Spatial habitat patterning of the freshwater pearl mussel, Margaritifera margaritifera, in the River Rede, North East England.

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Spatial habitat patterning of the freshwater pearl mussel, *Margaritifera margaritifera,* in the River Rede, North East England.

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Abstract

Habitat degradation is prevalent in freshwater ecosystems and acts at multiple scales to impact biodiversity. It has severe consequences for the endangered freshwater pearl mussel (*Margaritifera margaritifera*). Due to this species' ecological importance, preservation of the declining population on the River Rede, NE England, is of interest to conservation organisations. Physical habitat parameters in the Rede were assessed across a series of scales relevant to the species' requirements. Water quality was assessed at the catchment scale. Depth measurements and remotely sensed data on grain size distributions were collected at the meso-scale. Substrate composition, flow type, proximity to the channel edge and adult mussel distribution data were observed at the microhabitat scale. Meso-scale and microhabitat surveys were performed within four 400 m river reaches. A significant contagious distribution of the 310 observed *M. margaritifera* was identified. All sampled habitat factors related significantly to mussel presence, although flow type displayed a more complex association. Logistic regression and preference modelling further allowed the species' habitat requirements to be refined, identifying areas of preferred habitat. Mussels were distributed as a function of substrate composition and depth, primarily in areas less than 20 cm deep (above summer low flow). Areas less than 3 m from the bank, run flows, and low turbulence flow types also contributed to the definition of preferred habitat. The Rede *M. margaritifera* population was found to respond to habitat patchiness. This is in accordance with patchy distributions, related to habitat character, found in recruiting populations and is promising for future conservation efforts. The multiple scale approach employed here could contribute to future catchment management methods.

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Declaration and Statement of Copyright

The content of this thesis has not previously been submitted for a degree in this or any other institution. All content drawn from the work of others has been credited appropriately.

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Chapter 1 Introduction and Literature Review

1.1. Introduction

Severe species decline is occurring at a global scale (Baillie *et al.* 2004). The world's species are susceptible to multiple pressures, however habitat loss and degradation in habitat quality are widely acknowledged as the most severe threats to global biodiversity (Fischer and Lindenmayer, 2007; Dent and Wright, 2009). Habitat loss can result from large scale threats that cause changes to habitat and indirectly result in species decline. An assessment by Sala *et al.* (2000) highlighted such a role for climate change, global atmospheric carbon dioxide concentrations and nitrogen deposition. Other threats also occur worldwide but have varying *local* significance and impacts on biodiversity can be both direct and indirect. These are frequently linked to anthropogenic actions. Patterns of threats, such as the introduction of non-native species, geographically follow patterns of human activity (Sala *et al.* 2000). Land use change, habitat modification and pollution pose major threats to species at a local level (Wilcove *et al.* 1998; Hamer and McDonnell, 2008; Jones-Walters, 2008).

Habitat degradation occurs across all ecosystems, as illustrated by Sala *et al.* (2000), yet their assessment identifies freshwater ecosystems as the most severely affected, experiencing more acute biodiversity declines than any *terrestrial* ecosystem. Habitat degradation in freshwater systems is particularly concerning as it is a relatively rare ecosystem in global terms (0.01% of Earth's water is freshwater), yet it supports 6% of recorded species (Dudgeon *et al.* 2006). Coupled with a high degree of local endemism in some freshwater species, the importance of conserving this ecosystem as a valuable environmental, scientific, economic and social resource becomes paramount (Dudgeon *et al.* 2006). Overexploitation, water pollution, flow modification and direct damage to habitat (for example through dredging, riparian clearance or increased siltation) can all cause degradation in the quality of the freshwater ecosystem (Díez *et al.* 2000; Sabater *et al.* 2000; Dudgeon *et al.* 2006; Mesa, 2010). The broader scaled climate or atmospheric threats identified by Sala *et al.* (2000) maintain their significance, but are overlain on these more freshwater-specific concerns.

Drivers of freshwater ecosystem decline will have differing levels of influence between catchments. Interaction between threatening forces exacerbates their effect on habitat (Dudgeon *et al.* 2006) and highlights the potency of threats acting across scales. Frissell *et al.* (1986) produced a schematic diagram illustrating the varying scales across which processes can act in a river system, including those which degrade the ecosystem (Figure 1.1).

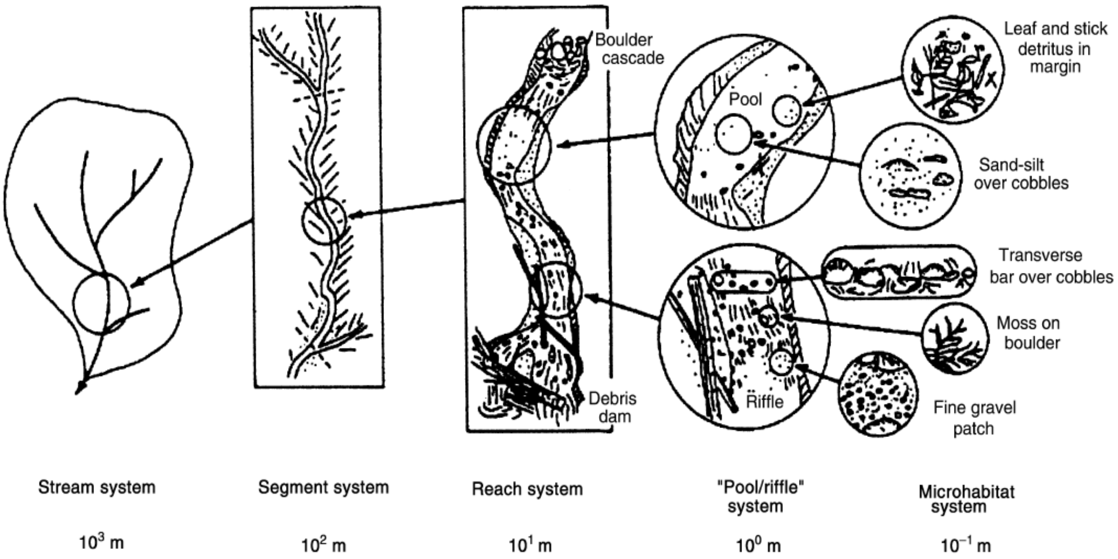


Figure 1.1 Diagram of nested habitat scales in a river ecosystem.
This hierarchical system of nested scales can be used as a basis to demonstrate how processes causing habitat degradation at one scale in a river ecosystem can have impacts at other scales. Furthermore, threats endangering biodiversity will accumulate across scales: an organism manifest at the smallest, microhabitat scale may experience the threats posed at all scales above that. The diagram was developed by Frissell *et al.* (1986).

Habitat damage occurs at many intersecting scales. The resultant ecological destruction at any point is a function of the cumulative effect of threats across the entire system, not just at a single scale (Fausch *et al.* 2002). An assessment of habitat degradation focused at a singe scale is unlikely to identify all causes of decline and our resultant understanding of the system will be incomplete (Fausch *et al.* 2002). Bolland *et al.* (2010) make this point in their conservation protocol: assessments of habitat suitability must address all scales relevant to the species under protection. In their examination of habitat degradation on the River Esk, different habitat parameters were accounted for at three nested scales (river reach, site and spot) each of which must be assessed if all factors influencing species decline are to be found and prevented.

1.2. Spatial and river ecology theory

The dynamism in environmental systems has long been recognised, whether naturally or anthropogenically induced (Pickett and Thompson, 1978). Various models for quantifying relationships in environmental patterning unite ideas of environmental processes, structure, function and change (Addicott *et al.* 1987). The influence of these factors on species distribution dynamics is also integral to the concept (Doak, 2000). Most importantly, the significance of scale within this sphere is fundamental (Bell *et al.* 1991). The increasing use of the idea of patches and a general landscape matrix or mosaic of patches, linked via appropriate corridors of flows, has moved spatial ecology theory to a more holistic vision (Bell *et al.* 1991).

In ecology, a scale hierarchy of mechanisms determine habitat distribution patterns (McAuliffe, 1983). At one level, a species will be regulated by large scale habitat parameters, such as overall water chemistry variation in freshwater. Within this range, further determinates of habitat will influence a species' distribution. In a river environment this may include flow velocities or substrate composition. At any relevant habitat scale a species must also contend with physical disturbances or predation. A culmination of all ideal circumstances across these scales results in an area, or patch, of suitable habitat (McAuliffe, 1983).

A patch can be defined as a homogenous area that is distinctly different in nature from surrounding areas (after Forman, 1995 and Thorp *et al.* 2006). Patch character is scale (spatial and temporal), organism and process dependent (Thorp *et al.* 2006). Within the patch, a degree of internal heterogeneity may exist but this is replicated throughout to form the homogenous patch character (Forman, 1995). Patches are dynamic features due to the ecosystem processes and flows that create them and, in turn, that are driven by their existence (Downing, 1991; Forman, 1995). Patches can therefore vary in their suitability for an organism's needs or vary in ecological importance. Where inter-species associations are part of the ecological system, this will also have a bearing on how patch dynamics function (Downing, 1991).

Due to the nested nature of the scale hierarchy, it is particularly important that all key ecosystem mechanisms are assessed. Evaluation of factors explaining change or degradation, for example, may be omitted from the investigation if irrelevant study scales are observed, or if important scales are ignored. There is a strong indication that details of autecology (biological relationship between a specific species and its environment) must be incorporated into an investigation to ensure that mechanisms, patch character and scales of assessment are all relevant to the species under study (Bell *et al.* 1991; McCoy *et al.* 1991; Thorp *et al.* 2006).

Patches, in the riverine system, are distinct from the concept of patch theory in spatial ecology in general, due to the prominent role of hydrological flows (Wiens, 2002). Flows of energy and organisms, for example, between patches in terrestrial landscapes, must rely on connectivity via corridors (Forman, 1995). In a river network, connectivity between patches is very high as a result of the water flow (Wiens, 2002; Fullerton *et al.* 2010). Patch boundaries will still exist though; an organism's perception of these being especially dependent on its mobility and particular habitat requirements. This heightened connectivity renders spatial and temporal scales even more significant.

Many authors have advanced towards combining these ideas from spatial ecology in contemporary river science (Thorp *et al.* 2006). Within the foundation of a whole river system, ideas of spatial patterning have been made a focus (Newson and Newson, 2000; Thorp *et al.* 2006). This drives progression towards including all relevant spatial and temporal scales in river assessments, to ensure interpretations drawn are as accurate a representation of the complete system as possible. Two main features of consequence can thus be drawn out of river ecology literature. Firstly, the changing approach to fluvial systems analysis and the increased assessment at different scales is very important. Secondly the incorporation of discontinuous patch hierarchies and dynamics has been a feature of recent river ecology models.

Ward (1989) introduced longitudinal, lateral, vertical (groundwater interactions) and temporal dimensions on which river systems function as essential for examination.

Persistent neglect to address scales beyond short, easily accessible sections (such as reaches and smaller, as defined by Frissell *et al.* 1986) in assessments of river systems was scrutinised by Fausch *et al.* (2002). They argued that small scale assessments have led to our restricted understanding of fluvial systems and that ideally a continuous assessment should be attained. This holistic view of processes and form, across relevant scales, allows key interactions to be assessed and it highlights the way the effects of disturbances and habitat degradation occurring at a large, catchment scale filter down to influence processes and structures at smaller scales (Ward, 1998; Fausch *et al.* 2002).

Since this call to expand scales of assessment, progression in river ecology has witnessed an increasing recognition of the use of patch theory and the idea of a heterogeneous river habitat mosaic. Appreciation of heterogeneity within and across river scales can advance management and conservation approaches (Fausch *et al.* 2002). In addition to assessing the river as a whole system, the internal structure must be incorporated (Forman, 1995; Wiens, 2002).

Thorp *et al.* (2006) developed the Riverine Ecosystem Synthesis (RES). The fundamental idea behind their cross-scale model of biocomplexity is that the river should be viewed as an along-stream “array” of hydrogeomorphic patches, with reoccurrence (Thorp *et al.* 2006). These large patches are termed Functional Process Zones (FPZs). These are conditioned by broad scale parameters including geology, climate, soils and vegetation. In turn, these govern water discharge routes, sediment and nutrient load to the specific functional process zone they feed. This will define the large scale patch character experienced by organisms inhabiting the zone, possibly rendering it unsuitable for some species, even at this scale. Processes inducing habitat degradation may occur at this scale, instigating species decline within the FPZ. This can include climate changes on a global scale proposed by Sala *et al.* (2000) or lower magnitude changes in land use (Wilcove *et al.* 1998; Fisher and Lindenmayer, 2007). FPZs are reminiscent of the upper levels of the mechanism hierarchy introduced by McAuliffe (1983) but perhaps gives more recognition to the discontinuities between FPZs created by changes in flow or substrate, than the less distinct hierarchical levels discussed in the earlier paper.

Thorp *et al.* (2006) have thus created one level of considering patches in the river ecosystem, already with discontinuities, boundaries, spatial and temporal context established (as directed by Wiens, 2002). Yet further scales, pertinent to causes of habitat decline, organism requirements and general river system study (Fausch *et al.* 2002) must be covered. This is achieved when Thorp *et al.* (2006) unite the work of Wu and Loucks (1995) with the FPZ model to create the overall RES. Wu and Loucks (1995) created the Hierarchical Patch Dynamics paradigm, which accommodates heterogeneity and scale differences within the system. Thorp *et al.* (2006) identify the key principles in the paradigm that, if applied, capture the complexity of the system and highlight the hierarchy of scales that produce habitat and patches, whether in decline or relatively undisturbed. Wu and Loucks (1995) propose that:

- Ecosystems are composed of "nested, discontinuous hierarchies of patch mosaics" and consideration of this allows analysis of small patches within larger ones, though they will be linked via multiple processes (Wu and Loucks 1995; Thorp *et al.* 2006).
- Random processes and a non-equilibrium balance have high significance in shaping patch dynamics at lower levels (Wu and Loucks 1995; Thorp *et al.* 2006).
- Such ephemerality at one level leads to a meta-stable state at higher hierarchical levels; viewed at a larger scale, the system may appear to display more equilibrium-like conditions (Wu and Loucks 1995; Thorp *et al.* 2006).

Taken together, these model intricacies can help establish how to view and assess the river ecosystem to define its internal heterogeneity and system of patches, which in turn define aquatic species and organism distribution. Equally, the inadequacy of patches is of interest, where ideal physical parameter conditions do not overlap at appropriate scales for a given species' requirements. This may happen if destructive processes occur at one of the many, interlinked scales.

The RES thus consists of large scale FPZ hydrogeomorphic patches and, nested within these, small scale patches governed by Wu and Loucks' Hierarchical Patch Dynamics paradigm mechanisms. In these smaller scale patches abiotic and biotic factors will interact to define organism distribution, including small scale variations in substrate or dissolved oxygen, competition and resource availability and the suitability of the patch for sustainable levels of reproduction (Thorp *et al.* 2006). These form the overall, heterogeneous, river habitat mosaic. A more complete understanding of the causes and impacts of habitat degradation and the resultant patterns of biodiversity decline could be attained by considering the river ecosystem in terms of the above RES model.

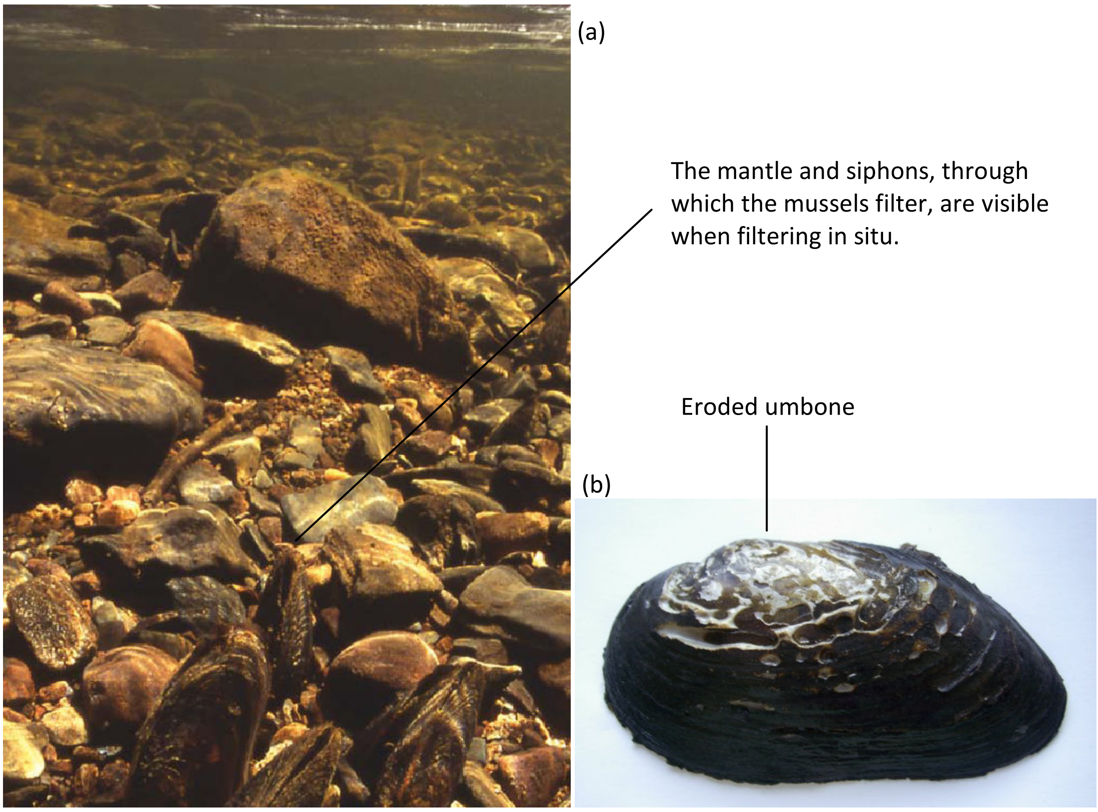
It is evident from the above review that certain factors are crucial. Wiens (2002) reiterates the importance of patch context: conditions beyond the specific patch occupied by an organism will still have influence, thus a full array of scales (Frissell *et al.* 1986) must be included in a habitat assessment (Pringle, 1988; Forman, 1995; Wiens, 2002). An overview of what should be considered, having reviewed the current ideals from both spatial and river ecology, is assembled in Pringle's (1988) study. Patch characteristics such as size, distribution, duration and interaction processes are significant to the organisms experiencing them. Correspondingly, the study organism's perception of space and time should be incorporated to fully appreciate the situation pertinent to them. Study scales and acknowledgement of the whole stream network and catchment are also fundamental features illuminated in relevant contemporary literature assessed here (Pringle, 1988). We can only achieve this holistic view with the expansion of assessment scales: broader and finer scales are needed in synergy to capture the nested hierarchy of patches and their interactions that will enlighten us to the current circumstances of habitat degradation and any species decline.

1.3. Habitat degradation and the freshwater pearl mussel, *Margaritifera margaritifera*

Habitat degradation has been established as a serious threat to global biodiversity (Sala *et al.* 2000), particularly in freshwater ecosystems (Dudgeon *et al.* 2006). This investigation focuses on presenting the case of the freshwater pearl mussel, *Margaritifera margaritifera*, as an example of an important species which is suffering critical decline due to habitat degeneration, among other threats.

Margaritifera margaritifera is an aquatic bivalve mollusc (Figure 1.2). The Unionida order of freshwater bivalves contains six families, including Margaritiferidae. *Margaritifera* is one of ten genera in this family (Bogatov *et al.* 2003) and *M.*

margaritifera is one of twelve species within the genus (Bogan, 2008). As approximately 800 unionid species exist (Bogan, 2008), the *Margaritifera* genus is a relatively small subset of the order (Bogatov *et al.* 2003). Literary accord suggests that freshwater molluscan fauna are in global decline, with *M. margaritifera* among these (Bogan, 2008). Habitat modification and deterioration is extensively acknowledged as the reason for this (Wilcove *et al.* 1998; Lydeard *et al.* 2004). Araujo and Ramos (2000) note that only three species of the *Margaritifera* genus are found in Europe. *M. margaritifera* is the most widespread. They are relatively immobile filter feeders, spending the entirety of their long lifecycles in freshwater and moving only short distances, if necessary (disregarding when entrained in high flows) (Aldridge, 2000; Araujo and Ramos, 2000). This species can live in excess of 100 years (Skinner *et al.* 2003; McLeod *et al.* 2005). As they develop slowly, taking up to fifteen years to reach maturity, habitat must remain suitable for long periods (Skinner *et al.* 2003) and any changes may impede population persistence.



<u>Figure 1.2 *M. margaritifera* viewed (a) *in situ* in river habitat and (b) *ex situ*.</u>
(a) Image by Sue Scott, from Skinner *et al.* (2003). The adult aquatic bivalve lives semi-buried in the finer bed substrates. The mantle edge and siphons remain exposed for filtering.
(b) Author's image. This adult mussel measures 110 mm on the longest axis. The bare umbone (oldest and thickest part of the shell) is caused by erosion and is visible on the fully exposed shell. This is often a feature on this species (Moorkens, 1999; Lewis, *pers. comm*.)

1.4. Geographical range of *Margaritifera margaritifera*

Margaritifera margaritifera is distributed throughout the Holarctic ecozone (Young and Williams, 1983). Populations exist in North America (Young and Williams, 1983; Bauer, 1987; Skinner *et al.* 2003) and Europe (Hartmut and Gerstmann, 2007; Englund *et al.* 2008), including the British Isles, within a latitudinal range of approximately 40 ^{o}N to regions approaching 70 ^{o}N (Bauer, 1992; Munch and Salinas, 2009). Throughout this species' range, severe population reductions have occurred (Cosgrove and Hastie, 2001), leaving remaining populations in localised pockets. In Central Europe, populations decreased by 90% over the twentieth century (Bauer, 1988) and later papers suggest this situation may have deteriorated further (Geist, 2010). Scotland is a global *M. margaritifera* “stronghold” (Hastie and Young, 2003a), harbouring more than fifty viable populations (McLeod *et al.* 2005). However, even in Scotland, they are declining or extinct in 70% of the sites they occupied only a century ago (Hastie and Young, 2003a). Only one recruiting population remains in England (McLeod *et al.* 2005) and the remaining populations are in local decline. Historically, populations of freshwater pearl mussels existed in dense beds of 1000 m^{-2}, yet densities of mussels are estimated to have fallen significantly in some areas (Bauer, 1987), leaving sparse populations.

Within the established geographical range, *M. margaritifera* occupy very specific areas of macrohabitat. These broad scale habitat features create a landscape context within which the fresh water pearl mussel’s historic distribution arose, before any changes from habitat degradation influenced the species’ range. They inhabit relatively undisturbed, unpolluted, oligotrophic, fast flowing streams and rivers with neutral or slightly acidic water pH and low calcium content (Strayer, 1993; Skinner *et al.* 2003; Hastie *et al.* 2004). The underlying geology must thus be suitable to maintain these conditions. Geology will also play a major role in defining large and medium scale stream geomorphology (Brainwood *et al.* 2008). The required stream gradient has been reported to be within the range of 0.5-5.0 m km^{-1} (Hastie *et al.* 2004). Coarse substrate should be a characteristic of the catchment, which is again linked to the character of the underlying geology (Hastie *et al.* 2004; Brainwood *et al.* 2008). On a moderate (reach) scale, this is often associated with the specificities of the microhabitat requirements (Hastie *et al.* 2004). Ideally riparian vegetation should feature highly within the catchment (Hastie *et al.* 2004). Freshwater pearl mussel rivers must have an adequate population of native salmonids for successful mussel reproduction and maintenance of the mussel population (Hastie and Young, 2003a). More detailed discussions of lifecycle complexities and finer scaled microhabitat preferences are undertaken in Sections 1.6 and 1.9.

1.5. *Margaritifera margaritifera* as a candidate for conservation

There is much support for the freshwater pearl mussels' position as a focus for conservation due to its ecological importance in the types of stream it inhabits

(Bolland *et al.* 2010; Geist, 2010). Many terms are in frequent use to identify species with roles that are significant to the ecological status or stability of an ecosystem, or where species form the foundation of larger conservation efforts (Simberloff, 1998). Geist (2010) identifies the freshwater pearl mussel as particularly noteworthy, as it embodies many of the principles behind *all* of these notions: ‘flagship’, ‘indicator’, ‘umbrella’ and ‘keystone’ species, unlike most other species (Geist, 2010).

In the freshwater pearl mussel’s guise as an ‘indicator’ of the quality of their harbouring catchments, it is well established that *M. margaritifera* is a stenoecious species (inhabits areas within only a narrow range of conditions), particular to clean oxygenated rivers. Excessive nutrient levels and a rising trophic status would lead to their decline (Geist, 2010). This determines that any river supporting a healthy freshwater pearl mussel population is considered near-pristine and is likely to represent a high quality river ecosystem. In light of this, *M. margaritifera* is frequently an icon of conservation campaigns, used as a ‘flagship’ species to lead remediation work towards ecosystem recovery (Bolland *et al.* 2010; Geist, 2010). The classification of *M. margaritifera* as an ‘umbrella’ species reinforces its value as a flagship species. Conservation efforts must recognise than an umbrella species requires, or is affected by, factors across a large area (Lambeck, 1997). The freshwater pearl mussel, despite being comparatively immobile and remaining in its aquatic habitat throughout its life, is affected by factors influencing its immediate habitat that may occur throughout the river catchment. For example, the river’s clean, oligotrophic status may be impaired if toxins are delivered to the water, even at a point source some distance away. To prevent the decline of *M. margaritifera,* restoration or conservation of entire catchments is necessary so that conditions remain suitable across all scales (Bolland *et al.* 2010). If this approach to conservation is taken, it is likely that suitable conditions for many other species will be preserved (Geist, 2010).

While conservation involving species specific action is common, there can be disadvantages where habitat restoration for one species hampers others or, in the case of umbrella species, the benefits brought from interactions with other, non-focal species can be small or overestimated (Simberloff, 1998). However, if the concept of umbrella and flagship species are combined with the values of keystone species, as is the case with *M. margaritifera,* conservation practices may be more satisfactory. Despite the severe decline in this species, the freshwater pearl mussel could remain an important keystone in the catchments where it persists (Aldridge *et al.* 2007). This status implies it is important in the sustainable functioning of its harbouring ecosystem or community. Dense mussel beds filter abundant amounts of water, adequate to purify the fluvial ecosystems they occupy (Smith and Jepsen, 2008): an adult mussel can filter fifty litres of water per day (Zuiganov *et al.* 1994, cited in Skinner *et al.* 2003), producing only harmless pseudo faeces (Downing, 1991; Hastie and Young, 2003a). Furthermore, while salmonid species thrive in many catchments where *M. margaritifera* do not exist, the relationship between the ecologically and economically important salmonids and *M. margaritifera* is thought to be symbiotic by some scientists (Hastie and Young, 2003a). The mussel bed area provides suitable conditions for other invertebrates to thrive (Hastie and Young, 2003a; Skinner *et al.* 2003). These will perform their own ecological functions and provide food for other species, again including salmonids and other fish species.

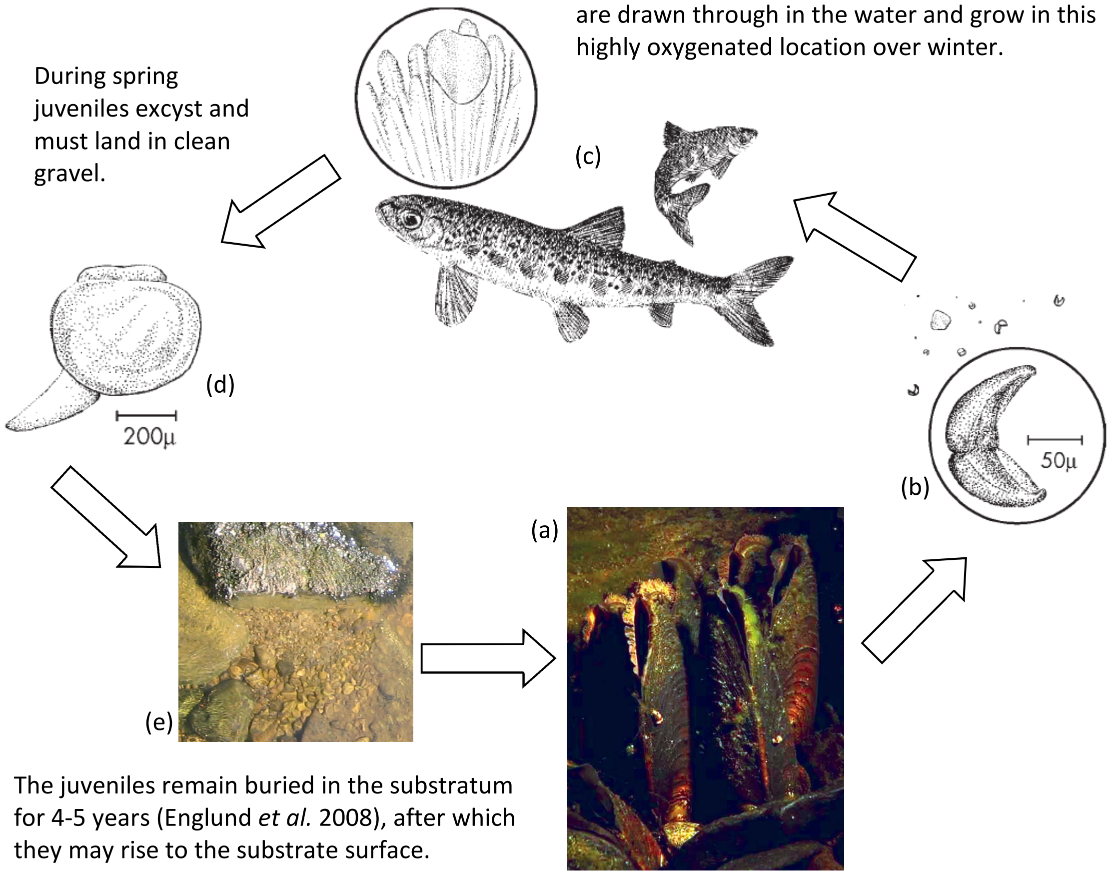
The importance of *M. margaritifera* as an indicator of good quality, functional river ecosystems, together with the severity of its global decline, confirms there is an urgent need to study key parameters that affect freshwater pearl mussels. One approach to this is to study the populations that are not recruiting, as sustaining all populations that remain should be a priority. Any investigation must firstly incorporate aspects of their seemingly precarious lifecycles. When conducting research in this sphere all pearl mussel life stages should be considered as they are all relatively long. (Bolland *et al.* 2010; Box and Mossa 1999). Furthermore, the evident need to maintain an approach that covers all pertinent scales should be accounted for.

Returning briefly to the idea that details of autecology must be incorporated in to the study to ensure that assessments are all relevant to the species under study (Bell *et al.* 1991; McCoy *et al.* 1991; Thorp *et al.* 2006), a review of the *M. margaritifera* lifecycle, threats to the species, its protection status and broad habitat preferences will be made. This will offer a foundation to the final aims of the investigation.

1.6. The lifecycle of *Margaritifera margaritifera*

1.6.1. The lifecycle

The lengthy lifecycle of the freshwater pearl mussel is complex (Figure 1.3). *Margaritifera margaritifera* mature at 10-15 years of age (Skinner *et al.* 2003) and remain reproductively active throughout life (Bauer, 1987). Reproduction requires little effort from the adult mussels (Österling *et al*. 2010). In June or July the adult, male mussels release sperm into the flowing water body (Hastie and Young, 2003b). The females take in the sperm as they filter water in the normal manner (Hastie and Young, 2003c) and their eggs are fertilised (Figure 1.3 (a)). The cycle continues with the spat (glochidial release, Figure 1.3 (b)) attributed to temperature increase (Hastie and Young, 2003b). M*argaritifera margaritifera* use salmonids as hosts (Figure 1.3 (c)). They are highly host specific: successful development is associated with Atlantic salmon, *Salmo salar* and brown trout, *Salmo trutta* (Hastie and Young 2003a). A sustainable level of glochidial attachment requires a density of age 0+ salmonids of 0.1 m^{-2} (Englund *et al.* 2008), though this density is disputed. *Margaritifera margaritifera* not only rely on the host species for successful recruitment, but also for dispersal of the population and colonisation in other areas of the river (Skinner *et al.* 2003). When juveniles excyst from the host (Figure 1.3 (d)) it is crucial that they settle in silt-free, stable sand and gravel substrates with high levels of oxygen in the interstitial spaces of the substratum (Figure 1.3 (e)) (Bolland *et al.* 2010). Furthermore, Buddensiek *et al.* (1993) demonstrated how crucial the quality of the interstitial environment is, particularly water quality, to successful mussel recruitment.



Glochidia must attach to the host fish. Successful glochidia encyst onto the salmonid's gills when they are drawn through in the water and grow in this highly oxygenated location over winter.

During spring juveniles excyst and must land in clean gravel.

The juveniles remain buried in the substratum for 4-5 years (Englund *et al.* 2008), after which they may rise to the substrate surface.

Fertilised eggs develop in the female and are released as glochidia (i.e. mussel larvae) into the water column between July and September (Skinner *et al.* 2003).

Figure 1.3 Representation of the *M. margaritifera* lifecycle.
The freshwater pearl mussels' lifecycle is complex, requiring specific habitat conditions at each stage and the presence of specific host fish. Adapted from diagram by S. Wroot, in Skinner *et al* (2003). Image a) by Sue Scott, from Skinner *et al.* (2003). Image b) Author's own.

1.6.2. Losses in the reproductive process

The losses in the freshwater pearl mussels' reproductive process are considerable. While the female will release 1-4 million glochidia in a single spat (Skinner *et al.* 2003), many are lost as a result of the parasitic manner of glochidial development (Hastie and Young, 2003c). Many authors cite the time between spat and attachment to the host as the first highly vulnerable life stage (Preston *et al.* 2007). Figure 1.4 indicates the most significant fall in survival at this stage. A further 95% of glochidia will not fully develop on the host (Hastie and Young, 2003c), though if they do, excysting from the host is another vulnerable stage in the *M. margaritifera* lifecycle. An estimated 95% of juveniles are lost between excysting from the host and settling in gravel substrate (Hastie and Young, 2003c). This is as a result of the specific substrate requirements in which the juveniles develop. Adults are more tolerant of habitat variation (Hastie *et al.* 2000); however, continued low rates of recruitment, or even recruitment failure, soon render a local population unsustainable.

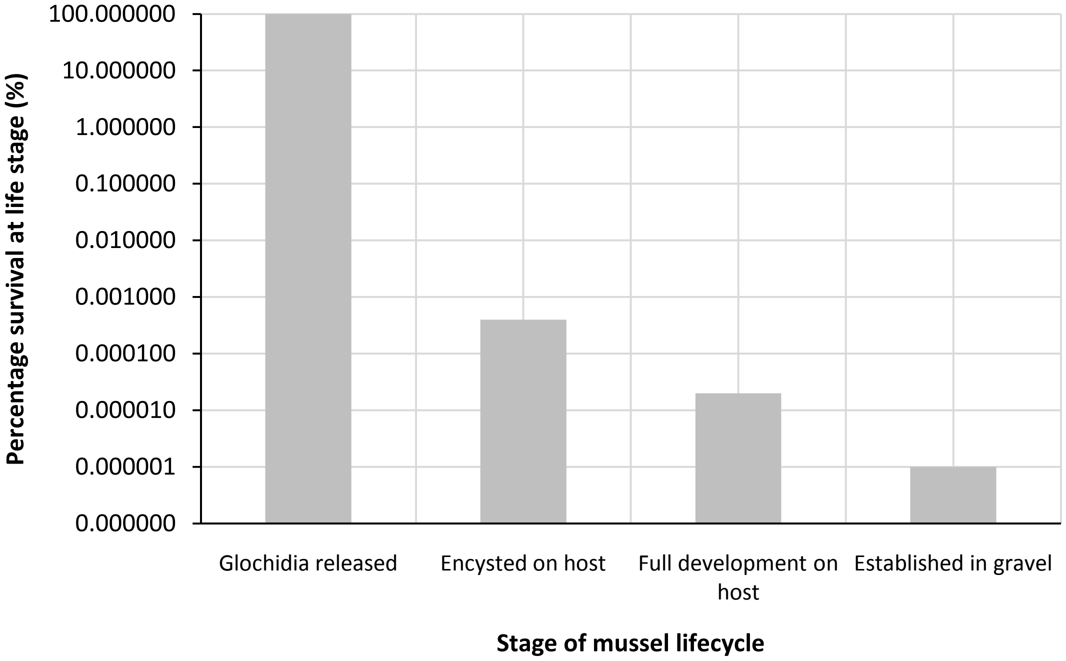


Figure 1.4 Survivorship curve for an average glochidial release.
Author's own graph based on estimations of percentages of mortality in released glochidia from Hastie and Young (2003c) (also Young and Williams, 1984a, 1984b and Bauer, 1987). This steeply declining survivorship curve demonstrates the extreme number of glochidia lost in the normal reproductive process of freshwater pearl mussels. To give an example, 2 million is an illustrative number of glochidia released by one female during the spat (Skinner *et al.* 2003). The number of surviving juveniles (established in gravel) from this would be 0.02; that is 0.000001% of the original 2 million. The most severe loss rate is between glochidial release and encystment.

Statistically, the established loss rates even in healthy populations (Figure 1.4) mean fifty spawning females will produce just one juvenile mussel, per annual spat, that will successfully establish in the substrate. The surviving juveniles that settle in suitable gravels will still face the threats posed by habitat degradation while in the interstices (Buddensiek *et al.* 1993) and those threats facing adult mussels throughout their lives. Consequently, while a population loses most individuals it invests in within the first year (stages shown on x axis), further losses are made after this period. To a certain extent, the long life expectancy and high female fecundity can negate the effects of an annual flux in the salmonid population or short term habitat disturbances. According to Bauer's results (1987), the freshwater pearl mussel's reproductive strategy allows females to produce glochidia an average of 47 times across their lifespan. If the survivorship rates demonstrated in Figure 1.4 are applied to Bauer's findings, approximately 0.94 juveniles will be produced per female that reach establishment in gravel. Over years of habitat degradation and other threats, there are major impacts on recruitment that the reproductive strategy cannot overcome (Ross, 1992; Hastie and Young, 2003c).

1.7. Threats to *Margaritifera margaritifera*

The freshwater pearl mussel suffers an extensive range of threats (McLeod *et al*. 2005). These function across a range of scales (Bolland *et al*. 2010) and impacts of the threats vary with an individual mussels' life stage (Box and Mossa, 1999; Bolland *et al*. 2010), meaning that effects across a river's population can differ. The complex relationship between the scale at which causes of decline transpire and the way the species reacts to threats makes them difficult to overcome, especially where long-term processes cause harm that is not immediately evident (Box and Mossa, 1999). Any indirect changes in habitat may, for example, induce slow changes to a river community structure. The resultant ecosystem deterioration may only gradually cause decline in other species or ecosystem processes. Alternatively, years of increased stress may make a population more susceptible to extinction via common or minimal disturbances (Mason, 1996). Many causes of *M. margaritifera* decline, among other unionids, are examined in the literature. These can broadly be classed into two main areas: exploitation and habitat degradation, though they may act in association across multiple scales.

1.7.1. Exploitation

Predation is not a major issue for adult mussels (Geist, 2010), though otters, *Lutra lutra*, muskrats, *Ondatra zibethicus*, (introduced in Central Europe) and occasionally birds may pose a risk (O'Sullivan, 1994; McLeod *et al.* 2005; Geist, 2010). Anthropogenic exploitation is of considerably more concern and has been cited as a major cause of decline. In early papers, where habitat change was only initially being recognised as a threat, pearl fishing for pearls and nacre was considered the most damaging of all pressures (Young and Williams, 1983). The practice is now illegal in the UK, yet before this species was fully legally protected in 1998 (UK Wildlife, 2010), pearl fishing was promoted as a leisure activity and divers were therefore able to access even the mussel beds in deeper pools, that traditionally maintained populations (Young and Williams, 1983). This practice can decimate mussel populations by rapidly reducing the adult mussel density. The *M. margaritifera* recruitment strategy will be inadequate to recover the population thereafter from the reduced number of adults and any juveniles that may be left in the gravels during the fishing episode, irrespective of the quality of the remaining habitat.

