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Crayfish in Scotland

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Abstract

Unlike the rest of Britain, Scotland has no native crayfish species. There are, however, two introduced species: the white-clawed crayfish (*Austropotamobius pallipes*) has inhabited Loch Croispol in Sutherland and Whitemoss Reservoir in Renfrewshire for several decades. *A. pallipes* is endangered in its native range and Scottish stocks may constitute an important conservation resource in the future. The other crayfish species in Scotland, the North American signal crayfish (*Pacifastacus leniusculus*) was first recorded in the wild in 1995 and has spread rapidly to inhabit many river catchments and standing waters, where it is considered a serious threat to native biodiversity. The purpose of this thesis was to conduct research into the distribution, control and impact of non-native crayfish in Scotland, with a main focus on *P. leniusculus.*

A comparative field study on the River Clyde in southern-central Scotland was used to test the efficacy of different sampling methods for detecting *P. leniusculus* in shallow, flowing waters. A combination of kick-sampling and three-run electrofishing was shown to be the most effective method and assisted in the development of a crayfish detection protocol. This protocol has been applied by fishery trusts across Scotland to determine the fine-scale distribution of *P. leniusculus* in rivers. Results of a radio-tracking study in the River Clyde catchment showed that *P. leniusculus* moves up to 195 m day $^{-1}$ in lotic habitats, with flow and in-stream barriers identified as potential impediments to movement.

In addition to studies of *P. leniusculus*, surveys were undertaken to assess the status of the two *A. pallipes* populations in Scotland. Results showed the occurrence of a low density and high density population at Loch Croispol and Whitemoss Reservoir respectively. These sites could serve as long-term "*ex-situ*" ark sites for *A. pallipes* but only if measures are taken to mitigate the current biosecurity threats of *P. leniusculus* and disease.

Laboratory studies were used to assess the potential threat of *P. leniusculus* to Atlantic salmon (*Salmo salar*) redds and the globally endangered freshwater pearl mussel (*Margaritifera margaritifera*). The burial of *Salmo salar* eggs in redds appears to afford protection from predation by *P. leniusculus* but other life stages may be at greater risk. Crayfish attempted to predate upon *M. margaritifera* but were unsuccessful, probably due to the thick, protective shell of the adult mussels tested; predation of juvenile mussels is predicted to be more likely.

Finally, the impact of a large-scale trapping programme on a population of *P. leniusculus* in a large lake was evaluated using mark and recapture methods at Loch Ken in southern Scotland. The programme significantly reduced the number of males in the population but its effect on females was complicated by seasonal variation in trappability and the bias of traps towards males. Depth was found to be a significant determinant of the catch of *P. leniusculus*. Animals were also found to make significant movements of 800 metres in two weeks. Crayfish occur in Loch Ken at densities which are high compared with other lakes and the loss of native biodiversity there is expected to be considerable.

Continued research into invasive species such as *P. leniusculus* will provide valuable data to support management decisions and help tackle what is one of the top five drivers of human-induced global change.

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Author's declaration

I declare that the work presented in this thesis is entirely my own except where specifically acknowledged or cited. No part of this thesis has been submitted for any other degree or qualification.

The following chapters have been published in co-authorship with my supervisors and others:

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Zara Gladman

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Chapter 1: General introduction

1.1 The growing threat of species invasions

> "We must make no mistake. We are seeing one of the great historical convulsions of the world's fauna and flora."

It has been over 50 years since Charles Elton pioneered the field of invasion ecology by publishing his book *"The Ecology of Invasions"*, which described and prophesised the accelerating threat of non-native, invasive species to biodiversity (Elton, 1958). Since the 1990s, the citation rate of his book has risen dramatically (Ricciardi and MacIsaac, 2008), reflecting the increasing problem of biotic invasions in today's age of globalisation. The deliberate or accidental introduction of non-native, invasive species to new ecosystems is now recognised as the second biggest threat to global biodiversity after habitat loss (Lowe *et al.*, 2000).

Since Elton's day, invasion ecology has been characterised by a plethora of terminology and definitions. "Non-native", "non-indigenous", "exotic", "foreign" and "alien" have been used interchangeably to describe the same concept (Colautti and MacIsaac, 2004). The International Union for Conservation of Nature (IUCN) defines an alien species as "a species, subspecies, or lower taxon occurring outside of its natural range (past or present) and dispersal potential (i.e. outside the range it occupies naturally or could not occupy without direct or indirect introduction or care by humans)" (IUCN, 1999). "Invasive" expands this definition to describe an alien species "that becomes established in natural or semi-natural ecosystems or habitat, is an agent of change, and threatens native biological diversity" (IUCN, 1999). A similar definition is offered by the Department for Environment, Food and Rural Affairs (DEFRA) which states that non-native invasive species "have the ability to spread causing damage to the environment, the economy, our health and the way we live" (DEFRA, 2011).

Not all alien species are invasive: the "tens rule" states that one in ten introduced species will appear in the wild, one in ten of these will become

established and one in ten of established species becomes a "pest" (i.e. "invasive") (Williamson and Fitter, 1996). Some taxa, however, appear to defy this rule (see 1.3, this chapter). A further consideration is the variable rates at which species invade: during the early stages of colonisation, the rates of population growth and expansion can vary considerably between species (Crooks and Soulé, 1999). While some species (such as zebra mussels, *Dreissena polymorpha* Pallas) invade at a rapid rate, others (such as the collared dove *Streptopelia decaocto* Frivaldszky) may persist at low levels for a long time (i.e. a "lag period") before undergoing a population explosion (Crooks and Soulé, 1999). Such lags have implications for effectively assessing the risks posed by certain invaders and may lead to misjudgements about the need for or timing of their management (Crooks, 2005).

The definitions of "invasive species" provided by the IUCN and other organisations tend to focus exclusively on those species invasions which have occurred as a result of human intervention. There is some discussion, however, about whether a difference exists between human-mediated species invasions and natural colonisation events (Lockwood *et al.*, 2007). Native species that undergo rapid range expansion may exert unwanted impacts on invaded areas that are comparable with the effects of non-native invaders: these natural invasions are known as "eruptions" and have been observed in a number of bird species. In North America, for example, eruptive invasions of boreal seed-eating birds results in large numbers of individuals appearing in areas outside of their usual range (Koenig and Knops, 2001). Natural invasions like this represent a branch of invasion ecology which receives less attention than that concerned with human-mediated invasions. The effects of human-induced climate change may, however, muddy the definition of "natural" with respect to species invasions or range expansions in the future (this is discussed further in Chapter 8).

The costs of non-native invasive species are considerable. Detrimental impacts are derived from competition, predation, disease spread, habitat modification and genetic pollution of native communities (Manchester and Bullock, 2000) and incur huge economic costs – in Great Britain alone, invasive species are estimated to cost £1.7 billion annually (Williams *et al.*, 2010).

In an ideal world, "an ounce of prevention is worth a pound of cure" would be the driving mantra behind invasive species management. In reality, however, many invaders are already here and have reached sufficient numbers to impose adverse impacts on the environment and economy. The focus for many environmental managers is therefore centred on finding ways to effectively control, eradicate or contain existing populations of invasive species.

In 2003, the Department for Environment, Food and Rural Affairs (DEFRA) published a review of non-native species policy and legislation in Great Britain (DEFRA, 2003). One of the key findings of this review was the general lack of strategic coordination in tackling invasive species in this country. This led to the formation of the GB Native Species Secretariat in 2005 and the publication of the Invasive Non-Native Species Framework Strategy in 2008 (DEFRA, 2008). The Species Action Framework (SAF) was set up by Scottish Natural Heritage (SNH) in 2007 with the aim of providing a strategic approach to species management in Scotland specifically (SNH, 2007). This framework lists six invasive non-native species as posing a significant threat to native biodiversity: the American mink (*Neovision vison* Schreber), grey squirrel (*Sciurus carolinensis* Gmelin), New Zealand pygmy weed (*Crassula helmsii* Kirk Cockayne), *Rhododendron ponticum* L., wireweed (*Sargassum muticum* Yendo Fensholt) and the North American signal crayfish (*Pacifastacus leniusculus* Dana) (SNH, 2007). These invaders have been the subject of targeted research and action over the past five years, with the chief objective being to mitigate their negative impacts on native biodiversity. This thesis presents the results of research into the only invertebrate species listed in the SAF: the North American signal crayfish.

1.2 The arrival of North American signal crayfish in Europe: a brief history

Crayfish or "Astacida" are decapod crustaceans of which there are more than 640 described species (Crandall and Buhay, 2008). In the Northern hemisphere, two families exist: the Astacidae and Cambaridae. The Southern hemisphere has one family, the Parastacidae (Holdich, 2002). Crayfish have been of interest to humans throughout history (Figure 1-1), namely as a food source for commercial harvest (especially in Sweden, see below) or subsistence, as model organisms for the study of anatomy or zoology (see TH Huxley's famous "The Crayfish: An

Introduction to the Study of Zoology", Huxley, 1880), bioindicators for heavy metal pollution (Kouba *et al.*, 2010), control agents for schistosome-carrying snails (Mkoji *et al.*, 1999), bait for predatory fish and not least, as valued components of the cultural heritage of many countries, providing inspiration for art, music and literature (Gherardi, 2011).
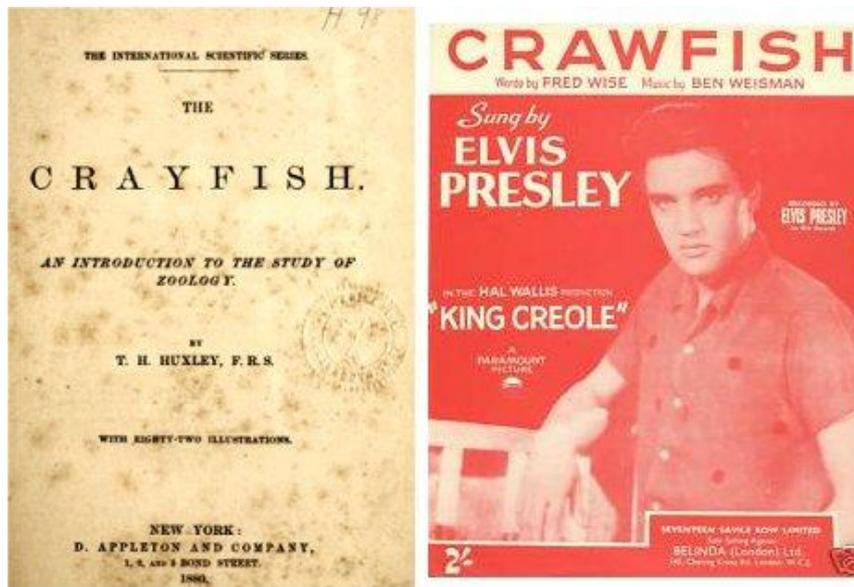
Figure 1-1 - Crayfish in science and culture: [left] Thomas Henry Huxley's 1880 book, "The crayfish: an introduction to the study of zoology" and [right] "Crawfish", as sung by Elvis Presley.

The numerous perceived benefits derived from crayfish populations might explain why so many human-mediated translocations of non-native crayfish have occurred all over the world. Unfortunately, such human intervention has led to the endangerment of many native crayfish species, which are listed in the International Convention for Conservation of Nature (IUCN) Red List. At the time of writing, 44 crayfish species as classified as critically endangered; 60 are endangered; 30 are vulnerable, 31 are near threatened and a further 106 are data deficient (IUCN, 2011). Invasive non-native species are cited as threatening 39% of crayfish species for which there are sufficient data (including 45.5% of critically endangered species).

In Europe, the number of non-native crayfish species now outnumbers that of native species 2:1 (Holdich *et al.*, 2009). The most widely distributed of these is *P. leniusculus* (Figure 1-2 and Figure 1-3) (Holdich *et al.*, 2009). This species is a member of the Astacidae family and native to western North America, between the Rocky Mountains and the Pacific Ocean (Lewis, 2002). In addition to Europe,

the range of *P. leniusculus* has expanded to new habitats in North America and Japan (Lewis, 2002). The species is easily identifiable from the white dorsal patch or "signal", present on the chela, where the movable and fixed finger parts join (Lewis, 2002).

Figure 1-2 - The North American signal crayfish (*Pacifastacus leniusculus* Dana).
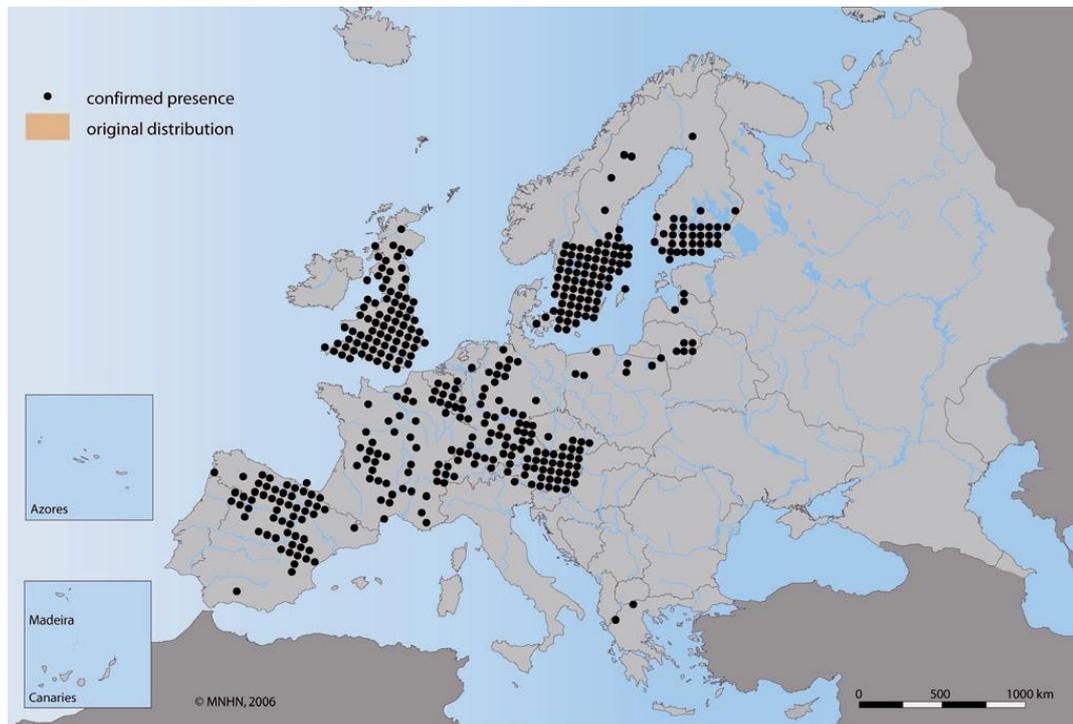
Figure 1-3 – Map of the distribution of signal crayfish in Europe, from the CRAYNET Atlas (Souty-Grosset *et al.*, 2006)

The introduction of *P. leniusculus* to Europe began in Sweden, where feasting on crayfish has been an integral part of the culture since the 16th Century (Swahn, 2004). To this day, Swedes continue the tradition of throwing "crayfish parties" on the 8th August each year, a unique celebration which has become a national institution (Swahn, 2004). Healthy populations of the native noble crayfish (*Astacus astacus* L.) supported Swedish demand until the mid-1800s, when a crayfish plague fungus (*Aphanomyces astaci* Shikora) devastated fisheries (Henttonen and Huner, 1999). The requirement for a plague-resistant stock to supplement the declining natives led to the import of *P. leniusculus* from Natoma Lake, California in December 1959 (Svärdson, 1995). *P. leniusculus* is not native to California but from further north, where it is well-adapted to life in cool, temperate water bodies and has a life cycle similar to that of the noble crayfish (Henttonen and Huner, 1999). This may explain the success of its transfer to Swedish waters. Subsequently, secondary introductions were made from Sweden to many other European countries. Primary introductions were also made directly from North America to Finland, France and Austria (Holdich *et al.*, 2009).

Signal crayfish were imported to the British Isles in the 1970s by aquacultural entrepreneurs (Holdich *et al.*, 1999a, Holdich and Reeve, 1991). Until 1982, there were no restrictions regarding the import of exotic crayfish species into the UK and so *P. leniusculus* was introduced into a variety of habitats with varying levels of security (Hogger, 1986). Hobbs *et al.* (1989) predicted correctly that the import of crayfish for aquaculture would almost certainly lead to breeding populations in the wild, as has been the case for species such as the red swamp crayfish (*Procambarus clarkii* Girard) elsewhere in Europe. Unlike in Sweden and most other European countries, the tradition of eating crayfish is not embedded in British culture (Holdich *et al.*, 1999c) and few farmers made profits from their aquaculture ventures. Many deregistered their sites as crayfish farms and instead of cultivating and rearing *P. leniusculus,* they harvested animals from the newly established wild populations (Holdich *et al.*, 1999c).

Other sources of crayfish in British waters may have included the disposal of unwanted specimens from the aquarium and pond trade (Holdich *et al.*, 1999a) or the use of live crayfish as fishing bait (now illegal in the UK) (Lodge *et al.*, 2000). Consequently, *P. leniusculus* has become well established in the wild in England, Wales (Holdich *et al.*, 1999a) and Scotland (Maitland, 1996) and its range has continued to increase due to natural and anthropogenic movements (Holdich *et al.*, 1999a).

1.3 The ecological implications of signal crayfish introductions

The signal crayfish was introduced to Europe by well-intentioned authorities (Henttonen and Huner, 1999) with little regard for the ecological implications (Lewis, 2002). Nowadays, the dangers of introducing exotic species to new ecosystems are well recognised and represent a major component of human-induced global change (Vitousek *et al.*, 1997).

Crayfish appear to defy the "tens" rule which states that only one in ten introduced species will become established, of which only one in ten becomes an invasive species (Buřic *et al.*, 2011). Aspects of the ecology of crayfish might

help explain their success as invaders and the wide-ranging impacts that they impose on the environment.

> "In fact, few things in the way of food are amiss to the crayfish; living or dead, fresh or carrion, animal or vegetable, it is all one."

As noted by Huxley (1880), crayfish have a wide dietary spectrum. As large, omnivorous macroinvertebrates, crayfish (Holdich, 2002) can affect several trophic levels by feeding on macrophytes, detritus, invertebrates (Crawford *et al.*, 2006, Nyström *et al.*, 1996) periphyton (Nyström *et al.*, 2001), amphibians (Axelsson *et al.*, 1997) and fish (Guan and Wiles, 1997a). Through this omnivory, their relatively large size and the high densities that populations may reach (Nyström *et al.*, 1996), crayfish have the potential to impose considerable environmental stress on freshwater ecosystems and often irreparable shifts in species diversity (Hobbs *et al.*, 1989). Previous studies that highlight the significant multitrophic level effects that crayfish have on freshwater ecosystems (Nyström *et al.*, 2001) are described below.

1.3.1 Impact on macrophytes

Macrophytes play a significant role in aquatic ecosystems by influencing water chemistry (i.e. nutrient dynamics, dissolved carbon, oxygen and pH) and also biological interactions (Jeppesen *et al.*, 1998). Any changes in macrophyte species composition or density will therefore have repercussions for the whole ecosystem (Nyström *et al.*, 1999).

The introduction of signal crayfish commonly leads to a reduction in both the biomass and species richness of macrophytes (Hogger, 1986, Nyström *et al.*, 1999, Nyström *et al.*, 1996, Nyström and Perez, 1998, Nyström *et al.*, 2001). *P. leniusculus* reduced large beds of *Elodea canadensis* to "a few sparse patches" after its introduction to a study lake in Hampshire (Hogger, 1986). Another study using artificial ponds in Sweden found high crayfish abundances to be associated with decreases in macrophyte coverage and species richness (Nyström *et al.*, 1996). In a replicated field experiment, signal crayfish reduced macrophyte coverage by both direct grazing and nonconsumptive fragmentation, leading to floating plant parts accumulating in the habitat (Nyström *et al.*, 2001). In comparison with other studies, Nyström *et al.*, (1999) found crayfish

to only moderately reduce macrophyte biomass in artificial pond littorals but suggested that stronger negative effects may only become apparent in the long term. Stable isotope analyses indicate that invertebrates are the preferred food item for crayfish; it is possible that crayfish will begin to more seriously impact macrophytes at higher densities after invertebrates are depleted (Nyström *et al.*, 1999).

Several studies have highlighted the indirect effects that a loss or change in macrophytes can have on the rest of an ecosystem, and in particular, invertebrate species (Nyström *et al.*, 1996, Nyström and Perez, 1998). Guan and Wiles (1998) commented that the consumption of macrophyte detritus and macroalgae would not only lead to a reduction in the abundance of detritus and algae but also have an important impact on benthic insects and nutrient cycling. Many invertebrate taxa are more likely to be associated with a particular macrophyte species, thus mixed stands of macrophytes will support greater invertebrate species richness than stands with fewer macrophyte species (Brown *et al.*, 1988). Given that crayfish reduce macrophyte species diversity and biomass, it is not surprising that a reduction in herbivorous and detritivorous invertebrates follows (Nyström *et al.*, 1996).

1.3.2 Impact on invertebrates

Crayfish can impact invertebrate communities both indirectly via the consumption of macrophytes and detritus (as above) (Guan and Wiles, 1998, Nyström *et al.*, 1996) and directly via predation. Invasion of stream communities by signal crayfish often leads to a reduction in macroinvertebrate taxon richness (Crawford *et al.*, 2006, Stenroth and Nyström, 2003) and changes in the invertebrate assemblage (Nyström *et al.*, 1999) as a result of selective predation. Slow-moving invertebrates such as gastropods are at a greater risk of predation than active and sediment dwelling taxa (Nyström *et al.*, 1999).

For many European countries, one of the foremost issues relating to *P. leniusculus* introductions is the devastating impact on native crayfish populations. Ironically, the signal crayfish – a species brought to Sweden because of its crayfish plague resistance – is itself a carrier of the disease, to which the native noble crayfish (*A. astacus*) remain susceptible (Lodge *et al.*,

2000). Native crayfish may also suffer directly as a result of interspecific competition for food and shelter, making them more susceptible to predation (Lodge *et al.*, 2000). In England and Wales, the native white-clawed crayfish (*Austropotamobius pallipes* Lereboullet) is suffering a similar fate to the noble crayfish in Scandinavia, with declines largely attributed to the spread of signal crayfish and plague (Holdich *et al.*, 1999c, Füreder *et al.*, 2010).

1.3.3 Impact on amphibians

The impact of signal crayfish on amphibians has not been studied extensively. There is evidence, however, that *P. leniusculus* will impact amphibians via predation of eggs and tadpoles (Axelsson *et al.*, 1997, Nyström and Åbjörnsson, 2000). In aquarium experiments, Axelsson *et al.* (1997) found that signal crayfish would consume the eggs of seven species of amphibian, with feeding rates increasing with temperature. Crayfish also consumed and caused sub-lethal damage to tadpoles, particularly in less complex habitats. Similarly, Nyström & Åbjörnsson (2000) found crayfish to have a negative effect on survival of tadpoles in experimental tanks and a positive indirect effect on periphyton biomass due to reduced grazing by tadpoles.

California has been invaded by two non-native crayfish species: the red swamp crayfish in the south (*P. clarkii*) and signal crayfish in the north. In southern California, the red swamp crayfish, has been shown to predate on California newt *Taricha toroso* [Rathke] eggs and larvae and repeatedly attack adults, driving them onto land and impeding breeding. As a result, an inverse relationship between the distribution of newts and introduced crayfish in streams has been observed (Gamradt *et al.*, 1997). There are no published studies on the impact of *P. leniusculus* on amphibians in northern California, although its presence has been cited as a potential cause of decline (Kats *et al.*, 2006).

Similar interactions might be expected for *P. leniusculus* should it be introduced to amphibian-colonised waters in the UK. Given the vulnerable status of amphibians worldwide (Beebee and Griffiths, 2005), such introductions would be highly undesirable.

1.3.4 Impact on fish

Several studies have investigated the effect of *P. leniusculus* introductions on fish (Bubb *et al.*, 2009, Degerman *et al.*, 2007, Griffiths *et al.*, 2004, Guan and Wiles, 1997a, Hayes, 2012, Light, 2005, Peay *et al.*, 2009, Stenroth and Nyström, 2003).   In surveys on the River Great Ouse, an inverse relationship between crayfish abundance and bullhead (*Cottus gobio* L.) and stone loach (*Barbatula barbatula* L.) was observed (Guan and Wiles, 1997a). This observation was tested further in laboratory experiments, which showed that crayfish outcompete both species for shelter and predate upon them. A recent study found signal crayfish to have a negative impact on the recruitment of salmonids in an English stream, although the mechanism of decline was not known (Peay *et al.*, 2009). Signal crayfish have been found to outcompete juvenile Atlantic salmon *Salmo salar* L. for shelter, leaving them more vulnerable to predation and forcing them to use up fat reserves by swimming in the water column (Griffiths *et al.*, 2004). Similarly, signal crayfish were shown to displace Paiute sculpin *Cottus beldingii* [Eigenmann and Eigenmann] from refuges in laboratory experiments (Light, 2005).   In the presence of crayfish, sculpin shifted into higher-velocity microhabitats and increased their activity rate by fleeing, leading to reduced growth rates.

In southern England, recent work has found signal crayfish to impact the growth rates of chub (*Leuciscus cephalus* L.) (Kevin Wood, pers. comm., 2009). This impact was size dependent: growth rates of young chub were significantly lower in the presence of *P. leniusculus*; the growth rates of older chub, however, were higher when crayfish were present, probably because these fish were big enough to utilise crayfish as a food source. Positive and negative effects of crayfish on bullhead have also been recorded, with crayfish serving as a prey item but also as a competitor (Hayes, 2012).

Not all studies have found crayfish introductions to have detectable effects on fish species. A study in a Swedish stream using *in situ* enclosures found introduced signal crayfish to have no effect on brown trout *Salmo trutta* L. fry survival (Stenroth and Nyström, 2003). In addition, results from long-term stream electrofishing surveys in Sweden showed that fish densities within sites were not significantly different before and after the introduction of crayfish

(Degerman *et al.*, 2007).   The streams surveyed by Degerman *et al.*, (2007) however, were mostly located in forests with stable banks and coarse substrata that would be difficult for crayfish to burrow into. As a result, the fish in these streams would not have been subjected to the potentially negative effects of crayfish burrowing (Degerman *et al.*, 2007). The observed effects of crayfish on fish are likely to vary considerably depending on the habitat composition, experimental approach and fish species (Degerman *et al.*, 2007).

1.4 Non-native crayfish in Scotland: project aims

Unlike the rest of Britain, Scotland has no native crayfish species. There are, however, two introduced species: *A. pallipes* has inhabited Loch Croispol in Sutherland and Whitemoss Reservoir in Renfrewshire for several decades.   *A. pallipes* is not thought to be invasive and the decline of populations in its native European range means that the two Scottish populations may constitute valuable refuge stocks for conservation in the future. The other crayfish species in Scotland, *P. leniusculus* was first recorded in the wild in 1995 in Galloway (Maitland, 1996).   Given the significant ecological impacts of *P. leniusculus* found in previous studies (reviewed above) this species may threaten the integrity of Scottish freshwater ecosystems. Little is known about the status of *A. pallipes* and *P. leniusculus* populations in Scotland or the consequences of crayfish introductions for native biodiversity. The two populations of *A. pallipes* have not been surveyed in over a decade. Previous research has highlighted the potential for *P. leniusculus* to impact Atlantic salmon stocks (Griffiths *et al.* 2004) and significantly alter the structure of invertebrate communities in Scottish rivers (Crawford *et al.* 2006). Information about potential impacts on other biota, or the fine-scale distribution of signal crayfish, however, is sorely lacking.

Research into non-indigenous crayfish can be grouped into three broad themes, which explore the status, control or impact of introduced species. This PhD aims to answer questions which relate to each of these themes, as illustrated in Figure 1-4, below.
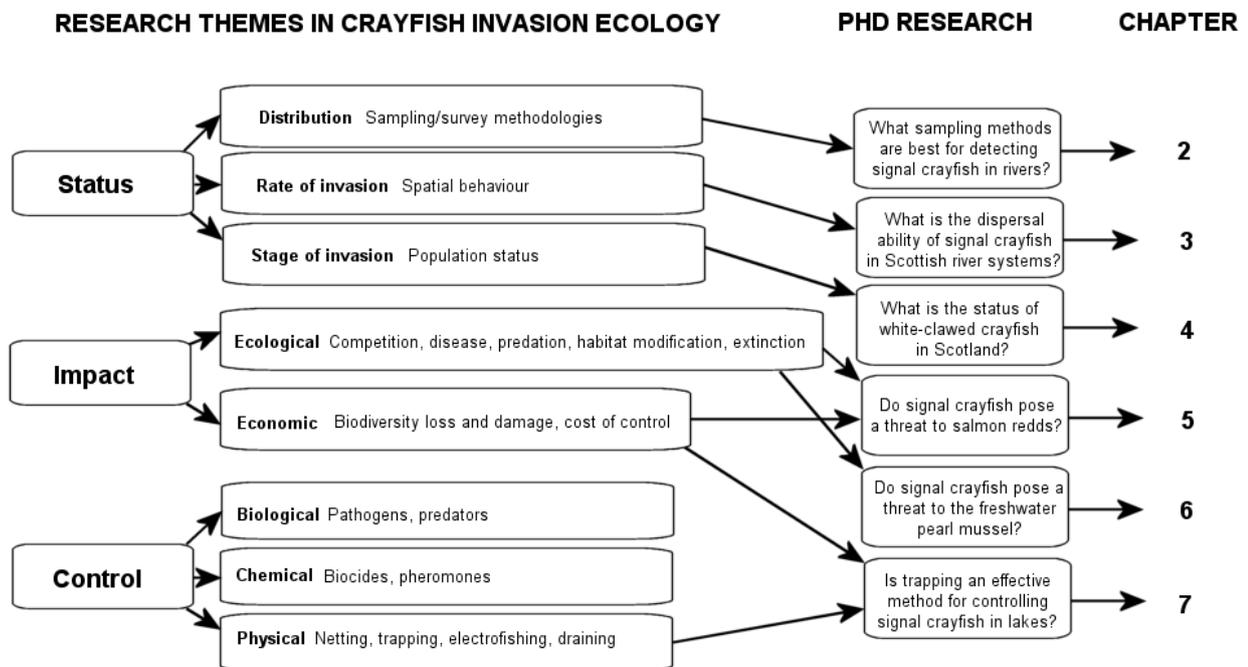
Figure 1-4 - Research themes in crayfish invasion ecology and the scope of this PhD research

The aims of this PhD are to:

☐ Develop methodologies for surveying signal crayfish, which can be used to assess the status of this species in Scotland (Chapter 2)

☐ Investigate the movements of signal crayfish in Scottish rivers, thus providing an insight into their dispersal ability (Chapter 3)

☐ Assess the status of white-clawed crayfish populations in Scotland (Chapter 4)

☐ Assess the potential impact of signal crayfish on native species of economic and conservation importance, namely Atlantic salmon (*Salmo*

salar L.) and the freshwatwer pearl mussel (*Margaritifera margaritifera* L.) (Chapters 5 and 6)

☐ Assess the efficiency of trapping as a control method for signal crayfish using a case study of an infested loch (Chapter 7)

With the exception of Chapter 4, this thesis will focus on the invasive non-native American signal crayfish and the implications of its establishment in Scotland.

Chapter 2: Detecting signal crayfish in riffles

2.1 Abstract

The spread of the invasive signal crayfish (*P. leniusculus*) outside of its natural range is of widespread concern due to the threats posed to native biodiversity. To date, there is no standard protocol for determining signal crayfish presence or absence in a watercourse. For the purposes of this investigation, the crayfish detection ability of active sampling methods — hand-netting, electrofishing (one, two and three runs), kick sampling and Surber sampling — was tested at 30 sites along the River Clyde, southern central Scotland. No single technique was successful in detecting crayfish in 100% of the sites known to contain crayfish and so the efficacy of combinations of techniques was examined. The combination of techniques that resulted in a 100% detection rate was electrofishing (three runs) together with kick sampling. These results suggest that three-run electrofishing and kick sampling are the best candidates for incorporation into a crayfish detection protocol. The mean time taken to apply electrofishing (three runs) was significantly greater than the mean time to apply kick sampling. Given the lower effort required for its application, kick sampling is recommended as the preliminary technique: if kick sampling yields a negative result, the application of electrofishing will decrease the chance of recording a false negative presence. If both kick sampling and electrofishing fail to detect crayfish, trapping may further decrease the risk of a false negative result. These findings have assisted in the development of a crayfish detection protocol, which has been applied across Scotland to determine the current distribution of signal crayfish.

2.2 Introduction

Since its discovery in Scotland in 1995 (Maitland, 1996) the North American signal crayfish *P. leniusculus* has extended its range to several localities across the country, including the upper reaches of the River Clyde (Bean *et al.*, 2006, Maitland *et al.*, 2001). Given its potential to damage ecosystems (for a review, see Holdich, 1999), the signal crayfish is listed under the Scottish Government Species Action Framework as a species posing a significant threat to biodiversity (SNH, 2007).

The fine-scale distribution of crayfish within catchments is largely unknown (Bean *et al.*, 2006) and there is a pressing need to develop methods to determine the distribution of signal crayfish populations, in order to inform containment or control programmes. Signal crayfish were first detected in the upper River Clyde area in 1999 but carapace lengths suggested that crayfish were introduced at least 10 years before this (Trudgill, 2000). It seems likely, therefore, that current distribution records underestimate the number of established populations owing to the apparent lag between the introduction and detection of crayfish (Hiley, 2003).

Although guidelines are available for monitoring white-clawed crayfish (*A. pallipes*) (Peay, 2003), there is no standard protocol available for determining signal crayfish presence or absence in a watercourse. Signal crayfish exhibit a different spatial behaviour (Bubb *et al.*, 2006a) and inhabit a wider range of habitats than native European species, thus necessitating the need for a species-specific method of sampling (Weinländer and Füreder, 2012). There is also a lack of empirical evidence to support selection of the most efficient method. Several techniques are available for sampling crayfish populations, which may differ in their efficiency. Trapping, hand-netting, quadrat sampling, kick sampling, electrofishing and Surber sampling have been used with varying degrees of success (Alonso, 2001, Byrne *et al.*, 1999, Gallagher *et al.*, 2006, Peay, 2003, Rabeni *et al.*, 1997, Smith *et al.*, 1996, Usio and Townsend, 2001, Waters *et al.*, 1993). This study tests the relative efficiency of four active sampling methods – hand-netting, electrofishing (one, two and three runs), kick sampling and Surber sampling – which have the potential to determine signal crayfish presence in a field setting.

2.3 Materials and methods

2.3.1 Study area

Previous survey and crayfish removal activities supported by Scottish Natural Heritage and carried out by local angling interests over the last nine years have identified the approximate limits of signal crayfish distribution in the River Clyde. For the present study, alternative techniques were tested in areas around the likely upper limit (i.e. where densities were sufficiently low to provide an indication of differential catch efficiencies among the methods). In total, 30 sampling sites in areas of riffle (fourth stream order) ranging from 15–30 metres in length and comprising habitat known to support crayfish in some parts of the catchment were identified along the River Clyde, southern-central Scotland (from Ordnance Survey Great Britain National Grid Reference NS 95879 18598 to 97243 09583).

The first site to be sampled was believed to be upstream of the signal crayfish distribution in the Clyde. By commencing work in a no-crayfish zone (Site 1) and progressing downstream into colonised areas, it was hoped to identify the most sensitive technique of the four and also minimise the risk of spreading the species further upstream via contaminated equipment.

Shallow riffles were selected as sampling sites on the basis that they were similar in terms of their channel shape and flow characteristics, abundant within the survey area, easy to sample using the chosen techniques and likely to contain small crayfish at higher densities than deep pools (Guan, 2000). At each site, mean depths were taken using a metre stick and the percentage of the site falling into each of six depth ranges was estimated (<10 cm, 11–20 cm, 21–30 cm, 31-40 cm, 41-50 cm and >50 cm); for most sites, the majority of depths were within the range 11–40 cm.

At each site, red flags and measuring tape were used to divide the area into three equal, adjacent sections. In two of the sections, hand searching and electrofishing were used to collect crayfish. The third section was further divided from bank to bank into two parts, with either kick sampling or Surber sampling used to collect samples. The order of application of techniques from

the upstream to downstream end was rotated sequentially between sites to ensure that each technique was used in a different section of the sample area.

Between sites, all equipment was checked for contamination and nets were thoroughly washed. Upon capture crayfish were killed on-site in 80% alcohol and transported to the laboratory for further analyses. All sites were sampled between 30 October and 26 November 2008. By sampling during cold conditions when crayfish were relatively inactive and thus difficult to detect, the most sensitive of the techniques could be determined.

2.3.2 Sampling procedure

2.3.2.1 Hand searching

The marked section was actively searched using a small handheld hoe to turn over rocks and vegetation and collect any exposed crayfish in a small hand-net of 1 mm mesh. Times taken to detect the first crayfish and search the whole section were recorded. Crayfish were preserved in 80% alcohol and transported to the laboratory.

2.3.2.2 Electrofishing

Electrofishing was carried out using an Electracatch WFC-11 backpack unit (200 V; smooth dc current). Two operatives worked together, moving the anode across and upstream through the section and collecting any stunned crayfish in a short-handled fry net of 1mm mesh; these were transferred to river water in a bucket until the whole section had been surveyed. This was repeated a further two times. Time taken to detect the first crayfish and complete each of the three sample runs was recorded. Crayfish were preserved in 80% alcohol and transported to the laboratory.

2.3.2.3 Kick sampling

In one half of the third section, a 3 minute kick-sample (the standard time for a kick sample as described by Freshwater Biological Association protocols, FBA 2012) was taken using a standard D-shaped pond net (250mm width frame, 1mm mesh). The surveyor moved around the site in order to sample all available

habitats; the total sampling time of 3 minutes was divided between all habitats. The pond net was placed on the river bed downstream of the kicking to collect the sample and also swept through weeds and undercut banks. The contents of the net were transferred into a white plastic tray and examined for the presence of crayfish. The time taken to find the first crayfish was recorded. The whole sample was then transferred into a pot, preserved with 80% alcohol and transported to the laboratory.

2.3.2.4 Surber sampling

Ten replicate Surber samples were taken in the other half of the third section, using a Surber sampler (330 x 310mm width frame, 1mm mesh). The Surber sampler was placed randomly within the section and the substrate disturbed using a three-pronged rake to a depth of approximately 10 cm for 30 seconds to generate each sample. Samples were checked on-site for crayfish and the time taken to find the first crayfish recorded. Whole samples were preserved in sealed pots with 80% alcohol and transported to the laboratory.

2.3.2.5 Laboratory treatment of samples

In the laboratory, preserved kick and Surber samples were searched a second time for crayfish. Times taken to find the first crayfish and sort each sample were recorded. Finally, the carapace length (CL) of all crayfish was measured from the tip of the rostrum to the posterior margin of the carapace using Vernier callipers (± 0.1 mm).

2.3.3 Statistical analysis

Differences in the crayfish detection abilities of the techniques were tested for significance by applying the Chi square test (with Yates' correction, to account for 1 degree of freedom). Student t tests were used to compare the mean times taken to apply the electrofishing and kick sampling techniques and to detect the first crayfish. The mean carapace lengths of crayfish caught by kick sampling and electrofishing were also compared using t tests. All analyses were carried out using SPSS statistical software (version 14.0).

2.4 Results

Crayfish were detected at 25 of the 30 sites sampled. Figure 2-1 shows the location of sites and the presence or absence of signal crayfish at each site.
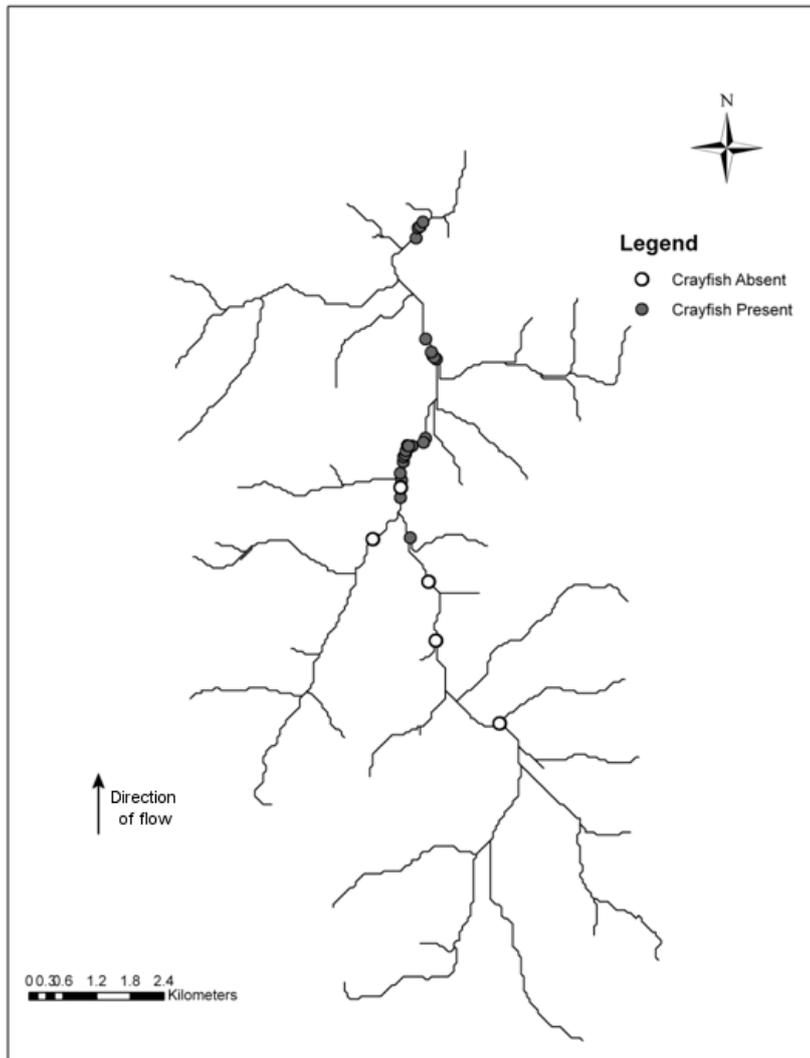
Figure 2-1 - River Clyde showing the presence or absence of crayfish at sample sites (based on all the results of all detection methods combined).

There was a significant difference between the methods in the number of sites at which signal crayfish were detected (Figure 2-2, χ^2 = 25.25, p < 0.0001, df = 3). For each of the methods, the number of sites where crayfish were detected was compared with the number of sites detected to contain crayfish when all six techniques were applied (i.e. the best available knowledge of crayfish presence, representing the 'expected' detection rate of 100%).   Hand searching, electrofishing (one run) and Surber sampling produced detection rates that differed significantly from the expected rate (χ^2_{Yates} = 58.41, p < 0.01, df = 1;

χ^2_{Yates} = 6.25, p < 0.05, df = 1; χ^2_{Yates} = 13.69, p < 0.001, df = 1, respectively). For kick sampling and electrofishing (two and three runs), however, the observed frequencies of crayfish detection did not differ significantly from those expected (χ^2_{Yates} = 3.61, p > 0.05, df = 1; χ^2_{Yates} = 1.69, p > 0.1, df =1; χ^2_{Yates} = 0.81, p > 0.1, df = 1, respectively). The proportion of sites found to contain crayfish by one run of electrofishing differed significantly from the proportion produced by three runs (χ^2_{Yates} = 5.97, p < 0.05, df = 1).
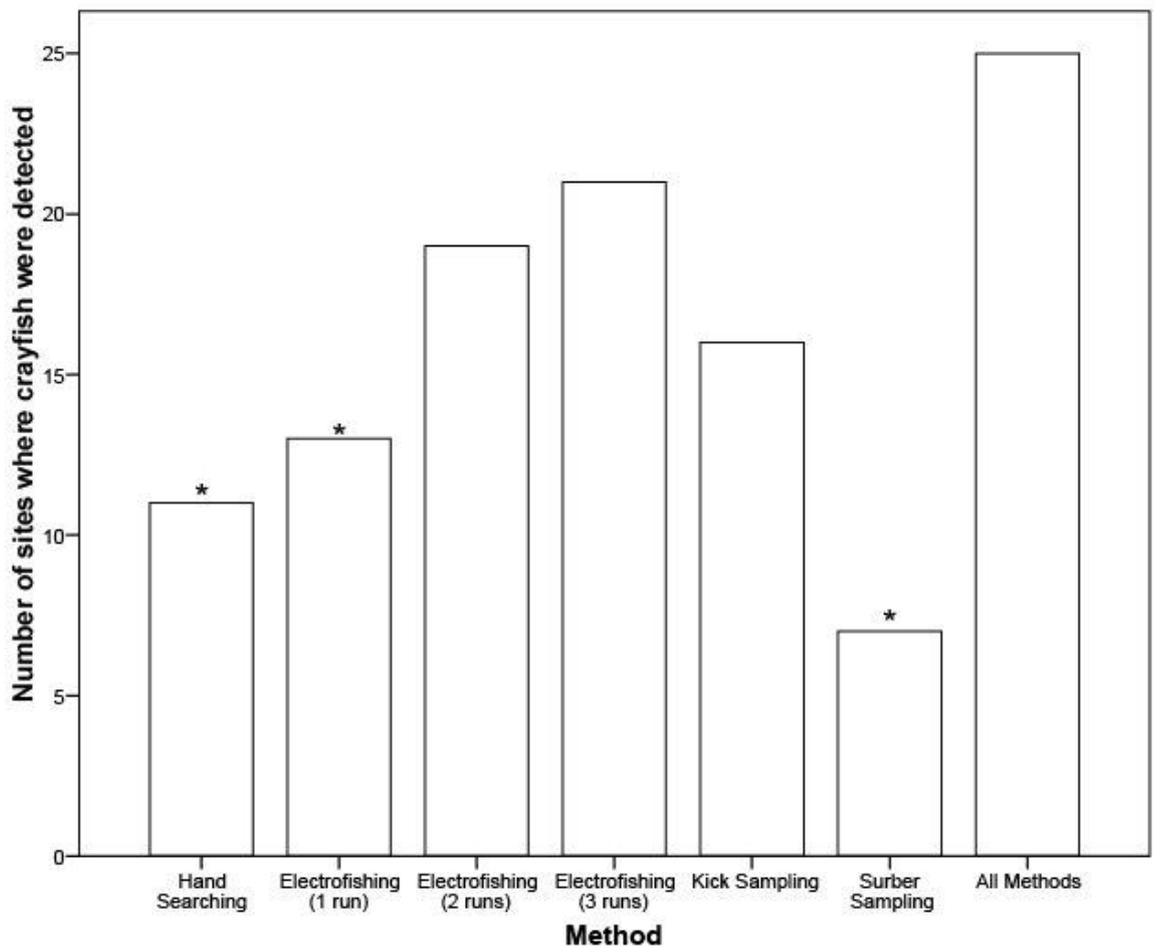
Figure 2-2 - Number of sites where crayfish were detected by each method (and all methods combined). Asterisks (*) denote methods that detected crayfish in a number of sites that differed significantly from the number of sites known to contain crayfish (i.e. where crayfish were detected by at least one method).

