Microhabitat use and recolonisation in white-clawed crayfish: application to conservation

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Abstract

White-clawed crayfish *Austropotamobius pallipes* are listed as vulnerable on the International Union for the Conservation of Nature (IUCN) red list and British populations represent one of the greatest concentrations of this species in Europe. White-clawed crayfish still remain at risk in Britain due to habitat degradation, pollution, and non-indigenous crayfish and disease. This thesis investigates the microhabitat use of juvenile and adult white-clawed crayfish and quantifies the rate and pattern of recolonisation into an unoccupied reach of river.

The microhabitat study was carried out over a 1 km stretch of the River Wansbeck, Northeast England in 2008 and a 3.5 km stretch on the Hart Burn, a tributary of the Wansbeck, in 2009. In September 2008 there was a 1 in 115 year flood and the data collected before and after the flooding were compared. Crayfish recorded in the Wansbeck were separated into three age groups, 0+ (0 - 9.4 mm carapace length (CL)), 1+ (9.5 - 16.4 mm CL) and 2+ and older (16.5 mm+ CL). Crayfish recorded in the Hart Burn were separated into four age groups 0+ (0 - 7.4 mm), 1+ (7.5 - 13.4 mm), 2+ (13.5 - 22.4 mm) and 3+ and older (22.4 mm+).

Crayfish of different ages had different microhabitat requirements where young crayfish, in particular 0+ crayfish, were more restricted in the microhabitats they inhabited. Young crayfish were found in closer proximity to the bank, in smaller substrata and slower velocities than older crayfish. In both study sites substratum heterogeneity created suitable habitat for all ages of crayfish and in the Hart Burn, root habitats were found to be important for all ages of crayfish. Extensive flooding in 2008 was found to significantly reduce densities of larger crayfish, probably reflecting large-scale mortalities or major redistribution.

Recolonisation was studied in the lower 3 km of the Hart Burn after a mass mortality event occurred in May 2004, apparently due to a one-off acute pollution incident. The recolonisation data highlights the impact mass mortality events can have on crayfish populations. It took 4 years of no further pollution for the population to recover which relates to a recolonisation rate of 750 m year^{-1}. The passive downstream drift of juveniles was the most rapid form of movement recorded and was responsible for the downstream population expansion in the first two years. Juvenile crayfish seem to have some control over their dispersal, as there was limited dispersal over periods of high flows and rapid dispersal over periods of low flows. Active upstream and downstream dispersal by adult crayfish was similar in rate.

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General Introduction

1.1 Introduction to conservation

"The universe would be incomplete without man; but it would also be incomplete without the smallest transmicroscopic creature that dwells beyond our conceitful eyes and knowledge" (Muir 1916).

The conservation of biodiversity is an important issue for many reasons. Natural diversity is good for our welfare and Wilson (1984) went as far as saying humans have a genetic predisposition to like biological diversity (Meffe and Carrol 1997). Greater biological diversity provides a greater variety of food and resources, which should help to buffer the human population from environmental catastrophes (Primack 1998). The environment provides many important resources for humans, including medicines, pollination of agricultural plants, recycling, replenishing oxygen, fertilisation by nitrogen fixation and aesthetic beauty (Meffe and Carrol 1997). Conservation is also important for scientific knowledge. Wallace (1863) believed that animals and plants are individual letters making up the volumes of Earth's history; if a few letters are lost it will make the sentence unintelligible and so extinctions will obscure the invaluable record of the past. This still holds true today, as scientists believe that extinctions make it harder to understand the ecological relationship between organisms (Sadava *et al*. 2008).

A global effort to conserve the natural environment may be a recent phenomenon, but the origins of conservation ideas can be traced back to ancient religious and philosophical beliefs (Meffe and Carrol 1997, Primack 1998). Conservation started to become an issue when the European states began to colonise the rest of the world, disrupting traditional resource use and causing rapid overexploitation (Hunter 1996). At the time, it was thought of as inconceivable that nature's resources would ever run out, an idea that was soon shattered with the extinction of European cattle *Bos primigenius* in 1627, and the dodo bird *Raphus cucullatus* in the 1680s (Primack 1998). Europeans started to show a concern for wildlife in the late nineteenth century, and in America the first ever national park, Yellowstone National Park, was created in

1872 (Hunter 1996, Primack 1998). Global biodiversity is changing at an unprecedented rate and currently 36% of all species listed on the IUCN red list are threatened (Primack 1998, Sala *et al.* 2000, IUCN 2010). Land use change is having a huge impact on global biodiversity, as grasslands and tropical forests are converted to cropland causing local extinction of plants and animals (Sala *et al*. 2000). After land use, change climate change is the next most important driver of biodiversity change, as it causes changes in habitat, leading to extinctions and changes in species distributions (Carpenter *et al.* 1992, Sala *et al.* 2000). Extinction rates are 100 to 1000 times their pre human levels and conservation has become an important global issue and an area that needs much attention (Meffe and Carrol 1997, Pimm *et al.* 1995, Primack 1998).

This thesis is the result of research carried out in the freshwater environment. Conservation of the freshwater environment is a subject that cannot be comprehensively covered in this thesis. However, an overview of the main threats to freshwaters and the strategies that could be employed to combat them will be outlined in the following two sections.

1.2 Threats to freshwater ecosystems

Freshwater ecosystems are some of the most endangered ecosystems in the world (Dudgeon *et al*. 2006). Humans have significantly modified freshwater ecosystems, as freshwater is fundamentally important to human welfare providing drinking water, irrigation, means of transport and even energy (NRC 1992). Freshwater ecosystems support 6% of all species yet only cover about 0.8% of the Earth's surface (Dudgeon *et al*. 2006). Even though the extinction rates in freshwater habitats are higher than those in terrestrial habitats, only 7% of the papers published in the journal "Conservation Biology" from 1997-2001 were concerned with freshwater species (Abell 2002, Dudgeon *et al.* 2006).

1.2.1 Human impacts on hydrology

Large-scale modifications of freshwater habitats have taken place over millennia, as for example at least 5,000 years ago on the River Nile (Smith 1971), and as civilisation has advanced so too have the threats to streams and rivers (Allan and Flecker 1993). Since the 20th century the damming of rivers has caused dramatic physical changes as it fragments the river ecosystem, converting a free flowing river into a reservoir habitat (Jager *et al.* 2001). Reservoirs impede the downstream migration of fish and outflows from reservoirs have altered chemical properties of rivers, while dams act as upstream barriers (Allan 1995). Dam constructions on the Volga River at Volgograd, Russia, ca 500 km upstream from the Volga river delta, for example has prevented beluga sturgeon *Huso huso* from accessing their usual spawning ground and has significantly reduced their migration distances (Birstein *et al.* 1997, Khodorevskaya *et al.* 1997). Juveniles no longer grow to their full size and do not become reproductively active, preventing natural reproduction (Khodorevskaya *et al*. 1997). On the River Danube a dam altered the biochemistry of the entire Black Sea basin, which included a reduction in silicate, which can cause species shifts in algae that control the alkalinity of the water and can also result in toxic algae blooms (Humborg *et al*. 1997).

The effects of straightening and channelising of rivers may not be as physically obvious as dams, but they have still contributed greatly to river degradation worldwide. Rivers have been channelised to aid navigation. This has created physical uniformity and led to the loss of native species through loss of habitat complexity (Allan 1995). Flood defence mechanisms such as levees prevent rivers discharging onto the floodplain periodically, which is an important cycle as it creates important spawning, nursery and foraging habitat for many fish species (Bunn and Arthington 2002). The Macquarie Marshes in Australia are of international importance and depend upon the water from the Macquaries River for their existence. Water reaching the marshes has been reduced, due to river regulation, leading to a reduction in marsh area and a consequent decline in water birds (Kingsford and Thomas 1995). Instead of replenishing wetlands and floodplains, the water is usually rapidly exported, causing further problems by lowering water tables and summer flows (Allan and Flecker 1993).

Abstraction of water for drinking and irrigation has become so intense that some rivers now contain no flowing water for several months of the year (Malmqvist and Rundle 2002). Poor water management is common in many countries and an extreme example of water extraction is the drying of the Aral Sea, regarded as one of the world’s worst environmental disasters (Allan 1995). The main tributaries supplying the Aral Sea lose most of their water to nearly 3 million hectares of cotton fields (Stone 1999). The Aral Sea, which used to be the world's fourth largest lake, lost 80% of its volume over four decades (Postel 2000, Stone 1999). It is now saltier than the ocean due to evaporation and agricultural run off and all 24 species of native fish have disappeared (Allan 1995, Stone 1999).

A more recent hydrological threat is the development of hydropower. As well as the flooding of biodiverse rainforests the generation of hydroelectricity has a huge impact on freshwater ecosystems. The fluctuating water levels cause erosion and desiccation which destroys the biota in the littoral zone (Smith *et al*. 1987). Brazil has hydroelectric plans to build 80 dams which would flood roughly 100,000 km^2 of Amazonia (Allan and Flecker 1993). Even in the UK where most hydroelectric energy resources have already been harnessed, the rapid expansion in small-scale hydroelectricity poses risks for some biota such as migrating Atlantic salmon and lamprey.

1.2.2 Human impacts on water quality

One of the main threats to freshwater ecosystems is pollution from agriculture, industry and domestic sources (Maitland and Morgan 1997). Intensive farming with heavy application of fertilisers results in enriched water entering rivers (Beasley and Roberts 1999). Fewer than 10% of rivers worldwide can be classified as pristine in terms of nitrates (WHO/UNEP 1987). Agricultural practices have resulted in higher levels of nitrogen and phosphorus flowing into enclosed seas such as the Adriatic and lagoons of the Nile (Turley 1999). This has led to eutrophication and the algal blooms and their decomposition can lead to deoxygenation of the water, which can cause fish extinctions like the extinction of vendance *Coregonus albula* and smelt *Osmerus eperlanus* in Scottish lakes (Beasley and Roberts 1999). Altering nutrients can also affect sensitive species such as the pearl mussel *Margaritifera margaritifera* (Beasley and Roberts 1999). Pearl mussels thrive in 'soft' waters which are poor in nutrients and experience reduced life expectancy under increasing nitrate concentration and eutrophication (Bauer 1988). The build up of silt also destroys pearl mussel habitat especially for juveniles. This has contributed to pearl mussels becoming listed as endangered on the International Union for the Conservation of Nature (IUCN) red list (Bauer 1988, IUCN 2010). Pollution from industries such as mining, involves the release of heavy metals into rivers, which elevates metal concentrations. This can cause changes in the community structures of aquatic insects (Roline 1988), and reduced richness in macroinvertebrates, which can take hundreds of years to recover (Malmqvist and Hoffsten 1999).

Atmospheric pollution is another source of pollution and the concept of ‘acid rain’ was born in the late 1960s (Baron *et al*. 2002). Acid rain is a trans-boundary pollutant which can affect large areas, where the decrease in pH and increase in aluminium concentration can cause dramatic declines in freshwater species diversity including zooplankton and macroinvertebrates (Driscoll *et al*. 2001). In Norway, Atlantic salmon *Salmo salar* became virtually extinct in 25 rivers due to the acidification of the water (Hesthagen and Larsen 2003). Kitamura and Ikuta (2001) found that brown trout *Salmo trutta* were extremely sensitive to acidification and spawning behaviour was severely inhibited even in slightly acidic waters.

1.2.3 Human impacts on habitat

As well as directly affecting the river flow and quality, humans have also altered the landscape through which rivers flow. Intensification of agriculture has led to the clearing of forests and removal of riparian vegetation, which accelerates the rainwater runoff causing severe erosion and degradation of the streambed and can have adverse effects on stream ecology (Maitland and Morgan 1997). The loss of riparian vegetation has severely affected species such as the southern river otter *Lontra provacax,* which is only found in the Argentine and Chilean Patagonia region (Lariviere 1999). The southern river otter needs adequate riparian habitat in order to survive and due to habitat loss is now listed as endangered by the IUCN (Sepulveda *et al*. 2007). In the Himalayas, forest removal has led to devastating flooding (Allan and Flecker 1993).

Afforestation with dense plantations also causes serious problems due to the impact of forest cultivation, drainage and road building (Nisbet 2001). Afforestation can also significantly reduce summer temperatures due to the shade provided by the canopy (Webb and Crisp 2006). Soil disturbance due to ploughing, drainage and harvesting causes large quantities of sediments to enter rivers, resulting in increased siltation and turbidity (Nisbet 2001). Afforestation, in particular with conifer trees, acidifies soils by capturing acidifying components from the atmosphere and depositing them in the soil (Friberg *et al.* 1998, Nisbet 2001). In Denmark, the soil is sandy and has a low buffering capacity, so large scale plantations of conifers in close proximity to lakes and streams has had devastating effects on some freshwater ecosystems due to acidification of waters (Friberg *et al*. 1998). Another devastating human impact is the introduction of non-native species and the consequences that follow.

1.2.4 Impacts of introduced species

A wide range of organisms have been introduced to freshwater ecosystems all over the world due to deliberate and accidental transfer of non-native (exotic) species (Bubb *et al*. 2006). Allan and Flecker (1993) stated that more than 160 species of exotic fishes from 120 countries have been introduced worldwide, and it is highly likely that the number has increased significantly since then. The devastation caused from some exotic species is well documented and their impacts can cascade through several trophic levels (Abell 2002). Zebra mussels *Dreissena polymorpha* have invaded all five of the North American Great lakes, are currently found in 23 US states, and are predicted to spread to the entire continental USA (Connelly *et al.* 2007, Malmqvist and Rundle 2002). Zebra mussels compete for food and over grow other molluscs resulting in the obliteration of many native molluscs (Pace *et al.* 1998, Schindler 2001). The invasions have also caused severe declines in phytoplankton and microzooplankton due to increased grazing pressure, which can have ramifications throughout the ecosystem, as many fish species depend on their availability during early life stages (Caraco *et al.* 1997, Pace *et al.* 1998).

Brown trout have been introduced throughout much of the world and were introduced to the South Island of New Zealand in 1867 (Townsend 1996). The competitive and predatory potential of brown trout has led to the extinction of the southern grayling *Prototroctes oxyrhynchus,* a species that was once abundant (McDowall 1990). The introduction of brown trout has caused severe declines of fish such as *Galaxias bulgaris.* The remaining populations are fragmented to regions inaccessible to the trout, which has led to genetic constraints such as inbreeding (Townsend and Crowl 1991). The main reason for the decline of the native galaxiids is due to direct predation from the trout, as the galaxiids have not evolved a predator escape response (Mcdowall 1990, Townsend and Crowl 1991).

Introduced species may not only outcompete the natives but can also carry deadly diseases and parasites (Allan and Flecker 1993). Introduced alien signal crayfish *Pacifastacus leniusculus* carry an oomycete fungus *Aphanomyces astaci* which is acutely pathogenic to the native European species (Edgerton *et al*. 2004). The introduction of signal crayfish led to the outbreak of crayfish plague in Europe in 1860 decimating many populations of native crayfish (Lozan 2000). Japanese eel *Anguilla japonica* were introduced to Europe in 1982 and carried with them a nematode *Anguillicola crassus.* The nematode may prevent European eels from reaching their spawning ground, as it attacks the swim bladder and reduces swimming performance and resistance to stress (Kirk 2003). This has had serious consequences for the European eel, and has contributed to their decline resulting in them now being listed as critically endangered on the IUCN red list (IUCN 2010).

1.2.5 Impacts of human population increase

All the threats to freshwater ecosystems will be exacerbated due to an increasing global human population. The population is set to increase to 9 billion by the year 2050 and 50% of the world's population will be experiencing waters stress by the year 2025 (Revenga *et al.* 2005). In developing countries the demands will lead to huge problems and large economic costs. In these circumstances it is doubtful that there will be much consideration for the ecological integrity of the systems that provide the much-needed water (Malmqvist and Rundle 2002). Increasing demand for water is already exerting immense pressure on freshwater environments, causing a decline in the quality and quantity of water and is leading to extinctions and severe biodiversity loss (United Nations. 2005). An expanding population is accompanied by an increase in the use of fertilisers, which was predicted to increase by 145% between 1990-2050 (Kroeze and Seitzinger 1998). The removal of accessible run off is predicted to rise to 70% by 2025. If this figure is realised it will lead to severe degradation of aquatic ecosystems, the extinction of beneficial species, and the decimation of many fish populations (Postel 2000).

1.2.6 Impacts of climate change

The increasing human population is having a dramatic effect on the world's climate and climate change is now a major topic on the political agenda (Walther *et al*. 2005). Anthropogenic induced climate change is expected to cause a warming of 0.2°C per decade (IPCC 2007). The effects of global climate change is the least predictable of all factors affecting freshwater ecosystems (Allan and Flecker 1993). Lotic systems will be highly sensitive to future changes, where there will be species shifts and changes in hydrology (Carpenter *et al*. 1992). Thermal limits of freshwater environments will be increased, which is likely to open them up to species invasions (Carpenter *et al*. 1992). Most of the invasive species affecting the North American Great Lakes originate from warmer waters, so an increase in global temperatures will give them a competitive advantage and accelerate their spread (Schindler 2001). Increasing temperatures will increase evapotranspiration, and along with a reduction in precipitation will lead to lower run off and decreased flows, converting permanent first order streams to short lived temporary streams (Murphy and Timbal 2008, Schindler 2001). Floodplains and wetlands will be especially vulnerable as the lack of flooding will lead to their demise, along with important fish populations that depend on them (Schindler 2001). Climate change is also causing a rise in sea levels, which results in saltwater intrusion into freshwater wetland systems (Hughes 2003). Lower than average rainfall in southeast Australia combined with an increase in evaporation is putting the floodplains and wetlands under serious threat and in some areas of Australia the extension of saltwater ecosystems is as rapid as 0.5 km yr^{-1} (Hughes 2003, Murphy and Timbal 2008).

Climate change can also increase river flows, where in Alaska a higher degree of summer warming results in increasing run off from glaciers, which can significantly affect flow regimes and sediments in downstream areas. The increase in glacial melt water will decrease the water temperature, which can significantly affect freshwater species, especially invertebrates which may be eliminated by the cold temperatures (Firth and Fisher 1992). Climate change can also lead to the acidification of rivers, as warming causes sulphur deposits to reoxidise and declining flows decreases the influx in base cations (Schindler 2001).

1.3 Conservation methods for freshwater ecosystems

Conservation of freshwaters is of vital importance to ensure their long-term viability and in order to conserve them the previously outlined threats need to be addressed with a sense of urgency.

1.3.1 Hydrology

Dam construction and operation is now coming under increasing scrutiny, with the complete removal of a growing number of dams whose environmental damage outweighs the social benefits (Postel 2000). Proposals have been made to operate dams in a way that protects the ecological functions of the river, by improving the upstream migration of fish and maintaining a minimum flow over the dam (Benstead *et al*. 1999). In the US there is a limit on the volume of water that can be diverted away from the San Francisco bay delta-estuary and Los Angeles has been made to reduce its water withdrawals from the tributaries feeding into Mono Lake (Postel 2000). Postel (2001) suggests a number of improvements that can be made in terms of water use in agriculture, which can help reduce water extraction. Drip irrigation is a technique where a network of perforated plastic tubing enables farmers to deliver water directly to the plant roots, eliminating water waste and doubling crop yield (Postel 1999, 2001). This technique can also be used to deliver fertilisers, which will help to regulate agricultural run off and improve water quality (Baron *et al.* 2002, Postel 1999). Crops can be improved so that they use water more efficiently, where the extra available water could be reserved for ecosystems (Postel 1999). Limits on water extraction could be enforced and the drainage of wetland and peat lands by means of moorland gripping could be reversed by filling in ditches and raising the water table (Holden *et al*. 2004).

1.3.2 Water quality

Point source pollution incidences are easier to control than diffuse pollution and thus it is easier to maintain a high water quality (Allan 1995). Agriculture represents the major source of diffuse pollution, but afforestation and atmospheric pollution are also important contributors (Baron *et al.* 2002, Nisbet 2001). Non point sources of nutrients and toxins now supply the majority of pollutants to freshwater ecosystems (Baron *et al*. 2002). Nevertheless, rivers have a natural recovery system, and diffuse pollution can be combated. After sewage improvements took place the tidal River Thames, south east England, went from containing no fish at all to 80 species within a decade (Gameson and Wheeler 1977). A spill of a highly toxic herbicide into the Upper Sacramento River in California virtually eradicated all forms of life over a 60 km stretch, but within 10 weeks algae and insect populations had made a remarkable recovery (Allan and Flecker 1993). In Norway and Sweden a strategy to improve water quality involves continuous liming with limestone powder (Hesthagen and Larsen 2003). It was found to be successful at re-establishing Atlantic salmon to previously acidified rivers; however, it is hard to say whether this method protects the whole aquatic community (Hesthagen and Larsen 2003). Controlling atmospheric emissions is another way to combat acidification. North American and European management has been successful in controlling the emission of sulphur nitrogen and other organic toxins (Baron *et al.* 2002, Driscoll *et al.* 2001). However, water quality improvements will be useless if physical habitat degradation renders the habitat unsuitable for the species to re-establish.

1.3.3 Habitat

One method of reversing habitat degradation is rehabilitating the in-stream habitat so it reflects a natural stream. Rivers that have been channelised pose a greater challenge and require river engineers to follow important ecological and geomorphic principles in order to make the channel design as natural as possible (Allan 1995). Features such as mini-wetlands, meanders, riffle-pool sequences, streamside vegetation and reducing the slope of the bank will help restore the stream and reduce the input of nutrients and pollutants (Petersen *et al*. 1992). It is important to look beyond the riverbanks where a buffer strip of uncultivated land can help filter out sediments, prevent erosion and protect important riparian habitat in both agricultural and forestry systems (Nisbet 2001). Buffer strips can range from a few metres to 30 metres, but a 10 metre buffer strip of rye grass was found to substantially reduce surface run off in central Illinois, U.S.A (Osborne and Kovacic 1993). Prato (1989) found that minimum tillage was the most economically effective method for reducing erosion rates. In Denmark, lakes have been restored by means of dredging, oxygenation and the reduction in external nutrient loading (Jeppesen *et al*. 1999). The restoration of river habitat and morphology also improves nutrient retention in the catchment, which aids in the recovery of lakes.

Full protection of aquatic habitat can only be achieved when there is complete control of the catchment. Freshwaters need to be viewed in a landscape context as many of the problems come from outside the river systems (Baron *et al*. 2002). A broad coalition of water users is needed where headwaters, riparian zones and habitats that meet special needs of species are preserved and the numerous protected areas involved are integrated into an effective network (Allan 1995, Baron *et al.* 2002).

1.3.4 Invasive species

Although with restoration efforts streams can recover from pollution and habitat modifications over time, alien species are virtually impossible to eradicate (Abell 2002). In spite of this, major efforts have been made to eradicate *Gyrodactylus salaris* from the rivers of Norway. *Gyrodactylus salaris* is a freshwater ectoparasite, which naturally infects Baltic stocks of Atlantic salmon without causing significant impacts on the population but is highly infectious to the Atlantic stocks (Peeler *et al*. 2006). *Gyrodactylus salaris* was first discovered in Norway in 1975 and has resulted in the collapse of salmon populations in 45 rivers (Peeler *et al.* 2006, Winger *et al.* 2008). Attempts to eliminate the parasites have been carried out using rotenone, which is an indiscriminate poison killing all fish species and gill-breathing invertebrates, and therefore indirectly eliminating the parasite (Winger *et al*. 2008). This method may be extreme but has successfully eliminated the parasite from 19 rivers, which the salmon have successfully recolonised, but several treatments have also failed (Peeler *et al.* 2006, Winger *et al.* 2008). Legislations have been passed enforcing European countries to control the introduction of invasive species, where the sixth community environment action plan states that there must be the “prevention and mitigation of impacts of invasive alien species and genotypes” (The European Parliament and the Council of the European Union 2002). However, prevention is always better than cure and tighter control over the aquarium trade and educating the fishing public can help prevent introductions and slow the spread of invasive species (Allan and Flecker 1993).

1.3.5 Reintroductions

One of the most positive areas of conservation lies in the establishment of new populations. The deliberate translocation of organisms by human means is becoming increasingly important for reinforcing declining populations (White *et al*. 2003). Research needs to be done into the quality of the habitat, the appropriate numbers to be released and the genetic structure of the population (White *et al*. 2003). Poorly researched reintroduction programmes can result in a waste of resources and money, as the populations fail to establish, and can even lead to reintroduced animals expanding into new areas and damaging the habitat (Macdonald *et al*. 2000). Once the populations have been established they need to be protected and their numbers must increase through natural reproduction (Birstein *et al*. 1997). For example, the Soviet Government introduced over 12.9 million beluga sturgeon into the Volga River. However, as there was no natural reproduction the beluga populations in the Volga River continued to decline despite the restocking (Khodorevskaya *et al*. 1997).

Reintroducing rare species within their natural range helps to increase their range and reduce the risk of extinction (Macdonald *et al*. 2000). The European beaver *Castor fiber* used to be widespread across Europe but suffered a severe reduction in numbers due to habitat loss and over hunting (Nolet and Rosell 1998). By the beginning of the 20th century only eight small populations remained (Macdonald *et al.* 1995, Nolet and Rosell 1998). The European beaver has been widely reintroduced across Europe with the first reintroduction in Sweden in the 1920s and has now been reintroduced into Britain (Macdonald *et al.* 1995, Macdonald *et al.* 2000, Nolet and Rosell 1998). Beavers are keystone species, so its reintroduction can promote freshwater ecosystems functions, improve water quality, aid flood control and recreate conditions that are favourable to certain species (Hodder and Bullock 1997, Macdonald *et al.* 2000). However, reintroductions carry certain risks and although beavers may maintain wetlands and create re-growth, which benefits the aquatic fauna, they may also damage forestry crops, agricultural land and riverbanks, and impede the migration of salmon (Macdonald *et al*. 1995). Reintroductions of nationally extinct populations are especially hard to predict and the alteration of habitat and possible transmission of pathogens and parasite may be harmful to other species (Hodder and Bullock 1997). Reintroduction may be an important management strategy but will always carry an air of uncertainty.

This thesis focuses on the white-clawed freshwater crayfish *Austropotamobius pallipes*, native in Britain, and an important crustacean which is threatened by all the previously discussed issues. As rivers and streams are the most mistreated and ignored natural resources, conservation of flagship species such as the iconic stream-dwelling crayfish will help to conserve important freshwater ecosystems (Schindler 2001). Flagship species play an important role in conservation as they can be used to educate the local people and creating a community ownership of such species can promote conservation of the local environment.

1.4 Crayfish Ecology

1.4.1 Introduction

Crayfish belong to the largest crustacean order, the Decapoda which divides into two super families the Astacoidea and the Parastacoidea (Hogger 1988, Holdich and Lowery 1988). Astacoidea contains the two families, Astacidae and Cambaridae, which occur in the Northern hemisphere and the Parastacoidea super family contains the Parastacidae family, which is confined to the southern hemisphere (Holdich and Lowery 1988). There are over 500 species of crayfish found throughout the world, many of which are found in temperate countries but some are also found in the tropics (Holdich *et al*. 2004). The tropical *Astacoides* consists of six species all found in Madagascar, confined to the high altitudes of the mountains (Horton and Hobbs 1988). The majority of crayfish species are native to North America but there is also a great diversity of species in Australasia (Holdich *et al.* 2004, Souty-Grosset *et al.* 2006, Taylor 2002). The widespread distribution of crayfish species is due to their behavioural, physiological and ecological adaptations. However, many species have been able to expand their range unnaturally due to human intervention (Hogger 1988).

1.4.2 Life cycle

Crayfish possess the basic arthropod body plan of a segmented body covered by a protective exoskeleton which must be shed in order for the crayfish to grow (Lowery 1988, Reynolds 2002). The moulting cycle dominates the life of these animals, as during moulting crayfish are vulnerable to attack from predators and cannibalism (Lowery 1988, Reynolds 2002). There is a strong tendency for adult crayfish to synchronise their moulting which may be an adaptation to reduce the risk of cannibalism (Brewis and Bowler 1983). Prior to moulting, the calcium from the existing exoskeleton is reabsorbed, leaving the crayfish soft and vulnerable. However, this only provides 10 % of the calcium required to harden the exoskeleton, so crayfish must reabsorb a considerable amount of calcium from the water (Lowery 1988). There are numerous moults during the first few months but they decrease as the crayfish increases in size eventually reaching one moult per year (Lowery 1988, Reynolds 2002).

When crayfish reach maturity, sexual dimorphism becomes more prominent as moult frequency varies between the sexes. Females have a lower moult frequency as they cannot moult when bearing eggs (Figure 1.1), and due to the energetic demands of reproduction they also have smaller moult growth increments (Reynolds 2002). As well as growing faster, males also have larger chelae once they reach sexual maturity and females have a broader abdomen in order to accommodate the eggs (Reynolds 2002, Thomas and Ingle 1987). Both the age and size at maturity is greatly affected by the environment. Crayfish mature at younger ages in areas of good growing conditions, but delay maturity in harsh environments (Matthews and Reynolds 1995).

The exoskeleton places constraints on internal fertilisation so males fertilise the eggs by the transfer of a spermatophore by the gonopods. Gonopods are modified anterior pleopods that function like a plunger, introducing the white spermatophore mass onto the underside of the female (Holdich 2003). Data on vas deferens weights has suggested that some males mate several times whilst others none at all. This implies there is a vertebrate-type dominance hierarchy in the mating system (Reynolds 2002). Females carry immature eggs in their ovaries which mature during a period of suitable temperature and photoperiod (Reynolds 2002). When they are newly hatched the females continue to carry the juveniles under her abdomen in order to protect them from predators. After the first moult they are released from the females but continue to cling to their ruptured eggs using their toothed chelae (Gherardi 2002, Thomas and Ingle 1987). After the second moult the juveniles begin to swim and walk away from the female until they leave the female entirely after their third moult (Gherardi 2002, Lowery 1988). The life cycle of the white-clawed crayfish can be seen in Figure 1.2.

The reproductive cycle of the Cambaridae family is quite different from other crayfish families, as cambarid crayfish exhibit cyclic dimorphism. After the production of young the mature females and males eat voraciously and then within weeks moult and assume immature appearance (Ackefors 1999).

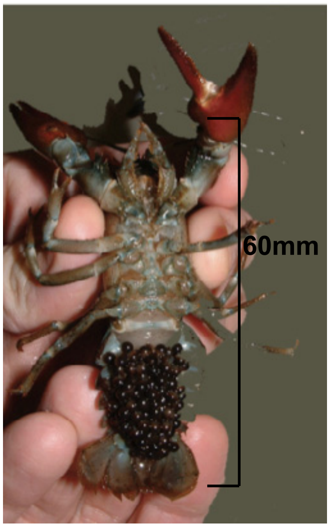


Figure 1.1- Photograph of a 'berried' female signal crayfish *Pacifastacus leniusculus*. The term berried is used to refer to mature females that carry the egg mass of newly hatched young

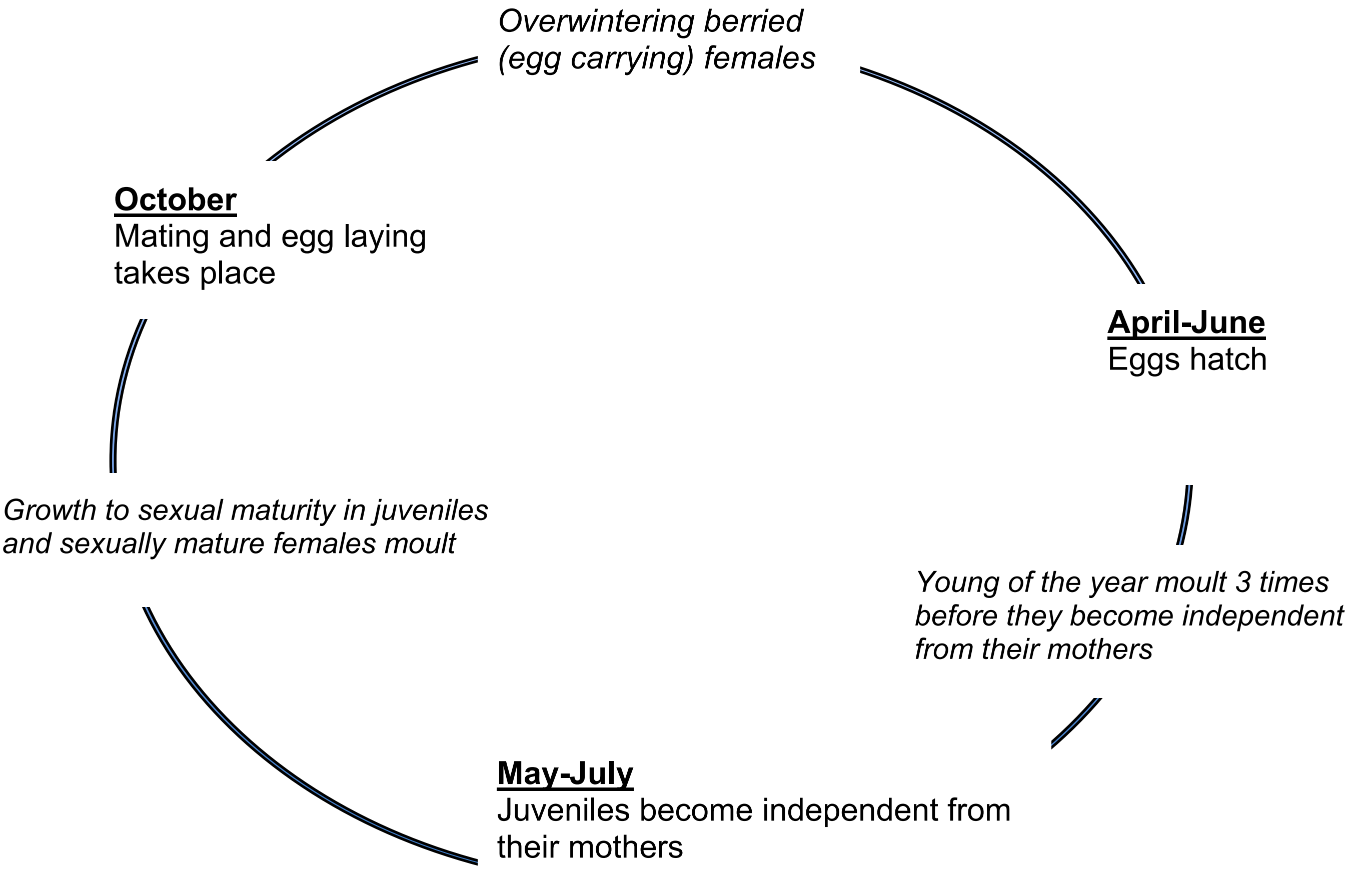


Figure 1.2- Schematic life cycle of a white-clawed crayfish

1.4.3 Habitat

1.4.3.1 Water chemistry

The chemical properties of water can affect processes such as moulting and reproduction and so, to some extent, determine which areas crayfish inhabit. Most crayfish cannot thrive in habitats where the pH ≤ 5. The water becomes too acidic and disturbs the calcium metabolism vital to the moulting process resulting in reproductive failure (Cukerzis 1988, Nystrom 2002). As crayfish are poikilothermic they can only survive at temperatures where important physiological process are able to take place. The ability to tolerate variability in temperature differs from species to species. The cambarids are restricted to warmer waters and although they have been found to survive in lakes in southern Sweden they cannot reproduce (Nystrom 2002). In contrast, the Astacidae family can cope with lower temperatures. For example, white-clawed crayfish *Austropotamobius pallipes* can survive in streams that experience low winter temperatures of 1.8°C and barely exceed 10°C in summer (Bubb *et al*. 2002, Souty-Grosset *et al*. 2006). These cool water astacid crayfish have a high requirement for dissolved oxygen whereas the cambarids and parastacids are less demanding (Reynolds 2002). Certain cambarid species display behavioural adaptations to low oxygen concentrations. For example the red swamp crayfish *Promcambarus clarkii,* climbs onto bushes and aquatic plants, exposing its gills to the air, allowing it to survive in waters with oxygen concentrations as low as 0.4 mg l^{-1} (Nystrom 2002). In comparison the astacid narrow-clawed crayfish *Astacus leptodactylus* can only survive temporary oxygen depletion of 3.97 mg l^{-1} (Koksal 1988).

