increased hyporheic oxygenation can increase the depth at which macroinvertebrates can burrow into the substrate, and can also benefit aquatic plants by allowing roots to penetrate deeper into the river bed (Boulton and Hancock, 2006; Boano *et al.*, 2014). More deeply-rooted macrophytes further facilitate nutrient exchange and microbial communities as a result of transpiration, active transport, and the release of root exudates (Boano *et al.*, 2014; Wu, Wang and He, 2017). Deep-rooted vegetation is also less-likely to be uprooted during flood events, thereby reducing bank erosion and sediment flux downstream (Swanson *et al.*, 2017).

Increased hyporheic oxygen levels can positively impact freshwater mussel populations by increasing the survival rate of juvenile mussels (Buddensiek, 1995; Geist and Auerswald, 2007; Klos, Rosenberry and Nelson, 2014; Scheder *et al.*, 2015), which may imply that recruitment is more likely to happen in areas of river bed previously bioturbated by mussels. This may depend on the grain-size distribution of the river bed sediment, as mussel bioturbation of the sand and gravel-dominated substrate used in the flume investigation did not result in improved hyporheic

oxygen levels. However, it is probable that sand and gravel-dominated rivers with very low percentages of fines would already show high levels of hyporheic exchange and substrate oxygen levels due to the relatively higher permeability of the substrate.

The increased percentage of fine-grained sediment in the substrate, observed with mussels in the flume, could potentially prove detrimental to host fish such as salmonids, which require well-oxygenated river sediments for spawning (Beschta and Jackson, 1979; Chapman, 1988; Malcolm *et al.*, 2009). However, the results of the river study found non-significant differences in the percentage of substrate fines, and the increased interstitial oxygen levels from bioturbation would likely provide more favourable conditions for spawning host fish. The maintenance of well-oxygenated hyporheic environments brought about by healthy unionid populations may therefore be critical to the long-term survival of freshwater mussels.

Due to the differing results for hyporheic oxygen observed in the flume and river investigations, the *Anodonta* species investigated in these studies may be of most benefit to improving oxygen levels in river beds with higher proportions of fine, cohesive sediment. The mean percentage of fines in the flume substrate throughout the investigation was only 0.8%, whereas the mean percentage of fines in both Markeaton Brook and the River Sence during the river investigation was 11-12%. The minimum percentage of substrate fines required for these species to beneficially impact hyporheic oxygen levels is therefore likely to fall between these two values, but should be tested in a natural river setting where other biotic and abiotic interactions impacting the results can be accounted for. More research is required to determine which river bed grain-size distributions respond most positively to the presence of mussels, in terms of oxygen levels, to provide a more targeted approach to river bioremediation and habitat restoration projects involving freshwater mussels.

5.3.2 Influence on sediment flux

The filtration and biodeposition of fine sediment from the water, and the increased mobilisation of sand and gravel by mussels, as evidenced in this research, has implications that are far-ranging in nature. Increased mixing and mobilisation of coarse sand and gravel could be of benefit to species that require gravel patches on the surface of the river bed for shelter and spawning. The improved water clarity would be of benefit to a variety of freshwater organisms such as macrophytes, phytoplankton, grazers and filter-feeders, whereas the biodeposition of organic matter on the river bed would provide food to benthic macroinvertebrates.

Increased mobilisation of sand and gravel may have implications for river channel morphology and flood management, as the erosion of sand and gravel may increase the channel capacity of reaches with mussels, thus reducing the risk of flooding in those areas. Conversely, accumulation of sand and gravel downstream of mussel beds could locally reduce channel capacity, resulting in an increased risk of flooding along those river reaches. Alterations to channel morphology in the vicinity of, and downstream of mussel beds would also have influences on stream velocities, bed shear stresses and turbulence that may affect rates of erosion and deposition, and the likelihood of mussels being entrained during high discharge events. As freshwater mussels tend to form patchy aggregations in river environments (Strayer, 1999; Daraio, 2010), the differential rates of erosion and deposition in mussel patches, compared with reaches free from mussels, may contribute to overall habitat heterogeneity.

Although mussels were found to increase critical boundary shear stresses in the flume, they also significantly reduced water velocities, which would ordinarily be associated with a reduction in boundary shear stresses. Observations of the mussels in the flume showed increased mobilisation of sand and gravel when the mussels where actively burrowing in the sediment, and very little movement of sediment when they were stationary, although this is not supported by empirical data. It is therefore possible that the lower velocities in the flume would have resulted in reduced

shear stresses when the mussels were inactive, and increased shear stresses when they were burrowing or moving around on the substrate. As the sediment trap was not analysed until the end of the study, periods of reduced shear stresses may not have been represented in the sediment trap data, as the episodes of rapid mobilisation of grains may have been followed by periods of significantly reduced sediment transport. However, the presence of fine laminations of organic matter in the sediment trap provide evidence that periods of low critical boundary shear stress did occur with mussels in the flume.

Due to the contrasting results for water velocities and critical boundary shear stresses in the flume investigation, it is difficult to predict exactly how macroinvertebrate species with different tolerances to flow would be effected by the presence of mussels. The reduced water velocities would likely benefit rheophobic species, but the increased critical shear stresses indicated by the sediment trap data may be detrimental to them. It is also uncertain from the sediment trap data precisely how much of the increased flux of sand and gravel was a direct result of higher bed shear stresses, and how much was due to reduced entrainment thresholds resulting from the mussels' disturbance of grain fabrics, sedimentary structures and biofilm development.

It is probable that during periods of active mussel bioturbation, macroinvertebrate species in the immediate vicinity of the mussels would be at an increased risk of entrainment. However, based on the sediment trap data, mean critical boundary shear stress with mussels in the flume did not exceed 0.48 Nm^{-2}. Experiments with the cased caddisfly *Potamophylax latipennis* (Curtis, 1834) have shown them unlikely to be entrained at bed shear stresses of 0.53 Nm^{-2}, with shear stresses of 3.69 Nm^{-2} required to significantly increase the probability and distance of entrainment (Lancaster *et al.*, 2006).

As the critical boundary shear stress data for this project were obtained using a substrate with different characteristics to natural river sediment, and subject to different biotic and abiotic influences, it is difficult to directly equate the data with sediment flux in a natural river

environment. Mussel behaviour may also have been affected by the different conditions in the flume compared with their natural habitat, which may have given rise to increased or decreased burrowing behaviour, or impacted rates of mussel filter-feeding. Additionally, the relatively short duration of the study could have affected the results for sediment flux, as rates of bioturbation may have changed over time. Therefore for the mussels influence on sediment flux to be more effectively applied in hydrological modelling or river management scenarios, it is recommended that sediment flux data be gathered from the mussels' natural river habitat to be more certain of true nature of the effects. Hydrological modelling should also account for variability in mussel behaviour in natural river settings, as burrowing activity is likely to fluctuate depending on hydrological conditions and time of year, thus sediment flux data encompassing periods of at least a year in duration would be preferable.

The reduced dissolved and suspended load in the water as a result of mussel filtration has a variety of potential applications, such as in water treatment, aquaculture, river restoration, and river management. Moreover, not only were unionids found to capture and deposit fine and organic sediment, they were also mixing it into the subsurface, thereby reducing the likelihood of its resuspension, and transport downstream. The capture and burial of organic matter by unionids could represent a key aspect of carbon sequestration in freshwater ecosystems, and thus deserves further investigation. As unionids can constitute a significant proportion of freshwater benthic biomass (Mann, 1964; Negus, 1966; Spooner and Vaughn, 2006), their potential importance in landscape-wide climate change mitigation strategies should be considered.

5.3.3 Improved habitat heterogeneity

The results of the river and flume investigations indicate that freshwater mussels increased the textural heterogeneity of river bed sediment. Mussels also increased the longitudinal profile length of the flume substrate, which suggests an increase in topographic roughness. The

combined effects of biodeposition and bioturbation by mussels created a complex patchwork of gravels and coarse sand, interspersed with areas of fine-grained sediments and mussel biodeposits. In a river environment, this substrate heterogeneity would provide a mosaic of microhabitats that could potentially increase the range of species that the river bed supports.

Higher levels of habitat complexity are associated with increased species diversity in rivers, due to the wider variety of ecological niches supported by the increased range refugia and substrates (Luck *et al.*, 2010; Reid, Brierley and Boothroyd, 2010; Platt, 2011; Wyżga *et al.*, 2012; Bellmore and Baxter, 2014; Wohl, 2016). Increased substrate complexity can also increase the resilience of freshwater communities to fluctuating hydrological conditions (Luck *et al*., 2010; Wyżga *et al*., 2012; Wohl, 2016). Additionally, the more uneven and heterogeneous river bed, and the protrusion of the mussels shells into the water column may provide zones of reduced water velocities enabling more rheophobic macroinvertebrate species to shelter and feed.

Scoured patches of gravel may provide more favourable substrate for juvenile mussels and spawning fish (Beschta and Jackson, 1979; Chapman, 1988; Buddensiek, 1995; Geist and Auerswald, 2007; Malcolm *et al*., 2009; Klos, Rosenberry and Nelson, 2014; Scheder *et al*., 2015), whereas accumulations of mussel biodeposits would provide nutrients for producers and detritivores, and thus benefit other trophic levels (Hakenkamp and Palmer, 1999; Vaughn and Hakenkamp, 2001; Beekey, Mccabe and Marsden, 2004; Vaughn, Nichols and Spooner, 2008). The development and enhancement of diverse patchworks of habitat-mosaics, within rivers, is considered an integral component of nature-based river restoration (Addy *et al.*, 2016). The ability of unionids to improve the heterogeneity of river-bed environments is indicative of their potential value in nature-based solutions to river management.

5.3.4 Relevance to freshwater mussel conservation and river remediation

To date, the main focus of freshwater mussels conservation, and river restoration projects involving mussels in Europe has been on *Margaritifera margaritifera* (Linnaeus 1758), as discussed in Section 1.2.5. However, the positive effects on water quality and habitat conditions demonstrated by this research indicate that *Anodonta* species may provide important ecosystem services in river habitats. Due to their relative abundance, and faster rates of recruitment compared with *M. margaritifera* (Quinlan *et al.*, 2015; Lavictoire *et al.*, 2020; IUCN, 2022), they could potentially be deployed more rapidly and extensively in river remediation projects than their more endangered counterparts. Furthermore, due to the increased tolerance of *Anodonta* species to a wider range of hydrological conditions (Killeen, Aldridge and Oliver, 2004, p. 49; Lopes-Lima *et al*., 2017), and their ability to utilise a broader range of host fish species (Bauer and Hochwald, 1991; Huber and Geist, 2017), they could provide benefit to rivers that are not suitable for *M. margaritifera.* This may be particularly pertinent given the current concerns over sewage discharge and water quality in UK rivers (The Rivers Trust, 2023).

The capacity for *Anodonta* to reduce water turbidity, TDS, and the downstream flux of fine and organic sediment could be of potential benefit in river restoration projects where reduction in suspended sediment loads or high dissolved nutrient loads are a management objective. *Anodonta* species may also positively influence hyporheic oxygen levels in rivers with grain-size distributions comparable to those found in Markeaton Brook and the River Sence, containing moderate amounts of silt and clay. It is possible that they may improve hyporheic oxygen levels in a broader range of river bed sediment types, although the results of the flume study suggest that oxygen levels in sediments containing very small quantities of silt and clay may not respond positively to the presence of mussels.

A key feature of many degraded river environments is a lack of habitat heterogeneity as a consequence of channelisation, dredging and siltation (Sime *et al.*, 2017; Frainer *et al.*, 2018;

JNCC, 2019). As a result, many river remediation projects have incorporated strategies to restore habitat heterogeneity, such as the reintroduction of gravel, cobbles, boulders and woody debris into the channel, removal of engineering works along river banks, planting of riparian vegetation, and working with landowners to reduce siltation (Natural England, 2014b, 2014a, 2022; Addy *et al.*, 2016; Sime *et al.*, 2017; Frainer *et al.*, 2018; JNCC, 2019). The results of the river and flume investigations indicate that *Anodonta* species may provide a valuable contribution to improving heterogeneity of rivers in such situations, by increasing sediment mobility and providing patches of coarser sand and gravel, as well as patches of finer sediments on the river bed. The more irregular and uneven river bed topography, and the protrusion of the mussels shells into the water column would also contribute to overall habitat heterogeneity, which may increase the range of species that the river can support (Luck *et al.*, 2010; Reid, Brierley and Boothroyd, 2010; Platt, 2011; Wyżga *et al.*, 2012; Bellmore and Baxter, 2014; Wohl, 2016).

Although the *Anodonta* species investigated in this project are classified as "Least Concern" according to the IUCN, their populations in Britain and Europe are in decline (IUCN 2022). The results of the river and flume investigations indicate that these species may be valuable ecosystem engineers in river environments, yet they lack the government protections and conservation resources directed at maintaining and restoring *M. margaritifera* populations. For these reasons, the translocation of *Anodonta* species, or other unionid species into rivers for the purpose of habitat remediation should be carefully considered in terms of the suitability of the river habitat for those species, and the probability of their long-term survival. Translocation of mussels may have an improved chance of success in rivers with historical populations of mussels, as that may indicate that hydrological and sedimentological conditions may be suitable for them. Alternatively, the suitability of a river environment could be tested with a pilot study involving a small number of mussels to assess their tolerance to varying hydrological conditions in the river.

5.4 Limitations of the research and opportunities for further study

The conclusions drawn from this thesis highlight a range of potential opportunities for further study, with regard to the impact of unionids on river sediment. The limited spatial scale of the studies in Markeaton Brook, the River Sence, and the flume investigation restricted the amount of sediment samples and macroinvertebrate kick samples that could be taken without causing significant disturbance to the substrate. The small sample sizes for the investigations reduced the reliability of the data, and thus reduced the probability of obtaining statistically significant results for the variables tested.

To avoid causing severe disturbance to the mussels and river bed sediment within each quadrat in the river study, kick samples were taken from the river bed immediately downstream of each quadrat rather than inside it. The rationale for this was that macroinvertebrates downstream of the mussel-containing quadrats may be affected by any changes to water velocity, water quality and sediment flux relating to the presence of mussels, and may have been influenced by downstream transport of mussel biodeposits. However, the macroinvertebrates sampled were not in direct proximity to the mussels, their bioturbation of the river bed, or their influence on bed shear stresses. Furthermore, there were mussels present in the kick sample area downstream of quadrats where mussels had been removed from the river bed. Those mussels would have been influencing conditions on that part of the river bed, which may also have affected the diversity and abundance of macroinvertebrates in those areas. This likely impacted the accuracy and reliability of the datasets for macroinvertebrate abundance and the BMWP scores.

The small size of the quadrats in the river study, and the small cross-sectional area of the flume channel, coupled with the requirement to take regular samples of the substrate also influenced the methods used for sediment sampling. Using the modified syringe to extract sediment cores limited the depth from which grain-size distributions, inorganic carbon and organic carbon could be sampled. A combination of fluvial processes and mussel bioturbation in the flume meant that

some fine and organic sediment appeared to be buried deeper in the substrate than the length of the core sampler, based on viewing the substrate through the glass walls of the flume. This meant that this sediment was not accounted for when assessing the mussels influence on the lateral and vertical flux of this material. The chosen coring method also failed to preserve the vertical profile of the river bed sediment, which would have been possible with freeze-coring methods (Petts *et al*., 1989; Young *et al*., 1991; Schuett-Hames *et al*., 1996). The use of freeze-corers would have provided improved understanding of how mussels influence the vertical distribution of sediment and organic matter. Freeze-coring methods may therefore be more appropriate for future investigations of mussel translocation projects involving more extensive reaches of rivers.

To avoid excessive disturbance to the river bed, the armour layer was only sampled at the beginning and the end of the river study. This provided a more limited dataset and made it less clear whether any observed differences between quadrats were as a direct result of mussel activity or a result of fluvial processes. This might have been resolved with the use of less invasive technologies such as laser scanning or optical granulometry (Heritage and Milan, 2009; Langhammer *et al.*, 2017; Pearson *et al.*, 2017; Woodget, Fyffe and Carbonneau, 2018), which would have enabled more regular sampling of surficial sediments. The use of laser scanners would also have enabled the generation of Digital Elevation Models (DEMs) of the river bed and flume substrate surface, which would have provided much more precise data to visualise three-dimensional changes to river bed topography, bed roughness and surface elevation (Milan, Heritage and Hetherington, 2007; Heritage and Milan, 2009; Milan *et al*., 2011;Woodget, Fyffe and Carbonneau, 2018).

River discharge was not measured during the river investigation, as the comparative quadrat method considered the relative change in variables between control and experimental quadrats, which were both subject to the same variations in discharge and sediment supply. However measuring discharge at each site throughout the study would have provided more insight into sedimentological and hydrological changes in each quadrat, and if quadrats responded differently

to fluctuations in discharge. The absence of any river gauging stations near the chosen sites also made it difficult to correlate changes observed in each river to varying levels of discharge.

The river investigation had to be terminated early as many of the translocated mussels were washed away during periods of increased discharge. This meant that the long-terms effects of the presence of mussels on river sediments may not have been fully realised. The use of metal cages or trays to prevent the mussels being washed away was avoided in this study due to the influence that such structures may have on mussel behaviour and sedimentary processes. However, if they had been used it may have been possible run the experiment for a longer duration, with less risk to the translocated mussels. Alternatively, the feasibility of translocating mussels to a new river site could be more thoroughly tested by comparing hydrological and sedimentological parameters at the site to those in established mussel beds over the course of a year or more to ensure the conditions are suitable for long-term mussel survival.

The data from the flume investigation indicated that *A. anatina* increased the erosion of sand and gravel, and increased critical boundary shear stresses compared with the control study. However, this may have been an artefact of the mussels being introduced to a new environment, causing them to move around the flume in an attempt to find the most favourable position. Continuing the experiment over longer periods of time with better quantification of how the rate of sediment flux varied over time would have improved the investigation. The reliability of the flume data could also have been improved with additional repeats of the investigation, and could have been expanded to test the effects of varying parameters such as substrate grain-size composition, water velocities, mussel density and mussel species. Additionally, more insight into how mussels interacted with the substrate and how behaviour changed over time could have been provided with the use of underwater video cameras, such as that described by Pledger (2017) for monitoring foraging fish.

The low percentage of fine, cohesive sediment, the lack of cobbles and boulders, and the homogenised nature of the substrate may have influenced the erosion and transport of sediment in the flume. The absence of stratification, cross-stratification and particle sorting in the flume substrate may have also affected critical entrainment thresholds. Furthermore, the lack of variability in discharge and sediment supply may have affected mussel behaviour and the development of bedforms in the flume.

Another limitation of the flume investigation was that the preliminary studies for the river and flume investigations were carried out in the reach of Markeaton Brook designated "Site 1" (Figure 2.2, Table 2.1, Section 2.2.2), whereas the river investigation was carried out at nearby Sites 2 and 4 (Figure 2.2, Table 2.1, Section 2.2.2). Site 1 was chosen as it was immediately downstream of Site 2 and contained similar densities of *A. anatina and A. cygnea*. It also appeared to have a similar depth and width, with similar hydrological conditions. However, the results of the preliminary study to determine grain-size distributions for the flume substrate (described in Section 2.3.2) indicated that the proportion of silt and clay in the river bed sediment was only 2.5%. The results of the subsequent river investigation indicated a mean percentage of 11-12% fines in the Markeaton Brook bed sediment, which was significantly different to the preliminary study.

The large discrepancy between the results of the preliminary study and the main river investigation suggest there were differences in hydrological and sedimentological conditions at these sites. It is also possible that grain-size distributions in the preliminary study may have been influenced by the different time of year that the samples were collected, as differing antecedent conditions could have resulted in differential scouring of fines from the river bed.

The results of the preliminary study influenced the sample sizes and methodologies chosen for the river investigation. However, given the sedimentological differences between the sites, the conclusions of the preliminary studies may have been different if they were carried out at Site 2 or

4. The low quantities of fine sediment shown in the results of the preliminary study also influenced the decision not to add any fine sediment to the flume substrate, with the exception of the introduced seston, which resulted in a mean percentage of fines of 0.8% over the duration of the study. If the proportion of fine sediment in the flume study was more comparable to that found in the river investigation, the conclusions from the flume study may have been different.

Biochemical effects associated with microbial, macroinvertebrate and plant communities in natural stream environments can influence sediment transport through electrostatic interactions, precipitation of minerals, cementation processes, and biofilm development (Battin and Sengschmitt, 1999; Battin *et al.*, 2016). The absence of mineralisation, biota and microbiota in the original flume substrate mixture could have affected sediment mobility and hyporheic oxygen levels. Although seston was introduced to the flume at regular intervals, the short duration of the study would have limited the amount of biofilm development that could have occurred during the investigation. Likewise, the use of relatively sterile dechlorinated tap water, rather than river water in the flume, may have hindered the development of a biofilm layer on the sediment surface. It is possible that greater biofilm development on the substrate surface would have increased sediment stability, which would have increased critical entrainment thresholds.

The potential impact of freshwater mussels on biofilm development would be of interest for further study, as their presence may accelerate the formation of biofilms, as a result of the biodeposition of organic matter on the river bed. Alternatively, bioturbation by mussels may inhibit the formation of stable biofilms. The use of sediment and water extracted from a natural river environment may have provided more insight into the influence of unionids on biofilm development and sediment stability.

Measurements of sediment transport in the river investigation would have improved confidence in the sediment flux data so that it could be more effectively applied in river management scenarios. It is probable that a wide variety of biotic and abiotic factors present in natural environments serve

to amplify or diminish the impact of mussels on rates of sediment flux. The presence of mussels may also encourage or deter other species with the capacity to modify sedimentological processes.

Sediment transport in the river investigation could have been quantified with the use of sediment traps and tracer particles on the river bed. Tracer particles and particle image velocimetry (PIV) could also have been utilised in the flume investigation to improve understanding of the mussels influence of sediment flux, hydrodynamics and turbulence (Kumar *et al*., 2019). Furthermore, the use of tracers could have helped quantify the vertical mixing of particles resulting from fluvial processes and mussel bioturbation (Mahaut and Graf, 1987; Solan *et al.*, 2004).