The number of sites where crayfish were detected by different combinations of techniques was also calculated (Figure 2-3). The two most successful combinations of techniques were kick sampling together with two runs of electrofishing (96% detection rate) and kick sampling together with three runs of electrofishing (100% detection rate).
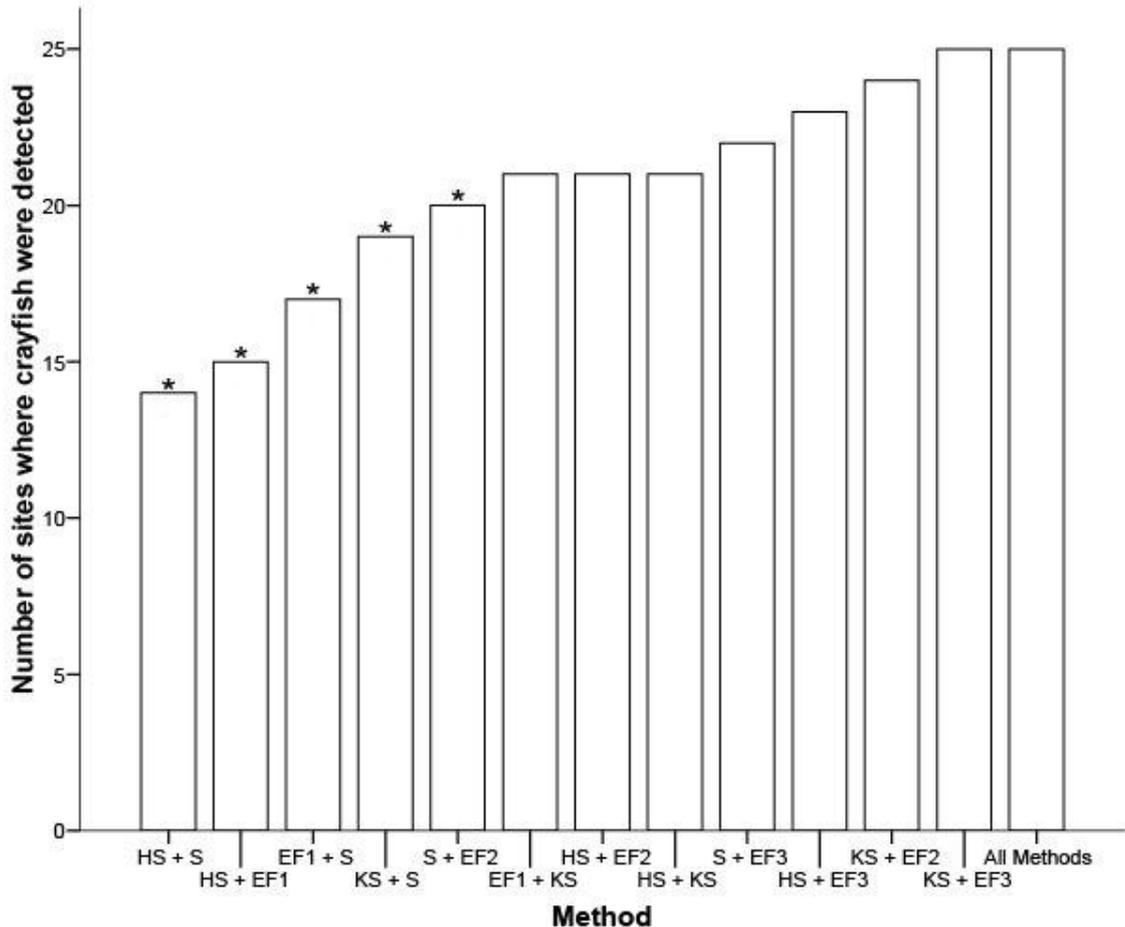
Figure 2-3 - Number of sites where crayfish were detected using combinations of two methods (HS = hand searching; S = Surber sampling; KS = kick sampling; EF1 = electrofishing (1 run); EF2 = electrofishing (2 runs); EF3 = electrofishing (3 runs). Asterisks (*) denote the combinations that detected crayfish at a frequency that differed significantly from the number of sites known to contain crayfish (i.e. where crayfish were detected by at least one method).

The mean time taken to carry out the electrofishing technique (three runs) was significantly longer than the time taken to obtain and sort a kick sample (t = 8.14, p < 0.001, df = 58), with a mean time of 26.10 ± 5.84 minutes compared with 10.88 ± 1.84 minutes. For sites in which both electrofishing and kick sampling detected crayfish, the mean time taken to detect the first crayfish by electrofishing was 6.61 ± 2.72 minutes and by kick sampling was 3.23 ± 1.18

minutes. These times were not significantly different (t = 24.07, p > 0.05, df = 26).

Crayfish caught by electrofishing were significantly larger than those caught by kick sampling (t = 24.07, p < 0.0001, df = 169), with a mean carapace length of 20.3 ± 1.82mm compared with 12.89 ± 1.27 mm.
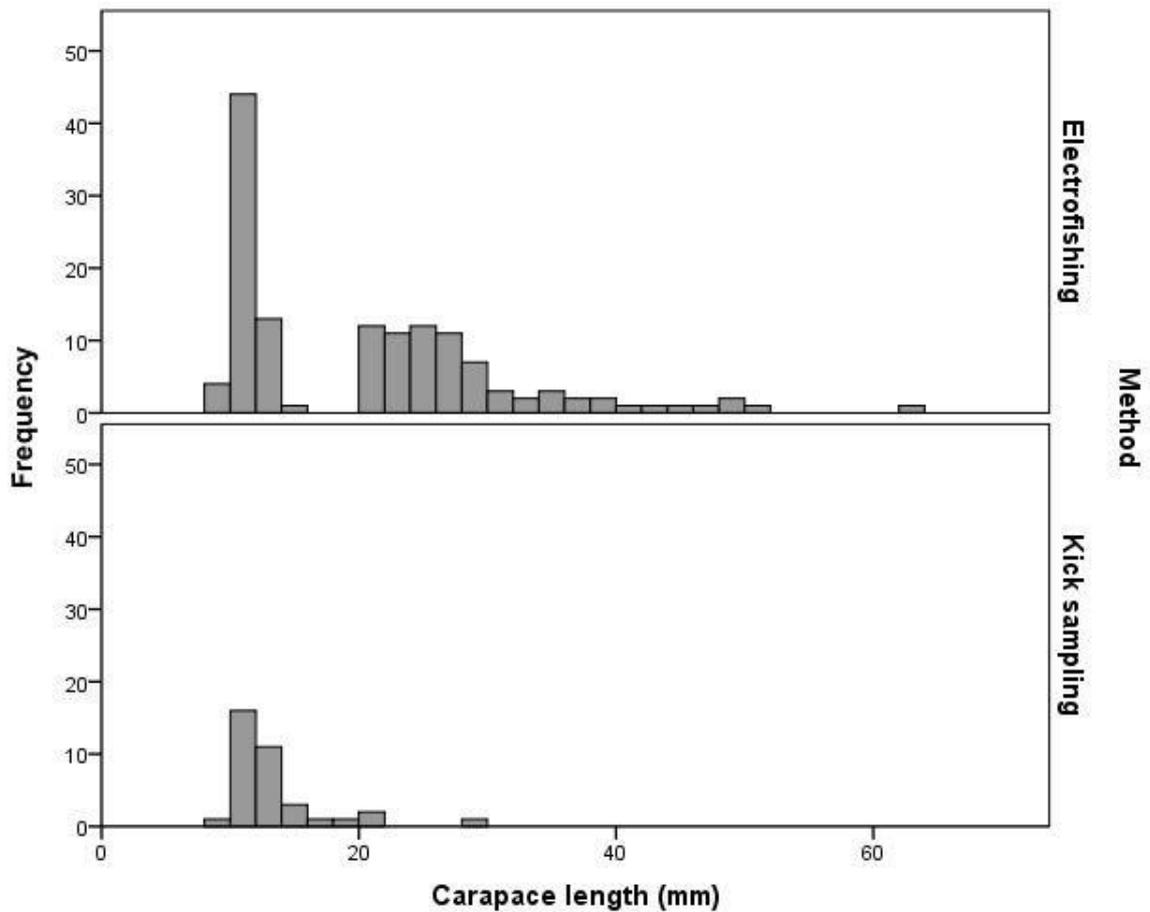
Figure 2-4 – Size frequency of crayfish captured by electrofishing and kick sampling. Crayfish captured by electrofishing were significantly larger than those captured by kick sampling (p <0.0001).

2.5 Discussion

All techniques used in this study were successful in capturing North American signal crayfish. Using data from all techniques combined, crayfish were found at 25 of the 30 sampling sites. Crayfish had not previously been recorded at three of the sites where a negative result was obtained (Matt Mitchell, 2009, pers. comm.). These sites are now known to lie outside the extreme limit of the crayfish distribution within the River Clyde. The two other negative results were obtained at locations adjacent to sites containing crayfish. Reasons for these negative results are unclear, although it is possible that either conditions were simply unsuitable for crayfish colonisation or that crayfish were present at such a low density that even extensive surveying using the multiple techniques employed in this study was insufficient to detect them.

Developing a suitable methodology for crayfish detection should take account of the detection ability of potential techniques but also the amount of effort, in terms of time and labour, which is required to apply them. The results described below have implications for developing a detection methodology.

2.5.1 Hand searching

Hand searching has been used previously to sample crayfish species. Most studies, however, aim to provide estimates of crayfish population size rather than a simple indicator of presence/absence. Rabeni *et al*. (1997) found hand-netting depletion to greatly underestimate population abundances of Northern koura (*Paranephrops planifrons* White) and only recommended its use if applied in conjunction with other capture methods. A recent survey of acuminate crayfish (*Cambarus acuminatus* Faxon) in Pennsylvania found hand searching to be less efficient than electrofishing over large stream reaches (US National Park Service, 2007).

In the present study, hand searching was similarly inefficient, detecting crayfish in only 44% of the sites known to have crayfish present. It is possible, however, that some of this low efficiency was derived from the way in which the method was applied. Searching of the section was indiscriminate, with an equal amount of time spent throughout the area, irrespective of habitat type. Surveyors

commented, however, that hand searching tended to be more successful in the same parts of the river, such as near the bank and in areas of high sediment deposition. In areas near the bank, surface waters may be slower and less broken compared with the mid-channel, thus improving visibility for capture. Peay (2003) recommended hand searching as a sampling technique to monitor *A. pallipes* but only if applied selectively. The results of this study suggest that using surveyors who are experienced in identifying likely crayfish habitat will increase the detection ability of this technique (Peay, 2003). Hand searching was much less efficient, however, than electrofishing and kick sampling, both of which were applied indiscriminately.

2.5.2 Electrofishing

Electrofishing was trialled as a method of eradicating signal crayfish from a Scottish river several years ago and shown to be inadequate, although it appeared to reduce the overall population size (Ribbens and Graham, 2004, Sinclair and Ribbens, 1999). Electrofishing surveys for crayfish differ from those used to assess salmonid populations in that the time taken to complete each run is longer and the capture efficiency is lower because crayfish do not behave in the same way as salmonids when exposed to an electric current. Although disturbed or stunned by the current, crayfish do not display the same degree of galvanotaxis as observed in most fish species and this adds to the time taken to capture affected individuals. The increased time taken to sample crayfish using this technique compared with sampling fish was previously described by Alonso (2001), who highlighted cheliped loss as a cause for concern when conducting electrofishing surveys of the endangered white-clawed crayfish. Cheliped loss by signal crayfish was commonly observed during electrofishing in the present study.

A single electrofishing run returned a poor crayfish detection rate (52% efficiency) at all population densities. The proportion of sites found to contain crayfish using one run of electrofishing was significantly lower than that obtained for multiple runs; on eight occasions, crayfish were not detected at all during the first run but detected in subsequent runs. Alonso (2001) conducted a minimum of three-run depletion estimates in an electrofishing survey of *A. pallipes* but wherever the trend of catches suggested it, a fourth effort was

made. The aim of the present study was not to estimate *P. leniusculus* population density but rather to develop a rapid detection technique. One run of electrofishing is clearly inadequate for this purpose. Reasons for this are unclear, although it is possible that a second pass is necessary to stun crayfish that have been drawn out of sheltered habitats during the first run. The efficiency of electrofishing as a detection technique may be low for crayfish that are occupying deep burrows (which may be common during low temperatures or high flow).

Two or three runs of electrofishing produced the highest detection rates of all the tested techniques. In both cases, the frequency of detection did not differ statistically significantly from the frequency of sites known to have crayfish, with detection rates of 76% for two-run electrofishing and 84% for three-run electrofishing.

2.5.3 Surber sampling

Surber sampling detected crayfish at the lowest number of sites of all the techniques, which differed significantly from the frequency of sites known to harbour crayfish and thus severely underestimated crayfish presence. Surber samples were taken at random throughout the whole section rather than focused on specific patches of habitat, which might have contributed to such a low rate of detection. Usio and Townsend (2001) noted the problems of underestimating crayfish population size associated with using Surber sampling. If data from only Surber samples taken in their study of the Southern koura *Paranephrops zealandicus* [White] had been considered in their analysis, crayfish biomass would have been underestimated by up to 32% of the figure calculated from electrofishing. In the present study it was also clear that Surber sampling was inefficient in comparison with electrofishing.

Guan (2000) used a modified, large Surber sampler to collect *P. leniusculus* from the River Great Ouse in England with some success, taking 12 arbitrarily selected samples per site in order to calculate relative abundance. This represents a much greater area and effort than was used in the investigation reported here. The River Great Ouse has a higher density, biomass and production of signal crayfish than other reported figures for this species elsewhere (Guan, 2000), and

so the combination of high density and increased sampling effort may have increased the success of this technique in Guan's study.

The crayfish captured by Surber sampling were all juveniles of a small size (mean carapace length: 11.45 mm, n = 18). This suggests that adult crayfish may be more difficult to dislodge during the 'raking' process or that they can move rapidly out of the sample area and are therefore less likely to be swept into the net. Juveniles may be less able to escape capture via this method, either because they have a poorer swimming ability or because they may be less likely to abandon in-stream refuges than larger individuals. Surber sampling works best in shallow riffles and is limited by depth (more so than the other tested methods). Given that juveniles tend to occupy shallow riffles at greater densities than adults (Guan, 2000), this habitat-dependent limitation on Surber sampling might further bias the capture efficiency, when more widely applied.

Given the bias of Surber sampling towards young crayfish, the time of year in which sampling is conducted could affect the chance of crayfish detection. In the periods immediately following the release of juveniles from incubation by females, the river is likely to support a greater number of juveniles than the rest of the year. Surber sampling may therefore be most successful in detecting crayfish during these periods. Surber sampling does not appear to represent a robust method of detecting crayfish presence, particularly if crayfish are present at low to moderate densities. Analysis of samples may, however, allow the potential impact of crayfish on invertebrate communities to be assessed.

2.5.4 Kick sampling

Kick sampling was found to be a relatively reliable technique. In this study, it detected crayfish presence on 64% of occasions where crayfish were known to be present. The method has been used in previous studies to sample *A. pallipes* (Gallagher *et al.*, 2006, Smith *et al.*, 1996). Gallagher *et al.* (2006) used kick sampling in conjunction with trapping to investigate the presence of *A. pallipes* and found that kick samples yielded fewer animals than traps. Another study used kick sampling and stone turning to sample *A. pallipes*, with the different methods applied depending on habitat type (Smith *et al.*, 1996). Hand searching was used in mid-channels with large stones; kick samples were taken in marginal

regions of the river with smaller stones. The average catch-per-unit-effort for kick sampling was found to be 3.2 times higher than that for stone turning. In the present study, kick sampling was also more productive than hand searching, detecting crayfish in 22% more of the sites known to contain crayfish. Again, it is likely that kick sampling has the potential to detect crayfish at a rate higher than that observed, since our method did not target sampling according to habitat. Kick sampling favoured capture of small crayfish over large ones, possibly due to differences in escape ability; it is also probable that small juveniles are more likely to be swept into a pond net than heavier adults, which are less easily dislodged, as suggested for Surber sampling.

2.5.5 Combinations of techniques

None of the techniques was successful in detecting crayfish at 100% of the sites known to have crayfish. The application of two techniques was therefore considered. The combination of techniques that detected crayfish in all of the 25 sites known to contain crayfish was kick sampling plus three runs of electrofishing. Electrofishing and kick sampling captured crayfish from a range of size classes. Electrofishing may be more biased towards the capture of large crayfish since it relies on the observer to spot and net the animal and larger crayfish are more easily spotted than small ones.

There is evidence of age-specific differential habitat use by signal crayfish (Blake and Hart, 1993). Juveniles are more likely to occupy shallow water (Guan, 2000), possibly as a response to predation by fish (Blake and Hart, 1993) and larger crayfish. Adults are more vulnerable to predation by terrestrial predators such as otters and mink (Englund and Krupa, 2000) than fish and this might explain their absence from shallower vegetation and leaf litter (Demers *et al.*, 2003) and preference for deeper pools (Guan, 2000).

The study presented here shows clear evidence of technique-dependent size selection. This, combined with different habitat limitations on each technique and size-specific habitat-use, suggests that kick sampling is more likely to be successful in detecting the presence of crayfish in shallow water with heavy vegetation cover (i.e. more complex habitats), where it is most efficient and where smaller crayfish are more abundant. By contrast, at sites with deeper

water and a higher proportion of larger individuals inhabiting simpler habitats, electrofishing may be more effective.

2.5.6 Conclusions and recommendations

This study suggests that hand searching, one-run electrofishing and Surber sampling do not represent efficient techniques for detection of signal crayfish. A combination of three runs of electrofishing plus kick sampling, however, provides the best chance of crayfish detection of all the active surveying methods.

Electrofishing, although more reliable, represents a lengthier, more physically demanding technique than kick sampling. As such, kick sampling should be used as the preliminary technique in any detection protocol, with one, two and three runs of electrofishing applied thereafter. Both methods allow crayfish presence to be detected on the same day as sampling, unlike trapping, which should be employed as a final detection technique only if both kick sampling and electrofishing yield a negative result.

In addition to sampling method, factors such as the time of sampling, weather conditions and habitat parameters (including stream order) are likely to be important in influencing the efficiency of crayfish detection. Trapping data, for example, indicate that crayfish are most easily caught on the River Clyde during the summer months (Matt Mitchell, 2009, pers. comm.) and so this would be the best time of year to undertake surveys. Targeting sampling to habitat favourable for crayfish (e.g. muddy banks for burrowing, vegetation cover and slow-moving water) is also important. For this study, the difficulty of sampling in deep pools and burrows led us to conclude that targeting small crayfish in riffles would be the best test of the competing methods. There are likely to be sites, however, that require crayfish surveys but which are too deep to allow kick sampling or electrofishing. A further consideration is the stream order of the site being sampled. For the present study, sampling was applied in a relatively low-order (fourth) stream channel; the total area surveyed by electrofishing varied depending on the width of the channel but averaged at ~80 m^2. Application of bank-to-bank electrofishing in higher order channels would lead to the sampling of much larger areas, which may be unnecessary. Again, targeting of sampling

towards areas containing habitat favourable for crayfish is recommended, to prevent undue effort. Detection protocols for crayfish (see Appendix II) should be modified to take account of these factors which will vary between sites and catchments, in order to maximize efficiency.

Chapter 3: Movements of signal crayfish in a Scottish river

3.1 Abstract

Crayfish are the largest mobile invertebrates in freshwater ecosystems with the capacity to invade new habitats. In the summer of 2009, the movements of non-native signal crayfish (*Pacifastacus leniusculus* Dana) at two sites (a first-order channel and a fourth-order channel) in the headwaters of the River Clyde were studied using radio-tracking. Crayfish were found to move up to 195 m day^{-1}. The cumulative distance moved ranged from 21.7 m to 645.5 m in 28 days. There was considerable variation in the movements of individual crayfish. Biotic factors (crayfish sex and size) had no significant effect on the distance or frequency of movements whereas abiotic factors (site, river flow and waterfalls) were found to be important. Crayfish moved significantly greater distances in the channel compared with the burn, where waterfalls and the steep gradient appeared to limit upstream dispersal. River flow had a negative impact on the number and distance of movements in the channel; in the burn, river flow had a negative impact on the distance of upstream movements. These observations provide an insight into the dispersal ability of non-native signal crayfish in new habitats, which may be considered when devising control or containment strategies.

3.2 Introduction

Crayfish are the largest mobile invertebrates in freshwater ecosystems (Holdich, 2002) with the capacity to move and exploit patchy resources, re-colonise disturbed habitats and expand their range to new areas (Robinson *et al.*, 2000). Observing such movements in the aquatic environment is difficult but has been achieved in previous studies using mark and recapture methods (e.g. Hazlett *et al.*, 1979, Kerby *et al.*, 2005, Light, 2003, Momot, 1966), PIT tagging (Bubb *et al.*, 2006b, Bubb *et al.*, 2008) and radio-tracking (e.g. Bubb *et al.*, 2004, Buřič *et al.*, 2009a, Gherardi *et al.*, 2002, Robinson *et al.*, 2000). Crayfish movements are highly complex and influenced by a wide range of factors including crayfish size (Gherardi *et al.*, 2002, Light, 2003, Robinson *et al.*, 2000, Webb and Richardson, 2004), sex or reproductive state (Bubb *et al.*, 2002, Buřič *et al.*, 2009a, Hazlett *et al.*, 1979, Light, 2003), water level (Hazlett *et al.*, 1979), river flow (Bubb *et al.*, 2004, Gherardi *et al.*, 2002, Kerby *et al.*, 2005, Light, 2003, Momot, 1966), gradient (Bubb *et al.*, 2004, Light, 2003), temperature (Bubb *et al.*, 2002, Bubb *et al.*, 2004, Buřič *et al.*, 2009b, Flint, 1977), light (Flint, 1977), season (Buřič *et al.*, 2009a, Flint, 1977, Henry, 1951, Light, 2003) and the presence of in-stream barriers (Bubb *et al.*, 2006b, Bubb *et al.*, 2008, Kerby *et al.*, 2005, Light, 2003). In addition to movements in water, some species have been found to move overland (Gherardi and Barbaresi, 2000).

Information on the movement patterns of crayfish has applications in both conservation and management. An estimation of the home range of crayfish helps ensure that an effective reserve size is allocated for protected species (Ryan *et al.*, 2008, Webb and Richardson, 2004) and allows identification of important habitats (Armitage, 2000). The movements of endangered species in response to floods, in-stream barriers, invasive species and other potential threats may also be assessed (Bubb *et al.*, 2006a). Equally, studies of invasive non-native crayfish movements provide an insight into their dispersal ability in new habitats which may be considered when devising control or containment strategies.

The North American signal crayfish *P. leniusculus* is the most widespread non-native crayfish species in Europe, with current records in 27 countries (Holdich *et al.*, 2009). Since its discovery in Scotland in 1995 (Maitland, 1996), *P.*

leniusculus has colonised over 174 km of river length spanning 13 different catchments (Sinclair, 2009). Although much of the spread of this species may be attributed to introductions by humans (Holdich *et al.*, 1999a), studies of native populations in western North America (Henry, 1951) and introduced populations in England and California have shown that *P. leniusculus* is also capable of moving significant distances naturally (Bubb *et al.*, 2002, Bubb *et al.*, 2004, Bubb *et al.*, 2006b, Bubb *et al.*, 2006b, Flint, 1977, Guan and Wiles, 1997b, Light, 2003).

Over the past decade, radio-tracking has emerged as a useful tool for providing fine-scale, continuous information about crayfish movements without causing significant disturbance to animals or the environment, which is a major limitation of mark and recapture studies. Radio-tracking studies of *P. leniusculus* have so far been restricted to a few rivers in England (Bubb *et al.*, 2002, Bubb *et al.*, 2004, Bubb *et al.*, 2006a). Furthermore, although information is available on *P. leniusculus* movements in large river channels (~30 metres width) there have been no radio-tracking studies to elucidate their behaviour in lower order streams. The present study investigated the movements of *P. leniusculus* at two upland sites in the headwaters of the River Clyde, central-southern Scotland, where it is a well established non-native species. The first site was the Crookedstane Burn, a first-order tributary of the River Clyde which is thought to have been the original source of the *P. leniusculus* introduction in the catchment. The second site was the Daer Water (a fourth-order channel) which is located on the main channel, which is upstream of Crookedstane Burn and about 2 km downstream of the known upper limit of the *P. leniusculus* distribution in the catchment (Sinclair, 2009). Crayfish activity is positively correlated with temperature (Bubb *et al.*, 2004) and so tracking was conducted during mid-summer after the release of young in order to gauge the maximum dispersal potential at the two sites. The importance of biotic factors (crayfish sex and size) and abiotic factors (site, river flow and waterfalls) in influencing crayfish dispersal was also examined.

3.3 Materials and methods

3.3.1 Study sites

Two study sites were used, within the catchment of the River Clyde, central southern Scotland (see Figure 3-1 for map and Figure 3-2 for photographs) at an elevation of around 270-290 metres above sea level; ~ 2 km separates the sites. Site A was located on the Crookedstane Burn (Ordnance Survey Great Britain National Grid Reference NS 96689 14902), a first-order tributary of the River Clyde which flows adjacent to Crookedstane Farm. This stream is relatively narrow (~1-4 metres wide) and flows at a high gradient (1: 15) over a substrate of gravel, cobbles and moss-covered boulders. The channel is characterised by pools and waterfalls. Bank vegetation comprises grasses and overhanging ferns; the stream also passes through a small forested area.

Site B (NS 95563 13178) was located on the Daer Water, upstream of where it joins the Potrail Water to form the main stem of the River Clyde. This fourth-order channel consists of a series of pools and riffles and is much wider (~ 8-10 metres), deeper and flatter than Site A. The gradient is approximately 1: 32. The channel is flanked by grazed grass banks and flows under a road bridge, past Watermeetings Farm.
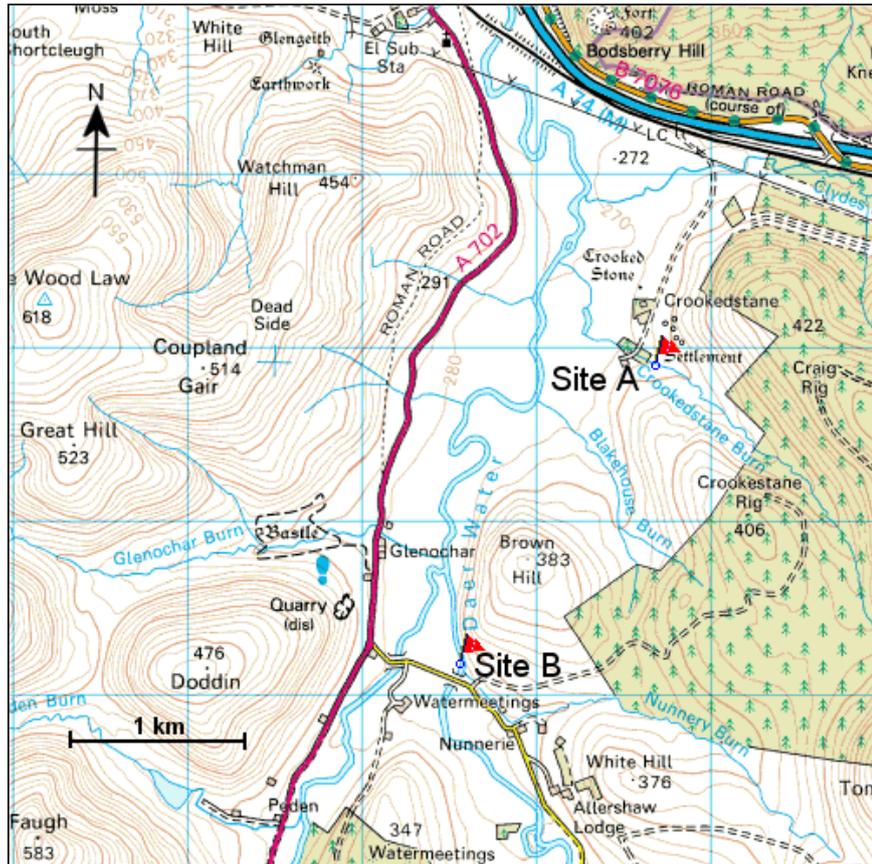
Figure 3-1 - Map showing the locations of Site A (Crookedstane Burn) and Site B (Daer Water) in the River Clyde catchment.

Figure 3-2 - Photographs of Site A (Crookedstane Burn) and Site B (Daer Water).

3.3.2 Crayfish and tagging

Crayfish were obtained from both sites using Swedish Trappy™ traps which were set overnight and baited with domestic cat food. The carapace length of each crayfish was measured from the tip of the rostrum to the posterior margin of the carapace using Vernier callipers (± 0.1 mm). Electronic scales were used to measure the mass of each crayfish (± 0.1 g). Only adult crayfish (carapace

length 32 - 44.9 mm) were tagged. There was no significant difference between the size of crayfish that were tagged at Site A and Site B (Mann Whitney U = 50.5, p > 0.05, n = 16) or between the size of males and females (Mann Whitney U = 41.5, p > 0.3, n = 16).   A radio-transmitter (PIP3 single-celled tag; dimensions: 14 x 7 x 4 mm with whip antenna of c. 10 cm; mass: 1.5g; Biotrack Ltd.) was attached to the top of the carapace using glue (Araldite® Rapid Super Strong Adhesive). Radio-transmitters represented 1.9-7.4% of the wet mass of the crayfish, which is within the range used in previous crayfish tracking studies (e.g. Bubb *et al.*, 2004, Buřič *et al.*, 2009b). Crayfish were held in a shallow tray of water (~ 3 cm depth) for about 25 minutes until the glue was dry and then returned to the site of collection. Crayfish were trapped and released at the two sites on consecutive days: on 24th July 2009, eight crayfish (four males and four females) were tagged and released at three locations within Site A. On 25th July 2009, nine crayfish (five males and four females) were tagged and released at three locations within Site B.

3.3.3 Daily tracking

Each radio-transmitter emitted a unique pulse signal at a frequency between 170.236 and 173.993 MHz. Crayfish were tracked using a lintec flexible 3-element Yagi antenna (Biotrack Ltd.) connected to a receiver (Sika, 4 MHz, Biotrack Ltd.) and headphones.   After the position of a crayfish was approximated, the Yagi antenna was replaced with a custom built rod antenna (Nosrat Mirzai, University of Glasgow) which was less sensitive than the Yagi antenna and by adjusting signal gain, allowed the position of a crayfish to be determined more precisely (within c. 0.3 metres). On a few occasions at Site A, crayfish were observed moving around outside of their burrows and so their positions could be pinpointed exactly. When water levels were too dangerous to enter the river, positions were estimated from the bank side and so tracking accuracy was reduced. After each crayfish was located, a grid reference was recorded (using a Garmin eTrex Venture HC GPS Unit) and a pre-labelled garden cane was inserted into the bank to mark the linear stream position.   The distance of crayfish movements (following the stream) was determined from the previous position (as marked by the cane) to the new position.

All tracking was conducted during daylight hours when crayfish were relatively inactive. At the start of the study, an underwater camera (Pentax Optio W30 Digital Compact) was used to confirm that crayfish were occupying refuges underneath the river bank during the day. Changes in crayfish position were therefore representative of nocturnal movements by crayfish between different refuges. Crayfish were allowed to acclimatise for five (Site B) or six (Site A) days following tagging before tracking commenced. Crayfish were tracked every day for nine days; after nine days, tracking was halted to due to faulty equipment. After a break of four days, daily tracking resumed and continued for 18 days, ending on 28 August 2009.

3.3.4 Overnight tracking

To confirm the nocturnal behaviour of *P. leniusculus*, night-time tracking was also conducted overnight on 30 August 2009. Three crayfish at Site A were monitored for 30 minutes during three different time periods: dusk (20:45-21:15), darkness (01:25-01:50) and dawn (06:05-06:35). The position and activity of each crayfish during each time period was recorded.

3.3.5 Environmental measurements

Data on river flow at Abington Gauge Station (NS 93271 22756) was obtained from the Scottish Environment Protection Agency (SEPA). This station lies about 12.5 km downstream of Site A and 15 km downstream of Site B on the main stem of the River Clyde. Mean nightly flows (between 20:00 and 08:00) were calculated in order to ascertain flow conditions during periods of nocturnal crayfish activity. Mean nightly flows were compared with the number and magnitude of crayfish position changes recorded on the following day.

At Site A, the movement of crayfish across three different waterfalls was recorded. The dimensions of each waterfall were measured using a measuring stick.

3.3.6 Statistical analysis

Statistical analyses were carried out using PASW Statistics (version 18.0.0) in order to test for the effects of crayfish size and sex, site and river flow on the

number and distance of crayfish movements. Where possible, data were normalised using square root or log transformations in order to apply parametric statistics (t-test, paired t-test, Pearson's product moment correlation); if this was not possible, non-parametric statistics (Mann Whitney U-test, Wilcoxon signed ranks test, Spearman's rank correlation coefficient or Chi Square test with Yates' correction) were applied.

3.4 Results

3.4.1 Tag retention

Of the 17 crayfish tagged, 12 were tracked for the maximum study period of 28 days (28 radio-fixes over 36 days). Tag loss or signal failure reduced the total tracking time to zero days for one crayfish (crayfish 17, excluded from these results), eight days for two crayfish (crayfish 5 and crayfish 10) and 10 days for one crayfish (crayfish 15). Tag loss was presumed to have occurred when repeated, exceptionally large (>500 metres) movements were observed in a downstream-only direction, followed by a loss of signal; these observations may be explained by detached tags being washed down the river. Tag loss was likely due to crayfish moulting, which occurs at a frequency of once or twice per year in adults during the summer months (Aiken and Waddy, 1992). It is also possible that mortality of crayfish and subsequent washing downstream led to a loss of signal. One other crayfish was discovered half-eaten on the bank-side after 16 days of tracking (crayfish 4).

3.4.2 Movements out of water

At Site B, crayfish were found stranded in waterlogged grass on top of the bank on several occasions. After stranding, crayfish 12 and crayfish 13 returned to the water within 24 hours. Crayfish 10 remained on the bank side for 48 hours, after which a signal could no longer be detected; it is possible that this individual was predated on due to its vulnerability out of water. Crayfish 14 remained on the bank side for 14 days (weather conditions were damp throughout) before returning to the water.

3.4.3 Overnight tracking and burrow occupation

Two of the three crayfish observed overnight were active during the dusk observations (conducted at 20:45-21:15); the third crayfish remained in its burrow. At the start of the darkness observations (01:25-01:50), none of the crayfish had moved from the positions recorded at dusk. No crayfish were observed moving during the darkness observations. Between the end of the darkness observations and the start of the dawn observations (06:05-06:35), two

of the crayfish changed positions. No crayfish were observed moving during the dawn observations. Photographs from an underwater camera confirmed that crayfish occupied refuges underneath the river bank during the day (Figure 3-3).

Figure 3-3 - Crayfish in daytime refuge.

3.4.4 Movement patterns

There was considerable variation in the movements of individual crayfish. The daily positions of crayfish relative to their point of release were calculated for Site A (Figure 3-4) and Site B (Figure 3-5).

After release, all crayfish at Site A moved downstream; thereafter, the direction of movements varied.   None of the crayfish moved above the uppermost waterfall (channel width: 2 m, fall height: 80 cm). The three crayfish (crayfish 1-3) that were released furthest upstream moved down the middle waterfall (channel width: 1.2 m, fall height: 46 cm) and two of the three (crayfish 2 and 3) also moved down the lowermost waterfall (channel width: 80 cm, fall height: 45 cm). Crayfish 1 was tracked moving back up and down the middle waterfall; on one occasion during daylight, this individual was observed using its chelipeds to cling onto overhanging vegetation and climb the waterfall. No other crayfish were either recorded or observed moving up waterfalls. At the middle release

point, where two crayfish were released, one (crayfish 5) moved down the lowermost waterfall; the other (crayfish 4) remained close to the release point. Crayfish at the lower release point (crayfish 6-8) moved downstream and did not move far enough upstream to re-enter the area of release. The maximum positions recorded upstream and downstream of any one release point at Site A were 14 metres (crayfish 1) and 78.7 metres (crayfish 2) respectively. Crayfish often remained in the same position for short (~2-4 days) or long (~1-2 weeks) periods before migrating overnight to a new position.

At Site B, crayfish moved in both upstream and downstream directions after release. During the tracking period, the maximum positions upstream and downstream of any one release point were 73 metres (crayfish 10) and 254.5 metres (crayfish 12) respectively. As observed in Site A, crayfish tended to remain in the same position for variable periods before embarking on overnight migrations to a new refuge. During the second half of the tracking period, several crayfish moved very little or not at all.

Figure 3-4 - Daily positions of crayfish at Site A relative to the points of release which are indicated by the dotted lines; solid lines denote the location of waterfalls.
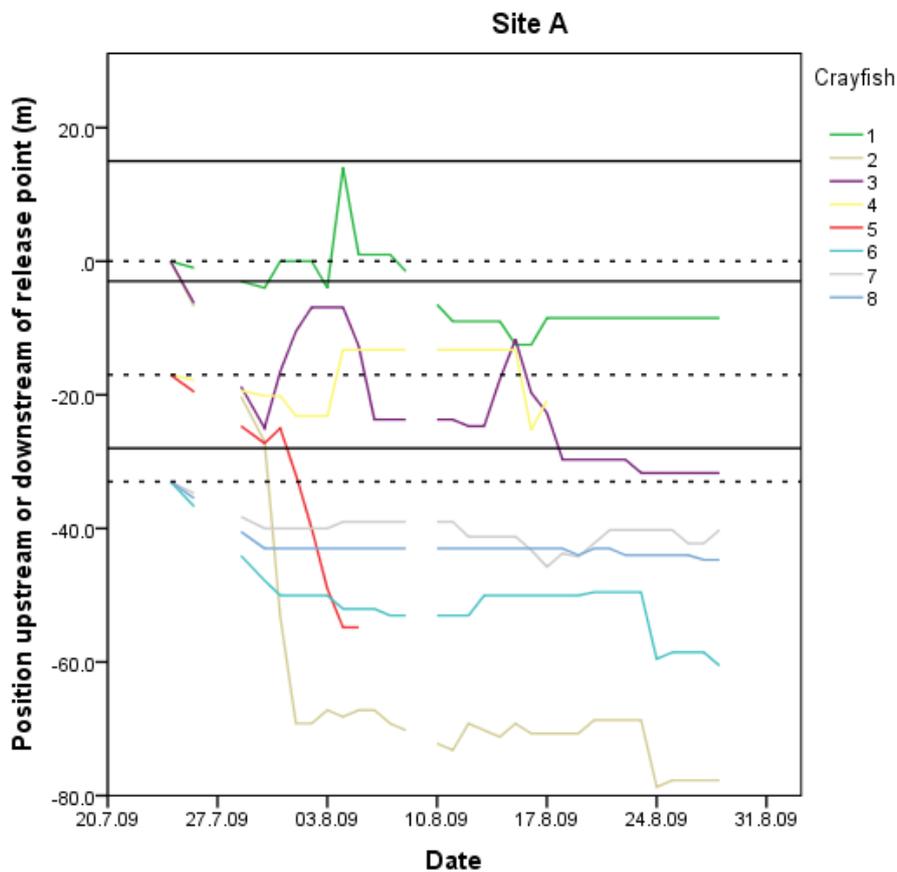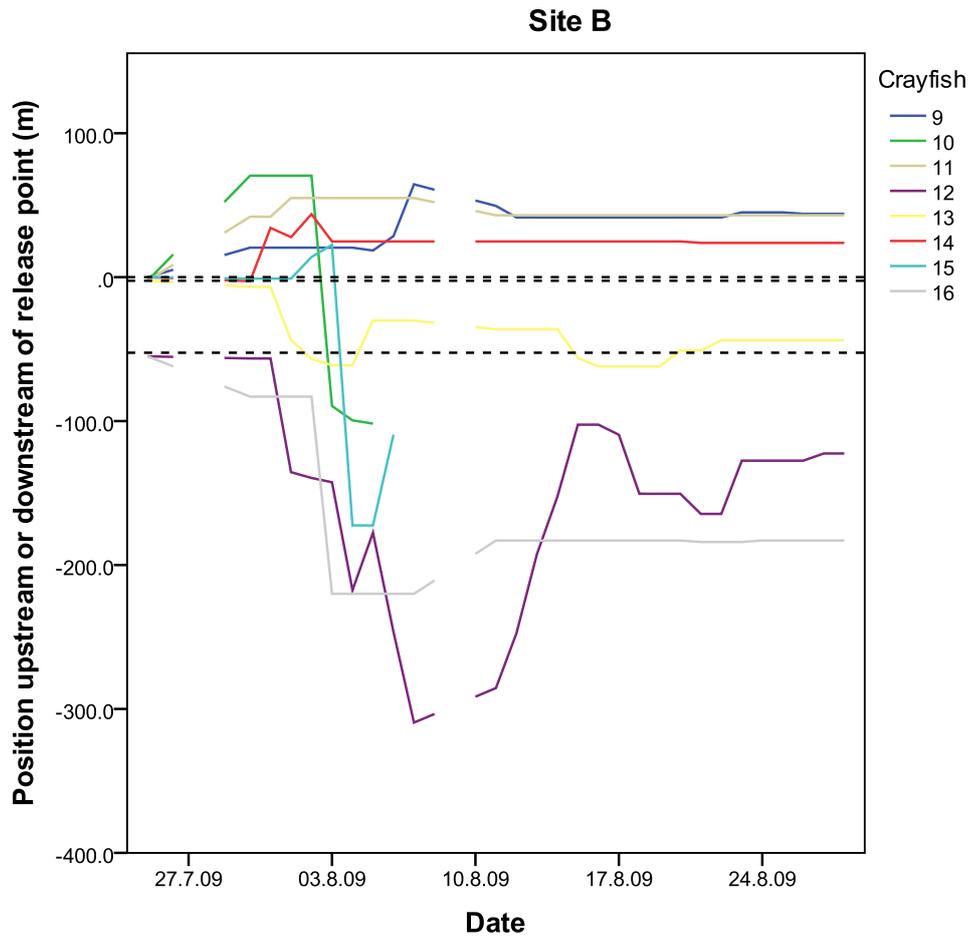
Figure 3-5 - Daily positions of crayfish at Site B relative to points of release which are indicated by the dotted lines.

The maximum distances moved upstream and downstream from the release point were used to calculate a linear range for each crayfish (Figure 3-6). To allow comparison of crayfish tracked for different lengths of time, the linear range was divided by the tracking period to give a range per day tracked (RPD), as described by Bubb *et al*. (2004). The cumulative distance moved by each crayfish was also calculated (Table 3-1).
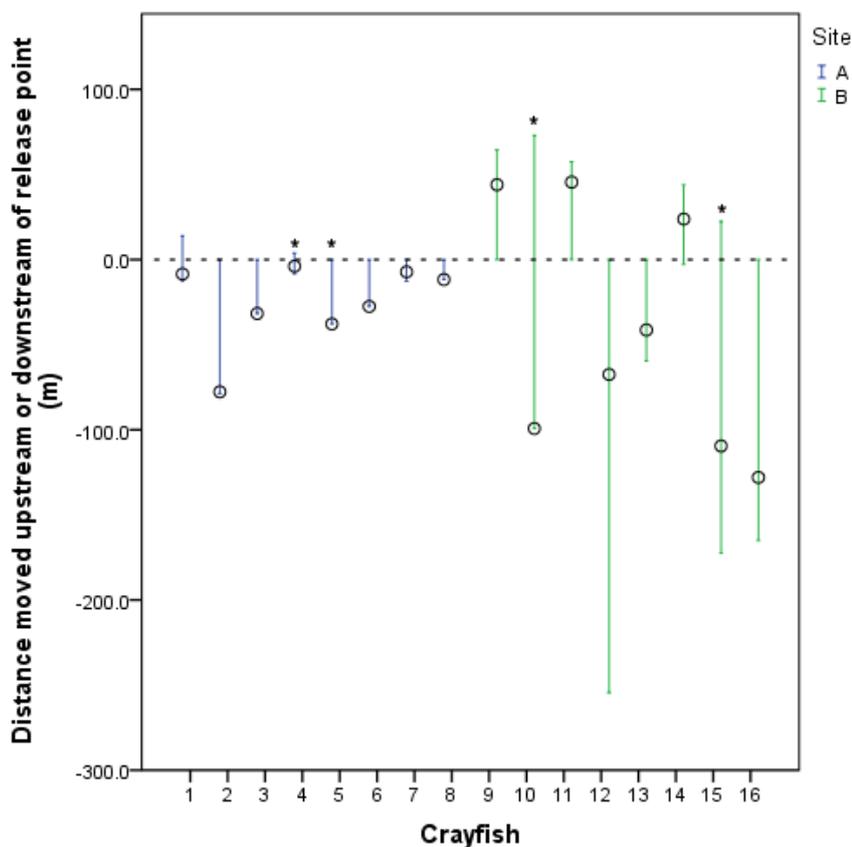
Figure 3-6 - Linear range of movements over the course of the whole study period by crayfish from Site A (crayfish 1-8) and Site B (crayfish 9-16); the dotted line at the origin represents the release point; circles represent the final position. All crayfish were tracked for 28 days apart from those marked with asterisks, which were tracked over shorter periods (see Table 3.1).

Table 3-1 - Cumulative distance moved by crayfish at Site A and Site B.