Although crayfish may be able to tolerate hypoxic conditions it was thought that relatively few are able to survive exposure to saline water. However, some crayfish species have demonstrated the ability to survive and grow in increased levels of salinity. Narrow-clawed crayfish inhabit brackish waters and can endure salinity fluctuations from four to 14 gl^{-1} and white-clawed crayfish, narrow-clawed crayfish and signal crayfish are able to survive for several weeks in a salinity of 21 gl^{-1} (Holdich *et al.* 1997, Koksal 1988, Nystrom 2002). Crayfish may be able to survive exposure to salinities of 21 gl^{-1} but reproduction and growth is disturbed above 7 gl^{-1} (Nystrom 2002). Therefore the presence of crayfish in brackish waters will be for feeding purposes only and colonisation of these habitats will be limited (Koksal 1988, Nystrom 2002).

1.4.3.2 Physical habitat

Crayfish distribution cannot be explained by water quality alone as physical characteristics play an important role (Broquet *et al.* 2002, Souty-Grosset *et al.* 2006). Crayfish are found in a wide range of habitats including lakes, rivers, swamps, wetlands, canals, reservoirs, and water-filled quarries but are usually highly localised, only inhabiting favourable sections within the habitat (Holdich 2003, Nystrom 2002). A common requirement of all crayfish species is suitable substratum in which to burrow or to find a refuge (Reynolds 2002). Unlike the astacids, the cambarids and parastacids are able to burrow into soft bank substratum, enabling them to exploit more habitats (Horton and Hobbs 1988). Some species like the southern prairie crayfish *Procambarus hagenianus* spend most of their life in burrows, leaving them occasionally in order to find food or a mate (Gherardi 2002). Other species like the common yabby *Cherax destructor* only burrow in winter or during droughts (Gherardi 2002). For the non-burrowing astacids refuges are usually in the form of logs, stones, tree roots and macrophytes (Peay 2000). Macrophytes and plant biomass provide important protection for many crayfish species; for example, wetland plants protect *Procambarus allenin* from the predatory largemouth bass *Micropterus salmoides* (Nystrom 2002). Cobbles and stones are another important refuge from predators, exemplified by rusty crayfish *Orconectes rusticus* in Wisconsin Lake, where use of cobble habitat was associated with a low predation risk (Kershner and Lodge 1995). Refuges are crucial for crayfish survival and so most aggressive behaviour between crayfish is centred around them.

1.4.4 Behaviour

When food or refuges are limited, crayfish will interact aggressively (Lodge and Hill 1994). The larger and older crayfish are more dominant and Bovjerg (1956) observed that within an hour a dominance hierarchy was established between the crayfish. Davis and Huber (2007) observed *O. rusticus* and found that larger individuals frequently displaced smaller individuals from refuges. As well as aggression cannibalism is also induced when there are low levels of food or in the presence of a moulting individual (Holdich 1991, Nystrom 2002). Cannibalism is usually directed at smaller conspecifics but aggression can occur between species. For example, the introduced signal crayfish is particularly aggressive towards the smaller native white-clawed crayfish and narrow-clawed crayfish (Nystrom 2002).

Crayfish activity, including conspecific interactions is concentrated during nocturnal hours where the absence of diurnal fish predators allows them to leave the protection of their refuges (Gherardi 2002). However, increased movement by night may be to avoid predators such as eels *Anguilla* sp. that prey by chemoreception, where refuges will provide no protection but increase their vulnerability to trapping (Blake and Hart 1993). Ingle (1976), found that juvenile *A. pallipes* were rarely seen during nocturnal hours and Davis and Huber (2007), found that smaller rusty crayfish individuals had a peak of activity in the afternoon. Juvenile crayfish may avoid nocturnal activity and forage under refuge to prevent aggressive interactions with larger conspecifics and predators. Juvenile crayfish may be able to avoid predators by identifying their chemical cues, where one study found that noble crayfish *Astacus astacus* juveniles were able to discriminate between chemical cues of several fish species (Blake and Hart 1993). This enables them to reduce encounters with predators by monitoring the habitat from within their shelter (Blake and Hart 1993, Nystrom 2002). Crayfish also use chemoreception to locate conspecifics. Crayfish species such as northern clear-water crayfish *Orconectes propinquus* and virile crayfish *Orconectes virilise* were found to be attracted to chemicals released from conspecifics of the opposite sex during the reproductive season (Hogger 1988, Nystrom 2002). Another use of chemoreception is in the location of food, since crayfish are stimulated to feed when compounds such as amino acids from animals and carbohydrates from plants are released (Nystrom 2002).

1.4.5 Feeding

Macrophytes, algae, snails, worms, insects and even small fish are all components of a crayfish diet (Holdich 2003, Nystrom 2002). Crayfish are able to consumer a wide range of food types due to the structure of their mouthparts and their ability to hold and grasp food items with their walking legs (Nystrom 2002). Unlike the omnivorous adults, juvenile crayfish are considered carnivores as they feed predominantly on invertebrates (Goddard 1988, Reynolds and O'Keefe 2005). Juveniles require the animal protein for rapid growth in order to become less vulnerable to gape-limited fish predators and so increase their likelihood of reaching adulthood (Momot 1995, Nystrom 2002). Adult crayfish suffer a loss in dexterity so invertebrates that are more active are lost from an adult's diet. Instead, adult crayfish feed on plants such as moss, where the periphyton associated with it is easy to handle and easily digestible (Goddard 1988, Momot 1995, Reynolds and O'Keefe 2005). This ontogenic shift towards greater herbivory may be in order to meet certain nutrient requirements, for example macrophytes such as *Chara* may be an important source of calcium (Holdich 2003, Momot 1995). However, adult crayfish are not as herbivorous as once thought, as the detritus ingested contains large communities of insects and soft-bodied metazoans and they are probably best described as opportunistic feeders (Momot 1995). This is reflected in the diet of white-clawed crayfish, where detritus was more prominent in their guts during autumn, coinciding with the peak litter fall, and insect larvae were more prominent in spring and summer, coinciding with the highest levels of insect biomass (Gherardi *et al*. 2004).

1.4.6 Crayfish as keystone species

Crayfish are the largest, mobile invertebrates inhabiting freshwaters and due to their large size and numbers (in suitable habitat) and their many trophic links are often considered as keystone species, as they can modify habitats and reduce or even eliminate plants and animals through predation (Momot 1995, Holdich 2003). Adult crayfish consume plants selectively, preferring to handle single-stemmed species such as *Elodea canadensis* and plants that lack buoyancy and cellulose such as *Chara* (Momot 1995, Nystrom *et al.* 1999). As well as consuming macrophytes, crayfish mechanically eliminate them while searching for other food items and can greatly reduce or even eliminate certain species (Momot 1995). The effect crayfish have on plant biomass is most obvious when they are eliminated from a habitat. Several studies found that the elimination of crayfish resulted in excessive weed growth and a significant increase in *Chara* (Abrahams 1966, Goddard and Hogger 1986, Matthews and Reynolds 1992). Macrophytes create habitat heterogeneity and decrease predation efficiency so their reduction will have consequences for invertebrates (Nystrom and Perez 1998). As well as indirectly affecting invertebrates through the decrease in plant biomass, crayfish also actively forage on invertebrates (Nystrom *et al*. 1999), and can influence the community structure in habitats in which they are present. Crayfish significantly reduce the number of leeches and molluscs, in particular snails, which leads to the domination of larger thicker-shelled snails and active swimming invertebrates (Lodge *et al.* 1994, Nystrom *et al.* 1999, Nystrom and Perez 1998). At high densities, competition occurs between crayfish and fish over food, and crayfish can directly impact on fish growth and fecundity by modifying shelters and spawning areas (Momot 1995). Small benthic fish may be at risk from interactions with crayfish. In British rivers, both the native white-clawed crayfish and the invasive signal crayfish were dominant over bullhead *Cottus gobio* and ousted them from refuges (Bubb *et al.* 2009). Guan and Wiles (1997) also found that the survival of bullhead and stone loach *Noemacheilus barbatulus* were significantly lower in the presence of signal crayfish. Crayfish not only control macrophyte growth and exert top-down influences on community structure; they also play an important role as prey. Crayfish are a source of food for large predators such as brown trout, European otter *Lutra lutra*, grey heron *Ardea cinerea*, and European eel *Anguilla anguilla* (Smith *et al.* 1996, Wiltshire and Reynolds 2006).

A less obvious effect of crayfish is through a form of non-trophic engineering, where they exert a physical effect on river and lakebed sediments (Statzner *et al.* 2003, Usio and Townsend 2004). The walking activities of crayfish promotes sand and gravel erosion and stirs up the layer of sediments, which can release phosphorus and nutrients into streams and lakes and affect the entire water body (Momot 1995, Usio and Townsend 2004). In addition, the burrowing behaviour of some species of crayfish can lead to considerable damage and even collapse of the riverbank (Guan 1994).

The important roles of crayfish in freshwater ecosystems are becoming increasingly recognised (Souty-Grosset *et al.* 2006, Usio and Townsend 2004). Unfortunately, these valuable species are in decline and several crayfish species are threatened or have already become extinct worldwide (Nystrom 2002).

1.5 Threats to crayfish

1.5.1 Pollution

As crayfish inhabit relatively clean waters, pollution is a serious threat (Hart and Fuller 1974, Holdich and Lowery 1988). Organic pollution results in siltation, weed growth and a decrease in dissolved oxygen, which can lead to the exclusion of crayfish from certain river stretches (Holdich and Lowery 1988, Reynolds *et al.* 2002). The addition of phosphorus and nitrogen from human sewage causes eutrophication. This can cause toxic algae blooms and signal crayfish experienced high mortality in a Swedish pond during a bloom of toxic cyanobacteria *Oscillatoria sancta* (Nystrom 2002). Insecticides are the most toxic group of chemicals to crayfish and these agricultural pollutants are particularly threatening as they usually affect stretches of river in unpolluted rural sites where crayfish are locally abundant (Nystrom 2002, Souty-Grosset *et al.* 2006). Synthetic pyrethroids were first introduced in the early 1990s as they posed a lower threat to humans. However, they are 100 times more toxic to invertebrates than previously used organophosphates, where they affect the neurological systems of crayfish leading to paralysis and death (Howells and Slater 2003, Morolli *et al.* 2006, Slater and House 2001). With the intensification of agriculture the use of synthetic pyrethroids is a serious threat to crayfish, which in comparison to other macroinvertebrates have a lengthy recovery time (Peay 2002, Slater and House 2001). A pollution incidence in Poland led to the disappearance and replacement of the less tolerant native noble crayfish with more tolerant alien spiny-cheek crayfish *Orconectes limosus* (Nystrom 2002).

1.5.2 Habitat degradation

Wilcove *et al* (1998) stated that in North America alone there are 67 threatened crayfish species; 4% due to alien species, 28% of the species are threatened because of pollution, but 52% were due to habitat degradation (categories are nonexclusive so do not add up to 100). Human activities such as draining, dredging and channelisation are the usual causes of habitat degradation. They have a significant impact on crayfish populations due to the removal of important cover and food sources (Goddard and Hogger 1986, Holdich and Lowery 1988). During river operations the existing populations can be removed unintentionally (e.g. by dredging) and the river is left in a state that seriously deters colonisation from neighbouring populations, so crayfish populations become segregated (Hart and Fuller 1974). Channel alterations and drainage methods in the Tyrol region of Italy are seriously threatening white-clawed crayfish populations (Fureder *et al*. 2002). River operations can also lead to the increase in barriers such as long culverts, which increase local velocities and prevent crayfish from entering, especially when the ends are vertically stepped

(Peay *et al*. 2006). The presence of barriers and fragmentation of populations into sub populations results in reduced genetic variation and increased risk of extinction due to demographic stochasticity (Peay 2002). Even small barriers can have dramatic effects on crayfish dispersal (Bubb *et al*. 2008). There are only a few well documented cases of habitat degradation eliminating a crayfish species but *Cambarellus alvarezi* became extinct when the spring where it was found was removed for agricultural purposes in Northern Mexico (Taylor 2002).

Intensification of agriculture has also lead to habitat degradation, where overstocking of riverside fields leads to severe poaching of the riverbank which increases turbidity and sediments entering the water (Nystrom 2002, Slater and House 2001). Mud and silt accumulation destroys crayfish habitat due to the loss of interstices between large sediment particles and reduced oxygen content in the interstitial water (Slater and House 2001, Taugbol and Skurdal 1999). This has greatly reduced crayfish populations in the Eastern United States (Hart and Fuller 1974, Nystrom 2002).

1.5.3 Invasive species

The greatest threat to European crayfish is the threat imposed by invasive crayfish species. Non-indigenous crayfish species have been introduced all over the world and the competition from the introduced signal crayfish was partly to blame for the extinction of the sooty crayfish *Pacifastacus nigrescens* in the Western United States (Taylor 2002). Crayfish were accidentally introduced through ballast water and deliberately implanted into fish farm ponds and lakes and sometimes into the wild in order to establish crayfish farming (Holdich et al. 1999b). In some areas of Europe the native crayfish have become endangered due to the introduction of alien crayfish species (Souty-Grosset *et al*. 2006). In Europe, unlike in other parts of the world, the alien crayfish carry a commensal organism *Aphanomyces astaci* that causes drastic declines in the native species (Taylor 2002).

1.5.4 Threats to European crayfish

There may be over 500 crayfish species worldwide but only five are native to Europe (Edgerton *et al*. 2004). These five species belong to the family Astacidae and in Europe there are two genera of crayfish: *Astacus* which consists of three species and *Austropotamobius* which comprises of two

(Holdich 2002a, Souty-Grosset *et al.* 2006). Three of these five European species are classed as vulnerable; the noble crayfish, which is widespread in eastern, central and northern parts of Europe; the stone crayfish, *Austropotamobius torrentium*, which is confined to central Europe and the white-clawed crayfish, which is found in southern, central and northern Europe (Lowery and Holdich 1988, Taylor 2002). The remaining two species are the narrow-clawed crayfish which is found in Asia and Eastern Europe and the thick-clawed crayfish *Astacus pachyous*, which is confined to areas of the Caspian and Asov sea (Lowery and Holdich 1988, Souty-Grosset *et al.* 2006).

1.5.4.1 Crayfish plague

Over the last two centuries the abundance and distribution of native freshwater crayfish in Europe has been subject to major changes. This is due to the arrival and consequent spread of crayfish plague caused by the oomycete fungus *Aphanomyces astaci* (Souty-Grosset *et al*. 2006). Crayfish from North America were introduced to Italy unintentionally through ballast water introductions in the 1860s (Holdich and Gherardi 1999). Since the introduction of alien crayfish from North America there have been widespread mortalities of native crayfish and by the 1930s the oomycete fungus *A. astaci* was implicated and became known as crayfish plague (Holdich and Gherardi 1999). Due to the rapid spread and destruction of the plague, alien crayfish from North America, in particular the invasive signal crayfish, were introduced to supplement native crayfish stocks for exploitation (Holdich *et al*. 1999a, Holdich and Gherardi 1999, Taylor 2002). However, North American crayfish carry the fungus as a sub-clinical infection so the introduced invasive signal crayfish became vectors of the plague and from 1960 onwards were responsible for the continuing spread of the disease (Edgerton *et al.* 2004, Holdich *et al.* 1999a, Holdich and Gherardi 1999). The oomycete fungus *A. astaci* infects the host through the uncalcified regions where the mycelia then grow within the host (Holdich 2003, Smith and Soderhall 1986). The fungus releases spores, which on contact with water, transform into zoospores that are chemotactically attracted to crayfish (Smith and Soderhall 1986). The condition is extremely contagious and the spores can remain viable for up to nine days in the water (Smith and Soderhall 1986, Taugbol *et al.* 1993). In Turkey, crayfish plague was responsible for reducing the native crayfish stocks by 85% and estimates found that roughly 5% of the original Swedish populations of native crayfish remain (Ackefors 1999, Taylor 2002).

Once infected it is difficult to see visually but the most obvious symptoms are behavioural abnormalities (Holdich 2003). Once the mycelium grows within the body it impairs mobility causing crayfish to walk on the tips of their legs as if walking on stilts (Holdich 2003, Smith and Soderhall 1986). Brown patches may also appear on the membrane between joints and infected individuals rarely live for more than two weeks (Holdich 2003, Smith and Soderhall 1986). The rapid spread of the plague is a direct result of human activity responsible for the movement of infected crayfish and equipment around Europe (Holdich 1991). Spores can be carried in the mud on boots, in the water, on fish and fishing equipment (Holdich 2003).

Other alien crayfish species have been introduced into Europe and currently there are ten alien species present in Europe (Souty-Grosset *et al*. 2006). One of these is the red swamp crayfish, which was introduced to Spain in the early 1970s (Ackefors 1999, Holdich 2002b). The red swamp crayfish is a tropical species but is now present in ten countries with temperate climates and is able to occupy a number of habitat types and adapt to temporarily dry conditions (Holdich 2002b). It is a well known vector of crayfish plague and is a major risk factor to the survival of white clawed crayfish in Spain (Gil-Sanchez and Alba-Tercedor 2006). Red swamp crayfish have also eliminated indigenous mollusc species in Spain and led to a 99% reduction in plant cover in Chozas lake in northwest Spain (Souty-Grosset *et al*. 2006). More recently the marbled crayfish *Procambarus spp.* has been introduced to Germany and as it belongs to the Cambaridae family is may also be a vector of the plague (Holdich *et al.* 2004, Souty-Grosset *et al.* 2006). Furthermore, it has been found to reproduce parthenogenetically and can release 120 juveniles every 8-9 weeks, so even the introduction of a single specimen can pose serious threats to natives (Scholtz *et al.* 2003, Souty-Grosset *et al.* 2006).

European crayfish are also affected by other diseases such as the protozoan *Thelohaniasis contejeani* which causes porcelain disease, where infected individuals have a porcelain white colouration in the tissue of their tail muscles (Holdich 2003). This protozoan rarely causes mass mortalities like crayfish plague, but if the prevalence in a population is above 10% it may cause population crashes (Holdich 2003, Holdich *et al.* 2004, Souty-Grosset *et al.* 2006).

1.5.5 Threats to white-clawed crayfish

This thesis considers white-clawed crayfish *Austropotamobius pallipes* (Figure 1.3) in Britain. Some of the largest remaining white-clawed crayfish populations in Europe are found in Britain and Ireland where it is the sole native species (Holdich 2003, Holdich *et al.* 1999b). White-clawed crayfish can be distinguished from other European species by the one post orbital spine (Laurent 1988). It has a pinkish white underside and the body is olive brown in colour (Goddard and Hogger 1986, Holdich 2003). Despite the dangers associated with the introduction of alien crayfish species in the late nineteenth and early twentieth century, Britain continued to import signal crayfish. Crayfish plague started to spread in Britain soon after 1981 and signal crayfish have become established as far north as the River Clyde in Scotland (Lowery and Holdich 1988, Peay 2000).

Figure 1.3- Photograph of a white-clawed crayfish

There are four other crayfish species that have been introduced to Britain; two of them, the red swamp crayfish and the spiny cheeked crayfish, have been found to be vectors of the plague (Holdich *et al.* 2004, Palmer 1994). The narrow clawed crayfish and the noble crayfish have been found in sites in the south of England and although they are native European species they are still considered a threat (Holdich *et al*. 1999a). White-clawed crayfish have been in severe decline in Britain since the 1970s due to habitat loss, pollution, crayfish plague and competition with signal crayfish (Holdich *et al*. 2004). Records of white-clawed crayfish were mapped according to their presence within 10 km squares and in the period 1997-2001 the white-clawed crayfish distribution had declined by 20.3%, in comparison to their distribution in 1990-1996 (Sibley *et al*. 2002).

1.5.5.1 Competitive exclusion

There are some cases in Britain where populations of signal crayfish do not harbour the plague and mixed populations of signal and white-clawed crayfish arise (Bubb *et al.* 2005, Holdich 2003, Holdich *et al.* 2004). However, these mixed populations may exist for a couple of years but eventually the signal crayfish eliminate the native populations through competitive exclusion (Bubb *et al.* 2006, Holdich 2003, Holdich *et al.* 2004). In Britain, all mixed populations of white-clawed crayfish and signal crayfish that have existed were entirely made up of signal crayfish within nine years (Holdich 2003). Signal crayfish have a higher growth rate, higher fecundity, they reach their sexual maturity earlier, and have a better physiological tolerance to changing environments than white-clawed crayfish (Holdich *et al*. 1999a, Lozan 2000, Nystrom 2002). In contrast white-clawed crayfish are a more K-selected species (live longer, have a lower fecundity and a slower growth rate) (Gherardi *et al*. 1997).

1.6 Ecology of the white-clawed crayfish

1.6.1 Life cycle

White-clawed crayfish usually become sexually mature in their third year although this is dependent on growth rate (Holdich 2003). Mating takes place in October and November once the temperature has dropped below 10^{o}C for an extended period (Holdich 2003, Matthews and Reynolds 1995, Peay 2000). As white-clawed crayfish cannot grow below 10^{o}C they reach their northern limits in England and Ireland (Pratten 1980). However, a population was introduced to a limestone loch in Sutherland northwest Scotland where the gulf stream ameliorates winter temperatures (Holdich 2003, Palmer 1994). During the mating season males show increased movements and aggression induced by a receptive female, when the males are only receptive for a few days (Villanelli and Gherardi 1998, Woodlock and Reynolds 1988). Males compete aggressively with one another and also remove competitors spermatophores from receptive females by eating them and then replacing them with their own (Villanelli and Gherardi 1998). After mating, the females retire to the deepest waters of the river and conceal themselves beneath rocks while they brood their eggs over winter, held beneath their tail (Holdich 2003, Ingle 1976, Villanelli and Gherardi 1998). Over winter, crayfish remain torpid in their refuges and for white-clawed crayfish, the harsh winter conditions can sometimes reduce a population by up to 40% (Brewis and Bowler 1983, Peay 2000). Hatching occurs in April and May in the south of their range and up to a month later in the north of England, where females start to become more aggressive and display territorial behaviour (Ingle 1976, Villanelli and Gherardi 1998). Around 20-25 days after hatching, the juveniles have moulted for the third time and the mother vibrates her abdomen while holding it out horizontally, releasing the hatchlings which are now completely independent from the mother (Ingle 1976, Thomas and Ingle 1987, Villanelli and Gherardi 1998). In the north of England, Brewis and Bowler (1985), stated that hatching does not occur until July-August. However, in the River Wansbeck, Northumberland almost all juveniles were released from the mothers by mid-July (H. Ream pers. obs).

In comparison to other astacids white-clawed crayfish have a low reproductive rate only producing around 60-80 eggs a year (Reynolds 1997). The growing season is reduced by as much as three months in the northerly British and Irish populations due to the colder summer temperatures (Laurent 1988). This further reduces their fecundity rendering them more vulnerable to disturbances than other white-clawed crayfish populations (Laurent 1988, Reynolds and Matthews 1997). Nevertheless, these northerly populations are important for the conservation of this species, where the most densely populated areas of white-clawed crayfish in Britain were found in the northeast of England (Souty-Grosset *et al.* 2006).

1.6.2 Origins and taxonomy

The origins of British and Irish populations of white-clawed crayfish have been disputed, where some believed that Irish populations were introduced from Britain relatively recently (Lucey 1999). However, analysis of haplotypes showed that populations in Ireland were not introduced from Britain but from western France, possibly by French monastic orders travelling to Ireland (Gouin *et al.* 2003, Reynolds 1997). British populations of crayfish may have also arrived from France via stream connections when the land bridge between

Britain and the rest of Europe existed (Gouin *et al.* 2003, Grandjean *et al.* 1997). However, molecular techniques showed that British populations were very similar to French, which suggests a relatively recent origin (Holdich 2002a). White-clawed crayfish may have arrived in Britain due to human introductions from France, as literature from the 18th century refers to the stocking of pools with white-clawed crayfish and there is little reference to crayfish in Britain earlier than the 17th century (Thomas and Ingle 1971, Grandjean *et al.* 1997). Molecular techniques such as nucleotide sequence analysis of mitochondrial DNA has also helped to solve the taxonomic problem of white-clawed crayfish, which has gone through several taxonomic revisions. Now it is widely accepted that *A. pallipes* is a species complex that consists of two genetically distinct species *A. pallipes* and *A. italicus,* where *A. italicus* consists of four sub species all found in Italy (Fratini *et al*. 2005).

1.6.3 Habitat of white-clawed crayfish

White-clawed crayfish are found in relatively hard, mineral rich waters on calcareous and rapidly weathering rocks (Holdich 1991). The majority are found in waters with chalk, limestone or sandstone deposits where the pH typically ranges from 6.5 - 9 (Holdich 2003). White-clawed crayfish are more sensitive to calcium than other crayfish species and are unable to survive in waters with less than 5mg l^{-1} of calcium (Holdich 2003, Jay and Holdich 1981, Smith *et al.* 1996). They can acclimatise to a range of temperatures and altitudes and have been found to inhabit high altitude lakes in the Alps, up to 1500 m (Souty-Grosset *et al*. 2006). They can survive in rivers in northern England, where the water temperature reaches 1.8°C in winter (Bubb *et al*. 2002), and in rivers in Spain, where the temperature reaches 23^{o}C in summer (Laurent 1988). Typically, they inhabit low order streams that are often in upland areas and are on average 0.75-1.25 metres deep, but they have been found in shallower waters (Bubb *et al.* 2008, Holdich 2003, Laurent 1988). They also occupy lakes; however, in Ireland they are usually found near the mouth of the inflowing river due to predation from eels and are scarce in lakes larger than 1,000 hectares (Hogger 1988, Reynolds *et al.* 2000, Reynolds 1997).

White-clawed crayfish are usually found in high quality waters either class A or B on the UK Environment Agency general quality assessment scale, which relates to very good or good water quality in terms of chemical pollutant levels

(Gallagher *et al.* 2006, Holdich 2003, Holdich *et al.* 1999b,). Nevertheless they have been known to exist below storm overflows and sewage works, where they seemed relatively tolerant to the pollution (Souty-Grosset *et al*. 2006). They are often found in relatively high gradient streams but require refuges to survive in the fast running waters (Bubb *et al.* 2008, Holdich 2003). Substratum is an important aspect of the environment for white-clawed crayfish, as it provides protection for juveniles from fish predators such as perch *Perca fluviatilis*, brown trout, and pike *Esox lucius,* but also protection for adults from mammals and birds such as grey heron, mink *Neovison vison*, otters and rats *Rattus norvegicus* (Armitage 2000, Hogger 1988, Holdich 2003). Studies have found that white-clawed crayfish often remain in one location for a number of days, where they display aggressive behaviour towards crayfish that they come into contact with (Bubb *et al.* 2008, Robinson *et al.* 2000). This may be so they can defend refuges, which are vital to their survival but are usually a limiting factor (Bubb *et al.* 2008, Statzner *et al.* 2003). Displacement from refuges by larger and more aggressive signal crayfish is a serious threat to white-clawed crayfish. It leaves them exposed to predators and may be one of the reasons they are competitively excluded by signal crayfish from plague free sites (Bubb *et al*. 2006).

1.7 Conservation of white-clawed crayfish

White-clawed crayfish are currently labelled as threatened on the IUCN red list (IUCN 2010). White-clawed crayfish have had a protected status in Britain since 1986 by the Wildlife and Countryside Act but more recently the international recognition of its vulnerability has led to its inclusion in the Bern convention (Kemp *et al.* 2003, Palmer 1994). This was later updated through the EU Habitats and Species Directive in 1992, where white-clawed crayfish are listed in Annexes II and V (Kemp *et al.* 2003, Palmer 1994, Souty-Grosset *et al.* 2006). Annex II requires the designation of special areas of conservation and Annex V makes it an offence to take white-clawed crayfish from the wild and sell it without a license (Kemp *et al*. 2003, Souty-Grosset *et al*. 2006).

1.7.1 Habitat management

In the UK, the Joint Nature Conservation Committee (JNCC) has drawn up an action plan for white-clawed crayfish and one of the aims is the designation and management of protected areas (Palmer 1994). Habitat for white-clawed crayfish needs to be protected and riparian corridors can play an important role by protecting freshwater environments from agricultural and industrial effluents (Fureder *et al*. 2002). During river works on the River Lea, southern England, complex bank-structure and tree roots were maintained as well as refuges in the form of rocks, which greatly improved the recolonisation (Peay *et al.* 2006).

1.7.2 Reintroductions

The JNCC also aims to publish guidelines on species translocations and re-establishments (Palmer 1994). Not all areas considered suitable for white-clawed crayfish populations are currently occupied by the species and re-introductions are regarded as crucial for the conservation and management of this species (Holdich 2003, Schulz *et al.* 2002). Reintroduction can restore recently lost stocks and buffer against extinction rate or extend the range of existing populations by aiding recolonisation processes (Gil-Sanchez and Alba-Tercedor 2006, Kemp *et al.* 2003, Reynolds 1997). Reintroductions have taken place in Irish lakes where populations were lost due to crayfish plague. Crayfish plague normally results in 100% mortality, but as it has no secondary host the fungus will eventually die out (Taugbol and Skurdal 1999). *In situ* tests can show whether the plague persists by holding crayfish in cages for a whole year and monitoring their health (Schulz *et al.* 2002, Spink and Frayling 2000). Reintroduction into the Boyne catchment and White lake in Ireland successfully resulted in good stocks after several years. These population may subsequently be important for possible reintroduction in Britain, where they will act as an offshore reservoir (Reynolds *et al*. 2002).

1.7.3 Protection from alien crayfish

Alien crayfish have to be accepted as part of the European fauna as it would be impossible to eradicate them, other than in small, isolated water bodies. However, it may be possible to prevent the spread of signal crayfish and other non-native crayfishes into water courses currently free from this species (Taugbol and Skurdal 1999). Isolation may help prevent signal crayfish and crayfish plague from reaching important native populations. High gradient barriers such as weirs and waterfalls can limit the spread of signal crayfish and crayfish plague in an upstream direction and protect populations of white-clawed crayfish situated upstream of the barrier (Light 2003, Peay 2002).

Electric barriers may be installed to prevent signal crayfish entering pipes and tributaries but this would have a high cost of installation (Sibley and Noel 2002). Sex pheromone baited traps have shown to be effective at attracting and trapping male signal crayfish, which could be used to create a non-breeding population (Stebbing *et al*. 2003). Stebbing *et al* (2003) also tested the use of repellent pheromones, with the idea that they could be used to restrict the expansion of a population, but the results were inconclusive.

1.7.4 Legislation and public awareness

Legislation can help restrict the movement of signal crayfish and other alien crayfish and help prevent situations where alien crayfish are accidentally introduced to more river courses. Effective legislation can only be passed if those who write the laws are well educated on the subject and there is a need to improve communications between experts and policy makers (Taylor 2002). As the live importation of crayfish is allowed into Britain, there are strict laws on what happens to crayfish once imported. Crayfish were included in the prohibition of keeping live fish (crayfish) order 1996, where it is an offence to keep live white-clawed crayfish in England and Wales and it is prohibited to farm alien signal crayfish in designated “no-go” areas unless you have a licence (Department for Environment Food and Rural Affairs 2010). However, people frequently ignore these laws and prosecutions need to be made. Previous prosecutions include a fishery owner who was prosecuted for illegally introducing rainbow trout into a watercourse in the River Wear catchment, northeast England (Environment Agency 2010). Legislation is a good measure to prevent further spread but much depends on the awareness of the public and their willingness to comply. Since 1988, and the severe decline in white-clawed crayfish, the British public have become much more aware of the situation (Holdich 1991). The public need to be educated on the threats and consequences of introducing alien crayfish into rivers (Souty-Grosset *et al*. 2006). Information campaigns and sterilising facilities for fishing gear can help limit the spread of crayfish plague (Reynolds *et al*. 2002). Carefully regulated recreational fishing of white-clawed crayfish can also enhance the awareness of this species and encourage the public to view white-clawed crayfish in term of aesthetic beauty and biological diversity (Holdich *et al.* 1999a, Reynolds 1997).

Most importantly, conservation can only be applied effectively with the backing of sound scientific knowledge. In this thesis, information is gathered on the microhabitat requirements and recolonisation processes of white-clawed crayfish, which will provide valuable information for the conservation of this species.

1.8 Research aims

Chapter 2 of this thesis sets out to expand the understanding of the microhabitat requirements of white-clawed crayfish, in particular juveniles. It is hypothesised that small, young crayfish use different microhabitats than larger, older crayfish. As mentioned in section 1.5, draining, dredging and channelisation of rivers along with intensification of agriculture is destroying crayfish habitat, which is having significant impacts on native crayfish populations. One of the conservation strategies outlined in section 1.7 to combat habitat degradation is the designation and management of protected areas. Microhabitat requirements of adults and juvenile white-clawed crayfish will provide invaluable information needed to identify areas that provide important habitat for all ages of crayfish.

Chapter 3 sets out to give a better understanding of recolonisation in crayfish and the processes that are involved. It is hypothesised that recolonisation of unoccupied habitat by white-clawed crayfish involves both active dispersal by large adults in upstream and downstream directions, as well as small individuals passively dispersing from upstream regions. Pollution incidences are a serious threat to stream-dwelling crayfish, in particular white-clawed crayfish, as section 1.6 highlights they have low fecundity and slow growth. Reintroductions are another crucial conservation strategy outlined in section 1.7, as they can buffer against extinctions and expand the range of existing populations. An understanding of the processes involved in recolonisation, through dispersal and the effects of pollution incidences, is crucial to the effective conservation of this species and success of reintroduction projects.

Microhabitat use in white-clawed crayfish

2.1 Introduction

In order to conserve native crayfish species a thorough understanding of their habitat requirements is needed. Populations of crayfish are usually highly localised, only inhabiting sections of a river where the habitat provides appropriate shelter from predation and drift (Edgerton *et al*. 2004). Many crayfishes, including sensitive and threatened species, are strongly affected by microhabitat features (Benvenuto *et al*. 2008). An understanding of habitat and microhabitat requirements is vital in order to correctly identify suitable areas for conservation and possible sites for successful reintroductions.