1.7.2. Habitat degradation

Habitat degradation, of some form, is cited as a threat to the freshwater pearl mussel almost without exception (Buddensiek *et al.* 1993; Beasley and Roberts, 1999; Araujo and Ramos, 2000; Hastie *et al.* 2000; Hastie and Young, 2003a; Harmut and Gerstmann, 2007; Englund *et al.* 2008; Bolland *et al.* 2010, among many others). Degradation has been shown to impact the freshwater pearl mussel both directly and indirectly, via various interlinked factors. These include salmonid decline, land use change and engineering works.

Salmonid decline

The importance of the host species has already been unambiguously established in Section 1.6. It therefore follows that a decline in Atlantic salmon or brown trout in freshwater pearl mussel rivers can pose a threat to mussel populations' survival. The magnitude of glochidia losses seen under normal host population conditions (>99.9%) is very large, but where there are too few hosts, even fewer glochidia will successfully attach, making losses at later stages of development yet more significant. While losses at this stage have been attributed to raised water temperatures at spawning times in some cases (Akijama and Iwakuma 2007), a lack of salmonids is frequently cited as a major threat in catchments (Hastie and Young, 2003a; Englund *et al.* 2008).

Many changes may make a river less suitable for salmonid hosts, even if these changes do not affect the mussel directly, such as new structures limiting anadromous fish migration in the wider catchment, so that the mussels must rely solely on non-migratory brown trout. The mussel population therefore comes under stress as an aging population develops: only glochidia are directly affected by the lack of a fish host, though the general population suffers. Eventually the population would become extinct through poor recruitment and relatively normal mortality levels but it may be accelerated by the occurrence of other threats, for example losses to flood events (Hastie *et al.* 2001) or small pollution events that may otherwise be tolerated by a healthy population. This demonstrates the importance the multiple, interacting issues occurring across spatial and temporal scales that may present a threat to a mussel population, both directly and indirectly (Englund *et al.* 2008).

Land use change

The origins of habitat degradation frequently derive from changes to the catchment via anthropogenic land use change (Wilcove *et al.* 1998). Activities in the catchment that constitute land use changes include increased intensity of agricultural practices (arable and livestock based practices), forestry, mining and industrial or urban development (Bauer, 1988; Warburton, 1997; Wilcove *et al.* 1998; Hartmut and Gerstmann, 2007; Moorkens *et al.* 2007). These all induce pollution of the environment in some forms that are detrimental to *M. margaritifera*. Water development is also noted by Wilcove *et al.* (1998) as a significant pressure to mussels of all species.

Chemical water quality deterioration

The stringent requirements the freshwater pearl mussel has of water quality in its environment have been broadly established in Sections 1.4 and 1.6: deviation from an oligotrophic, clean river habitat thus poses a risk to mussel survival.

Mining and industry can introduce metals to the river channel and increase conductivity. High levels of some metals, such as copper and zinc are directly toxic to molluscs (Young, 2005; Hartmut and Gerstmann, 2007) so could cause immediate mortality in a mussel population. Increases in conductivity (representing higher concentrations of iron, sulphates and any heavy metals in mine outflow or leached from quarry workings etc.) are less obviously harmful, though Buddensiek *et al.* (1993) found that increased conductivity was associated with a lack of juveniles in freshwater pearl mussel populations. They did not establish the source of the increased ion content however, which may also come from organic pollutant sources. Areas of plantation forestry have been found to cause stream acidification, as run-off from these areas is acidic (Neal *et al.* 2010). *Margaritifera margaritifera* can only tolerate a pH between 6.5-7.5 (Bauer, 1983; Oliver, 2000; Skinner *et al.* 2003). Acidification in the catchment can cause the lower threshold to be surpassed and the species will decline (Englund *et al.* 2008).

Increased trophic status can arise where nitrate and phosphate pollution occur. This is common where agricultural activity intensifies. The application of fertilisers on agricultural land can pollute waterways with excessive nutrient loads if it is allowed to enter the channel (directly or leaching from the land in the catchment). Nitrates in particular are noted to have a very significant impact on the freshwater pearl mussel, causing harm at all life stages (Bauer, 1988), whereas phosphates have particularly deleterious effects on juvenile mussels (Bauer, 1988; Buddensiek *et al.* 1993). The impact of nitrates, whether indirect or whether they are directly toxic is unknown. Phosphates are thought to act indirectly by increasing organic production and detritus (Bauer, 1988). The damaging effect of eutrophication has long been recognised, even when the abovementioned effects of exploitation were still paramount, as it causes such as significant diversion from the oligotrophic conditions in which *M. margaritifera* sustainably thrive (Young and Williams, 1883).

Increased sedimentation

Intensive grazing, extensive cultivation and direct sediment delivery via runoff from the land, mine workings and engineering works (Cosgrove and Hastie, 2001; Allan, 2004) can increase the input of fine sediments to the river channel. These are highly detrimental to freshwater pearl mussels (Moorkens *et al.* 2007; Österling *et al*. 2010). Fine sediment causes siltation of the gravels needed by juveniles. Sedimentation is an issue stemming from the catchment land use and diffuse pollution sources, for example, deforestation and agriculture. This will affect all mussels in the river if water quality parameters exceed tolerable levels. Point sources of sediment pollution (small scale, local livestock access, for example) are only an issue at certain sites.

While adult mussels can tolerate some siltation of the substrate, juvenile *M. margaritifera* are impacted heavily by increased fine sediment deposition. A hardpan layer created by fines among the sands and gravels will cause elevated juvenile mortality (Box and Mossa, 1999) as high levels of oxygen and nutrient exchange within the gravel interstices and interstitial water are required for survival (Buddensiek *et al.* 1993). Siltation of gravels prevents salmonid spawning, reducing host numbers (Hastie and Young, 2003a), lessens the mussels' foraging ability, reduces oxygen levels and increases pH (Österling *et al*. 2010). Furthermore, a detrimental positive feedback is set up whereby increased sedimentation allows increased macrophyte and macroalgal growth. This will decay, absorbing oxygen, and introducing more nutrients and sediment, all of which create adverse conditions for *M. margaritifera* (Moorkens *et al.* 2007), but will further improve conditions for plants.

River engineering works

Engineering works can cause direct damage to *M. margaritifera* populations. Dredging, for example, will move mussels to the channel edge with the silt detritus (Aldridge, 2000). The mussels may be directly damaged by the works or become buried in silt and suffocate. If they survive initially, they cannot manoeuvre out of the silty habitat and other local threats will cause population decline (Aldridge, 2000). Indirect effects also cause issues, depending on the scale and location of the works in relation to the mussel population (Cosgrove and Hastie, 2001). Large scale work such as dam, road or flood defence construction can pose threats, even if remote from the mussel beds.

This examination of the threats to *M. margaritifera* populations highlights the complexity of the interaction between reasons for decline and the lifecycle stages. Some threats mentioned above, such as engineering works or acute pollution events, can eradicate all mussels and simultaneously render local habitat unsuitable. In other cases a slower rate of decline will occur, for example in rivers where low levels of siltation prevent *juvenile* survival or pearl fishing gradually reduces *adult* density to unsustainable levels. The variable impacts of factors on each life-stage partition of a *M. margaritifera* population have implications for the resulting spatial patterning within the remaining population. Consequently, interpretations of mussel's spatial patterning must be within the context of threats that are relevant to a study area. For example, false negatives are likely where adult mussels have been removed *en masse* via pearl fishing, as mussel absence may not indicate an area of unsuitable habitat; it could be that exploitation has reduced the population density in that area, rather than habitat decline. On the other hand, if no pearl fishing has occurred, low mussel density may indicate an area of less suitable habitat. Patterning of freshwater pearl mussels can be associated with the patterning of available habitat but there are limits to the extent of this connection. The age structure and density of the observed mussels, together with threat prevalence must be considered in interpretations of spatial patterning to fully appreciate the response of *M. margaritifera* to habitat patterning and patchiness.

1.8. Conservation status and protection of *Margaritifera margaritifera*

A wide range of threats, generally from anthropogenic sources, are evidently affecting *M. margaritifera* populations. The importance of this species affords them an extensive protected status. The International Union for Conservation of Nature (IUCN) red list of threatened species classifies the species as 'endangered' in the 1996 assessment (most recent inclusion *M. margaritifera*, Mollusc Specialist Group, 1996). In the previous five assessments, beginning in 1983, it was considered 'vulnerable', indicating the increasing gravity of their situation.

Margaritifera margaritifera is an Annex II species listed under the EU Habitats Directive (JNCC website, 2011). Annex II features species that are in urgent need of conservation in Europe. A total of forty protected Special Areas of Conservation (SACs) have been established within the UK to contribute to the conservation of *M. margaritifera* specifically, in accordance with the Habitats Directive. These are primarily in Scotland as designations are made based on functional populations.

Special Area of Conservation status accounts for the prevention of riparian damage, thus the mussels’ wider habitat as well (O’Keeffe and Dromey, 2004), but they may not extend to the prevention of indirect threats. The mussels’ reliance on its host fish means salmonid habitat must also be protected to prevent mussel decline (O’Keeffe and Dromey, 2004).

In addition to SACs formed in accordance with European policy, implementation of the UK Biodiversity Action Plan in 1994 (DEFRA report, 2007) gave rise to a specific freshwater pearl mussel Species Action Plan (SAP) and fourteen Local Biodiversity Action Plans (LBAPs) designed for *M. margaritifera* protection, such as in the Northumberland National Park (Biodiversity: The UK Steering Group Report, 1995). Within the UKBAP, *M. margaritifera* is listed as a priority species and, constructively, rivers are priority habitats (UKBAP website, 2007). The freshwater pearl mussel SAP aims to maintain or increase the mussels’ UK population and to encourage re-colonisation of the species at certain sites. The focus is on the improvement of water quality, land and catchment management and the development of reintroduction and monitoring programmes (Biodiversity: The UK Steering Group Report, 1995). Protection of the existing populations is a vital element of policy at all scales and the species is legally protected (UKBAP website, 2007).

Active conservation measures are being undertaken. The age of the above legislative plans demonstrates a long standing attempt at conserving this species. The Freshwater Biological Association (FBA) ‘Pearl Mussel Ark Project’ is one such scheme, developed in 2007. The FBA have created a facility to house and rear juvenile mussels which can be released once they are less susceptible to habitat deterioration (FBA website, 2010). In order to improve the success of conservation efforts, studies such as that by Bolland *et al.* (2010) suggest that certain protocols should be followed to ensure that conservation programmes are sustainable and do not require continual remediation work. For example, they recommend that potential restocking sites must be suitable for all mussel life stages, or the problem will continue once a certain point is reached: it will not be a sustainable practice to return captive-bred mussels or to stock glochidia-infected salmonids to unsuitable sites, even if they were historically viable.

Reservations are held by some, despite the extent of *M. margaritifera*'s protected status. The JNCC Report (2007) 'Conservation status assessment' for the species warns that some areas of our knowledge of the freshwater pearl mussel are inadequate and, in light of evident multifactorial reasons for decline, conservation measures should be precautionary (JNCC website, 2011).

1.9. Habitat preferences of *Margaritifera margaritifera*

The broad requirements *M. margaritifera* make of their habitat is established in Section 1.4. These macrohabitat features set the context for the historic distributions of the species. However, as seen in Section 1.7, pressures are now placed on the ecosystems where the freshwater pearl mussel would normally thrive sustainably. An assessment of microhabitat features is required, in addition to the larger scale parameters, for a full study of the effect of habitat degradation.

The geographic range of *M. margaritifera* is highly extensive (see Section 1.4). Within such a range, variations in habitat preferences occur. Freshwater pearl mussels show local adaption to water quality and depth parameters in particular, as these will change with the character of the wider environment (Gittings *et al.* 1998; Young, 2005). A general consensus on which other habitat parameters are important is evident in the literature (Hastie *et al.* 2000; Young, 2005). Further complications in the assessment of local scale habitat preferences stem from *M. margaritifera*'s need for different environments at certain life stages, typically in that juveniles' requirements are far more stringent than that of adults (Hastie *et al.* 2000). For this reason the juvenile freshwater pearl mussel preference envelopes should be represented in a potential habitat, as this will ensure that a recruiting, sustainable population can be maintained (Bolland *et al.* 2010).

1.9.1. Water quality

As a purely aquatic invertebrate, the maintenance of suitable water chemistry values is essential to freshwater pearl mussel survival. The literature reports threshold values for the key water quality parameters that have significant influences on mussels. If these thresholds are exceeded, freshwater pearl mussel survival, particularly juvenile survival, and reproduction will be inhibited (Bauer, 1988). Bauer has undertaken many studies into the water quality requirements of freshwater pearl mussels, but Purser's (1985) recommendation that only local studies should be used as guidance for habitat ideals implies that Bauer's studies in central Europe may not accurately define the requirements of British *M. margaritifera*. It should be mentioned however, that in Young's (2005) comparison of Bauer's (1988) values and those of Oliver (2000), it appears that Oliver's samples of Scottish *M. margaritifera* tolerate higher levels of most water chemistry indicators.

Mussel water quality tolerances in this study were taken from Beasley and Roberts (1999), Skinner *et al.* (2003) and Oliver (2000, cited in Young, 2005). Skinner *et al.* and Oliver's studies reflect the favourable conditions found where populations are recruiting in Scotland. Specific values for water quality parameters are given in Table 1.1. Juvenile survival relies on maintaining low levels of calcium, phosphate and biological oxygen demand (Skinner *et al.* 2003). Nutrient levels should be low for survival at all life stages, in accordance with their preferred 'oligotrophic' river status. Near saturation levels of dissolved oxygen are also crucial for survival at all stages.

With particular reference to juvenile *M. margaritifera*, the quality of the interstitial water is crucial. A comparison of Redox potential in the free flowing water column and at depth in the substrate gives an indication of the permeability of the substrate. In streams with recruiting freshwater pearl mussel populations, Redox potential has been found to be at similar levels in the water column and at depth (for example Geist and Auerswald (2007) found Redox potential to be 0.53 V and 0.47 V respectively in the water column and at 10 cm depth in the substrate, compared with a difference of 0.2 V between the two measurements in sites harbouring non-recruiting populations). Though the observed Redox potential values may vary between rivers, the significance of this parameter as an indicator or substrate permeability is important in assessing juvenile *M. margaritifera* habitat.

Table 1.1 Water quality parameters and requirements for *M. margaritifera.*
Adapted from Young (2005), the table displays the upper limits or ideal levels of water parameters that influence *M. margaritifera*'s survival. Adult mussels are generally more tolerant of variation in conditions; therefore some deviance from these values would not necessarily cause mussel death. It may affect the viability of a population, however, as juveniles could be affected.

Water quality parameter	**Value (After Oliver, 2000)**	**Notes**
Nitrate	<1 mg l^{-1}	High levels significantly increase adult mortality (Bauer, 1988)
Phosphate	<0.03 mg l^{-1}	Buddensiek *et al.* (1993) suggest this significantly influences juvenile survival in particular.
pH	6.5-7.2	May increase to pH 7.5 (Skinner *et al.* 2003)
Conductivity	<100 µs/cm	May increase in limestone areas (Skinner *et al.* 2003).
Calcium	<10 mg l^{-1} as $CaCO_3$ (~4 mg l^{-1} as Ca)	Highly disputed in the literature: 'ideal' calcium concentrations range from 2 mgl^{-1} (Bauer, 1988) to 10-11 mg l^{-1} (Boycott, 1936; Beasley and Roberts, 1999) and even up to 50 mg l^{-1} in some rivers (Boycott, 1936).
Biological Oxygen Demand (BOD)	<1.3 mg l^{-1}	This is considered high by some other studies, but local variation will occur. It is important for juvenile survival to have low BOD levels as interstitial water must be highly oxygenated.
Dissolved Oxygen	90-110% saturation	

1.9.2. Depth

Depth is considered a key feature of *M. margaritifera* habitat. Hastie *et al.* (2000) refined the ideal habitat to 30-40 cm in depth in their specific study river in Scotland. This preference is based on a comparison of the depth of available habitat and proportional use. In the River Kerry, Hastie *et al.* (2000) recorded areas up to a maximum depth of 0.95 m. However, utilisation of habitat at this depth was very rare.

This is in agreement with other regions of Western Europe: Gittings *et al.* (1998) found the depth of *M. margaritifera* habitat in Ireland to be correspondingly low, at around 20 cm. Similarities in climate may explain this: in mild climates, such as that found in Britain, rivers freeze very infrequently (there is thus little resultant ice damage to biota and habitat). This enables *M. margaritifera* to survive at the shallow depths described in the literature from these regions. At higher latitudes, for example in Finland or Sweden, mussels only inhabit much deeper channel areas, as Scandinavian rivers are likely to freeze to greater depths (Hendelberg, 1961). Freshwater pearl mussel populations at higher latitudes have commonly been found at depths of up to 3 m (Gittings *et al.* 1998), with channel areas under 0.3-0.5 m considered wholly unsuitable (Hendelberg, 1961). In Scotland, *M. margaritifera* have been reported at 3 m (Young, *pers. obs*. cited in Hastie *et al.* 2000). This review of depth preferences suggests a full range of habitat depths have been sampled and the ideal, shallow water depth of 30-40 cm is likely to be a representative preference for British freshwater pearl mussel populations, rather than a result biased by sampling designs weighted to accessible areas. Hastie *et al.* (2000) found mussels were often found within 3 m of the bank, which also correlates to shallow depth.

1.9.3. Vegetation

The role of vegetation in freshwater pearl mussel habitat is dependent on the scale at which vegetated areas exist within the mussel's perceived environment. In channel vegetation, with a significant influence on *M. margaritifera*'s immediate environment, has been identified as a negative habitat feature; it increases BOD, siltation and nutrient levels (Hastie *et al.* 2004; Moorkens *et al.* 2007). In channel vegetation is established as a threat to survival in Section 1.7.2. Hastie *et al*. (2000) could only relate this to siltation; they found no direct significant link between vegetation cover and mussel presence.

Conversely, the role of riparian vegetation, in channel shading, is more positive. The results of Gittings *et al.* (1998) implied shaded areas of channel are preferred by *M. margaritifera* in their Irish study river and mussels are frequently observed in this environment: close to the bank under shading trees (Gittings *et al.* 1998; Hastie *et al.*

2004). This could be a function of channel temperature where overhanging riparian vegetation is found. Moorkens *et al.* (2007) assess the habitat of *M. durrovensis.* This species inhabits lime-rich waters in Ireland, but its other habitat preferences bear relation to other members of the *Margaritifera* genus. Moorkens *et al.* note that in rivers with a high suspended sediment load, or that have a high propensity to turbidity in summer spate flows, oxygen depletion is a major risk for the mussel population. Mussels overcome turbidity by closing the valves as ingestion of sediment is lethal. The mussel is protected in this state but after a number of days it will die of oxygen starvation (Moorkens *et al.* 2007). This process is faster at high temperatures and it is possible that cooler areas under trees could slow the process of oxygen depletion by slowing the rate of metabolism. However, cool water temperatures may be the definitive mussel preference (Buddensiek, 1995). A potential preference for shaded areas due to reduced algal growth in these locations (due to a lack of light) has also been suggested (Hastie *et al.* 2004).

1.9.4. Flow and substrate

Flow and substrate are the remaining features defining freshwater pearl mussel habitat preferences. Hastie *et al.* (2000) give a specific range of flow velocities at which mussels in the River Kerry (Scotland) are found: a velocity preference range of 0.25-0.75 m s^{-1} is given by computed habitat suitability curves developed in the study. This value is very specific and therefore potentially applicable only to the River Kerry and rivers and catchments of similar morphology and quality. In a broader sense, flow velocity must consistently be adequate to bring nutrients to the mussels and allow nutrient, oxygen and waste exchange between the water column and substrate interstices (Bolland *et al.* 2010) to permit juvenile survival. Mussels are not associated with slow flowing or smooth, laminar flows but are positively associated with faster flowing sections: rippled flows, with broken or unbroken standing waves (Hastie *et al.* 2003).

Substrate and flow demonstrate established associations (Gomez, 1991). Substrate as a feature of mussel habitat must be assessed across spatial scales and, again, life stages. It is considered highly related to mussel preferences: substratum-based discriminant function models developed by Hastie *et al.* (2000) successfully predict mussel existence in <92% of cases. They propose that the patchy spatial distribution of mussels in the River Kerry may be related to variation in substrate compositions. A mix of clast sizes is required (demonstrated in Figure 1.2 (a)). These can be boulder or cobble dominated (Hastie *et al*. 2000) but must include patches of finer material such as gravels and sand (Gittings *et al.* 1998) so that mussels can burrow into the sediment for stability. Large clasts prevent the sands in which the mussels are secured from entrainment in high flow events. Pebble dominated substratum is considered poor habitat as these present no areas for purchase, nor are they stable.

While sand is required, silt or clay substrates are not suitable (Hastie *et al.* 2003; Moorkens *et al.* 2007). Silts are tolerated by adults (Hastie *et al*. 2000) but can be dangerous as mussels cannot move out of them and if they sink and the siphons are blocked, they will die. Adults will also inhabit fissures in bedrock as these environments are still stable and provide fast flowing water. However, neither excessively silty channel areas, nor bedrock are suitable for juvenile development (Hastie *et al.* 2003). A suitable habitat must have clean, aerated, fine gravels for juveniles to spend the post-parasitic stage in (Buddensiek *et al.* 1993). Substrate can thus be considered on two scales: Hastie *et al.* (2000) suggest adult substrate preferences function within a 1-10 m scale, whereas interstitial cleanliness in an area of less than 1 m^2 is crucial to juvenile survival. While substrate is acknowledged as extremely important, deficiencies in any of the habitat parameters mentioned could have a detrimental effect on a *M. margaritifera* population, particularly the viability of recruitment mechanisms.

1.10. Presenting the case of the freshwater pearl mussel in the River North Tyne catchment, Northumberland

The severity of the plight of *M. margaritifera*, combined with their evident value, confirms there is an urgent need to study key parameters that affect freshwater pearl mussels. One approach to this is to study the populations that are not recruiting, as sustaining all remaining populations should be a priority. The populations in the Tyne network are an appropriate study system, as initial remediation work has been carried out and there is existing interest in this population. The River Rede is a tributary of the

North Tyne and sustains a depleted *M. margaritifera* population that is no longer thought to be recruiting (Environment Agency/E_3 Ecology report, 2006). Three surveys of the contemporary freshwater pearl mussel population have been undertaken in recent decades, thus some useful background information is available (Oliver and Killeen, 1996a; Rooksby, 1997; Environment Agency/E_3 Ecology report, 2006). Despite these extensive surveys, no evidence of recruitment is apparent in the Rede, as shown by a lack of juvenile mussels (Environment Agency/E_3 Ecology report, 2006) and glochidia-infected salmonids (Lewis, *pers. comm*.). However adult mussels from the Tyne catchment have successfully produced viable glochidia and juveniles at the FBA hatchery and at the Environment Agency Kielder Hatchery, the latter in River North Tyne water (Environment Agency website, 2010; Lewis, *pers. comm*.; Miles, *pers. comm*.).

1.10.1. Habitat degradation in the River Rede: threats faced by the *Margaritifera margaritifera* population

A broad examination of factors that threaten *M. margaritifera* populations in any catchment where the species thrived historically has been undertaken in Section 1.7. These threats are not all applicable to the population in the River Rede. Two key threats appear to apply pressure instigating decline (Environment Agency/E_3 Ecology report, 2006): exploitation through pearl fishing and habitat degradation due to pollution, specifically in terms of increased siltation of gravels.

The primary, historic cause of the decline in the Rede population is thought to have been extensive pearl fishing (Oliver and Killeen, 1996a; Rooksby 1997). There was a Roman pearl fishery on the River Rede in Rochester (Environment Agency/E_3 Ecology report, 2006) and pearl fishing continued into the 20th century as late as the 1960s (Lewis, *pers. comm*.; Environment Agency/E_3 Ecology report, 2006). Pearl fishing is now illegal and is no longer an active threat. Consideration of a wider temporal scale suggests *M. margaritifera's* long life expectancy, high age of sexual maturity and low fecundity may mean that the effects of this threat are still felt today (Österling *et al*. 2010), thus all ongoing pressures will induce more stress than if they were the sole issue.

The most significant contemporary threat is thought to be bed siltation, derived from forestry and agricultural (livestock grazing) sources. Some areas of extensive bank erosion also exist (Environment Agency/E_3 Ecology report, 2006). The impacts of siltation are covered in Sections 1.6 and 1.7.2. Furthermore, localised disturbances have occurred in the Rede as a result of engineering work: channelization for the prevention of floods occurs near Otterburn and creation of artificial pools for the leisure fishing industry. They cover only small areas and thus are not major threats to the Rede population (Environment Agency/E_3 Ecology report, 2006). Metal pollution is not thought to be considerable in the Rede, despite the presence of many former mines and quarries (Environment Agency/E_3 Ecology report, 2006).

The availability of the salmonid host is not thought to be a current reason for decline in the Tyne network (Environment Agency/E_3 Ecology report, 2006). However, it may have been a contributor to the persistence of population decline during the early part of the 20th century. During industrial development in North East England, water quality in the Tyne catchment deteriorated rapidly. As a consequence of pollution from industrial effluents, mining effluents and sewage discharges (Warburton, 1997; Milner *et al.* 2004), key species' populations, such as Atlantic salmon, waned. In years when the salmonid population was very low, the Rede freshwater pearl mussel population would have needed to rely only on non-migratory brown trout as hosts in recruitment. After the amelioration of water quality, particularly dissolved oxygen content, further down the Tyne catchment and a programme of restocking, salmon stocks have recovered effectively (Milner *et al.* 2004). The extensive losses from the reproductive strategy (outlined in Section 1.6.2) would have been all the greater if fewer hosts were available for an extended period of time (Hastie and Young, 2003a). This episode may have the potential to explain some of the historic decline in this local population.

1.10.2. Current conservation efforts

The Tyne Rivers Trust, the Environment Agency and the Freshwater Biological Association all have current interests in the Tyne and Rede mussel populations, with the former managing extensive efforts in river restoration and improvement. To aid the progression towards rebuilding the Rede population, as one of the few English freshwater pearl mussel populations still in existence, an assessment of freshwater pearl mussel habitat will be valuable both for the general understanding of the nature of available habitat on the Rede and for the conservation bodies' management approaches.

Sedimentation has already been identified as one of the major problems affecting the Rede, research and work into reducing this has begun. Consequently this project will examine other physical habitat factors in the Rede that are pertinent to *M. margaritifera* survival. This can aid the identification of aspects that may require future research and management effort. Studies have already identified a patchy distribution in *M. margaritifera* populations. Gittings *et al.* (1998) concluded that their distribution corresponded to appropriate conditions in certain habitat variables, namely the degree of channel shading and depth. Hastie *et al.* (2000) distinguish a "highly contagious, non-random spatial distribution pattern" in the River Kerry population, patchiness that they attribute to variation in substrate composition at the sub-10 m scale. The evidence that this species responds to habitat patches in other rivers is acknowledged in this study. Confirmation of whether *M. margaritifera* distribution in the Rede still relates to habitat patchiness, or whether their distribution is more random in relation to habitat parameters could help river management approaches. Information concerning the Rede mussels' distribution may help to identify areas of habitat degradation and establish whether the Rede *could* harbour a larger, viable population, thus informing remediation work.

1.11. Aims, research questions and objectives

1.11.1. Aim

The aim of this study is to examine the distribution of suitable freshwater pearl mussel (*Margaritifera margaritifera*) habitat existing in the River Rede. It further aims to assess the current dispersion of freshwater pearl mussels as a function of the physical habitat. Outputs will aid the stakeholders' endeavours to maintain and recover this species' population.

1.11.2. Research questions

In light of the above examination of the literature associated with *M. margaritifera* and habitat degradation, the following research questions have been devised:

1. What is the spatial distribution of freshwater pearl mussels and physical habitat variables on the River Rede?
2. Can areas of preferred habitat be identified or do physical environmental parameters demonstrate no relationship to *M. margaritifera* presence?
3. Is habitat character patchiness relevant to *M. margaritifera* in terms of whether this species' distribution on the Rede is a function of physical habitat?

1.11.3. Objectives

1. To collect contemporary information on the physical habitat parameters in the River Rede through combined *in situ*, field based and remote sensing techniques.
2. To perform a ground survey of present mussel distribution and habitat parameters associated with areas of mussel habitation.
3. To collate images acquired via remote sensing techniques to extend the data acquired in ground surveys and consider the extent to which these can supplement traditional methods.
4. To examine the relationship between mussel location and physical habitat parameters to discern whether mussel distribution bears relation to habitat patchiness.
5. To deliver the findings of the project to the Tyne Rivers Trust and provide an information basis for management decisions relating to this species.

Chapter 2 Methodology

2.1. Study location

2.1.1. Study location: River Rede

The River Rede is a major tributary of the River North Tyne in Northumberland. It is 48 km in length with a catchment area of 18 km^2 (Heritage and Milan, 2004). The Rede's source rises in the Cheviots, at Carter Bar, near the England - Scotland border. It feeds Catcleugh Reservoir approximately 4 km downstream. The catchment geology comprises carboniferous limestones in the Alston formation and the Tyne Limestone formation (Figure 2.1) (Lawrence *et al.* 2007). These are overlain by peat glacial till (Heritage and Milan, 2004). Coal measures and ironstone shales have been mined since the Roman times. Moorland and conifer woodland (including extensive forestry workings) dominate the land use in the headwaters. In the lower catchment areas of rough, semi-improved and improved pasture are stocked with sheep and cattle. Small areas of deciduous woodland are in evidence. The catchment is sparsely populated with small villages and numerous farmsteads. Local mean annual rainfall is 1026 mm (Heritage and Milan, 2004).

Channel width varies between 2 m and 36 m. Bankfull discharge is at 8.5 m^3 s^{-1} (Heritage and Milan, 2004). The Rede is hydrologically flashy with a high bed load conveyance, according to Heritage and Milan (2004), though their study suggests substrate packing may reduce initial levels of gravel entrainment in high flow events. The flashy nature means that fine upland sediments are commonly in suspension, colouring the Rede water for several days after heavy rain events. The Rede substrate comprises primarily cobble sized clasts; though extensive areas of sand and gravel substrates exist where channel management has occurred. Flows on the Rede include sparse pools and frequent riffle sections of considerable length (Harvey *et al.* 1994). Areas of glide are also extensive.

In the upper and lower reaches the riparian margins generally include mature trees. There is a high degree of channel shading, though tunnelling is rare and this is restricted to the upper, narrower reaches. The middle reaches are more open with riparian margins of grass. Where the adjacent land is grazed this is often short and of the same composition as the pasture (Harvey *et al.* 1994). River banks are generally high (<20 m) and steep (30-80^{o}), most notably in the middle sections. 90^{o} river cliffs are seen throughout the Rede (Harvey *et al.* 1994).

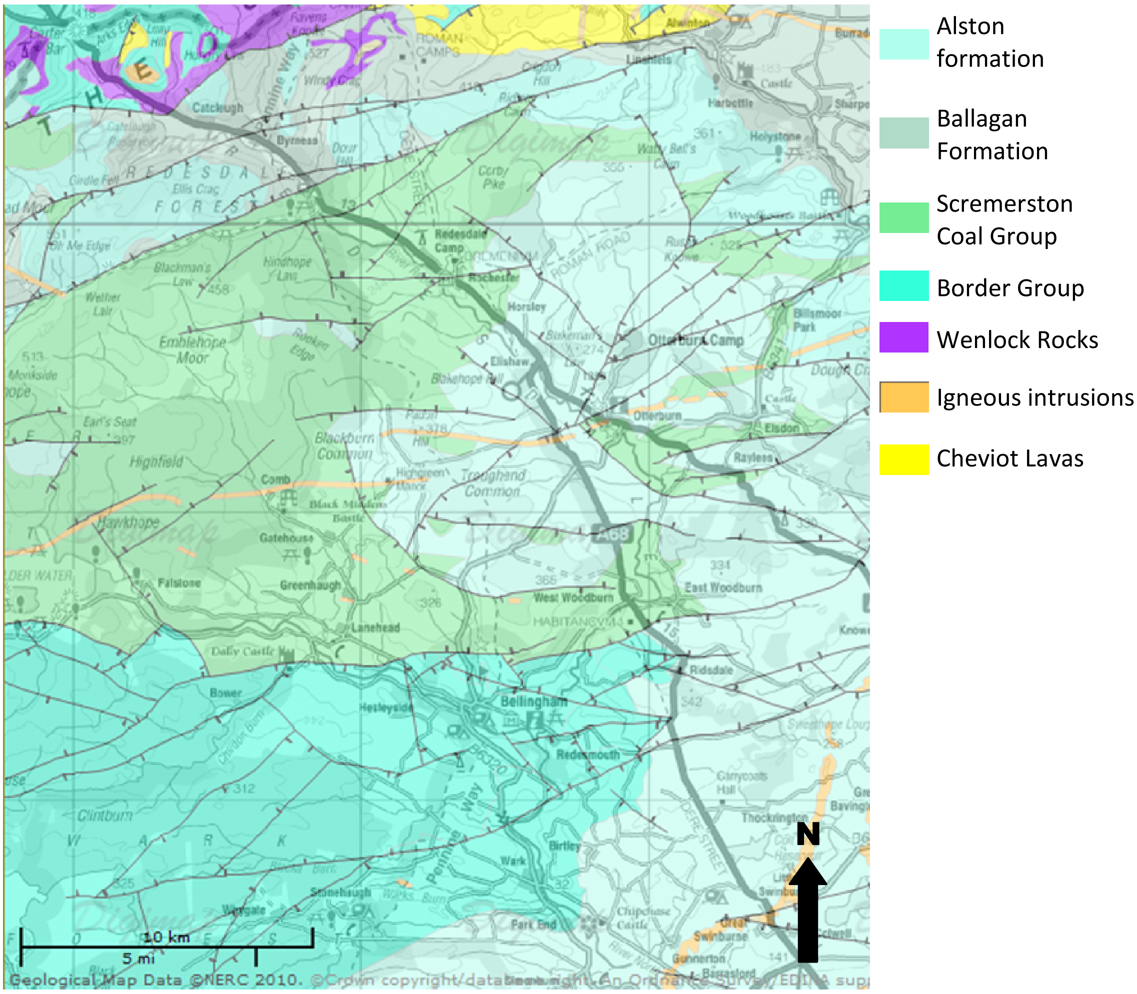


Figure 2.1 Geological map of the Redesdale area

Source: Edina Digimap (2010). The catchment is dominated by calcium rich limestones, such as those found in the Alston Formation, the Scremerston Coal Group (part of the Tyne Limestone formation), the Ballagan Formation and the Wenlock Rocks in the far north of the catchment. There are also small igneous intrusions evident throughout the catchment.

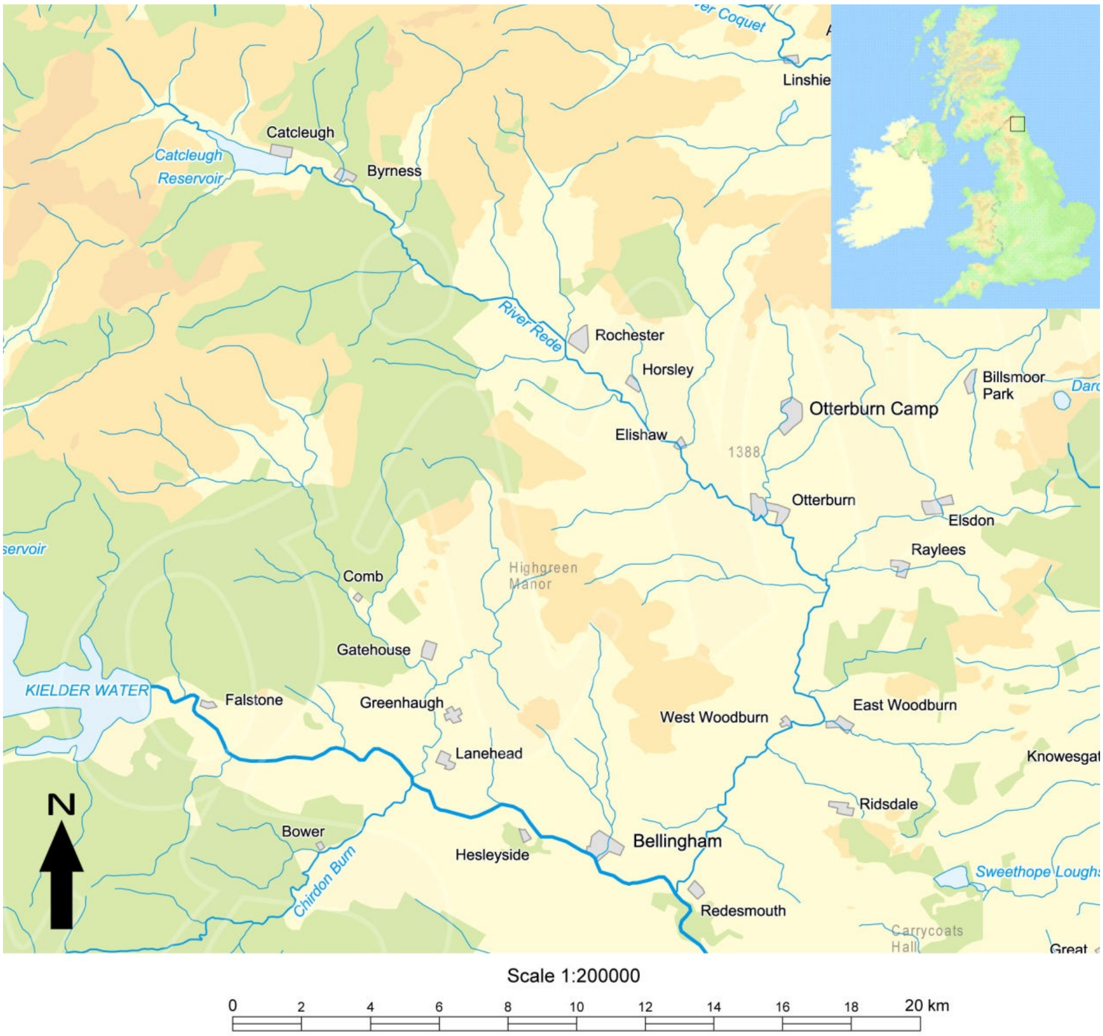


Figure 2.2 Location map of the River Rede.
Source: Ordnance Survey Edina Digimap (2010). The River Rede is in Northumberland (inset). At nearly 50 km in length, it is a major tributary of the River North Tyne.

The Rede population of *M. margaritifera* is aged with little, if any, recruitment. The most recent survey suggested a total of 2,461 mussels in the river (Environment Agency/E_3 Ecology report, 2006). This is 60% more than the 1997 survey but may still be a conservative figure. These are all situated in approximately the lower third of the river, downstream of Otterburn, but are well spread with only four beds identified containing more than 100 individuals. No evidence of juvenile mussels was found in any of the previous three surveys though improved methods and coverage meant that a higher mussel count was obtained each time (Oliver and Killeen, 1996; Rooksby, 1997; Environment Agency/E_3 Ecology report, 2006). Studies unanimously agree that the current population is considerably degraded in comparison to historical records, primarily due to pearl fishing. Over the twentieth century the decline heightened but this is attributed to multiple causes (Section 1.10.1).

Oliver and Killeen (1996) suggest that the Rede contains a significant number of mussels for its size but the beds are sparsely populated and some mussels may be located where they were deposited by floods (Environment Agency/E_3 Ecology report, 2006). There is acknowledgement of some downstream movement of beds and individuals do wash out into the North Tyne, where the river environment eventually becomes unsuitable. The contemporary *M. margaritifera* population is considered unsustainable if the current conditions do not improve (Environment Agency/E_3 Ecology Report, 2006).

2.1.2. Study sites

Four sites were used in this study, spread along the section of the River Rede that contains the *M. margaritifera* population (see previous section). The species is strictly protected by national and international law, yet unfortunately populations are still susceptible to illegal pearl fishing, which can eliminate whole river populations. In this work, the actual locations of mussel populations have therefore been kept anonymous at the request of the Environment Agency, North East Region. As a result the locations of the study sites, mussel surveys and imagery areas will remain undisclosed and no specific location maps have been included. The four locations are named A-D with sites within them numbered 1-5, as explained in Section 2.7. The four study sites vary in character. This ensures a representative sample of the River Rede habitat variation is surveyed and that the variation in areas supporting mussel populations is included. Site descriptions are given in Table 2.2.

