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**Impacts of signal crayfish**  
**on stream fishes**

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**Thesis submitted for the degree of Master of  
Science (by research)**

**School of Biological and Biomedical Sciences**

**Durham University**

**2012**

## **Abstract**

Invasive species cause biodiversity and economic loss globally. Invasive crayfish have a wide range of effects as a result of their high densities and biomass, feeding at multiple trophic levels, aggressive competition for shelter and ecosystem engineering. In Britain, the invasive signal crayfish *Pacifastacus leniusculus* has displaced the native white-clawed crayfish *Austropotamobius pallipes* from much of its former range and occupied previously crayfish-free habitats. Signal crayfish could affect fish populations by preying upon eggs, juvenile and adult fish and competing for food and shelter.

This thesis assesses the extent to which signal crayfish can affect densities of the commercially important brown trout *Salmo trutta*, and a species of benthic fish of conservation concern, the European bullhead *Cottus gobio*, in upland streams. This thesis also investigates the extent to which signal crayfish predation could affect salmonid egg survival through a combination of controlled field and laboratory experiments.

Electric fishing was used to estimate fish densities from tributaries of the upper River Tees, north east England. Model selection was then used to determine which factors most affected bullhead and 0+ (less than one year old) brown trout densities. Signal crayfish density was negatively related to both bullhead density and the density of 0+ brown trout. Substrate composition and flow variables were also selected as predictors of 0+ brown trout and bullhead densities. Small (10-16.2 mm carapace length (CL)) crayfish and fine material apparently reduced buried sea trout *S. trutta* egg survival in controlled field experiments. However, in laboratory studies, only larger crayfish were found to significantly reduce the survival of salmon *S. salar* eggs, and this reduction only affected eggs on the surface of the gravel and not buried eggs.

These results suggest that signal crayfish pose a threat to both salmonid and bullhead populations, and that, where possible, the sites chosen for reintroduction or habitat enhancement for salmonids and sculpins should be free of invasive crayfish. Evidence for signal crayfish predation upon buried salmonid eggs remains equivocal, although this study provides definite evidence that large signal crayfish will prey upon exposed Atlantic salmon eggs. Both egg predation, and other mechanisms by which signal crayfish may affect salmonid and bullhead populations, are worthy of further investigation. Structural equation models should be used to further investigate relationships between the densities of a range of fish and crayfish species.

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## **Declaration**

Unless otherwise stated the work presented in this thesis is the author's individual contribution. The fieldwork described at the beginning of chapter 3 (Section 3.3.1) was conducted by the author's supervisor, Dr. Martyn Lucas, and a previous Master's student, Miss Nicola Dale, who did not complete her Master's. The raw data produced by this fieldwork has not previously been analysed, save for a single graph presented in appendix 4, or published, and all analysis of this data, with the sole exception of the aforementioned graph, is the author's sole work. No other part of this thesis is the work of any person or group other than the author.



## **Statement of copyright**

The copyright of this thesis rests with the author. No quotation from it should be published without the author's written consent and information derived from it should be acknowledged.

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## **Chapter 1 - Introduction**

### **1.1.1 Invasive species**

Humans have been introducing species either deliberately or accidentally for thousands of years, with early examples of introductions including the dingo, *Canis lupus dingo* probably originally introduced to Australia as a domesticated or feral dog between 3500 and 4000 years ago (Savolainen *et al.* 2004), and the rabbit *Oryctolagus cuniculus*, probably originally introduced to Great Britain by the Normans in 1066. Species may be imported for a number of reasons including for: pest control, food, familiarity, pets, other recreational uses and by accident (Pimentel *et al.* 2000). The majority of imported species do not become invasive, because they fail to enter the wild, fail to establish a reproducing population once in the wild, or establish only small populations which do not then spread to become invasive. Williamson's 'tens rule' although not without exceptions (Williamson & Fitter 1996), provides a fairly good indication of the likelihood of a species becoming an invasive pest, stating that the likelihood of each progression (from captive to wild to a self sustaining population to invasive) is about 10 (5-20) percent and thus that the likelihood of a newly imported species becoming invasive is about  $0.1 \times 0.1 \times 0.1 = 0.001$  or 0.1% (Williamson & Brown 1986). Once established, however, invasive species often exclude native species, and are consistently recognised as one of the most serious threats to biodiversity, often second only to climate change and/or loss of habitat (Lodge *et al.* 2000; Sala *et al.* 2000; IUCN 2010). Invasive species can also cause serious economic damage, with estimates for the economic cost (including losses, damages and control costs) of such species in the US ranging up to almost \$137bn per year (Pimentel *et al.* 2000). A more recent estimate of the economic cost of non-native species suggests that the total cost of invasive species in Great Britain is £1.7bn per year (Williams *et al.* 2010).

### **1.1.2 Invasive crayfish**

Humans have been introducing crayfish to new environments for hundreds of years, although the vast majority of (known) introductions have occurred in the past 120 years (Hobbs, Jass & Huner 1989). As large, invertebrate omnivores, crayfish may have a number of ecological effects, potentially radically altering freshwater communities by predation upon vulnerable algae, plants and invertebrates, and the eggs, larvae and occasionally adults of some fish and amphibian species (Guan & Wiles 1997; Nystrom *et al.* 2001; Dorn & Wojdak

2004; Renai & Gherardi 2004; Cruz *et al.* 2006; Mueller, Carpenter & Thornbrugh 2006). Crayfish may also compete with a number of fish species for food (Wilson *et al.* 2004) and/or shelter (Griffiths, Collen & Armstrong 2004; Bubb *et al.* 2009). Through a combination of consumption and clipping of macrophytes (Hobbs, Jass & Huner 1989; Wilson *et al.* 2004), bioturbation (thus increasing the amount of suspended material and nutrients), and burrowing, crayfish can also dramatically alter the physical structure of habitats and communities (Matsuzaki *et al.* 2009; Usio *et al.* 2009). Their burrowing may contribute to river bank collapse in some areas (Guan 1994). The burrowing and herbivorous activities of invasive crayfish, most notably red swamp crayfish *Procambarus clarkii* can also cause serious and costly damage, especially in rice fields (Correia & Ferreira 1995; Lodge *et al.* 2000). Invasive Crayfish may help to support unnatural densities of large predators, including other damaging invasive species, by taking energy from several trophic levels (including plants and detritus) and making it more directly available to predators at higher trophic levels (Geiger *et al.* 2005).

Invasive crayfish may cause damage to native crayfish species as a result of competitive exclusion, predation, the introduction of diseases (Holdich, Rogers & Reynolds 1999; Edgerton *et al.* 2004) and hybridisation (Perry *et al.* 2001; Perry, Feder & Lodge 2001). Invasive crayfish are often also more fecund and aggressive, eat more food per individual (Olsen *et al.* 1991; Haddaway *et al.* 2012), and reach higher densities than native crayfish, potentially making them far more damaging to other species (Hill, Sinars & Lodge 1993; Lozan 2000; Wilson *et al.* 2004; Bubb *et al.* 2009). Many invasive crayfish species are more aggressive and switch more readily to novel prey (Renai & Gherardi 2004) than their native competitors and these traits may enable invasive crayfish to colonise previously crayfish-free habitats, unsuitable for the native species as a result of low food availability. Invasive crayfish may also be able to occupy previously crayfish-free areas as a result of greater niche width (Olsson *et al.* 2009), and/or mobility over land (Cruz & Rebelo 2007), potentially making them damaging to a number of species, particularly many amphibians, that would previously have been able to avoid crayfish predation by utilising habitats inaccessible to the native crayfish (Renai & Gherardi 2004).

### **1.1.3 Thesis aims and introduction outline**

This thesis seeks to address the effects of signal crayfish upon upland stream fish. To this end the remainder of the first part of the introduction covers crayfish biology and the history of signal crayfish, and research into their effects, in Great Britain. Throughout the

introduction previous research is considered critically, to enable the thesis to highlight, and address some of, the key deficiencies in current knowledge and previous experimental work.

The second part of the introduction addresses the likely effects of signal crayfish upon benthic fish. To this end it investigates the means by which signal crayfish might affect stream fish populations, namely: competition for shelter, predation and competition for food. This section of the introduction concludes by briefly discussing the extent to which benthic fish could affect signal crayfish populations.

The third part of the introduction addresses salmonid life cycles and population declines in order to contextualise the subsequent discussion of possible crayfish effects upon different salmonid life stages.

The potential effects of signal crayfish upon salmonid eggs and juvenile fish are addressed in parts four and five of the introduction respectively. Egg predation is considered in the context of likelihood and possible constraints. For juvenile salmonids the effects considered are similar to those considered for benthic fish.

The introduction concludes with a brief summary of research to date and its limitations. This information is used to identify gaps in current knowledge and produce detailed research aims for the thesis.

#### **1.1.4 Crayfish biology**

Crayfish are large, typically freshwater, decapod crustaceans. Crayfish are divided into three major families: Astacidae native to Europe and the western coast of North America, Cambaridae native to eastern and central North America, Japan and parts of Eastern mainland Asia, and Parastacidae native to parts of South America, Australia, New Zealand and New Guinea. Crayfish taxonomy is subject to a degree of uncertainty (Fratini *et al.* 2005), but there are known to be over 300 species of North American Cambarid crayfish, accounting for the majority of extant crayfish diversity, with the 100 or so Parastacid species making up most of the remainder, and probably fewer than twenty Astacid species.

Structurally crayfish have a body plan broadly typical of the Malacostraca (higher crustaceans) with a rigid carapace covering the cephalothorax, which houses most of the internal organs, and well developed musculature in the tail allowing for powerful ventral

movement of the tail fan. Crayfish have a head (cephalon) made up of five segments, anterior to, but fused with the thorax and bearing the antennae, antennules, eyes and labrum. The mouthparts are borne on a separate gnathorax beneath the head. The anterior end of the carapace forms a pointed rostrum that largely covers the head. The carapace length of a crayfish is measured dorsally from the tip of the rostrum to the back of the carapace where it meets the first segment of the abdomen (Figure 1.1). The thorax of a crayfish is composed of eight segments and bears three pairs of segmented maxillipeds and five pairs of segmented pereopods. The maxillipeds are used in food handling, with the tips used to cut and tear food, whilst setae (hairs) further up the maxillipeds may be used to sift food from the substrate, aid in food handling or, especially in young crayfish, filter feed (Holdich 2002). The first three of a crayfish's pereopods are chelate (bear claws) although these are far larger on the first pereopod (cheliped). The first three pairs pereopods are used for feeding and chemoreception (Holdich 2002). Pereopod pairs two, four and five are used in ordinary locomotion, assisted by pairs one and three on difficult terrain or in strong currents (Holdich 2002). The abdomen of a crayfish is composed of six segments each surrounded by a separate calcified tube. These tubes are joined to each other and the back of the carapace by flexible membranes allowing for the ventral flexing of the thorax. Thoracic segments 2-5 bear feathery pleopods (swimmerets) to which eggs and young crayfish attach in females, while in males segment 1 bears pleopods and segment 2 gonopods modified for copulation. The sixth thoracic segment has flattened uropod appendages, that, along with the telson, form a tail fan with a large surface area, which can be moved quickly and powerfully, using the muscles in the abdomen, to move the crayfish rapidly backwards, typically as an escape response (Holdich 2002).

Crayfish undergo direct development with all the larval stages taking place in the egg, and juveniles being very similar in form to adults with the exception of the lack of uropods in first stage juveniles. Female crayfish protect their eggs and juveniles until the second (Astacidae) or third (Cambaridae and Parastacidae) juvenile stage. In many species mated females show reduced activity until the point at which their young hatch and feeding activity is suppressed until after the young have begun to forage independently (Reynolds 2002).

Crayfish are ectothermic, and initially show a roughly linear decrease in metabolic rate as temperature decreases, although some acclimation does occur over time (McWhinnie & Oconnor 1967). The rate of feeding (Croll & Watts 2004) and juvenile development (Reynolds 2002) are also, understandably, therefore, highly temperature dependent.

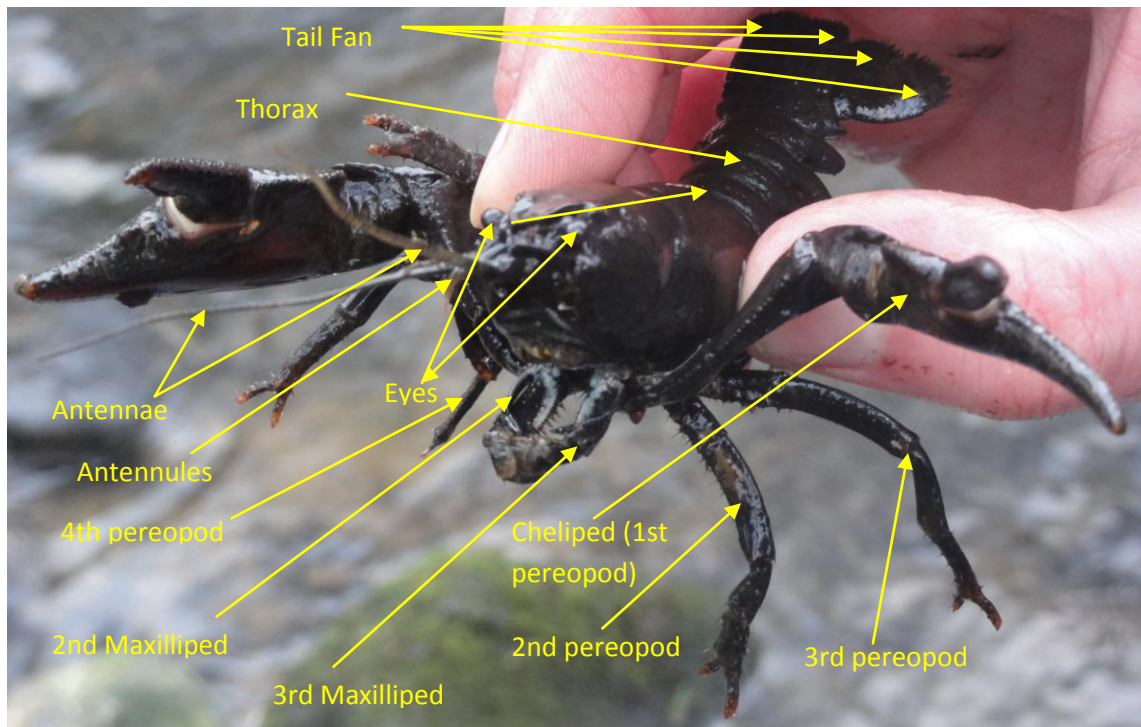


Figure 1.1: A large (c. 50mm CL) signal crayfish, held by the author, with key features labelled. The two ended arrow indicates the measured carapace length. The 5th pereopods and 1st maxillipeds are not visible in this photograph. Photo credit William Foulds.

### **1.1.5 Signal crayfish in Great Britain**

Signal crayfish *Pacifastacus leniusculus* were first imported into Great Britain in the 1970s with a trial in 1976 followed by the import and stocking of 150000 juveniles into 245 ponds and lakes during 1977 and 1978, with subsequent imports also taking place (Lowery & Holdich 1988). Introductions occurred both into ponds and lakes for captive aquaculture, and as direct additions into wild systems (Holdich, Rogers & Reynolds 1999). By the mid 1980s a combination of successful introductions to wild systems and escapes from aquaculture had led to the signal crayfish being widely distributed in the wild (Lowery & Holdich 1988).

Since its introduction the signal crayfish has extirpated white-clawed crayfish *Austropotamobius pallipes*, the only native crayfish species, from many river and lake systems either directly through competition, predation and mating with female white-clawed crayfish (Holdich & Domaniewski 1995), or indirectly by acting as a vector for parasites and diseases, most notably the fungal disease crayfish plague *Aphanomyces astaci* (Goddard & Hogger 1986; Holdich & Gherardi 1999). Similar patterns have been observed elsewhere in Europe, with both signal, and other crayfish introductions, resulting in the loss of populations of white-

clawed and other endemic crayfish species (Taugbol & Skurdal 1999; Lodge *et al.* 2000; Westman & Savolainen 2001). It is noted that white-clawed crayfish are naturally absent from large parts of Great Britain, in particular, the whole of Scotland, although a single introduced population exists in Loch Croispol (Maitland 1996), yet signal crayfish are rapidly colonising several Scottish catchments such as the Clyde and the Tweed (Gladman *et al.* 2009).

Signal crayfish demonstrate burrowing activity thereby contributing to river bank erosion and even collapse in some areas (Guan 1994). Signal crayfish predate a range of macroinvertebrates (Guan & Wiles 1998; Stenroth & Nystrom 2003; Crawford, Yeomans & Adams 2006) and probably exert greater predation pressure upon native invertebrate species than white clawed crayfish as a result of their higher densities, greater individual predation rates and lower susceptibility to some parasites (Haddaway *et al.* 2012). Williams *et al.* (2010) estimate a total annual economic cost to Great Britain from signal crayfish of £2689000.

It is possible that signal crayfish are able to occupy previously crayfish-free habitats (in catchments where they are known to occur) where insufficient food is available to support white-clawed crayfish populations. A similar process has been observed with signal crayfish invading habitat that Shasta crayfish *Pacifastacus fortis* had been unable to utilise, probably as a result of limited food availability (Pintor, Sih & Bauer 2008). In that study, signal crayfish invading habitats without Shasta crayfish, or any other crayfish species, were more aggressive, bolder and better at consuming novel prey, than signal crayfish invading habitats with Shasta crayfish (Pintor, Sih & Bauer 2008). This implies that signal crayfish may not only be able to invade previously crayfish free habitats, but that they might actually be more damaging to the native fauna in such habitats, than they would be in habitats that were previously occupied by native crayfish species. In this context it is worth observing that both white-clawed (Holdich & Domaniewski 1995) and Shasta crayfish (Pintor, Sih & Bauer 2008) are relatively non-aggressive and shy by comparison to signal crayfish, with Shasta crayfish also being less active and white-clawed crayfish moving shorter distances between refuges (Bubb, Thom & Lucas 2006). Evidence that signal crayfish are better able to exploit food resources than white-clawed crayfish comes from their greater feeding rate in laboratory experiments (Haddaway *et al.* 2012), and the faster individual growth, and greater: densities, individual sizes and population biomass of signal crayfish where they have replaced white-clawed crayfish (Guan & Wiles 1996; Guan 2000).

Much of the research involving signal crayfish in Great Britain during the 1980's and 1990's focused on their spread and interactions with white clawed crayfish. More recent



papers have attempted to address their interactions with benthic fish (Bubb *et al.* 2009), effects on benthic invertebrate populations (Crawford, Yeomans & Adams 2006) and predation of salmonid eggs (Edmonds, Riley & Maxwell 2011; Gladman *et al.* 2012). However, significant gaps remain in understanding of the effects of signal crayfish as ecosystem engineers and the effects of signal crayfish upon salmonid and benthic fish population densities. Furthermore, questions still remain regarding predation of salmonid eggs by small crayfish, and the ability of bullhead to defend their eggs from crayfish predation.

## **1.2 Crayfish effects upon benthic fish**

Benthic fish may be keystone species in some habitats, through their regulation of certain species in the invertebrate community (Woodward *et al.* 2008). Additionally the bullhead *Cottus gobio*, a benthic sculpin locally common in stony streams across Europe, is listed on the European habitats directive (92/43/EEC) and spined loach *Cobitis taenia* populations in Great Britain are of potential conservation concern because of their restricted distribution (Maitland & Campbell 1992). Some studies investigating the densities of benthic fish in relation to signal crayfish density in rivers have found them to be negatively correlated (Guan & Wiles 1997; Bubb *et al.* 2009; Peay *et al.* 2009). One study (Degerman *et al.* 2007), however, found no significant evidence of any correlation between the density of signal crayfish and the density of any fish species in Swedish streams and rivers, although this study might have been flawed by the fact that it used the crayfish numbers caught by electric fishing as its only measure of crayfish abundance, and electric fishing might not give an effective measure of relative crayfish abundance between sites (Cowx & Lamarque 1990). Two further studies have also failed to demonstrate any effect of signal crayfish on benthic fish densities (Hayes 2012; Ruokonen *et al.* 2012).

### **1.2.1 Competition for shelter**

Signal crayfish have been shown to be superior competitors for shelter to bullhead and better at excluding them from shelter than white-clawed crayfish, because bullhead are less likely to share a shelter with signal crayfish than white-clawed crayfish (Bubb *et al.* 2009). Exclusion of bullhead from shelter by signal crayfish has also been demonstrated in another study, which also showed that signal crayfish can exclude stone loach *Barbatula barbatula*

from shelter (Guan & Wiles 1997). A study in North America involving signal crayfish and the Paiute sculpin *Cottus beldingi* also found evidence of reduced shelter use, and increased sculpin activity and fleeing behaviour in the presence of crayfish (Light 2005). Exclusion from shelter would be likely to greatly increase the predation risk to which small benthic fish are exposed from predatory birds, mammals and fish (Guan & Wiles 1997). Light's (2005) study is also interesting in that it found that the total density of signal crayfish and Paiute sculpin was closely correlated to, and typically also roughly equal to the density of unembedded rocks. This indicates that shelter may be a factor limiting sculpin density and that an increase in crayfish density, resulting in a reduction in shelter availability to sculpins, could reduce sculpin population densities. Crayfish densities in Light's (2005) study were, however, relatively low ( $0.89 \pm 0.09$  (mean  $\pm$  SD) one year old or older (1+) crayfish/m<sup>2</sup>), and almost invariably fell below the density of unembedded rocks, leaving some shelter available for sculpins. Signal crayfish densities in Great Britain may be far higher ( $\geq 20$  crayfish/m<sup>2</sup> in riffles (Guan & Wiles 1997)), ( $>8$  but  $<25$  crayfish/m<sup>2</sup> (Bubb *et al.* 2009)), likely increasing the exclusion of fish from shelters, as at such densities the density of crayfish alone is frequently likely to approach or equal the density of unembedded rocks. Both crayfish and benthic fish may also use and probably also compete for, alternative shelters such as woody debris, overhanging banks, macrophytes and tree roots.

Spiny cheek crayfish *Orconectes limosus* are capable of excluding young of the year burbot *Lota lota* from shelter (Hirsch & Fischer 2008). In this context the authors suggest that the reason for this exclusion was a gross overestimation of predation risk by the juvenile burbot. However, crayfish might be capable of successfully preying upon fish of greater body length than their own, and are probably capable of inflicting significant harm upon fish with their chelae, so actual predation or injury risks may also be possible explanations for the exclusion of burbot from shelter, especially given that the authors did observe aggressive activity from the crayfish directed towards the young of the year burbot. Stress hormone (cortisol) levels were elevated in young of the year burbot following exposure to crayfish, and this may constitute another harm caused by crayfish by increasing the fish's energy use and, thus, reducing growth and/or increasing predation risk due to a need to spend more time foraging. Assuming that benthic fish also show a stress response to the presence of crayfish, the increased energy use from this response would compound the problems caused by increased energy use from greater activity and fleeing behaviour. Although white-clawed crayfish are also dominant over bullhead, and probably also other benthic fish, in competition

for shelter, signal crayfish may be more damaging in these contexts because of their greater aggression and population densities (Bubb *et al.* 2009).

Laboratory studies involving predatory fish have suggested that crayfish increase the predation risk to which small benthic fish are exposed by excluding them from shelter (Rahel & Stein 1988), whilst also finding that the presence of predatory fish increased the vulnerability of the small benthic fish to crayfish predation by forcing them to seek shelter. Another study (Bryan, Robinson & Sweetser 2002) produced similar results, suggesting that the presence of crayfish significantly reduced prey fish (in this study Little Colorado spine dace *Lepidomeda vittata*) entrance rates into and exit rates from refuges as well as overall activity (measured as number of movements per minute) relative to predator free controls. When the spinedace were exposed to both crayfish and a native fish predator (Apache trout *Oncorhynchus apache*) their response was broadly similar to that observed in the presence of the trout alone with reductions in entrance and exit rates from shelters, but not activity as observed with crayfish alone. When exposed to crayfish and a non-native fish predator (Rainbow trout *Oncorhynchus mykiss*) the spinedace reacted in the same way as they did to the crayfish alone, with reduced entrance and exit rates from shelter and overall activity, as opposed to only reducing activity as they had in the presence of rainbow trout alone. Spinedace appear to react to both fish and crayfish predators, although in the case of crayfish and rainbow trout together it is not clear whether the spinedace were responding purely to the crayfish, or to both species. The differences in reactions to different combinations of fish predator and crayfish also demonstrate that the effects of crayfish upon fish are likely to be influenced by the other predators present in the environment of the prey fish species.

In one study the presence of crayfish actually reduced the vulnerability of a sculpin (*Cottus bairdi*) to fish predation (McNeely, Futrell & Sih 1990). This study differed from those of Bryan, Robinson & Sweetser (2002) and Rahel and Stein (1988) in that the crayfish used were smaller and the fish predator larger, with the result that the crayfish were vulnerable (albeit only to a very limited extent) to predation from the fish predator. In this context the authors argue that the crayfish distracted the predatory fish's attention from the sculpin allowing it to enter shelter, and that although the crayfish routinely displaced the sculpin from shelter in the absence of the predator, in the presence of the predator they became far more resistant to such displacement. Bryan, Robinson & Sweetser (2002) and Rahel and Stein (1988) both used crayfish species known to be highly aggressive: virile crayfish *Orconectes virilis* and rusty crayfish *O. rusticus* respectively, whilst Mcneely, Futrell & Sih (1990) used phallic crayfish

*O. putnami*, a species for which behavioural data are lacking. If virile and rusty crayfish are more aggressive than phallic crayfish this might also contribute to their ability to increase rather than decrease the predation risk to which benthic fish housed with them in the presence of a fish predator are exposed. On balance with signal crayfish being a large and aggressive species capable of reaching high densities, although they might distract fish predators from small benthic fish on occasions, it appears likely that the effects of exclusion from shelter would in most cases outweigh those of such distractions, increasing the overall predation risk to which small benthic fish would be exposed.

### **1.2.2 Predation**

Signal crayfish have also been found to be predators of benthic fish (Guan & Wiles 1997) and probably also predate the eggs of sculpins (Light 2005; Bubb *et al.* 2009). Signal crayfish are likely to be a greater threat to benthic fish in these contexts than white-clawed crayfish because of their greater aggression and maximum densities. Breeding bullhead males establish a nest under stones, and the female(s) then laying eggs onto the ceiling of the nest, which the male will then fertilise and defend until they hatch (Marconato, Bisazza & Fabris 1993). This pattern of spawning might make bullhead eggs vulnerable to crayfish predation, as the eggs are clustered and, thus, would be a highly profitable source of food for crayfish if the male bullhead was unable to defend them. Male bullheads engaged in nest defence might also be more vulnerable to injury or predation from crayfish.

Johnny darter *Etheostoma nigrum* are typically displaced from shelter by crayfish (Rahel & Stein 1988), but it has been demonstrated that they are able to reproduce successfully in the presence of crayfish, including the highly aggressive rusty crayfish, through a combination of selecting nest sites with openings that restrict the maximum size of crayfish that can enter and aggressive nest defence (Rahel 1989). Aggressive nest defence against crayfish in johnny darter takes the form of a display, followed by nipping of the posterior of the abdomen if the crayfish does not retreat (Rahel 1989). The success of Nest defence appears to be negatively related to crayfish size, with johnny darter successfully preventing about 40% of the largest crayfish able to enter nests, of the average entrance size found in the wild, from entering their nest in laboratory experiments (Rahel 1989). Another darter *Etheostoma crossopterum* has also been shown to be capable of significantly reducing the number of eggs lost from its nest to crayfish predation, with an average of >95% of the eggs remaining after 48

hours with three *Orconectes margorectus* and the male darter present, but no eggs remaining in any trials with crayfish in which the male was removed (Knouft & Page 2004). No information on the sizes of the animals involved in this experiment is given. Bullhead nest defence against crayfish has not been observed, and as such the ability of male bullhead to defend their egg masses from crayfish remains unknown. An investigation into the ability of male bullhead to defend their nests from both white-clawed and signal crayfish, combined with investigations into the extent of predation upon bullhead eggs in environments both with and without crayfish would be worthwhile. Filial cannibalism has, however, been reported in male bullhead, potentially complicating such studies (Marconato, Bisazza & Fabris 1993).

Dietary studies might also be of use in understanding the extent of crayfish predation upon benthic fish and their eggs. For example, stable isotope studies indicate trophic level from nitrogen fractionation (typically a 3ppt fractionation per trophic level) and can indicate carbon sources from C fractionation. In this context the fact that signal crayfish tissues have been found to be  $\delta^{15}\text{N}$  enriched to approximately the same extent as those of bullhead and stone loach suggests that they are not major predators of these species (or others with a similar trophic level), a result supported by the dietary modelling in that study which suggested that signal crayfish were preying primarily on benthic macro invertebrates (Ruokonen *et al.* 2012). Other studies might suggest a more serious threat from signal crayfish, with fish remains reported in the gut contents of up to 13.3% of large (>45mm carapace length (CL)) signal crayfish (Guan & Wiles 1998) in the river Great Ouse in July. That study, however, failed to find any evidence of crayfish predation upon fish eggs, in any season, suggesting this might not be a serious threat, although fish eggs might not survive the crayfish gastric mill and could have formed part of the amorphous matter found in crayfish guts and presumed to be of animal origin.

In conclusion signal crayfish are almost certain to predate benthic fish and their eggs where they are found together. However, the extent of such predation is the subject of partially contradictory evidence (Guan & Wiles 1998; Ruokonen *et al.* 2012) and its effects upon benthic fish populations remain unclear.

### **1.2.3 Competition for food**

In lake environments crayfish apparently feed at similar trophic levels to bullhead or stone loach, but, as mentioned above, this did not apparently result in a reduction in the

population densities of these fish, although it might have narrowed the width of their feeding niches slightly (Ruokonen *et al.* 2012). In this context a population's niche width is defined as the range of trophic levels occupied by the prey of individuals within that population. Another study, by contrast, found that only some bullhead and signal crayfish populations in chalk streams showed overlapping trophic niches, and that where they did, this overlap was limited (Hayes 2012). Hayes (2012) also found that the  $\delta^{15}\text{N}$  range of bullhead was positively correlated with small, but negatively correlated with large signal crayfish density, suggesting that predation by bullhead on small crayfish may serve to widen the niche width of bullhead, whereas competition with large crayfish narrows it. To better represent a population's niche space, stable isotope data can be given as a SEA value, an area effectively equivalent to the standard deviation of bivariate data, of the total niche space represented by the differing levels of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  found in the tissues of a sample of a population. Hayes (2012) found that the  $\text{SEA}_c$  (the SEA with a correction applied to account for small sample sizes) occupied by bullhead was proportionately reduced by increasing densities of large crayfish. This would suggest that large signal crayfish may compete with bullhead for preferred prey items (Hayes 2012). Signal crayfish have been shown to significantly reduce the total biomass and diversity of benthic macroinvertebrates in enclosures, even at moderate densities (5 crayfish per 1.26  $\text{m}^2$  enclosure) (Stenroth & Nystrom 2003), suggesting that they certainly have the potential to compete with small benthic fish, such as bullhead and stone loach which feed predominantly on benthic macroinvertebrates (Ruokonen *et al.* 2012). In one field study, however, although signal crayfish were found to reduce invertebrate diversity and increase their average size, there was no statistically significant evidence that crayfish reduced invertebrate biomass (Crawford, Yeomans & Adams 2006).

Abundances of some small fish such as the sunfish *Lepomis macrochirus* and *L. gibbosus* which feed on macroinvertebrates in North American lakes are negatively correlated with the abundance of invasive rusty crayfish, and this may result in part from reductions in macroinvertebrate numbers, resulting from rusty crayfish predation (Wilson *et al.* 2004). However, the lower fish numbers could also result from nest predation by rusty crayfish or the loss of suitable macrophyte habitat as a consequence of rusty crayfish damage to macrophytes resulting in reduced macrophyte cover and diversity (Wilson *et al.* 2004).

In lotic environments in Europe signal crayfish apparently shift with age from a predominantly carnivorous diet to a more mixed diet including a substantial amount of plant detritus (Guan & Wiles 1998). However, when the amorphous matter found in crayfish guts

and presumed to be of animal origin was included in this analysis animal matter remained about half of the crayfish diet for much of the year, including the summer when feeding rates were highest, even in crayfish of the largest size class. Stable isotope analysis of crayfish tissues suggests that in many populations much of the energy used for growth (roughly 30-50% in adults, and typically slightly more in juveniles) comes from animal sources, even where the majority of the gut contents by weight consists of plant detritus (Whitledge & Rabeni 1997). One study on signal crayfish has, however, suggested that, with the exception of cannibalism, signal crayfish eat only a very small percentage of animal matter at any life stage, although this study involved signal crayfish in their native habitat in North America rather than in Europe (Bondar *et al.* 2005).

A laboratory study comparing the effects of crayfish competition with those of intraspecific competition on fish concluded that increasing the density of either conspecific competitors or adding virile crayfish resulted in a reduction in fish growth over the course of the experiment (Carpenter 2005). This study found that crayfish had a greater effect on the growth rate of fish (at an equal total animal density) than additional conspecific competitors on flannelmouth sucker *Catostomus latipinnis*, whilst additional conspecific competitors had a greater effect on Gila chub *Gila intermedia* growth, suggesting that the effects of crayfish competition are highly species specific. This study might have been confounded by the fact that crayfish were found to have eaten two of the sucker in one trial, and although this trial was excluded, it suggests that crayfish are predators of the sucker. The reduction in sucker growth rate might, therefore, not be entirely due to competition for food, as escaping from predation, and the stress from being kept with potential predators probably increased sucker energy use. Another study, however, found that in competition for fish eggs, the presence of slimy sculpin *Cottus cognatus* (of total length roughly equal to the carapace length of the crayfish used) dramatically reduced the feeding rate of virile crayfish, whereas the presence of crayfish had no detectable effect on sculpin feeding rate (Miller, Savino & Neely 1992).

It is reasonable to conclude that signal crayfish may compete with benthic fish for food, especially given evidence of dietary niche narrowing in bullhead related to the density of large crayfish present (Hayes 2012). The extent of competition for food, and its effects upon benthic fish densities, are harder to assess, but such competition, nevertheless, remains a mechanism by which signal crayfish might reduce benthic fish populations.

### **1.2.4 Benthic fish as predators of crayfish**

Although a number of fish species have been demonstrated to be predators of crayfish of a variety of ages (Neveu 2001) sculpins such as bullhead are unlikely to be predators of adult crayfish owing to the fact that they are typically of similar size to or smaller than adult crayfish. A dietary study has, however, shown that juvenile (age 0+) signal crayfish may form a major part of the diet of larger bullhead (Dahl 1998), evidence supported by the stable isotope results from Hayes (2012). This may contribute to observed negative correlations between crayfish and bullhead numbers (Guan & Wiles 1997; Bubb *et al.* 2009). However, no such correlations have yet been documented with bullhead and white-clawed crayfish (or other native European crayfish) even though bullhead would presumably also be predators of juvenile white-clawed crayfish, which would remain at a more manageable size for longer on account of their slower growth rate. This indicates that, in line with previous interpretations, in most cases negative relationships between signal crayfish and bullhead densities are probably primarily the result of crayfish exclusion of bullhead rather than vice-versa, although it remains possible that bullhead predation of juvenile crayfish may contribute to such observations.

### **1.2.5 Conclusions**

There are a number of mechanisms by which signal crayfish may affect benthic fish populations. Some of these, in particular predation upon bullhead eggs require further testing. Other mechanisms, notably predation and competition for shelter have been demonstrated in experimental conditions although it is hard to assess their individual effects upon wild benthic fish populations. Given the conflicting evidence, and the number of potential mechanisms by which signal crayfish may reduce benthic fish densities, further research, covering a number of sites, would be worthwhile to determine whether or not signal crayfish reduce benthic fish densities.

### **1.1.3 Life history, importance and population decline of Atlantic salmon and brown trout**

In the UK, fishing for trout and salmon is a major business with the total value of migratory salmonid (referring to Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* that migrate to sea and return to freshwater to spawn) fishing in England estimated to be £86



million per annum (Radford, Riddington & Tingley 2001). The total expenditure by anglers targeting Atlantic salmon and sea trout (migratory *S. trutta*) on the River Spey in Scotland alone is thought to be about £10.8 million per annum (Butler *et al.* 2009). From here on unless otherwise stated, I use the vernacular name 'brown trout' to refer to all *S. trutta* including both migratory and stream/lake-resident fish, and not just the 'freshwater-resident' form to which the term is sometimes applied, in conjunction with the term 'sea trout' for the anadromous form. While both Atlantic salmon and brown trout are widespread in cool, oxygen-rich freshwaters in Britain, Atlantic salmon have declined markedly and in Europe are protected under the European Habitats directive 92/43/EEC.

Atlantic salmon and brown trout populations have declined dramatically across much of the species' natural ranges as a result of numerous factors including river dams which obstruct both adult upstream and smolt downstream migrations (Stefansson *et al.* 2003). Historic overfishing has dramatically reduced some European salmonid populations (Parrish *et al.* 1998). Changes in land use frequently reduce the quality of in-stream and riparian habitat, reducing the quality and availability of salmonid habitat and resulting in reductions in the availability of invertebrate prey, reducing the growth and survival of juvenile salmonids (Poff & Huryn 1998). Dams and changes in land use may also increase the levels of silt and fine sand in streams (Poff & Huryn 1998); deposition of excess silt on top of eggs results in oxygen starvation and increased egg mortality (Soulsby *et al.* 2001; Levasseur *et al.* 2006). Pathogens and parasites may pose a serious threat to salmonid populations, especially where salmonids are farmed at the entrances of rivers important for wild salmonids, allowing for the transfer of parasites and diseases from the farmed fish to wild fish (Bakke & Harris 1998). The introduction of novel pathogens to populations with fish translocated from other regions has also proved very damaging to some Atlantic salmon populations (Youngson & Verspoor 1998). The introduction of hatchery reared conspecific salmonids may also be damaging to native populations through competition for resources and breeding between hatchery reared fish and the natural population reducing the fitness and genetic diversity of the natural population (Youngson & Verspoor 1998). Pollution, including acid rain may make rivers systems entirely unsuitable for salmonids resulting in local extinctions, or contribute to declines through its direct effects on fitness (Parrish *et al.* 1998) and indirect immunosuppressive effects in combination with a range of pathogens (Bakke & Harris 1998). Climate change is likely to have adverse effects upon Atlantic salmon populations resulting in reduced growth and juvenile survival in the south and centre of the species' range, although this may be partially

compensated for with an increase in growth and survival in the north of the species' range (Graham & Harrod 2009).