Site	Crayfish	Sex	Tracking period (days)	Cumulative distance (metres)
A	1	F	28	56.5
	2	F	28	104.1
	3	M	28	96.9
	4	M	16	38.7
	5	M	8	42.3
	6	M	28	55.7
	7	F	28	25.2
	8	F	28	21.7
B	9	F	28	81
	10	M	8	245.3
	11	M	28	69.5
	12	F	28	645.5
	13	F	28	149.7
	14	M	28	82.2
	15	M	10	292.5
	16	F	28	204

3.4.5 Number and distance of movements

The effects of site, sex, size, flow and temperature on crayfish movements were as follows:

3.4.5.1 Site

Number of movements

There was no significant difference between the total number of movements (i.e. changes in position between sampling days) by crayfish that were tracked for the full study period at Site A and Site B (Mann Whitney U = 15, p > 0.6, n = 12). The frequencies of upstream and downstream movements by crayfish did not differ significantly between sites (Figure 3-7, χ^2_{Yates} = 0.303, p > 0.5, df = 1). Within Site A, individual crayfish showed a statistically significant bias towards downstream movements (Paired t-test, t = -2.736, p < 0.05, df = 7). At Site B, there was no significant difference between the number of upstream and downstream movements by crayfish (Paired t-test, t = -1.667, p = 0.139, df = 7).
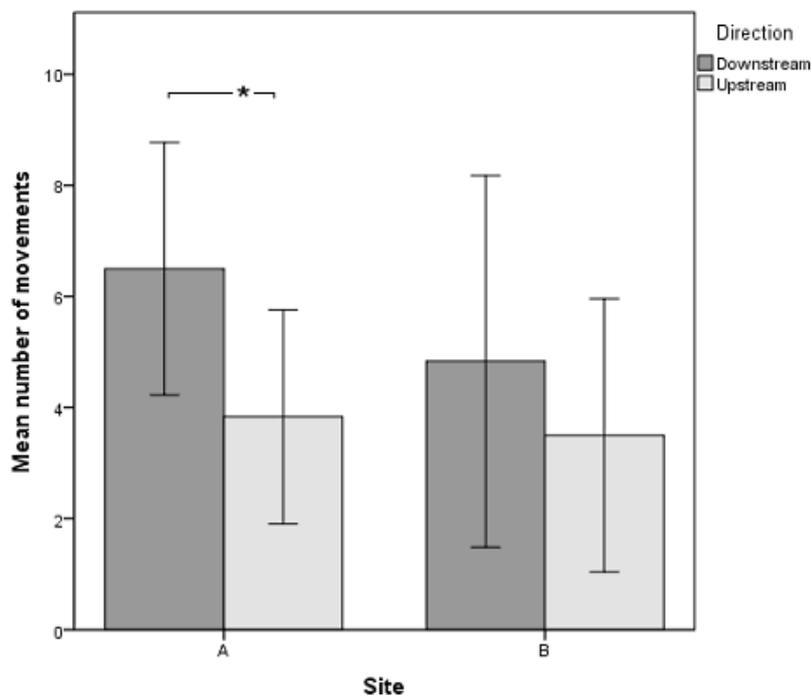
Figure 3-7 - Mean (± 95% CI) number of movements upstream and downstream by crayfish at Site A and Site B; asterisks denote a significant difference between groups.

Crayfish crossed the channel to occupy refuges on the opposite bank more frequently at Site A than at Site B (Figure 3-8, Wilcoxon signed-rank test, Z = -3.688, p < 0.001, n = 27). This was unsurprising, given the differences in stream dimension between the sites. Over the course of the study period, seven of the eight crayfish at Site A crossed the width of the channel at least once. At Site B, four of the eight of the crayfish crossed the bank at least once.
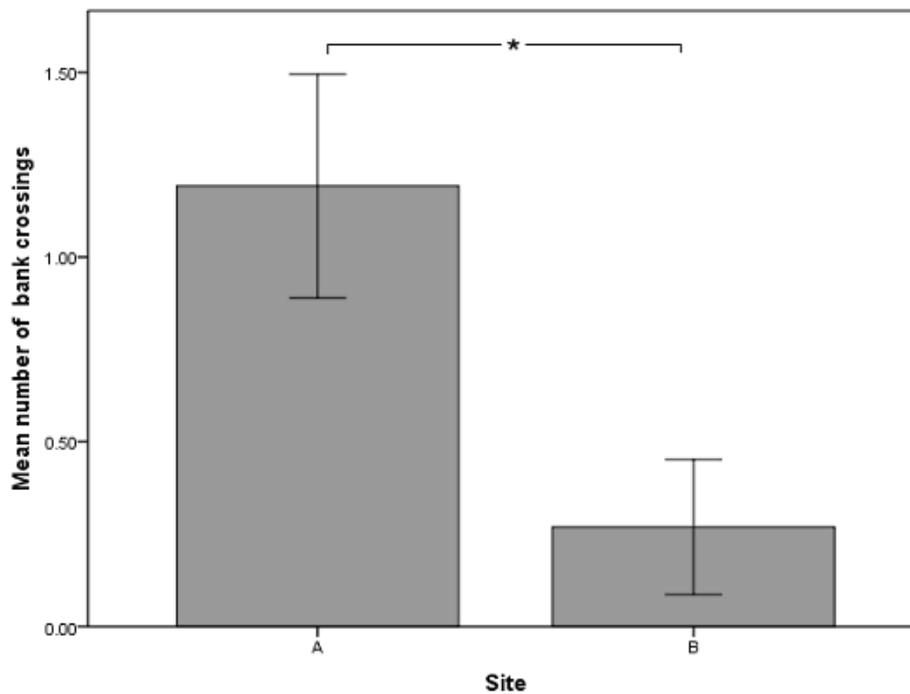
Figure 3-8 - Mean (± 95% CI) number of bank crossings per night by crayfish at Site A compared with crayfish at Site B; asterisks denote a significant difference between groups.

Distance of movements

At Site A, the maximum distances moved upstream and downstream in any one night were 18 metres (crayfish 1) and 26 metres (crayfish 2) respectively. At Site B, the maximum distances moved upstream and downstream in any one night were 63 metres and 195 metres (crayfish 15) respectively.

The distances of daily movements (excluding values of zero) were significantly greater at Site B than at Site A (Mann Whitney U = 844.5, p < 0.001, n = 131). This was true for both upstream and downstream movements (Figure 3-9, t-test: $t_{upstream}$ = -7.566, p < 0.001, df = 47; $t_{downstream}$ = -3.721, p < 0.001, df = 58).
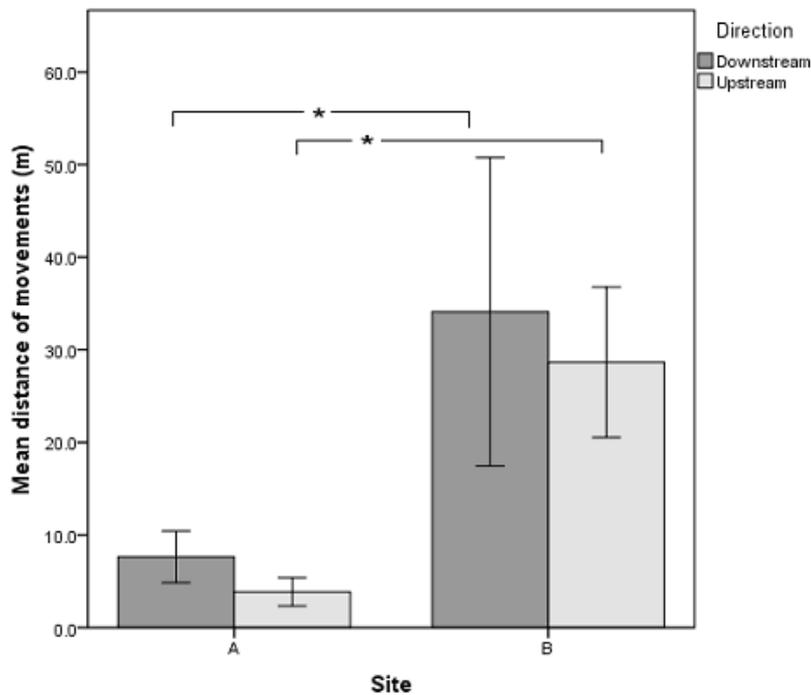
Figure 3-9 - Mean (± 95% CI) distance of daily downstream and upstream movements by crayfish at Site A and Site B; asterisks denote significant differences between groups.

Within sites, the mean distance of daily downstream movements was greater than the mean distance of upstream movements. However, this difference was not statistically significant (t-test, Site A: t = -1.928, p > 0.05, df = 64; Site B: t = 1.294, p > 0.2, df = 57). At both sites, the majority of large movements (≥ 10 metres) were in a downstream direction: 87.5% at Site A and 62.5% at Site B.

Linear range

The RPD of crayfish was significantly greater at Site B than at Site A (Figure 3-10, Mann Whitney U = 8 p < 0.05, n = 16).
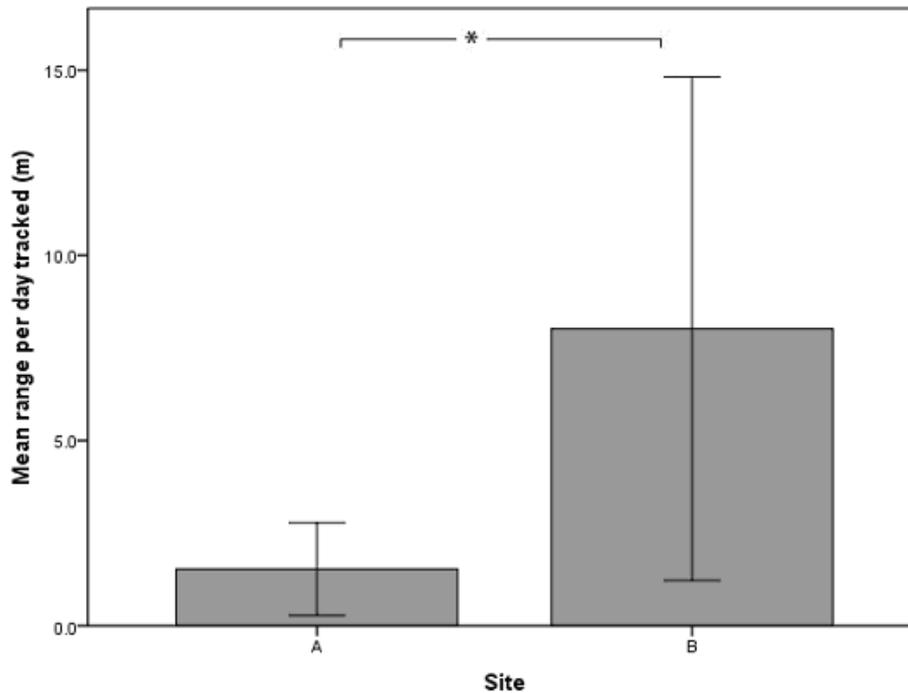
Figure 3-10 - Mean (± 95% CI) range per day of crayfish at Site A and B; the asterisk denotes a significant difference between sites.

Due to the significant differences between the distances of crayfish movements at Site A and Site B, the effects of sex, size and river flow on distances moved were analysed separately at the two sites.

Cumulative distance

The cumulative distance moved by individual crayfish ranged from 21.7 to 104.1 metres at Site A and 69.5 to 645.5 metres at Site B.

For crayfish that were tracked for the full tracking period, the cumulative distance did not differ significantly between Site A and Site B (Mann Whitney U = 6, p > 0.05, n = 12) and so crayfish from the two sites were pooled before testing for the effects of sex and size. There was no significant relationship between

sex (Mann Whitney U = 13, p > 0.6, n = 12) or size (Pearson's product-moment correlation, r = -0.061, p > 0.8, n = 12) and cumulative distance.

3.4.5.2 Sex

Due to the small sample size of the two sexes, it should be noted that the statistical power of the tests described below was low:

Number of movements

There was no significant relationship between sex and the total number of movements by crayfish that were tracked for the full study period at Site A (Mann-Whitney U = 4.5, p > 0.8, n =6), Site B (Mann-Whitney U = 1. p > 0.1, n = 6) or for both sites combined (Mann-Whitney U = 11, p > 0.3, n =12).

When the direction of movements was considered, there was no significant relationship between sex and the frequency of upstream and downstream movements at Site A (χ^2_{Yates} = 0.093, p > 0.7, df = 1) or at Site B (χ^2_{Yates} = 0, p > 0.9, df = 1) or when crayfish from both sites were combined (χ^2_{Yates} = 0.131, p > 0.7, df = 1).

Distance of movements

There was no significant relationship between sex and the mean daily distance moved by crayfish at Site A (t-test, t = -0.587, p > 0.5, df = 69) or at Site B (t-test, t = -0.472, p > 0.6, df = 30). When the direction of movement was considered, there was no significant relationship between sex and the distance of daily upstream or downstream movements by crayfish at Site A (t $_{upstream}$ = 1.390, p > 0.4, df = 19; t $_{downstream}$ = -1.335, p > 0.05, df = 43) or at Site B (Mann Whitney U $_{upstream}$ = 82, p > 0.4, n = 25; t $_{downstream}$ = -0.715, p > 0.5, df = 11).

Linear range

There was no significant difference between the RPD of males and females at either Site A (Mann Whitney U = 6, P > 0.6, n = 8) or at Site B (Mann Whitney U = 8, p = 1, n =8).

3.4.5.3 Size

Number of movements

There was no correlation between crayfish size and the total number of movements by crayfish that were tracked for the full study period at Site A (Spearman's rank correlation coefficient, $r_s = 0.116$, $p > 0.8$, $n = 6$), Site B ($r_s = -0.086$, $p > 0.8$, $n = 6$) or for both sites pooled ($r_s = -0.067$, $p > 0.8$, $n = 12$).

When the direction of movement was considered, the total number of upstream or downstream movements was not correlated with crayfish size at Site A (Spearman's rank correlation coefficient, $r_{s\ upstream} = -0.319$, $p > 0.5$, $n = 6$; $r_{s\ downstream} = 0.435$, $p > 0.3$, $n = 6$), Site B ($r_{s\ upstream} = -0.03$, $p > 0.9$, $n = 6$; $r_{s\ downstream} = -0.029$, $p > 0.9$, $n = 6$) or for both sites combined (Pearson's product-moment correlation, $r_{upstream} = -0.178$, $p > 0.5$, $n = 12$; $r_{downstream} = 0.023$, $p > 0.9$, $n = 12$).

Distance of movements

It should be noted that the following analyses suffered from pseudoreplication, since multiple distance measurements were recorded for each individual crayfish. There was no significant correlation between crayfish size and the distance of daily movements at either Site A (Pearson's product-moment correlation, $r = 0.125$, $p > 0.1$, $n = 131$) or at Site B (Spearman's rank correlation coefficient, $r_s = -0.006$, $p > 0.9$, $n = 59$).

When the direction of movements was considered, there was no significant correlation between crayfish size and the distance of daily movements upstream or downstream at either site (Site A, Pearson's product-moment correlation, $r_{upstream} = 0.264$, $p > 0.05$, $n = 50$; $r_{downstream} = 0.038$, $p > 0.7$, $n = 80$; Site B, Spearman's rank correlation coefficient, $r_{s\ upstream} = -0.019$, $p > 0.9$, $n = 25$; Pearson's product-moment correlation, $r_{downstream} = -0.162$, $p > 0.3$, $n = 34$)

Linear range

There was no significant correlation between RPD and the size of crayfish at either Site A (Spearman's rank correlation coefficient, r_s = -0.286, p > 0.4, n = 8) or at Site B (r_s = -0.381, p > 0.3, n = 8).

3.4.5.4 Flow

Mean nightly flow rate (Q), as measured at Abington Gauging Station, ranged from 4 to 73.46 m^3/s over the study period.

Number of movements

There was a significant negative correlation between the mean night time river flow (m^3/s) and the proportion of tracked crayfish that were active (i.e. changed positions) at Site B (Figure 3-11, Spearman's rank correlation coefficient, r_s = -0.457, p < 0.05, n = 25) but not at Site A (Spearman's rank correlation coefficient, r_s = 0.062, p > 0.7, n = 25). When crayfish from both sites were pooled, there was no significant correlation between flow and the proportion of active crayfish (Spearman's rank correlation coefficient, r_s = --0.172, p > 0.2, n = 50). Only consecutive nights of tracking were considered to ensure that the number of crayfish movements was not underestimated.
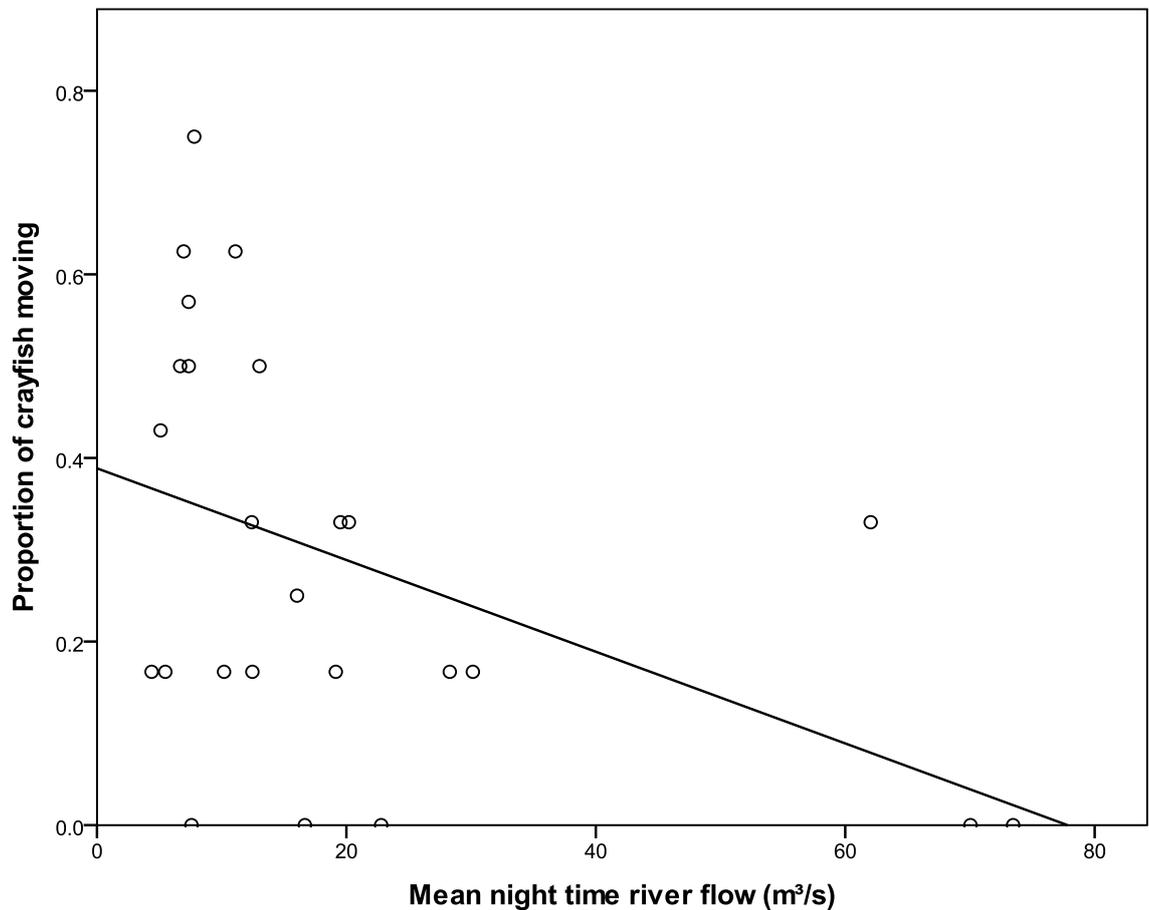
Figure 3-11 – At Site B there was a significant negative relationship (r_s = -0.457) between mean night time river flow and the proportion of crayfish that moved during that night

When the direction of movements was considered, there was no significant relationship between mean night time flow and the number of upstream or downstream movements at either Site A (Spearman's rank correlation coefficient, $r_{s\ upstream}$ = -0.012, p > 0.9, n = 25; $r_{s\ downstream}$ = -0.165, p > 0.4, n = 25), Site B ($r_{s\ upstream}$ =-0.297, p > 0.1, n = 25; $r_{s\ downstream}$ = -0.344, p > 0.05, n = 25) or for both sites combined ($r_{s\ upstream}$ = -0.146, p > 0.4, n = 25; $r_{s\ downstream}$ = -0.331, p > 0.1, n = 25).

Distance of movements

There was a significant negative correlation between mean night time river flow and the distances moved overnight by crayfish at Site B (Figure 3-12, Spearman's rank correlation coefficient, r_s = -0.338, p < 0.05, n = 164) but not at Site A (Spearman's rank correlation coefficient, r_s = -0.101, p > 0.3, n = 79).
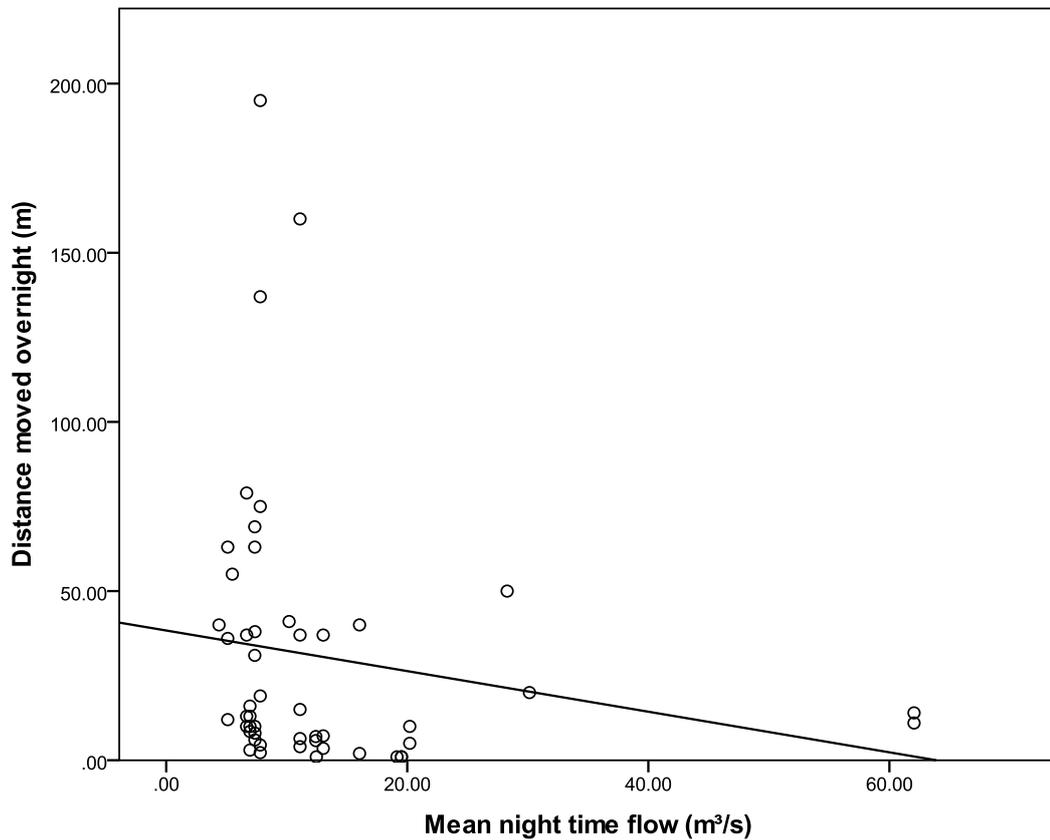
Figure 3-12 - There was a significant negative relationship (r_s = -0.338) between mean night time flow and the distance moved by crayfish at Site B.

When the direction of movements was considered, there was a significant negative correlation between flow and the daily distances moved upstream (Figure 3-13, Spearman's rank correlation coefficient, r_s = -0.575, p < 0.001, n = 26) but not downstream (r_s = 0.026, p > 0.8, n = 37) at Site A. At Site B, there was no significant negative correlation between flow and daily distances moved upstream (Spearman's rank correlation coefficient, r_s = -0.295, p > 0.1, n = 28) or downstream (Spearman's rank correlation coefficient, r_s = -0.328, p > 0.1, n = 18).
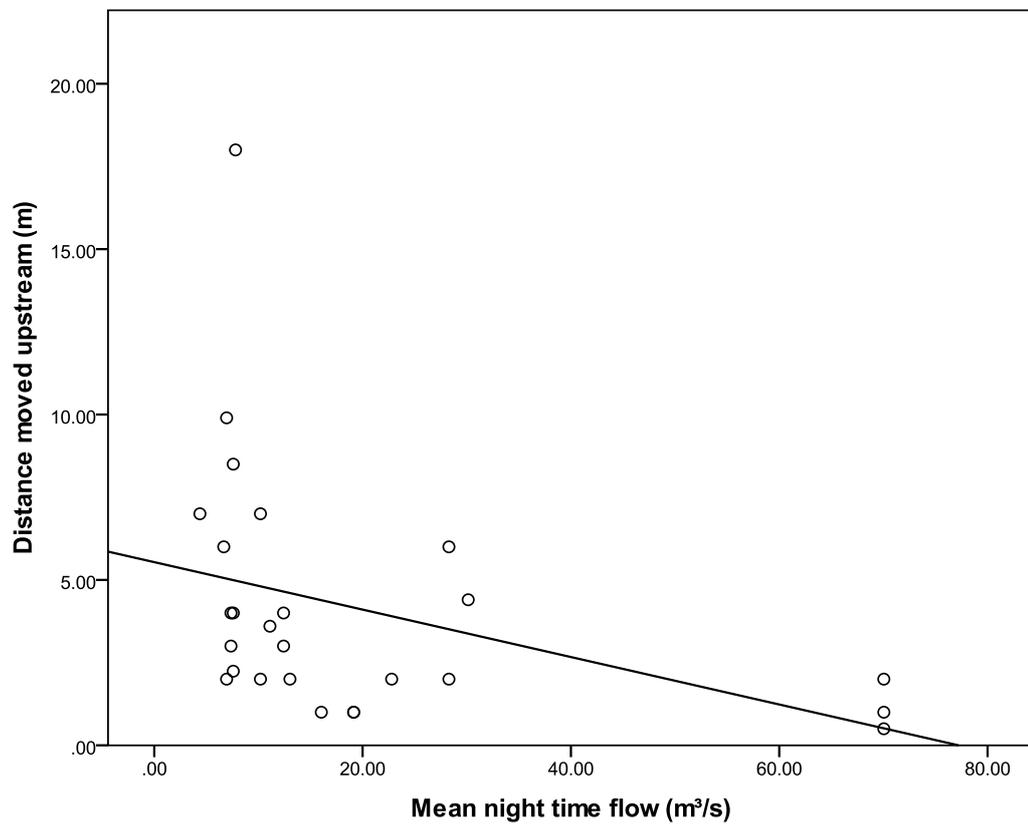
Figure 3-13 - There was a significant negative correlation (r_s = -0.575) between mean night time flow and the distance that was moved upstream by crayfish overnight at Site A.

Discussion

3.4.6 Movement patterns

The movement patterns of *P. leniusculus* observed here were similar to those observed in previous studies of this species (Bubb *et al.*, 2004) and of other crayfish species (Buřič *et al.*, 2009b, Gherardi and Barbaresi, 2000, Gherardi *et al.*, 2000). Individuals often remained in the same place for days or weeks before moving overnight to a new position, where they would reside for another extended period before migrating again. *P. leniusculus* occasionally returned to previously occupied refuges; this contrasts with the results of previous studies that found no evidence of burrow re-occupation by *P. leniusculus* (Bubb *et al.*, 2004, Bubb *et al.*, 2008).   Such studies were conducted in much larger watercourses than the present study, however. The number of refuges may be more limited in small watercourses compared with large ones, leading to a greater chance of reoccupation; it is also possible that smaller sites are more easily navigated, facilitating homing behaviour.   Other incidences of homing behaviour by crayfish have been recorded in small watercourses: the spiny cheek crayfish (*Orconectes limosus* Rafinesque) displayed homing behaviour in a small reservoir tributary (Buřič *et al.*, 2009b).

P. leniusculus moved up to 26 m day^{-1} at Site A and 195 m day^{-1} at Site B. Previous studies have also shown *P. leniusculus* to be capable of significant movements: in a mark and recapture study of *P. leniusculus* in Californian streams, individuals moved a maximum of 120 m day^{-1} (Light, 2003); in the River Wharfe in England, *P. leniusculus* moved up to 341 m in two days (Bubb *et al.*, 2006a).   Such variation indicates that *P. leniusculus* is flexible in its spatial behaviour between habitats, as has been observed in other invasive crayfish species (Barbaresi *et al.*, 2004). The red swamp crayfish *P. clarkii*, for example, was found to move 0.3-76.5 m day^{-1} in an Italian irrigation ditch (Barbaresi *et al.*, 2004) but up to 4 km day^{-1} in Spanish rice fields (Gherardi *et al.*, 2000). During the present study, the frequency and cumulative distance of movements by *P. leniusculus* did not differ significantly between Site A and Site B, indicating that *P. leniusculus* were equally active at the two sites. There were, however, significant differences between the daily distances and direction of *P. leniusculus* movements at the two sites. *P. leniusculus* moved significantly

greater distances at Site B than at Site A, which translated to longer linear ranges; there was a greater frequency of bank crossings at Site A than at Site B; and there was a greater frequency of downstream than upstream movements at Site A but not at Site B.

Such differences may be largely attributable to the physical characteristics of the two sites. Previous studies have highlighted the importance of habitat features including gradient, water velocity and in-stream barriers in influencing crayfish movements. The lack of waterfalls and lower gradient at Site B may have enabled *P. leniusculus* to move greater linear distances at this site. The greater channel width at Site B may also explain the reduced frequency of bank crossings compared with Site A. In order to cross the channel and occupy refuges on the opposite bank, *P. leniusculus* would be required to move a much greater distance at Site B than at Site A; such a move may incur a higher energetic cost or elevate predation risk due to the greater amount of time spent exposed in the middle of the stream.

At Site B, the frequency of upstream and downstream movements did not differ significantly. Other studies of *P. leniusculus* have either found no difference in the frequency of upstream or downstream movements (Bubb *et al.*, 2004, Wutz and Geist, In Press), as recorded here, or a tendency for crayfish to move upstream (Bubb *et al.*, 2006b). Even if downstream movements by *P. leniusculus* are no more frequent than upstream ones, the distance of downstream movements is often greater and biases colonisation towards the downstream, especially in upland rivers (Bubb *et al.*, 2004). At both sites in the present study, most of the largest movements (≥10 metres) by *P. leniusculus* occurred in a downstream direction. At Site A, a bias towards downstream colonisation was also derived from the greater frequency of downstream movements; this observation was unsurprising given the steeper gradient and presence of waterfalls at this site, which have been found to limit the upstream movement of crayfish in other watercourses (Bubb *et al.*, 2004, Bubb *et al.*, 2006b, Bubb *et al.*, 2008, Kerby *et al.*, 2005, Light, 2003). Light (2003) found *P. leniusculus* to be absent from stream sites with gradients > 3% but present in reservoirs and low-gradient streams. Barriers such as culverts with a drop-off of > 0.25 m and waterfalls have been found to prevent upstream colonisation by *P. leniusculus* (Bubb *et al.*, 2006b, Light, 2003). Similarly, Kerby *et al.* (2005)

observed that high water velocity and the presence of height barriers reduced or eliminated upstream migration by the invasive *P. clarkii* in mountain streams. During the present study, no *P. leniusculus* moved upstream of the steepest waterfall, confirming the potential for in-stream barriers to limit or delay upstream invasion. The presence of overhanging vegetation may, however, limit the effectiveness of some barriers by providing a climbable surface for crayfish to overcome the fall, as observed in the present study.

Although habitat parameters were of clear importance, differences between the movement patterns of *P. leniusculus* from Site A and Site B may also have resulted from density-dependent responses to resource availability. At high densities, increased competition for resources might stimulate greater dispersal of animals into lower density areas. An investigation of the effect of density on crayfish movements was not made during the present study, although *P. leniusculus* is believed to occur at a higher density at Site A compared with Site B. In any case, the study sites are probably too physically dissimilar to permit a meaningful investigation of density effects. Field studies by Bubb *et al.* (2004) found no difference in the spatial strategies of *P. leniusculus* from a high-density and low-density population in two physically similar upland rivers. Nonetheless, laboratory studies have found evidence for density-dependent dispersal by crayfish. Bovbjerg (1959) hypothesised that animals displaying intra-specific aggression would disperse in a density-related fashion. This was supported by observations of the crayfish *C. alleni,* which showed faster rates of dispersal from a release point when the initial density of crayfish was higher. Laboratory studies of *P. leniusculus* found that increases in density led to increases in aggressive interactions between conspecifics as well as increases in foraging activity and dispersal rate (Pintor *et al.*, 2009). Pintor *et al.* (2009) proposed the existence of an aggression syndrome for *P. leniusculus* whereby some individuals are more aggressive or active than others across different contexts. Such an aggression syndrome might help to explain the high degree of variation in the dispersal rate of individuals, as observed in the present study and in other studies of *P. leniusculus* (Bubb *et al.*, 2004). More aggressive individuals may be the drivers of invasion.

In addition to site-related influences, the impact of flow on the spatial behaviour of *P. leniusculus* were investigated. High flow was believed to be the

cause of mortality of white-clawed crayfish that were found downstream after a spate in Dalton Beck, an English stream (Robinson *et al.*, 2000). Spates have also been negatively associated with *P. leniusculus* abundance in Californian streams (Light, 2003). During the present study, *P. leniusculus* at Site B were occasionally discovered on top of the bank in waterlogged grass after spates. Although the radio signal of one stranded individual was lost after 48 hours, stranding did not appear to cause mortality of *P. leniusculus*, which can survive out of water for up to three months in a humid atmosphere (Hiley, 2003); nor did stranding instigate overland dispersal, with individuals maintaining the same position on land before returning to the water. High flow has been found to reduce the number and distance of movements by *P. leniusculus*, suggesting that crayfish remain in refuges as protection from passive dispersal downstream (Bubb *et al.*, 2004). Rises in water level led to a reduction in catches of the virile crayfish *Orconectes virilis* [Hagen], again indicating that crayfish are less active during high discharges (Hazlett *et al.*, 1979). In the present study, high flow rates at the gauging station were associated with a reduction in the proportion of crayfish moving and the and distance of movements by *P. leniusculus* at Site B. On the two nights in which the highest mean discharges occurred (70 m^3/s on 20 August and 73.5 m^3/s on 24 August), movements of *P. leniusculus* at Site B halted completely. This suggests that during high flows, *P. leniusculus* remain in refuges, as suggested by Bubb *et al.* (2004). At Site A, there was no significant impact of flow on the proportion of *P. leniusculus* moving but the distance of upstream movements was reduced. The relationship between the discharges at each of the sites relative to the discharge at the gauging station is not known. Factors to explain the observed disparity between sites are open to speculation and may include differences in habitat structure, as before: compared with Site A, Site B is a larger, more exposed channel which is fed by several tributaries; as a result, discharge is expected to be greater, which may lead to greater reductions in *P. leniusculus* activity at this site. During periods of high flow at the gauging station, *P. leniusculus* at Site A remained active but the distance of upstream movements was reduced, reflecting the difficulty of moving against the stronger current.

Numerous studies of *P. leniusculus* have failed to find any effect of sex on their movements (Bubb *et al.*, 2002, Bubb *et al.*, 2004, Bubb *et al.*, 2006b, Bubb *et*

al., 2006a, Guan and Wiles, 1997b) but this may be due to the timing and scope of each study. A few authors have noted some differences: a recent mark-and-recapture study observed male *P. leniusculus* to be more mobile than females; males were also more likely to move downstream, whereas females tended to move upstream (Wutz and Geist, In Press). Another study found *P. leniusculus* females to show a seasonal pattern of migration that was not observed in males (Light, 2003). Flint (1977) also observed sex-related differences in movements by *P. leniusculus* in Lake Tahoe, with females migrating into shallow water later in the summer than males. This lag in activity was attributed to the females still being ovigerous, which is widely acknowledged as reducing crayfish movements (Bubb *et al.*, 2002, Hazlett *et al.*, 1979, Merkle, 1969). Differences in the activity patterns of crayfish have also been related to the mating season (Buřič *et al.*, 2009a). In the present study, tracking was conducted outside of the mating season and none of the females were ovigerous, which might explain why no sex-related differences in movements were detected.

Body size had no apparent influence on *P. leniusculus* movements, which concurs with the results of many other studies (Bubb *et al.*, 2002, Bubb *et al.*, 2004, Bubb *et al.*, 2006a, Bubb *et al.*, 2006b, Guan and Wiles, 1997b). Only large adults were tracked, however, and the size range (carapace length: 32 - 44.9 mm) was probably too narrow to allow adequate detection of size effects. Bubb *et al.* (2004) suggested that at high densities, small, sub-dominant crayfish might be more likely to disperse than large crayfish due to the potentially stronger effects of competition. This suggestion is challenged, however, by the results of a recent study which investigated the immigration of *P. leniusculus* from high density stream sections into sections that had a relatively lower density from trapping; nearly all of the *P. leniusculus* that immigrated were large, with a carapace length > 50 mm (Moorhouse and Macdonald, 2011). In another study, Light (2003) found that large *P. leniusculus* moved greater distances than small ones. Similarly, Wutz and Geist (In Press) found large male *P. leniusculus* to be the most mobile individuals and suggested that this was because of a reduced need to seek refuge or a greater level of foraging activity. Large crayfish may also be more aggressive and perhaps thwart the dispersal of less aggressive animals, as suggested by Mobberley and Owens (1966). Counter to these observations is a study by Bubb *et al.* (2006a) which used PIT tagging to

investigate the movements of a wide range of size classes of *P. leniusculus* and found no pattern in relation to size, suggesting that crayfish of all sizes contribute to dispersal. Effective methods for tracking the movements of juvenile crayfish are currently lacking. In any case, size may be less important than abiotic factors in explaining the movements of *P. leniusculus*.

3.4.7 Dispersal

Between their arrival in the main channel of the River Clyde in c. 1991 and 2001, *P. leniusculus* spread to inhabit about 5 km of river (Reeve, 2004), translating to a dispersal rate of 0.5 km year^{-1}. By 2009, *P. leniusculus* had invaded a further 12 km of river, suggesting an increase in dispersal rate to 1.5 km year^{-1}(Sinclair, 2009). Variable rates of expansion by *P. leniusculus* have been reported in other rivers: 1.27-2.4 km year^{-1} in the River Wharfe (Bubb *et al.*, 2005), 0.12-0.55 km year^{-1} in the River Ure (Bubb *et al.*, 2005), 1 km year^{-1} in Gaddesby Brook (Sibley, 2000) and 2.88 km year^{-1} in the upper River Stour (Wright and Williams, 2000).

During the present study which was conducted during the two warmest months of the year, *P. leniusculus* moved up to 2.58 m day^{-1} (0.39 m day^{-1} upstream and 2.19 m day^{-1} downstream) at Site A and 9.36 m day^{-1} (2.09 m day^{-1} upstream and 7.27 m day^{-1}downstream) at Site B. Midsummer movements of *P. leniusculus* in the River Wharfe was found to be larger at 13 m day^{-1} downstream (Bubb *et al.*, 2004), perhaps due to warmer mean temperatures at a lower latitude. Bubb (2004) suggested that maintenance of such a rate over just four summer months would be enough to explain the observed rate of annual dispersal (i.e. range expansion) of 1.5 km year^{-1}. By this logic, the daily dispersal rates observed in the present study would translate to annual rates of 0.31 km year^{-1} (0.05 km year^{-1} upstream and 0.26 km year^{-1} downstream) at Site A and 1.12 km year^{-1} (0.25 km year^{-1} upstream and 0.87 km year^{-1} downstream) at Site B. These rates are slower than the suggested rate of 1.5 km year^{-1} on the main stem of the River Clyde. It is possible that the dispersal potential of *P. leniusculus* increases with stream order due to reductions in gradient and associated height or velocity barriers.

There is a growing urgency to identify effective methods for preventing the observed range expansion and subsequent impacts of non-native *P. leniusculus* in lotic ecosystems. Two barriers were recently constructed close to where the headwaters of the River Clyde (which is infested with *P. leniusculus*) and the River Annan (which is free of *P. leniusculus*) meet in an attempt to block the spread of *P. leniusculus* between catchments. This study suggests that the upstream dispersal of *P. leniusculus* may be reduced or halted within catchments by the presence of waterfalls. In sites where introduced *P. leniusculus* have yet to penetrate far upstream, construction of artificial barriers may be worthwhile. This may be particularly effective and easy to implement in small watercourses like streams, where the rate of dispersal by *P. leniusculus* appears to be lower, as observed here. In-stream barriers were recently constructed at the upper Fall River and Spring Creek in California to protect the endemic Shasta crayfish *Pacifastacus fortis* [Faxon] from invading *P. leniusculus* (US Fish and Wildlife Service, 2009). Extensive surveys have also been carried out to identify additional sites where installation of barriers would be beneficial (US Fish and Wildlife Service, 1998). Similar strategies may be useful for tackling invasive crayfish in Europe but the potential impacts of barrier construction on other mobile taxa such as anadromous fish will require careful consideration.

Chapter 4: The paradox of a non-native but high conservation value species

4.1 Abstract

Non-native species are often held in negative regard by ecologists due to the detrimental impacts that biotic invasions can have on native biodiversity. It is difficult to envisage a situation in which such species might be considered valuable or, more extremely, deliberately introduced to new ecosystems. In recent years, however, the notion that all non-native species are inherently "bad" has been subject to debate. The white-clawed crayfish (*A. pallipes*) has suffered serious population declines throughout its native European range. A new approach to conserving *A. pallipes* in England and Wales is to identify and establish "ark sites", isolated bodies of water that can support healthy populations in the long term. Scotland has two populations of *A. pallipes*, at Loch Croispol and Whitemoss Reservoir, where it is paradoxically a non-native but protected species. Surveys were undertaken to assess the status of these crayfish populations and to determine the suitability of Loch Croispol and Whitemoss Reservoir as ark sites. The results revealed a high-density population at Whitemoss Reservoir and a low-density population at Loch Croispol, where crayfish were significantly smaller. Factors such as latitude, habitat availability and predation pressure may help explain the observed differences in crayfish density and size between sites. Loch Croispol and Whitemoss Reservoir could serve as long-term "*ex-situ*" ark sites for *A. pallipes* but only if measures are taken to mitigate the current biosecurity threats of non-native crayfish introductions and disease. The value now ascribed to *A. pallipes* populations in Scotland demonstrates how a non-native species may actually constitute an important conservation resource. Although controversial, the introduction of endangered species to non-endemic regions may emerge as a viable conservation strategy in the future.

4.2 Introduction

Many non-native species are held in negative regard by ecologists due to the detrimental impacts that biotic invasions can have on native biodiversity. Although species introductions tend to be made with the best intentions, they can have unforeseen and often disastrous consequences. The introduction of the notoriously invasive American cane toad (*Bufo marinus* L.) to Australia, for example, was an unsuccessful attempt to control sugar cane pests (Shine, 2010). Lethal toxicity from toad predation has led to the endangerment of a range of native species, from crocodiles to marsupials (Shine, 2010). In addition to biological control, species have been introduced to new ecosystems as sources of food, sport and for ornamental purposes.

Introduced species that establish new populations may adversely affect native species through predation, competition, introduction of diseases and parasites, habitat alteration and hybridisation and they may play a major part in driving species extinctions (Clavero and García-Berthou, 2005, Vitousek *et al.*, 1997). Introduced species contributed to the extinction of 68% of 40 North American fish taxa (Miller *et al.*, 1989) and 52% of the world's Critically Endangered bird species are threatened by invasive species (BirdLife International, 2008). Changes in community diversity or in extreme cases, extinctions, can have knock-on effects on ecosystem functioning by disrupting primary productivity, nutrient cycling, decomposition and other processes (Vitousek *et al.*, 1997). Such ecological damage inevitably incurs economic costs when efforts are made to remove aliens and restore the natural environment. Biotic invasions in the United States, United Kingdom, Australia, South Africa, India and Brazil are estimated to cost over US$314 billion annually (Pimentel *et al.*, 2001). The problem of invasive species has necessitated the formation of dedicated management groups and steering committees: the GB Non Native Species Secretariat, for example, was established in 2005 to ensure a co-ordinated approach to tackling invasive non-native species in Great Britain (DEFRA, 2008). The Global Invasive Species Programme aims to address the growing threat on the largest scale (McGeoch *et al.*, 2010).

Given the immense efforts made by conservationists and environmental managers to mitigate the impacts of alien invasions, it is difficult to envisage a

situation in which such species might be considered valuable or, more extremely, deliberately introduced to new ecosystems. In recent years, however, the notion that all non-native species are inherently "bad" has been challenged (Sagoff, 2005), sparking fierce debate amongst ecologists (Simberloff, 2005). Not all non-native species become "invasive" and have unwanted ecological impacts. An audit of non-native species in Scotland recorded at least 988 alien species but only about a tenth of these could be considered naturalised (SNH, 2001). According to Manchester and Bullock (2000), most non-native species that are established in the UK do not significantly affect native fauna and flora and may actually confer major benefits to society, in agriculture, horticulture and forestry (Genovesi and Shine, 2003). There is also increasing uncertainty about what constitutes a native or non-native species. Until recently, populations of the pool frog *Rana lessonae* [Camerano, 1882] in the UK were thought to have originated from introductions made from central and southern Europe during the 1800s. Recent research using archival, genetic, bioacoustic and archaeozoological techniques has however, provided compelling evidence for the species' native status (Beebee *et al.*, 2005). Some "non-native", endangered plant species are excluded from the British Red Data Book of threatened or near-extinct species despite being present in Britain for at least 500 years (Preston *et al.*, 2004). There is no defined length of time for which a species must be resident in order to qualify as native (Willis and Birks, 2006) and this has led to a lack of continuity in the classification of native and non-native species across Europe (Colin Bean 2012, pers. comm.). Classifying a species as native or non-native is of importance since it may have implications for its management i.e. whether it could be subject to conservation action or an eradication programme (Beebee *et al.*, 2005).

Recent investigations by Holdich *et al.* (2009) concluded that the white-clawed crayfish (*A. pallipes*) became established in the wild in England and Wales prior to 1500 and so should be regarded as native to these areas (a view now endorsed by the IUCN) (Holdich *et al.*, 2009). In Scotland, however, *A. pallipes* is naturally absent, possibly due to the presence of the Southern Uplands fault (Jay and Holdich, 1981). Streams and rivers south of the fault flow over hard-weathering, acidic substrata and these may be unfavourable for colonisation by white-clawed crayfish (Jay and Holdich, 1981). An unverified report of white-clawed crayfish

was made in the 1960s in the River Whiteadder, a tributary of the Tweed in southern Scotland (Jay and Holdich, 1981). Some individuals were also introduced to a fish farm in West Lothian in the mid-1970s but the population was wiped out by a pollution event in 1978 (Jay and Holdich, 1981). White-clawed crayfish are currently thought to be present in only two localities in Scotland, at Loch Croispol, Durness (Thomas, 1992) and Whitemoss Reservoir in Renfrewshire (Maitland *et al.*, 2001). The Loch Croispol population may have originated from the introduction of crayfish to a feeder stream in 1945 (Thomas, 1992). According to anecdotal information, Whitemoss Reservoir has also been inhabited by crayfish for several decades (Maitland *et al.*, 2001).

The white-clawed crayfish has suffered serious population declines and local extinctions throughout most of its native range as a result of the introduction of the non-native North American signal crayfish *P. leniusculus*, epidemics of crayfish plague (*A. astaci*) which is frequently carried by signal crayfish and pollution (Holdich and Lowery, 1988). Data from England, France and Italy suggest that 50-80% of populations have been lost in the past decade (Füreder *et al.*, 2010). The species was recently upgraded from "threatened" to "endangered" on the IUCN Red List of Threatened Species (Füreder *et al.*, 2010) and native populations could face extinction in Britain in the next 30 years if no action is taken to halt its decline (Sibley, 2002).