2.2. Water quality samples and walkover survey

2.2.1. Walkover survey

The Rede river corridor survey by Harvey *et al.* (1994) gives an excellent overview of the channel, riparian environment and the wider catchment characteristics. The mussel population and habitat reports give very detailed information of the Rede ecosystem but they are only extensive in areas where mussels exist. The river corridor survey is extensive across the catchment but observations are only undertaken as a series of 500 m sections. A total of 59 sections encompass the River Rede (Harvey *et al.* 1994). This study requires a higher data resolution on which to base the choice of sample sites. It was thus deemed important to examine the River Rede to reinforce the information in the existing literature.

A walkover survey was conducted between Catcleugh Reservoir and the Rede-Tyne confluence. In most areas the channel was observed continuously. However, due to physical access restrictions or where the landowner could not be identified, this intensity was not maintained. In these cases the river was accessed at a minimum of once every kilometre with additional points where tributaries joined the main Rede channel and the river corridor survey data were used as a supplement.

2.2.2. Water quality sampling

In an assessment of any river ecosystem it is crucial to consider the entire river network and catchment (Fausch *et al.* 2002). A wealth of literature has identified the importance of certain water quality parameters in respect to *M. margaritifera* (Young, 2005, Moorkens *et al.* 2007), including early studies written at the initial stages of the recognition of this species' decline (Young and Williams, 1983). The need for water quality amelioration to sustain the mussel population is also accredited in policy documents (UKBAP Species Action Plan, 1995).

Margaritifera margaritifera require very specific chemical conditions (section 1.9.1) but in such a dynamic environment as a river, water chemistry can vary considerably. It is justifiable to consider all spatial and temporal changes as relevant to freshwater pearl mussel habitat. For example, Bolland *et al.* (2010) identify phosphorous concentrations as particularly variable, often displaying seasonal peaks. In order to record such episodic maximum concentrations in chemical parameters, continuous sampling should be undertaken, as any acute pollution event could be terminally damaging to the mussel population. However, utilising equipment to do this was not feasible for such a small scale project where water quality was not the main feature of the investigation. In this study, water chemistry parameters contributed to habitat quality assessment at the catchment scale. As a result a compromise was made and water chemistry analysis was indicative only of the background chemical conditions. Thus the important temporal component, identified by Bolland *et al.* (2010), of water quality variation can be addressed using Environment Agency historical data (Environment Agency, 2009). This only addressed seasonal means, not maximum values.

Secondary data from the Environment Agency is only provided for certain points on the Rede. The temporal variation this provides is useful but the spatial context in which data are set is minimal. As a result a spatially extensive water sampling strategy was employed to expand knowledge of downstream variation, comparing sites across the catchment. This overcame the need to repeat point samples multiple times, yet still represented the complexity of water quality variation downstream (Mason, 1996). A study made by Rushton *et al.* (1989) in the local area of Otterburn examined agricultural fertiliser applications, leaching of which could lead to an increase in trophic status. It provides locally relevant information on likely timings for application of chemicals to the catchment. Sampling was therefore carried out both when the nutrient status of the Rede was low and when likely to be high.

Winter water quality sampling was completed in January to capture minimal nutrient conditions in the water column. The summer period would also have been appropriate in light of high seasonal productivity reducing nutrient levels but the time was designated to other fieldwork. The closed period on grassland for the application of manufactured nitrogen fertiliser is between mid-September and early February (DEFRA website, 2010); therefore it is very unlikely that any will have been applied before winter sampling. Natural fertilisers (manure etc.) may be applied after November but this is unlikely to have occurred in the sampling period as antecedent conditions were very wet and it would not be feasible or effective to make any applications. Spring samples were taken in April to capture changes in nutrient levels reflecting the fertiliser applications at this time. Days when the river was in spate were avoided for reasons of safety and to avoid capturing peak leaching episodes. Adult mussels can avoid damage during short, pulse deliveries of pollutants by closing the valves (Young, 2005) therefore these events do not need to be addressed in the sampling design.

Spatial context is crucial in establishing the relationship between water quality and ecological and physical parameters (Mason, 1996). Winter water samples were taken approximately every kilometre along the full length of the Rede. This scale of assessment follows the approach of Bolland *et al.* (2010). Additional samples were taken at significant locations that may influence the quality of the water column immediately downstream. These included tributary confluences and cattle access points.

As the Rede water quality was not the primary focus of the investigation, it was not practical to assess interstitial water quality, including the degree of bed siltation. Furthermore, as juvenile mussels require open gravels, where water flows freely in the interstitial spaces, the assessments made of the main water column should be indicative, to some extent, of the environment experienced by any juveniles in the gravels (Buddensiek *et al.* 1993). While some studies (for example Bauer, 1983) looked at chemical parameters in the water column and others in sediment and the mussels' body tissues, the primary transfer of pollutants that may cause eutrophication or harm the mussel population is in solution in the water body (Leeds-Harrison, 1995).

Of the factors crucial to *M. margaritifera* survival, no facilities were available in this study to assess biological oxygen demand (advocated by Bauer, 1988 and Young, 2005) or Redox potential, despite their relevance. As Rooksby (1997) suggests metal poisoning is not an issue in the area, no specific assessments of toxic metals were made. However, due to evidence of historical mining and quarrying activity, it is possible that drainage from spoil heaps transfers dissolved metals to the Rede channel (Bradley, 1995) to some degree. Conductivity is measured to validate this issue, with acknowledgement of sites where organic pollution may be prevalent (Chapman, 1996). Table 1.1 displays the water quality parameters assessed in water quality sampling. Although the two key methods used to analyse the water samples (portable probe measurements and Dionex analysis - see Section 2.2.3) give values for many aspects of water chemistry, only those relevant to the requirements of *M. margaritifera* were used in this investigation (see section 1.9.1).

A simple water sampling method was derived with reference to the literature and established sampling methods. A calibrated portable probe meter (YSI 556 MPS multi probe system) was used to obtain values for parameters in Table 2.1. This is preferential to laboratory methods as a certain amount of sample degradation occurs between laboratory analysis and sample collection (Bartram and Ballance, 1996). While some anions and cations will also degrade in this time, the facilities do not exist to assess these in the field therefore adequate cold-storage arrangements were made to ensure the samples remained as viable as possible before analysis could be performed. This method's simplicity ensured that samples could be collected along the entire river length within a practical time frame so as to minimise variance attributable to changing weather patterns and discharge.

Table 2.1 Water quality parameters sampled.
While readings of additional factors were given in-situ from the portable probe meter, conductivity, pH and dissolved oxygen were considered most valuable as indicators of the water quality suitability for mussel habitation. Equally Dionex analysis gave many outputs but those listed were deemed the most useful indicators in previous studies.

Parameter	**Method**	**Relevant literature**
Conductivity	Portable probe meter – in situ	Bauer, 1988; Chapman, 1996; Young, 2005.
pH	Portable probe meter – in situ	Bauer, 1983; Englund *et al.* 2008; Bolland *et al.* 2010.
Dissolved oxygen	Portable probe meter – in situ	Young, 2005; Bolland *et al.* 2010.
Nitrates, Phosphates, Calcium	Dionex- laboratory analysis from samples frozen on day of collection.	Bauer, 1983; Young and Williams, 1983; Bauer, 1988; Young, 2005.

2.2.3. Sampling procedure

On arrival at a sampling site the portable probe meter was lowered into the water about 1-2 m from the edge. Once the meter readings had settled, or after 5 minutes, values for temperature, pH, conductivity and dissolved oxygen were recorded. Gloves were worn to obtain an uncontaminated 40 ml sample of water from flowing water at the channel edge (approximately 50 cm from the bank) in a new 50 ml vial. If the banks were steep and the river level too low to collect the sample by hand, the vial was attached to a 2 m plastic pole to reach the water level safely. To ensure the sample was as representative as possible, the vial was rinsed with river water first.

The sample was kept cool to minimise degradation. On return from the field samples were stored in a freezer until analysis. After the water sample had been obtained, qualitative notes on antecedent weather conditions and the sample location (GPS reading, land use, cattle access areas, tributary influences etc.) were taken.

Spring variation in sampling design

After the winter water sampling had been completed, it was evident that the spring water sampling need not be as spatially extensive. While there was some variation about the average trend in water quality variation in the upper 15 km of the River Rede, no major change was evident between areas where mussels were present and those reaches where they were not. The spring sampling effort was thus changed to be more intensive, starting approximately 15 km downstream. This is still over 10 km upstream of the most northerly mussel beds to maintain a good representation of the quality of water reaching them. Contracting the survey effort allowed extra sampling points to be incorporated within the shorter sampling frame.

2.2.4. Laboratory method- Dionex analysis

Deriving the concentration of anions and cations in the samples to give values for nitrates, phosphates and calcium was carried out using ion chromatography. Water samples collected in the field were fully defrosted before analysis to ensure the actual concentration of ions in the water was assessed, rather than in a concentrated solution. In preparation for Dionex analysis, a subsample of at least 10 ml of the original sample was filtered using a GD/X 0.2 μm pore size cellulose acetate filter to remove the majority of suspended sediment. This was then passed through a separating column using a Dionex Automatic Sampler and filtered through a 10 μl sample loop for anions or a 25 μl loop for cations. Based on a gradient system of ion size, different ions are sequentially taken from the sample. Conductivity changes are monitored to give the amount of each ion in the sample.

2.3. Assessment of habitat and *M. margaritifera* distributions

The requirement to move beyond the traditional scales of fluvial system assessment (Fausch *et al.* 2002) necessitates the use of new techniques. There is consensus that remote sensing techniques meet the need to expand scales of assessment from discontinuous, reach level data to the network and catchment scale (Carbonneau *et al.* 2004a; Legleiter *et al.* 2004; Marcus and Fonstad, 2008), in accordance with current river ecology theory (Fausch *et al.* 2002; Thorp *et al.* 2006). Remote sensing is often the most cost effective and viable method of data collection that can obtain the detail required (Dugdale *et al.* 2010). As extensive habitat data coverage is essential for the assessment of habitat patchiness, remote sensing provides a highly relevant tool for this investigation.

Remote sensing techniques can supply details of catchment scale habitat patterning in terms of substrate distribution, depth and flow type variation (Carbonneau *et al.* 2004b). It is not possible to acquire such detailed data at this scale via traditional sampling techniques. Aerial imagery was obtained and analysed (Section 2.4) with this intention, however, the resulting data were unfit for purpose due to poor image quality and a high suspended sediment load obscuring the bed view (Section 3.1). As time constraints and weather conditions prohibited the collection of new aerial images, low level terrestrial images were obtained (Section 2.6). This method was time consuming hence only the pool/riffle system scale could be assessed. These were done within four 400 m reaches (Section 2.5). Grain size distributions and depth measurements were made at this scale. Microhabitat was also assessed within these four reaches using more traditional survey methods (Chalmers and Parker, 1989; Gittings *et al.* 1998), alongside adult mussel distribution. Despite the initial loss of the detailed but extensive dataset from aerial imagery, the assessment of habitat was made as comprehensive as possible by sampling representative areas of channel. A total of 3500 m^2 was covered by aerial imagery and 5134 m^2 was covered in the microhabitat survey. In the endeavour to cover as much of the Rede habitat character as possible, these datasets often cover different channel areas. They are thus analysed separately, but conclusions from each scale are used in conjunction to draw conclusions on mussel habitat in the Rede across scales. Ideally these methods would be used to complement each other. For example, substrate was assessed at both the pool/riffle system and microhabitat scales and comparisons of data for this key habitat feature (Hastie *et al.* 2000) were used in corroborating the relationship between *M. margaritifera* and habitat patterning at multiple scales. Due to the necessary misalignment of sampling areas, this could only be done to a certain extent.

2.4. Remotely sensed aerial images

2.4.1. Remotely sensed images - collection and analysis

A series of 312 images, obtained in August 2009 by Apem, Ltd. were used as there was not scope for direct image collection of this scale in the project timeframe. The platform used was a Vulcanair P68 Observer 2 aircraft equipped with a Canon EOS- 1Ds Mark II camera. This outfit allows high resolution images (pixel resolution of about 3 cm) of an extensive area to be acquired efficiently (Apem Ltd. website, 2008). Images were 4992 x 3328 pixels which amounts to approximately 150 x 100 m ground area, based on the optimal pixel resolution of 3 cm. This resolution is required for analysis using the Fluvial Information System (FIS) with images in the standard red-green-blue colour bands (Fluvial Information System User Manual, 2009).

2.4.2. Fluvial Information System analysis

The images were georeferenced on receipt thus it was possible to begin image classification immediately. The ‘Image classification tool’ and ‘unsupervised classification’ method was chosen. This classifies areas in an image according to pixel attributes: similar pixels are statistically clustered (Fluvial Information System User Manual, 2009). Using signature areas for supervised classification produced unreliable results. As recommended in the User Manual, all images were classified with a 25% resize and a filter of 10 pixels into three classes. The first class is the wetted river area and the second exposed substrate with a third showing all other, non-channel areas. An example of a classified image can be seen in Figure 2.3.

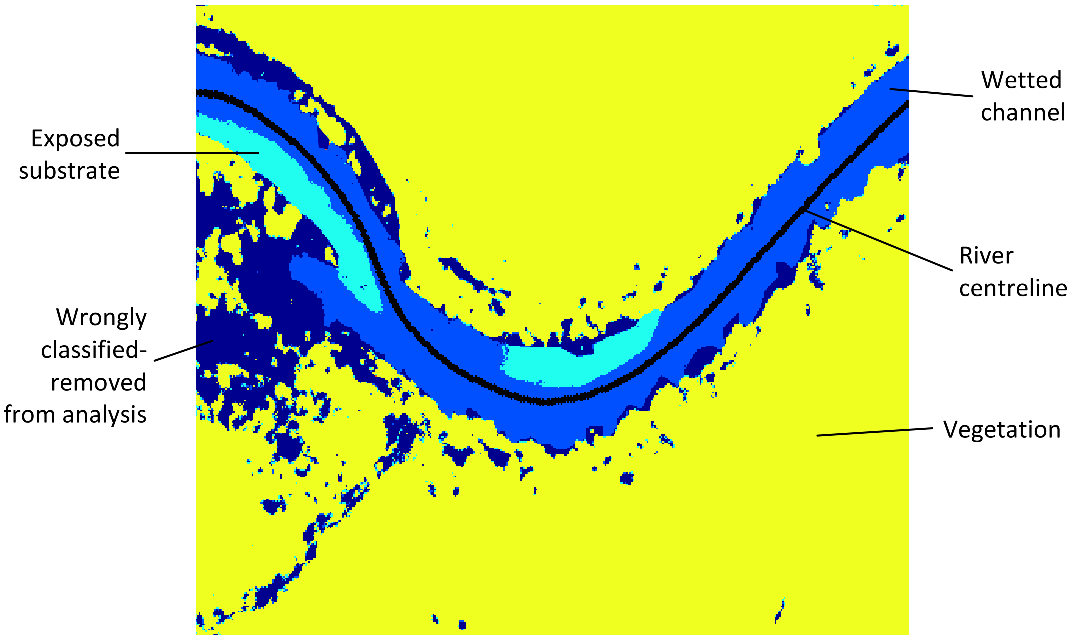


Figure 2.3 A classified image from the River Rede.
This image is classified into 3 classes- wetted channel, exposed gravels and vegetation. The other areas are where the analyst has removed areas that were incorrectly classified.

The ‘Centreline production tool’ was used to analyse all images and the most representative centreline was achieved using a moving average smoothing filter with a filter size of 60. The chosen filter size was slightly nearer the lower end of the recommended bracket (between 30 and 100) as the River Rede maintains some tight meander bends (Fluvial Information System User Manual, 2009). Larger filters over-smoothed the centreline and several meanders were missed (Figure 2.4). The image digitisation method used was ‘minimum distance line tracing’, which creates a centreline by bisecting exactly the classified wetted channel. This method was again chosen due to the extreme meander bends, which can bias the ‘image skeletonisation line tracing’ method (Fluvial Information System User Manual, 2009).

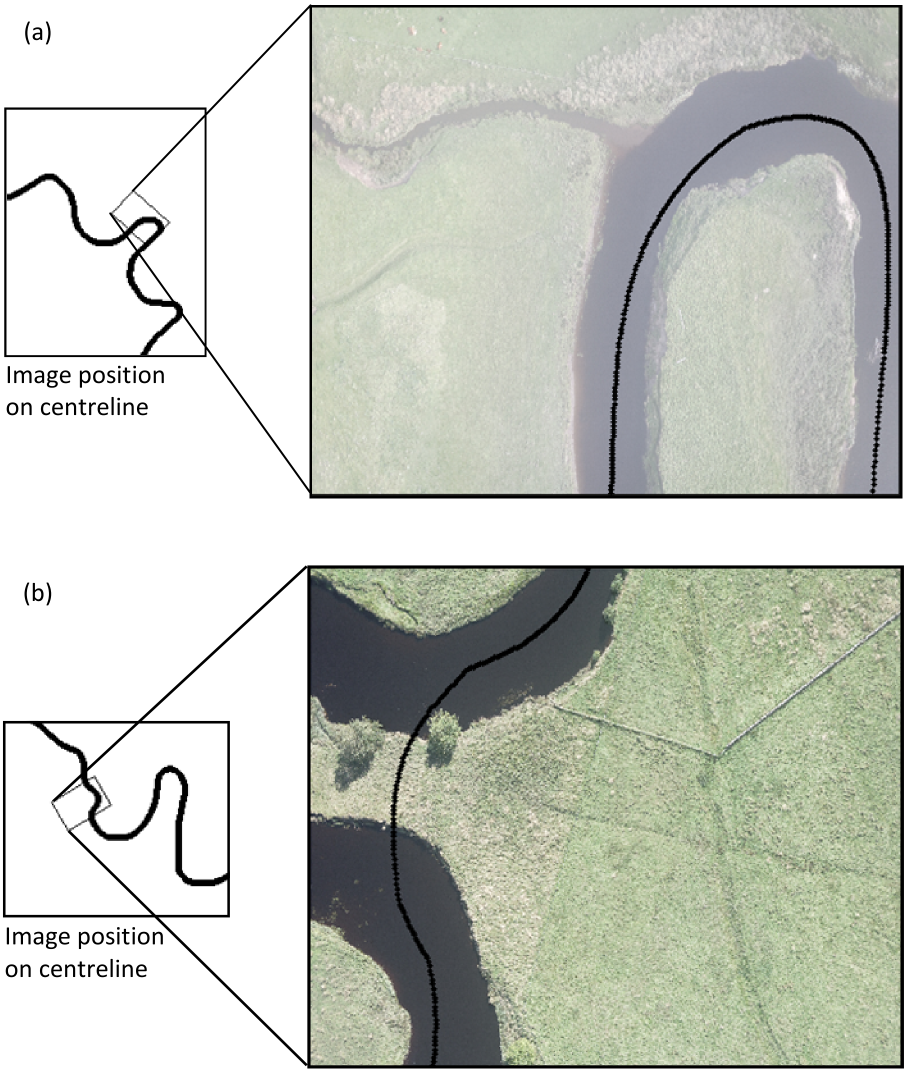


Figure 2.4 Centreline over-smoothing of a meander bend.
If the smoothing filter on the 'Centreline production tool' is too large then tight meander bends are missed, thus shortening the centreline and creating an inaccurate river coordinate system. The filter used was most appropriate for the River Rede. Image (a) displays a correctly calculated centreline, with appropriate filter settings, which gives accurate values for a River Coordinate System. Outputs such as image (b) suggest further alterations should be made to the calculated centreline.

This initial level of analysis produced a channel fitted coordinate system, the river coordinate system (RCS), which is carried through the study to express spatial context. It is based on the orthogonal curvilinear coordinate system defined by Smith and McLean (1984) and developed by Legleiter and Kyriakidis (2006) as a spatial referencing system for river channels where transformations are used to move between this spatial reference system and a Cartesian system such as the British National Grid (BNG).

Generation of a width profile was achieved using the centreline and RCS as a baseline. All images were assessed though some data points, such as where meanders were not traced properly by the centreline, were removed. The width long profile was developed for the entire photographed length at sample frequencies of 100 m, 10 m and 1 m. The latter is used in the study as the greatest level of detail can be attained with equal efficiency.

2.5. Sampling design

For the smaller scale surveys a sampling method was required that captures the spatial heterogeneity of both the mussel distribution and habitat parameters. Continuous sections were observed to allow successful identification of patches in terms of habitat heterogeneity and complexity and the scales across which mechanisms work (Bell *et al.* 1991). There was a need to capture the interrelation of both the habitat variables *in situ* and the relevance of these parameters to the organism's perception of habitat (McCoy at al. 1991; Downing, 1991). The scale on which the sampling was based involved both a large catchment scale approach (Fausch *et al.* 2002) in order to portray habitat variance as it changes along the Rede and, at the suggestion of Hastie *et al.* (2000), allowed close observation of the 1-10 m scale. The sampling design revealed the distribution of the mussel populations as a function of physical habitat parameters.

Based on these requirements the following sampling strategy was developed. Four main areas were identified that encompassed the differing physical river habitat features found on the Rede. These were all located in areas where some mussel populations were known to exist, based on the findings of previous Environment Agency reports. This was necessary, rather than defining new, random locations, as the mussel population on the Rede is very sporadic and it was crucial to ensure that an adequate number of samples would return positive mussel findings within the finite time available to perform the surveys. The four locations were chosen specifically to include the majority of habitat characteristic variation found in the Rede (Table 2.2).

At each of the four locations, 400 m long sections of channel were delimited as the areas in which sampling would occur. This section length was chosen as based on the Frissell *et al.* (1986) hierarchical stream classification system. These sections approximate the ‘reach’ scale. The reach scale is frequently employed in assessments of population parameters and distribution patterns. While the reach scale is not often very physically discrete, it offers and effective display of medium and long term changes in the river, again relevant to the mussel population in the Rede. Other points considered included channel accessibility and access permission.

Table 2.2 Distinguishing physical features of each sample location.
Source information: *Pers. obs*. and Harvey *et al.* (1994) - River Corridor Survey.

Location	Flow types	Riparian vegetation	Management	Notable features
A	Glide Run Riffle Marginal deadwater	Minimal. One tree, reeds and steep grassed banks.	Considerable. Channel dredging. Banks frequently underpinned with logs.	Cattle access. Sand and gravel dominated. Deep channel relative to other locations. Adjacent improved pasture. Extensive bank erosion.
B	Glide Run Riffle Marginal deadwater Spill	Very extensive but not continuous. Mature trees, grassed banks.	None notable. One area of concrete bank reinforcements adjacent to buildings (10 m).	Small areas of livestock access. Generally cobble dominated, some exposed bedrock. Largest mussel beds. Adjacent improved pasture.
C	Glide Run Riffle Marginal deadwater	Continuous; no tunnelling (complete shading by trees). Mature trees line banks. Grassed banks, some areas of reeds.	None notable.	Cobble dominated. Adjacent improved pasture and drained wetland. Sandy earth banks, occasional erosion.
D	Glide Run Riffle Marginal deadwater	Continuous; no tunnelling. Very high, steep (70^o) banks ensure extensive channel shading. Mature trees, reeds, grassed banks. Vegetation on some mid-channel bars/islands.	None notable. Bridge piers in vicinity but not at sample sites.	Cobble dominated. Exposed gravel bars feature here. Adjacent improved pasture and broad-leaf woodland. Area of widest channel but most shaded.

2.6. Terrestrial imagery

Grain size and depth are key features of the river environment defining mussel habitat and must be accounted for. The literature reveals the developments made over more than three decades in the practice of ‘photo sieving’, whereby grain size is measured from imaged substrate (Ibbeken and Schleyer, 1986). While an extensive range of platforms have been used to capture images, from satellite imagery (Luck *et al.* 2010) to cameras attached to tethered balloons (Morche *et al.* 2008), the most viable for use in this project is an adaption of the hand-held pole used by Bird *et al.* (2010). Bird *et al.* use a vertically mounted, non-metric camera on an aluminium pole. The monopod suspends the camera 10 m above the channel, thus positioning it under the tree canopy to gain a clear view of the channel. Conquering issues posed by the tree canopy was the main motivation behind the development of this method and this functionality renders it particularly valuable for use in the Rede. Much of the study river is lined by mature trees and in places high banks exacerbate the issues of overhang and shadowing that limit the quality and comprehensive coverage of high-level aerial images. This riparian characteristic may also limit the value of the high level image coverage due to the predominance of mussel presence in shaded areas of channel, adjacent to the bank (section 1.9.3). Accordingly, selection of the pole-mounted camera is highly feasible as a method of obtaining images to assess mussel habitat as it can be used under the overhanging canopy and overcomes this issue (the pole used in this investigation could be lowered to >2 m). The hand-held pole is very mobile, serviceable by only two field workers and allows the rapid attainment of extensive, high-resolution datasets. A ground resolution of 3 cm was achieved in the Bird *et al.* 2010 study and was surpassed in the Rede images.

A reasonably mobile tripod platform, with suspended camera system, was used by Ibbeken and Schleyer (1986). This early development in remote sensing does remove the need for the arduous task of traditional, in-channel grain sieving and achieves equal grain size measurement accuracy. The distribution bias derived from traditional methods, where the large boulder and small clay particle sizes are missed, is also rectified to some extent (Ibbeken and Schleyer, 1986). While these advantages in measurement range and mobility are retained in the larger, monopod platform method, gains are made in data coverage by using a taller pole to achieve a larger image footprint. Ibbeken and Schleyer (1986) suspend the camera only 2 m from the ground, meaning coverage at useful scales, providing continuous data, is difficult to achieve. The 10 m pole of Bird *et al*. (2010) gave an image ground footprint of 96 m^2. Clearly following the method with a longer monopod can give more extensive coverage of the river area “without sacrificing the local spatial detail” (Carbonneau *et al.* 2005), using the limited time available to maximum efficiency. Issues pertaining to remotely sensed data collection still remain however, such as weather conditions, lighting and water clarity, which can affect image quality (Legleiter *et al.* 2004; Carbonneau *et al.* 2006; Marcus and Fonstad, 2008).

Water depth was a potential issue in using the hand-held pole platform. As depth measurements were taken manually in the field, the problems involved in remotely sensing depth are only briefly considered here. However, the problem of limited functionality and the practicality of using the pole in deep water can have a bearing on study site selection. Aerial photosieving analysis on images from deeper water is often more difficult as reflectance in the red band (found by Carbonneau *et al.* 2006, among others, to be best correlated to depth changes) is inversely proportional to depth (Legleiter *et al.* 2004). As the water column absorbs the light reflected from the substrate, in deep water all reflected light is absorbed and the substrate is not visible. This is a continual issue with remotely sensing river systems, but was not a major problem with this chosen method. The channel water depth accessible by the operators, where full boom function can be maintained, was surpassed before excessive depth began to limit the substrate view providing good lighting conditions are sustained.

2.6.1. Sampling method- location of image transects

Time constraints and practicality, combined with unsuitable weather conditions during the planned fieldwork period, did not allow continuous image coverage to be achieved. While the monopod boom platform and method allowed the greatest possible image coverage, survey sites still had to be chosen carefully to attain data for a representative area of both mussel presence and absence. The conditions in areas where mussels cannot survive is as useful in terms of river management as areas that are suitable for habitation. The images collected still allowed the compilation of datasets that represent the downstream changes in physical factors initially introduced by Vannote *et al.* (1980) as a continuum. They further allowed analysis of these changes in terms of ecosystem patchiness; not as a directional series of changes but as a dynamic series of conditions, after Thorp *et al.* (2006).

The sampling method for imagery transects is based on the system explained in Section 2.5. All imagery transects were located within the four main 'reach' sections (defined in accordance with the classifications of Frissell *et al.* 1986). The imagery transects moved into the finer classifications offered by Frissell *et al.* (1986) and were located according to 'Pool/Riffle Systems'. These are characterised by bed topography, depth and water velocity. They are not solely restricted to pool or riffle bed forms but include side channels, runs, glides and rapids etc. and may be related to structures such as woody debris in the channel (Frissell *et al*. 1986). The paper also notes that this classification should play a key role in habitat study. These broad habitat features, appearing within the 'Pool/Riffle Systems' classification, were deemed appropriate to initially define areas of mussel habitat.

On this basis, the exact location of imagery transects was identified by walking along the bank or moving into the channel within the 400 m reach and locating areas of differing character in accordance with the classification features above, for example depth or flow velocity. Some of the features that were identified in each area and used as a basis for site selection are given in Table 2.2. The advantage of locating transects in this manner, using the Frissell *et al.* (1986) foundation, is that the habitat parameters of interest are nested within this scale. Microhabitats at the sub-metric scale, changes in which may give rise to mussels' preferential use or non-use of the area, are clearly captured within the extent of image transects. Analysis of these "patches within pool/riffle systems that have relatively homogenous substrate type, water depth, and velocity" may reveal the pearl mussels' response to habitat patches (Frissell *et al.* 1986, p208). This is a major aim of the study. This approach was established, with successful results, by Morche *et al.* (2008). Although a different platform was used, their sampling design identified seven sites for image collection that were representative of the characteristic hydromorphological units in the reach.

Furthermore this approach allowed false negatives to be accommodated to a certain extent. Areas of suitable physical and chemical character featured in some of the sample areas, though the mussels have now been removed. Former surveys suggest the Rede population was far more extensive before pearl fishing (Rooksby, 1997; Lewis, A. *pers. comm.*) and the locations of some formerly large mussel beds were covered within the sample locations. Once surveyed, these areas, amalgamated into the results, can still indicate good conditions in terms of the habitat parameters assessed. Modelling may thus indicate a positive result, despite them returning a negative result in direct observations. There are limitations to the extent this can be interpreted however, as there is no way of assessing the spatial extent of this type of area without further imaging and survey work. This does also rely on the assumption that they are ‘suitable’ because they display the same characteristics as contemporary pearl mussel habitat. Information on the presence of false negative returns may be useful to conservation bodies on the Rede.

A record of the types of features in the images was kept and also the proportion of transects that displayed a mussel presence. This ensured reasonably equal coverage of areas of mussel presence and absence. A random sampling system may not have achieved this due to the very sparse and sporadic mussel population in the Rede. Mussel presence and absence was observed directly at the time of image capture. Assessing mussel presence from the image, during analysis, was deemed inaccurate as many mussels are mostly buried or adjacent to large boulders and thus difficult to identify in the images.

2.6.2. Surveying method

A Canon IXUS 50, 5 megapixel, digital camera was used throughout image collection. It was mounted on a 6 m telescopic, aluminium pole (at full extent). The actual attachment mechanism allowed the camera alignment to be adjusted to ensure the lens was angled directly towards the ground but was then fixed in position by tightening bolts. This ensured all images were taken at the same viewing angle. One operator stood outside the imaged area to operate the camera. In order to achieve consistently high quality photographs, the camera remained on an automatic setting. This counteracts the impact of both changing atmospheric light conditions and local channel lighting, as advised by Ibbeken and Schleyer (1986). An automatic winder was used, after Bird *et al.* (2010), to allow time for the second operator to elevate the boom and for all boom movement to stop before the camera shutter was opened. This ensured all images were as clear as possible, with no motion blur.

Image transect locations were established using a Garmin Oregon GPS hand held unit. As the channel was often lower than surrounding land and tree cover was extensive, the GPS units took some time to establish an accurate, consistent grid reference. The need to maintain efficiency meant that the GPS unit remained on the bank, parallel to the linear image transect, throughout image collection and the reading was recorded before moving on. Locating the images allows the analyst to correlate between the various datasets (water quality/ mussel survey etc.) if necessary at a later date.

The correct elevation angle of 60^{o} was maintained by referencing a clinometer attached to the boom handle (Figure 2.5). In theory this assured the operator that, at 6 m extension, the camera was 5.2 m from the river bed and gave a consistent image resolution of less than 2 mm. In practice this was not always the case. After Morche *et al.* (2008) a scale board of known dimensions was placed in every image. This could be measured electronically to determine individual image pixel resolutions in analysis. The use of a scale board also enabled use of the boom at a 4 m extension, where trees prevented the use of a full 6 m pole and guaranteed consistently accurate results where the lower end of the boom was in a depression in the channel bed, however severe.

Most imagery transects were full width transects, perpendicular to the channel and flow. Some were a single traverse of the channel, others multiple, depending on the shape of the study feature or mussel bed. Transects were marked by a 30 m measuring tape (after Ibbeken and Schleyer, 1986) to ensure that an appropriate distance across the channel was moved each time. While Ibbeken and Schleyer (1986) used the tape to restrict overlap, Morche *et al.* (2008) included ample overlap to remove the effect of image distortion and to ensure no gaps between photos. At a tripod height of 2 m, it was perhaps possible for Ibbeken and Schleyer to ensure no gaps existed between images. However, in this study slight variations in boom height (due to an uneven channel bed) meant that it was better to follow the Morche *et al.* (2008) method as gaps were more likely. The errors this could have induced may have led to inaccuracies in any results drawn as missing or skewed data could have had a bearing on such a diminished mussel population.

Transects were traversed in a series of 4.5 m steps. If multiple upstream transects were taken, these were 3 m apart and the boom operator moves 3 m directly upstream of the previous transect. The operator will therefore be standing on the centreline of the previous imagery transect for the subsequent transect (Figure 2.6). This spacing left no gaps, ensuring an overlap of 50 cm on each side of a photograph. The overall layout can be seen in Figure 2.6.

Clinometer- allows a boom angle of 60° to be maintained while the photo is being taken.

Camera- attached to adjustable camera mount.

Height of camera from river bed is approximately 5.2 m. This gives an image footprint of approximately 5 x 3.5 m. Slight overlap is ensured to create a continuous image dataset.

Figure 2.5 Using the hand-held boom.

Once a transect location had been established, the boom operator moved across the transect, repeating image collection. The camera timer was initiated and 10 seconds were available for boom elevation and stabilisation before 2 images were taken consecutively. An alarm on the camera gave the boom operator notice of current functions. Images were checked and repeats made, if necessary, before the operator moved along to the next station. Note: this is an example diagram to demonstrate the boom method, not a true site transect from the study. Dimensions are approximate.

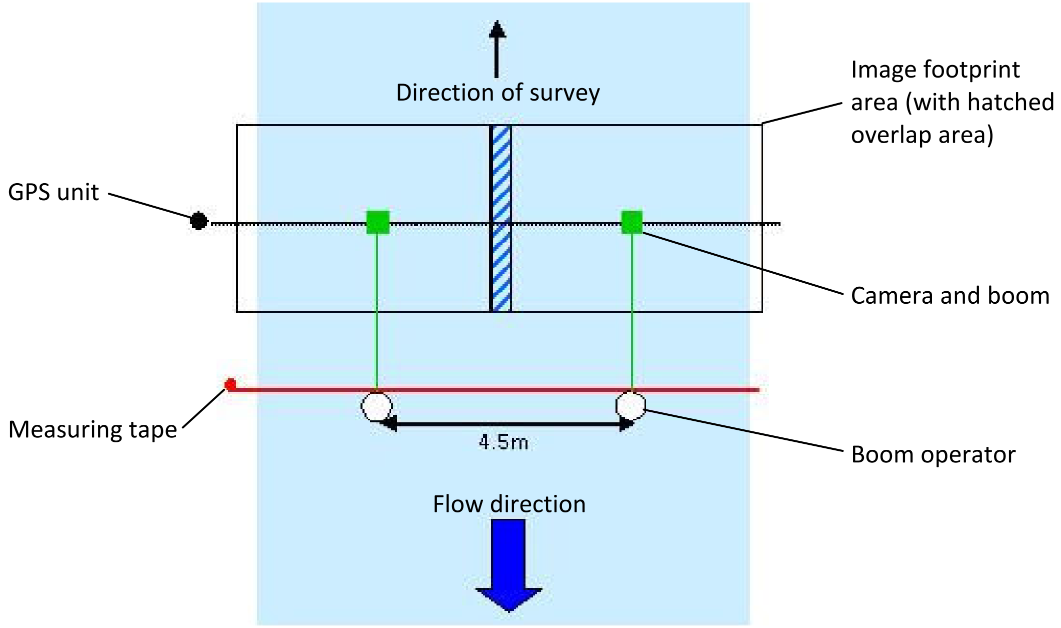


Figure 2.6 Example imagery transect layout with boom operator's first and second positions, shown left and right respectively.

The diagram shows a single traverse of the channel. The section is worked upstream to prevent disturbed sediment washing into the imaged area. The measuring tape allows the boom operator to move across the transect in 4.5 m steps to ensure 0.5 m overlap on each side of the image. This overlap is clearly seen in the centre of the diagram and at the banks. A hand held GPS unit marks the image transect centreline location. It is on this centreline that the operator would position themselves if a second traverse were made.

2.6.3. Image analysis

The terrestrial images were analysed using image processing software in the laboratory. Using this method, minimal processing yielded extensive, accurate grain size distributions yet made good use of suitable field conditions to acquire extensive image coverage. In recent years, progress in *automated* grain size analysis has been made and employed and greatly aids advancement in expanding scales of assessment in gravel-bed rivers (Butler *et al.* 2001; Carbonneau *et al*. 2005; Verdú *et al.* 2005).

Important substrate stability data were ascertained from the overall distributions of large clasts in terms of D_{50}, D_{84} and D_{90}, that is the 50^{th}, 84^{th} and 95^{th} percentile of the substrate size distribution. Some error came from inherent difficulties in measuring vertical and surface grain size variability across all size ranges (silt to boulder) and from automated photo sieving of smaller particles. Due to the minimal topographical effect of fine particles, grain edges are not usually defined as well as for coarser particles by the automated techniques (Butler *et al.* 2001). There is also a perpetual issue with automated recognition of the *a* and *b* axes (Butler *et al.* 2001) due to effects of perspective and shadowing, which fully automatic photo sieving cannot account for.

In some cases automatic approaches are not possible, as in Morche *et al.* (2008). The authors ascribe this to an anticipated error increase due to continual changes in light conditions between the images. They therefore use a semi-manual method after Ibbeken and Schleyer (1986). A full analysis of the substrate is given by these techniques but it can be time consuming, computationally heavy (Butler *et al.* 2001) and the required programmes were not available for use in this project.

Consequently, a contemporary photo sieving method developed by Carbonneau *et al.* (2004a) and modified by Dugdale *et al.* (2010) for use in deriving median grain size from close range imagery, was chosen as the basis for grain size analysis in this investigation. A MATLAB (Mathworks, 2009) based graphical user interface was used to determine particle sizes on-screen: a technique labelled 'aerial photo sieving'. The Dugdale *et al.* (2010) paper successfully uses this method to overcome the need for time-consuming field calibration data, which is normally needed for the automated photo sieving methods mentioned previously. However, it is convenient, cost effective and very applicable to the extent of photo coverage obtained for this project. Use of more advanced photo sieving methods would need further equipment and software, whereas MATLAB (Mathworks, 2009) was available for use. Dugdale *et al.* (2010) acknowledge a slight over prediction of grain size using the aerial photo sieving method of 0.5-3.5 mm. If a similar error is assumed here, all grains classed as coarse sand or larger should be accurately identified. This is a good level of detail for the study of mussel habitat, reinforcing the suitability of this method for this study both in terms of the extent and accuracy of the obtainable data and the practicality of obtaining the images.

Nonetheless there are certain limitations that should be considered. While the error in measurements is small, this is large relative to the smallest clast sizes (medium and fine sand or silt). Determining the presence of these substrates would be crucial to establishing the viability of juvenile habitat, as these fines inhibit their survival (section

1.7.2). It was not possible to accurately define or measure the presence of particles smaller than coarse sand using this method. Adult mussels can survive in this substrate type though and the focus of this study is based on adult mussel presence. This photo sieving method was thus deemed most appropriate for use, though interpretations of this investigation's results for habitat extent or suitability should not be extended to juvenile mussel habitat as the data resolution was not adequate.