In the UK brown trout spawn in the beds of shallow streams and rivers between mid October and mid December, with the fry then emerging between mid March and early May, whilst Atlantic salmon normally spawn in a similar fashion between late October and January, with the fry typically emerging in early May (Maitland & Campbell 1992). Different salmonid species spawn in different ways with some species such as Atlantic salmon and brown trout digging redds (trenches) in gravel beds, in which they bury their eggs (Crisp & Carling 1989; Barlaup *et al.* 1994), whilst others such as lake trout *Salvelinus namaycush*, from North America, scatter their eggs over gravel/cobble substrates, such that they will sink into the interstices between stones, but make no active effort to bury them (Savino & Miller 1991). In both Atlantic salmon and brown trout, alevins (recently hatched fish, still bearing a yolk sac) hatch prior to emergence but remain in their redd gaining nutrition from the yolk sac until it is almost entirely depleted before emerging as fry. This period lasts several weeks in Atlantic salmon and potentially longer in brown trout (97 days at 4.8°C) (Maitland & Campbell 1992).

Young brown trout (Frost & Brown 1967) and Atlantic salmon (Mills 1989; Maitland & Campbell 1992) are highly territorial and competitive. The result of this competitiveness is that increased egg and thus fry densities do not necessarily result in increased recruitment to later life stages; instead recruitment either increases no further, or decreases (Elliott 1984; Elliott 2006) beyond a (habitat specific) 'optimum' egg density (see Mills (1989) for a review). Although common, and generally accepted, this pattern is not, however, universally observed (Elliott & Hurley 1998). Explanations for the discrepancy between the flat-lining of recruitment beyond the optimum egg density in some studies and the decrease in recruitment recorded in others include environmental conditions. Whether recruitment declines or remains stable above the optimal egg density for recruitment, the effects of the loss of 'excess' eggs will not be negative, and as such, provided eggs are overproduced relative to the numbers required for optimum recruitment, populations should be able to tolerate some level of egg loss without a reduction in recruitment. In heavily depleted populations (such as many of those in Europe), however, eggs are already likely to be being produced at below the number required to generate optimum recruitment and as such any egg loss is likely to reduce recruitment to later life stages. In cases where egg and consequently juvenile production is limited by competition among adult trout (Elliott & Hurley 1998) the effect of a reduction in egg and juvenile survival is harder to predict because although it would be likely to reduce recruitment

to the limiting life stage it could then indirectly increase survival and/or egg production of adult fish.

The young Atlantic salmon and brown trout which successfully establish territories grow for one or more (usually two to three) years, feeding on a diet consisting primarily of drifting invertebrates, predominantly of benthic origin, although insects on the surface of the water are taken, especially by brown trout (Frost & Brown 1967; Mills 1989; Maitland & Campbell 1992). After attaining a critical size (typically 10-20cm in Atlantic salmon and 15-22cm in sea trout), and probably also receiving environmental cues, sea trout and the vast majority of Atlantic salmon smoltify, becoming silvery in colour and undergoing physiological changes which enable them to tolerate salt water and migrate out to sea (Mills 1989; Maitland & Campbell 1992). Stream or lake living brown trout do not smoltify, but do typically begin to shift to a more piscivorous diet with increasing size (Frost & Brown 1967). The life cycle of Atlantic salmon is shown in figure 1.2.

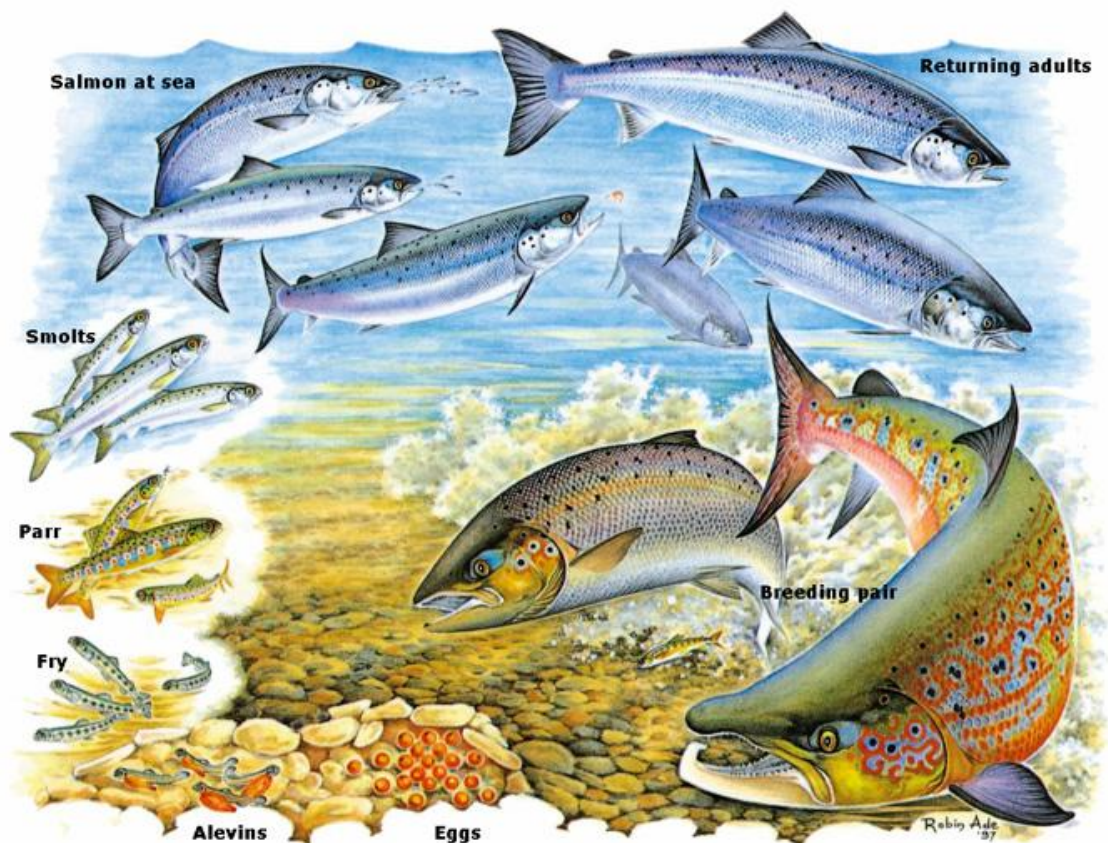


Figure 1.2: An illustration of the life cycle of the Atlantic salmon. Brown trout have a broadly similar life cycle although some individuals remain in fresh water for their entire life. Note that the Alevins (also known as sac fry) of most salmonid species remain in the redd until their yolk sac is almost depleted. Illustration from <http://www.nasco.int/atlanticsalmon.html> courtesy of the Atlantic Salmon Trust and Robin Ade.

#### **1.4 Crayfish predation upon salmonid eggs**

Signal crayfish are potential predators of the eggs of brown trout and Atlantic salmon, and salmonid egg predation has been demonstrated in signal (Nyberg & Degerman 2009; Setzer, Norrgard & Jonsson 2011) and other crayfish species globally, notably northern clearwater crayfish *Orconectes propinquus* and rusty crayfish in North America (Fitzsimons *et al.* 2006; Ellrott *et al.* 2007). However, the eggs used in at least three of these experiments (including those with signal crayfish) were 'settled' onto the gravel rather than being buried in redds under eight or more centimetres of gravel as the eggs of *S. salar* and *S. trutta* typically are in the wild (Crisp & Carling 1989). Eggs settled into interstices between stones are likely to be more vulnerable to predation than those buried under several centimetres of gravel (Fitzsimons *et al.* 2006). Experiments which have demonstrated egg predation upon European salmonid eggs by signal crayfish to date have used Arctic charr eggs which are generally smaller than those of brown trout and Atlantic salmon and scattered on the surface of the substrate rather than buried in redds (Maitland & Campbell 1992).

In laboratory conditions virile crayfish have been shown to prefer rainbow trout eggs to zebra mussels *Dreissena polymorpha* (Love & Savino 1993). Red swamp crayfish also appear to be a highly efficient predator of razorback sucker *Xyrauchen texanus* eggs even when alternative food sources are available (Mueller, Carpenter & Marsh 2005). The high energy value of fish eggs, in combination with their short handling time suggests that fish eggs are likely to be a highly profitable food source for crayfish, and thus that predation of fish eggs would be expected when crayfish encounter them (Love & Savino 1993).

Rusty crayfish can rapidly learn to associate food and egg odour cues (Weisbord, Callaghan & Pyle 2012), and, thus, given their similar feeding patterns (omnivorous, opportunistic and polytrophic), it is likely that a similar response exists in signal crayfish. This response would allow newly invading populations with no prior experience of salmonid eggs as a food source to learn that they were palatable even if they were not initially recognised as a food source. Other sources also suggest that crayfish may learn to associate novel cues with food and, that, once formed, such associations last for at least three weeks (Hazlett 1994). Both studies do, however, pose a problem for a learning hypothesis in that for learning to occur either both egg and food odours were needed together (Weisbord, Callaghan & Pyle 2012), or the crayfish had to be able to touch the food item whilst exposed to the odour (Hazlett 1994), and this would appear to be unlikely in the wild whilst the eggs were buried in redds. However, eggs in redds may die and decompose, potentially producing more 'attractive'

odours, or eggs may become exposed by the digging of other salmonids excavating their redds, or substrate movement, potentially allowing for co-localisation of food and egg odours, or direct contact between crayfish and eggs.

The potential severity of the threat posed by egg predation is illustrated by studies on lake trout in the North American Great Lakes which attribute the lack of measurable recruitment in some populations to a combination of low initial egg density and excessive egg predation from a number of predators including (especially invasive) crayfish (Jonas *et al.* 2005). Some egg predation might not be harmful, and might even be beneficial to a population, if it reduced the density of emerging fry to such a level as to reduce competition to such a level as to produce optimal recruitment to later life stages. In salmonid populations that are already depleted, such as those in the UK (see section 1.3) this is, however, unlikely to be the case. Lake trout populations in many of the Great Lakes are similarly depleted, primarily as a result of overfishing and sea lamprey *Petromyzon marinus* predation (Jonas *et al.* 2005). Jonas *et al.* (2005) has stated that generally in excess of 80 lake trout eggs per predator per m<sup>2</sup> are required to ensure that recruitment is not totally prevented by egg predation, with the minimum egg density from which he recorded recruitment being 24 eggs per predator per m<sup>2</sup>. This is important because it suggests that egg predation (from crayfish and other predators) could contribute to the extinction of heavily depleted salmonid populations, or prevent the recovery or restoration of depleted or extinct populations. If the presence of signal crayfish increases community egg predation rates, they could increase the critical number of spawning fish required for successful recruitment, endangering larger salmonid populations than those that would be threatened by native predator communities. Stocking densities would also have to be higher for successful restorations of depleted or extinct salmonid populations.

#### **1.4.1 Access to eggs**

One study into the ability of signal crayfish to predate the eggs of salmonid fish has suggested that signal crayfish will not excavate and eat salmonid eggs in mesocosm-type trials in captivity (Gladman *et al.* 2012). In this study eggs were buried in mesh boxes at a depth of 5 cm in an experimental arena with a single signal crayfish. After 14 days none of these boxes were fully excavated and across 12 test arenas only one excavation (depth 2 cm) was observed within 3 cm of a box. A subsequent experiment using 12 identical test arenas observed no digging within 5 cm of any box containing buried eggs over a seven day period (Gladman *et al.*

2012). Gladman *et al.* (2012) did, however, find that crayfish would readily excavate boxes containing pieces of herring *Clupea harengus* with three of the 12 crayfish producing excavations greater than 5 cm deep within a 5 cm radius of the box and a further three completely excavating the box containing the fish on the first night. After 14 days with the herring 10 of 12 crayfish had either completely excavated the box or produced an excavation greater than two centimetres deep within three centimetres of the box. These results are important because they demonstrate a lack of signal crayfish digging activity for eggs, but also that large crayfish are very capable of digging to depths of at least 5 cm seeking food. In a laboratory experiment involving the eggs of brown trout and noble crayfish *Astacus astacus* it was also observed that the crayfish failed to excavate the trout eggs from under 8 cm of gravel (Rubin & Svensson 1993).

Partially contradictory evidence comes from observations of white-clawed and signal crayfish digging more frequently and to greater depths when in enclosures with Atlantic salmon eggs, suggesting that they can detect eggs buried under gravel (Edmonds, Riley & Maxwell 2011). The maximum depths reached by digging crayfish in these experiments were 107 mm and 99 mm for signal and white-clawed crayfish respectively, although there was no evidence of predation on buried eggs or alevins which were at depths of 130-150 mm (Edmonds, Riley & Maxwell 2011). Signal crayfish dug an average of 13 mm deeper than white-clawed crayfish in the presence of eggs, with the average excavation depth being 74 mm for signal crayfish and 61 mm for white-clawed crayfish (Edmonds *et al.* unpublished). Similarly the average maximum depth reached in the presence of eggs was 97 mm for signal crayfish and 81 mm for white clawed crayfish (Edmonds *et al.* unpublished). If crayfish in the wild dig to these depths, they would probably succeed in excavating the eggs of many of the smallest salmonids (non-migratory brown trout of 200-300 mm total length), which typically bury their eggs at depths of around 100 mm (Crisp & Carling 1989). The difference in average maximum digging depths between crayfish species could also be very important in this context, with signal crayfish being likely to reach far more of such eggs, many of which appear to be clustered at depths very close to 100 mm (Crisp & Carling 1989). Another paper roughly corroborates these burial depths for salmonids suggesting that burial depths are correlated with length, and that brown trout bury their eggs at a mean ( $\pm$  SD) depth of 167 ( $\pm$  52) mm, while the larger Atlantic salmon bury their eggs at a mean depth of 270 ( $\pm$  39) mm (Barlaup *et al.* 1994). Other studies have, however, reported modal egg burial depths for small non-migratory brown trout redds as low as 40 mm (Elliott 1984) at which point they would be buried at shallower depths than the average depths to which either white-clawed or signal

crayfish have been observed to dig in the presence of eggs. On the basis of the studies presented above, brown trout, especially in populations where the average adult size is small, are probably more vulnerable to crayfish (and especially signal crayfish) predation of their eggs, than Atlantic salmon, on account of the shallower depths at which they bury their eggs.

Although very little, if any, literature exists on the subject, it is also possible that small crayfish could access salmonid eggs by moving through the interstices between stones, potentially giving smaller crayfish better access to salmonid eggs. This advantage would, however, be dependent upon the grain size of the material in which salmonids were spawning, with a larger grain size being likely to favour small crayfish that could move between the interstices associated with a larger grain size, and making digging to access the eggs more difficult. By contrast a smaller grain size would be expected to favour predation by larger crayfish since such a grain size would reduce the size of the interstices, making digging the only means by which crayfish could access the eggs, and thus favouring larger crayfish which almost certainly have a greater ability to dig.

#### **1.4.2 Effects of water temperature and time of year**

The native white-clawed crayfish apparently shows very little activity during the period when salmonid eggs are likely to be available, becoming torpid for a 30 week period during the winter (Brewis & Bowler 1982). However, evidence from dietary studies suggests that at least juvenile signal crayfish (CL up to 33 mm) feed actively by winter, even down to water temperatures as low as 4 - 6 °C (Guan & Wiles 1998). Investigation of local movements by radio tracking suggests that larger signal crayfish (mean carapace length 43.2 mm for females and 49.8 mm for males) may also be active by winter, with a linear increase in the proportion of signal crayfish showing movement at least once within a ten minute period, from less than 20% at temperatures of 1.8 °C to between 65% and 80% across multiple samples at temperatures of about 7°C (Bubb, Lucas & Thom 2002). In this study, the frequency of large scale movements declined dramatically in mid December with an associated fall in mean water temperatures from 7.9 °C to 3.9 °C, corresponding to the end of the spawning season in brown trout and over half way through the spawning season of Atlantic salmon. This suggests that, even if feeding was restricted to the period when long distance movements were made frequently, at least some salmonid eggs (including most brown trout eggs) might be at risk from signal crayfish predation immediately following spawning. Local activity, which might be

associated with feeding (Bubb, Lucas & Thom 2002) continued at similar levels to that recorded during the warmer period, during the cooler period, making it impossible to know whether the reduction in long distance movements would be associated with a reduction in the predation risk to salmonid eggs. Activity of 3 - 5 year old signal crayfish at 4 °C has also been demonstrated under laboratory conditions, albeit at far lower levels than those recorded at higher temperatures (Lozan 2000), although this study was restricted to males. However, during sampling in Scotland between January and March 2008, in spite of the use of detection methods including baited trapping, Gladman *et al.* (2009) failed to detect signal crayfish at multiple sites, where they had previously been found, suggesting that feeding activity is far lower by winter, or even entirely suspended in some populations.

Even if low temperatures do not altogether prevent signal crayfish feeding they may still significantly reduce food consumption rates, as demonstrated in northern clearwater crayfish and rusty crayfish when feeding upon rainbow trout eggs (Ellrott *et al.* 2007). Another study (Fitzsimons *et al.* 2006) also reports an exponential decline in northern clearwater crayfish consumption of lake trout eggs with decreasing temperatures. Ellrott *et al.* 2007 also report that the effect of temperature was more pronounced in rusty crayfish than in northern clearwater crayfish, probably as a result of physiological differences between the species, although this effect was not significant. As ectotherms, crayfish food consumption is generally proportionally lower at colder temperatures, although this effect is probably subject to some degree of acclimation over time (Guan & Wiles 1998; Croll & Watts 2004). Given the above information, it would be useful to compare the egg predation rates of white-clawed and signal crayfish at several temperatures.

Salmonid eggs might also be vulnerable to predation during the spring (March, April, May), with feeding by signal crayfish of all size classes having been demonstrated in May, crayfish of up to 33 mm carapace length continuing to feed year round and some limited evidence of overwinter feeding by crayfish of carapace length 33-45 mm (Guan & Wiles 1998). In this context, Atlantic salmon with their later hatching dates might be expected to be more vulnerable to predation than brown trout, although this might be offset, or even outweighed, by the greater depths at which they bury their eggs. The extended period of spring activity in signal crayfish, relative to white-clawed crayfish, is another period during which salmonid eggs may be vulnerable to increased predation pressure in areas with signal crayfish populations.

Water temperature is heavily dependent upon the source of the stream water, with ground-water fed systems having far more stable (and during the colder months warmer)



temperatures than surface-water fed systems. This probably results in a longer period of crayfish activity, and possibly even year round activity, in ground-water fed systems such as many chalk streams. This in turn means that the source of stream is likely to be a significant determinant of the vulnerability of salmonid eggs in that system to crayfish predation.

Crayfish breed in the autumn, with mating beginning in September in signal crayfish and October in white-clawed crayfish; hatching then occurs at any time between late April and late May in signal crayfish in southern England, with hatching in white-clawed crayfish occurring about one month later, depending upon water temperature (Guan & Wiles 1999; Bubb 2004). Based on the lower water temperatures and observational evidence hatching in both species probably occurs about one month later (June in signal crayfish and July in white-clawed crayfish) in Northern England (Ream 2010). After hatching female signal and white-clawed crayfish continue to defend their young until around their third moult; a period of at least two, but less than four, weeks. Female feeding is suppressed to some extent until the end of the period of juvenile defence, and females are relatively quiescent until shortly before their eggs hatch (Reynolds 2002; Bubb 2004). A winter movements study supports this assertion to some extent finding that berried female signal crayfish moved a median distance of 20.5 m, compared to a median distance of 99.5 m for male and non-berried females, although this difference was not significant, probably as a result of the small sample size (4 animals) of berried females (Bubb, Lucas & Thom 2002). Breeding female crayfish of both species are, therefore, unlikely to be major predators of salmonid eggs, given that both crayfish species begin to breed in advance of brown trout and Atlantic salmon, and that both brown trout and Atlantic salmon fry will have emerged before breeding female crayfish of either species return to normal levels of feeding activity.

### **1.4.3 Other factors affecting rates of crayfish predation upon salmonid eggs**

Salmonid species also appears to affect the rate of crayfish egg predation, with lower observed predation rates for lake trout eggs (0.5-1.5 eggs crayfish<sup>-1</sup> day<sup>-1</sup>) relative to those of rainbow trout eggs (1.0-5.5 eggs crayfish<sup>-1</sup> day<sup>-1</sup>) for both rusty and northern Clearwater crayfish (Ellrott *et al.* 2007). This difference might result from the crayfish having difficulty handling the larger eggs (4-5 mm diameter for lake trout compared to 2-3 mm diameter for rainbow trout) of lake trout (Ellrott *et al.* 2007), although given the size of the crayfish used (carapace length 21-34 mm) this appears unlikely. The lower consumption rates of the larger

lake trout eggs more likely results from that fact that crayfish would have to eat fewer of them to achieve the same level of energy intake: this conclusion would be supported by a calculation, based on the mid-point of the egg size ranges given (average sizes are not given), which would suggest that a lake trout egg ( $\frac{4}{3} \times 0.225^3 \times \pi = 4.77 \times 10^{-2}$  ml) would have a volume about six times greater than that of a rainbow trout egg ( $\frac{4}{3} \times 0.125^3 \times \pi = 8.18 \times 10^{-3}$  ml). These same authors also failed to find a significant temperature effect upon consumption of lake trout eggs by either rusty or northern clearwater crayfish, in spite of having found a temperature effect upon both species' consumption of rainbow trout eggs. Since the eggs of Atlantic salmon (5-7 mm diameter) and brown trout (4-5 mm diameter) differ in size (Maitland & Campbell 1992) and might also differ in odour, the effects of these differences on crayfish predation rates would be worthy of investigation, at a range of temperatures.

One author has attempted to set a minimum size for crayfish to be viable predators of lake trout eggs, stating that crayfish with a carapace length >19 mm were considered to be viable predators for lake trout eggs (diameter 5-5.6 mm) (Jonas *et al.* 2005). However, it is not clear from the paper how this size estimate has been made. The only paper to describe the process of crayfish egg handling simply states that eggs 'required no handling prior to consumption' (Love & Savino 1993), implying that eggs might have been broken and eaten entirely with the maxillipeds and other mouthparts. This study, however, involved large (37-50 mm carapace length) crayfish feeding on rainbow trout eggs. Rainbow trout eggs are smaller than lake trout, Atlantic salmon or Brown trout eggs (see above), and thus, particularly smaller crayfish, might have greater difficulty handling the larger eggs of some other salmonid species. When signal crayfish predate pond snails *Lymnaea stagnalis*, they are known to hold the snail using their walking legs and then crush the shell at the aperture using their maxillipeds, in contrast to crabs which typically use their chelae to crush prey (Nystrom & Perez 1998). However, signal crayfish are known to use their chelae in the handling of other prey such as fish (Guan & Wiles 1997), so it is difficult to predict how they would handle or break into salmonid eggs.

Negative density dependence has been demonstrated in predation rates of rusty crayfish feeding on trout eggs, but not of northern Clearwater crayfish (Ellrott *et al.* 2007). Given that, in common with rusty crayfish, signal crayfish are a highly aggressive species, and their densities in the UK far exceed the densities at which they are reported to occur in their natural environment (Lozan 2000), a high level of competition and thus negative density dependence appears very possible; as such, the effect of density upon individual egg

consumption is worthy of investigation. Given the difference in the effects of density between the aggressive rusty crayfish and the less aggressive northern Clearwater crayfish it might also be worth investigating whether a density effect exists in white-clawed crayfish which are known to be a less aggressive species than signal crayfish (Bubb *et al.* 2009).

Egg density might also influence predation rates, with northern Clearwater crayfish predation upon trout eggs being higher at intermediate egg densities of around 3125 eggs/m<sup>2</sup> than at either higher or lower egg densities (Fitzsimons *et al.* 2006). This suggests that egg density is another variable that will need controlling and, if possible, investigating.

#### **1.4.4 Relative effects of crayfish and other egg predators**

Fitzsimons *et al.* (2006) found that the northern Clearwater crayfish was generally a less efficient egg predator than the slimy sculpin. At least one other study involving North American fish and crayfish species has also found similar results (Miller, Savino & Neely 1992). A study investigating salmonid egg predation by bullhead, found that, in large substrates (62 ± 10 mm (mean ± SD) diameter pebbles) two bullhead ate 82.8 ± 5.9% (mean ± SD) of 50 Atlantic salmon eggs over a period of 15 days, even when provided with an alternative food source (Palm *et al.* 2009). This predation rate was greatly reduced when a smaller substrate was used, thus demonstrating the potential importance of substrate size in determining salmonid egg predation rates. The potential impact of bullhead predation on salmonid populations was also demonstrated in this study, with the juvenile Atlantic salmon recruitment rate being about one tenth as high in the River Vindelälven in the stretch in which bullhead were present as it was in the area in which bullhead were absent (Palm *et al.* 2009); this effect probably also results from bullhead predation upon salmonid fry, and possibly also from competition for food and or shelter between bullhead and salmon fry. The effect could also result in part or in full from environmental variables unrelated to the presence of bullheads and not accounted for in this study. Although subject to limitations, the above work makes clear the importance of considering crayfish consumption of salmonid eggs in the context of the level of consumption native and non-native fish species.

The only studies to have attempted to directly compare the effects of European fish and crayfish predation on salmonid eggs relate to Arctic charr. Both studies report that fish rapidly eat the majority of exposed eggs and are likely to have a greater effect in this regard than crayfish (Nyberg & Degerman 2009; Setzer, Norrgard & Jonsson 2011). However one of

the studies goes on to suggest that the combined effects of fish and crayfish is greater than either individually and that crayfish may ultimately cause greater egg losses than fish (Setzer, Norrgard & Jonsson 2011). This experiment is, however, flawed in that the crayfish only treatment they used for experimental enclosures allowed access by fish up to at least 35-40 mm (the maximum size of fish recovered within an enclosure) in length. The authors have claimed that such fish would be unable to predate Arctic charr eggs on the basis of the fact that related sculpins up to 42 mm in length in the Great Lakes were unable to feed upon lake trout eggs. Size data from the eggs used in their predation experiment contradicts this idea however, with the Arctic charr eggs used by these authors being 3 - 4 mm in diameter, while lake trout eggs are reported variously as being c. 4.5 mm (Fitzsimons *et al.* 2006) 4 - 5 (Ellrott *et al.* 2007) or 5 - 5.6 mm in diameter (Jonas *et al.* 2005). This in turn means that their estimates of egg predation by signal crayfish may overestimate their impact given that this effect might actually include some predation by small fish. The authors' also imply that 2.8 cm diameter holes in the sides of the enclosures allowed access for crayfish of up to 10 cm in total length, but prevented access by any fish more than about 40 mm long on the basis of the fact that no larger fish were recovered from the enclosures. Given that crayfish generally have larger and more rigid bodies than fish of the same length this seems very unlikely. However, in support of their conclusions the authors' also note that a previous study failed to find eggs in the stomachs of any of the fish species found in any of their enclosures and, thus, it is possible that their crayfish treatments were effectively preventing access by fish likely to predate eggs, and probable that they were preventing a substantial proportion of such access. Additionally, this study remains useful in that it demonstrates that, where access was possible for both fish and crayfish egg loss rates were higher than if either group was 'excluded'.

A relatively low level of crayfish egg predation may be significant if that predation is upon eggs that are not naturally predated by any of the other species present. This in turn makes it important to consider whether both bullhead and signal crayfish are capable of accessing and preying upon buried eggs.

## **1.5 Crayfish effects upon juvenile salmonids**

### **1.5.1 Predation upon emerging fry**

In laboratory conditions the presence of a signal crayfish in a 75 L hatching aquarium reduced the percentage of juvenile Atlantic salmon successfully emerging from the gravel and

escaping into an overflow trough (escapement) to a mean of 74.4% of control levels (Edmonds, Riley & Maxwell 2011). In the same experiment the presence of a white-clawed crayfish was shown to reduce escapement to a mean of 87.6% of control levels. Only two instances of crayfish predation on emerging fry, both by signal crayfish, were detected (Edmonds, Riley & Maxwell 2011). However, given the scale of reductions in fry escapement in both white-clawed and signal crayfish treatments, it appears likely that both crayfish species were preying upon emerging salmonid fry in this experiment. Far greater differences between the numbers of emergent fry found dead on the gravel were detected between species with 32 dead fry found across the three signal crayfish enclosures, but only three found across the three white clawed crayfish enclosures. The greater (possibly non-predatory) aggression of signal crayfish toward other species, might account for the proportionately greater discrepancy in number of dead fry found on the gravel, than the percentage reduction in escapement. Such aggression, might, result in more fry being killed with the chelae but then not eaten. The difference in escapement between crayfish species does, however, indicate that signal crayfish are likely to be a greater threat to emerging salmonids than white-clawed crayfish. The outcome of the experiment may have been affected to some degree by the fact that egg survival to hatching in the control, which was applied as a correction factor to escapement rates, might have been higher than that of eggs in the crayfish trials, because control eggs were on the surface of the gravel, whereas the eggs with the crayfish were buried at depths of 130-150 mm. A lack of shelter for emerging fry probably also increased fry predation rates in this experiment. The potential effects of a lack of shelter on predation upon juvenile fish by crayfish have been demonstrated by a study in which the presence of deep cover reduced predation loss of larval razorback sucker to red swamp crayfish over 72 hours from 96.7% to 70% (Mueller *et al.* 2003). The crayfish used in the deep cover experiment were substantially larger (mean carapace length 48.9 mm) than those used in the experiment without cover (mean carapace length 34 mm), and the effect that this increase in size will have had upon predation rates is unknown. A study in the river Great Ouse, England, found that larger crayfish had fish in their gut contents far more often than smaller crayfish suggesting that they are more effective as predators of fish (Guan & Wiles 1998). This suggests that the use of larger crayfish in the second of the Mueller *et al.* (2003) experiments might have resulted in an underestimation of the beneficial effects of cover upon juvenile fish survival.

Predation of emerging sea trout fry by noble crayfish has also been reported, resulting in a 68.6% reduction in the number of emerging fry that survived until the end of the experiment, although in that study fry were housed with crayfish until all the fry had emerged

rather than being allowed to escape (Rubin & Svensson 1993). Crayfish densities in Rubin and Svensson's experiment (1 crayfish per 0.5x0.4 m tank or 5 crayfish per m<sup>2</sup>) are within the range of densities recorded for wild signal crayfish in Britain (yearly peaks of 15-20+ m<sup>-2</sup> across all size classes in riffles (Guan & Wiles 1997; Guan 2000)). Rubin and Svensson (1993) did, however, use a higher density than that recorded for wild signal crayfish of the size range they used (total length 9.2-10.4 cm) in Britain (e.g. peak yearly abundance estimate of 1.2 m<sup>-2</sup> with carapace length >30mm in pools, and the vast majority of crayfish caught in riffles were smaller than 9.2cm total length (Guan & Wiles 1996)) and this probably increased predation rates. Increases in the level of predation observed are also likely to have resulted from a lack of space in the tanks restricting the ability of fry to move away from crayfish and the fact that the crayfish only had only one alternative food source (*Chara fragilis* algae), as opposed to the variety of food sources they would have available in the wild (Rubin & Svensson 1993). A lack of habitat segregation, which might exist in the wild and the fact that less shelter was provided in these experiments than would typically be available in the wild probably also contributed to the high predation rates observed in this experiment (Rubin & Svensson 1993).

In the wild, in addition to their greater aggression, signal crayfish may also be more dangerous to emerging salmonid fry than white-clawed crayfish because they have the potential to reach greater population densities (Guan & Wiles 1996; Guan 2000). This suggests that both further laboratory experiments investigating crayfish predation upon emerging salmonid fry, and field experiments to assess population densities of juvenile salmonids in habitats with both white-clawed and signal crayfish, are worthwhile.

### **1.5.2 Direct effects on free swimming juvenile salmonids**

The ability of signal crayfish to predate salmonid fry (except whilst they are emerging) is unknown, although experiments have suggested that other crayfish species (specifically *Austropotamobius italicus* (common name white clawed crayfish, so taxonomic name used when referring to this species) and red swamp crayfish) are efficient sit and wait predators capable of capturing mobile prey with fast escape reactions such as amphibian larvae and brown trout fry (Renai & Gherardi 2004). White-clawed crayfish and *A. italicus* are very closely related and, although probably distinct species (Grandjean *et al.* 2000; Fratini *et al.* 2005), their taxonomic status is debated (Trontelj, Machino & Sket 2005). The close relationship between the white-clawed crayfish and *A. italicus* implies that white-clawed crayfish are probably

capable of capturing prey, including juvenile salmonids, in the same sit and wait fashion observed in *A. italicus*. Further evidence for the potential effectiveness of crayfish as predators of salmonid fry comes from the lack of any significant difference between predation rates of virile crayfish upon lake trout eggs and sac fry (alevins) (Savino & Miller 1991). However, the sensory capabilities and locomotor performance (Frost & Brown 1967) of salmonids increase greatly as they progress through the alevin to fry transformation. It is, therefore, likely that the susceptibility of young salmonids to capture by crayfish would also decline markedly along this developmental trajectory.

There is some anecdotal evidence of attacks by signal crayfish upon larger juvenile brown trout (the only individual for which details are given was 95mm long) (Peay *et al.* 2009). Peay *et al.* (2009) also found a negative correlation between juvenile brown trout and signal crayfish densities, with increases in signal crayfish density either between sites, or on the same site over time, resulting in a decrease in the numbers of juvenile brown trout found. This study, however, covered only a single short stretch of river with limited independence between sites and, therefore, provides useful, but inconclusive, evidence for a wider effect of signal crayfish on brown trout densities. An aquaculture experiment has also demonstrated predation by noble crayfish upon Atlantic salmon parr (mean fork length 72.1 mm) (Holm 1989).

Taking the evidence above, in conjunction with evidence of signal crayfish predation upon benthic fish (Guan & Wiles 1997) and emerging fry (Edmonds, Riley & Maxwell 2011), it is likely that both signal and white-clawed crayfish are able to recognise and capture juvenile salmonids in the wild. To date no information is available on the frequency of crayfish predation upon juvenile salmonids under natural conditions or its effects upon salmonid populations. Additionally, although Peay *et al.* (2009) provide some evidence that signal crayfish may reduce juvenile brown trout densities, this evidence alone is not sufficient to assume such an effect across a range of habitats.

The exclusion of one year old Atlantic salmon parr from shelters by signal crayfish (Griffiths, Collen & Armstrong 2004) has been observed and this could increase their vulnerability to predation from piscivorous fish, birds and mammals. Competition for shelter is likely to be particularly important when signal crayfish reach high densities, such as those described earlier in this chapter, as at such densities they may occupy all, or almost all available refuges, preventing excluded fish from finding alternative refuges. Exclusion from shelters might also increase energy usage directly, as a result of the need to expend more

energy swimming against the current, and/or indirectly, as a result of increased stress levels, as observed in young of the year burbot excluded from shelters by crayfish (Hirsch & Fischer 2008). Greater energy use might in turn reduce growth and/or increase the amount of time spent to foraging, further increasing predation risk. However, in an enclosure-exclosure experiment, no significant effects of signal crayfish density, or presence/absence upon the survival of brown trout fry were found (Stenroth & Nystrom 2003), although this experiment might have been confounded by small sample sizes and high variation in survival within treatment types. It might be worth investigating the effects of signal crayfish exclusion from shelter upon juvenile salmonid stress levels and energy use in laboratory conditions. Competition with signal crayfish, for shelter may result in a reduction in the growth and/or density of juvenile salmonids in wild populations.

### **1.5.3 Competition for food**

Crayfish appear to be capable of reducing both the biomass and diversity of other benthic macroinvertebrate species (Stenroth & Nystrom 2003). It is, therefore, possible that, by reducing the overall mass of other benthic invertebrates in a system, crayfish could reduce the availability of benthic invertebrates in the drift. If this is the case, then the effects might be expected to include reduced growth and survival of juvenile salmonids, as observed when juvenile salmonids compete with other predators capable of reducing the biomass of benthic invertebrates such as the Siberian sculpin *Cottus poecilopus* (Amundsen & Gabler 2008). The effects of this reduction could be especially pronounced in the relatively oligotrophic, low food supply streams in which salmonid populations are often found (Maitland & Campbell 1992), where crayfish had not previously been found, or had previously occurred only at very low densities. Signal crayfish may substantially increase the average mass of individual benthic invertebrates (Crawford, Yeomans & Adams 2006), with potentially dramatic effects upon the number and type of prey items available to juvenile salmonids which are initially very restricted in the range of prey items they can consume. Similarly Crawford, Yeomans and Adams (2006) also found that crayfish reduced the diversity of some invertebrate families present, although they did not detect any effect of signal crayfish density upon the total benthic invertebrate biomass. Stenroth and Nyström (2003) did not detect an effect of crayfish presence upon trout survival in spite of finding a reduction in invertebrate diversity in the presence of crayfish, in enclosure experiments including both brown trout fry signal crayfish. It is, however, possible that much of the food available to trout of this size ( $31.6 \pm 1.4$  mm (mean



± SD) total length) came from drift, with many of the smallest invertebrate food items likely to have passed through the 2mm steel mesh with which the enclosures were surrounded, and thus it is possible that crayfish might not have affected food availability in this study in the way they would do when established throughout a stream.

Because of the potential impact of crayfish competition for food, and the lack of studies on the subject to date this represents an area where further research is urgently needed, in particular focusing on the effects of crayfish species and density on the amount and types of invertebrate prey in the drift. The effects of such competition would likely also manifest themselves as a reduction in juvenile salmonid growth and survival, ultimately resulting in lower densities of juvenile salmonids.

#### **1.5.4 Bullheads and other predators and competitors of salmonid fry and parr**

Juvenile salmonids are predated by a range of mammalian, fish and avian species, (see Mills (1989) for a review), the effects of which may be enhanced if crayfish exclude juvenile salmonids from shelter. Bullhead might, however, be of special significance in this context as sculpins could have a similar mode of action to crayfish upon juvenile salmonid survival by preying upon small fry (Patten 1975) and excluding juvenile salmonids of a range of sizes from shelters (Hesthagen & Heggenes 2003). Bullhead might also reduce juvenile salmonid populations through competition for food as evidenced in Siberian sculpin (Amundsen & Gabler 2008), and egg predation (Palm *et al.* 2009). However, several studies have failed to find any evidence for any population level effects of the presence of bullhead upon Atlantic salmon (Pihlaja *et al.* 1998; Amundsen & Gabler 2008), although Palm *et al.* (2009) suggests that the presence of bullhead can reduce the recruitment index (0+ juveniles 100 m<sup>-2</sup> per spawning female) for Atlantic salmon tenfold. The term 0+ in this thesis is used to refer to individuals of less than one year old, 1+ is used to refer to individuals between one and two years old and 2+ to refer to individuals two to three years old. One further study has also suggested that competition with bullhead may reduce brown trout densities, with evidence of a strong negative correlation (after correcting for density dependence) between 0+ brown trout and bullhead densities, and weaker negative correlations between different age groups of the two species or 1+ age groups of both species (Elliott 2006). Given that the strongest correlation between trout and bullhead reported in Elliott's (2006) study is between 0+ trout

and 0+ bullhead, it appears likely that this is the result of a competitive interaction rather than predation since 0+ individuals of both species would likely be too similar in size for either to predate the other. The evidence in regard to bullhead predation of juvenile salmonids suggests that bullhead may reduce trout densities in some circumstances, with demonstrable mechanisms by which bullhead could reduce salmonid populations (although many of these have been demonstrated in other, albeit similar, sculpin species), but only some studies having managed to find evidence of negative relationships between salmonid and bullhead densities.