An area of research priority would be an assessment of the carbon sequestration capacity of unionids. The burial of organic (and inorganic) carbon captured from the water, as well as burial of carbonate shell material by mussels, may represent a significant component of carbon cycling, and provide a potential tool in the mitigation of climate change. The modification of the composition, texture and oxygen levels of river sediments by unionids may also have implications with regard to taphonomy, and the fossil record, which would be worthy of future study.

5.5 Closing remarks

The results and conclusions drawn from this research provide greater insight into the role of freshwater mussels as ecosystem engineers of freshwater environments. Freshwater mussels were shown to facilitate the exchange of sediment, oxygen and carbon from the pelagic to the benthic and hyporheic environments, and significantly influence water velocities, critical boundary shear stresses, and the erosion, transport and deposition of sediment. Improved water clarity, hyporheic oxygen availability, and the biodeposition of fine and organic matter by mussels would be of benefit to a range of freshwater species, and may have important implications with regard to carbon cycling and the management and restoration of river habitats.

Freshwater mussels were shown to improve habitat heterogeneity by altering the spatial distribution of sediment grain-sizes on the river bed. The increased topographic and textural complexity of the river bed resulting from mussel bioturbation and biodeposition, would likely increase the availability of microhabitats in fluvial benthic environments. The findings of this project demonstrate the intrinsic value of our more common, yet declining unionid species, and highlight the importance of maintaining healthy populations of these mussels. The positive influence of freshwater mussels on river bed conditions, as demonstrated by this research, validates their status as ecosystem engineers, and underlines their important role in the sediment and nutrient dynamics of fluvial systems.

References

Addy, S., Cooksely, S., Dodd, N., Waylen, K., Stockan, J., Bigg, A. and Holstead, K. (2016) *River restoration and biodiversity: Nature-based solutions for restoring rivers in the UK and Republic of Ireland.* Available at: http://www.crew.ac.uk/sites/default/files/sites/default/files/publication/River Restoration and Biodiversity_for web_high.pdf (Accessed: 21 September 2019).

Akhurst, D.J., Clark, M.W., Reichelt-Brushett, A. and Jones, G.B. (2011) Grain size normalization: A case for post extraction normalization and inclusion of selective extraction procedures, *Limnology and Oceanography: Methods*, 95(5), pp. 215–231.

Aldridge, D.C. (1999) The morphology, growth and reproduction of Unionidae (bivalvia) in a fenland waterway, *Journal of Molluscan Studies*, 65, pp. 47–60.

Aldridge, D.C., Fayle, T.M. and Jackson, N. (2007) Freshwater mussel abundance predicts biodiversity in UK lowland rivers, *Aquatic Conservation-Marine and Freshwater Ecosystems*, 17, pp. 554–564.

Aldridge, D.C. and McIvor, A.L. (2003) Gill evacuation and release of glochidia by *Unio pictorum* and *Unio tumidus* (Bivalvia : Unionidae) under thermal and hypoxic stress, *Journal of Molluscan Studies*, 69, pp. 55–59.

Aldridge, D.W., Payne, B.S. and Miller, A.C. (1987) The effects of intermittent exposure to suspended solids and turbulence on three species of freshwater mussels, *Environmental Pollution*, 45(1), pp. 17–28.

Allen, D.C. and Vaughn, C.C. (2011) Density-dependent biodiversity effects on physical habitat modification by freshwater bivalves, *Ecology*, 92(5), pp. 1013–1019.

Allen, J.R.L. and Thornley, D.M. (2004) Laser granulometry of Holocene estuarine silts: Effects of hydrogen peroxide treatment, *Holocene*, 14(2), pp. 290–295.

Almedeij, J. and Diplas, P. (2005) Bed load sediment transport in ephemeral and perennial gravel bed streams, *Eos, Transactions American Geophysical Union*, 86(44), p. 429.

Ashley, G.M. (1990) Classification of large-scale subaqueous bedforms: a new look at an old problem, *Journal Of Sedimentary Petrology*, 60(1), pp. 160–172.

Atkinson, C.L., First, M.R., Covich, A.P., Opsahl, S.P. and Golladay, S.W. (2011) Suspended material availability and filtration-biodeposition processes performed by a native and invasive bivalve species in streams, *Hydrobiologia*, 667(1), pp. 191–204.

Atkinson, C.L. and Forshay, K.J. (2022) Community patch dynamics governs direct and indirect nutrient recycling by aggregated animals across spatial scales, *Functional Ecology*, 36(3), pp. 595–606.

Augspurger, T., Keller, A.E. and Black, M.C. (2003) Water quality guidance for protection of freshwater mussels (Unionidae) From ammonia exposure, *Environmental Toxicology and Chemistry*, 22(11), pp. 2569–2575.

Barber, I., Nairn, D. and Huntingford, F.A. (2001) Nests as ornaments: revealing construction by male sticklebacks, *Behavioral Ecology*, 12(4), pp. 390–396.

Barnhart, M.C., Haag, W.R. and Roston, W.N. (2008) Adaptations to host infection and larval parasitism in Unionoida, *Journal of the North American Benthological Society*, 27(2), pp. 370–394.

Battin, T.J., Besemer, K., Bengtsson, M.M., Romani, A.M. and Packmann, A.I. (2016) The ecology and biogeochemistry of stream biofilms, *Nature Reviews Microbiology*, 14(4), pp. 251–263.

Battin, T.J. and Sengschmitt, D. (1999) Linking sediment biofilms, hydrodynamics, and river bed clogging: Evidence from a large river, *Microbial Ecology*, 37(3), pp. 185–196.

Baudinet, D., Alliot, E., Berland, B., Grenz, C., Plantecuny, M.R., Plante, R. and Salen-Picard, C. (1990) Incidence of mussel culture on biogeochemical fluxes at the sediment-water interface, *Hydrobiologia*, 207, pp. 187–196.

Bauer, G. and Hochwald, S. and W.S. (1991) Spatial distribution of freshwater mussels: the role of host fish and metabolic rate, *Freshwater Biology*, 26, pp. 377–386.

Beckett, D.C., Green, B., Thomas, S. and Miller, A. (1996) Epizoic invertebrate communities on upper Mississippi River unionid bivalves, *American Midland Naturalist*, 135(1), pp. 102–114.

Beekey, M.. Mccabe, D.J. and Marsden, J.E. (2004) Zebra mussel colonization of soft sediments facilitates invertebrate communities, *Freshwater Biology*, 49, pp. 534–545.

Bellmore, J.R. and Baxter, C. (2014) Effects of geomorphic process domains on river ecosystems: A comparison of floodplain and confined valley segments, *River Research and Applications*, 30, pp. 617–630.

Benelli, S., Bartoli, M., Rachetti, E., Moraes, P.C., Zilius, M., Lubiene, I. and Fano, E.A. (2017) Rare but large bivalves alter benthic respiration and nutrient recycling in riverine sediments, *Aquatic Ecology*, 51(1), pp. 1–16.

Benito-Reyes, E., Alvarez-Codesal, S. and Sweeting, R. (2015) Variability within pearl mussels populations and their lifecycle (in captivity), *The Freshwater Biological Association.* Available at: https://www.researchgate.net/publication/281448568 (Accessed: 18 June 2017).

Beschta, R.L. and Jackson, W.L. (1979) The intrusion of fine sediments into a stable gravel bed, *Journal of the Fisheries Research Board of Canada*, 36(2), pp. 204–210.

Best, J. (2005) The fluid dynamics of river dunes: A review and some future research directions, *Journal of Geophysical Research*, 110, pp. 1–21.

Beukema, J. J. & Cadee, G.C. (1996) Consequences of the sudden removal of nearly all mussels and cockles from the Dutch Wadden sea, *Marine Ecology*, 17 (1-3), pp. 279-289.

Beuselinck, L., Govers, G., Poesen, J., Degraer, G. and Froyen, L. (1998) Grain-size analysis by laser diffractometry: Comparison with the sieve-pipette method, *Catena*, 32(3–4), pp. 193–208.

Bhattacharyya, A., Ojha, S.P. and Mazumder, B.S. (2013) Evaluation of the saltation process of bed materials by video imaging under altered bed roughness, *Earth Surface Processes and Landforms*, 38(12), pp. 1339–1353.

Bially, A. and MacIsaac, H.J. (2000) Fouling mussels (*Dreissena* spp.) colonize soft sediments in Lake Erie and facilitate benthic invertebrates, *Freshwater Biology*, pp. 85–97.

Binelli, A., Magni, S., Soave, C., Marazzi, F., Zuccato, E., Castiglioni, S., Parolini, M. and Mezzanotte, V. (2014) The biofiltration process by the bivalve *D. polymorpha* for the removal of some pharmaceuticals and drugs of abuse from civil wastewaters. *Ecological Engineering*, 71, pp. 710–721.

Black, E.M., Chimenti, M.S. and Just, C.L. (2017) Effect of freshwater mussels on the vertical distribution of anaerobic ammonia oxidizers and other nitrogen-transforming microorganisms in upper Mississippi river sediment, *PeerJ*, 5, p. e3536.

Boano, F., Harvey, J.W., Marion, A., Packman, A.I., Revelli, R., Ridolfi, L. and Wolman, A. (2014) Hyophreic flow and transport processes: Mechanisms, models, and biogeochemical implications, *Reviews of Geophysics*, 52(4), pp. 603–679.

Boeker, C., Lueders, T., Mueller, M., Pander, J. and Geist, J. (2016) Alteration of physico-chemical and microbial properties in freshwater substrates by burrowing invertebrates, *Limnologica*, 59, pp. 131–139.

Bogan, A.E. (1993) Freshwater bivalve extinctions (Mollusca: Unionoida): A Search for Causes, *American Zoologist*, 33, pp. 599–609.

Bolden, S.R. and Brown, K.M. (2002) Role of stream, habitat, and density in predicting translocation success in the threatened Louisiana pearlshell, *Margaritifera hembeli* (Conrad), *Journal of the North American Benthological Society*, 21(1), pp. 89–96.

Bontes, B.M., Verschoor, A., Dionisio Pires, L.M., Van Donk, E. and Ibelings, B.W. (2007) Functional response of *Anodonta anatina* feeding on a green alga and four strains of cyanobacteria, differing in shape, size and toxicity, *Hydrobiologia*, 584(1), pp. 191–204.

Boulton, A.J. and Hancock, P.J. (2006) Rivers as groundwater-dependent ecosystems: A review of degrees of dependency, riverine processes and management implications, *Australian Journal of Botany*, 54(2), pp. 133–144.

Brainwood, M., Burgin, S. and Byrne, M. (2008) The role of geomorphology in substratum patch selection by freshwater mussels in the Hawkesbury-Nepean River (New South Wales) Australia, *Aquatic Conservation-Marine and Freshwater Ecosystems*, pp. 1285–1301.

Brim Box, J., Dorazio, R.M. and Liddell, W.D. (2002) Relationships between streambed substrate characteristics and freshwater mussels (Bivalvia : Unionidae) in coastal plain streams relationships between streambed substrate characteristics and freshwater mussels (Bivalvia :

Unionidae) in coastal plains, *Journal of The North American Benthological Society*, 21(2), pp. 253–260.

Brim Box, J. and Mossa, J. (1999) Sediment, land use, and freshwater mussels : prospects and problems, *Journal of The North American Benthological Society*, 18(1), pp. 99–117.

British Geological Survey (2019) *BGS Geological map data*. Available at: https://digimap.edina.ac.uk/roam/map/geology (Accessed: 11 October 2019).

Brush, L.M. and Nolan, T.B. (1961) Drainage basins, channels, and flow characteristics of selected streams in Central Pennsylvania. *U.S. Geological Survey Professional Paper 282-F*. Washington D.C, pp. 145-162.

Buddensiek, V., Fleischauer-Rossing, S. and Wachtler, K. (1990) Studies on the chemistry of interstitial water taken from defined horizons in the fine sediments of bivalve habitats in several northern German lowland waters, *Archiv Für Hydrobiologie*, 127, pp. 151–166.

Buddensiek, V. (1995) The culture of juvenile freshwater pearl mussels *Margaritifera margaritifera* L. in cages: A contribution to conservation programmes and the knowledge of habitat requirements, *Biological Conservation*, 74(1), pp. 33–40.

Bunte, K. and Abt, S.R. (2001) Sampling surface and subsurface particle-size distributions in wadable gravel-and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring, *General technical report, United States Department of Agriculture, Forest Service. 1-430* [Preprint].

Burge, C.A., Closek, C., Friedman, C., Groner, M., Jenkins, C., Shore-Maggio, A. and Welsh, J. (2016) The use of filter-feeders to manage disease in a changing world, *Integrative and Comparative Biology*, 56(4), pp. 573–587.

Burlakova, L.E., Karatayev, A.Y. and Karatayev, V.A. (2012) Invasive mussels induce community changes by increasing habitat complexity, *Hydrobiologia*, 685, pp. 121–134.

Cairngorms National Park (2005) *Cairngorms National Park News, Pearl mussels re-introduced at secret sites in the Cairngorms National Park*. Available at: https://cairngorms.co.uk/pearl-mussels-re-introduced-at-secret-sites-in-the-cairngorms-national-park/ (Accessed: 7 February 2022).

Carbonneau, P.E., Bergeron, N. and Lane, S.N. (2005) Automated grain size measurements from airborne remote sensing for long profile measurements of fluvial grain sizes, *Water Resources Research*, 41(11), pp. 1–9.

Carla L. Atkinson, Matt R. First, Alan P. Covich, S.P.O. and Golladay, S.W. (2011) Suspended material availability and filtration – biodeposition processes performed by a native and invasive bivalve species in streams, *Hydrobiologia*, 667, pp. 191–204.

Cesari, P. and Pellizzato, M. (1990) Biology of *Tapes philippinarum*, in *Tapes philippinarum: Biologia e Sperimentazione*, Ente di Sviluppo Agricolo, Venice, pp. 21-46.

Chamberlain, J.A. (2007) The Devonian Bivalve, *Archanodon catskillensis* : A status report on the first freshwater mussel from New Jersey, in *Contributions to the Paleontology of New Jersey (II)*, pp. 24–41.

Chapman, D.W. (1988) Critical review of variables used to define effects of fines in redds of large salmonids, *Transactions of the American Fisheries Society*, 117(1), pp. 1–21.

Church, M. (2006) Bed material transport and the morphology of alluvial river channels, *Annual Review of Earth and Planetary Sciences*, 34(1), pp. 325–354.

Clements, E.A. (2015) Host choice and behavioural responses to flow change in the freshwater pearl mussel, *Margaritifera Margaritifera*, in Scotland, *University of Glasgow*, MSc Thesis.

Cohen, R.R.H., Dresler, P., Phillips, E. and Cory, R. (1984) The effect of the Asiatic clam, *Corbicula fluminea*, on phytoplankton of the Potomac River, Maryland, *Limnology and Oceanography*, 29(1), pp. 170–180.

Conant, B., Cherry, J.A. and Gillham, R.W. (2004) A PCE groundwater plume discharging to a river: Influence of the streambed and near-river zone on contaminant distributions. *Journal of Contaminant Hydrology*, 73(1–4), pp. 249–279.

Cope, W.G. and Waller, D.L. (1995) Evaluation of freshwater mussel relocation as a conservation and management strategy, *Regulated Rivers: Research & Management*, 1, pp. 147–155.

Corenblit, D., Steiger, J., Gurnell, A. and Tabacchi. E. (2007) Darwinian origin of landforms, *Earth Surface Processes and Landforms*, 32, pp. 2070–2073.

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

Coulter, B. (2011) *LS 13 320 Laser Diffraction Particle Size Analyzer Instructions For Use*. PN B05577A. Beckman Coulter, Inc. 250 S. Kraemer Blvd, Brea, CA 92821.

Crooks, J.A. (2002) Characterizing ecosystem-level consequences of biological invasions: the role of ecosystem engineers, *Oikos*, 297(2), pp. 153–166.

Cushway, K.C., Ring, N., Patton, D and Woolnough, D. (2022) Landscape associations with native and invasive freshwater mussels, *Hydrobiologia*, 849, pp. 2449-2462.

Dame, R., Dankers, N., Prins, T., Jongsma, H. and Smaal, A. (1991) The influence of mussel beds on nutrients in the Western Wadden Sea and Eastern Scheldt Estuaries, *Estuaries*, 14(2), pp. 130–138.

Daraio, J., Weber, L.J. and Newton, T.J. (2010) Hydrodynamic modelling of juvenile mussel dispersal in a large river: the potential effects of bed shear stress and other parameters, *Journal of the North American Benthological Society*, 29(3), pp. 838–851.

DEFRA (2021) *England | Catchment Data Explorer, Department for Environment Food and Rural Affairs, Crown Copyright*. Available at: https://environment.data.gov.uk/catchment-planning (Accessed: 18 June 2023).

Devon Wildlife Trust (2021) *Freshwater pearl mussel project*. Available at: https://www.devonwildlifetrust.org/what-we-do/our-projects/freshwater-pearl-mussel-project (Accessed: 22 May 2022).

Dey, S. (2014) Fluvial hyrodynamics: Hydrodynamic and sediment transport phenomena, *GeoPlanet: Earth and Planetary Sciences*. Edited by P. Rowinski. Berlin: Springer-Verlag, pp. 189-259.

Dinis, P. and Castilho, A. (2012) Integrating sieving and laser data to obtain bulk grain-size distributions, *Journal of Sedimentary Research*, 82(9), pp. 747–754.

Diplas, P. and Fripp, J.B. (1992) Properties of various sediment sampling procedures, *Journal of Hydraulic Engineering*, 118(7), pp. 955–970.

Diplas, P. and Sutherland, A.J. (1988) Sampling techniques for gravel sized sediments, *Journal of Hydraulic Engineering*, 114(5), pp. 484–501.

Dittmann, S. (1990) Mussel beds—amensalism or amelioration for intertidal fauna?, *Helgoländer Meeresuntersuchungen*, 352(3), pp. 335–352.

Dobler, A.H., Hoos, P. and Geist, J. (2022) Distribution and potential impacts of non-native Chinese pond mussels *Sinanodonta woodiana* (Lea, 1834) in Bavaria, Germany, *Biological Invasions*, 24, pp. 1689-1706.

Duan, X., Wang, Z. and Tian, S. (2008) Effect of streambed substrate on macroinvertebrate biodiversity, *Frontiers of Environmental Science and Engineering in China*, 2(1), pp. 122–128.

Dück, Y., Lorke, A., Jokiel, C. and Gierse, J. (2019) Laboratory and field investigations on freeze and gravity core sampling and assessment of coring disturbances with implications on gas bubble characterization, *Limnology and Oceanography: Methods*, 17(11), pp. 585–606.

Dunbar, M.J., Warren, M., Extence, C., Baker L., Cadman, D,. Mould, D., Hall, J. and Chadd, R. (2010) Interaction between macroinvertebrates, discharge and physical habitat in upland rivers, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20 (S1), S31-S44.

Eckman, J. and Nowell, A. (1984) Boundary skin friction and sediment transport about an animal-tube mimic, *Sedimentology*, 31, pp. 851–862.

Engelund, F. and Hansen, E. (1967) A monograph on sediment transport in alluvial streams, *Teknisk Forlag*, p. 65.

Environment Agency (2019a) *Catchment Data Explorer*. Available at: https://environment.data.gov.uk/catchment-planning/RiverBasinDistrict/9 (Accessed: 18 October 2019).

Environment Agency (2019b) *Main River Map*. Available at: https://environment.maps.arcgis.com/apps/webappviewer/index.html?id=17cd53dfc524433980cc333726a56386 (Accessed: 11 October 2019).

Evans, E. and Wilcox, A.C. (2014) Fine sediment infiltration dynamics in a gravel-bed river following a sediment pulse, *River Research and Applications*, 30(3), pp. 372–384.

FBA (2022) *FBA Ark*. Available at: https://www.fba.org.uk/FBA/Public/Discover-and-Learn/Projects/The_Ark.aspx (Accessed: 6 February 2022).

FBA (2023) *Freshwater Pearl Mussel Ark — Freshwater Biological Association*. Available at: https://www.fba.org.uk/freshwater-species-research-and-recovery-programme/freshwater-pearl-mussel-ark (Accessed: 3 February 2023).

Fogelman, K.J., Stoeckel, J., Abdelrahman, H. and Helms, B. (2022) The relative importance of suspended versus benthic food resources to freshwater mussels in central Texas, U.S.A., *Freshwater Biology*, 67(6), 1063-1078.

Ford, N.B., Gullett, J. and May, M.E. (2009) Diversity and abundance of unionid mussels in three sanctuaries on the Sabine River in northeast Texas, *Texas Journal of Science*, 61(4), pp. 279–294.

Frainer, A., Polvi, L., Jansson, R. and McKie, B. (2018) Enhanced ecosystem functioning following stream restoration: The roles of habitat heterogeneity and invertebrate species traits, *Journal of Applied Ecology*, 55(1), pp. 377–385.

Friend, P.F. (1993) Control of river morphology by the grain-size of sediment supplied, *Sedimentary Geology*, 85(1–4), pp. 171–177.

Frostick, L.E., Lucas, P.M. and Reid, I. (1984) The infiltration of fine matrices into coarse-grained alluvial sediments and its implications for stratigraphical interpretation, *Journal of the Geological Society of London*, 141(8), pp. 955–965.

Fuentes, J., Gregorio, V, Giráldez, R. and Molares, J. (2000) Within-raft variability of the growth rate of mussels, *Mytilus galloprovincialis*, cultivated in the Ria de Arousa (NW Spain), *Aquaculture*, 189(1–2), pp. 39–52.

Gascho Landis, A.M., Haag, W.R. and Stoeckel, J.A. (2013) High suspended solids as a factor in reproductive failure of a freshwater mussel, *Freshwater Science*, 32(1), pp. 70–81.

Gasparini, N.M., Tucker, G.E. and Bras, R.L. (2004) Network-scale dynamics of grain-size sorting : implications for downstream fining, stream-profile concavity, and drainage basin morphology, *Earth Surface Processes and Landforms*, 29, pp. 401–421.

