Consequences for lotic ecosystems of invasion by signal crayfish

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Consequences for lotic ecosystems of

invasion by signal crayfish

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A thesis submitted to the

University of London for the

Degree of Doctor of Philosophy

February, 2012

Statement of originality

I certify that this thesis, and the research presented within it, are the product of my own work. In Chapters Five and Six, sections of the work presented are a result of collaboration with MSc students. Their input is clearly acknowledged on the first pages of these chapters. The guidance I received from my supervisors is acknowledged in a section dedicated to this purpose. Throughout the thesis, the ideas of other people are cited using a referencing format typical of that seen in the biological sciences. Other views and opinions given are those of the author.

Richard Hayes

February, 2012

Abstract

Non-native invasive species are major drivers of biodiversity loss and ecosystem-level modification. The signal crayfish (*Pacifastacus leniusculus*) is a highly successful invasive species and demonstrates traits often seen in keystone species, including top-down predatory effects, a high degree of omnivory, and an ability to physically modify its habitat. From field surveys, and *in situ* and artificial channel experiments, I show that signal crayfish have direct and indirect impacts on the benthos, as well as ecosystem process rates, in lowland, chalk stream ecosystems. Furthermore, I show that these effects are often dependent on crayfish life stage. I demonstrate that two native fish species (chub, *Leuciscus cephalus* and bullhead, *Cottus gobio*) may be affected positively, as well as negatively, by signal crayfish invasion. In addition, population genetics reveals overall high levels of genetic diversity in populations of signal crayfish in the UK.

Acknowledgements

The acquisition of various techniques was required in order for me to complete this thesis. Thanks to Dr Nicola Ings for her mass spectrometer tuition, and thanks to Serena Dool, Hao-Chih Kuo and Kalina Davies for their guidance in molecular techniques.

I formally acknowledge the input of Adrian Hards and Kevin Wood at the beginning of the chapters relevant to their work. However, I would also like to express my gratitude to them less formally, for their high quality input.

In addition, I would like to thank all of my dear family, for their constant and continuing support. In particular, thanks to Marion Henshaw, whose proof reading and 'commerations' greatly improved the readability of this thesis. Finally, and most of all, special thanks go to Hikari Osaki, for keeping me together.

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Chapter One: Introduction

Non-native and invasive species:

Over millions of years natural barriers to the migration of flora and fauna have enabled speciation to occur, forming diverse and distinct biota the world over. Barriers include mountain ranges and the bodies of water, some of which vast, that separate the continents. Not only can the spatial separation of organisms lead to their speciation, but once evolved, the movement of species is often restricted. Amongst other factors, this can lead to relatively localised distributions of species at the world-wide scale.

Since pre-historic times *Homo sapiens* (Linnaeus) has overcome geographic and continental barriers. A consequence of this dispersal, and the subsequent connectivity of mankind at broad spatial scales, was the effective breakdown of geographic barriers for all manner of flora and fauna, through the human mediated translocation of species. These translocations were and continue to be both intentional and unintentional. Plants of agricultural and medicinal value are thought to have been traded for approximately 10,000 years and certainly since the ancient Egyptian civilisation (Reichard and White, 2001).

Whilst the human mediated translocation of species undoubtedly shares a history almost as long as that of our own species, the development of transport technology and thus the scale, speed and extent of introductions has increased in an accelerating fashion over the last two centuries (Cohen and Carlton, 1998, Mack et al., 2000, Mack, 2003). Although the vast majority of introduced taxa fail to establish upon arrival at a location outside of their native range (Mack et al., 2000),