The overall habitat requirements of white-clawed crayfish *Austropotamobius pallipes* (outlined in 1.6.3) are similar to many other crayfish species, especially native European astacids (Lowery and Holdich 1988). White-clawed crayfish tend to shelter under and between stones and Peay (2000) believes that crayfish prefer to refuge under larger stones, as they are more stable and stand up to high flows. Bubb *et al* (2006) also found that large substrate was important for creating suitable refuges and only recorded crayfish in locations with boulders and large cobbles. Boulders and cobbles are generally agreed to provide suitable habitat for white-clawed crayfish, but the importance of finer substrate is disputed. Some studies found that fine substrate such as silt was unsuitable for white-clawed crayfish and they were found not to inhabit areas covered in it (Blake and Hart 1993, Holdich 2003). However, Peay *et al* (2006) found that in a study on the River Ivel, southeast England, crayfish were frequently found in habitats that were largely dominated by silt. It may be the case as Nystrom (2002) states, that an optimal habitat for crayfish is one that contains a diversity of substrate sizes.

Several studies on white-clawed crayfish found that steep banks and exposed tree roots were present in the most widely used habitats (Smith *et al*. 1996, Peay *et al*. 2006, Benvenuto *et al*. 2008). Benvenuto *et al* (2008) found that exposed tree roots were particularly important for smaller crayfish, as they provided structural complexity at the stream edge. Nystrom (2002) explains that tree roots may also be important for juveniles in the form of flood protection. Studies on white-clawed crayfish also found vegetation cover to be important. Smith *et al* (1996) found that, in particular, over hanging vegetation was associated with favourable crayfish habitat. Usio (2007) found that early succession species, in particular alder *Alnus glutinosa* and willow *Salix spp*, were associated with high crayfish abundance. This may be because their leaves are not only a food source but also a good source of nitrogen (Usio 2007). However, Peay *et al* (2006) found that too much shading from trees caused crayfish numbers to drop due to the lack of emergent vegetation. Emergent and submerged vegetation, where emergent vegetation grows and emerges from the water and submerged vegetation only grows underwater, can create favourable habitat within the channel (Holdich 2003), but if the vegetation becomes too dense, it can impede the movement of the crayfish and create unfavourable habitats (Peay *et al.* 2006).

While there is a good deal of information concerning habitat and some information on microhabitat requirements of the white-clawed crayfish, few studies have examined potential differences in habitat use by young, small crayfish (Carapace length < 25 mm) compared with those of more commonly studied larger adult crayfish. Benvenuto *et al* (2008) found that predation pressures could lead to segregation in the distribution of juveniles and adults. Large adults are more susceptible to predation from terrestrial predators, so they refuge in the deeper waters under boulders, whereas juvenile crayfish are heavily predated on by fish, so they take refuge in the shallower waters under finer substrate. It is hypothesised that different sizes of crayfish will inhabit different microhabitats, at least partly on the basis of small crayfish being able to exploit small refuges. In considering reintroductions, conservation, or mitigation, it is important to ensure that habitat and microhabitat characteristics are suitable for all life stages, including juveniles. This study used quantitative sampling of crayfish across and range of microhabitats to determine the microhabitats used by juveniles as well as adult white-clawed crayfish.

2.1.1 Population structure

The main difficulty in ascribing microhabitat use by juveniles and adults is deciding which of the crayfish caught are juveniles and which are not. The definition of a juvenile is an individual which has not yet reached sexual maturity. Age at maturity is dictated principally by growth rate to a critical size. In reality, the age crayfish reach sexual maturity is strongly affected by environmental conditions. They can delay maturity in harsher environments and crayfish which have smaller moult increments, due to limb regeneration and disease, will reach critical size later in their life (Englund and Krupa 2000).

A female crayfish can be identified as sexually mature if it bears eggs. Rhodes and Holdich (1982) found females as small as 23 mm carapace length bearing eggs in the River Leen, England. Brewis and Bowler (1982, 1985) concluded that in Northumberland, the minimum carapace length of sexual maturity in females is 22 mm and all are mature by 27 mm carapace length. Determining whether a male is sexually mature is significantly harder and the data on the sizes of sexually mature males is sparse and less reliable. One method that can be used to determine sexual maturity in males is to look for whitened gonopods (Reynolds 2002). Males have been recorded to mature at smaller sizes than females (Thomas and Ingle 1971, Brown and Bowler 1977). However, Lowery (1988) found that the females reached sexual maturity at 28 mm carapace length and males at 33 mm.

White-clawed crayfish in Britain are said to reach the critical size in which they become sexually mature in their third year, and this age class is referred to as 2+ (Peay 2000). When released 8 - 10 months after reproduction in May - July, they are referred to as 0+ crayfish. They then enter their first mating season in October as 0+ crayfish, as although the eggs were laid a year ago they are only two - four months old. These 0+ crayfish then become 1+ crayfish once the young of the following year are released. Following this method of age classification, crayfish entering their third mating season, where they have usually reached sexual maturity, will be classed as 2+.

2.2 Study site

Microhabitat data collection was carried out on the River Wansbeck and the Hart Burn, a tributary of the Wansbeck in northeast England (Figure 2.1). The Wansbeck system is located in Northumberland, where the source is centred on 55° North, 2° West. The source is 300 m above sea level and is located at OS grid reference NY914829 and the river enters the North Sea at NZ305854 (Worrall *et al*. 1998). In the Wansbeck white-clawed crayfish are the only crayfish species and densities are among the highest recorded in the UK (Rogers 2005). The bedrock in the Wansbeck catchment consists of Millstone grit, which was deposited during the Carboniferous age (Abesser *et al*. 2005). The River Wansbeck is 53 km long and has a width of 1 m near its source and 20 m at the lower end (Thom 1990). The Hart Burn is an eroding upland stream whose width rarely exceeds

10 m and joins the Wansbeck after flowing for around 20 km from its source.

In the Wansbeck, the data was collected over a 500 m stretch of the river. The site is situated just over 6 km downstream of the confluence with the Hart Burn, where the river runs through open pastures. The average gradient of the river section was 4.75 m km^{-1}. The river section was lined with trees, mostly early succession species, especially alder. The bank-side vegetation consisted mostly of tall grasses including canary grass *Phalaris arundinacea*, branched bur reeds *Sparganium erectum* and butterbur *Petasites hybridus.* In the Hart Burn, the data was collected over a 3.5 km stretch, starting from a point 4 km upstream of the confluence of the Wansbeck and working downstream ending at 0.5 km upstream of the confluence. The average gradient of the river section was 7.14 m km^{-1}, where the river passed through mostly deciduous woodlands and unimproved pasture. The bank-side vegetation was mostly made up of deciduous trees such as alder and ash *Fraxinus excelsior.*

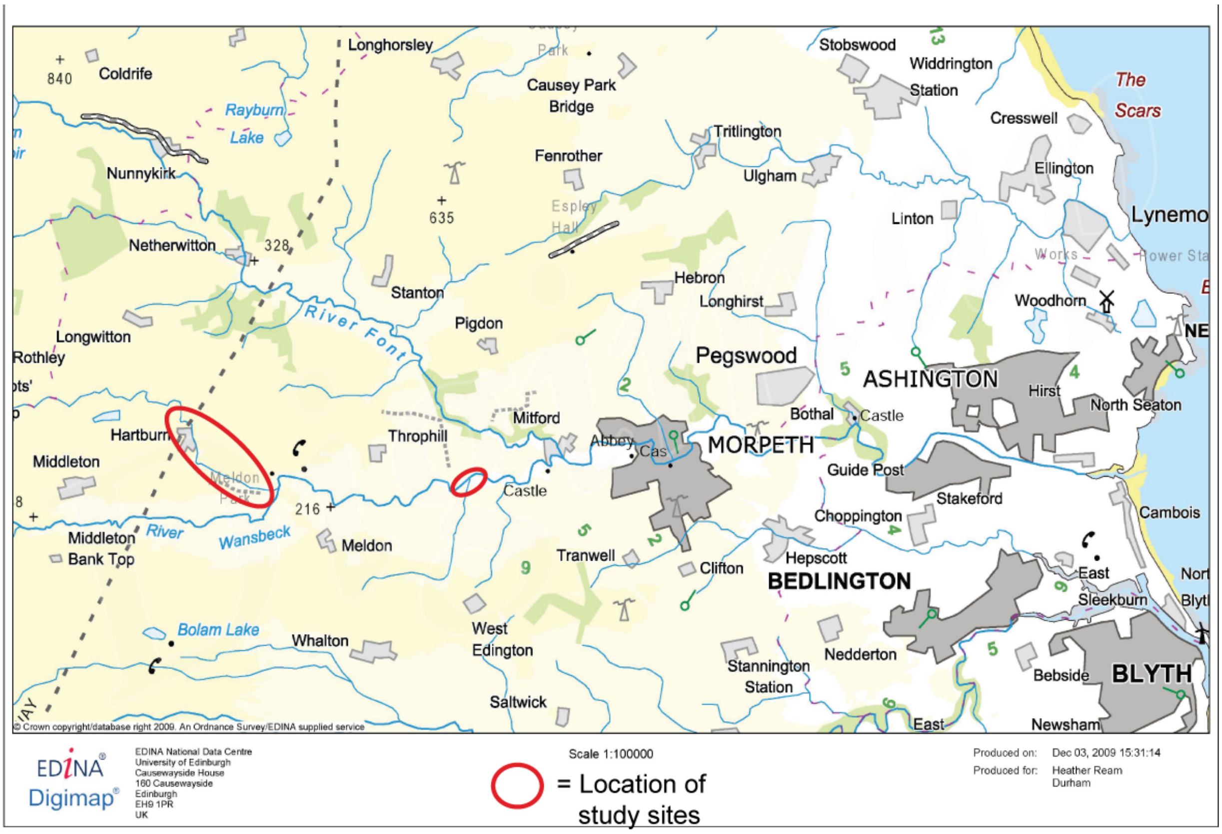


Figure 2.1- Location of study sites in the Hart Burn and Wansbeck

2.3 Methods

2.3.1 Collection of microhabitat data

Data collection took place over the period 5th August - 29th September 2008 and 29th July - 15th September 2009, when young of that year (0+) had been at liberty for several weeks. Sampling was by way of quadrats in which the crayfish were sampled using a Surber sampler together with microhabitat (Figure 2.2; see also section 2.3.2). Quadrats were placed quasi-randomly and all visible habitat types that were within the constraints on the depth were sampled. Crayfish were sampled from water depths up to 0.5 m. Deeper water could not be sampled due to the constraints of hand searching, where areas deeper than arms length could not be effectively searched. Sampling progressed in an upstream direction to avoid disturbance to subsequent sampling areas. A total of 75 quadrats were taken in the Wansbeck and 101 in the Hart Burn. The quadrat measured 0.70 m by 0.70 m creating an area of 0.49 m^2. The quadrat was placed in the river and the microhabitat within the quadrat was recorded in detail.

Figure 2.2- Photograph of the Surber sampler used to capture crayfish and delimit the area for microhabitat recording

The water velocity was measured using an OHIO Great Atlantic Flow Meter in 2008, and a Valeport electromagnetic flow meter Model 801, in 2009. OHIO Great Atlantic Flow Meter uses propeller revolutions to measure velocity in m/s and the Valeport electromagnetic flow meter uses the Faraday principle to measure the flow of water past a flat sensor. The propeller and flat sensor are suited for shallow and slower moving waters, so it is suited for measuring velocities in appropriate crayfish microhabitat. As crayfish are found on the streambed, the velocity was recorded 10 cm from the bottom. Water depth was measured using a meter ruler. Both the velocity and depth were measured within the quadrat at all four corners and the centre, in order to give average values as well as divergence. Velocity heterogeneity was calculated as the standard deviation of the five readings. To avoid disturbance to the sediment, which would result in the movement of crayfish away from the area, depth and velocity were not recorded prior to the placement of the Surber sampler. The Surber sampler may affect the velocity of the water but it did not seem to have a dramatic effect, and measuring the depth and velocity at set points within the quadrat created consistency between samples.

The substrate composition was classified according to particle size by using a modified Wentworth Scale (Table 2.1). The Wentworth scale is based on the diameter of the particle and each category is twice the preceding one (Giller and Malmqvist 1999). Substrate index was calculated from the percentage cover of different substrate categories. Substrate index = ∑nS where n is the percentage cover and S is the substrate category, which in this case ranges from one to six, with bedrock being zero. The cover of embedded substrate, where there were no crevices between the substrate and the riverbed, was also recorded. When creating the substrate index, cover of embedded substrate was given the value of zero, as like bedrock embedded substrate provides no refuges for crayfish. No Bedrock was recorded in the Wansbeck. Canopy cover over the quadrat was recorded in six categories based on the height and density of the nearby vegetation (Table 2.2).

Table 2.1- Modified version of the Wentworth scale

Size category	Particle diameter (mm)	Category
Sand	< 2	1
Gravel	2 - 16	2
Pebble	16 - 64	3
Small Cobble	64 - 128	4
Large Cobble	128 - 256	5
Boulder	> 256	6

Table 2.2- Canopy cover scale based on the cover provided by bank-side vegetation

Canopy cover category	Description
0	Vegetation height of less than 1 m on both banks
1	Vegetation height of less than 2 m on both banks
2	Vegetation height of less than 2 m on closest bank only
3	Vegetation height of over 2 m on both banks
4	Vegetation height of over 2 m on both banks and low branches overhanging
5	Dense overhead cover

The percentage cover of organic matter, tree roots and aquatic vegetation was recorded. Organic matter included twigs, leaves and organic detritus and aquatic vegetation fell into the category of moss, algae or macrophyte. Although organic matter was recorded in the Wansbeck, the cover in the quadrats was not substantial enough to include it as a variable. Due to the lack of accessible root habitats, cover of roots was also not included as a variable in the Wansbeck. The distance of the quadrat to the closest bank was also recorded to the nearest metre.

2.3.2 Sampling crayfish

The Surber sampler was made of netting fitted around the 0.70 x 0.70 m frame. At the downstream end, there was a large netting bag, which captured any crayfish swept into it by the flow or by an escape response (Figure 2.2). The netting was also extended to create a skirt around the bottom of the quadrat. The skirt was weighed down with leaded cord, in order to prevent crayfish from escaping through the gaps between the bottom of the quadrat and the riverbed.

Once the sampler was in place, stones within the sampler were progressively removed from the bed, carefully checking for crayfish as each was removed. Crayfish observed were captured by hand with the assistance of an aquarium net. Once all the stones were removed an aquarium net was used to scour the remaining substrate for smaller crayfish. Finally, the sampler was removed and the net bag was checked for crayfish. Sampled crayfish were measured by carapace length (from the rostral apex to the posterior median edge of the cephalothorax) to the nearest 0.1 mm using vernier callipers. The sex and any damaged limbs or evidence of disease was also recorded. Once the data had been collected, the stones were replaced and the crayfish were released at the site of capture. Sampling was carried out under a licence from Natural England.

2.3.3 Determining age groups

Some animals exhibit clear growth rings, which can be used to estimate age; however, such patterns do not occur in crayfish. The frequency distribution of carapace lengths was used to determine age groups. The frequency distribution obtained from a sample of lengths is often skewed and polymodal, where the modes refer to different age groups (Bhattacharya 1967). In general, variation in growth rates between individuals is lower earlier in life and results in a narrow range of sizes in the first cohorts, and typically progressively wider variation in subsequent cohorts. This usually enables the first few cohorts to be distinguished reliably. The Bhattacharya method identifies distinct normal distributions which may represent age groups (Bhattacharya 1967, Gayanilo 1998). Once one has been identified it is removed from the total distribution and the process is repeated until it is no longer possible to identify normal distributions (Bhattacharya 1967, Gayanilo 1998). The separation index is used to separate the modes, but separation is generally unreliable if the separation index is lower than 2 (Gayanilo *et al*. 2005). The boundaries between age classes were defined as where the lines of the normal distributions cross. FISAT II (Fisheries and Aquaculture Department) was used to run the Bhattacharya method.

2.3.4 Logistic regression

To see whether it is possible to predict the presence of crayfish from the variables recorded, logistic regression was carried out. Quadrats were separated into two categories, quadrats in which crayfish were present and quadrats in which crayfish were absent. Logisitc regression predicts the probability of a quadrat containing crayfish. Each variable recorded was analysed, where the log-likelihood determines if the variable had a significant effect on the outcome.

2.3.5 Principal components analysis

As multiple variables were recorded in order to understand the pattern of distribution in crayfish, multivariate analysis was used. Principal components analysis (PCA) was used as it reveals the relationships between variables, where interacting variables form factors. Factors can become the axis on a graph, where each crayfish caught can be plotted. This allows for the visualisation of how the relationships between variables affect the presence of different crayfish ages. The variables for PCA were selected by removing all the variables with diagonal elements in the anti-correlations less than the minimum of 0.5. For both data sets, the Kaiser-Meyer-Olkin measure of sampling adequacy (KMO statistic) was above the minimum recommended by Kaiser (1974) of 0.5 and Bartlett's test was highly significant ($p < 0.001$). This establishes that there is a relationship between the variables and PCA is appropriate for the data sets. Variables were considered for each crayfish caught so the sample size for the Wansbeck was 223 and for the Hart Burn 315. According to Steven (1992) when the sample size is 200 a loading of 0.364 and over is considered significant, and for a sample size of 300 and loading of 0.298 and over is considered significant. For the Wansbeck factor loadings of 0.364 and less were ignored and for the Hart Burn factor loadings of 0.298 and less were ignored. The factors were rotated using oblique rotation to improve the interpretability of the factors. Oblique rotation was used as the component correlation matrix showed correlation between factors. The values were taken from the pattern matrix, although the structure matrix was consulted to make sure the pattern matrix had not suppressed any values (Field 2005).

2.3.6 Canonical correspondence analysis

Another multivariate analysis, Canonical correspondence analysis (CCA), was used to test for relationships between the assembly of age groups and the environmental variables (Terbraak and Verdonschot 1995), and these can be visualised in an ordination diagram. CCA was performed using CANACO 4.5 for Windows, where the data was transformed using square root transformation. Manual forward selection step was used, with the number of permutations set to 999 which is the standard number used in the literature, as it ensure all ambiguous and weak effects are also identified (Buja & Eyuboglu, 1992).

2.3.7 Non-parametric tests

Kolmogorov-Smirnov tests were used to measure the normality of the data. Where data was not normally distributed, Kruskal Wallis tests were carried out for the continuous variables in order to make multiple comparisons. If a significant difference between age groups was found, Mann-Whitney tests were used to identify between which age groups the significant difference existed. As multiple Mann-Whitney tests were used, in order to make comparisons between age groups, the Bonferroni correction was applied. For the Hart Burn, six different tests were carried out and for the Wansbeck only three, so the modified critical levels for were 0.0083 and 0.017 respectively. For the categorical data, which in this case is distance from bank as it was recorded to the nearest metre, and canopy cover, a chi-square test was used. As all the continuous variables were found to have a non-normal distribution no parametric tests were carried out.

2.3.8 The effects of flooding

Data collection on the Wansbeck was interrupted due to a 1 in 115 year flood 6th - 8th September 2008. Previous to the flooding 45 quadrats had been collected. Once the river had become safe enough to collect data, 30 more quadrats were collected from the river over the period 25th -29th September 2008. Quadrats were placed in habitats that were newly formed from the flooding, as well as habitats that existed before the flooding. The data collected before and after the flooding were compared to see if the extensive flooding had any effect on the population of crayfish at the site.

After the flooding took place, 12 quadrats were taken from areas that were immediately adjacent to previously placed quadrats taken before the flooding. These 12 pairs can allow direct comparisons to be made of conditions before and after the flood. The size distributions before and after the flooding were not normally distributed. Non-parametric Mann-Whitney tests were carried out when comparing all quadrats and a Wilcoxon test was carried out for the 12 pairs of quadrats taken from the same position.

2.4 Results

2.4.1 Population structure

In the Wansbeck a total of 203 crayfish were caught, the carapace lengths of which ranged from 5.5 - 46.2 mm. In the Hart Burn a total of 283 crayfish were caught, of which the carapace lengths ranged from 3.8 - 40.3 mm. The output from FISAT II, in which the Bhattychara's method is used to assign age classes, can be seen in Appendix 1. For the Wansbeck the Bhattacharya method found four age groups all with significant separation indexes above 2 (4.5 - 9.4 mm carapace length (CL), 9.5 - 16.4 mm CL, 16.5 - 30.5 mm CL, 30.5 - 40.5 mm CL). As the fourth age group only consists of a very small number of crayfish, it was included in the third group, which became 16.5 mm+ CL. The three age groups used were as follows 0+ = 0 - 9.4 mm CL, 1+ = 9.5 - 16.4 mm CL, 2+ and older = 16.5 mm+ CL (Figure 2.3).

For the Hart Burn data set the Bhattacharya method found five different age groups all with separation indexes higher than 2 (3.5 - 7.4 mm CL, 7.5 - 13.4 mm CL, 13.5 - 22.5 mm CL, 15.5 - 29.4 mm CL, 29.5 - 35.5 mm CL). However, the fourth age group overlaps the third age group and the fifth age group only consists of a few crayfish. The first three age groups were used and all the rest were grouped as 22.5 mm+ CL. The four age groups used were as follows 0+ = 0 - 7.4 mm CL, 1+ = 7.5 - 13.4 mm CL, 2+ = 13.5 - 22.5 mm CL, 3+ and older = 22.5 mm + CL (figure 2.4).

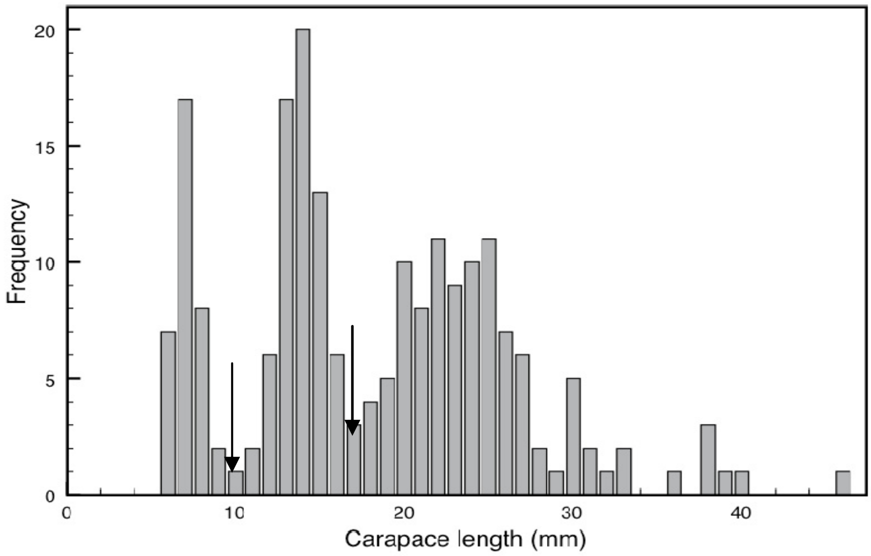


Figure 2.3- Size distribution of crayfish caught in the Wansbeck, where the arrows signify the upper size limit of the first two age classes

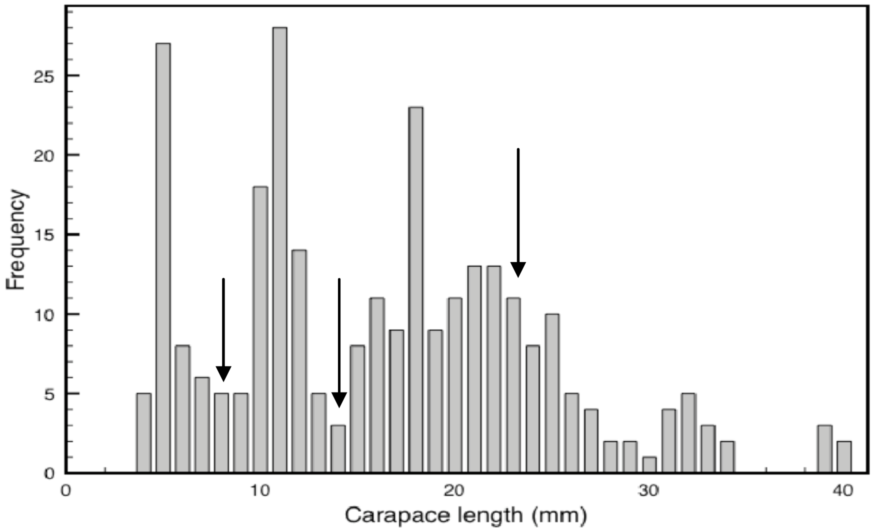


Figure 2.4- Size distribution of crayfish caught on the Hart Burn, where the arrows signify the upper size limit of the first three age classes

2.4.2 Logistic regression

The logistic regression on the Wansbeck data found that the percentage cover of small cobbles (χ^2 = 4.973, p = 0.026), moss (χ^2 = 6.776, p = 0.009) and canopy cover (χ^2 = 4.159, p = 0.041) positively affect the presence of crayfish. The logistic regression lines in Figures 2.5 - 2.7 show that the effects of the variables are the same, where an increase of canopy cover, moss and smaller cobbles increases the probability of finding a crayfish. Depth was found to affect the presence of crayfish (χ^2 = 4.973, p = 0.026) but as the depth increased the probability of finding a crayfish decreased (Figure 2.8). Sampling bias may have impacted on this association, where it is easier to find crayfish in shallower water. All other variables were found to have no significant effect on crayfish presence.

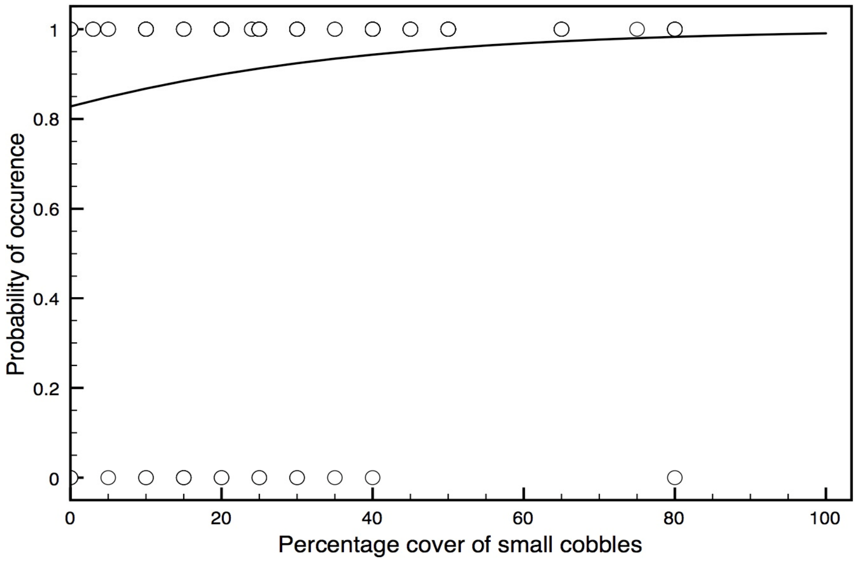


Figure 2.5- Logistic regression for crayfish presence/absence dependent on the cover of small cobbles in the Wansbeck

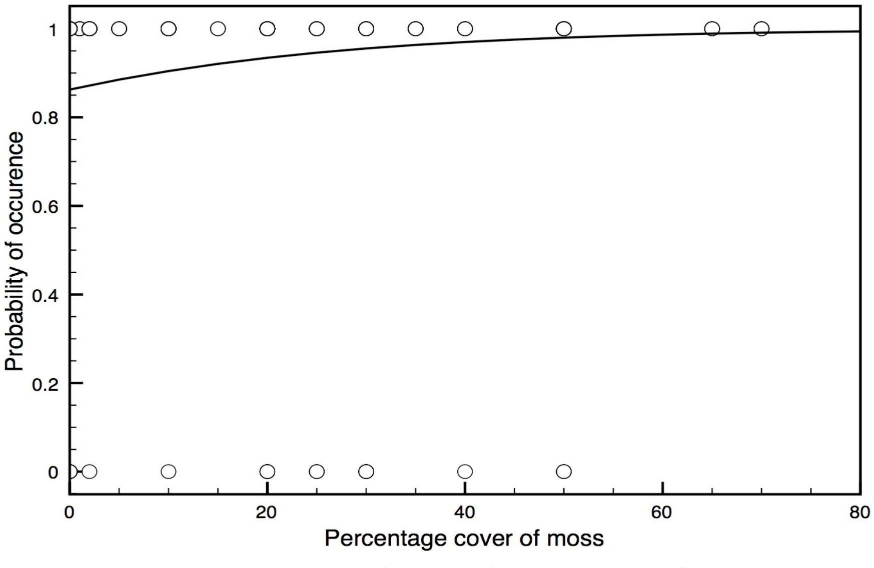


Figure 2.6- Logistic regression for crayfish presence/absence dependent on the cover of moss in the Wansbeck

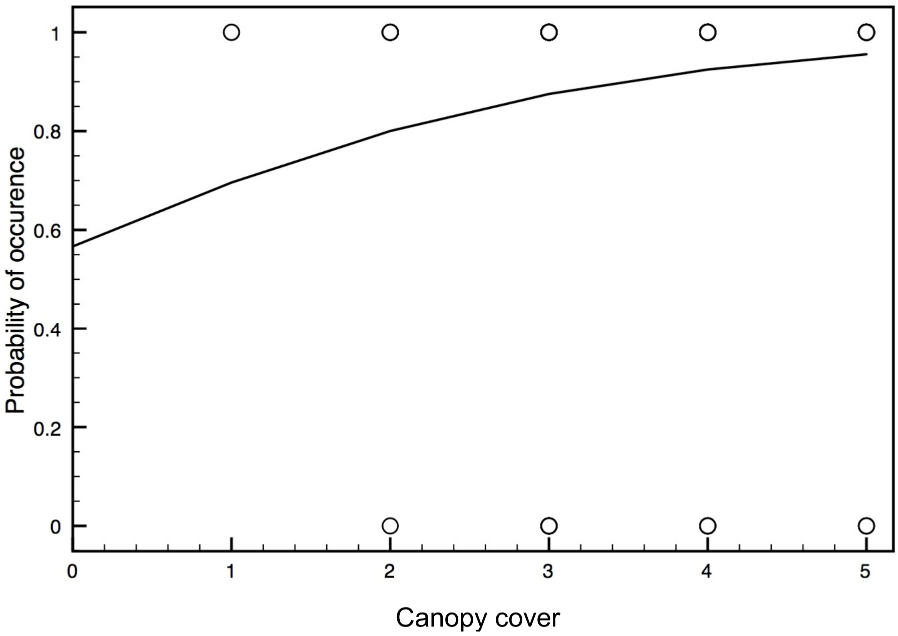


Figure 2.7- Logistic regression for presence/absence of crayfish dependent on canopy cover in the Wansbeck

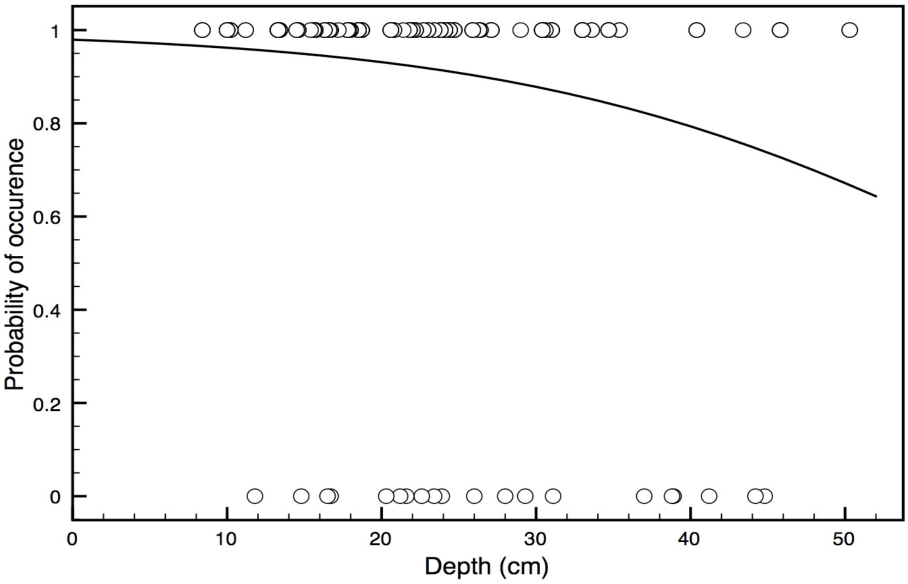


Figure 2.8- Logistic regression for presence/absence of crayfish dependent on depth in the Wansbeck

For the Hart Burn substrate index was found to positively affect the presence of crayfish (χ^2 = 8030, p = 0.005; see also Figure 2.9). Presence of bedrock was found to have a negative effect on crayfish presence (χ^2 = 7.718, p = 0.005; see also Figure 2.10). Distance from bank showed a significant but weaker negative relationship (χ^2 = 10.225, p = 0.036; see also Figure 2.11). Velocity was expected to be important but was not quite significant (χ^2 = 13.753, p = 0.056). Figure 2.12 shows the logistic regression for velocity where at higher velocities there was a reduced probability of finding a crayfish.