Geist, J. and Auerswald, K. (2007) Physicochemical stream bed characteristics and recruitment of the freshwater pearl mussel (*Margaritifera margaritifera*), *Freshwater Biology*, 52(12), pp. 2299–2316.

Gerino, M. (1990) The effects of bioturbation on particle redistribution in Mediterranean coastal sediment. Preliminary results, *Hydrobiologia*, 207(1), pp. 251–258.

Gibson, C. (FBA) (2018) Restoring freshwater mussel rivers, in. *19th Annual Network Conference Engaging with Rivers; River Restoration for Biodiversity*. Available at: https://www.therrc.co.uk/sites/default/files/files/Conference/2018/WorkshopC/fba_rfmrie_final.pdf (Accessed: 14 March 2023).

Giles, H. and Pilditch, C.A. (2006) Effects of mussel (*Perna canaliculus*) biodeposit decomposition on benthic respiration and nutrient fluxes, *Marine Biology*, 150, pp. 261–271.

Gillis, P.L., McInnis, R., Salerno, J., de Solla, S., Servos, M. and Leonard, E. (2017) Freshwater mussels in an urban watershed: Impacts of anthropogenic inputs and habitat alterations on populations, *Science of the Total Environment*, 574, pp. 671–679.

Gong, W., Jia, L., Shen, J. and Liu, J. (2014) Sediment transport in response to changes in river discharge and tidal mixing in a funnel-shaped micro-tidal estuary, *Continental Shelf Research*, 76, pp. 89–107.

Gopko, M., Mirnova, E., Pasternak, A., Mikheev, V. and Taskinen, J. (2017) Freshwater mussels (*Anodonta anatina*) reduce transmission of a common fish trematode (eye fluke, *Diplostomum pseudospathaceum*), *Parasitology*, 144(14), pp. 1971-1979.

Gordon, N.D., McMahon, T.A. and Finlayson, B.L. (1992) *Stream Hydrology: an introduction for ecologists*. John Wiley and Sons.

Gorrick, S. and Rodríguez, J.F. (2014) Scaling of sediment dynamics in a laboratory model of a sand-bed stream, *Journal of Hydro-Environment Research*, 8(2), pp. 77–87.

Gottesfeld, A.S. (1998) Bedload transport during sockeye redd excavation and by floods in the Stuart-Takla experimental watersheds, British Columbia, in *Proceedings of the Forest-fish conference: land management practices affecting aquatic ecosystems,* May 1-4, 1996, Calgary, Alberta., pp. 249–258.

Gottesfeld, A.S., Hassan, M.A., Tunnicliffe, J.F. and Poirier, R.W. (2004) Sediment dispersion in salmon spawning streams: The influence of floods and salmon redd construction, *Journal of the American Water Resources Association*, 40(4), pp. 1071–1086.

Graf, D.L. and Cummings, K.S. (2007) Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida), *Journal of Molluscan Studies*, 73(4), pp. 291–314.

Grant, J., Emerson, C.W. and Shumway, S.E. (1993) Orientation, passive transport, and sediment erosion features of the sea scallop *Placopecten magellanicus* in the benthic boundary layer, *Canadian journal of zoology*, 71, pp. 953–959.

Gray, A.B., Pasternack, G.B. and Watson, E.B. (2010) Hydrogen peroxide treatment effects on the particle size distribution of alluvial and marsh sediments, *Holocene*, 20(2), pp. 293–301.

Green, J. (2007) Stock assessment of the littoral mussel (*Mytilus edulis*) beds on Fenham Flats (Holy Island), *The Northumberland Sea Fisheries Committee*, pp. 1-7.

Greenbaum, N., Schwartz, U., Carling, P., Bergman, N., Mushkin, A., Zituni, R., Halevi, R., Benito, G. and Porat, N. (2020) Frequency of boulders transport during large floods in hyperarid areas using paleoflood analysis – An example from the Negev Desert, Israel, *Earth-Science Reviews*. 202, 103086.

Grenz, C., Hermin, M., Baudinet, D. and Daumas, R. (1990) In situ biochemical and bacterial variation of sediments enriched with mussel biodeposits, *Hydrobiologia*, 207(1), pp. 153–160.

Grimm, N.B. and Fisher, S.G. (1984) Exchange between interstitial and surface water: Implications for stream metabolism and nutrient cycling, *Hydrobiologia*, 111(3), pp. 219–228.

Gum, B., Lange, M. and Geist, J. (2011) A critical reflection on the success of rearing and culturing juvenile freshwater mussels with a focus on the endangered freshwater pearl mussel (*Margaritifera margaritifera*), *Aquatic Conservation: Marine and Freshwater Ecosystems*, 21(7), pp. 743–751

Gutierrez, J.L., Jones, C., Strayer, D. and Iribarne, O. (2003) Mollusks as ecosystem engineers: the role of shell production in aquatic habitats, *Oikos*, 101(1), pp. 79–90.

Haag, W.R. and Stoeckel, J.A. (2015) The role of host abundance in regulating populations of freshwater mussels with parasitic larvae, *Oecologia*, 178(4), pp. 1159–1168.

Hakenkamp, C.C. and Palmer, M.A. (1999) Introduced bivalves in freshwater ecosystems: The impact of *Corbicula* on organic matter dynamics in a sandy stream, *Oecologia*, 119(3), pp. 445–451.

Hamilton, H., Brim Box, J. and Dorazio, R.M. (1997) Effects of habitat suitability on the survival of relocated freshwater mussels, *Regulated Rivers Research Management*, 13, pp. 537–541.

Han, X., Fang, H.W., Johnson, M.F. and Rice, S.P. (2019) The impact of biological bedforms on near-bed and subsurface flow: A laboratory-evaluated numerical study of flow in the vicinity of pits and mounds, *Journal of Geophysical Research: Earth Surface*, 124(7), pp. 1939–1957.

Hardison, B.S. and Layzer, J.B. (2001) Relations between complex hydraulics and the localized distribution of mussels in three regulated rivers, *Regulated Rivers: Research & Management*, 17(1), pp. 77–84.

Harriger, K., Moerke, A. and Badra, P. (2009) Freshwater mussel (Unionidae) distribution and demographics in relation to microhabitat in a first-order Michigan stream, *Methods*, pp. 149–162.

Harvey, G.L., Henshaw, A., Clifford, N., Holah, H. and Grey, J. (2014) Invasive crayfish as drivers of fine sediment dynamics in rivers: Field and laboratory evidence, *Earth Surface Processes and Landforms*, 39(2), pp. 259–271.

Hassan, M.A., Gottesfeld, A.S., Montgomery, D.R., Tunnicliffe, J.F., Clarke, G.K.C., Wynn, G., Jones-Cox, H., Poirier, R., MacIsaac, E., Herunter, H. and Macdonald, S.J. (2008) Salmon-driven bed load transport and bed morphology in mountain streams, *Geophysical Research Letters*, 35(4), pp. 1–6.

Hastie, L.C., Cooksley, S.L., Scougall, F., Young, M.R., Boon, P. J. and Gaywood, M.J. (2003) Characterization of freshwater pearl mussel (*Margaritifera margaritifera*) riverine habitat using River Habitat Survey data, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 13(3), pp. 213–224.

Heritage, G.L. and Milan, D.J. (2009) Terrestrial Laser Scanning of grain roughness in a gravel-bed river, *Geomorphology*, 113(1–2), pp. 4–11.

Hey, R.D. and Thorne, C.R. (1983) Accuracy of surface samples from gravel bed material, *Journal of Hydraulic Engineering*, 109(6), pp. 842–851.

Hirst, S. (2009) *The Esk pearl mussel and salmon recovery project*. Available at: https://www.northyorkmoors.org.uk/looking-after/our-projects-and-partnerships/natural-heritage-and-land-management/pearl-mussels/Project-newsletter.pdf (Accessed: 22 May 2022).

Hoellein, T.J., Zarnoch, C., Bruesewitz, D. and DeMartini, J. (2017) Contributions of freshwater mussels (Unionidae) to nutrient cycling in an urban river: filtration, recycling, storage, and removal, *Biogeochemistry*, 135(3), pp. 307–324.

Hoke, E. (2011) The Freshwater Mussels (Mollusca: Bivalvia: Unionoida) Of Nebraska, *Transactions of the Nebraska Academy of Sciences & Affiliated Societies*, 32, pp. 1–46.

Holden-Brown, M. and Tolley, N. (2011) *Derby City Council Preliminary Flood Risk Assessment*. Derby, pp. 3-38.

Holdich, D.M., Peay, S., Foster, J., Hiley, P. D. and Brickland, J. H. (2006) Studies on the white-clawed crayfish (*Austropotamobius pallipes*) associated with muddy habitats, *BFPP - Bulletin Francais de la Peche et de la Protection des Milieux Aquatiques*, (380–381), pp. 1055–1078.

Hooke, J. (2003) Coarse sediment connectivity in river channel systems: A conceptual framework and methodology, *Geomorphology*, 56(1–2), pp. 79–94.

Hornbach, D.J. and Deneka, T. (1996) Freshwater mussel assemblages: A comparison of a qualitative and a quantitative collection method for examining freshwater mussel assemblages, *Journal of North American Benthological Society*, 15(4), pp. 587–596.

Horton, M., Keys, A., Kirkwood, L., Mitchell, F., Kyle, R. and Roberts, D. (2015) Sustainable catchment restoration for reintroduction of captive bred freshwater pearl mussels *Margaritifera margaritifera*, *Limnologica*, 50, pp. 21–28.

Howard, J.K. and Cuffey, K.M. (2006) The functional role of native freshwater mussels in the fluvial benthic environment, *Freshwater Biology*, 51(3), pp. 460–474.

Huber, V. and Geist, J. (2017) Glochidial development of the freshwater swan mussel (*Anodonta cygnea*), Linnaeus 1758) on native and invasive fish species, *Biological Conservation*, 209, pp. 230–238.

Huehner, M.K. (1987) Field and laboratory determination of substrate preferences of unionid mussels, *The Ohio Journal of Science*, 87(1), pp. 29–32.

IUCN (2022) *IUCN Red List of Threatened Species*. Available at: http://www.iucnredlist.org (Accessed: 22 March 2022).

JNCC (2019) *European community directive on the conservation of natural habitats and of wild fauna and flora (92/43/EEC).* Fourth report by the United Kingdom under Article 17 (England). Supporting documentation for the conservation status assessment for the species: S1029 - Freshwater pearl mussel *(Margaritifera margaritifera)*, pp. 3-15.

Johnson, G.C., Krstolic, J.L. and Ostby, B.J.K. (2014) Influences of water and sediment quality and hydrological processes on mussels in the clinch river, *Journal of the American Water Resources Association*, pp. 878–897.

Johnson, M.F., Reid, I., Rice, S.P. and Wood, P.J. (2009) Stabilisation of fine gravels by net-spinning caddisfly larvae, *Earth Surface Processes and Landforms*, 34(3), pp. 413–423.

Jones, C.G., Lawton, J.H. and Shachak, M. (1994) Organisms as ecosystem engineers, *Oikos*, 69(3), pp. 373–386.

Jones, C.G., Lawton, J.H. and Shachak, M. (1997) Positive and negative effects of organisms as physical ecosystem engineers, *Ecology*, 78(7), pp. 1946–1957.

Jones, J.B. and Holmes, R.M. (1996) Surface-subsurface interactions in stream ecosystems, *Trends in Ecology and Evolution*, 11(6), pp. 239–242.

Jones, J.I., Murphy, J.F., Collins, A.L., Sear, D.A., Naden, P.S. and Armitage, P.D. (2011) The impact of fine sediment on macro-invertebrates, *River Research and Applications*, 28(8), pp. 1055–1071.

Jørgensen, C.B., Kiørboe, T., Møhlenberg, F. and Riisgård, H.U. (1984) Marine ecology progress series, *Marine Ecology - Progress Series*, 15(3), pp. 283–292.

Kat, P.W. (1984) Parasitism and the Unionacea (Bivalvia), *BioScience*, 59(2), pp. 189–207.

Kelly, M. (2023) *River Clun Recovery Project*, *The National Association for Areas of Outstanding Natural Beauty*. Available at: https://landscapesforlife.org.uk/about-aonbs/aonb-case-studies/river-clun-recovery-project (Accessed: 31 January 2023).

Killeen, I., Aldridge, D. and Oliver, G. (2004) *Freshwater Bivalves of Britain and Ireland*. Field Studies Council.

Killeen, I. and Moorkens, E. (2016) *The translocation of freshwater pearl mussels: a review of reasons, methods and success and a new protocol for England*, Natural England Commissioned Report NECR229, pp. 2-27.

Kirkegaard, J., Wolters, G., Sutherland, J., Soulsby, R.L., Frostick, L.E., McLelland, S.J., Mercer, T. and Gerritsen, H. (2011) *Users Guide to Physical Modelling and Experimentation: Experience of the HYDRALAB Network*. Edited by L. E Frostick, S. J McLelland, and T.G. Mercer. CRC Press/Balkema, Leiden, The Netherlands.

Kjelland, M.E., Woodley, C., Swannack, T. and Smith, D. (2015) A review of the potential effects of suspended sediment on fishes: potential dredging-related physiological, behavioural, and transgenerational implications, *Environment Systems and Decisions*. Kluwer Academic Publishers, pp. 334–350.

Kleinhans, M.G. (2002) Sorting out sand and gravel : sediment transport and deposition in sand-gravel bed rivers, *The Royal Dutch Geographical Society/Faculty of Geographical Sciences, Utrecht University*. PhD Thesis.

Klerks, P.L., Fraleigh, P.C. and Lawniczak, J.E. (1996) Effects of zebra mussel (*Dreissena polymorpha*) on seston levels and sediment deposition in western Lake Erie, *Canadian Journal of Fisheries and Aquatic Sciences*, 53, pp. 2284–2291.

Klos, P.Z., Rosenberry, D.O. and Nelson, G.R. (2014) Influence of hyporheic exchange, substrate distribution, and other physically linked hydrogeomorphic characteristics on abundance of freshwater mussels, *Ecohydrology*, pp. 1284–1291.

Krieger, K.A. (2007) Distribution and abundance of burrowing mayflies (*Hexagenia* spp.) in Lake Erie, 1997-2005, *Journal of Great Lakes Research*, 33(1), pp. 20–33. Available at: https://doi.org/10.3394/0380-1330(2007)33.

Krumbein, W.C. (1934) Size frequency distributions of sediments, *Journal of Sedimentary Petrology*, 4(2), pp. 65–77.

Kumar, S.S., Kozarek, J., Hornbach, D., Hondzo, M. and Hong, J. (2019) Experimental investigation of turbulent flow over live mussels, *Environmental Fluid Mechanics*, 19(6), pp. 1417–1430.

van de Lageweg, W.I., van Dijk, W., Baar, A., Rutten, J., and Kleinhans, M.G. (2014) Bank pull or bar push: What drives scroll-bar formation in meandering rivers?, *Geology*, 42(4), pp. 319–322.

Lai, R., Wang, M., Yang, M. and Zhang, C. (2017) Method based on the Laplace equations to reconstruct the river terrain for two-dimensional hydrodynamic numerical modeling, *Computers and Geosciences*, 111, pp. 26–38.

Lancaster, J., Buffin-Bélanger, T., Reid, I. and Rice, S. (2006) Flow- and substratum-mediated movement by a stream insect, *Freshwater Biology*, 51(6), pp. 1053–1069.

Landmeyer, J.E., Bradley, P., Trego, D., Hale, K. and Haas, J. (2010) MTBE, TBA, and TAME attenuation in diverse hyporheic zones, *Ground Water*, 48(1), pp. 30–41.

Langhammer, J., Lendzioch, T., Miřijovskỳ, J. and Hartvich, F. (2017) UAV-based optical granulometry as tool for detecting changes in structure of flood depositions, *Remote Sensing*, 9(3).

Larsen, B.M. (2013) The freshwater pearl mussel *Margaritifera margaritifera* in Norway – status and conservation strategies, in *Norweigan Institute for Nature Research International meeting – Kefermarkt, November 2013*.

Lavictoire, L., Moorkens, E.A, Ramsey, A.D., Sinclair, W. and Sweeting, R.A. (2015) Effects of substrate size and cleaning regime on growth and survival of captive-bred juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Linnaeus, 1758), *Hydrobiologia*, 766(1), pp. 89–102.

Lavictoire, L., Ramsey, A.D., Moorkens, E.A., Souch, G. and Barnhart, C. (2018) Ontogeny of juvenile freshwater pearl mussels, *Margaritifera margaritifera* (Bivalvia : Margaritiferidae), *PLoS ONE*, 13(3), p. e0193637.

Lavictoire, L., Notman, G., Pentecost, A., Moorkens, E.A., Ramsey, A.D and Sweeting, R.A. (2020) Substrate parameters affecting propagation of juvenile freshwater pearl mussels

Margaritifera margaritifera (Bivalvia: Margaritiferidae), *Journal of Conchology* , 43(5), pp. 467–480.

Lavrentyev, P.J., Gardner, W.S. and Yang, L.Y. (2000) Effects of the zebra mussel on nitrogen dynamics and the microbial community at the sediment-water interface, *Aquatic Microbial Ecology*, 21(2), pp. 187–194.

Layzer, J.B. and Madison, L.M. (1995) Microhabitat use by freshwater mussels and recommendations for determining their instream flow needs, *Regulated Rivers: Research & Management*, 10(2–4), pp. 329–345.

van Leeuwen, B., Augustijn, D., van Wesenbeeck, B., Hulscher, S. and de Vries, M. (2010) Modelling the influence of a young mussel bed on fine sediment dynamics on an intertidal flat in the Wadden Sea, *Ecological Engineering*, 36(2), pp. 145–153.

Lewis, J.B. and Riebel, P.N. (1984) The effect of substrate on burrowing in freshwater mussels (Unionidae), *Canadian Journal of Zoology*, 62(10), pp. 2023–2025.

Libois, R.M. and Hallet-Libois, C. (1987) The unionid mussels (Mollusca, Bivalvia) of the Belgian upper River Meuse: An assessment of the impact of hydraulic works on the river water self-purification, *Biological Conservation*, pp. 115–132.

Lisle, T.E. and Hilton, S. (1999) Fine bed material in pools of natural gravel bed channels crest, *Water Resources Research*, 35(4), pp. 1291–1304.

Lopes-Lima, M., Sousa, R., Geist, J., Aldridge, D.C., Araujo, R., Bergengren, J., Bespalaya, Y., Bódis, E., Burlakova, L. and Van Damme, D. (2017) Conservation status of freshwater mussels in Europe: state of the art and future challenges, *Biological Reviews*, 92(1), pp. 572–607.

Lopes-Lima, M., Burlakova, L., Karatayev, A., Mehler, K., Seddon, M. and Sousa, R. (2018) Conservation of freshwater bivalves at the global scale: diversity, threats and research needs, *Hydrobiologia*, 810, pp. 1–14.

Lopez, J.W. and Vaughn, C.C. (2021) A review and evaluation of the effects of hydrodynamic variables on freshwater mussel communities, *Freshwater Biology*, 66(9), pp. 1665–1679.

Luck, M., Maumenee, N., Whited, D., Lucotch, J., Chilcote, S., Lorang, M., Goodman, D., Mcdonald, K., Kimball, J. and Stanford, J. (2010) Remote sensing analysis of physical complexity of North Pacific Rim rivers to assist wild salmon conservation, *Earth Surface Processes and Landforms*, 35(11), pp. 1330–1347.

Lummer, E.M., Auerswald, K. and Geist, J. (2016) Fine sediment as environmental stressor affecting freshwater mussel behavior and ecosystem services, *Science of the Total Environment*, 571, pp. 1340–1348.

Luzier, C. and Miller, S. (2009) Freshwater mussel relocation guidelines written and edited by Christina Luzier and Shelly Miller. *A product of the Pacific Northwest Native Freshwater Mussel Workgroup*. pp. 1-7.

M. Piedra, Haynes, H. and Hoey, T. (2012) The spatial distribution of coarse surface grains and the stability of gravel river beds, *Sedimentology*, 59(3), pp. 1014–1029.

Madej, M.A. (1999) Temporal and spatial variability in thalweg profiles of a gravel-bed river, *Earth Surface Processes and Landforms*, 24(12), pp. 1153–1169.

Magni, S., Parolini, M., Soave, C., Marazzi, F., Mezzanotte, V. and Binelli, A. (2015) Removal of metallic elements from real wastewater using zebra mussel bio-filtration process, *Journal of Environmental Chemical Engineering*, 3(2), pp. 915–921.

Mahaut, M.-L. and Graf, G. (1987) A luminophore tracer technique for bioturbation studies, *Oceanologica Acta*, 10(3), pp. 323–328.

di Maio, J. and Corkum, L.D. (1995) Relationship between the spatial distribution of freshwater mussels (Bivalvia: Unionidae) and the hydrological variability of river, *Canadian Journal of Zoology*, 73, pp. 663–671.

Majdi, N., Bardon, L. and Gilbert, F. (2014) Quantification of sediment reworking by the Asiatic clam *Corbicula fluminea* Müller, 1774, *Hydrobiologia*, 732(1), pp. 85–92.

Malcolm, I.A., Soulsby, C., Youngson, A.F. and Tetzlaff, D. (2009) Fine scale variability of hyporheic hydrochemistry in salmon spawning gravels with contrasting groundwater-surface water interactions, *Hydrogeology Journal*, 17(1), pp. 161–174.

Mann, K.H. (1964) The pattern of energy flow in the fish and invertebrate fauna of the River Thames, *Verhandlungen der Internationalen Vereinigung fur theoretishce und angewandte Limnologie*, 15, pp. 485–495.

Mason, C. (2016) *NMBAQC's Best practice guidance particle size analysis (PSA) for supporting biological analysis*. Available at: http://www.nmbaqcs.org/media/1255/psa-guidance_update18012016.pdf (Accessed: 05 August 2018).

Mason, R.J., Rice, S.P., Wood, P.J. and Johnson, M.F. (2019) The zoogeomorphology of case-building caddisfly: Quantifying sediment use, *Earth Surface Processes and Landforms*, 44(12), pp. 2510–2525.