As long as rivers continue to be threatened by the spread of signal crayfish and disease, restocking with white-clawed crayfish is a risky conservation strategy (Holdich *et al.*, 2004). A new approach to conserving the white-clawed crayfish in England and Wales is to identify and establish refuge populations in "ark sites": isolated and self-contained bodies of water that can support healthy populations in the long-term (Peay, 2009). A small number of ark sites have already been established and there are aspirations to create many more (Whitehouse *et al.*, 2009). These new populations have recently been established in private lakes in southern England and an appeal has been made to the public to identify ponds, lakes or gravel pits that could be used to establish ark sites (Environment Agency, 2010). The reintroduction of organisms to habitats where they have been rendered extinct is unlikely to be successful if the pressures that originally threatened the population are still extant (Conant,

1988). The establishment of white-clawed crayfish populations in new isolated sites may offer better prospects for the long-term survival of the species.

Given the plight of white-clawed crayfish in the rest of Europe, it has been suggested that the two populations in Scotland may serve as important "refuge" stocks in ready-made ark sites that are already excellent candidates for conservation management. As a function of the fact that white-clawed crayfish are protected under Schedule 5 of the Wildlife & Countryside Act 1981 and are listed on the annexes of the EC Habitats Directive (Annexes IIa and Va) and the Bern Convention (Appendix III), it is paradoxical that this species, despite being non native to Scotland, is also specifically protected by law.

Although their presence has been known in Scotland for some time, there have been no published data on the status of white-clawed crayfish at either Loch Croispol or Whitemoss Reservoir in over a decade (Maitland *et al.*, 2001). In this study I assess the status of the two Scottish populations of white-clawed crayfish and examine Loch Croispol and Whitemoss Reservoir against the currently accepted criteria for selecting "ark sites", as described by Peay (2009). The findings are discussed in light of the "*ex situ*'" conservation of white-clawed crayfish in Scotland.

4.3 Materials and methods

4.3.1 Study sites

Whitemoss Reservoir (Figure 4-1) is located in the catchment of the lower River Clyde in Renfrewshire, west central Scotland (National Grid Reference: NS 415718). It is a small reservoir which is approximately 0.23 km long, 0.23 km wide and has a surface area of 0.04 km^2. Much of the perimeter is shallow and lined with cobbles; deeper areas are dominated by a fine silt substrate. There is a small overflow into a burn on the east side of the reservoir and no inflow; the reservoir is spring-fed. Whitemoss Reservoir is a popular angling site owned by the nearby community of Inchinnan and regulated by the Inchinnan Angling Club which stocks it regularly with rainbow trout *Oncorhynchus mykiss* [Walbaum].

Figure 4-1 - Whitemoss Reservoir

Loch Croispol (Figure 4-2) is a coastal, freshwater loch located near Durness, Sutherland in the north-west of Scotland (National Grid Reference: NC 390680). It is about 0.7 km long, 0.24 km wide and has a surface area of 0.12 km^2. The loch is generally shallow, with depths under 3 m. The loch lies in a basin of

Cambrian limestone formed from ice erosion and solution (Spence *et al.*, 1984) at an elevation of 14 metres above sea level. The shallow littoral zones are lined with an abundance of limestone rubble (Thomas, 1992) and the dominant macrophyte is *Chara* (Holdich and Reeve, 1991). Submerged plants grow down to 6 metres due to good water clarity and a high euphotic depth (Spence, 1972). An afferent stream carrying the outflow of a field drainage system enters at the southerly end; the outflow leaves the north of the loch and continues for about 350 metres to the sea at Balnakeil Bay. The loch supports populations of brown trout (*S. trutta*), three-spined stickleback (*Gasterosteus aculeatus* L.) and eel (*Anguilla anguilla* L.). The loch formerly hosted a population of Arctic charr (*Salvelinus alpinus* L.).

Figure 4-2 - Loch Croispol

4.3.2 Survey procedure

A variety of sampling techniques were deployed at each site to assess the abundance of white-clawed crayfish. The sex and carapace length of all captured crayfish were recorded. Carapace length was measured from the tip of

the rostrum to the posterior margin using Vernier callipers (± 0.1mm). A small number of crayfish evaded capture during hand searching and so could not be measured.

4.3.2.1 Trapping

In May 2009 at Loch Croispol, 21 Swedish Trappy™ traps were baited with mackerel (*Scomber scomber* L.) and set overnight. Traps were set singly or in lines of three to five. The following morning, traps were retrieved and inspected for crayfish.

In August 2009 at Whitemoss Reservoir, ten white-clawed crayfish traps (Alana Ecology Ltd.) made from green 4 mm plastic mesh were baited with domestic cat food and set singly overnight at ten different locations. The following morning, traps were retrieved and inspected for crayfish.

4.3.2.2 Hand searching

Timed hand searches were conducted along all accessible parts of the shoreline perimeter, during daylight hours. Refuges were actively searched by turning over unembedded cobbles and boulders and catching any disturbed crayfish using hand nets.

4.3.2.3 Night viewing

Timed searches were conducted in darkness, using torches to view and hand nets to capture any active crayfish away from their refuges.

4.3.2.4 Electrofishing

Timed electrofishing was carried out along the shoreline. Electrofishing was conducted using an Electracatch WFC11 backpack unit with 200V smooth DC current. Two operatives worked together, moving the anode along the shoreline and netting any crayfish or fish using a pond net.

For hand searching, night viewing and electrofishing, catch per unit effort (CPUE) was calculated as the total number of crayfish captured divided by the total sampling time (crayfish which evaded capture during hand searching were

not included). For trapping, CPUE was calculated as the total number of crayfish caught per trap per night fished.

4.3.2.5 Ark site assessment

The checklist of criteria (Peay, 2009) was used to assess the eligibility of both Loch Croispol and Whitemoss Reservoir for 'ark site' status.

4.3.3 Statistical analysis

A Student t-test was applied using R statistical software (version 2.10.0) to compare the mean carapace length of crayfish from the two sites.

4.4 Results

4.4.1 Abundance

Figure 4-3 shows the catch per unit effort for each sampling method at Whitemoss Reservoir and Loch Croispol. At Whitemoss Reservoir, the CPUE was high for trapping, electrofishing and night viewing, with values of 0.70, 0.68 and 0.64 respectively; hand searching had a lower CPUE of 0.2. At Loch Croispol, no crayfish were caught in traps or by electrofishing. The CPUE was 0.04 for hand searching and 0.02 for night viewing.
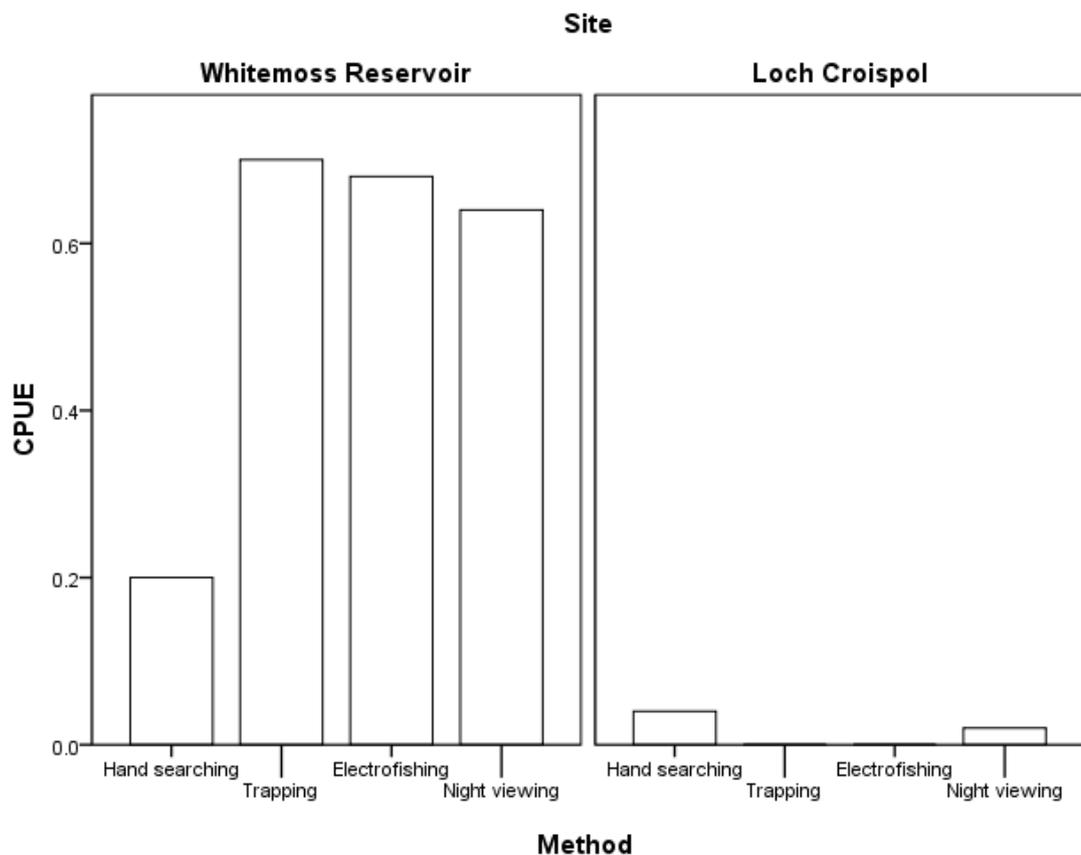
Figure 4-3 - Catch per unit effort (CPUE) for each sampling method at Whitemoss Reservoir and Loch Croispol. For hand searching, electrofishing and night viewing, CPUE was defined as the total number of crayfish captured divided by the sampling time in minutes; for trapping, CPUE was defined as the total number of crayfish captured per trap per night fished.

4.4.2 Size distribution

Figure 4-4 shows the size distribution (carapace length, CL) of crayfish caught at Whitemoss Reservoir and Loch Croispol.
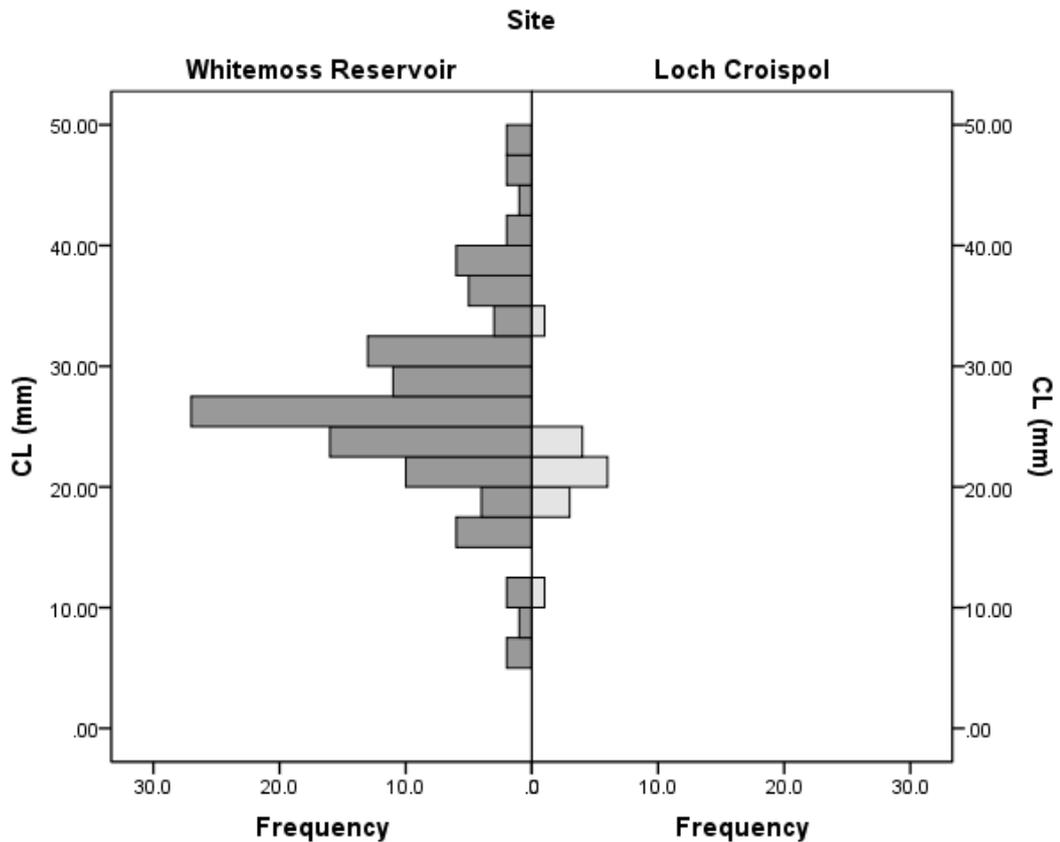
Figure 4-4 - Size distribution (carapace length, CL) of crayfish captured at Whitemoss Reservoir and Loch Croispol.

At Whitemoss Reservoir, the carapace length of crayfish ranged from 7.2 mm to 49.1 mm. At Loch Croispol, the range was 11.9 mm to 32.8 mm. The crayfish caught at Whitemoss Reservoir were significantly larger than those caught at Loch Croispol ($t = 4.22$, $p < 0.01$, $df = 37$).

4.4.3 Sex ratios

At Loch Croispol, the total sex ratio of captured crayfish ($n = 15$) was 1 male: 1.1 females. One female was carrying eggs.

At Whitemoss Reservoir, five individuals were too small to be sexed. The sex ratio of the remaining catch ($n = 113$) was 1 male: 0.8 females. No berried females were captured.

4.4.4 Ark site suitability

Table 4-1 shows the ratings for Whitemoss Reservoir and Loch Croispol when compared to the ark site criteria set out by Peay (2009). The rationale behind obtaining the "Best" ark site status is provided in Table 4-2. Based on these ratings, both sites were classed as a "Possible go" for starting an ark site. This means that the sites have at least some risks and require improvements to reduce these risks. According to the criteria, the sites may only be effective refuges in the short- or medium- term and so alternative or additional sites would be recommended as insurance. The time scale of a "short" or "medium" term refuge is not defined by the criteria.

Table 4-1 - Ark site eligibility ratings for Whitemoss Reservoir and Loch Croispol.

Ark site criteria	Rating – Whitemoss Reservoir (Best/Good/Possible/Poor)	Rating – Loch Croispol (Best/Good/Possible/Poor)
Degree of enclosure	Good	Possible
Aquatic barriers	Possible	Best
Habitat suitability	Possible	Best
Non-native crayfish/ plague	Possible	Best
Angling	Poor/Bad	Good/Possible
Usage and risks from access	Possible	Best/Good
Ownership	Poor	Poor
Size	Good	Good
Stage of development	Best	Best
Bank profile	Good	Poor
Substrate (submerged)	Best	Best
Bankside vegetation	Best	Possible
Rationale/risk assessment	Possible	Good/Best
Ark site? Go/Improve and go/Possible go/No go	Possible go	Possible go

Table 4-2 - Criteria and qualification for "Best" status, as stipulated in Peay *et al.*, 2009.

Criteria	Qualification for "Best" status
Degree of enclosure	Site is a wholly enclosed still water, no watercourse in or out.
Aquatic barriers	Site very unlikely to flood from a watercourse, (e.g. frequency <1in 1000 years) and is separated from watercourse or other waterbody that has potential for colonisation by non-native crayfish by >100 m dry habitat.
Habitat suitability	Site with water quality of high ecological status (equivalent to Biological GQA Grade A), fed by groundwater, or surface drainage from land with semi-natural habitat or low intensity agriculture. Perennial water, little variation in water level, or slow seasonal variation and abundant refuge habitats at all water levels.
Non-native crayfish/ plague	No or few populations of non-native crayfish in the catchment. No cases of crayfish plague in this or adjacent catchments in >5 years. Non-native crayfish not carrying crayfish plague.
Angling	No angling allowed and low likelihood of illegal access for angling
Usage and risks from access	Isolated, away from housing or areas with high public access. Nature conservation is the main or one of the main objectives.
Ownership	Owned by conservation agency or public body with a commitment to conservation objectives and the resources to implement them.
Size	Optimum size for ark sites is currently unknown.
Stage of development	Site more than 15 years old (but note old sites may have high biodiversity already, a possible constraint)
Bank profile	> 50% bank steep, >60°, including some submerged bank that is vertical, undercut, or of complex structure, e.g. fissured rock, clay/earth, large stone revetting, or tree roots.
Substrate (submerged)	Abundant, overlapping irregular boulders (>25 cm) on > 20% of the bed, overlying sand, gravel or clay; deeply cracked and fissured rock in margins and on up to 50% of bed; submerged unmortared rough stone revetting along banks or structures. Refuge potential in > 75% of margin and > 20% overall.
Bankside vegetation	10 - 75% margin partly shaded by shrubs or trees (optimum unknown and may differ for streams and still waters).
Rationale/risk assessment	Very isolated site, good barriers present already or can be created. Site usage means risk of crayfish plague is low and the risk of introduction of non-native crayfish is very low. A very good candidate for ark site if water quality and habitat are acceptable.

4.5 Discussion

4.5.1 Population status

The last survey of white-clawed crayfish at Loch Croispol was conducted in the summer of 1990, when ten crayfish were captured by hand and four were sighted but evaded capture (Thomas, 1992). Surveys conducted between 1984 and 1989 also yielded very small numbers of crayfish, despite significant sampling effort. The sparse catches suggested the presence of a small population but it was not possible to estimate the population size or age groups from such a limited sample. A similarly small number of crayfish were obtained during the present survey. After extensive hand searching, night viewing, trapping and electrofishing only 15 specimens were caught, giving a very low CPUE. The range of sizes (11.9-32.8 mm) found in the catch and the capture of a berried female indicates that the population is, however, continuing to breed. Levels of actual recruitment are unknown and low numbers of adults may be reflective of poor survivorship.

Although the low CPUE is probably indicative of a small population, the potential impacts of survey timing and methodology on capture rate should be considered before any firm conclusions about the status of the Loch Croispol population are drawn. Seasonality in crayfish activity, for example, may have contributed to the low catch: the present survey was conducted in May but white-clawed crayfish are most active from June – November (Reynolds *et al.*, 2010). This might explain why trapping, which is a passive survey method, was poor at detecting crayfish. Active survey methods, however, such as hand searching and electrofishing may be less affected by fluctuations in crayfish activity (although crayfish may be buried deeper in the substrate during cold periods, necessitating a high search effort). This is supported by the results of previous hand searches at Loch Croispol that were conducted between June and August (i.e. within the 'active crayfish' time bracket) but which yielded similar numbers of crayfish to those observed during the present survey in May.

Limitations in the sampling methodology may further explain why such a small number of crayfish were observed at Loch Croispol. No crayfish were caught by trapping or electrofishing. The failure of trapping may be partly attributed to

the use of Swedish Trappy traps which are designed for trapping large crayfish species like the American signal crayfish. The large mesh size and aperture may have led to poor retention of the smaller white-clawed crayfish. Nonetheless, Trappy traps have been used to successfully sample white-clawed crayfish in other surveys (e.g. Matthews and Reynolds, 1995, Spink and Rowe, 2002). Trapping is only recommended as a survey method for high density populations (Peay, 2003) and it is possible that the population at Loch Croispol is simply too small for this technique to be effective. The use of Trappy traps at Whitemoss Reservoir would have verified the effectiveness of this method for sampling a high density population.

Electrofishing has also been used to survey white-clawed crayfish (e.g. Alonso, 2001, Bernardo *et al.*, 1997) and is particularly recommended for sampling lakes of high conductivity (Reynolds *et al.*, 2010). Despite its favourable limestone geology, no crayfish were detected by electrofishing at Loch Croispol. Electrofishing efficiency may have been limited by the abundant fissured rock, boulders and rubble available to the crayfish as cover. Furthermore, this technique may be more difficult to apply in still water than in rivers due to the increased risk of turbidity and therefore reduced visibility for catching the animals.

Night viewing at Loch Croispol led to the capture of just one individual. No other active crayfish were observed during the search. Crayfish activity varies at different times of the day and may be influenced by predation risk (Robinson *et al.*, 2000) or abiotic factors including temperature (Barbaresi and Gherardi, 2001). Consequently, the success of night viewing as a survey method is likely to vary considerably depending on the time, date or season in which sampling occurs. Due to variation in sampling conditions and the bias of night searching towards active adult crayfish, it is only recommended as a supplementary survey method (Peay, 2003).

Hand searching was the most successful method, which accords with the findings of other white-clawed crayfish surveys and protocols for monitoring the species (Peay, 2003). In a recent survey of 13 lakes in Ireland, hand searching was the most common method employed (O'Connor *et al.*, 2009). Hand searching is recommended for lakes with abundant stony cobble in the shallows (Reynolds,

2006) and so was easily applied to this type of habitat in Loch Croispol. Despite its success over the other methods, however, a considerable amount of time was required in order to capture crayfish by hand searching, giving a low CPUE (five times lower than that obtained at Whitemoss Reservoir). This suggests that in the past 20 years, the population of white-clawed crayfish at Loch Croispol has remained at a relatively low density.

The population of white-clawed crayfish at Whitemoss Reservoir was last sampled after its discovery over a decade ago, when a wide range of sizes were recorded and the population was described as "thriving" (Maitland *et al.*, 2001). During the present survey, a large number of crayfish encompassing a wide size range (7.2-49.1mm) were captured. The survey was conducted too late in the season for observation of berried females but the presence of young-of-the-year in catches indicates that this is a breeding population.

In comparison with Loch Croispol, the CPUE at Whitemoss Reservoir was high for all methods. The survey was conducted in August during a period of high crayfish activity, which might help explain the higher catch, at least for the passive survey methods: both trapping and night viewing were more successful at Whitemoss Reservoir than at Loch Croispol. Trapping efficacy may also have been improved at Whitemoss Reservoir due to the use of specialised white-clawed crayfish traps, which have a finer mesh and smaller apertures than the Swedish trappy traps. Night viewing had a CPUE that was over 30 times greater than that observed at Loch Croispol: again, it is difficult to attribute this difference to seasonal variation in crayfish activity or actual differences in population density. The CPUE values obtained for the active survey methods, hand searching and electrofishing, may give a better indication of differences in population density between the two sites. For both of these methods, the CPUE was higher at Whitemoss Reservoir than at Loch Croispol, suggesting the population is larger. Based on these results, the Whitemoss Reservoir population appears to be still "thriving".

The factors that have limited the crayfish population at Loch Croispol or promoted the high population density at Whitemoss Reservoir are of interest since they will be relevant to white-clawed crayfish sites elsewhere. Previous studies have highlighted abiotic factors (such as temperature, water chemistry

and substratum type), and biotic factors (including competition and predation) as being key determinants of crayfish abundance (Lodge and Hill, 1994). The relative importance of all of these parameters in affecting the populations at Loch Croispol or Whitemoss Reservoir was not investigated during the present study but some general observations were made, which may provide a basis for further investigation.

Given its position at high latitude, temperature is likely to be a major limiting factor for the crayfish population at Loch Croispol. The northern limit for natural populations of white-clawed crayfish in Great Britain is in Northumberland (Jay and Holdich, 1981), where crayfish exhibit slower growth and attain a smaller size than those further south due to the lower temperatures (Brewis and Bowler, 1982). This agrees with the observation that crayfish at Loch Croispol were significantly smaller than those found at Whitemoss Reservoir, which is over 250 miles due south. The timing of the survey at Loch Croispol in spring may, however, have biased this result by under-representing larger, egg-bearing female crayfish and contributing to the small sample size.

Despite its relatively large size, most of the crayfish captures at Loch Croispol were localised in one part of the loch, on the southern edge, as occurred during the last survey in 1992 (Thomas, 1992). This area largely consisted of loose slabs and cobbles lying on a soft bed of marl, as well as large sections of fissured rock. This habitat type was less common on the northern side of the loch, which was not inhabited by crayfish. In a study of white-clawed crayfish in Ireland, a greater abundance of bedrock was observed in areas where crayfish were present than where they were absent (Gallagher *et al.*, 2006). Gallagher *et al.* (2006) hypothesised that bedrock provides a stable habitat and contains crevices and cracks in which crayfish can seek shelter from predators and high flows. The presence of boulders/cobbles as a substrate was also found to be associated with crayfish presence by Naura and Robinson (1998). In contrast to Loch Croispol, the crayfish at Whitemoss Reservoir were widely distributed throughout its perimeter, which is characterised by abundant pebbles and cobbles as well as leaf litter from overhanging trees. Abundant crevices in the dam wall of the reservoir also provide effective refuges. Although the site is relatively small, Whitemoss Reservoir appears to contain a high proportion of good quality crayfish habitat.

Predation pressure is a potentially important stressor on crayfish populations. Loch Croispol is inhabited by both eels and brown trout, that may help maintain the low density of crayfish here. The gut contents of a small number of brown trout (n = 8) were dissected but no crayfish were found; crayfish predation by trout can be seasonal, however, and is highest during the summer (Hepworth and Duffield, 1987). Sampling of fish later in the season might yield different results. In addition to influencing the overall density of a population, predation can impact the distribution of crayfish within an ecosystem. Crayfish may shift their habitat preference according to the presence or absence of a predator (Englund and Krupa, 2000). In a study of crayfish in lakes in Wisconsin, predation risk was found to differ significantly among habitat types, with crayfish on sand experiencing much higher mortality rates than those in cobbles (Kershner and Lodge, 1995). The use of cobble habitat by crayfish was positively correlated with lake-wide predator density (Kershner and Lodge, 1995). It is possible that high levels or risk of predation at Loch Croispol have restricted the distribution of crayfish to areas with enough cover to confer protection. Predation risk may be augmented by the pale, limestone rich substrate of the loch, against which a white-clawed crayfish (which is dark brown in colour) will appear obvious to predators. Predation by fish is also possible at Whitemoss Reservoir, which is stocked with rainbow trout. The increased predation risk from stocking may be compensated, however, by the abundant cover at this site.

Terrestrial predators are another source of pressure on crayfish populations. Both Loch Croispol and Whitemoss Reservoir are within the range of the European otter *Lutra lutra* L. and the non-native American mink *N. vison*. Examination of otter spraints or mink scats could help determine the importance of crayfish as a prey item. Crayfish may also be susceptible to predation by birds. Future work at Loch Croispol and Whitemoss Reservoir should aim to identify the main predators at these sites.

4.5.2 Ark site suitability

Based on the criteria established by Peay (2009), Loch Croispol and Whitemoss Reservoir qualify as potential "ark sites". Both contain long-established, breeding populations of white-clawed crayfish. Furthermore, they are currently free from the main threats to white-clawed crayfish: that is, signal crayfish and

the fungal disease crayfish plague for which the signal crayfish is a vector. The degree to which signal crayfish and plague threaten Whitemoss Reservoir or Loch Croispol will affect their effectiveness as safe ark sites. Signal crayfish and plague may be introduced accidentally by a variety of means, including the use of live crayfish as bait (Lodge *et al.*, 2000) or with deliveries of hatchery trout during stocking (Bean *et al.*, 2006). Deliberate introductions of crayfish for harvesting or disposal of unwanted animals from the aquarium and pond trade are also possible (Holdich, 1999).

Loch Croispol is relatively isolated from the nearest population of signal crayfish, which inhabits the River Nairn catchment over 160 km away. There is no stocking of fish at Loch Croispol and angling for brown trout is managed by local crofters. Even if the risk of a signal crayfish invasion is low, however, crayfish plague remains a threat since the disease may also be transmitted via infected angling equipment and footwear, as has occurred in Ireland (Reynolds, 1988). None of the crayfish captured at Loch Croispol showed any signs of disease, although the sample size was limited. Should crayfish plague arrive at Loch Croispol, the population will be particularly vulnerable to extirpation, due to its small size and localised distribution within the water body.

The nearest population of signal crayfish to Whitemoss Reservoir is less than 35 km away in the River Kelvin, making this site much more vulnerable to an invasion than Loch Croispol. Whitemoss Reservoir is also heavily used by anglers, who may fish in signal crayfish infested sites. Fish stocking could further increase the risk of introduction of crayfish or disease. Although there was no evidence of crayfish plague during the present survey, one animal was found to be suffering from a parasitic infection. The abdominal muscle of this individual was coloured milky-white and examination of a sample under the microscope suggested infection by a microsporidian (Nicholas Beevers, pers. comm.) The pathogen was not formally identified but is likely to have been porcelain disease *Thelohania contejeani* [Henneguy], which is common among white-clawed crayfish populations.

There is likely to be some subjectivity in qualifying what the "Best" conditions are for white-clawed crayfish survival. For example, the optimum size for an ark site is currently unknown; classification of Whitemoss Reservoir and Loch

Croispol as "Good" sizes should be considered arbitrary until evidence becomes available from other case studies. Nonetheless, the ark site checklist provided a useful framework for identifying the main threats at these sites. In light of the results, Loch Croispol and Whitemoss Reservoir could serve as valuable, long-term "*ex-situ*" ark sites for the white-clawed crayfish but only if measures are taken to mitigate the threats highlighted above, the most important of which is the management of biosecurity.

4.5.3 "Ex situ" conservation: a controversial strategy?

Although both populations are protected by law, there have been no efforts to manage, conserve or monitor the white-clawed crayfish at Loch Croispol or Whitemoss Reservoir. As populations continue to decline elsewhere in Europe, there may be increasing pressure to take action to protect the Scottish sites. Conservation of a technically "non-native" species could, however, present a dilemma for some ecologists, whose traditional aim is to protect native biodiversity. Should resources be channelled into conserving an alien species – albeit a globally endangered one – when there are many other native species that are also under threat? It is also possible that white-clawed crayfish have had a detrimental impact on native biodiversity, which would further weaken the cause for their protection.

A similar dilemma has arisen relating to the conservation of banteng *Bos javanicus* [d'Alton], an endangered bovid species which is native to Southeast Asia (Timmins *et al.* 2008). Hunting for horns, the bushmeat trade, habitat loss and genetic pollution through interbreeding with other cattle species have led to major reductions in Asian populations, with further declines projected for the future (Timmins *et al.*, 2008). Recent genetic analyses have confirmed the genetic purity of an introduced population of banteng in Australia, which has been established in the wild without human control for over 150 years (Bradshaw *et al.*, 2006). If Australian banteng negatively impact the environment (e.g. through overgrazing and trampling) then land managers may choose to control or eradicate the population. Bradshaw *et al.* (2006) argue, however, that the negative impacts caused by banteng are minimal in comparison with those caused by other non-native mammals in Australia.

Crayfish are omnivorous and affect organisms at multiple trophic levels by feeding on detritus, macrophytes, invertebrates and fish (Reynolds and O'Keefe, 2005). They are also prey for otters (Breathnach and Fairley, 1993), trout (Momot, 1967), eels (Blake and Hart, 1995) and other predators. Given their polytrophic interactions, introduced crayfish have the potential to cause major changes to native communities (Crawford *et al.*, 2006, Hobbs *et al.*, 1989), including local species extinctions (Holdich, 1999). The spread of the signal crayfish for example, has caused widespread concern in Europe due to the threats posed to biodiversity (Gladman *et al.*, 2010), including fundamental changes to invertebrate communities (Crawford *et al.*, 2006) and negative interactions with juvenile salmon (Griffiths *et al.*, 2004). To the author's knowledge there are no available data on the conditions at Loch Croispol and Whitemoss Reservoir prior to the introduction of crayfish. Thus, it is difficult to judge whether these introductions have had detrimental effects on native biota. Both populations have persisted at the two sites for several decades and so any ecosystem changes resulting from their establishment have probably already occurred. Even if it was possible to demonstrate that white-clawed crayfish have had negative effects, it would be very difficult to attempt to restore either site to its original state. Furthermore, Whitemoss Reservoir is an artificial site and so the threat to native species is not a relevant concern. At this stage, maintenance of these increasingly rare white-crayfish populations seems the most logical and beneficial move.

Bradshaw *et al.* (2006) suggest that the introduction of endangered species to non-endemic regions, although controversial, is a viable conservation strategy. The IUCN guidelines for setting up areas of conservation strongly suggest that they should be located within or as close as possible to the natural range of the target species (IUCN, 2002). In the case of the white-clawed crayfish, however, establishment of an ark site far from its native range actually confers an advantage, since it will also be distant from the threats of signal crayfish and disease.

The introduction of white-clawed crayfish to Scotland some decades ago was not for conservation purposes. The success of these populations and the failure of traditional conservation methods to mitigate the white-clawed crayfish extinction crisis elsewhere in Europe, however, may encourage conservationists

to consider introducing the species to other "*ex situ*" sites. Climate change is expected to increase the advantage of signal crayfish over white-clawed crayfish in the future (Sibley *et al.*, 2009). This may provide an additional incentive for the translocation of non-native *A. pallipes* to new sites.

At present, ark sites for white-clawed crayfish have only been established within the native range of the species. However, unless these sites have previously been inhabited by white-clawed crayfish, they still represent naïve ecosystems with the potential to undergo significant changes following the introduction of this keystone species. Careful ecological assessments should be undertaken at potential ark sites to ensure that crayfish introductions are not made at the expense of other species of conservation concern. The creation of entirely new ecosystems for white-crayfish by flooding former quarries and gravel pits, as has been initiated in England (Whitehouse *et al.*, 2009), may be a safer way of promoting biodiversity than introducing animals to pre-existing sites. Reservoirs like Whitemoss Reservoir, which are already "artificial" ecosystems that lack native fish assemblages may also prove useful.

The value now ascribed to the white-clawed crayfish populations in Scotland demonstrates how a non-native species may actually constitute an important conservation resource. The present surveys at Loch Croispol and Whitemoss Reservoir have provided baseline data on two populations occurring at a low and a high density, respectively, that have survived without management for several decades. Future work should aim to monitor the crayfish at these sites and raise awareness of good practice for biosecurity (e.g. disinfection of angling equipment and footwear), to ensure the continued survival of these vulnerable refuge stocks, and of the white-clawed crayfish species as a whole.

Chapter 5: Investigating the threat of signal crayfish to salmon redds

5.1 Abstract

North American signal crayfish (*P. leniusculus*) were exposed to three successive laboratory treatments in order to test their ability to detect and excavate Atlantic salmon eggs (*S. salar*) or dead fish (herring, *Clupea harengus* L.) buried in the gravel of an artificial stream. There was a significant difference between the number of excavations made by signal crayfish when exposed to salmon eggs or dead fish. Crayfish successfully detected and excavated buried fish but were unable to detect buried salmon eggs, despite significant opportunity. The results strongly suggest that signal crayfish do not present a threat to Atlantic salmon via egg predation. In the wild, however, the vulnerability of redds may vary according to a range of biotic and abiotic factors. These findings will be of interest in Europe, where *S. salar* is considered an important species for conservation and in particular, countries such as Scotland and Norway, where recreational and commercial salmonid fisheries form an important economic mainstay.

5.2 Introduction

The invasion of freshwater ecosystems by non-native species is increasingly recognised as one of the most significant threats to biodiversity (Chornesky and Randall, 2003), presenting another challenge to the conservation of native fish populations (Dudgeon *et al.*, 2006). Among these invaders are non-indigenous crayfish species, including *Pacisfastacus leniusculus*, which has been shown to have far-ranging ecological impacts (see Chapter 1).

Recently, research has focussed on the interactions between crayfish and fish (Bubb *et al.*, 2009, Degerman *et al.*, 2007, Griffiths *et al.*, 2004, Hayes, 2012, Peay *et al.*, 2009). Such interactions are complex (Peay *et al.*, 2009) and likely to vary according to their ecological context. In some cases, crayfish may serve as prey items for fish species such as the European eel (*A. anguilla*) (Blake and

Hart, 1995) and perch (*Perca fluviatilis* L.) (Blake and Hart, 1995, Söderbäck, 1994). A number of introductions of *P. leniusculus* in Scotland can be traced to commercial fisheries, in which crayfish may have been introduced as a food source for trout (Bean *et al.*, 2006). Alternatively, fish may be predated upon by crayfish themselves. *P. leniusculus* can, for example, consume small benthic fish such as bullheads and stone loach (Guan and Wiles, 1997a, Guan and Wiles, 1998). Laboratory studies have also shown that fish eggs, including those of Arctic charr and whitefish (*Coregonus* spp.) are palatable to *P. leniusculus* (Nyberg and Degerman, 2000).

In addition to predator-prey interactions, crayfish can compete with fish for food and shelter. *P. leniusculus* has been shown to negatively impact invertebrate abundance and community structure, thereby reducing prey availability for salmonids and other fish species (Crawford *et al.*, 2006). One important species with which *P. leniusculus* may interact is the Atlantic salmon (*S. salar*), which is widely distributed across Europe. *S. salar* is considered to be a species of conservation concern and is listed under Annexes II and V of the European Union's Habitats Directive 92/43/EEC as a species of European importance. In Scotland, salmon are valued as an integral part of the natural heritage (JNCC, 2007) and for the significant contributions that recreational and commercial fisheries make to the economy (Radford *et al.*, 2004).

Given the complexity of crayfish-fish interactions, identifying the mechanisms by which crayfish can affect fish populations is difficult. Fluvarium trials have shown that *P. leniusculus* can outcompete juvenile *S. salar* for shelter, forcing them to spend more time swimming in the water column and thus increasing both the use of energy reserves and their risk of predation (Griffiths *et al., 2004*). Recent research in England has shown that *P. leniusculus* can reduce the abundance of salmonid and benthic fish species where they co-exist (Bubb *et al.*, 2009, Peay *et al.*, 2009) but the relative importance of predation, competition and other interactions in contributing to these losses has not been quantified. *P. leniusculus* is known to readily predate fish eggs, including those of salmonids, when available (Nyberg and Degerman, 2000). A recent field experiment showed that *P. leniusculus* in Lake Vattern are more important predators of great Arctic charr (*Salvenlinus umbla* L.) eggs than native fish and may reduce the recruitment and recovery of these endangered stocks (Setzer *et al.*, 2011).

While some salmonids, including charr and grayling (*Thymallus thymallus* L.) tend to lay their eggs on or near the gravel surface, many other species, like *S. salar* construct nests or "redds", comprising depressions in the gravel into which they lay then cover their eggs. Eggs may be buried in gravel at depths of 15-25 cm (Bardonnet and Bagliniere, 2000). Little is known about the propensity of crayfish to detect and excavate salmonid nests and thus the level of protection from these predators that is afforded by nest building. The aim of the present study was to determine the ability of *P. leniusculus* to detect and excavate artificial salmonid redds.

5.3 Materials and methods

5.3.1 Crayfish

Adult, male, size-matched (53.5–61.5mm carapace length) *P. leniusculus* were collected from the River Dee (Kirkcudbrightshire, south-west Scotland) using Swedish Trappy™ traps baited with brown and rainbow trout (*S. trutta* and *O. mykiss*, respectively) carcasses. Prior to experiments, crayfish were kept in secure holding tanks which contained plastic piping to provide shelter and minimise the potential for agonistic interactions. Crayfish were starved for 72 hours prior to the commencement of experiments and for their duration. Before introduction to test arenas, all experimental animals were blotted dry and weighed (± 0.1 g) using an electronic balance. Carapace length was measured from the tip of the rostrum to the posterior margin of the carapace using Vernier callipers (± 0.1mm). After experiments had finished, all crayfish were euthanised by freezing.

5.3.2 Experimental setup

Experiments were conducted at the Marine Scotland Science fluvarium (Almondbank, Perthshire, central Scotland) which was supplied with water (temperature range: 2.4–9.6 °C) from the River Almond, in which conditions are naturally suitable for the survival of both crayfish and salmon. The stream was divided transversely into 16 test arenas (46 x 28 x 24 cm), as described in Griffiths *et al.* (2004), which could be observed through the glass side. Each arena was filled with gravel (10–20mm), with larger particles (30–50mm) scattered over the gravel surface. These particles were within the range of sizes

found in natural redds (Kondolf and Wolman, 1993). Two pieces of plastic tubing (15 cm long, 5.5cm diameter) were placed in each half of the arena to provide shelter. Arenas were secured with clear plastic lids which were weighted with bricks and covered in black plastic sheeting to minimize light. Plastic overhangs and a flow regulation device were fitted in the area surrounding the stream as secondary containment measures.

To test their ability to detect and excavate two potential prey types buried in gravel, 16 crayfish, housed individually, were subject to each of three conditions in the following sequence:

Egg I

Plastic mesh boxes (40 x 60 x 40 mm, mesh size 5 mm) containing c. 250 *S. salar* eggs were buried to a depth of 5 cm in 12 experimental arenas (one in each). In four arenas, empty boxes were buried, which served as controls. One adult male *P. leniusculus* was placed in each arena. The trial was terminated after 14 nights.

Fish

The setup was similar to the Egg I trial but 12 boxes contained an equivalent volume of fresh herring (*C. harengus*) instead of eggs. Crayfish from the egg trial were re-assigned to new arenas to ensure that different animals were used as controls. The trial was terminated after two nights.

Egg II

The purpose of this trial was to verify that observed differences in crayfish behaviour between the Egg I and Fish trials were not due to temporal changes in crayfish foraging activity. The setup was identical to the Egg I trial but observations were terminated after seven (rather than 14) nights.

For all trials, experimental boxes containing eggs or fish were buried in arenas located downstream of those containing empty boxes. Between trials, crayfish

were held temporarily in buckets and the gravel in each arena was mixed vigorously to remove any residual scents.

Observations

Observations were made under a darkened cover from the side of the stream. A red-light torch was used to locate and observe the crayfish. Preliminary observations made during the day and overnight footage from an infrared camera (AquaCam, System Q Ltd.) revealed that crayfish were most active at night. To avoid disturbing the crayfish during these active periods, observations were made once per day during daylight hours. During each observation, the number and location of excavations in each arena was recorded. A ruler was used to estimate the dimensions of any excavations.

At the end of each treatment, the number of arenas falling into each of four categories was recorded: no excavations in box area; minor excavations (≤2 cm depth) in box area; major excavations (>2 cm depth) in box area; and complete excavations (removal of enough gravel to expose the box). Box area was defined as being within a 5 cm radius of the gravel surrounding the box.

5.3.3 Statistical analysis

To determine whether there was a significant relationship between box contents (salmon eggs or fish) and the number of arenas containing excavations in the box area, a Chi-square test (with Yates' correction, to account for 1 degree of freedom) was applied using software (SPSS vers.17.0.1).

5.4 Results

In all trials, crayfish made no excavations near the empty control boxes. The number of arenas containing excavations in the box area was significantly lower when eggs were used as prey than when fish were the prey ($\chi^2_{Yates} = 20.05$, d.f. = 1, $p < 0.001$), (Figure 5-1 and below).
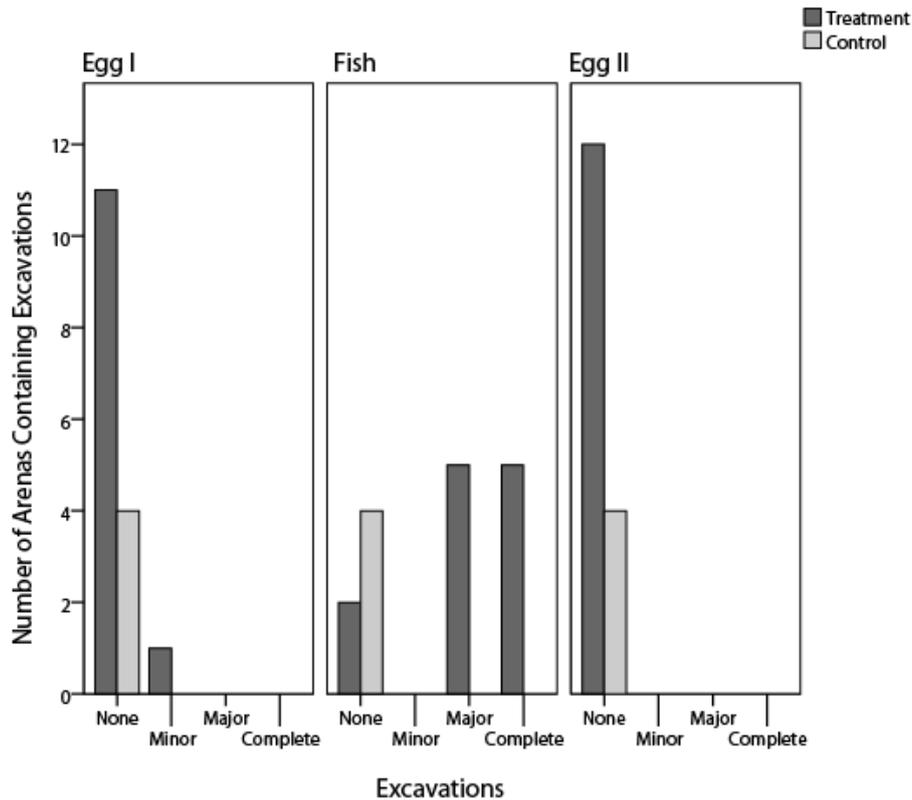
Figure 5-1 - Number of arenas containing no excavations, minor excavations (≤ 2 cm depth), major excavations (> 2 cm) and complete excavations (≥ 5cm) in the box area at the end of each trial.

Egg I

After 14 nights, none of the empty boxes had been excavated by crayfish. There was one minor excavation of 2 cm depth, at 3 cm distance from one of the boxes containing eggs; there were no excavations near the boxes in the remaining 11 arenas containing eggs.

Fish

After one night, excavations within the box area occurred in six of the 12 arenas containing fish, half of which were complete excavations and the other half major excavations.

After two nights, there were excavations in 10 of the 12 arenas containing boxes with fish: new excavations were made in four arenas and existing excavations were deepened. At the end of the trial, there were five arenas with major excavations near the boxes and five arenas in which boxes were completely excavated.

Egg II

After seven nights, there were no excavations near the boxes containing eggs. The results of the Egg I and Fish trials indicated that crayfish would respond to the buried bait, if at all, within the first 48 h of exposure and so seven nights was deemed sufficient for this final trial.

5.5 Discussion

This study found that *P. leniusculus* did not excavate the boxes that contained *S. salar* eggs despite considerable opportunity. By contrast, *P. leniusculus* excavated boxes containing fish in the same positions and at the same depth after just one (50% of boxes) or two (83%) nights of exposure.

Given the order of the trials, one interpretation is that the excavations made during the Fish trial were due to an increase in the hunger levels and thus foraging activity of the crayfish rather than the change in prey type. Crayfish did not forage during the Egg II trial, however, after a starvation period of 23 days. It is therefore more likely that the negative results obtained for the Egg I and Egg II trials were due to a failure of the crayfish to detect the eggs. These results strongly indicate that *P. leniusculus* do not present a predation risk to eyed salmon eggs buried in redds.

A recent study by Edmonds *et al.* (2011) found that *P. leniusculus* spent more time occupying incubator quartiles that contained the buried eggs/embryos of *S. salar* than quartiles without eggs/embryos. In addition, digging activity by *P. leniusculus* was greater in incubators containing *S. salar* eggs than controls. This suggests that *P. leniusculus* is in fact capable of detecting buried *S. salar* eggs, although there was no evidence of this in the present study. Edmonds *et al.* (2011) used video cameras to constantly monitor crayfish position whereas the present study only recorded crayfish position once a day, which may explain the apparent lack of egg detection reported here. However, even if *P. leniusculus* is capable of detecting *S. salar* eggs, Edmonds *et al.* (2011) found no evidence of predation upon them, in agreement with the present study.