The graphical user interface used here was opened in MATLAB (Mathworks, 2009) and each image was loaded and photo sieved individually. A total of 200 images were suitable for use while 48 images were withdrawn as it was not possible to clearly define particle edges due to shadow or poor lighting conditions and obscuration by vegetation or white water for example. Once loaded, the pixel resolution for the individual image was calculated from scale board of known dimensions. The pixel resolution for most images in this study was 1.7-2 mm. Occasionally the boom was used at 4 m, which results in a higher resolution of 0.7 mm. A 7x8 grid was superimposed on the image by the aerial photosieving interface, creating 56 nodes at intersections (Figure 2.7). The cursor was used to measure the semiminor (*b*) axis of the clast directly under each node in turn. A grain of 2 mm diameter (very coarse sand) or above could be identified in this study in well lit images, as the resolution achieved using the boom method was very high. Once complete, values were given for D_5, D_{16}, D_{50}, D_{84} and D_{90}, percentiles commonly used in grain size analysis (Morche *et al.* 2008). These values were then taken on for further analysis.

Images were taken from a very low height compared to studies using aircraft platforms, for example, therefore the edge distortion was minimal and was not thought to greatly impact the resulting measurements. Despite the overlap between images, suggested by Morche *et al.* (2008) to avoid gaps, repeated clast measurements at the edges of adjacent images are not thought to occur. The overlap is only 50 cm so does not cover all of the grid area. As some adjustment in the exact centreline location on each image is expected through slightly changing boom placement, combined with the precision with which clasts are selected for measurement, it is assumed very few repeats are made, if any.

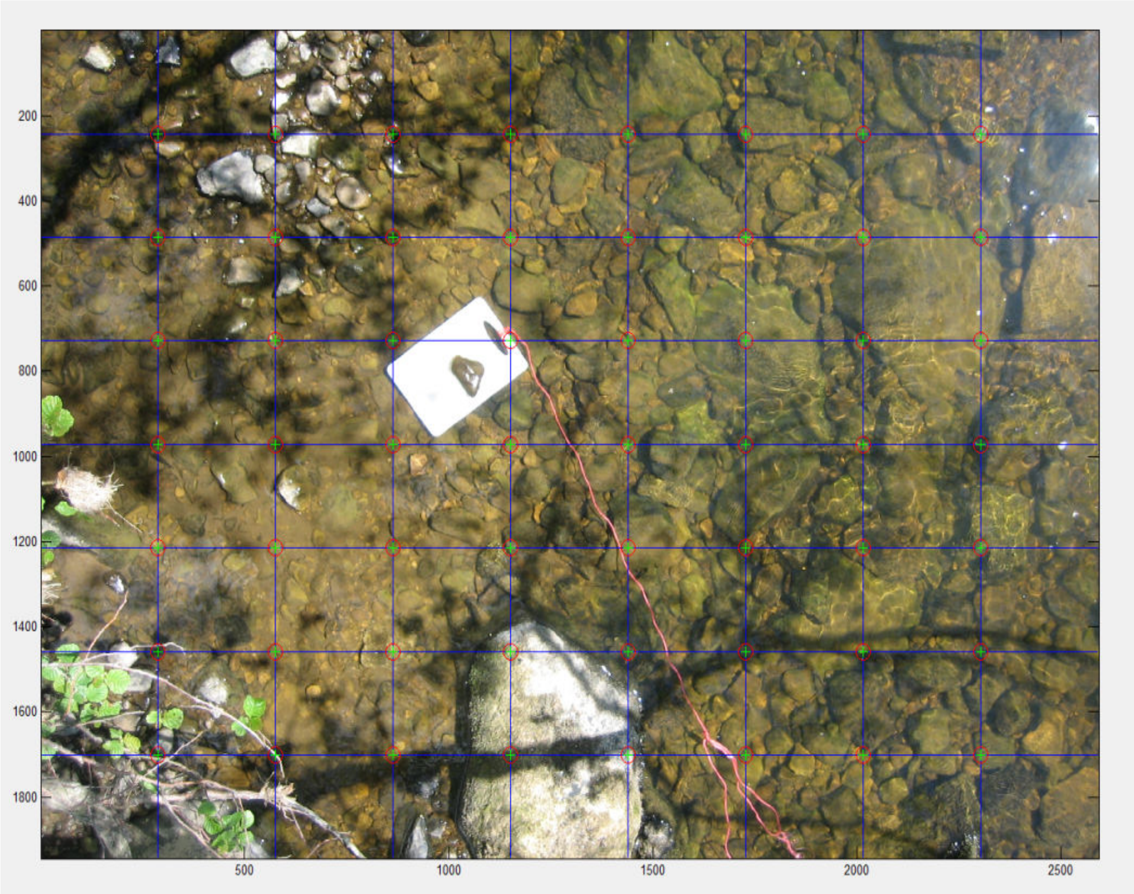


Figure 2.7 Aerial photo sieving graphical user interface.

The graphical user interface used in aerial photo sieving was similar to that used by Dugdale *et al.* (2010) to overcome the need for field based grain size calibration data. The interface allows the image pixel resolution to be calculated and therefore exact grain size measurements are calculated. Only the clasts under nodes on the superimposed grid were measured and at this resolution, grains of 2 mm can be identified. Some evidence of common remote sensing issues is evident here: small amounts of specular reflectance appear in the top right corner and there is some obscuration by vegetation. These cause no problems for photo sieving here.

2.6.4. Depth measurements

Five depth measurements were taken across each imagery transect centreline. They were spaced at equal intervals across the channel. This results in one measurement for the channel centre, two bank-side depths and two intermediate ones. This limited number of measurements would create only a crude intimation of the overall cross sectional channel geometry. However, this was not the aim. The depth measurements were not considered in isolation; instead a general depth measurement for the areas where mussels were and were not found was adequate for the purposes of this investigation. Averaged across the multiple areas of mussel presence and absence, an adequate idea of appropriate depths for suitable mussel habitat could be ascertained.

Many studies have developed methods of deriving depths from aerial imagery based on pixel brightness and light absorbance values (Fonstad and Marcus, 2005; Carbonneau *et al.* 2006), in addition to grain size. It was intended that more detailed depth data would be available from the terrestrial images but various software based issues meant that this could not be done in this study. It was thus deemed most efficient and practical to use basic measurements taken in the field, at the time of image collection, rather than undertaking further computer analysis of the images. This explains the coarseness of the depth data.

2.7. Mussel survey

Since 1995, three surveys of the Rede *M. margaritifera* population have been undertaken. These were in 1995, 1997 and 2006. Actual mussel counts were made to firmly establish the population size. The irregular nature of the Rede mussel population distribution meant that random point sampling was not suitable to establish the full population size (Rooksby, 1997; Environment Agency/E^3 Ecology report, 2006) and the whole river was surveyed. However, the spatial extent of these surveys was dictated only by former surveys and local knowledge. This allows the possibility of omitting some areas of mussel habitation, though the reports are confident this did not occur. Further inaccuracies may have arisen from the sampling method: paired surveyors progressed along the channel within 5 m of the banks, as this was the area most commonly harbouring mussels. While regular cross-channel transects were also surveyed and where mussels were found a more detailed assessment was made, this may have led to the omission of individual mussels from the overall population count. As a result the data from the existing Rede surveys were not deemed appropriate for use in this project and was used only as a guide for the mussel survey undertaken here.

For the purposes of this study a formal population estimate was not required; instead details of presence and absence in sections, in relation to habitat conditions, were employed to analyse habitat use. While it was appropriate for previous surveys to concentrate on areas of mussel beds, rather than individuals, the depleted nature of the Rede population means that *any* area where the physical habitat parameters may be suitable for mussel habitation could be important to the assessment of mussel habitat in this study. Recording the location even of single mussels may be crucial to gain a greater idea of suitable mussel habitat in the Rede.

Methods used in freshwater pearl mussel population surveys undertaken on other rivers where the whole channel has not been surveyed consecutively also have potential to miss some areas of mussel presence. Gittings *et al*. (1998) made 2-3 m wide transects every 15-20 m downstream in order to comply with habitat patchiness identified in River Corridor Surveys. However, these are done at the reach scale. Deriving a sampling design from this method for a mussel habitat assessment at the micro scale, as in this project, may incur inaccuracies. For the depleted Rede population, where only a small portion of suitable habitat may be in use, it is important to survey all areas continuously and capture all areas in use. This includes areas of physical parameter transition which may occur between the Gittings *et al*. (1998) transects, yet which may still be suitable for mussels.

The extent of relationships between mussel presence and habitat distributions and thus the character of 'suitable' *M. margaritifera* habitat cannot be achieved if the above methods are followed, where possible key areas of channel are outside the sample area.

2.7.1. Sampling design

Time constraints did not allow sampling of the whole 400 m section so a subsample of the channel section was identified. It was considered more effective to cover a higher proportion of a shorter section, representing the full array of the local physical habitat variation, than a smaller fraction of a longer section. Therefore approximately 13-23% of the 400 m sections were sampled, a similar amount to the 20% coverage made in Hastie and Young's 2003(a) study in the Rivers Kerry and Moidart. These sections were also covered by the terrestrial image locations and allowed direct comparisons between variables from the two datasets.

Within the four 400 m sample locations established for all reach and sub-reach scale observations (Section 2.5), five sites were identified for sampling. These were systematically placed within the section as shown in Figure 2.8, though some freedom was allowed in the actual placement of survey areas when in the field. Of these five survey areas, three were chosen in the field for data collection. This decision was based on accessibility with available equipment (some sites were too deep to access and/or to view the river bed) and relevance to study in terms of offering an original habitat character to the survey.

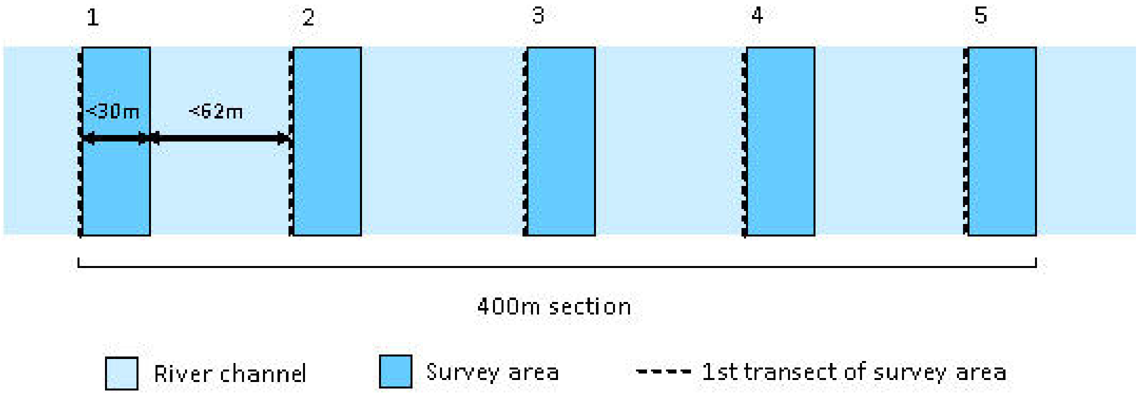


Figure 2.8 Survey areas within the 400 m channel sections.
The 400 m section delimited at each of the four locations (A-D) was divided into 5 sections. The spacing was designed for <30 m to be surveyed in a set of contiguous transects (dark blue). Survey areas are numbered 1-5 moving upstream. At the time of sampling these survey areas were occasionally moved up or downstream by 10-20 m in order to ensure variation in all physical characteristics was captured. Time constraints would not have allowed the acquisition of data covering the full variation if sampling had been done completely randomly. Therefore sampling had to be systematic.

Ideally 100-300 samples of mussel *presence* were needed from the sparse population (Wolcott and Church, 1991; Box and Mossa, 1999). To complete an adequate number of samples in suitable weather conditions and within a practical time frame, the survey areas were sampled on the basis that no more than 1 day would be spent in one area. If 30 transects or around 500, 1 m^2 quadrats were sampled before a full day's field work had been completed, this was deemed appropriate to represent the study area in question and, in the interests of time, the survey effort moved to the next area. The survey area covered the full width of the river channel in accordance with the justifications shown by Gittings *et al.* (1998), yet areas were sampled continuously to improve on this method and that of the Environment Agency surveys. The method thus covered both extensive areas of the Rede catchment (Figure 2.8) and extensive areas of the channel in contiguous samples (Figure 2.9) quickly enough for a large number of samples to be taken, both of mussel presence and absence.

The mussel survey was undertaken in August and September 2010 under very low flow conditions. A river level of under 10 cm above normal summer lows was required at Otterburn and Rede Bridge gauging stations. Occasionally there were several days between surveys (at different sites) after any rain event to allow the river to fall and suspended sediment to settle. Raised water levels and brown, peaty water inhibited the view of the river bed and it was likely mussels would have been missed.

2.7.2. Survey method

Sampling along the contiguous, cross river transects was carried out in a series of consecutive 1 m^2 quadrats. The use of transects and quadrats is well established in ecological studies (Brown and Harrison, 1970; Gittings *et al.* 1998; Hastie and Young, 2003a). No frame was used in this study, instead the quadrat was demarcated by a 30 m tape and a 1 m rule on the bank immediately perpendicular to the transect line (see Figure 2.9). The size of the quadrat is important (Brown and Harrison, 1970; Chalmers and Parker, 1989). While a 0.25 m^2 quadrat is normally used in studies of freshwater mussels (Hardison and Layzer, 2001; Outeiro *et al.* 2008), a 1 m^2 quadrat was chosen after Hastie and Young (2003a), as it is easily divisible into 100, 10 cm^2 sections to estimate the substrate percentages (Chalmers and Parker, 1989). It is also appropriate to the mussel population in terms of representing their immediate environment at the relevant scale (Hastie *et al.* 2000). The use of square quadrat areas across the transect means that, unlike the linear approach in the Environment Agency/E$_3$ Ecology (2006) report and other similar studies, it should be easy to define patches of habitat when the assessment is made on a grid pattern (Figure 2.9). This should improve the accuracy of sampling mussel location in the field, which is acknowledged as difficult due to mussels’ clumping behaviour (Hastie *et al*. 2000; Hastie and Young, 2003a).

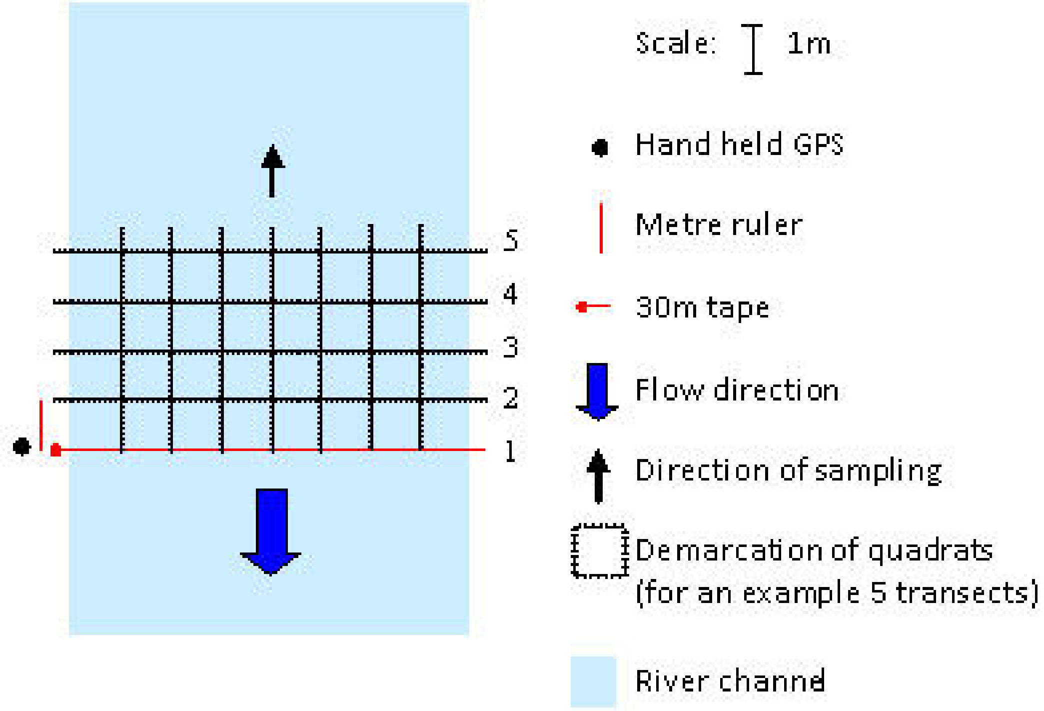
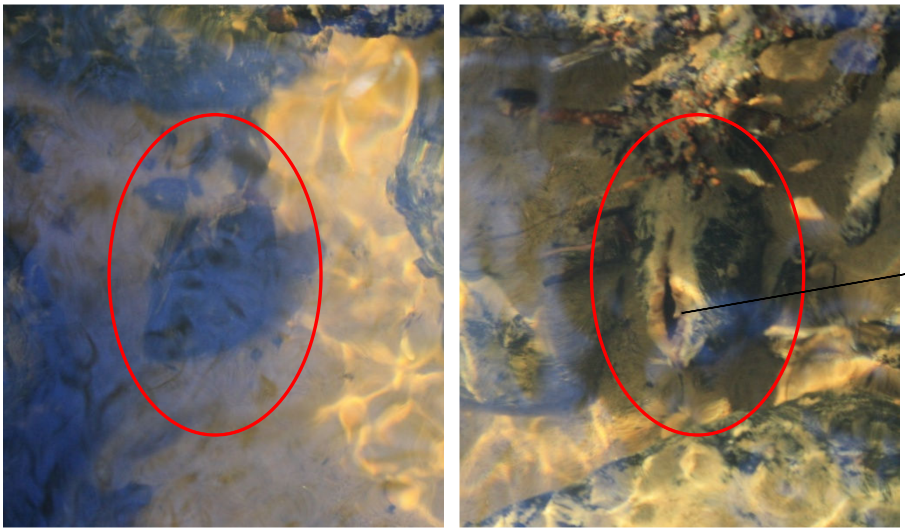


Figure 2.9 Diagrammatical representation of transect and quadrat layout across river channel.

Sampling was carried out moving upstream. The GPS marking the exact location of the transect was placed on the bank at the transect location. The surveyor moved along the 30 m tape and sampled each square metre quadrat using the metre rule and tape for reference to the boundaries of the quadrat. Once transect ‘1’ was done, survey effort moved upstream 1 m and the process was repeated. An example of the surveying pattern is shown for five transects and 32 full 1 m^2 quadrats in dotted lines.

A bathyscope (acrylic bottomed bucket/drawer) was used to view the mussels effectively in water up to approximately 1.3 m deep as advocated in many previous studies (Oliver and Killeen, 1996b; Gittings *et al.* 1998; Hastie and Young, 2003a). The bathyscope was moved back and forth, systematically across the whole quadrat area to ensure the mussel search and substrate assessment was thorough. Only the adult mussels visible on the surface were counted; no sub-surface searches were made to avoid disturbance. As a result the pearl mussels were viewed as in Figure 2.10. A bank assistant took records of the number of mussels in each quadrat. If mussels were on the upper or right perimeter of the quadrat they were counted in the next one. Care was taken not to count these perimeter mussels twice, nor to stand on mussels in previously sampled areas.

While this survey technique was likely to omit records of juveniles from the results, this was accepted as a reasonable compromise for several reasons. While the presence of juveniles is fundamental to a sustainable population, the focus of the study does not rely on an assessment of juvenile mussel presence specifically, especially as numbers were likely to be very low as the previous survey found no juvenile mussels in the Rede the (Environment Agency/ E_3 Ecology report, 2006). Notwithstanding the additional time requirements for sieve sampling the substrate for juveniles, the established low numbers in this river meant that it was not practical or justifiable to spend the limited fieldwork time on a more accurate mussel sampling technique. This does however have implications for interpretation of the results, in that any conclusions on habitat use and availability will not apply to the juvenile population in the Rede.



'Black slit' effectively identified mussels from stones on first observation.

Figure 2.10 Examples of *M. margaritifera* as seen during surveying.
The two mussels in the images below are nestled among cobbles and burrowed into a coarse sand substrate. In these examples only half of the mussel is buried below the sand thus they are reasonably easy to see. In some instances the 'black slit' of the mantle edges forming the siphons (Oliver and Killeen, 1996b), is the only visible part. This, and that they are often mistaken for stones, means that a careful search of the quadrat must be made.

In addition to a count of pearl mussel presence per quadrat, several other physical habitat parameters were sampled within the quadrats. These are outlined in Table 2.3, including details of how they were recorded. Although several studies have found that flow velocity is an important factor in defining pearl mussel habitat (Hastie *et al.* 2000), defining the flow for each quadrat would have been impractical. To use a flow meter for each of several thousand quadrats would have exceeded available time limits and, furthermore, a representative flow velocity would not have been obtainable for a $1m^2$ area unless several recordings were made. As there seems to be a wide variation in mussel flow velocity preferences reported in the literature (Skinner *et al.* 2003) and some acknowledge that mussels are found in unexpected areas (Oliver and Killeen, 1996) a broader, faster approach to flow classification was used: flow type. This was based on the classifications derived by Padmore (1998) and took one of ten forms, as seen in Table 2.3. An overall type for the dominant category in the quadrat was recorded.

The substrate composition of each quadrat is a crucial part of the assessment as the channel bed is a major component of the physical environment forming the mussels' habitat. This is stressed in the literature covering many studies (Gittings *et al.* 1998; Hastie *et al.* 2000; Moorkens *et al.* 2007). Assigning substrate to a meaningful number of size classes in the field via observation alone is impractical and subjective (Box and Mossa 1999). Substrate sieving is also time-consuming. The general consensus in the literature is that the stability of the substrate matrix is vital (Vannote and Minshall, 1982; Hastie *et al.* 2000). This can be inferred from the relative proportions of small, mobile sands and gravels and larger stabilising clasts. Therefore three substrate size classes were used in classification, which fully represented the substrate matrix composition. The assessment of substrate involved estimation of the relative proportions of sand, gravel and cobble-boulder substrates to the nearest 20%. To estimate to a smaller percentage would be too subjective and excessive error would build between observations. An adaption of the Wentworth scale (1922) was used to classify clasts. 'Sand' was defined as all sediment less than 2 mm in size, 'gravel' was defined as any substrate between 2 mm and 64 mm. The 'cobble/boulder' category represented all clasts above 64 mm, including boulders (>256 mm) and bedrock and therefore included all clasts considered large enough to stabilise the bed in all but the highest flow events. Bedrock is normally an unsuitable channel substrate for extensive mussel colonisation, as no interstitial gravel is present. While some mussels in the

Rede do inhabit the fissures in bedrock areas (Lewis, *pers. comm.*), use of this habitat type was not deemed extensive enough to warrant classification into a separate category in the Rede.

Table 2.3 Variables observed for each quadrat and format of data.
At each quadrat observations of the following eight variables were collected, including the three observations for substrate.

Variable	Data type	Range	Values taken
Transect number	Continuous, integer scale	1+	Any (positive) on integer scale
Pearl mussel number	Continuous, integer scale	0+	Any integer, absolute count
Metre from edge	Continuous, integer scale	1+	Any (positive) on integer scale
Substrate- sand, gravel and cobble+	Discrete category	1-100	0, 20, 40, 60, 80, 100
Flow type	Categorical	Any of the 10 biotopes associated with the flow classifications identified by Padmore (1998).	Marginal deadwater, pool, glide, boil, run, riffle, rapid, cascade, spill, waterfall.
Time	Continuous	Generally between 09.00 and 17.30- good daylight hours.	Time of transect survey- this was later correlated with the GPS device to give precise locations.

2.7.3. Locating the transects

In order to assess the patchiness of mussel distribution and habitat variation it was necessary to know the location of the transects and quadrats in relation to others in the dataset. Tight time constraints restricted many aspects of the methods applied and this meant that using a Total Station to obtain accurate transect positions was impractical. An adequate level of precision and accuracy was gained by using a Garmin

Oregon 300 hand held GPS. It was not turned off during the fieldwork day. Positions from the same transect were therefore accurate relative to each other, though less so between sites. Time recorded on the track log was used to correlate the location to the transect number.

2.8. Analysis methods

Much of the data obtained from the methods outlined in Chapter 2 were analysed directly. The results of this analysis are displayed in Chapter 3. However, the initial analytical methods were developed further via the creation of logistic regression models and preference models to predict mussel presence and absence in various areas of differing habitat character. The analysis methods employed in the development of these models are given here.

2.8.1. Logistic regression models

Logistic regression was applied to the datasets collected during the ground surveys and terrestrial imaging to produce two models to infer the likelihood of pearl mussel presence. The chosen outcomes of these models were the most parsimonious, yet biologically accurate accounts of the relationships between mussel presence and the habitat variables observed (Hosmer and Lemeshow, 2000). Logistic regression was valuable as it combined a range of habitat variables and accounted for the variation in their characteristics. Therefore it can predict the most suitable habitat patches by assigning a high probability of presence. Where some characteristics of habitat are sub-optimal the probability will be lower. However, all observations in the input data must be complete therefore terrestrial imagery and ground survey data could not be combined. Logistic regression requires that the data are prepared according to the formats described in Table 2.4. Substrate was in discrete categories but if all three substrate categories were included they related by maintaining a constant sum: the three variables combined to 100% for each quadrat. Therefore a maximum of two of these three variables was employed in the model. Remotely sensed habitat data were all continuous thus needed no transformation, however, the coarseness of the depth data meant that only transect average, maximum and minimum depth readings were considered in the models.

These models were based on data collected at the microhabitat scale (quadrat data for mussel presence and absence and other habitat variables) and at the pool/riffle scale (terrestrial images and depth measurements). It was not possible to perform logistic regression at the catchment scale, using water chemistry data, as no data for mussel presence was available at this scale.

Table 2.4 Logistic regression ground survey model: predictor variables.
Input/Predictor variables were re-coded from the survey collection format for use in logistic regression. Flow categories are derived from the classification system derived by Padmore (1998).

Variable	**Data type**	**Range**	**Values taken**
Pearl mussel number	Dichotomous	0-1	Presence ‘1’ Absence ‘0’
Metre from edge	Discrete	1-18	1,2,3...
Substrate	Discrete category	1-100	0, 20, 40...
Flow (rate)	Discrete	1-5	1,2,3...
Flow (type)	Categorical, transformed into binary	4 variables, 0-1	Each of Marginal deadwater/Pool, Glide, Run and Riffle: Yes ‘1’; No ‘0’

Logistic regression analysis was performed in Stata 11 (StataCorp, 2009). Separate models were run for mussel survey data and terrestrial image data. All habitat variable data were compiled into one model. This initial model was improved by the omission of variables displaying a low z statistic (-2≤ Z ≤2), an insignificant p-value (above 0.05), or where the 95% confidence interval displayed a large range or crossed 0 (levels of acceptance outlined by Hosmer and Lemeshow, 2000 and UCLA website, 01/10/10). Through this iterative process statistically significant and biologically viable models were developed.

2.8.2. Preference models

Preference models give a quantitative analysis of habitat preferences. The degree of habitat tolerance and avoidance can be derived from these models. Model partitioning was based on the observed values used in data collection for the mussel ground survey (Table 2.3). Partitioning for terrestrial imagery photo sieved grain sizes was based on Wentworth's (1922) classification of grain sizes. A total of five partitions were made to derive preferences for sand, gravel, cobble, boulder and large boulder substrates. The latter partition was an addition to the Wentworth scale for clasts over 1000 mm. Differentiation between boulders and the preference for the presence of particularly large boulders gives additional, useful detail. Depth readings from the terrestrial imagery dataset were partitioned into 10 cm sections, from 10-110 cm deep. The preference models are derived from Jacobs (1974) method:

$$P_i = \frac{S_i - A_i}{(S_i + A_i) - 2(S_i \times A_i)} \tag{1}$$

where P is the preference for habitat partition i, S is the proportional utilisation of that partition by *M. margaritifera* and A is the overall proportional availability of that partition of habitat. Equation 1 must be applied to each partition of each habitat parameter. This gives a number in the range $1 > P_i > -1$ for each partition. Those partitions of a habitat parameter where $P = 1$ are highly favoured by the freshwater pearl mussel. Partitions where $P = -1$ are avoided. An interim P confers a degree of mussel tolerance for each partition. The outcomes have been represented graphically in Section 3.5.2.

Chapter 3 Results

The results from both the FIS and water chemistry analysis gave an initial, large scale presentation of the River Rede catchment environment. This was developed further with more specific reach scale details of downstream variation and habitat parameters based on the terrestrial imagery and pearl mussel ground survey data. The mussel survey variables were flow type, proportion of sand, gravel and cobble/boulder and an adult mussel count for each quadrat. Terrestrial image variables included depth and average photo sieved grain size distributions (D_5, D_{16}, D_{50}, D_{84} and D_{95}) with mussel presence or absence for each transect. Models of habitat location predictors and pearl mussel habitat preferences were constructed via logistic regression and the preference model equation derived by Jacobs (1974).

3.1. Aerial imagery

Aerial imagery demonstrated that the River Rede displayed a gradual increase in width downstream (Figure 3.1), from approximately 7 m at the start of the sample, to an average of 17 m as it approached the confluence with the North Tyne. There was a great deal of variability in width with a minimum of 2 m and a maximum of 36 m. Minimal error may have arisen from the classification system in FIS whereby overhanging vegetation, obscuring the aerial channel view, may have caused artificially small width readings.

Fluvial Information System analysis should have provided channel maps of D_{50} and depth variation, from which habitat maps can be generated. This was attempted but with poor results. A calibration was performed to relate image red band brightness to the observed depth measurements (taken on site, at time of flight). The proportion of variability explained by the resulting equation of the exponential trend line was $R^2 = 0.0001$ ($P > 0.1$). Minimal information could be gleaned from the images due to poor light conditions, which caused the images to appear veiled, and an excessively high sediment load obstructed the substrate view. These analyses were thus disregarded.

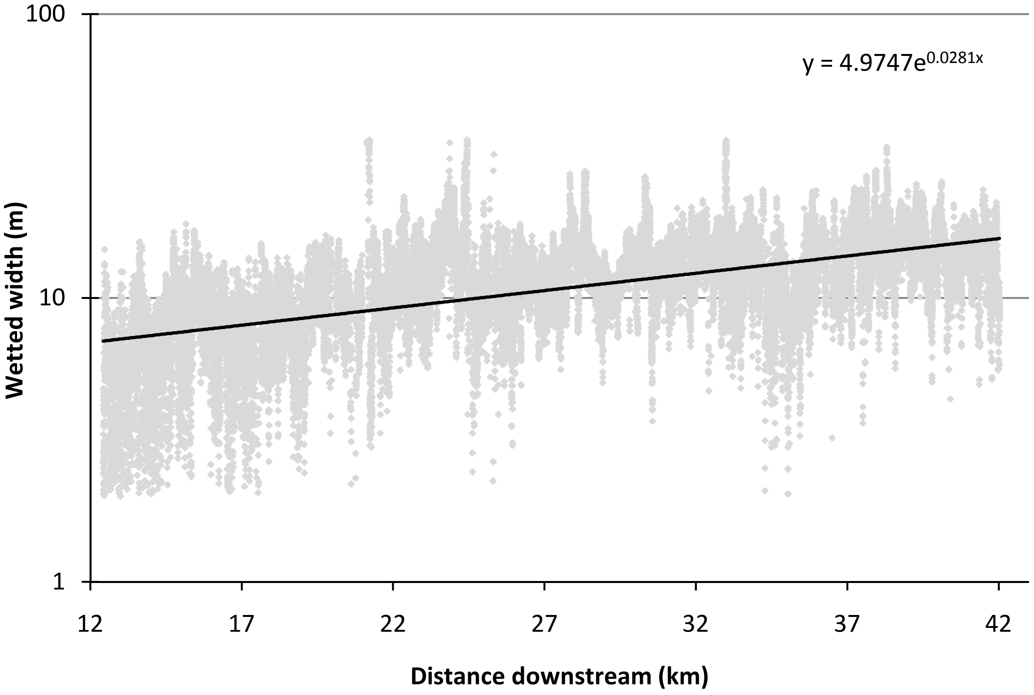


Figure 3.1 FIS Generated River Rede width profile.
Profile of width variation produced in FIS using the Channel Width Long Profile Builder tool. This graph depicts wetted width as this is relevant to mussel habitat preferences. Individual width measurements are represented by the grey points. A regression line for these data is displayed in black. The equation defining the relationship between width and distance downstream suggests a very gradual increase downstream (R^2 = 0.3, *P* <0.01).

3.2. Catchment water chemistry

A total of 43 winter samples and 26 spring samples were collected. Of the six parameters listed in Table 2.1, representative results were achieved for five of these: conductivity, pH, dissolved oxygen, nitrates and calcium concentration. While efforts were made to retain the integrity of the water samples for Dionex analysis, phosphate readings were all below the detection limit of 0.02 mg l^{-1}. As it was possible that the phosphate samples had deteriorated, rather than phosphate truly existing only at very low concentrations, this water chemistry parameter is not included here.

The downstream variations in conductivity, pH and dissolved oxygen are displayed in Figure 3.2 in relation to thresholds for sustainable freshwater pearl mussel tolerance given by Young (2005). Winter conductivity readings (Figure 3.2 (a)) remained below the mussel preference threshold for nearly 40 km downstream. At 39.2 km downstream the conductivity rose above the preference threshold. This sample point was immediately after a tributary joins the Rede. There is some variation around the average of 68.4 µs cm^{-1} (standard deviation of 13.1 µs cm^{-1} with the last five, post-tributary points omitted) but it remains generally constant. In spring, conductivity was consistently higher and permanently above the preference threshold along the survey length. A constant level of conductivity was maintained until a rapid increase in conductivity at 30 km downstream. After this peak, again at a tributary confluence, it fell again. Two anomalous low points existed at 25.8 km and 34.6 km downstream.

The variation in pH again showed raised, more alkaline levels in spring compared to winter (Figure 3.2 (b)). Both datasets displayed a similar trend: the water body became more acidic towards the Tyne confluence until a sharp rise back towards a more alkaline state. This change occurred in the same places as conductivity changes were seen. In winter the majority of the sampled sites were within a suitable pH range for freshwater pearl mussel preference.

Dissolved oxygen values represented the measurement taken approximately 1-2 m from the channel edge. As a general overview, dissolved oxygen remained at a fairly constant level downstream (Figure 3.2 (c)). Spring readings were withdrawn from analysis as dissolved oxygen saturation was measured as only 40% throughout the sample sites. With no evidence of a pollution incident this was likely to be due to equipment failure such as a damaged electrode membrane. Winter values generally ranged from approximately 100-135%, with few higher anomalous readings. Overall all winter sample sites contained adequate dissolved oxygen to meet mussel preferences.

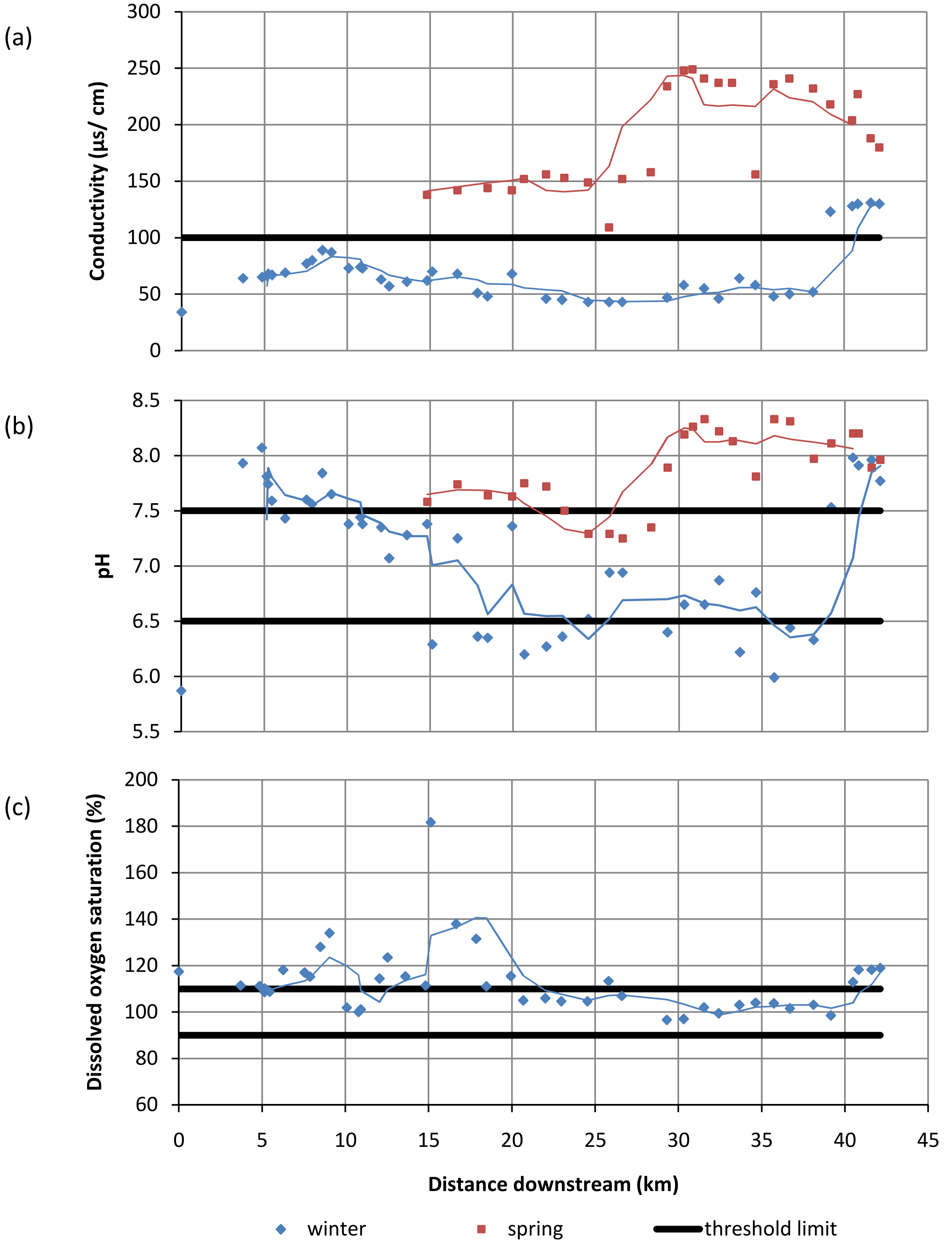


Figure 3.2 Downstream variation in water chemistry parameters: (a) conductivity (b) pH and (c) dissolved oxygen.

The black lines show thresholds of each parameter, beyond which conditions may become detrimental to *M. margaritifera* survival (Young, 2005, after Oliver, 2000). For conductivity, there is an upper preference limit but for pH and dissolved oxygen there is a preference band. Trend lines are four-point moving averages, which are suitable as the sampling density means focus should be on overall variance.

Having established the spatial variation in water chemistry, the temporal variation should be addressed further. Secondary data from an Environment Agency monitoring station approximately half way along the 42 km sampled reach gave water chemistry readings for the last 20 years in some cases (Environment Agency, 2009, see Appendix A). A comparable conductivity dataset was not available; however, pH and dissolved oxygen values were available. The average value for pH over the last 20 years is 7.7 at this single site (Environment Agency, 2009, Appendix A), with no significant directional trend shown via Pearson product-moment correlation ($r = 0.03$, $P > 0.05$). Thus pH remained constantly slightly above the upper preference limit. Dissolved oxygen appears to have remained within the preference threshold when viewed over an extended period, especially in the last decade where fewer extreme results were seen. With a mean dissolved oxygen saturation of 99.7%, ± 7.7% SD, conditions since 1994 should have been ideal for *M. margaritifera* survival. These results support the findings in this study.

Two other valuable water chemistry datasets were derived from the water sampling programme: nitrate and calcium concentrations. These are established as determining factors for mussel habitat preferences (Young, 2005; Bauer, 1988). The downstream trends in nitrate and calcium concentrations can be seen in Figure 3.3 (a) and (b) respectively.