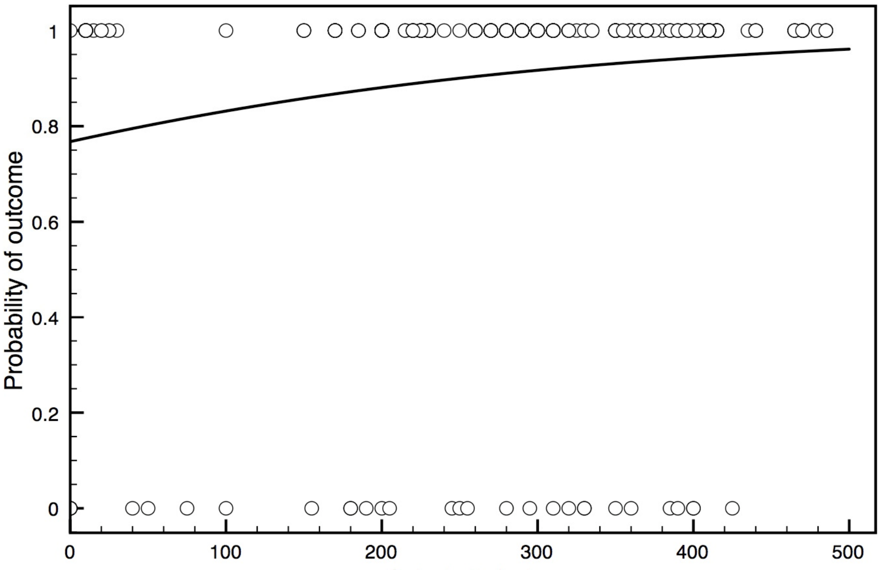


Figure 2.9- Logistic regression for presence/absence of crayfish dependent on substrate index in the Hart Burn

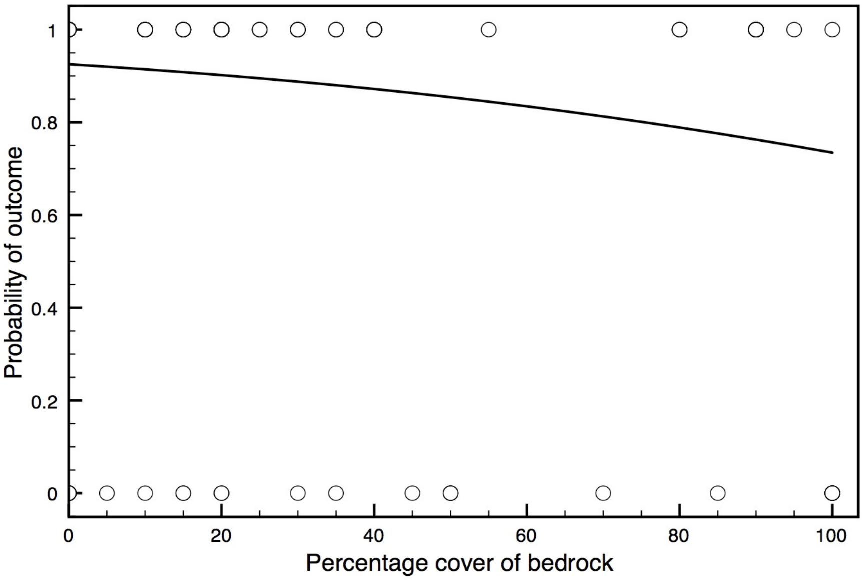


Figure 2.10- Logistic regression for presence/absence of crayfish dependent on cover of bedrock in the Hart Burn

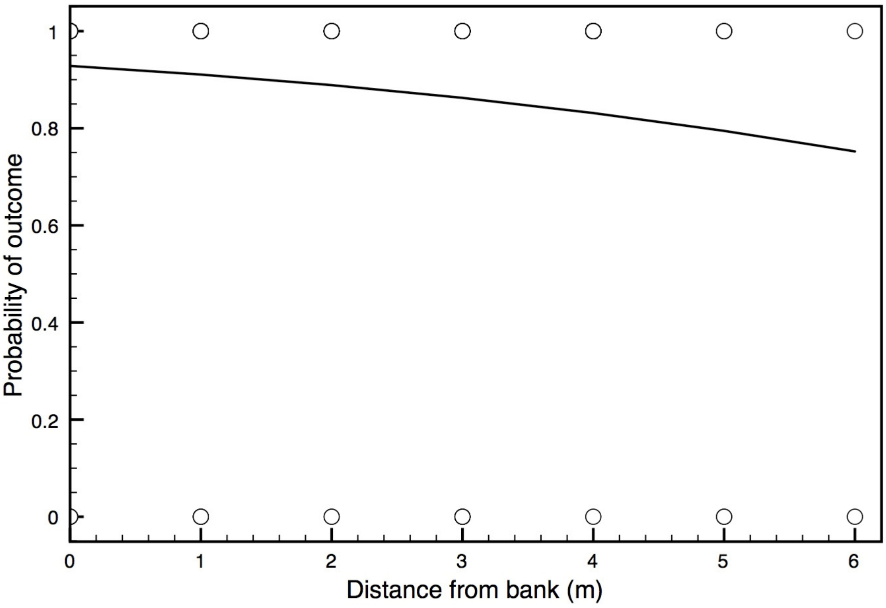


Figure 2.11- Logistic regression for presence/absence of crayfish dependent on distance from the bank in the Hart Burn

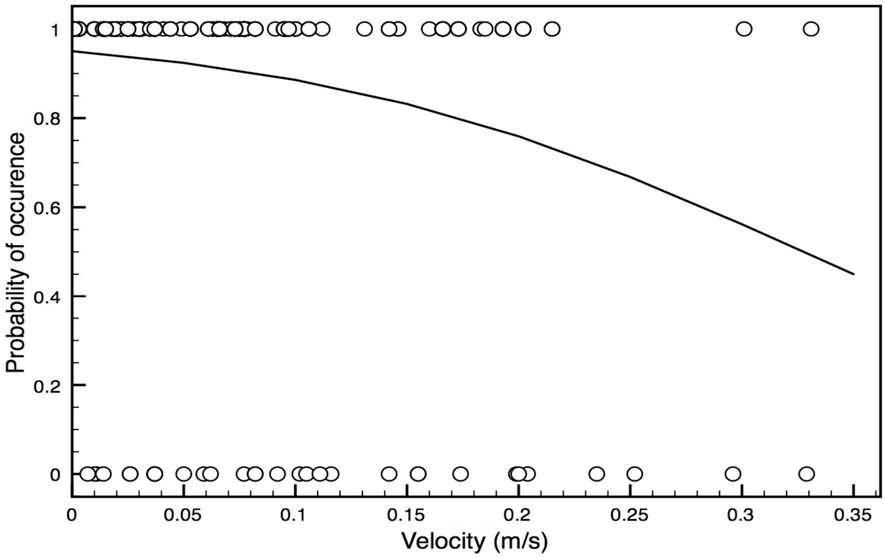


Figure 2.12- Logistic regression for presence/absence of crayfish dependent on the velocity in the Hart Burn

It is possible to determine whether a combination of variables can influence crayfish presence by inputting more than one variable to the logistic regression. However, this was not deemed necessary, as the multivariate analyses will determine how a combination of variables influences the occurrence of crayfish.

2.4.3 Multivariate analysis

2.4.3.1 Principal components analysis

Four variables, distance, velocity, velocity heterogeneity and substrate index were incorporated into a principal components analysis for the Wansbeck data.
Two factors were extracted, which explained 75% of the total variance, with factor one explaining 49.5% of the variance and factor two explaining 25.5%.
Table 2.3 presents the pattern matrix, where only the significant factor loadings of variables are shown.

Table 2.3- Pattern matrix for principal components analysis for the Wansbeck

Variable	Loading on factor 1	Loading on factor 2
Velocity	0.942	
Velocity heterogeneity	0.923	
Substrate index		0.885
Distance		0.643

Five variables, distance, velocity, substrate index, percentage cover of algae and moss and percentage cover of roots were incorporated into a principal components analysis for the Hart Burn data. The two factors extracted explained 69% of the total variance, with factor one explaining 46% of the variance and factor two explaining 23%. Table 2.4 presents the pattern matrix.

Table 2.4- Pattern matrix for principal components analysis for the Hart Burn

Variable	Loading on factor 1	Loading on factor 2
Velocity	0.862	
Distance	0.732	
% cover of algae/moss	0.703	
% cover of roots		0.896
Substrate index		-0.817

For each data set the factor analysis scores for each individual distinguished by age group were plotted, where the score for factor one was the X coordinate and the score for factor two was the Y coordinate. The resulting graphs are shown in Figures 2.13 and 2.14. Both PCA graphs show little separation in the microhabitats inhabited by different age groups of crayfish.

Both graphs also show clustering of points at relatively large substrate indexes and low velocities. By joining the outermost points on the graph maximum convex polygons were drawn, which represent the microhabitat breadth of the age groups. When the sizes of the convex polygons were defined they were not controlled for sample size. The maximum convex polygon for 0+ crayfish in both graphs is smaller in comparison to the older age groups, which implies that 0+ individuals occupy a smaller range of microhabitats.

There was no significant difference between the X coordinates for the age groups in the Wansbeck but there was a significant difference between the Y coordinates (Kruskal Walls, $n = 203$, $p < 0.0001$). Points that represent the microhabitat use of 0+ crayfish (Mann-Whitney U, $n = 136$, $p = 0.002$) and 1+ crayfish (Mann-Whitney U, $n = 168$, $p < 0.0001$) were recorded significantly lower down the Y-axis than points representing the microhabitat use of 2+ crayfish. This implies that 2+ individuals tend to be found in microhabitats that are composed of larger substrate and found further away from the bank in comparison to 0+ and 1+ crayfish.

For the Hart Burn there was a significant difference between the points that represent microhabitat use by different age groups in both X (Kruskal Wallis, n = 315, p < 0.0001), and Y coordinates (Kruskal Wallis, n = 315, p = 0.014). Points that represented microhabitat use of 0+ crayfish were recorded significantly lower down the X-axis in comparison to 2+ (Mann-Whitney U, n = 146, p < 0.0001) and 3+ and older crayfish (Mann-Whitney U, n = 108, p < 0.0001). Points that represented microhabitat use of 1+ crayfish were also recorded significantly lower down the X-axis in comparison to 2+ (Mann-Whitney U, n = 175, p = 0.002) and 3+ and older crayfish (Mann-Whitney U, n = 137, p = 0.001). This implies that crayfish aged 2+ and 3+ and older were found in microhabitats with higher velocities, found further away from the bank and with a larger cover of moss and algae in comparison to the younger 0+ and 1+ crayfish. Points that represent microhabitat use of 0+ crayfish were also recorded significantly higher up the Y-axis in comparison to 3+ and older crayfish (Mann-Whitney U, n = 108, p = 0.005). This implies that 0+ crayfish were found in finer substrate and in microhabitats with a higher percentage cover of roots

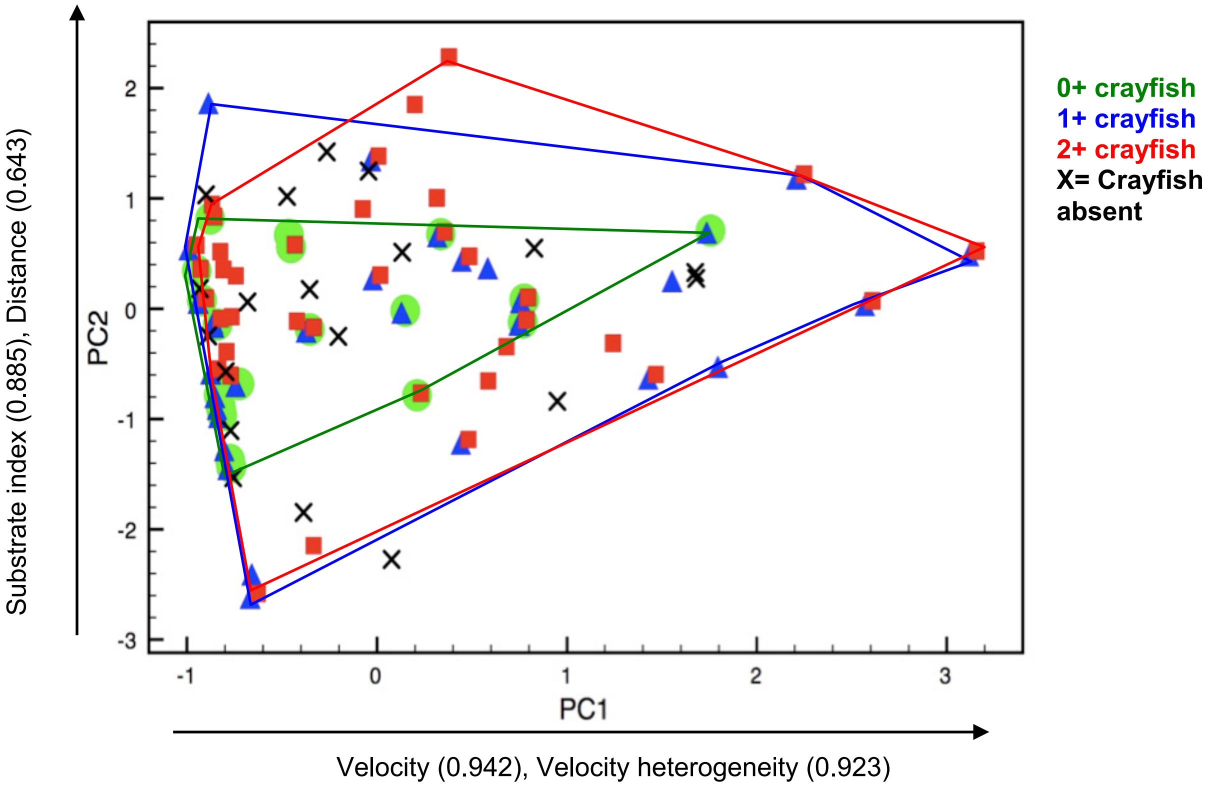


Figure 2.13- PCA biplots of microhabitat use by different age groups of crayfish in the Wansbeck. The overall microhabitat breadth is expressed for each age group by the maximum convex polygon. Individual points are shifted slightly to enhance visibility

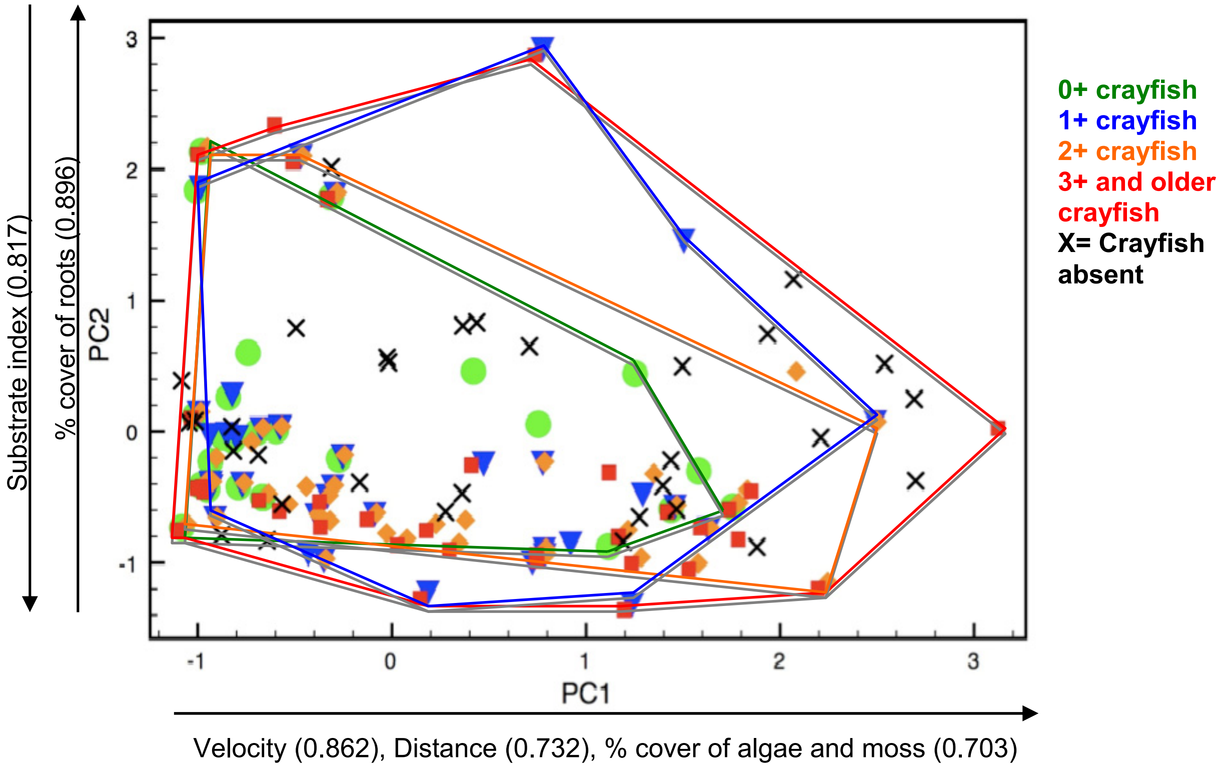


Figure 2.14- PCA biplots of microhabitat use by different age groups of crayfish in the Hart Burn. The overall microhabitat breadth is expressed for each age group by the maximum convex polygon. Individual points are shifted slightly to enhance visibility

2.4.3.2 Canonical correspondence analysis

For the CCA, no significant variables were found for the Wansbeck, but five were found for the Hart Burn: velocity, substrate index, roots, organic matter, and depth. There is a degree of similarity with the PCA, as velocity, substrate index and roots were also found to be important variables. However, unlike in the PCA, depth and organic matter were found to be important variables and not distance and cover of moss and algae.

The ordination diagram in figure 2.15 shows that the older crayfish of 2+ and 3+ and older are associated with each other in that they inhabit similar habitats, especially in terms of depth, velocity and substrate index. The percentage cover of roots seems particularly important to crayfish of age 3+ and older. The crayfish aged 0+ and 1+ were also found in similar habitats that are low in velocity, are shallow, have a low substrate index and contain organic matter. In particular, aged 0+ crayfish were found in areas with organic matter. The microhabitats where no crayfish were found have no associations with the microhabitats inhabited by all ages of crayfish, suggesting that these microhabitats are different in terms of the environmental variables included. It seems uninhabited microhabitat have a lower cover of roots, higher velocity and lower substrate index.

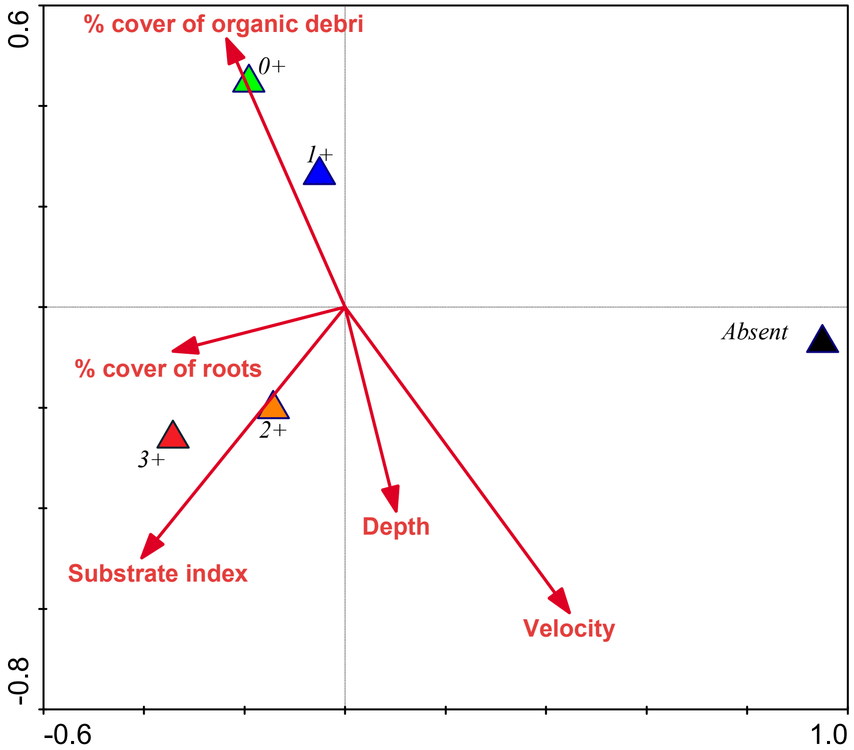


Figure 2.15- Canonical correspondence analysis ordination diagram, which includes the average vectors of the significant variables, and the symbols represent the centroids for the different age classes

The results from the multivariate analysis and the logistic regression were very different between the Wansbeck and the Hart Burn, which highlights the differences between the data sets.

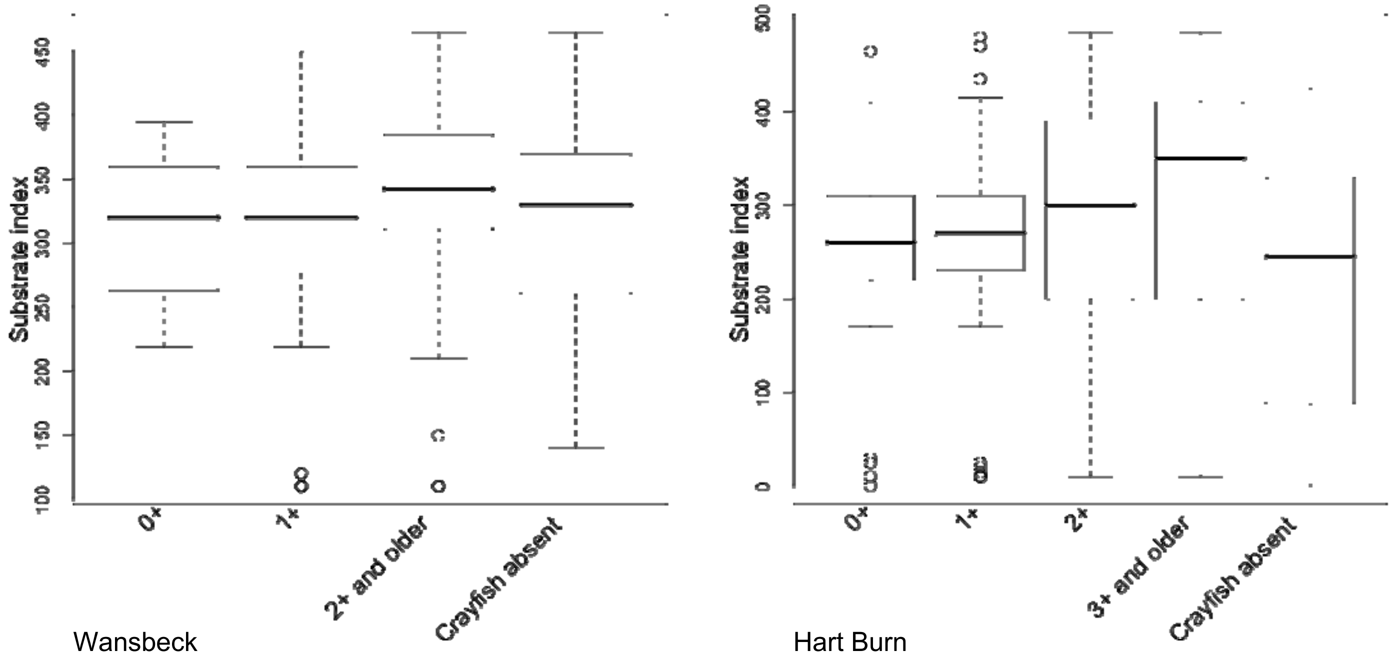
2.4.4 Univariate analysis

After examining all the variables together in the multivariate analysis, each individual variable will be explored in detail in order to identify important variations between age groups.

2.4.4.1 Substrate

Substrate index was found to be an important variable in the multivariate analysis for both the Wansbeck and the Hart Burn. Substrate index was also found to be important in influencing the presence of crayfish in the Hart Burn and small cobbles were important in influencing crayfish presence in the Wansbeck. larger crayfish in both the Wansbeck and the Hart Burn tended to occur in microhabitats with larger substrate indexes (Figure 2.16). Median substrate index inhabited was significantly different between age groups in both the Wansbeck (Kruskal Wallis test, n = 203, p = 0.014) and the Hart Burn (Kruskal Wallis test, n = 315, p = 0.002). In the Wansbeck the crayfish aged 2+ were found in significantly larger substrates than 1+ crayfish (Table 2.5). In the Hart Burn the crayfish aged

3+ and older were found in significantly larger substrates than 0+ crayfish (Table 2.6).



Wansbeck

Hart Burn

Figure 2.16- Box plots including median, upper and lower quartiles, 95% upper limit and 5% lower limit of substrate index in crayfish microhabitats for the Wansbeck and the Hart Burn.

Table 2.5- P values for Mann-Whitney U tests for use of substrate indexes by different age groups of crayfish in the Wansbeck, where the highlighted cells signify a significant result

	0+ (n=35)	1+ (n=67)	2+ and older (n=101)
0+ (n=35)		0.969	0.035
1+ (n=67)			0.009

Table 2.6- P values for Mann-Whitney U tests for use of substrate indexes by different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.437	0.06	0.004
1+ (n=75)			0.28	0.014
2+ (n=100)				0.118

Each individual substrate category was compared to determine the origin of the significant difference in overall substrate index. The Kruskal Wallis tests for all substrate types in both the Wansbeck and Hart Burn are shown in Table 2.7. For the Wansbeck the pebble substrate and the large cobble substrate showed significant differences in percentage cover between age classes. For the Hart Burn sand, pebbles, small cobbles, large cobbles, boulders, and bedrock all showed significant differences in percentage cover between age classes. Box plots of substrate size across age groups for the Wansbeck are presented in Figures 2.17 A-E.

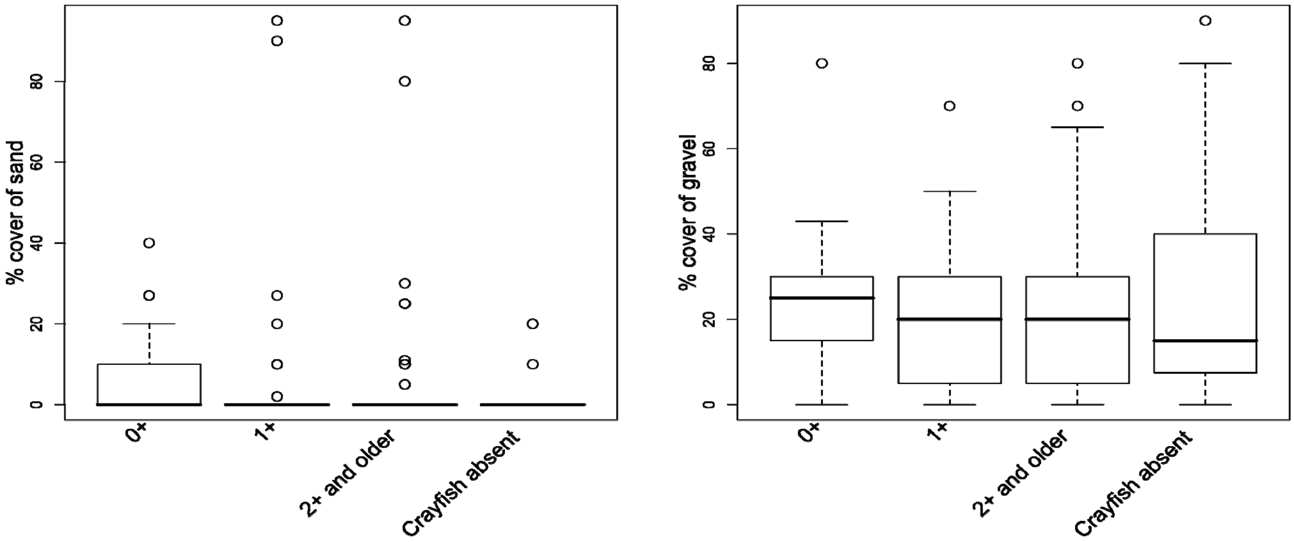
For the Wansbeck, 0+ and 1+ crayfish tended to occur in microhabitats with the greatest cover of pebbles, where they were found in microhabitats with a significantly higher cover of pebbles than 2+ and older crayfish (Table 2.8; see also Figure 2.17, C). Microhabitats inhabited by crayfish of 2 years and older tended to contain a higher proportion of large cobbles, where they were found in microhabitats with significantly greater cover of larger cobbles in comparison to 1+ crayfish (Table 2.8; see also Figure 2.17 E). Box plots of substrate size across age groups for the Hart Burn are presented in Figure 2.18, A-E.

Table 2.7- P values for Kruskal Wallis tests for use of seven different substrate types by different age groups of crayfish in the Wansbeck and the Hart Burn, where the highlighted cells signify a significant result

	Kruskall Wallis Hart Burn (n=283)	Kruskall Wallis Wansbeck (n=203)
Sand (<2mm)	<0.0001	0.318
Gravel 2-16mm	0.646	0.413
Pebbles 16-64mm	0.016	<0.0001
Small cobbles 64-128mm	0.028	0.057
large cobbles 128-256mm	<0.0001	0.029
Boulders >256mm	0.001	0.374
Bedrock	0.02	n/a

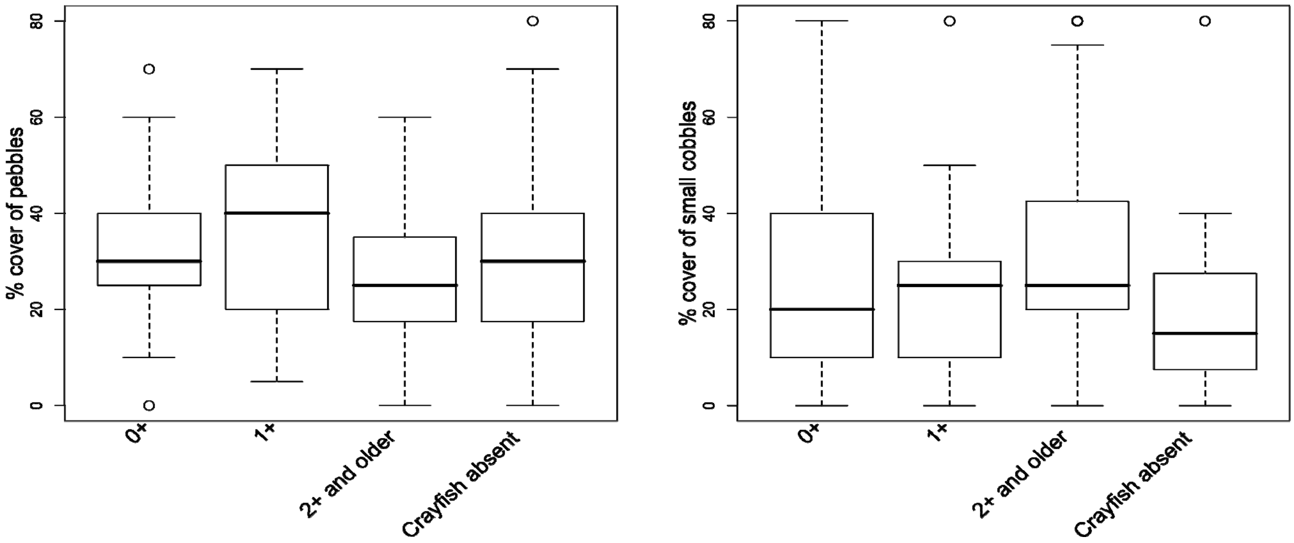
Table 2.8- P values for Mann-Whitney U tests for use of two different substrate types by different age groups of crayfish in the Wansbeck, where the highlighted cells signify a significant result

		0+ (n=35)	1+ (n=67)	2+ and older (n=101)
% cover of pebbles	0+ (n=35)		0.493	0.005
	1+ (n=67)			<0.0001
% cover of large cobbles	0+ (n=35)		0.696	0.13
	1+ (n=67)			0.011



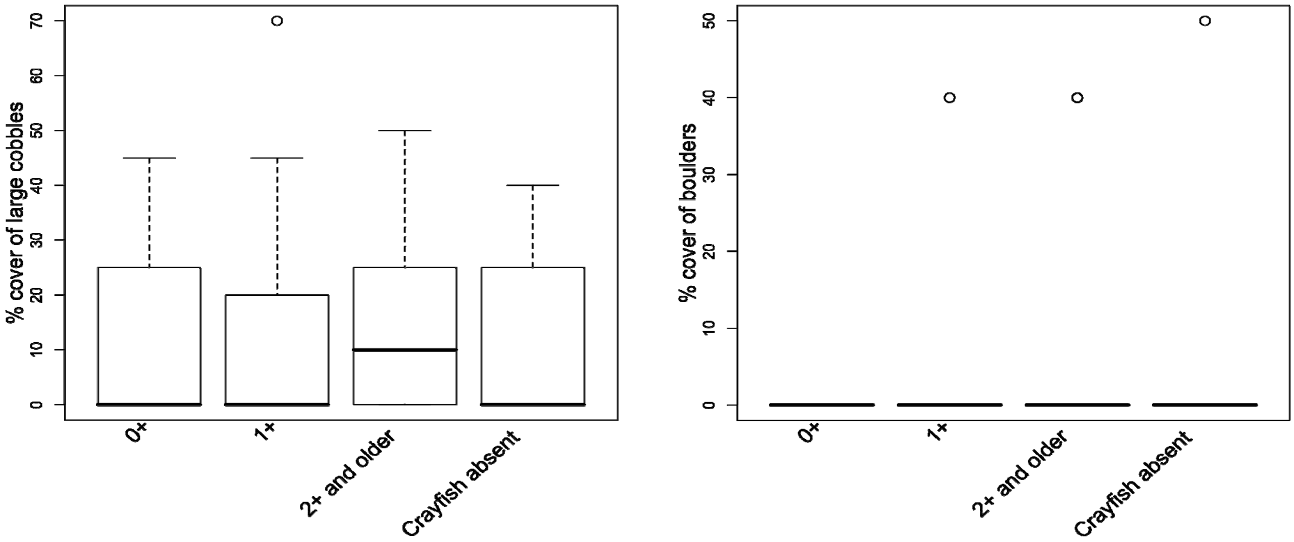
A) Sand < 2 mm diameter

B) Gravel 2 - 16 mm diameter



C) Pebbles 16 - 64 mm diameter

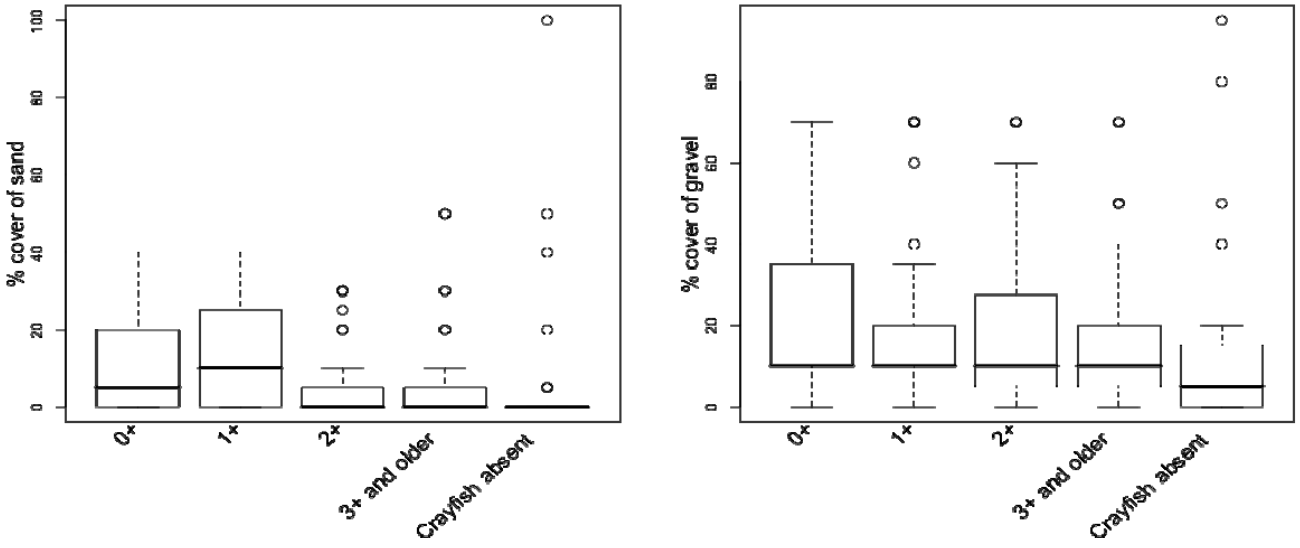
D) Small cobbles 64 - 128 mm diameter



E) Large cobbles 128 - 256 mm diameter

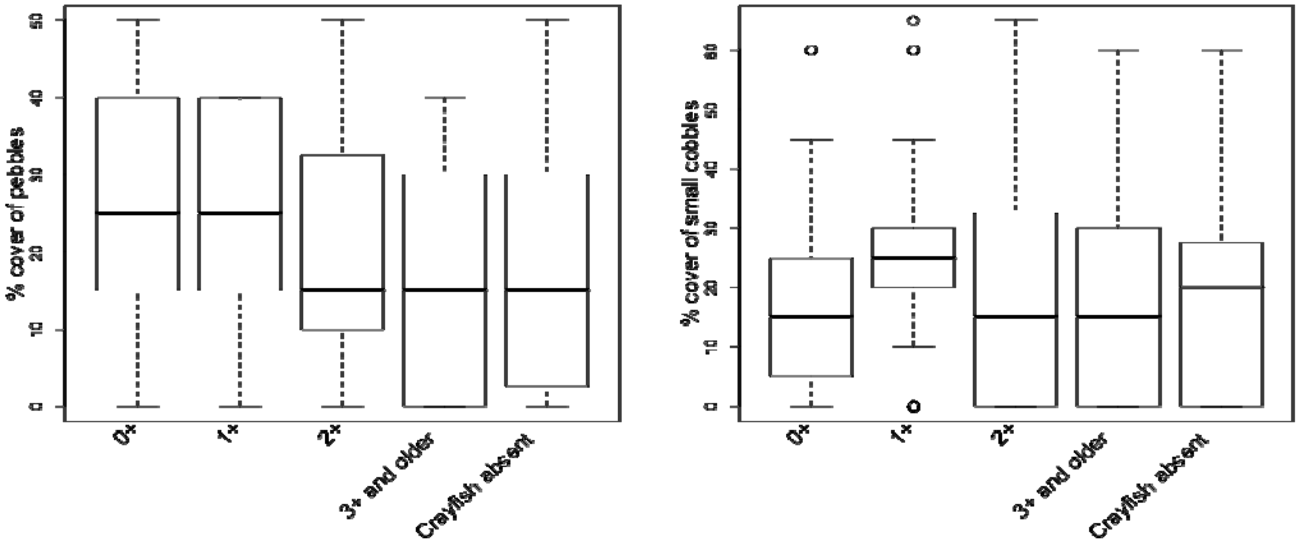
F) Boulders > 256 mm diameter

Figure 2.17- Box plots including median, upper and lower quartiles, 95% upper limit and 5% lower limit of the substrate composition of crayfish microhabitats on the Wansbeck