If possible, the relative effects of signal crayfish and bullhead upon salmonid populations should, therefore, be considered. Given that increases in signal crayfish populations might reduce bullhead populations, the relationships between these species could potentially also complicate attempts to analyse the effects of both species upon salmonid populations.

## **1.6 Discussion and research direction**

Invasive crayfish species have the potential to alter native ecosystems through their direct and indirect effects on native biota. In the case of the invasive signal crayfish in the UK, evidence already exists of this species' ability to extirpate populations of the native white-clawed crayfish. Signal crayfish may reduce bullhead and stone loach densities (Guan & Wiles 1997; Bubb *et al.* 2009; Peay *et al.* 2009), although not all studies investigating the relationships between signal crayfish and fish densities have found evidence of such a reduction (Degerman *et al.* 2007; Ruokonen *et al.* 2012). The mechanisms behind signal crayfish induced reductions in the densities of these species are uncertain, although it appears likely that competition for shelter and direct predation are both factors (Guan & Wiles 1997; Bubb *et al.* 2009), with other possible mechanisms such as egg predation being worthy of investigation. Dietary niche overlaps between signal crayfish and both stone loach and bullhead have been demonstrated, but no evidence of a detrimental effect upon population densities from this overlap (Hayes 2012; Ruokonen *et al.* 2012) has yet been found.

Increases in direct and indirect predation resulting from non-native crayfish are likely to be a factor in benthic fish population declines in some conditions, but the extent of this predation is difficult to quantify in natural systems, and artificial environments might increase the frequency of such predation. The various means by which signal crayfish could reduce benthic fish populations are very difficult to separate, especially in field based studies, and

distinguishing the effects of competition, especially for food, from those of predation risk on small fish species growth rates would be difficult even in laboratory studies. Further work investigating the relationship between signal crayfish and benthic fish densities across a number of sites could help to validate or refute evidence of a crayfish effect upon benthic fish population densities. Specific laboratory investigations into the ability of bullhead to defend their nests from crayfish would be useful in understanding the level of harm likely to be done by crayfish nest predation. Native white-clawed crayfish occupy a similar ecological niche to signal crayfish, and are, therefore, also potential competitors for shelter and predators of benthic fish and their eggs (Bubb *et al.* 2009), although they are less aggressive and typically occur at lower densities; as such, if possible they should also be included in the studies suggested above for comparison to signal crayfish and crayfish-free trials. Bullhead may be a keystone species in some habitats (Woodward *et al.* 2008) and are of some conservation concern in Europe, emphasising the need for further research into the effects of both white-clawed and signal crayfish upon benthic fish.

Signal crayfish are undoubtedly potential predators of salmonid eggs and in common with other crayfish species (Savino & Miller 1991; Jonas *et al.* 2005; Fitzsimons *et al.* 2006; Ellrott *et al.* 2007; Nyberg & Degerman 2009) will predate exposed Arctic charr eggs. However, the extent of crayfish predation upon salmonid eggs in Britain remains unknown as a result of potentially conflicting evidence about signal crayfish winter feeding activity (Guan & Wiles 1998; Bubb, Lucas & Thom 2002; Gladman *et al.* 2009), and willingness to excavate salmonid eggs (Edmonds, Riley & Maxwell 2011; Gladman *et al.* 2012). Signal crayfish (with the exception of breeding females) are likely to be active in the spring before most salmonid eggs have hatched, and in the autumn until the majority of salmonids have spawned. However, questions remain in relation to their ability to access salmonid eggs, whether learning is necessary in order for salmonid eggs to be recognised as food, and the length of these 'windows of opportunity'. The location and source of rivers is likely to be an important factor in determining the risk to which eggs are exposed as these factors are important determinants of the water temperature. Given the importance of salmonids to the UK economy (Radford, Riddington & Tingley 2001; Butler *et al.* 2009), and the fact that Atlantic salmon are listed on the European habitats directive (92/43/EEC) further investigation into the predaceous activity of signal crayfish upon salmonid eggs across a range of temperatures is necessary.

Signal crayfish are potential predators of salmonid fry, and possibly larger juveniles, with predation upon emerging fry having been demonstrated in signal (Edmonds, Riley &

Maxwell 2011) and other (Rubin & Svensson 1993) crayfish species, and with other crayfish species being effective predators of juvenile salmonids (Holm 1989; Renai & Gherardi 2004), at least in laboratory conditions. Evidence of the effects of signal crayfish on salmonid populations in nature is lacking; one study has linked increasing signal crayfish populations to a reduction in the numbers of brown trout found (Peay *et al.* 2009), although other studies have not found any evidence for such effects (Stenroth & Nystrom 2003; Degerman *et al.* 2007). It appears likely that crayfish predate juvenile salmonids to some extent in nature, but this would be difficult to quantify. Wider scope studies accounting for environmental variables and looking for any relationships between the density of signal crayfish and salmonid densities should be conducted in order to validate or refute suggestions that signal crayfish reduce salmonid densities. White clawed crayfish almost certainly predate at least some juvenile salmonids, since the very closely related *Austropotamobius italicus* will predate brown trout fry in laboratory experiments (Renai & Gherardi 2004). Very few studies have been conducted comparing the effects of signal crayfish upon salmonids to those of white-clawed crayfish, and in many cases white-clawed crayfish presence might be the natural state. Given the above information, where possible, studies should aim to include trials or sites both without crayfish and with white-clawed and signal crayfish.

Hence this study's aims were to:

1. Investigate the relationships between white-clawed and signal crayfish and stream fish densities with a focus on bullhead and (particularly juvenile) salmonids
2. Investigate the ability of signal crayfish to predate buried salmonid eggs in both relatively natural and artificial settings
3. Investigate the ability of male bullhead to defend their eggs against predation by white-clawed and signal crayfish.

## **Chapter 2 - The effects of crayfish density upon benthic fish and salmonid densities**

### **2.1 Research aims**

This thesis component aimed to determine whether or not the density of crayfish can influence the density of salmonids and small benthic fish in upland hard water streams. Although other studies have attempted to investigate these relationships in the past, they have generally omitted environmental variables (Degerman *et al.* 2007) and/or focused on a single study site (Peay *et al.* 2009). Additional studies have investigated the relationship between signal crayfish and bullhead densities, but although these have attempted to select similar sites, they have not explicitly modelled bullhead densities in relation to a complete range of environmental variables (Guan & Wiles 1997; Bubb *et al.* 2009; Hayes 2012).

### **2.2 Hypotheses**

Signal crayfish were expected to reduce 0+ brown trout and bullhead densities as a result of the direct and indirect effects of crayfish competition and predation. The density of 0+ brown trout was also expected to increase in response to increased availability of juvenile salmonid feeding, and/or salmonid spawning, habitat. Bullhead were expected to reduce 0+ brown trout densities, as a result of predation and competition. Bullhead densities were expected to be positively related to the proportion of the substrate that provided suitable shelter.

### **2.3 Materials and methods**

#### **2.3.1 Study area**

Streams of the upper to middle River Tees catchment in North-East England were used as an upland, hard-water study system to determine the relationship between densities of crayfish, juvenile salmonids and benthic fishes, especially bullhead. The hard-water system is suitable for crayfish and the streams are appropriate for salmonids (see below) making it a suitable study system.

The River Tees flows approximately eastwards from its source on the slope of Cross Fell in the North Pennines to its estuary slightly to the North East of Middlesbrough draining an area of roughly 1906 km<sup>2</sup> (Hudson-Edwards, Macklin & Taylor 1997). The geology of the river Tees consists primarily of carboniferous limestones, sandstones and shales in its upper reaches, with Permian marl and limestone, and Triassic and Jurassic sandstone and mudstone lower down the catchment (Hudson-Edwards, Macklin & Taylor 1997). Mining activity has taken place at the top end of the catchment from the Roman period, with lead extraction peaking between 1815 to 1880, while the majority of zinc extraction is thought to have taken place between 1880 and 1920 (Dunham 1948). The last metal mine in the area closed in 1955, although some downstream pollution as a result of historic mining, and reworking of downstream sediments from this mining creates a continued source of some lead, copper, zinc and cadmium pollution (Hudson-Edwards, Macklin & Taylor 1997). However, due to the limestone catchment and resultant high pH, levels of heavy metals in solution are low, and well below toxic levels to fish and invertebrates in the streams surveyed in this thesis (Environment Agency unpublished data; M. Lucas, pers. comm.).

The lower reaches of the river around Darlington, Stockton on Tees and Middlesbrough have been used extensively for disposal of industrial waste from the iron and chemical industries since 1841, with untreated sewage also being discharged into the tidal section of the river until 1985 (Shillabeer & Tapp 1988). By the early 1930s the high levels of cyanide contained within this pollution in combination with low oxygen availability in the estuary was causing high levels of migratory fish mortality among salmon and sea trout smolts and returning adults (Alexander, Southgate & Bassindale 1935). The result of this mortality was that by 1935 Atlantic salmon (and probably also sea trout) were effectively extinct in the Tees' catchment. After 1935, no further catches of salmon or sea trout were reported from the River Tees until the late 1960s, when a few small, intermittent, sea trout catches were recorded (Williams *et al.* 2009). Since 1970, however, steps have been taken by various agencies (Shillabeer & Tapp 1988; Whitfield 2002) to reduce the amount of pollution entering the Tees. The first recorded migratory salmon in the river since 1933 was caught by rod and line in 1982 and a restocking program ran from 1985 to 1999 (Williams *et al.* 2009). Salmon rod catches have recovered substantially since 1982, peaking in 2004, although they remain well below the numbers typically caught between 1905 and 1913 (Williams *et al.* 2009). Sea trout rod catches also recovered during the 1990s to peak in 2004 at a level roughly comparable to that recorded in the historic data from the early (Pre 1914) 1900s. An estuarine barrage was placed in the river at Stockton on Tees in 1995. Although a fish pass is present at this barrage, there is

ongoing debate that the barrage may be having a limiting effect on migration success, in that part of the river (M. Lucas pers. comm.). Nevertheless, trends in recorded salmon numbers at the logger for the Tees Barrage fish pass appear to confirm the general upward trend in Atlantic salmon numbers, with sea trout numbers apparently remaining roughly stable (Williams *et al.* 2009). Riverine brown trout stocks in the River Tees appear for the most part to be healthy (Environment Agency electric fishing data, unpublished), although some populations may still be affected by residual pollution from mining activity or localised agricultural pollution.

Five major tributaries enter the River Tees, in the forms of the Rivers Lune, Balder, Greta, Skerne and Leven along with numerous more minor tributaries. The River Tees and its major tributaries are impounded in a number of areas, notably the Cow Green Reservoir on the main river, the Selset and Grassholme reservoirs on the River Lune and the Balderhead, Blackton and Hury Reservoirs on the River Balder (Figure 2.1). The flow and area of the River Tees is also regulated to some extent by the River Tees barrage to the East of Middlesbrough.

The chosen study area was tributaries of the middle and upper sections of the River Tees. This area was chosen because of its hard, slightly alkaline waters suitable for bullheads, juvenile salmonids and crayfish. Although all three groups can tolerate a range of habitat conditions, brown trout and Atlantic salmon are dependent upon cool, well oxygenated streams, with suitable substrate for spawning (Frost & Brown 1967; Mills 1989; Elliott 1991; Armstrong *et al.* 2003). Although signal crayfish are broadly habitat generalists, it is likely that, in common with most other crustaceans, they are intolerant of water with a pH much below 6.0, which reduces juvenile survival and inhibits post-moult recalcification (France 1993). One study found no signal crayfish on any site where the water pH was less than 6.5, even though they were found on otherwise similar sites throughout the study system (Usio *et al.* 2006). The River Tees catchment is partially colonised by signal crayfish, with signal crayfish spreading up tributaries from the colonised area of the main river, and was therefore an ideal study region because it meant that it was possible to find sites with signal crayfish at a range of densities or totally absent. The date and site/s of signal crayfish introduction into the river Tees are not known, although it is believed that they were introduced into ponds on or near one or more of the tributaries (including Deepdale Beck, Figure 2.1) and escaped and spread down-stream to enter the River Tees, before spreading along the river and up into further tributaries. Until recently the river had extensive white-clawed crayfish populations (Environment Agency, unpublished) although as of the beginning of this study the current state of these populations

was largely unknown. It was hoped that remnant populations of white-clawed crayfish might be found in a number of tributaries to allow their effects upon fish populations to be compared to those of signal crayfish.

Study sites were chosen to be as functionally independent as feasible, with multiple sites on the same tributary or tributary system being at least 2 km apart and/or with fished tributaries entering the main stem downstream of any main stem study sites. The areas fished were generally small, shallow nursery streams or forks of larger rivers suitable for juvenile salmonids and relatively easy to electric fish with only two people present. Signal crayfish are apparently currently in the process of invading a number of tributaries and thus towards the end of the study, when more sites with crayfish present were needed, the sites chosen tended to be towards the downstream end of tributaries near where they joined the river Tees.



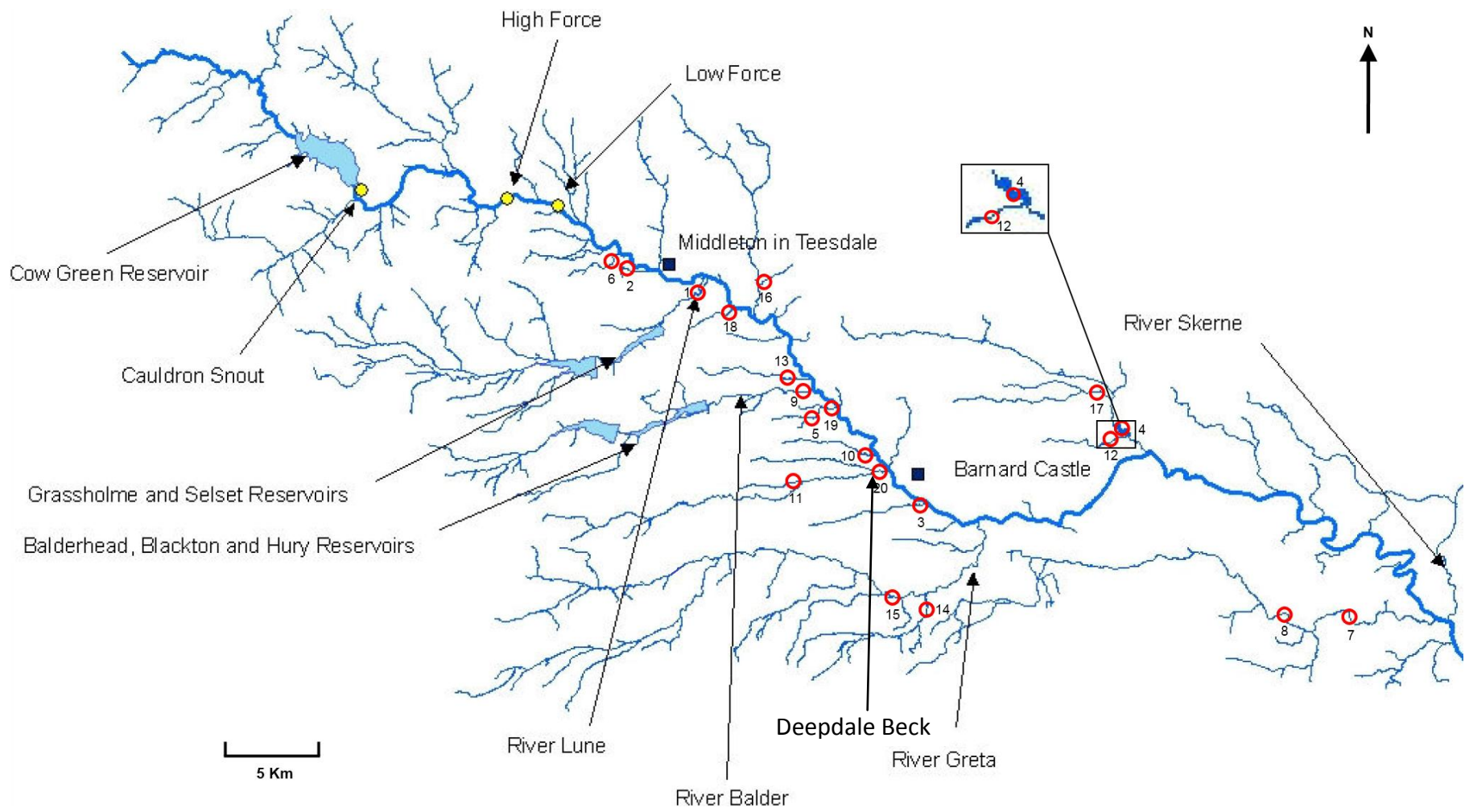


Figure 2.1: The middle and upper river Tees catchment, red circles, enlarged relative to actual study sites sizes for clarity and numbered in date order, show the approximate locations of study sites (Table 2.1), yellow circles show major waterfalls (> 5 m high) on the main stem. Site 12 is on tributary of Langley Beck, whereas site 4 is on the main stem of Langley Beck upstream of the confluence with said tributary. Map modified from Williams *et al.* (2009).

### **2.3.2 Sampling protocol**

Sites covered an area of 98-212 m<sup>2</sup>, resulting in a length sampled of between 16 and 100 m depending upon the width of the river. This resulted from an aim to achieve a study site area greater than, but as close as possible to 100 m<sup>2</sup>. Sites were also chosen to have a width less than 10 m (ideally less than 5 m), so that fishing was possible with two people and single anode electric fishing gear. All the sites used in this study had an average water depth (on the basis of transect data) of less than 300 mm, and the bottom visible across over half (typically over 80%) of the site area. This allowed for efficient sampling of fish, and in particular benthic fish such as bullhead, which on account of their lack of swim bladder do not drift in the water column following stunning and, therefore, frequently had to be picked up off the bottom of the river. Additionally fishing was only conducted in near base flow conditions. Accessibility was also vital and, thus, all sites chosen were within about 250 m of vehicular access and had at least one point at which the banks were reasonably low. The site fished at each location was chosen to have the largest possible proportion of the riverbed covered by unembedded stones of 2-256 mm diameter (gravel, pebble and cobble). These substrates were expected to provide suitable salmonid spawning and nursery habitat (Crisp & Carling 1989; Armstrong *et al.* 2003) and shelter for benthic fish (Tomlinson & Perrow 2003) and crayfish (Light 2005). The names and locations of sites are given in Table 2.1, with their approximate locations shown in Figure 2.1.

Fish numbers were assessed by placing stop nets at the upstream and downstream boundaries of the site and then performing three electric fishing runs of the enclosed area using pulsed DC electric fishing gear (Electracatch WFC4 and a portable generator). Fish caught during electric fishing were released outside of the fished area as soon as biometrics had been taken after each run. At least 15 minutes were left between finishing an electric fishing run and the starting the next one.

Table 2.1: Site numbers, names, waterbodies, grid references and survey dates for the sites sampled for fish and crayfish densities.

Site number	Site name	Water body	OS grid reference	Date surveyed
1	Mickleton Mill	River Lune	NY 965 247	14/07/2011
2	Tarn Farm	Unnamed tributary	NY 929 254	15/07/2011
3	Thorsgill	Thorsgill Beck	NZ 063 153	18/07/2011
4	Alwent	Alwent Beck	NZ 147 184	19/07/2011
5	U/S Lance	Lance Beck	NZ 013 189	25/07/2011
6	Parkend	Parkend Beck	NY 927 259	26/07/2011
7	Clow	Clow Beck	NZ 246 106	28/07/2011
8	Aldbrough	Aldbrough Beck	NZ 197 122	29/07/2011
9	Balder	River Balder	NZ 012 200	02/08/2011
10	Scur	Scur Beck	NZ 040 173	03/08/2011
11	Raygill	Ray Gill	NZ 025 172	08/08/2011
12	Newsham	Westholme Beck	NZ 143 179	09/08/2011
13	Wilden	Wilden Beck	NZ 004 206	13/08/2011
14	Gill	Gill Beck	NZ 063 104	15/08/2011
15	Greta	River Greta	NZ 047 113	16/08/2011
16	Blackton	Blackton Beck	NY 990 247	17/08/2011
17	Sudburn	Sudburn Beck	NZ 137 201	30/08/2011
18	Icaron	Icaron Beck	NY 977 236	31/08/2011
19	D/S Lance	Lance Beck	NZ 024 194	09/09/2011
20	Deepdale	Deepdale Beck	NZ 044 166	22/09/2011

Electric fishing was chosen as a means of estimating fish abundance because of its relatively high efficacy for sampling fish in small streams when compared to seine netting techniques (Wiley & Tsai 1983) and the fact that it can be used without the need to leave nets in for extended time periods. It was important that the surveys could be conducted without the need to leave equipment on site for extended periods because of the unpredictable and sometimes very high flows in some tributaries of the river Tees and the difficulty of obtaining land owner permission to visit some sites on multiple occasions. The original intention was to use depletion electric fishing, where a regression is fitted to the declining catch from across multiple fishing runs (Carrier, Rosenfeld & Johnson 2009) to estimate total fish numbers of each species and where appropriate age class, on each site. However, this was unsuccessful because fish numbers of one or more species rose between the first or second run and one of the subsequent runs on multiple sites. Depletion electric fishing may also be a flawed method if multiple runs are conducted on the same day because of decreases in fish catchability between the first and subsequent runs as fish stressed by the first run seek shelter and,

therefore, become harder to catch on subsequent runs (Carrier, Rosenfeld & Johnson 2009). For these reasons, the data used are the total catch of each species from three electric fishing runs, which allows minimum fish density on each site to be calculated, but may be biased by differential fish catchability between sites.

Crayfish were also caught by electric fishing, but because the accuracy of crayfish density assessments based on electric fishing alone has been questioned (Cowx & Lamarque 1990), each site was also hand searched for one man hour (two researchers, myself included searching for 0.5 hour each) for crayfish. This involved searching likely refuges in a range of mesohabitats and capturing crayfish by hand with the aid of an aquarium net. Hand searching effort was subsequently adjusted to one man hour / 100 m<sup>2</sup> site area by multiplying the number of crayfish caught during the hand search by site area/100. This figure was then added to the crayfish catch from the electric fishing to give an estimate of the minimum crayfish density for the site (Equation 2.1).

$$\widehat{CF}m^{-2} = \frac{\frac{CF_h A}{100} + CF_e}{A} \quad (2.1)$$

$CF$  represents the number of crayfish, hence  $\widehat{CF}m^{-2}$  is the estimated minimum number of crayfish per m<sup>2</sup>,  $CF_h$  is the number of crayfish caught by hand searching and  $CF_e$  is the number of crayfish caught by electric fishing.  $A$  is the site area in m<sup>2</sup>.

Fish and crayfish caught were counted, measured (total length for benthic fish, fork length for midwater fish and carapace length for crayfish) and identified to species level. Fork length is defined here as the distance from the front of the head to the centre of the tail fork. If over 50 of any fish species were captured then they were all counted, but only the animals caught in the runs up to and including the one in which the 50th fish was captured were measured; any further salmonids caught were placed in an age class (e.g. 0+) on the basis of an estimate of their length and the age-date rules described on page 45.

Flow velocity has been identified as a relevant factor in juvenile salmonid survival (Stenroth & Nystrom 2003). Flow velocity was, therefore, recorded at 60% depth by a series of transects with points at 25%, 50% and 75% of the width of the river. Transects were made at the downstream end of the site, 25%, 50% and 75% of the site length upstream (u/s) and at the u/s boundary of the site. Flow was classified into one of four categories, similar to those used by Semple (1991); cascade (water flowing vertically or almost vertically), riffle (shallow and fast flowing water with a broken, rippling surface), glide (flowing water with a relatively

smooth, unbroken surface) and pool (still or near still water, deeper than the surrounding areas) and the percentage of the site's area covered by each was recorded. The percentage cover of substrate types by size was estimated, using a modified version of the Wentworth scale (Table 2.2) (Wentworth 1922), similar to that used in the past for salmonid (Semple 1991) and crayfish (Ream 2010) habitat assessment. The percentage cover of bedrock was also estimated. The extent to which stones in the substrate were embedded was estimated (as a percentage embeddedness for the substrate on each site as a whole), following hand searching, with embedded stones defined as those which had to be pulled free from sediments on the river bed. Sediment build-up around stones reduces water flow through the substrate, increasing salmonid egg mortality (Poff & Huryn 1998), and thereby reducing expected 0+ salmonid densities. A build up of sediment around larger stones in the substrate might also restrict the use of such stones for cover and, thus, affect the availability of refuges for salmonids, crayfish and benthic fish. It is important to record the substrate sizes present, as these will affect trout spawning habitat (Crisp 1996), and shelter availability, and might also affect predator access to salmonid eggs, and or alevins, for both crayfish (Savino & Miller 1991), and other predators (Palm *et al.* 2009). The approximate level of shading at each site was recorded, as again this appears to affect salmonid density (Eklov *et al.* 1999). The maximum and minimum shading on each site was estimated visually, using a modified version of the canopy cover scale used in Ream (2010), where cover is ranked on a scale from zero to five (Table 2.3).

Oxygen concentrations, pH and water temperature were recorded (in the centre of the river channel, between 12:00 and 13:00) as these have been found to be significantly related to brown trout *Salmo trutta* density in a previous study (Eklov *et al.* 1999). Organic pollutants, such as ammonia, are difficult to measure, because they are hard to quantify at low levels. Additionally, spot measurements of factors such as oxygen and pollution concentrations do not reflect the range observed in these factors over time. The benthic macroinvertebrate community present is an accurate indicator of water quality, integrating varying water quality conditions over the extended time periods relevant to fish lifecycles (Bargos *et al.* 1990; Whitehurst & Lindsey 1990). The biological monitoring working party (BMWP) 3 minute kick sampling method (Armitage *et al.* 1983) was used to sample the benthic macroinvertebrate diversity present at each site. This system provides scores for families of macroinvertebrates according to their pollution sensitivity, with sensitive groups scoring more highly. The BMWP score is the sum of the scores of all families present, thereby providing a fairly accurate indicator of the water quality and reliably detecting substantial differences in water quality as

a result of pollution (Cao, Bark & Williams 1996) by integrating the diversity and sensitivity of the taxa present. The average score per taxon (ASPT) is the mean of individual taxa BMWP scores present on a site. Both BMWP and ASPT have been criticised as measures of water quality when used alone, BMWP because a high diversity of tolerant and semi-tolerant taxa can imply good water quality and ASPT because small numbers of very sensitive or very tolerant taxa can dramatically alter the score given to a site (Extence *et al.* 1987). The LQI takes a composite of the BMWP and ASPT indices (Extence *et al.* 1987) and although it loses some of the precision of these indices, by including both it helps mitigate the individual flaws of each and provides a useful guideline value for comparison to published water quality requirements for salmonid fish. It was, therefore, decided to use the LQI value when comparing water quality to the reference values required for fish community types, whilst the more precise ASPT and BMWP values were considered for modelling.

The BMWP scores assigned to families have proved contentious because the original scoring system gave 'safe minimum' scores to each family, assigned primarily on the basis of the most pollution tolerant species within that family; thus, a family with one or more relatively rare tolerant species could be dramatically undervalued (Walley & Hawkes 1996). The original score system also 'lumped' the Coleopteran families together giving them all a score of five in spite of marked differences between their pollution sensitivity (Walley & Hawkes 1996). Walley and Hawkes (1996) also criticise the artificial upper bound and rounding to the nearest whole number, both of which artificially alter the scores given to a range of particularly very sensitive families. On these bases and using a computational method to recalculate scores a revised BMWP scoring system has been proposed (Walley & Hawkes 1996); however, many groups continue to use systems based upon the original scores with minimal modification (e.g. water framework directive classification (WFD-UKTAG 2008)) and, thus, both sets of scores are presented in the results to allow their comparison.

Table 2.2: Substrate size categories, a modified version of the Wentworth scale, adapted from Ream (2010), and similar to that used by Semple (1991).

Size category	Particle diameter (mm)
Sand	< 2
Gravel	2 - 16
Pebble	16 - 64
Cobble	64 - 256
Boulder	> 256

Table 2.3: Canopy cover scale, modified from Ream (2010)

Canopy cover category	Description
0	Vegetation height < 1 m on both banks
1	Vegetation height < 2 m on both banks
2	Vegetation height > 2 m on one bank only
3	Vegetation height > 2 m on both banks
4	Vegetation height > 2 m on both banks and overhanging branches
5	Dense overhead cover

### **2.3.3 Data analysis**

The effects of signal crayfish, on benthic fish densities, may vary between benthic fish species. Two benthic fish species were captured on sample sites. Stone loach densities were not modelled because stone loach were only found at six sites, and at low densities. Bullhead, by contrast, occurred at 13 sites, at a range of densities, and, thus, were suitable for modelling.

The number of bullhead caught was considered as a variable in the modelling of salmonid density, as some authors (see sections 1.4.4 and 1.5.4) have reported that bullhead might influence juvenile salmonids through competition for food, egg and fry predation, or exclusion from shelter, although this is disputed.

Salmonid densities were refined to consider only brown trout densities because Atlantic salmon were only found on three sites and grayling on two. Brown trout, by contrast, were relatively ubiquitous (found on 18 sites), with 0+ brown trout found on 14 sites. Brown trout fry, parr and adults differ dramatically in their habitat requirements, with the densities of 0+ fish typically being highest in areas of relatively shallow water near suitable spawning gravels, whereas adults are typically found in deeper water, possibly with less dependency upon the substrate, although undercut banks and other cover are probably favoured (Crisp 1996). For this reason, brown trout were broken down to approximate age classes for analysis, with 0+ fish defined (on the basis of length-frequency data) as those  $\leq 75$  mm in length in July,  $\leq 90$  mm in length from 1st August until 31st August and  $\leq 100$  mm in length from then on. 0+ trout were a good choice for understanding the effects of crayfish, as eggs and 0+ fish are the least mobile age groups (Armstrong *et al.* 2003) and probably the most vulnerable to predation by and/or competition with crayfish (Rubin & Svensson 1993; Crawford, Yeomans & Adams 2006; Edmonds, Riley & Maxwell 2011). 0+ trout densities, therefore, are likely to most closely reflect the number of trout that survived incubation, hatching and emergence (Armstrong *et al.* 2003), assuming that initial densities are not so high as to result in a substantial negative

density dependence effect upon survival (Elliott 2006). Had the data permitted, the relationships between signal crayfish and older trout densities would also have been investigated; however, far fewer 1+ and older trout were caught, thereby making it unlikely that modelling or statistical testing would have produced meaningful results.

Having decided upon focal groups (bullhead and 0+ brown trout densities), the next step was to determine which environmental variables were affecting their densities, and in particular if signal crayfish were among them. To this end, model selection used the AIC (Akaike Information Criterion) approach. This information theoretic approach has several advantages over traditional null hypothesis testing approaches (Richards, Whittingham & Stephens 2011). From the perspective of the results presented here the key advantages were the ability to account for overdispersion (Richards 2008) and to identify all supported models (Burnham, Anderson & Huyvaert 2011). AIC approaches also offer advantages through the production of effect sizes for each factor included in a model, which allow the scale, and thus, the likely biological importance of any effects to be determined (Burnham, Anderson & Huyvaert 2011; Richards, Whittingham & Stephens 2011). Finally, AIC approaches do not suffer from a known problem with stepwise multiple regression, whereby forward (selection) and backward (elimination) regression may produce different results (Whittingham *et al.* 2006).

All subsets modelling was beyond the coding ability of the author. As such it was necessary to eliminate some variables prior to modelling. Variables were considered for elimination if they were thought unlikely to influence focal species density, or the data recorded for those variables were unlikely to be of adequate quality for modelling. Some predictor variables were also eliminated because they were inherently related to alternative predictor variables, which were better related to focal species density. Full statistical analyses were not conducted at this stage because the proposed relationship between 0+ brown trout or bullhead densities and the factors that might affect them was non-linear and multifactorial (Equation 2.2). An  $R^2$  value greater than 0.1 (10% of the variation explained by a linear relationship between x and y) was taken as evidence that the predictor used should be included in modelling. For a sample size of 20, an  $R^2$  value of 0.1 has a significance of about 0.17. The critical  $R^2$  value of 0.1 was chosen to have a  $p$  value greater than the usual 0.05 to allow for the fact that the model proposed was non-linear and involved multiple effects, thereby necessitating a considerable safety margin because of the reduced power of a linear regression test to detect a relationship in such circumstances.

Correlations between factors can influence the modelled effect size of, and support for those factors. It was, therefore, also important to be aware of potential relationships between



two factors that appeared to influence brown trout or bullhead densities. Where evidence of a correlation was observed the potentially correlated factors were not included together in models. Correlations of  $r \geq 0.44$ , which produce a p value of 0.05, when conducting a Pearson correlation test with  $n = 20$ , were taken as evidence that two factors were related and, thus, should not be included in a model together. The limited coding ability of the author prevented the error structure applied in the models from being applied to the analysis at this stage. This method generated a large number of scatter plots for consideration and ecological interpretation, but was considered appropriate for analysis at this level. Alternatives, such as the generation of correlation matrices of all variables, although possible, were not adopted here.

The effect of environmental variables on mean 0+ brown trout or bullhead density,  $y$  was assumed to have the form:

$$y = e^{(\beta_0 + A\beta_1 + B\beta_2 + C\beta_3)} \quad (2.2)$$

Here, A, B and C represent environmental predictors from the raw data,  $EXP(\beta_0)$  represents the density when all other values are zero and  $\beta_i$  for  $i = 1, 2$  and  $3$  represent the effect sizes of environmental variables A, B and C respectively. A number of different variables were trialled for A, B and C (Tables 2.9-2.12). Factors were removed from the model as necessary by setting their  $\beta$ -value to zero.

The log likelihood of each model, given the data, was calculated for each model based on a negative binomial distribution of width  $1/\phi$ , calculated from the extent of dispersion observed in the data. The log likelihood value of each model was maximised using the solver function in Microsoft Excel which altered  $\beta_0$ ,  $\phi$  and  $\beta_i$  as appropriate. The models varied in complexity from a two factor null model (including only  $\beta_0$  and  $\phi$  as variables) to five factor models including three additional variables. The AIC value for each model was then calculated using

$$AIC(M) = -2\ln L(M) + 2K \quad (2.3)$$

where  $M$  is the model of interest,  $L(M)$  is the maximum-likelihood of the model, given the data and  $K$  is the number of estimated model parameters. Lower AIC values reflect greater model parsimony. For each model considered, AIC differences, value denoted  $\Delta$ , were calculated as the difference between the AIC of the model and the lowest AIC amongst all models. Thus, the best AIC model is associated with  $\Delta = 0$ . It has been shown that all models with a  $\Delta$ -value less than six should be selected to have an approximately 95% chance of selecting the model which is actually most parsimonious with the data (Richards 2008). An additional refinement is

necessary to avoid selecting overly complex models; namely excluding all models with an AIC value greater than or equal to that of a simpler (nested) version of the same model (Richards 2008).

## **2.4 Results**

### **2.4.1 Initial results and variable selection for modelling**

A total of 20 sites, of which nine had signal crayfish and 11 did not, were included in the analysis. One additional site (How Beck, Appendix 1, 2) with white-clawed crayfish was also surveyed but this site was exceptional in having a waterfall almost 1 m high half way up the site. One site would also have constituted an inadequate sample size ( $n=1$ ) for the analysis of the effects of white-clawed crayfish upon fish densities, and thus this site was excluded. All fish and crayfish caught during the study, were successfully identified to species level, with the exception of larval *Lampetra sp.* lamprey less than 12cm total length, which cannot be identified to species level by external features (Harvey & Cowx 2003). Several fish species were caught that were not expected to affect focal species densities, or to be affected to the same extent as bullhead or 0+ brown trout (based on the evidence presented in sections 1.2, 1.4 and 1.5) by signal crayfish density. These were minnow *Phoxinus phoxinus* (eight sites), three-spined stickleback *Gasterosteus aculeatus* (six sites), lamprey *Lampetra sp.* (three sites), grayling *Thymallus thymallus* (two sites) and roach *Rutilus rutilus* (one site). Full details of number and density of each fish species caught on each sites are provided in Appendix 1.

Bullhead were the dominant species in terms of number of individuals (1506) caught (Table 2.4). Brown trout were the second most abundant fish species (274 individuals caught) and the most commonly detected species (captured on 18 sites). Bullhead were detected on the second greatest number of sites of any fish species (13). Young of the year (0+) brown trout were captured on 14 of the 18 sites at which brown trout were captured.

A summary table of the raw environmental data recorded on each site, but not presented in this chapter can found in Appendix 2. Water quality suitability for salmonid presence was assessed using biotic indices derived from the invertebrate sampling to provide longer term measures of its suitability for salmonids. With one exception (Newsham Beck) LQI values from the sites were all greater than or equal to five (Table 2.5), reflecting excellent water quality, a level of water quality greater than the reference value of 4.5 considered necessary for a salmonid fishery (Extence *et al.* 1987). There is little published information on

bullhead water quality requirements of bullhead, although they often occur sympatrically with brown trout, and are thought to have broadly similar water quality requirements (Tomlinson & Perrow 2003). The one site on which the LQI was less than that considered to meet the reference value required for a salmonid fishery only missed this value marginally, with an index value of 4.0. This site also had high bullhead densities, possibly indicating good water quality. Subsequent modelling (Section 2.4) adequately predicted the lack of 0+ brown trout on the site as a result of high substrate embeddedness and signal crayfish densities.

Table 2.4: Focal fish and signal crayfish counts (sum of three fishings for fish and three fishings plus hand search corrected for area (number caught during hand search x site area in m<sup>2</sup>/100) for signal crayfish) and densities from the sites sampled. Signal crayfish counts following correction are rounded to the nearest whole number. Abbreviations are as follows: SC - signal crayfish, BH - Bullhead, BT - brown trout.

Site name	Site area (m <sup>2</sup> )	SC count	SC density m <sup>-2</sup>	BH count	BH density m <sup>-2</sup>	BT count	0+ BT count	0+ BT density m <sup>-2</sup>
Mickleton Mill	147	0	0.000	113	0.768	3	2	0.014
Tarn Farm	127	0	0.000	13	0.102	79	77	0.606
Thorsgill	148	0	0.000	42	0.284	3	1	0.007
Alwent	212	85	0.494	61	0.288	1	0	0.000
U/S Lance	108	0	0.000	0	0.000	13	11	0.101
Parkend	106	0	0.000	144	1.357	4	1	0.009
Clow	166	0	0.000	396	2.381	1	0	0.000
Aldbrough	151	0	0.000	363	2.408	5	3	0.020
Balder	98	33	0.342	17	0.174	3	0	0.000
Scur	177	111	0.628	0	0.000	3	2	0.011
Raygill	139	89	0.636	0	0.000	12	8	0.057
Newsham	121	83	0.687	92	0.761	0	0	0.000
Wilden	137	137	0.998	0	0.000	0	0	0.000
Gill	109	0	0.000	0	0.000	27	13	0.119
Greta	126	0	0.000	91	0.720	12	1	0.008
Blackton	119	0	0.000	0	0.000	57	55	0.461
Sudburn	146	0	0.000	143	0.980	1	0	0.000
Icaron	107	2	0.020	0	0.000	41	41	0.382
D/S Lance	130	54	0.413	13	0.100	8	8	0.061
Deepdale	121	15	0.126	18	0.149	7	5	0.041

Table 2.5: Original (Armitage *et al.* 1983) and revised (Walley & Hawkes 1996) biological monitoring working party (BMWP) and average score per taxon (ASPT) scores for each site and the Lincoln quality indices (LQI) derived from those scores. Sites are ordered by date surveyed.