with increasing propagule pressure comes the increasing likelihood of introductions that lead to successful establishment (Veltman et al., 1996). Successful establishment of non-native species has increased within the past century in particular; for example, in the last one hundred years approximately two hundred non-native species established in the San Francisco bay and delta ecosystem (Cohen and Carlton, 1998). A similar trend is seen in the UK conurbation of London; the number of established non-indigenous species in the Thames basin has also displayed a marked increase in the past century (Jackson and Grey, in prep, Figure 1.1).
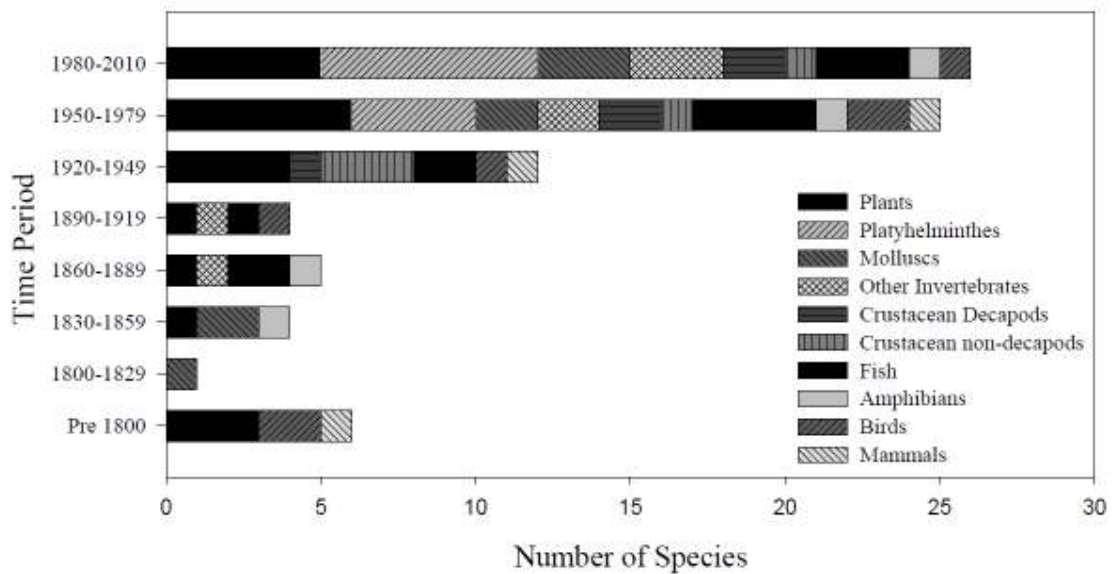
Figure 1. 1. Established non-native species of the Thames basin, UK, separated into 30 year periods. Numbers represent newly established species per 30 year period. Species are divided into taxonomic groups. (Reproduced from Jackson and Grey, in prep)

The classification of non-native and invasive species is essentially dependant on making a distinction between the natural and human mediated migration of organisms. This is a distinction that might be debated, owing to difficulty in

determining whether the two types of colonisation are fundamentally different. A further distinction of important note is seen within the terminology used concerning non-native species, especially when considering that the terminology used in the field of invasion ecology is often inconsistent (Colautti and MacIsaac, 2004). 'Invasive species' is a loaded term, often used to imply negative effects caused by an organism, whether direct, indirect, or both, and the connotations of such terms as 'invasion' have opened the field to criticism (Simberloff, 2003). While it has been argued that the term 'invasive' should pertain to the invasiveness of introduced species (their potential to colonise an area), and should not connote impact (Ricciardi and Cohen, 2007), Lockwood *et. al.* use the term 'invasive' to describe non-native species with a demonstrable ecological or economic impact (Lockwood et al., 2007), and it is in this sense that it shall be used throughout this thesis. It is therefore distinct from a term such as 'non-native species', which does not have the same connotations as 'invasive' and might be used when an organism has established outside of its range, but may have relatively minor implications for the recipient ecosystem.

Impacts of invasive species:

Invasive species can cause ecological and / or economic damage at a range of scales (Mooney, 2004). Perhaps the most insidious impacts of non-native species are those manifested at the genetic level. Where historic barriers between closely related or sub-species are lost, hybridisation and introgression of genes can threaten a rare species' existence (Rhymer and Simberloff, 1996). Introgression is the process whereby interspecific genes enter a gene pool through hybridisation, followed by back-crossing of hybrids with one or both of the parental species. When

introgression proceeds asymmetrically, there is a possibility of the extinction of local gene pools. The best known examples of gene pools threatened by introgression with introduced species are seen in avian taxa, for example various duck species, such as the grey duck (*Anas superciliosa superciliosa* Gmelin) in New Zealand and the white-headed duck (*Oxyura leucocephala* Scopoli) in Spain (Rhymer et al., 1994, Munoz-Fuentes et al., 2007).

The more obvious effects of invasive species are those that occur at individual, community and whole ecosystem levels. Extinctions, local extirpations and reductions in abundance of native species have all been shown to result from invasion by non-native species. For the 170 extinct species on the IUCN Red List database where a cause of extinction was stated, an interaction with invasive species contributed to their extinction in 91 (54%) cases; and invasive species were *the only* given cause in 34 (20%) cases (Clavero and Garcia-Berthou, 2005).

Small island communities represent perhaps the most well known examples of invasions and associated extirpations. Of particular note, introductions of predatory mammals have led to extirpation of native species on islands, largely attributable to a lack of predator avoidance when previously no predator species had been present (Courchamp et al., 2003). In fact, the probability of a bird species having been extirpated on an oceanic island correlates with the number of non-native mammal predators established (Blackburn et al., 2004).

Losses of species can have important consequences for ecosystems. In terrestrial systems, plant primary productivity increases positively with plant species and functional group diversity (Loreau et al., 2001). Meta-analyses of marine experimental work and long term regional and fisheries data demonstrated that all

ecosystem processes examined were positively associated with the diversity of both primary producers and consumers (Worm et al., 2006). Across all biomes, increased biodiversity can buffer ecosystem functioning against environmental fluctuations according to the Insurance Hypothesis: if many species are present before perturbation of a community occurs, it is more likely that some species will maintain ecosystem functioning when others fail, than if the community was depauperate to begin with (Yachi and Loreau, 1999).