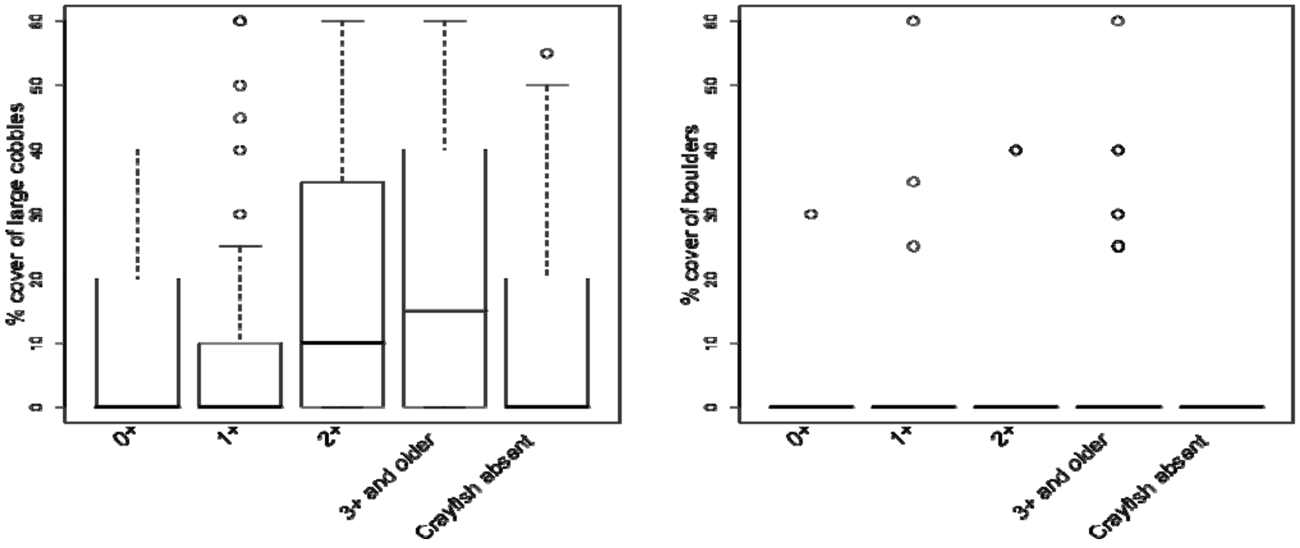
A) Sand < 2mm diameter

B) Gravel 2 - 16mm diameter



C) Pebbles 16 - 64mm diameter

D) Small cobbles 64 - 128mm diameter



E) Large cobbles 128 - 256mm diameter

F) Boulders > 256mm diameter

Figure 2.18- Box plots including median, upper and lower quartiles, 95% upper limit and 5% lower limit of the substrate composition of crayfish microhabitats on the Hart Burn

The P values for the Mann-Whitney tests for each variable, where there was a significant value in the Kruskal Wallis test, are shown in Table 2.9. Quadrats where crayfish were absent were not compared, as the sample size was low and introducing another group would reduce the strength of the statistical test. Age 0+ and 1+ crayfish had a significantly higher cover of sand compared to 2+ and 3+ and older crayfish (Table 2.9; see also Figure 2.18, A). Age 3+ crayfish were recorded in microhabitats with a significantly higher cover of large cobbles in comparison to 1+ and 2+ crayfish (Table 2.9; see also Figure 2.18, E). Crayfish aged 3+ and older also had an association with boulders, although it is unclear from the boxplot they were recorded in microhabitats with a significantly higher cover of boulders than microhabitats used by 2+ crayfish (Table 9; see also Figure 2.18,F).

Bedrock was recorded in the Hart Burn only and there seems to be little variation in the cover of bedrock between microhabitats of different age groups (Figure 2.19). However, the Kruskal Wallis test reported a significant difference (Table 2.7), and age 2+ crayfish were recorded in microhabitats with a significantly higher cover of bedrock than 1+ crayfish (Table 2.9).

Table 2.9- P values for Mann-Whitney U tests for use of six different substrate types by different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

		0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
% cover of sand	0+ (n=46)		0.524	<0.0001	0.001
	1+ (n=75)			<0.0001	<0.0001
	2+ (n=100)				0.324
% cover of pebbles	0+ (n=46)		0.892	0.165	0.011
	1+ (n=75)			0.15	0.005
	2+ (n=100)				0.076
% cover of small cobbles	0+ (n=46)		0.004	0.759	0.748
	1+ (n=75)			0.017	0.042
	2+ (n=100)				0.961
% cover of large cobbles	0+ (n=46)		0.171	0.03	0.003
	1+ (n=75)			<0.0001	<0.0001
	2+ (n=100)				0.343
% cover of boulders	0+ (n=46)		0.581	0.76	0.012
	1+ (n=75)			0.731	0.009
	2+ (n=100)				0.002
% cover of bedrock	0+ (n=46)		0.055	0.354	922
	1+ (n=75)			0.002	0.06
	2+ (n=100)				0.265

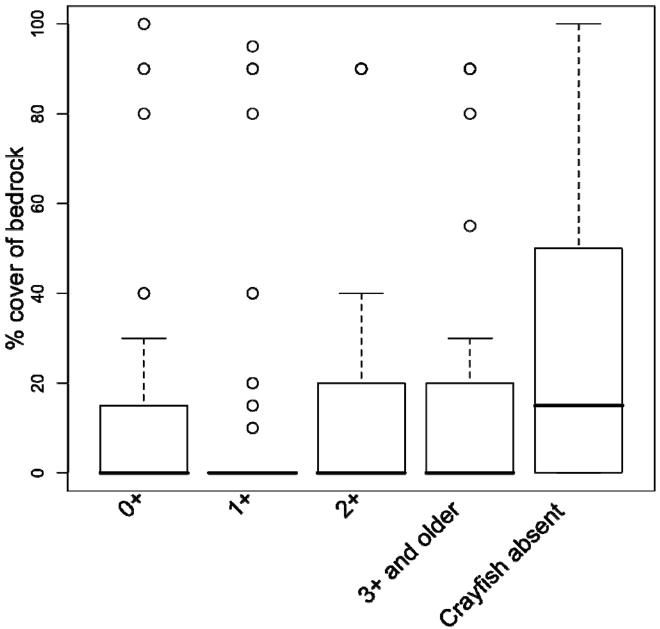


Figure 2.19 – A box plot of the percentage cover of bedrock found in microhabitats of different age groups in the Hart Burn

In the Wansbeck microhabitats inhabited by 2+ and older crayfish had significantly higher substrate indexes than 1+ crayfish (Figure 2.16), due to a higher cover of large cobbles and a lower cover of pebbles. For the Hart Burn, the significantly higher substrate indexes found in microhabitats inhabited by 3+ and older crayfish in comparison to 0+ crayfish (Figure 2.16) was due to a higher cover of large cobbles and lower cover of sand.

2.4.4.2 Water velocity

Water velocity was found to be a significant variable explaining the microhabitat use of crayfish in the PCA for both the Wansbeck and the Hart Burn. Larger crayfish tended to inhabit microhabitats with higher velocities in both the Wansbeck and the Hart Burn (Figure 2.20). In the Hart Burn there was a significant difference in median velocities between age groups (Kruskal Wallis, $n = 283$, $p < 0.0001$), but not for the Wansbeck (Kruskal Wallis, $n = 203$, $p = 0.274$). Hart Burn crayfish of age 2+ and 3+ and older were recorded in microhabitats with significantly higher velocities than 0+ and 1+ crayfish (Table 2.10).

Velocity heterogeneity was found to be important in explaining the microhabitat use of different age groups of crayfish on the Wansbeck, as it was included in the PCA. However, it was not found to be a significant variable in the PCA for the Hart Burn. There is little variation between age groups (Figure 2.21), and there was no significant difference in velocity heterogeneity between the age groups in the Wansbeck (Kruskal Wallis, $n = 203$, $p = 0.186$) or the Hart Burn (Kruskal Wallis, $n = 283$, $p = 0.165$).

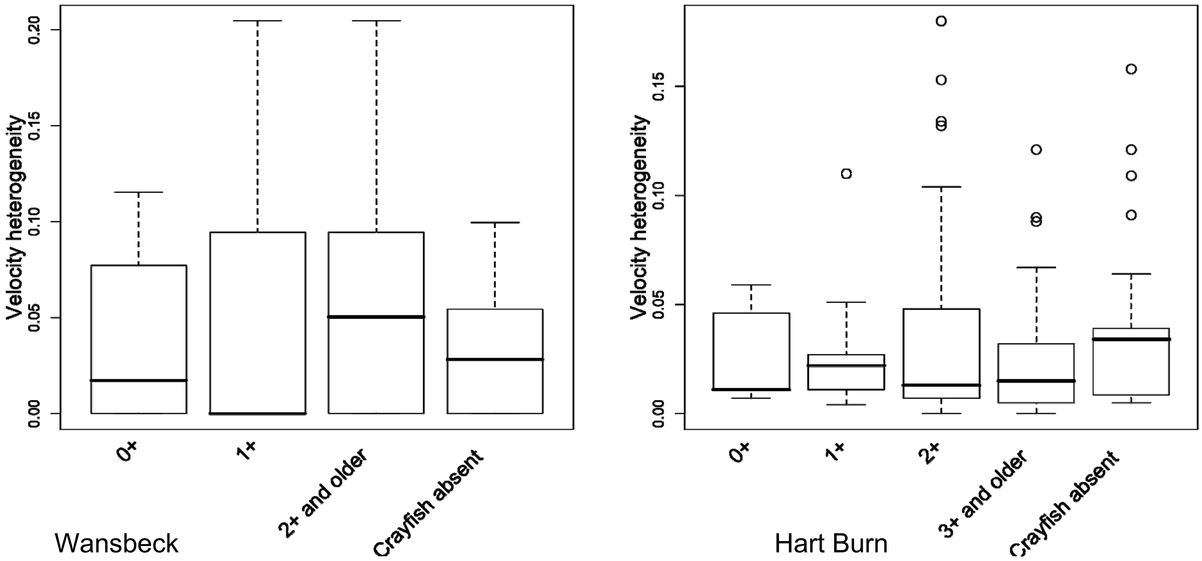


Figure 2.20- Box plots of the velocities found in microhabitats of different age groups in the Wansbeck and the Hart Burn

Table 2.10- P values for Mann-Whitney U tests for the water velocities recorded in microhabitats of different age groups of crayfish in the Wansbeck, where the highlighted cells signify a significant result

	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.245	0.001	<0.0001
1+ (n=75)			0.006	0.002
2+ (n=100)				0.324

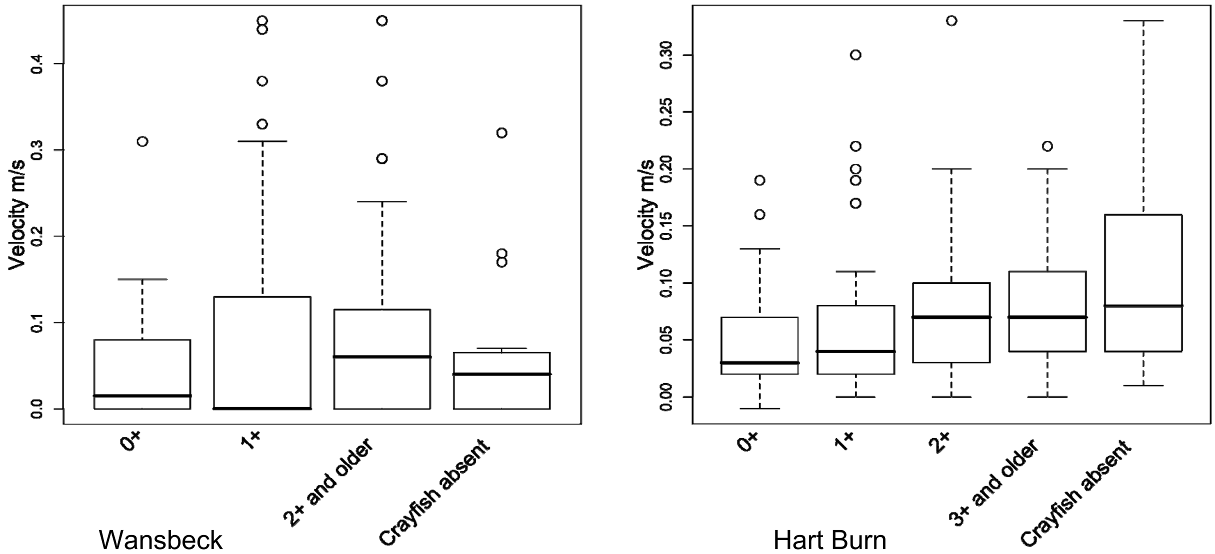


Figure 2.21- Box plots of the velocity heterogeneity found in microhabitats of different age groups in the Wansbeck and the Hart Burn

2.4.4.3 Distance from bank

Distance from bank was also found to be a significant variable in the PCA for describing crayfish distribution in both the Wansbeck and the Hart Burn. For both the Wansbeck and the Hart Burn distance from the bank, expressed in distance classes of <1 m, 1 - 3 m, >3 m, was found to be significantly different between the age classes of crayfish (Wansbeck $\chi^2 = 28.844$, $p < 0.0001$, Hart Burn $\chi^2 = 21.773$, $p < 0.0001$). In the Wansbeck, more aged 0+ and 1+ crayfish were found within 1 m of the bank than would be expected if there was no effect of age (Figure 2.22). For microhabitats found 1 - 3 m from the bank, the observed values were similar to the expected. In microhabitats 3 m and further from the bank, there were more 2+ and older crayfish found than expected and less 1+ and especially 0+ crayfish.

In the Hart Burn more 0+ and 1+ crayfish and less 2+ and 3+ and older were observed within 1 m of the bank than would be expected if age had no effect (Figure 2.23). The same pattern was observed for microhabitats found 1 - 3 m from the bank. For the microhabitats found 3 m and further from the bank there were less 0+, 1+ and 2+ than expected and more 3+ and older crayfish.

2.4.4.4 Cover of moss and algae

Moss was found to be a significant variable in the multivariate analysis for the Hart Burn. Larger crayfish tended to be found in microhabitats with a higher cover of moss and algae in both the Hart Burn and the Wansbeck (Figure 2.24). In the Wansbeck the 2+ individuals had the largest median for percentage cover of moss. In the Hart Burn, the effect is less obvious where the medians are all zero, as the values for percentage cover of algae and moss were not as large as in the Wansbeck. There was a significant difference in the cover of moss and algae between microhabitats of different aged crayfish in both the Wansbeck (Kruskal Wallis, n = 203, p = 0.034) and the Hart Burn (Kruskal Wallis, n = 283, p = 0.002). Wansbeck crayfish of age 2+ and older were found in microhabitats with signficantly more algae and moss in comparison to 0+ crayfish (Table 2.11). Hart Burn crayfish of age 3+ and older were found in microhabitats with signficantly more algae and moss in comparison to both 0+ and 1+ crayfish (Table 2.12).

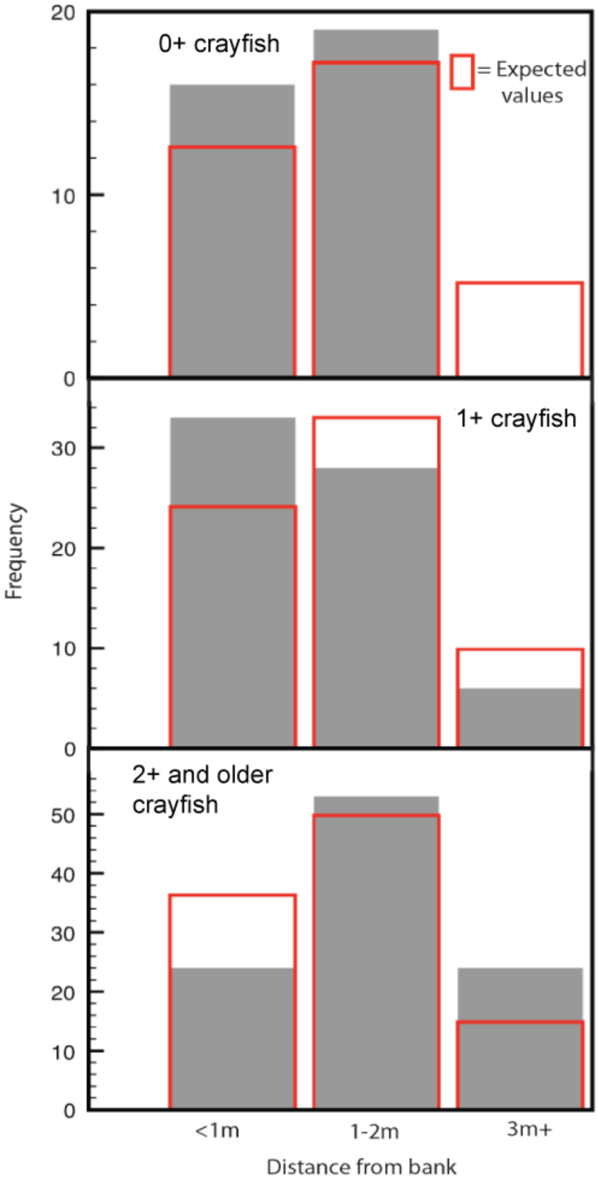


Figure 2.22- A bar chart of expected and observed numbers of crayfish of different age groups, in microhabitats with varying distances from the bank, recorded in the Wansbeck

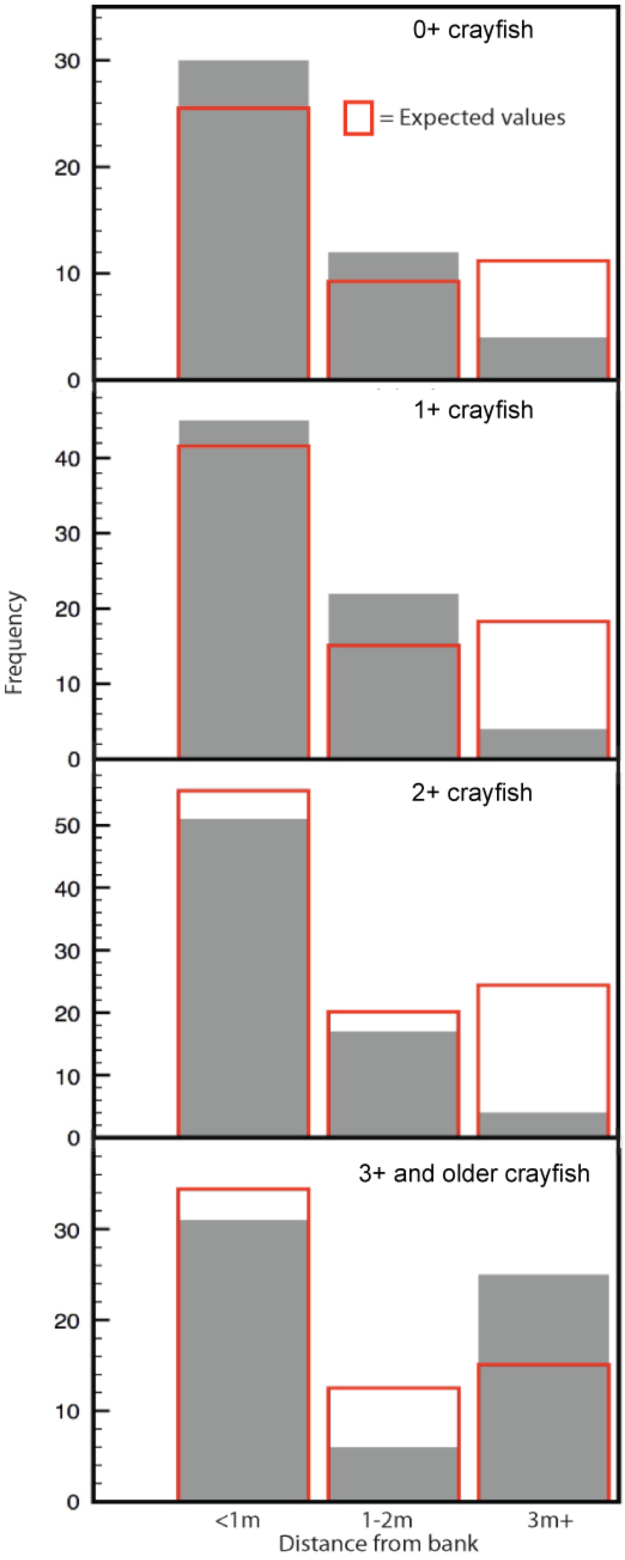


Figure 2.23- A bar chart of expected and observed numbers of crayfish of different age groups, in microhabitats with varying distances from the bank, recorded in the Hart Burn

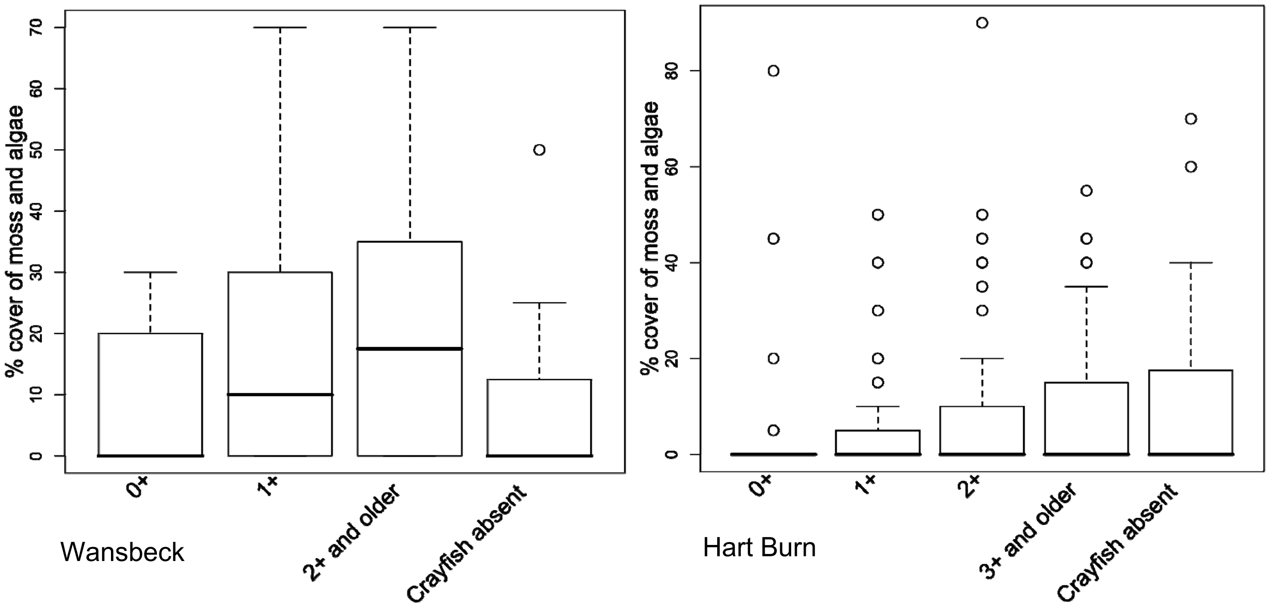


Figure 2.24- Box plots of the cover of moss and algae found in microhabitats of different age groups in the Wansbeck and the Hart Burn

Table 2.11- P values for Mann-Whitney U tests for cover of moss and algae recorded in microhabitats of different age groups of crayfish in the Wansbeck, where the highlighted cells signify a significant result

	0+ (n=35)	1+ (n=67)	2+ and older (n=101)
0+ (n=35)		0.028	0.006
1+ (n=67)			0.596

Table 2.12- P values for Mann-Whitney U tests for cover of moss and algae recorded in microhabitats of different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.206	0.014	0.001
1+ (n=75)			0.092	0.004
2+ (n=100)				0.155

2.4.4.5 Cover of roots

Cover of roots was a variable that was recorded in the Hart Burn only, as it contained a high number of accessible roots that could be sampled, unlike in the Wansbeck. The variable was found to be important in describing crayfish distribution in the PCA and CCA. Univariate analysis showed there to be a significant difference in cover of roots between age groups (Kruskal Wallis, n = 283, p = 0.025). The box plot in Figure 2.25 suggests that older crayfish are found in microhabitats with a higher cover of roots. Age 2+ and 3+ and older crayfish were found in microhabitats with significantly more root cover than 1+ crayfish (Table 2.13).

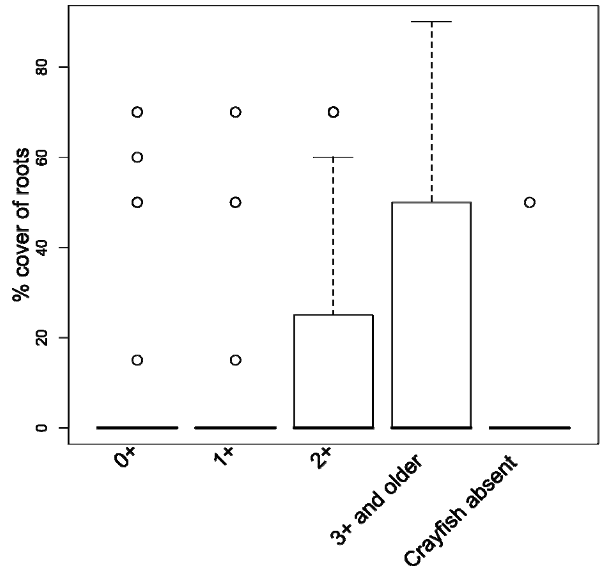


Figure 2.25- A box plot of the cover of roots found in microhabitats of different age groups in the Hart Burn

Table 2.13- P values for Mann-Whitney U tests for cover of roots recorded in microhabitats of different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

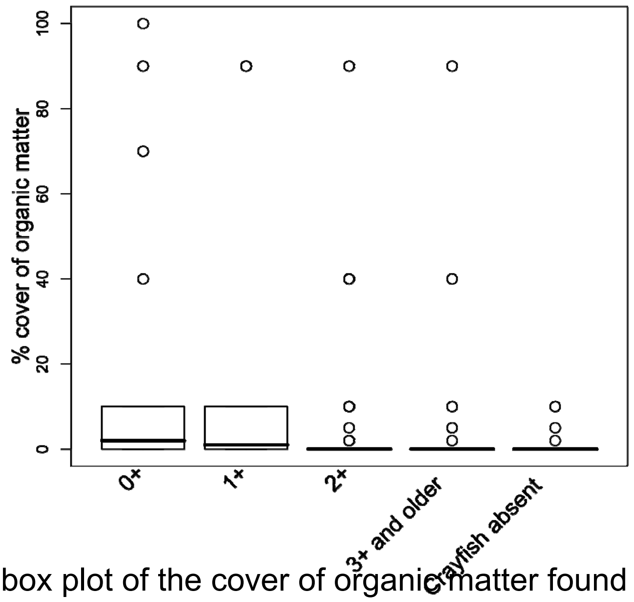
	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.096	0.399	0.354
1+ (n=75)			0.005	0.006
2+ (n=100)				0.828

2.4.4.6 Cover of organic matter

Organic matter was another variable that was only included in the analysis for the Hart Burn, as it contained a high number of accessible areas of leaf litter, so provided a large enough sample size unlike in the Wansbeck. Organic matter was extracted as a factor in the CCA for the Hart Burn. Univariate analysis identified differences in extent of organic matter cover between age groups (Kruskall wallis, n = 283, p < 0.0001). There was a significantly higher cover of organic matter in microhabitats of the two youngest age groups (Table 2.14; see also Figure 2.26).

Table 2.14- P values for Mann-Whitney U tests for cover of organic matter recorded in microhabitats of different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.584	<0.0001	0
1+ (n=75)			<0.0001	<0.0001
2+ (n=100)				0.565



igure 2.26- A box plot of the cover of organic matter found in microhabitats of different age groups in the Hart Burn

2.4.4.7 Water depth

Depth was not found to be an important variable in the PCAs but was included in the CCA output for the Hart Burn. Logistic regression also identified it as a significant variable for influencing the presence of crayfish in the Hart Burn. For the Wansbeck there was no significant difference in depth between ages (Kruskal Wallis, n = 203, p = 0.056), but there was for the Hart Burn (Kruskal Wallis, n = 283, p = 0.001). Box plots in Figure 2.27 suggest a small influence on depths used by different age groups. In the Hart Burn age 2+ crayfish were found in microhabitats that were significantly deeper than 1+ crayfish (Table 2.15)

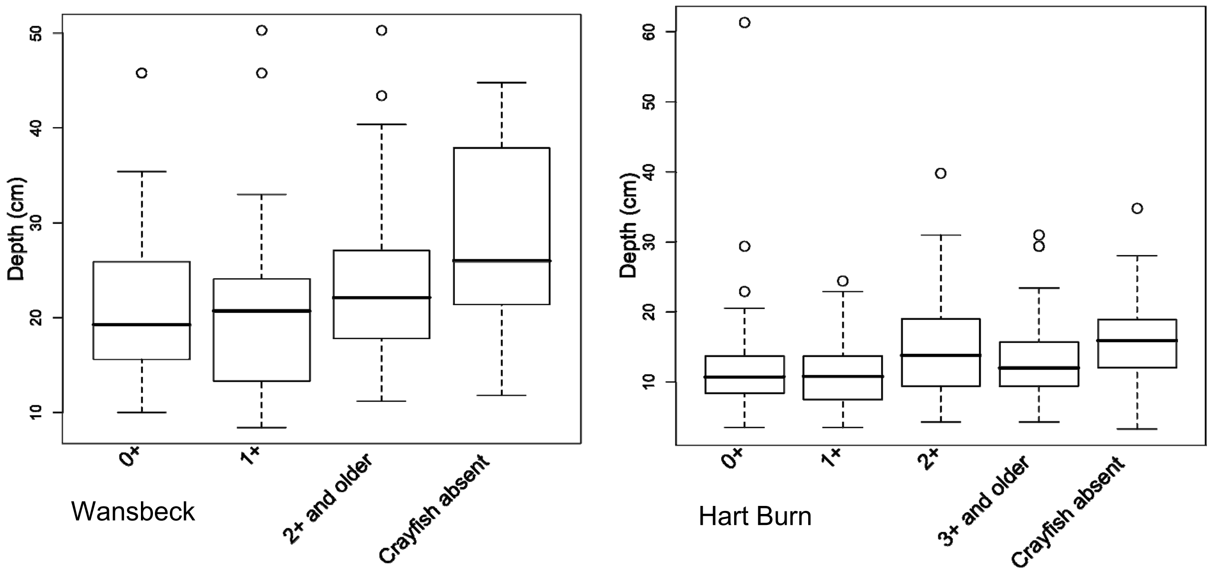


Figure 2.27- Box plots of the depth in microhabitats of different age groups in the Wansbeck and the Hart Burn

Table 2.15- P values for Mann-Whitney U tests for depth recorded in microhabitats of different age groups of crayfish in the Hart Burn, where the highlighted cells signify a significant result

	0+ (n=46)	1+ (n=75)	2+ (n=100)	3+ and older (n=62)
0+ (n=46)		0.582	0.014	0.084
1+ (n=75)			<0.0001	0.009
2+ (n=100)				0.365

2.4.4.8 Canopy cover

Canopy cover was classified in categories between 0 and 5, but due to the high abundance of trees there were few quadrats with little canopy cover, so categories 0 - 3 were grouped together. Canopy cover was not found to be an important variable in either of the multivariate analyses for both the Hart Burn and the Wansbeck, but was identified by logistic regression as a factor which influences the presence of crayfish on the Wansbeck. For the Wansbeck there was found to be a significant difference between age groups ($\chi^2 = 9.694$, $p = 0.046$), but no relationship was found for the Hart Burn ($\chi^2 = 5.464$, $p = 0.141$). The expected and observed values for the Wansbeck are shown in Figure 2.28. More 0+ crayfish were found in relatively open sites than would be expected if age had no effect. More 0+ and 1+ crayfish were found in sites with dense overhead cover and more 2+ and older crayfish were found in the high cover category 4 than would be expected.

2.4.5 The effects of flooding on crayfish populations.

The density of crayfish recorded in the Wansbeck before and after the 2008 flooding was similar, where the mean density recorded before the flooding was 2.6 crayfish/quadrat compared to 2.8 crayfish/quadrat after the flooding. There was no significant difference between overall densities of crayfish before and after the flooding (Mann-Whitney U, n=75, p=0.367), but the oldest age group of 2+ and older saw significant declines in densities after the flooding (Table 2.16). There was no significant difference in densities in other age groups (Table 2.16).

The crayfish caught before the flooding were significantly larger than the crayfish caught after the flooding (Mann-Whitney U, n = 203, p = 0.002; see also Figure 2.30). This may be a reflection of the reduction in abundance of large adult crayfish. In the matched pairs of quadrats before and after the flooding (Figure 31), there was no significant difference (Wilcoxon, n=12, p= 0.272). However, this may be a reflection of the small sample size. There is always the possibility of sampling bias, as adult habitat may have been harder to search after the flooding due to the increased depth of channel sections.