Site name	BMWP	Revised BMWP	ASPT	Revised ASPT	LQI	LQI from revised scores
Mickleton Mill	109	106.0	7.27	7.07	6.0	6.0
Tarn Farm	80	74.2	7.27	6.75	5.5	5.5
Thorsgill	101	107.7	5.94	6.34	5.5	6.0
Alwent	121	121.1	6.37	6.37	6.5	6.5
U/S Lance	72	68.9	7.20	6.89	5.5	5.5
Parkend	93	96.0	7.15	7.38	6.0	6.0
Clow	92	88.2	5.75	5.13	5.5	5.0
Aldbrough	98	97.6	6.53	6.51	6.0	6.0
Balder	113	111.5	7.53	7.43	6.0	6.0
Scur	68	68.0	6.80	6.80	5.5	5.5
Raygill	89	86.3	6.85	6.64	5.5	5.5
Newsham	64	63.1	4.92	4.85	4.0	4.0
Wilden	84	83.7	7.00	6.98	5.5	5.5
Gill	123	126.5	7.24	7.44	6.5	6.5
Greta	109	109.4	6.81	6.84	6.0	6.0
Blackton	96	94.5	6.86	6.75	6.0	6.0
Sudburn	85	84.3	5.67	5.62	5.0	5.0
Icaron	109	114.2	7.27	7.61	6.0	6.0
D/S Lance	78	78.5	7.09	7.14	5.5	5.5
Deepdale	76	82.3	6.91	7.48	5.5	5.5

The percentage of dissolved oxygen and water temperature and pH were not considered for incorporation into models because they were measured on only one occasion and might vary markedly daily and seasonally. Maximum shade score was considered for elimination by the use of graphs with  $R^2$  values. Maximum shade score was considered for elimination because all sites had at least some areas with vegetation greater than 2m high on both banks, producing a score of 3, 4 or 5 for all sites. This single shade score was, therefore, unlikely to fully reflect differences in shading between sites, which related more to the area covered than the maximum or minimum shade scores. Regression analyses on graphs produced  $R^2$  values less than 0.1 for the relationships between maximum shade score and both 0+ brown trout and bullhead densities. Shade was, therefore eliminated as a variable from modelling. The rate of development and thus hatching date in fish is temperature dependent. Unrecorded differences in spring temperature between sites probably, therefore, resulted in unrecorded differences in 0+ brown trout emergence dates between sites. A similar effect was

also expected for young of the year bullhead. This meant that the number of days since the start of the study, or an estimated date of fry hatching/emergence date, was likely to be of limited value in estimating the populations of these groups. Regression analysis produced an  $R^2$  value of less than 0.1 for the relationship between days since the start of the study and either 0+ brown trout or bullhead density. Days since the start of the study were, therefore, not used in modelling.

BMWP and ASPT are both indicators of water quality, based upon invertebrate diversity and sensitivity. It was, therefore, decided to use only whichever of ASPT and BMWP had a higher  $R^2$  value, for its relationship with the focal species density, to model that species. The original and revised BMWP and ASPT values produced very similar total ASPT and BMWP scores and identical LQI scores in all but two cases (Table 2.5). From here on in, therefore, only the more widely used original scores have been used. Regressions produced larger  $R^2$  values for the relationships between ASPT and 0+ brown trout  $R^2 = 0.115$  or bullhead  $R^2 = 0.188$  densities, than the relationships between BMWP and 0+ brown trout  $R^2 = < 0.0001$  or bullhead  $R^2 = 0.0024$  densities. As such, from here on, only ASPT was considered as a predictor of focal species density.

Age 0+ salmonids are generally found in the areas of lower flow, behind rocks in flowing water, where they feed predominantly by darting out to catch passing invertebrates from the drift (Frost & Brown 1967; Armstrong *et al.* 2003). Percentage cover of different flow types (see section 2.2.2) was, therefore, likely to be a good predictor of 0+ brown trout densities. In addition to percentages of cascade, glide, riffle and pool, an additional variable, Rifcas was defined as riffle + cascade. Rifcas was intended to represent the most suitable conditions for juvenile trout, being the areas where fast flowing and relatively slower water were expected to be in close proximity. This produced a large number of linked habitat variables as the total percentage of cascade, glide, riffle and pool was always 100%. An increase in the area covered by one habitat type, therefore, meant a decrease in the area covered by one or more other habitat types. This in turn meant that if one habitat type had any predictive power, all of the other habitat types might also be expected to have, some proportion, of that predictive power. It was, therefore, decided to eliminate any habitat variables with an  $R^2$  value less than 0.1 for their relationship with 0+ brown trout density. Depth was also considered as a predictor of 0+ trout densities, as 0+ trout are generally thought to favour shallow water (Armstrong *et al.* 2003).

Four habitat metrics (H1-4) were considered as possible representations of the amount of brown trout spawning habitat available (Table 2.4). These variables were also

considered as predictors of bullhead density alongside a variable intended to represent shelter availability (shelter) (Table 2.6). The density of 0+ brown trout was not expected to respond to shelter availability as 0+ brown trout typically shelter in areas of slower water behind, rather than underneath, rocks (see previous paragraph). The availability of this habitat was expected to be better represented by the percentage cover of riffle, cascade or Rifcas.

Table 2.6: Descriptors of the metrics used for riverbed habitat types

Habitat name	Description
H1	Percentage unembedded gravel + pebble + cobble
H2	Percentage unembedded gravel
H3	Percentage unembedded gravel + pebble
H4	Percentage unembedded gravel + pebble + cobble/3
Shelter	Percentage unembedded pebble + cobble + boulder

Bullhead density was also expected to be related to flow type, although it was unclear from the literature which flow types would be the best predictors of bullhead density. As such all flow type variables with an  $R^2$  value greater than 0.1 for their relationship with bullhead density were considered as predictors of bullhead density.

Although bullhead were expected to respond to the availability of shelter, given the limited information available on their habitat preferences, H1, H2, H3 and H4 were also considered as habitat predictors, if the  $R^2$  value for their relationship with bullhead density exceeded 0.1.

Table 2.7, below, shows a summary of the variables originally considered for modelling of bullhead and/or 0+ brown trout, indicating those eliminated as a result of regression analysis.

Table 2.7: The variables considered for modelling bullhead and 0+ brown trout densities. Ticks indicate variables included in modelling. Other variables were eliminated on the basis of biological reasoning (B), regression (R) or a combination of biological reasoning and regression analysis (BR).

Variable	Use in bullhead density modelling	Use in 0+ brown trout density modelling
Dissolved oxygen	B	B
Temperature	B	B
pH	B	B
Maximum shade	BR	BR
Days since beginning of study	BR	BR
ASPT	BR	✓
BMWP	R	R
H1	R	✓
H2	✓	✓
H3	R	✓
H4	R	✓
Shelter	✓	B
Glide	✓	✓
Riffle	R	✓
Cascade	✓	R
Pool	R	R
Rifcas	✓	✓
Mean depth	✓	✓
Mean flow	✓	✓
Signal crayfish density	✓	✓
Bullhead density	N/A	✓

The results of simple correlation tests to ensure that correlated variables were not included in models together are shown in table 2.8. Where two variables were found to be correlated, they were both modelled in all possible combinations, with other non-correlated variables, but models including the correlated variables together were not considered.

Table 2.8: The relationships between the predictor variables for bullhead and/or 0+ brown trout density. Ticks show significant correlations ( $r \geq 0.444$ ,  $p \leq 0.05$ ), crosses correlations that were tested for and not found and N is used to show cases where it was not appropriate to include both factors because they were inherently related in some way. SC refers to signal crayfish density and BH to bullhead density.

	H1	H2	H3	H4	Shelter	Glide	Riffle	Cascade	Rifcas	Depth	Flow	ASPT	SC	BH
H1	N	N	N	N	N	✓	✓	x	x	x	x	✓	x	x
H2		N	N	N	N	x	x	x	x	x	x	x	x	x
H3			N	N	N	x	✓	x	x	x	x	x	x	x
H4				N	N	✓	✓	x	x	✓	x	✓	x	x
Shelter					N	✓	x	x	x	x	x	✓	x	x
Glide						N	N	N	N	✓	x	✓	x	✓
Riffle							N	N	N	✓	x	✓	x	x
Cascade								N	N	x	✓	x	x	x
Rifcas									N	✓	✓	✓	x	x
Depth										N	x	x	x	x
Flow											N	x	x	x
ASPT												N	x	x
SC													N	x
BH														N

### **2.4.2 Modelling**

In order to determine whether or not signal crayfish density affected bullhead density, bullhead density was modelled using a range of factors. This was also a useful further way to test whether any of the factors identified as potential predictors of both 0+ brown trout and bullhead density could actually predict bullhead density and thereby create an interaction effect when attempting to model brown trout density.



AIC values for the models of bullhead density with a  $\Delta$  value less than or equal to six are shown in Table 2.10 below along with descriptions of each of the factors (Table 2.9).

The final models provide strong support for the use of a flow characteristic as a means of predicting bullhead density (Table 2.10). Glide is the best supported flow characteristic, although Rifcas is also well supported (appearing in three models, two within a  $\Delta$  value of two of the best model) and cascade receives some support (appearing in one model).

Signal crayfish density appears in three of the six supported models including the AIC best model indicating good support for signal crayfish density as a variable for use in predicting bullhead density. Percentage cover of shelter appears in one, well supported, model, indicating reasonable support for its use as a predictor of bullhead density. The AIC best model predicted a bullhead density 3.85 times higher in the absence of signal crayfish than at the highest observed signal crayfish density. The AIC best model had the lowest signal crayfish effect size; the largest signal crayfish effect size among supported models predicted a bullhead density 14.7 times higher in the absence of signal crayfish, than at the highest observed signal crayfish density. The observed values, and values predicted by the AIC best model for bullhead density on each site are shown below (Figure 2.2), along with the predicted bullhead density across the observed range of signal crayfish densities (Figure 2.3) and percentage cover of glide (Figure 2.4). The AIC best model for bullhead density had an  $R^2$  value of 0.402, indicating that it explained about 40% of observed variation in bullhead densities.

Models using ASPT as a predictor of bullhead density were not ultimately considered to be of predictive value. Some such models were within a  $\Delta$  value of six (and even two) of the AIC best model, however, ASPT always appeared as a negative predictor of bullhead density in such models. Given that bullhead are thought to require good water quality and predate aquatic invertebrates (Tomlinson & Perrow 2003), a negative relationship with ASPT makes little sense as a means of predicting bullhead densities. The probable cause and implications of this effect are discussed further in section 2.5.1.

Table 2.9: The parameters included in models of bullhead density and the environmental variables they affected (A, B and C in equation 2.1) with the means and ranges for those variables.

Parameter	Environmental variable affected	Mean	Range
$\beta_0$	None (Bullhead density when $\beta_{1-4} = 0$ )	N/A	N/A
$\beta_1$	Signal crayfish density $m^{-2}$	0.217	0.00 - 0.998
$\beta_2$	Percentage glide	27.3	0 - 70
$\beta_3$	Percentage Rifcas	48.3	5 - 90
$B_4$	Percentage shelter	43.9	10 - 60
$B_5$	Percentage cascade	12	0 - 50

Table 2.10: AIC and  $\Delta$  values for the alternative models with a  $\Delta$  value less than six to explain bullhead density.

Model	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$	$\beta_5$	$\phi$	K	AIC	$\Delta$
$\beta_1 + \beta_2$	-1.11	-1.35	0.0401	x	x	x	1.02	4	176.47	0
$\beta_2$	-1.07	x	0.0447	x	x	x	1.13	3	176.81	0.34
$B_1 + \beta_3 + \beta_4$	-1.29	-2.69	x	-0.0563	0.0420	X	2.00	5	177.44	0.967
$\beta_1 + \beta_3$	-1.04	-1.88	x	-0.0353	x	x	1.23	4	178.10	1.63
$\beta_3$	-0.904	x	x	-0.0334	x	x	1.56	3	180	3.95
$B_5$	-0.881	x	x	x	x	-0.0618	1.74	3	181	4.45

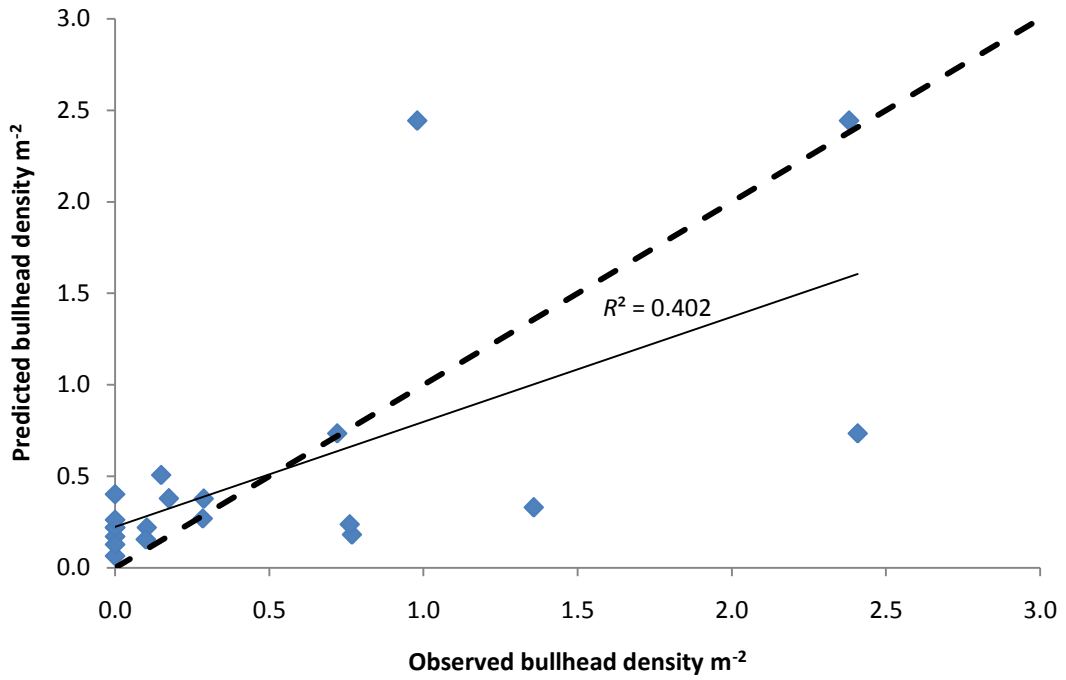


Figure 2.2: The observed density of bullhead at each site against the predicted density of bullhead for that site from the AIC best model (crayfish density and % cover of glide).  $R^2 = 0.402$  (solid line), a perfect fit between the predicted and observed models (dashed line) would produce an  $R^2$  value of 1.0.

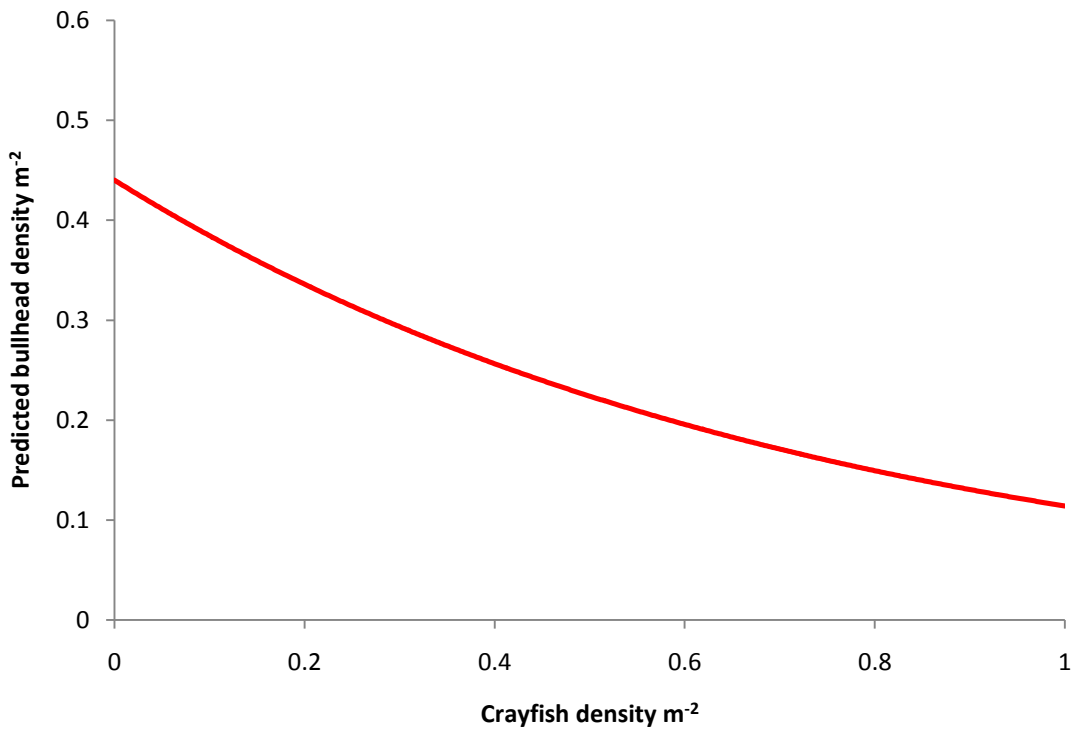


Figure 2.3: The predicted bullhead density over the observed range of crayfish densities (with all other variables held at their mean value) from the AIC best model (crayfish density and percentage cover of glide).

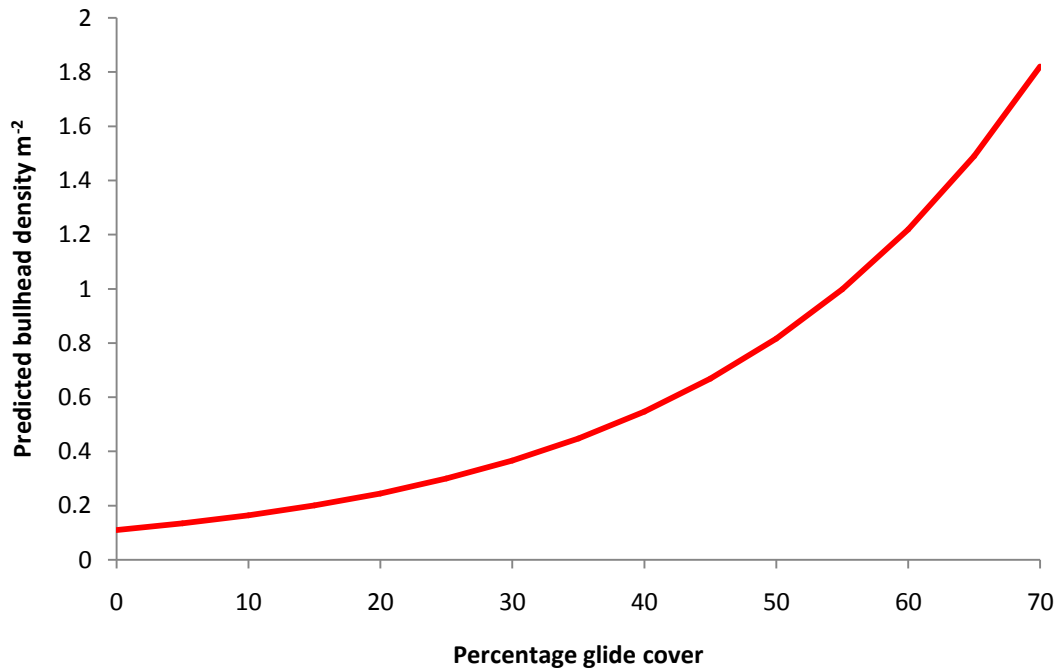


Figure 2.4: The predicted bullhead density over the ranges of habitat cover for glide (with all other variables held at their mean value from the AIC best model (crayfish density and percentage cover of glide).

There was evidence, from models of bullhead density, that bullhead density was related to Rifcas, ASPT and signal crayfish density. Bullhead density was not, therefore, included as a predictor of 0+ brown trout density in models with Rifcas, ASPT or signal crayfish density. Bullhead density was, however, considered in combination with: H1, H2, H3, H4, depth and flow.

Models were produced for all combinations of three or fewer variables, excluding those that were correlated, or showed predictive value for other variables in previous modelling.

The AIC best model uses H2, signal crayfish density and the percentage cover of Rifcas to model 0+ brown trout density. This is also the only model within a  $\Delta$  value of two of the AIC best value and, thus, the only model to receive strong support.

Across their observed density range signal crayfish may have a dramatic effect upon predicted 0+ brown trout densities, with the best model predicting a 0+ brown trout density 9.27 times higher in the absence of signal crayfish than at their highest observed density (range in this differential for models including a signal crayfish effect, 5.72 - 42.7 times). The best AIC model predicts 0+ brown trout densities 84.0 times higher on sites with the highest

observed percentage of Rifcas cover than those with the lowest. The best AIC model also predicts that sites with the highest percentage cover of H2 will have 0+ brown trout densities 8.90 times higher than those with the lowest percentages of H2 cover.

Of the flow characteristics modelled, only Rifcas and glide ultimately appeared in any models within an AIC  $\Delta$  value of six of the best model (Tables 2.11, 2.12). Riffle was also considered in combination with H2 and signal crayfish or bullhead density, but was not supported. Signal crayfish appeared as a factor in five of the nine substantively supported models, including the AIC best model (Table 2.12). Bullhead density and ASPT did not appear in any substantively supported models.

A number of different spawning habitat metrics were trialled, and all received some support, although H2 was the best supported appearing in the model with the lowest AIC value and three of the nine models within an AIC  $\Delta$  value of six of the best model (Table 2.12). Other habitat variables were moderately supported, appearing in one to two models each, but no models within a  $\Delta$  value of two of the AIC best model. A substrate variable appeared in seven of the nine models within an AIC value of six of the best model.

Depth and flow, both of which were correlated with Rifcas (Table 2.8), appeared together in several of the less well supported models, providing moderate support for their use when modelling 0+ brown trout densities.

Figure 2.5 shows the predicted density of 0+ brown trout against the observed density for each site. Figures 2.6 and 2.7 show the effects of signal crayfish density and percentage cover of Rifcas and H2 respectively, over their observed ranges, as predicted by the AIC best model.

Table 2.11: The parameters included in models of 0+ brown trout density and the environmental variables they affected with the means and ranges for those variables.

Parameter	Factor affected	Mean	Range
$\beta_0$	None (0+ brown trout density when $\beta_{1-4} = 0$ )	N/A	N/A
$\beta_1$	Percentage cover H2	12.1	2 - 25.5
$\beta_2$	Percentage cover riffle + cascade (Rifcas)	48.3	5 - 75
$\beta_3$	Signal crayfish density ( $m^{-2}$ )	0.217	0 - 0.998
$\beta_4$	Mean flow ( $ms^{-1}$ )	0.212	0.0241 - 0.483
$\beta_5$	Mean depth (mm)	149	47.1 - 280
$\beta_6$	Percentage cover H1	50.6	12 - 72.3
$\beta_7$	Percentage cover H3	33.3	8 - 59.5
$\beta_8$	Percentage cover glide	27.3	5 - 70
$\beta_9$	Percentage cover H4	39.1	9.33 - 63.8

Table 2.12: AIC and  $\Delta$  values for the alternative models with a  $\Delta$  value less than six to explain 0+ brown trout density.

Model	$\beta_0$	$\beta_1$	$\beta_2$	$\beta_3$	$\beta_4$	$\beta_5$	$\beta_6$	$\beta_7$	$\beta_8$	$\beta_9$	$\phi$	K	AIC	$\Delta$
$\beta_1 + \beta_2 + \beta_3$	-3.50	0.0930	0.0521	-2.23	x	x	x	x	x	x	0.0567	5	107.84	0
$\beta_1 + \beta_2$	-3.27	0.129	0.0446	x	x	x	x	x	x	x	0.0729	4	111.15	3.30
$\beta_3 + \beta_4 + \beta_5$	-3.35	x	x	-2.53	8.69	-0.0210	x	x	x	x	0.0918	5	111.70	3.86
$\beta_4 + \beta_5 + \beta_6$	-3.65	x	x	x	9.70	-0.0207	0.0752	x	x	x	0.679	5	112.42	4.56
$\beta_2 + \beta_3 + \beta_7$	-3.39	x	0.0584	-2.14	x	x	x	0.0370	x	x	0.0959	5	112.53	4.68
$\beta_1 + \beta_3 + \beta_8$	-3.31	0.120	x	-1.74	x	x	x	x	-0.0484	x	0.0761	5	113.11	5.26
$\beta_2 + \beta_3$	-3.55	x	0.0691	-3.75	x	x	x	x	x	x	0.107	4	113.39	5.55
$\beta_2 + \beta_7$	-3.13	x	0.0476	x	x	x	x	0.0593	x	x	0.118	4	113.60	5.76
$\beta_4 + \beta_5 + \beta_9$	-3.38	x	x	x	9.02	-0.0163	x	x	x	0.0699	0.0865	5	113.79	5.95

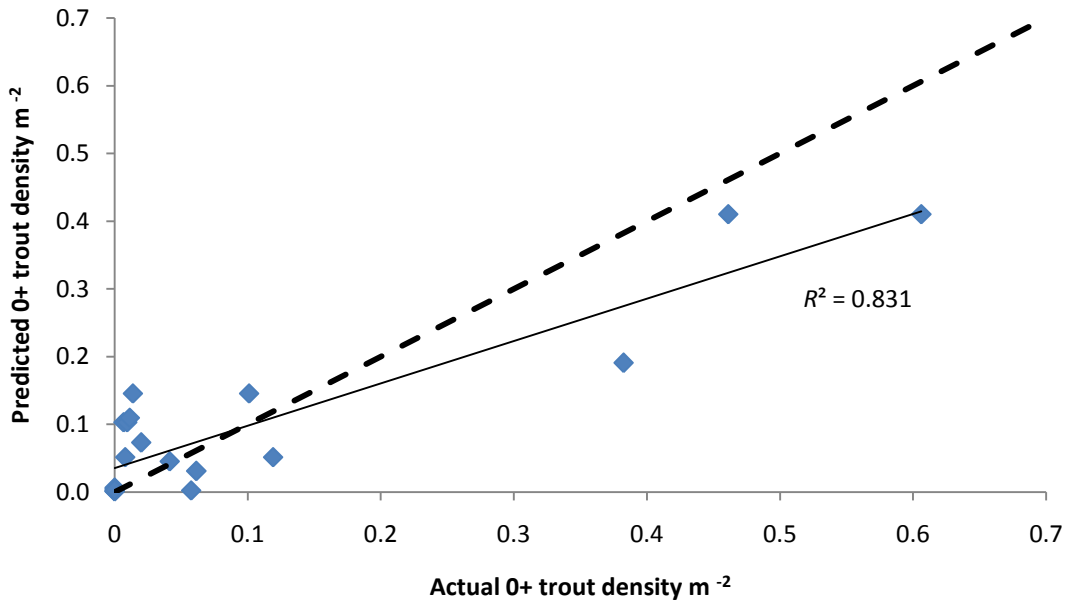


Figure 2.5: The actual and predicted 0+ brown trout densities at each site from the AIC best model using percentage cover of H2 and Rifcas and signal crayfish densities to predict 0+ brown trout densities.  $R^2 = 0.831$  (solid line), a perfect fit between the predicted and observed models (dashed line) would produce an  $R^2$  value of 1.0.

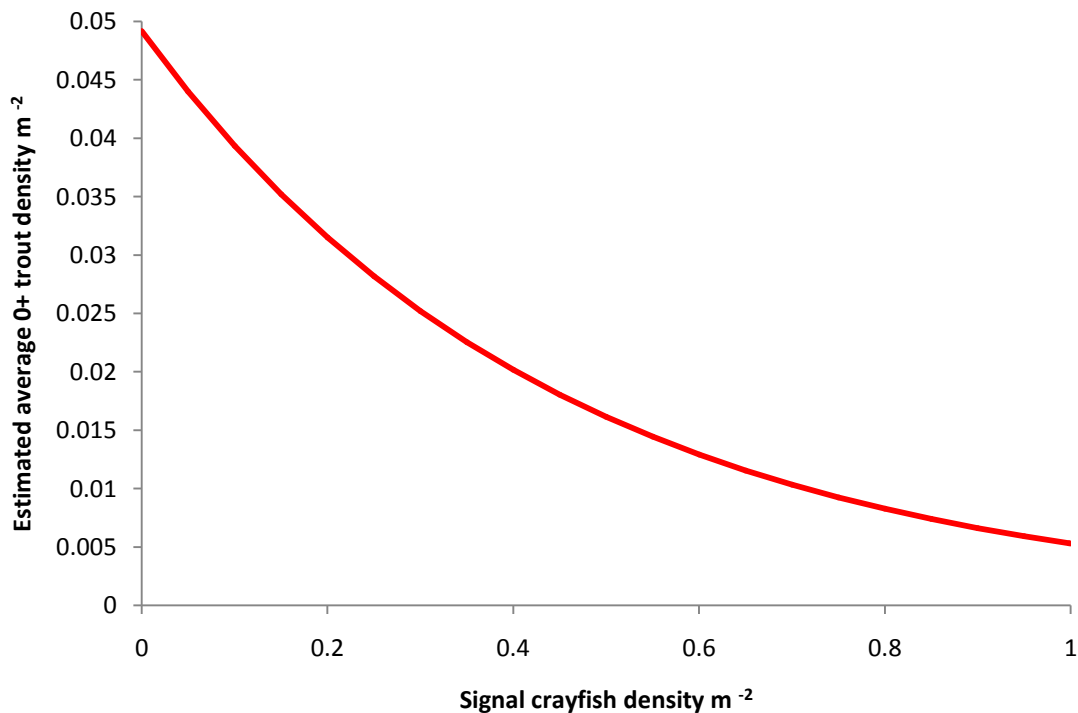


Figure 2.6: The predicted 0+ brown trout density over the observed range of signal crayfish densities (with all other variables held at their average value) from the AIC best model (signal crayfish densities and percentage cover of Rifcas and H2).

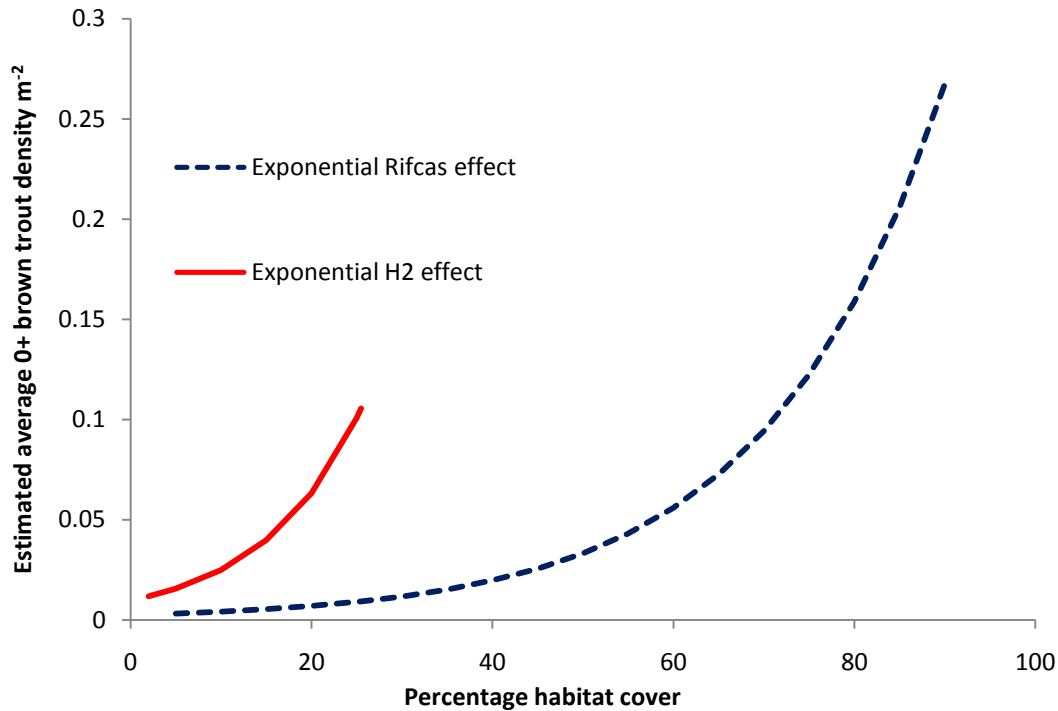


Figure 2.7: The predicted 0+ brown trout density over the observed range of percentage cover of Rifcas and H2 habitat types from the AIC best model (signal crayfish density and percentage cover of Rifcas and H2).

## **2.5 Discussion:**

Modelling demonstrated that, across 20 sites with varied habitat, signal crayfish density may predict both bullhead and 0+ brown trout densities. The AIC best model, of 0+ brown trout densities, included a large signal crayfish effect, with predicted 0+ brown trout densities over 9 times higher in the absence of signal crayfish, than at the highest observed signal crayfish density. The AIC best model of bullhead densities, predicted bullhead densities 3.85 times higher in the absence of signal crayfish, than at the highest recorded signal crayfish density. Models also helped to suggest habitat variables which might be used to predict 0+ brown trout or bullhead densities. The percentage cover of H2 and Rifcas, in combination with signal crayfish density, allowed for relatively accurate ( $R^2 = 0.831$ ) prediction of 0+ brown trout densities. Modelling also suggested some possible predictors of bullhead density, including the percentage of the riverbed covered with rocks that might provide shelter and the percentage cover of Rifcas or glide flow types.



This discussion begins by addressing the findings and implications of the bullhead and 0+ brown trout models. It will then briefly discuss the signal crayfish densities recorded here, and alternative means of measuring signal crayfish density. The potential limitations of this study are then addressed. This discussion concludes with a brief summary of the key findings and their management implications.

### **2.5.1 Bullhead model**

A number of factors received good support as predictors of bullhead density. Percentage cover of glide type habitat and signal crayfish density were the best supported predictors of bullhead density, with the percentage cover of Rificas type habitat and shelter also being well supported. Percentage cover of cascade type habitat received limited support.

That signal crayfish may have a substantial negative effect on bullhead densities is expected, given that signal crayfish are known to be capable of excluding both bullhead and other sculpins from shelter (McNeely, Futrell & Sih 1990; Light 2005; Bubb *et al.* 2009) and that some studies have reported that sculpin densities may approach the densities of available shelter (Light 2005). In previous studies signal crayfish effects have generally been found in upland river systems (Bubb *et al.* 2009; Peay *et al.* 2009), while evidence from other systems is more equivocal (Guan & Wiles 1997; Degerman *et al.* 2007; Hayes 2012; Ruokonen *et al.* 2012). As was observed here, where a signal crayfish effect was found, it generally resulted in a substantial reduction in projected bullhead densities on sites with the highest signal crayfish densities, relative to those without signal crayfish (Guan & Wiles 1997; Bubb *et al.* 2009).

Crayfish might also compete with sculpins for food, given that there is some evidence that both groups may decrease at least some measures of invertebrate diversity (This study)(Dahl 1998; Stenroth & Nystrom 2003; Crawford, Yeomans & Adams 2006). Two studies have found evidence for limited dietary niche overlap, between bullhead and signal crayfish, in at least some populations (Hayes 2012; Ruokonen *et al.* 2012), although neither found evidence for an effect of this overlap upon the density of either species. Upland waterways, however, typically have lower productivity than many other freshwater systems (Moss 2010). It is, therefore, possible that signal crayfish could affect bullhead densities through competition for food in upland systems, even though such an effect has not been demonstrated in lakes (Ruokonen *et al.* 2012) or lowland chalk streams (Hayes 2012). Signal crayfish predation upon bullhead eggs, juvenile or adult fish, although unproven in the wild, may also explain the observed negative relationship.

Bullhead predate juvenile signal crayfish (Dahl 1998) and bullhead niche width may increase in proportion to the density of small signal crayfish, in contrast to the decrease reported with increasing densities of large crayfish (Hayes 2012). Any competition for food would also affect signal crayfish to some extent and, thus, it is possible that any observed negative relationship between bullhead and crayfish densities may result, in part or in full, from bullhead effects on crayfish. No evidence of negative relationships between bullhead and white-clawed crayfish densities has yet been published (section 1.2.4), although bullhead presumably also predate juvenile white-clawed crayfish, which probably remain vulnerable to predation for longer on account of their slower growth rate. This suggests that, although both species might reduce the density of the other, especially during the signal crayfish colonisation phase, through competition for food and predation of juveniles, for the most part signal crayfish ultimately reduce bullhead densities, rather than vice-versa.

Glide appears as a positive predictor of bullhead density in the two best models of bullhead density. Other models suggested bullhead density was negatively related to the percentage of Rifcas or cascade. Glide habitats typically have lower flows than riffle or cascade type habitats and thus are probably favoured by adult bullhead on account of the fact that they are relatively weak swimmers (Tomlinson & Perrow 2003). Riffle and cascade type habitats may be used by juvenile bullhead, which could occupy interstitial spaces, but would be difficult to sample efficiently in, especially shallow, riffle by electric fishing.

Percentage cover of unembedded pebble, cobble and boulder (shelter) appeared in one well supported model, indicating good support for its use when modelling bullhead densities. Sculpins are generally thought to prefer unembedded stony substrate with larger cobbles/boulders for use as shelter (Tomlinson & Perrow 2003; Light 2005). Bullhead are, however, known to occur in other habitats (Tomlinson & Perrow 2003) including at high densities in a variety of chalk stream habitats (Prenda, Armitage & Grayston 1997) such as gravel-pebble substrate typical of chalk streams. Woody debris, macrophytes and tree roots may also provide effective shelters for bullhead (Carter, Copp & Szomlai 2004).

The negative relationship between bullhead density and ASPT probably results from bullhead predation of aquatic invertebrates, which has been demonstrated to significantly reduce the abundance of a number of aquatic invertebrate species in enclosures (Dahl 1998). This relationship was not considered appropriate for predicting bullhead densities, as it was thought to be an effect of bullhead density on ASPT not vice versa. The relationship observed here was also thought very unlikely to persist in lower quality water, where the ASPT value would probably be a positive predictor of bullhead density.