The exclusion of egg predation by *P. leniusculus* as a major threat to *S. salar* offers some hope for the conservation of this species in the face of crayfish invasions. The experimental design used here was simplified, however, compared with conditions in the wild. In natural redds, the density, position and depth of eggs varies (de Gaudemar *et al.*, 2000) and this may have implications for the risk of detection by crayfish. Fitzsimons *et al.* (2006) found that the Northern Clearwater crayfish *Orconectes propinquus* [Girard] was inefficient at finding and consuming the exposed eggs of lake trout *Salvelinus namaycush*

[Walbaum] at a low egg density (<600 eggs m^{-2}) but showed peak consumption at a density of 3000 eggs m^{-2}. The present study used an intermediate egg density (1938m^{-2}) and egg burial further hindered their detection by crayfish. Eggs were buried at a shallower depth (5 cm) than that typical of natural *S. salar* redds (15–25cm) (Bardonnet and Bagliniere, 2000), suggesting that in the wild, redds are sufficiently deep to evade predation. Crayfish could, however, disturb or predate exposed eggs or the shallower redds constructed by other fish species such as brown trout (*S. trutta*, depth range: 8–25 cm) (DeVries, 1997). Even if crayfish are unable to detect and seek out eggs, the incidental discovery of a redd might lead to its consumption.

All eggs used in the present study were several weeks post-spawn. Although redds may be safe during this stage of the incubation period, crayfish could affect other stages of development. A study of salmonid egg predation by sculpin (*Cottus cognatus* Richardson and *C. bairdi*) found that fish could detect eggs using chemical cues but only during a limited period of around 24 h post-spawning (Fitzsimons *et al.*, 2006). The ability of crayfish to detect eggs at different developmental stages or eggs that are dead or damaged is unknown. Newly hatched alevins may also be at risk; the emergence of fish from the gravel occurs as water temperature increases, which is likely to coincide with an increase in foraging activity by crayfish (Rubin and Svensson, 1993). During laboratory experiments, noble crayfish (*A. astacus*), did not excavate trout eggs but were observed chasing and preying upon fry (Rubin and Svensson, 1993). Similarly, Edmonds *et al.* (2011) observed piscivorous behaviour by *P. leniusculus* upon emerging *S. salar* fry in incubators.

Given the high densities that invasive crayfish may reach, the potential effect of predator abundance on egg predation rate should also be considered. Predation of trout eggs by rusty crayfish (*Orconectes rusticus* Girard) and Northern clearwater crayfish (*O. propinquus*) was found to increase with crayfish density (Ellrott *et al.*, 2007). At high densities, greater competition for food and space might lead to increased foraging or burrowing activity by crayfish, increasing the chance of redd disturbance. Although the present study tested only adult crayfish, juvenile crayfish may also disturb redds. There is evidence that the burrowing activity of *P. leniusculus* is influenced by size. Laboratory experiments found that the majority of small (< 50 mm carapace length) crayfish

introduced to containers of clay would excavate burrows, whereas two-thirds of large crayfish (> 50 mm carapace length), and in particular large males with bigger chelae, did not (Guan, 1994). Larger males are better equipped to defend themselves from predators and so may be less inclined to seek shelter and burrow (Guan, 1994). Smaller crayfish may also be more adept at accessing the interstitial spaces in the substrate than large crayfish (Setzer *et al.*, 2011). It is possible, therefore, that juvenile crayfish are more likely to encounter fish eggs than adult crayfish. The risk of egg predation by different size classes of *P. leniusculus* remains unknown.

In addition to the biotic variables mentioned above, future studies should consider the impact of abiotic factors such as sediment composition and temperature on predation risk. The metabolic rate and thus foraging activity of crayfish has been shown to increase with temperature (Ellrott *et al.*, 2007). Sediment composition may be important since it affects both the burrowing capabilities of crayfish (Grow, 1982) and the selection of redd sites by fish (Crisp and Carling, 1989).

Despite the increasing sympatry of invasive crayfish and native fish populations, our understanding of the complex interactions between these animals remains limited, perhaps because of the suite of variables that can influence such behaviours. The present study allows egg predation to be rejected as a major threat to Atlantic salmon but only under a specific set of conditions. Continued field and laboratory experiments will be crucial in identifying the impacts of crayfish introductions. Such impacts will have implications not only for the conservation of native fish but also for the sustainability of fisheries that form an important economic mainstay in Scotland and other countries.

Chapter 6: Disturbance and attempted predation of the freshwater pearl mussel by signal crayfish

6.1 Abstract

The continued expansion of the North American signal crayfish (*P. leniusculus*) outside of its native range is predicted to cause major changes in native invertebrate assemblages. Laboratory trials were used to investigate the potential impact of *P. leniusculus* on a globally endangered mollusc, the freshwater pearl mussel (*Margaritifera margaritifera* L.). Adult *M. margaritifera* from three size classes were half-buried in gravel and exposed to five nights with and without *P. leniusculus*. The daily position of *M. margaritifera* changed more frequently in the presence of *P. leniusculus*. The distances of position changes did not differ significantly when *P. leniusculus* were present or absent, although the maximum distance moved was much larger when they were present. There was no effect of mussel size on the number or distance of movements. Using overnight video footage, *P. leniusculus* were observed attempting to predate on *M. margaritifera*. All predation attempts were unsuccessful, probably due to the inability of *P. leniusculus* to penetrate the thick shell of *M. margaritifera*. *P. leniusculus* were also observed walking over and digging near *M. margaritifera*. Although adult *M. margaritifera* appear safe from predation, it is hypothesised that *P. leniusculus* may pose a threat to juvenile mussels due to their thinner shell thickness. Disturbance of *M. margaritifera* by *P. leniusculus* is likely to incur energetic costs through valve closure and reduced feeding opportunities. Prolonged valve closure due to predation threat and displacement from holding substrates may cumulatively reduce the fitness and long-term survival of *M. margaritifera*.

6.2 Introduction

The continued expansion of the North American signal crayfish *P. leniusculus* outside of its native range is predicted to cause major changes in native invertebrate assemblages. Even at moderate densities, *P. leniusculus* can significantly reduce the abundance and diversity of invertebrates in freshwater ecosystems (Crawford *et al.*, 2006, Stenroth and Nyström, 2003). The effects of *P. leniusculus* may be indirect, through competition for macrophytes and detritus (Nyström *et al.*, 1996) or direct, via predation. Crayfish exhibit selective predation by favouring slow-moving species (e.g. leeches and molluscs) over more mobile ones (e.g. stoneflies) leading to profound changes in the prevailing structure of invertebrate communities (Gherardi, 2007, Nyström *et al.*, 1999).

Determining the impact of *P. leniusculus* on species of high conservation value is a priority for conservationists. In Europe, *P. leniusculus* introductions have led to major declines in the native white-clawed crayfish (*A. pallipes*) as a result of interspecific competition and transmission of crayfish plague (*A. astaci*) (Holdich and Lowery, 1988); considerable efforts will now be required to save *A. pallipes* from extinction. Another endangered invertebrate species of global conservation concern is the freshwater pearl mussel (*M. margaritifera*), a critically endangered mollusc which has suffered significant declines in every part of its range (Skinner *et al.*, 2003). The biggest threat to *M. margaritifera* in the past has been pearl harvesting but this was made illegal in the UK in 1998 (Cosgrove *et al.*, 2000) and the species has also been protected through European legislation (Annexes II and V of the EU Habitats Directive; Appendix III of the Bern Convention). More recently, habitat disturbance, deteriorating water quality and reduced stocks of host salmonids have been cited as the main causes of decline (Beasley *et al.*, 1998, Cosgrove *et al.*, 2000). Scotland contains half of the world's remaining functional populations of *M. margaritifera* (Cosgrove *et al.*, 2000). Unfortunately, the range of *P. leniusculus* in Scotland is expanding, although *P. leniusculus* has yet to invade sites that support *M. margaritifera*. However, the two species already occupy some of the same river catchments and *P. leniusculus* is continuing to spread by natural and human-aided movements. *P. leniusculus* potentially represents a new, uncharacterised threat to the persistence of *M. margaratifera* populations in Scotland and consequently, the survival of this endangered species on a global scale.

Based on our knowledge of *P. leniusculus* dietary preferences, *M. margaratifera* may constitute an attractive new prey source. Gastropods and bivalves are particularly vulnerable to predation by *P. leniusculus*, as evidenced by numerous field (Bjurstrom *et al.*, 2010), enclosure (Stenroth and Nyström, 2003) and laboratory studies (Covich *et al.*, 1981, Ermgassen and Aldridge, 2011, Nyström and Perez, 1998, Olden *et al.*, 2009). A recent field study in Germany found *M. margaratifera* with shell damage that may have resulted from attacks by *P. leniusculus* living in sympatry with the mussel population (Schmidt and Vandré, 2009). However, there are no published accounts of predation or attacks by *P. leniusculus* on *M. margaratifera*, and the potential interactions between the species have not been observed directly nor tested experimentally. The present study used laboratory trials to investigate whether *P. leniusculus* will predate upon or damage a range of sizes of adult *M. margaratifera*. Previous studies have found that *P. leniusculus* exhibit size-selective predation, preferring smaller molluscs to large ones (e.g. Nyström and Perez, 1998). It is hypothesised, therefore, that *P. leniusculus* will choose to predate upon small *M. margaratifera*, in preference to large mussels. The results and implications for *M. margaritifera* survival following *P. leniusculus* invasions are discussed.

6.3 Materials and methods

6.3.1 *Animals*

6.3.1.1 Crayfish

In February 2011, *P. leniusculus* were collected, under licence, from Loch Ken, south-west Scotland, using Swedish Trappy™ traps baited with fish carcasses. There are no *M. margaritifera* in Loch Ken and so *P. leniusculus* were naïve to this species. For biosecurity reasons, only adult males (carapace length: 48.2 – 60.3 mm) were selected for experiments; all other crayfish were killed, as required by law, immediately on-site. Crayfish were transported to the laboratory in cool boxes and kept in holding tanks, which contained shelters to minimise agonistic interactions. To encourage foraging behaviour, crayfish were starved for 72 hours prior to experiments and for their duration. A total of 22 crayfish were used in experiments. The carapace length of each experimental animal was measured using Vernier calipers (± 0.1 mm), from the tip of the rostrum to the posterior margin of the carapace.

6.3.1.2 Mussels

In February 2011, adult *M. margaritifera* were collected, under licence, from the River South Esk, east Scotland. Mussels were collected by hand from the river bed and transported in cool boxes of river water to the laboratory, where they were allowed to settle in holding tanks. Vernier calipers were used to measure the length of each mussel (± 0.1 mm) and mussels were divided into small (54-76 mm), medium (77-90 mm) and large (>90 mm) size classes. A total of 39 mussels were used in experiments. After the experiments were complete, all mussels were returned to the site of collection.

6.3.2 *Experimental setup*

Unavailability of the Marine Scotland Science Fluvarium (as used in Chapter 5) meant that all experiments were conducted in a constant temperature room (15°C) at the Institute of Aquaculture, University of Stirling, Scotland. This temperature is within the range of temperatures known to support both mussels (Hastie and Young, 2003) and crayfish (Guan and Wiles, 1998) in the wild.

Fourteen black, opaque plastic tanks (dimensions 43 x 31 x 20 cm) were filled with gravel (particle size 10-50 mm) to a depth of 5 cm and connected to one of two water recirculation systems (seven tanks in each). The water recirculation systems were topped up with fresh tap water every day. Only thirteen tanks were used for experiments; the remaining tank was used to house spare mussels. All tanks were aerated using airstones and pumps, their open tops covered with green mesh netting that was held in place by elastic string. Mussels and crayfish were not fed for the duration of each trial.

For all trials, each tank was divided transversely into four sections and longitudinally into three sections, to form a grid (Figure 6-1). The divisions were marked on the side of the tank using coloured tape. In positions B1-B3, one small, one medium and one large mussel were half-buried in the gravel, in an orientation similar to that observed in the wild. One piece of plastic tubing (15 cm long, 5.5 cm diameter) was placed in B4 and served as a crayfish shelter. The position of the three different sized mussels in B1-B3 was altered sequentially between tanks.

Figure 6-1 - Position of the mussels (M) and crayfish shelter (S) in each tank

6.3.2.1 Predation trial

A total of fifteen mussels were placed in five tanks as above and allowed to acclimate for 24 hours. One crayfish was then introduced to each tank. To avoid disturbing the nocturnal activities of crayfish, observations were made in daylight hours when crayfish were inactive; daily observations provided evidence, therefore, of the previous night's activities. During each daily observation, mussels were inspected for signs of predation by crayfish. Observations were made for seven days, after which all crayfish were removed

and euthanised.   No mussels were predated on by crayfish and these animals were retained for the disturbance trial.

6.3.2.2 Disturbance trial (five nights of crayfish exposure plus five nights without crayfish)

The disturbance trial commenced one week after termination of the predation trial.   Mussels from the predation trial were reused.   An additional 24 mussels were distributed between eight tanks to give a total sample size of 39 mussels. All mussels were allowed to acclimate for 24 hours.   Six crayfish (that had not been used in Trial 1) were added to six of the tanks.   No crayfish were placed in the seven remaining tanks.   To prevent the scent of crayfish from influencing mussels in other tanks, crayfish-free and crayfish-occupied tanks were connected to different water circulation systems. Observations of all thirteen experimental tanks were made for five nights, after which all crayfish were removed and euthanised.   The water circulation systems were topped up with fresh water.   Mussels were returned to their original positions and allowed to acclimate for 24 hours.   One crayfish was added to each of the seven tanks that had not received crayfish previously.   Observations of all tanks were made for five nights, after which the crayfish were removed and euthanised.

During each observation, the alphanumeric position and burial depth of each mussel and the position of the crayfish were recorded *in situ*, using the marked grid (Figure 6-1).   A photograph was also taken to allow measurement of daily mussel positions.   The marked grid provided a scale against which the distances of mussel position changes could be measured using a ruler; measurements were taken from the centre of the mussel's original position to the centre of its new position.   The number and distance of daily mussel position changes were calculated.

6.3.2.3 Crayfish behavioural observations

The nocturnal behaviour of crayfish in the presence of mussels was further investigated by taking video recordings using an infrared camera (Precision Lens Night Shot, 1:1.8/1:2.0) linked to a video recorder (Daewoo DV-K611) and monitor.   Infra-red lights (Monacor LED-IR) provided illumination.   Mussels from the previous trials were reused but all crayfish were fresh specimens.   One

crayfish was introduced to a transparent plastic tank (dimensions: 17.5 x 33.5 x 18.5 cm) that contained one medium-sized mussel, half-buried in gravel and a piece of plastic tubing for shelter. The camera was suspended above the tank using a tripod. Recordings were made in darkness (between 22:30 and 04:00) for three consecutive nights. The experiment was repeated for three more crayfish, allowing analysis of twelve nights of footage. All crayfish were removed and euthanised after the experiment.

Video footage was analysed by recording the dominant activity and position of the crayfish every five minutes, for 30 seconds. The activity of the crayfish was recorded as either: inactive and not in contact with the mussel; inactive and in passive contact with the mussel (i.e. sitting on/touching the mussel but not moving); active and not in contact with the mussel; active and in passive contact with the mussel (i.e. walking or climbing over the mussel); or active and in direct contact with the mussel. Crayfish activity was further categorised as: moving, walking/climbing, digging or attacking mussel. Mussel attacks were defined as aggressive encounters in which the crayfish attempted to crack open the shell of the mussel using the mandibles and chelae. Crayfish position in the tank was categorised as: top, middle or bottom and inside or outside of the shelter.

6.3.3 Statistical analysis

All statistical analyses were conducted in R (version 2.13.1) using the glmmADMB package (http://glmmadmb.r-forge.r-project.org). Count data from the Disturbance Trial was tested for its fit with a Poisson distribution (a standard distribution for count data) but was found to have a better fit for a negative binomial distribution. This distribution was used to test for the effect of crayfish presence or mussel size on the frequency of mussel movements: the response variable was the total number of mussel position changes during the trial; crayfish presence and mussel size category were fixed factors; mussel ID and time (exposure to the crayfish treatment in the first or second half of the trial) were random effects. Non parametric tests (Mann Whitney U-test, Kruskal-Wallis test) were used to test the effect of crayfish presence and mussel size on the distance of mussel movements.

6.4 Results

6.4.1 Predation trial

After seven days, there was no predation of mussels by crayfish, nor any evidence of shell damage. It was noted that mussels often changed positions in the gravel between observations. The effect of crayfish presence on the number and distance of mussel position changes was investigated further during the disturbance trial.

6.4.2 Disturbance trial

6.4.2.1 Number of mussel position changes

The number of mussel position changes over five nights was significantly greater in the presence of crayfish (Figure 6-2, negative binomial model, $z = 2.73$, $p < 0.001$, $n = 78$) than when mussels were kept alone. There was no effect of mussel size on the number of position changes.
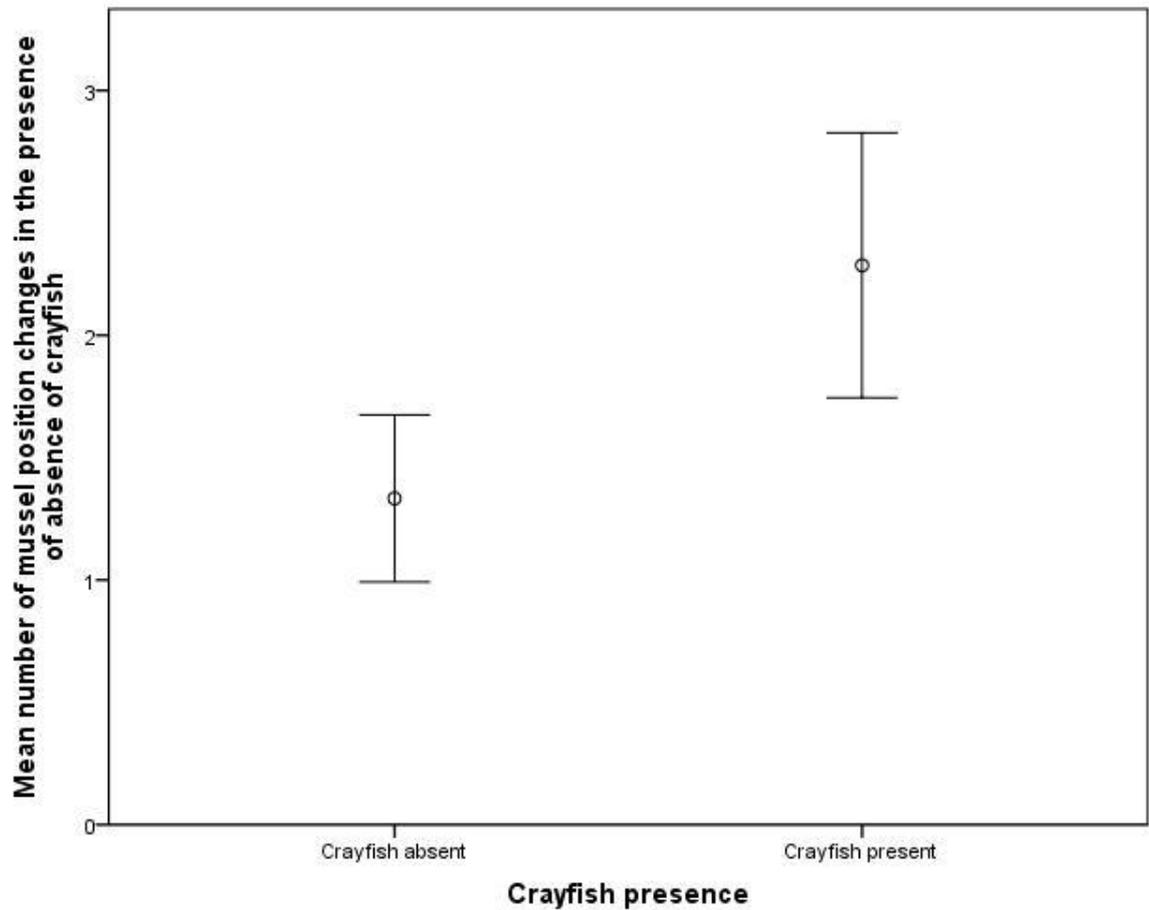
Figure 6-2 - Number of mussel position changes in the presence and absence of crayfish (number of mussels = 39). Data are presented as mean number of position changes per treatment ± 95% confidence intervals.

6.4.2.2 Distance of mussel position changes

There was no significant difference between the distance of daily mussel position changes in the presence and absence of crayfish (Mann Whitney U = 497, p > 0.932, n = 69).   There was no effect of mussel size on the distance of position changes (Kruskal-Wallis Test, x^2 =1.166, df = 2, p > 0.6).   The maximum distance moved by mussels in any one night was 17 cm in the absence of crayfish and 32 cm when crayfish were present.   The distance moved was more variable in the presence of crayfish (SD = 6.95 with crayfish, compared with 5.68 without).

6.4.3 Crayfish behavioural observations

All crayfish attacked mussels within the first night of exposure (Figure 6-3). Attacks on mussels were observed on all nights for all crayfish, apart from Crayfish 1 which remained in its shelter on the second night.
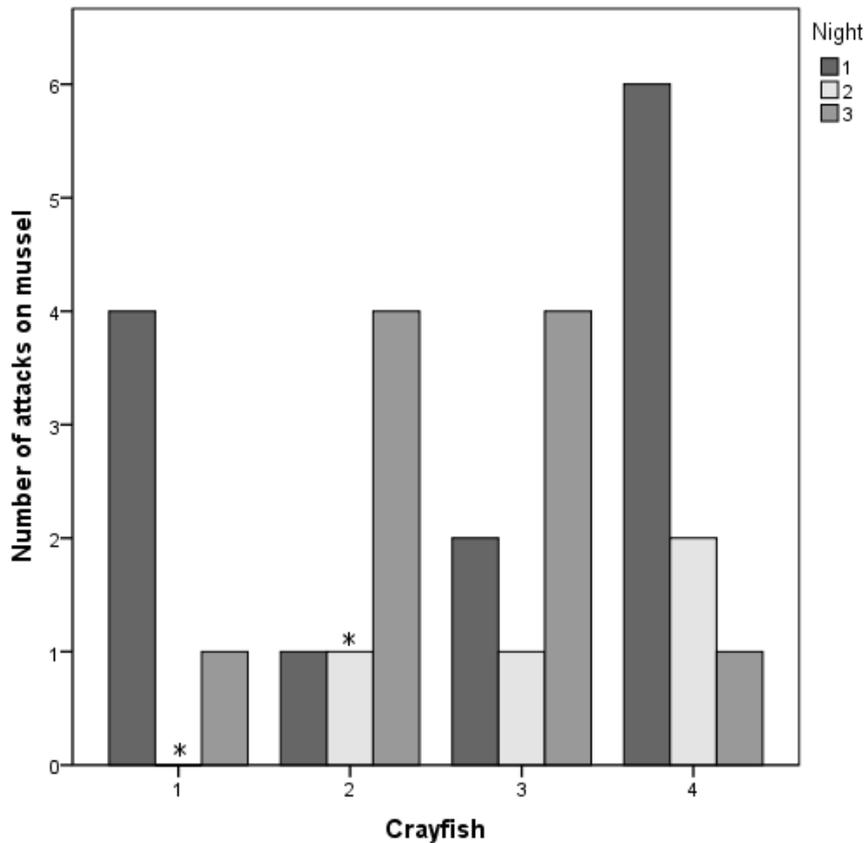
Figure 6-3 - Number of attacks on the mussel by each of four crayfish on three consecutive nights. Observations were conducted every five minutes between 2230 and 0400 hours (number of observations = 67 per night). Asterisks denote nights in which a recording failure reduced the number of observations to 43.

During a typical attack (for screen captures, see Figure 6-4), crayfish used the chelae and walking legs to dislodge the mussel from the gravel; the chelae were often used to manipulate the position of the mussel by rolling it over or carrying it around the tank. Both the mandibles and chelae were then used to try and crack open the shell. The chelae held the mussel in place while the mandibles made contact with the edge of the shell. Mussel valves remained closed throughout the attack. None of the attacks resulted in successful predation of mussels by crayfish.
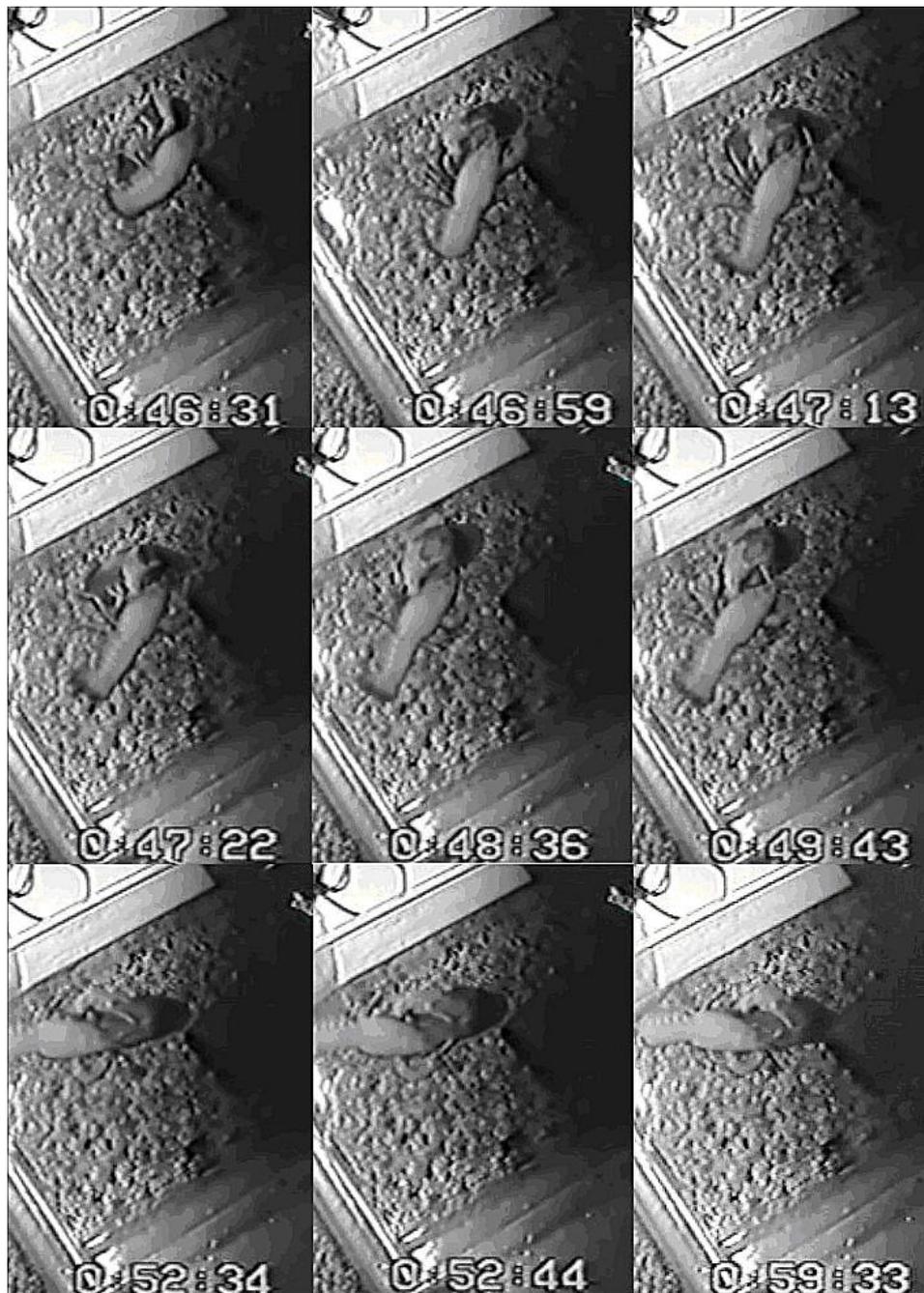
Figure 6-4 – Screen capture of crayfish attacking mussel

Figure 6-5 shows the activity of the four crayfish during the three nights. Crayfish were active at least some of the time each night, apart from one individual (Crayfish 1) which was completely inactive on Night 2. A recording failure on this night reduced the number of observations of Crayfish 1 and Crayfish 2 from 67 to 43 however, so the activity of these individuals may have been underestimated.
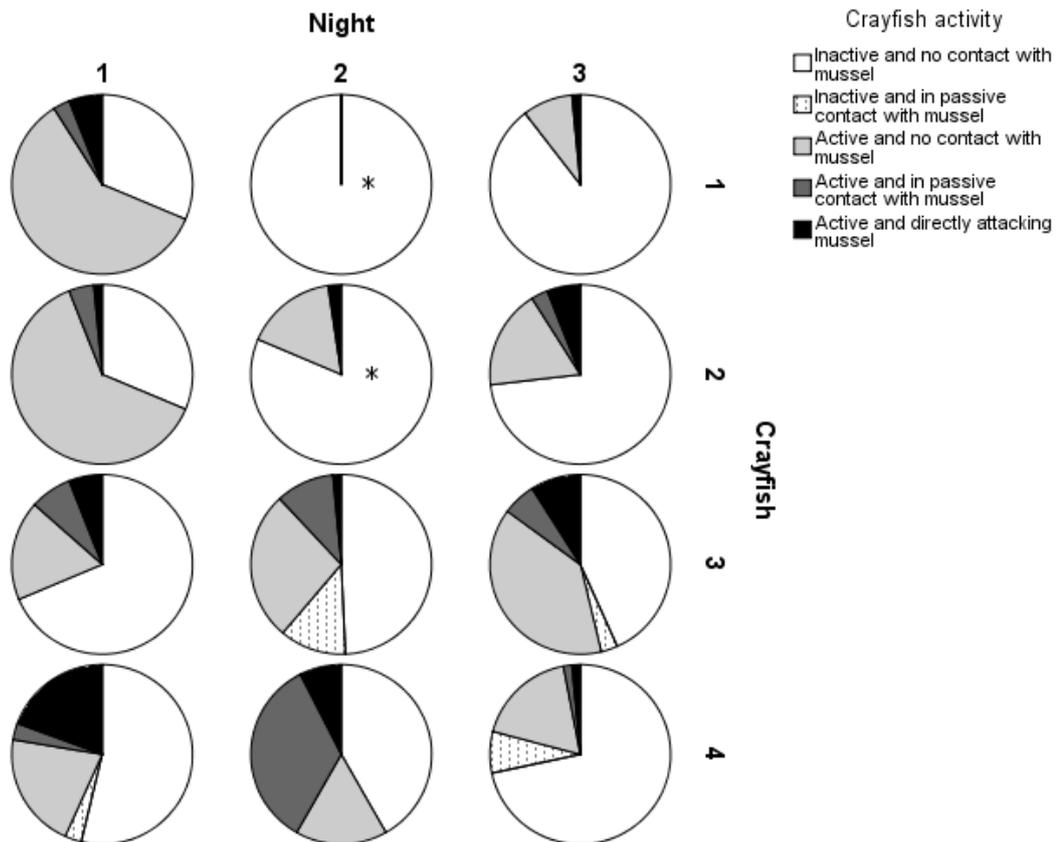
Figure 6-5 - Proportion of time in which crayfish were in contact with mussels on three consecutive nights. Observations were conducted every five minutes between 2230 and 0400 hours (number of observations = 67 per night). Asterisks denote nights in which a recording failure reduced the number of observations to 43.

When active, crayfish spent 8.7-71.8% of their time in contact with the mussel, either passively (by walking/climbing over the mussel) or directly (by deliberately attacking the mussel) (Table 6-1). Direct attacks constituted 12.6-100% of contacts during active periods. Even during inactive periods, crayfish were sometimes observed in contact with (i.e. sitting on or touching) the mussel.

Table 6-1- Percentage (%) of active and inactive periods which were spent in contact with the mussel. Numbers in brackets are the % of contacts which were direct attacks. Observations were conducted every five minutes between 2230 and 0400 hours (number of observations = 67 per night). Asterisks denote nights in which a recording failure reduced the number of observations to 43.

Crayfish	Night	% of active period spent in contact with mussel, of which ( ) % were direct attacks	% of inactive period spent in contact with mussel
1	1	13.1 (66.7)	0
	2*	Inactive	0
	3	14.3 (100)	0
2	1	8.7 (25.1)	0
	2*	12.4 (100)	0
	3	34 (66.6)	0
3	1	43 (44.4)	0
	2	30.7 (12.6)	19.4
	3	27.8 (60.2)	0.6
4	1	51.7 (86.7)	5.3
	2	71.8 (17.9)	0
	3	14.4 (49.8)	9.5

6.5 Discussion

6.5.1 Predation

This study demonstrates that under laboratory conditions, *P. leniusculus* will attempt to predate on *M. margaritifera*. Despite repeated efforts, all predation attempts were unsuccessful. This lack of success may be due to a lack of familiarity with the prey type or a basic inability of *P. leniusculus* to overcome the physical or behavioural defences of *M. margaritifera* of the size range 54-110 mm that was available in this study.

Previous studies have found that invasive crayfish only require a short learning time to identify and exploit new prey. Juveniles of the red swamp crayfish *P. clarkii* needed less than 12 hours to learn how to efficiently predate novel larvae prey (*Chaoborus* sp.) (Ramalho and Anastácio, 2011). Another study showed that *P. clarkii* that were naïve to *Corbicula* clams would consume them within the first few days of exposure and that consumption rate increased after four days, suggesting a familiarisation process (Covich *et al.*, 1981). Despite being novel prey for the virile crayfish *O. virilis*, zebra mussels (*Dreissena polymorpha* Pallas) were readily consumed during feeding trials, with no change in feeding rate (i.e. no familiarisation process) through time (Love and Savino, 1993). During the present study, *P. leniusculus* were naïve to *M. margaritifera* yet clearly recognised it as prey, attacking within the first night of exposure. The ability to rapidly recognise new prey may in part explain the success of non-native crayfish when invading new ecosystems (Ramalho and Anastácio, 2011). Despite this quick predatory response, *P. leniusculus* were not able to consume *M. margaritifera*, even after seven nights of exposure. Given this generous exposure period, it is likely that factors other than learning time are responsible for the observed lack of predation.

The most likely conclusion from the results of this study is that *P. leniusculus* is simply incapable of consuming adult *M. margaritifera*. Natural predation of *M. margaritifera* is rare and restricted to a few opportunistic species: hooded crows (*Corvus corone cornix* L.) have been observed dropping mussels from a height to smash open the shells before consumption; oystercatchers (*Haematopus ostralegus* L.) predate mussels by using their bills to prise the valves apart or by

hammering through the shell; American mink (*N. vision*) and the European otter (*L. lutra*) are also occasional predators (Cosgrove *et al.* 2007). In comparison with these natural predators, *P. leniusculus* is smaller and probably weaker and more restricted in the behavioural techniques available to crack open the shell of *M. margaritifera*. In a field study of *M. margaritifera* in a stream infested with *P. leniusculus*, Schmidt and Vandré (2009) found mussels with shell perforations that were presumed to be derived from crayfish attacks. This observation is unusual in light of the present study in which no shell damage by *P. leniusculus* was observed, even after a generous familiarisation period. This suggests that the shell injuries observed by Schmidt and Vandré (2009) were not derived from *P. leniusculus* attacks or that *P. leniusculus* is capable of causing injury to *M. margaritifera* but under a different set of conditions from those provided in the present study.

Numerous studies have found size-selective predation of molluscs by crayfish and it was hypothesised that *P. leniusculus* would choose to predate on small *M. margaritifera* rather than medium or large mussels. In laboratory experiments, *P. leniusculus* was shown to consume a greater number of small zebra mussels (7-12 mm) than medium (16-21 mm) or large (25-30 mm) ones (Ermgassen and Aldridge, 2011). The number of attacks on medium and large mussels was, however, greater than on small mussels, perhaps due to a higher encounter rate. The apparent preference for small mussels may reflect the inability of crayfish to predate larger mussels, rather than an innate preference for small ones. Similarly, Klocker and Strayer (2004) observed that *Orconectes spp.* would only predate on freshwater mussels (Unionidae) smaller than 8.9 mm but found many mussels larger than this with extensive shell damage. Small bivalves are easier to manipulate than large ones (Warner, 1997); they also have thinner shells which may be more easily cracked open by crayfish (Covich *et al.*, 1981). In the current study, even the smallest *M. margaritifera* evaded predation by *P. leniusculus*. There was no effect of mussel size on the number of mussel position changes, suggesting that crayfish were not size-selective in their attacks, or that different-sized mussels responded similarly to crayfish. The smallest *M. margaritifera* tested here were 54-76 mm and thus considerably larger than the bivalves used in previous studies, which have focussed on smaller species. The maximum size of prey that *P. leniusculus* can exploit has not been

rigorously quantified but it is likely that the mussels tested here exceeded this critical size. The Appalachian brook crayfish *Cambarus bartonii* [Fabricius] has been found to consume relatively large *Corbicula* (24-35 mm) but only if shells have been previously damaged (Covich *et al.*, 1981). All *M. margaritifera* in the present study had intact shells; it is conceivable that shell damage would increase the predation risk for *M. margaritifera* as it does for *Corbicula*. In the wild, organic pollution has been linked to lighter, more brittle shells in *M. margaritifera* which might also be expected to elevate predation risk (Frank and Gerstmann, 2007).

These observations suggest that adult *M. margaritifera* are protected from predation by *P. leniusculus*, probably due to the thick protective shell. The width of the inner and outer shell layers of *M. margaritifera* increases with age (Helama and Valovirta, 2007) and so large mussels may be at a lower risk of predation than small ones. Predation of small, thin-shelled juvenile mussels may be more likely and this requires investigation. The loss of juveniles would be particularly undesirable given their rarity in the wild: surveys of Scottish *M. margaritifera* found that only 17 of 52 functional populations contained juvenile mussels below 20 mm (Cosgrove *et al.*, 2000). Most European populations are overaged, with the youngest individuals frequently being 30-50 years old (Geist, 2010). Protection of the few sites that still contain *M. margaritifera* juveniles from the threat of *P. leniusculus* invasions would be a sensible precaution.

6.5.2 Disturbance

A greater number of *M. margaritifera* position changes occurred when *P. leniusculus* were present than when they were absent; this was probably due to the manipulation of *M. margaritifera* by *P. leniusculus* during predation attempts. However, *M. margaritifera* can also move of its own accord and so it is possible that position changes are indicative of predator avoidance behaviour, or a search for more favourable habitat.

Predator avoidance behaviour in *M. margaritifera* has not been widely studied. Like other bivalves, *M. margaritifera* closes its valves when startled to protect the vulnerable inner tissues and this was observed in the video footage during crayfish attacks. A recent study showed that *M. margaritifera* take longer to

reopen their valves after startling (e.g. tapping the shell) when they are also exposed to the scent of crayfish *A. pallipes* (Wilson *et al.*, 2012). After handling, small *M. margaritifera* show prolonged periods of inactivity, which is thought to be a predator avoidance strategy for the species (Wilson *et al,.* 2012). Reducing movements or increasing attachment to the sediment may be advantageous for sedentary animals in the presence of predators, since escape by movement is unlikely (Toomey *et al.*, 2002). During the video recordings in the present study, *M. margaritifera* did not move; instead of righting themselves and burrowing back into the gravel after excavation by *P. leniusculus*, *M. margaritifera* remained horizontal and motionless in the tank. Based on the current observations and those of previous studies, there is no evidence, therefore, that *M. margaritifera* increases its movements in response to predators; in fact, the opposite appears to be true. The increased number of *M. margaritifera* position changes in the presence of *P. leniusculus* was most likely due to the direct action of *P. leniusculus*.

Overall, there was no significant difference between the distances of *M. margaritifera* position changes in the presence and absence of *P. leniusculus*; distances were, however, slightly more variable in the presence of *P. leniusculus* and the maximum distance was almost twice as big as when *P. leniusculus* were absent. As sedentary animals, mussels have a limited capacity for movement; the higher maximum distance of position change in the presence of *P. leniusculus* further suggests interference by *P. leniusculus*.

The disturbance of *M. margaritifera* by *P. leniusculus* may have adverse impacts on the survival of *M. margaritifera*. If *M. margaritifera* is excavated by *P. leniusculus* then this may increase the risk of predation by other, more adept predators. It may also result in larger-scale displacement by river currents, causing physical damage and movement away from suitable habitats. Following excavation, *M. margaritifera* must expend energy if it is to burrow back into its preferred position. Valve closure in response to contact with *P. leniusculus* is also energetically expensive and forces *M. margaritifera* to reduce the amount of time spent feeding, respiring and excreting waste (Wilson *et al.*, 2012). Previous studies have demonstrated the impact of disturbance on other bivalve species such as the hard clam (*Mercenaria mercenaria* Linné) and bay scallop (*Argopecten irradians concentricus* Say) (Irlandi and Mehlich, 1996). The growth

rate of juvenile scallops was 25% faster in experimental cages containing low numbers of browsing fish, compared with high numbers. Laboratory experiments confirmed that scallops spend significantly less time with their valves open (i.e. feeding) in the presence of browsing fish, compared with when fish are absent (Irlandi and Mehlich, 1996). Similar disturbance effects might be expected to cumulatively reduce the feeding, growth and long-term survival of *M. margaritifera*.

Further disturbances may be derived from the burrowing activity of *P. leniusculus*. Video footage showed *P. leniusculus* digging in the gravel close to *M. margaritifera*. In the wild, burrowing by crayfish accelerates bank and river bed erosion (Guan, 1994) leading to increases in the bedload flux (Johnson *et al.*, 2010) and water turbidity (Angeler *et al.*, 2001). An increase in sediment load may be detrimental to the filtering ability of *M. margaritifera*, which closes its valves and stops feeding if loads are too high (Beasley *et al.*, 1998). Recent research has indicated that high turbidity and sedimentation in streams are strongly associated with recruitment failure in *M. margaritifera* (Österling *et al.* 2010). Laboratory studies have also shown that *P. leniusculus* significantly modifies the topography of gravel substrates, reducing the stability of the bed (Johnson *et al.*, 2010). Such changes to gravel bed structure would be expected to adversely impact *M. margaritifera*, which prefers to colonise stable substrata (Hastie *et al.*, 2000).

Finally, although not investigated here, *P. leniusculus* has the potential to indirectly impact *M. margaritifera* through adverse interactions with salmonids. *P. leniusculus* adversely impacts juvenile Atlantic salmon (*S. salar*) through competition for shelter (Griffiths *et al.*, 2004) and has also been linked to the reduced recruitment of salmonids in streams in England (Peay *et al.*, 2009). *M. margaritifera* is dependent on salmonids for a fundamental part of its life cycle (Young and Williams, 1984). If *P. leniusculus* initiates or exacerbates the loss of fish stocks in areas inhabited by *M. margaritifera*, then this could further imperil *M. margaritifera* populations.

6.5.3 Conclusions

This study makes a number of preliminary deductions about the potential impact of *P. leniusculus* on *M. margaritifera*. It is clear that *P. leniusculus* recognises *M. margaritifera* as prey and launches attacks on proximal mussels. No predation attempts were successful, probably due to the thick, protective shell of *M. margaritifera*; smaller, thin-shelled juvenile mussels may be more vulnerable than the adult mussels tested here. Finally, although no mussels were predated, *M. margaritifera* may be subject to disturbance and indirect effects of *P. leniusculus.*

These observations, although informative, should be regarded with caution before making extrapolations to the wild, in which a range of biotic and abiotic factors will influence crayfish and mussel behaviour. Feeding trials are limited in their ability to mimic the full assemblage of prey available to a predator in natural conditions. When prey types were offered singly, the virile crayfish *O. virilis* was found to consume zebra mussels and rainbow trout eggs at the same rate; when both prey were present, however, *O. virilis* preferred to consume trout eggs, which had a much lower handling time (Love and Savino, 1993). Similarly, predation of zebra mussels by *O. propinquus* was reduced when crayfish were also offered macrophyte foods (MacIsaac, 1994). No alternative food source was offered to P. leniusculus in the current study.

The omnivorous and opportunistic nature of *P. leniusculus* means that predicting the feeding behaviour of crayfish in the wild is difficult but factors such as handling time and prey availability are expected to be important. The large effort required to penetrate the shell of *M. margaritifera* may make it a less attractive prey choice than other items, at least when adult mussels are concerned. Other factors not investigated here include the potential effects of crayfish size (Guan and Wiles, 1998), sex (Pérez-Bote, 2004), season (Correia, 2002) and density (Ellrott *et al.*, 2007) on feeding behaviour.

The water chemistry of areas inhabited by *M. margaritifera* may be an important abiotic factor in determining the likelihood of a successful invasion by crayfish. *M. margaritifera* prefers to inhabit oligotrophic, nutrient-poor waters that are low in calcium. In a study of *M. margaritifera* in Germany, Bauer (1988)

recorded favourable calcium levels of 2 ppm and suggested that at levels above this, populations will not reproduce successfully. Crayfish, by contrast, require high levels of calcium for post-moult mineralization of the exoskeleton, with growth rates generally increasing with water hardness (Aiken and Waddy, 1987). It might be supposed, therefore, that differences in the calcium requirements of the two species will negate the co-occurrence of *M. margaritifera* and *P. leniusculus* in the wild. The two species already co-occur in a German river, however, demonstrating that overlap is possible (Schmidt and Vandré, 2009). This is further supported by the observation that signal crayfish have successfully established in water bodies with very low calcium levels (e.g. 1.6 ppm in Finland, Westman and Savolainen, 2002); in addition, some atypical populations of *M. margaritifera* in England and Ireland have been found to tolerate calcareous waters (Skinner *et al.*, 2003).

Future studies should aim to determine the relative importance of the biotic and abiotic factors mentioned above and help clarify the threat posed by *P. leniusculus* to *M. margaritifera*. Knowledge of potential stressors to *M. margaritifera* populations will be essential in facilitating effective conservation of this highly endangered species.

Chapter 7: The effectiveness of trapping as a control method for signal crayfish at Loch Ken

7.1 Abstract

In the summer of 2009, a four-month trapping programme was conducted on Loch Ken, in order to assess the scale of the infestation of *P. leniusculus* and the feasibility of controlling the population. The effects of this removal programme on the population were investigated using mark and recapture methods. The removal programme was found to significantly reduce the number of males in the population but its effect on females was complicated by seasonal variation in trappability and the bias of traps towards males. By the end of the programme the sex ratio of the catch was skewed towards females. Trapping also reduced the mean size of crayfish and it is hypothesised that continual trapping would further depress this further. Crayfish were shown to make significant movements of up to 800 m in two weeks, providing another insight into their invasive capabilities in lochs. Depth was an important determinant in the distribution of crayfish within the loch, with greater numbers captured in shallow water. Finally, the total catch of crayfish was shown to be potentially misleading as an indicator of population size in comparison with mark and recapture data. Crayfish occur in Loch Ken at densities which are high compared with other lakes and the loss of native biodiversity is expected to be considerable. It is also suggested that trapping may have a greater impact if used in conjunction with other control methods that reduce the number of juvenile crayfish and if factors such as seasonality in crayfish activity and habitat preference are taken into consideration.