While the loss of species resulting from invasions is important, a far more common outcome of invasion is that the introduced species will alter the abundance and / or distributions of native species, rather than bringing about their outright exclusion (Lockwood et al., 2007). For example, removal of the dingo (*Canis lupus dingo* Meyer), an alien top-predator, was associated with increased activity of herbivores and of an introduced mesopredator, the red fox (*Vulpes vulpes* Linnaeus) (Letnic et al., 2009). Salmonids introduced into stream ecosystems can alter the distribution and foraging behaviour of mayflies, resulting in reduced diurnal grazing of algae by mayflies (Simon and Townsend, 2003). These more subtle impacts of invasive species can still have functionally significant consequences for ecosystems. The increased herbivore and red fox activity associated with dingo removal was linked to decreased grass cover and decreased small mammal diversity – providing strong evidence that a trophic cascade had occurred (Letnic et al., 2009). Trophic cascades occur when the direct influence of an organism on an adjacent trophic level has an indirect effect on subsequent trophic levels (Hairston, 1960, Threlkeld, 1988). Trophic cascades can have profound effects on aquatic ecosystems in particular (Strong, 1992).

Predatory effects aside, there are numerous examples of invasive species competitively displacing or even excluding native species (Mooney and Cleland, 2001): *Impatiens glandulifera* (Royle) invades riparian zones and excludes native plant species (Hulme and Bremner, 2006); North American native ants have been competitively displaced by an invasive Argentine ant (Holway, 1999); and a well studied example from freshwater ecosystems is the widespread replacement of native unionid mussels by invasive zebra mussels (*Dreissena polymorpha* Pallas) (Strayer and Malcom, 2007). The means of displacement and / or exclusion of a native by an invasive competitor are not necessarily restricted to trophic interactions. Competition for territory / shelter is a non-trophic interaction which can have negative impacts on native predators. On tropical Pacific islands, native gecko (*Lepidodactylus lugubris* Duméril and Bibron) abundance has been drastically reduced by the non-native common house gecko (*Hemidactylus frenatus* Duméril and Bibron) and evidence suggests that the mechanism may be behavioural exclusion (Case et al., 1994).

Competitive effects between a native and an invasive species do not occur in isolation. Where these competitive interactions are between consumers and / or predators, there are potential consequences for the lower levels of food webs. Interference between competing predators might lead to decreased impacts on prey assemblages; however, in the absence of interference, impacts may remain unchanged or even be increased (Sih et al., 1998, Snyder and Evans, 2006). In Californian vineyards, a dominant invasive spider suppressed herbivore abundance while native predatory spiders did not (Hogg and Daane, 2011). Synergistic effects can also result from predator-predator interactions. For example, suppression of

aphid populations was shown to double in a mesocosm experiment, where the

foraging method of two predators was complementary (Losey and Denno, 1998).

Whether such synergistic effects occur between competing invasive and native

predators is, as of yet, largely unknown.

Owing to impacts on ecosystem goods and services, the costs sustained by invasive

species are not only ecological, but economic as well. This includes losses in crops,

fisheries, forestry and human wellbeing and health (Pimentel et al., 2005).

Combining financial costs attributable to both direct losses and those incurred

through measures implemented to control invasive species, the annual cost to the US

economy alone is estimated at $120 billion per annum (Pimentel et al., 2005).

Non-native species in freshwater ecosystems:

Owing to a long historic separation at the continental scale, freshwater habitats are

particularly susceptible to extinctions and extirpations resulting from exotic

invasions (Rahel, 2007). In North America alone, 3 genera, 27 species and 13

subspecies of fish are recorded as having become extinct in the last century. The

second most frequently cited cause of extinction (in 68% of cases) was the effect of

invasive species, second only to habitat alteration (73%) (Miller et al., 1989).

Although clichéd, cichlid extinctions in Lake Victoria following the introduction of

Nile perch (*Lates niloticus* Linnaeus) represent a powerful example of the most

dramatic impacts invasive species are capable of inflicting. Between 1979 and 1990,

data suggests that approximately 200 of 300 + endemic cichlids had disappeared or

were threatened with extinction (Witte et al., 1992).

Various aquatic invaders have impacted freshwater ecosystems profoundly via mediating trophic cascades or through ecosystem engineering effects. For example brown trout (*Salmo trutta* Linnaeus) appeared to cause a shift from bottom-up, to top-down regulated stream invertebrate communities in New Zealand. This apparently resulted in a cascade whereby periphyton accrual increased dramatically (Huryn, 1998). Introduced opossum shrimp (*Mysis diluviana* Audzijonyte and Väinölä) provide a rare example of long term multiple cascading effects caused by an introduced species (Ellis et al., 2011). These effects shifted community composition drastically, extending from primary producers all the way through the food web, ultimately leading to the local disappearance of a non-aquatic species, the bald eagle (*Haliaeetus leucocephalus* Linnaeus). There are also multiple examples of aquatic invasive species inducing ecosystem level change when their presence within a recipient ecosystem causes modification of the physical environment. Ecosystem engineers are defined as "organisms that directly or indirectly control the availability of resources to other organisms by causing physical state changes in biotic or abiotic materials" (Jones et al., 1997). Invasive ecosystem engineers include dreissenid mussels, which shift energy flow in ecosystems from pelagic-profundal to benthic littoral (Higgins and Vander Zanden, 2010) and North American beavers (*Castor canadensis* Kuhl) (Choi, 2008). So great were the consequences of 'engineering' by beavers in the Tierra del Fuego archipelago, that scientists planned the largest eradication project ever attempted (Choi, 2008).