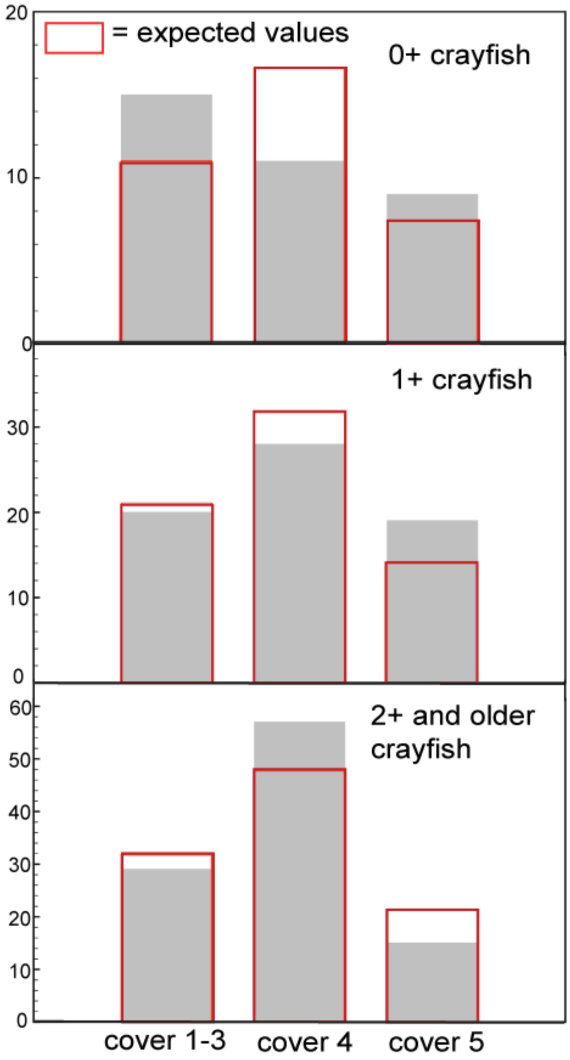


Figure 2.28- A bar chart of expected and observed numbers of crayfish of different age groups, in microhabitats with varying degrees of canopy cover, recorded in the Wansbeck

Table 2.16- P values for Mann-Whitney U test for densities of different crayfish age groups before and after a major flooding event in September 2008, where the highlighted cells signify a significant result

Age group	P value
0+	0.194
1+	0.274
2+ and older	0.007

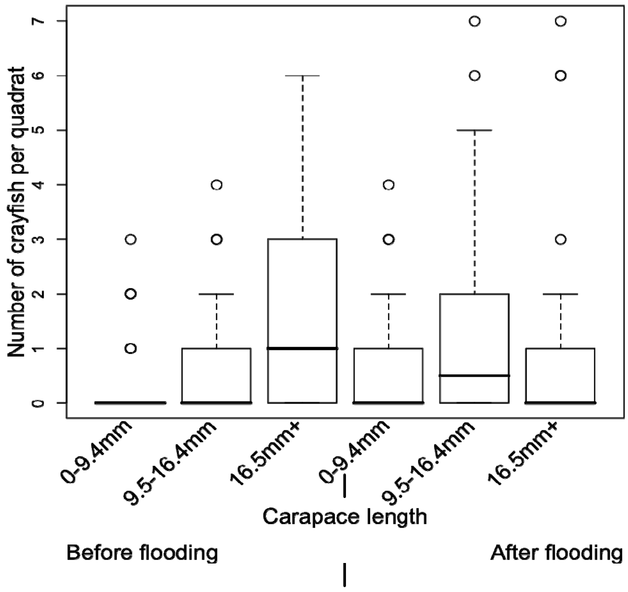


Figure 2.29- Densities of different crayfish age groups recorded in the 75 quadrats collected in the Wansbeck in 2008, before and after the major flooding event in September

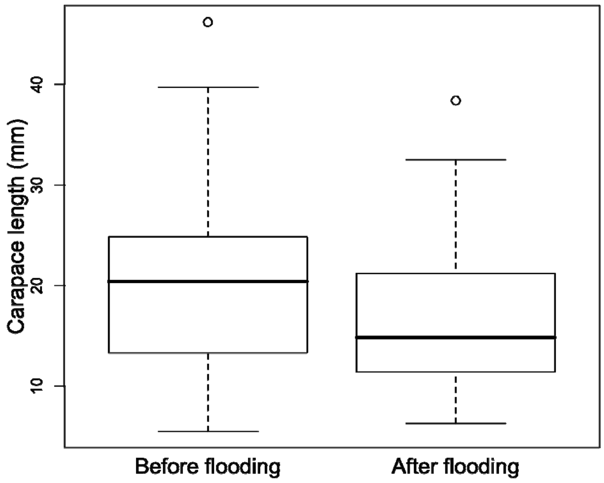


Figure 2.30- Size distribution of crayfish caught from all 75 quadrats taken in the Wansbeck 2008, before and after the major flooding event in September

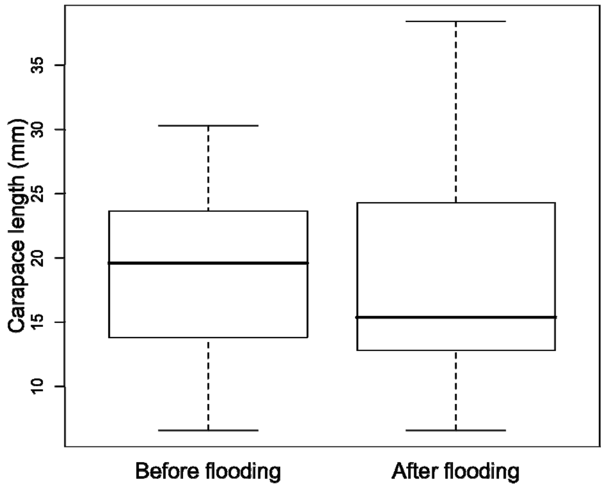


Figure 2.31- Size distribution of crayfish caught from 12 matched pairs taken in the Wansbeck 2008, before and after the major flooding event in September

2.5 Discussion

2.5.1 Factors influencing crayfish presence

Different variables influenced crayfish presence on the Wansbeck and the Hart Burn. For the Wansbeck, crayfish were more likely to be present in areas with a high cover of small cobbles, moss and canopy cover and less likely to be present in deep waters. Previous studies have also recognised that crayfish are usually absent from deeper waters (Holdich 2003), but this may be because deeper waters are harder to sample. Other studies have also found tree shading to be important in creating suitable crayfish habitat (Naura and Robinson 1998). It is surprising that small cobbles in particular would be important for influencing crayfish presence, as crayfish have been found to favour larger stones, where cobbles only provide suitable habitat for juveniles (Peay 2000, Holdich 2003). However, in the Wansbeck a large number of adult crayfish were found under small cobbles, so they may represent an intermediate substrate that is widely used by a broad range of crayfish age groups, and therefore important in creating favourable habitats. Cover of moss and algae was found to be important in influencing crayfish presence, which is surprising as white-clawed crayfish are found in many rivers where moss is largely absent, for example the low lying River Ivel in Bedfordshire, England (Peay 2000). It may be that the cover of moss and algae represents the size of the substrate, where there is generally a higher cover on larger substrates such as cobbles and boulders. If this is the case then it is the substrate size that is influencing crayfish presence where there is a higher chance of finding a crayfish in larger substrates.

In the Hart Burn, crayfish were more likely to be present in larger substrates, close to the bank, in slower velocities and in areas with a minimal cover of exposed bedrock. Bedrock is important for influencing crayfish absence, as it provides little refuge for crayfish, except where it is deeply fissured. Crayfish may generally be found close to the bank due to the added protection from high flows and rivers tend to get deeper further away from the bank. Large substrates such as boulders and large cobbles are important for crayfish, especially larger crayfish, which need a stable and solid substrate to refuge under. However this result is somewhat contradictory to the Wansbeck results, which found small cobbles to be important refuges. Crayfish are probably associated with slower velocities as it may make it easier for crayfish to forage, where less energy will be expended when moving. Higher velocities may also create unstable environments, which would be unfavourable to crayfish as they are easily injured by moving rocks. Benvenuto *et al* (2008) agrees with this finding, as they found that white-clawed crayfish avoided streams with velocities over 0.1 m s^{-1}. Streissl and Hodl (2002) also carried out logistic regression when looking at stone crayfish *Austropotamobius torrentium* in Austria. Similarly they found that the probability of finding a stone crayfish increases with increasing substrate size and decreased with increasing velocity.

Overall it seems the important variables that influence crayfish presence vary, depending on the river. The output of the CCA showed a distinct separation between microhabitats where different ages of crayfish were present and where crayfish were absent. The separation of microhabitats was in terms of velocity, depth, substrate index, cover of organic matter and roots, which implies these are also important variables that influence the presence of crayfish. Unlike the Wansbeck, the variables important for influencing crayfish presence in the Hart Burn reflected the variables incorporated into the multivariate analysis.

2.5.2 Microhabitat use by different age groups

The PCA found velocity, substrate index and distance from bank to be good predictors of the presence of the different age groups, as they were found to be important in both rivers. These three variables showed interactions with one another, where microhabitats in close proximity to the bank had slower velocities, due to the projecting bank creating slower pools, and slower velocities resulted in finer substrate. In both rivers the influence of these variables were similar.

The areas covered by the maximum convex polygons on the PCA graphs imply that the 0+ crayfish are much more restricted by their microhabitat requirements than the older crayfish, in both the Wansbeck and the Hart Burn. There is always the possibility that smaller 0+ crayfish are harder to catch in different environments, restricting the environments in which they can be caught and recorded, but the use of a Surber sampler should have overcome this bias. The Wansbeck PCA suggests that 0+ crayfish are found in microhabitats with lower velocities than older crayfish. However the univariate velocity results found no significant difference between age groups. This is surprising but may be due to the lower sensitivity of the flow meter used that year, an impellor driven meter which exhibits low sensitivity at low velocities, which may have masked subtle differences in velocities. This relationship, where younger crayfish are found in slower velocities, is also apparent in the Hart Burn PCA graph. It is also reinforced by the univariate results for the Hart Burn, where 0+ and 1+ crayfish inhabited microhabitats with significantly lower velocities than 2+ and 3+ crayfish. The younger crayfish are not capable of substantial active movements and are much more susceptible to the flow. Slower velocities may allow them to control their movement more effectively, enabling them to remain in suitable microhabitats and refuges.

From the PCA graphs, 0+ and 1+ crayfish in the Hart Burn were found significantly closer to the bank. These results are reinforced by the chi-square results for the Hart Burn and the Wansbeck, where 0+ crayfish were absent from microhabitats 3 m and further from the bank. The importance of distance from bank for the younger age classes may not be due to the direct effect of that variable, but the outcome of other variables such as velocity and substrate. The fact that there are slower velocities and finer substrates closer to the bank creates suitable habitat for juveniles. However, Benvenuto *et al*, (2008) found that the structural complexity at the stream edge was important for juveniles in terms of protection. Features such as roots may be a reason younger crayfish are associated with the stream edge, where the roots may provide invaluable protection to juveniles, especially during flooding.

The PCA graphs also show that in both the Wansbeck and the Hart Burn 0+ crayfish were absent from quadrats with large substrate indexes, but positively associated with finer substrate indexes. This idea that smaller crayfish are found in finer substrate is reinforced by the univariate results for substrate index, and the univariate results for the individual substrate categories. However, it was not only 0+ crayfish which were found in finer substrates. It has often been thought that crayfish are not found in sandy or muddy habitats, as they can only refuge in hard substrate, except for those species and sites were burrows could be made (Blake and Hart 1993, Holdich 2003). Nevertheless, in the Wansbeck microhabitats that were mostly covered in sand contained 1+ and 2+ and older crayfish. In the Hart Burn 0+ crayfish were found inhabiting a microhabitat where the substrate cover was 100% sand and all the age groups were found in quadrats where sand and gravel made up the majority of cover. This implies that although 0+ crayfish are absent from larger substrate, larger crayfish are not necessarily absent from finer substrate. Larger crayfish may be able to refuge in the finer substrate by burrowing into it, although the permanence of any such burrow depends greatly on the stability of the fine substrate. Generally white-clawed crayfish can only create ‘open’ burrows in compacted clay.

The PCA graph from the Hart Burn shows a clustering of points around larger substrate, where 89% of all points signifying presence of crayfish represented microhabitats with a large substrate size. This implies that although all substrates are available to them to refuge in, larger substrate seem to be preferable. From the two study sites, it was found that in general 0+ and 1+ crayfish are found in microhabitats with a higher cover of sand and pebbles in comparison to other age groups, and 1+ crayfish are found to inhabit microhabitats with a higher cover of small cobbles. Older crayfish are found in microhabitats with a higher cover of large cobbles and boulders in comparison to other age groups. This suggests that age groups are substantially segregated by substrate, which implies habitats with a range of substrates will be important in order to accommodate all ages of crayfish.

Foster (1993) also found that larger crayfish inhabited larger refuges, as a significant positive relationship between stone area and carapace length was found. The reason for the segregation may be due to the intra-specific competition between crayfish. The more stable refuges will be provided by the larger rocks, which the more dominant, larger crayfish will inhabit. These older crayfish will oust out the smaller crayfish to the less favourable finer substrate. However, smaller crayfish may also actively decide to take refuge in finer substrate found in shallower waters, where they can protect themselves from cannibalism and predation. Blake and Hart (1993) concluded that differential mortality and not juvenile behaviour explained the high abundance of juvenile signal crayfish in small substrates, where potential predators including conspecifics were less successful in smaller substrates and shallower water.

Adult crayfish primarily consume vegetal items so moss and algae provides a major nutritional benefit. This may explain why adult crayfish were found in microhabitats with a larger cover of moss and algae. Gheradi *et al* (2004) found that white-clawed crayfish showed a preference to moss over other plant materials, and Foster (1995) found that there was a positive association of crayfish with moss cover. Cover of moss and algae was not found to be an important variable in the Wansbeck. This may be because the majority of recordings of moss and algae in the Wansbeck were on the aerially exposed surface of the rocks, where its inaccessibility may make it an unsuitable food source. Alternatively, algae and moss cover may not be directly important for crayfish, but may reflect the size of the substrate, where larger substrates usually have a higher cover of moss and algae. The univariate results for moss exhibit the same pattern as the substrate index, where 0+ and 1+ crayfish were found in microhabitats with significantly less cover of moss and smaller substrates, compared to the 3+ and older crayfish. If the significant effect of moss and algae cover on the distribution of age classes was purely down to the underlying factors of substrate size, it means that cover of moss and algae is not an important variable.

Cover of roots was included in the analysis of the Hart Burn data but not the Wansbeck. The PCA implies that roots were important to 0+ crayfish as the points that represent microhabitats used by 0+ crayfish were found higher up the axis. However, according to the univariate results root cover is important for the older crayfish of age 2+ and 3+ and older, where they inhabit microhabitats with the highest cover of roots. The refuge areas provided by the roots in the Hart Burn are usually quite large so suitable for larger crayfish. Root habitats create very stable and effective refuges and act as leaf litter traps, which means they provide an important source of food for the omnivorous adults. The larger crayfish may take advantage of these high quality refuges and exclude the smaller crayfish from the root habitats. Other studies found that roots were important for juveniles where Smith *et al* (1996) concluded that marginal habitats with roots are important nurseries for juveniles and Benvenuto *et al* (2008) also found that roots give juveniles important additional protection at the stream edge. Holdich (2003) found that roots are important shelters for juveniles, but also adults if the roots are larger. In our study sites, the tree species of alder and ash may produce larger roots in comparison to other rivers, which contain finer roots systems e.g. willow that may be more suitable for juveniles. Nevertheless, there were limitations in the use of a Surber sampler, as it did not easily allow the sampling of roots. Only the edges of roots could be sampled and the more compacted roots closer to the bank and in the under cut of the bank, which may have been more suitable for juveniles, could not be sampled. This may explain why root habitats were not found to be important for juvenile crayfish, as the root habitats important for juveniles were not sampled.

For the Wansbeck PCA, velocity heterogeneity was included as a significant variable; however, there were no significant difference found in the univariate analysis. Velocity heterogeneity may purely be reinforcing the importance of velocity, as larger velocities are generally associated with larger substrate, which will have local effects on the flow and so create more velocity heterogeneity. It is not surprising that velocity heterogeneity seems of limited relevance, because crayfish do not need to rely on slow pools of flow created by substrate, instead they refuge in the substrate itself.

Cover of organic matter and depth were included as significant variables in the CCA for the Hart Burn. The CCA ordination plot (Figure 2.15) showed that the cover of organic matter is important for 0+ and 1+ crayfish but not for 2+ and 3+ and older crayfish. Organic debris may be an important variable for 0+ and 1+ as it can provide refuge for the smaller crayfish, but not for the larger, older crayfish. Also, organic matter will provide an ideal habitat for invertebrates, which the carnivorous juveniles prefer to feed upon (Momot 1995). The organic matter itself is not a good food source for juveniles but it is widely consumed by the older omnivorous adults (Goddard 1988). However, high cover of organic matter was usually found close to the bank where the substrate was small, making it unsuitable for adult crayfish.

Depth is important for larger crayfish, as they need deeper water to protect themselves from terrestrial predators such as heron *Ardea cinerea* and otter *Lutra lutra*, which hunt by sight so the predation risk is high in the shallower clearer waters (Englund and Krupa 2000, Benvenuto *et al*. 2008). The younger smaller crayfish tend to avoid deeper waters due to threats from fish predators, which only pose a threat in depths where they can manoeuvre and hunt successfully (Englund and Krupa 2000, Benvenuto *et al*. 2008). It is also possible that juveniles are mainly found in shallower waters due to the resources it provides such as good habitat for invertebrates, which juveniles solely feed upon. Whereas, the wider range of food items consumed by the adults including plant material, requires them to venture into deeper waters. This possible segregation due to predation and resource use is reflected in the CCA plot (Figure 2.15), where the 0+ and 1+ crayfish are found in shallower water in comparison to 2+ and 3+ and older crayfish. This pattern is also shown in the univariate results for Hart Burn, where the larger 2+ crayfish are found in significantly deeper water than the 1+

crayfish. However, on the Wansbeck there was no significant difference in depth between age classes and other studies such as Englund and Krupa (2000), found that water depth was an insignificant variable for crayfish. Whether depth plays an important role in habitat selection may depend on how strong the predation pressures are in the environment. It is also possible that the limitations of the method used, where water deeper than 0.5 m could not be sampled, may mask the overall pattern of distribution. However, it is unlikely that juveniles will be found in deeper waters than were sampled so the conclusions drawn are still valid.

The CCA plot shows an association between 0+ and 1+ crayfish and between 2+ and 3+ and older crayfish. The Hart Burn 2+ crayfish are still too small to be sexually mature. This implies that a potential change in behaviour after becoming sexually mature does not seem to affect microhabitat requirements. It seems the difference in microhabitat requirements is a direct result of size where after reaching a threshold size (around 13 mm carapace length for the Hart Burn) the microhabitat requirements of the crayfish changes.

Finally, the remaining variable of canopy cover was not found to be an important variable in the multivariate analyses, but there was a significant chi-square result for the Wansbeck. In the Wansbeck, the younger crayfish of age 0+ and 1+ are found in the most covered areas, probably because the younger crayfish inhabit microhabitats close to the bank, which will be highly covered. Overhead vegetation has little impact on stream dwelling crayfish as they take refuge under substrate so the added protection of vegetation will be minimal. However, canopy cover may help protect older crayfish, which are preyed on by terrestrial predators, and this may explain why the larger crayfish were not found in exposed areas. Nevertheless, the majority of quadrats taken in the more exposed sites were in close proximity to the bank, which is most likely the main reason adult crayfish were not recorded in those locations. The trees that create the canopy cover may be more important for crayfish than the canopy cover itself, as their leaves provide important nutrition (Foster 1995). The main reason for the lack of significant results is that both study sites had extensive amounts of tree cover, so there was not enough canopy cover variability to determine its importance.

2.5.3 The effects of flooding

After the 1 in 115 year flood on the Wansbeck 6th- 8th September 2008, there was not a significant reduction in overall crayfish density, but there was a significant reduction in the larger crayfish. The smaller crayfish may be less vulnerable from moving boulders during high flows, as they can fit into smaller crevices so have less chance of becoming injured. However, on Tuesday 16th September 2008, the Environment Agency reported a mass stranding of crayfish in the fields beside the River Wansbeck. The mass stranding event occurred just 2.5 km upstream of the study site. Overall roughly 6,150 crayfish were returned safely to the river but about 10,300 died. There was reported to be a higher survival of juveniles, probably due to the fact they could survive in pools of water for longer. This may be one of the reasons for the reduction in large adult crayfish caught after the flooding.

After the flooding, a couple of quadrats contained very high densities of crayfish (over 10 crayfish/quadrat), densities that had not been recorded in the Wansbeck study area previous to the flooding. There were fewer areas suitable for crayfish due to newly deposited mounds of gravel or exposed bedrock with no crevices. This may have led to high densities of crayfish collecting in the remaining suitable habitat.

It is important to consider that the microhabitat use of crayfish may change by night when the crayfish become active. There is the possibility of radio tracking and night observations but these methods are biased towards adults, as only adult crayfish can carry a radio transmitter and smaller crayfish will be even harder to find at night and by torchlight. The main purpose of this study was to identify the microhabitat requirements of juveniles and how they compare to adult crayfish. It is unlikely that juveniles will move into new microhabitats by night as they tend to remain under refuge (unlike adults who feed at night), so only recording the microhabitat use during the day will have little effect on the conclusions drawn.

2.5.4 Conservation implications

The Wansbeck is believed to have the largest density of white-clawed crayfish in Britain, which implies there is excellent crayfish habitat present (Rogers 2005). The habitat recorded in the Wansbeck could create a model to be applied to areas where river restoration is required. The most basic yet fundamental requirement of a white-clawed crayfish is access to a suitable refuge (Peay 2002). Substrate heterogeneity is vital in order to provide refuges for all age groups, as although larger substrate is important, finer substrate is still accessible to larger crayfish and pebbles provide important refuges for smaller crayfish. In order to maintain these important refuges, land management is needed to reduce excessive siltation, which leads to the embedding of potential refuges, where interstices are filled with silt and become unavailable to crayfish (Peay 2000). Many studies have concluded that enhancing bank-side features is important in the conservation of crayfish (Smith *et al*. 1996, Peay *et al*. 2006, Benvenuto *et al*. 2008). Bank-side features such as tree roots and riparian vegetation, maintains a complex bank structure, which creates habitat heterogeneity, including slower pools for the juveniles and roots for all ages of crayfish. Engineering work that damages or alters the bank-side features should be prevented. Strong flows cause instability in the sediment, where shifting beds of gravel and cobbles is unlikely to present suitable microhabitat. Therefore, patterns of microhabitat suitability are also linked to channel geomorphology, where the risk of severe flooding should be low as this may have a mass mortality effect on the larger reproductively active crayfish.

Recolonisation by white-clawed crayfish

3.1 Introduction

Dispersal is fundamental to the survival of a species and individuals are always under a selective pressure to disperse in order to avoid extinction risks, competition, inbreeding, and unfavourable variability in habitat quality (Bilton *et al.* 2001, Bowlby *et al.* 2007). Dispersal is especially important in freshwater environments, where the conditions are changing constantly (Lucas and Baras 2001). Dispersal is a density dependent process, where individuals move away from high-density areas in search of a new site with few resource competitors (Bovbjerg 1959, Elliott 2003, Bubb *et al.* 2006). This can lead to the colonisation of new areas and recolonisation of areas which have become depopulated.

Recolonisation in riverine invertebrates can occur via downstream drift, active migration, and from aerial sources (Lancaster *et al*. 1996). Aerial dispersal may be the most prominent mechanisms of colonisation in insects, however, is it not possible for other lotic macroinvertebrates (Wallace 1990). For macroinvertebrates such as white-clawed crayfish, which have no terrestrial life form, dispersal is limited by the physical parameters of the stream (Detenbeck *et al*. 1992). Dispersal can only occur through active upstream and downstream movements and passive downstream drift from the young of the year (0+ crayfish) once they have been released from their mothers (see section 1.6.1).

The physical medium of flowing water allows organisms to be transported in the water column, where in river systems the transport is always downstream (Lancaster *et al*. 1996). Downstream drift is an important source of recolonisation in macroinvertebrates (Townsend and Hildrew 1976, Lancaster *et al.* 1996). This includes crayfish, where in Britain, in the summer months of June-August, newly independent juveniles can be transported over considerable distances downstream (Robinson *et al*. 2000). As the crayfish become larger and more dense, they are less easily transported in the water column, so downstream drift is less likely to occur (Bubb *et al*. 2004). Instead, the larger, more mobile adult crayfish are able to make active movements in order to disperse (Robinson *et al.* 2000, Bubb *et al.* 2004). The upstream dispersal ability of white-clawed crayfish is largely dependent on the mobility of these actively moving adults, but downstream movement may occur by passive transport of young (< 10 mm) and active movement of adults.

Many adult decapod crustaceans are highly mobile animals at least partly due to their size. American lobsters *Homarus americanus* were recorded to have a maximum movement of 2.5 km day^{-1} (Bowlby *et al*. 2007), and freshwater crabs *Potamon fluviatile* of 134 m day^{-1} (Gherardi *et al*. 1998). In comparison, crayfish seem to move relatively slowly, where the highly invasive red swamp crayfish *Procambarus clarkii* only has a maximum rate of movement of 11 m day^{-1} (Gherardi *et al.* 2002). In comparison to other decapods white-clawed crayfish appear to be poor dispersers; they cover small areas when foraging and move relatively slowly (Gherardi *et al.* 2001, Bubb *et al.* 2008). Adult white-clawed crayfish reside in the same area of the stream for long periods of time (Gherardi *et al.* 1998, Robinson *et al.* 2000, Bubb *et al.* 2008). However, stationary phases are interspersed between nomadic phases, where they can move up to 200 m in one night (Brown 1979, Gherardi *et al.* 1998). These large movements are particularly common after moulting and during the mating season (Brown 1979, Gherardi *et al.* 1998).

The limited dispersal ability of white-clawed crayfish implies that it may take a substantial amount of time to recolonise a stretch of river after a disturbance event. However, the time taken for recolonisation may depend on the location of the dispersal pools to the depleted zone. Dispersal by passive and active means from an upstream source could result in rapid recolonisation, whilst active upstream dispersal to a depleted zone would be much slower. This makes it vitally important to understand the spatial behaviour and recolonisation ability of white-clawed crayfish, in order to apply this to reintroduction and restocking schemes. This Chapter aims to increase the understanding of the movement and population growth of crayfish, by studying a population of white-clawed crayfish while they recolonise a stretch of river after a pollution event. It also seeks to determine the relative importance of active dispersal of adults and passive drift of juveniles in the recolonisation process.

3.2 Study site

The data was collected from the Hart Burn, a tributary in the Wansbeck catchment, previously described in Chapter 2. On 14 May 2004, there was a mass mortality event on the Hart Burn caused by unidentified pollution. The pollution source was located by the Environment Agency in the Hart Burn woods (NGR: NZ 08908652). Surveys were carried out by the EA and it was concluded that a 3 km stretch downstream of the source was affected, where an estimated 30,000 crayfish died along with huge mortalities of other invertebrates such as mayflies (Ephemeroptera) and stoneflies (Plecoptera). No fish or higher vertebrate mortality was recorded and healthy populations of crayfish were found upstream of the source and in the River Wansbeck, where the affected portion of the Hart Burn meets. The specific pollutant was not identified, but appears to have been highly temporary. No pollution events have been reported there since.

3.3 Methods

White-clawed crayfish reproduce in October - November and the females lay their eggs soon after mating has occurred. Females bear their eggs over winter, which hatch in northern England around June. The juveniles remain attached to their mothers for several weeks and once they have moulted three times their mother releases them, usually around mid-July. Following the incident in May 2004, three surveys were carried out in 2004 (carried out by D.H. Bubb and M.C. Lucas), in the affected zones as well as upstream and downstream. The surveys in 2004 (carried out by D.H. Bubb and M.C. Lucas) were in June/early July (before the release of the young), August (after release of young from the females) and finally October (prior to egg laying). Sequential surveys were carried out in May, August and September 2005, June and August 2006, May and September 2007 (carried out by D.H. Bubb and M.C. Lucas), September 2008 and August 2009 (carried out by M.C. Lucas, H.M. Ream and V. Louca). The surveys were timed so they were prior to egg laying in autumn, where females become inactive, and with decreasing temperatures crayfish become harder to find. All surveys were carried out by standardised timed-effort hand searching of streambed refuges by experienced surveyors during clear, low-water conditions. Each survey took several days, but in similar conditions, where the crayfish were counted, sexed and measured. Stones were moved aside and any crayfish that were seen were caught if possible and a note was made if they escaped. The carapace length (from the rostral apex to the posterior median edge of the cephalothorax) of each crayfish that was caught was measured to the nearest 0.1 mm using vernier callipers. Hand searching refuges is the most effective and robust method for rapidly assessing presence and relative densities in shallow stream environments, especially when populations are present in low densities (Peay 2000, Bubb 2004).

Standardised effort searches were carried out for a minimum of one-person hour, where several two-person hour searches were carried out at each selected site. The catch per unit effort for each survey was calculated as the total number of crayfish caught and seen in one hour and this was used as a measure of abundance and compared across sites. Sites were selected along the river, which provided suitable habitat for crayfish. Suitable crayfish habitat included slow flowing stony sections, boulder riffles, cobbles and leaf litter, and among bank-structures including overhanging vegetation and submerged roots (Smith *et al.* 1996, Peay 2000, Holdich 2003, Benvenuto *et al.* 2008). The sites selected also had areas of low turbulence and were relatively shallow so they could be easily searched. Sites were selected across the whole reach of the affected area, including upstream of the pollution and downstream in the River Wansbeck (Figure 3.1). Surveys carried out prior to 2008 did not include the author, but raw data was provided for analysis.

Surber sampler quadrats of 0.7 x 0.7 m dimensions were collected in the Hart Burn in 2009 from seven of the sites surveyed (A,B,C,D,E,F,H; see Figure 3.1). The data from these quadrats was used to compare densities and microhabitats between the sites and for comparison to hand-search data from that year. Macrohabitat data was also recorded at 11 of the sites in 2009 (A,B,C,D,E,F,H,K,M,N,O; see Figure 3.1). Habitat data was recorded to aid in interpretation of observed patterns of recolonisation. Velocity, depth, channel substrate, bank-side vegetation, bank-structure, flow types, the size of the buffer strip, adjacent land use and canopy cover were recorded. Nine values for velocity and depth were recorded at each site, where they were measured at three points along three transects of the stream. The velocity was measured with a Valeport electromagnetic flow metre model 801. The modified Wentworth scale shown in Chapter 2 (Table 2.1) was used to calculate the substrate composition of each site.

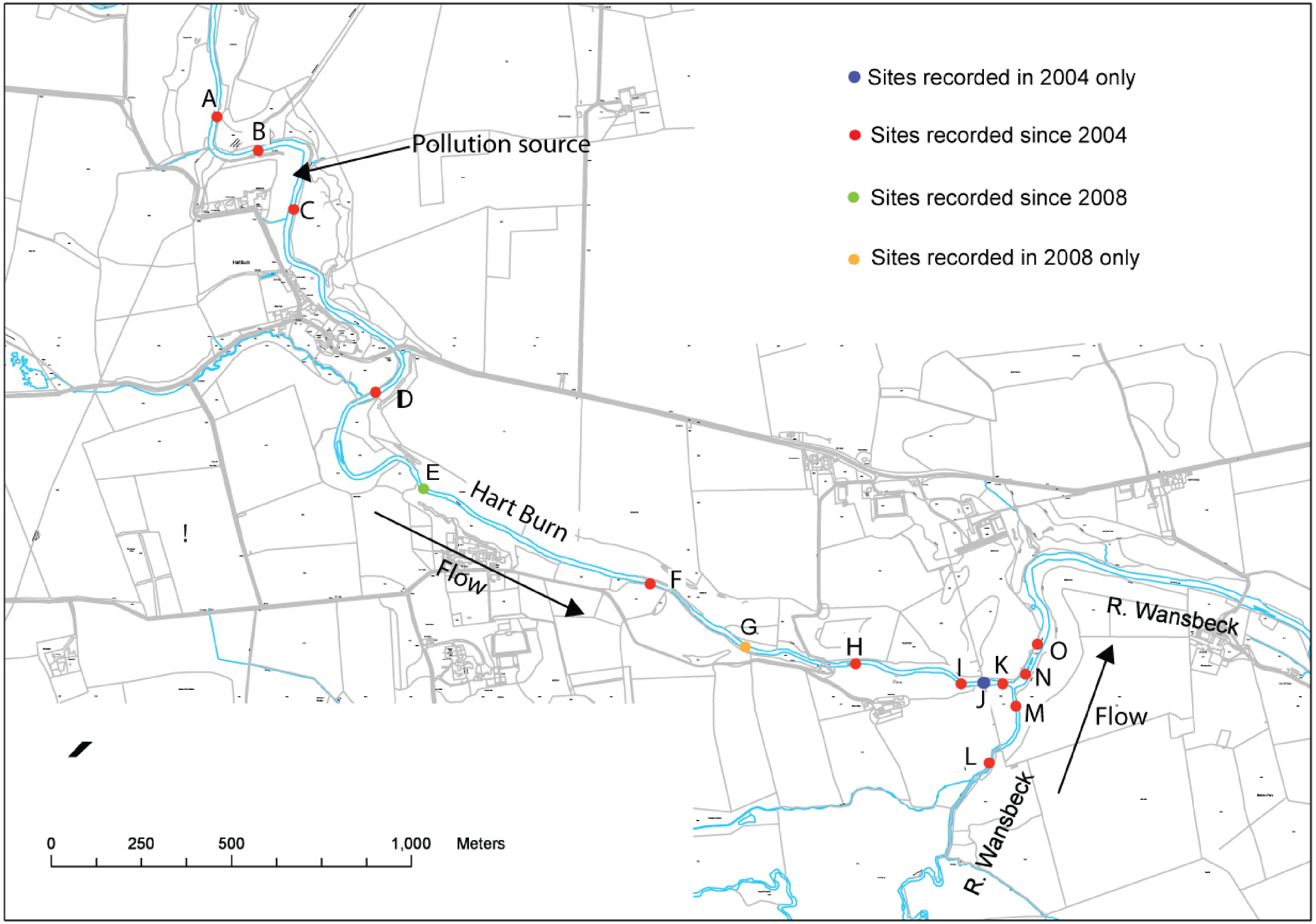


Figure 3.1- Map of sites where the standardised fixed-time effort hand searches took place

The bank-side vegetation was categorised into cover of woody bushes, reeds, herbaceous vegetation, evergreen trees and deciduous trees. The bank-structure was categorised into tree roots, overhang, herbaceous vegetated slope, eroding bank, vertical bank, steep rocky bank and beach, where the definitions of each category is shown in Table 3.1. The percentage cover of flow type was categorised into rapid, riffle, glide and pool in accordance to Padmore (1998), with the definition of each category presented in Table 3.2. Canopy cover was recorded as a percentage of the river section covered by the canopy of trees.