7.2 Introduction

There are now several well-established populations of non-native *P. leniusculus* in Scotland, occurring in a range of habitats including ponds, streams, rivers and lochs. The largest population is thought to inhabit Loch Ken near Castle Douglas in southern Scotland where it originated from the introduction of *P. leniusculus* to feeder streams in the Water of Ken catchment (Maitland, 1996). Previous studies have highlighted the adverse impacts of introduced *P. leniusculus* on native biodiversity including macrophytes (Nyström and Strand, 1996), invertebrates (Crawford *et al.*, 2006) and fish (Peay *et al.*, 2009). Given the sizeable population of *P. leniusculus* at Loch Ken, significant environmental impacts are expected, as experienced in other invaded lakes (e.g. Josefsson and Andersson, 2009). Although there have been no studies to formally assess the impacts of *P. leniusculus* at Loch Ken, local knowledge has suggested adverse effects on native fish and there is also visible bank erosion and vegetation loss in areas where the density of crayfish burrows is high (Figure 7-1, Andrew Blunsum, 2011, pers. comm.). In addition, the infestation is having a detrimental effect on local businesses that are reliant on angling activities for income. Crayfish cause nuisance to anglers by taking bait intended for fish and as a result, fishermen are increasingly reported as taking their trade elsewhere.

Figure 7-1 – Damage to banking and associated vegetation as a result of crayfish burrows. Photo courtesy of Andrew Blunsum, Loch Ken Ranger.

In order to mitigate the major ecological and economic impacts imposed by non-native *P. leniusculus*, attempts have been made to control nuisance populations using a variety of mechanical, chemical and biological methods (for a review, see Freeman *et al.*, 2010). The appropriateness of different control strategies will vary according to the numerous habitat types populated by crayfish (Freeman *et al.*, 2010). Chemical control using biocides for example, which has had some success in eradicating crayfish from ponds (Peay *et al.*, 2006, Sandodden and Johnsen, 2010) would be impractical in a large river or a lake such as Loch Ken. Other methods like electrofishing and netting are also expected to be ineffective in large water bodies. The introduction of exotic predators as a means of biological control has been used to manage populations of other pest species but remains a controversial approach (Freeman *et al.*, 2010). Options for the removal of crayfish from Loch Ken are therefore limited. One potential method is the use of trapping, which has been employed in the commercial harvest and control of crayfish in other lakes (Bills and Marking, 1988, Hein *et al.*, 2006, Hein *et al.*, 2007, Karen *et al.*, 2004).

Between June and September 2009, the Scottish Government (Marine Scotland) funded an intensive four-month trapping programme on Loch Ken in order to assess the scale of the crayfish infestation and the feasibility of controlling the population by this method. The following study aimed to build on this work by determining the impact of the removal programme on the crayfish population. The effects of intensive trapping on the number, size and sex ratio of crayfish were investigated. Crayfish movements and the influence of depth on catch were also examined. The findings and implications for control of the *P. leniusculus* population at Loch Ken are reported here.

7.3 Materials and methods

Between 23 June and 1 September 2009, approximately 659, 300 crayfish were removed from Loch Ken by intensive trapping. In order to assess the impact of this intensive trapping on the adult crayfish population, two mark and recapture studies were conducted, as described below.

7.3.1 Study sites

Sampling was conducted along three different transects (Figure 7-2): Site 1 (National Grid Reference NX 64793 74628 to NX 65029 74363), Site 2 (NX 64158 75122 to NX 64394 74850) which was north of Site 1 and Site 3 (NX 65200 73981 to NX 65363 73658) which was south of Site 1. The sites were located approximately 400 metres apart and within the designated area for the trapping programme. At each site, a 400 metre line consisting of 15 equally-spaced creels (dimensions: 0.56 m x 0.41 m x 0.3 m, mesh size 22 x 22 mm) was placed parallel to the shore line. Creels were deployed from a boat (9.95m Catamaran) which was fitted with a hydraulic creel hauler and self-shooting system. The position and depth of each creel was recorded using a GPS system and hydroacoustic depth finder, available on the boat. Surface water temperature at the time of trap setting was recorded using a thermometer and ranged from 11.4 to 12.2°C during the first mark and recapture session and from 12.9 to 14.2°C during the second mark and recapture session.
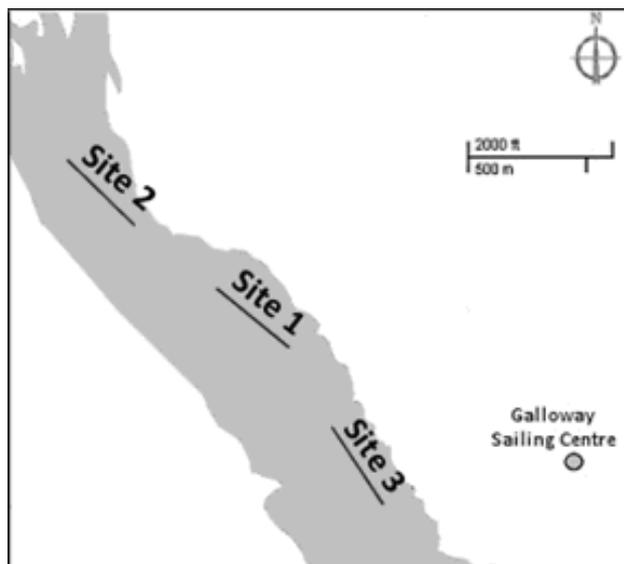
Figure 7-2 - Location of sampling sites.

7.3.2 Mark and recapture methods

Estimates of crayfish population abundance at the three sites were made via the mark and recapture method. Crayfish were captured, marked, released (under licence) and later resampled during two different mark-recapture sessions, conducted before and after the removal programme. The first session was completed on 19th-21st May 2009 (capture, mark and release) and 2nd-4th June 2009 (resampling), prior to commencement of the removal programme. The second session was completed on 10th-12th September 2009 (capture, mark and release) and 24th-26th September 2009 (resampling), after termination of the removal programme.

The capture, marking and release of crayfish at the three sites was conducted over three consecutive days (one site per day) during both sessions. At each site, a line of creels was baited with herring (*C. harengus*) and set overnight. The following morning, creels were retrieved and all crayfish emptied into buckets of water, which were covered to provide shelter from sunlight.

The sex and carapace length of all animals were recorded. Carapace length was measured in millimetres, from the rear of the eye socket to the edge of the carapace using Vernier callipers (± 0.1 mm). The number of berried females was also determined. All crayfish caught were batch marked by punching two holes in the telson and uropods with a needle (Guan, 1997) (Figure 7-3) and by applying coloured acrylic varnish to the carapace (Figure 7-4). The combination of holes and varnish colour used to mark the crayfish was varied both spatially (between sites) and temporally (between the May and September sessions).
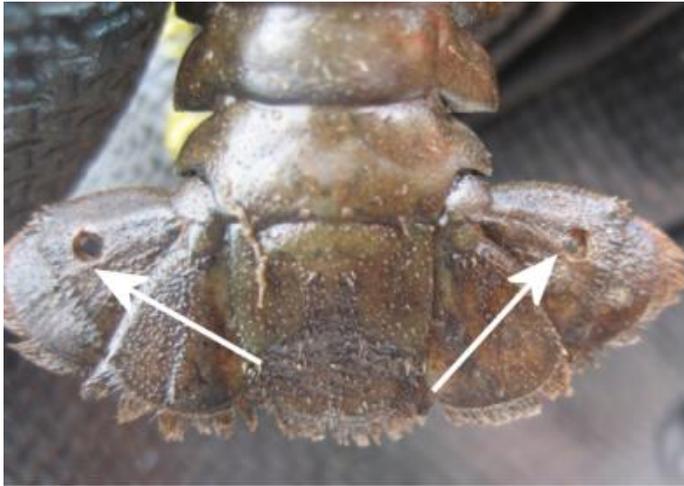
Figure 7-3 - Example of holes punched in the uropods; the position of the holes was varied among sites and between sampling periods.

Figure 7-4 - Example of a crayfish marked with acrylic varnish; different colours were used according to site and sampling period.

Resampling was conducted after two weeks to allow dispersal and mixing of marked animals, as recommended by Nowicki *et al*. (2008).   The number of marked crayfish recaptured was counted, and all animals were measured and sex determined as before.   After processing, all resampled crayfish were killed in a boiler of hot water on board the boat.

7.3.3 Statistical analysis

Due to the bias of traps towards catching male crayfish, data for the sexes were analysed separately.

Estimates of the size of the trappable population were calculated using a modification of the Peterson formula, as used in previous studies of crayfish populations (e.g. Skurdal *et al.*, 1992) and described by Krebs (1998) as:

$$N = \frac{(M + 1)(C + 1)}{(R + 1)} - 1$$

Where:

N = Estimate of population size

M = Total number of crayfish marked during the first visit

C = Total number of crayfish captured during the second visit

R = Total number of crayfish marked during the first visit that were captured during the second visit

The Peterson formula has the following assumptions:

☐ The population is closed such that N is constant

☐ All animals have an equal chance of being captured during the first visit

☐ Marking has no impact on catchability

☐ Marks are retained between the sampling periods

☐ Marked animals become randomly mixed with unmarked animals

Although the population at Loch Ken is not strictly 'closed', this method was deemed adequate for the purposes of this study, which aimed to assess the relative numbers of crayfish before and after the removal programme rather than obtain an accurate figure for population size. The Poisson distribution was used to estimate upper and lower 95% confidence limits for each population estimate. Population estimates were converted into population densities based on an effective sampling area of 13 m^2 per creel, as applied in previous studies of crayfish in lakes (in which trapping efficiency was estimated in lakes

containing a known crayfish density, based on SCUBA surveys) (Abrahamsson and Goldman, 1970, Kirjavainen and Westman, 1999, Lodge and Lorman, 1987).

Counts of the number of crayfish captured per creel were square-root transformed for normality and Student t-tests were used to test for significant differences between the number of crayfish caught before and after the removal programme. Student t-tests were also used to test for differences between the carapace lengths of males and females and of crayfish caught before and after the removal programme.

The effect of depth on the number and size of crayfish caught was tested for significance using Spearman's rank correlation test.

Statistics were applied using PASW Statistics (v.18) software.

7.4 Results

7.4.1 Marking methods

Of the two methods of marking employed, punching holes in the telson and uropods (Guan, 1997) proved a more reliable marking method. All crayfish were marked with two holes but examination of recaptured animals showed that in 4.7% of animals recaptured after two weeks, only one hole remained. All recaptured crayfish marked with acrylic varnish were also marked with one or more holes. 11.3% of recaptured crayfish marked with holes, however, were no longer marked with varnish.

7.4.2 Effect of the removal programme on total catch

A total of 3879 crayfish (2352 in capture and 1527 in recapture sessions) were caught before the removal programme (in May/June) and 3205 crayfish (1489 in capture and 1716 in recapture sessions) after the removal programme (in September). Figure 7-5 shows the total catch of males and females before and after the removal programme. No berried females were captured in September.
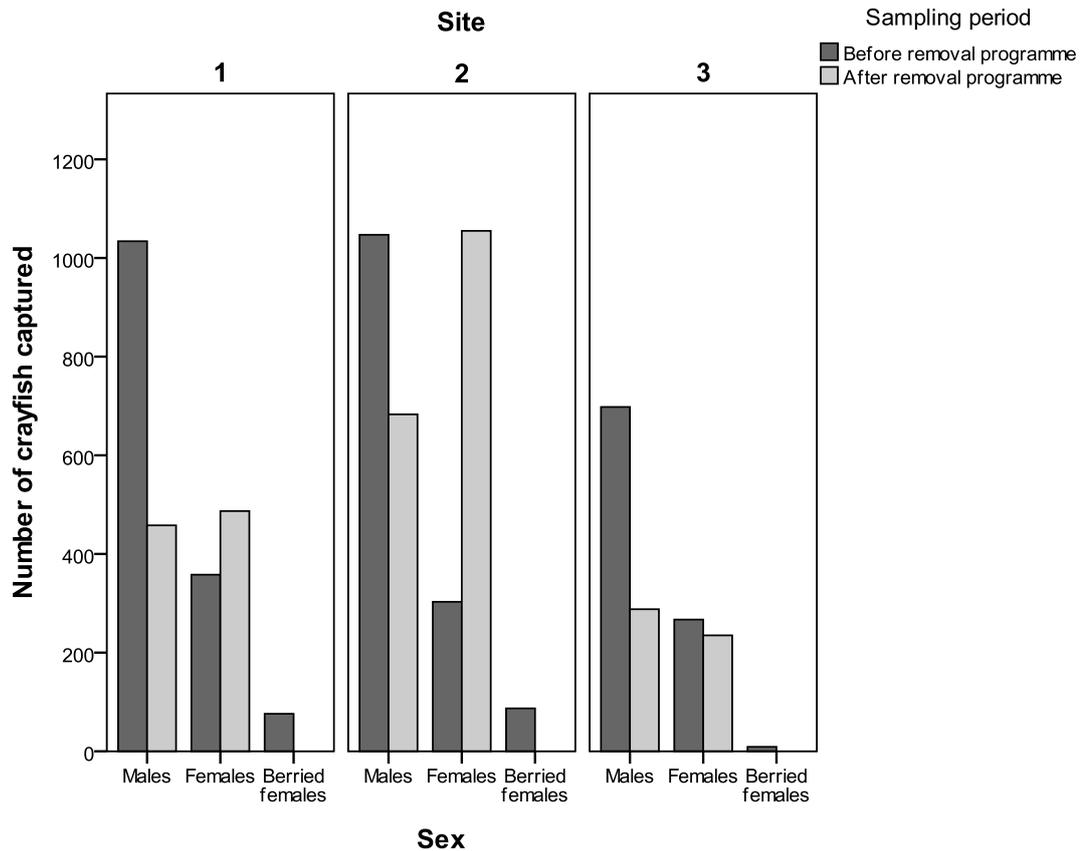
Figure 7-5 - Total numbers of male, non-berried female and berried female crayfish caught during the two mark and recapture sessions before (May/June) the removal programme and after (September) the removal programme.

Figure 7-6 shows the mean number of male and female (berried and non-berried females combined) crayfish captured per creel before and after the removal programme. At all sites, there was a significant reduction in the number of males captured per creel after the removal programme (Site 1: $t = 6.260$, $p < 0.001$, $df = 58$; Site 2: $t = 2.656$, $p < 0.01$, $df = 58$; Site 3: $t = 3.726$, $p < 0.001$, $df = 58$), with reductions of 55.9%, 34.3% and 56.5% at Sites 1-3 respectively. This was not the case for females: at Site 1 and Site 3, the catch of females did not change significantly after the removal programme (Site 1: -0.886, $p > 0.3$, $df = 58$; Site 3: $t = 0.008$, $p > 0.9$, $df = 58$), with increases of 12.2% and 11.1% respectively. At Site 2 there was a significant increase of 169.2% in the number of female crayfish captured per creel after the removal programme ($t = -6.053$, $p < 0.001$, $df = 58$).
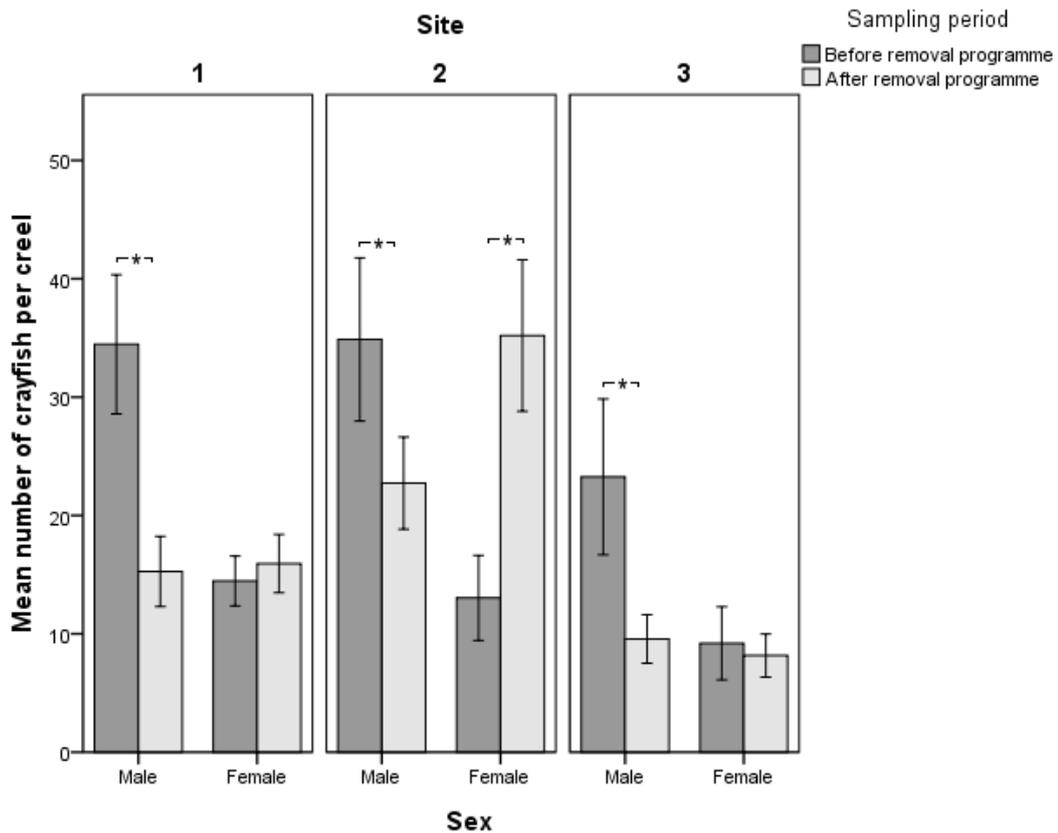
Figure 7-6 - Mean number of crayfish per creel caught before and after the removal programme. Data for non-berried and berried females were combined. Error bars are 95% confidence intervals. Asterisks denote pairwise statistically significant differences.

7.4.3 Sex ratios

The sex ratios of the catch taken before and after the removal programme at each site are shown in Table 7-1. In all cases, there was an increase in the proportion of females caught after the removal programme.

Table 7-1 - Sex ratio of catch from each site, before and after the removal programme.

Site 1		Site 2		Site 3	
Before	After	Before	After	Before	After
1 male to 0.42 females	1 male to 1.04 females	1 male to 0.37 females	1 male to 1.54 females	1 male to 0.40 females	1 male to 0.85 females

7.4.4 Effect of the removal programme on crayfish size

The mean carapace length of male crayfish was greater than that of female crayfish both before and after the removal programme (t_{before} = 19.467, $p < 0.001$, df = 3919; t_{after} = 2.660, $p < 0.01$, df = 3203).

Figure 7-7 shows the mean carapace lengths of crayfish at each site before and after the removal programme. At all sites, the mean carapace length of male

crayfish was significantly reduced after the removal programme (Site 1: t = 17.618, p < 0.001, df = 1490; Site 2: t = 4.763, p < 0.001, df = 1728; Site 3: t = 4.257, p < 0.001, df = 983); for females, however, the carapace length was only significantly reduced at Site 1 (t = 8.664, p < 0.001, df = 909). At Site 2 there was a significant increase in the carapace length of females after the removal programme (t = -4.974, p < 0.001, df = 1443); at Site 3, there was no significant difference in the mean carapace length of females before or after the programme (t = -1.034, p > 0.3, df = 519).
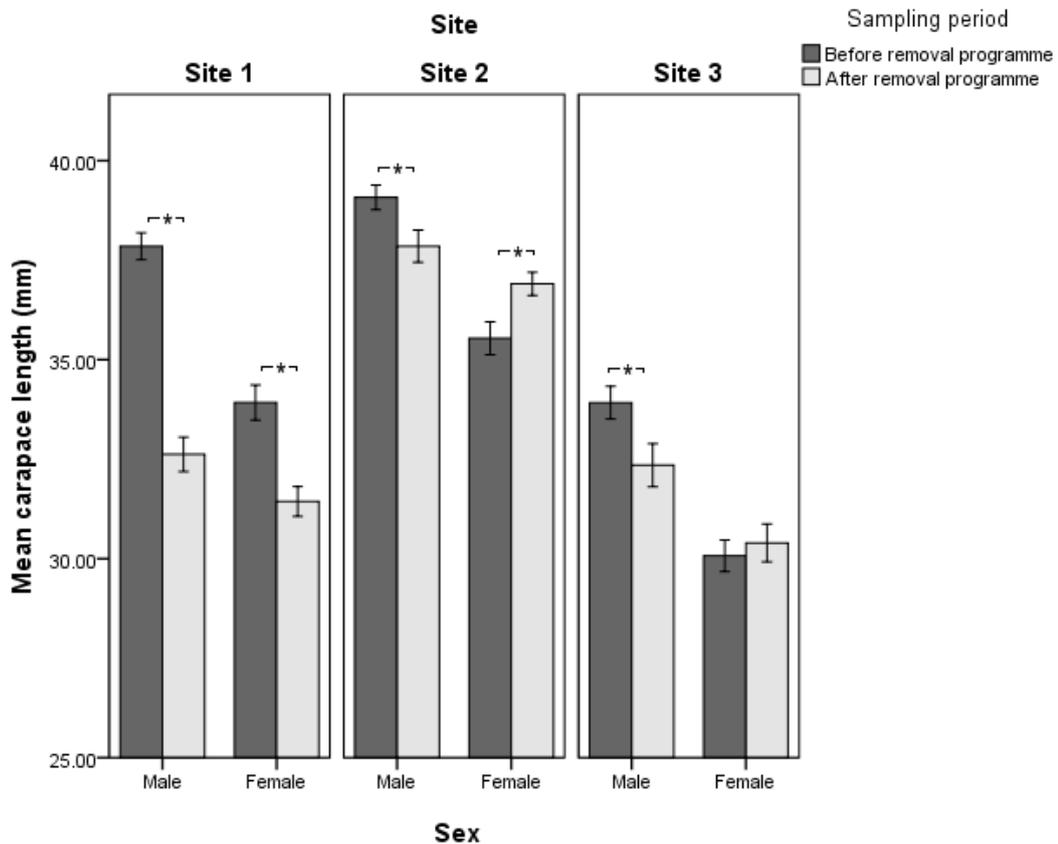
Figure 7-7 - Mean carapace lengths of crayfish before and after the removal programme. Data for non-berried and berried females was combined. Error bars are 95% confidence intervals. Asterisks denote pairwise statistically significant differences.

7.4.5 Effect of depth on catch

The effect of depth on the number and size of crayfish captured before and after the removal programme was investigated.

Before the removal programme, there was a significant negative correlation between creel depth and the number of male, non-berried female and berried

female crayfish captured (Figure 7-8, Spearman's rank correlation coefficient, r_s $_{males}$ = -0.299, p < 0.01, n = 90; r_s $_{females}$ = -0.248, p < 0.05, n = 90; r_s $_{berried\ females}$ = -0.519, p < 0.001, n = 90). This correlation was strongest for berried females.

There was also a significant negative correlation between creel depth and the carapace length of males and non-berried females (Spearman's rank correlation coefficient, r_s $_{males}$ = -0.299, p < 0.001, n = 2807; r_s $_{females}$ = -0.247, p < 0.001, n = 936) before the removal programme.   There was no significant correlation between depth and the carapace length of berried females (r_s $_{berried\ females}$ = 0.012, p > 0.8, n = 178).
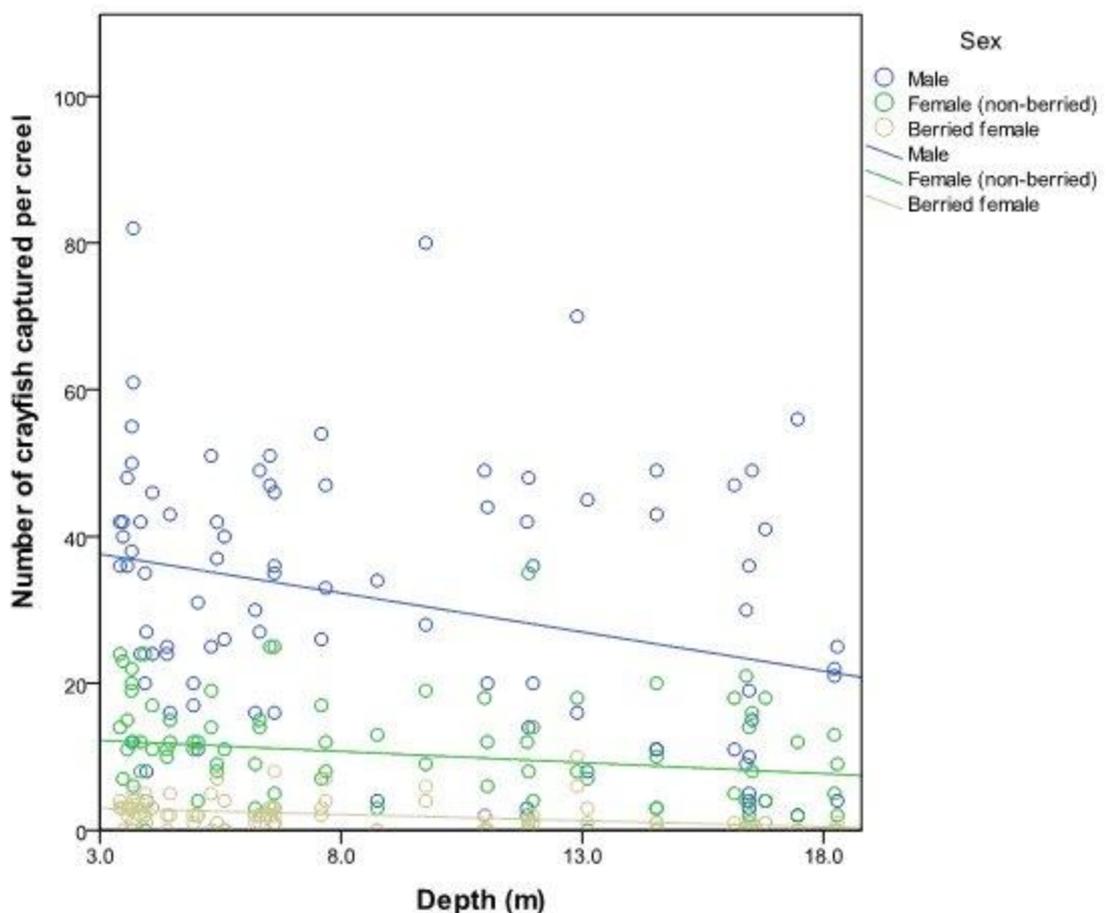
Figure 7-8 - Correlation between creel depth and the number of crayfish captured per creel before the removal programme (in May/June).

After the removal programme, there remained a significant negative correlation between creel depth and the number of male (Spearman's rank correlation coefficient, r_s $_{males}$ = -0.242, p < 0.05, n = 90) and female (r_s $_{females}$ = -0.298, p <

0.01, n = 90) crayfish captured (Figure 7-9). No berried females were captured in September.
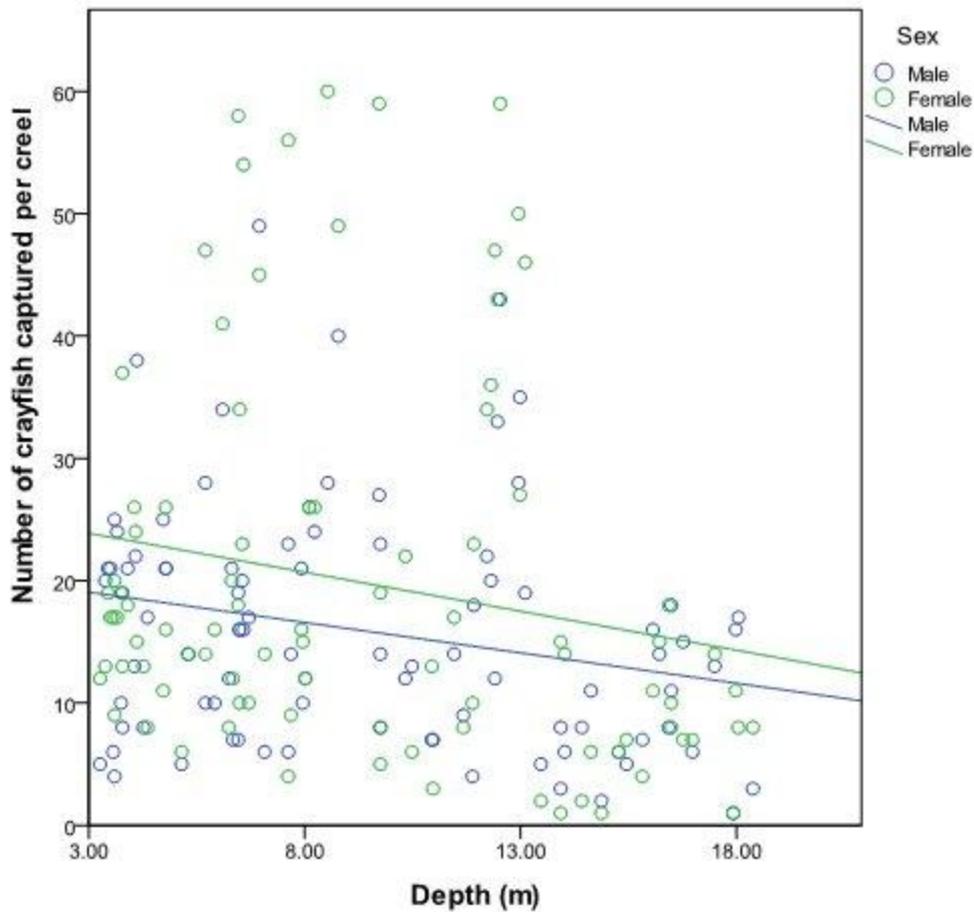
Figure 7-9 - Correlation between creel depth and the number of crayfish captured per creel after the removal programme (in September).

Unlike before the removal programme, there was a significant positive correlation between creel depth and the size of male (Spearman's rank correlation coefficient, $r_{s\ males}$ = 0.098, p < 0.001, n = 1428) and female ($r_{s\ females}$ = 0.088, p < 0.001, n = 1777) crayfish captured.

7.4.6 Mark and recapture results and population estimates

Mark and recapture results and population estimates for males and females before and after the removal programme are given in Table 7-2 and Table 7-3. Due to variation in the trappability of females and the bias of traps towards catching males, it should be noted that the population estimates provided for females are not a true representation of population size; instead, these estimates indicate the size of the "trappable" population of female crayfish at the time of sampling.

The sex ratio of an unexploited crayfish population is expected to be 1:1 and previous authors have recommended doubling the population estimates obtained for males as a means of calculating the total population size (Nowicki *et al.*, 2008), thus overcoming the problem of variability in the trappability of females. On this basis, it was possible to use the figures obtained for males to calculate estimates of the total population at each site before the removal programme (when the sex ratio was assumed to be 1:1); these figures are provided in an additional column in Table 7-2. At Site 3 low numbers of crayfish were captured during the June recapture session. A large proportion of crayfish in this catch had very soft carapaces, suggesting the occurrence of a synchronous moulting event. Recently moulted crayfish are vulnerable to predation and less likely to venture into traps, which may explain the low catch. As a result, the population estimates obtained for Site 3 prior to the removal programme are unreliable and should be interpreted with caution (these estimates are shaded grey in Table 7-2 and Table 7-3).

Table 7-2 - Mark and recapture results, population estimates and population densities for males; population estimates for the total population before the trapping programme are also given.

Site	Before Removal Programme					After Removal programme			
	Captures	Captures during resampling (marked)	Population estimate* (Poisson 95% confidence limits)	Population density** (crayfish m^{-2})	Population estimate for males and females, assuming a 1:1 ratio	Captures	Captures during resampling (marked)	Population estimate* (Poisson 95% confidence limits)	Population density** (crayfish m^{-2})
1	517	517 (30)	8655 (6126 – 12657)	44 (31-65)	17312 (12252 – 25314)	134	324 (10)	3988 (2261.6-7697)	21 (40-116)
2	644	403 (20)	12408 (8194-19741)	64 (42-101)	24818 (16388 – 39482)	281	402 (22)	4940 (3323-7679)	25 (17-39)
3	560	138 (15)	4874 (3022-8296)	25 (16-43)	9748 (6044 – 16592)	159	128 (1)	10319 (3127-18764)	53 (16-96)

*N=(M+1)(C+1)/R+1
**Based on a sampling radius of 13m^2 per creel (Abrahamson and Goldman 1970, Capelli 1975, Lodge and Lorman 1987)

Table 7-3 - Mark and recapture results, population estimates and population densities for females.

Site	Before Removal Programme				After Removal Programme			
	Captures	Captures during resampling (marked)	Trappable population estimate (Poisson 95% confidence limits)	Population density** (crayfish m-²)	Captures	Captures during resampling (marked)	Population Estimate (Poisson 95% confidence limits)	Population Density** (crayfish m-²)
1	236	198 (13)	3368 (1645 – 5599)	15 (8-29)	203	274 (8)	6232 (3339 - 12750)	32 (17-65)
2	176	214 (2)	12684 (3496 - 20976)	59 (18-108)	616	439 (18)	14287 (2320- 9234)	73 (47-12)
3	219	57 (7)	1594 (785- 3180)	8 (4-16)	96	149 (4)	2909 (1299- 7275)	15 (7-37)

*N=(M+1)(C+1)/R+1

**Based on a sampling radius of 13m² per creel (Abrahamson and Goldman 1970, Capelli 1975, Lodge and Lorman 1987)

Figure 7-10 shows the population estimates of males and females at the three sites before and after the removal programme. The data presented here suggests that the removal programme reduced the male population at Sites 1 and 2 by 54% and 60.2% respectively but there was an increase of 111.8% at Site 3. The apparent increase at Site 3 is likely to be an artefact, however, of the unreliable estimate obtained before the removal programme due to the moulting event. For females, the data suggests population increases of 114.8% at Site 1, 23.9% at Site 2 and 92.7% at Site 3; these increases are likely to be reflective of an increase in the trappability of females in September following the release of young rather than true increases in population size.
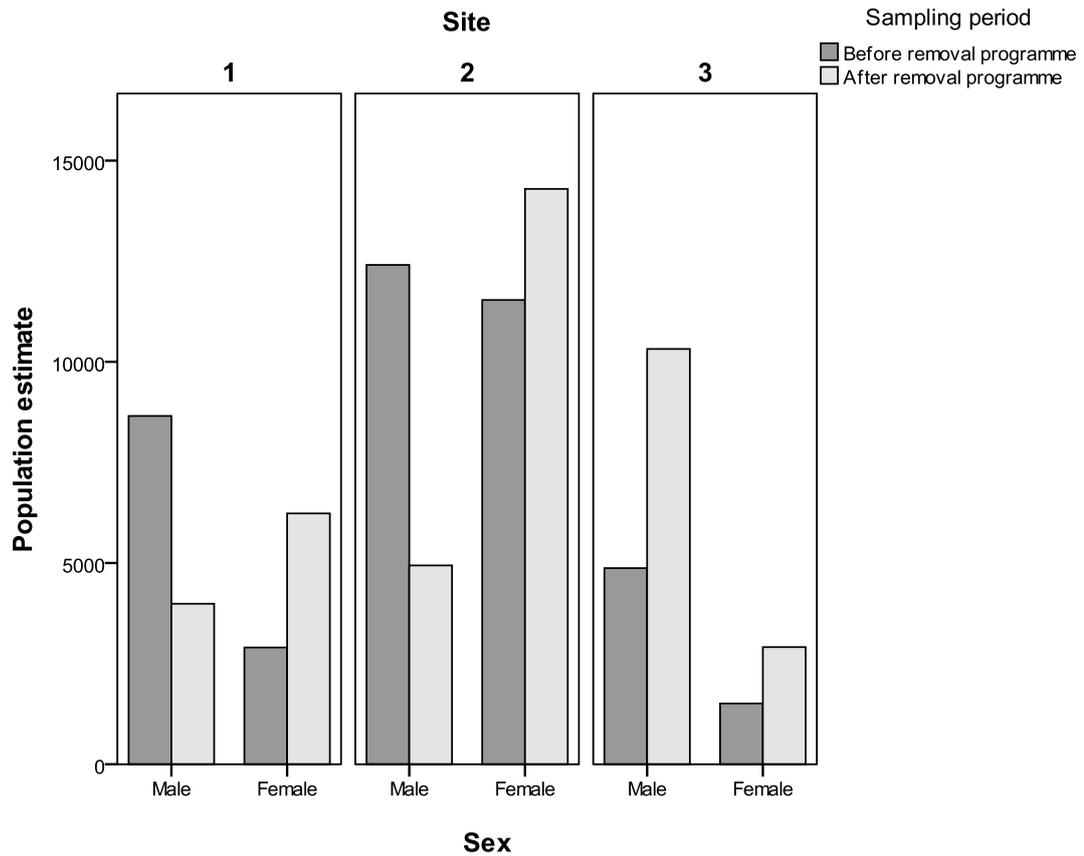
Figure 7-10 - Population estimates for males and females at Sites 1-3 before and after the removal programme. Data for non-berried and berried females was combined.

7.4.7 Inferring changes in population size from catch data and mark and recapture data

Catch data and mark and recapture data were used to make inferences about changes in the population after the removal programme (Table 7-4).

Making inferences about changes in the population size is not possible if animals vary in their trappability between sampling periods. As a result, it was not possible during the present study to determine the impact of the removal programme on female crayfish. Although both catch data and the mark and recapture data suggest variable increases in the female population, these increases are more likely to reflect an increase in the trappability of females in September (following the release of young and reduced competition from males) than an increase in the population. Similarly, a reduction in the trappability of crayfish at Site 3 during the June resampling (due to a synchronous moulting event) means that inferences about changes in the population at this site will be flawed.

In light of these considerations, making a robust assessment of the impact of the removal programme on the population may be restricted to male crayfish at Site 1 and Site 2. Catch data suggested a reduction in the male population of over 50% at Site 1 and over 30% at Site 2. The large confidence intervals associated with the mark and recapture data mean that it is difficult to make inferences about population change, both in terms of magnitude and the direction of change.

Table 7-4 - Change in total catch vs. change in population estimates after the removal programme.

	Catch data		Mark and recapture data	
	Males	Females	Males	Females
Site 1	-55.7%	+14.3%	-54% (-82.1% to +25.6%)	+114.8% (-40.4% to +675%)
Site 2	-34.8%	+170.5%	-60.2% (-83.2% to -6.3%)	+23.9% (-88.9% to +308.7%)
Site 3	-58.7	+14.9%	+ 111.8% (62.3% to +520.9%)	+92.7% (-8.5% to +356.4%)

7.4.8 Crayfish movements

This study found evidence of significant movements by crayfish in this loch system. During both mark-recapture sessions, crayfish released at Site 1 were recaptured at Site 2, and vice versa (Table 7-5), indicating movements of at least 800 metres in two weeks. Crayfish from Site 3 were also recaptured at Site 1. At Site 3, only crayfish originating from Site 3 were recaptured. During the first recapture session in June, nine "vagrant" crayfish were captured; during the September recapture session, five "vagrant" crayfish were captured. Additionally, in March 2011, one crayfish that was marked and released at Site 1 in September was recaptured at Low Park (NX 64231 75763), suggesting a minimum dispersal of 1.5 km in 6 months.

Table 7-5 - Number, sex, carapace length and minimum distance travelled by 'vagrant' crayfish recaptured at sites other than their site of release, before (May/June) and after (September) the removal programme.

BEFORE	Sex	Carapace length (mm)	From	To	Minimum distance travelled in 14 days (m)
	M	41.6	Site 2	Site 1, Creel 11	667
	M	45.3	Site 2	Site 1, Creel 9	613
	F	33.3	Site 3	Site 1, Creel 10	533
	M	44.3	Site 3	Site 1, Creel 9	560
	M	41.2	Site 3	Site 1, Creel 5	667
	F	35.3	Site 1	Site 2, Creel 2	747
	M	38.7	Site 1	Site 2, Creel 13	453
	M	37.2	Site 1	Site 2, Creel 2	747
	F	30.7	Site 1	Site 2, Creel 11	507
AFTER	F	32.5	Site 2	Site 1, Creel 1	400
	F	31.5	Site 2	Site 1, Creel 7	560
	F	35.3	Site 2	Site 1, Creel 8	587
	M	36.1	Site 2	Site 1, Creel 8	587
	M	33.6	Site 1	Site 2, Creel 1	800

7.5 Discussion

7.5.1 Marking method

Crayfish were marked using both acrylic varnish and hole-punching in order to increase the chance of mark-retention and allow a comparison of the effectiveness of the two methods. Both methods were relatively successful. Acrylic varnish was easy to apply, and more immediately recognisable than the holes, which made processing animals during the study less time-consuming. Hole-punching was also simple to apply and careful inspection of the telson/uropods allowed holes to be identified successfully during the recapture sessions. All recaptured crayfish that were marked with varnish were also marked with one or more holes; 11.3% of recaptured crayfish that were marked with holes, however, were no longer marked with varnish. This loss of varnish may be attributed to crayfish moulting, or perhaps mechanical damage. Moulting by *P. leniusculus* typically occurs during the summer months and so would be expected during the present study. Juvenile crayfish moult frequently, at a rate of up to 11 times in their first year (Mason, 1975). The present study, however, focussed exclusively on sexually mature crayfish, which only moult once or twice a year (Aiken and Waddy, 1992). Even after moulting, holes or scars left by holes were still visible. The effect of applying coloured acrylic varnish to the carapace on the predation risk of crayfish in Loch Ken is unknown and should not be discounted when drawing conclusions from population estimates. Given the reduced rate of retention due to moulting and potential effects on predation risk, acrylic varnish application should be rejected in favour of hole-punching in future mark-and-recapture studies.

7.5.2 Effect of trapping on catch

There was a significant reduction in the total catch of males after the removal programme at all sites; for females, however, the trapping had no obvious effect on crayfish numbers. The observed difference between the impact of the removal programme on males and females is likely due to the sex-related bias of sampling crayfish with traps and seasonal variation in the catchability of females. The bias of traps towards catching large males rather than females and juveniles is well documented (e.g. Bills and Marking, 1988, Lawrence *et al.*,

2006). After large males occupy traps, smaller males and females avoid them, in order to evade cannibalism (Holdich *et al.*, 1999b). As a result, trapping is expected to alter the sex ratio of a population by decreasing the proportion of larger, catchable males but increasing the proportion of females and smaller males (Lawrence *et al.*, 2006). Trapping of females may be more effective after the removal of male crayfish due to reduced competition and cannibalism (Hein *et al.*, 2007). Lawrence et al. (2006) compared the sex ratio of the yabby (*Cherax albidus* Clark) from a wild population that had not previously been trapped to a commercially-harvested population and found the sex ratios to be 1 male to 0.82 females and 1 male to 1.2 females respectively. Similarly, in the present study, the sex ratio of total catch of crayfish (for all sites combined) was 1 male: 0.44 females prior to the intensive removal programme and 1 male: 1.24 females afterwards. This lends support for the hypothesis that trapping can lead to skewed sex ratios by removing more males than females. It is possible that altering the sex ratio in this way may increase the reproductive potential and therefore density of the population (Lawrence *et al.*, 2006).

The vulnerability of crayfish to capture by trapping may also vary as a result of changes in behaviour that relate to reproductive state. Higher catches of females are likely to occur, for example, after they have released their young (Richards *et al.*, 1996). Prior to this, berried females are trap shy (Holdich *et al.*, 1999b). During the May/June capture sessions, many of the captured females (15.6%) were still bearing eggs. During the September sessions, however, no berried females were captured, indicating that release of juveniles had already occurred. This behavioural effect is likely to have further contributed to the male-bias of traps in May and June. The impact of the removal programme on numbers of females might have been greater if it had started later in the season after the release of young, as suggested by Hein *et al.* (2007). Although trapping berried females may be an effective way of significantly reducing recruitment, there is a risk that their removal will lead to feedback mechanisms such that crayfish start to mature earlier and produce more eggs (Holdich *et al.*, 1999). There is some evidence for this from the River Clyde where a long-term trapping programme has led to females reproducing at a smaller size (Matt Mitchell, 2009, pers. comm.). There is a risk that continued

trapping will result in a population which is characterised by small, reproducing animals that will evade capture by trapping.

At all sites, there was a reduction in the mean carapace length of male crayfish after the removal programme. Previous studies have suggested that intensive trapping removes the larger, more catchable crayfish from the population (Lawrence *et al.*, 2006), allowing smaller individuals to benefit from reduced competition (Skurdal and Ovenild, 1986). Continual removal of the largest individuals, via trapping, may eventually lead to the establishment of a population structure which is dominated by large numbers of small, untrappable individuals (Holdich *et al.*, 1999b) which is a concern for commercial fisheries, since the majority of crayfish remaining will be below market size (Lawrence *et al.*, 2006). For the present study however, the primary concern is the potential ecological damage that crayfish may cause at Loch Ken. More research is required into the relative impacts made by small and large crayfish, which may differ in their magnitude and nature (crayfish diet, for example, may vary according to age; ontogenetic differences in habitat use may also occur).

If large crayfish have a greater negative impact on ecosystems than small crayfish, then continual trapping might be a useful means of reducing this damage. Size-dependent impacts of introduced *P. leniusculus* were documented in enclosure experiments in a Japanese marsh (Usio *et al.*, 2009). Impacts of crayfish on macrophytes, invertebrate biomass and invertebrate taxa richness were 3-27 times greater in large crayfish compared with small ones (Usio *et al.*, 2009). Nonetheless, small crayfish were found to have strong negative impacts when they reached high densities. In light of this, Usio *et al.* (2009) recommend using a control method that reduces juveniles alongside trapping of large crayfish for effective mitigation of negative impacts. Rogers *et al.* (1997) suggested that regular trapping of large individuals might have allowed fish predation on smaller individuals to eventually eliminate a signal crayfish population in carp ponds in England. More recently, a combination of intensive trapping and increased fish predation of the invasive rusty crayfish *O. rusticus* in Sparkling Lake, Wisconsin was found to cause a massive decline in the crayfish population (Hein *et al.*, 2006, Hein *et al.*, 2007). Crayfish were trapped from late June to late August as part of a five year removal programme. While trapping removed large crayfish, fishing regulations were used to protect

predatory fish that consumed the smaller age classes. The subsequent decline of the crayfish population led to the recovery of macrophytes in the lake, with benefits for native sunfish *Lepomis* sp (Hein *et al.*, 2007). There are currently no data available on predation of crayfish in Loch Ken, although predatory fish such as pike (*Esox lucius* L.), perch (*P. fluviatilis*) and the European eel (*A. anguilla*) are known to be present. Protection of these predatory species in conjunction with sustained trapping may be a potential strategy for controlling crayfish in Loch Ken in the future.