McDonald, J.H. (2014) *The Handbook of Biological Statistics*. Available at: http://www.biostathandbook.com/twosamplettest.html (Accessed: 27 May 2017).

McIvor, A. (2004) Freshwater Mussels As Biofilters, *University of Cambridge*, PhD Thesis.

Méndez, G., Péréz-Arlucea, M., Stouthamer, E. and Berendsen, H. (2003) The TESS-1 suction corer: A new device to extract wet, uncompacted sediments, *Journal of Sedimentary Research*, 73(6), pp. 1078–1081.

Met Office (2019) *UK climate averages 1981-2010*. Available at: https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages#?tab=climateTables (Accessed: 18 October 2019).

Miall, A.D. (1985) Architectural-element analysis: A new method of facies analysis applied to fluvial deposits, *Earth-Science Reviews*, 22, pp. 261–308.

Milan, D.J. (1994) Preliminary results of a nitrogen freeze-coring methodology for determining the vertical distribution of benthic invertebrates from the lowland River Babingley, UK, *Internationale Vereinigung für Theoretische und Angewandte Limnologie*, 25(3), pp. 1406-1409.

Milan, D.J. (1996) The application of freeze-coring for siltation assessment in a recently regulated stream, *Hydrologie dans les Pays Celtiques*, 79, pp. 253–266.

Milan, D.J., Heritage, G.L., Large, A.R.G. and Fuller, I.C. (2011) Filtering spatial error from DEMs: Implications for morphological change estimation, *Geomorphology*, 125(1), pp. 160–171.

Milan, D.J., Heritage, G.L. and Hetherington, D. (2007) Application of a 3D laser scanner in the assessment of erosion and deposition volumes and channel change in a proglacial river, *Earth Surface Processes and Landforms*, 32, pp. 1657–1674.

Miller, M.C., McCave, I.N. and Komar, P.D. (1977) Threshold of sediment motion under unidirectional currents, *Sedimentology*, 24, pp. 507–527.

Mirto, S., La Rosa, T., Danovaro, R. and Mazzola, A. (2000) Microbial and meiofaunal response to intensive mussel-farm biodeposition in coastal sediments of the Western Mediterranean, *Marine Pollution Bulletin*, 40(3), pp. 244–252.

Miura, T. and Yamashiro T (1990) Size selective feeding a phytoplanktivorous and viability of *Anodonta* freshwater of egested algae, *Japanese Journal of Limnology*, 51(2), pp. 73–78.

Monaco, P., Famiani, F. and la Iacona, F. (2016) Bulldozing and resting traces of freshwater mussel *Anodonta woodiana* and substrate characteristics in lake-margin and river settings of Umbria, Italy, *Rivista Italiana di Paleontologia e Stratigrafia*, 122(1), pp. 53–62.

Moore, J.W. (2006) Animal ecosystem engineers in streams, *BioScience*, 56(3), p. 237.

Moore, J.W., Schindler, D.E. and Scheuerell, M.D. (2004) Disturbance of freshwater habitats by anadromous salmon in Alaska, *Oecologia*, 139(2), pp. 298–308.

Moorkens, E.A. and Killeen, I.J. (2014) Assessing near-bed velocity in a recruiting population of the endangered freshwater pearl mussel (*Margaritifera margaritifera*) in Ireland, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 24(6), pp. 853–862.

Mosley, M.P. and Tindale, D.S. (1985) Sediment variability and bed material sampling in gravel-bed rivers, *Earth Surface Processes and Landforms*, 10(5), pp. 465–482.

Mueller, R.P., Tiller, B., Bleich, M., Turner, G. and Welch, I. (2011) Assessment of the species composition, densities, and distribution of native freshwater mussels along the Benton County shoreline of the Hanford Reach, Columbia River, 2004, *U.S. Department of Energy under Contract DE-AC05-76RL01830 Pacific Northwest Laboratory*.

Murray-Bligh, J.A.D. (1999) Procedure for collecting and analysing macro-invertebrate samples. *Quality Management Systems for Environmental Monitoring: Biological Techniques BT001. Version 2.0.* Bristol.

Nanson, G.C. (1980) Point bar and floodplain formation of the meandering Beatton River, northeastern British Columbia, Canada, *Sedimentology*, 27(1), pp. 3–29.

Nanson, G.C. and Huang, H.Q. (2017) Self-adjustment in rivers: Evidence for least action as the primary control of alluvial-channel form and process, *Earth Surface Processes and Landforms*, 42(4), pp. 575–594.

National Biodiversity Network (2016) *NBN Gateway*. Available at: https://nbn.org.uk/the-national-biodiversity-network/archive-information/nbn-gateway/ (Accessed: 6 March 2017).

National Biodiversity Network (2021) *NBN Atlas*. Available at: https://species.nbnatlas.org/ (Accessed: 23 October 2021).

National Trust (2019) *A history of the Park at Kedleston Hall*. Available at: https://www.nationaltrust.org.uk/kedleston-hall/features/a-history-of-the-park-at-kedleston-hall (Accessed: 11 October 2019).

Natural England (2014a) *Planning for the future improvement programme for England's Natura 2000 Sites (IPENS). Site Improvement Plan: River Clun SAC. UK0030250*. Available at: http://publications.naturalengland.org.uk/publication/6216527934128128?category=4879822899642368 (Accessed: 31 January 2023).

Natural England (2014b) *Planning for the future improvement programme for England's Natura 2000 Sites (IPENS). Site Improvement Plan: River Ehen SAC. UK0030057*. Available at: http://publications.naturalengland.org.uk/publication/6203335036108800?category=6329101765836800 (Accessed: 1 February 2023).

Natural England (2022) *Life R4ever Kent: Project information*. Available at: https://www.gov.uk/government/publications/life-r4ever-kent-restoring-and-revitalising-to-make-a-more-resilient-river-kent-and-its-species/life-r4ever-kent-project-information-note-1 (Accessed: 1 February 2023).

Negus, C.L. (1966) A quantitative study of growth and production of unionid mussels in the River Thames at Reading, *Journal of Animal Ecology*, 35(3), pp. 513–532.

Nelson, J.M., Shreve, R., McLean, S. and Drake, T. (1995) Role of near-bed turbulence structure in bed load transport, *Water Resources Research*, 31(95), pp. 2071–2086.

NERC (CEH) (2017) *Land Cover Map 2015*. Available at: https://digimap.edina.ac.uk/roam/map/environment (Accessed: 16 October 2019).

Nicholas, A.P., Sambrook Smith, G., Amsler, M., Ashworth, P., Best, J., Hardy, R., Lane, S., Orfeo, O., Parsonsm, D. and Reesink, A. (2016) The role of discharge variability in determining alluvial stratigraphy, *Geology*, 44(1), pp. 3–6.

Nichols S. J. and Garling D. (2000) Food-web dynamics and trophic-level interactions in a multispecies community of freshwater unionids, *Canadian Journal of Zoology*, 78, pp. 871–882.

Nogaro, G., Datry, T., Mermillod-Blondin, F., Foulquier, A. and Montuelle, B. (2013) Influence of hyporheic zone characteristics on the structure and activity of microbial assemblages, *Freshwater Biology*, 58(12), pp. 2567–2583.

North York Moors (2019) *Pearl mussels*. Available at: https://www.northyorkmoors.org.uk/looking-after/our-projects-and-partnerships/natural-heritage-and-land-management/pearl-mussels (Accessed: 22 May 2022).

Nuova Rade (2019) *Products | Fishing | Angling Accessories.* Available at: https://www.nuovarade.com/products.php?categoryId=267/ (Accessed: 30 January 2019).

Nuttle, W.K. (2002) Eco-hydrology's past and future in focus, *Eos*, 83(19), pp. 205–212.

Ochrana Prirody (2022) Monitoring and seminatural breeding of the freshwater pearl mussel *(Margaritifera margaritifera)* in the Aš region. Available at: https://www.ochranaprirody.cz/en/our-projects/eea-grants/sgsii-2-the-freshwater-pearl-mussel-as-region/ (Accessed: 7 February 2022).

O'Hare, M.T., Tree, A., Neale, M.W., Irvine, K., Gunn, I., Jones, J. and Clarke, R. (2007) *Lake benthic macroinvertebrates I: improving sampling methodology. Science Report SC030294/SR1*. Bristol: Environment Agency.

Ordnance Survey (2015) *Derby, Uttoxeter, Ashbourne and Cheadle, sheet 259, 1:25,000.* Ordnance Survey (Explorer Series).

Ordnance Survey (2016a) *Leicester, Coventry and Rugby, sheet 140, 1:50,000*. Ordnance Survey (Landranger Series).

Ordnance Survey (2016b) *Nottingham and Loughborough, sheet 129, 1:50,000*. Ordnance Survey (Landranger Series).

Ortega, J.C.G., Figueiredo, B., da Graça, W., Agostinho, A. and Bini, L. (2020) Negative effect of turbidity on prey capture for both visual and non-visual aquatic predators, *Journal of Animal Ecology*, 89(11), pp. 2427–2439.

Partnership for the Delaware Estuary (2013) Freshwater mussel recovery strategy for the Delaware Estuary and river basin, *Partnership for the Delaware Estuary*. Available at: http://www.delawareestuary.org/ (Accessed: 20 May 2022).

Pearl Mussel Project (2020) *Freshwater Pearl Mussel Programme.* Available at: https://www.pearlmusselproject.ie/farm-programme/ (Accessed: 22 May 2022).

Pearson, E., Smith, M., Klaar, M. and Brown, L. (2017) Can high resolution 3D topographic surveys provide reliable grain size estimates in gravel bed rivers?, *Geomorphology*, 293(March), pp. 143–155.

Petts, G.E., Thoms, M., Brittan, K. and Atkin, B.. (1989) A freeze-coring technique applied to pollution by fine sediments in gravel-bed rivers, *Science of the Total Environment*, 84(C), pp. 259–272.

Platt, J. (2011) Habitat complexity and species diversity in rivers, *Cardiff University*, PhD Thesis.

Pledger, A.G. (2014) Foraging fish as zoogeomorphic agents : their effects on the structure and composition of gravel-bed river sediments with implications for bed material transport, *Loughborough University*, PhD Thesis.

Pledger, A.G., Rice, S.P. and Millett, J. (2017) Foraging fish as zoogeomorphic agents: An assessment of fish impacts at patch, barform, and reach scales, *Journal of Geophysical Research: Earth Surface*, 122(11), pp. 2105–2123.

Quinlan, E. (2014) Ecogeomorphological dynamics of the River Ehen prior to its restoration, *University of Aberdeen,* PhD Thesis.

Quinlan, E., Gibbins, C., Malcolm, I.., Batalla, R., Vericat, D. and Hastie, L. (2015) A review of the physical habitat requirements and research priorities needed to underpin conservation of the endangered freshwater pearl mussel *Margaritifera margaritifera*, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 25(1), pp. 107–124.

Quinlan, E., Gibbins, C.N., Batalla, R. J. and Vericat, D. (2015) Impacts of small scale flow regulation on sediment dynamics in an ecologically important upland river, *Environmental Management*, 55(3), pp. 671–686.

Quinlan, E., Malcolm, I.A. and Gibbins, C.N. (2014) Spatio-temporal variability of dissolved oxygen within the shallow subsurface zone of a freshwater pearl mussel bed, *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, 185(3), pp. 281–294.

Ragnarsson, S. and Raffaelli, D. (1999) Effects of the mussel *Mytilus edulis* L. on the invertebrate fauna of sediments, *Journal of Experimental Marine Biology and Ecology*, 241(1), pp. 31–43.

Rasmussen, J.B. (1988) Habitat requirements of burrowing mayflies (Ephemeridae:*Hexagenia*) in lakes, with special reference to the effects of eutrophication, *Journal of the North American Benthological Society*, 7(1), pp. 51–64.

Reeders, H.H. and Bij de Vaate, A. (1990) Zebra mussels (*Dreissena polymorpha*): a new perspective for water quality management, *Hydrobiologia*, 200–201(1), pp. 437–450.

Reform Rivers (2011) *Restoration of the freshwater pearl mussel and its habitats*. Available at: https://wiki.reformrivers.eu/index.php/Sweden-_Restoration_of_the_Freshwater_Pearl_Mussel_and_its_habitats_(LIFE04/NAT/SE/000231) (Accessed: 7 February 2022).

Reid, H.E., Brierley, G.J. and Boothroyd, I.K.G. (2010) Influence of bed heterogeneity and habitat type on macroinvertebrate uptake in peri-urban streams, *International Journal of Sediment Research*, 25(3), pp. 203–220.

Reid, I., Frostick, L.E. and Layman, J.T. (1985) The incidence and nature of bedload transport during flood flows in coarse-grained alluvial channels, *Earth Surface Processes and Landforms*, 10(1), pp. 33–44.

Reid, I. and Laronne, J. (1995) Bed load sediment transport in an ephemeral stream and a comparison with seasonal and perennial counterparts, *Water Resources Research*, 31(3), pp. 773–781.

Reid, N., Thompson, D., Hayden, B., Marnell, F. and Montgomery, W. (2013) Review and quantitative meta-analysis of diet suggests the Eurasian otter (*Lutra lutra*) is likely to be a poor bioindicator, *Ecological Indicators*, 26, pp. 5–13.

Reinhardt L., Jerolmack D., Cardinale B., Vanacke, V. and Wright, J. (2010) Dynamic interactions of life and its landscape: feedbacks at the interface of geomorphology and ecology, *Earth Surface Processes and Landforms*, 35, pp. 78–101.

Rice, S., Stoffel, M., Turowski, J. and Wolf, A. (2012) Disturbance regimes at the interface of geomorphology and ecology, *Earth Surface Processes and Landforms*, 37(15), pp. 1678–1682.

Rice, S., Pledger, A., Toone, J. and Mathers, K. (2019) Zoogeomorphological behaviours in fish and the potential impact of benthic feeding on bed material mobility in fluvial landscapes, *Earth Surface Processes and Landforms*, 44(1), pp. 54–66.

Rice, S. and Church, M. (1998) Grain size along two gravel-bed rivers: statistical variation, spatial pattern and sedimentary links, *Earth Surface Processes and Landforms*, 23(4), pp. 345–363.

Roditi, H.A., Strayer, D.L. and Findlay, S.E.G. (1997) Characteristics of zebra mussel (*Dreissena polymorpha*) biodeposits in a tidal freshwater estuary, *Archiv Fur Hydrobiologie*, 140(2), pp. 207–219.

Rogers-Lowery, C.L. and Dimock, R. (2006) Encapsulation of attached ectoparasitic glochidia larvae of freshwater mussels by epithelial tissue on fins of naïve and resistant host fish, *Biological Bulletin*, 210(1), pp. 51–63.

Roseberry, J.C., Schmeeckle, M.W. and Furbish, D.J. (2012) A probabilistic description of the bed load sediment flux : 2. Particle activity and motions, *Journal of Geophysical Research*, 117, pp. 1–21.

Rosgen, D.L. (1994) A classification of natural rivers, *Catena*, 22(3), pp. 169–199.

Rzodkiewicz, L.D., Annis, M.L. and Woolnough, D.A. (2022) Contaminants of emerging concern may pose prezygotic barriers to freshwater mussel recruitment, *Journal of Great Lakes Research*, 48(3), pp. 768–781.

Sansom, B.J., Bennett, S., Atkinson, J. and Vaughn, C. (2020) Emergent hydrodynamics and skimming flow over mussel covered beds in rivers, *Water Resources Research*, 56(8).

Sauder, L.A., Engel, K., Stearns, J., Masella, A., Pawliszyn, R. and Neufeld, J. (2011) Aquarium nitrification revisited: Thaumarchaeota are the dominant ammonia oxidizers in freshwater aquarium biofilters, *PLoS ONE*, 6(8).

Scheder, C., Lerchegger, B., Flodl, R., Csar, D., Gumpinger, C. and Hauer, C. (2015) River bed stability versus clogged interstitial: Depth-dependent accumulation of substances in freshwater pearl mussel (*Margaritifera margaritifera* L.) habitats in Austrian streams as a function of hydromorphological parameters, *Limnologica*, 50, pp. 29–39.

Schuett-Hames, D., Conrad, B., Pleus, A. and Smith, D. (1996) Field comparison of the McNeil sampler with three shovel-based methods used to sample spawning substrate composition in small streams. *Northwest Indian Fisheries Commission*, pp. 1–22.

Schulte, P., Lehmkuhl, F., Steininger, F., Loibl, D., Lockot, G., Protzea, J., Fischer, P. and Stauch, G. (2016) Influence of HCl pretreatment and organo-mineral complexes on laser diffraction measurement of loess-paleosol-sequences, *Catena*, 137, pp. 392–405.

Schumacher, B.A., Shines, K., Burton, J. and Papp, M. (1990) Comparison of three methods for soil homogenization, *Soil Science Society of America Journal*, 54(4), pp. 1187–1190.

Schwalb, A.N. and Pusch, M.T. (2007) Horizontal and vertical movements of unionid mussels in a lowland river, *Journal of the North American Benthological Society*, 26(2), pp. 261–272.

Shah, M.F., Al Mamun, M.A., Hossain, M.T., Moniruzzaman, M., Yeasmine, S., Uddin, M.H. and Jasim U.M. (2022) Clearance of *Escherichia coli* by the freshwater mussel *Lamellidens marginalis* in laboratory conditions, *Molluscan Research*, pp. 1–7.

Shrivastava, S., Stewardson, M.J. and Arora, M. (2021) influence of bioturbation on hyporheic exchange in streams: Conceptual model and insights from laboratory experiments, *Water Resources Research*, 57(2).

da Silva, J.P., Gonçalves, D., Lopes-Lima, M., Anastácio, P., Banha, F., Frimpong, E., Gama, M., Miranda, R., Reis, J., Filipe, A. and Sousa, R. (2022) Predicting climatic threats to an endangered freshwater mussel in Europe: The need to account for fish hosts, *Freshwater Biology*, 67(5), pp. 842–856.

Silverman, H., Cherry, J., Lynn, J., Dietz, T., Nicholas, S. and Achberger, E. (1997) Clearance of laboratory-cultured bacteria by freshwater bivalves: differences between lentic and lotic unionids, *Canadian Journal of Zoology*, 75(11), pp. 1857–1866.

Sime, I., Webley, J., O'Leary, E., Watkin, N., Wilkie, L., Ferguson, S. and Grigor-Taylor, F. (2017) Rewilding and species reintroductions: Pearls in Peril – Conserving and reintroducing freshwater pearl mussels, *Bulletin of the Chartered Institute of Ecology and Environmental Management*, 95, pp. 37–41.

Skinner, A., Young, M. and Hastie, L. (2003) *Ecology of the freshwater pearl mussel*, *Conserving Natura 2000 Rivers, Ecology Series No. 2* English Nature, Peterborough.

de Smit, J.C., Brückner, M., Mesdag, K., Kleinhans, M.G. and Bouma, T.J. (2021) Key bioturbator species within benthic communities determine sediment resuspension thresholds, *Frontiers in Marine Science*, 8, 726238, pp. 1-11.

Smith, J. (2019) Bream (*Abramis brama* L.) as zoogeomorphic agents and ecosystem engineers: Implications for fine sediment transport in lowland rivers, *Loughborough University*, PhD Thesis.

Solan, M., Wigham, B., Hudson, I., Kennedy, R., Coulon, C., Norling, K., Nilsson, H. and Rosenberg, R. (2004) In situ quantification of bioturbation using time-lapse fluorescent sediment profile imaging (f-SPI), luminophore tracers and model simulation, *Marine Ecology Progress Series*, 271, pp. 1–12.

Somerfield, P.J. and Warwick, R.M. (2013) Meiofauna techniques, in *Methods for the Study of Marine Benthos*. Plymouth, UK: Plymouth Marine Laboratory, pp. 253–284.

Sousa, R., Ferreira, A., Carvalho, F., Lopes-Lima, M., Varandas, S. Teixeira, A. (2018) Die-offs of the endangered pearl mussel *Margaritifera margaritifera* during an extreme drought, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 28(5), pp. 1–5.

South Cumbria Rivers Trust (2021) *Life R4ever Kent Project*. Available at: https://scrt.co.uk/life-r4ever-kent-project/ (Accessed: 1 February 2023).

Spooner, D.E. and Vaughn, C.C. (2006) Context-dependent effects of freshwater mussels on stream benthic communities, *Freshwater Biology*, pp. 1016–1024.

Štambuk, A., Pavlica, M., Vignjević, G., Bolarić, B. and Klobučar, G. (2009) Assessment of genotoxicity in polluted freshwaters using caged painter's mussel, *Unio pictorum*, *Ecotoxicology*, 18(4), pp. 430–439.

Statzner, B., Vet, E., Champagne, J., Morel, R. and Herouin, E. (2000) Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams, *Limnology and Oceanography*, 45(5), pp. 1030–1040.

Statzner, B. (2012) Geomorphological implications of engineering bed sediments by lotic animals, *Geomorphology*, 157–158, pp. 49–65.

Statzner, B., Peltret, O. and Tomanova, S. (2003) Crayfish as geomorphic agents and ecosystem engineers: Effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams, *Freshwater Biology*, 48(1), pp. 147–163.

Statzner B, Sagnes P, Champagne J-Y, V.S. (2003) Contribution of benthic fish to the patch dynamics of gravel and sand transport in streams, *Water Resources Research*, 39(11), pp. 1-17.

Stevcic, C., Pulkkinen, K. and Pirhonen, J. (2018) The use of biological traps for water treatment in Recirculating Aquaculture Systems. *5th European Congress of Conservation Biology, 2018, University of Jyvaskyla, Finland.*

Stoyanova, T., Vidinova, Y., Yaneva, I., Tyufekchieva, V., Parvanov, D., Traykov, I. and Bogoev, V. (2014) Ephemeroptera, Plecoptera and Trichoptera as indicators for ecological quality of the Luda Reka River, Southwest Bulgaria, *Acta Zoologica Bulgarica*, 66(2), pp. 255–260.

Strayer, D.L. (1999) Use of flow refuges by unionid mussels in rivers, *Journal of the North American Benthological Society*, 18(4), pp. 468–476.