### **2.5.2 Age 0+ brown trout model**

Signal crayfish density was well supported as a negative predictor of 0+ brown trout density. It was expected that signal crayfish would reduce 0+ brown trout densities, given that Peay *et al.* (2009) reported a similar result, albeit from a more limited study, and the range of mechanisms by which crayfish might affect salmonid populations (Sections 1.5.1-1.5.3). Of these mechanisms, given that the habitat types selected in the supported models would have been more suitable for spawning than shelter, competition for shelter seems unlikely to have had a dramatic direct effect up to the point of this study. Competition for shelter may, however, reduce the survival rate of overwintering parr (Griffiths, Collen & Armstrong 2004), thereby reducing the number of 1+ and 2+ fish in the system and this could ultimately reduce the number of spawning adults and thus the number of 0+ trout. ASPT was not supported as a predictor of 0+ brown trout densities and was not significantly correlated with signal crayfish densities. Although ASPT is intended primarily as an indicator of water quality, this does provide limited evidence that competition for food might not be the primary cause of the negative relationship between signal crayfish and 0+ brown trout densities. However, many upland trout streams have relatively low productivity (Frost & Brown 1967; Moss 2010), and crayfish could potentially reduce the availability of food resources to (gape limited) 0+ salmonids by increasing the average size of benthic macroinvertebrates (Crawford, Yeomans & Adams 2006). Other hypotheses to explain the negative relationship between 0+ brown trout and signal crayfish densities such as predation upon eggs, fry and/or larger fish are impossible to test from the data presented here although they do appear very possible (Section 1.5).

It is unsurprising that Rificas was the only well supported predictor of flow conditions, given that it was intended to represent areas where fast flowing water for feeding would be near slower water where fish could rest between feeding forays, thereby providing suitable habitat for 0+ brown trout (Maitland & Campbell 1992; Armstrong *et al.* 2003). Glide was only supported as a negative predictor of 0+ brown trout densities in a single model (Table 2.10), with a relatively high AIC  $\Delta$  value and probably, therefore, predicts an absence of suitable habitat for 0+ brown trout.

That the best supported habitat metric (H2) consisted only of unembedded gravel is slightly surprising as brown trout spawn in substrates of a range of sizes (Armstrong *et al.* 2003). However, given that the number of sea trout returning to the Tees most years remains relatively low (Williams *et al.* 2009), it is likely that the majority of brown trout spawning in the river Tees are smaller, riverine brown trout. Salmonids are known to select spawning habitat in relation to their own size (Crisp & Carling 1989), and studies have reported considerable

variation in brown trout spawning preferences, with some stating that spawning brown trout favour gravel less than 20 mm in diameter (Armstrong *et al.* 2003). In light of this information, and given that trout fry tend to remain close to the spawning habitat from which they emerged for the first few months of their life (Armstrong *et al.* 2003) it appears reasonable that H2 is the AIC best spawning habitat metric for this study area.

Mean depth received limited support as a negative predictor of bullhead density. Similarly mean flow received limited support as a positive predictor of bullhead density. Juvenile trout have been reported to favour shallow water with faster flowing areas (Armstrong *et al.* 2003). These predictors were, however, both correlated with Rificas (mean depth negatively and flow positively) and, thus, their predictive power might come in part from their relationship to Rificas. Percentage cover of Rificas might be a more suitable predictor variable as riffle and cascade type habitats are also typically associated with areas with a stony substrate, which create the broken water surface, and also areas of high and low flow in close proximity. By contrast, shallow, fast flowing areas could have a bedrock substrate, providing few suitable areas for 0+ brown trout to feed or shelter from predators.

It was not ultimately possible to include bullhead density in models alongside signal crayfish density and/or percentage cover of Rificas because they were both supported as predictors of bullhead density in modelling (Table 2.10). Bullhead may affect 0+ salmonid densities through egg predation (Palm *et al.* 2009), although this has yet to be demonstrated in the wild. Some studies have found evidence that bullhead may reduce 0+ salmonid densities (Elliott 2006; Palm *et al.* 2009), but at least one study has also failed to find any evidence of such an effect (Gabler, Amundsen & Herfindal 2001).

### **2.5.3 Potential limitations**

The signal crayfish densities recorded in this chapter came from a combination of electric fishing and hand searching and this combination of methods has not previously been used together in the fashion used here to assess signal crayfish densities and as such no directly comparable data is available. The densities recorded in this chapter are lower than those recorded from the hand searching the area enclosed by mesh sided Surber samplers with a mesh bag at the downstream end (bullhead up to about 15 per m<sup>2</sup> and crayfish up to about 25 per m<sup>2</sup>)(Guan & Wiles 1997; Bubb *et al.* 2009). Crayfish densities recorded at Wilden Beck from Surber searching, in advance of the enclosure experiments described in chapter 3 varied between 4.08 and 32.7 per m<sup>2</sup> (Appendix 3) in contrast to the 0.998 m<sup>-2</sup>

recorded from electric fishing in the subsequent summer (Appendix 1). Surber searches catch almost all crayfish and bullhead within the sampled area, and are far more efficient than electric fishing for catching (particularly 0+) benthic fish and crayfish. Surber samplers are, however, only appropriate for use in water up to about 50 cm deep) and thus would not have enabled the same variety of habitat types, or total area, to be covered as electric fishing.

The bullhead densities presented in this study are not directly comparable to those presented elsewhere, because this study used minimum densities from three electric fishing runs. By contrast Degerman *et al.* (2007) used data from a mixture of three run, depletion electric fishing and single run electric fishing. The majority of other published studies used either single run, or three run depletion electric fishing to estimate bullhead densities. Bullhead densities were not reported in any of the Environment Agency reports the author had access to.

The minimum densities of 0+ brown trout reported in this chapter were similar to the lowest estimates reported for such densities in the literature of 2.5-66.1 per 100 m<sup>2</sup> (Lund, Olsen & Vollestad 2003). Given that the densities reported here are minimum densities, not estimates of the total density, and the sites sampled were not, for the most part, known trout spawning tributaries this was as expected. The densities of 0+ trout observed in this chapter were consistently below 1 fish per m<sup>2</sup>, a level below that typically observed by Elliott (1984) in August/September following substantial density dependent mortality. The sites sampled for this chapter typically also only had relatively small areas of the AIC best spawning habitat (H2) (Figure 2.9), and the sea trout population of the River Tees is thought to be below historic levels (section 2.2.1). It is, therefore, unlikely that initial egg densities were high enough to result in a high level of density dependent 0 + brown trout mortality on many, if any, of the sites sampled for this chapter.

It was not possible to consider a number of potentially important, but related (either by correlation or by modelling) variables together in models. This problem was probably most pronounced for bullhead in 0+ brown trout modelling, where the number of variables that could be included alongside bullhead density was ultimately very limited. Other combinations of variables such as glide and shelter together when modelling bullhead density, or depth and/or flow with Rifcas when modelling 0+ brown trout density would also be very worthy of consideration. The effects of related variables on focal species density could be investigated using structurally explicit models including a variety of possible relationships between potentially related factors to determine which best fit the data. A structurally explicit

modelling approach was not ultimately adopted here because a larger data set would have been needed in order to reliably consider the large number of variables in such a model.

This study was slightly limited by a relatively small sample size ( $n=20$ ) and some unrecorded variation between sites (for instance in stream gradient, percentage cover of each level of shading and length of undercut banks) as well as limitations on the accuracy of estimates of the percentage cover of substrate and flow types. The fact that several fish species failed to deplete during successive electric fishings across multiple sites has also prevented the use of depletion modelling to estimate fish numbers meaning that the fish numbers presented are minimum densities. These minimum densities might be prone to issues of differential fish catchability between sites, although the site selection and sampling protocol (section 2.2.2) should have helped to mitigate this by ensuring that all focal fish species were similarly catchable on all sites. To fully validate the models used here they would need proofing against real data from similar but entirely independent upland stream system(s).

#### **2.5.4 Conclusions and implications**

The observed effect sizes and their implications for the effects of signal crayfish over their observed range of densities on 0+ brown trout density suggest that signal crayfish density is a potentially important determinant of 0+ salmonid density. Signal crayfish density is, in the AIC best model, the predictor with the second largest effect size on, 0+ brown trout densities. Signal crayfish density was also well supported as a predictor of bullhead density, although models of bullhead density were far less effective at explaining the observed variation in bullhead densities.

Were the model of brown trout density to retain its (> 80%) explanatory power in other systems it could be a useful tool for predicting brown trout densities. Some of the key predictor variables for 0+ brown trout density might also be priorities when conducting habitat enhancement for riverine brown trout. In particular the importance of the H2 habitat type suggests that habitat enhancement involving the addition of gravel to spawning habitat poor streams, as is being conducted in parts of the River Tees, might be effective. The habitat variables used in this study were based on unembedded stones and, thus, also emphasise the known (Parrish *et al.* 1998) importance of minimising fine sediment inputs into salmonid spawning streams. The support for Rificas and to lesser extent mean flow as positive predictors of juvenile salmonid density suggest the importance of maintaining shallow areas with variable flow, including sheltered areas behind stones for juvenile salmonids. These results, along with

the, albeit weaker, support for mean depth and percentage cover of glide as negative predictors of 0+ brown trout density also support the theory that channelisation may reduce salmonid populations (Poff & Huryn 1998).

The model of bullhead density explains only around 40% of the variation in bullhead densities and, thus, its usefulness is largely limited to providing evidence that specific variables may predict bullhead densities. The support for shelter as a predictor of bullhead density suggests that habitat enhancement (Knaepkens *et al.* 2004) might increase bullhead populations. The positive relationship between bullhead density and the percentage cover of glide and negative relationship to the percentage cover of Rifcas, also suggest that although bullhead are often sympatric with brown trout they probably have quite different habitat requirements, favouring a slower and more constant flow.

When selecting sites for habitat enhancement for bullhead (Knaepkens *et al.* 2004) or brown trout, the density (or potential density if crayfish are currently invading the system) of signal crayfish (and probably other, especially invasive, crayfish) on those sites should be considered. This is especially the case in upland streams where there is increasing evidence that signal crayfish density may influence bullhead and 0+ brown trout densities (Bubb *et al.* 2009; Peay *et al.* 2009).

The relationship between bullhead density and ASPT found in this study should be investigated further. If this relationship is found elsewhere, this may constitute a limitation upon the use of ASPT to estimate water quality. It is, however, very likely that such a limitation would only apply in streams with the good water quality thought necessary for bullhead to occur at high densities.

It would be possible to investigate the effects of the proposed relationships between predictor variables on bullhead and 0+ brown trout densities through explicit (structural) modelling, of the alternative relationships between them, followed by AIC analysis of the quality of the competing models produced. Unfortunately, however, an insufficiently large sample size and lack of time prevented such analysis from being conducted in this study.

## **Chapter 3 - Crayfish predation upon salmonid eggs**

### **3.1 Research aims**

This research aimed to assess the ability of signal crayfish to predate salmonid eggs buried under the gravel in the period from salmonid spawning in late autumn to hatching in early spring. Controlled field enclosure-exclosure experiments were used to determine whether or not small (large 0+ and small 1+) crayfish, which are often found in gravel during the winter (M. Lucas and J. Findlay, personal observation), reduce the survival of buried eggs. Subsequent laboratory work focused on signal crayfish feeding activity at varying temperatures, as crayfish are ectotherms, and the ability of crayfish of a range of sizes to access salmonid eggs by digging and or moving through interstices between stones. This work included crayfish of a range of size classes, as previous research has focused only on the ability of large crayfish to access buried salmonid eggs by digging.

### **3.2 Hypotheses**

Fish eggs are protected by a tough membranous shell which crayfish would have to break to access the contents of the egg. The mechanism crayfish use to break this shell (break into) fish eggs, is not known, although it may involve the chelae or maxillipeds and/or other mouthparts. Given that salmonid eggs are likely to be a highly profitable energy source, all crayfish large enough to break into eggs were expected to consume eggs. A lack of data made it impossible to reliably predict the minimum size of crayfish that would be capable of breaking into eggs. It was hypothesised that crayfish egg predation rates would increase with increasing temperature, as expected from an ectotherm and in line with the results from previous experiments involving crayfish predation rates at differing temperatures (Section 1.4.2). Predation rate was also expected to be higher in larger crayfish. Finally, it was anticipated that egg burial would reduce crayfish access to eggs and, thereby, reduce the egg predation rate.



### **3.3 Materials and methods**

#### **3.3.1 Enclosure-exclosure study of the effects of signal crayfish on salmonid egg survival under field conditions**

The study site, Wilden Beck, chosen for the enclosure experiment is a tributary of the river Tees (confluence at NZ 015 206). Wilden Beck is a stream (typically about 2 m wide at the study site) broadly typical of the catchment, with the exception of the very high signal crayfish density (0.998 m<sup>-2</sup> from electric fishing in Chapter 2 (Appendix 1), the highest density recorded on any site). Wilden Beck flows over farmland for most of its length, although it passes through Wilden Woods, a small area of woodland about 700 m long immediately downstream of Wilden Bridge. The hard, slightly basic water (pH 7.53 recorded during sampling for chapter 2), is broadly typical of that observed in tributaries of the River Tees. Water quality in Wilden Beck is sufficient to support salmonids on the basis of a BMWP of 84, ASPT of 7.0 and resultant LQI of 5.5, indicative of excellent water quality (again from sampling in Chapter 2). Wilden beck has very low densities of fish and, uniquely among the sites sampled for Chapter 2, no bullhead or brown trout were recorded in the beck (Appendix 1). The lack of fish in Wilden Beck may result from the high crayfish densities and/or sediment and nutrient inputs from livestock on the surrounding farmland. Signal crayfish were taken from, and enclosures placed, about 100 m up and down stream of Wilden Bridge (NZ 004 206) (Figure 2.1 downstream side of site 13). The area upstream of Wilden Bridge was in a livestock field, while the area downstream of Wilden Bridge was in Wilden Woods. This site was chosen for its ease of access, and because it already had a large population of signal crayfish, meaning that any crayfish escapes would be of minimal significance to the beck's ecology. It was not realised, at the point of site selection, that Wilden Beck had a high fine sediment input; indeed, Wilden Beck appeared to have a lower fine sediment load than some alternative sites.

An enclosure-exclosure approach was used in order to determine whether or not juvenile crayfish, which can move through the sediment, could affect the survival of salmonid eggs buried in typical spawning substrate through the egg development stage. The enclosures in this experiment served to maintain the appropriate crayfish density and ensure that additional crayfish, or other large egg predators from the surrounding environment, could not access the eggs within the enclosure. Enclosure-exclosure studies are a widely applied technique in field ecology for examining predation or competition effects (Rosenheim, Wilhoit & Armer 1993; Dahl 1998; Stenroth & Nystrom 2003) under semi-controlled field conditions.

Eighty 0.0912 m<sup>2</sup> enclosures were made using folding, plastic meshed boxes (38 x 24 x 19 cm) lined with 3 mm hexagonal woven nylon mesh netting to prevent crayfish larger than 10 mm carapace length from escaping, or additional crayfish entering from the surrounding environment (tested in preliminary trials). Locations were chosen for enclosures on the basis of their suitability for trout spawning, with an un-embedded gravel substrate, and flowing water deep enough to cover the gravel in the enclosures at low flows; enclosures were not placed in riffles or cascades. Each enclosure was double-tagged with numbered plastic identification tags. Loose bed material was removed from an area the size of the enclosure and about half its depth. The enclosure was placed in this depression, long axis aligned to the main flow. It was anchored with metal poles up and downstream and a rope line attached it to a nearby tree.

Once enclosures were in position, material from the surrounding riverbed (from which any crayfish and fish were first removed) was added to fill each enclosure to approximately the same level as the surrounding gravel. Using substrate from the surrounding river bed had two advantages, it meant that: natural food resources such as algae and invertebrates would be available to the crayfish as alternatives to the trout eggs and a natural range of substrate sizes and, thus, as far as possible interstitial space sizes were present. Manual removal and re-addition of the substrate may have resulted in a degree of sorting and, thus, influenced the sizes of interstitial spaces to some extent, but this was unavoidable. Sixty brown (sea) trout eggs, obtained from the Environment Agency's Kielder Hatchery in Northumberland, and fertilised on the day of egg transfer, were then added to the gravel in each enclosure with a funnel and buried under a second layer of substrate at a total depth of 5-10 cm (within the range of depths at which brown trout bury their eggs in the wild (Crisp & Carling 1989). Egg tubes were added to 64 of the 80 enclosures. Tubes were made of a 2 mm square wire mesh, closed at each end with a plastic bung, which was fixed in place by a small cable tie passing through the bung and the mesh. Tubes were about 8 cm long and were buried vertically in the gravel with the bottom at a depth of about 8 cm such that the top was roughly level with the surface of the gravel. Each tube contained 10 eggs, rendered inaccessible, by the tube, to most (if not all) predators, including the crayfish in the enclosures. All eggs were introduced to the enclosures on 25th November 2010. It was assumed that the environment in each tube would be the same as that in the cage in which it was placed, with the exception of crayfish predation. It was, however, possible that the egg tubes could reduce water flow over the eggs, and/or increase sediment build up around the eggs and, thus, tubes were also placed in crayfish free enclosures to enable the effect of the tubes on egg survival to be tested.

Crayfish were added to enclosures at densities of zero (control), one ( $11.0 \text{ m}^{-2}$ ), two ( $21.9 \text{ m}^{-2}$ ) or three ( $32.9 \text{ m}^{-2}$ ) per enclosure to reflect the densities of crayfish observed in the beck (2-16 crayfish/ $0.49 \text{ m}^2$  Surber sampler (Appendix 3)). Twenty enclosures were used for each treatment, and a treatment was assigned to each enclosure at random. Crayfish came from an area of Wilden Beck upstream of the study site and were the smallest individuals captured, unable to fit through the 3 mm mesh surrounding the boxes, with carapace lengths of between 10.0 and 16.2 mm. This range of sizes corresponded to large age 0+ and small age 1+ crayfish, on the basis of previous length-frequency distribution data from Wilden Beck (Appendix 4). Measured crayfish were added to enclosures from 27th November to 3rd December 2010. Even though signal crayfish were present on Wilden Beck throughout the study, the study was carried out under licence from Natural England as technically signal crayfish cannot legally be captured and then introduced (even to enclosures and/or on the same site) without licence.

The original experimental design involved removing half of the boxes 1 month after the experiment commenced (early January) and the other half in early April, after hatching but shortly before the estimated date of fry emergence from the gravel. However, due to the departure of the student originally responsible for this work (See Declaration) the first removal was not carried out. In early February 2011 Wilden Beck flooded, displacing some of the enclosures short distances, and resulting in complete loss of some others. The flooding of Wilden Beck also necessitated the recovery of all the remaining enclosures as soon as the river level had fallen sufficiently to make this possible (between 14<sup>th</sup> and 18<sup>th</sup> February 2011). When the enclosures that had not been washed away by the flooding were recovered, some were found to have been either beached, or buried with silt and debris (high enough to cover any part of the top of the enclosure); as such, whether each enclosure was beached or buried was recorded to enable such enclosures to be excluded from parts of the analysis as necessary. As each enclosure was recovered, the gravel was removed to a white sorting tray and the abundance of six orders or families of the dominant benthic macroinvertebrates (fresh water shrimps [Gammaridae], hog lice [Asellidae], mayfly larvae [Ephemeroptera], caddisfly larvae [Trichoptera], stonefly larvae [Plecoptera] and true fly larvae [Diptera]) were recorded using a semi-quantitative scale. For each enclosure, this scale ranked each family as either absent or present at one of three densities: low (1-5 individuals), medium (6-10 individuals) or high (>10 individuals). The numbers of live and dead trout eggs recovered from each enclosure and egg tube were counted and recorded. Finally, following recovery, the sorted sediment from each enclosure was transferred to a bag, labelled, dried in an oven and sieved. This allowed the

percentage of fine sediment (<2 mm diameter) by dry weight to be measured, although the figure this produced represents a minimum estimate for the actual percentage of fine sediment in the enclosure at the end of the experiment, as some fine material will have escaped from the enclosures during their recovery.

### **3.3.2 The effects of crayfish size, water temperature and egg burial on Atlantic salmon egg predation by signal crayfish**

All of the laboratory experiments were carried out in a secure room of the Durham University Life Sciences Support Unit, under DEFRA licence. The room in which work was conducted was designed to be temperature and photoperiod controlled.

#### **3.3.2.1 Animal husbandry:**

Crayfish were collected from Wilden Beck over an area of the beck extending about 300 m upstream of Wilden Bridge between late November 2011 and late January 2012. Crayfish were of both sexes, although berried females were avoided because of their lower levels of activity (Bubb, Lucas & Thom 2002). Age 0+ crayfish (carapace length 8-14 mm) were stored in a tank of 760 x 300 x 380 mm (length x width x depth) with undergravel filtration and 1+ crayfish (CL 16-22 mm) were stored in a large water storage container of 960 x 600 x 580 mm with filtration from a large external canister filter with spray-bar. Age 2+ crayfish (CL  $\geq$  24 mm) were kept individually in small transparent plastic tanks (320 x 220 x 200 mm) with undergravel filtration. The putative age classes were defined based on length frequency data from N. Dale (Appendix 4), although the gaps between age classes were placed at the top end of the gaps between classes observed in N. Dale's data to account for the fact that the laboratory work was conducted later in the year. The undergravel filtration system was of the traditional design, with an uplift pipe attached to a porous undergravel plate. Air bubbling up the uplift pipe draws water up that pipe, thereby drawing water down through the gravel, causing the gravel to function as a filter.

Crayfish in storage tanks were provided with a 30 mm deep, gravel (10 mm diameter) substrate and shelter in the form of sections of drainpipe cut in half along their length and of roughly appropriate size for the crayfish. Whilst being stored before experiments crayfish were

fed *ad libitum* on chopped carrot, with sinking fish pellets provided biweekly. Crayfish used for experiments were last given fish pellets between 48 and 72 hours prior to the beginning of the experiment. The photoperiod throughout the study was 8 hours light, 16 hours darkness, with a shifted photoperiod such that the light period ran from 06:00 to 14:00. Experimental animals were acclimated to this altered photoperiod for a minimum of 72 hours after being bought into the laboratory. The salmon eggs used in experiments were obtained from the Environment Agency's Kielder Hatchery in Northumberland, and maintained in a single layer in a tank of 535 x 345 x 163 mm with a large external canister filter and spray bar to provide sufficient filtration and aeration. Eyed (approximately 260 to 350 degree day) eggs were collected from Kielder Hatchery every week to ten days to replace those used in experiments, although a small number of eggs from each batch were kept back to ensure that they survived and hatched normally. Eggs developed rapidly in the relatively warm conditions of the experimental room and, thus, in order to run all experiments with eggs at a roughly equivalent developmental stage, it was necessary to collect them frequently. Dead eggs were removed at least once every 48 hours to prevent the spread of (especially fungal) infections; survival to hatching of unused eggs was typically greater than 95%.

### **3.3.2.2 Experimental procedures**

For experimental work, crayfish were placed, one to a tank, in small tanks, the majority of which were similar to those used for the storage of large crayfish (transparent plastic 320 x 220 x 200 mm). Two crayfish per feeding trial were video recorded for behavioural observation, with the intention of recording activity and confirming that any egg loss above the control rate resulted from crayfish predation. These crayfish were initially housed individually in glass tanks of similar sizes (355 x 205 x 205 mm) to the aforementioned plastic tanks; however, on 1 February 2012 one of these glass tanks was broken and thereafter replaced with a plastic tank, reducing visibility of crayfish at the front of the tank. Recorded tanks were lit from above with an infrared light source, and filmed from above and slightly in front of the tanks using a low light video camera (ABCA systems vista VPM 6130). Videos were recorded onto a digital video recorder designed to record video footage from security cameras (Addonics Advantage ANDVR4-500 4 Way Digital CCTV recorder). The distance covered by crayfish in video recorded tanks was estimated, using the fact that crayfish tended to follow the sides of the tank. This allowed the numbers of lengths and widths and approximate (estimated) fractions (half and quarter lengths and half widths), covered by crayfish, in the course of an experiment, to be

counted and used as an estimate of the distance covered by that crayfish. The tip of the crayfish's rostrum (the front most point of its carapace) was used to judge its position. Age 0+ crayfish proved impossible to follow in video footage and, thus, video analysis was not conducted for this age group. Although video analysis allowed feeding on and handling of the piece of carrot to be observed, poor recording quality and the gravel substrate made salmon eggs difficult to see and, thus, it was not possible to observe crayfish predation upon eggs.

All experimental tanks had an under gravel plate and uplift pipe in place in a similar fashion to that found in the storage tanks used for large crayfish, but with the uplift pipe blocked, and the airstone underneath the under gravel plate rather than in the uplift pipe. This caused air to bubble up through the gravel, with the undergravel plate helping to spread the air flow slightly and so caused some local water movement up through and out of the surface of the gravel. Each experimental tank also contained one crayfish shelter consisting of a half drainpipe of a size appropriate for entry of crayfish being used in the trial. A 5-6 g disc of chopped carrot was provided alongside fish eggs in experimental set ups, so that crayfish had an alternative food source available. This quantity of carrot was deemed to be *ad libitum* because crayfish of the largest size class in holding tanks never fully consumed a piece of carrot of this size in fewer than five days, while all experiments lasted less than 3 days.

Egg recovery was achieved by first using a pipette to pick out any eggs on the surface of the gravel, and then sieving the gravel. When sorting eggs from 10 mm gravel, a small metal sediment sorting sieve with mesh sizes of 8 mm and 4 mm was used. This was highly effective as the vast majority of the 10 mm gravel was stopped by the 8 mm mesh, while a small amount of finer material and any eggs present fell through and were stopped by the 4 mm mesh. The sediment sorting sieve was too small for use in sorting the large volumes of larger gravel used in the buried egg experiments and as such a large sieve was made. This sieve used 13 mm square chicken wire to form the mesh, with a second c. 3 mm square plastic mesh sieve underneath to catch eggs as they fell through the larger sieve. Eggs recovered from tanks were classified into one of six categories defined in table 3.1.

Table 3.1: Classifications for recovered eggs. Note that hatched eggs were only recovered in the final (long term, age 1+) trial.

Name	Description	Notes
Healthy	Normal pinkish-yellow colour, intact membrane	
Unbroken dead	White colour, intact membrane	Not known if crayfish involved (e.g. by scratching surface of egg increasing susceptibility to infection)
Dead and broken	Broken membrane or fragments thereof, with whitish material attached	Almost certainly predated.
Damaged	Broken membrane, alevin damaged in some way (e.g. leaking yolk sac)	Only recorded from experiments where hatching had not yet started.
Hatched (live alevins)	Alevin recovered alive and healthy with no obvious damage	
Hatched (dead alevins)	Dead alevin, most appeared damaged and were missing yolk sac	Not known if damaged individuals were predated before, during or after hatching, or if they were healthy when they hatched.

For experimental work on unburied eggs, clean 10 mm diameter gravel about 30 mm deep, was used to cover the undergravel plate and provide a fairly natural substrate for the crayfish. Experimental tanks used for experiments on buried eggs were filled to a depth of about 100 mm with 20 - 40 mm diameter gravel, a size similar to that used in a previous investigation of signal crayfish predation upon salmonid eggs (Edmonds, Riley & Maxwell 2011), and within the range of substrate sizes used for egg burial by wild Atlantic salmon and brown trout (Crisp & Carling 1989). Controls, identical to the experimental set ups but without the crayfish present were also run for both the surface and buried egg experimental designs to determine rates of egg loss as a result of eggs not being re-found during sorting of the tanks contents. To avoid any experience effects no crayfish was used more than once. Experiments lasted approximately 20 hours (range 20 h 0 m - 20 h 32 m) encompassing less than 1 hour of light, 16 hours of darkness and then less than 4 further hours of light, reflecting the fact that signal crayfish are principally nocturnal. All crayfish of a given size class and treatment were typically run on the same night. This meant that the differences in experimental duration between treatments were consistent. However, given the relatively small size of differences (maximum 32 minutes difference between any two trials) and the fact that they took place in the light period when signal crayfish are less active (Lozan 2000), and that no activity was observed between the end of the 20th hour and the termination of experiments in any video recorded tank, they were unlikely to be of any significance.

The effects of crayfish size upon their ability to handle fish eggs were tested using 10 crayfish of each of the three size classes. Ten eyed eggs were offered to each crayfish in a small depression on the surface of the gravel at a nominal water temperature of 9 °C (actual range in recorded tanks 8.9-9.6 °C), chosen to be within the range at which salmon eggs develop naturally. The depression was close to the area where water bubbled up through the gravel to create a flow of oxygenated water over the eggs, thereby simulating the conditions in a redd.

To investigate the ability of crayfish to access buried eggs, 10 crayfish of each size class were placed individually in separate tanks. The temperature during this experiment was nominally 9 °C (actual range in recorded tanks 9.0 - 9.5 °C). Ten eyed eggs were then buried in each tank at a depth of 80mm. This was the same depth as that used by Rubin and Svensson (1993) when investigating egg and fry predation by noble crayfish and towards the shallower end of egg burial depths used by riverine brown trout (Rubin & Svensson 1993), but not as shallow as the modal depth of 4 cm reported in one study of brown trout (Elliott 1984). Eggs were buried in close proximity to, but not on top of, the area in which air was bubbling up through the gravel. The eggs were placed in close proximity to the area where air was bubbled up through the gravel to simulate the constant flow of oxygenated water in the redds in which trout and salmon naturally bury their eggs.

To investigate the effects of water temperature on crayfish predation of salmonid eggs, following the completion of other experimental work, the room was cooled to the lowest temperature it was capable of maintaining, which should have been 5 °C according to the technical specification. With the target air temperature for the room set to 5°C, the actual water temperature in experimental tanks ranged from to 7.0 - 7.5 °C, a markedly higher temperature than planned or expected. It was originally intended that there be greater separation between the highest and lowest temperatures and possibly an intermediate temperature for example 5, 7 and 9 °C trials. The nominal 9 °C work is, therefore, hereafter referred to as high temperature and the nominal 7 °C work as low temperature. Two days after the low temperature had been achieved stably 10 crayfish of each of the 1+ and 2+ size classes were placed individually in tanks with 10 salmon eggs in a set up that, with the exception of temperature, was otherwise identical to that used with eggs on the surface of the substrate at the higher temperature range. No control was conducted at this temperature; instead data from the high temperature control was used as control data for this experiment. Given that no definite egg mortality was observed in any control, the sub 100% recovery rate was probably down to a failure to re-find eggs; hence, there was no reason to suspect that temperature



would affect the recovery rate from control experiments. Crayfish in the 0+ age class exhibited no evidence of salmon egg predation in the high temperature experiment with eggs on the surface of the gravel and, thus, were excluded from the low temperature experiments in which predation rate was expected, with reasonable certainty, to be equal or lower.

Following completion of the low temperature work, the room temperature was raised to produce a water temperature of about 9 °C, which was maintained for two days prior to any further experiments. To ensure that a lack of time was not responsible for the failure to detect any evidence of predation by 0+ crayfish or any significant evidence of predation by 1+ crayfish (section 3.4.2), longer term trials with eggs on the surface of the substrate were conducted. The long term trials used a set up similar to that in the first experimental trials (nominal temperature 9 °C, recorded temperature range 9.2 - 9.9 °C), but over a nominal time period of 68h (68 h 19m to 68h 30m), including three complete nights.

### **3.3.3 Data analysis**

Given the simplicity of the data, with the number of factors having been kept to a minimum, modelling probably wouldn't have substantially increased the amount of information that could be inferred (Richards, Whittingham & Stephens 2011) and, as such, the data were analysed using null hypothesis testing approaches. For significance testing, in line with normal statistical conventions the difference between two groups has been deemed to be significant if  $p < 0.05$ . Data analysis was conducted using IBM SPSS statistics version 19. Visual examination of frequency distribution graphs was used to check the data for approximate normality because, in some cases, group sizes were too small for Kolmogorov-Smirnov or other statistical tests for normality to produce meaningful results. Where three or more pairwise tests were applied together a Bonferroni correction was used to account for the increased likelihood of producing a false positive result. This test was chosen because, although conservative, it is easy to apply and subject to relatively few restrictions (Narum 2006).

Non-parametric statistical tests have been used throughout most of section 3.4.2 because in all treatments except those using 2+ crayfish with eggs on the surface of the substrate, or 1+ crayfish in long term trials, 10 healthy eggs were recovered from at least half of the replicate tanks and, thus, the data were skewed in such a way as to make transformation to remove the skew impossible.

The effects of crayfish in laboratory experiments were investigated by significance testing on the numbers of healthy eggs and dead and broken eggs recovered. Significance testing on the number of damaged and unbroken dead eggs recovered was pointless as only a very small number of such eggs were recovered from across all experiments. Where predictions have been made, these have used purely the number of healthy eggs recovered as the proportion of dead and broken eggs recovered was not known. It is also likely that in some cases little or no evidence of an egg remained following predation, further undermining the use of dead and broken eggs as predictors of the level of egg predation. Dead and broken eggs were still subject to all significance tests as their recovery provided the most definite evidence of egg predation, and their numbers were expected to increase as egg predation increased.

### **3.4 Results**

#### **3.4.1 Enclosure-exclosure experiment**

A total of 72 enclosures were recovered of which 16 had become either buried or beached prior to recovery. A total of five enclosures had fewer live crayfish in at the point of recovery than they had contained at the beginning of the experiment, of which three were buried or beached. No enclosures contained more crayfish at the end of the experiment than at the start and there was no evidence of the exchange of any crayfish between the enclosure and the surrounding environment, with the largest apparent change in the carapace length of a single crayfish recorded being a loss of 0.2mm, a figure almost certainly within the margins of error created by the use of different sets of callipers and human error on part of the measurer. A summary of the enclosures recovered is presented in Appendix 5. The data presented here excludes beached and buried enclosures. The data were also analysed with the beached and buried enclosures included and proved broadly similar to with the beached or buried enclosures excluded, but with greater variability, which was probably unrelated to crayfish numbers and these results were, therefore, not presented.

Enclosures contained between one and six macroinvertebrate groups with Diptera being recorded in all but one recovered enclosure and Gammaridae and Ephemeroptera also being found in the majority of enclosures (Appendix 5). Diversity analysis has not been conducted for invertebrates because the groups sampled represented only an (unknown) portion of total invertebrate diversity, being only a sample of the invertebrate groups most commonly present. Algae were also present on gravel recovered from the surface of the

substrate in all enclosures. Nevertheless, the data demonstrate that crayfish had food, other than trout eggs, available through the experimental period.

The number of live eggs remaining in enclosures at the end of the experiment was negatively related to both the percentage of fine material by dry weight and the number of crayfish present (Figure 3.1). Analysis of covariance (ANCOVA), suggested no evidence of an interaction between the effects of the number of crayfish and the percentage of fine material affecting the number of live eggs recovered, but egg survival was negatively related to both the number of crayfish and the percentage of fine sediment in the enclosure (Table 3.2).

Table 3.2: The outcomes of ANCOVA tests for the effects of crayfish numbers, the percentage of fine material by dry weight and the interaction between crayfish numbers and the percentage of fine material upon the number of live eggs recovered from enclosures.

Test	Interaction			Number of crayfish			Percentage fine material		
	df	F	p	df	F	p	df	F	p
ANCOVA	3	0.016	0.997	3	<b>7.24</b>	<b>&lt;0.001</b>	1	<b>8.35</b>	<b>&lt;0.01</b>

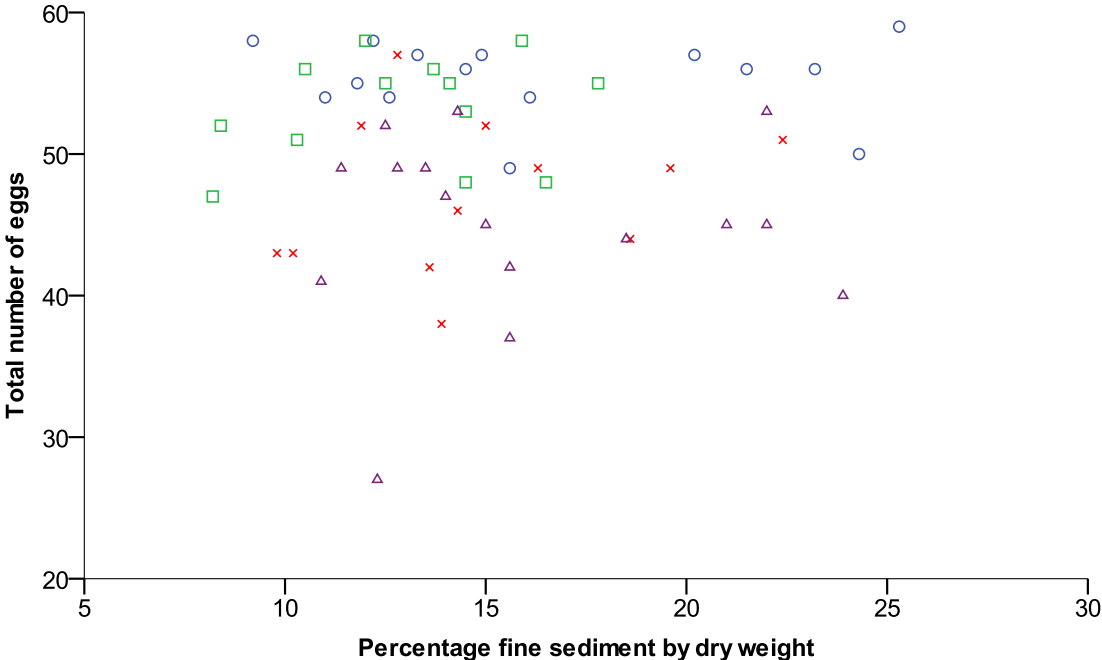


Figure 3.1: The number of live eggs remaining in enclosures against the percentage of fine sediment (<2mm diameter) by dry weight, for each crayfish density: zero crayfish (circles), one crayfish (squares), two crayfish (crosses), and three crayfish (triangles). Lines are not shown on this graph because of limits in the author's coding ability and the SPSS graphical package.

The number of dead eggs found in enclosures was positively related to the percentage of fine material present (Figure 3.2). ANCOVA produced no significant evidence of an interaction between the effects of the number of crayfish and the percentage of fine material affecting the number of dead eggs recovered (Table 3.3). The number of dead eggs recovered was positively related to the percentage of fine material in the enclosures, but there was no evidence for an effect of the number of crayfish in an enclosure on the number of dead eggs recovered (Table 3.3).

Table 3.3: The outcomes of ANCOVA tests for the effects of crayfish numbers, the percentage of fine material by dry weight and the interaction between crayfish numbers and the percentage of fine material upon the number of dead eggs recovered from enclosures.