7.5.3 Effect of depth on catch

In May and June, depth was found to have a negative impact on the catch of crayfish. Berried females in particular were more likely to be captured in shallow than deep water. Previous studies have also found a reduction in the catch of crayfish with increasing depth (e.g. Abrahamsson and Goldman, 1970, Hein *et al.*, 2007). In Sparkling Lake, catch rates of *O. rusticus* were lowest at deep sites where water temperatures were colder and where the habitat was mostly comprised of sand or muck (Hein *et al.*, 2007). Abrahamson and Goldman (1970) observed a sharp decline in the number of *P. leniusculus* at depths below 40 m in Lake Tahoe, California, even if the bottom substrate was favourable habitat. The eggs of *P. leniusculus* are unable to hatch in cold, deep water (Abrahamsson and Goldman, 1970) which might explain the stronger apparent preference of berried females for warm, shallow depths than deep water in the present study.

Also during May and June, there was a negative correlation between depth and the size of male and non-berried females. In spring/summer, the productivity of the loch may be greater at shallow depths than in deep water, leading a greater abundance of food and perhaps enhanced growth rates for crayfish at these sites. It is also possible that larger, more competitive crayfish exclude smaller crayfish from favourable habitats at these shallow depths.

In September, there remained a negative correlation between depth and the number of crayfish captured but this association was not as strong as in May and June, suggesting an increase in the number of animals inhabiting deeper water. This was coupled with the observation that more large crayfish were found in

deep water, unlike in May/June when the reverse was the case. Abrahamson and Goldman (1970) observed that strong wave action during cold weather caused significant mortality of *P. leniusculus* in shallow areas of Lake Tahoe due to collision with moving boulders and a presumably reduced food supply. A later study by Flint (1977) showed that *P. leniusculus* move into deeper water during the winter months to avoid storms. Loch Ken is also subject to major storms during the winter and so migration of crayfish to deep water in the autumn may be an effective survival strategy in this ecosystem.

7.5.4 Mark and recapture

Catch data alone is not necessarily a good indicator of population size (Goldman and Rundquist, 1977). In a comparison of crayfish density at Lake Donner and Lake Tahoe, for example, mean catch/trap data suggested that populations from the two sites were of very similar densities (Goldman and Rundquist, 1977). Mark and recapture data, however, showed that this was not the case, with a significantly lower population density in Lake Donner.

In the present study, there were differences between the changes in population size inferred from the raw catch data and the estimates obtained from mark and recapture data (Table 7-4). The impact of trapping on males at Site 2, for example, may have been underestimated by the catch data, which suggested a percentage decrease that was almost half that of the mean value obtained from the mark and recapture results (-34.8% compared with -60.2%). Such gross differences would suggest that trapping data is not sufficient in evaluating population abundance. However, given the large confidence intervals associated with the mark and recapture data (e.g. -83.2% to -6.3% around the mean of -60.2% for Site 2), this may not be the case.

Both raw catch data and mark and recapture data were complicated by the effects of season, reproductive state and moulting. Although both methods suggested increases in the number of females after the programme, such a scenario is highly unlikely. In order to make robust inferences about changes in the female population, sampling should be conducted during the same season; unfortunately, logistics did not permit for this during the present study. The synchronous moulting event and subsequent low catch during the June recapture

at Site 3 illustrates the importance of sampling on multiple occasions in order to obtain a reliable picture of the state of the population: if sampling is only conducted once and happens to coincide with a period of moulting then this could lead to an underestimation of crayfish abundance.

Translating the population estimates to population densities is difficult due to the lack of information on the factors influencing trap collection radius (Goldman and Rundquist, 1977). The area covered by one creel roughly equated to 0.23 m^2 but we would obviously expect the effective sampling area to be much greater than this. Variables such as habitat type, temperature, substrate, type of trap, distance between traps, bait, crayfish species and density may influence the effective sampling area (Goldman and Rundquist, 1977) Accosta and Perry (2000) estimated the effective sampling area of baited minnow traps used to capture blue crayfish *Procambarus alleni* [Faxon] to be about 56.3m^2 in flooded marsh habitat. This figure was estimated by measuring the recapture rate of marked crayfish in traps which were set at various radial distances away (Accosta and Perry, 2000). By contrast, studies of crayfish in lakes have suggested an effective trapping area of 12.5-12.7 m^2 per trap (Abrahamsson and Goldman, 1970, Lodge and Lorman, 1987). For these studies, effective trapping area was calculated according to the efficiency of traps in lakes where crayfish density had been previously estimated using SCUBA survey methods. If a 13 m^2 effective trapping area is applied to the present study, some tentative figures for crayfish density may be calculated, with a mean range of 21 – 53 male crayfish m^{-2} (excluding female and juvenile crayfish) after the removal programme. Given the high mobility of crayfish in the present study (see Chapter 3 and Section 7.55, this Chapter), 13 m^2 is, however, likely to be an underestimate of the true effective trapping area. If Accosta and Perry's value of 56.3 is applied, then this gives a more conservative mean range of 4.7 – 12.2 male crayfish m^{-2} after the removal programme.

These densities far exceed the range of densities obtained for *P. leniusculus* in other lakes. Across three transects at Lake Tahoe, for example, the density of crayfish ranged from 0.53-8.38m^{-2} (Goldman and Rundquist, 1977). Lower densities ranging from 0-1.15 m^{-2} were obtained at transects on Donner Lake (Goldman and Rundquist, 1977). Flint and Goldman (1977) found signal crayfish

density to be correlated with the amount of stone cover and stone size. Similarly, in Lake Billy Chinook, densities of large trappable signal crayfish were estimated to be between 0.24 m^{-2} and 1.13 m^{-2} depending on habitat, with lower densities present on vertical basalt cliffs compared with boulder/cobble habitat elsewhere (Lewis and Horton, 1997). Abundant boulders and gentle slopes were also found to be the preferred habitat in a small Finnish lake, where densities of 0.2-0.4 crayfish m^{-2} were recorded in the most favourable areas (Kirjavainen and Westman, 1999). In a lake in Hampshire, crayfish had a clumped distribution with densities of around 1.8 m^{-2} in inhabited areas (Hogger, 1986). There are currently no data available on the habitat available for crayfish at Loch Ken and the densities provided are unlikely to be representative of the whole lake. Comparisons of populated sites with those that have not been populated may help identify preferred habitats and determine the rate at which crayfish establish, which would provide a significant insight into the invasion and establishment process elsewhere. During surveys in the present study, fishermen observed that creels hauled from areas of Loch Ken that were not occupied by crayfish were covered in macrophytes; creels hauled in areas inhabited by crayfish, however, were coated in muddy substrate and were macrophyte-free (DeeFish, 2009, pers. comm.). Previous studies have found high crayfish abundances to be associated with decreases in macrophyte coverage and species richness (Elser *et al.*, 1994, Flint and Goldman, 1975, Hogger, 1986, Nyström, 1999, Nyström and Strand, 1996, Nyström *et al.*, 2001). Stable isotope analyses indicate that invertebrates are the preferred food item for crayfish; crayfish at high densities, however, may seriously impact macrophytes after invertebrates are depleted (Nyström *et al.*, 1999). Given the high densities of crayfish observed at Loch Ken, it is likely that macrophytes are being readily depleted, which will have indirect effects on the rest of the ecosystem, for example by impacting benthic invertebrates and nutrient cycling (Flint and Goldman, 1975, Guan and Wiles, 1998).

7.5.5 *Crayfish movements*

The observation that signal crayfish will disperse significant distances (at least 800 metres) in a relatively short time (two weeks) concurs with previous studies of crayfish movements. Radiotracking and PIT tagging of signal crayfish in rivers, for example, found crayfish to be capable of movements of over 340

metres in two days (Bubb *et al.*, 2006a). Movements of signal crayfish in lakes however, have not been widely researched. In a study of crayfish in Lake Tahoe, individually tagged animals were found to move a maximum distance of 400 metres over a 4-week period (Flint, opt. cit.). Another study of crayfish in a Finnish lake found that almost 75% of crayfish moved less than 100 metres over a period of 15 months, with only a few individuals making more substantial movements of up to 580 metres (Kirjavainen and Westman, 1999). In the present study, a greater number of vagrant crayfish were captured during the May/June recapture session than in September; this may be due to potentially higher temperatures earlier in the season, which are correlated with greater crayfish activity levels (Bubb *et al.*, 2002). Maximum movements might be expected, therefore, during midsummer. Vagrants consisted of both males and females and so movement did not appear to be sex-related. None of the vagrant females, however, were berried and reproductive state has been shown to influence crayfish movements in previous studies (Bubb *et al.*, 2002). Radio-tracking of signal crayfish in the River Wharfe, for example, found a reduction in movement by berried females in comparison with non-berried females (Bubb *et al.*, 2002). Given the small sample size (n = 14) it is difficult to make many solid deductions about the factors influencing crayfish dispersal in this loch.

Although there are no data available for lakes, a recent study investigated the impact of removal of crayfish by trapping on the movements of *P. leniusculus* in the River Windrush (Moorhouse and Macdonald, 2011). Four 1 km stretches of river were divided into sections: one middle 500 m section and two 250 m sections upstream and downstream. At two removal sites, crayfish were removed from the middle section by trapping; at two non-removal sites, crayfish in the middle section were trapped but returned after marking. At all sites, crayfish captured in the upstream and downstream sections were marked and returned. The authors found that although immigration rates into the middle section were similar at removal and non-removal sites, the distances moved by crayfish were greater at removal sites. Increasing body size was also found to increase the likelihood of immigration. It was suggested that the absence of large crayfish in removal sites reduced the likelihood of interference competition and thus facilitated the immigration of large individuals over greater distances. The authors concluded that the impact of trapping will not

only reduce the abundance of crayfish at the site of removal but extend beyond it by at least 200 m upstream and downstream. It is possible that the removal programme in the present study encouraged greater movements of crayfish than would be expected in an unexploited population.

7.5.6 Conclusions

This study attempted to assess the impact of the removal programme on the population of crayfish at Loch Ken. Several factors, however, should be considered when interpreting the results, including the sex and size-related bias of trapping, seasonal fluctuations in catch due to temperature and reproductive state, moulting events, and gaps in knowledge about effective trapping area, predation levels and habitat composition in the loch.

What is clear is that crayfish occur in Loch Ken at densities which are very high compared with other lakes and the loss of native biodiversity will be considerable. It should also be noted that the present study did not consider juvenile crayfish which were excluded from sampling (due to the large creel mesh size) but which may also exert ecological impacts. Losses in biodiversity can have direct economic impacts (Pimentel *et al*. 2001): at Loch Ken, an area renowned for its fishing, infestation of the loch by signal crayfish is already incurring major costs for local hoteliers and businesses, as anglers are reported to take their trade elsewhere. There is a pressing need to investigate the impact of signal crayfish on the native flora and fauna of Loch Ken and the effectiveness of long-term trapping in reducing this impact. Previous studies have indicated that trapping alone is unlikely to be an effective control method for crayfish (Bills and Marking, 1988) and will not completely eradicate a population. The methods available for controlling crayfish in water bodies as large as Loch Ken remain limited, however. The use of trapping in conjunction with other control methods such as enhanced fish predation (via protection of predatory species from fishing pressure) may offer a better chance of effectively reducing the environmental impacts of this invader, as is the strategy for the control of crayfish in Sparkling Lake (Hein *et al.*, 2006, Hein *et al.*, 2007). However, to ensure success, trapping would have to be sustained in the long term as its cessation would likely result in a quick population recovery and subsequent return of adverse impacts. Any trapping programmes should also

take account of seasonal changes in crayfish activity and habitat preferences, including the influence of water depth, which was shown to be an important determinant of crayfish catch during the present study. A cost-benefit analysis to weigh up the ecological and economic benefits of removal by trapping against the costs of this strategy would be strongly recommended before embarking on future projects.

Chapter 8: General discussion

8.1 Summary

This PhD has contributed to the ever-growing field of invasion ecology and it is hoped that the results provided will have useful applications in the management of invasive non-native species in Scotland and further afield. The studies presented here aimed to provide information under three broad themes, namely the status (Chapters 2-4), impact (Chapters 5 and 6) and control (Chapter 7) of introduced crayfish in Scotland. The discussion which follows will summarise the findings of each chapter in relation to these themes, address the limitations of the studies and make recommendations for future research.

8.1.1 Status of crayfish populations in Scotland

There is a pressing need to develop effective methods for the rapid detection of non-native invasive species, including crayfish. Data pertaining to the distribution of invaders must be kept up to date to ensure that an early warning of populations is obtained whilst the opportunity still exists to initiate an eradication or management programme (Gladman *et al.*, 2009). The results presented in Chapter 2 showed that a combination of three-run electrofishing and kick sampling can be used to rapidly detect *P. leniusculus* in rivers. These findings were used to develop a signal crayfish "detection protocol" which was applied by seven Fishery Trusts across Scotland and allowed the distribution of *P. leniusculus* to be determined much more precisely than was previously known (Gladman *et al.*, 2009 , see Appendix I and Appendix II). Based on these surveys and more recent records, *P. leniusculus* is now estimated to inhabit at least 174 km of river length in Scotland. An improved knowledge of the *P. leniusculus* distribution has also allowed potential sites for eradication of localised populations to be identified.

Although effective in riffles, it should be noted that electrofishing and kick sampling are likely to be of limited use for surveying crayfish in deep or lentic habitats, where trapping may be more appropriate. Survey methods will also be ineffective if applied during periods of low crayfish activity in cold temperatures

or where crayfish densities are very low. In order to maximise efficiency, detection protocols for *P.leniusculus* should be modified to take account of such factors (see Appendices I and II).

In the future, more advanced techniques such as the identification of DNA fragments from water samples may allow the detection of invasive species in environments where sampling is difficult or where densities of the invader are low (Ficetola *et al.*, 2008), thus overcoming many of the limitations of the sample methods described here. Another avenue not explored in the present study is the potential use of remotely operated vehicles (ROVs) to conduct surveys in deep water habitats. However, until more efficient methods are developed, full use should be made of the current protocol to ensure that the best knowledge of the *P. leniusculus* distribution is maintained and that management strategies are employed where appropriate.

The results of the *P. leniusculus* surveys described in Gladman *et al.* (2009) have revealed the rapid and extensive spread of the species in many Scottish rivers. While much of this spread may be attributed to human-mediated movements, *P. leniusculus* is a large, mobile invertebrate capable of expanding its range naturally. Chapter 3 aimed to investigate the degree to which *P. leniusculus* disperses naturally in two riverine habitats, a first-order stream and a larger, fourth-order channel. Radiotracking was the preferred method for this study since it allows crayfish movements to be monitored with minimum disturbance to the animals or the environment. The results showed that *P. leniusculus* will move up to 195 day^{-1}, which goes some way in explaining its rapid penetration of river habitats. Biotic factors such as crayfish sex and size appeared to be less important in influencing crayfish movements than abiotic factors like flow and habitat, although the study did not provide a sufficient investigation of these factors: data on the local discharge, water velocities, and water temperatures (via the use of temperature loggers) would have been useful. An accurate knowledge of the density of crayfish occurring at the two sites would also have been desirable, although the sites are probably too physically dissimilar for a meaningful investigation of density-dependent effects. The study was also limited by only tracking the movements of large crayfish; the degree to which small crayfish play a part in range expansion remains uncertain.

The most significant observation arising from the radiotracking study was that the upstream dispersal of *P. leniusculus* may be reduced or halted by the presence of waterfalls. This suggests that the construction of in-stream barriers may be a useful strategy for reducing the rate at which *P. leniusculus* invades linear watercourses. Prevention of the spread of *P. leniusculus* in this way may be an easier feat than attempting to control established populations. With this in mind, surveys to identify sites where the installation of such barriers would be beneficial are recommended. Any plans for barrier construction should of course consider unintended impacts on other migratory animals, including anadromous fish.

One matter which is certainly related to the spread of invasive crayfish is the urgent need to conserve dwindling populations of native crayfish species, and this has become another important area of research. Chapter 4 aimed to determine the status of an endangered but non-native crayfish in Scotland, *A. pallipes*, at two lentic sites. Surveys conducted at Loch Croispol and Whitemoss Reservoir revealed two reproducing populations at a low and high density respectively, with latitude, habitat availability and predation pressure suggested as potential limiting factors. The study showed that the population at Loch Croispol was confined to a small proportion of the lake; if conservation management of this stock becomes a priority in the future, then the introduction of artificial refuges to areas where shelters are currently limiting may encourage expansion of this population. Surveys also identified potential threats to *A. pallipes* at both sites, through the spread of disease and *P. leniusculus*. Raising awareness of the value of these stocks and promoting disinfection of angling equipment are recommended for mitigating such threats.

Although this study has provided much-needed information on the status of *A. pallipes* in Scotland, the scope of the work was rather limited. Firstly, the data only give a brief "snapshot" of the state of the populations at one point in time. This is not ideal: where possible, surveys should be conducted on several occasions every year for a robust population assessment. The establishment of a monitoring programme for *A. pallipes* at these sites is therefore recommended. Furthermore, at Loch Croispol, the use of traps that are designed for the capture of large crayfish may have reduced the chance of capturing *A. pallipes* at this site; future surveys should avoid their usage. Similarly conducting the surveys in

early spring may have impeded the detection of crayfish at this high altitude site; futures surveys in mid-summer are recommended   This study has highlighted the opportunity for future research into the diseases and parasites present in these isolated populations through the discovery of one crayfish at Whitemoss Reservoir which was suffering from a microsporidian infection, believed to be Porcelain disease (*T. contejeani*).   There is also scope for research into the origins of *A. pallipes* populations in Scotland using genetic techniques, as has been done for *A. pallipes* in Ireland (Gouin *et al.*, 2003).   A further topic of interest is the trophic role that *A. pallipes* has come to occupy in these communities.   Although outwith the remit of this thesis, stable isotopes analysis is currently underway which aims to address this question (the author, in prep.).

The persistence of *A. pallipes* in Scotland while populations in its native range continue to decline means that Loch Croispol and Whitemoss Reservoir may constitute valuable sites for "*ex situ*" conservation of the species.   This raises issues of conservation philosophy and suggests changing attitudes towards non-native species.   Where threats show no sign of abating in the native habitat of endangered species, conservationists may be forced to consider *ex situ* conservation as a viable strategy for preventing extinction.   With climate change expected to condemn many species to extinction in their native ranges (Thomas *et al.*, 2004), the ethics of non-native species introductions may become even more blurred in the future.

8.1.2 Impact of crayfish on Scotland biodiversity

One of the utmost aims of research into invasive non-native species is to determine their impacts on native communities.   There is particular demand for information about the potentially adverse interactions between non-native crayfish and species of high economic or conservation value, including *S. salar* (Chapter 5) and *M. margaritifera* (Chapter 6).

Aquatic ecosystems provide a difficult setting for disentangling the complex relationships between crayfish and fish due to the scarce opportunity for direct observations (Finlay and Kendall, 2008).   Although field studies can provide evidence of population-level effects, determining the underlying mechanisms of

change at the individual level is difficult. For this reason, the potential threat of *P. leniusculus* to the eggs of a commercially important fish species, *S. salar* was investigated using an artificial stream (Chapter 5). This stream is fed by the River Almond, therefore allowing the behaviour of animals to be observed under almost natural conditions. The key finding from this study was that *P.leniusculus* does not predate the eggs of *S. salar* buried in shallow redds and therefore the risk of egg predation in the wild is predicted to be low. Other recent studies using different experimental designs also found no evidence of the predation of eggs in redds by *P. leniusculus* (Edmonds *et al.*, 2011, Findlay, 2012 pers. comm.). Although these results offer some hope for the persistence of *S. salar* in the face of crayfish invasions, it is important to consider that this fish species may be subject to adverse impacts during other stages of its life cycle, such as the emergence of fry from gravel after hatching, as witnessed by Edmonds *et al.* (2011) or during overwintering, as suggested by Griffiths *et al.* (2004). The threat of *P. leniusculus* to salmonids has not been fully characterised and further research would be welcome, including an investigation of non-predatory impacts such as burrowing.

While *P. leniusculus* and *S. salar* are already living in sympatry in the wild, Chapter 6 explored the potential for crayfish to impact a species which it has not yet encountered in Scottish waters, *M. margaritifera*. The lack of range overlap in the field necessitated the use of laboratory experiments for this investigation. The results showed that *P. leniusculus* is unable to predate on adult *M. margaritifera*. However, this observation is of little comfort given the significant amount of disturbance that crayfish were found to impose on the mussels. Furthermore, this study was limited by only investigating the impact of *P. leniusculus* on adult mussels. Future research which investigates the impact of *P. leniusculus* on juvenile mussels is recommended. Information on the maximum shell thickness that *P. leniusculus* is able to crack open would also be useful. Ultimately, this study has shown the potential for *P. leniusculus* to adversely impact a globally endangered species, adding further impetus to the need to prevent the spread of crayfish to mussel-inhabited sites.

There is much scope for future research into the impact of *P. leniusculus* on other native species. Practical issues during the present course of study prevented an investigation of the potential impact of crayfish on lamprey, of

which there are three species in Scotland (*Lampetra fluviatilis* L., *L. planeri* and *Petromyzon marinus* L.). All three species are listed on Annex II of the European Habitats Directive (92/43/ECC) as requiring conservation. Juvenile lamprey (ammocoetes) that inhabit silt beds along the edge of rivers may be at particular risk from crayfish burrowing activity and/or predation.

As part of the Species Action Framework, translocation attempts of the rarest fish in the UK, the vendace (*Coregonus albula* L.) have been made to the Daer Reservoir, a short distance from the Daer Water (SNH, 2007) where *P. leniusculus* is well established. If *P. leniusculus* is introduced to the Daer Reservoir or other translocation sites, this may have unknown effects on *C. albula* and potentially thwart attempts to conserve this rare fish. Determining the complex interactions between crayfish and valuable fish species should remain a focus for research and allow identification of threats.

Another sizeable knowledge gap surrounds our understanding of the impact of *P. leniusculus* on amphibians, which has been restricted to a small number of studies (Axelsson *et al.*, 1997, Nyström and Åbjörnsson, 2000, Nyström *et al.*, 2001). With amphibian populations already in decline, the potential impact of *P.leniusculus* on this group of vertebrates requires addressing.

8.1.3 Control of crayfish

Preventing the introduction and spread of non-native species is the surest way of mitigating their impacts. Once an invader has become established, its eradication is unlikely. In such cases, the best that environmental managers can hope for may be to effectively control the population and reduce it to a level that no longer causes significant harm.

Chapter 7 provided a case study of the impact of an intensive four-month trapping programme on a population of *P. leniusculus* present in a large lake, Loch Ken. The findings confirmed the impossibility of eradicating a crayfish population of this size using traps, which are biased towards catching large, male individuals. Depth and season were found to have important influences on crayfish catch and mark and recapture data showed crayfish to be very mobile, as has been found in rivers by radiotracking (Chapter 3). The implications of

these results are that the use of traps to control *P. leniusculus* in large lochs like Loch Ken is unlikely to be efficient, although it may have more impact if habitat and seasonality in crayfish activity are taken into account and if used in conjunction with other control methods that remove juvenile crayfish, such as fish predation. Future research to identify predatory fish resident in the loch and take measures to enhance their numbers is recommended, although fish predation alone will be an ineffective control method. This study was restricted to an investigation of the impact of the removal programme on the crayfish population but a more important question which remains unanswered is the effectiveness of trapping in reducing adverse ecological impacts. For future trapping programmes, an assessment of the impact of trapping on native communities would be prudent.

The case of Loch Ken has raised questions about the socio-economic implications of controlling crayfish on a large scale. Recent proposals have been made to establish a crayfish fishery on the loch. At present, it is illegal to trap, keep or transport live signal crayfish without a licence. Even if trapping is found to alleviate the adverse ecological impacts of crayfish (which is as yet unproven, see above) or generates revenue in the local area, there is a risk that such a venture will encourage the introduction of *P. leniusculus* to other water bodies and exacerbate the loss of biodiversity elsewhere.

Loch Ken provides a sobering example of the extreme difficulty of tackling *P. leniusculus* after its establishment. Prospects for the control or eradication of *P. leniusculus* in smaller water bodies are better, however, and are discussed in a recent review by Freeman *et al.* (2010). Chemical control using biocides has been the most effective method to date (Peay *et al.*, 2006, Sandodden and Johnsen, 2010) and is set to be applied in ponds in Lochaber this summer. Research into the development of other methods to enable the effective control of *P. leniusculus* in the diversity of habitats currently occupied is greatly needed.

8.2 Final thoughts and the future of species invasions

The North American signal crayfish is not the first and will certainly not be the last invader to threaten Scotland's native aquatic biodiversity. In addition to *P.*

leniusculus, England is host to several non-indigenous crayfish species: the noble crayfish *A. astacus*, narrow clawed crayfish *Astacus leptodactylus* [Eschscholtz], spiny-cheek crayfish *O. limosus,* red swamp crayfish *P. clarkii*, and virile crayfish *O. virilis* (Holdich *et al.*, 2009).   In the River Thames catchment, some of these species are now co-occurring and their interactions have been the subject of recent study (Jackson, 2012, pers. comm.).   It is conceivable that one day these species will arrive in Scotland and become as ubiquitous as *P. leniusculus.*   Other non-native species of immediate concern include the Chinese mitten crab *Eriocheir sinensis* [H. Milne-Edwards] and the killer shrimp *Dikerogammus villosus* [Sowinsky], both of which have invaded in England and are capable of causing significant environmental damage.

Predicting how climate change will interact with species invasions is an emerging challenge in ecological research. The consequences for aquatic ecosystems have been speculated by Rahel and Olden (2008). Rising temperatures could, for example, facilitate the expansion of outdoor aquaculture and other human activities (such as angling) to new areas which were previously too cold. This may exacerbate the spread of invasive species to new areas due to escapes or deliberate introductions (Rahel and Olden, 2008). New invasion routes may also be derived from increases in floods and changes in streamflow regimes (Rahel and Olden, 2008).

The rate at which invaders competitively displace native species may be accelerated by climate change, since native species tend to be more sensitive to environmental fluctuations than their invasive counterparts. The signal crayfish, for example, has a temperature tolerance which is between 1.3 and 3°C greater than that of the white-clawed crayfish (Firkins and Holdich, 1993). In addition, temperature rises have the potential to magnify the impacts of invaders, by increasing their food consumption rate (as shown in studies of *Orconectes* spp. by Elrott *et al.*, 2007) (Rahel *et al.*, 2008).

As described in Chapter 1, the term "invasive" traditionally refers to non native species which have been introduced to new areas by humans and have undesirable impacts. In the face of climate change, however, certain native species may increase in abundance and spread to new regions, leading to negative impacts on native communities which are reminiscent of those caused

by invasive species (Rahel and Olden, 2008). One such example is the upstream migration of Northern pike (*Esox lucius* L.) into Lake Stavlussukjavri in Sweden, which has been linked to the extinction of Arctic char (*Salvelinus alpines* L.) and reduced densities of nine spined stickleback (*Pungitus pungitus* L.) (Byström *et al.*, 2007). Although not directly observed, temperature rises derived from climate change were suggested as facilitating the migration of pike into the lake, which was previously a suboptimal temperature for colonisation (Bystrom *et al.*, 2007). Cases like this have led Rahel and Olden (2008) to suggest that climate change will force a redefinition of "invasive species" (Rahel and Olden, 2008). Other authors have called for greater linkage between climate change and invasive research: existing studies of invasive species may be useful for identifying important life history traits for predicting the invasive potential of other species in response to climate change (Ward and Masters, 2007).

As well as complicating invasion ecology theory and research, climate change may force conservationists to develop new approaches for managing endangered species. The concept of *ex situ* conservation was explored in Chapter 3 using a case study of non native white-clawed crayfish in Scotland. These northerly populations may become even more important in the future if southerly, native populations are thermally stressed by rising temperatures. Assisted migration or translocation of imperilled species to higher latitudes may allow climate driven extinction to be avoided (McLachlan *et al.*, 2007). This is a controversial strategy, however, given the numerous unprecedented impacts that have resulted from previous human mediated species introductions (McLachlan *et al.*, 2007). Policy about species introductions should always be informed by careful ecological research.

In the face of these accelerating threats, tightening legislative controls and raising the profile of the dangers of invasive species through education are of clear importance. Finally, co-operation between scientists, land managers, government agencies and the general public will be essential in the fight against the "great historical convulsions of the world's fauna and flora" and in dealing with the effects of man-made climate change.

Appendix I

The paper which follows was published as Gladman ZF, Adams CE, Bean CW, Sinclair CA, Yeomans WE. 2009. Signal crayfish in Scotland. In: *Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009.*, Brickland J, Holdich DM, Imhoff EM (eds). Leeds, UK.

Signal crayfish in Scotland

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ABSTRACT

The spread of the non-indigenous North American signal crayfish (*Pacifastacus leniusculus*) in Scottish freshwater ecosystems is of major concern due to the threats that this species poses to biodiversity. In 2007, the Scottish Government listed signal crayfish under the Species Action Framework (SAF), a five-year strategy for species management in Scotland. One of its foremost objectives is to determine the distribution of signal crayfish in Scotland, thereby allowing control or containment efforts to be targeted appropriately. This paper outlines the recent work undertaken to fulfil this objective. Existing records of crayfish distribution were collated and validated prior to extensive field surveys. A standard crayfish detection protocol involving kick sampling, electrofishing and baited-traps was applied at all sites. Signal crayfish are now known to occupy at least 58 km of river length in Scotland. They are also present in a small number of standing waters, ranging in size from small ponds to large lochs. Field surveys confirmed and refined crayfish distribution records and identified sites where eradication of localised populations might be possible. At some sites the protocol failed to detect crayfish despite previous records. This lack of detection may be attributed to the completion of fieldwork at a time of year when crayfish activity is low and also the difficulty of detecting crayfish at low densities. Future surveys should take account of these limitations and where appropriate, modify the survey timings or methodologies to maximise the likelihood of crayfish detection.

Keywords: crayfish, distribution, Scotland, survey

INTRODUCTION

Unlike the rest of the British Isles, no crayfish species occur naturally in Scotland (Maitland 1996, Holdich et al. this volume). There are, however, two known introduced species. The white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet), inhabits Loch Croispol, Sutherland (Thomas 1992) and Whitemoss Reservoir, Renfrewshire (Maitland et al. 2001). The Loch Croispol population is thought to have originated from the introduction of crayfish to a feeder stream in 1945 (Thomas 1992). Local information suggests that the population at Whitemoss Reservoir has also been present for many decades (Maitland et al. 2001). The other crayfish species in Scotland, the North American signal crayfish, *Pacifastacus leniusculus* (Dana), was first recorded in the wild in Galloway in 1995 (Maitland 1996) and has become established in at least eight localities across the country (Bean et al. 2006).

White-clawed crayfish are protected under Schedule 5 of the Wildlife & Countryside Act 1981, Appendix III of the Bern Convention, Annexes IIa and Va of the EC Habitats Directive and The Nature Conservation (Scotland) Act 2004. Given the plight of white-clawed crayfish in the rest of Britain resulting from signal crayfish introductions and the subsequent spread of crayfish plague, these Scottish populations are likely to represent valuable refuge stocks for conservation in the future. Recent survey work has assessed and confirmed the suitability of Loch Croispol as an ark site for white-clawed crayfish, which is sufficiently isolated from the threat of invading signal crayfish and supports a healthy, recruiting population (the author 2009, pers. obs.). Future surveys will assess the status of the white-clawed crayfish population at Whitemoss Reservoir.

To date, there is no obvious evidence to suggest that white-clawed crayfish populations have negatively impacted native biota or have dispersed or been translocated from their sites of introduction. By contrast, the introduction and continued spread of signal crayfish has been highlighted as cause for concern in Scotland and is likely to have a significant impact on freshwater ecosystems. Previous research in Scotland has highlighted the potential for signal crayfish to impact Atlantic salmon stocks (Griffiths et al. 2004) and significantly alter the structure of invertebrate communities (Crawford et al. 2006). In 2007, the Scottish Government listed signal crayfish under the Species Action Framework (SAF) as an invasive species posing a significant threat to native freshwater biodiversity. This framework, developed and implemented by Scottish Natural Heritage (SNH) and partners, sets out a five-year long strategy for species management in Scotland (SNH 2007). One of the foremost objectives of the SAF Signal Crayfish Implementation Plan is to assess the distribution and status of signal crayfish in Scotland, which will allow control and containment programmes or other mitigation measures to be targeted appropriately.

The purpose of this paper is to outline the most recent efforts made by SNH and the Rivers and Fisheries Trusts of Scotland (RAFTS) to determine the fine-scale distribution of signal crayfish in Scotland. The merits and problems associated with this programme of work and implications for future projects are discussed.

MATERIALS and METHODS

Collating records

Prior to field surveys, existing records of signal crayfish distribution were collected from published and unpublished literature. Additionally, Fisheries Trusts and District Salmon Fishery Boards (DSFBs) were contacted by letter to appeal for up-to-date information. In December 2008, a workshop was held to allow verification of crayfish records by delegates from the Rivers and Fisheries Trusts of Scotland (RAFTS), the United Clyde Angling Protective Association Ltd (UCAPA), SNH and seven different Fisheries Trusts. Marine Scotland (formerly the Fisheries Research Services, FRS) and the Scottish Environment Protection Agency (SEPA) were also asked to provide access to any data held by them.

Field surveys

The locations of sites to be surveyed for crayfish were finalised based on the validity of previous records and the expert opinions of participating Fishery Trust and DSFB biologists. A list of sites surveyed during the exercise is provided in Table 1. In March 2009, a standard protocol for detecting signal crayfish (Gladman et al. *in prep.*) was applied at all survey sites. This active-search protocol was based upon the results of previous field experiments on the River Clyde and involved the sequential application of kick sampling, up to three runs of electro-fishing and baited-trap setting to determine crayfish presence. Sample timings and equipment including nets, traps, baits and field-recording sheets were standardised. Before and after use, equipment was thoroughly disinfected. All crayfish captured during the survey work were counted and killed on-site, prior to storage in 100% (Analar grade) alcohol.

Table 1. Locations of sites surveyed for signal crayfish in Scotland during 2008/9.

LOCATION	RIVER CATCHMENT	FISHERY TRUST UNDERTAKING THE SURVEY
Upper Clyde	Clyde	Clyde River Foundation
River North Esk (ponds); Lugar Burn/main stem	North Esk	Esks DSFBs
Pow Burn	South Esk	Esks DSFBs
Rankeillour Burn (Fife)	Eden	Forth Fisheries Trust
River Teith (pond and ditches)	Forth	Forth Fisheries Trust
River Tyne (Stillwater fishery, East Lothian	East Lothian Tyne	Forth Fisheries Trust
Tiel Burn (Fife)	Tiel	Forth Fisheries Trust
Murray Burn	Water of Leith	Forth Fisheries Trust
Kirkcudbrightshire	Dee	Galloway Fisheries Trust
Skyre Burn	Fleet	Galloway Fisheries Trust
River Nairn	Nairn	Ness and Beauly Fisheries Trust
Dighty Water (Dundee)	Dighty	Tay DSFB
River Earn	Earn	Tay DSFB
River Ardle (pond and small stream)	Ericht	Tay DSFB
Shee Water (pond and small stream)	Ericht	Tay DSFB
Rivers Ettrick and Till	Tweed	Tweed Foundation
Kirkbank (Teviot Water)	Tweed	Tweed Foundation

RESULTS and DISCUSSION

Fine-scale maps showing the distribution of signal crayfish in Scotland, based on the current surveys, are provided in Sinclair (2009). The main findings are summarised below:

Signal crayfish are now known to occupy at least 58 km of river length in Scotland. This figure represents a minimum estimate of crayfish distribution and does not include populations in large still waters such as Loch Ken in Galloway. Loch Ken is thought to contain the largest population of signal crayfish in Scotland and the Scottish Government has recently provided funding to undertake a major trapping research programme, which will include an assessment of crayfish distribution, population size and overall density. The present project has successfully confirmed and delimited signal crayfish distribution at a number of sites. It has also provided some indication of the relative density of crayfish within and between catchments and, in the case of the Clyde, helped determine the approximate upstream and downstream limit of crayfish distribution on the main stem and associated tributaries. New records of crayfish presence have been verified by surveys on the Arvie Burn in the Kirkcudbrightshire Dee catchment and on the Tiel Burn and its tributaries in Fife.

Based on the results, potential sites for eradication of localised populations on the Forth, Fleet, Tweed and Nairn catchments have been

identified. By contrast, surveys have demonstrated that the cost-effective eradication of crayfish populations in some areas, such as the Clyde and Kirkcudbrightshire Dee, is now impossible. The upstream spread from the main stem to adjoining burns in these well-established populations appears to be relatively slow. The reasons for this are unknown and require investigation. On the Clyde there is a pressing need to apply targeted control of the crayfish currently occupying headwaters and take preventative measures to avoid cross-catchment spread to the nearby River Annan.

The sequential use of kick sampling, electrofishing and trapping as part of the crayfish detection protocol has proven effective, with electrofishing generally detecting crayfish in sites where kick sampling failed (but requiring greater effort in terms of the time taken to obtain the positive result), thus providing information on the relative density of crayfish within catchments. Very few crayfish were caught in traps, supporting the decision to favour active search methods over passive ones, such as trapping. Kick sampling and electrofishing were also shown to be adaptable for use in still water, detecting crayfish in ponds on the Forth catchment. Electrofishing was unsuitable, however, for use in deep, turbid water or areas with very strong currents. Practitioners regarded the protocol as cost and time-effective: kick sampling does not require expensive or specialist equipment (i.e. only pond nets and trays), nor does it require specialist training for surveyors to implement; electrofishing is already an integral part of fishery surveys that are carried out by Fisheries Trusts throughout Scotland and so equipment and trained staff were readily available; traps were easily assembled and deployed. For a team of two or three people, the estimated time to apply all three methods at one site was one hour.

Despite its practicalities, the protocol failed to detect signal crayfish on several occasions. In two catchments, the Esk and the Tweed, no crayfish were found during surveys despite previous records. The Esk Rivers and Fisheries Trust reported the capture of a single crayfish during juvenile fish surveys in the Pow Burn in 2008; crayfish were also found at Drumtochty pond in the same year. No crayfish were found at either location during the present study which involved taking six replicate kick samples at each site and setting five traps which were checked daily for eight days (Pow Burn) and seven days (Drumtochty). Similarly, surveys failed to confirm previous records of crayfish presence in areas within the Tweed, Tay and Kirkcudbrightshire Dee catchments. Ponds on the Tay and Esk, which were previously subject to chemical control trials (Peay et al. 2006), did not yield crayfish during the current surveys (Peay 2009, pers. comm.).

Reasons for the lack of positive records at sites where signal crayfish were previously found are likely to relate primarily to the time of year in which sampling was undertaken and also the difficulty of detecting crayfish low densities. Due to external pressures, this programme of fieldwork was completed in Quarter 1 of 2008, during a time of year that is suboptimal to crayfish detection. At Knocknairling Burn in the Kirkcudbrightshire Dee catchment, for example, a local landowner reported crayfish as being easily visible during low summer flows two years ago but no crayfish were detected during the present surveys in March. The efficacy of surveying is likely to increase, therefore, during the summer months when water temperatures and subsequent crayfish activity are higher. Detecting crayfish at low densities, particularly in larger water bodies is difficult, as observed on the Tweed. Variation in weather

conditions and habitat type between catchments may also have impacted the efficiency of crayfish detection. During surveys on the Tay catchment, for example, sampling conditions were poor due to snowmelt and at some sites electrofishing was not possible due to high water. At two sites on the Nairn, kick sampling was not possible due to excessive depth within the sampling area or the presence of deep silts within the main river channel.

It is hoped that this project marks the beginning of a long-term monitoring plan for signal crayfish in Scotland. Data collected this year using the standardised method will serve as a baseline against which future changes in crayfish distribution can be assessed. Such work will provide a useful body of knowledge for use by SNH, SEPA and others involved in the monitoring and management of invasive non-native species in Scotland and other parts of the UK. Feedback from practitioners will aid improvements in the design of the signal crayfish detection protocol, which may already require modification to take account of variation in water body or habitat type, weather conditions and crayfish density between catchments. Increasing the number of kick sample and electrofishing replicates might help improve the reliability of the protocol as a detection method. The feasibility of incorporating crayfish surveying into routine fishery monitoring work during the summer, when crayfish activity is highest and most detectable, should be considered. Data relating to the distribution of this species must be kept up to date to ensure that an early warning of new populations is obtained whilst the opportunity still exists to initiate a rapid management or eradication programme. Developing and implementing the best strategy for such programmes has been the focus of previous research (Reeve 2002, Ribbens and Graham 2004) and is currently under review (Freeman et al. 2009); this will form the next step in fulfilling the aims of the SAF Signal Crayfish Implementation Plan.

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REFERENCES

Bean CW, Maitland PS and Collen P (2006). Crayfish in Scotland: A review of current status and legislative control. *Freshwater Crayfish* 15: 220-228.

Crawford L, Yeomans WE and Adams CE (2006). The impact of introduced signal crayfish

Pacifastacus leniusculus on stream invertebrate communities. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16: 611-621.

Freeman MA, Turnbull JF, Yeomans WE and Bean CE (2009). Prospects for the control of crayfish in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems* DOI: 10.1002/aqc.1065.

Griffiths SW, Collen P and Armstrong JD (2004). Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology* 65: 436-447.

Maitland PS (1996). The North American signal crayfish, *Pacifastacus leniusculus* (Dana), established in the wild in Scotland. *Aquatic Conservation*:   *Marine and Freshwater Ecosystems* 6(2): 107-110.

Maitland PS, Sinclair C and Doughty CR (2001). The status of freshwater crayfish in Scotland in the year 2000. *Glasgow Naturalist* 23:26-32.

Peay S (2009). Scott Wilson Ltd/University of Leeds, England. *Personal communication*.

Peay S, Hiley PD, Collen and Martin I (2006). Biocide treatment of ponds in Scotland to eradicate signal crayfish. *Bulletin Français de la Pêche et de la Pisciculture* 380-381(1-2): 1363-1379.

Reeve ID (2002). The removal of the North American signal crayfish (*Pacifastacus leniusculus*) from the River Clyde. Scottish Natural Heritage Commissioned Report No. 020 (ROAME No. F) LI12).

Ribbens JCH and Graham JL (2004). Strategy for the conservation and possible eradication of the American signal crayfish (*Pacifastacus leniusculus*) in the River Dee catchment and Skyre Burn catchment, Dumfries and Galloway. Scottish Natural Heritage Commissioned Report No. 014. (ROAME No. FO2lk05).

Scottish Natural Heritage (2007). A Five Year Species Action Framework: Making a Difference for Scotland's Species.

Sinclair CA (2009). Fine scale mapping of signal crayfish distribution in Scotland. Scottish Natural Heritage Commissioned Report, Project 26686.

Thomas WJ (1992). A note on the Crayfish of Loch Croispol. *Glasgow Naturalist* 22:102-109.

Appendix II

The following "crayfish detection protocol" was used by the Rivers and Fisheries Trusts of Scotland, as described in Gladman *et al.* (2010).

(1) Prior to sampling:
- Photograph the site
- Note the grid reference
- Disinfect all equipment (1% Virkon and drying recommended)

(2) Check the watercourse for:
- Muddy substrate
- Burrows
- Vegetation cover
- Slower flowing water
- Diggings in front of rocks
- Deep water (trapping only)

(3) Focus kick sampling in an area that fits one or more of criteria set out in (2), particularly along the river margins as follows:
- Move around the sample area, kicking vigorously for 30 seconds
- Hold a pond net on the riverbed, downstream of the kicking
- Sweep the net through areas of dense weed/tree roots and undercut banks
- After 30 seconds, empty the sample into a large white tray and search thoroughly for crayfish
- Return to the sample area and kick for another 30 seconds
- Empty the second sample into a white tray and search thoroughly for crayfish
- Repeat the above until a total of 6 samples (equivalent to 3 minutes kicking time) have been collected and subsequently searched for crayfish

(4a) Crayfish Present

(4b) Crayfish Absent

- Count and note the total number of crayfish caught
- Kill the crayfish by placing them in a container of alcohol; label the container with the date, site description and method of sampling
- Cease sampling
- Ensure all equipment is free of crayfish before leaving the site:
➡ Check underside of footwear (felt-soled waders are not recommended)
➡ Check and wash out nets

Conduct electrofishing as follows:
- Set the electrofishing equipment to an output optimum for the site to be sampled (e.g. 200V smooth dc current is recommended for the Upper Clyde)
- Choose the bank which best fits the criteria set out in (2) and begin sampling from this side
- A shocker and netter should work together, systematically moving the anode through the sample site for 5 minutes
- To disturb crayfish resting on the river bed, bounce the anode along the bottom of the substrate
- Catch crayfish using a hand net held close to the anode (banner nets are not recommended)

(5a) Crayfish Present

(5b) Crayfish Absent

If one 5-minute pass does not yield crayfish, complete a second 5-minute pass

(6a) Crayfish Present

(6b) Crayfish Absent

If two 5-minute passes do not yield crayfish, complete a third 5-minute pass

(7a) Crayfish Present

(7b) Crayfish Absent

(8a) Crayfish Present

If the third pass of electrofishing does not yield crayfish:
- Select a suitable location for trapping, according to the habitat criteria set out in (2)
- Bait a Swedish trappy trap with previously frozen rainbow trout
- Attach plastic string to the trap and secure it to the bank using a stake
- Ensure the string is of adequate length, with enough slack to allow movement and prevent damage in the event of changing water levels
- In fast flowing water, stones may be used to provide the trap with additional weight for sinking
- Launch the trap into the water body
- Set further traps between this location and the down (or upstream) location where crayfish were last detected by kick sampling or electrofishing, as an extension to the no crayfish limit
- Check each trap after 24-48 hours for crayfish, recording the period between trap setting and checking; in cold periods when crayfish are less likely to be active, a 48 hour period may be preferable

(8b) Crayfish Absent

Record the limit of distribution (Grid reference and/or GPS co-ordinates) based on the furthest up or downstream where crayfish are captured in traps

Bibliography

Abrahamsson SAA, Goldman CR. 1970. Distribution, density and production of the crayfish *Pacifastacus leniusculus* Dana in Lake Tahoe, California - Nevada. *Oikos* 21: 83-91.

Acosta CA, Perry SA. 2000. Effective sampling area: a quantitative method for sampling crayfish populations in freshwater marshes. *Crustaceana* 73: 425-431.

Aiken DE, Waddy SL. 1987. Molting and growth in crayfish: a review. Canadian Technical Report of Fisheries and Aquatic Sciences 1587. New Brunswick, Canada. 1-34.

Aiken DE, Waddy SL. 1992. The growth process in crayfish. *Reviews in Aquatic Sciences* 6 (3, 4): 335-381.

Alonso F. 2001. Efficiency of electrofishing as a sampling method for freshwater crayfish populations in small creeks. *Limnetica* 20(1): 59-72.