Freshwater crayfish:

Freshwater crayfish are a monophyletic group belonging to the largest crustacean taxon, the Decapoda, and are made up of two superfamiles, the Astacoidea and the

Parastacoidea (Crandall et al., 2000). The Astacoidea are further broken down into two families, the Astacidae (39 species) and the Cambaridae (420 species) (Hobbs 1989 in Crandall and Buhay, 2008). The native distribution of the Parastacoidea is limited to the southern hemisphere, whereas the Astacoidea are found in the northern hemisphere. Over 640 crayfish species are currently described (Crandall and Buhay, 2008). A graphical summary of the international distribution of freshwater crayfish is seen in Figure 1.2. The highest radiation of crayfish diversity has occurred in North America, with 382 species recorded, while Australasia has 151 confirmed species; however these numbers are still increasing with new species identified each year (Crandall and Buhay, 2008). Contrastingly, only five native species of freshwater crayfish are extant in Europe (Holdich et al., 2009).
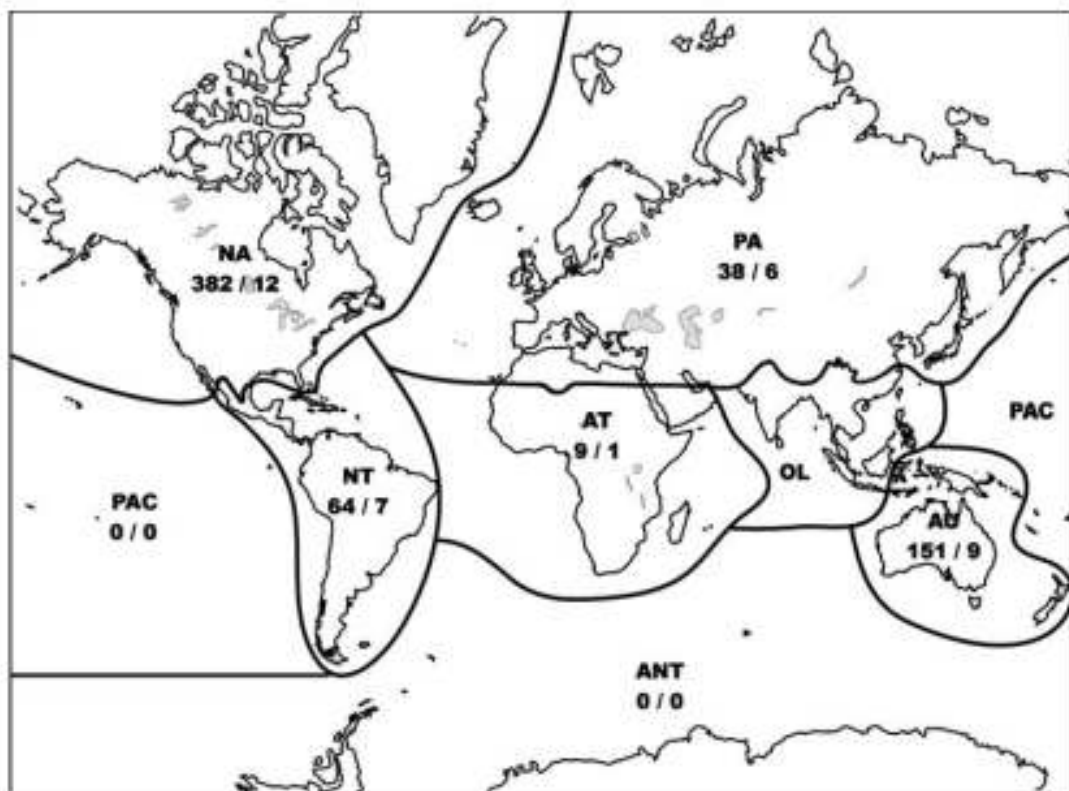
Figure 1. 2. The worldwide distribution of freshwater crayfish within their native ranges (species number / genus number). NA, Nearctic; PA, Palaearctic; PAC,

Pacific Oceanic Islands; NT, Neotropical; AT, Afrotropical; OL, Oriental; AU, Australasian; ANT, Antarctic (Reproduced from Crandall and Buhay 2008).