Nitrate concentration represented nutrient content in the Rede. In accordance with *M. margaritifera* preferences, the River Rede had a low nutrient status. While there was a gradual downstream escalation of the nitrate concentration (with an average of under 0.1 mg l^{-1} at the upstream sampling sites, rising to an average of approximately 0.3 mg l^{-1} at 40 km downstream), it remained under half the stated threshold limit for freshwater pearl mussels (threshold delimited by Oliver, 2000 cited in Young, 2005). There was one anomalously high reading at 39 km, again after the tributary that caused disturbance in the winter samples of pH and conductivity. The Environment Agency data (2009) revealed that a similar level of nitrate concentration in the Rede had been sustained over time (Appendix A). Since 1990, nitrate concentration in the Rede (at this single point) was usually under the threshold level of 1 mg l^{-1}. Two higher readings suggest the water chemistry in the Rede may sporadically become less suitable for mussel habitation, but the threshold was surpassed only once in the 20 year dataset, in June, 2007.

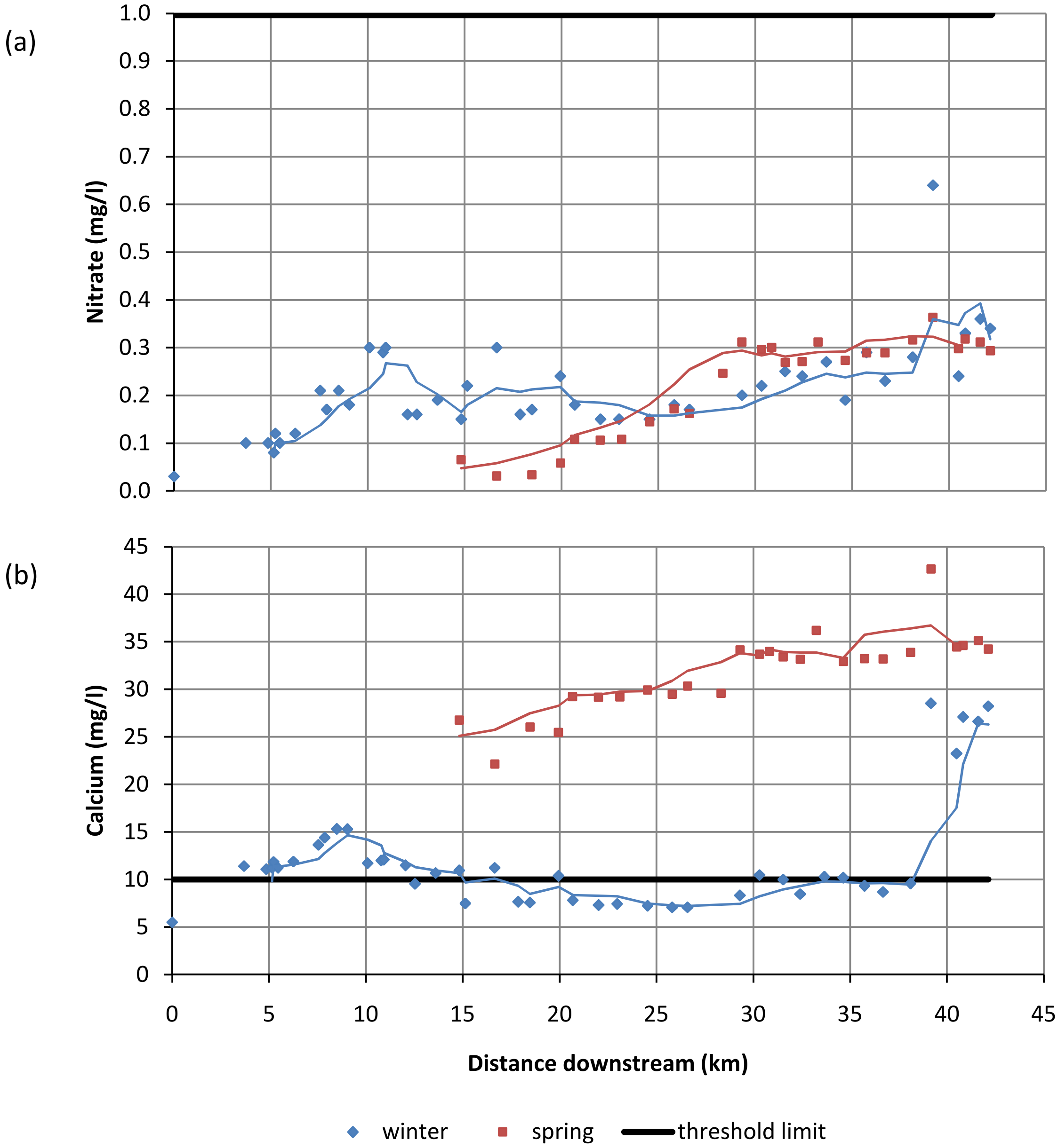


Figure 3.3 Downstream variation in water chemistry parameters: (a) nitrate concentration and (b) calcium concentration.
Despite a downstream increase in nitrates, they remained under the threshold limit (Oliver, 2000). Conversely, calcium measurements were frequently above the desired concentration limit (Beasley and Roberts, 1999), particularly in spring and at the most downstream sampling sites in winter. Trend lines are four-point moving averages.

Calcium concentrations in the Rede, both over space and time, suggested that this factor may reduce the suitability of conditions for the freshwater pearl mussel. This was based on the preference threshold of 10 mg l^{-1} established by Beasley and Roberts (1999). This was considered a more suitable limit than that given by Oliver (2000) or Bauer (1988) in Table 1.1 as Redesdale geology is dominated by limestone (see Figure 2.1). Elevated levels of calcium may thus be expected. In spring, calcium concentrations were consistently above the preference threshold and levels increase downstream. In the winter dataset, levels were much lower and through the middle section of the Rede (from 15-37 km downstream) were below the mussel tolerance limit. The limit was however exceeded after the tributary at 39 km and in the higher reaches of the Rede. The situation over time also indicated that water hardness was unsuited to freshwater pearl mussel preferences. Since 1990, calcium concentrations have only been recorded below the 10 mg l^{-1} threshold six times. The overall spread of concentrations was highly variable (mean, 20.5 mg l^{-1}; range 1.3-38.9 mg l^{-1}) with no significant change over time ($r = 0.16$, $P > 0.05$, Appendix A).

Of the key water quality indicators that have a significant influence on mussel presence, only nitrate concentration and dissolved oxygen saturation were consistently at a suitable level relative to freshwater pearl mussel preference thresholds. The others were all more variable but the influence of tributaries seemed to be noteworthy.

3.3. Mussel distribution and habitat variables

3.3.1. Sub-reach scale variation

Following the initial introduction to the Rede from the width profile and overall water chemistry variation, this section relates physical habitat to mussel counts made during the ground surveys. The final site selection, as outlined in Chapter 2, consisted of twelve sites across four river locations (labelled A-D). Their characteristics varied in order to capture a representative picture of the River Rede environment (Table 2.2). The data from all locations have been amalgamated to draw results from the river as a whole, combining all the differing areas into the mussel distributions and habitat models. Table 3.1 gives details of the mussel distributions across the four locations.

Table 3.1 Pearl mussel distribution overview.
At each of the four locations A-D, three sites (approximately 30 m of channel sampled in 1 m^2 quadrats) were surveyed for mussel presence. The results are given by site. Out of a total of 5134 quadrats sampled, a total of 310 mussels were found in 135 quadrats. These are referred to as positive quadrats i.e. containing mussels. (PM- Pearl Mussel)

Location	**Site**	**No. of PM at site**	**No. positive quadrats**	**No. negative quadrats**	**Max. PM count (in one quadrat)**
A	1	8	8	403	1
	3	3	3	253	1
	5	0	0	403	0
B	3	1	1	410	1
	4	191	46	368	21
	5	15	12	459	2
C	1	41	25	368	6
	3	14	10	197	4
	5	17	14	742	3
D	1	9	8	542	2
	3	5	2	421	3
	4	6	6	433	1
Total		310	135	4999	

The depleted status of the Rede freshwater pearl mussel population was evident considering the relative ratio of positive to negative quadrats (Table 3.1). Despite the sparse distribution demonstrated at location A, for example, a potential trend arose when all results were considered concurrently. Calculation of the Index of Dispersion as 6.5 (χ^2 = 33376, 5133 d.f., $P < 0.05$) (Fowler *et al.* 1998) suggested a contagious distribution. However, due to the large sample size the observations were grouped into a frequency distribution. The shape of the distribution was compared with a negative binomial probability distribution (suggested most appropriate by Fowler *et al.*

1998). The distribution of the *M. margaritifera* population observed in the Rede fitted very closely to the negative binomial distribution (r = 0.97, $P < 0.01$), thus the null hypothesis, that the mussel population was randomly distributed can be rejected and a contagious dispersion was accepted. The contagious distribution demonstrated by this is supported by Figure 3.4. As the number of positive quadrats increased, the maximum number of mussels found in any one quadrat increased. While a quadratic trend line fitted to this data yields an R^2 of 0.9, there was clearly a lack of data points representing the upper end of the mussel and positive quadrat counts. This curve could thus display a pattern which may not be supported if a greater number of data were available. However, if the extreme data point at a maximum mussel count of 21 was excluded, the quadratic relationship remained and the data exhibited a Pearson's correlation coefficient of r = 0.86 where $P < 0.01$, thus a highly significant relationship is seen. Clearly data from larger mussel aggregations should be sought to verify the strength of this relationship further, to reduce the reliance on the two largest mussel counts (per quadrat). However, the likelihood of a contagious distribution is very high in light of the observed distribution's close fit to the negative binomial distribution.

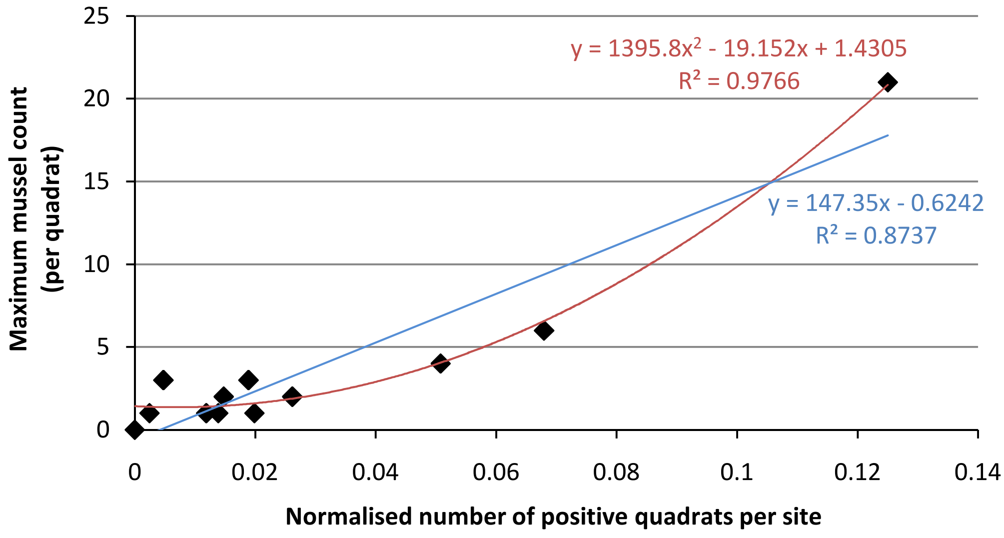


Figure 3.4 Association between the number of positive quadrats and maximum mussel count.

It appears that at sites where only a few positive quadrats are found the mussel counts per quadrat were generally low. However, where more positive quadrats were seen, the maximum mussel count increased at a faster rate. This has been demonstrated as a significant trend. The trend line shown is a polynomial trend line, order two and the number of positive quadrats has been normalised to remove error introduced from differing quadrat numbers between sites.

The average proportions of sand, gravel and cobble and boulder per microhabitat transect varied downstream (Figure 3.5). Sand constituted approximately 30% of the substrate composition in the upper sample area, A (data up to approximately 27 km downstream). From 33 km it fell substantially to under 10% of the overall substrate make up for the remainder of the Rede's length. The gravel fraction represented the majority of the substrate composition from 26.5-27 km downstream. Following a similar trend to the sand partition, the gravel proportion fell after 33 km downstream to under 20% in the middle reaches. At location D the gravel partition rose proportionally to the fall in larger clast predominance, to 15-40%. In the upper reach, cobble and boulder generally constituted less than 30% of the substrate, although a small rise was seen at 26.97 km downstream. Cobbles and boulders constituted over 50% of the substrate composition throughout the remaining river length (beyond 33 km downstream) with the exception of one transect at 35.135 km downstream.

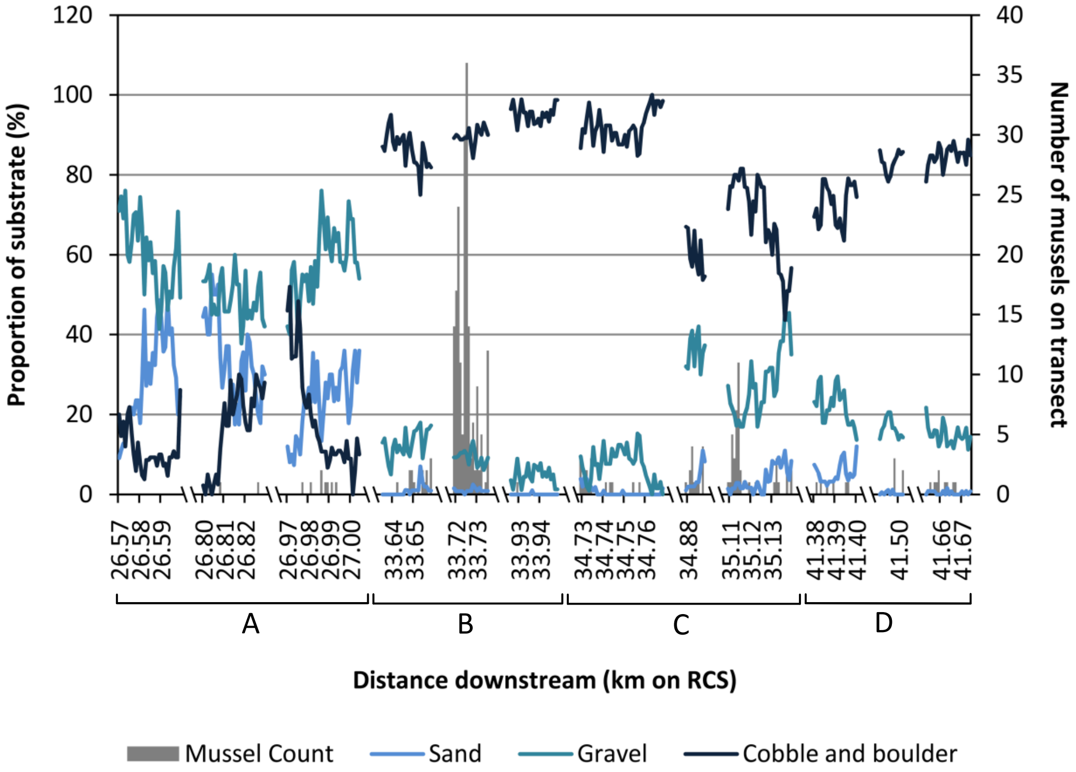


Figure 3.5 Substrate variation downstream.
Three distinct sections of substrate proportion variation were evident in the plot of substrate proportions downstream. Data from each sampling sites are present on the graph, with each separated by axis breaks (x axis) for clarity in graphical representation. Note the false origin of the x axis on this graph, though it still relates to the full RCS. Study locations A, B, C and D are marked.

Initial observations were drawn when the mussel dispersal was mapped onto this variation. The largest numbers of mussels were found in areas where the stable, large substrates dominated at over 60-80%. Further analysis of specific habitat variables may clarify reasons for the high, yet unsustained mussel counts from 33.723-33.739 km downstream. A secondary, smaller peak in mussel counts was seen at 35.113 km which again seemed to mirror a rise in cobble and boulder proportion.

Flow type was recorded as categorical data, rather than an accurate flow velocity in the interests of time during surveying. For presentation of variation this was transformed to a set of ordinal data, 'flow type,' based on the relative levels of turbulence seen in each flow type given by Padmore (1998). Therefore low turbulence areas with a 'scarcely perceptible flow' (Padmore, 1998), such as marginal deadwaters, were coded as 1. Increasingly turbulent glides, runs, riffles and spills were represented by increasing discrete values from 2-5. A spill is 5, for example, as Padmore (1998) described its character as 'fast, smooth boundary turbulent flow'.

All mussels in location A, above ~27 km, were found around a flow type of 2 (glide), though areas of higher turbulence were found here (Figure 3.6). Further downstream the mussel distribution appeared sporadic. If focus was placed on the areas of most dense mussel distribution, it could be inferred that mussels are found where the flow type is 2.5-3. The smaller mussel concentration mentioned above at 35.113 km also featured in an area where the average flow type fell briefly to within these parameters. Some intimation of a relationship between mussel distribution and flow is detected from Figure 3.6. This can be examined further relative to the flow conditions in their immediate environment.

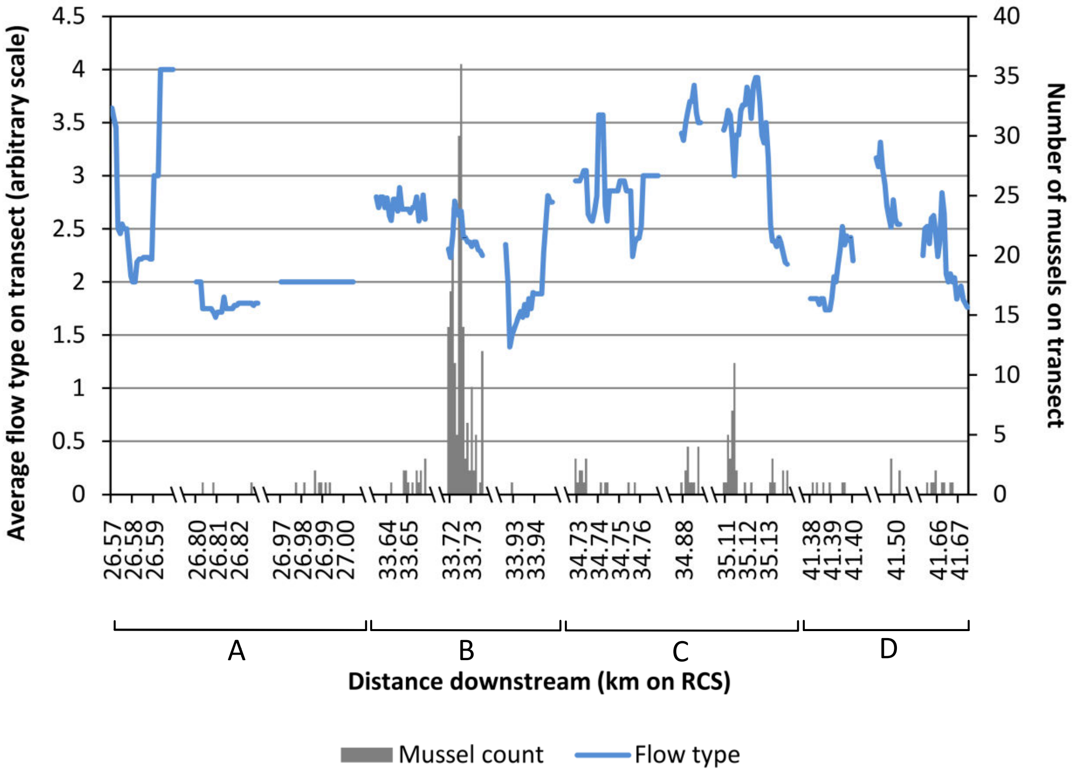


Figure 3.6 Flow type variation downstream.

The subjective variable 'flow type' was highly variable throughout the river's length. For presentation of variation an average of the ordinal flow categories (based on turbulence levels described by Padmore, 1998) was used here. This gives a basic impression of overall flow type in each site, by microhabitat transect. Note the false origin of the x axis on this graph, though it still relates to the full RCS. Study locations A, B, C and D are marked.

3.3.2. Quadrat scale variation

Results from the brief assessment of catchment and reach scale datasets preliminarily suggest relationships existed between pearl mussel distribution and the physical fluvial environment. Narrowing the scale of observation towards the sub-reach scale, a significant relationship was identified between mussel presence and distance from the channel margin. Figure 3.7 displays two important trends. Firstly, the majority of pearl mussels found during the ground survey were close to the bank/channel margin and numbers reduced towards mid-channel quadrats. Secondly, the modal distance from edge was at 2 metres, rather than immediately against the edge of the channel and suggested a more complex relationship than initially observed. This was supported by the probability values given via the conditional mean (based on values normalised against sampling effort) for each metre across the channel. Both trends displayed this unimodal distribution and a threshold of mussel preference, or tolerance, was reached at 10 m: no mussels were found in any quadrat beyond 10 m distance from the channel margin. This negative relationship was statistically highly significant (Spearman's rank correlation, r_s = -0.95, significance level 0.01).

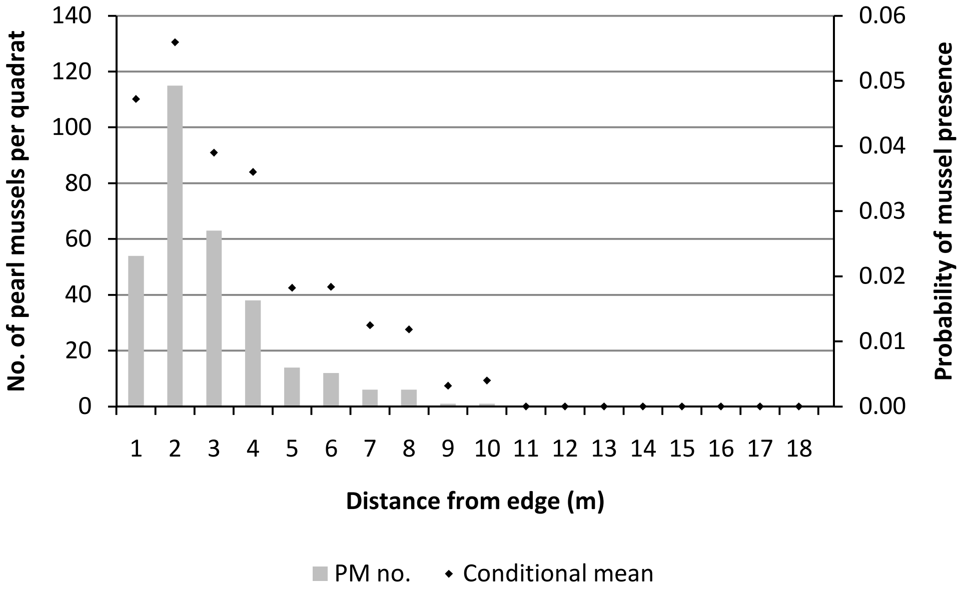


Figure 3.7 Pearl mussel dispersal as a function of distance across channel.
The column graph is founded on mussel count data. The conditional mean is based on presence and absence of mussels in quadrats at each position from the channel edge, representative of the edge of the wetted area at very low flows (all sampling was undertaken when flow level was 4-18 cm *below* the 'typical range' identified by the Environment Agency). The number of mussels found in a quadrat increased to a majority at 2 metres from the bank. This decreased gradually away from the bank.

The larger scale habitat features presented above condition the microhabitat that the fresh water pearl mussel ultimately perceives due to its limited mobility. In order to fully assess the questions posed in this study regarding pearl mussel distribution and habitat patchiness, these smaller scales must be assessed. This was achieved at the quadrat scale.

The histograms presented in Figure 3.8 (a)-(c) display mussel distributions relative to normalised numbers of quadrats found with varying proportions of sand, gravel and cobble and boulder. Standardising the sample effort for the categories made the results from each quadrat composition more comparable.

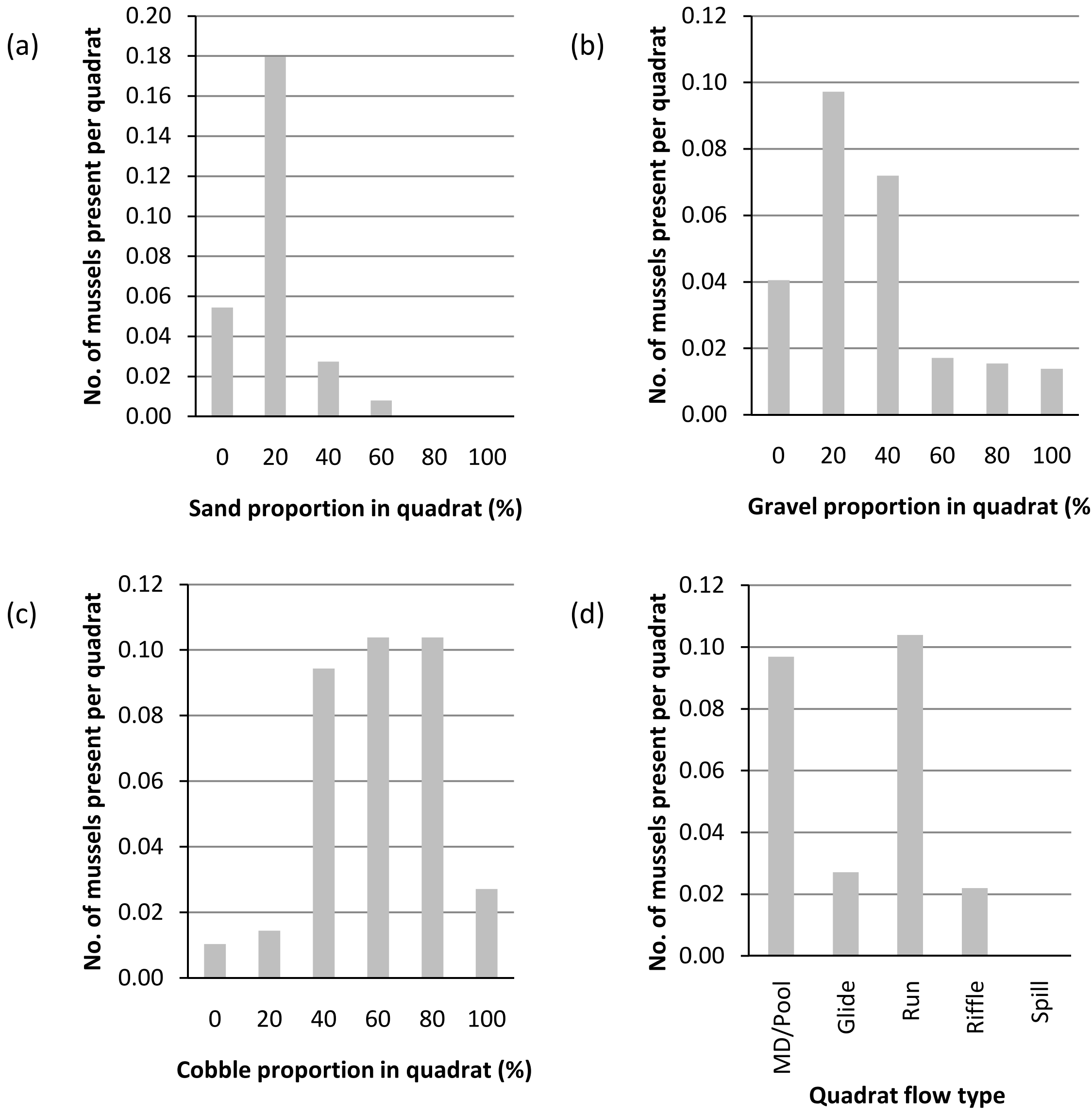


Figure 3.8 Mussel distribution by individual habitat variables.
Distributions with variance in (a) sand, (b) gravel, (c) cobble and boulder and (d) by flow type. Distinct differences in mussel prevalence existed with all variables. The x axis gives the maximum proportion for the category. Substrate proportions are given to the nearest 20% in the sampling method. MD is marginal deadwater.

Sand was the only substrate category where mussels displayed a threshold of tolerance, or preference. This occurred at 60%, beyond which no mussels were found (Figure 3.8 (a)). The majority of mussels were found where sand constituted 20% of the quadrat substrate and the distribution was unimodal with a positive skew of 2. Gravel also followed this unimodal, positively skewed distribution but suggested greater degrees of pearl mussel tolerance. Although the number of mussels found was 0.08 per quadrat fewer in the modal value of gravel compared to sand, they were spread across the full range of gravel proportions. This suggests any proportion of gravel could be suitable for mussel habitation, though there was still a preference for 20-40% gravel. Cobbles and boulders displayed a different distribution pattern. The spread shown in Figure 3.8 (c) indicated that fewer mussels were found where cobble/boulder proportions were at both high and low extremes. The similar mussel counts at 40, 60 and 80% cobble and boulder suggested a wide habitat envelope was suitable and a greater variation in cobble and boulder proportion was tolerable, relative to the values required for smaller clast sizes.

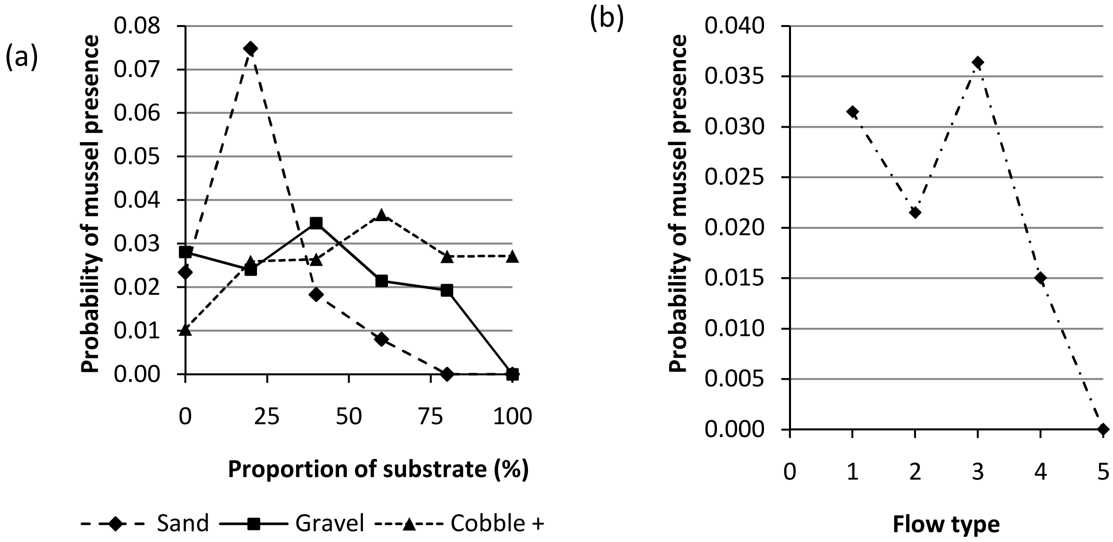


Figure 3.9 Probability of pearl mussel presence conditional on (a) the proportion of each substrate and (b) flow type.

Conditional means demonstrate how the substrate constituents and flow influence the probability of mussel presence. The probability was most distinctly altered when conditional on sand proportion. A preferred sand fraction of 20% was plain. When conditional on flow, the slower, less turbulent three variants give a greater probability of mussel presence.

Substrate has appeared as a crucial factor relating to the current dispersion of mussels in the Rede at both the catchment and the quadrat scale. Conditional probability calculations gave further support of the importance of sand in particular as a predictor of the probability of mussel presence (Figure 3.9 (a)). The conditional probability of mussel presence based on proportions of gravel and cobble/boulder followed the trends described for the actual mussels counts obtained during ground surveys. The probability of positive mussel quadrats rose with the cobble and boulder percentage up to 60%. It was inversely proportional to the amount of gravel in the quadrat after 40%. The marked increase in the probability of mussel presence at 20% sand was twice that of the next highest predictor: cobble and boulder at 60%. This can be assessed further in terms of how the different substrates interact relative to mussel prevalence. All probabilities seemed very low and it could be interpreted that none of the available variables bear considerable relevance to mussel presence. However, the low adult population density in the Rede meant that the ratio of quadrats searched to positive quadrats was very low; the probability of positive observations was simply unlikely. The number of adult mussels may have been reduced through pearl fishing, pollution events or just a gradual decline after successive years of failed recruitment but these events may not render the habitat unsuitable thereafter, rather that sampling reveals multiple false negatives: habitat is of good quality but there are no mussels left to populate it. These observations may imply that the Rede population could be so depleted that any statistical relationships would always indicate that the probability of finding a mussel was low, despite the presence of abundant ‘suitable’ habitat. Models will later be employed to further clarify the relationship and to give insight to the significance of the findings.

Figure 3.8 (d) is a normalised histogram of mussel frequency relative to the five flow types sampled. This dataset was bimodal with the largest number of mussels seen in marginal deadwater (MD) or pool areas and run flows. The mussel presence probability conditional on flow type (Figure 3.9 (b)) supported this. The relative difference in mussel counts between the two modal flow types and the others observed was very distinct and may be related to the different influences of each flow type on environmental stability experienced by the mussels.

Assessments of the individual substrate variables’ influence on mussel presence were based on the ground survey samples for individual substrate types. However, the relationships uncovered, together with those identified in the literature, suggested a more holistic view of the quadrat data should be taken, as perceived by the mussels. The column graph in Figure 3.10 displays both mussel count per quadrat and the percentage of positive quadrats found by substrate proportion composition. This was derived from the 21 possible combinations of each substrate proportion composing the quadrat. For example, category 1 represented quadrats with 100% cobble sized (and larger) clasts. Category 2 represented quadrats with fewer cobbles (80%) and with the remaining 20% comprising gravel. The full category list and all proportion compositions are shown in Appendix B and the overall availability of each partition of the substrate categories is seen in Figure 3.11. The four highest ranked categories are in Table 3.2. Categories were numbered primarily according to an increasing proportion of sand and secondly by increasing proportions of gravel (see Appendix B).

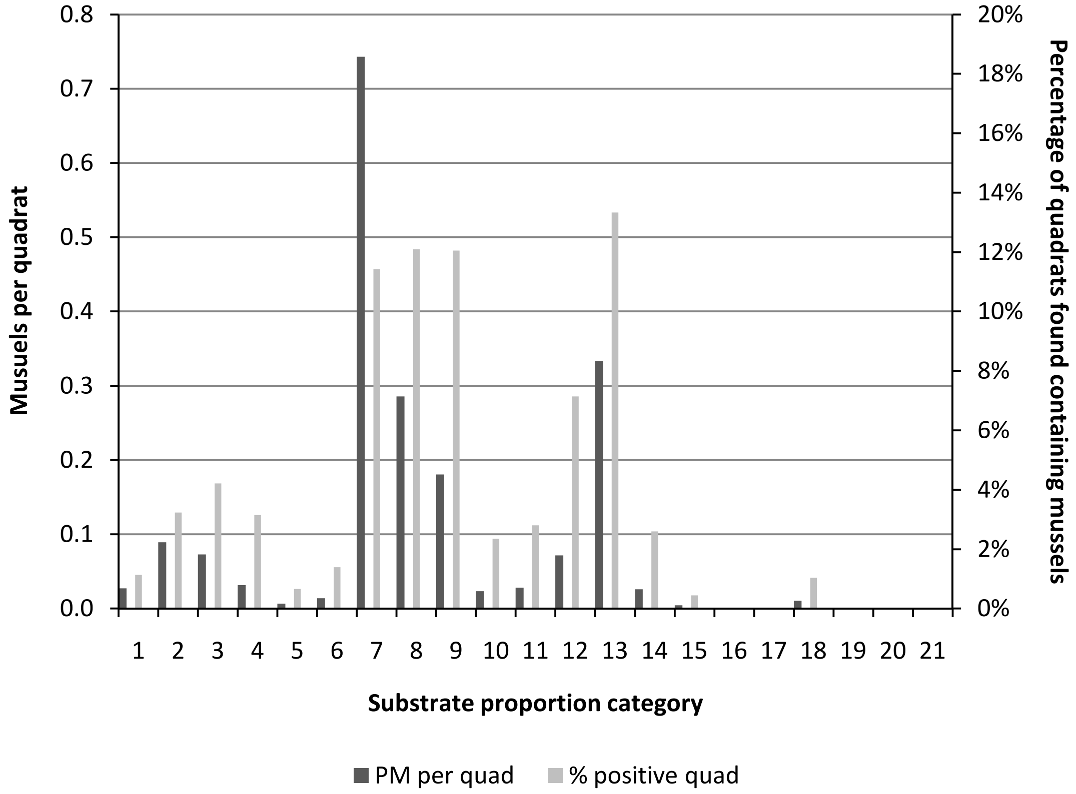


Figure 3.10 Mussel distribution by substrate proportion category.

The 21 categories represent varying amounts of each substrate type. The sand and gravel proportions generally increase by category. Substrate proportion categories are defined in full in Appendix B. Four distinct highest ranked categories (7, 8, 9 and 13) represented the proportion categories that both supported most mussels and gave the highest percentages of positive quadrats. These all displayed a high proportion of cobble/boulder substrates but with approximately half of the quadrat area containing finer substrates suitable for burrowing.

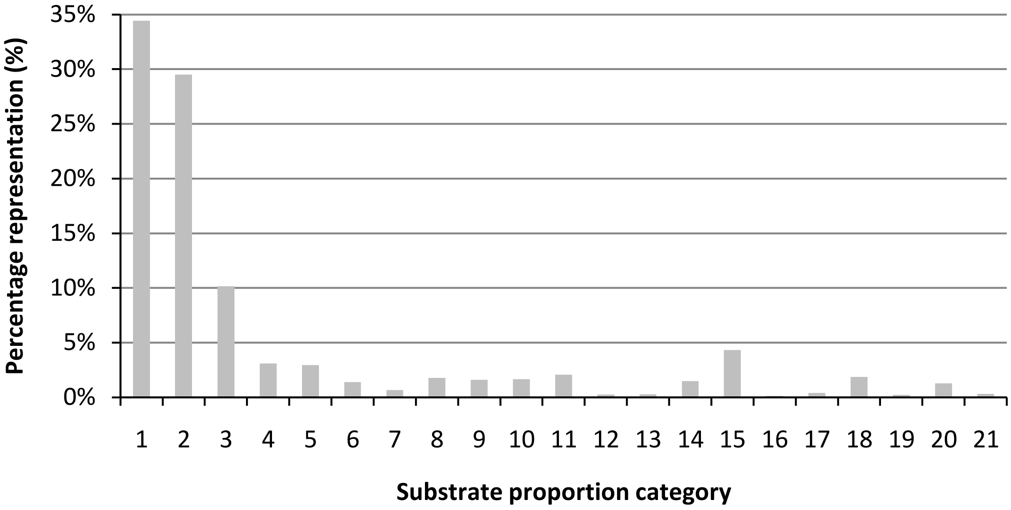


Figure 3.11 Relative proportions of substrate compositions available in the Rede**.** Each potential substrate composition category was represented in the River Rede microhabitat surveys, but not to equal levels. Categories 1 and 2 were represented to a much greater degree than other substrate composition partitions. Substrate proportion categories were defined in Appendix B.

Table 3.2 Substrate categories with predominant mussel presence. Of the 21 possible combinations of the three substrate proportions, these four represent the substrate matrix compositions that harboured the majority of the Rede pearl mussels.

Category	**Sand**	**Gravel**	**Cobble and boulder**
7	20	0	80
8	20	20	60
9	20	40	40
13	40	20	40
Average	25	20	55

The development of these 21 substrate categories is similar to a simple, ordinal index that Box and Mossa (1999) suggested was suitable for defining substrate proportions in areas surveyed for mussels, though theirs additionally included a ‘fines’ category. This method allowed a full range of compositions to be identified easily and was sensitive to changes in all substrate partitions individually. As an alternative approach, Bubb's (2004) use of a substrate index was applied as this results in easily comparable values between 100 and 600 being assigned to each quadrat, where substrate index values increased with overall substrate size. However, the index used in Bubb (2004) related to an average grain size for a quadrat and was not, therefore, sensitive to differences between a quadrat with 'ideal' mussel habitat (a mix of large clasts and fines) and 'poor' habitat (an area of highly mobile, mid-sized pebbles, for example), as the average for these was similar. The Box and Mossa (1999) approach was considered more suitable.