Test	Interaction			Number of crayfish			Percentage fine material		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
ANCOVA	3	0.159	0.924	3	0.105	0.957	1	<b>24.2</b>	<b>&lt;0.001</b>

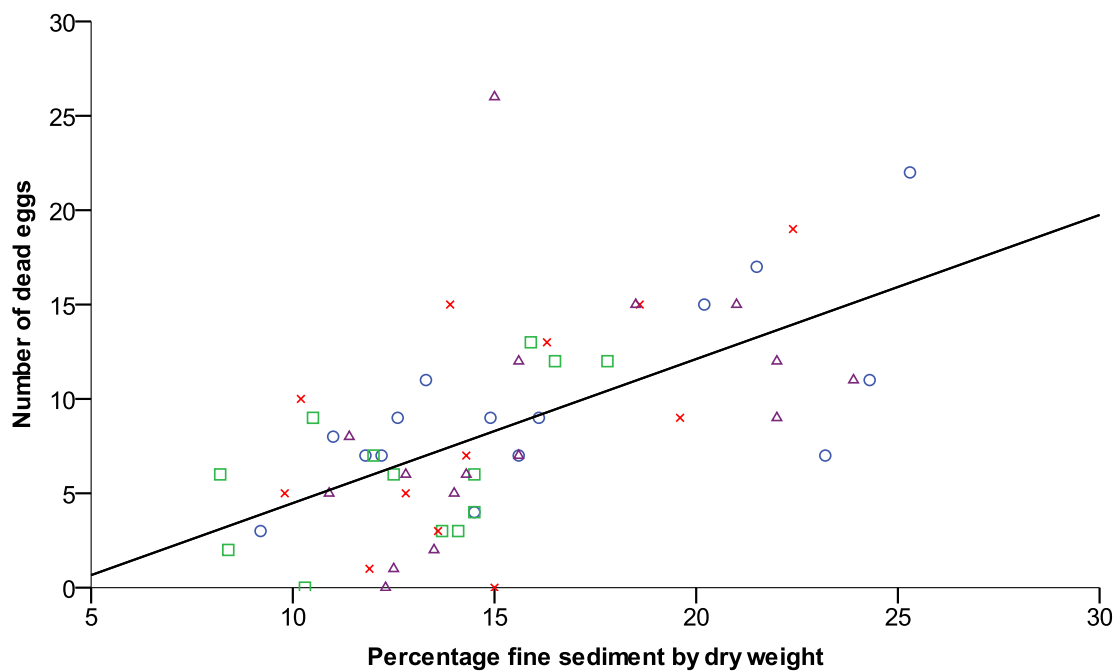


Figure 3.2: The number of dead eggs remaining in enclosures against the percentage of fine sediment (<2mm diameter) by dry weight, for each crayfish density: zero crayfish (circles), one crayfish (squares), two crayfish (crosses), and three crayfish (triangles). The regression line is for percentage fine sediment against number of dead eggs for all crayfish densities,  $R^2 = 0.351$ .

ANCOVA showed no statistically significant evidence of an interaction between the effects of the number of crayfish and the percentage of fine material affecting the total (live and dead) number of eggs recovered (Table 3.4). The total number of eggs recovered was negatively affected by the number of crayfish in the enclosure, but there was no evidence was for an effect of fine material on the total number of eggs recovered (Figure 3.3). Post-hoc testing revealed that enclosures with two or three crayfish had significantly fewer eggs remaining at the end of the experiment than those with zero or one crayfish, but that there were no significant differences within these two groups (Table 3.5) (Figure 3.3).

Table 3.4: The outcomes of ANCOVA tests for the effects of crayfish numbers, the percentage of fine material by dry weight, and the interaction between crayfish numbers and the percentage of fine material upon the total number of eggs recovered from enclosures.

Test	Interaction			Number of crayfish			Percentage fine material		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
ANCOVA	3	0.238	0.869	3	<b>14.6</b>	<b>&lt;0.001</b>	1	0.273	0.604

Table 3.5: The results of a Tukey HSD post-hoc test for significance in the differences between the total numbers of eggs recovered from enclosures containing different numbers of crayfish.

Number of crayfish in enclosure (I)	Number of crayfish in enclosure (J)	Mean difference in total number of eggs remaining (I-J)	Standard Error of the mean	<i>p</i>
0	1	2.103	1.873	0.678
	2	8.167	1.915	<b>&lt;0.001</b>
	3	10.458	1.777	<b>&lt;0.001</b>
1	2	6.064	1.979	<b>&lt;0.05</b>
	3	8.356	1.846	<b>&lt;0.001</b>
2	3	2.292	1.888	0.621

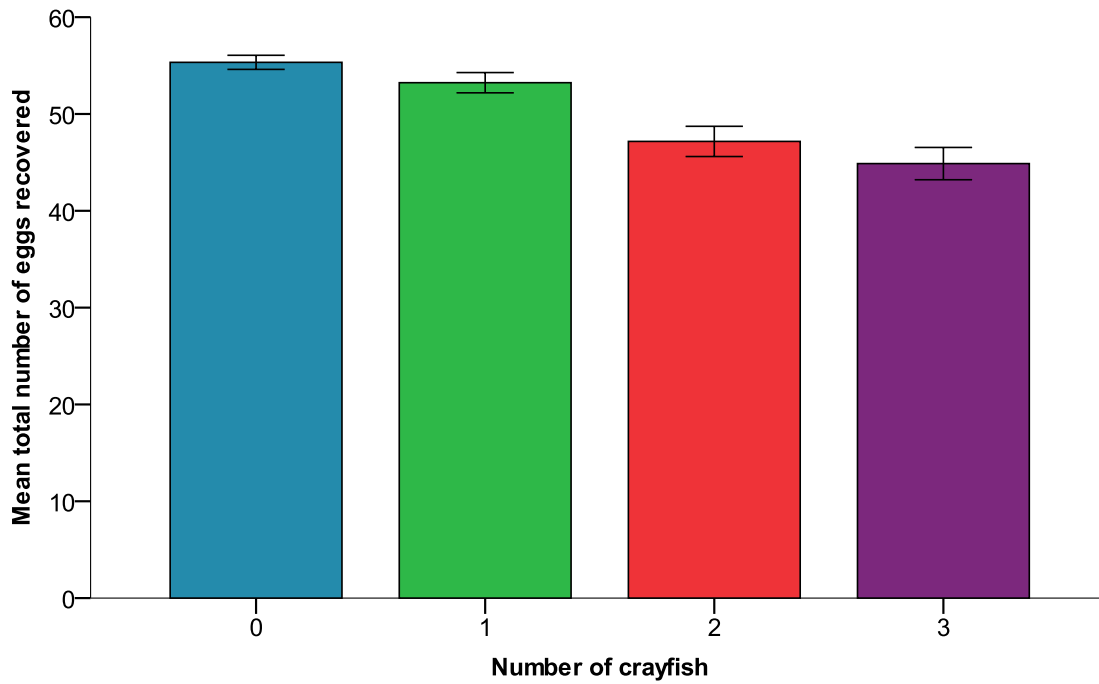


Figure 3.3: The mean total number of eggs (dead and alive) recovered from enclosures against the number of crayfish in those enclosures. Error bars show one standard error of the mean.

ANCOVA showed no significant evidence of an interaction between crayfish numbers and the percentage of silt in enclosures affecting egg survival in tubes (Table 3.6). The percentage egg survival in tubes was closely related to the amount of silt in the enclosures in which the tubes were contained (Figure 3.4), but there was no significant evidence of an effect of crayfish numbers upon egg survival in tubes (Table 3.6). Dead eggs in tubes tended to rot into a single mass, making it impossible to count the number of dead eggs remaining in tubes, and thus preventing any analysis from being conducted upon the total number of eggs or the number of dead eggs remaining in egg tubes.

Table 3.6: The outcomes of ANCOVA tests for the effects of crayfish numbers, the percentage of fine material by dry weight and the interaction between crayfish numbers and the percentage of fine material upon the number of live eggs recovered from egg tubes.

Test	Interaction			Number of crayfish			Percentage fine material		
	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>	df	<i>F</i>	<i>p</i>
ANCOVA	3	0.044	0.987	3	2.56	0.067	1	<b>65.3</b>	<b>&lt;0.001</b>

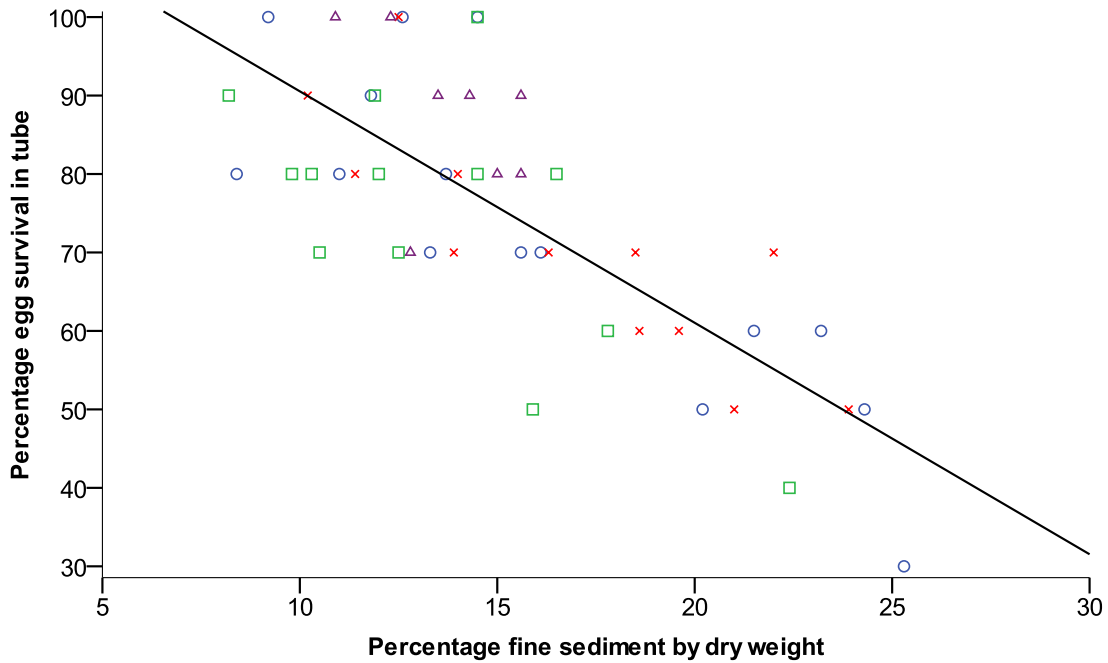


Figure 3.4: The number of live eggs remaining in tubes against the percentage of fine sediment (<2mm diameter) by dry weight in the enclosure from which the tube was recovered, for each crayfish density: zero crayfish (circles), one crayfish (squares), two crayfish (crosses), and three crayfish (triangles). The regression line is for percentage fine sediment against the number of live eggs in the tube for all crayfish densities in the associated enclosures,  $R^2 = 0.584$ .

Linear regression indicated that egg survival was closely related between enclosures and their associated tubes for enclosures without crayfish, but not in enclosures with crayfish (Table 3.7, Figure 3.5).

Table 3.7: The results of linear regression tests for relationships between the total numbers of eggs remaining for enclosures and the tubes within those enclosures. Significant outcomes after applying Bonferroni corrections are shown in bold text.

Number of crayfish in enclosure	N	$R^2$	$p$
0	13	<b>0.635</b>	<b>&lt;0.005</b>
1	12	.0111	0.744
2	8	0.174	0.304
3	15	.117	0.211
All crayfish densities	48	0.0847	0.011

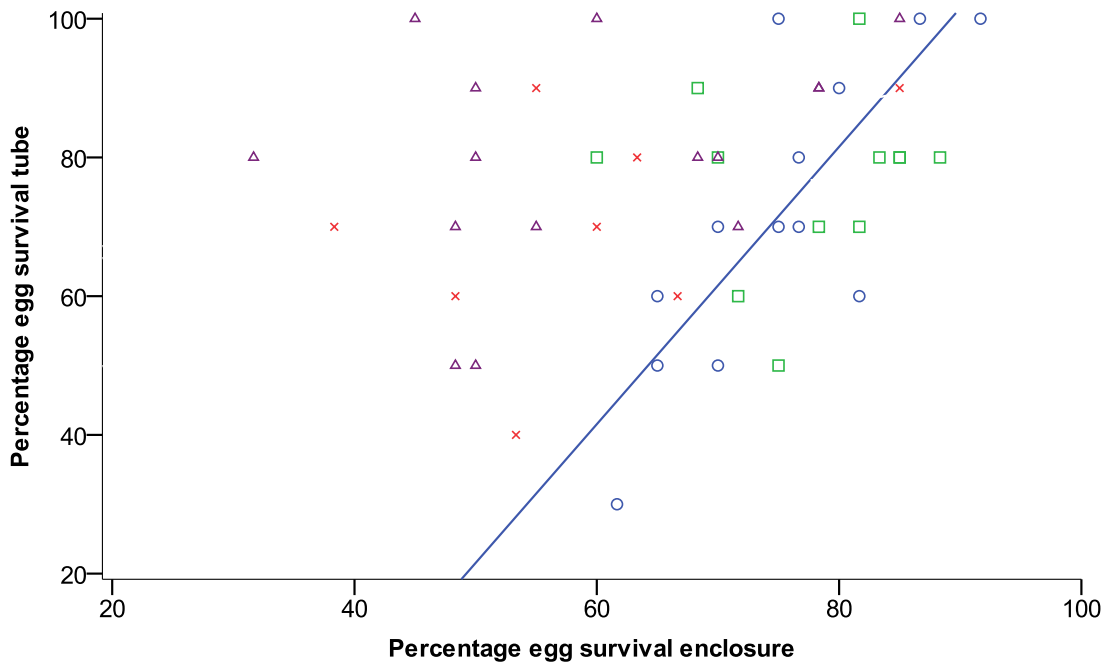


Figure 3.5: The percentage of live eggs remaining in enclosures, and the tubes within those enclosures, for each crayfish density: zero crayfish (circles), one crayfish (squares), two crayfish (crosses), and three crayfish (triangles). The regression line is for percentage egg survival in the tube against percentage egg survival in the associated enclosure, for enclosures without crayfish  $R^2 = 0.635$ .

Percentage egg survival was higher in tubes from enclosures containing three crayfish, but there was no significant evidence of any differences in egg survival between tubes and enclosures at lower crayfish densities, paired samples T-test (Table 3.8, Figure 3.6).

Table 3.8: The results of paired T tests to determine percentage egg survival differed significantly between enclosures and the tubes within them for each crayfish density. Significant outcomes after applying Bonferroni corrections are shown in bold text.

Number of crayfish in enclosure	Total N	<i>t</i>	<i>p</i>
0	13	0.446	0.480
1	12	0.163	0.814
2	8	-1.894	0.123
3	15	<b>-4.471</b>	<b>&lt;0.005</b>



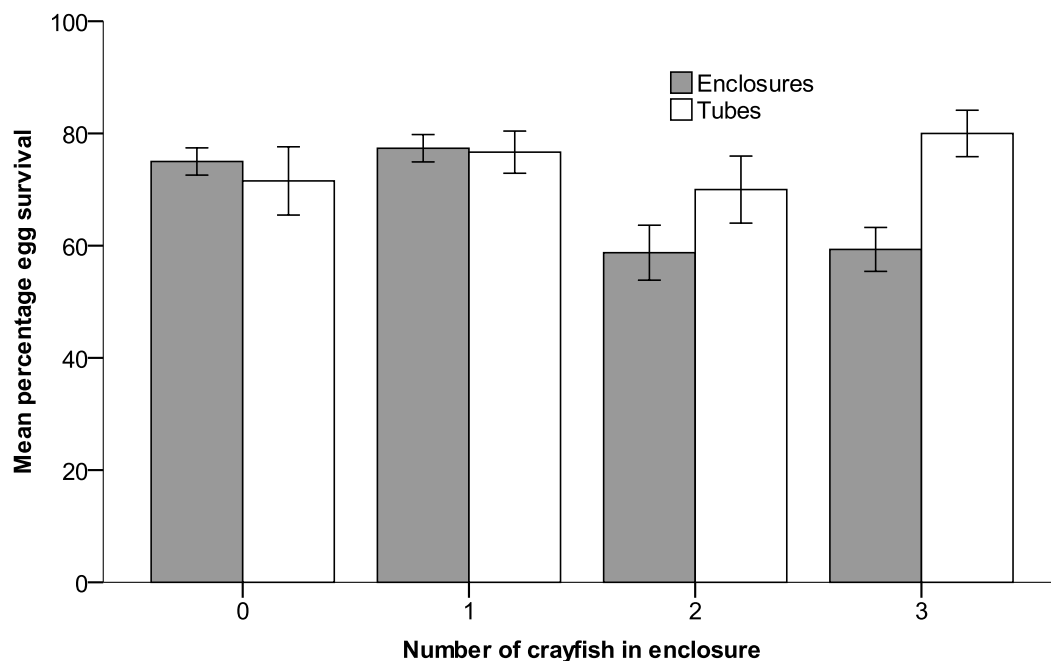


Figure 3.6: The mean percentage of eggs surviving in enclosures and the tubes within those enclosures for enclosures containing each of zero to three crayfish. Error bars show one standard error of the mean.

### **3.4.2 The effects of crayfish size, egg burial and water temperature on Atlantic salmon egg predation by signal crayfish**

For the first three batches of eggs (those used in the 20 hour high and low temperature trials with eggs on the surface of the substrate and the trials with buried eggs), survival to hatching was estimated to be greater than 95% for those eggs not used in experiments, with approximately five to ten deaths and unfertilised eggs from each batch of around 400 eggs. Survival to hatching in the final batch (used for low temperature and long term trials) was lower, although still greater than 90%. Most of the eggs that did not survive, especially from the final batch, were seemingly unfertilised eggs, lacking the visible eye that appears after around 250 degree days of development. These eggs were removed one night after the final batch of eggs was bought into the temperature controlled room for storage and before any eggs were used in experiments. After the removal of these eggs survival to hatching in the final batch of eggs was similar to that in the previous three batches.

No dead and broken, unbroken dead, damaged or hatched eggs were recovered from any of the control experiments suggesting that the technique for burying and recovering eggs was not breaking or damaging eggs, or dramatically increasing their susceptibility to infection.

Although crayfish were classified into size classes, where direct comparisons between treatments were made it was necessary to verify that the crayfish within these size classes did not differ significantly in carapace length. The median carapace lengths of age 1+ and age 2+ crayfish used at high and low temperatures appeared similar (Figure 3.7), and Mann-Whitney U tests produced no evidence that the median values for these groups differed (Table 3.9).

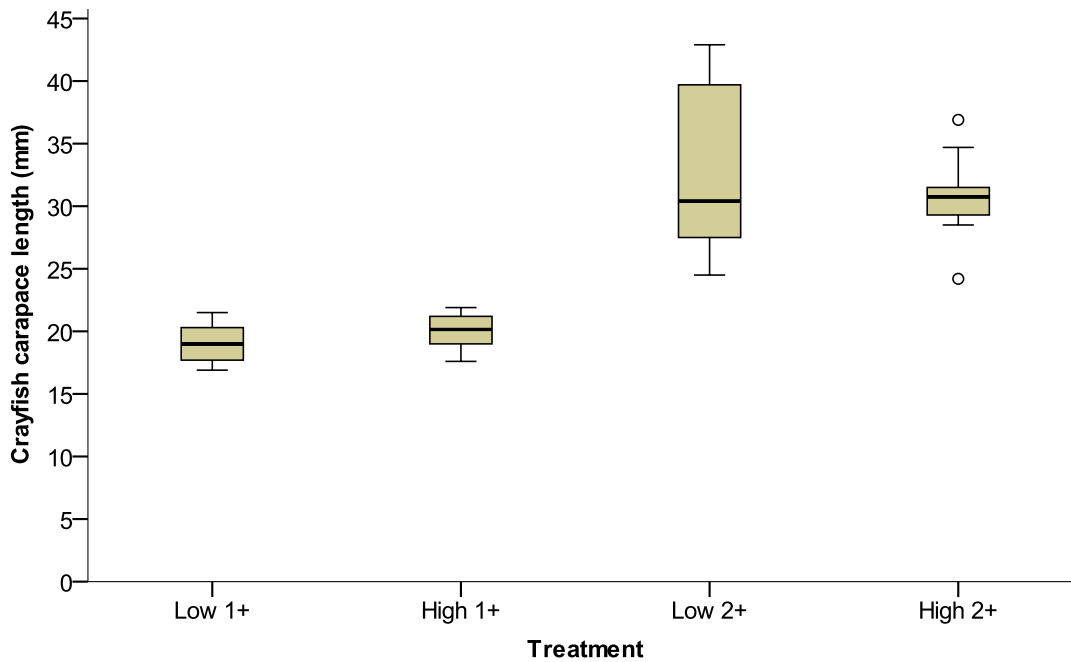


Figure 3.7: The carapace length of crayfish in each size class used in high and low temperature 20 hour trials. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

Table 3.9: Mann-Whitney U tests for differences in median carapace length between the crayfish of the 1+ and 2+ age classes used in high and low temperature treatments.

Test	Groups tested	N	Degrees of freedom	Test statistic	<i>p</i>
Mann-Whitney U	High and low temperature 1+ crayfish	20	1	31.0	0.165
Mann-Whitney U	High and low temperature 2+ crayfish	20	1	48.0	0.912

The high temperature trials with unburied eggs revealed differences in the number of eggs eaten by crayfish of different size classes, with a reduction in egg survival apparent in tanks with 2+ crayfish and possibly also 1+ crayfish (Figure 3.8), but not in those with 0+ crayfish. The number of dead and broken eggs recovered was apparently greater in tanks with 1+ or 2+ crayfish than in control or 0+ crayfish tanks (Figure 3.9). The carapace length of the smallest crayfish in a tank from which dead and broken eggs were recovered was 17.6 mm. A Kruskal-Wallis test was used to determine if there was evidence of a difference in the median values of egg survival and the number of dead and broken eggs recovered between groups. To test whether individual differences between groups were significant Mann-Whitney U tests were used to compare groups in a pair wise fashion. Only two unbroken dead eggs (one from a 1+ crayfish tank and one from a 2+ crayfish tank) and no damaged or hatched eggs were recovered from this experiment. No dead and broken eggs were found in any 0+ or control tanks.

Statistical testing revealed significant differences between crayfish age classes in the numbers of both healthy eggs and dead and broken eggs recovered (Table 3.10). Subsequent Mann-Whitney U tests showed that the only significant differences between groups were between the 2+ and control or 0+ groups, with a greater median number of dead and broken eggs and a lower median number of healthy eggs recovered from the 2+ group in each case.

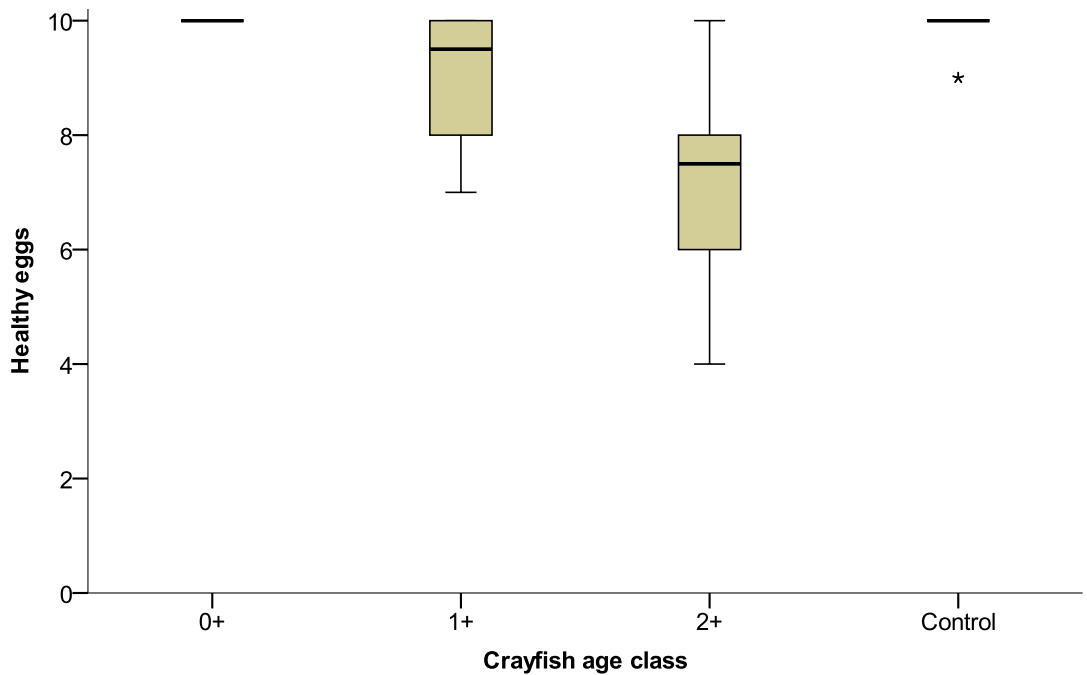


Figure 3.8: The number of healthy eggs recovered from tanks containing crayfish of each age class, from high temperature 20 hour trials with eggs on the surface of the gravel. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

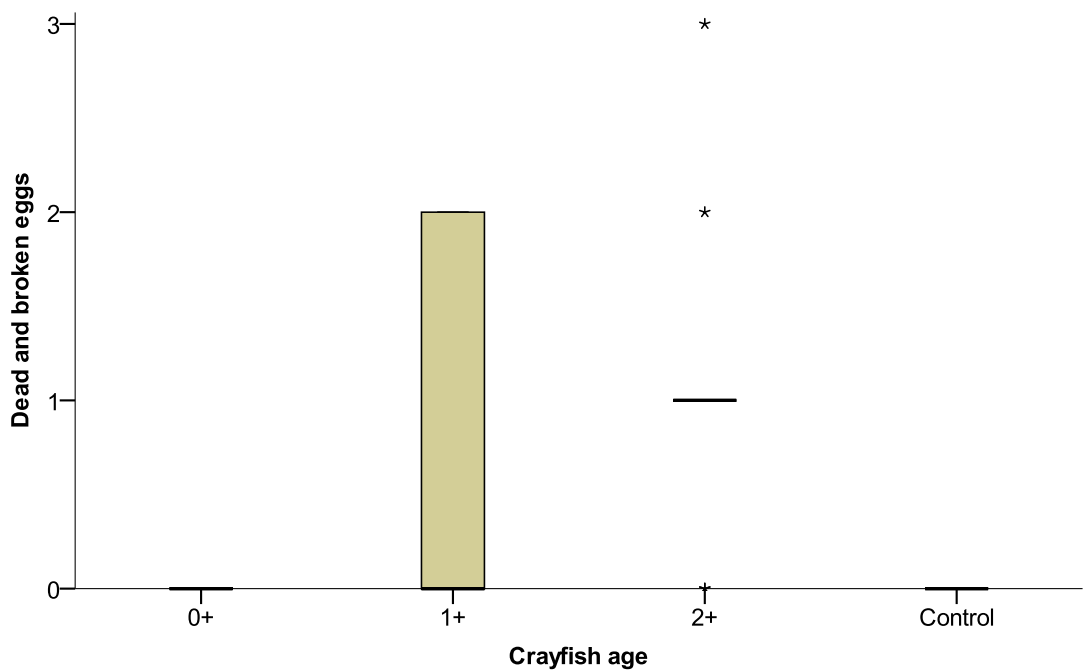


Figure 3.9: The number of dead and broken eggs recovered from tanks containing crayfish of each age class, from high temperature 20 hour trials with eggs on the surface of the gravel. Elements of the graph are as described for figure 3.8.

Table 3.10: The outcomes of statistical tests for differences in the number of healthy and dead and broken eggs recovered between different crayfish size groups and controls with eggs on the surface of the substrate in high temperature short term trials. No dead and broken eggs were found in any 0+ or control tanks. Significant outcomes after applying Bonferroni corrections are shown in bold text.

Test	Groups tested	N	Degrees of freedom	Test statistic	<i>p</i>
Kruskal-Wallis	All crayfish ages and controls, healthy eggs	40	3	<b>18.2</b>	<b>&lt; 0.001</b>
Kruskal-Wallis	All crayfish ages and controls, dead and broken eggs	40	3	<b>18.7</b>	<b>&lt; 0.001</b>
Mann-Whitney U	0+ and 1+ crayfish, healthy eggs	20	1	25.0	0.063
Mann-Whitney U	0+ and 2+ crayfish, healthy eggs	20	1	<b>10.0</b>	<b>&lt; 0.005</b>
Mann-Whitney U	1+ and 2+ crayfish, healthy eggs	20	1	24.5	0.052
Mann-Whitney U	0+ crayfish and control, healthy eggs	20	1	10.0	0.481
Mann-Whitney U	1+ crayfish and control, healthy eggs	20	1	32.0	0.190
Mann-Whitney U	2+ crayfish and control, healthy eggs	20	1	<b>12.0</b>	<b>&lt; 0.005</b>
Mann-Whitney U	0+ and 1+ crayfish, dead and broken eggs	20	1	30.0	0.143
Mann-Whitney U	0+ and 2+ crayfish, dead and broken eggs	20	1	<b>10.0</b>	<b>&lt; 0.005</b>
Mann-Whitney U	1+ and 2+ crayfish, dead and broken eggs	20	1	36.5	0.315
Mann-Whitney U	1+ crayfish and control, dead and broken eggs	20	1	30.0	0.143
Mann-Whitney U	2+ crayfish and control, dead and broken eggs	20	1	<b>10.0</b>	<b>&lt; 0.005</b>

For buried eggs, only the number of healthy eggs was considered for statistical analysis as, with the exception of a single unbroken dead egg recovered from a 0+ crayfish trial, only healthy eggs were recovered from these trials. The distributions and median values of the data for each crayfish age group appeared similar (Figure 3.10). A Kruskal-Wallis test found no significant evidence of any differences in the median number of healthy eggs recovered (Table 3.11) between any of the crayfish age groups and, as such, no further statistical testing was conducted on these data.

No evidence of any substantial excavations was observed in any buried egg trial and no crayfish were found buried in any experimental trial, although large crayfish sometimes made excavations up to about the height of their own carapace (no more than 2 cm deep) in which they appeared to shelter.

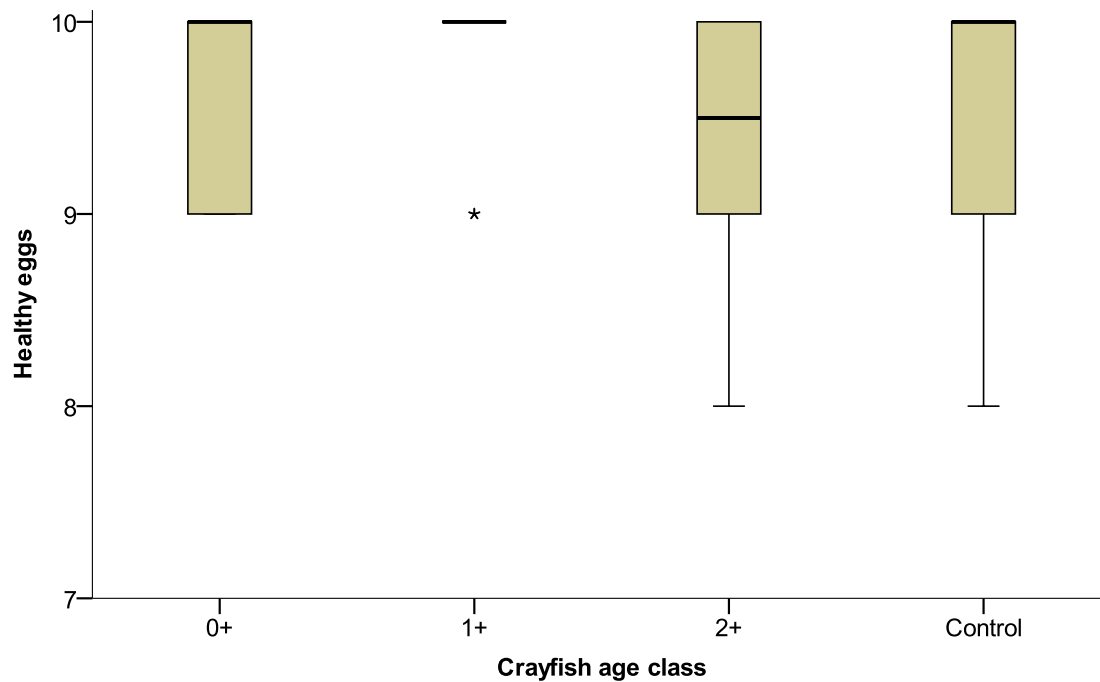


Figure 3.10: The number of healthy eggs recovered from tanks containing crayfish of each age class, from high temperature 20 hour trials with eggs buried under the gravel. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

Table 3.11: The outcomes of statistical tests for differences in the number of healthy eggs recovered between different crayfish size groups and controls with eggs buried under the gravel in short term trials.

Test	Groups tested	N	Degrees of freedom	Test statistic	<i>p</i>
Kruskal-Wallis	All crayfish ages and controls, healthy eggs	40	3	2.32	0.131

No unbroken dead eggs, and only two damaged eggs (both from 2+ crayfish tanks) were recovered from low temperatures trials with the eggs on the surface of the substrate. As

stated previously 0+ crayfish were not included in this experiment (as there was no evidence of an effect in the high temperature substrate surface trials) and the control data used for this experiment is that collected for the high temperature trial with eggs on the surface of the gravel. Fewer healthy eggs survived in the 2+ group compared to the age 1+ and the control groups (Figure 3.11). The 2+ group also differed notably from the control group in the number of dead and broken eggs recovered, while differences between the 1+ and 2+ groups or the control and 1+ groups were smaller (Figure 3.12). The carapace length of the smallest crayfish in a tank from which dead and broken eggs were recovered was 19.0 mm

There were significant differences between crayfish age classes in the numbers of both healthy eggs and dead and broken eggs recovered (Kruskal-Wallis test, Table 3.12). The median number of healthy eggs recovered differed significantly between the 2+ group and the control or 1+ groups (Mann-Whitney U test, Table 3.12). No individually significant differences in the number of dead and broken eggs recovered between any groups were detected (Mann-Whitney U test, Table 3.12).

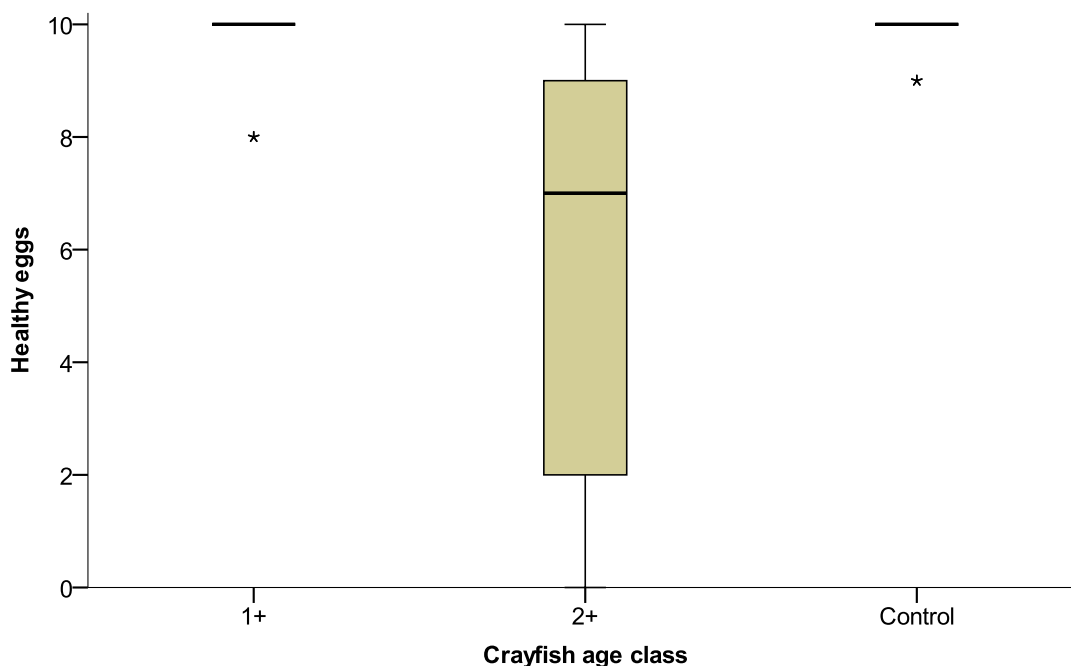


Figure 3.11: The number of healthy eggs recovered from tanks containing crayfish of each age class, from low temperature 20 hour trials with eggs on the surface of the gravel. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

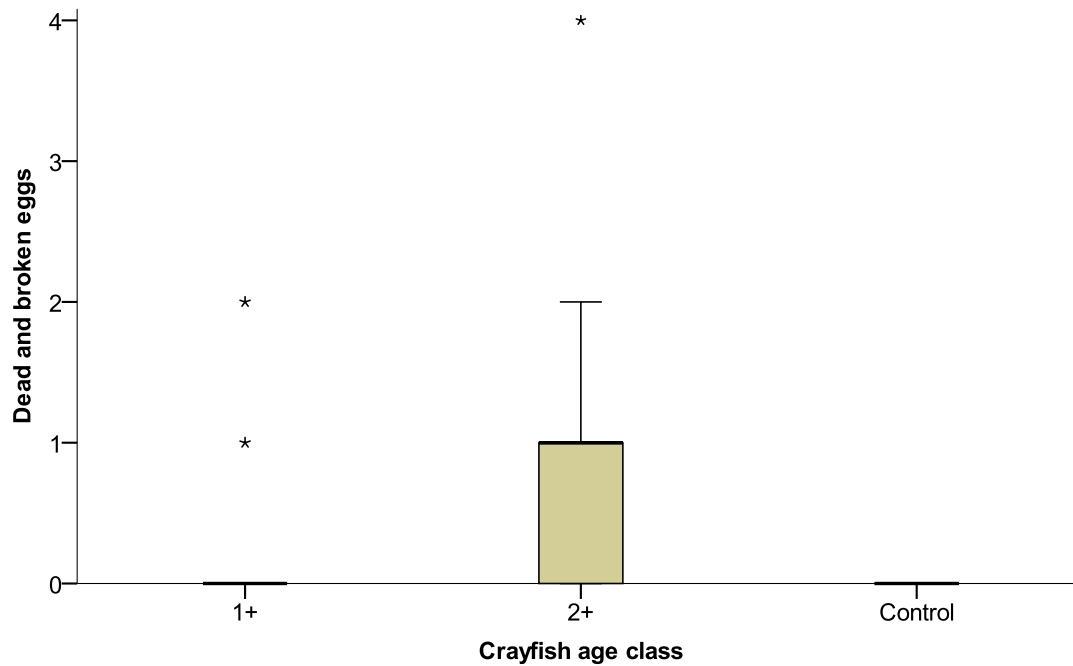


Figure 3.12: The number of dead and broken eggs recovered from tanks containing crayfish of each age class, from low temperature 20 hour trials with eggs on the surface of the gravel. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

Table 3.12: The outcomes of statistical tests for differences in the number of healthy and dead and broken eggs recovered between different crayfish size groups and controls with eggs on the surface of the substrate in low temperature short term trials. Significant outcomes after applying Bonferroni corrections are shown in bold text.