Angeler DG, Sánchez-Carrillo S, García G, Alvarez-Cobelas M. 2001. The influence of *Procambarus clarkii* (Cambaridae, Decapoda) on water quality and sediment characteristics in a Spanish floodplain wetland. *Hydrobiologia* 464: 89-98.

Armitage V. 2000. Observations of radio tracked crayfish (*Austropotamobius pallipes*) in a northern British river In *Crayfish Conference*, Rogers D, Brickland J (eds). Leeds; 58-74.

Axelsson E, Nyström P, Sidenmark J, Bronmark C. 1997. Crayfish predation on amphibian eggs and larvae. *Amphibia-Reptilia* 18: 217-228.

Barbaresi S, Gherardi F. 2001. Daily activity of the white-clawed crayfish, Austropotamobius pallipes (Lereboullet): a comparison between field and laboratory studies. *Journal of Natural History* 35: 1861 - 1871.

Barbaresi S, Santini G, Tricarico E, Gherardi F. 2004. Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History* 38: 2821-2832.

Bardonnet A, Bagliniere JL. 2000. Freshwater habitat of Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* 57: 497-506.

Bauer G. 1988. Threats to the freshwater pearl mussel *Margaritifera margaritifera* L. in Central Europe. *Biological Conservation* 45: 239-253.

Bauer G. 1998. Allocation policy of female freshwater pearl mussels. *Oecologia* 117: 90-94.

Bean CW. 2012. Scottish Natural Heritage. Personal communication.

Bean CW, Maitland PS, Collen P. 2006. Crayfish in Scotland: a review of current status and legislative control. *Freshwater Crayfish* 15: 220-228.

Beasley CR, Roberts D, Mackie TG. 1998. Does the freshwater pearl mussel, *Margaritifera margaritifera* L., face extinction in Northern Ireland? *Aquatic Conservation: Marine and Freshwater Ecosystems* 8: 265-272.

Beebee TJC, Buckley J, Evans I, Foster JP, Gent AH, Gleed-Owen CP, Kelly G, Rowe G, Snell C, Wycherley JT, Zeisset I. 2005. Neglected native or undesirable alien? Resolution of a conservation dilemma concerning the pool frog *Rana lessonae*. *Biodiversity and Conservation* 14: 1607-1626.

Beebee TJC, Griffiths RA. 2005. The amphibian decline crisis: A watershed for conservation biology? *Biological Conservation* 125: 271-285.

Bernardo JM, Ilheu M, Costa AM. 1997. Distribution, population structure and conservation of *Austropotamobius pallipes* in Portugal. *Bulletin Français de la Pêche et de la Pisciculture* 347: 617-624.

Bills TD, Marking LL. 1988. Control of nuisance populations of crayfish with traps and toxicants. *The Progressive Fish-Culturist* 50: 103-106.

BirdLife International. 2008. Critically Endangered birds: a global audit. Cambridge, UK.

Bjurstrom L, Ruokonen T, Pursiainen M, Jones RI, Hämäläinen H. 2010. Impacts of the invasive signal crayfish on littoral macroinvertebrates of large boreal lakes: A pilot study in Lake Päijänne, Finland. *Freshwater Crayfish* 17: 177-182.

Blake M, Hart PJB. 1993. Habitat preferences and survival of juvenile signal crayfish, *Pacifastacus leniusculus* - the influence of water depth, substratum, predatory fish and gravid female fish. *Freshwater Crayfish* 9: 318-332.

Blake MA, Hart PJB. 1995. The vulnerability of juvenile signal crayfish to perch and eel predation. *Freshwater Biology* 33: 233-244.

Blunsum, A. 2011. Personal communication.

Bovbjerg RV. 1959. Density and dispersal in laboratory crayfish populations. *Ecology* 40: 504-506.

Bradshaw CJA, Isagi Y, Kaneko S, Bowman DMJS, Brook BW. 2006. Conservation value of non-native banteng in Northern Australia. *Conservation Biology* 20: 1306-1311.

Breathnach S, Fairley JS. 1993. The diet of otters *Lutra lutra* (L.) in the Clare River system. *Biology and Environment: Proceedings of the Royal Irish Academy* 93B: 151-158.

Brewis JM, Bowler K. 1982. The growth of the freshwater crayfish *Austropotamobius pallipes* in Northumbria. *Freshwater Biology* 12: 187-200.

Brown CL, Poe TP, French III JRPF, Schloesser DW. 1988. Relationships of phytomacrofauna to surface area in naturally occurring macrophyte stands. *Journal of the North American Benthological Society* 7: 129-139.

Bubb DH, Lucas MC, Thom TJ. 2002. Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in an upland river, determined by radio telemetry. *Hydrobiologia* 483: 111-119.

Bubb DH, O'Malley OJ, Gooderham AC, Lucas MC. 2009. Relative impacts of native and non-native crayfish on shelter use by an indigenous benthic fish. *Aquatic Conservation: Marine and Freshwater Ecosystems* 19: 448-455.

Bubb DH, Thom TJ, Lucas MC. 2004. Movement and dispersal of the invasive signal crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology* 49: 357-368.

Bubb DH, Thom TJ, Lucas MC. 2006a. Movement, dispersal and refuge use of co-occurring introduced and native crayfish. *Freshwater Biology* 51: 1359-1368.

Bubb DH, Thom TJ, Lucas MC. 2006b. Movement patterns of the invasive signal crayfish determined by PIT telemetry. *Canadian Journal of Zoology-Revue Canadienne de Zoologie* 84: 1202-1209.

Bubb DH, Thom TJ, Lucas MC. 2008. Spatial ecology of the white-clawed crayfish in an upland stream and implications for the conservation of this endangered species. *Aquatic Conservation: Marine and Freshwater Ecosystems* 18: 647-657.

Bubb DH, Thom TJ, Lucas MC. 2005. The within-catchment invasion of the non-indigenous signal crayfish *Pacifastacus leniusculus* (Dana), in upland rivers. *Bulletin Français de la Pêche et de la Pisciculture* 665-673.

Buřic M, Hulák M, Kouba A, Petrusek A, Kozák P. 2011. A successful crayfish invader is capable of facultative parthenogenesis: a novel reproductive mode in decapod crustaceans. *PLoS ONE* 6: 1-5.

Buřič M, Kouba A, Kozák P. 2009a. Spring mating period in *Orconectes limosus*: the reason for movement. *Aquatic Sciences - Research Across Boundaries* 71: 473-477.

Buřič M, Kozak P, Kouba A. 2009b. Movement patterns and ranging behavior of the invasive spiny-cheek crayfish in a small reservoir tributary. *PhD Thesis, University of South Bohemia*.

Byrne CF, Lynch JM, Bracken JJ. 1999. A sampling strategy for stream populations of white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet) (Crustacea, Astacidae). *Biology and Environment: Proceedings of the Royal Irish Academy* 99B: 89-94.

Byström P, Karlsson J, Nilsson P, Van Kooten T, Ask J, Olofsson F. 2007. Substitution of top predators: effects of pike invasion in a subarctic lake. *Freshwater Biology* 52(7): 1271-1280.

Chornesky EA, Randall JM. 2003. The threat of invasive alien species to biological diversity: setting a future course. *Annals of the Missouri Botanical Garden* 90: 67-76.

Clavero M, García-Berthou E. 2005. Invasive species are a leading cause of animal extinctions. *Trends in Ecology & Evolution* 20: 110-110.

Conant S. 1988. Saving endangered species by translocation: are we tinkering with evolution? *Bioscience* 38: 254-257.

Correia AMa. 2002. Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). *Acta Oecologica* 23: 421-429.

Cosgrove PJ, Young MR, Hastie LC, Gaywood M, Boon PJ. 2000. The status of the freshwater pearl mussel *Margaritifera margaritifera* Linn. in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems* 10: 197-208.

Cosgrove PJ, Hastie LC, Syme I. 2007. Recorded natural predation of freshwater pearl mussels *Margaritifera margaritifera* (L.) in Scotland. *Journal of Conchology* 39: 469-472.

Covich AP, Dye LL, Mattice JS. 1981. Crayfish predation on *Corbicula* under laboratory conditions. *American Midland Naturalist* 105: 181-188.

Crandall KA, Buhay JE. 2008. Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae—Decapoda) in freshwater. *Hydrobiologia* 595: 295-301.

Crawford L, Yeomans WE, Adams CE. 2006. The impact of introduced signal crayfish *Pacifastacus leniusculus* on stream invertebrate communities. *Aquatic Conservation: Marine and Freshwater Ecosystems* 16: 611-621.

Crisp DT, Carling PA. 1989. Observations on siting, dimensions and structure of salmonid redds. *Journal of Fish Biology* 34: 119-134.

Crooks JA. 2005. Lag times and exotic species: the ecology and management of biological invasions in slow-motion. *Ecoscience* 12(3): 316-329.

Crooks JA, Soulé ME. 1999. Lag times in population explosions of invasive species: causes and implications. In *Invasive Species and Biodiversity Management*, Sandlund OT, Schei PJ, Viken A (eds). Kluwer Academic Publishers, The Netherlands. 103-125.

de Gaudemar B, Schroder SL, Beall EP. 2000. Nest placement and egg distribution in Atlantic salmon redds. *Environmental Biology of Fishes* 57: 37-47.

DeeFish. 2009. Personal communication.

DEFRA. 2003. Review of non-native species policy. Report of the working group. *Department for Environment Food and Rural Affairs*.

DEFRA. 2008. The Invasive Non-Native Species Framework Strategy for Great Britain. *Department for Environment Food and Rural Affairs*.

DEFRA, 2011. What are invasive non-native species? URL: https://secure.fera.defra.gov.uk/nonnativespecies/index.cfm?sectionid=15 Accessed: 03/02/13

Degerman E, Nilsson PA, Nyström P, Nilsson E, Olsson K. 2007. Are fish populations in temperate streams affected by crayfish? - A field survey and prospects. *Environmental Biology of Fishes* 78: 231-239.

Demers A, Reynolds JD, Cioni A. 2003. Habitat preference of different size classes of *Austropotamobius pallipes* in an Irish river. *Bulletin Français de la Pêche et de la Pisciculture* 127-137.

DeVries P. 1997. Riverine salmonid egg burial depths: review of published data and implications for scour studies. *Canadian Journal of Fisheries and Aquatic Sciences* 54: 1685-1698.

Dudgeon D, Arthington AH, Gessner MO, Kawabata Z-I, Knowler DJ, Lévêque C, Naiman RJ, Prieur-Richard A-H, Soto D, Stiassny MLJ, Sullivan CA. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews* 81: 163-182.

Edmonds NJ, Riley WD, Maxwell DL. 2011. Predation by *Pacifastacus leniusculus* on the intra-gravel embryos and emerging fry of *Salmo salar*. *Fisheries Management and Ecology* 18: 521-524.

Ellrott BJ, Marsden JE, Fitzsimons JD, Jonas JL, Claramunt RM. 2007. Effects of temperature and density on consumption of trout eggs by *Orconectes propinquus* and *O. rusticus*. *Journal of Great Lakes Research* 33: 7-14.

Elser JJ, Junge C, Goldman CR. 1994. Population structure and ecological effects of the crayfish *Pacifastacus leniusculus* in Castle Lake, California. *Great Basin Naturalist* 54: 162-169.

Elton CS. 1958. *The Ecology of Invasions by Animals and Plants*. The University of Chicago Press: Chicago and London.

Englund G, Krupa JJ. 2000. Habitat use by crayfish in stream pools: influence of predators, depth and body size. *Freshwater Biology* 43: 75-83.

Environment Agency. 2010. "Not too late for crayfish on the ark." URL: http://www.environment-agency.gov.uk/news/132082.aspx Accessed: 05/07/12.

Ermgassen P, Aldridge D. 2011. Predation by the invasive American signal crayfish, *Pacifastacus leniusculus* Dana, on the invasive zebra mussel, *Dreissena polymorpha* Pallas: the potential for control and facilitation. *Hydrobiologia* 658: 303-315.

Ficetola GF, Miaud C, Pompanon Fo, Taberlet P. 2008. Species detection using environmental DNA from water samples. *Biology Letters* 4: 423-425.

Findlay JDS. 2012. Personal communication.

Finlay JC, Kendall C. 2008. Stable isotope tracing of temporal and spatial variability in organic matter sources to freshwater ecosystems. In *Stable Isotopes in Ecology and Environmental Science*. Blackwell Publishing Ltd: 283-333.

Firkins I, Holdich DM. 1993. Thermal studies on three species of freshwater crayfish. *Freshwater crayfish* 9:241-248.

Fitzsimons J, Williston B, Williston G, Bravener G, Jonas JL, Claramunt RM, Marsden JE, Ellrott BJ. 2006. Laboratory estimates of salmonine egg predation by round gobies (*Neogobius melanostomus*), sculpins (*Cottus cognatus* and *C-bairdi*), and crayfish (*Orconectes propinquus*). *Journal of Great Lakes Research* 32: 227-241.

Flint RW. 1977. Seasonal activity, migration and distribution of crayfish, *Pacifastacus leniusculus*, in Lake Tahoe. *American Midland Naturalist* 97: 280-292.

Flint RW, Goldman CR. 1977. Crayfish growth in Lake Tahoe - effects of habitat variation. *Journal of the Fisheries Research Board of Canada* 34: 155-159.

Flint RW, Goldman CR. 1975. The effects of a benthic grazer on the primary productivity of the littoral zone of Lake Tahoe. *Limnology and Oceanography* 20: 935-944.

Frank H, Gerstmann S. 2007. Declining populations of freshwater pearl mussels (*Margaritifera margaritifera*) are burdened with heavy metals and DDT/DDE. *Ambio* 36: 571-574.

Freeman MA, Turnbull JF, Yeomans WE, Bean CW. 2010. Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 211-223.

FBA, 2012. Freshwater Biological Association. Collecting freshwater macrophyte samples. URL: http://www.fba.org.uk/recorders/publications_resources/sampling-protocols/contentParagraph/01/document/CourseInvertSamplingProtocol.pdf. Accessed: 28/11/12.

Füreder L, Gherardi F, Holdich DM, Reynolds J, Sibley P, Souty-Grosset C. 2010. *Austropotamobius pallipes*. In *IUCN 2010. IUCN Red List of Threatened Species*.

Gallagher MB, Dick JTA, Elwood RW. 2006. Riverine habitat requirements of the white-clawed crayfish, *Austropotamobius pallipes*. *Biology and Environment: Proceedings of the Royal Irish Academy* 106B: 1-8.

Gamradt SC, Kats LB, Anzalone CB. 1997. Aggression by non-native crayfish deters breeding in California newts. *Conservation Biology* 11: 1042-1042.

Geist J. 2010. Strategies for the conservation of endangered freshwater pearl mussels (*Margaritifera margaritifera* L.): a synthesis of conservation genetics and ecology. *Hydrobiologia* 644: 69-88.

Genovesi P, Shine C. 2003. European Strategy on Invasive Alien Species. *Convention on the Conservation of European Wildlife and Natural Habitats.* Council of Europe.

Gherardi F. 2011. Towards a sustainable human use of freshwater crayfish (Crustacea, Decapoda, Astacidea). *Knowledge and Management of Aquatic Ecosystems* 401: 1-22.

Gherardi F. 2007. Understanding the impact of invasive crayfish. In *Biological invaders in inland waters: profiles, distribution, and threats*. Gherardi F (ed). Springer, Dordrecht. 507-542.

Gherardi F, Barbaresi S. 2000. Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv fuer Hydrobiologie* 150: 153-168.

Gherardi F, Barbaresi S, Salvi G. 2000. Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences - Research Across Boundaries* 62: 179-193.

Gherardi F, Tricarico E, Ilhéu M. 2002. Movement patterns of an invasive crayfish, *Procambarus clarkii*, in a temporary stream of southern Portugal. *Ethology Ecology & Evolution* 14: 183-197.

Gladman ZF, Adams CE, Bean CW, Sinclair CA, Yeomans WE. 2009. Signal crayfish in Scotland. In *Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009.*, Brickland J, Holdich DM, Imhoff EM (eds). Leeds, UK. 43-48.

Gladman ZF, Yeomans WE, Adams CE, Bean CW, McColl D, Olszewska JP, McGillivray CW, McCluskey R. 2010. Detecting North American signal crayfish (*Pacifastacus leniusculus*) in riffles. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20 (5): 588-594.

Goldman CR, Rundquist JC. 1977. A comparative ecological study of the California crayfish, *Pacifastacus leniusculus* (Dana) from two subalpine lakes (Lake Tahoe and Lake Donner). *Freshwater Crayfish* 3: 51-80.

Gouin N, Grandjean F, Pain S, Souty-Grosset C, Reynolds J. 2003. Origin and colonization history of the white-clawed crayfish, *Austropotamobius pallipes*, in Ireland. *Heredity* 91: 70-77.

Griffiths SW, Collen P, Armstrong JD. 2004. Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology* 65: 436-447.

Grow L. 1982. Burrowing soil-texture relationships in the crayfish, *Cambarus diogenes diogenes* Girard (Decapoda, Astacidea). *Crustaceana* 42: 150-157.

Guan RZ. 2000. Abundance and production of the introduced signal crayfish ina British lowland river. *Aquaculture International* 8: 59-76.

Guan RZ. 1994. Burrowing behaviour of signal crayfish, *Pacifastacus leniusculus* (Dana), in the River Great Ouse, England. *Freshwater Forum* 4: 155-168.

Guan RZ. 1997. An improved method for marking crayfish. *Crustaceana* 70: 641-652.

Guan RZ, Wiles PR. 1997a. Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation Biology* 11: 641-647.

Guan RZ, Wiles PR. 1998. Feeding ecology of the signal crayfish *Pacifastacus leniusculus* in a British lowland river. *Aquaculture* 169: 177-193.

Guan RZ, Wiles PR. 1997b. The home range of signal crayfish in a British lowland river. *Freshwater Forum* 8: 45-54.

Hastie LC, Young MR, Boon PJ, Cosgrove PJ, Henninger B. 2000. Sizes, densities and age structures of Scottish *Margaritifera margaritifera* (L.) populations. *Aquatic Conservation: Marine and Freshwater Ecosystems* 10: 229-247.

Hastie LC, Young MR. 2003. Timing of spawning and glochidial release in Scottish freshwater pearl mussel (*Margaritifera margaritifera*) populations. *Freshwater Biology* 48: 2107-2117.

Hayes RB. 2012. Consequences for lotic ecosystems of invasion by signal crayfish. *PhD Thesis, Queen Mary University of London*.

Hazlett B, Rittschof D, Ameyaw-Akumfi C. 1979. Factors affecting the daily movements of the crayfish *Orconectes virilis* (Hagen, 1870) (Decapoda, Cambaridae). *Crustaceana. Supplement* 121-130.

Hein CL, Roth BM, Ives AR, Zanden MJV. 2006. Fish predation and trapping for rusty crayfish (*Orconectes rusticus*) control: a whole-lake experiment. *Canadian Journal of Fisheries and Aquatic Sciences* 63: 383-393.

Hein CL, Vander Zanden MJ, Magnuson JJ. 2007. Intensive trapping and increased fish predation cause massive population decline of an invasive crayfish. *Freshwater Biology* 52: 1134-1146.

Helama S, Valovirta I. 2007. Shell morphometry, pre-mortal taphonomy and ontogeny-related growth characteristics of freshwater pearl mussel in northern Finland. *Annales Zoologici Fennici* 44: 285-302.

Henry KA. 1951. Spring creek crayfish migrations 1949 and 1950. *Oregon Fish Commission Research Briefs* 3: 48-55.

Henttonen P, Huner JV. 1999. The introduction of alien species in Europe: a historical introduction. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 13-22.

Hepworth DK, Duffield DJ. 1987. Interactions between an exotic crayfish and stocked rainbow trout in Newcastle Reservoir, Utah. *North American Journal of Fisheries Management* 7: 554 - 561.

Hiley PD. 2003. The slow quiet invasion of signal crayfish (*Pacifastacus leniusculus*) in England — prospects for the white-clawed crayfish (*Austropotamobius pallipes*). In *Management and conservation of crayfish: Proceedings of a conference held in Nottingham on 7 November 2002*, Holdich DM, Sibley PJ (eds). Environment Agency: Bristol; 127-138.

Hobbs HH, Jass JP, Huner JV. 1989. A review of global crayfish introductions with particular emphasis on two North-American species (Decapoda, Cambaridae). *Crustaceana* 56: 299-316.

Hogger JB. 1986. Aspects of the introduction of signal crayfish *Pacifastacus leniusculus* (Dana), into the Southern United-Kingdom .1. Growth and Survival. *Aquaculture* 58: 27-44.

Holdich DM. 2002. Background and functional morphology. In *Biology of freshwater crayfish*, Holdich DM (ed). Blackwell Publishing Ltd.: Oxford; 720.

Holdich DM. 1999. The negative effects of established crayfish introductions. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 31-47.

Holdich DM, Palmer M, Sibley PJ. The indigenous status of *Austropotamobius pallipes* (Lereboullet) in Britain. In *Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009.*, Brickland J, Holdich DM, Imhoff EM (eds). Leeds, UK. 1-11.

Holdich DM, Reynolds JD, Souty-Grosset C, Sibley PJ. 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 11: 394-395.

Holdich DM, Sibley P, Peay S. 2004. The white-clawed crayfish - a decade on. *British Wildlife* 15: 153-164.

Holdich DM, Ackefors H, Gherardi F, Rogers D, Skurdal J. 1999a. Native and alien crayfish in Europe: some conclusions. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 281-291.

Holdich DM, Gydemo R, Rogers WD. 1999b. A review of possible methods for controlling nuisance populations of alien crayfish. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 245-270.

Holdich DM, Lowery R. 1988. *Freshwater crayfish: biology, management and exploitation*. Croom Helm Ltd, Beckenham.

Holdich DM, Reeve ID. 1991. Distribution of fresh-water crayfish in the British Isles, with particular reference to crayfish plague, alien introductions and water-quality. *Aquatic Conservation: Marine and Freshwater Ecosystems* 1: 139-158.

Holdich DM, Rogers WD, Reynolds JD. 1999c. *Native and alien crayfish in the British Isles*. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 221-234.

Huxley TH. 1880. *The Crayfish: an introduction to the study of zoology*. D. Appleton and Company: New York.

Irlandi EA, Mehlich ME. 1996. The effect of tissue cropping and disturbance by browsing fishes on growth of two species of suspension-feeding bivalves. *Journal of Experimental Marine Biology and Ecology* 197: 279-293.

IUCN, 1999. IUCN Guidelines for the Prevention of Biodiversity Loss caused by Alien Invasive Species. 24 pp. *International Union*

IUCN. 2011. IUCN Red List of Threatened Species. Crayfish. Version 2011.2. *International Union for Conservation of Nature*.

IUCN. 2002. Technical Guidelines on the Management of Ex-situ populations for Conservation. Approved at the 14th Meeting of the Programme Committee of Council, Gland, Switzerland, 10 December 2002. *International Union for Conservation of Nature*.

Jackson M. 2012. Personal communication.

Jay D, Holdich DM. 1981. The distribution of the crayfish, *Austropotamobius pallipes*, in British waters. *Freshwater Biology* 11: 121-129.

Jeppesen E, Søndergaard M, Søndergaard M, Christoffersen K, eds. 1998. *The structuring role of submerged macrophytes in lakes*. Berlin: Springer-Verlag.

JNCC. 2007. Second Report by the UK under Article 17 on the implementation of the Habitats Directive from January 2001 to December 2006. *Joint Nature Conservation Committee*.

Johnson MF, Rice SP, Reid I. 2010. Topographic disturbance of subaqueous gravel substrates by signal crayfish (*Pacifastacus leniusculus*). *Geomorphology* 123: 269-278.

Josefsson M, Andersson B. 2009. The environmental consequences of alien Species in the Swedish Lakes Mälaren, Hjälmaren, Vänern and Vättern. *AMBIO: A Journal of the Human Environment* 30: 514-521.

Karen AW, John JM, David ML, Anna MH, Timothy KK, William LP, Theodore VW. 2004. A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. *Canadian Journal of Fisheries and Aquatic Sciences* 61: 2255-2266.

Kats L, Pintor L, Sih A, Kerby J. 2006. Aquatic nuisance species: a multi-stage approach to understanding the invasion ecology of exotic crayfish in Northern and Southern California. *Research Final Reports*, California Sea Grant College Program, UC San Diego. URL: http://www.escholarship.org/uc/item/9vv2r9t4 Accessed: 03/12/12.

Kerby JL, Riley SPD, Kats LB, Wilson P. 2005. Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. *Biological Conservation* 126: 402-409.

Kershner MW, Lodge DM. 1995. Effects of littoral habitat and fish predation on the distribution of an exotic crayfish, *Orconectes rusticus*. *Journal of the North American Benthological Society* 14: 414-422.

Kirjavainen J, Westman K. 1999. Natural history and development of the introduced signal crayfish, *Pacifastacus leniusculus*, in a small, isolated Finnish lake, from 1968 to 1993. *Aquatic Living Resources* 12: 387-401.

Klocker CA, Strayer DL. 2004. Interactions among an invasive crayfish (*Orconectes rusticus*), a native crayfish (*Orconectes limosus*), and native bivalves (Sphaeriidae and Unionidae). *Northeastern Naturalist* 11: 167-178.

Koenig WD, Knops JMH. 2001. Seed-crop size and eruptions of North American boreal seed-eating birds. *Journal of Animal* Ecology 70: 609-620.

Kondolf GM, Wolman MG. 1993. The sizes of salmonid spawning gravels. *Water Resources Research* 29: 2275-2285.

Kouba A, Buřič M, Kozák P. 2010. Bioaccumulation and effects of heavy metals in crayfish: a review. *Water, Air, & Soil Pollution* 211: 5-16.

Krebs CJ. 1998. *Ecological methodology (second edition)*. Benjamin Cummings: University of British Columbia.

Lawrence CS, Morrissy NM, Vercoe PE, Williams IH. 2006. Harvesting freshwater crayfish (*Cherax albidus* Clark) by trapping contributes to high densities and stunted animals - a preliminary population model. *Freshwater Crayfish* 15: 56-62.

Lewis SD. 2002. Pacifastacus. In *Biology of Freshwater Crayfish*, Holdich DM (ed). Blackwell Science: Oxford; 511-540.

Lewis SD, Horton HF. 1997. Life history and population dynamics of the signal crayfish, *Pacifastacus leniusculus*, in Lake Billy Chinook, Oregon. *Freshwater Crayfish* 11: 34-53.

Light T. 2005. Behavioral effects of invaders: alien crayfish and native sculpin in a California stream. *Biological Invasions* 7: 353-367.

Light T. 2003. Success and failure in a lotic crayfish invasion: the roles of hydrologic variability and habitat alteration. *Freshwater Biology* 48: 1886-1897.

Lockwood JL, Hoopes MF, Marchetti MP. 2007. Invasion Ecology. Blackwell Publishing: Oxford; 304 pp.

Lodge D, Hill A. 1994. Factors governing species composition, population size, and productivity of cool-water crayfishes. *Nordic Journal of Freshwater Research* 69: 111-136.

Lodge DM, Lorman JG. 1987. Reductions in submersed macrophyte biomass and species richness by the crayfish *Orconectes rusticus*. *Canadian Journal of Fisheries and Aquatic Sciences* 44: 591-597.

Lodge DM, Taylor CA, Holdich DM, Skurdal J. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. *Fisheries* 25: 7-20.

Love J, Savino JF. 1993. Crayfish (*Orconectes virilis*) predation on zebra mussels (*Dreissena polymorpha*). *Journal of Freshwater Ecology* 8: 253-259.

Lowe S, Browne M, Boudjelas S, De Poorter M. 2000. 100 of the world's worst invasive alien species: a selection from the Global Invasive Species Database. *Aliens 12, The Invasive Species Specialist Group (ISSG)*.

MacIsaac HJ. 1994. Size-selective predation on zebra mussels (*Dreissena polymorpha*) by crayfish (*Orconectes propinquus*). *Journal of the North American Benthological Society* 13: 206-216.

Maitland P, Sinclair C, Doughty C. 2001. The status of freshwater crayfish in Scotland in the year 2000. *Glasgow Naturalist* 23: 26-32.

Maitland PS. 1996. The North American signal crayfish, *Pacifastacus leniusculus* (Dana), established in the wild in Scotland. *Aquatic Conservation: Marine and Freshwater Ecosystems* 6: 107-110.

Manchester SJ, Bullock JM. 2000. The impacts of non-native species on UK biodiversity and the effectiveness of control. *Journal of Applied Ecology* 37: 845-864.

Mason JC. 1975. Crayfish production in a small woodland stream. *Freshwater Crayfish* 2: 449-479.

Matthews MA, Reynolds JD. 1995. A population study of the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet) in an Irish Reservoir. *Biology and Environment: Proceedings of the Royal Irish Academy* 95B: 99-109.

McGeoch MA, Butchart SHM, Spear D, Marais E, Kleynhans EJ, Symes A, Chanson J, Hoffmann M. 2010. Global indicators of biological invasion: species numbers, biodiversity impact and policy responses. *Diversity and Distribution* 16: 95-108.

McLachlan JS, Hellmann JJ, Schwartz MW. A framework for debate of assisted migration in an era of climate change. *Conservation Biology* 21(2): 297-302.

Merkle EL. 1969. Home range of crayfish *Orconectes juvenalis. American Midland Naturalist* 81: 228-235.

Miller RR, Williams JD, Williams JE. 1989. Extinctions of North American Fishes during the past century. *Fisheries* 14: 22-38.

Mitchell, M. 2009. Personal communication.

Mkoji GM, Hofkin BV, Kuris AM, Stewart-Oaten A, Mungai BN, Kihara JH, Mungai F, Yundu J, Mbui J, Rashid JR, Kariuki CH, Ouma JH, Koech DK, Loker ES. 1999. Impact of the crayfish *Procambarus clarkii* on *Schistosoma haematobium* transmission in Kenya. *The American Journal of Tropical Medicine and Hygiene* 61: 751-759.

Mobberly WC, Jr., Owens JV. 1966. Movement of the crawfish *Faxonella Clypeata* in a linear maze. *Ecology* 47: 313-316.

Momot WT. 1967. Effects of brook trout predation on a crayfish population. *Transactions of the American Fisheries Society* 96: 202 - 209.

Momot WT. 1966. Upstream movement of crayfish in an intermittent Oklahoma stream. *American Midland Naturalist* 75: 150-159.

Moorhouse TP, Macdonald DW. 2011. Immigration rates of signal crayfish (*Pacifastacus leniusculus*) in response to manual control measures. *Freshwater Biology* 56: 993-1001.

Nakata K, Hamano T, Hayashi K-I, Kawai T. 2002. Lethal limits of high temperature for two crayfishes, the native species *Cambaroides japonicus* and the alien species *Pacifastacus leniusculus* in Japan. In. Blackwell Science Pty: 763-767.

Naura M, Robinson M. 1998. Principles of using River Habitat Survey to predict the distribution of aquatic species: an example applied to the native white-clawed crayfish *Austropotamobius pallipes*. *Aquatic Conservation: Marine and Freshwater Ecosystems* 8 (4): 515-527.

Nowicki P, Tirelli T, Mussat Sartor R, Bona F, Pessani D. 2008. Monitoring crayfish using a mark-recapture method: potentials, recommendations, and limitations. *Biodiversity and Conservation* 17: 3513-3530.

Nyberg P, Degerman E. 2000. The predation of the signal crayfish on the roe and young of the Arctic char, a laboratory experiment. *Lake Vättern's Water Care Organisation's Annual Report. (In Swedish).*

Nyström P. 1999. Ecological impact of introduced and native crayfish on freshwater communities: European perspectives. In *Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation*, Gherardi F, Holdich DM (eds). Crustacean Issues II, Balkema, Rotterdam. 63-85.

Nyström P, Åbjörnsson K. 2000. Effects of fish chemical cues on the interactions between tadpoles and crayfish. *Oikos* 88: 181-190.

Nyström P, Bronmak C, Graneli W. 1999. Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* 85: 545-553.

Nyström P, Bronmark C, Graneli W. 1996. Patterns in benthic food webs: A role for omnivorous crayfish? *Freshwater Biology* 36: 631-646.

Nyström P, Perez JR. 1998. Crayfish predation on the common pond snail (*Lymnaea stagnalis*): the effect of habitat complexity and snail size on foraging efficiency. *Hydrobiologia* 368: 201-208.

Nyström P, Strand JA. 1996. Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater Biology* 36: 673-682.

Nyström P, Svensson O, Lardner B, Bronmark C, Graneli W. 2001. The influence of multiple introduced predators on a littoral pond community. *Ecology* 82: 1023-1039.

O'Connor W, Hayes G, O'Keeffe C, Lynn D. 2009. Monitoring of white-clawed crayfish *Austropotamobius pallipes* in Irish lakes in 2007. *Irish Wildlife Manuals* 37.

Olden JD, Larson ER, Mims MC. 2009. Home-field advantage: native signal crayfish (*Pacifastacus leniusculus*) out consume newly introduced crayfishes for invasive Chinese mystery snail (*Bellamya chinensis*). *Aquatic Ecology* 43: 1073-1084.

Österling ME, Arvidsson BL, Greenberg LA. 2010. Habitat degradation and the decline of the threatened mussel *Margaritifera margaritifera*: influence of turbidity and sedimentation on the mussel and its host. *Journal of Applied Ecology* 47: 759-768.

Peay S. 2003. Monitoring the white-clawed crayfish *Austropotamobius pallipes*. *Conserving Natura 2000 Rivers Monitoring Series* 1: 53.

Peay S. 2009. Selection criteria for "ark sites" for white-clawed crayfish. In *Crayfish conservation in the British Isles. Proceedings of a conference held on 25th March 2009.*, Brickland J, Holdich DM, Imhoff EM (eds). Leeds, UK. 63-69.

Peay S, Guthrie N, Spees J, Nilsson E, Bradley P. 2009. The impact of signal crayfish (*Pacifastacus leniusculus*) on the recruitment of salmonid fish in a headwater stream in Yorkshire, England. *Knowledge and Management of Aquatic Ecosystems* 12: 394-395.

Peay S, Hiley PD, Collen P, Martin I. 2006. Biocide treatment of ponds in Scotland to eradicate signal crayfish. *Bulletin Français de la Pêche et de la Pisciculture* 380-381: 1363-1379.

Pérez-Bote JL. 2004. Feeding ecology of the exotic red swamp crayfish, *Procambarus Clarkii* [Girard, 1852] in the Guadiana River (SW Iberian Peninsula). *Crustaceana* 77: 1375-1387.

Pimentel D, McNair S, Janecka J, Wightman J, Simmonds C, O'Connell C, Wong E, Russel L, Zern J, Aquino T, Tsomondo T. 2001. Economic and environmental threats of alien plant, animal, and microbe invasions. *Agriculture, Ecosystems & Environment* 84: 1-20.

Pintor LM, Sih A, Kerby JL. 2009. Behavioral correlations provide a mechanism for explaining high invader densities and increased impacts on native prey. *Ecology* 90: 581-587.

Preston CD, Pearman DA, Hall AR. 2004. Archaeophytes in Britain. *Botanical Journal of the Linnean Society* 145: 257-294.

Rabeni CF, Collier KJ, Parkyn SM, Hicks BJ. 1997. Evaluating techniques for sampling stream crayfish (*Paranephrops planifrons*). *New Zealand Journal of Marine and Freshwater Research* 31: 693-700.

Radford A, Riddington G, Anderson J, Gibson H. 2004. The economic impact of game and coarse angling in Scotland. *Report commissioned by the Scottish Executive Environment and Rural Affairs Department*. 70 pp.

Rahel FJ, Olden JD. 2008. Assessing the effects of climate change on aquatic invasive species. *Conservation Biology* 22(3): 521-533.

Rahel FJ, Bierwagen B, Taniguchi Y. 2008. Managing aquatic species of conservation concern in the face of climate change and invasive species. *Conservation Biology* 22(3): 551-561.

Ramalho R, Anastácio P. 2011. Crayfish learning abilities: how does familiarization period affect the capture rate of a new prey item? *Ecological Research* 26: 53-58.

Reeve ID. 2004. The removal of the North American signal crayfish *(Pacifastacus leniusculus)* from the River Clyde. *Scottish Natural Heritage Commissioned Report No. 020 (ROAME No. F00LI12)*.

Reynolds JD. 1988. Crayfish extinctions and crayfish plague in central Ireland. *Biological Conservation* 45: 279-285.

Reynolds JD. 2006. Manual for monitoring Irish lake stocks of white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet). In *National Parks and Wildlife Service*.

Reynolds JD, O'Connor W, O'Keeffe C, Lynn D. 2010. A technical manual for monitoring white-clawed crayfish *Austropotamobius pallipes* in Irish lakes. *Irish Wildlife Manuals* 45: 23.

Reynolds JD, O'Keefe C. 2005. Dietary patterns in stream- and lake-dwelling populations of *Austropotamobius pallipes*. *Bulletin Français de la Pêche et de la Pisciculture* 376-377: 715-730.

Ribbens J, Graham J. 2004. Strategy for the containment and possible eradication of American signal crayfish (*Pacifastacus leniusculus*) in the River Dee catchment and Skyre Burn catchment, Dumfries and Galloway. *Scottish Natural Heritage, Commissioned Report No. 014 (F02LK05)*.

Ricciardi A, MacIsaac HJ. 2008. In Retrospect: The book that began invasion ecology. *Nature* 452: 34-34.

Richards C, Kutka FJ, McDonald ME, Merrick GW, Devore PW. 1996. Life history and temperature effects on catch of northern orconectid crayfish. *Hydrobiologia* 319: 111-118.

Robinson CA, Thom TJ, Lucas MC. 2000. Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. *Freshwater Biology* 44: 509-521.

Rogers WD, Holdich DM, Carter E. 1997. Crayfish eradication. *Report for English Nature, Peterborough.*

Rubin JF, Svensson M. 1993. Predation by the noble crayfish, *Astacus astacus* (L.), on emerging fry of sea trout, *Salmo trutta* (L.). *Nordic Journal of Freshwater Research* 68: 100-104.

Ryan KA, Ebner BC, Norris RH. 2008. Radio-tracking interval effects on the accuracy of diel scale crayfish movement variables. *Freshwater Crayfish* 16: 87-92.

Sagoff M. 2005. Do non-native species threaten the natural environment? *Journal of Agricultural and Environmental Ethics* 18: 215-236.

Sandodden R, Johnsen SI. 2010. Eradication of introduced signal crayfish *Pacifastacus leniusculus* using the pharmaceutical BETAMAX VET.®. *Aquatic Invasions* 5: 75-81.

Schmidt C, Vandré R. 2009. Do signal crayfish harm freshwater pearl mussels? Particular field observations. In *Aquatic Conservation Conference*. Sundsvall, Sweden.

Setzer M, Norrgård JR, Jonsson T. 2011. An invasive crayfish affects egg survival and the potential recovery of an endangered population of Arctic charr. *Freshwater Biology* 56: 2543-2553.

Shine R. 2010. The ecological impact of invasive cane toads (*Bufo marinus*) in Australia. *The Quarterly Review of Biology* 85(3): 253-291.

Sibley P. 2000. Signal crayfish management in the River Wreake catchment. In *Proceedings of the Crayfish Conference held on 26th/27th April 2000 in Leeds*, Rogers D, Brickland J (eds). Environment Agency, International Association of Astacology, English Nature: Leeds. 84-96.

Sibley PJ. 2002. The distribution of crayfish in Britain. In *Management and conservation of crayfish*, Holdich DM, Sibley PJ (eds). Nottingham: 64-72.

Sibley PJ, Holdich DM, Lane MR. 2009. Invasive crayfish in Britain – Management and Mitigation. In *International Urban Ecology Review* 4: 105-118.

Simberloff D. 2005. Non-native Species DO Threaten the Natural Environment! *Journal of Agricultural and Environmental Ethics* 18: 595-607.

Sinclair C, Ribbens J. 1999. The distribution of American signal crayfish (*Pacifastacus leniusculus*) in the Kirkcudbrightshire Dee (Dumfries and Galloway) and an assessment of the use of electrofishing as an eradication technique for crayfish populations. *Scottish Natural Heritage, Commissioned Report* F99AC601.

Sinclair CA. 2009. Fine scale mapping of signal crayfish distribution in Scotland. *Scottish Natural Heritage, Commissioned Report* 26686.

Skinner A, Young M, Hastie L. 2003. Ecology of the freshwater pearl mussel. *Conserving Natura 2000 Rivers Ecology Series, English Nature, Peterborough* No. 2.

Skurdal J, Ovenild T. 1986. Growth, maturity and fecundity of *Astacus astacus* in Lake Steinsfjorden, SE Norway. *Freshwater Crayfish* 6: 182-186.

Skurdal J, Qvenild T, TaugbØL T. 1992. Mark-recapture experiments with noble crayfish, *Astacus astacus* L., in a Norwegian lake. *Aquaculture Research* 23: 227-233.

Smith GRT, Learner MA, Slater FM, Foster J. 1996. Habitat features important for the conservation of the native crayfish *Austropotamobius pallipes* in Britain. *Biological Conservation* 75: 239-246.

SNH. 2001. An audit of alien species in Scotland. Scottish Natural Heritage.

SNH. 2007. A five year Species Action Framework: making a difference for Scotland's species. Scottish Natural Heritage.

Söderbäck B. 1994. Interactions among juveniles of two freshwater crayfish species and a predatory fish. *Oecologia* 100: 229-235.

Souty-Grosset C, Holdich DM, Noël PY, Reynolds JD, Haffner P. 2006. Atlas of Crayfish in Europe. *Muséum national d'Histoire naturelle, Patrimoines naturels* 64: 187.

Spence DHN. 1972. Light on freshwater macrophytes. *Botanical Journal of Scotland* 41: 491 - 505.

Spence DHN, Barclay AM, Allen ED. 1984. Limnology and macrophyte vegetation of a deep, clear limestone lake, Loch Borralie. *Botanical Journal of Scotland* 44: 187 - 204.

Spink J., Rowe J. 2002. The current distribution of signal and native crayfish in Broadmead Brook, Wiltshire. *Freshwater Forum* 19: 3-10.

Stenroth P, Nyström P. 2003. Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshwater Biology* 48: 466-475.

Strayer DL. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55: 152-174.

Svärdson G. 1995. The early history of signal crayfish introduction into Europe. *Freshwater Crayfish* 8: 68-77.

Swahn JÖ. 2004. The cultural history of crayfish. *Bulletin Français de la Pêche et de la Pisciculture* 372-373: 243-251.

Thomas CD, Cameron A, Green RE, Bakkenes M, Beaumont LJ, Collingham YC, Erasmus BFN, de Siqueira MF, Grainger A, Hannah L, Hughes L, Huntley B, van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Townsend Peterson A, Phillips OL, Williams SE. 2004. Extinction risk from climate change. *Nature* 427: 145-148.

Thomas W. 1992. A note on the crayfish of Loch Croispol. *Glasgow Naturalist* 22: 107-109.

Timmins RJ, Duckworth JW, Hedges S, Steinmetz R, Pattanavibool A. 2008. *Bos javanicus*. In *IUCN Red List of Threatened Species*. Version 2012.1.

Toomey MB, McCabe D, Marsden JE. 2002. Factors affecting the movement of adult Zebra mussels (*Dreissena polymorpha*). *Journal of the North American Benthological Society* 21: 468-475.

Trudgill N. 2000. North American signal crayfish (*Pacifastacus leniusculus*) in the River Clyde. In. Contract Report for the United Clyde Angling Protective Association. Lanark, UK.

US Fish and Wildlife Service. 1998. Recovery plan for the Shasta crayfish (*Pacifastacus fortis*).

US Fish and Wildlife Service. 2009. Shasta crayfish (*Pacifastacus fortis*) 5 year review: summary and evaluation.

US National Parks Service. 2007. Crayfish survey and discovery of a member of the *Cambarus acuminatus* complex (Decapoda: Cambaridae) at Valley Forge National Historical Park in Southeastern Pennsylvania. Technical Report: NPS/NER/NRTR-2007/084. 46 pp.

Usio N, Kamiyama R, Saji A, Takamura N. 2009. Size-dependent impacts of invasive alien crayfish on a littoral marsh community. *Biological Conservation* 142: 1480-1490.

Usio N, Townsend CR. 2001. The significance of the crayfish *Paranephrops zealandicus* as shredders in a New Zealand headwater stream. *Journal of Crustacean Biology* 21: 354-359.

Vitousek PM, Dantonio CM, Loope LL, Rejmanek M, Westbrooks R. 1997. Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology* 21: 1-16.

Warner GF. 1997. Factors affecting the selection of pond snail prey by signal crayfish. *Freshwater Crayfish* 11: 194-202.

Waters TF, Kaehler JP, Polomis TJ, Kwak TJ. 1993. Production dynamics of smallmouth bass in a small Minnesota stream *Transactions of the American Fisheries Society* 122: 588-598.

Webb M, Richardson AMM. 2004. A radio telemetry study of movement in the giant Tasmanian freshwater crayfish, *Astacopsis gouldi*. *Freshwater Crayfish* 14: 197-204.

Weinländer M, Füreder L. 2012. Associations between stream habitat characteristics and native and alien crayfish occurrence. *Hydrobiologia* 693: 237-249.

Westman K, Savolainen R. Growth of the signal crayfish *Pacifastacus leniusculus*, in a small forest lake in Finland. *Boreal Environment Research* 7: 53-61.

Whitehouse AT, Peay S, Kindemba V. 2009. Ark sites for white-clawed crayfish - guidance for the aggregates industry. *Buglife - The Invertebrate Conservation Trust, Peterborough* 1-11.

Williams FE, Eschen R, Harris A, Djeddour DH, C.F. P, Shaw RS, Varia S, Lamontagne-Godwin JD, Thomas SE, Murphy ST. 2010. The economic cost of invasive non-native species on Great Britain *CABI.org* CABI Project No. VM10066.

Williamson M, Fitter A. 1996. The varying success of invaders. *Ecology* 77: 1661-1666.

Willis KJ, Birks HJB. 2006. What is natural? The need for a long-term perspective in biodiversity Conservation. *Science* 314: 1261-1265.

Wilson CD, Arnott G, Elwood RW. 2012. Freshwater pearl mussels show plasticity of responses to different predation risks but also show consistent individual differences in responsiveness. *Behavioural Processes* 89: 299-303.

Wood, K. 2009. Personal communication.

Wright R, Williams M. 2000. Long term trapping of signal crayfish at Wixoe on the River Stour, Essex. In *Proceedings of the Crayfish Conference held on 26th/27th April 2000 in Leeds*, Rogers D, Brickland J (eds). Environment Agency, International Association of Astacology, English Nature: Leeds. 75-78.

Wutz S, Geist J. In Press. Sex- and size-specific migration patterns and habitat preferences of invasive signal crayfish (*Pacifastacus leniusculus* Dana). *Limnologica - Ecology and Management of Inland Waters*.

Young MR, Williams J. 1984. The reproductive biology of the freshwater pearl mussel in Scotland I & II. *Archiv Fur Hydrobiologie* 99, 100: 405-422 and 429-442.