The ecological role of crayfish:

Owing to a predominance of vegetable matter in gut content analyses, crayfish have, in the past, been considered herbivores and detritivores (Momot, 1995). They are, however, omnivorous and furthermore represent key predators of freshwater invertebrates in many ecosystems (Momot, 1995, Nystrom and Strand, 1996, Usio and Townsend, 2004). These concurrent but fairly disparate roles have led to their classification as 'functional omnivores but trophic predators' (Parkyn et al., 2001). With 14 or more distinct food items found in the gut content of crayfish in multiple studies (Whitledge and Rabeni 1997; Guan and Wiles 1998; Stenroth and Nystrom 2003), crayfish are clearly generalist omnivores. Although the majority of gut content is generally vascular plant material (Momot, 1995), stable isotope approaches have repeatedly shown the importance of this resource is greatly exaggerated on the basis of gut content analysis alone. Aquatic invertebrates are consistently the second most dominant group within gut content, but seem to be as important if not more so for growth in many crayfish species (Whitledge and Rabeni, 1997, Parkyn et al., 2001, Olsson et al., 2008, Giling et al., 2009).

The predatory role of crayfish has in some scenarios led to their being regarded as keystone predators (Momot, 1995, Nystrom et al., 1996), including where they have instigated cascading effects. A keystone species is defined as having a disproportionate impact on its community relative to its abundance / biomass (Power et al., 1996). For example, crayfish led to an increase in periphyton abundance

following direct suppression of macrophyte and snail densities (Lodge et al., 1994). However, the importance of consumptive and non-consumptive destruction of algae and macrophytes by crayfish should not be underplayed, as such effects have also led to cascades and assignation of keystone status; for example, crayfish can facilitate epilithic diatoms and sessile, grazing insects via exclusion of filamentous algae (Creed, 1994, Rodriguez et al., 2005).

Across the lifetime of an individual, crayfish exhibit a relatively large size range. Starting at several millimetres as juveniles, and generally reaching total lengths of around 15 cm, crayfish are the largest mobile freshwater invertebrates (Skurdal and Taugbøl, 2002, Holdich, 2002a). Using gut content analysis and / or stable isotope data, crayfish ontogeny has been shown both to be important (Whitledge and Rabeni, 1997, Guan and Wiles, 1998, Correia and Anastacio, 2008, Stenroth et al., 2008) as well as unimportant (Whitledge and Rabeni, 1997, Stenroth et al., 2008, Bondar and Richardson, 2009) in determining their diet. Findings appear contradictory across species and even within species. Red swamp crayfish (*Procambarus clarkii* Girard) progressing through juvenile, sub-adult and adult life-stages shift increasingly from carnivory to detritivory (Correia and Anastacio, 2008). Similarly, in a UK river, larger signal crayfish (*Pacifastacus leniusculus* Dana 1852) have been shown to feed proportionately more on vascular detritus than smaller conspecifics (Guan and Wiles, 1998). Contrastingly, stable isotope analysis of signal crayfish revealed larger individuals occupying a higher trophic position than smaller individuals in lakes with wide littoral zones (Stenroth et al., 2008).

Crayfish can act as ecosystem engineers by decreasing sand in gravel interstices, suppressing the growth of filamentous algae on gravel and reducing biofilm cover on 'sand dunes' (Creed and Reed, 2004, Statzner et al., 2000, Statzner et al., 2003, Usio and Townsend, 2004, Zhang et al., 2004). Bioturbation by crayfish can have knock-on effects on fine sediment caught in leaf packs, with resultant impacts on invertebrates associated with this substrate (Usio and Townsend, 2004). Clever experimental design teased apart the relative impacts of sediment accrual and crayfish predation on invertebrate abundances and revealed that Tanypodinae were selectively preyed upon by crayfish, whereas three other taxa responded more dramatically to sediment bioturbation (Usio and Townsend, 2004).

To date, most published studies of fish-crayfish interactions have focussed on the impact of fish on crayfish and not vice versa. Crayfish can be an important prey item of fish in lentic and lotic environments (Didonato and Lodge, 1993, Garvey et al., 1994, Fortino and Creed, 2007). Fish predation on crayfish has been implicated in regulating crayfish population density; for example, the distribution of young-of-year crayfish in headwaters (Fortino and Creed, 2007). While correlative, numerous studies have shown crayfish populations to be negatively correlated with fish populations (Mather and Stein, 1993, Usio and Townsend, 2000, Olsson et al., 2006).

Interactions between crayfish and fish are not one sided however, and crayfish have been implicated in negatively affecting fish populations, including through direct predatory effects. Crayfish collected from a river in England have been found to include fish in their gut content (Guan and Wiles, 1998). Furthermore, benthic fish mortality increased in artificial channels containing crayfish, and crayfish were observed attacking and consuming bullhead (*Cottus gobio* Linnaeus) in

aquaria (Guan and Wiles, 1997). A further mechanism by which crayfish may negatively affect fish populations is predation on fish eggs. Successful reproduction of two fish species was negatively affected by crayfish via egg predation in pond experiments (Dorn and Mittelbach, 2004).