Strayer, D.L. (2014) Understanding how nutrient cycles and freshwater mussels (Unionoida) affect one another, *Hydrobiologia*, pp. 277–292.

Strayer, D.L., Geist, J., Haag, W., Jackson, J. and Newbold, J. (2019) Essay: Making the most of recent advances in freshwater mussel propagation and restoration, *Conservation Science and Practice*, 1:e53, pp. 1–9.

Strayer, D.L., Caraco, N.F., Cole, J.J., Findlay, S., Pace, M.L. (1999) Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River, *BioScience*, 49, pp. 19–27.

Swan, C.M. and Palmer, M.A. (2000) What drives small-scale spatial patterns in lotic meiofauna communities?, *Freshwater Biology*, 44(1), pp. 109–121.

Swanson, S., Kozlowski, D., Hall, R., Heggem, D. and Lin, J. (2017) Riparian proper functioning condition assessment to improve watershed management for water quality, *Journal of Soil and Water Conservation*, 72(2), pp. 168–182.

Tankersley, R. and Dimock Jr., R. (1993) The effect of larval brooding on the filtration rate and particle-retention efficiency of *Pyganodon cataracta* (Bivalvia: Unionidae), *Canadian Journal of Zoology*, 71(10), pp. 1934–1944.

Tanner, W.F. (1960) Helicoidal flow, a possible cause of meandering, *Journal of Geophysical Research*, 65(3), pp. 993–995.

Taskinen, J., Berg, P., Saarinen-Valta, M., Välilä, S., Mäenpää, E., Myllynen, K. and Pakkala, J. (2011) Effect of pH, iron and aluminum on survival of early life history stages of the endangered freshwater pearl mussel, *Margaritifera margaritifera*, *Toxicological and Environmental Chemistry*, 93(9), pp. 1764–1777.

Taskinen, J. and Salonen, J.K. (2022) The endangered freshwater pearl mussel *Margaritifera margaritifera* shows adaptation to a local salmonid host in Finland, *Freshwater Biology*, 67(5), pp. 801–811.

The Rivers Trust (2023) *Raw sewage in our rivers | The Rivers Trust*. Available at: https://theriverstrust.org/key-issues/sewage-in-rivers (Accessed: 22 July 2023).

Thomas, G.R., Taylor, J. and de Leaniz, C.G. (2010) Captive breeding of the endangered freshwater pearl mussel *Margaritifera margaritifera*, *Endangered Species Research*, 12(1), pp. 1–9.

Thoms, M.C. (1992) A comparison of grab-and freeze-sampling techniques in the collection of gravel-bed river sediment, *Sedimentary Geology*, 78, pp. 191–200.

Turner, C.B. (2010) Influence of zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis*) mussel invasions on benthic nutrient and oxygen dynamics, *Canadian Journal of Fisheries and Aquatic Sciences*, 67, pp. 1899–1908.

Tuttle-Raycraft, S., Morris, T.J. and Ackerman, J.D. (2017) Suspended solid concentration reduces feeding in freshwater mussels, *Science of the Total Environment*, 598, pp. 1160–1168.

Tyrrell, M. and Hornbach, D.J. (2010) Selective predation by muskrats on freshwater mussels in 2 Minnesota rivers. *The North American Benthological Society*, 17(3), pp. 301–310.

UK CEH (2023) *National River Flow Archive*. Available at: https://nrfa.ceh.ac.uk/data/search (Accessed: 22 July 2023).

Valeport Limited, St Peter's Quay, Totnes, Devon, U. (2013) Valeport Models 001 & 002 Flow Meters, *Datasheet Reference: Model 001 or 002 version 2b.*, p. 2013.

Vaughn, C. and Hakenkamp, C. (2001) The functional role of burrowing bivalves in freshwater ecosystems, *Freshwater Biology*, 46, pp. 1431–1446.

Vaughn, C.C. (2017) Ecosystem services provided by freshwater mussels, *Hydrobiologia*, pp. 1–13.

Vaughn, C.C., Gido, K.B. and Spooner, D.E. (2004) Ecosystem processes performed by unionid mussels in stream mesocosms: Species roles and effects of abundance, *Hydrobiologia*, 527(1), pp. 35–47.

Vaughn, C.C., Nichols, S.J. and Spooner, D.E. (2008) Community and foodweb ecology of freshwater mussels, *Journal of the North American Benthological Society*, pp. 409–423.

Vaughn, C.C. and Spooner, D.E. (2006) Unionid mussels influence macroinvertebrate assemblage structure in streams, *The North American Benthological Society*, 25(3), pp. 691–700.

Vaughn, C.C., Spooner, D.E. and Hoagland, B.W. (2002) River weed growing epizoically on freshwater mussels, *The Southwestern Association of Naturalists*, 47(4), pp. 604–605.

Viles H.A., Naylor, L., Carter, N. and Chaput D. (2008) Biogeomorphological disturbance regimes: progress in linking ecological and geomorphological systems, *Earth Surface Processes and Landforms*, 33, pp. 1419–1435.

Walker, T.R., Grant, J., Weise, A., McKindsey, C., Callier, M. and Richard, M. (2014) Influence of suspended mussel lines on sediment erosion and resuspension in Lagune de la Grande Entree, Iles-de-la-Madeleine, Quebec, Canada, *Aquaculture*, 433, pp. 450–457.

Walley, W.J. and Hawkes, H.A. (1997) A computer-based development of the biological monitoring working party score system incorporating abundance rating, site type and indicator value, *Water Research*, 31(2), pp. 201–210.

Wang, Q., Li, Y. and Wang, Y. (2011) Optimizing the weight loss-on-ignition methodology to quantify organic and carbonate carbon of sediments from diverse sources, *Environmental Monitoring and Assessment*, 174(1–4), pp. 241–257.

Watt, J., Hastie, L.C. and Cosgrove, P.J. (2018) Monitoring the success of freshwater pearl mussel reintroductions. *Scottish Natural Heritage Research Report No. 956.*

Way, C.M., Hornbach, D., Miller-Way, C., Payne, B. and Miller, A. (1990) Dynamics of filter feeding in *Corbicula fluminea* (Bivalvia: Corbiculidae), *Canadian Journal of Zoology*, 68(1), pp. 115–120.

Wentworth, C.K. (1922) A scale of grade and class terms for clastic sediments, *The Journal of Geology*, 30(5), pp. 377–392.

West Cumbria Rivers Trust (2021a) *Irt Freshwater Mussel Project*. Available at: https://westcumbriariverstrust.org/projects/irt-freshwater-mussel-project (Accessed: 6 February 2022).

West Cumbria Rivers Trust (2021b) *Pearls in Peril*. Available at: https://westcumbriariverstrust.org/projects/pearls-in-peril (Accessed: 22 May 2022).

Wiberg, P. and Smith, J.D. (1987) Calculations of the critical shear stress for motion of uniform and heterogeneous sediments, *Water Resources Research*, 23(8), pp. 1471–1480.

Williams, J.D., Warren, M., Cummings, K., Harris, J. and Neves, R. (1993) Conservation status of freshwater mussels of the United States and Canada, *Fisheries*, 18:9, pp. 6–22.

Wohl, E. (2016) Spatial heterogeneity as a component of river geomorphic complexity, *Progress in Physical Geography*, 40(4), pp. 598–615.

Wolman, M.G. (1954) A method of sampling coarse river-bed material, *EOS, Transactions American Geophysical Union*, 35(6), pp. 951–956.

Wood, P. and Armitage, P. (1997) Biological effects of fine sediment in the lotic environment, *Environmental Management*, 21(2), pp. 203–17.

Woodget, A.S. and Austrums, R. (2017) Subaerial gravel size measurement using topographic data derived from a UAV-SfM approach, *Earth Surface Processes and Landforms*, 42(9), pp. 1434–1443.

Woodget, A.S., Fyffe, C. and Carbonneau, P.E. (2018) From manned to unmanned aircraft: Adapting airborne particle size mapping methodologies to the characteristics of sUAS and SfM, *Earth Surface Processes and Landforms*, 43(4), pp. 857–870.

Wotton, R., Malmqvist, B., Muotka, T. and Larsson, K. (1998) Fecal pellets from a dense aggregation of suspension-feeders in a stream: An example of ecosystem engineering, *Limnology and Oceanography*, 43(4), pp. 719–725.

Wu, H., Constantinescu, G. and Zeng, J. (2020) Flow and entrainment mechanisms around a freshwater mussel aligned with the incoming flow, *Water Resources Research*, 56, pp. 1–24.

Wu, H., Wang, X. and He, X. (2017) Effects of selected root exudate components on nitrogen removal and development of denitrifying bacteria in constructed wetlands, *Water*, 9, 430, pp. 1–13.

Wyżga, B., Oglęcki, P., Radecki-Pawlik, A., Skalski, T. and Zawiejska, J. (2012) Hydromorphological complexity as a driver of the diversity of benthic invertebrate communities in the Czarny Dunajec River, Polish Carpathians, *Hydrobiologia*, 696(1), pp. 29–46.

Yager, E.M., Kenworthy, M. and Monsalve, A. (2015) Taking the river inside: Fundamental advances from laboratory experiments in measuring and understanding bedload transport processes., *Geomorphology*, 244, pp. 21–32.

Young, M.K., Hubert, W. and Wesche, T. (1991) Biases associated with four stream substrate samplers, *Canadian Journal of Fisheries and Aquatic Science*, 48, pp. 1882–1886.

Young, M.R., Cosgrove, P., Hastie, L. and Henninger, B. (2001) A standardised method for assessing the status of freshwater mussels in clear, shallow rivers, *Journal of Molluscan Studies*, 67(3), pp. 395–396.

Zaiko, A., Daunys, D. and Olenin, S. (2009) Habitat engineering by the invasive zebra mussel *Dreissena polymorpha* (Pallas) in a boreal coastal lagoon: impact on biodiversity, *Helgoland Marine Research*, pp. 85–94.

Zajac, K. (2014) Size-Dependent Predation By Otter *Lutra Lutra* on swan mussels *Anodonta Cygnea* (Linnaeus 1758) - Observations and radiotelemetry experiment, *Journal of Conchology*, 41(5), pp. 559–563.

Zanetell, B.A. and Peckarsky, B.L. (1996) Stoneflies as ecological engineers – hungry predators reduce fine sediments in stream beds, *Freshwater Biology*, 36(3), pp. 569–577.

Zapitis, C., Huck, M., Mehra, A., Bulling, M.T., Ramsey, A.D. (2021) Behavioural responses of *Anodonta anatina* and *Unio pictorum* to temperature and algal concentration, *Journal of Molluscan Studies*, 87, eyab036, pp. 1–9.

Zapitis, C., Huck, M. and Ramsey, A.D. (2021) Oxygen consumption during digestion in *Anodonta anatina* and *Unio pictorum* in response to algal concentration, *Hydrobiologia*, 848(12–13), pp. 3209–3217.

Zieritz, A., Geist, J. and Gum, B. (2014) Spatio-temporal distribution patterns of three stream-dwelling freshwater mussel species: towards a strategy for representative surveys, *Hydrobiologia*, 735(1), p. 123.

Zimmerman, G.F. and de Szalay, F. (2007) Influence of unionid mussels (Mollusca: Unionidae) on sediment stability: an artificial stream study, *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, 168(4), pp. 299–306.

Ziuganov, V., Miguel, E., Neves, R., Longa, A., Amaro, R., Beletsky, V., Popkovitch, E., Kaliuzhin, S., Miguel, S. and Neves, J. (2000) Life span variation of the freshwater pearl shell: A model species for testing longevity mechanisms in animals, *Ambio*, 29(2), pp. 102–105.

Appendices

Appendix 1: Graphs of the running mean and SEM of variables measured in preliminary studies to determine optimum sample sizes for the river and flume investigations.

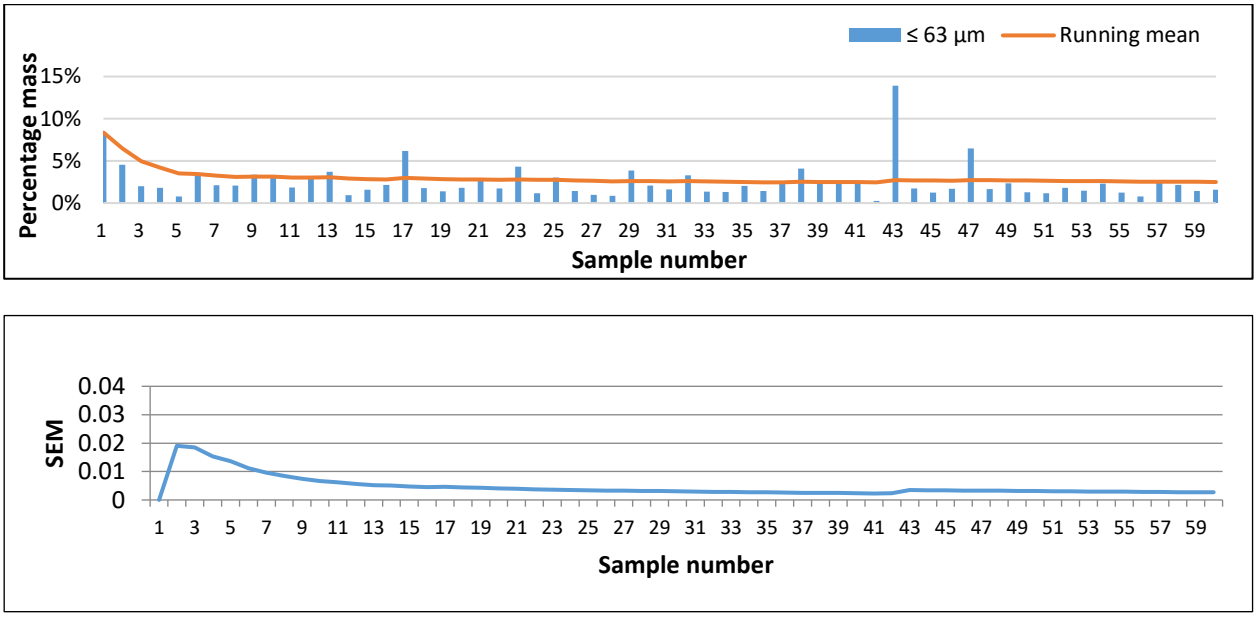


Figure A: Graphs to show the running mean and standard error of the mean (SEM) for the percentage dry mass of the < 63 µm grain-size fraction in sediment samples from Markeaton Brook.

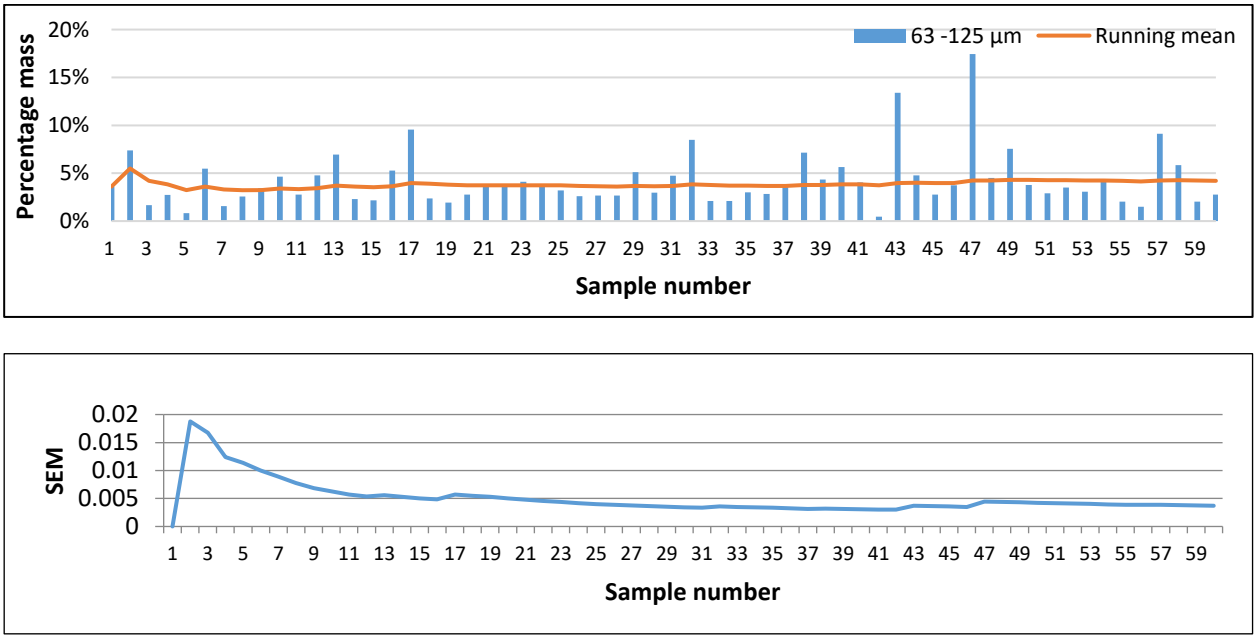


Figure B: Graphs to show the running mean and the standard error of the mean (SEM) for the percentage dry mass of the 63-125 µm grain-size fraction in sediment samples from Markeaton Brook.

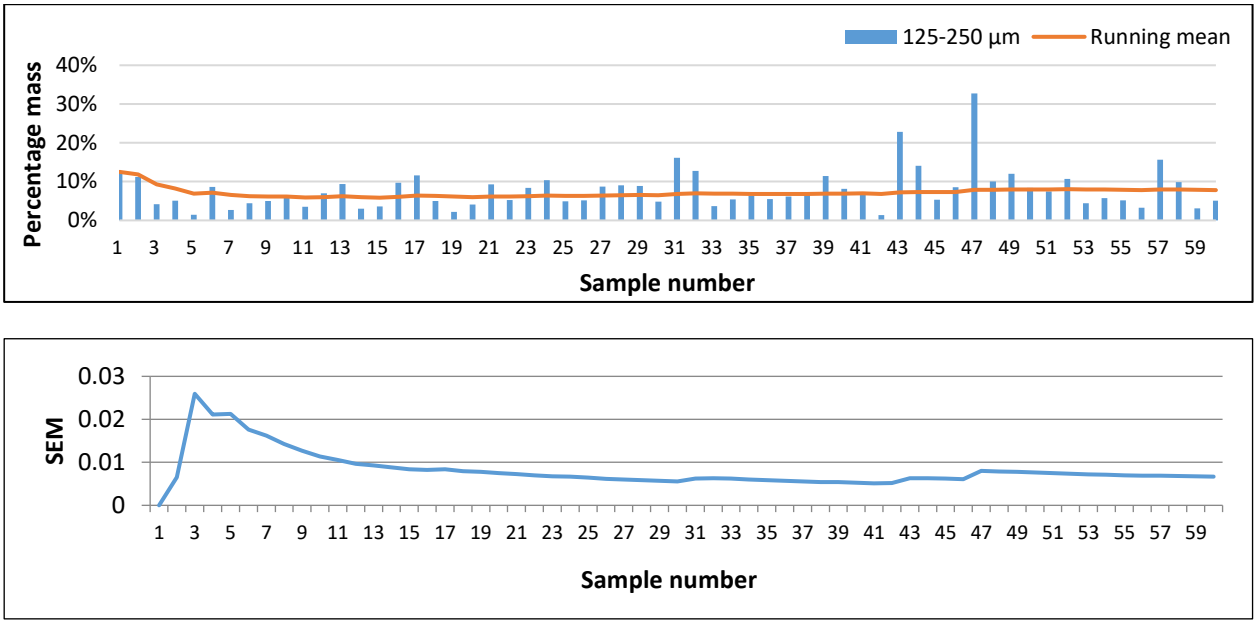
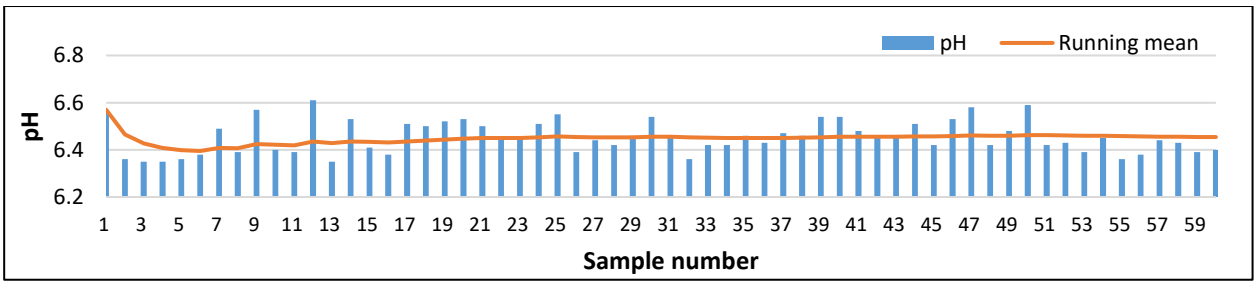


Figure C: Graphs to show the running mean and the standard error of the mean (SEM) for the percentage dry mass of the 125-250 µm grain-size fraction in sediment samples from Markeaton Brook.



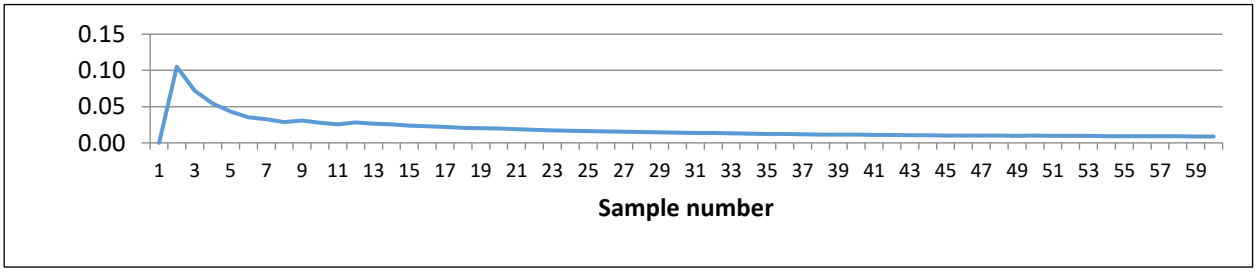


Figure D: Graphs to show the running mean and the standard error of the mean (SEM) for the pH of the sediment samples taken from Markeaton Brook.