Table 3.1- Definition of bank-structure categories recorded in the Hart Burn

Category	Definition
Tree roots	Tree roots projecting into the water from the bank
Overhang	Overhanging bank above the water or undercut bank below the water
Herbaceous vegetated slope	Self explanatory
Bare eroding bank	Crumbling bank with no vegetation
Vertical bank	No overhang, erosion or vegetation, but too steep to classify as beach
Steep rocky vertical bank	Self explanatory
Beach	Usually in the form of rocks and gravel and includes mounds of rocks and gravel which form the bank

Table 3.2- Definition of flow types recorded in the Hart Burn

Flow type	Definition
Broken standing water (rapid)	White water and tumbling waves with the crest facing upstream
Unbroken standing water (riffle)	Undular standing waves with crest facing upstream and without breaking
Smooth boundary turbulent (glide)	Very little turbulence occurs, reflection is distorted and floating debris moves in a downstream direction
Scarcely perceptible flow (pool)	Floating debris appears stationary and reflection is not distorted

In order to compare the sizes of crayfish at the sites during the first two years of recolonisation, pair-wise comparisons between sites were carried out. Non-parametric tests were used as the sizes were non-normally distributed (Kolmogorov-Smirnov test statistic, $p < 0.05$). For the first two years (2004 - 2005) the sizes of crayfish at sites C, D, F, and I were compared to each other and the unaffected Wansbeck sites combined, and then separately to the unaffected Hart Burn reach. A site was only included in analysis if there were a sufficient number of crayfish present (n=5). A sample size of five was chosen, as it was a compromise between looking for differences and minimising the number of sites excluded, but a sample size close to five may lack the power to detect differences. In 2004, site I was the only site that had high enough numbers for comparisons to be feasible. In 2005, all sites had high enough numbers, so the Bonferroni correction was applied, where the significance level became 0.05/10 (where the number of comparisons determines the denominator). Pair-wise comparisons were only carried out for the first two years, as it would be unlikely if any further significant differences would occur as the population increases and becomes demographically similar.

Daily water discharge data was also obtained for the years 2004 – 2005, to determine if discharge had any effect on the initial recolonisation. The data was obtained by the Environment Agency at a recording station on the Wansbeck in Mitford (NGR: NZ 1745885790), just under 10 km downstream from the confluence with the Hart Burn. Although flow in the Hart Burn sub-catchment may be affected by local rainfall not received by the upper Wansbeck catchment, normally patterns of flow in the two sub-catchments are similar on any given day. Discharge at Mitford is therefore regarded as an adequate measure of crude patterns of discharge in the Hart Burn.

3.4 Results

3.4.1 Long-term trends in recolonisation

Figure 3.2 presents the relative abundance of crayfish caught at each of the survey sites from 2004 until 2009. There were some fluctuations in catch per unit effort (CPUE) between different times of the year, with highest values normally in summer, but the same spatial patterns were evident. There were markedly reduced numbers over a 3 km stretch, in comparison to the controls a month after the pollution event.

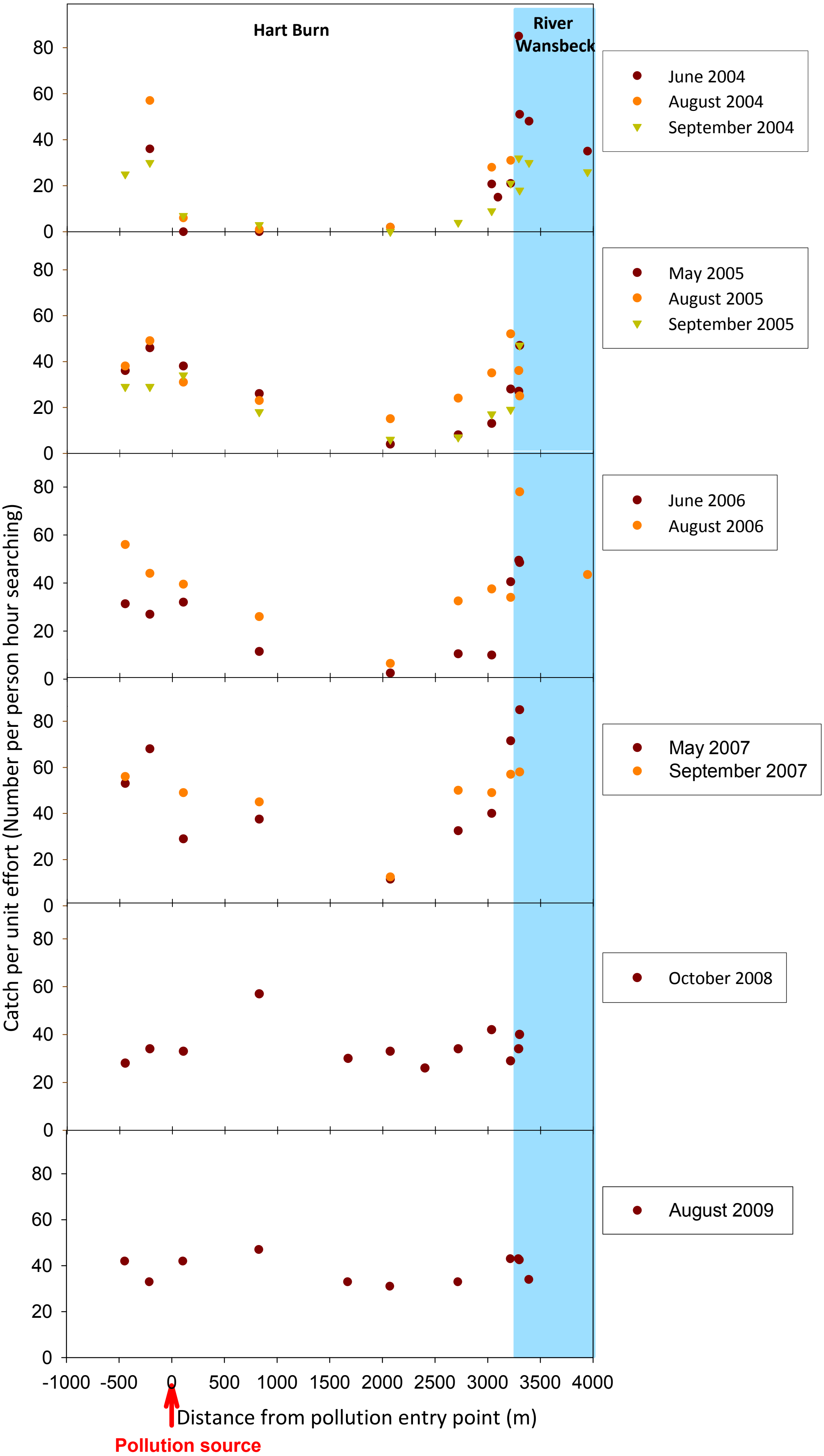


Figure 3.2- Catch per unit effort of white-clawed crayfish on the Hart Burn, showing timescale of recolonisation. Negative values refer to sites upstream and positive values downstream

The two most downstream Hart Burn sites, K (3214 m downstream of pollution), and I (3034 m downstream of pollution), had a relatively high number of crayfish in 2004. However, it still took three years for the sites to reach similar abundances to those of the unaffected controls. There were early signs of recolonisation at site C (105 m downstream) in August and September 2004, and the population there seemed to recover in terms of relative density within a year. The two middle sites D (826 m downstream of pollution), and F (2070 m downstream of pollution), took the longest to recover. Site, D, took three years for relative abundances to reach similar levels to that of the controls, while site F, took four years. Overall, even though there was suitable habitat available and a lack of competition, it took four years for the crayfish population to recolonise the 3 km stretch of river to relative densities similar to those in unaffected control reaches.

3.4.2 Initial recolonisation and demography

To ensure there is no difference in the demography of the two separate unaffected control reaches, the size distribution in the control sites upstream in the Hart Burn and downstream in the Wansbeck were compared in Figures 3.3 and 3.4.
There seems to be little variation in sizes and there was no significant difference between sites (Independent T test, $n_1 = 180$, $n_2 = 119$, $p = 0.160$).

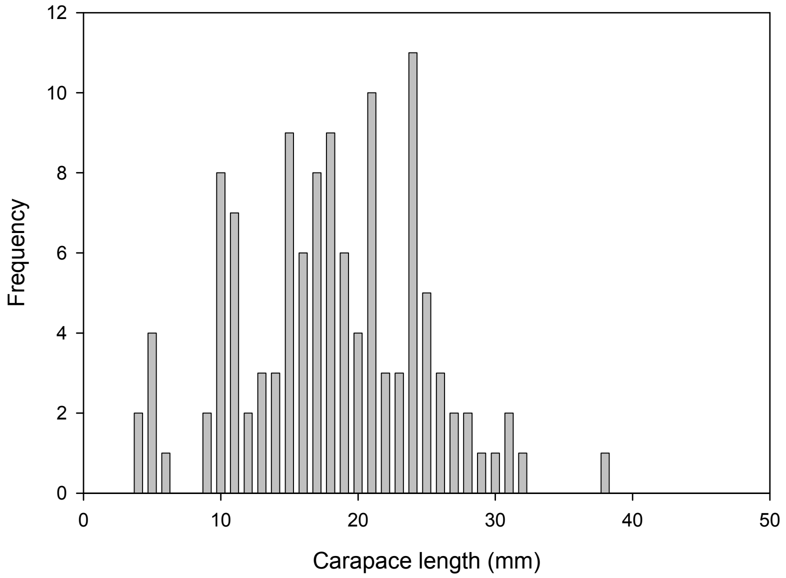


Figure 3.3- Size distribution of crayfish caught in the Hart Burn control sites on the 30th/31st July 2009

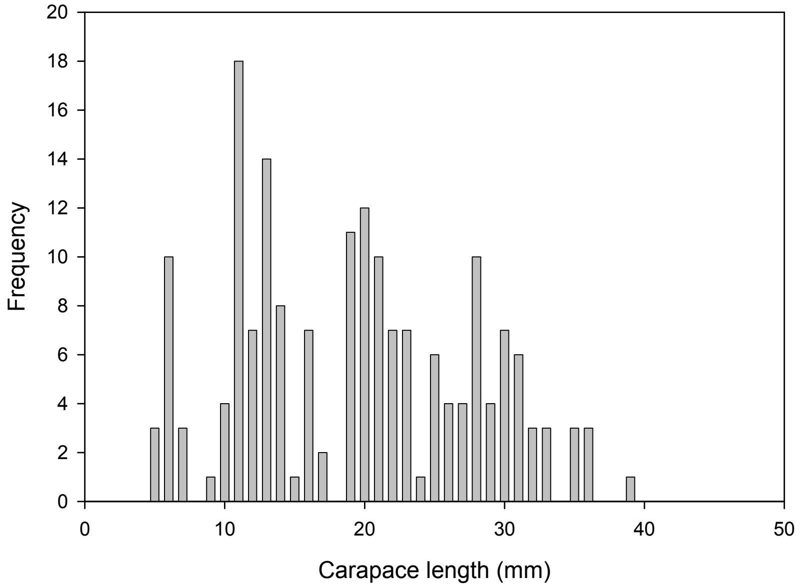


Figure 3.4- Size distribution of crayfish caught on the Wansbeck on the 5th August 2009

Distinction between age classes was achieved by monthly length frequency analysis (Figure 3.5). In May and June, before the young have been released in that calendar year, the smaller juveniles of carapace lengths as small as 7 mm would have been released the previous year. Once the young of the year have been released in July, there is a clear size separation between the 1+ and 0+ crayfish. In July-August crayfish with a carapace length less than 10 mm will be aged 0+, and those with a carapace length greater than 10 mm will be aged 1+.

If crayfish with carapace lengths smaller than 10 mm are recorded at a site in August, and reproductively active crayfish (> 25 mm carapace length (CL)) have also been recorded at the site, then it suggests that a reproductively active population is present.

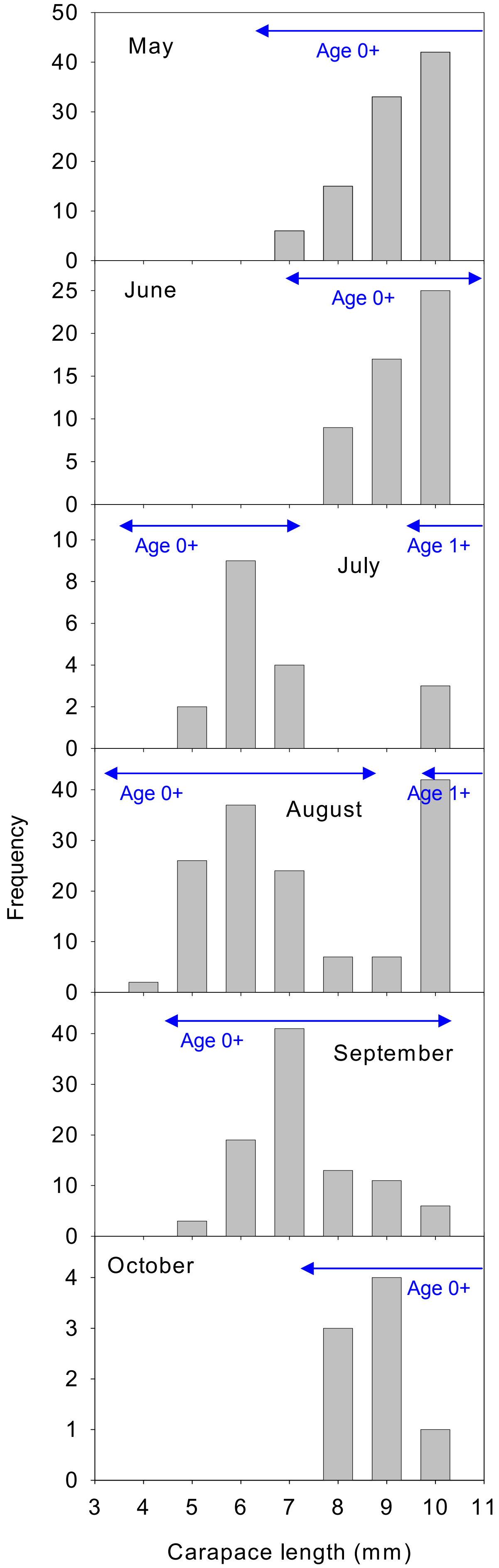


Figure 3.5- Size distribution of crayfish with carapace lengths 10 mm and less, across months, showing the separation of 0+ and 1+ age groups by month. Release of young from females occurs in July

The size distribution of crayfish caught in every survey is presented in Figure 3.6 as box plots. In June/July 2004, about a month after the pollution incident, a relatively high abundance of crayfish was caught at sites K and I. The presence of individuals around 10 mm carapace length (Age 0+, 2003 year class), suggests that these may have survived the pollution incident.

In August 2004, there were no young of the year (0+ crayfish, 2004 year class) present at the two downstream sites K and I. The crayfish found at a site I, were significantly larger than those found in the control sites in the Hart Burn (Mann-Whitney U, n = 23, p = 0.046). This suggests an influx of larger individuals but no influx of smaller individuals. From the 2004 discharge data (Appendix 2) there was a high flow from the 8th August 2004 lasting until the 29th August 2004, yet surveys carried out on 31st August, immediately after the high flows, found no influx of juveniles in the depleted zone.

In October 2004, there was an influx of 0+ and 1+ juveniles in the upper parts of the depleted zones, where they reached as far downstream as site D (826 m from pollution source), despite the fact that there had been low flows since the last survey in August (Appendix 2). Age 1+ crayfish ranging from 18 - 20 mm carapace length were present at site H.

In May 2005, there was an influx of larger individuals (ca. 20 - 30 mm CL) to site C (105 m downstream from pollution source), and the downstream movement of adult crayfish reached as far as site D (826 m). There was more downstream dispersal of 0+ crayfish (released the previous July), which now reached as far as site I (3034 m). Site C and D were still mainly being colonised by juveniles, where site D was made up of significantly smaller crayfish than the control sites in the Hart Burn (Mann-Whitney U, n=91, p=0.001). No large crayfish were recorded at site F.

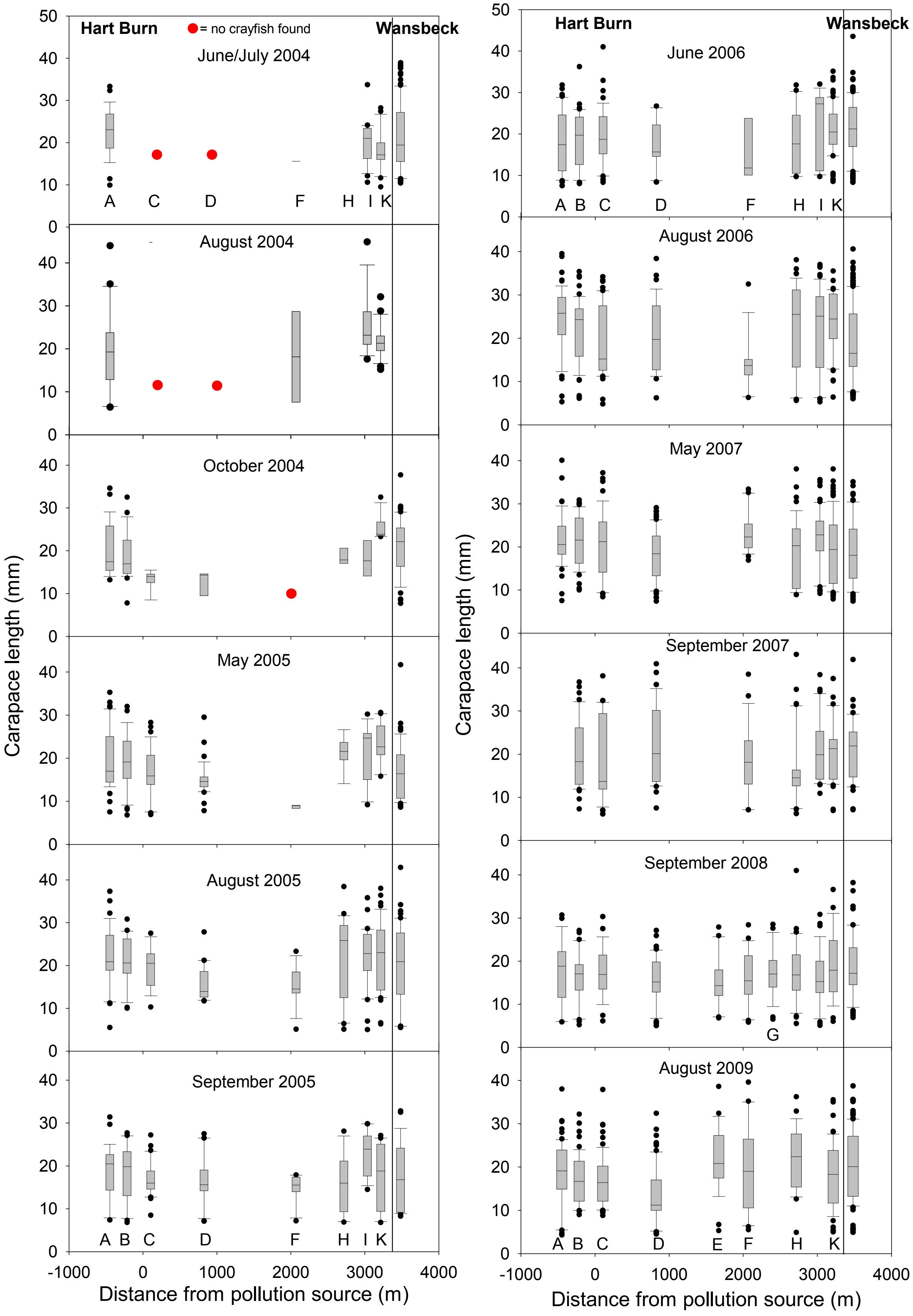


Figure 3.6- Size distribution of crayfish caught in the standardised fixed-timed effort hand searching of the survey sites on the Hart Burn from 2004 - 2009, not all sites were surveyed every year. Negative distances refer to sites upstream and positive values downstream of the pollution source

In August 2005, there was an influx of larger individuals to the downstream sites H, K, and I. An influx of larger individuals was also seen at site F. The influx of larger crayfish was still much greater in the downstream sites than upstream, where site I had significantly larger crayfish than D (Mann-Whitney, n = 44, p = 0.003). However, the influx of crayfish at site F was comprised of smaller crayfish than those found in the Hart Burn control sites (Mann-Whitney, n = 64, p = 0.01), but this difference was not significant after the Bonferroni correction. The upstream sites (C and D) also had an influx mainly comprising smaller crayfish, where crayfish in site D were still significantly smaller than those found in the Hart Burn control sites (Mann-Whitney, n = 72, p = 0.004). As a few berried females were recorded at site H and I in May 2005, it may mean that the populations at these sites are starting to increase through reproduction.

By September 2005, a further influx of larger individuals to C and D resulted in all sites except F having crayfish larger enough to be reproductively active (> 25 mm CL). The crayfish in site I were still significantly larger than upstream sites such as site C (Mann-Whitney U, n=27, p=0.003). Crayfish in site I were also larger than crayfish at site D (Mann-Whitney U, n=28, p=0.015), and F (Mann-Whitney U, n=20, p=0.015); however, after the Bonferroni correction these differences were not significant.

In June 2006, there was a further influx of larger individuals to F and there is now a similar number of large crayfish at all sites, which can be seen from the upper quartiles on the box plots. However, the abundances are still low in sites D, F, H and I. In August there is an influx of 0+ crayfish and a dramatic increase in abundance at all sites except F, which suggests the populations at all sites except F are reproductively active. A further influx of larger individuals has led to a substantial amount of large reproductively active crayfish finally arriving at site F.

By 2007, all sites except F had reached similar abundances to the controls. In September 2007 there was an influx of 0+ crayfish to site F, which suggest the population is reproductively active and the size distribution of site F is similar to all other sites. However, the abundances at site F are still very low in comparison to all other sites. It is not until 2008 that abundances at site F finally increase to that similar to the controls and all other sites.

3.4.3 Habitat and recolonisation

Since it is possible that prolonged periods of recolonisation (e.g. site F) will be due to poor habitat, surveys of habitat and microhabitat quality across sites were made, together with quadrat estimates of density. There was a significant difference in the densities of crayfish in quadrats (taken at sites A, B, C, D, E, F, H, K, M, N, O) in 2009, found between the sites (Kruskal Wallis, n = 76, p = 0.035). Mann-Whitney tests were not carried out due to the large number of comparisons and low statistical power of comparison after the Bonferroni correction. The densities found at each site are shown in Figure 3.7.

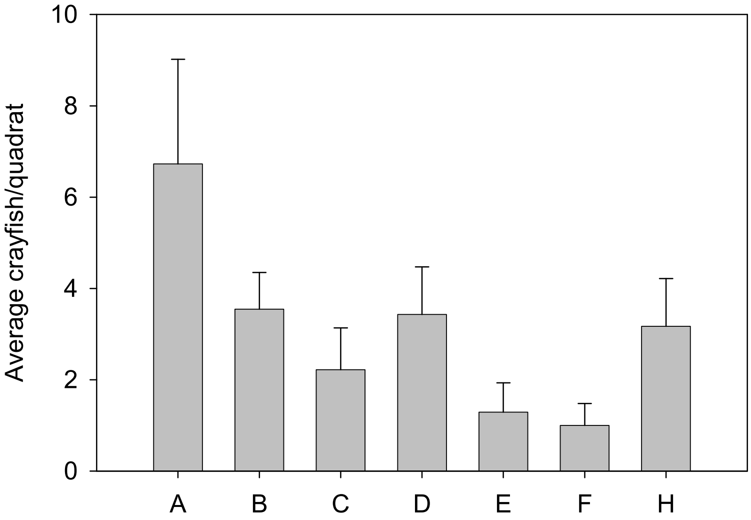


Figure 3.7- Average densities of crayfish, including standard error bars, calculated from densities recorded in the quadrats, at seven of the sites in the Hart Burn

The low densities found in sites F and E are most likely to be the cause of the significant difference. The relatively low densities at site F were less evident in the standardised timed-effort searches in 2008 and 2009, although in 2005 - 2007 this site exhibits the lowest relative abundance of those sampled. Kruskal Wallis tests were carried out in order to compare the microhabitat and macrohabitat variables of velocity and depth between the sites, and the microhabitat variable of substrate index. However, no significant differences were found in the microhabitat or macrohabitat variables (microhabitat variables: velocity p = 0.193, depth p = 0.532 and substrate index p = 0.237; macrohabitat variable: velocity p = 0.077, depth p = 0.796). Buffer strips, land use, cover of different substrates, and bank-side vegetation were not amenable to statistical analysis but were similar between sites.

Two variables, flow type and bank-structure, did highlight some important differences between sites. Figure 3.8 shows the composition of flow types found at each site, and Figure 3.9 shows the bank-structure at each site. Although site F shows similar composition of flow type to the rest of the sites, site E (immediately upstream) had a higher proportion of rapids than any other site. The presence of rapids implies a higher gradient and stronger flow, which may have led to a smaller number of suitable refuges for the crayfish. The major difference seen at site F was in the bank-structure. The banks at site F are almost entirely steep and rocky, with a small cover of vegetated slope. The steep sides of site F imply that there is a high gradient and an unstable environment, also linked to high flows at site E. A large cover of steep rocky slope was also present at site B; however, unlike site F it was not steep on both sides but contained large areas of beach. Site B was also entirely composed of glides and pools, which suggest a low gradient and a more stable environment.

During high flows, the river will erode away the bank and deposit it in the river channel, leading to unsuitable habitat. The amount of substrate that was embedded was recorded in the quadrats and the percentage of rocks that were embedded in finer sediment (i.e. without gaps around the periphery of large particles) for each site is presented in Figure 3.10. Site F had the highest recording of embedded substrate, where although all the medians were zero the mean cover was 12% in site F, 5% in site E, 3% in site D and all the other sites contained no embedded substrate. The zone that took the longest to recover (containing sites F and E) contained the largest amount of embedded substrate.

3.4.4 Comparison of methods

The use of the Surber sampler allows crayfish of all sizes to be caught in a given area. Whereas, with timed hand searches the smallest crayfish may be harder to locate and catch. The size distribution of crayfish from the hand searches in 2009 and the quadrats collected in the seven sites in the same year were compared and the results are shown in Figure 3.11. Overall the size distributions appear broadly similar, although the crayfish caught in the quadrats were significantly smaller at site B (Mann Whitney U, $n=96$, $p=0.007$) and site C (Mann Whitney U, $n=85$, $p=0.001$). This implies that hand searching may misrepresent the smaller crayfish.

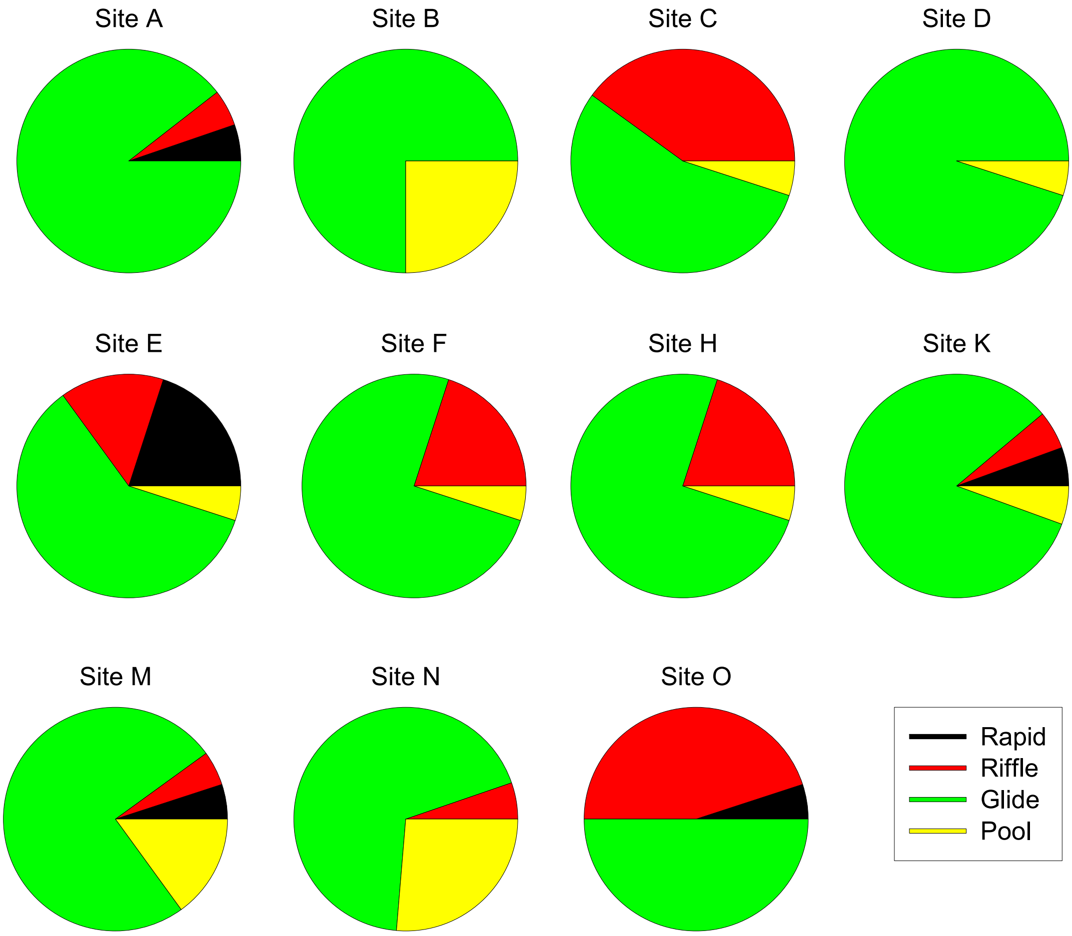


Figure 3.8- Composition of flow types at 11 of the sites on the Hart Burn

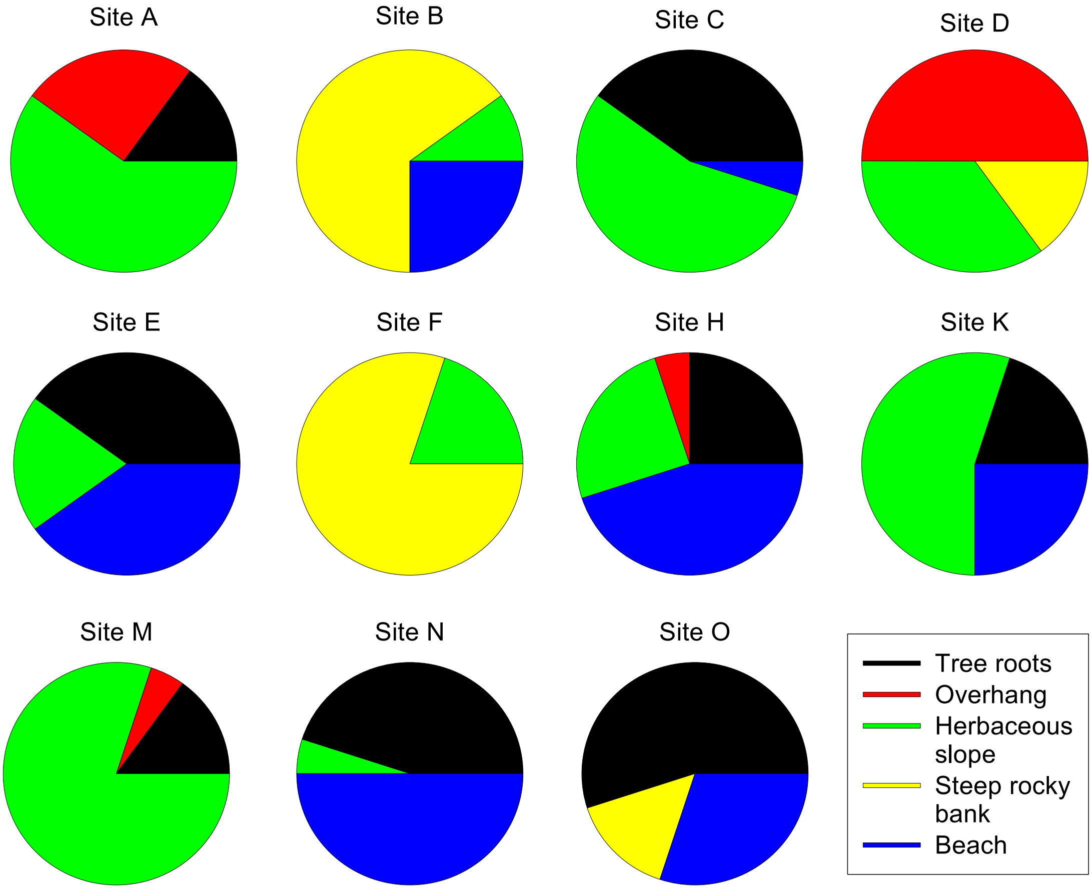


Figure 3.9- Bank-structure observed at 11 of the sites on the Hart Burn

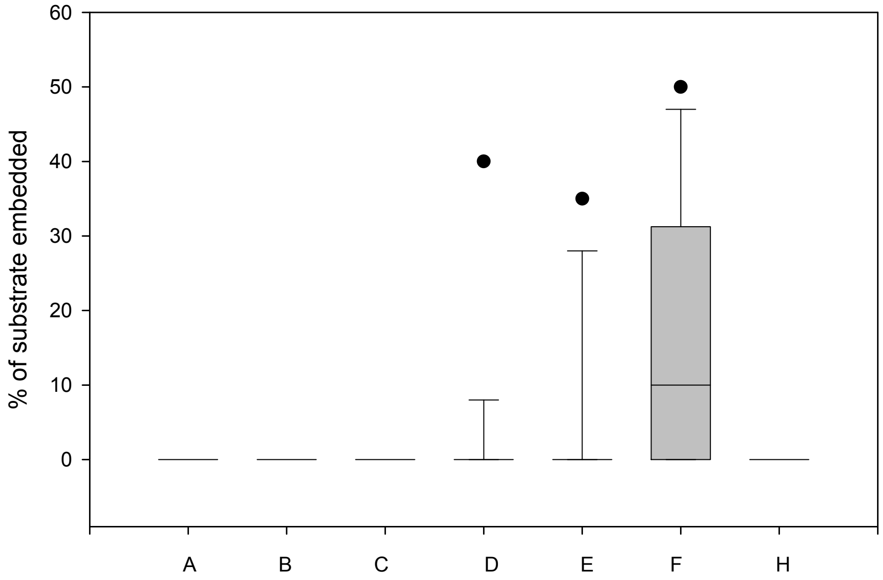


Figure 3.10- Percentage of embedded substrate found in seven of the sites on the Hart Burn

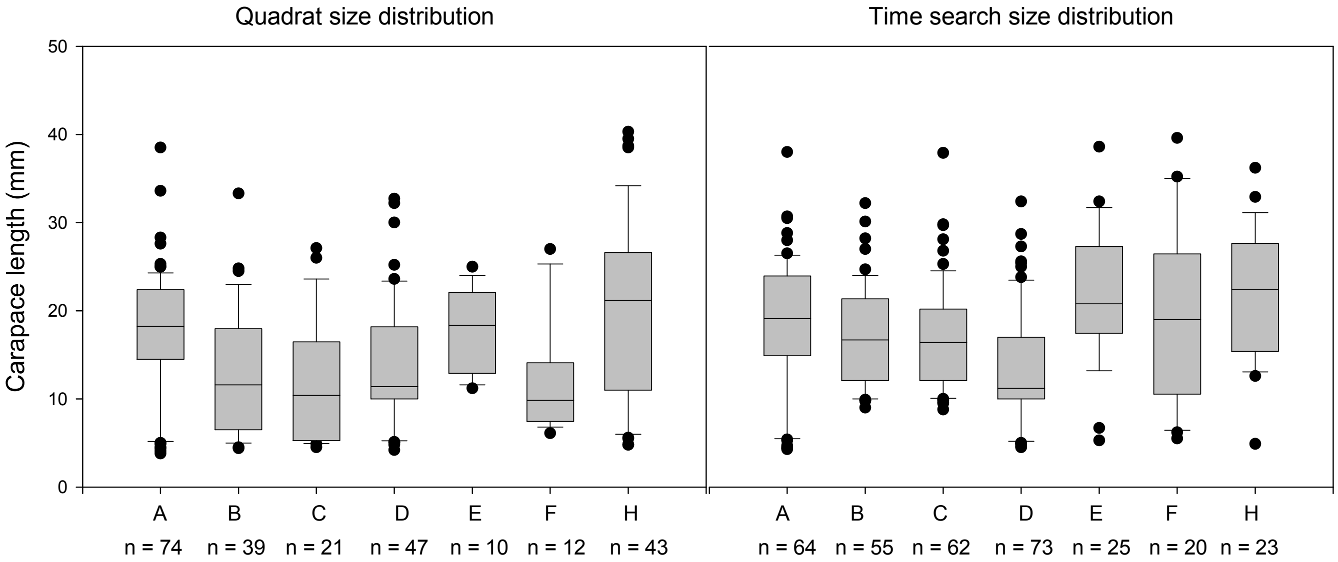


Figure 3.11- Comparison of crayfish sizes caught by hand and in the Surber sampler (quadrat)

3.5 Discussion

In order to recolonise a 3 km stretch of river, it took the white-clawed crayfish population four years, based upon relative abundances and the occurrence of similar demographic composition to the control reaches. This equates to a recolonisation rate of 0.75 km year^{-1}. During this period there is no evidence of any further acute pollution episodes in the reach, nor any reports of conspicuous crayfish mortality episodes. This is fairly rapid in comparison to previously recorded rates of colonisation by white-clawed crayfish. Gil-Sanchez and Alba-Tercedor (2006), recorded a population expansion rate after restocking 52 and 43 crayfish in the Guadalquivur River basin, south east Spain, of 0.075- 0.2 km year^{-1}. This implies that natural recolonisation from a dense population is a faster process than colonisation by a relatively small restocked population, as might be expected. Restocking usually involves the introduction of several populations dispersed along a stretch of river, where each population provides only a single source of crayfish to recolonise the local area. In natural populations there will be a large population surrounding the depopulated area, creating several recolonisation sources from upstream and downstream and from connecting tributaries along the stretch of river. The number of population sources depends on the location of the disturbance in relation to the geography of the river. Passive dispersal from upstream combined with active movement from both upstream and downstream appears to have increased the rate of population expansion and recolonisation. The higher densities found in the Hart Burn and Wansbeck populations in comparison to previous studies and restocking schemes will also create a higher propagule pressure, forcing the crayfish to disperse away from the population, in order to escape from the competition.