In five of the categories in Figure 3.10 (16, 17, 19, 20, 21) no mussels were found. These were quadrats dominated by sand and were likely to be too mobile for mussel habitation. The four modal categories each accounted for over 10% of the quadrats observed containing mussels, a cumulative value of 48.9%. These substrate compositions also harboured higher adult mussel densities at the quadrat scale. The majority of quadrats containing mussels were in a substrate composition of 40% each of sand and cobble and 20% gravel. Category 7, 20% sand and 80% cobble, harboured the most mussels at 0.74 mussels per quadrat of that type. The interaction between the large, stabilising substrates and the smaller sands and gravels, suitable for penetration by the mussel foot, was clearly a defining factor in habitat suitability. A very large proportion of sampled mussels were observed in only four of the possible substrate compositions. The average ratio of these substrate compositions was 25:20:55 sand, gravel and cobble/boulder respectively. This matrix suggested a requirement in accordance with the preferences outlined by previous studies (Oliver and Killeen, 1996; Hastie *et al.* 2000; Environment Agency/E_3 Ecology report, 2006) and, in addition to adequate stability, may provide appropriate conditions for higher mussel population densities as these categories clearly represent habitat with larger sandy spaces for more mussels per square metre. The lower mussel count per quadrat for category 9, relative to the others, supports this, as this category has most area comprising gravel (a more mobile substrate, additionally less suitable for burrowing than sand).

The remaining categories which harboured mussels, but to a lesser extent than 7-9 and 13, were often cobble and boulder dominated categories (for example 1-4). The number of mussels found per quadrat was smaller as, as an extension of the above point, there are fewer finer substrates offering space for mussel habitation. However, approximately 64% of the sampled Rede substrates were cobble dominated, with very little sand or gravel (Figure 3.11) and this type of quadrat accounted for nearly 12% of the total percentage of positive quadrats. This type of substrate composition therefore represents an important, abundant resource as mussel habitat in the Rede. It may not be of such high quality as the categories with the modal mussel counts, but many mussels still utilise this resource. Chi-squared testing revealed that the distribution of mussels per quadrat across the substrate categories showed a significant departure from the frequency distribution expected if mussels were distributed by chance (Chi-squared = 113, 20 d.f. P <0.001). The relationships identified between mussel presence and substrate composition at the microhabitat scale were clearly noteworthy.

Many of the substrate proportion categories represented a substantial percentage of the observed positive quadrats but those representing <1% could indicate locations where mussels were found by chance, in a location where they were deposited in recent high flow events. Categories with very low mussel counts and where few quadrats were found to contain mussels (e.g. categories 10, 11 and 18) must though be set within the context of the specific sample sites. Mussels found in these categories were in substrates containing very high proportions of sand and gravel but at location A these substrates were relatively stable as bed armouring was widespread. This habitat was therefore observed to be functional, though the low counts may be indicative of its unsuitability for juveniles or may be due to other threats in this area, such as high rates of disturbance. Further analysis of the variation in mussel presence between different substrate compositions is undertaken in section 4.2.5.

3.4. Terrestrial imagery and habitat variables

Data from the traditional ground surveys identified substrate composition as a major element in determining pearl mussel habitat. The distance from the channel edge was also related but may be significant as a proxy for both degree of channel shading and as a parameter which is proportional to depth. Flow was seen to have less impact on mussel presence, though may still play a part in habitat preference if not distinctly in habitat selection. The interpretation of some of these variables' relationship with mussel presence can be developed further using data from the terrestrial imagery. A total of 49 imagery transects were made across the four locations (A-D), in areas with mussel presence and absence. From these, 200 images were suitable for use, from 45 transects. This included 102 images with mussel presence. The remaining images were removed due to image obscuration by in-channel vegetation, excessive depths or specular reflectance. This may have led to a bias towards mussel presence in the dataset as deep areas, typically unsuitable for *M. margaritifera* (Section 1.9.2), were often withdrawn from use. Photo sieving for grain size distributions at D_5, D_{16}, D_{50}, D_{84} and D_{95} (at the 5th, 16th etc. percentile) yielded an accurate range of grain sizes for each of the images.

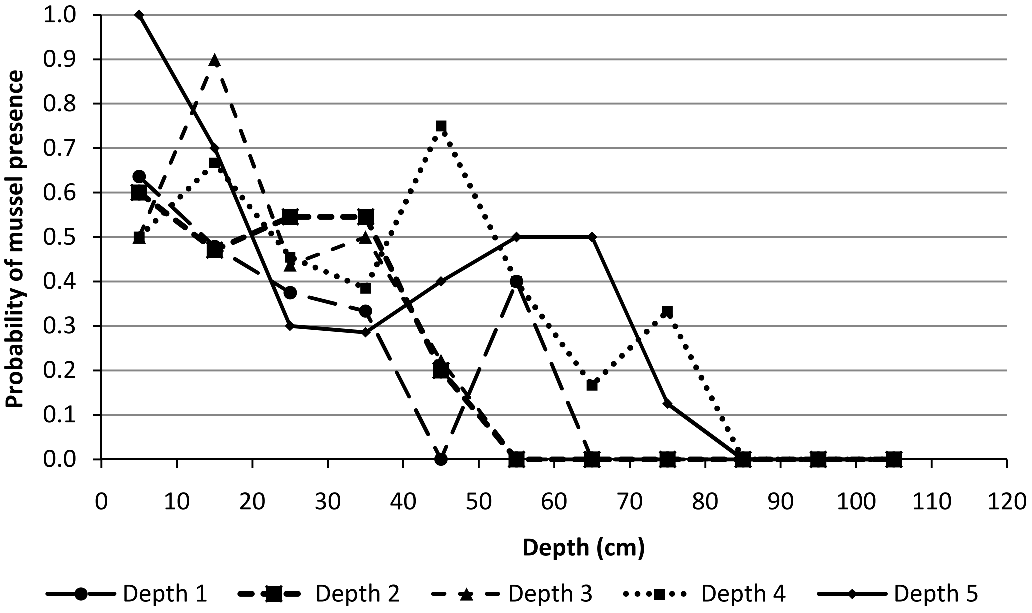


Figure 3.12 Probability of mussel presence conditional on depth.
The depth measurements were from 5 equally spaced points across the channel. Depth 3 was therefore always the middle of the channel with 1 and 5 equally close to the bank. Probabilities are plotted against the mid-point of the depth categories.

The relationship between depth and the probability of mussel presence was demonstrated in Figure 3.12. The general trend for the depth measurements suggested mussel presence was highly negatively correlated to depth and this was supported by significant Pearson's correlation coefficients (r = -0.8 for all depth measurements, $P < 0.01$). This trend applied to both edge measurements (Depth 1 and

5) and to the channel centre measurement (Depth 3) which may be used to represent an area of channel that was shallow across much of its width.

The grain size results from photo sieving produced trends that matched those deduced from the ground survey data (Figure 3.13). It indicated that the substrate matrix should ideally have been a mixture of small and large sediment. Where the y axis displayed a high probability, it can be seen that the conditional means for the upper grain size percentiles were large (≥256mm, cobble/boulder on the Wentworth scale, 1922) while the D_5, D_{16}, and D_{50} were in the sand and gravels range of grain size. This is likely to have related to the stability of the immediate environment but with areas suitable for the mussel foot to penetrate the sands and gravels.

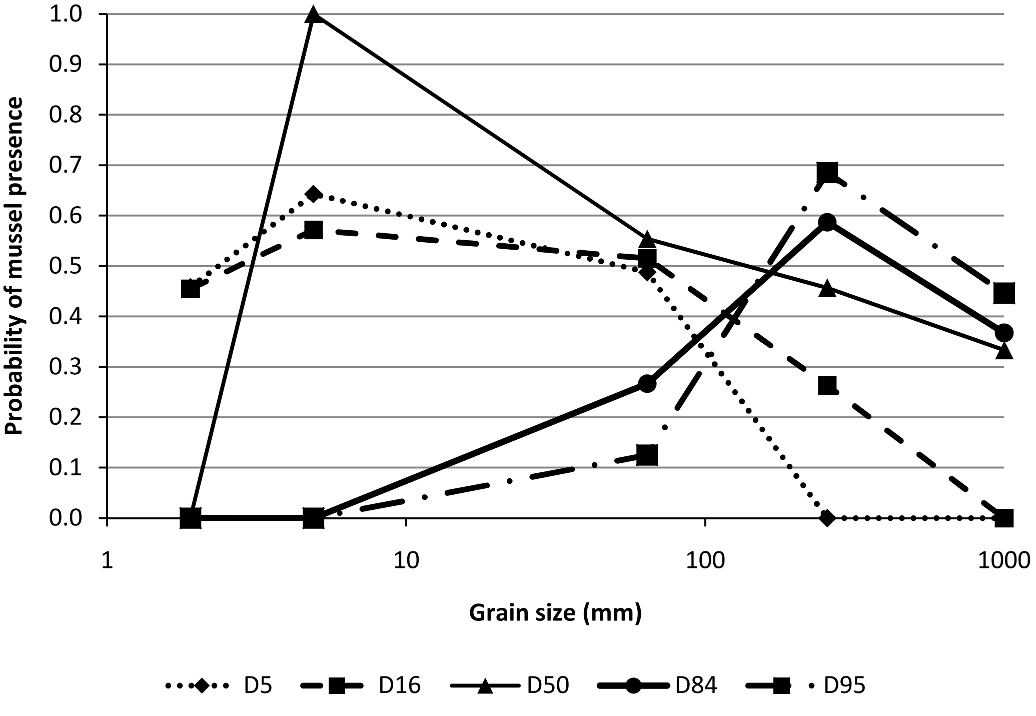


Figure 3.13 Probability of mussel presence conditional on average substrate size at D_5, D_{16}, D_{50}, D_{84} and D_{95}.

Values plotted on the x axis represent the upper limit of grain size for the percentile shown, though the largest grain size is plotted at 1000 mm for clarity. It represents all clasts over 256 mm. Grain sizes are derived from partitions used by the Wentworth scale (1922).

The variables observed as part of the terrestrial imagery data collection offered more aspects of the physical fluvial environment for assessment. Actual depth values and, more importantly, a more detailed assessment of grain size than was possible in the ground survey, due to time constraints, will be incorporated into models of *M. margaritifera* habitat preferences and tolerances. These will attempt to define the habitat preferences of the Rede mussel population.

3.5. Habitat preferences

In order to move towards addressing the final points of the investigation, the data were collated in to models of habitat use and preference. This was attempted through the use of preference modelling (e.g. as used by Hedger *et al.* 2006) and logistic regression modelling. Modelling was originally performed using the full dataset. From this it was evident that the extreme ratio of positive to negative quadrats in the ground survey data (only 135 of 5134 observations featured at least one freshwater pearl mussel) was causing considerable bias in the results. The full dataset preference models suggested a situation of perpetual tolerance, where the small mussel population avoided all habitat types, but some to a lesser degree than others. Equally, the full dataset logistic regression models consistently predicted mussel absence and all instances of positive quadrats were missed, again suggesting that none of the habitat parameters were significant in defining mussel presence. These original results were not representative of the initial analyses presented above, where certain factors are clearly significant in characterising mussel habitat.

As a result it was necessary to assemble a smaller subset of data from the original 5134 ground survey observations. It should be noted here that the terrestrial imagery dataset was more balanced in terms of mussel presence and absence, thus analysis of terrestrial imagery data continued with the full dataset. In order to obtain a representative sample of the total ground survey dataset, a subsample of 500 negative quadrat observations was extracted by generation of random numbers from the set of quadrats in which mussels were absent (random number generation was performed using Microsoft Office Excel, 2007). These records were combined with all 135 positive quadrat observations to create a new subset of 635 results. This smaller subset was representative of the original dataset. A comparison of the means of each dataset, for each parameter, showed that the maximum deviation from the original mean was ±0.8% (in this case percentage gravel cover). This is less than one full unit change in every case and means remain equivalent. Furthermore, there is an even spread of data from each of the four locations (A-D) with 10% of the new data subset from locations A and D, 11% from B and 9% from C. Subsequent analysis was carried out using the ground survey results based on this subset of results.

3.5.1. Logistic regression models

Logistic regression models were produced for the ground survey and terrestrial image data. Certain variables were consequently identified as statistically significant predictor variables to infer the likelihood of pearl mussel presence, linking specific habitat characteristics to mussel requirements.

Using the ground survey data as a foundation, the final, strongest model contained four predictor variables: the distance from the channel edge, whether the quadrat was defined as a run and the proportions of sand and cobble. The full model is:

$$Log\left(\frac{p}{1-p}\right) = 1.145 - 0.368e + 0.838r - 0.032s - 0.014c \qquad (2)$$

where p is the probability of mussel presence, e is distance from the channel edge, in metres, r is the presence or absence of flow type ‘run’ (coded in binary), s is the proportion of sand, as a percentage and c is the proportion of cobble, as a percentage. Chi-squared testing indicated that the model was statistically significant (Chi-squared = 96.1, 4 d.f., P <0.001). The statistical significance of the individual factors which are included in the model is seen in Table 3.3.

Table 3.3 Logistic regression 'ground survey subset' model: independent variable significance and odds ratios.
The model was refined until all variables were significant to a critical value of at least 0.01, with narrow confidence intervals.

Variable	Odds ratio	Standard error	Z statistic	P value	95% Confidence interval	
Distance from edge	0.692	0.033	-7.67	0.000	0.630	0.760
Run	2.312	0.522	3.72	0.000	1.486	3.598
Sand proportion	0.969	0.009	-3.51	0.000	0.952	0.986
Cobble proportion	0.986	0.005	-3.00	0.003	0.977	0.995

While the four parameters defined by the model as significant predictors of mussel presence were biologically viable, according to the literature concerning habitat preferences and the statistical significance was high, the model may not be as powerful as initial assessment suggests. The pseudo R^2 established in Stata for this model was only 0.1. The low odds ratios (Table 3.3) and small coefficients assigned to each parameter (in equation 2) suggested that each had only a small influence on the probability of finding areas of mussel presence. For example, for a unit (1 m) increase in distance from the bank, there was only a 0.7 change in the odds of mussel presence. The model can be run back into the full dataset (5134 quadrats) with only a small degree of circularity incurred, as the model is derived from only a small subset of the total results. This exercise indicated that this logistic regression model was not very successful at predicting *M. margaritifera* presence. Of the total 5134 quadrats, 3% were predicted as likely to contain a mussel (based on a probability of 0.6) though 6% of the quadrats' mussel presence or absence was incorrectly identified. Where *p* (probability of predicting presence) is greater than 0.6 (thus there is still a 40% chance that the prediction may be wrong) only 11 quadrats are correctly predicted to contain a mussel. This means that just 8% were correctly assigned as a 'positive' quadrat containing at least one mussel. There were no correct predictions if the certainty of the probability is raised above 0.6. Of the 11 correct predictions of pearl mussel presence, the quadrats all contained 60% cobble or above, no sand, were in an area of ‘run’ and were within the first two metres from the bank.

A model for predicting the presence of *M. margaritifera* was also developed for data gleaned from the remotely sensed terrestrial imagery. The strongest model included three parameters: D_{50}, D_{95} and the maximum depth on the transect:

$$Log\left(\frac{p}{1-p}\right) = 3.078 - 0.016D_{50} + 0.002D_{95} - 0.055d_{max} \qquad (3)$$

where p is the probability of mussel presence, D_{50} is median grain size, in mm, D_{95} is grain size D_{95}, in mm, and d_{max} is the maximum depth reading for the transect, in cm. The model generated was highly significant (Chi-squared = 51.36, 3 d.f., $P < 0.001$). Table 3.4 gives an overview of the significance of each parameter which went towards justifying their inclusion.

In contrast to the logistic regression model built on the ground survey data, even with the improvements brought by using a subset of data, this model seemed more robust. The pseudo R^2 gives a value of 0.2. The model was again tested on the original dataset, though the consequences of a circular argument arising was much more pertinent as the whole test dataset was also used to create the model. This was necessary as the original dataset only consisted of 200 observations and withdrawing any may have led to an even weaker model. This testing did, however, suffice to demonstrate the model success to a certain extent. The terrestrial imagery logistic regression model produced slightly more certain probabilities of correctly predicting mussel presence. At a level of probability $p > 0.9$, 53% of the terrestrial image observations’ mussel presence or absence were correctly identified. Within this, 2% of positive (mussel presence) observations were correctly identified.

Table 3.4 Logistic regression 'terrestrial imagery' model: independent variable significance and odds ratios.

The model was refined until all variables were significant to a critical value of at least 0.05, with narrow confidence intervals.

Variable	Odds ratio	Standard error	Z statistic	P value	95% Confidence interval	
D_{50}	0.984	0.004	-3.35	0.001	0.975	0.993
D_{95}	1.002	0.001	2.29	0.022	1.000	1.004
Maximum depth for transect	0.946	0.009	-5.57	0.001	0.928	0.965

3.5.2. Preference envelopes

During the univariate analyses (Sections 3.3 and 3.4) several physical habitat variables emerged with significant associations with pearl mussel presence. Most significant of these were distance from edge and the substrate matrix composition. The latter was confirmed by the terrestrial imagery analysis. Substrate composition in terms of the varying proportions of small and larger clasts and the way these interact was important and analysis of depth data clearly implied that *M. margaritifera* avoid deep channel areas. Preference modelling was used to develop these findings. They gave a quantitative measure of habitat use relative to availability and from this habitat selection behaviour could be identified.

The first assessment of habitat preferences was based on the ground survey results. Initial observations of the preference values as a whole suggested that the model partitioning was adequate to reflect *M. margaritifera* preferences. Across the range of parameters that were assessed in the ground surveys it was clear that some areas of habitat were preferentially selected by populations of the freshwater pearl mussel. Beginning with their location in the channel it was seen that areas close to the bank were more frequently used than mid-channel areas, with preferential use falling as distance from edge increases (Figure 3.14). The first metre from the channel edge was not as favoured as metres 2 and 3 though. Not all areas of channel will have featured sections over 10 m from the bank, but where these existed they were clearly highly avoided by the mussel population with a consistent preference value of -1. The complex message from the median distances from the channel edge (for example at 7 and 8 m) where preference values rise, contrary to the overall trend, may be noise or a function of channel morphology on bends or differing riparian features. Significance testing undertaken during univariate analysis showed this as a strong relationship (see Section 3.3).

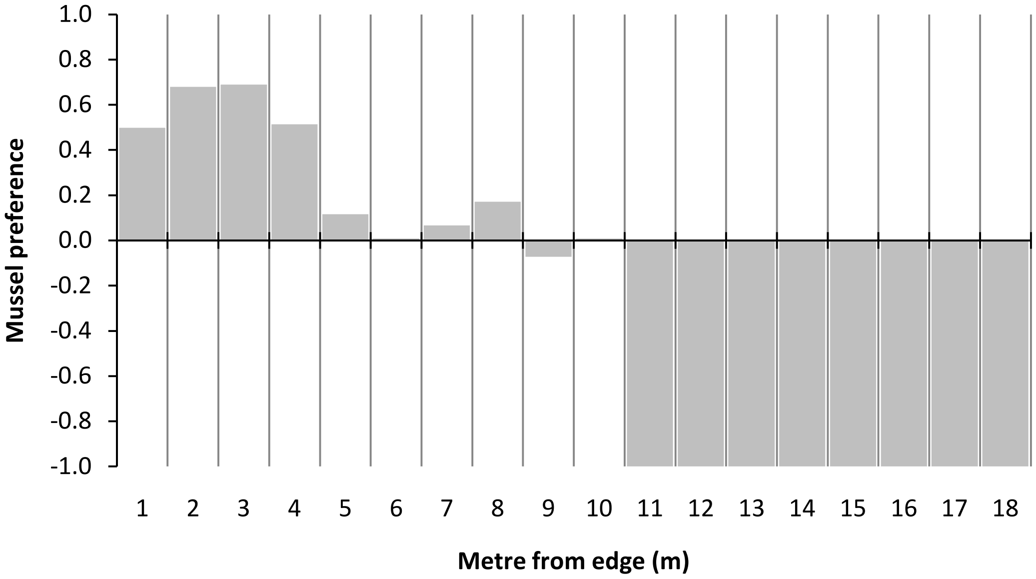


Figure 3.14 Preference model of *M. margaritifera* for distance from the channel edge. Preference values were calculated from a subset of the ground survey data. A clear trend is evident here whereby mussels strongly selected areas of habitat close to the edge of the channel, though areas within the first metre show a slightly more neutral stance. Central channel areas were strongly avoided.

The reduced magnitude of the mussel preference values seen in Figure 3.15 suggested it was a less defining factor in characterising *M. margaritifera* habitat use. Areas of low flow turbulence (such as pools and marginal deadwaters) seemed to represent a slight preference, with little evidence of selection or avoidance for riffle and run flows. Areas of glide were selected least frequently for habitation, with a preference value of -0.4.

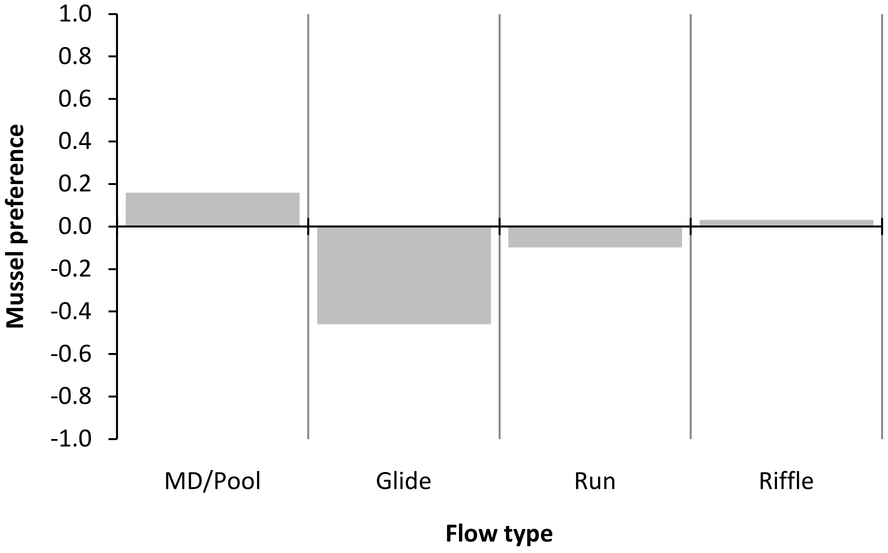


Figure 3.15 Preference model of *M. margaritifera* for quadrat flow type.
The lower preference values calculated for all partitions of this habitat parameter suggest it had less influence on freshwater pearl mussel habitat selection than some of the other parameters in this assessment. However, glide appears to have been most commonly avoided while slow flowing, low turbulence areas and their opposite, riffle sections, were more frequently selected for use. MD = marginal deadwater.

With regard to substrate proportion, Figure 3.16 demonstrates that mussels avoided areas that were composed solely of highly mobile substrates: in habitats where over 80% sand and 100% gravel occurred, preference was -1. Mussels also strongly avoided microhabitats without any sand, but showed positive selection for mixed substrates (Figure 3.16 and Figure 3.17). The highest magnitude value for cobble and boulder substrate preference was 0.66, compared to gravel at -1 and sand, where all were above ± 0.58.

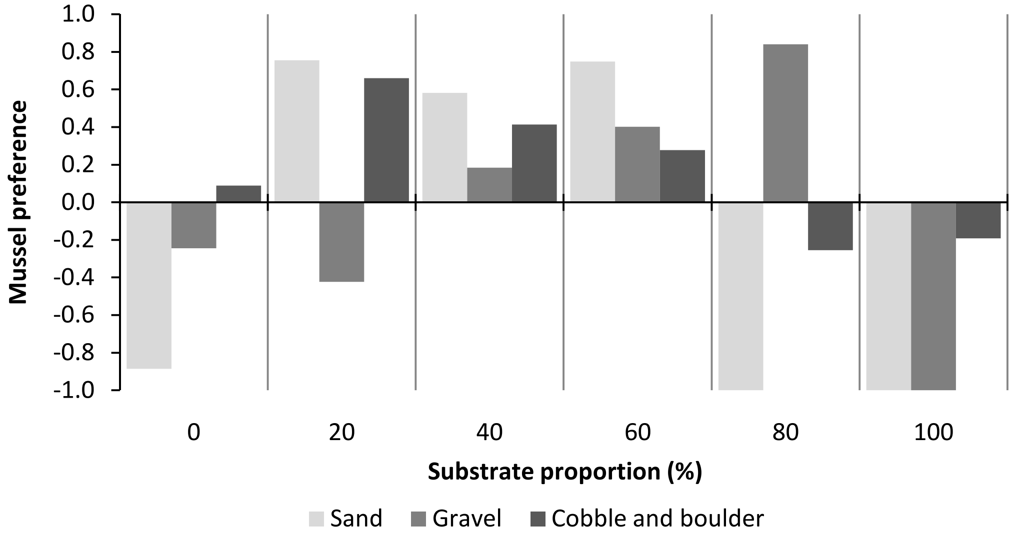


Figure 3.16 Preference model of *M. margaritifera* for quadrat substrate proportion.
The freshwater pearl mussel avoided habitat areas where sand represents both 0% and very high proportions of the substrate. Cobble and boulder was the only parameter of substrate which showed any indication that mussels will select the area for use when it constitutes 0% of the substrate.

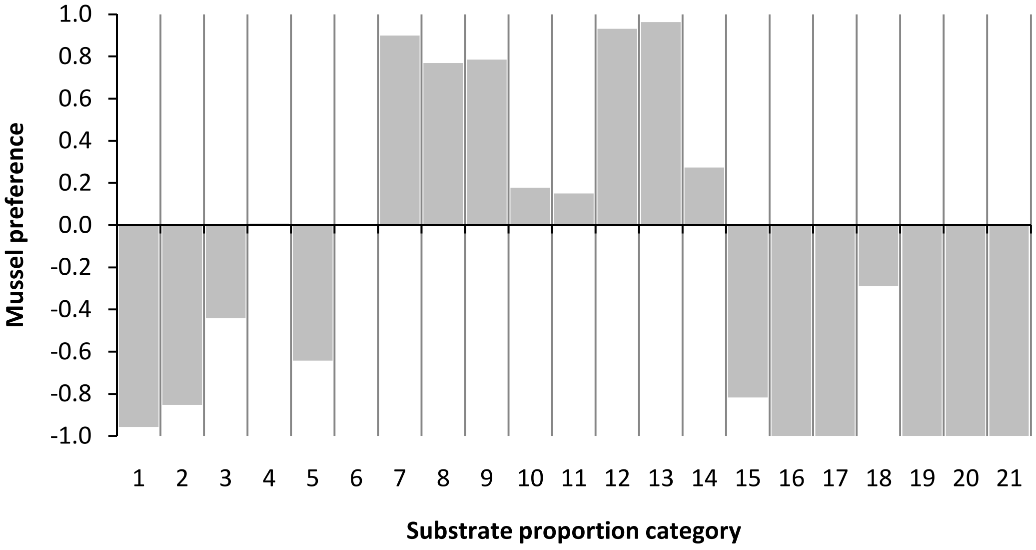


Figure 3.17 Preference model of *M. margaritifera* for quadrat substrate proportion category.
Taking account of the combined proportions of sand, gravel and cobble and boulder, the five most frequently utilised substrate categories remained the same as those identified in the raw results.

A similar assessment of substrate composition preferences was made using the remotely sensed data from the photo sieved terrestrial imagery. As habitat from this dataset was assessed at a different scale, slightly different patterns can be identified. A culmination of the transect scale and quadrat scale data (equivalent to the 'pool/riffle' system scale and microhabitat scale of Frissell *et al.* 1986) thus revealed the overall quality of the Rede physical habitat and the impact of habitat degradation across these scales. *M. margaritifera* preference values showed that ideally the smaller partitions of grain size (D_5 and D_{16}) should be sands or gravels (according to Wentworth's 1922 grain size classification system) for a high proportional use (Figure 3.18). Where any grain size parameter was between 4.9-64 mm (pebble partition), preferences were more neutral across the range of the five parameters. However, even here there was a degree of avoidance of the smaller grain size division. Habitat where pebbles make up the larger divisions of grain size (D_{50} D_{84} and D_{95}) seemed to be tolerated, though not selected to a great degree with a preference value of 0.4.

Concerning the larger substrate partitions, mussel preference values were highest where D_{95} represented cobbles (clasts from 64-256 mm diameter on the *b* axis) and where large clasts constituted most of the substrate composition. Where all clasts above D_{16} (the remaining 84%) fell in the category 'cobble', sized 64-256 mm, a high degree of habitat selection was seen. An even higher degree of preference (preference value of 0.94) is demonstrated where the D_{50} (50%) of the substrate is over 256 mm (boulder). While a range of substrate compositions could comprise suitable habitat, with an accumulation of various combinations of partitions showing high preference and selection rates, the partitions featuring the highest preference values were where D_{50} represented both gravels (clasts between 1.9-4.9 mm) and boulders (clasts between 256-2000 mm).

Previous studies of *M. margaritifera* habitat requirements have identified mussels' preference for a mixed substrate composition, comprising both large clasts and fines in reasonable quantity. Analysis of habitat must therefore be able to accommodate this dual preference and recognise its presence in the Rede. This is achieved on multiple levels. Firstly, the logistic regression models incorporated data on grain size as an indicator of the probability of mussel presence and substrates of more than one size category are within each model. There are limits to this analysis, in that only a certain range of substrate parameters were available for inclusion in the models, but with this dataset, this variable has been included as representatively as possible. While preference models have also been created for establishing the mussels' requirements of substrate composition, this has been done for the clast sizes/partitions individually (Figure 3.16 and Figure 3.18), thus interpretations must consider the preferences displayed for each partition in context and not on and individual basis. This is logical as mussels will never experience the influence of one substrate type at the microhabitat level if other substrates are present within that environment. Finally, and most pertinently, the use of the substrate proportion categories is most applicable to the assessment of mussels' preferences for a mixed substrate composition, as appropriate to their lifestyle requirements. This parameter collates the composition of substrates into distinct categories (see section 3.3.2) which allows analysis to discern immediately between different habitat character without having to consider several variables at once (all substrate proportions or size distributions for example). As there is a substrate proportion category to account for each proportional representation of substrate type (up to the level of the initial sampling resolution), interpretations on preferences regarding all habitat conditions that the mussels may experience in the Rede can be made within the full context of the microhabitat environment.

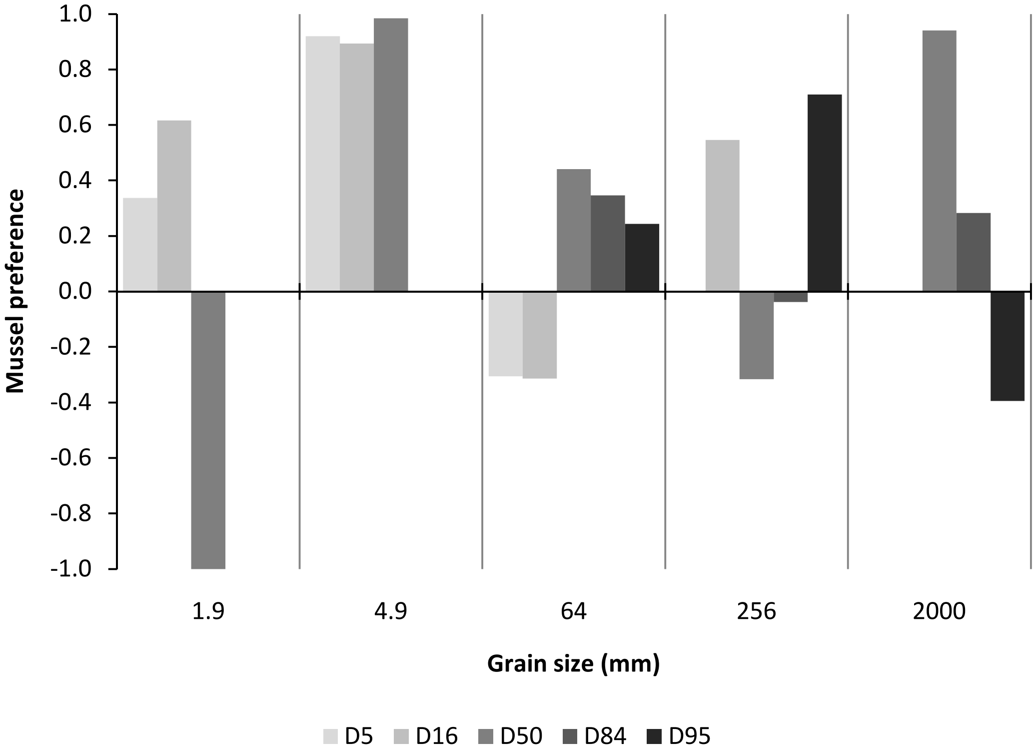


Figure 3.18 Preference model of *M. margaritifera* for grain size distributions.
This preference model was derived from the photo sieved grain size distribution from remotely sensed terrestrial imagery. Habitat where the lower percentiles of the grain size distribution (D_5 D_{16} and, to a certain extent, D_{50}) represented small clast sizes such as sands and gravels were preferentially selected. Also, habitat where 50-84% of the area comprises large clast sizes (cobbles and boulders) was strongly selected. The partitioning here is based on Wentworth (1922) classifications.

The final preference model related to water depth on the terrestrial imagery transects. Figure 3.19 was based on average channel depth taken from all five depth measurements across all transects. While depth measurements 1-5 could be attributed to a certain area of the channel (see Section 2.6.4), when amalgamated, the differing morphology of the channel and thalweg position, among other influences, will alter the parts of the transect and depth reading that is shallower, regardless of the precise mussel locations. The average therefore seemed the most reliable of all the possible representations of depth taken alongside the photo sieved grain size results. *Margaritifera margaritifera* preferentially selected habitat in shallower areas of channel and preference decreased with depth (Figure 3.19). However, this trend was not always maintained. For example, there was a small rise in preference for areas that were, on average, 31-40 cm deep compared to areas of 21-30 cm in depth, but this fell again at 41-50 cm in depth. There was also a marked diversion from the trend at 51-60 cm, where the preference value rose again to 0.8, very close in magnitude to the most strongly selected shallower areas. This was likely to be an artefact of the data: only three transects featured an average depth of 51-60 cm and one of these supported a mussel population. This was therefore construed as a favourable habitat partition. This was, however, likely to be unrealistic as too few data exist. No preference was shown for an average depth of 61-70 cm, but this was also due to a lack of data (zero observations for this partition), and should not be interpreted as a representative, true neutral preference.

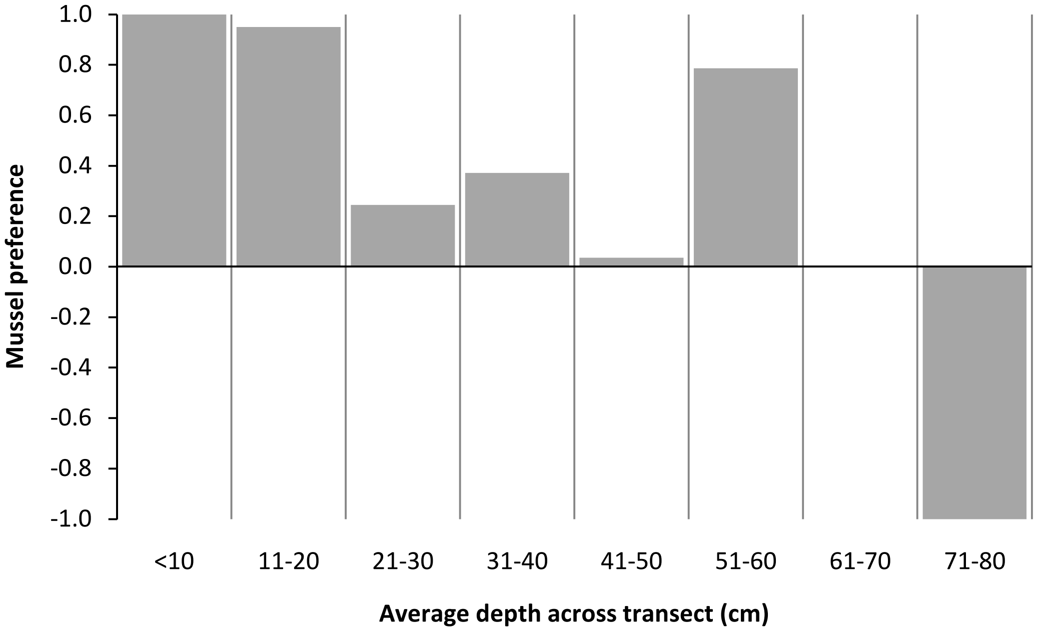


Figure 3.19 Preference model of *M. margaritifera* for average transect depth.
Average values were taken from the average of depth readings 1-5 for each transect. Freshwater pearl mussels preferentially select shallower sections of the channel and preference values generally decreased with increasing depth.

Chapter 4 Discussion and Interpretation

Interpretations employed data from all sources displayed in Chapter 3 to gain an understanding of adult *M. margaritifera* distributions and habitat patterning in the River Rede, across relevant scales. It seems appropriate at this point to remind the reader that all distances downstream on the RCS are given relative to a randomly placed, undisclosed location upstream of all known mussel locations. This represents 0 km downstream throughout the investigation and preserves the security of the population. Furthermore, it should be remembered that only adult mussels were surveyed. Interpretations therefore apply to adult mussels, other than where juvenile mussels are referred to specifically.

4.1. Spatial distribution of *M. margaritifera* in the River Rede

The River Rede freshwater pearl mussel population displayed an aggregated pattern of dispersion at each scale of assessment, including the reach scale, ‘pool/riffle’ scale and at the microhabitat scale. Distributions were firstly assessed at the reach scale (Figure 3.5 and Figure 3.6). Locations B and C (from 33.700 km to 35.115 km downstream) demonstrated significantly larger quantities of mussels per transect than other locations. The significance of the disparities between sites harbouring very few mussels and those inhabited by larger aggregations was supported by the analysis of quadrat data at the Frissell *et al.* (1986) ‘microhabitat’ scale. The main confirmation of a contagious distribution came from the calculation of the Dispersion index and the significance of the fit between the mussel distribution and a negative binomial model (Section 3.3.1). It was further established from the results displayed in Figure 3.4 that this propensity to form aggregated distributions at the microhabitat scale of more than three mussels per square metre (Hastie *et al.* 2004) was demonstrable in the Rede.

Where only a few positive quadrats were present at a site, the maximum mussel count per quadrat was correspondingly low. As the number of positive quadrats increased, the maximum mussel count increased. If a linear correlation were observed it would indicate that mussels spread evenly over the area of channel where more habitat becomes available. However, the quadratic relationship shown in the data was stronger than the linear fit (Figure 3.4) and suggests that more mussels congregate together where more suitable areas are found. This trend was shown to maintain a significant Pearson's correlation coefficient ($r = 0.86$, $p < 0.01$) and was in full agreement with the accounts of distributions in other rivers (Gittings *et al.* 1998; Hastie *et al*. 2000). *Margaritifera margaritifera* have been observed in "highly aggregated distribution patterns" (Hastie *et al.* 2000). While it should be remembered that this study's trends were based on only 12 sites, there were 135 positive quadrats and a total of 5134 quadrats within the dataset. Based on this data it was assumed to be an accurate account of freshwater pearl mussel distribution in the sampled areas of the Rede. This was an indication that some sites were more appropriate for adult *M. margaritifera* habitation than others and high mussel prevalence may thus have been dependent on habitat, as the literature suggests (Hastie *et al.* 2004).

In light of this agreement with the literature standard for other populations of *M. margaritifera*, this is a positive outcome for the Rede population as it suggests that these mussels still respond to features that favour the formation of dense beds, as in a healthy population (Smith and Jepson, 2008). Hastie *et al.* (2004) attribute high mussel densities to macroscale habitat features, such as bed substrate and riparian woodland, and also to microhabitat features as in Hastie *et al.* (2000). Their use of a (mussel) dispersion index, developed to show the "highly contagious patterns" (Hastie *et al.* 2004) in the population, led to a conclusion that where density is over one mussel per square metre, optimal conditions may be represented. In the River Rede only five sites harboured consistently low densities (inclusive of all transects at a site, see Table 3.1) suggesting that at the majority of sample sites, areas of suitable adult *M. margaritifera* habitat remained. This is promising for the efforts to maintain a sustainable population in the Rede. A correlation with the literature regarding population aggregation was not adequate, alone, to establish the impact and the possibility of reconciling habitat degeneration in the Rede, nor to establish if the patchiness of mussel distributions in the Rede was a function of physical parameters.