Test	Groups tested	N	Degrees of freedom	Test statistic	<i>p</i>
Kruskal-Wallis	All crayfish ages and controls, healthy eggs	40	3	<b>18.5</b>	<b>&lt;0.001</b>
Kruskal-Wallis	All crayfish ages and controls, dead and broken eggs	40	3	<b>14.0</b>	<b>&lt;0.001</b>
Mann-Whitney U	1+ and 2+ crayfish, healthy eggs	20	1	<b>17.0</b>	<b>&lt;0.0167</b>
Mann-Whitney U	1+ crayfish and control, healthy eggs	20	1	48.0	0.912
Mann-Whitney U	2+ crayfish and control, healthy eggs	20	1	<b>14.0</b>	<b>&lt;0.01</b>
Mann-Whitney U	1+ and 2+ crayfish, dead and broken eggs	20	1	30.50	0.143
Mann-Whitney U	1+ crayfish and control, dead and broken eggs	20	1	40.0	0.481
Mann-Whitney U	2+ crayfish and control, dead and broken eggs	20	1	20.0	0.0232



For age 1+ and 2+ crayfish the average number of both healthy (Figure 3.13) and dead and broken (Figure 3.14) eggs recovered was similar between high and low temperature treatments. Mann-Whitney U tests provided no evidence of a temperature effect on the numbers of eggs of healthy eggs or dead and broken eggs recovered from experiments with crayfish of either size class (Table 3.13).

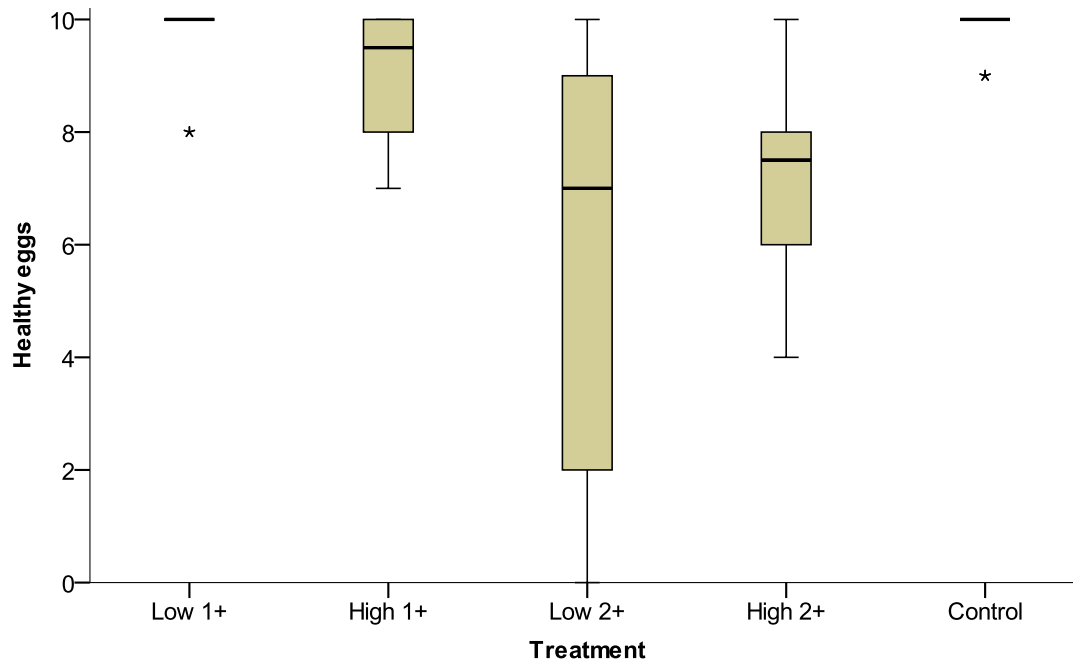


Figure 3.13: The number of healthy eggs recovered from 20 hour high and low temperature treatments with each crayfish size class. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

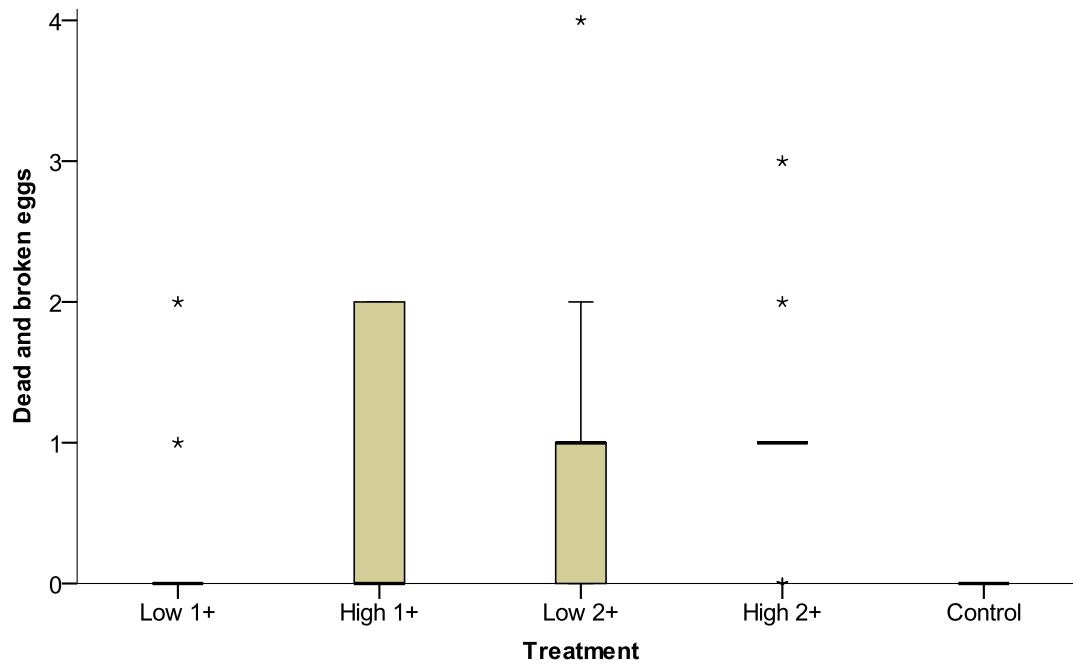


Figure 3.14: The number of dead and broken eggs recovered from 20 hour high and low temperature treatments with each crayfish size class. Thick black lines show the median value, boxes the interquartile range (IQR), T bars the 95% confidence limits, circles the outliers (1.5 - 3 times the IQR above or below the boxes), and stars extreme cases (greater than three times the IQR above or below the boxes).

Table 3.13: The outcomes of Mann-Whitney U tests to determine whether the number of healthy eggs and the number of dead and broken eggs differed significantly between high and low temperature treatments with 1+ and 2+ crayfish.

Test	Groups tested	N	Degrees of freedom	Test statistic	<i>p</i>
Mann-Whitney U	Healthy eggs, high and low temperature 1+ crayfish	20	1	35.0	0.280
Mann-Whitney U	Healthy eggs, high and low temperature 2+ crayfish	20	1	45.5	0.739
Mann-Whitney U	Dead and broken eggs, high and low temperature 1+ crayfish	20	1	39.0	0.436
Mann-Whitney U	Dead and broken eggs, high and low temperature 2+ crayfish	20	1	42.5	0.579

Given that there was no evidence for any difference in egg survival or predation between the two groups (Table 3.13) the high and low temperature treatment groups of crayfish of each size class were combined for the purposes of further data analysis. It was then possible to perform a test with a larger sample size to directly investigate the effect of crayfish

size rather than interpreted age upon the numbers of eggs recovered. By treating each egg as a single trial with two possible outcomes (recovered healthy or not, or dead and broken or not), it was possible to use binomial logistic regression tests and, thereby, perform parametric statistical tests upon a bounded data set. The number of healthy eggs recovered appeared to be negatively related to crayfish carapace length (Figure 3.15). A Wald test of the relationship fitted by binomial logistic regression provided highly significant evidence of this relationship (Table 3.14). Similarly, the number of dead and broken eggs recovered appeared to be positively related to crayfish carapace length (Figure 3.16) and, this relationship was also highly statistically significant (Table 3.14).

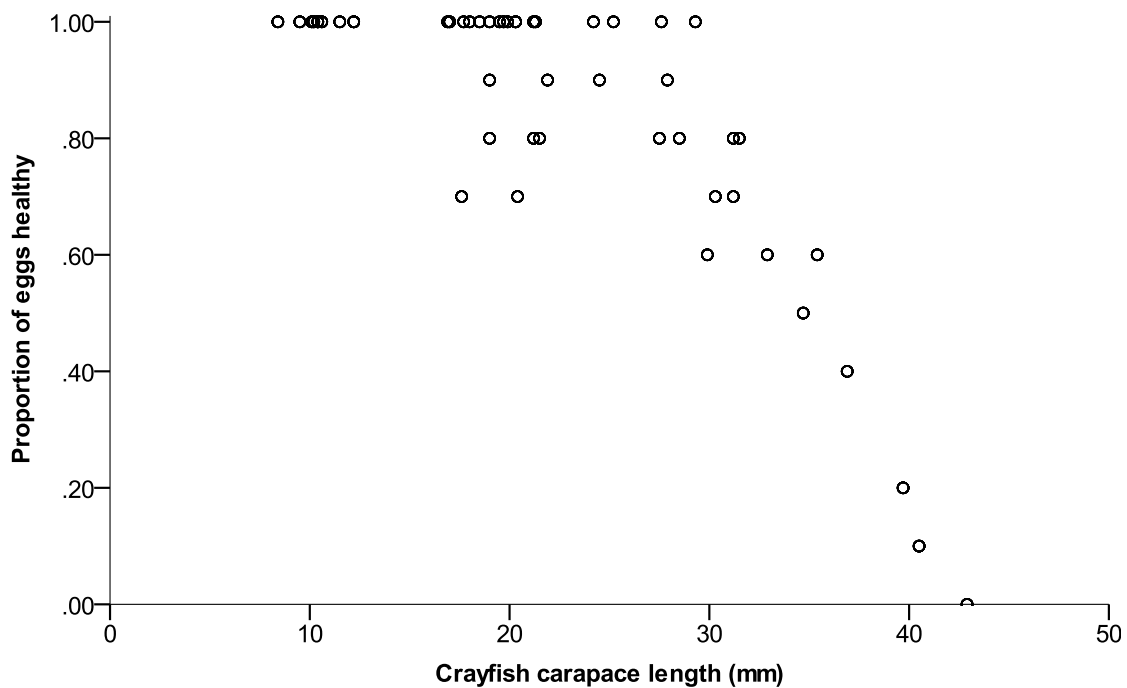


Figure 3.15: The proportion of eggs recovered healthy from each trial against the carapace length of the crayfish used in that trial. For crayfish over 16 mm CL the data includes both high and low temperature trials. Crayfish less than 16 mm CL are all from high temperature trials.

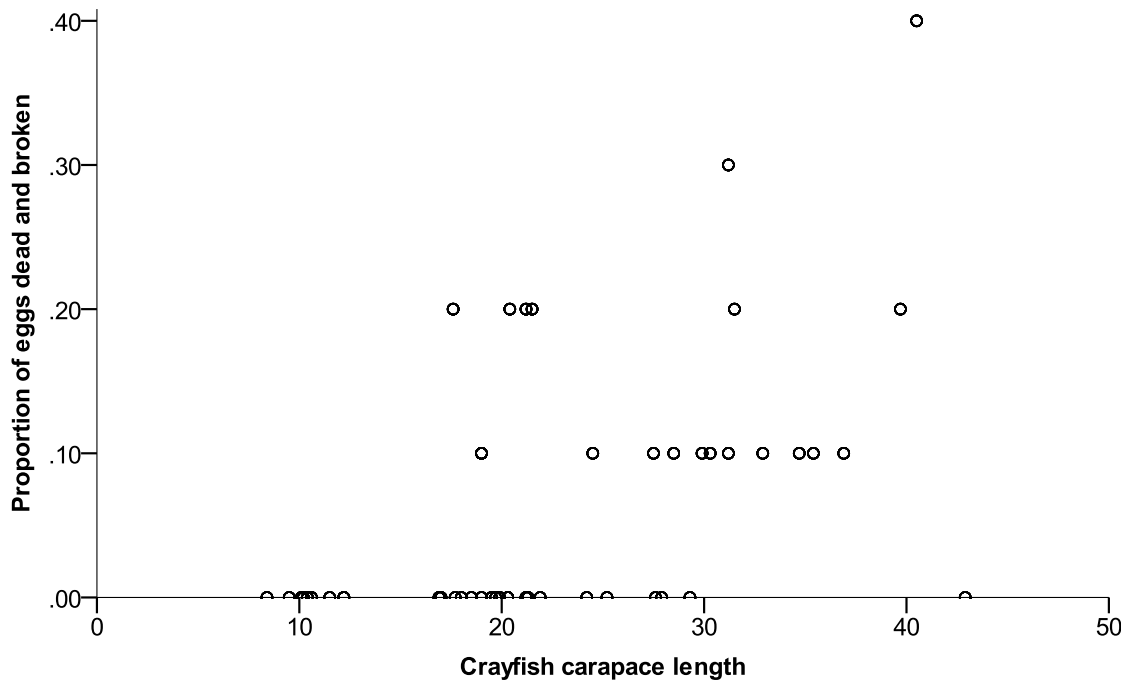


Figure 3.16: The proportion of eggs recovered dead and broken from each trail against the carapace length of the crayfish used in that trial, for crayfish over 16 mm CL the data includes both high and low temperature trials. Crayfish less than 16 mm CL are all from high temperature trials.

Table 3.14: The outcomes of binomial logistic regression tests to determine whether the number of healthy eggs and/or the number of dead and broken eggs recovered were correlated with the carapace length of the crayfish to which they were exposed in the experiments. The data used is from trials with eggs on the surface of the substrate at both high and low temperatures.

Test	Groups tested	N	$\beta$	S.E.	Test statistic ( <i>W</i> )	<i>p</i>
Binomial logistic regression	Number of healthy eggs recovered against crayfish carapace length	500	-0.210	0.0223	<b>88.0</b>	<b>&lt; 0.001</b>
Binomial logistic regression	Number of dead and broken eggs recovered against crayfish carapace length	500	0.0910	0.0216	<b>17.8</b>	<b>&lt; 0.001</b>

Using the values for  $\beta$  produced by binary logistic regression, it was possible to predict the carapace length at which the mean proportion of healthy eggs recovered would fall below that observed in control experiments with eggs on the surface of the substrate (0.98). A carapace length of 16.3 mm predicted that each egg had a 0.98 change of being recovered

healthy, suggesting that crayfish with a CL greater than 16.3 mm are capable of preying upon Atlantic salmon eggs (figure 3.17).

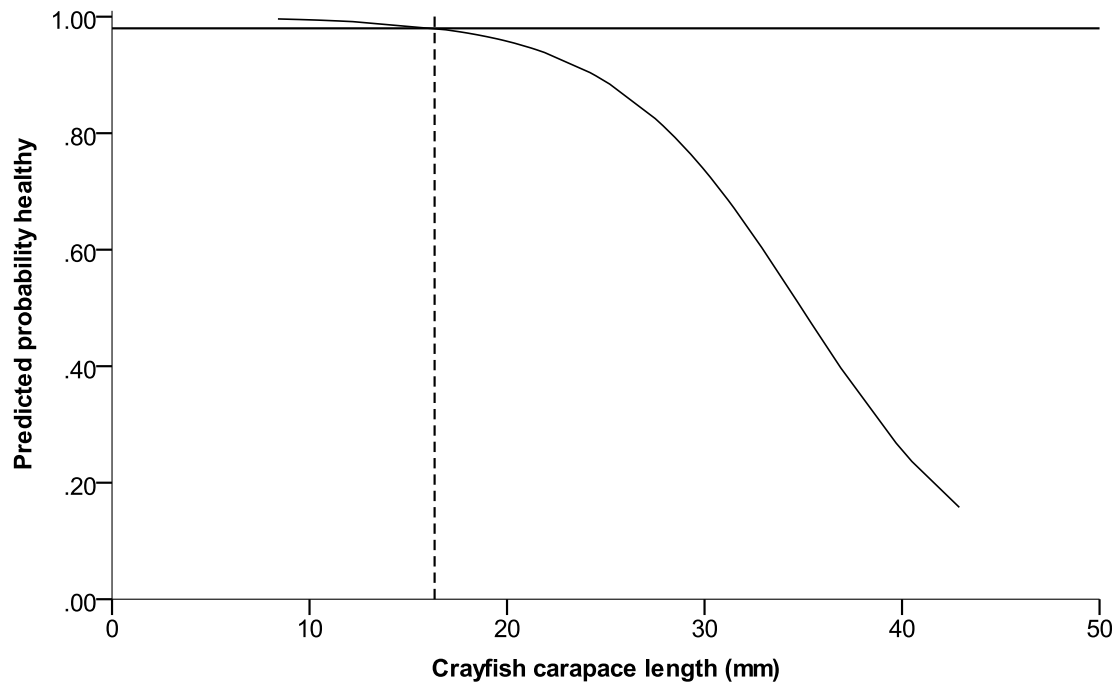


Figure 3.17: The predicted probability of each egg being recovered healthy against crayfish CL. The solid reference line shows the mean proportion of healthy eggs recovered in controls (0.98) and the dashed line the CL at which the likelihood of each egg being recovered healthy falls below that level (16.3mm).

The recovery rate of healthy eggs in the long term experiment with 0+ crayfish was 100% across all trials thereby providing no evidence of any predation upon eggs by 0+ crayfish. The number of healthy eggs recovered from long term trials with 1+ crayfish was generally lower, but this was confounded by the fact that the eggs used in this trial had started to hatch, meaning that the number of healthy eggs recovered was reduced. A total of three hatched fish were recovered, but the recovery rate for hatched fry was unknown, as was the change in egg detectability and ease of predation by crayfish in the lead up to hatching. A total of four dead and broken eggs were recovered from 1+ crayfish tanks. The carapace length of the smallest crayfish in a tank from which dead and broken eggs were recovered was 16.9 mm. The fact that eggs were hatching makes it unclear whether dead and broken eggs were predated, or simply misinterpreted empty egg membranes from which fish had hatched, although those described as dead and broken did appear to contain remnants of white material and have

membranes damaged in multiple places typical of dead and broken eggs from other experiments. Additionally, even if the dead and broken eggs had been predated it would still be impossible to know whether this had happened whilst the eggs were still intact, or during hatching. For these reasons the numbers of eggs, in any condition, recovered from the long term 1+ trials are not directly comparable with those recovered from other experiments and, thus, meaningful statistical analysis of the data from these trials is not possible. Of the hatched fish, two were dead at the time of recovery, although it was not clear whether crayfish predation was responsible for this mortality.

Only a limited number (two for each crayfish size and temperature treatment) of crayfish tanks were filmed and, as such, it was not possible to use statistical methods to compare the distances moved by crayfish subject to different temperature regimes. The total distance moved over the first 20 hours of the trials (none of the crayfish recorded moved outside of this period), and the maximum distance moved in an hour by each crayfish was recorded and is given below (Table 3.15). The only apparent differences in the movement data were that 2+ crayfish generally moved further both in their most active hour and over the course of the night than 1+ crayfish, and that male crayfish generally moved greater total and maximum hourly distances than females. Recorded digging activity was limited to creating small scrapes similar to those described previously, and up to about the height of the crayfish's carapace ( $\leq 2$  cm deep) in which the crayfish apparently sheltered.

Table 3.15: The total and maximum hourly distances moved by video recorded crayfish at high and low temperatures over 20 h recording periods

Treatment	Crayfish sex	Crayfish CL (mm)	Total distance moved (m)	Maximum distance moved in one hour (m)
1+ crayfish, High temperature	Male	21.2	13.96	4.54
1+ crayfish, High temperature	Male	19.5	11.15	5.30
2+ crayfish, High temperature	Male	29.9	101.21	10.93
2+ crayfish, High temperature	Female	29.3	28.56	6.93
1+ crayfish, Low temperature	Female	20.3	6.76	2.90
1+ crayfish, Low temperature	Male	21.5	27.47	8.64
2+ crayfish, Low temperature	Male	42.9	16.97	9.03
2+ crayfish, Low temperature	Male	40.5	76.33	15.95

### **3.5 Discussion**

Enclosure-exclosure experiments demonstrated that both signal crayfish and fine sediment can almost certainly influence egg survival. The presence of high densities of crayfish significantly reduced both the total number of eggs and the number of live eggs recovered (Table 3.1, 3.3), but had no effect upon the number of dead eggs recovered (Table 3.2). Increased levels of fine sediment in the substrate resulted in reductions in the number of live eggs recovered (Table 3.1) and increases in the number of dead eggs recovered (Table 3.2), but had no effect upon the total number of eggs recovered (Table 3.3). Egg survival in tubes was related only to the percentage of fine sediment in the enclosure (Table 3.5). The percentage egg survival in tubes was significantly related to the percentage egg survival in the associated enclosure for enclosures without crayfish, but not in enclosures containing crayfish at any density (Table 3.6). Egg survival differed significantly between enclosures and their associated tubes for enclosures with the highest crayfish density (Table 3.7).

Laboratory experiments demonstrated that signal crayfish will prey upon exposed Atlantic salmon eggs, but provided no evidence of excavation of, or predation upon, buried eggs. Only the largest (2+ and older) signal crayfish significantly reduced egg survival (defined as the number of healthy eggs recovered) relative to controls or 0+ crayfish trials (Table 3.9). Egg survival in trials with 1+ crayfish was not significantly different to that in any other trials (Table 3.9). No significant evidence of an effect of crayfish of any size upon buried egg survival was detected (Table 3.10) and no dead and broken eggs were recovered from any experiments with buried eggs. In low temperature trials 2+ and older crayfish also reduced egg survival relative to control levels (Table 3.11). Egg survival in low temperature 1+ trials was not significantly different to that in control trials (Table 3.11). Egg survival was not significantly different between high and low temperature 1+ or 2+ trials (Table 3.12).

After combining the high and low temperature data sets, binomial logistic regression provided significant evidence of a negative relationship between crayfish CL and the number of healthy eggs recovered (Table 3.13). Binomial logistic regression also provided significant evidence of a positive relationship between crayfish CL and the number of dead and broken eggs recovered (Table 3.13). By rearranging the equation produced by binomial logistic regression, it was possible to calculate that egg survival was predicted to fall below control levels when crayfish CL exceeded 16.3 mm. This result was in line with the fact that the smallest crayfish in a trial from which a dead and broken egg was reliably recorded had a carapace length of 17.6 mm, whilst a probable dead and broken egg was recovered from a trial with a 16.9 mm CL crayfish.

This discussion begins by discussing the findings, limitations and implications of the enclosure-exclosure and laboratory experiments in isolation. The discussion then compares and contrasts the results of the two experiments, with the aim of explaining any apparent discrepancies in the results. The discussion concludes by addressing the current understanding of crayfish predation upon salmonid eggs, and what this research has contributed to it.

### **3.5.1 Enclosure-exclosure experiments**

It appears probable that small 1+ and possibly also large 0+ signal crayfish are capable of preying upon salmonid eggs, which is interesting in that previous studies on other species of crayfish have only considered crayfish to be predators of salmonid eggs from sizes of 19mm carapace length and upwards (Jonas *et al.* 2005; Fitzsimons *et al.* 2007). The maximum carapace length of crayfish used in the study presented here was 16.2mm (Appendix 5), suggesting that crayfish are probably capable of preying upon salmonid eggs from smaller sizes than previously assumed, although this might, in part, relate to the fact that the lake trout eggs (5-5.6mm diameter) used in the majority of crayfish salmonid egg predation studies to date (Jonas *et al.* 2005) are larger than the brown trout eggs (4-5mm diameter) (Maitland & Campbell 1992) used in this study. There was no significant evidence of a difference in the number of dead eggs recovered between any crayfish density or the control (Table 3.2), implying that crayfish were preying upon live eggs rather than scavenging dead eggs. This indicates egg predation, rather than incidental mechanical damage, from the movements of crayfish, which would be expected to result in an increase in the number of dead eggs recovered as the number of crayfish increased. This suggests that, even at a small size, crayfish can probably break the protective chorion around live eggs, rather than simply scavenging dead eggs where the chorion would be softer. It is unlikely that the presence of crayfish would benefit other macroinvertebrates that might predate salmonid eggs, with the presence of crayfish in previous enclosure-exclosure studies having been found to reduce the density of some groups of macroinvertebrates that include potential predators of salmonid eggs (E.g. Trichoptera, Hirudinea)(Stenroth & Nystrom 2003). The effects of crayfish on more mobile macroinvertebrate predators are less clear, with some studies suggesting that crayfish reduce their abundance (Stenroth & Nystrom 2003), whilst others suggest that crayfish have little effect on the abundance of many such species (Nystrom, Bronmak & Graneli 1999).



Egg survival in tubes was significantly greater than that in the associated enclosures for enclosures containing three crayfish (Table 3.7). This provides further evidence that crayfish at this density predated eggs. It is possible that differences might also have existed in survival between tubes and enclosures in the case of enclosures containing two crayfish but that such differences were not detected as a result of the small sample size.

Evidence of a relationship between egg survival in enclosures and the tubes within them was only found for enclosures with zero crayfish (Table 3.6); although egg survival was expected to be lower in enclosures containing crayfish than in their associated tubes, the relationship was expected to persist at higher crayfish densities. The lack of evidence for such a relationship might be, to some extent, because of the small sample sizes involved once both beached and buried enclosures and enclosures without tubes had been excluded. Differences between crayfish predation rate in different enclosures with the same crayfish density could also have masked an effect of silt on egg survival, and thereby weakened the evidence of an effect by increasing unexplained variation. Crayfish predation rates probably differed between enclosures containing the same number of crayfish because crayfish size distributions differed slightly between enclosures (Appendix 5) as a result of the range of crayfish sizes used, and because of other individual differences between crayfish. Given that eggs were buried over a range of depths rather than at an exact depth, differences in egg burial depth between enclosures might also have contributed to differences in crayfish predation rates.

It is unlikely that there was a lack of alternative food sources available during the experiments as a variety of macroinvertebrate species were observed during sampling of the enclosures, and all enclosures contained algae and Diptera and/or Ephemeroptera, all of which are known to form part of the diet of crayfish elsewhere in the UK (Guan & Wiles 1998).

Where three crayfish were used in an enclosure the crayfish density was effectively roughly equal to the highest crayfish density observed in any habitat, and thus such enclosures could only be considered to represent a worst case scenario. In natural populations signal crayfish are found in a mixture of age/size classes. Crayfish in the size range 7 - 18 mm carapace length occurred at densities of up to eight per Surber sampler in Wilden Beck (Appendix 3), a density intermediate between the one and two crayfish per enclosure treatments. This range of lengths was intended to represent, on the basis of length frequency distributions produced by N. Dale for Wilden Beck (Appendix 4), the full size range of 0+ and small 1+ crayfish. Larger crayfish may also suppress the growth and feeding activity of smaller crayfish (Reynolds 2002). It should be observed, however, that even in a Surber sampler, some

of the smallest crayfish may have been missed during sampling. Predation by 0+ signal crayfish upon salmonid eggs in the wild is, therefore, likely, at least in high density populations, where, the density of 0+ and 1+ crayfish can approach that used in the two crayfish per enclosure treatment. Data from enclosures with three crayfish demonstrates that crayfish of the size range used are almost certainly capable of preying upon brown trout eggs. These enclosures should not, however, be used to estimate the rate of predation in wild populations because the density of small crayfish in these enclosures far exceeds that observed in the wild.

The effects of larger signal crayfish on salmonid egg survival have yet to be tested in a natural or semi-natural system. Larger crayfish would generally have to dig to access salmonid eggs, rather than moving through the interstices between stones, and although some studies have reported evidence of such digging (Edmonds, Riley & Maxwell 2011), others have failed to demonstrate it (Rubin & Svensson 1993; Gladman *et al.* 2012).

Crayfish are ectothermic, and the rates of salmonid egg consumption by some crayfish species have been found to be positively correlated with water temperature (Ellrott *et al.* 2007). This experiment was intended to run until April, when Wilden Beck would have been warmer. If this experiment had run to April, it is, therefore, likely that the effects of crayfish on egg survival would have been larger and, perhaps, detectable at lower crayfish densities.

As expected the number of live eggs recovered from enclosures (Table 3.1) and the tubes within them (Table 3.5) was negatively correlated with the percentage of fine material found in the enclosures by dry weight. This effect results from the fact that fine material reduces water flow through the substrate, and thus oxygen availability, resulting in increased egg mortality (Poff & Huryn 1998). The decomposition of fine organic material may also have reduced oxygen availability for the eggs. The fine mesh around the tubes could have contributed to reducing water flow and increased the build up of fine material in and around the tubes. However, although mean egg survival was higher in crayfish free enclosures, than in their associated tubes, this difference was small and non-significant. The fact that this study took place at low temperatures is probably responsible for the failure to detect an effect of silt levels on the total number of eggs recovered (Table 3.3), as dead eggs would have decomposed relatively slowly at the temperatures at which the study took place. The cool temperatures during the study period will have reduced the oxygen demand of both the eggs and any decaying organic matter within the fine sediment. This will have reduced the importance of the reduction in water movement, and potential increase in biological oxygen demand, associated with a build up of fine material.

Sediment loads during this study were unexpectedly high, probably for the most part because of very high flows in early February. These high flows resulted in a large amount of erosion of the banks which, in places, are heavily grazed down to the water. Swift flows and the moderate gradient of the beck, in combination with natural sediment traps such as woody debris, mean that most of the sediment in Wilden Beck is probably not normally entrained in the gravel. During the experimental period, however, a combination of the slower water flow in enclosures, and exceptionally high flows in the beck as a whole, meant that fine sediment levels in some enclosures were far higher than originally anticipated. The amounts of sediment in enclosures were measured and accounted for in statistical analysis and, thus, it is unlikely that the high sediment levels represent a serious flaw in this study.

### **3.5.2 The effects of crayfish size, egg burial and water temperature on Atlantic salmon egg predation by signal crayfish**

Crayfish age group was significantly related to the numbers of both healthy and dead and broken eggs recovered in all experiments with eggs on the surface of the substrate. Predation by 2+ signal crayfish on salmonid eggs was clearly demonstrated, with significant reductions in egg survival, relative to the control and 0+ groups, in high and low temperature trials and increases the number of dead and broken eggs in unburied egg trials at high temperatures. No evidence was observed of 0+ crayfish predation upon salmon eggs. Why 0+ crayfish didn't predate eggs on the surface of the substrate is not clear, but it is possible that they were either unable to break the eggs' chorion or that given the relatively greater energetic investment they would need to do so when compared with larger crayfish they would not do so without a definite food cue.

Given that dead and broken eggs were not found in any control or 0+ crayfish trial, their presence could indicate the smallest length at which crayfish predated salmon eggs. The smallest crayfish in a trial from which a dead and broken egg was recovered had a carapace length of 16.9 mm, but there is some uncertainty associated with this result as it came from a trial in which eggs were hatching and, thus, might have been the membrane from a hatched fish or an egg predated during hatching. The smallest crayfish in a trial from which a dead and broken egg was definitely recovered had a carapace length of 17.6 mm and this could, therefore, be taken as the implied minimum size at which signal crayfish can predate Atlantic salmon eggs although further experiments would be necessary to confirm this result.

Binomial logistic regression provided significant evidence that the number of healthy eggs recovered was negatively related to crayfish CL, whilst the number of dead and broken eggs recovered was positively related to CL. In the model of healthy eggs, the predicted proportion of eggs recovered first fell below that observed in control experiments at a crayfish CL > 16.3 mm. This provides further evidence that, even small, 1+ crayfish will predate exposed Atlantic salmon eggs.

No significant evidence of a temperature effect on egg survival in the presence of crayfish was found (Table 3.12), but this is probably a result of the small temperature difference between our temperature treatments, a constraint imposed by the limited capability of the experimental rooms cooling system. Data from previous laboratory work with other crayfish species suggests that egg predation rate would be lower at lower water temperatures (see section 1.4.2). The effect of water temperature on the rate of signal crayfish predation upon Atlantic salmon eggs cannot be accurately predicted as the effect of water temperature on egg predation rate differs between crayfish species (Ellrott *et al.* 2007).

No significant evidence of predation upon buried eggs by crayfish of any size class was observed in the laboratory work, and no crayfish was observed to reach a depth more than 2 cm below the surface of the gravel. The lack of evidence for predation upon buried eggs has to be considered in the context of a broader situation in which some sources have demonstrated digging by crayfish for buried fish eggs (Edmonds, Riley & Maxwell 2011), whilst others have failed to demonstrate any such digging (Rubin & Svensson 1993; Gladman *et al.* 2012). It is unlikely that a lack of time was responsible for the failure to detect digging, with a previous study having found that, with buried pieces of herring, after one night six of 12 crayfish had produced excavations over 2 cm deep, half of which were deep enough to expose the mesh sided box containing the fish, which was buried at a depth of 5 cm (Gladman *et al.* 2012).

A possible explanation for the discrepancies in digging behaviour may come from experience effects with the crayfish used here having come from a river in which no trout were caught during electric fishing (Appendix 1) whilst those used by Edmonds, Riley and Maxwell (2011) came from 'an area also occupied by wild brown trout'. Previous studies on dietary learning in crayfish, have suggested that either contact with the foodstuff, or co-association of the novel odour and a known food odour are necessary to produce a predatory response (Hazlett 1994; Weisbord, Callaghan & Pyle 2012). Even if signal crayfish are naturally predators of salmonid eggs, it is possible that being a species from the West coast of North America they would not recognise egg odour from Atlantic salmonid species as being indicative of a food

source, as their recent evolutionary history has probably been with salmonids of the *Oncorhynchus* and possibly *Salvelinus* genera. Specific response trials would be necessary to test whether or not this is the case, and indeed whether native European crayfish respond to the odour of the eggs of Atlantic salmonid species.

The rate of egg predation by wild crayfish might, therefore, be limited by the proportion of individuals within a population that had prior experience of an association between salmonid eggs and food odour or successful feeding, as suggested by the lack of a feeding response from naive crayfish to potential food items or cues including fish eggs in previous laboratory trials (Hazlett 1994; Weisbord, Callaghan & Pyle 2012). Crayfish predation upon salmonid eggs is probably also limited by redd depth (Edmonds, Riley & Maxwell 2011).

That eggs on the surface of the gravel were predated would indicate that contact is sufficient for larger crayfish to identify salmon eggs as a food source. It is also possible the crayfish were responding to the presence of the known food odour from the carrot and that this in combination with contact with the eggs would have been sufficient to induce predation upon the eggs. This mechanism would have been aided by the fact that at the beginning of the experiment the carrot and eggs were deliberately placed in fairly close proximity to each other in order to avoid either one being much closer to the exit of the shelter than the other. Carrot is considered to be a known food in this context because signal crayfish were given, and observed to feed upon, carrot whilst in holding tanks.

### **3.5.3 Comparing and contrasting enclosure-exclosure and laboratory results**

It is possible that crayfish apparently accessed eggs in the enclosure-exclosure experiments but not in the laboratory work because the former lasted much longer (> 1 month). This, in combination with the numbers of eggs used, would allow time for eggs to die and decompose to the point where they would release molecules that might be more easily recognised by crayfish. This, in turn, could provide the egg odour in combination with a food odour and/or attract the crayfish to an area where they could come into direct contact with the eggs, although the lack of evidence for an effect of crayfish upon the number of dead eggs recovered weakens this theory.

It is possible that predation occurred in the enclosure-exclosure work, but not in laboratory experiments with 0+ crayfish, because the crayfish used in the enclosure work

included larger individuals. The crayfish used in enclosure-exclosure experiments included a few with a CL > 16 mm, which would have fallen into the 1+ class in the laboratory experiments, and several others in the ambiguous (large 0+ or small 1+) 14 - 16 mm CL size range not used in laboratory experiments. If 16.4 mm CL is the minimum size at which signal crayfish can predate (5 - 7 mm diameter)(Maitland & Campbell 1992) Atlantic salmon eggs then it is likely that at least the larger of the 10 - 16.2 mm carapace length signal crayfish in the enclosure-exclosure experiments might be capable of preying upon 4 - 5 mm diameter (Maitland & Campbell 1992) sea trout eggs. In order to validate this theory, signal crayfish of a range of sizes should be filmed, using a high resolution camera, with Atlantic salmon and brown trout eggs, on a flat, white background. This is necessary because both poor recording quality and gravel prevented eggs from being seen during the laboratory experiments.

### **3.5.4 Conclusions**

Where signal crayfish co-occur with one or more salmonid species, egg predation by crayfish is highly likely as a result of predation by small individuals, which appear to reduce salmonid egg survival in near-natural conditions. The rate of such predation cannot be predicted accurately from the enclosure-exclosure study, however, because it used unnaturally high densities of small crayfish in the absence of larger crayfish, which may alter their behaviour. Egg predation by larger wild signal crayfish is also likely given that multiple studies have demonstrated that signal crayfish have the capacity to dig to the depths at which at least some salmonids bury their eggs (Edmonds, Riley & Maxwell 2011; Gladman *et al.* 2012) and are now known to predate exposed Atlantic salmon (this study) and Arctic charr (Nyberg & Degerman 2009; Setzer, Norrgard & Jonsson 2011) eggs. That crayfish from populations with prior experience of brown trout will dig for brown trout eggs adds further credence to this theory (Edmonds, Riley & Maxwell 2011). Laboratory work was unable to verify predation by crayfish as small as those used in the enclosure-exclosure experiments, but, it has demonstrated that 1+ crayfish will predate salmonid eggs. This in turn means that salmonid eggs are vulnerable to predation from a far larger proportion of most crayfish populations than that considered in previous work, which focussed on large crayfish.

## **Chapter 4 - General discussion**

This study has demonstrated that signal crayfish density can be a key predictor of both bullhead and 0+ brown trout densities in upland streams. Modelling also suggested a range of predictors which may help in deciding how best to enhance nursery habitat for brown trout and perhaps even improve future modelling of 0+ brown trout densities. Enclosure-exclosure experiments have demonstrated that small signal crayfish almost certainly reduce the survival of sea trout eggs in near natural conditions. Laboratory work has demonstrated that signal crayfish of a range of sizes equating to 1+ and older individuals, will predate exposed Atlantic salmon eggs. This discussion will address the implications of these findings before concluding with suggestions for future research.

This study provides strong evidence that signal crayfish can reduce 0+ brown trout densities in streams across multiple near independent sites after accounting for environmental covariates. Peay *et al.* (2009) previously reported a negative effect of signal crayfish density upon brown trout populations, but sampled only a single stretch of river and failed to account for environmental covariates. The possible causes of an effect of signal crayfish on 0+ brown trout density include egg predation (this thesis)(Edmonds, Riley & Maxwell 2011), competition for food (Crawford, Yeomans & Adams 2006) or shelter (Griffiths, Collen & Armstrong 2004) and direct predation upon emerging or free swimming juvenile fish (Rubin & Svensson 1993; Renai & Gherardi 2004; Edmonds, Riley & Maxwell 2011). The size of the signal crayfish effect upon juvenile brown trout densities is noteworthy because, if observed elsewhere it suggests that signal crayfish may have the potential to dramatically reduce the densities of 0+ trout found in an area. This in turn could accentuate salmonid population declines or prevent populations from recovering. Given the ecological similarity of 0+ brown trout and some other salmonid fish, signal crayfish could potentially also reduce the densities of other 0+ salmonid fish such as Atlantic salmon.