A recolonisation rate of 0.75 km year^{-1} translates to a daily movement of around 2.05 m day^{-1}. This is significantly higher than the average daily movement for adult white-clawed crayfish in an upland stream in northern England, recorded throughout the year by Bubb *et al* (2008) of 0.233 m day^{-1}. However, several studies have recorded much faster rates of movements. For example, Gherardi *et al* (1998) recorded daily movements in adult white-clawed crayfish of 6 m day^{-1} in autumn and in June, McCreesh (2000) recorded rates as high as 7.5 m day^{-1}. The variation in movement rates is partly due to the time period in which they were recorded. Rates of movement recorded over the whole year include the winter months, when crayfish have reduced mobility and will not be as high as those recorded in the summer and autumn months. As recolonisation rate represents the average movement throughout the year the value of 0.233 m day^{-1} is the most accurate to compare against. Although the recolonisation rate was 2.05 m day^{-1} the average distance covered by an individual crayfish may not be that far, due to the fact that in this study, the depleted zone of 3 km was recolonised from two sources (upstream and downstream). However, the recolonisation value is almost 10 times the movement rates within well-populated zones, recorded by McCreesh

(2000), Gherardi *et al* (1998), and Bubb *et al* (2008), which implies that recolonisation, where there is movement into a depopulated zone, is faster than within population dispersion. Other factors may have played a role such as the habitat in the Hart Burn. The substrate may have been easier to move through (e.g. fewer large expanses of bedrock) and the Hart Burn may have a smaller gradient, which would reduce the frequency of high flows.

This high rate of dispersal from a stretch of river abundant with refuges is somewhat contradictory to predictions. Bovbjerg (1959), Barbaresi and Gherardi (2001), Sint and Fureder (2004), all found that crayfish released in sites with a high density of shelters moved the smallest distance and had the lowest activity. Due to the widely available refuges in this reach of study stream one would expect more limited movement, as there would be limited competition and less need to disperse. However, Bovbjerg (1959) and Barbaersi and Gherardi (2001) recorded movements in laboratory conditions that may not reflect natural dispersal behaviour. Also, Barbaresia and Gherardi (2001) recorded dispersal behaviour of crayfish in isolation and the largest group of crayfish recorded by Bovbjerg (1959) was 20. Although Sint and Fureder (2004) measured movement in the field, they were looking at a reintroduced population of only 63 crayfish. The conditions during recolonisation may be very different, where the presence of a large natural population may provide a large amount of competition, resulting in a relatively fast rate of movement even when refuges are abundant.

As well as the average rate of movement of the population through the depleted zone, specific rates of movement can be calculated. Site, I, is roughly 256 m from the confluence and large adult crayfish were present at the site I on the 22nd June. If these had moved upstream from the Wansbeck then the minimum rate of movement of the crayfish would be 6.56 m day^{-1}. This is a very rapid rate of movement, especially in an upstream direction. Previously recorded rates of upstream movement by adult crayfish are substantially lower than the average movement rates of adult crayfish in all directions, recorded by McCreesh (2000), Gherardi *et al* (1998), and Bubb *et al* (2008). The maximum upstream movement of adult white-clawed crayfish recorded by Bubb *et al* (2008), was 734 m in 335 days, which equates to 2.19 m day^{-1}. Robinson *et al* (2000), recorded an upstream movement in adult crayfish of 1.36 m day^{-1} and 0.9 m day^{-1}. However, there is evidence that some crayfish may have survived the pollution incident. It is unlikely that the small individuals (ca. 10 mm CL) recorded at site I could have reached the site by upstream movement within two months (around 256 m upstream from the closest Wansbeck site), and there was no sign of passive dispersal to the other sites upstream. The presence of a 15.6 mm carapace length crayfish at site F (2070 m downstream from pollution) also suggests that a small number of crayfish survived, as it is unlikely that a 1+ crayfish (2002 year class) of this size would be passively dispersed over 2 km, or actively moved over 1 km in two months. Nevertheless, if the larger crayfish found in June 2004 had survived the pollution incident and remained in that site, then crayfish of that size should release young in late July. As there were no young of the year found at site K and I in August 2004, it suggests that they did in fact actively move upstream from the Wansbeck.

In August 2004, there were large individuals of 31.5 and 44.9 mm carapace length at site I, where no crayfish close to that size were found in the first sampling period. This suggests they must have moved in from the Wansbeck, the nearest colonising source. These large individuals were found at site I 109 days after the pollution event, which would indicate an upstream movement rate of 2.36 m day^{-1}. This is still higher than all the other recordings, especially as a minimum rate of movement. This suggests that white-clawed crayfish may be capable of rapid upstream movements. However, this is only one possible interpretation. It may be that during the standardised fixed-timed effort hand searching, crayfish were not recorded due to the low numbers present in the affected reach. Due to the low densities of crayfish after the pollution incident, only a very large sample effort would allow one to be confident about the presence of crayfish at particular sites.

The rate of active downstream dispersal by adults can also be calculated, where it is assumed that due to their size, they are not subject to unintentional downstream drift and it has been recorded that they never intentionally enter fast water flows in order to disperse downstream (Bubb *et al.* 2002, Bubb *et al.* 2004). In May 2005, large adult crayfish were recorded at site D that had not been present the previous year. Site D is 826 m from the pollution source and they were recorded 369 days after the pollution incident, equating to a minimum dispersal rate of 2.24 m day^{-1}. Robinson *et al* (2000), recorded similar rates of downstream active movement by adult white-clawed crayfish of 2.4 m day^{-1} and 1.67 m day^{-1}, and Bubb *et al* (2008), recorded a maximum downstream movement of adult crayfish of 918 m in 304 days, which translates to 3 m day^{-1}.

When comparing active downstream and upstream rates of movement in adult crayfish, several studies found that there was no bias in one direction (Brown 1979, McCreesh 2000, Robinson *et al.* 2000). However, Bubb *et al* (2004, 2005) recorded a strong bias towards downstream colonisation in signal crayfish populations. This was attributed to the geology of the upland river, where the high gradient of the upland stream created riffles and falls, which reduced upstream movement. In this study it seems that even though the upland river has a high gradient there is little bias towards active downstream movement of adults, as the upstream dispersal rates were also fairly rapid. However, it did take longer for the relative abundances and population size structure to increase to normal levels in the region of site F, which is in the downstream section of the affected reach. This may suggest that upstream colonisation of crayfish is slower than downstream. This would be expected, as colonisation involves the whole population, where downstream colonisation includes the passively dispersing juveniles and upstream colonisation does not.

The passive dispersal of juvenile crayfish is thought to occur over considerable distances (Robinson *et al.* 2000, Elliott 2002). In October 2004, individuals around 10 mm carapace length were found at site D, where it is assumed they had been passively dispersed from upstream, as they could not realistically have moved there actively. Site, D, is 826 m from the pollution source, so the minimum rate of movement by these juveniles in the 152 days since the pollution is 5.5 m day^{-1}. The following year, in May 2005, juveniles were present as far as site I, which were known to be passively dispersed due to the lack of reproductively active crayfish (>25 mm CL) in the population at site I in 2004. Site, I, is 3034 m from the pollution source, and as they arrived there within 369 days this equates to a minimum rate of 8.2 m day^{-1}. This suggests that passive downstream dispersal of juvenile crayfish is relatively rapid compared to active movements of the adults. Nevertheless, colonisation by juvenile 0+ and 1+ crayfish by passive drift requires a further two-three years of growth in the Hart Burn before they are reproductively mature.

When the passive dispersal rates are compared to the flow data, it seems that there is evidence of limited passive dispersal after a high flow event, but rapid passive dispersal during low flow periods. Due to the low numbers of reproductively active crayfish at sites H and I in May 2005, it is likely that the majority of the 0+ were passively dispersed from upstream. This suggests a substantial dispersal distance despite the low flows recorded since the young were released (appendix 2). This suggests that juvenile crayfish have some control over when and how far they are passively dispersed. There is evidence that benthic invertebrate juveniles have some control over their passive dispersal, where they can actively enter the flow and their behaviour can control their drift response (Elliott 1971, Lancaster *et al.* 1996). Invertebrates can increase their drift response in low velocities by actively swimming upwards, in order to remain in the flow for as long as possible (Campbell 1985). It certainly seems that in the case of white-clawed crayfish low flows are not a barrier to drift. In terms of the lack of dispersal in elevated flows, the juvenile crayfish may be able to avoid being washed out of the substrate and dispersed due to the microhabitats in which they are found. The interstices between gravel and small pebbles may protect them against high flows, allowing them to remain in the same position until they actively decide to enter the flow. Nevertheless, during extreme flood events, when more substrate is mobilised, it is possible they are washed out.

As crayfish recolonise a stretch of river by active upstream and downstream movement and passive downstream drift, the position of the population source affects the population structure. Initially the upstream sites that were recolonised by an upstream source had a population made up of significantly smaller crayfish, due to an influx of juveniles from passive downstream dispersal. Larger crayfish actively moved in a short while afterwards and modified the population size structure. In the downstream sites, where the closest population source was from downstream, larger crayfish initially dominated the population, as they are the most effective at actively moving upstream. Crayfish in site I were significantly larger than crayfish in upstream sites in September 2005, due to a lack of small juveniles. Juveniles appeared in the downstream stream sites through reproduction in the local population and eventually downstream drift. A lack of passive dispersal from upstream sources will delay the recolonisation process, as juveniles will not appear until a year after the larger crayfish have arrived and mated. Also, the annual *in situ* influx of juveniles will not be substantial until the reproducing population has increased back to normal levels.

White-clawed crayfish are known to have a low fecundity (Gherardi *et al*. 1997), so the presence of nearby large populations is important to recolonisation and population recovery. It was not the establishment of reproductively active populations, but the continuous influx of crayfish, through passive and active dispersal, that resulted in crayfish abundances in the affected river stretch reaching similar levels to those in the unaffected control reaches. This reliance on an influx of crayfish by dispersal lasted for two years for all sites except F where it lasted four. Upstream and especially downstream dispersal is therefore a driving force behind initial recovery in denuded sites, as natural reproduction by a few crayfish does not have a substantial effect on densities. This conclusion was also reached by Momot (1966), who stated that due to the low reproduction of crayfish, the repopulation of a denuded stream reach must occur through recolonisation not *in situ* reproduction.

Finally, habitat was found to play an important role in recolonisation. Although site F was the furthest away from a population source all sites except F were showing a steady increase since 2005. The numbers at Site F only started to increase three years later despite being only 646 m away from the downstream site H, which reached normal densities in 2006. The delay was mainly in the arrival of larger crayfish as juveniles were arriving within a year. This may suggest the occurrence of a barrier to active dispersal, particularly in an upstream direction. There were large expanses of bedrock upstream and downstream of site F (H. Ream, pers. obs.). The large expanses of bedrock may have provided a barrier, as crayfish would have been deterred from dispersing over large stretches of bedrock, which exceeded 50 m in length. The site was also found to contain the highest percentage of embedded substrate, possibly due to substantial bank erosion and collapse immediately upstream. Site F was unique to other sites in the sense it had steep rocky sides to both banks. Reduced availability of refuges, due to embedding and instability of the habitat at high flows, due to channelisation by the steep bank, may have prevented larger crayfish from establishing here as they actively choose their settlement location, unlike the passively dispersed juveniles. Gradually as densities increased elsewhere the pressure from competition may have forced some crayfish into less favourable habitats, leading to the delayed recolonisation of site F.

Although the results suggest that the standardise fixed-time effort hand searching may misrepresent smaller crayfish, this is still the most effective method to use when calculating abundances in areas where crayfish are patchily distributed, due to the larger areas that can be covered in a given time in comparison to placing quadrats.

3.5.1 Implications for conservation

Point source pollution events in rivers have dramatic effects on the biota, where the recovery time is related to the mobility of the species. Winged insects are at less risk due to rapid rate of aerial recolonisation. However, crustaceans such as shrimps, and other invertebrates that have no aerial life stage, will have a substantially longer recovery time in comparison to crayfish, which are considered the most mobile freshwater invertebrate. The presence of an upstream population, where downstream drift can originate from, is an important feature that will increase the recolonisation speed. Disturbance events in the top of catchments should be prevented at all costs, as it will be more damaging than if an event occurred further downstream, due to the importance of an upstream source of recolonisation in recovery. There should be concentration on keeping good quality habitat in upstream reaches, in order to sustain important upstream populations. Habitat suitability is likely to have a major effect on recolonisation ability and unsuitable habitat may delay recolonisation by several years. The availability of suitable habitat is essential for recolonisation projects. Abundant refuges at the edges of the denuded population were not found to decrease crayfish movement markedly, possibly due to the high competition found in large natural populations. The results of this study highlight the importance of propagule pressure as it represents a study where there is a dense population surrounding the affected stretch encouraging colonisation of unoccupied habitats. Many reintroduction schemes for endangered animals utilise small numbers of individuals, due to difficulties in obtaining them or the ethical need to minimise impacts on donor populations. The results of this study suggest that, release of small numbers of crayfish in reintroduction exercises will take a long period of time for establishment, with large risks of extinction during that period.

In conclusion, rates of recolonisation in white-clawed crayfish in this study stream are rapid, in comparison to within population dispersion and population expansions after restocking measured elsewhere. Passive downstream dispersal is the most rapid form of recolonisation for white-clawed crayfish in streams, with evidence obtained that juvenile crayfish have control over when and how far they are passively dispersed. Active movements of adults also contributed to recolonisation, with upstream and downstream active dispersal found to be similar in rate.

General Discussion

This thesis found that white-clawed crayfish *Austropotamobius pallipes* exhibit ontogenic microhabitat segregation. The issue of differing habitat requirements between life stages or ages has important implications for conservation of animals in general, since there is a general bias towards conserving areas that are known to be suitable for adults. Yet, juveniles are the most susceptible life stage in many species and studies on crayfish have found that limiting juvenile mortality is vital for the survival of the population (Meyer *et al*. 2007). Failing to identify important juvenile habitats may lead to the failure of conservation strategies. There are many examples of ontogenic habitat segregation, where the energy requirements and survival strategies vary between age classes (Loseto *et al*. 2006). For example, newly hatched pond turtles *Emys orbicularis* require shallower water to avoid risk of drowning (Ficetola *et al*. 2004). *Thermoosphaeroma thermophilum* is a freshwater isopod, where the juveniles were found above the substrate in the grass to avoid cannibalism from the adults (Jormalainen and Shuster 1997). The reef fish, Nassau grouper *Epinephelus straitus* has three different habitat associations, due to the increasing metabolic demand and reduction in predation risk as they mature (Eggleston 1995). In some fish species the juveniles prefer lagoon habitat, whilst the adults are found on the reefs (Gratwicke *et al*. 2006). Such differences imply that a lack of knowledge on juvenile habitat requirements could affect the conservation of many species. Juveniles are often overlooked in relation to habitat, as they are harder to locate due to their size and cryptic nature. Improved sampling efforts will be needed in order to effectively sample juveniles of crayfish and other animal species and increase the understanding of their ecology.

Identification of suitable habitat for white-clawed crayfish and its appropriate protection and rehabilitation through management, is crucial for conservation of this species. This thesis identified suitable white-clawed crayfish habitat as river sections with a heterogenous cover of substrate types, where the varying sizes of interstices provides refuge for all crayfish ages. Complex bank-structures including roots are also important along with heterogenous flow patterns. However, strong flows, which are exacerbated by channelisation, create unstable and unsuitable environments for crayfish due to shifting sediments. Silt accumulation in the interstices of substrate destroys important crayfish refuges (Taugbol and Skurdal 1999). A common cause of silt accumulation is poaching of riverbank edges by livestock, especially cattle, where cattle trample on and break up the bank, causing it to enter the river. In fields bordering important river stretches there could be a limit on the number of cattle that can be stocked and an obligatory upkeep of fences (Palmer 1994, Peay 2000). Preventing woodland clearance and creating substantial buffer strips could eliminate erosion sources, which contributes to silt accumulation (Palmer 1994). The presence of trees is important in preventing erosion, but they also create good bank-side habitat. Where they are lacking, trees could be planted close to the waters edge, in particular alder *Alnus glutinosa* and willow *Salix spp*, and a possible alternative to the removal of trees along side the riverbank is coppicing (Palmer 1994, Peay 2000). Also, the use of concrete in bank stabilising should be avoided, as it leads to the permanent loss of important crayfish bank-side habitat. Instead, un-mortared stones could be used, which would create refuges for crayfish (Peay 2000). In order to maintain a heterogenous flow pattern dredging and channelisation should be avoided (Holdich 2003). Land practices such as moorland gripping should be prevented in the upland catchments containing white-clawed crayfish, in order to decrease the chance of flash flooding. Restoration and protection of good quality crayfish habitat should be concentrated in important areas such as headwaters, which are important in recolonisation and the recovery of a population in the case of a disturbance event, such as pollution or extreme flooding.

Habitat also plays an important role in determining recolonisation success of crayfish and most other species. Suitable unoccupied habitat with abundant refuges, where low competition and, therefore, limited density dependent dispersal, might be expected to occur was, in-fact, not found to hinder the dispersal-driven recolonisation. Juvenile white-clawed crayfish play an important role in recolonisation as it was found they have a rapid rate of dispersal in the form of downstream drift. This reinforces the need to identify suitable habitat for all life stages, as in order for a population to expand, the dispersing juveniles need to settle in suitable habitat. This also implies that microhabitat heterogeneity is important, as in order for a population to survive in the newly colonised area, there needs to be suitable microhabitat accessible for all life stages.

The rapid downstream dispersal of juveniles also has serious implications for the spread of plague *Aphanomyces astaci* by invasive crayfish e.g. *Pacifastacus leniusculus*. As juveniles also carry the plague, the potential downstream spread in a system is substantial. However, this rapid downstream dispersal also has positive implications in terms of reintroduction and recolonisation of threatened crayfish species. This thesis found that there was not only rapid dispersal, relative to other dispersal rates recorded for white-clawed crayfish, in juveniles, but in adults and the population as a whole. This rapid dispersal in a species, which is deemed to be a poor disperser, may have only been possible due to the large natural population that was present. Although the number of individuals required for a successful reintroduction was not specifically tested in this thesis, the rapid dispersal and successful recolonisation, as a results of a large population, implies that in order to successfully reintroduce a population of white-clawed crayfish large numbers are likely to be needed.

Several reintroduction programmes have been carried out for native European crayfish, all with varying degrees of success. The first reintroduction programme for white-clawed crayfish in the UK, took place on the Bristol Avon, south west England, and started in 1982. Juveniles and a mixture of female and male adult crayfish were released at several sites, where the maximum number released was 150 (Spink and Frayling 2000). By 1998, the population at the site where 150 crayfish were released, had only moved a maximum of 150 m and at many of the other sites, which were all around 1 km apart, there was no expansion and no connectivity between the populations (Spink and Frayling 2000). However, larger scale reintroductions of noble crayfish *Astacus astacus* have been carried out in Norway, where 3,580 juveniles and 13,832 adults were reintroduced to the Glomma watercourse over a period of 10 years, and 26,404 juveniles and 18,916 adults were reintroduced to the Halden watercourse over a period of six years (Taugbol 2004). Even after 10 years densities had still not reached normal levels. This suggests that recolonisation takes a substantially longer time on a large scale, as they were repopulating whole watercourses, or there may have been unsuitable habitat available. Many reintroduction programmes fail, either due to a lack of suitable habitat, or if the reintroduced population is too small. The population has to be large enough in order to buffer against demographic stochasticity. Mortality events are especially common in the highly variable freshwater environment, where in winter, white-clawed crayfish populations can be reduced by 40 - 60% (Brewis and Bowler 1983).

Even though relatively rapid rates of recolonisation were recorded in the Hart Burn the point source pollution still had devastating effects, where it took over four years for the 3 km stretch to recover. Full recolonisation within this timeframe was only possible as there were no repeats of the pollution. For maintenance of strong, white-clawed crayfish populations, great effort needs to be made to ensure repeat pollution events do not reoccur, as they would fragments the existing population, which would seriously hinder recolonisation and recovery. Small populations will not expand at a rapid rate, so fragmented populations may be isolated permanently, leaving them at risk from extinction. When implementing reintroduction programmes, careful considerations should be made when deciding the location of introductions, in order to prevent population fragmentation.

An important tool in reintroduction programmes is population viability analysis (PVA). This method has mainly focused on the charismatic megavertebrates, but PVA can help increase the probability of success in reintroduction schemes (Marshall and Edwards-Jones 1998, Murphy *et al.* 1990). An important output of the PVA is the minimum viable population (MVP). PVA was used in the reintroduction of capercaille *Tetrao urogallus* in Scotland, where the MVP was calculated as 60 birds (Marshall and Edwards-Jones 1998). MVP has also been used to reintroduce tree squirrels *Sciurus spp* (Wood 2007); however, they may be difficulties in modelling highly fecund taxa such as invertebrates (Lindenmayer *et al.* 1995). Nevertheless, Meyer *et al* (2007) carried out PVA on noble crayfish and found that restocking would only be successful if at least 75 adults were introduced every five years. This number is substantially smaller than the numbers of noble crayfish released in the Glomma and Halden watercourses. However, Meyer *et al* (2007) was looking at a single restocking event and this number would have to be extrapolated if large-scale reintroduction programmes into whole watercourses, as outlined by Taugbol (2004), were to be attempted. Reintroducing large numbers is costly, but with the use of PVA, the increased confidence in success may allow large-scale reintroduction programmes to take place.

In order to reintroduce large populations of endangered freshwater invertebrates, it may be necessary to set up breeding programmes in order to provide the numbers. Breeding crayfish in particular will also reduce catchment-to-catchment movements, which would increase the risk of spreading plague. Artificial incubation of eggs has been carried out in white-clawed crayfish and was found to produce similar results to maternal incubation (Perez *et al*. 1999). Jones (1995), successfully reared juvenile red-clawed crayfish *Cherax quadricarinatus,* where the tanks contained similar sized juveniles, and there was frequent feeding and artificial shelters in order to decrease the risk of cannibalism and increase production. Breeding would be especially important in species with a low fecundity, such as white-clawed crayfish, where removing large numbers from the wild may have detrimental effects on the population.

Once the reintroduction scheme or recolonisation has been initiated the population needs to be monitored. Radio tracking is an effective method for monitoring adult crayfish, but in order to monitor juveniles Surber samplers or hand searching will have to be used. Surber samplers were found to be more effective at locating juveniles than hand searching. However, hand searching can cover larger areas in less time and snorkelling or SCUBA equipment can be used to search deeper waters.

Overall, identifying the habitat requirements of juvenile crayfish is vital to their conservation, as in chapter two it was found that the microhabitats may be substantially different from those of the adults and perceived habitat requirements of the species. If the importance of juvenile habitat is overlooked it could lead to serious consequence like population crashes. Conserving the important habitat features identified in chapter two is vital, especially in the headwaters, where the populations are an important recolonisation source. Juvenile white-clawed crayfish are especially important in recolonisation, where in chapter three passive downstream drift was found to be the most effective form of dispersal. If the conditions are right, recolonisation can be very rapid, even in a species which is known to usually have poor dispersal. If there is a large population and suitable habitat then rapid dispersal can lead to the fast colonisation of new sites, as was observed in chapter three. Creating these conditions will be vital in recolonisation and reintroduction projects for white-clawed crayfish

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Appendix 1

FISAT II output

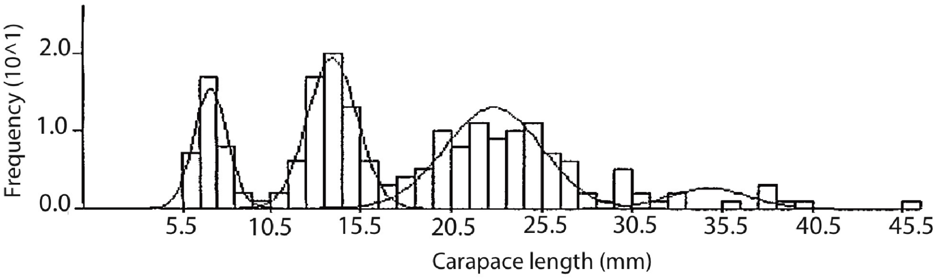

Figure 1- Size distribution of crayfish taken from the Wansbeck in August/September 2008, modelled to identify age groups

Table 1- Separation index for the four modal groups identified on the Wansbeck, where separation indexes above 2 represents a reliable separation from the adjacent group.

Group	Mean	s.d	Population	S.I
1	7.13	0.94	36	n.a
2	13.92	1.38	67	3.11
3	22.8	2.65	86	2.63
4	34.76	2.21	14	2.56

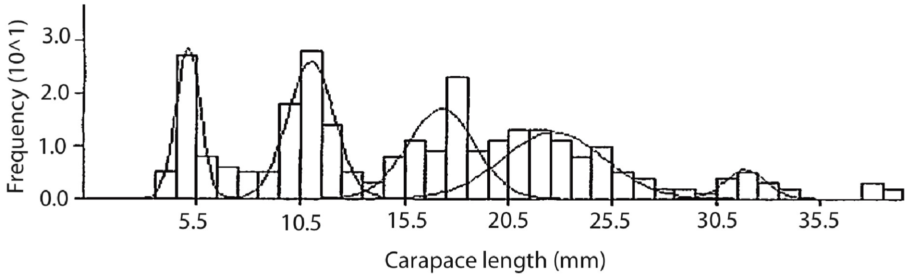

Figure 3-Size distribution of crayfish taken from the Hart Burn in July/August 2009, modelled to identify age groups

Table 2-Separation index for the five modal groups identified on the Hart Burn, where separation indexes above 2 represents a reliable separation from the adjacent group.

Group	Mean	s.d	Population	S.I
1	5.08	0.59	42	n.a.
2	11	1.11	72	3.49
3	17.24	1.61	69	2.58
4	22.67	2.45	78	2.15
5	31.91	0.99	14	2.46

Appendix 2

Flow data

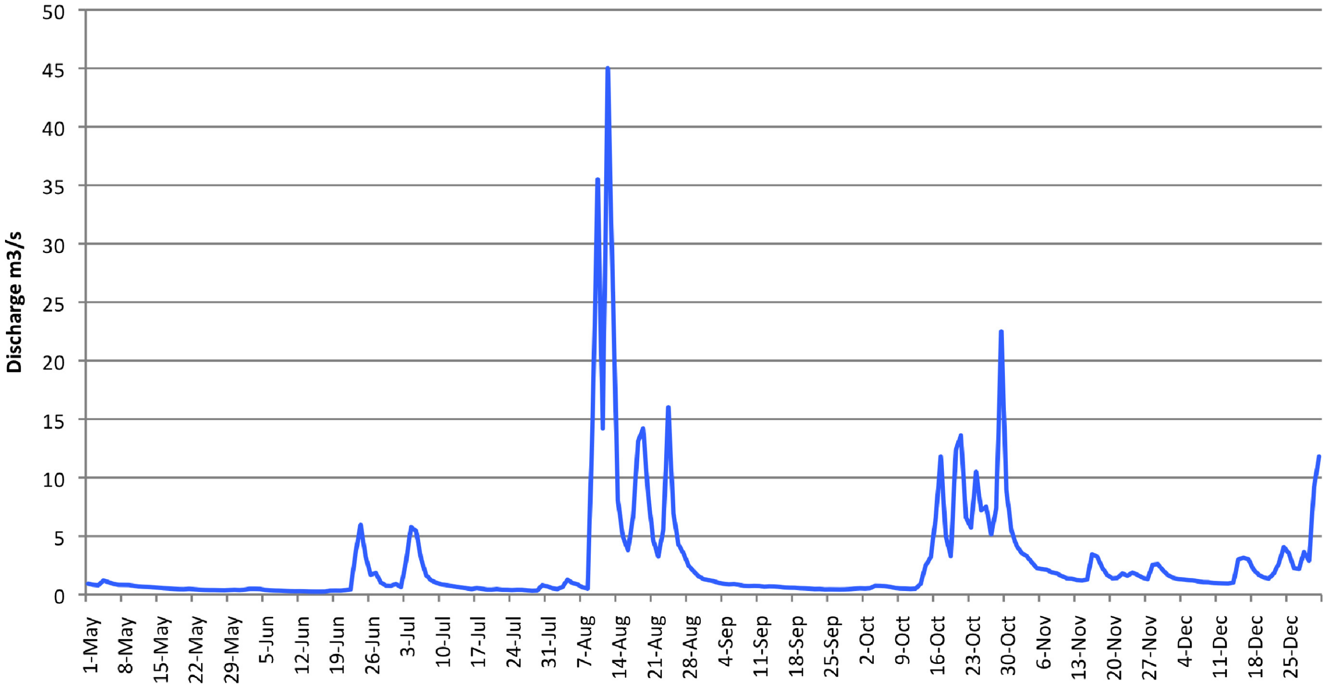

Figure 1- Daily discharge readings for the Wansbeck from the Mitford recording stations in 2004

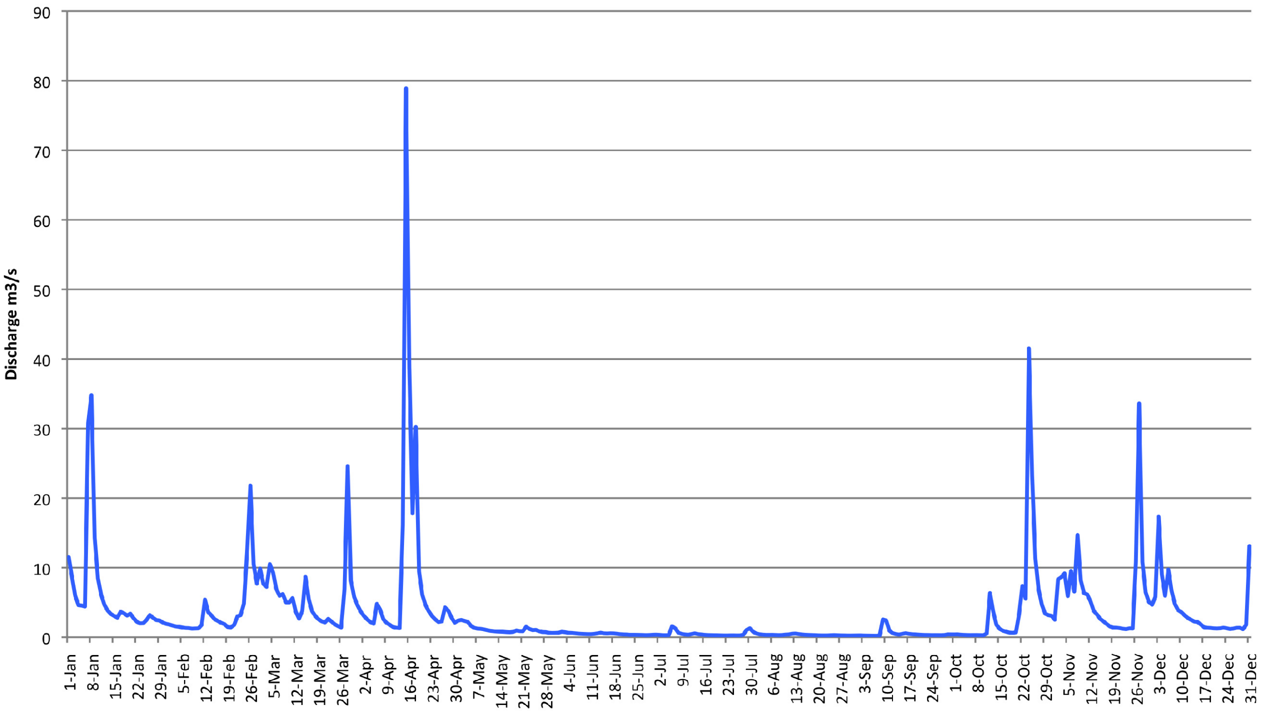

Figure 2- Daily discharge readings for the Wansbeck from the Mitford recording stations in 2005