Alternatively, non-trophic interactions, such as competition for shelter, have been proposed as a means by which crayfish might negatively influence fish populations. Crayfish were shown to force small fish from shelter in the presence of a large predatory fish using tank experiments, whereas in the control treatment without crayfish, the small fish spent more time under shelter (Rahel and Stein, 1988).

Although the predominance of effects reported in the literature appears to be negative, there are also studies where no effect of crayfish on fish was found. For example, crayfish had no impact on juvenile trout survival in an enclosure / exclosure experiment (Stenroth and Nystrom, 2003), and at a regional scale, no negative effects were found for any of eight commonly recorded fish species in Swedish streams where crayfish species were present (Degerman et al., 2007).

Crayfish as introduced species:

Owing to their popularity as a food source, crayfish have a long history of being introduced outside their native ranges for human consumption (Swahn, 2004). Additionally, the aquarium trade is to blame for introductions in more recent times (Holdich, 2003). Combined, these factors have led to the establishment of non-native crayfish populations at an international scale (Hobbs et al., 1989). Non-indigenous crayfish are fast becoming a ubiquitous group throughout Europe, with ten different species recorded among 37 countries / territories (Holdich et al., 2009).

Of these 10 species, eight originated in North America, whilst two were introduced from Australia (Holdich, 2003).

Crayfish as invasive taxa have been the focus of much research. Their impact on native crayfish has been an area of particular interest, having been implicated in local extirpations of several indigenous crayfish species (Gherardi and Holdich, 1999). Whilst it has not been demonstrated that invasive crayfish commonly lead to the local extirpation of non-crayfish taxa (but see Rodriguez et al., 2005, Jackson et al., in prep), various impacts of invasive crayfish on plant and especially macroinvertebrate communities have been demonstrated. In a large scale, multi-lake survey macrophyte species richness and abundance declined in crayfish (*Orconectes* spp.) invaded lakes as compared to non-invaded lakes (Rosenthal et al., 2006). Meta-analysis of 14 cage experiments covering seven non-indigenous crayfish species, revealed significantly lower total densities of the zoobenthos (primarily Gastropoda and Diptera) in invasive crayfish treatments relative to controls (McCarthy et al., 2006).

The keystone roles described in the previous section, whereby crayfish induced cascading effects via predation and engineering are likely to be important for ecosystems which receive non-indigenous crayfish. In fact, two of the experiments cited in the above section as examples of crayfish exerting keystone roles were studies conducted with non-native crayfish outside of their native ranges (Nystrom et al., 1996, Rodriguez et al., 2005). Such effects are likely to be of particular significance in localities with no history of crayfish presence. For example, a shift from clear to turbid waters in a shallow lake ecosystem, with associated loss of taxa

at all trophic levels, has been attributed to the introduction of red swamp crayfish (Rodriguez et al., 2005).

The potential impacts of invasive crayfish on fish are in general poorly understood; however, a number of studies have been published on the subject in recent years. Non-native crayfish have been associated with reduced numbers of larval crucian carp (*Carassius auratus* complex) (Matsuzaki et al., 2011) and, contrastingly, have been shown to suffer heavy predation pressure from native fish species (Nystrom et al., 2006, Tetzlaff et al., 2011). This appeared to result in the limitation of crayfish density in one case (Tetzlaff et al., 2011), but appeared to have no impact on numbers in the other (Nystrom et al., 2006). In line with discussion in the above section on the ecological role of crayfish, impacts of invasive crayfish on fish are unlikely to be limited to direct trophic interactions. The ability of crayfish to reduce macrophyte cover (e.g. Chambers et al., 1990, Nystrom et al., 2001), for example, is likely to have behavioural consequences for various species of fish. Multiple impacts resulting from introductions of the focal species of this thesis, signal crayfish, have been suggested and will be outlined in the following section.

Not only do some native fish species exploit non-native crayfish subsequent to their introduction, but consumption of the introduced red swamp crayfish in Spain by various terrestrial vertebrate predators and avian species has been recorded (Delibes and Adrian 1987; Peris et al. 1994; Beja 1996). Such consequences of introduction represent potential 'positive' impacts of invasive crayfish. Potential positive effects are further considered in subsequent chapters.

Signal crayfish:

Signal crayfish (Figure 1.3) belong to the family Astacidae, superfamily Astacoidea. Their natural range encompasses seven states of the USA and one province of Canada, extending from northern California to southern British Columbia, and east as far as regions of Utah and Montana (Crandall, 2001, Bondar, 2005 and references therein). On the basis of morphological characteristics three subspecies have been described; *Pacifastacus leniusculus leniusculus* (Dana 1852), *Pacifastacus leniusculus trowbridgii* (Stimpson 1857) and *Pacifastacus leniusculus klamathiensis* (Stimpson 1857). However, doubts have been raised as to the validity of these distinctions owing to morphological overlap within introduced ranges both in North America and in Japan (Riegel, 1959, Kawai et al., 2004).