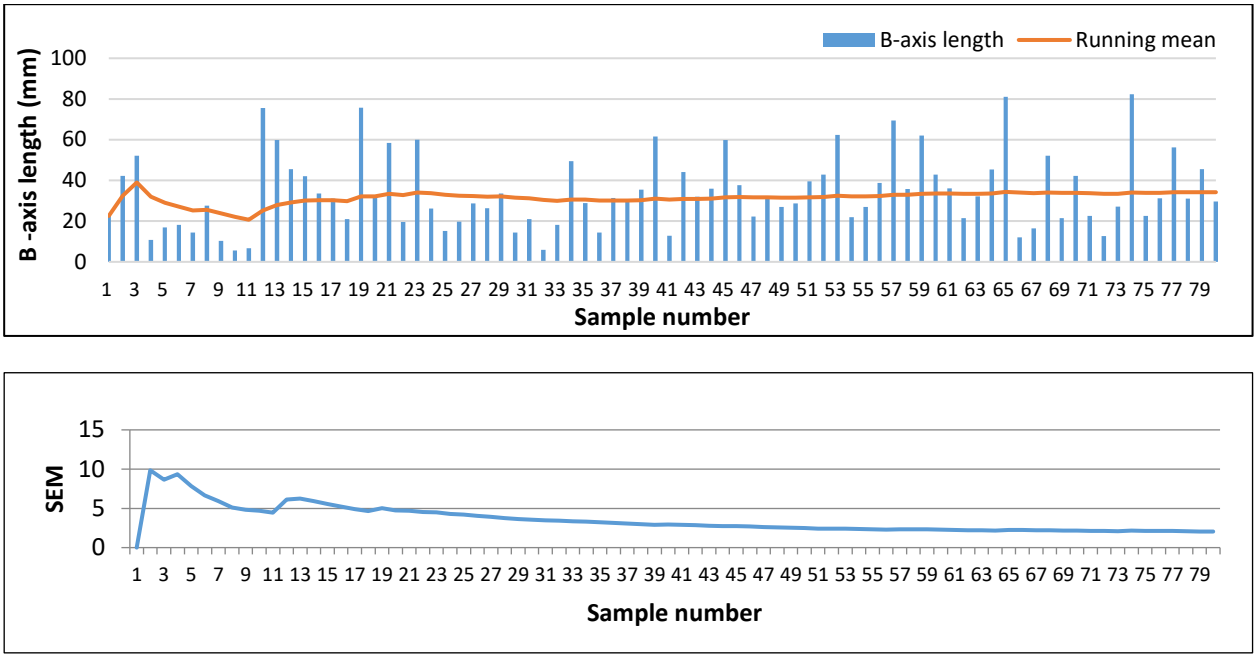


Figure E: Graphs to show the running mean and the standard error of the mean (SEM) for pebble B-axis length within a 2 m^2 area of Markeaton Brook.

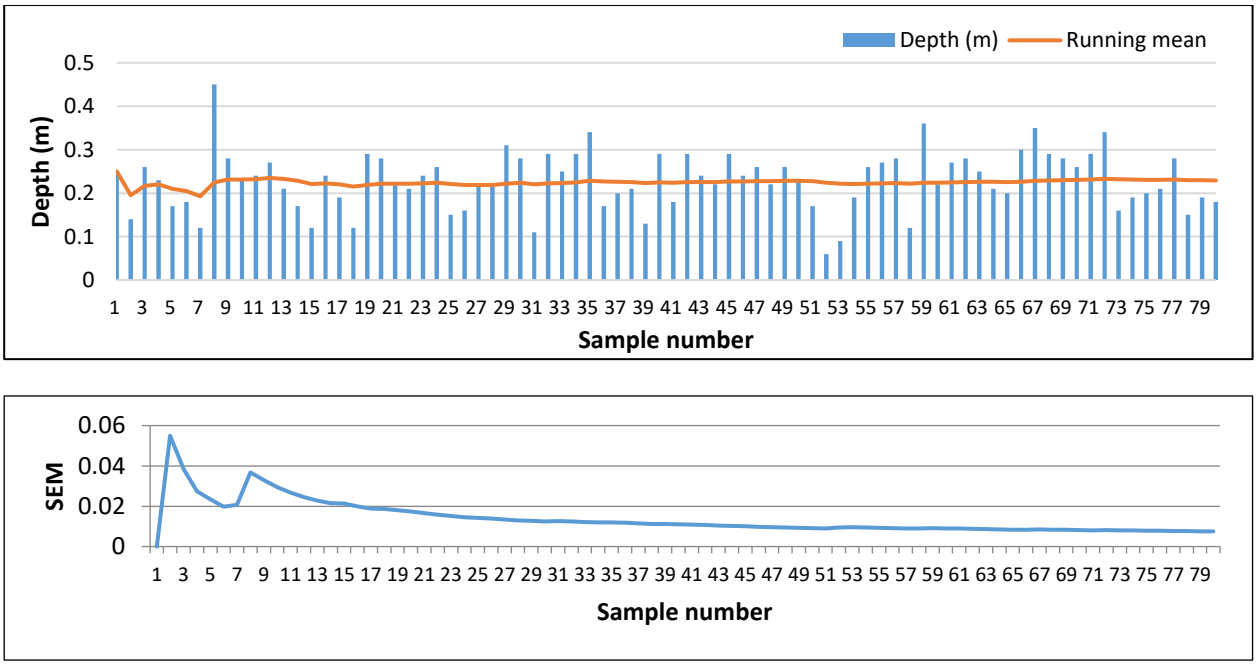


Figure F: Graphs to show the running mean and the standard error of the mean (SEM) for the measurements of water depth in the sampled stretch of Markeaton Brook.

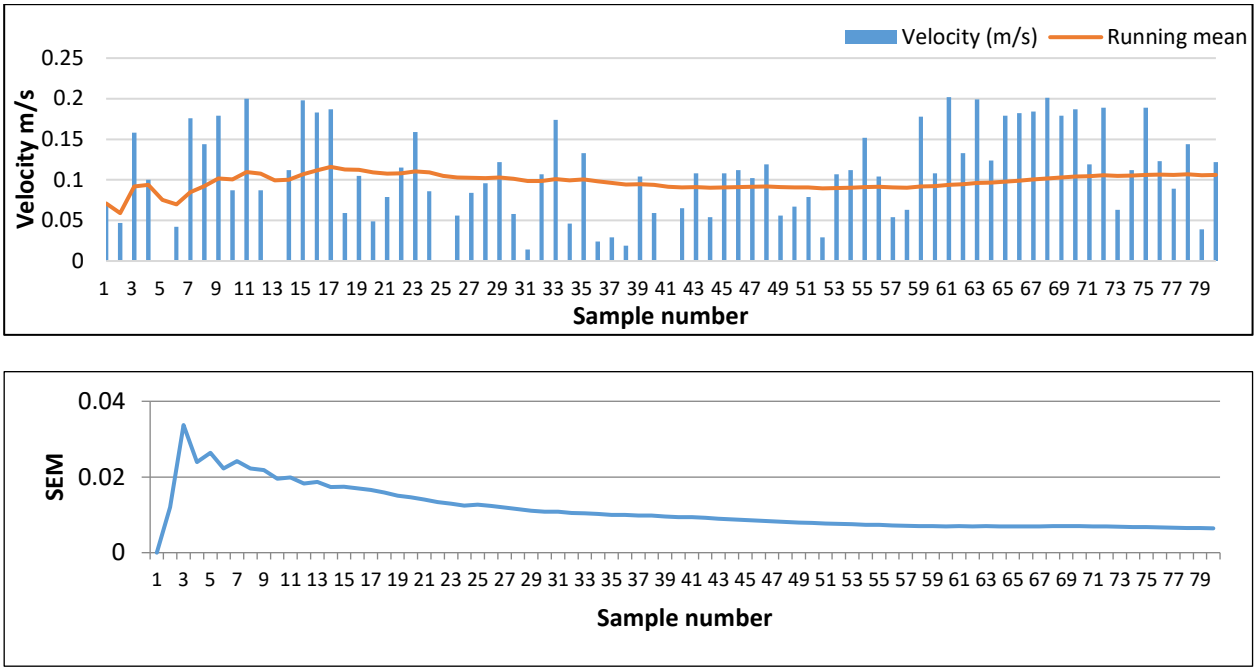


Figure G: Graphs to show the running mean and the standard error of the mean (SEM) for the measurements of near-bed (0.9 depth) water velocity from the sampled stretch of Markeaton Brook.

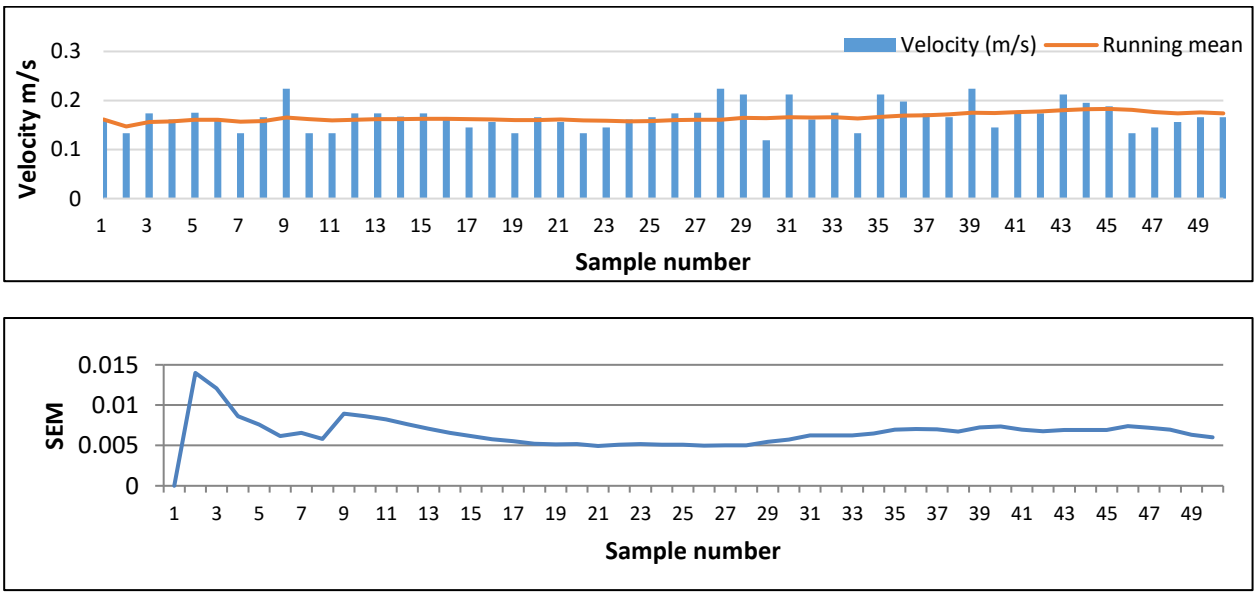


Figure H: Graphs to show the running mean and the standard error of the mean (SEM) for water velocity at 0.6 depth within a 2 m^2 area of Markeaton Brook.

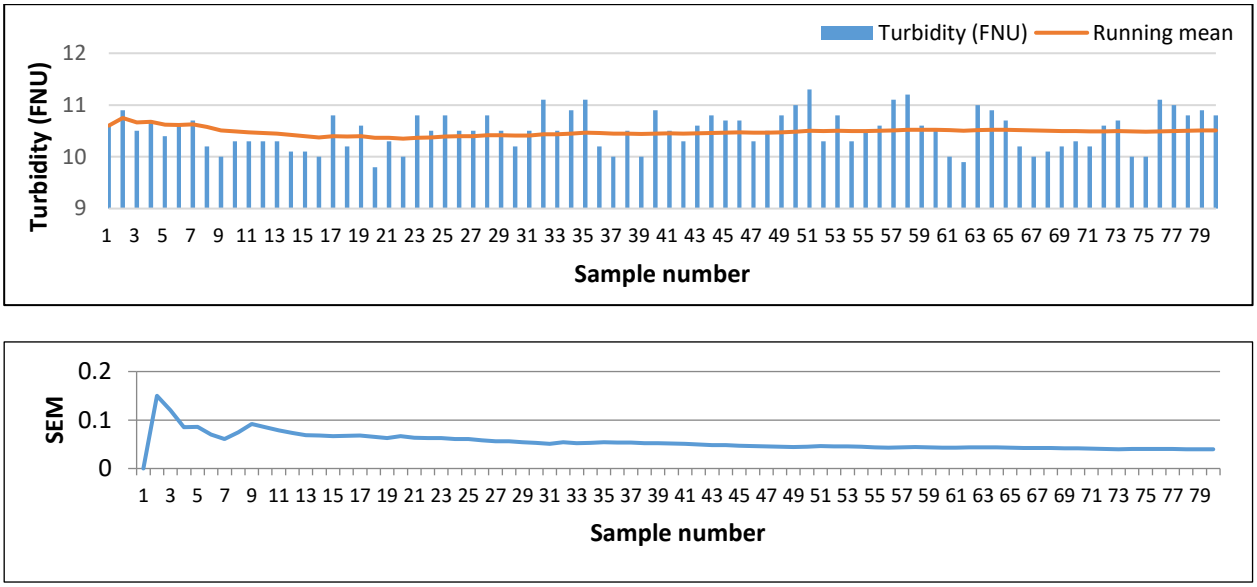


Figure I: Graphs to show the running mean and the standard error of the mean (SEM) for water turbidity in Formazin Nephelometric Units (FNU) within a 2 m^2 area of Markeaton Brook.

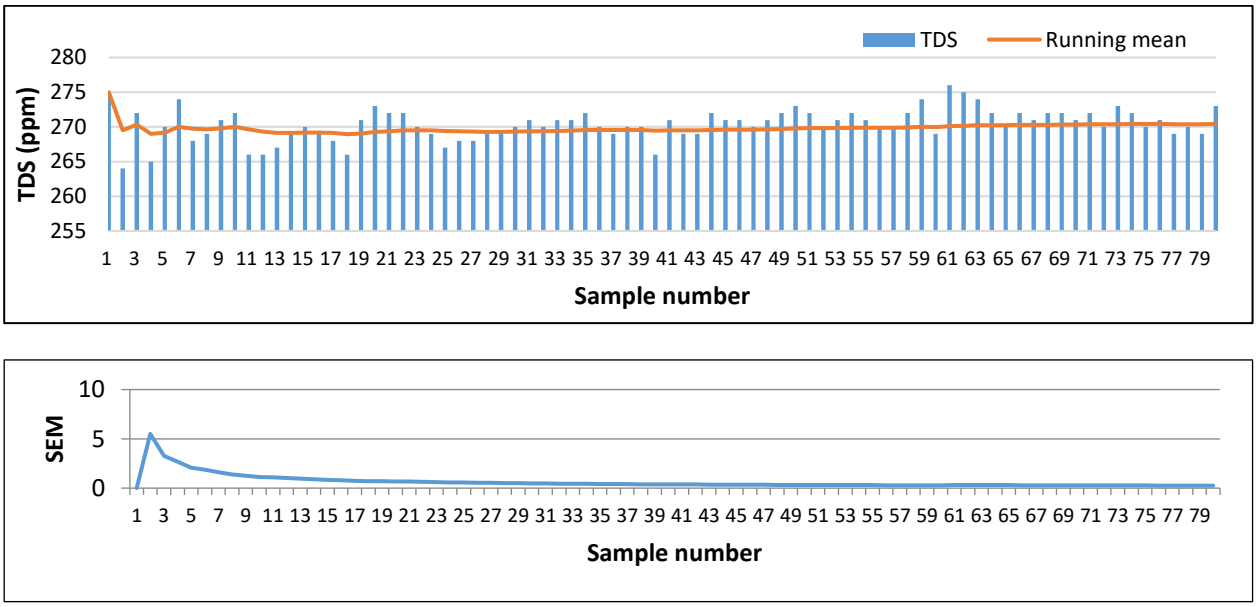


Figure J: Graphs to show the running mean and the standard error of the mean (SEM) for total dissolved solids (TDS) in ppm within a 2 m^2 area of Markeaton Brook.

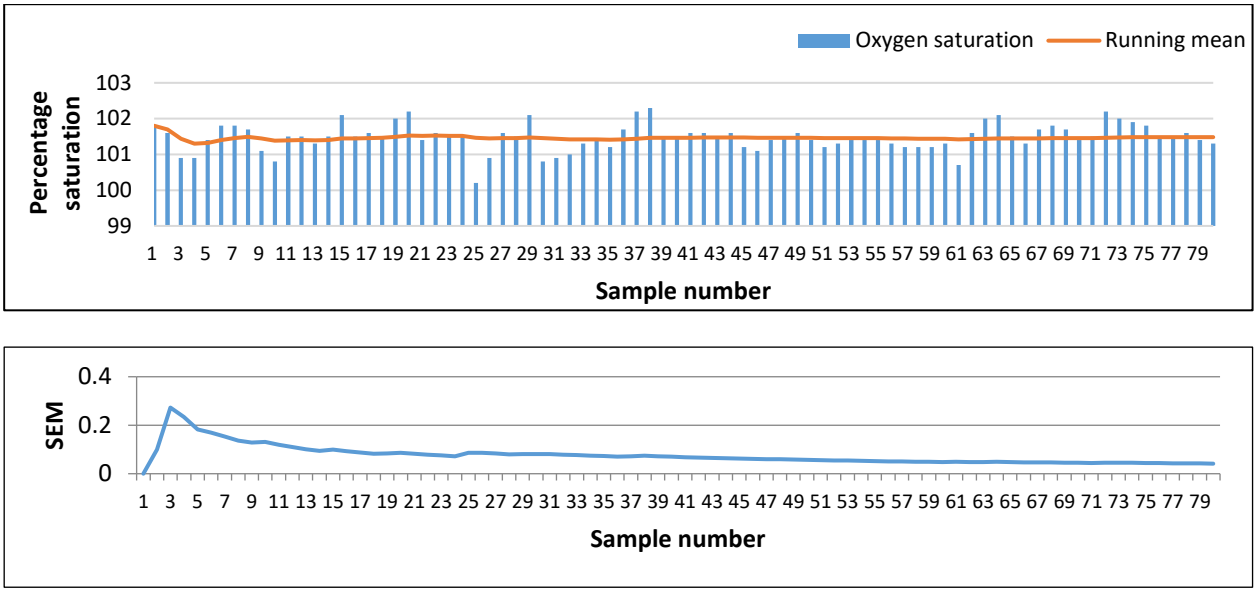


Figure K: Graphs to show the running mean and the standard error of the mean (SEM) for hyporheic oxygen saturation (%) within a 2 m^2 area of Markeaton Brook.

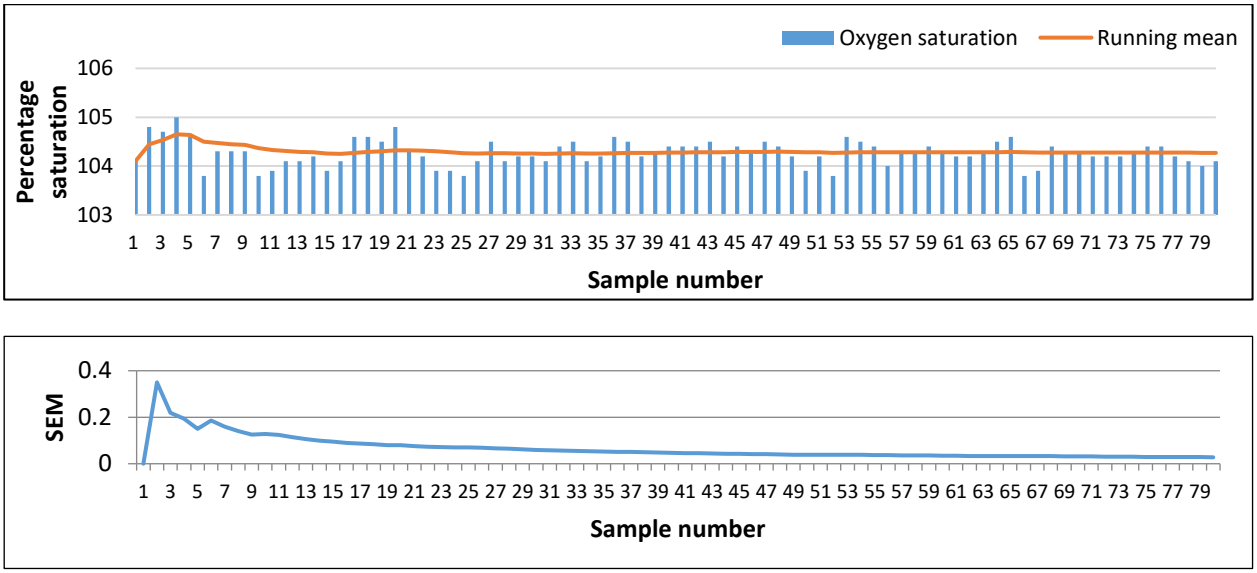


Figure L: Graphs to show the running mean and the standard error of the mean (SEM) for water oxygen saturation (%) within a 2 m^2 area of Markeaton Brook.