Areas of minimal mussel distribution were also of interest. According to Hastie *et al.* (2004), areas of mussel absence or low density presence are likely to represent sub-optimal conditions. Areas of mussel absence may correspond to areas of habitat that are historically persistently unsuitable, such as those with conditions that naturally contradict the 'ideal', as described in Chapter 1 (Hastie *et al.* 2000; Hastie *et al.* 2004; Young 2005; Bolland *et al* 2010). Sites demonstrating only *minimal* mussel presence however, may offer more complex and pertinent reasons as to why densities are so low. A lack of local recruitment can arise if conditions are not adequate for the more sensitive juvenile *M. margaritifera* (Buddensiek *et al.* 1993) or through early adult mortality due to habitat degradation (Wilcove *et al.* 1998; Cosgrove and Hastie, 2001; Moorkens *et al.* 2007). Habitat degradation through engineering works is known to have occurred on the Rede (Environment Agency/E$_3$ Ecology report, 2006) and has had an impact at certain sites. High magnitude flood events may entrain mussels and deposition sites will be in accordance with shear stress and stream power laws, rather than habitat preferences (Vannote and Minshall, 1982; Hastie *et al.* 2001; Environment Agency/E$_3$ Ecology report, 2006). This may account for some quadrats occupied by only a single mussel, explaining why conditions in these areas may be sub-optimal or why larger beds have not developed. Where mussels are subsequently observed in these sites, distributions are likely to be more random as mussels can move only short distances (Aldridge, 2000).

Areas of high mussel densities will be indicative of the most suitable local habitat, as advised in contemporary literature (Hastie *et al.* 2004). Low mussel density or areas of mussel absence will indicate sub-optimal habitat. With these assumptions it will be possible to determine any relationship between mussel distribution and the distribution of habitat on the Rede, in accordance with the project aims.

4.2. Relationships between adult *M. margaritifera* distribution and environmental parameters: the identification of preferred habitat

The aim of this study was not to identify where in the River Rede the freshwater pearl mussel could survive but to examine the current adult population's distribution as a function of the physical habitat and establish if any preferred habitat types exist and which parameters the adult mussels relate to. Assessment will combine results across the range of scales addressed in this study to give an account of the precise habitat experienced by adult *M. margaritifera* in the Rede. The concept of habitat patterning and quality is always applied in relation to a specific species (Bell *et al*. 1991). Therefore this assessment is restricted to the parameters which have been linked to freshwater pearl mussel requirements and observed accordingly (Section 1.9).

4.2.1. Water quality

Water quality data were represented at the most extensive spatial scale in this investigation. Water 'quality' must be relative to a certain purpose and in this case was in terms of the requirements of the freshwater pearl mussel for which various tolerance thresholds have been derived (Bauer, 1988; Buddensiek *et al.* 1993; Beasley and Roberts, 1999; Oliver, 2000; Young, 2005). It appears that water quality in the River Rede may be of some concern but analysis was only based on a broad assessment of parameters at an intensity of approximately 1 km sample spacing, thus some ideal areas may exist at a finer scale, such as would be experienced by *M. margaritifera*. However, the pervasive influence of water chemistry in the riverine environment cannot be ignored (Fullerton *et al.* 2010).

The parameters of least concern were dissolved oxygen and nitrate concentrations. While the most suitable reaches in terms of *M. margaritifera* requirements were found beyond 22 km downstream (in winter sampling), the high dissolved oxygen saturation needed by this species (90-110% is suggested by Oliver, 2000) is commonly found in the turbulent rivers with cobble dominated substrate such as this, as a high degree of air and water mixing occurs. This is supported by the long term data, which places readings within Oliver's (2000) thresholds during all months (as an average value across two decades) thus this water chemistry parameter was considered to be of little concern in the Rede.

Nitrate concentrations appeared satisfactory in the Rede, with the average level remaining below half the tolerance level of *M. margaritifera*. This is in accordance with the oligotrophic status required by freshwater pearl mussels (Skinner *et al.* 2003). This parameter was the only factor that Bauer (1988) found to significantly affect adult *M. margaritifera*. This is a good indicator for the Rede as it suggests the existing adult population is not under stress from increased nutrient concentrations. However, calcium concentrations in the water body may command a complex influence as a factor of the physical environment. While suitable levels existed between 17 and 37 km downstream in the winter sampling, outside this stretch and throughout the spring sample set, levels of calcium were above the tolerance thresholds reported in the literature (Bauer, 1988; Beasley and Roberts 1999; Oliver, 2000). While nitrate levels are suitable for mussel habitation in all areas of the Rede, elevated calcium levels may signify an increased propensity for eutrophication and higher productivity, particularly when combined with high levels of phosphates (Bauer, 1988). No data were available for phosphate levels in this study, thus this is only speculative. Further evaluation of the Rede water chemistry could clarify whether this relationship, identified by Bauer in Central European rivers, is of concern in the study catchment. This is important as it has ramifications for juvenile survival in particular. Overall, while the assessed nutrient (nitrate) levels in the Rede confirm satisfactory conditions for adults, initial indications from secondary macronutrient concentrations (calcium) suggest nutrient levels may be detrimental to juvenile survival (Bauer, 1988).

Conversely, thresholds for this parameter are contested (Boycott, 1936; Bauer, 1988; Beasley and Roberts, 1999; Young, 2005). The most likely reason for calcium concentration to be above *M. margaritifera* preference limits throughout the Rede catchment is the underlying limestone geology (Lawrence *et al.* 2007). More calcium leaches from the catchment geology or abandoned quarry areas (Moorkens *et al.* 2007) in the spring, after weathering and snowmelt, than is seen in the winter sampling. In light of the historic natural occurrence of high levels of calcium in the Rede catchment and that, formerly, a healthy population of *M. margaritifera* existed (Environment Agency/E$_3$ Ecology Report, 2006), there may be a degree of local adaption. Boycott (1936) observed *M. margaritifera* in rivers with calcium concentrations of up to 50 mg l^{-1} (this is exclusive of rivers acknowledged in this early paper that were later found to harbour a more calcium-tolerant subspecies of freshwater pearl mussel). It may be possible that local adaption to calcium is common, thus increased calcium concentrations should not be considered a major sign of habitat degradation in the Rede. The variability in calcium concentrations found in the literature would suggest this may the case (Boycott, 1936; Bauer 1988; Beasley and Roberts, 1999; Oliver, 2000; Young, 2005).

Whatever the source and implications of elevated calcium concentrations in the Rede, the influence of this factor on other, more detrimentally active water quality parameters cannot be ignored. This includes pH and conductivity levels (Bauer, 1988). Water leaching calcium carbonate from the Alston and Tyne limestone formations (Lawrence *et al.* 2007) is alkaline, thus we can expect raised pH in the Rede catchment. This was evident in the spring sampling data, coinciding with raised calcium concentrations, meaning that conditions were rendered too alkaline for freshwater pearl mussel preferences in most areas. During winter, observed pH was more appropriate for mussel habitation beyond 11 km downstream. The initial, rapid increase in pH from 0 km downstream, at pH 5.9, to the next measurements at around pH 8 can potentially be explained by the influence of Catcleugh Reservoir. The first data point is taken from the Rede as it passes through the peat moorland. After water has been held in the reservoir it may acquire the alkaline characteristics of the underlying clays and limestone (Edina Digimap, 2011). Moving downstream, pH was seen to return to ideal ranges. This may be as a result of the convergence of tributaries with the Rede supplying more acidic waters draining peat and forestry land (Neal *et al.* 2010).

High conductivity readings could also be attributed to the naturally elevated concentrations of calcium: again conductivity readings were higher in spring than winter, corresponding with the raised calcium levels. If this concurrence did not exist and conductivity alone was high, it would be indicative of the presence of other dissolved solids and mineral salts signifying a degree of pollution (Bartram and Balance, 1996; Chapman, 1996) as seen by Beasley and Roberts (1999) in rivers unsuitable for the freshwater pearl mussel. If high conductivity can be explained by naturally occurring (geological) environmental factors, water quality can be interpreted as suitable for freshwater pearl mussels.

For all three parameters (calcium, pH and conductivity), the established thresholds were consistently surpassed in spring sampling. This can be explained by the specific geological conditions in the Rede catchment but it could still result in minimal areas of suitable habitat being available to the Rede *M.* margaritifera population. As water is such an all-encompassing medium in their environment, this cannot be ignored as a possible negative feature of Rede habitat character. In particular, it is likely to have detrimental consequences for juvenile survival (Bauer, 1988; Buddensiek *et al.* 1993).

The suitability of some water quality parameters was uncertain. Agreement in the literature would generally suggest these results should be indicative of poor habitat throughout the Rede in spring and in all but the middle sections during winter. However, implications of the Rede catchment geology can often explain why calcium, pH and conductivity were observed at elevated levels and local adaption may have occurred (Purser, 1985 in Young, 2005). A significant point that repeatedly arose in Chapter 3, which may be a stronger indication of unsuitable freshwater pearl mussel habitat however, is at 39 km downstream. Here a tributary confluences with the Rede, causing a sharp rise in all but dissolved oxygen. These elevated levels persist downstream, with the exception of only a point rise in nitrates. This tributary may be a source of pollution as it drains areas of quarry and mining spoils where leaching of metalliferous compounds is likely to cause the observed rise (Chapman, 1996). This sort of input should potentially also be assessed, in conjunction with conditions on the main Rede, as influences from the entire catchment may affect the river where mussels can be found, even though none are recorded in Rede tributaries (Atkinson, *pers. comm*.).

The discussion of the water quality status of the Rede has revealed that many areas are likely to be outside the preference limits of *M. margaritifera*, however, the species' persistence suggests conditions are tolerated. Historically, conditions may have been similar as they can be linked to the geological character of Redesdale. Assuming this is the case, similar conditions, of a suitable quality, were available to the freshwater pearl mussel population throughout the sampled reaches of the Rede. It may be that upstream of the sample sites and in the headwaters, where water chemistry was seen to be different for some parameters, unsuitable large scale patch habitat existed. This cannot be verified as part of this study, however, as evidence is only anecdotal that the mussels do not exist in these reaches (Environment Agency/E_3 Ecology report, 2006; Lewis, *pers. comm*.). A more extensive appraisal of water quality was not within the scope of this study thus local adaption was assumed, though with the recommendation for further investigation in this area.

Assuming that water chemistry is appropriate for adult mussel survival at the meso-scale, habitat at smaller scales will now be assessed. The main parameters for study were distance from the channel edge, depth, flow and substrate character (combining data for substrate as individual proportions, exact grain size distributions and proportional composition as categories). These have been assessed to quantify the strength of the influence of individual parameters so that potential areas of preference could be inferred.

4.2.2. Distance from channel edge

A highly significant negative relationship was identified between the distribution of adult *M. margaritifera* and distance from the bank. Beyond 10 m from the bank no mussels were found. Some past studies identified distance from the channel edge as a significant factor influencing mussel presence, for example in the River Kerry, Scotland (Hastie *et al.* 2000). The freshwater pearl mussel selects shaded habitat where water temperature is regulated (Gittings *et al.* 1998; Akijama and Iwakuma, 2007). It is sensible to suggest that distance from the bank (on the Rede in particular, where there is often extensive, mature riparian vegetation) acted as a proxy for the degree of channel shading. Where trees overhang the channel, quadrats closer to the bank can be expected to be in shaded areas. These cooler areas may be preferentially selected as suitable habitat.

Distance from the channel edge may also be related to depth. Many authors note mussels' preferences for a certain depth envelope constituting ideal, or even tolerable, habitat. In the Kerry this was established at 30-40 cm (Hastie *et al.* 2000). Depth preference varies by river and geographical location, as established for Scandinavian rivers in Chapter 1 (Hendelberg, 1961). The Rede's customary channel shape means that the area next to one or both banks is shallow and depth increases towards the centre (*pers. obs.*). Mussels are usually found in shallow water in Britain (Gittings *et al.* 1998, Environment Agency/E$_3$ Ecology Report, 2006). The distance from edge variable could represent this aspect of habitat in addition to the degree of channel shading. A combination of these factors goes towards explaining the threshold reached at 10 m from the bank. The quadrats beyond this present a more hostile environment with little shading, higher water temperatures and intolerable depths. Freshwater pearl mussel survival would be limited in such environments and it could be suggested that mid-channel habitat is deliberately avoided for these reasons. The cited literature supports this interpretation.

The modal pearl mussel count at 2 m from the bank, rather than at the immediate edge, could be due to the specific hydrological regime of the Rede. While it is well established that *M. margaritifera* inhabit channel edge areas, this additional complexity could be particular to the Rede and similar rivers in terms of the significant temporal flux of water levels. The River Rede is very 'flashy', even with moderate rainfall events. The average range in river level can be up to 2.45 m (Otterburn) and 1.39 m (Redesmouth) with lows of 0.35 m and 0.21 m respectively (Environment Agency website, 2010). During summer months the area closest to the bank often emerges above the waterline and mussels can desiccate (*pers. obs*.). This justifies the observed distribution, despite the extent of suitable habitat adjacent to the edge.

Though width was not considered as a habitat feature in itself, it is relevant to habitat availability and corresponds to the findings associated with distance from edge. The width of the River Rede increases downstream, as is expected in an alluvial river in response to increasing discharge from an ever larger catchment (Richards, 1982). Correspondingly, this results in an increase in mid-channel microhabitat, though evidently the preferred areas of channel margin habitat remain constant. While depth is variable, even in channel margin areas, the freshwater pearl mussels' potential preference for shaded channel areas (Gittings *et al.* 1998) could mean that the available habitat for the species does not necessarily increase with increasing channel area.

4.2.3. Depth

In addition to the relationship between depth and mussel presence inferred above, a significant relationship between adult *M. margaritifera* presence and depth was found from direct analysis of observations. The probability of mussel presence is negatively correlated with depth. This trend is expected in accordance with other assessments of the species' preferences (Gittings *et al.* 1998; Hastie *et al.* 2000), though even some relatively local British studies have found them to prefer intermediate depths (Hastie *et al.* 2004). While patterning may be viably different in the Rede, this result's authenticity may be restricted due to practical limitations on sampling: sample areas had to be accessibly shallow for measurements to be taken. However, the maximum depth partition used in the calculation of conditional presence probabilities (120 cm) was less than the maximum measurable depth, thus the relationship's significance is accepted.

While it may be expected that where the edge of the channel was shallow there would be a higher probability of mussel presence, this is also the case for centre channel depths. If the channel centre is shallow it may indicate a generally shallow channel transect. This would render it more likely that mussels could inhabit the entirety of that area of channel, thus more positive quadrats could be expected across the area. However, due to the coarse sampling density of the depth measurements (Section 2.6.4) it is deemed justifiable to draw conclusions only from the overall relationship trend. A specific preferred depth range can therefore not be established from this representation of the data, though it may be viable to suggest that the observed adult mussel preference for shallower areas is relative to the Rede as a small river; they can be found in deeper areas in other, larger rivers.

4.2.4. Flow type

Flow was inherently variable within the location and site scales but location A seemed to display less variation than locations B, C and D. This was likely to be as a result of the homogenous channel character created by engineering at this site. The channel here had been deepened and straightened with frequent use of logs and pins for bank stabilisation. According to the literature, *M. margaritifera* habitat should comprise fast flowing sections, such as riffles and run flows (Hastie *et al.* 2003). In location A, only 23% of the section (as quadrats) was classified as these turbulent, fast flows compared to 70% as glide as a result of engineering works. It is likely that habitat in location A was limited at the reach scale by the extent of habitat change induced by engineering. Consequently the total available habitat in this area was likely to be small as, although suitable microhabitat may exist, nested within the reach, the reach scale conditions could render the majority of microhabitat unsuitable. Conversely, more habitat classed as ‘suitable’ exists in the remaining locations, with an average of 57% of quadrats as run or riffle flows. The least turbulent, slowest flows (as marginal deadwaters and pools) are most prevalent in location D (23%). The prevalence of suitable flow types in the middle reaches of the Rede indicated that flow is unlikely to have been a limiting factor here at the reach level.

At the microhabitat scale the relationship between flow and adult freshwater pearl mussel presence was less distinct than for the habitat variables discussed thus far. The spearman’s rank correlation coefficient suggests the negative relationship between mussel presence and the ordinal dataset based on turbulence is reasonably poor at $r_s = -0.4$. Furthermore, the patterns found on the Rede do not wholly agree with the standards found in other studies, though the relationship found can be defended.

Microhabitat identified as containing the ‘run’ flow type offered the highest probability of adult mussel presence. Though the probability was considerably lower than other variables’ value as predictors, this is in accordance with past studies (Hastie *et al.* 2004). However, marginal deadwater or pool areas were also identified as good predictors of adult mussel presence. This is in conflict with the conventional habitat standards of the species (Hastie *et al.* 2004 among others; refer to Chapter 1). While the observed relationship may be an artefact of deposition in conjunction with flood events (Environment Agency/E_3 Ecology report, 2006) it may also be in relation to the Rede’s characteristic ‘flashy’ regime and refugia from high flows. Marginal deadwaters have very slow, low turbulence flows (Padmore, 1998), thus there is less risk of mussels being entrained in the flow, as in riffles, yet adequate dissolved oxygen must remain to ensure survival. Whether the Rede adult mussel population requires any more protection than populations in other rivers, owing to its regime, is not clear from available evidence. It could be researched further in future studies but Howard and Cuffey (2003) suggested for other species in the *Margaritifera* genus that riffle areas may be too high stress with the extreme range of flows available in a river, thus they may not use riffle sections as frequently. In this situation, areas of refuge such as pools and marginal deadwaters may be preferred. High adult mussel densities may also be found in these areas as an artefact of the population depletion that has occurred:

mussels remain in marginal deadwaters but have been lost from the more turbulent areas as dense beds no longer exist to provide stability.

This unusual relationship may also be a function of depth. Marginal deadwaters are frequently on the edge of the channel where the shallowest areas are found. Preferences for these have been established. While the relationship with low turbulence flow areas as refuge would suggest that glides would show the next highest mussel prevalence, these are often deep areas in the Rede which have been shown to be avoided. Run areas are not excessively turbulent (Padmore 1998) and are considered suitable habitat (Hastie *et al.* 2004) but are not too deep to restrict mussel use of the channel area. Amalgamation of the above evidence confers that the Rede population's relation to both conventional run flows *and* marginal deadwaters or pools can be explained, though why different parts of the population are found in different areas is not definable. A likely interpretation is that the interrelation between all of these habitat parameters (flow, depth and the distance from the channel edge) drives selection of particular channel areas over others: superior habitat conditions for each variable may not overlap, but the most suitable habitat areas will combine the best the Rede environment has to offer.

4.2.5. Substrate composition

Substrate in the sampled areas of the River Rede were primarily cobble and boulder dominated (Figure 3.11) with smaller proportions of sands and gravels suitable for adult mussels to establish a stable purchase with their foot. This is in accordance with high quality habitat requirements confirmed in Chapter 1 and suggests that the majority of (sampled) areas in the Rede offer suitable habitat, particularly in locations B, C and D. However, throughout location A (from 26.5 km to 27.0 km downstream) gravel proportions generally remained above 50% and sand was more prevalent than cobble and boulder substrates at all but a 100 m section in site five. Location A can thus be expected to have greater substrate mobility with the lack of stabilising larger clasts (Hastie *et al.* 2000). However, personal observations did reveal that, in some areas, river bed armouring occurred acting as sheets of highly stable substrate material, though they comprised many smaller sized clasts (Richards, 1982). The development of an armoured section, where fines are removed leaving a tightly packed surface veneer, is likely to be as a result of channel modification reducing sediment delivery to the reach (Richards, 1982). This explains the presence of a small number of adult mussels that tolerated this unconventional mussel habitat: the armour layer is stable while allowing mussel foot burrowing and purchase. Cobble and boulder dominated habitat was restricted to the lower sampled reaches but the physical modifications to the gravel dominated location A rendered it suitable, though not ideal, for mussel habitation. Evidence from Box and Mossa (1999) suggests this would only be suitable for adult mussels though. The potential losses at the time of channelization via direct damage to mussel beds (Cosgrove and Hastie, 2001) and the reduced availability of juvenile habitat in location A may explain the small mussel count in this reach. Areas harbour more mussels where large scale habitat patterning displays the preferred mixed substrate characteristics, both in terms of positive quadrats and denser aggregations.

The most realistic representation of the habitat the individual mussels experience was portrayed in the combined substrate proportions through the use of the substrate category data, as no partition of the substrate will be available to the mussel population without the influence of other types unless it represents 100% of the quadrat substrate. Figure 3.11 tells us this was a rare occurrence for both sand and gravel substrates. As the literature also confirms that the interrelation between substrate types is important to *M. margaritifera* survival (Skinner *et al.* 2003), the substrate proportion category data will form the basis of the identification of a relationship between substrate and mussel distribution.

The importance of the substrate character for the maintenance of a sustainable *M. margaritifera* population is well established (Gittings *et al.* 1998; Hastie *et al.* 2000; Hastie *et al.* 2003). However, while the broad, location scale substrata character is important at one dimension, the actual prevalence of suitable substrate microhabitat is also of interest. Cobble and boulder dominated microhabitat (<1 m^2) constitute nearly 64% of the total areas sampled. Peaks in substrate categories representing mobile, fines dominated substrates (at the opposite extreme of Figure 3.11) are supplemented by quadrat counts from location A, where most large clasts have been lost downstream or possibly removed. While the most prevalent substrate compositions do not represent the ideal character described in the literature, as too few fines appeared to be present (according to compositions defined by Hastie *et al.* 2000), some sands will be present in the hollows between the larger clasts (Hastie *et al.* 2000; Wetzel, 2001) and this patterning may be of potential value in the Rede environment in light of its predominance.

The highest percentages of positive quadrats *and* quadrats with the largest mussel counts were within only 4 of the 21 substrate categories. These modal categories represent an average of 25% sand, 20% gravel and 55% cobble and boulder. This pattern of small amounts of sand or gravel and higher proportions of large clasts shows preference relationships that were echoed to a certain extent in the results for individual substrate types. The individual results suggest that wherever the substrate matrix includes sand, gravel or cobble and boulder to the appropriate proportions, the environment is suitable for adult mussels. If this were true then 13 of the 21 substrate combination categories would yield higher positive mussel returns than currently seen, rather than the 4 identified. This is based on the category meeting one of the modal criteria outlined in Figure 3.8 (a)-(c): sand or gravel at 20% and cobble from 40-80% inclusive. However, 3 of these 13 (categories 16, 17 and 20) did not contain any mussels, despite meeting the modal criteria and others displayed only small mussel counts. It is noticeable though that where more than one of the modal criteria is met (categories 2, 7, 8, 9 and 13) there is often a higher mussel count and more positive quadrats. Category 2 is the only exception. This may be because no sand is incorporated in this category matrix and Figure 3.9 (a) has shown that this variable has greater weight in increasing mussel presence probability than gravel. The suggestion that the interaction between substrates created an ideal stable environment explains this phenomenon (Hastie *et al.* 2000) and all substrate proportions must be assessed in synergy if adult mussel habitat is to be identified in the Rede. Interesting points can be drawn from this about the relationship between adult mussel distribution and substrate patterning.

Firstly the overall distribution is in accordance with that identified in the literature (Hastie *et al.* 2000; Hastie *et al.* 2003; Skinner *et al.* 2003; Hastie *et al.* 2004):

freshwater pearl mussels require a suitable substrate for burrowing but must also be stable via the presence of larger clasts. If this is not available, the mussels are vulnerable to entrainment in fast, turbulent flows (Vannote and Minshall, 1982). The difference between the amount of mussels located in the four modal categories (nearly 50%) and other areas is very distinct and the distribution of mussels between categories is significantly different from random (Section 3.3.2). While Spearman's rank- order correlation was applied to the individual substrate proportions, only sand's strong negative correlations with adult mussel presence was derived as highly significant ($P < 0.01$), the strong negative and strong positive relationship for gravel and cobble/boulder substrates with mussel presence (respectively) were significant only to $P < 0.1$. This is likely to be because mussels were shown to be more tolerant of *any* proportion of gravel and larger clasts. Nonetheless, as the relationship between quadrat scale substrate patterning and adult freshwater pearl mussel presence on the Rede was significant and agrees with the patterning found in other study rivers, the null hypothesis that substrate composition does not relate mussel presence can be rejected.

The relationship between freshwater pearl mussel distributions and substrate patterning on the Rede has been established here, but two further complexities exist that may be of interest in consideration of the sustainability of the remaining population in the face of habitat degradation. The importance of the presence of sand in a quadrat in terms of both the probability of it containing mussels and the number it will contain is undeniable: where there is more sand, there is more room for mussels. These areas are likely to be important for the formation of larger beds of *M. margaritifera*. However, mussel tolerance does display a threshold for this parameter (both in ground survey and terrestrial imagery results). Areas where the proportion of small clasts was too high were unsuitable as they were too mobile (Hastie *et al.* 2000; Venditti *et al.* 2010). No such threshold was evident for the larger clasts. What is more, there were a limited number of areas that fall into the key mussel harbouring categories (under 5% of the sampled area) with an 'ideal' sand proportion. Consequently, although cobble dominated areas do not show a high degree of mussel presence *or* large densities of mussels in them, they are still a very valuable habitat due to its prevalence in the Rede (65% of the area sampled contain over 80% cobble and boulder). As these areas are tolerated, it means some of these very abundant habitat areas are used. Areas where there are *no* small substrates (sand or gravels) would be unsuitable. They cannot sustainably inhabit these areas and would soon be lost downstream during high flow events. However, the reduction in flow velocity in the interstices between the cobbles and larger clasts allows the smaller substrates that would otherwise be susceptible to entrainment, to remain *in situ* (Hastie *et al*. 2000; Wetzel, 2001). The mussels can thus use these pockets of sand and gravels to maintain a suitable position in the river environment (Hastie *et al.* 2003). This type of habitat is restricted in the area it offers mussels for habitation but cumulatively it represents an important resource for the depleted Rede population and its value should not be underestimated.

The relationship between adult *M. margaritifera* distributions and habitat features is complex. At the catchment scale, water quality is uncertain. The literary evidence would suggest that all areas of the Rede are unsuitable for mussel habitation due to elevated levels of calcium, pH and conductivity (Bauer, 1988; Young, 2005). However, with the exception of the influence of tributaries, this inferior quality may be explained by the Rede's geological character and the species' presence here historically may indicate a degree of local adaption (Purser, 1985 in Young, 2005). In light of the former population's success and without further evidence, this is assumed to be the case. Therefore, at this largest scale of assessment the Rede habitat appears suitable for adult *M. margaritifera* habitation in the majority of locations. A more definitive assessment cannot be made due to the low density water chemistry sampling and the observed influence of tributaries.

The spatial distribution of *M. margaritifera* demonstrated relationships with all of the physical environmental parameters sampled at the microhabitat scale. These were distance from the channel margin, depth, flow, and substrate composition, though interpretations of relationships with wider scale habitat features were inferred from some of this, such as degree of channel shading via distance from the channel edge. The correlations between adult mussel presence and the conditions presented by the character of each of the four parameters were invariably robust, convincing and statistically significant. This applies in particular to distance from channel edge, depth and substrate character. That these three parameters are in accord with the spatial patterning of *M. margaritifera* populations observed in other locations (Gittings *et al.* 1998; Hastie *et al.* 2000; Hastie *et al.* 2003; Hastie *et al.* 2004; McLeod *et al.* 2005; Bolland *et al.* 2010) is worthy of note, as the least significant relationship was demonstrated by the parameter that did not conform fully to the literary standards: flow. With the lack of the stabilising effect of the habitual dense populations this species forms and the Rede's flashy regime (Smith and Jepsen, 2008), the refugia preference discussed above (Howard and Cuffey, 2003) may be a reasonable explanation for this, specific to the depleted Rede population conditions.

The extent of the significance of the identified relationships can lead us to reject the null hypothesis (that there is no relation between adult *M. margaritifera* presence and physical habitat character). Thus, in answer to the research question 'can areas of preferred habitat be identified or do physical environmental parameters demonstrate no relationship to *M. margaritifera* presence?' a conclusion can be drawn that preferred habitat character can be inferred from the results and *M. margaritifera* presence does display significant relationships with physical habitat parameters, in particular variation in substrate, distance from the channel edge and depth.

A considerable point of interest is the interrelation of the four habitat features. It has been indicated above that depth related to distance from edge, which in turn related to flow (both dependent on channel shape) which is intrinsically related to grain size distributions (Richards, 1982; Gomez, 1991). Richards (1982) also identifies how (specifically perimeter) sediments can be linked to depth. This should be taken forward in considering the relative importance of these and how they act together to create the overall ideal, preferred habitat. 'Habitat' inherently combines all parameters that are experienced by the species under study (McCoy *et al.* 1991). The character of each individual parameter will vary across scales and the interrelating processes identified here that lead to the development of certain characteristics desired by *M. margaritifera,* will also vary across these scales. No acknowledgement of relations between the areas sampled and spatial habitat patterning, in particular between adjacent quadrats, has been made. Some progress can be made towards this by addressing how the habitat features overlap within a quadrat to form the best available habitat. It must thus be remembered that areas where *some* of the ideal conditions are met could be useful habitat resources for the Rede mussel population as it is not certain that true optimal habitat exists. This may be as a result of habitat degradation but the lack of recruitment clearly suggests conditions are not ideal.

Many of the relationships identified here were highly significant. Progressing from this section of the discussion, the relative importance of each habitat feature and how these act in synergy to create ‘preferred’ habitat character can be clarified via consideration of the habitat models created and displayed in Section 3.5. The significant habitat features identified in the above assessment are condensed into the logistic regression model to define the precise character required of suitable *M. margaritifera* habitat. In reference to the ideas of ‘best available habitat’ discussed above, whereby some areas will be tolerated to a greater degree than others, preference models refine the logistic regression model to a level where the relative role of key factors in determining habitat suitability can be defined. This will further determine whether the mussel population is responding in the expected manner to environmental conditions or whether it is so degraded it is simply tolerating any conditions on the Rede and bears no relation to variation in the physical environment.

4.2.6. Logistic regression modelling

The logistic regression models are compiled only from habitat parameters that show a significant relationship to the dependent variable, mussel presence ($p < 0.05$). The model for the parameters observed in the ground survey showed less success than that derived from remotely sensed data, suggesting the input data needed to be as detailed as possible for substrate in particular. The photosieved grain size distributions used in the model from the remotely sensed data produced an overall model with a more significant pseudo R^2 than the ground survey model (Section 3.5.1). Both models are significant, however the predictive success of the ground survey data model is poor. This is likely to be due to the small proportion of positive quadrats, despite the use of a data subset for analysis.

Nonetheless, the model based on ground survey data assigned most predictive power to the variable ‘run’. In areas of run flows, there is a 2.3 increase in the odds of finding a positive quadrat. The importance of the other parameters can be seen in Chapter 3 but distance from the channel edge and the amounts of sand and cobble in the quadrat were also used to predict mussel presence by the model. The substrate variables both show negative relationships but the small coefficients suggest each unit change in these (in terms of percent coverage) has only a small effect on the chance of mussel presence. In the remotely sensed data model, grain size distributions at the 95th and 50th percentiles represented the most significant variables dictating the likelihood of mussel presence, followed by maximum depth of the transect. The negative correlation for D_{50} and positive correlation for D_{95} reiterate the preference for mixed substrate composition (Hastie *et al.* 2000).

The logistic regression models show that a number of factors need to overlap to create the ‘ideal’ habitat patch. Indeed, each of the environmental parameters considered in the study, as advocated by relevant literature, were represented in the models. This even extends to an attribute of flow, which did not relate fully to the literature standard (Hastie *et al.* 2004; Section 4.2.4). For a high probability of mussel presence it is crucial that these variables display appropriate character. If not enough of the predictors are in compliance with habitat requirements, the predicted outcome will not support a high probability of mussel presence.

It must be noted however, that the logistic regression models invariably displayed only small changes in the odds of mussel presence between different habitat conditions. The difference between 1 m from the edge and 6 m, which in some areas would be the difference between a supposedly preferred marginal habitat and an area in the centre of the channel, proffers only a 3.5 change in the odds of mussel presence, for example. While the inclusion of such a range of parameters is informative in terms of the range of factors that influence mussel habitat preference, the minimal impact they appear to have suggests it is highly justifiable to include the results of the preference modelling to verify the relative importance of each factor and that certain partitions (values) of these parameters are actively selected over others.

4.2.7. Preference modelling

The preference models again show cases of strong selection or avoidance for certain characteristics of each parameter, though some appear less significant when acknowledgement of proportional use of available habitat is included, as in these models. This applies to flow variables, for example.

A very high degree of selection is shown for habitat that is less than 20 cm in depth and within 2-3 m from the edge of the channel. The same substrate proportion categories appear to be actively selected as those presented above, where high mussel counts and a high degree of positive quadrats are seen (7, 8, 9 and 13). Category 12 is an additional preference contender, due to a high rate of positive quadrats, though they generally held fewer mussels. Understandably the proportions of individual substrate partitions and the grain size distributions are in accordance with the patterning represented by these categories. Presence of both fines and larger clasts are necessary, as a mixed composition, though the only areas strongly avoided are those containing a high percentage of fines (sands *or* gravels). It is interesting to note that substrate and depth related variables show the most extreme rates of selection and avoidance and these also constitute the variables included in the most successful logistic regression model (based on remote sensing data). Flow is the only variable that represents only small degrees of preference and avoidance; all flow partitions are close to representing neutral preference, which could be interpreted as an indication that this variable has least influence in defining patch character as preferred by *M. margaritifera.* Some areas are also consistently avoided, in addition to areas of high fines content, as mentioned. These include central areas of the channel and deep areas.

The similarities in preferences displayed by the *M. margaritifera* population on the Rede and those on the River Kerry (Hastie *et al.* 2000) are resolute. The study by Hastie *et al.* (2000) identifies habitat up to 3 m from the bank as “most heavily utilised” alongside depths of 30-40 cm. The survey in the Rede was carried out at a time of very low flow, anecdotally the lowest seen for many years following the dry spring. It is possible that a survey at other times would have found depth preferences slightly higher than those displayed in the preference modelling here but nonetheless, the similarity to the Kerry population is noteworthy in that mussels were found more commonly in the shallower areas available (maximum depth in the Kerry was 83 cm). Gittings *et al.* (1998) also found depths of 20 cm to hold the highest mussel densities. The substrate preferences are also highly concurrent with those identified by Hastie *et al.* (2000) at the sub-metric (quadrat) scale, both in terms of the preferred compositional matrix (mixed substrata, cobble/boulder dominated) and the strong predictive power of data on substrate composition on mussel presence. The Kerry study (Hastie *et al.* 2000) found substrate to be most closely related to mussel density. Using this variable in discriminant function models, they achieved high rates of success in predicting mussel presence. The significance of the substrate parameters in this Rede study was also evident; both in the strength of the degree of selection and avoidance motivated by varying substrate character and the inclusion of substrate as significant predictor variables in both of the logistic regression models.

4.3. Relevance of habitat patchiness to the contemporary River Rede population of *M. margaritifera*

Environmental parameters have been assessed across various scales and it is evident from the variation in character in the five observed parameters alone that the Rede habitat displays a certain degree of patchiness in character. Many areas of differing character are available for use but what is pertinent to this final research question is that certain habitat characteristics must overlap to form the preferred *M. margaritifera* habitat.

The existence of patchiness in the freshwater pearl mussel dispersal was demonstrated in Section 4.1. A significant contagious distribution was confirmed. However, this analysis implies only the existence of an aggregated distribution. More data would be required to establish the spatial scale of patchiness and the degree of aggregation at more extensive scales. Nonetheless, within the sampled reaches, relationships between these locations of mussel presence and habitat character were established as statistically significant.

The interpretation of the logistic regression results suggested that habitat character was important to the *M. margaritifera* population in the Rede. Each of the physical environment parameters were identified as very significant predictors of mussel presence. The inclusion of this range of habitat factors, which must all display the appropriate character in combination to give a high likelihood of adult mussel presence, is a good indication that only certain, tightly constrained habitat character is appropriate for *M. margaritifera*. The character of this habitat must be of an exacting depth and substrate composition, within 3 m the bank and ideally be situated in a run. In this respect it can be considered homogenous as any departure from this character would reduce the probability of mussel presence. It would thus be distinct from any other area and consequently fit the description of a patch from Forman (1995) and Thorp *et al.* (2006) as adopted in this study.

The key feature of these models that is that they combine a number of habitat factors. Individually these factors will vary over spatial contexts creating a matrix of habitats of differing character (McAuliffe, 1983; Wu and Loucks, 1995; Thorp *et al.* 2006). In some areas these habitat parameters will exhibit characteristics that are compliant with *M. margaritifera* preferences. Where the appropriate habitat characteristics are fed into the models, the interaction of these values creates a point within the channel matrix that displays a character in accordance with *M. margaritifera* preferences.

A degree of accord is observed between this study and that of Hastie *et al.* (2000), where mussel distributions were recognised as patchy. The associations discerned between freshwater mussel distributions and physical habitat on the Rede were also consistent with other studies' findings. Notably this includes the work on the River Spey (Hastie *et al.* 2003) where channel shading (associated with distance from edge on the Rede) and stable substrates demonstrated positive associations with mussel distribution and mobile, gravel-pebble substrates the opposite trend. Interestingly, while run features were positively associated with mussel distribution on the Spey, as found here, there is disagreement between mussel presence as a function of low turbulence flows in the Rede and that in other rivers (Hastie *et al.* 2003; Hastie *et al.* 2004). Gittings *et al.* (1998) found flow type to have no significant influence over mussel distribution densities beyond that described by depth and channel shading, which constituted their main related features.

The strength of the selection and avoidance values given in the preference models reiterates *M. margaritifera* preferences for certain qualities in the habitat parameters. This particularly applies to depth and substrate composition. Furthermore, there is literary support that the character of these parameters is key in defining an area that would be suitable for mussel presence (Gittings *et al.* 1998; Hastie *et al.* 2000). These interpretations lead to the conclusion that, as appropriate conditions must be met at the microhabitat scale in parameters that inherently vary across multiple scales, habitat patchiness is relevant to the remaining *M. margaritifera* population in the Rede. The features of habitat that influence habitat quality vary in significance: substrate composition and depth were found to be most influential; therefore mussel distributions can be expected to respond primarily to patchiness in these parameters.

4.4. Implications of findings for the River Rede

The original motivation for undertaking this investigation was in response to the decline of *M. margaritifera* as a result of habitat degradation in the Rede (Environment Agency/E$_3$ Ecology report, 2006). A study of available habitat and freshwater pearl mussel distributions in four representative locations on the river has been made and analysis has led to the conclusion that the habitat character does appear to be relevant to the Rede mussel population, as it is in other populations (Hastie *et al.* 2000). The Rede mussel population clearly shows preferential use of areas close to the channel edge, with appropriate depth and substrate composition. The strong relationships between habitat parameters and mussel presence indicate a positive response to habitat, as in healthy, functioning populations. However, the value of this finding must be considered relative to conditions in the study river. While the observed patterning of habitat and the robust links to mussel distribution are promising, there is clearly some evidence of adaption or tolerance as a larger than expected range of habitat conditions are utilised (in terms of water quality and flow for example) relative to those seen in other populations. This may be in relation to habitat degradation but implications for recruitment success and population sustainability are certainly of concern. These findings can be applied to the current situation in the River Rede and indicate where threats to survival may be arising: if any disturbance is causing one or more of the required habitat standards to be changed, the mussel population will respond to this and may avoid using the affected area of the Rede. These should be addressed to improve the chances of maintaining this mussel population.

Evaluation of the relevance of habitat character patchiness involved the analysis of parameters that vary across many scales and ultimately it is the culmination of appropriate habitat qualities at one scale, that experienced by individual freshwater pearl mussels, that represents patches of suitable or preferred habitat (McCoy *et al.* 1991; Fausch *et al.* 2002; Thorp *et al.* 2006). However, because the parameters that influence these patches (substrate composition, depth etc.) are driven by processes at much wider scales (Thorp *et al.* 2006), these are still important to the interpretation of the findings in terms conditioning physical river character and the resulting patchiness or heterogeneity of available microhabitat. The extent to which this occurs cannot be fully assessed as spatial context data are not available, however, some suggestions can be made regarding the impact of habitat degradation processes.