Figure 1. 3. A large adult signal crayfish (*Pacifastacus leniusculus*) with approximate scale (Reproduced from Holdich et al., 2009).

Ponds, lakes, streams and rivers are all suitable habitats for signal crayfish (Abrahamsson, 1970, Shimizu, 1983, Dahl, 1998, Johnsen et al., 2007). Densities and size structure appear to be determined in large part by the nature of benthic substrate and the availability of suitable shelter (Abrahamsson, 1970, Kirjavainen and Westman, 1999, Mueller, 2002, Savolainen et al., 2003, Nystrom et al., 2006). Although signal crayfish were not previously considered as a burrowing crayfish taxon in their native range, they have been shown to burrow when the substrate is suitable within their introduced range (Guan, 1994). The diet of signal crayfish is typical of omnivorous crayfish. Vascular detritus, algae, macrophytes, aquatic invertebrates, fish, amphibian eggs and amphibian larvae have all been identified from feeding studies and gut content analyses (Axelsson, 1997, Guan and Wiles, 1998). Signal crayfish themselves represent a prey item, and suffer predation by a number of fish predators, such as smallmouth bass (*Micropterus dolomieu* Lacépède), European perch (*Perca fluviatilis* Linnaeus) and European eel (*Anguilla anguilla* Linnaeus) (Blake and Hart, 1995, Lewis, 1997).

Of the six species of non-indigenous crayfish currently established in UK waters signal crayfish are by far the most widespread (Holdich et al., 2009). Not only are they particularly prevalent in the UK but they are also the most widespread invasive crayfish species in Europe; with a current range encompassing 27 territories (Figure 1.4). Further to their presence in Europe they have also successfully established in Japan (Kamita, 1970 in Ohtaka et al., 2005). Owing to their extensive distribution, the scope for this species to impact invaded waters is far greater than that of the remaining non-native crayfish found in the UK. Signal crayfish are therefore of particular interest and are the focal species of this PhD.
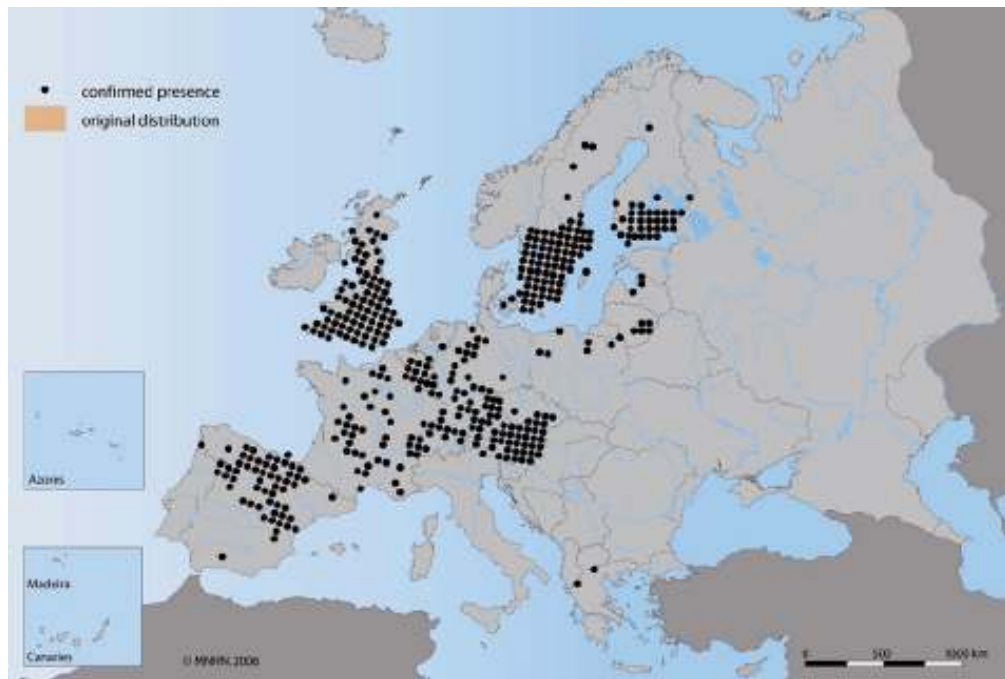
Figure 1. 4. The distribution of signal crayfish in Europe, based on 2006 records, with assignment to a 50 km^{-2} Universal Transverse Mercator grid (Reproduced from Souty-Grosset et al., 2006).