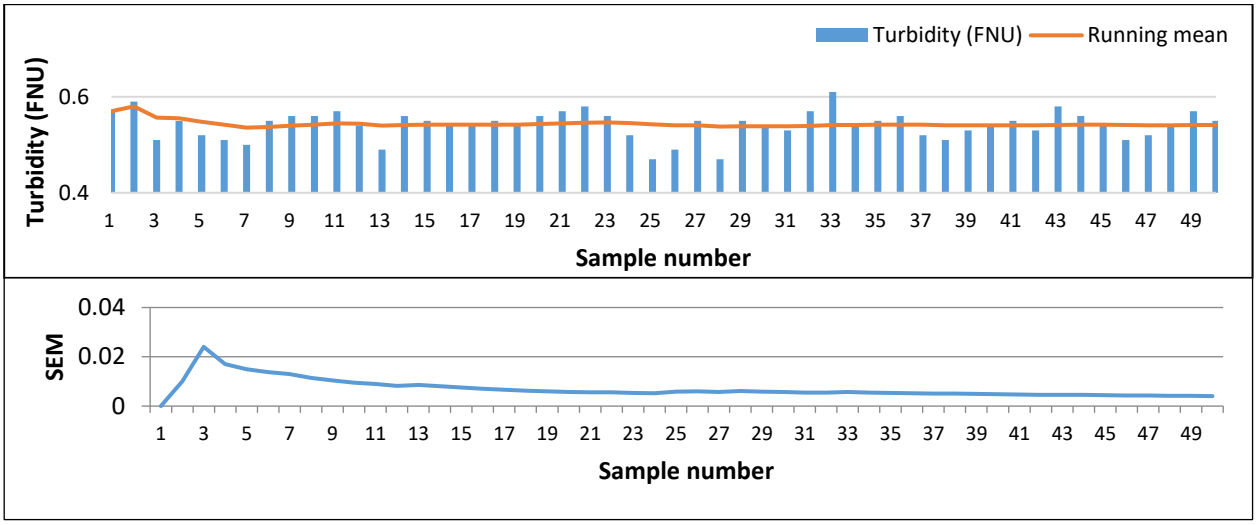


Figure M: Graphs to show the running mean and the standard error of the mean (SEM) for the turbidity of water in the flume's channel.

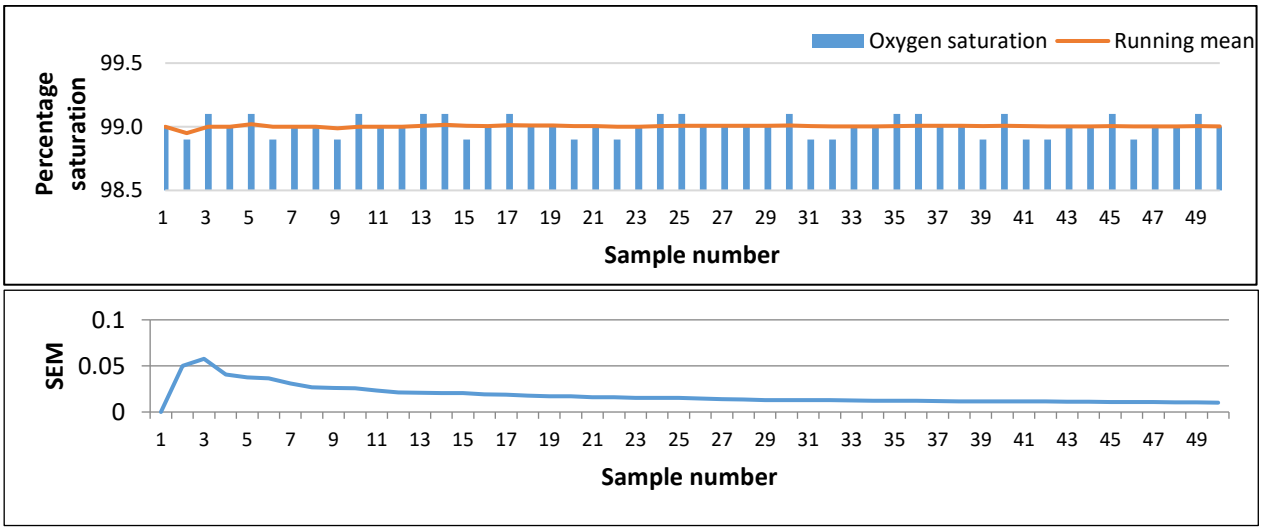


Figure N: Graphs to show the running mean and the standard error of the mean (SEM) for water oxygen saturation in the flume.

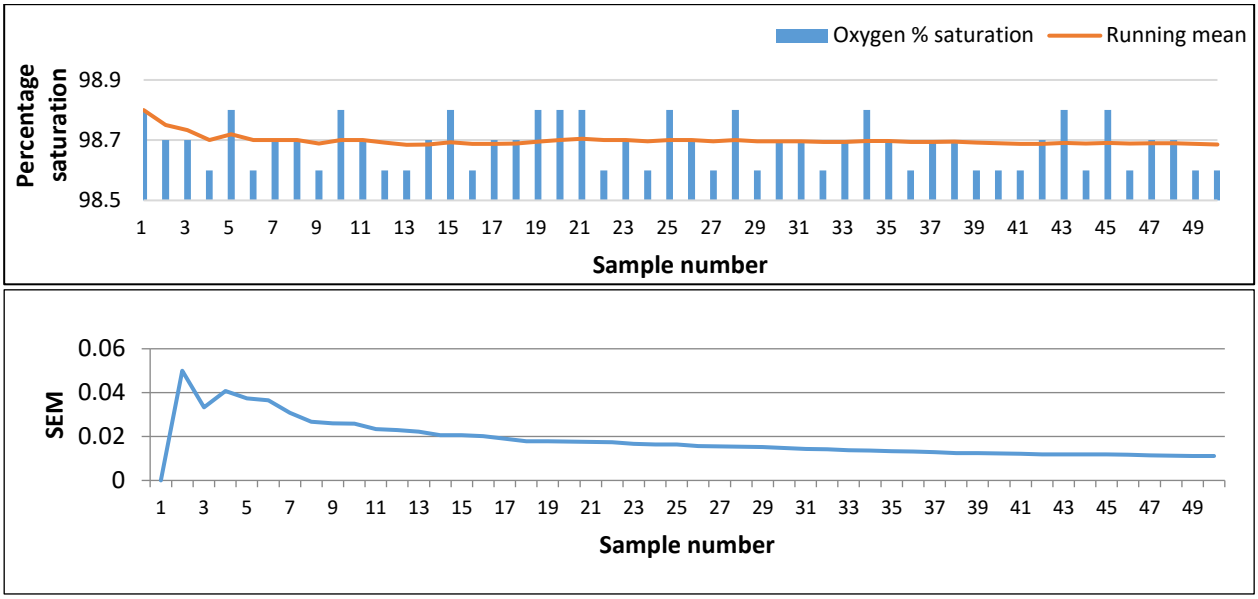


Figure O: Graphs to show the running mean and the standard error of the mean (SEM) for hyporheic oxygen saturation in the flume.

Appendix 2: Scoring system used in the calculation of BMWP values

Common Name	Family	Habitat Specific Scores		
		Riffles	Riffle/ Pools	Pools
Flatworms	Planariidae	4.5	4.1	3.7
	Dendrocoelidae			
Snails	Neritidae			
	Viviparidae			
	Valvatidae			
	Hydrobiidae			
	Lymnaeidae	3.2	3.1	2.8
	Physidae			
	Planorbidae			
Limpets and Mussels	Ancylidae			
	Unionidae	4.7	4.8	5.5
	Sphaeriidae	3.7	3.7	3.4
Worms	Oligochaeta	3.9	3.2	2.5
Leeches	Piscicolidae			
	Glossiphoniidae			
	Hirudididae			
	Erpobdellidae	2.8	2.8	2.6
Crustaceans	Asellidae	1.5	2.4	2.7
	Corophiidae			
	Gammaridae	4.7	4.3	4.3
	Astacidae			
Mayflies	Siphlonuridae			
	Baetidae	5.5	4.8	5.1
	Heptageniidae	9.7	10.7	13
	Leptophlebiidae			
	Ephemerellidae	7.6	8.1	9.3
	Potamanthidae			
	Ephemeridae	9	9.2	11
	Caenidae			
Stoneflies	Taeniopterygidae			
	Nemouridae			
	Leuctridae			
	Capniidae			
	Perlodidae			
	Perlidae			
	Chloroperlidae			
Damselflies	Platycnemidae			
	Coenagriidae			
	Lestidae			
	Calopterygidae			

Continued on next page….

The calculation of BMWP (biological monitoring working party) scores used a revision of the BMWP scoring system with habitat-specific scores adapted from Walley and Hawkes (1997) by Martin (2004). The habitat-specific scores are based on the following substrate compositions:

Riffles: ≥ 70% boulders and pebbles.

Pool: ≥ 70% sand and silt.

Riffle/Pool: the remainder.

River sites classified as a riffle:

RS sites A2, A4, B1, B2, B3, B4.

MB sites A4, B2, B4.

River sites classified as riffle/pool:

RS sites A1, A3.

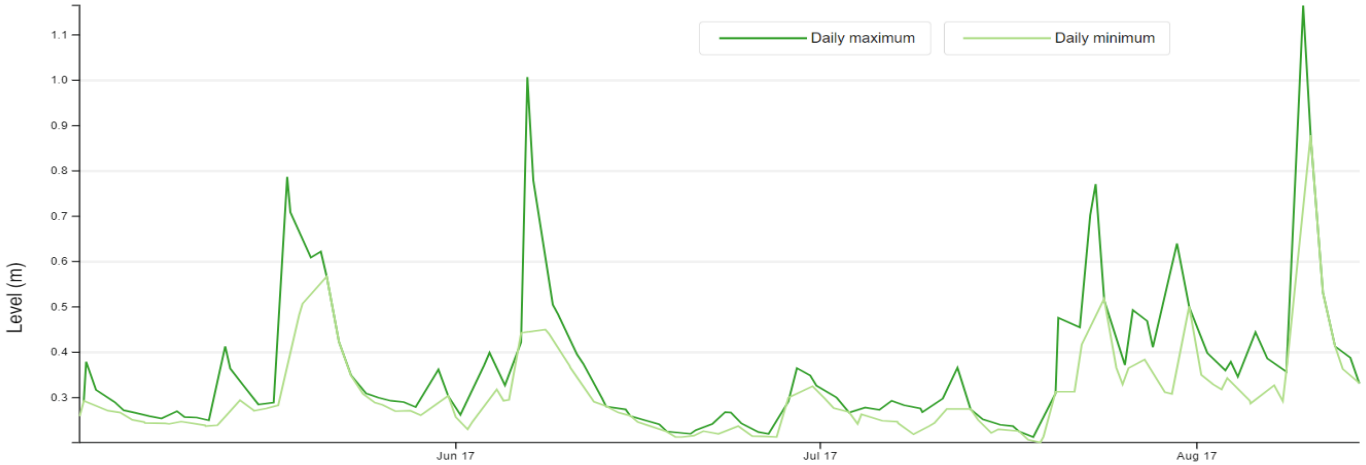
MB sites A1, 12, 13, B1, B3.

No sites used in the river study were classified as a pool.

Family groups found in the river study have been highlighted in green. Scores for family groups not found in the river study have not been included. The BMWP score is based on the presence of each family group in the kick samples for each site and their habitat-specific scores. The sum of the scores for the occurrence of each family group represents the total BMWP score for each site. The number of

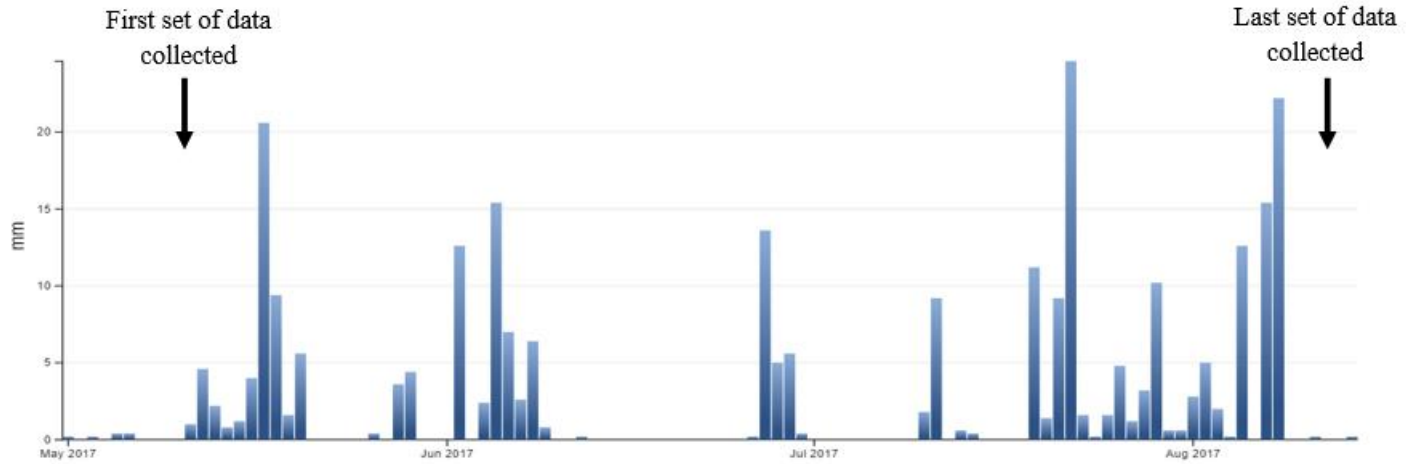
	Family	Riffle	R/P	Pool
Dragonflies	Gomphidae			
	Cordulegasteridae			
	Aeshnidae			
	Corduliidae			
	Libellulidae			
Bugs	Mesoveliidae			
	Hydrometridae			
	Gerridae			
	Nepidae			
	Naucoridae			
	Aphelocheiridae			
	Notonectidae			
	Pleidae			
	Corixidae			
Beetles	Haliplidae			
	Hygrobiidae			
	Dytiscidae			
	Gyrinidae	8.1	7.4	6.8
	Hydrophilidae			
	Clambidae			
	Scirtidae			
	Dryopidae			
	Elmidae			
	Chrysomelidae			
	Curculionidae			
Alderflies	Sialidae			
Caddisflies	Rhyacophilidae	8.2	8.6	9.6
	Philopotamidae	10.7	9.8	
	Polycentropidae			
	Psychomyiidae			
	Hydropsychidae	6.6	6.5	7.2
	Hydroptilidae			
	Phryganeidae			
	Limnephilidae			
	Molannidae			
	Beraeidae	8.3	7.8	10
	Odontoceridae			
	Leptoceridae			
	Goeridae			
	Lepidostomatidae	10.3	10.7	11.6
	Brachycentridae	9.3	9.7	11
	Sericostomatidae	9.1	9.3	10.3
True flies	Tipulidae	5.6	5	5.1
	Chironomidae	4.1	3.4	2.8
	Simuliidae	5.9	5.1	5.5

Appendix 3: River level, river discharge and rain fall data for the period of the investigation

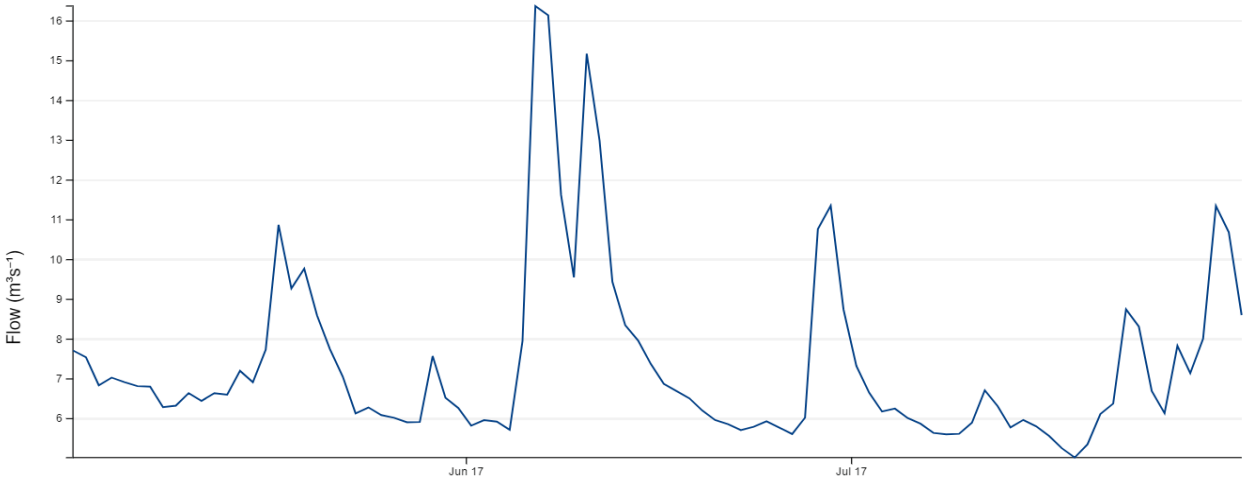


Data and graphs from DEFRA (2021). The nearest river gauging station to the River Sence sites is Temple Mill, which is downstream of RSA and RSB. River level data is provided, as discharge data is not available for this station. Rainfall data for the study period is from the Mount St Bernards rain gauge, which is located outside the catchment, just beyond the headwaters of the River Sence. © Crown Copyright 2021 Open Government Licence v3.0.

Temple Mill, Leicestershire, River Sence river level 01/05/17 - 14/08/17. Grid Reference **SK35590351** (downstream of RSA and RSB)**.**

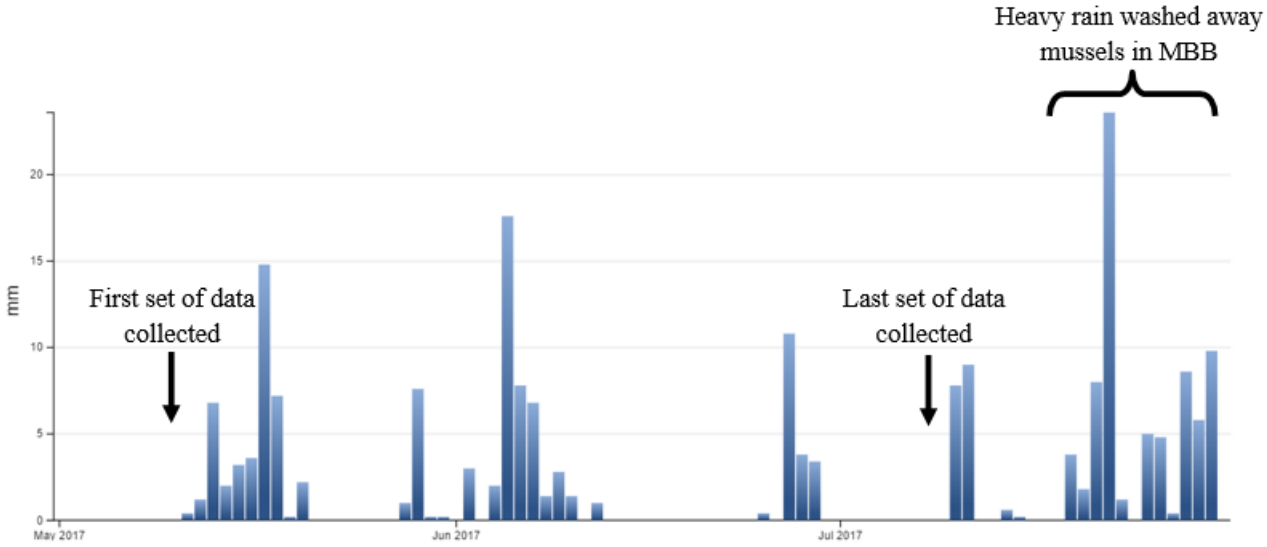


Mount St. Bernards, Leicestershire precipitation 01/05/17 - 14/08/17. Grid Reference **SK4597015880** (located outside the catchment, just beyond the headwaters of the River Sence)**.**



Data and graphs from DEFRA (2021). River discharge information is shown for the nearest river gauging station to the Markeaton Brook sites, at Derby St. Marys, which is downstream of MBA and MBB. The rainfall data is from the Meynell Langley rain gauge, which is upstream of MBA and MBB. © Crown Copyright 2021 Open Government Licence v3.0.

Derby St. Marys, River Derwent discharge 01/05/17 - 31/07/17. Grid Reference **K35393688** (downstream of MBA and MBB)**.**



Meynell Langley, Derbyshire precipitation 01/05/17 - 31/07/14. Grid Reference **SK2840040260** (upstream of MBA and MBB).

Appendix 4: Dimensions of individual mussels from the river-based investigation.

Measurements of the length (anterior to posterior), height (umbo to valve margin) and diameter (width across both valves) of mussels in Markeaton Brook. The length : diameter (L:D) ratio was calculated to aid identification of the species, using typical ranges described in Aldridge (1999) and Killeen, Aldridge and Oliver (2004).

Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D		Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D
1	71	43	26	2.7		51	81	47	26	3.1
2	78	47	29	2.7		52	65	40	16	4.1
3	70	42	23	3.0		53	70	43	21	3.3
4	71	43	27	2.6		54	75	44	23	3.3
5	82	49	32	2.6		55	67	39	22	3.0
6	59	37	20	3.0		56	71	44	23	3.1
7	64	42	25	2.6		57	74	42	23	3.2
8	72	44	25	2.9		58	78	47	23	3.4
9	64	37	26	2.5		59	70	40	21	3.3
10	63	40	21	3.0		60	72	44	21	3.4
11	55	37	20	2.8		61	73	45	22	3.3
12	74	43	25	3.0		62	70	41	18	3.9
13	61	40	21	2.9		63	59	41	15	3.9
14	63	35	22	2.9		64	56	33	16	3.5
15	61	37	22	2.8		65	77	46	29	2.7
16	56	40	22	2.5		66	82	49	23	3.6
17	69	42	24	2.9		67	74	44	26	2.8
18	72	40	25	2.9		68	94	53	31	3.0
19	56	34	22	2.5		69	76	44	25	3.0
20	55	35	20	2.8		70	57	37	17	3.4
21	58	34	19	3.1		71	90	50	24	3.8
22	59	38	20	3.0		72	70	40	22	3.2
23	68	38	22	3.1		73	80	49	25	3.2
24	59	35	21	2.8		74	84	48	25	3.4
25	68	41	25	2.7		75	71	44	20	3.6
26	74	43	23	3.2		76	75	41	23	3.3
27	61	34	24	2.5		77	79	49	25	3.2
28	70	40	20	3.5		78	72	46	26	2.8
29	70	41	21	3.3		79	76	48	24	3.2
30	80	52	30	2.7		80	58	39	16	3.6
31	60	35	21	2.9		81	70	40	18	3.9
32	83	48	25	3.3		82	69	46	20	3.5
33	72	43	21	3.4		83	71	42	24	3.0
34	78	46	29	2.7		84	59	39	18	3.3
35	64	29	19	3.4		85	55	36	16	3.4
36	77	46	28	2.8		86	73	41	23	3.2
37	60	40	20	3.0		87	77	46	21	3.7
38	83	45	24	3.5		88	76	41	20	3.8
39	77	48	28	2.8		89	73	43	21	3.5

40	76	42	23	3.3		90	70	40	18	3.9
41	57	35	16	3.6		91	64	38	19	3.4
42	77	44	27	2.9		92	58	36	17	3.4
43	69	42	24	2.9		93	77	40	21	3.7
44	67	38	25	2.7		94	57	32	18	3.2
45	74	46	27	2.7		95	64	37	20	3.2
46	66	42	26	2.5		96	77	44	21	3.7
47	68	45	27	2.5		97	69	45	24	2.9
48	80	46	28	2.9		98	78	48	26	3.0
49	68	39	27	2.5		99	69	46	20	3.5
50	76	47	27	2.8		100	75	41	23	3.3

	Length (mm)	Height (mm)	Diameter (mm)	L:D
Min	55.0	29.0	15.0	2.46
Max	94.0	53.0	32.0	4.06
Mean	69.6	41.9	22.7	3.12

Typical range of L:D ratio
A. anatina 3.1 (2.6-3.5)
P. complanata 3.5-4.5
A. cygnea 3.5-4.1 (some 2.5)

The majority of the sample population from Markeaton Brook falls within the typical range of *Anodonta anatina* with some individuals within in typical range of *A. cygnea.*

Measurements of the length (anterior to posterior), height (umbo to valve margin) and diameter (width across both valves) of a sample of mussels from the River Sence. 217 mussels were found in total. To minimise the length of time they were out of the water only 20 were measured to aid identification of the species present. The length : diameter (L:D) ratio was calculated using typical ranges described in Aldridge (1999) and Killeen, Aldridge and Oliver (2004).

Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D		Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D
1	120	60	34	3.5		11	94	57	31	3.0
2	90	52	29	3.1		12	89	52	26	3.4
3	97	54	37	2.6		13	81	48	24	3.4
4	104	58	35	3.0		14	114	60	37	3.1
5	97	55	33	2.9		15	123	67	47	2.6
6	108	58	32	3.4		16	111	61	33	3.4
7	84	46	28	3.0		17	88	50	27	3.3
8	114	56	40	2.9		18	109	60	36	3.0
9	92	55	30	3.1		19	107	60	35	3.1
10	90	52	25	3.6		20	75	44	21	3.6

	Length (mm)	Height (mm)	Diameter (mm)	L:D
Min	75.0	44.0	21.0	2.62
Max	123.0	67.0	47.0	3.60
Mean	99.4	55.3	32.0	3.14

The sample population from the Sence falls within the typical range of *A. anatina* and *A. cygnea.*

Appendix 5: Dimensions of individual mussels used in the flume-based investigation.

Measurements of the length (anterior to posterior), height (umbo to valve margin) and diameter (width across both valves) of mussels used in the flume-based investigation. The length : diameter (L:D) ratio was calculated to aid identification of the species, using typical ranges described in Aldridge (1999) and Killeen, Aldridge and Oliver (2004).

Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D		Mussel	Length (mm)	Height (mm)	Diameter (mm)	L:D
1	80.1	44.4	22.3	3.59		26	73.0	46.3	24.5	2.98
2	70.3	42.0	25.3	2.78		27	67.6	40.2	23.2	2.91
3	76.2	42.9	25.4	3.00		28	81.4	45.5	26.1	3.12
4	78.4	43.3	25.3	3.10		29	82.8	46.1	26.7	3.10
5	70.3	47.4	23.9	2.94		30	71.1	43.3	27.3	2.60
6	71.0	37.9	25.0	2.84		31	88.7	50.2	28.4	3.12
7	72.4	44.3	25.7	2.82		32	79.2	46.3	27.9	2.84
8	73.7	43.1	25.2	2.92		33	80.1	49.9	28.8	2.78
9	73.9	41.7	26.1	2.83		34	71.8	43.8	27.3	2.63
10	74.1	38.8	25.0	2.96		35	71.4	40.4	24.8	2.88
11	74.8	43.0	26.3	2.84		36	72.5	42.1	24.4	2.97
12	88.2	52.4	29.3	3.01		37	76.1	46.4	24.3	3.13
13	71.7	44.4	25.4	2.82		38	73.2	42.1	26.2	2.79
14	81.3	52.1	28.2	2.88		39	67.9	40.3	23.9	2.84
15	91.3	52.8	30.1	3.03		40	79.9	43.6	26.1	3.06
16	71.6	41.6	25.4	2.82		41	65.5	39.7	21.0	3.12
17	77.0	48.3	27.8	2.77		42	84.4	48.4	28.7	2.94
18	77.5	47.1	27.0	2.87		43	70.2	39.8	23.3	3.01
19	75.9	45.4	27.3	2.78		44	86.0	51.3	29.4	2.93
20	75.2	44.3	25.9	2.90		45	80.3	47.0	28.4	2.83
21	77.7	44.8	29.9	2.60		46	76.4	44.7	26.8	2.85
22	76.3	46.7	27.4	2.78		47	70.4	40.1	21.6	3.26
23	84.1	46.6	29.0	2.90		48	72.5	42.5	24.4	2.97
24	72.8	48.0	26.1	2.79		49	73.2	44.2	24.8	2.95
25	84.2	49.1	27.3	3.08		50	75.1	46.3	28.3	2.65

	Length (mm)	Height (mm)	Diameter (mm)	L:D
Min	65.5	37.9	21.0	2.60
Max	91.3	52.8	30.1	3.59
Mean	76.2	44.9	26.2	2.92

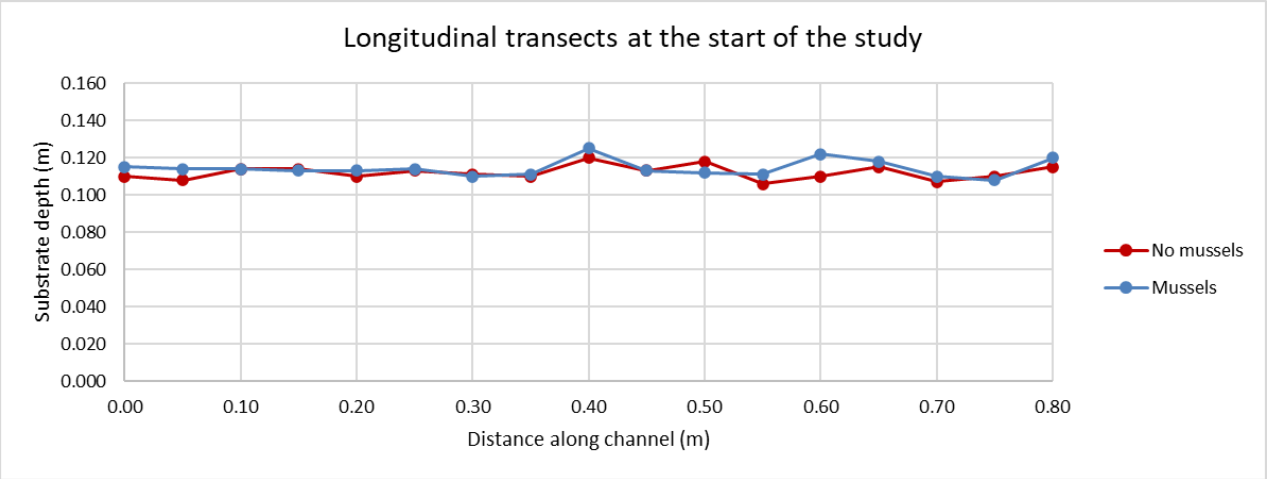
Typical range of L:D ratio
A. anatina 3.1 (2.6-3.5)
P. complanata 3.5-4.5
A. cygnea 3.5-4.1 (some 2.5)

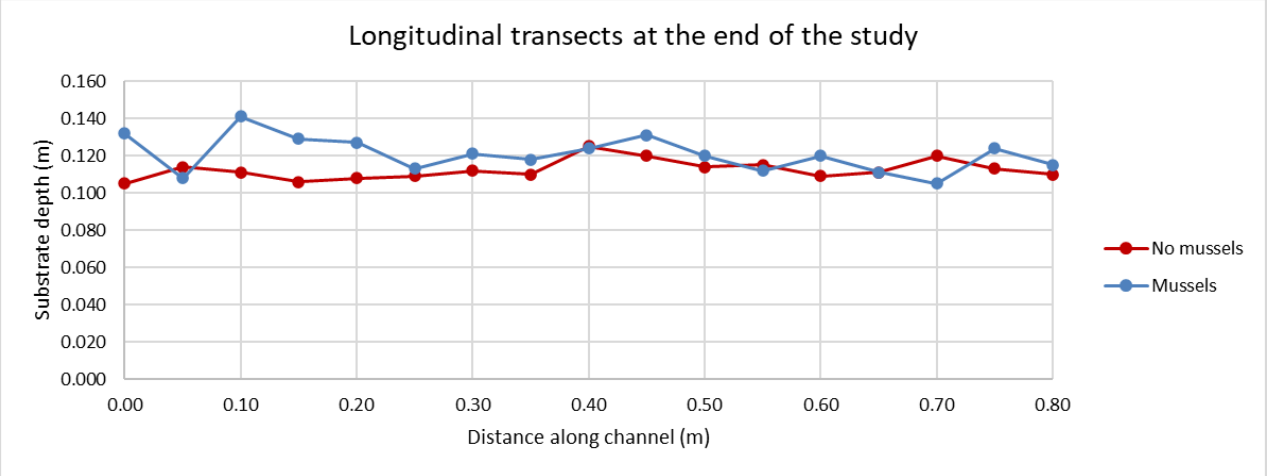
The majority of the sample population from Markeaton Brook falls within the typical range of *Anodonta anatina* with some individuals within in typical range of *A. cygnea.*

Appendix 6: Topographic profiles of the flume substrate

Longitudinal topographic profiles of the flume substrate at the beginning and end of the study:

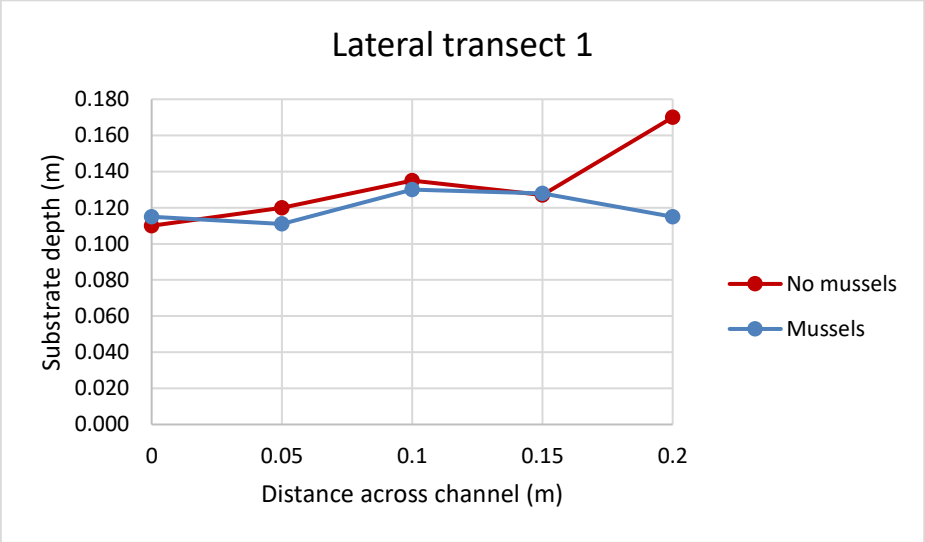
(The transects were positioned along the full length of the straight section of flume channel that did not contain the sediment trap. Distance 0.00 m is the upstream end the transect. Distance 0.80 m is the downstream end of the transect).

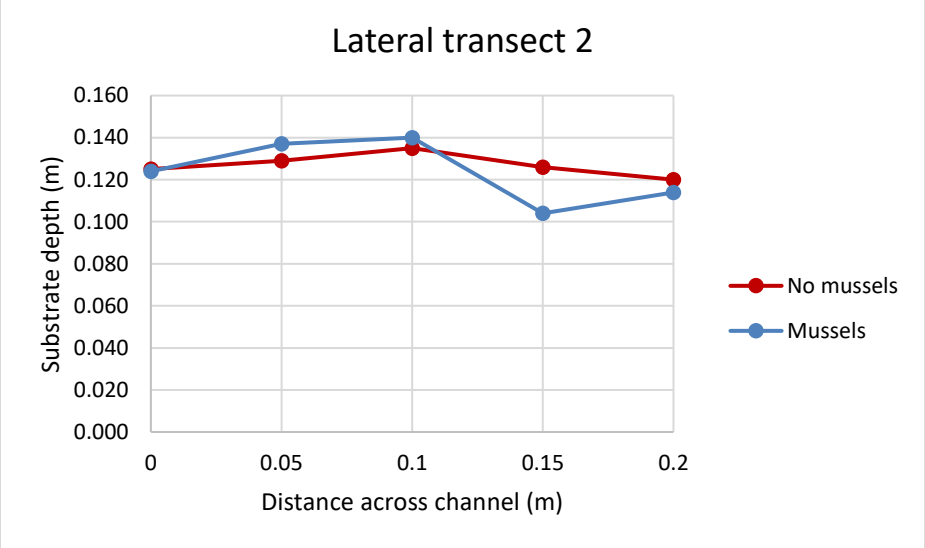


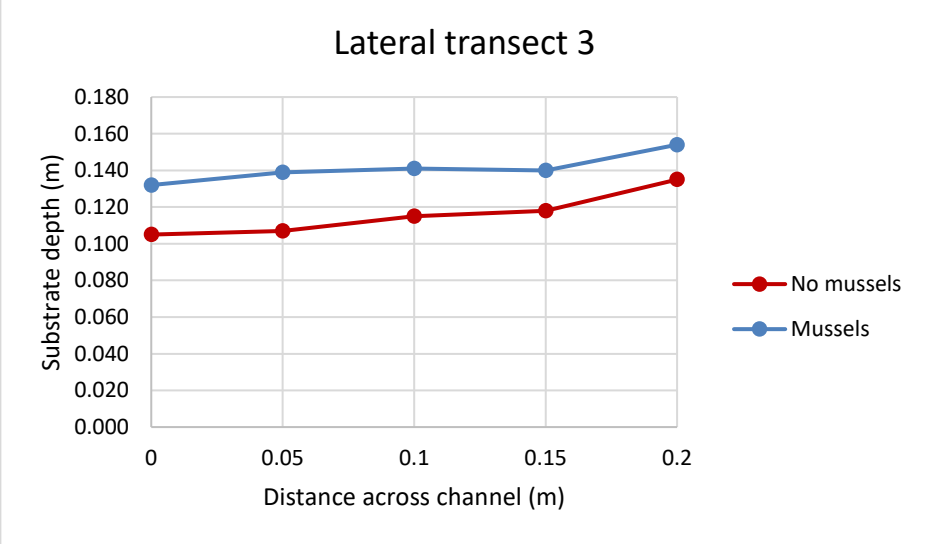


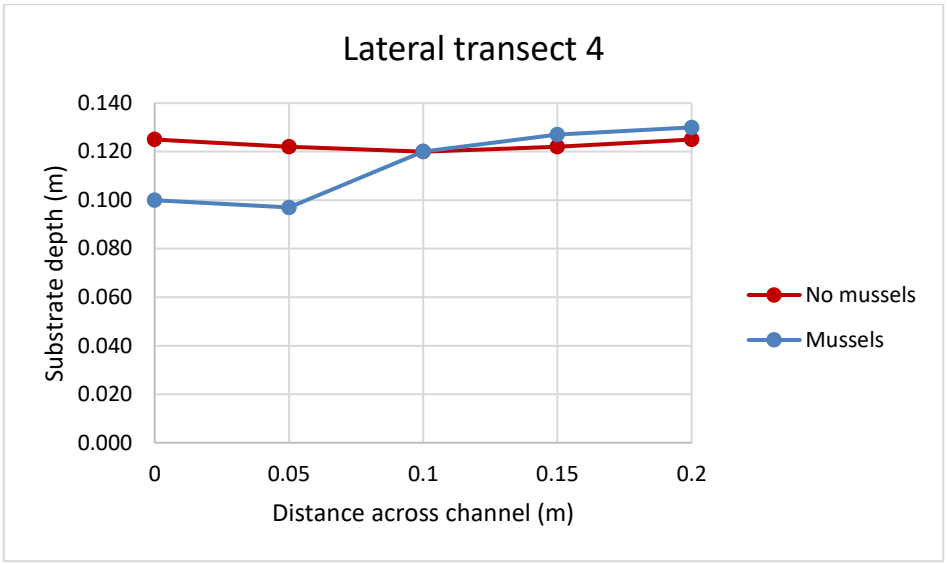
Lateral topographic profiles of the flume substrate at the end of the study:

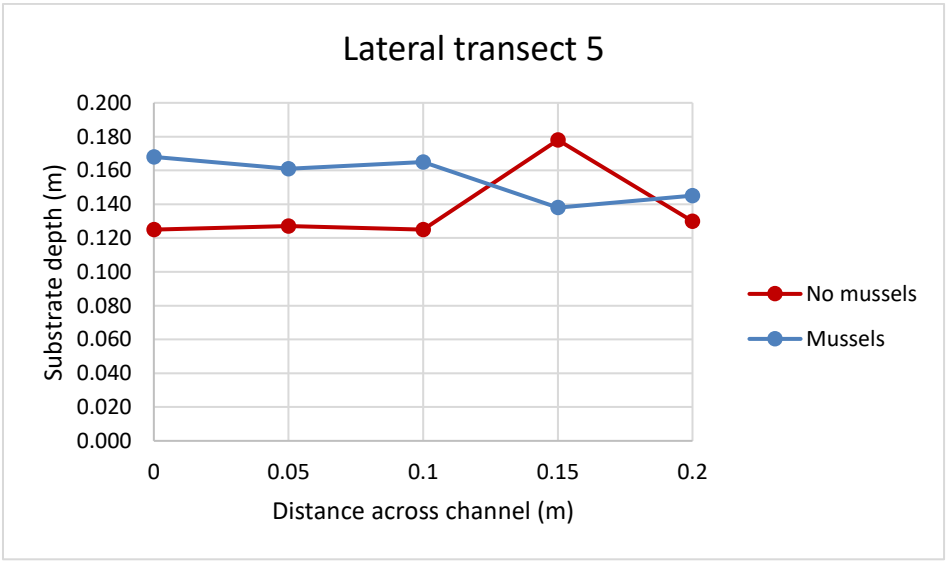
(Distance 0.0 m is the outside edge of the flume channel. Distance 0.2 m is the inside edge of the flume channel. Lateral transects 1 and 4 were taken from the upstream end of each curved section of flume channel. Lateral transects 3 and 6 were from the downstream end of each curved section of flume channel. Lateral transects 2 and 5 were from mid-way along each of the straight sections of flume channel).

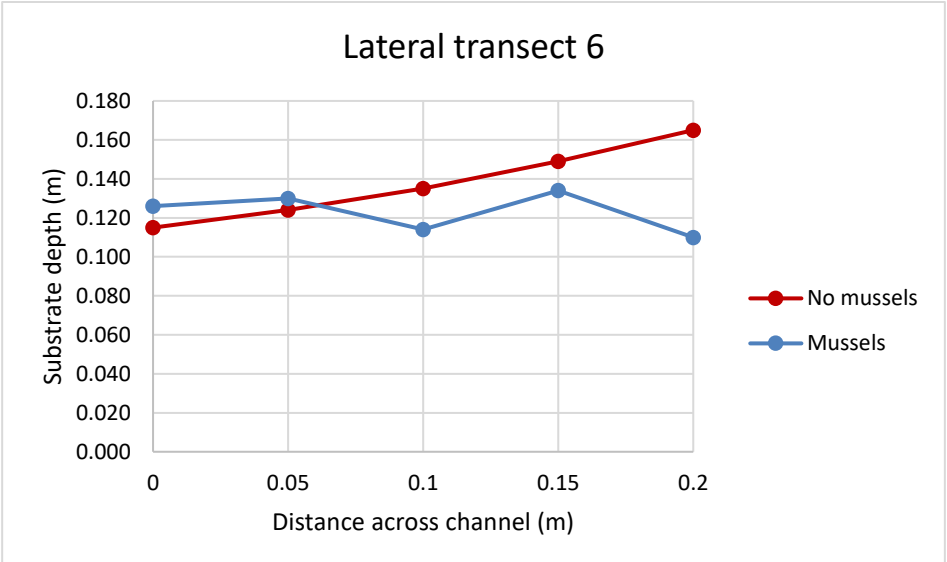







Lateral transect 4
Substrate depth (m)
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
0.05
0.1
0.15
0.2
Distance across channel (m)
No mussels
Mussels


Lateral transect 5
Substrate depth (m)
0.200
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
0.05
0.1
0.15
0.2
Distance across channel (m)
No mussels
Mussels


Lateral transect 6
Substrate depth (m)
0.180
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0
0.05
0.1
0.15
0.2
Distance across channel (m)
No mussels
Mussels

References for Appendices:

Aldridge, D.C. (1999) The morphology, growth and reproduction of Unionidae (bivalvia) in a fenland waterway, *Journal of Molluscan Studies*, 65, pp. 47–60.

DEFRA (2021) *England | Hydrology Data Explorer*, Department Food and Rural Affairs, Crown Copyright. Available at: http://environment.data.gov.uk/hydrology/ (Accessed: 15 June 2023).

Killeen, I., Aldridge, D. and Oliver, G. (2004) *Freshwater Bivalves of Britain and Ireland*, Field Studies Council.

Martin, R. (2004) Revision of the BMWP scoring system, *Centre for Intelligent Environment Systems.* Available at: http://www.cies.staffs.ac.uk/index.htm (Accessed: 08 August 2017).

Walley, W. J. and Hawkes, H. A. (1997) A computer-based development of the Biological Monitoring Working Party score system incorporating abundance rating, site type and indicator value, *Water Research*, 31(2), pp. 201-210.