Habitat degradation can occur at any scale and will have implications for the type of habitat available and, in turn, the extent of suitable *M. margaritifera* habitat that is available (Bolland *et al*. 2010; Österling *et al*. 2010). In location A, conditions in the Rede are seen to be different in character to the other sampled areas. The high proportion of sands and gravels and the more extensive areas of greater depth following channel engineering throughout this section (Environment Agency/E_3 Ecology report, 2006) will mean that patches of preferred habitat character are uncommon. Very little, if any, suitable *M. margaritifera* habitat will exist in this section according to the models utilised in this study. Channel adjustment in this section will also have impacts in other areas. For example, increased bank erosion here and the decreased bed stability, where armouring does not occur, will result in the ingress of fines in the substrate downstream. Again this habitat degradation will result in too high a proportion of fines in the substrate for (juvenile) mussel tolerance. In this situation, patches of habitat with characteristics in accordance with the established mussel preferences will be rare in the Rede (Moorkens *et al.* 2007). Furthermore, this will have implications for aspects of physical habitat that were not considered in this study, but are nonetheless cited as crucial in defining suitable mussel habitat (Buddensiek *et al.* 1993).

Many other threats to *M. margaritifera* exist, such as impacts of quarry works, forestry, certain agricultural practices and human activities (Moorkens *et al.* 2007; Österling *et al.* 2010) which apply equally to the Rede catchment (Environment Agency/E_3 Ecology report, 2006). These have the potential to affect the depth of the channel, substrate composition and flow patterning in particular; parameters which must all be appropriate to mussel requirements for a sustainable population to flourish. Other aspects of the river environment (for example water quality), not included within the variables observed to influence patch occurrence in this study, may also change (Bauer, 1988; Buddensiek *et al.* 1993). The processes causing degradation can occur at many scales and may induce changes to habitat character at any scale. Environmental processes occurring at different scales are instrumental in defining habitat patchiness and this means that if degradation continues to occur, the extent of suitable habitat in the Rede may decline or change across both spatial and temporal scales (McCoy *et al.* 1991). This would limit the sustainability of the Rede *M. margaritifera* population. It is evident that an holistic approach to management across all relevant scales is needed to improve the situation on the Rede, as amelioration of conditions across scales is needed for suitable habitat character to form and remain (Fausch *et al*. 2002). This could involve a high degree of landowner or public engagement to ensure progress is catchment-wide (Mostert, 2003; Tippett *et al*. 2005). The Tyne Rivers Trust is already making progress with this approach (Atkinson, *pers. comm.*). Indications from this study that the majority of the freshwater pearl mussel population responds with positive selection to certain, available habitat characteristics are a positive sign for the River Rede freshwater pearl mussels.

4.5. Limitations and further recommendations

4.5.1. Limits of spatial coverage

While this investigation has attempted to incorporate physical habitat parameters at as many relevant scales as possible within the time constraints of the project, limitations generally relate to the extent of the applicability of the findings. Many of the habitat parameters presented highly significant relationships with *M. margaritifera* presence and one of the primary pieces of evidence indicating the importance of habitat patchiness was the logistic regression modelling. The statistical significance and success of the remote sensing data model in particular remains evident but this was only based on a series of 200 images on 45 transects. The images were representative of the types of habitat available on the Rede but still covered an insubstantial amount of the Rede as a whole. As the character and quality of other areas cannot be defined from the available data, the patterns of association between habitat and *M. margaritifera* distributions cannot be quantitatively extrapolated beyond the sampled areas.

A further limitation to this project is the lack of spatial correlation between habitat factors in terms of the real space they occupy in the Rede. No account of autocorrelation of factors has been made. While the character and basic existence of suitable habitat has been identified, there is no demarcation of the extent to which each of the significant habitat characteristics overlap, thus no idea of the extent or number of appropriate patches that exist. Future geo-referenced data should therefore be put in a more formal spatial context to give more constructive results. The extent to which this was done in this project was primarily restricted by the scope of the aims and available data but also, to a certain degree, by the restriction of the use of maps for security reasons.

If the extent of habitat patchiness, the degree of aggregation across scales and the scale and recurrence of habitat patchiness (Thorp *et al.* 2006) were established, it would allow progression from establishing the relevance of habitat patchiness, to establishing the applicability of patch theory. As established in Section 1.2, this must include implications of habitat patch *dynamics* and adds a spatial (and temporal) context that would allow a better understanding of links between population persistence and patch availability. This will also aid the quantification of habitat degradation.

In light of these potential benefits, future enquiries should focus on expanding the scales of assessment. While the results of this investigation cannot be extrapolated across the catchment, the techniques employed are applicable to extension of the work. The possibility exists for spot checking some known larger beds to see if both the *M. margaritifera* distribution trends and the strength of the association between habitat character patchiness and mussel presence continue. This would be particularly valuable for assessment of areas of dense mussel habitation as these were not well represented in this study. Upper areas of the Rede should also be sampled, where former *M. margaritifera* populations existed (Environment Agency/E_3 Ecology report, 2006).

With a full overview of current habitat patch and mussel distributions, areas and sources of severe habitat degradation could be identified and, if the assessment of these factors were made via the accumulation of data across all relevant scales, the outcomes would be more representative of the actual situation in the Rede (Fausch *et al.* 2002). On a scale by scale basis, remediation of the Rede freshwater pearl mussels' circumstances could potentially be made.

4.5.2. Recommendations for future approaches

Application of methods

After the success of the logistic regression modelling derived from remotely sensed (terrestrial image) data, the expansion of sampling in the Rede could continue to use remote sensing techniques to rapidly acquire more extensive river habitat data. Both the boom method used here (and in studies such as that of Bird *et al.* 2010) and methods involving a UAV, as utilised in this study for width data and far more extensively by other studies (Dugdale, 2004; Dugdale *et al.* 2010) would advance knowledge of habitat availability on the Rede considerably by expanding the scale of assessment (Carbonneau *et al.* 2005). Quantification of the full extent of available habitat will be useful now that it has been established that the Rede freshwater pearl mussel population responds to habitat character patchiness. There are several considerations associated with these methods, such as those of atmospheric and river conditions (amount of suspended sediment etc.). Implications of these should not be underestimated in terms of time and cost efficiency but while these have already been found problematic on the Rede, during this study, the value of the remotely sensed data is considerable and was found to be worth the required effort.

Development of knowledge of the key habitat parameters

Assessment of the relationships between *M. margaritifera* distributions and habitat parameters revealed some traits seemingly specific to the Rede population. In certain cases in this study more specific requirements of the data were needed. Future work should consider observing flow in terms of actual flow velocity, rather than a broad classification of flow type. The analysis of the more comprehensive data from remote sensing indicated that a greater level of detail yields more information on distribution associations. More clarification of the influence of flow will explain the relevance of the refugia theory (Howard and Cuffey 2003) and, combined with temporal data could identify whether the presence of single mussels is indicative of distributions associated with flood events (Vannote and Minshall, 1982). A finer sampling scale would also benefit the quantification of water quality. This factor was not a major feature in the design of this study due to the restricted time available. However, the broad assessment that was made highlighted some potential issues on the Rede and this should be investigated as any issue at this scale will have profound impacts on the narrower scales in the hierarchy (Thorp *et al.* 2006).

To further define *M. margaritifera* distributions in respect to habitat variables, the close-range remote sensing in particular could be applied to areas of the Rede harbouring larger mussel beds. In these areas a more distinct ‘edge’ could be evident between used (preferred) habitat and that which seems to be avoided. Identifying what physical or chemical changes occur across this ‘edge’ habitat may be enlightening in terms of both the extent of useful habitat in the Rede and what limiting factors there are to this. Its value to conservation could therefore be considerable. This strategy is more appropriate to the study of larger beds as the occurrence of false negatives (where habitat is suitable, but not in use due to the deliberate removal of mussels) is less likely and the edge is thus easier to identify. It would require a survey of slightly different areas to those used in this study (areas of mussel presence would be targeted specifically) but it would be a worthwhile use of the methods employed here.

Assessment across temporal scales

Consideration of habitat and *M. margaritifera* distribution variation across temporal scales was not within the scope of this investigation. However, in terms of securing a sustainable population, it is necessary to further these findings by ascertaining details of patch longevity (Pringle *et al.* 1988). While the evidence presented in this study suggests that mussel distribution is related to habitat patchiness, the persistence and stability of these habitat characteristics must be adequate for mussel survival over their long lives. Research into the long term stability of habitat on the Rede, combining spatial and temporal assessments, will contribute to understanding of the impact of habitat degradation and thus drive appropriate management.

Further testing of models

The models derived from these datasets have been valuable in defining the relationships between habitat character patchiness and mussel distributions on the Rede. However, these outcomes may be very specific to the character of the Rede population in terms of the way it responds to habitat. For example, one interpretation of the relationship between flow and mussel distributions is that mussels in depleted, low density populations require refugia from high magnitude flow events. This is in contrast to healthy populations where the stability of large mussel beds counteracts this disturbance. In order to test the findings observed at this site and to establish the extent of the value of the models created in this study, further testing should be carried out using data from larger, recruiting populations and in rivers with different characteristics.

Chapter 5 Conclusions

5.1. Conclusions

In accordance with scales of assessment proposed in the literature, information on mussel distributions and habitat patterning was collated across the River Rede catchment, with a focus on four locations at the reach scale. Data collection at the catchment scale was unsuccessful, providing only the river coordinate system used as spatial context data. Habitat at the catchment scale is represented in terms of water quality data. Habitat at the reach and sub-reach scales was assessed via the amalgamation of data from the pool/riffle system scale and at the microhabitat scale (Frissell *et al.* 1986). These were obtained via the analysis of remotely sensed images and through more traditional ground survey methods respectively. A survey of freshwater pearl mussel presence was also undertaken at the microhabitat scale.

The ground survey effort yielded approximately 3% positive, 'mussel containing' quadrats, resulting in a total of 310 mussels, found across 135 quadrats. Examination of *M. margaritifera* distributions revealed significant levels of contagiousness, fitting to a negative binomial probability distribution (r = 0.97, $P < 0.01$). This patchiness was found to be highly correlated to the distribution of certain habitat characteristics.

The degree of variation observed in habitat parameters conferred evidence of habitat character patchiness. Water quality was assessed in relation to established *M. margaritifera* tolerances (Beasley and Roberts, 1999; Oliver, 2000; Young, 2005). Nitrate and dissolved oxygen saturation levels were suitable throughout the Rede. Calcium, conductivity and pH observations were within tolerance parameters throughout the areas of known mussel presence during winter sampling but all rose above tolerance thresholds in spring. This may however be attributed to catchment geology and a degree of local adaption may exist among the Rede *M. margaritifera* population (Purser, 1985 in Young, 2005). Depth and distance from channel edge both displayed significant negative correlations with mussel presence. Flow type demonstrated the weakest relationship with mussel presence, though the association was complex and not fully in accordance with patterning found in past studies. Mussels were associated with both runs and, against convention, low turbulence areas. This may be due to the flashy regime of the Rede necessitating the depleted population to use areas of lower turbulence as refugia (Howard and Cuffey, 2003). Mussel distributions were significantly related to certain compositions of substrate, preferring cobble and boulder dominated areas with some sand and gravel presence. These areas are stable but provide suitable burrowing substrates for the mussels' stabilising foot. This was in accordance with the literature (Gittings *et al.* 1998; Hastie *et al.* 2000) although areas of more frequently occurring microhabitat character, comprising over 80% cobble and boulder, were also utilised to a certain extent as they offer a stable environment. These areas were not associated with areas of high mussel aggregation but their relative abundance suggests they are an important habitat resource.

The mussel distributions were shown to relate strongly to physical environmental parameters but furthermore to occur as a function of physical habitat character patchiness. The features that contribute to the formation of appropriate habitat vary across a hierarchy of nested scales, but this is not exclusive of the other factors acting at adjacent scales. Logistic regression modelling demonstrated that, cumulatively, the patchy character of these environmental features must overlap, displaying appropriate characteristics, for mussel presence. A depth of less than 20 cm (in low summer flow conditions) and substrate composition as a mixture of sand and large clasts were principle features of habitat preference, relating most convincingly to areas of freshwater pearl mussel presence. A high probability of mussel presence was associated with small distances from the channel edge (less than 3 m); this feature also has implications for the degree of channel shading. Flow displayed some relevance in defining suitable habitat patches, with mussel presence most likely in 'run' flows.

From the strength of selection and avoidance shown in the preference models, it was inferred that the Rede *M. margaritifera* population responded to habitat based on the degree of suitability of its character. While logistic regression modelling can identify the best available habitat, when considered in combination with preference models it appeared that a range of amenable habitat is utilised in the Rede. Though this was still within the constraints of the definition of the *preferred* patch and areas retained their distinction from the surrounding matrix (it must be remembered that some habitat areas were actively avoided), it is likely that some degree of tolerance existed within the population, so that they could take advantage of the available habitat. This tolerance may have developed as a result of deteriorating habitat quality. Interrelation between habitat features across spatial scale contexts is a crucial feature in the consideration of *M. margaritifera* habitat patchiness.

Valuable conclusions presented here could contribute to habitat management. Rede mussels still maintain contagious distributions, as seen in other functioning populations (Hastie *et al.* 2000). The Rede *M. margaritifera* populations also appear to respond to habitat character patchiness. This is promising for the maintenance of the current population, although the fact that the surveyed adult mussels must tolerate some sub-optimal habitat is less encouraging for potential juvenile survival. There were reservations that Rede freshwater pearl mussels may exist only as deposited by floods or only in areas of historic habitation where conditions have changed and are now seemingly arbitrary. While this may be the case for some individual mussels, this study suggests this is not the case throughout the Rede: statistically significant patterning shows aggregations in distinctly defined habitat.

As habitat degradation is frequently a serious cause of *M. margaritifera* decline in rivers around the world, survey approaches employed here could be applied in other systems where the population has become depleted. If a freshwater pearl mussel population is found to be responding to habitat patchiness, as has been identified in healthy populations, working to manage change and habitat quality at appropriate scales, considering valid interrelating features, could be successful. In this approach there is an improved chance that the habitat features essential for *M. margaritifera* survival will be remediated at meaningful levels. Although the microhabitat scale is the final stage at which ideal habitat conditions should be represented for *M. margaritifera* habitation, it is clear that habitat quality at every stage of the scale hierarchy is relevant to sustainable *M. margaritifera* survival. The development of the use of terrestrial images to aid the collection of data at intermediate scales has proven to be a valuable progression from traditional survey techniques. In addition to the positive conclusions regarding the Rede mussel population, the efficiency of data collection and the additional detail achieved using terrestrial imagery can be considered a further positive advance.

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Appendix A Environment Agency Rede Water Quality Monitoring Data

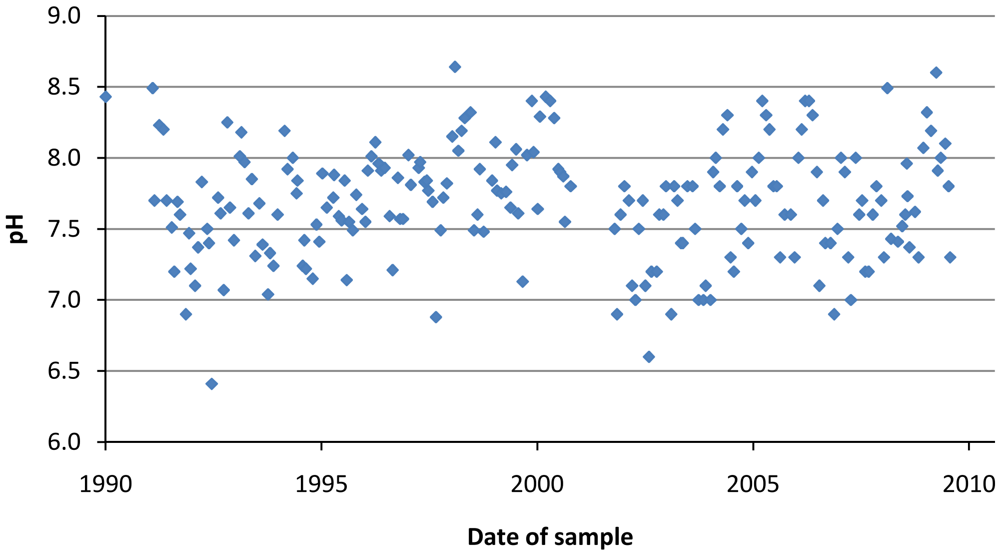

Figure A.1 Variation in pH since 1990.
Source: Environment Agency, 2009.

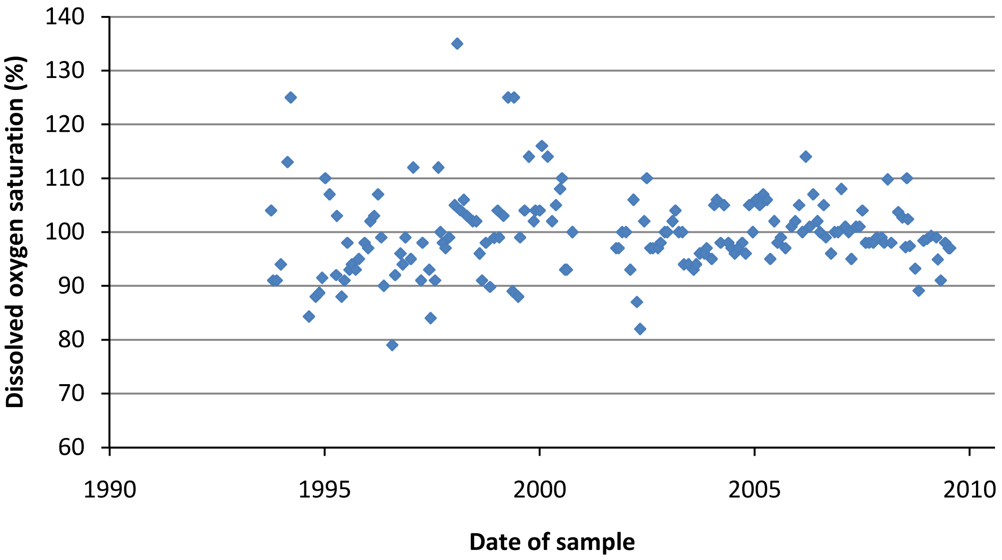

Figure A.2 Variation in dissolved oxygen saturation since 1994
Source: Environment Agency, 2009.

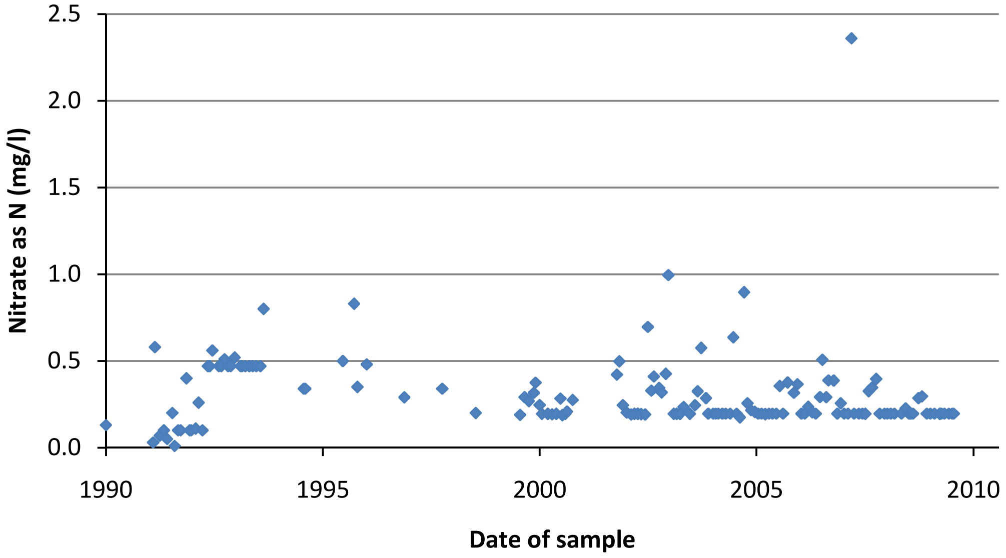

Figure A.3 Variation in Nitrates since 1990

Source: Environment Agency, 2009.

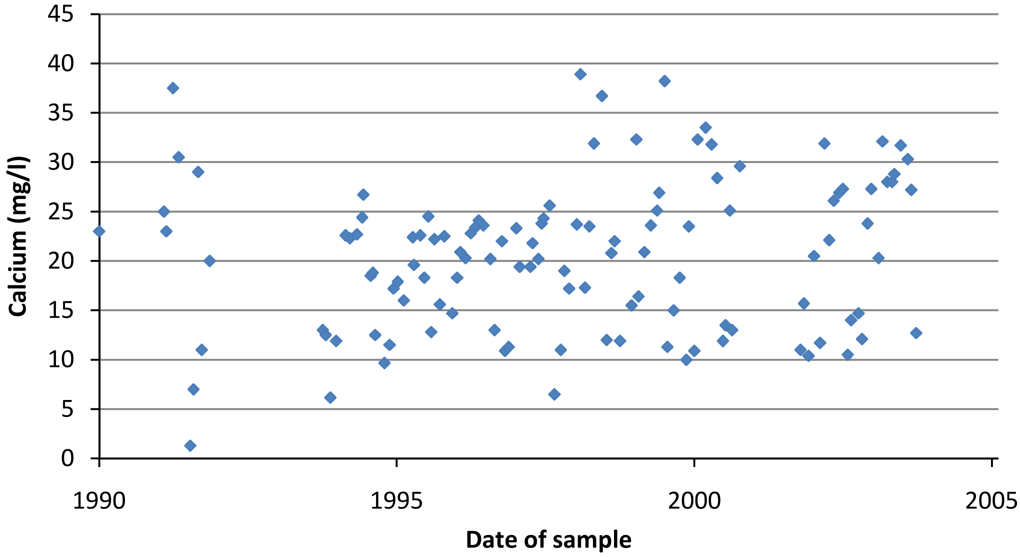

Figure A.4 Variation in Calcium since 1990

Source: Environment Agency, 2009.

Appendix B Substrate Index

Table B.1 Proportion categories for the 21 different combinations of substrate proportions

The modal categories are highlighted. These represent both the compositions harbouring the majority of mussels and the quadrat compositions in which the most positive quadrats were identified.

Sand	Gravel	Cobble and boulder	Proportion Category
0	0	100	1
0	20	80	2
0	40	60	3
0	60	40	4
0	80	20	5
0	100	0	6
20	0	80	7
20	20	60	8
20	40	40	9
20	60	20	10
20	80	0	11
40	0	60	12
40	20	40	13
40	40	20	14
40	60	0	15
60	0	40	16
60	20	20	17
60	40	0	18
80	0	20	19
80	20	0	20
100	0	0	21

Appendix C Terrestrial Imagery Data

The table comprises only images that were photosieved and included in the analysis. Images that were unsuitable for use have been withdrawn.

Transect	Image	Distance downstream	Grain sizes (mm)					Depths (cm)					Average transect depth (cm)	Minimum transect depth (cm)	Maximum transect depth (cm)	Mussel presence
			D5	D16	D50	D84	D95	1	2	3	4	5				
5	IMG_7012	42031	0.0	3.1	7.8	17.4	28.8	12.5	29	41	56	76	42.9	12.5	76	0
	IMG_7018		0.0	0.0	9.7	20.2	33.7									0
	IMG_7020		1.0	3.5	12.5	27.1	129.5									0
6	IMG_7025	42025	1.0	2.4	9.1	20.8	35.1	16	20	36	40	64	35.2	16	64	0
	IMG_7027		0.0	17.9	104.5	229.4	334.3									0
7	IMG_7034	42021	0.0	13.8	69.0	137.4	480.9	8	20	26	34.5	38.5	25.4	8	38.5	0
	IMG_7038		2.7	23.1	83.7	238.8	381.4									0
8	IMG_7049	42019	8.0	25.1	75.1	161.7	249.4	6	15	26	33	38	23.6	6	38	0
	IMG_7050		11.3	40.8	126.6	263.0	365.0									0
	IMG_7052		8.5	30.9	139.9	324.5	479.6									0
9	IMG_7057	42021	0.0	32.2	106.6	206.4	375.9	26.5	36	29	27	16.5	27	16.5	36	0
	IMG_7061		20.2	55.8	112.1	245.6	334.8									0
	IMG_7067		45.3	72.0	210.0	337.3	417.1									0
10	IMG_7075	41999	0.0	44.5	89.9	153.9	261.7	14	34	55	69	78	50	14	78	0
	IMG_7078		30.2	64.0	179.2	363.0	585.7									0
11	IMG_7405	41673	19.6	46.5	100.5	195.9	292.9	12	41	41	73	55	44.4	12	73	0
	IMG_7408		32.8	67.6	175.5	303.5	440.4									0
	IMG_7416		37.1	69.0	162.4	295.9	417.2									0
	IMG_7418		40.8	99.3	200.7	337.3	531.8									0
12	IMG_7422	41676	3.1	20.5	58.2	193.8	425.2	13	23	60	55	40	38.2	13	60	0
	IMG_7426		8.8	25.4	125.6	313.2	480.8									0
	IMG_7428		32.0	44.5	100.6	310.0	524.9									0
	IMG_7431		16.4	39.2	126.5	226.3	314.9									0
	IMG_7436		53.0	78.4	116.1	264.5	410.7									0
13	IMG_7438	41671	3.1	19.2	53.9	658.6	849.1	7	16	10	28	37	19.6	7	37	1
	IMG_7445		8.2	20.6	107.1	381.7	827.9									1
	IMG_7447		3.3	26.0	63.1	158.8	568.2	3	9	17	37	49	23	3	49	1
14	IMG_7449	41669	20.7	36.2	67.2	242.5	532.2									1
	IMG_7450		20.4	33.3	120.0	272.8	377.5									1
	IMG_7453		5.8	23.3	94.8	268.8	387.6									1
15	IMG_7455	41669	5.4	23.8	71.2	225.9	517.0	7	32	44	46	55	36.8	7	55	1
	IMG_7457		6.4	29.7	98.1	312.5	494.6									1
	IMG_7459		4.2	20.1	54.1	188.0	513.9	14	25.5	22	38.5	53	30.6	14	53	1

16	IMG_7461	41669	9.4	23.0	73.9	151.3	395.4									1
	IMG_7465		17.4	40.1	78.6	173.5	278.0									1
	IMG_7468		3.7	24.2	95.8	257.3	354.0									1
17	IMG_7478	41503	5.2	20.0	53.9	100.8	163.2	11	15	11	15	14	13.2	11	15	1
	IMG_7480		0.0	4.2	51.1	89.8	135.9									1
	IMG_7481		0.0	3.4	57.4	131.9	316.8	8	17	12	7	16	12	7	17	1
	IMG_7487		5.0	18.3	44.7	87.2	118.9									1
18	IMG_7493	41512	11.7	19.1	52.6	95.1	127.1	9	18	7	20.5	42	19.3	7	42	0
	IMG_7495		5.0	16.7	57.5	113.6	243.5									0
	IMG_7498		2.3	14.5	61.6	123.4	289.4									0
	IMG_7499		0.0	9.5	85.2	188.7	272.3									0
	IMG_7502		9.8	35.9	87.3	216.8	281.9									0
	IMG_7504		6.4	14.2	91.7	203.2	297.8									0
	IMG_7507		8.2	17.1	105.4	228.5	407.7									0
	IMG_7509		14.2	39.0	119.4	233.7	441.9									0
19	IMG_7513	41450	12.3	25.1	78.1	167.9	444.4	8	10	30	69	74	38.2	8	74	0
	IMG_7515		16.0	41.1	125.2	257.6	547.2									0
	IMG_7522		78.3	119.2	206.5	337.2	421.6									0
	IMG_7524		13.0	54.3	122.0	244.2	388.6									0
20	IMG_7528	41393	0.0	1.6	51.2	132.8	188.9	16.5	31	44	51	49	38.3	16.5	51	1
	IMG_7529		0.0	0.0	78.5	205.4	356.8	15	29	16	28	28	23.2	15	29	1
	IMG_7531		0.0	0.0	82.0	203.9	222.4									1
	IMG_7533		0.0	5.3	123.0	264.5	569.1									1
	IMG_7535		7.3	98.6	264.0	446.9	550.5									1
	IMG_7543		0.0	1.4	30.4	107.6	251.6									1
	IMG_7547		0.0	4.6	59.9	147.5	221.4									1
	IMG_7549		0.0	3.6	38.0	101.3	172.2									1
	IMG_7552		0.0	7.4	64.3	145.8	221.0									1
21	IMG_7555	26901	0.0	0.0	6.2	18.7	33.9	52	45	35	25	18	35	18	52	0
	IMG_7558		0.0	0.0	22.5	34.2	51.4	57	62	50	39	23	46.2	23	62	0
	IMG_7559		0.0	0.0	11.4	35.1	35.1	56	47	41	35	36	43	35	56	0
	IMG_7561		0.0	0.0	6.7	26.9	70.7									0
	IMG_7563		0.0	2.3	16.1	52.1	79.3									0
	IMG_7570		0.0	0.0	6.2	31.1	47.8									0
	IMG_7571		0.0	0.0	6.6	21.8	68.0									0
	IMG_7574		0.0	0.0	11.5	29.0	65.6									0
22	IMG_7577	26888	0.0	0.0	0.0	12.9	30.3	65	76	70	74	77	72.4	65	77	0
	IMG_7580		0.0	0.0	0.0	17.4	58.0									0
	IMG_7581		0.0	0.0	5.8	28.4	36.4									0
	IMG_7583		0.0	0.0	12.3	33.3	63.7									0
	IMG_7586		0.0	3.0	17.7	41.6	125.5									0
23	IMG_7590	26995	0.0	0.0	6.2	30.2	69.3	54	48	20	54	64	48	20	64	1
	IMG_7592		0.0	0.0	13.5	31.8	46.8									1
	IMG_7595		0.0	2.6	2.6	48.0	65.9									1
	IMG_7596		0.0	0.0	26.3	53.3	95.2									1
	IMG_7598		0.0	0.0	24.5	80.6	142.0									1

	IMG_7603		0.0	0.0	24.1	76.0	153.3									1
	IMG_7604		0.0	0.0	11.6	167.0	294.0									1
	IMG_7608		0.0	0.0	10.0	30.7	50.3									1
	IMG_7612		0.0	0.0	22.2	34.8	71.8									1
	IMG_7614		0.0	0.0	18.7	49.4	96.4									1
24	IMG_7619	26585	0.0	9.4	17.9	31.3	45.6	14	40	63	62	53	46.4	14	63	0
	IMG_7621		0.0	0.0	24.6	46.2	76.0									0
	IMG_7623		0.0	0.0	23.5	105.8	335.9									0
	IMG_7625		0.0	0.0	17.4	76.9	214.4									0
	IMG_7627		0.0	0.0	1.9	54.6	100.9									0
25	IMG_7633	26602	0.0	2.2	15.2	32.9	52.0									0
	IMG_7634		0.0	0.0	15.4	29.6	55.9									0
	IMG_7636		5.3	10.6	30.0	90.4	159.7									0
	IMG_7641		0.0	5.2	33.9	82.7	139.2									0
26	IMG_7644	34618	36.1	58.0	104.1	157.1	307.8	30	20	43	24	12	25.8	12	43	0
	IMG_7646		48.9	66.4	144.2	240.4	407.5									0
	IMG_7651		21.1	54.3	128.3	214.9	358.2									0
	IMG_7655		23.5	50.6	119.4	368.0	457.4									0
	IMG_7658		16.4	47.9	117.9	281.9	448.1									0
27	IMG_7666	34676	16.5	47.6	105.9	248.8	621.1	14	40	21	34	25	26.8	14	40	1
	IMG_7668		56.6	69.1	108.3	234.8	582.0	17	16	23	40	25	24.2	16	40	1
	IMG_7670		38.6	57.7	103.4	172.7	241.8									1
	IMG_7671		42.6	54.1	101.5	197.5	300.1									1
	IMG_7682		33.8	60.5	141.0	277.4	369.4									1
	IMG_7683		39.3	56.2	110.2	216.6	289.2									1
	IMG_7686		30.5	68.4	117.1	232.5	283.0									1
28	IMG_7687	34705	11.2	31.6	72.4	210.6	518.1	14	7	12.5	7	7	9.5	7	14	1
	IMG_7690		9.2	18.7	71.6	211.5	388.8									1
	IMG_7692		6.9	26.9	54.4	220.7	334.5									1
	IMG_7694		6.3	24.8	75.4	137.0	314.7									1
	IMG_7695		17.3	34.0	69.1	181.3	324.1									1
	IMG_7698		13.1	23.1	65.2	219.8	296.5									1
	IMG_7700		15.2	26.9	92.2	161.3	227.3									1
	IMG_7702		11.8	40.1	91.3	200.9	253.9									1
	IMG_7703		8.2	21.1	76.4	210.2	307.5									1
29	IMG_7707	34732	0.0	4.8	39.5	91.4	143.9	27	19	23	24	29	24.4	19	29	1
	IMG_7709		7.7	16.5	47.9	83.3	145.4									1
	IMG_7710		6.4	20.7	72.9	149.5	198.8									1
	IMG_7712		26.0	38.3	93.1	187.7	288.6									1
	IMG_7714		21.1	65.8	134.2	253.9	322.7									1
	IMG_7717		15.6	26.7	125.3	241.9	312.1									1
	IMG_7721		22.1	36.8	85.7	186.5	292.3									1
	IMG_7724		9.1	27.4	51.0	118.8	196.6									1
	IMG_7726		15.0	26.5	68.7	127.3	175.8									1
30	IMG_7730	34756	46.5	62.3	138.4	259.7	367.5	19	30	23	39	59	34	19	59	0
	IMG_7731		35.2	68.2	154.4	261.5	348.9									0

31	IMG_7739	34779	0.0	5.3	21.5	86.0	394.4	30	33	39	44	6	30.4	6	44	1
	IMG_7741		0.0	0.0	48.6	119.7	270.9									1
	IMG_7743		0.0	4.9	60.3	104.9	149.7									1
	IMG_7745		15.1	59.2	100.6	151.6	208.4									1
32	IMG_7750	34866	5.4	44.0	114.0	195.5	294.6	15	11	11	15	12	12.8	11	15	1
	IMG_7752		14.3	21.0	58.5	141.3	191.5									1
	IMG_7761		4.3	12.2	56.5	135.7	215.0									1
	IMG_7764		5.4	10.6	78.9	160.6	249.1									1
33	IMG_7771	34896	13.6	30.4	121.7	286.3	434.9	40	37	28	22	23	30	22	40	0
	IMG_7774		10.6	26.5	49.4	140.2	315.6	32	42	32	23	21	30	21	42	0
	IMG_7781		12.9	28.2	64.4	171.3	279.9									0
	IMG_7782		9.1	21.6	80.2	218.9	303.9									0
	IMG_7784		18.3	34.2	89.1	249.2	458.5									0
	IMG_7786		19.7	44.1	91.0	175.1	273.0									0
34	IMG_7790	35441	27.1	52.4	89.7	148.4	181.1	18	27	61	66	77	49.8	18	77	0
	IMG_7792		56.6	68.1	125.8	200.9	291.4	46	68	49	57	48	53.6	46	68	0
	IMG_7794		33.2	48.9	84.6	125.6	170.1									0
	IMG_7797		42.3	57.1	93.2	144.2	187.9									0
35	IMG_7809	34661	0.0	0.0	4.0	38.3	168.9	31	25	27	65	78	45.2	25	78	1
	IMG_7810		0.0	4.2	35.2	206.7	430.8									1
	IMG_7813		0.0	5.2	25.2	79.1	237.0									1
	IMG_7815		3.1	10.3	39.1	76.9	179.7									1
	IMG_7817		2.7	11.2	35.5	85.3	261.3									1
	IMG_7821		32.0	60.0	127.4	288.0	647.9									1
36	IMG_8002	34349	40.8	60.0	127.8	251.2	310.7	12	17	25	30	37	24.2	12	37	1
37	IMG_8010	34323	15.1	38.7	112.3	212.8	359.1	13	30	27	18	26	22.8	13	30	0
	IMG_8012		13.2	51.9	105.4	230.7	407.4									0
	IMG_8014		6.0	43.4	134.6	289.7	650.1									0
38	IMG_8029	34247	16.7	56.9	104.4	164.4	228.7	19	22	15	32	17	21	15	32	1
	IMG_8032		27.7	39.5	78.5	156.1	251.3									1
	IMG_8036		20.5	42.4	90.7	200.1	539.0									1
	IMG_8037		23.0	48.4	104.0	221.5	454.5									1
	IMG_8042		9.5	42.2	72.6	123.1	245.3									1
	IMG_8043		41.8	55.8	114.1	296.9	952.1									1
	IMG_8046		27.4	46.1	79.3	122.2	210.2									1
	IMG_8048		22.6	46.8	100.6	223.8	307.2									1
39	IMG_8050	34157	3.7	14.6	45.2	73.6	282.1	8	12	18.5	17	17	14.5	8	18.5	1
	IMG_8052		16.8	25.1	79.4	387.5	1847.4									1
	IMG_8066		4.6	17.0	56.0	85.8	155.1									1
	IMG_8069		6.8	15.8	55.5	108.0	347.2									1
40	IMG_8071	34162	10.8	27.4	96.4	217.3	479.5	26	20	14	9	35	20.8	9	35	0
	IMG_8072		7.4	34.6	123.6	287.8	394.9									0
	IMG_8074		5.0	38.5	144.4	263.7	514.2									0
	IMG_8076		11.9	29.0	103.2	254.6	451.5									0
	IMG_8079		10.5	25.3	69.1	200.8	364.2									0

41	IMG_8092	34072	15.2	63.8	130.0	330.0	426.1	20	36	63	69	78	53.2	20	78	0
	IMG_8093		0.0	0.0	22.2	205.0	361.7									0
42	IMG_8103	34001	3.0	66.8	135.5	211.0	273.5	14	15	41	34	23	25.4	14	41	0
	IMG_8106		23.6	50.0	131.8	242.2	310.8									0
	IMG_8122		43.4	59.3	103.3	263.6	637.6									0
43	IMG_8126	33926	32.7	56.8	501.8	1515.3	1728.3	103	54	23	3	22	41	3	103	0
	IMG_8127		13.1	54.4	162.4	412.5	939.9									0
	IMG_8131		65.3	91.3	175.2	304.5	427.7									0
44	IMG_8141	33874	0.0	7.6	68.2	229.7	338.7	24	10	24	47	75	36	10	75	0
	IMG_8149		18.2	70.7	262.4	1317.6	1794.6									0
45	IMG_8448	33765	12.0	30.9	122.1	251.0	390.3	9	26	36	44	18	26.6	9	44	1
	IMG_8450		12.1	37.5	126.2	244.6	343.2									1
	IMG_8452		15.9	67.1	164.3	410.7	632.9									1
	IMG_8457		7.8	27.8	176.8	324.3	529.1									1
	IMG_8458		8.1	46.0	146.6	348.8	417.2									1
	IMG_8460		11.4	27.1	117.4	435.2	840.6									1
	IMG_8462		12.3	31.9	90.6	434.8	707.5									1
46	IMG_8465	33733	18.5	34.6	114.4	241.7	475.6	15	29	34	30	19	25.4	15	34	1
	IMG_8467		17.5	51.8	178.0	299.7	388.1									1
	IMG_8472		11.2	33.3	101.6	197.3	289.8									1
	IMG_8474		13.4	47.7	105.7	227.1	354.4									1
	IMG_8479		12.3	19.0	90.7	221.3	369.5									1
47	IMG_8483	33732	14.8	29.7	79.2	190.5	983.6	10	7	7	7	7	7.6	7	10	1
48	IMG_8488	33648	5.2	20.5	124.8	379.4	994.8	26	35	29	16	9	23	9	35	1
	IMG_8492		22.0	28.4	85.1	253.6	531.3									1
49	IMG_9438	33640	20.6	45.6	137.8	281.5	429.9	24	11	70	29	24	31.6	11	70	0
	IMG_9442		39.9	76.5	174.1	376.8	1279.0									0
	IMG_9447		14.8	55.5	161.8	303.4	427.4									0
	IMG_9450		31.4	65.1	143.6	305.8	367.9									0
	IMG_9454		33.7	55.5	93.2	227.3	373.4									0