The model used here had a very high predictive value for 0+ brown trout densities. If the predictive value of this model was maintained in other systems it could be of value for predicting 0+ brown trout densities. The habitat variables selected in this model may also be useful for deciding how best to enhance habitat for stream dwelling 0+ salmonids. In particular the percentage cover of Rfcas and H2. H2 represents an estimate of the percentage cover of unembedded gravel and thereby emphasises the importance of minimising fine sediment inputs. The percentage cover of H2 could potentially also be increased by the addition of

gravel to otherwise suitable habitat. Rifcas habitat represents areas of variable flow, suitable for juvenile salmonid feeding. Such areas are typically found in shallow, fast flowing water with a stony river bed. This implies that, as has been previously suggested (Poff & Huryn 1998), channelisation will reduce 0+ brown trout densities. On the basis of the 0+ brown trout model, adding gravel to otherwise suitable spawning habitat and reversing channelisation should result in an increase in 0+ brown trout densities. Further research is necessary to confirm the effectiveness of such restoration for improving juvenile salmonid densities, although juvenile Chinook salmon, *Oncorhynchus tshawytscha* appear to grow faster at the downstream end of end of enhanced spawning habitat than in unenhanced habitat (Utz, Zeug & Cardinale 2012).

It is very likely that signal crayfish reduce bullhead densities in upland stream environments, with this study being the third to report such an effect (Bubb *et al.* 2009; Peay *et al.* 2009), and building on previous studies by including more sites and accounting for environmental variables. Possible causes for a negative effect of signal crayfish upon bullhead densities include predation on eggs and/or fish at any later life stage (Guan & Wiles 1997), competition for shelter (Bubb *et al.* 2009) and competition for food. This effect may have implications for bullhead conservation in Europe, suggesting that sites selected for bullhead re-introduction or habitat improvement should be free (and likely to remain free) of exotic crayfish. That signal crayfish appear to reduce bullhead densities in small upland streams, but not in other environments, may be because of the relatively low nutrient and productivity status of these streams (Moss 2010), increasing the vulnerability of bullhead to competition for food. Small upland streams are also likely to be spatially constrained, potentially increasing the frequency of interactions between bullhead and signal crayfish.

Although this study was unable to test the effects of sculpins upon juvenile salmonid survival, this has been a topic of some controversy in published literature, with some authors reporting a negative effect of sculpins on juvenile salmonid survival (Elliott 2006; Amundsen & Gabler 2008), while others have reported no such effect (Gabler, Amundsen & Herfindal 2001). Salmonids coexist with a range of sculpins across much of the northern hemisphere (Frost & Brown 1967; Patten 1975; Elliott 2006), and, thus bullhead alone clearly do not pose a serious threat to the survival of salmonid populations, although their presence may have implications for stocking success (Ward, Nislow & Folt 2008) and the fate of populations already subject to other pressures.

The data presented here provide some of the first published proof that signal crayfish will predate exposed Atlantic salmon eggs. Results from the laboratory work also provide clear



evidence that most 1+ and older crayfish, may predate Atlantic salmon eggs and, thus, that a wider range of crayfish size classes should be included in future experiments. The results from enclosure-exclosure experiments suggest that even smaller crayfish may predate brown trout eggs. The egg predation data presented here are ambiguous, with small crayfish apparently capable of reducing the survival of buried sea trout eggs in the field, but no evidence of predation upon buried eggs being observed in the laboratory study. Insights into the role of learning and crayfish responses to novel egg and food odour cues might help to account for these results (Hazlett 1994; Weisbord, Callaghan & Pyle 2012). Several crayfish species will predate the eggs of a range of salmonid species upon contact (Savino & Miller 1991; Nyberg & Degerman 2009)(this study), but the extent to which signal crayfish will dig for salmonid eggs remains ambiguous, with different studies reporting conflicting results (Edmonds, Riley & Maxwell 2011; Gladman *et al.* 2012)(this study). What does appear likely, given the results of research by Hazlett (1994) and Weisbord, Callaghan and Pyle (2012), is that crayfish would be attracted to any odours released by salmonid eggs if they learned to associate them with food. This, in combination with the range of scenarios under which crayfish could be attracted either to a redd (see section 1.4), or could otherwise encounter eggs and learn to associate them with food, suggests that crayfish pose at least a potential threat to buried salmonid eggs. However, given the number of studies in which crayfish have failed to dig for buried eggs (Rubin & Svensson 1993; Gladman *et al.* 2012)(this study), and that this behaviour has only been observed in a single study (Edmonds, Riley & Maxwell 2011), it is difficult to predict the frequency or impact of such digging behaviour in wild populations.

Appendix 6 presents details of an attempted investigation into the ability of male bullhead to defend eggs from crayfish. This investigation was not completed for logistical reasons (discussed further in appendix 6). Similarly, although this thesis originally intended to investigate the effects of white-clawed crayfish on salmonid and bullhead densities in the wild, this investigation was not conducted because of a lack of suitable sites with white-clawed crayfish within the study catchment and a lack of time in which to fully explore other possible river systems. Problems with temperature regulation in the temperature controlled room used for laboratory experiments in chapter 3 resulted in the work being behind schedule and, thereby, prevented experiments from being repeated with white-clawed crayfish or bullhead.

#### **4.1 Future research direction**

There is little need for further investigations of the effects of signal crayfish on bullhead densities in upland streams, with three studies now having reported a consistent result. Further research in other environments may, however, be beneficial in order to validate or refute evidence suggesting that bullhead densities are not affected by signal crayfish in these environments. Further work should aim, over the course of a longer and more extensive study, to build detailed structural models of crayfish and other potentially important species interactions with a range of environmental variables and then use these to assess the effects of native and invasive crayfish species upon the densities of a range of fish species. Such models could also offer insights into the relative effects of other predator and competitor species on focal fish species densities. Other salmonid species are an important area for investigation, given the life history similarities between brown trout and other species such as Atlantic salmon. It is also worth investigating whether exotic crayfish densities affect the densities of species with different life history strategies such as cyprinids and lamprey (Maitland & Campbell 1992). Although there is little need to further investigate the effects of signal crayfish alone on bullhead in upland streams, comparative studies of the effects of signal and other crayfish species would still be worthwhile. Structural models of ASPT should also be built to determine if bullhead, or other predators, can influence ASPT in other systems.

Laboratory work on male bullhead nest defence against a range of crayfish species and its outcomes would be worthwhile to understand the mechanisms by which crayfish might reduce bullhead densities. It may also be advantageous to investigate whether the presence of signal crayfish affects bullhead feeding, given that, in spite of their dominance over benthic fish including slimy sculpin in competition for shelter (Miller, Savino & Neely 1992; France 1993), virile crayfish are apparently outcompeted for food resources by slimy sculpin in laboratory trials (Miller, Savino & Neely 1992). Care would have to be taken in such trials to ensure as nearer natural environment as possible, since a lack of shelter and appropriate substrate may influence crayfish behaviour and feeding rate (Miller, Savino & Neely 1992).

At least six exotic crayfish species are now established in the wild in Great Britain (signal crayfish, narrow-clawed crayfish, spiny cheek crayfish, red swamp crayfish, noble crayfish (Holdich, Rogers & Reynolds 1999) and virile crayfish (Ahern, England & Ellis 2008)) at least three of which (signal, red swamp and virile crayfish) (Savino & Miller 1991; Love & Savino 1993; Mueller, Carpenter & Thornbrugh 2006) are known predators of fish eggs. Thus, it is necessary for future studies to consider not only the relative impacts of the native and signal

crayfish, but also the relative effects of these other invasive species. Further research is also required to establish the threat posed by many of these crayfish species to the later life stages of a range of fish and other species. Data on the predation efficiency of crayfish on especially older fish and fish in natural or semi-natural environments are still lacking, although data on salmonid fry predation are available for a number of species (Savino & Miller 1991; Rubin & Svensson 1993; Renai & Gherardi 2004; Edmonds, Riley & Maxwell 2011). Stable isotope analysis may be useful for detecting egg predation by wild crayfish, although care would be necessary to distinguish a salmonid or lamprey egg signal from that of adult fish that had died after spawning and then been scavenged by crayfish.

Further work is needed to ascertain if crayfish of a number of species respond to egg odour cues without the need for prior exposure to these cues in combination with contact with eggs or odour cues typical of food. If these experiments do not demonstrate attraction to egg cues, experiments should be conducted using cues from dead eggs followed (if attraction to dead egg cues is demonstrated) by exposure to healthy eggs to determine whether exposure to the cues released by decomposing eggs could attract crayfish to redds, and/or cause crayfish to associate egg cues with food. Future work should also examine the ability of crayfish to access and predate eggs in artificial redds, as studies to date have not accurately recreated the structure or pattern of water flow typically observed in wild salmonid redds, instead using eggs buried under flat gravel in a flow through system, or rising water which would carry egg odours directly up to crayfish, rather than displacing them downstream as would occur in a natural redd structure. The effects of temperature on salmonid egg predation differ between crayfish and salmonid egg species (Ellrott *et al.* 2007), and should therefore be investigated to compare the predation efficiency of a range of crayfish species at a range of temperatures.

It is also important to determine which salmonid life stages are affected by signal crayfish as this will have major implications for the appropriate strategy for dealing with such effects. This could be done with long term enclosure-exclosure experiments similar to those used by Elliott (2006) to investigate the effects of bullhead densities on 0+ brown trout survival. If signal crayfish predate eggs, stocking may be an appropriate strategy to mitigate signal crayfish effects upon trout densities. By contrast, if signal crayfish compete with juvenile fish, stocking is unlikely to improve the situation, and may even worsen it as a result of competition between wild hatched and stocked fish. A summary of recommendations for environmental managers and suggested future research is given in table 4.1.

Table 4.1: A summary of recommendations for environmental managers and future research.

Thesis component	Key recommendations for managers	Suggested future research
Bullhead model	<ul style="list-style-type: none"> <li>• When introducing bullhead, or enhancing bullhead habitat prioritise areas without alien crayfish and which alien crayfish are unlikely to invade.</li> <li>• Exercise caution when interpreting ASPT data from streams with high bullhead densities.</li> </ul>	<ul style="list-style-type: none"> <li>• Investigate the effects of a range of crayfish species on bullhead densities in a range of habitats using structural models of bullhead density incorporating environmental covariates.</li> <li>• Use structural models to investigate whether bullhead density can influence ASPT across a range of sites.</li> </ul>
0+ brown trout model	<ul style="list-style-type: none"> <li>• Focus reintroductions and habitat enhancements for salmonids on areas where alien crayfish are not present and are not likely to invade.</li> <li>• Avoid and where possible reverse channelisation to enhance the availability of shallow water habitats with areas of fast and slow flowing water in close proximity.</li> <li>• Reduce sediment inputs into salmonid spawning habitats.</li> <li>• Adding gravel to otherwise suitable habitat where spawning habitat availability is low might be beneficial.</li> </ul>	<ul style="list-style-type: none"> <li>• Investigate whether other salmonids and fish groups are affected similarly by signal and other crayfish species using structural models of the relationships between focal species, crayfish, other species and environmental variables.</li> <li>• Where channelisation is reversed, monitor salmonid populations in order to determine whether or not they increase.</li> <li>• Investigate whether the addition of gravel to otherwise suitable habitat increases 0+ Atlantic salmon and/or brown trout population densities.</li> </ul>
Enclosure-exclosure experiments and laboratory work	<ul style="list-style-type: none"> <li>• When reintroducing salmonids avoid habitats invaded by, or likely to be invaded by, alien crayfish.</li> <li>• Reduce fine sediment inputs to salmonid spawning habitat.</li> </ul>	<ul style="list-style-type: none"> <li>• Film the interactions of crayfish of a range of sizes and species with salmonid eggs using high quality recording equipment and a plain background.</li> <li>• Investigate whether crayfish are attracted to dead salmonid eggs, especially buried under gravel and, if experience of feeding on dead eggs causes crayfish to be attracted to healthy eggs.</li> <li>• Investigate the effects of egg species and temperature on the egg predation rates of a variety of crayfish species.</li> </ul>

## Appendices

### Appendix 1 - Counts and densities of fish and crayfish caught during fishing and hand searching

Appendix 1: Numbers (#) and densities ( $\#/m^2$ ) of all fish and crayfish species caught (fish caught by electric fishing, crayfish by electric fishing and hand searching). How Beck (How) was the only site on which white-clawed crayfish were found and had a notable (c. 1m high) waterfall on the site and was therefore omitted from analysis. Site numbers reflect the date order in which sites were surveyed and correspond to those in Table 2.1.

Site	Area ( $m^2$ )	Signal crayfish		White- clawed crayfish		Brown trout		O+ Brown trout		Bullhead		Atlantic Salmon		Stone loach		Minnow		Stickleback		Lamprey ( <i>Lampetra sp.</i> )		Grayling		Roach	
		#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$	#	$\#/m^2$
1	147.2	0	0	0	0	3	0.020	2	0.014	113	0.768	3	0.020	8	0.054	2	0.014	0	0	0	0	0	0	0	0
2	127.0	0	0	0	0	79	0.622	77	0.606	13	0.102	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	147.8	0	0	0	0	3	0.020	1	0.007	42	0.284	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	212.2	105	0.494	0	0	1	0.005	0	0	61	0.288	0	0	0	0	11	0.052	7	0.033	0	0	0	0	0	0
5	108.9	0	0	0	0	13	0.119	11	0.101	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	106.1	0	0	0	0	4	0.038	1	0.009	144	1.357	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	166.3	0	0	0	0	1	0.006	0	0	396	2.380	0	0	0	0	32	0.192	83	0.499	6	0.036	1	0.006	1	0.006
8	150.7	0	0	0	0	5	0.033	3	0.020	363	2.408	0	0	0	0	0	0	4	0.027	1	0.007	0	0	0	0
9	97.8	33	0.342	0	0	3	0.031	0	0	17	0.174	0	0	0	0	12	0.123	0	0	0	0	0	0	0	0
10	176.8	111	0.628	0	0	3	0.017	2	0.011	0	0	0	0	1	0.006	1	0.006	0	0	0	0	1	0.006	0	0
11	139.2	89	0.636	0	0	12	0.086	8	0.057	0	0	0	0	0	0	69	0.496	91	0.654	0	0	0	0	0	0
12	121.0	83	0.687	0	0	0	0	0	0	92	0.761	0	0	0	0	0	0	21	0.174	0	0	0	0	0	0
13	136.8	137	0.998	0	0	0	0	0	0	0	0	0	0	10	0.073	0	0	0	0	0	0	0	0	0	0
14	109.2	0	0	0	0	27	0.247	13	0.119	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	126.4	0	0	0	0	12	0.095	1	0.008	91	0.720	0	0	5	0.040	0	0	0	0	0	0	0	0	0	0
16	119.3	0	0	0	0	57	0.478	55	0.461	0	0	11	0.092	0	0	0	0	0	0	0	0	0	0	0	0
17	145.9	0	0	0	0	1	0.007	0	0	143	0.980	0	0	0	0	26	0.178	14	0.096	54	0.370	0	0	0	0
18	107.2	2	0.019	0	0	50	0.466	41	0.382	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
19	130.3	54	0.413	0	0	8	0.061	8	0.061	13	0.100	0	0	8	0.061	1	0.008	0	0	0	0	0	0	0	0
20	121.0	15	0.126	0	0	7	0.058	5	0.041	18	0.149	0	0	0	0	0	0	0	0	0	0	0	0	0	0
How	166.0	0	0	109	0.655	1	0.006	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

## **Appendix 2 - Environmental variables recorded from sites sampled for chapter 2**

Appendix 2: Environmental variables recorded for sampled sites, % O<sub>2</sub> refers to percentage oxygen saturation and % EMB to the percentage of the substrate that was embedded in the river bed. MB in the pH column refers to the fact that the pH meter was broken so pH was not measured. With the exception of % O<sub>2</sub> percentages refer to the percentage of the site covered. How Beck (How) was omitted from analysis as the only site with white-clawed crayfish, and a notable (c. 1m high) waterfall, on the site.

Site	Days into study	Water temp. (°C)	pH	% O <sub>2</sub>	Max. shade score	Min. shade score	Site area (m <sup>2</sup> )	Mean depth (mm)	Mean flow (ms <sup>-1</sup> )	% pool	% riffle	% glide	% cascade	% < 2 mm	% 2 -16 mm	% 16 - 64 mm	% 64 - 256 mm	% > 256 mm	% Bed rock	% EMB
1	1	15.7	8.67	117.6	5	2	147.2	181.2	0.246	35	50	5	10	10	15	30	40	5	0	20
2	2	15	8.20	118.0	5	1	127.0	88.1	0.233	15	60	10	15	10	30	40	15	5	0	15
3	5	13.9	8.20	112.0	4	4	147.8	111.7	0.152	30	40	15	15	15	10	40	30	5	0	30
4	6	12.7	8.14	113.6	4	4	212.2	249.9	0.195	45	15	40	0	15	20	35	25	5	0	30
5	12	13.3	7.86	113.5	5	1	108.9	47.1	0.084	30	60	10	0	20	20	35	20	5	0	30
6	13	11.8	8.07	108.0	5	3	106.1	74.9	0.086	25	50	20	5	15	5	50	25	5	0	25
7	15	17.7	7.98	146.2	4	3	166.3	217.9	0.191	15	15	70	0	25	15	35	20	5	0	35
8	16	14.5	8.55	150.0	3	3	150.7	124.0	0.188	10	50	40	0	30	20	30	15	5	0	45
9	20	16.2	8.12	113.5	5	5	97.8	222.2	0.164	35	15	35	15	10	25	25	20	20	0	30
10	21	15.8	7.73	117.2	5	5	176.8	206.9	0.483	10	40	0	50	20	10	20	30	20	0	50
11	26	15	7.01	113.0	4	1	139.2	105.9	0.133	40	30	25	5	30	10	40	15	5	0	25
12	27	13.6	7.63	108.5	5	3	121.0	84.0	0.131	35	25	35	5	35	15	20	20	10	0	25
13	32	16.2	7.53	110.1	5	4	136.8	125.1	0.269	20	30	30	20	30	15	20	30	5	0	25
14	33	12.6	7.75	117.5	5	5	109.2	147.9	0.157	30	35	25	10	15	30	30	20	5	0	30
15	34	14.7	7.77	117.0	5	3	126.4	206.5	0.350	15	20	40	25	5	20	25	35	15	0	30
16	35	13.1	7.35	119.2	3	1	119.3	103.2	0.377	15	35	10	40	15	30	25	25	5	0	30
17	48	11.7	7.70	107.1	5	3	145.9	279.6	0.024	25	5	70	0	40	5	15	10	0	30	60
18	49	10.5	8.07	119.8	5	5	107.2	81.9	0.180	20	50	15	15	5	15	15	50	15	0	25
19	58	12.5	MB	112.5	3	2	130.3	68.5	0.198	25	60	15	0	25	20	35	15	5	0	20
20	71	9.3	MB	119.8	5	5	121.0	244.2	0.406	15	40	35	10	15	10	35	30	10	0	30
How	43	114.7	MB	114.7	5	3	166.0	67.5	0.290	30	35	20	15	20	10	25	15	5	25	20

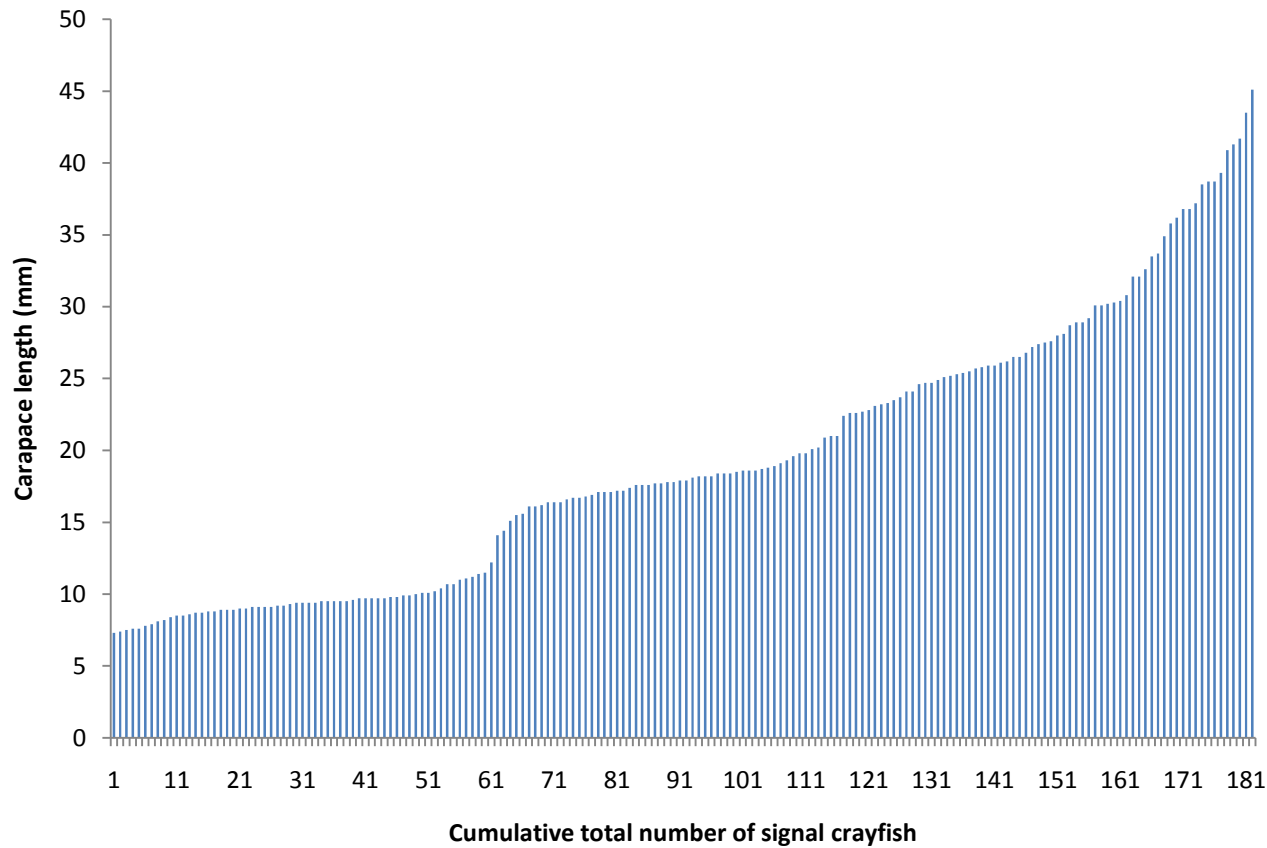
### **Appendix 3 - Crayfish caught from Surber sampler searches of Wilden Beck 12 October 2010**

Appendix 3: The numbers, densities and average carapace lengths of crayfish caught from Surber sampler searches (performed by M. Lucas and N. Dale) of Wilden Beck on 12th October 2010, about one month prior to the commencement of the field enclosure-exclosure experiments. Note that these densities from Surber sampler searches are far higher than the minimum crayfish density estimates produced by electric fishing.

Quadrat	Number of crayfish caught	Crayfish density m <sup>-2</sup>	Mean carapace length (mean ± SD (mm))	Median carapace length (mm)	Number of 7 - 18 mm CL crayfish caught	7-18 mm CL crayfish density m <sup>-2</sup>
1	10	20.4	15.0 ± 7.46	13.1	7	14.3
2	13	26.5	18.1 ± 9.61	16.8	8	16.3
3	10	20.4	16.0 ± 8.25	11.4	6	12.2
4	10	20.4	17.5 ± 9.72	14.8	4	8.16
5	3	6.12	20.4 ± 0.849	20.4	0	0
6	2	4.08	17.4 ± 12.1	17.4	1	2.04
7	8	16.3	18.6 ± 7.73	18.2	3	6.12
8	9	18.4	20.7 ± 8.56	20.1	3	6.12
9	7	19.0	19.0 ± 8.44	17.9	4	8.16
10	16	32.7	17.4 ± 6.56	17.6	9	18.4
11	8	16.3	16.8 ± 7.96	16.6	4	8.16
12	5	10.2	19.4 ± 9.57	17.7	3	6.12
Average	8.42	17.2	17.7 ± 7.98	17.7	4.33	8.84

## **Appendix 4 - Length frequency distribution of crayfish from Wilden Beck 12 October 2010**

Appendix 4: Cumulative frequency of crayfish by carapace length (graph produced by N. Dale and modified by J. Findlay) caught by M. Lucas and N. Dale in Surber samplers and hand searches of Wilden Beck from 12 October 2010. Relatively flat areas indicate likely age classes whilst steeper areas indicate the 'gaps' between these age classes in which few crayfish were caught.





## Appendix 5 - Enclosure data from crayfish predation on salmonid eggs enclosure-exclosure experiments

Appendix 5: Raw data from the enclosure-exclosure experiment. Enclosures are numbered in ascending order from upstream to downstream. Each enclosure initially contained 60 eggs and each tube 10 eggs. Abbreviations are as follows: Encl # - enclosure number, # CF - number of signal crayfish, Beach - Beached, Bury - buried, CF CL - crayfish carapace length, % fine - percentage fine (< 2mm diameter) sediment by dry weight, # alive/dead eggs - number of eggs recovered alive/dead, # eggs tube - number of eggs recovered (alive) from the egg tube, Gam - Gammaridae, Ase - Asellidae, Eph - Ephemeroptera, Tri - Trichoptera, Dip - Diptera. Invertebrate numbers were recorded on a semi quantitative scale # = 1-5, ## = 5-10, ### = >10. Black rows indicate enclosures that were not recovered and grey rows beached or buried enclosures.

Encl #	# CF added	CF CL (mm)	CF CL (mm)	CF CL (mm)	Beach	Bury	# CF alive	CF CL (mm)	CF CL (mm)	CF CL (mm)	# CF dead	% Fine	# alive eggs	# dead eggs	# eggs tube	Gam	Ase	Eph	Tri	Plec	Dip
1	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	0	9.2	55	3	10	#		#	#		##
2	1	11.7	N/A	N/A	N	N	1	11.7	N/A	N/A	0	8.4	50	2	8	#			#	#	#
3	3	16	10	10.2	N	N	3	15.9	10.1	10	0	11.4	41	8	8	#		#		#	#
4	2	15.1	11.0																		
5	2	10.3	16.2	N/A	N	N	2	10.3	16.2	N/A	0	22.4	32	19	4	##		#	#	#	##
6	1	10.6	N/A	N/A	N	N	1	10.6	N/A	N/A	0	14.1	52	3		#		#			#
7	2	10.4	10.4	N/A	N	N	2	10.4	10.5	N/A	0	11.9	51	1	9	#		#	#		##
8	3	10.4	12.2	11	N	N	3	12.2	10.4	11.1	0	22	33	12	7					#	#
9	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	0	11	46	8	8	#	#	#		#	#
10	0	N/A	N/A	N/A	Y	N	0	N/A	N/A	N/A	0	9.8	36	22	5			#	#		##
11	2	10	10.5	N/A	Y	N	2	10.5	10.2	N/A	0	13.5	42	10	9	#		#	#		#
12	3	10.2	10.7	11.4	N	N	3	10.2	10.6	11.4	0	22	44	9		#	#				###
13	3	16	14.8	11.2	N	N	3	11.1	15.9	14.8	0	21	30	15	5	#		#			#
14	3	11	11.3	10.8	N	N	3	10.8	11.3	11.3	0	12.5	51	1	10					#	#
15	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	0	11.8	48	7	9	##		##	#		##
16	2	10.5	10.5	N/A	N	N	2	10.6	10.5	N/A	0	15	52	0		##		#	#		###
17	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	0	13.3	46	11	7			#	#		#
18	2	10.0	10.3																		
19	1	10.9	N/A	N/A	N	Y	1	10.8	N/A	N/A	0	24	33	23	6	#		#		#	#
20	3	10.7	10.5	14.6	N	N	3	14.5	10.7	10.5	0	23.9	29	11	5	#			#		#
21	3	10.4	10	10.8	N	N	3	10.8	10	10.4	0	14	42	5	8	#		#		#	##
22	3	11.1	11.7	10.4	Y	N	3	11.6	11.1	10.4	0	19.9	28	20	4		#				#
23	1	10.8	N/A	N/A	Y	N	1	10.9	N/A	N/A	0	26.7	18	33	3	#		##	#		##
24	3	11.2	14.9	11	N	N	3	11.1	14.7	11.1	0	18.5	29	15	7	##			#		##

Appendix 5, continued: Raw data from the enclosure-exclosure experiment. Enclosures are numbered in ascending order from upstream to downstream. Each enclosure initially contained 60 eggs and each tube 10 eggs. Abbreviations are as follows: Encl # - enclosure number, # CF - number of signal crayfish, Beach - Beached, Bury - buried, CF CL - crayfish carapace length, % fine - percentage fine (< 2mm diameter) sediment by dry weight, # alive/dead eggs - number of eggs recovered alive/dead, # eggs tube - number of eggs recovered (alive) from the egg tube, Gam - Gammaridae, Ase - Asellidae, Eph - Ephemeroptera, Tri - Trichoptera, Dip - Diptera. Invertebrate numbers were recorded on a semi quantitative scale # = 1-5, ## = 5-10, ### = >10. Black rows indicate enclosures that were not recovered and grey rows beached or buried enclosures.

Encl #	# CF added	CF1 CL (mm)	CF2 CL (mm)	CF3 CL (mm)	Beach	Bury	# CF alive	CF1 CL (mm)	CF2 CL (mm)	CF3 CL (mm)	# CF dead	% Fine	# alive eggs	# dead eggs	# eggs tube	Gam	Ase	Eph	Tri	Plec	Dip
25	2	10.2	10.6	N/A	N	N	2	10.6	10.2	N/A	None	13.6	39	3		#		#		#	##
26	1	14.3	N/A	N/A	Y	N	1	14.3	N/A	N/A	None	20.2	38	14	6			#			##
27	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	12.6	45	9	10	#		#	#	#	###
28	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	23.2	49	7	6	#		#		#	#
29	1	10.9	N/A	N/A	N	N	1	10.8	N/A	N/A	None	13.7	53	3	8	#		#	#		#
30	2	11.2	10.6	N/A	Y	N	2	11.2	10.5	N/A	None	19.8	41	15	5	#		#	#		##
31	3	10.1	10.1	13.3	N	N	3	13.3	10.1	10.1	None	12.8	43	6	7	#		#		#	#
32	0	N/A	N/A	N/A	N	Y	0	N/A	N/A	N/A	None	25.6	27	31	2	###	#		#		#
33	1	11	N/A	N/A	N	N	1	11	N/A	N/A	None	10.5	47	9	7			##			#
34	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	12.2	51	7		#		#	#	#	##
35	2	15.4	11.2	N/A	N	N	2	15.4	11.2	N/A	None	9.8	38	5	8	#	#	##		#	#
36	3	11.1	10.8	13.9	Y	N	2	11.7	10.8	N/A	1	18.3	18	33	5						##
37	1	14.5	N/A	N/A	N	N	1	14.5	N/A	N/A	None	10.3	51	0	8	#		#	#		#
38	1	10.7	N/A	N/A	N	N	1	10.7	N/A	N/A	None	14.5	42	6	8	#		#		#	##
39	2	10.7	11.8	N/A	N	N	2	11.9	10.6	N/A	None	19.6	40	9	6	##		##		#	#
40	3	11.2	10.6	10.8	N	N	3	11.2	10.6	10.8	None	13.5	47	2	9	#	#	#			##
41	2	10	11.1	N/A	N	N	1	N/A	11.1	N/A	None	12.8	52	5		#		##		#	#
42	1	12.3	N/A	N/A	N	N	1	12.3	N/A	N/A	None	8.2	41	6	9			#	#		#
43	3	11.8	15.5	10.9	N	Y	1	11.8	N/A	N/A	2	26.9	3	54	0	#					#
44	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	21.5	39	17	6			#			#
45	1	10.8	N/A	N/A	N	N	1	10.8	N/A	N/A	None	17.8	43	12	6	#		##	#	#	#
46	1	10.4	N/A	N/A	N	N	0	N/A	N/A	N/A	None	15.9	45	13	5	#		##			#
47	2	10.2	10.7	N/A																	
48	0	N/A	N/A	N/A																	
49	2	10.8	11.9	N/A	N	N	2	10.8	11.9	N/A	None	13.9	23	15	7	#		#		#	#
50	1	13.2	N/A	N/A	N	N	1	13.1	N/A	N/A	None	16.5	36	12	8	#		##	#		#
51	2	13.5	11	N/A	N	N	2	13.6	10.8	N/A	None	10.2	33	10	9	#		##			#
52	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	13.6	39	11	5		#	##		#	##

Appendix 5, continued: Raw data from the enclosure-exclosure experiment. Enclosures are numbered in ascending order from upstream to downstream. Each enclosure initially contained 60 eggs and each tube 10 eggs. Abbreviations are as follows: Encl # - enclosure number, # CF - number of signal crayfish, Beach - Beached, Bury - buried, CF CL - crayfish carapace length, % fine - percentage fine (< 2mm diameter) sediment by dry weight, # alive/dead eggs - number of eggs recovered alive/dead, # eggs tube - number of eggs recovered (alive) from the egg tube, Gam - Gammaridae, Ase - Asellidae, Eph - Ephemeroptera, Tri - Trichoptera, Dip - Diptera. Invertebrate numbers were recorded on a semi quantitative scale # = 1-5, ## = 5-10, ### = >10. Black rows indicate enclosures that were not recovered and grey rows beached or buried enclosures.

Encl #	# CF added	CF1 CL (mm)	CF2 CL (mm)	CF3 CL (mm)	Beach	Bury	# CF alive	CF1 CL (mm)	CF2 CL (mm)	CF3 CL (mm)	# CF dead	% Fine	# alive eggs	# dead eggs	# eggs tube	Gam	Ase	Eph	Tri	Plec	Dip
53	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	20.2	42	15	5	##	#	#	#	#	#
54	3	10.3	10.5	10.6	N	N	3	10.3	10.7	10.6	None	15	19	26	8	##	#	#	#	#	#
55	0	N/A	N/A	N/A																	
56	1	10.2	N/A	N/A																	
57	2	11.6	16.1	N/A	N	Y	2	11.4	16	N/A	None	23.5	29	10	5	#	#		#		#
58	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	14.9	48	9		#		#		#	##
59	1	11.7	N/A	N/A	N	N	1	11.5	N/A	N/A	None	12.5	49	6	7	#		##	#		#
60	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	25.3	37	22	3			#			#
61	2	11	12.2	N/A	Y	N	2	11	12.2	N/A	None	12.2	0	40	0	#			#		###
62	2	10.7	10.4	N/A	N	N	2	10.7	10.6	N/A	None	16.3	36	13	7	#		#		#	#
63	3	10.9	10.5	13.8	N	N	3	13.9	10.9	10.5	None	15.6	30	7	9	#		##			##
64	3	10.9	10.1	10.3	Y	N	2	11	10.2	N/A	1	15.3	12	32	3	#	#	#	#		##
65	2	10.2	11.4	N/A	N	N	2	10.2	11.5	N/A	None	14.3	39	7		##		#		#	##
66	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	14.5	52	4	10	#		##	#		###
67	3	11.1	10.8	13.9	N	N	3	13.8	10.8	11.3	None	10.9	36	5	10	#					##
68	1	12.8	N/A	N/A																	
69	1	10.2	N/A	N/A																	
70	3	10.9	11	10.5	N	N	3	11.2	11	10.5	None	14.3	47	6	9	#		#		#	###
71	0	N/A	N/A	N/A	Y	N	2	N/A	N/A	N/A	None	18.2	3	52	1	#		#	#		#
72	1	10.3	N/A	N/A	Y	N	1	10.3	N/A	N/A	None	15.6	45	11	8		#	##	#	#	##
73	1	10	N/A	N/A	N	N	1	9.9	N/A	N/A	None	12	51	7	8	#		##	#		##
74	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	16.1	45	9	7	#		#			##
75	3	10.8	11.3	15.1	N	N	3	15	11.3	11	None	15.6	30	12	8	#		#	#		#
76	0	N/A	N/A	N/A	N	N	0	N/A	N/A	N/A	None	15.6	42	7	7			#		#	##
77	2	11.7	10.5	N/A	Y	N	2	11.7	10.4	N/A	None	14.3	40	2	9	#	#	#		#	###
78	3	10.7	10.9	10.3	N	N	3	11	10.8	10.3	None	12.3	27	0	10	##			#		##
79	2	10.9	14.2	N/A	N	N	2	14.2	10.9	N/A	None	18.6	29	15	6	#	#	##			###
80	1	11.7	N/A	N/A	N	N	1	11.7	N/A	N/A	None	14.5	49	4	10	#		##	#		##

## **Appendix 6 - Crayfish predation upon bullhead eggs and male bullhead nest defence**

### **Research Aims**

This work aimed to assess the ability of male bullhead to defend their eggs from predation by signal crayfish. Bullhead eggs might be especially vulnerable to crayfish predation as they are clustered. Additionally male bullhead engaged in nest defence might be more vulnerable to crayfish predation, or injury during interactions with crayfish than they would otherwise be (Bubb *et al.* 2009)(see section 1.1.2). It is, however, also worth noting that some small benthic fish species are capable of defending their eggs from crayfish predation, in spite of being excluded from shelter by crayfish in other circumstances (see section 1.1.2), and this implies that bullhead might also be able to defend their eggs successfully, in at least some cases. If time permitted it was intended to repeat this research for bullhead nest defence against white-clawed crayfish so as to assess the relative success of male bullhead defending their nests from the two crayfish species.

### **Materials and methods**

Bullhead were to be bought into captivity and kept in large, mixed sex holding tanks with artificial shelters in which a nesting male bullhead with eggs could easily be captured and removed for experimental work. The aim was to induce bullhead to spawn in captivity, or if this was not possible to bring in bullhead with their egg masses from the field. Initial trials would be observed to ensure that interactions resulting in damage to either the bullhead or the crayfish were rare or preferably non-existent. Bullhead could then be placed in individual experimental arenas and their interactions with crayfish videoed over the course of a night. Two shelters were to be provided in each experimental set up to avoid competition for shelter. The work was to be conducted in a temperature and photoperiod controlled room of the Durham University life sciences support unit (LSSU).

### **Abandonment**

Initial field surveys were conducted and breeding bullhead were found on dates between 30/3/2011 and 12/4/2011, with the number of egg masses found in one hours' hand

searching peaking at 12 on 12/4/2011. However, at this time no crayfish could be found on either the site from which I was searching for bullhead, or other field sites. Additionally at this time the Durham University LSSU building was undergoing refurbishment so it was not possible to bring in bullhead for captive breeding trials. The bullhead breeding season was shorter than expected, and about 1 month after the start of surveying, on 27/4/2011 no egg masses were found in a 1 hour hand search and the work was abandoned.

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