The introduction of signal crayfish into Europe was large in scale and systematic in approach. In 1960, an initial introduction of a small number of individuals was made from three Californian catchments to Sweden (Souty-Grosset, 2006). Owing to the success of the initial introduction, between 1967 and 1969 large numbers were imported from lakes Tahoe and Hennessey to Sweden (10,000 in 1969 alone) and Finland. Furthermore, illegal stockings were made into Austria from California and into France from Oregon. In the UK, signal crayfish were introduced into fish farms from Sweden at around 300 sites in the late 1970s until around 1990 (Defra, 2011). Stocking was made at 17 sites in the Thames basin in 1981 alone (Ellis (Environment Agency), personal communication). Whilst their farming was largely unsuccessful,

signal crayfish established successfully in the wild and are now found from Cornwall to the Highlands region of Scotland (Figure 1.5).
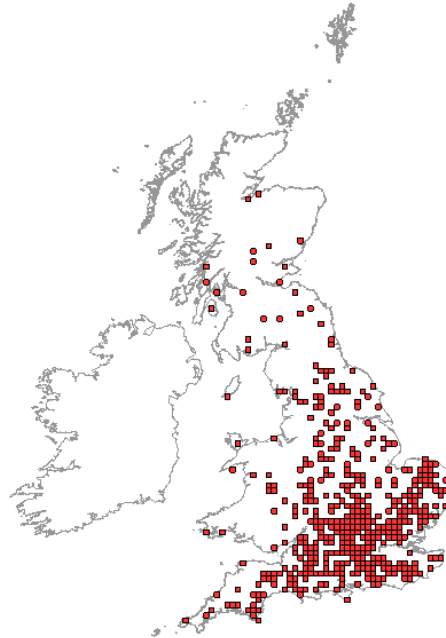
Figure 1. 5. The distribution of signal crayfish in the UK, based on 2004 records, representing presence within 10 km^{-2} squares (National Biodiversity Network. © Crown Copyright. All rights reserved NERC 100017897 2004).

Natural dispersal of crayfish between catchments appears uncommon (Fetzner and Crandall, 2003, Smith and Smith, 2009). For this reason, the large scale spread of signal crayfish across the UK must be attributable to human mediated introductions. However, it is likely that a contributory factor to the success of establishment in the UK is the relatively great dispersal ability of signal crayfish within river networks (Bubb et al., 2006).

Signal crayfish are perhaps best known to both the scientific and non-scientific community of Europe for their role in the decline of native crayfish, including the

white-clawed crayfish (*Austropotamobius pallipes* Lereboullet) and noble crayfish (*Astacus astacus* Linnaeus). In the UK, evidence indicates that signal crayfish are capable of competitively displacing white-clawed crayfish (Holdich and Domaniewski, 1995, Holdich et al., 1995, Bubb et al., 2005, Bubb et al., 2006, Dunn et al., 2009). However, a far more commonly cited cause for the decline of native crayfish is the spread of the North American crayfish plague, caused by the oomycete *Aphanomyces astaci* (Schikora) (e.g. Kemp, 2003, Holdich et al., 2009). Through co-evolution with the pathogenic oomycete, North American crayfish show an inherent immune response and increased resistance to the plague (Cerenius et al., 2003). White-clawed crayfish, however, lack an adequate immune response and populations rapidly extirpate once infected (Holdich, 2003, BBC, 2011). Signal crayfish are a known vector of crayfish plague and therefore not only are they capable of out-competing white-clawed crayfish, but they appear to have been a critical factor in extirpations of numerous populations of white-clawed crayfish (Alderman et al., 1990, Holdich, 2003).

Aside from interactions with native crayfish, there are numerous examples in the literature of the negative effects invasive signal crayfish have on the benthic macroinvertebrate community. Their presence in the river Clyde, Scotland, was associated with reduced total abundances of macroinvertebrates and reduced taxon richness of Plecoptera, Chironomidae and Crustacea (Crawford et al., 2006). A weak but significant negative effect on predatory invertebrate biomass was seen in signal crayfish treated cages of a pond littoral community, relative to controls (Nystrom et al., 2001) and the most abundant grazers, snails, were greatly reduced by crayfish. Total invertebrate biomass and taxon richness were reduced with increasing densities of signal crayfish in both artificial pond and stream enclosure / exclosure

experiments in Sweden (Nystrom et al., 1996, Stenroth and Nystrom, 2003). The total biomass of invertebrates and the biomass of herbivorous / detritivorous invertebrates were reduced in ponds, whereas in the stream enclosure study slower moving invertebrate biomass was reduced in the presence of crayfish (specifically Hirudinea, Odonata, Trichoptera, Isopoda (*Asellus*) and Bivalvia), while more mobile prey and sediment dwelling organisms were less affected.

Interactions with small benthic taxa represent the most studied aspect of the possible impacts of non-indigenous signal crayfish on fish. It has been shown that signal crayfish out-compete bullhead and stone loach (*Barbatula barbatula* Linnaeus) for shelter (Guan and Wiles, 1997, Light, 2005 (*Cottus beldingi* Eigenmann & Eigenmann) Bubb et al., 2009), which is likely to have consequences for fish mortality through increased exposure to predators at sites where refugia are limited. Negative impacts of signal crayfish on bullhead and stone loach populations have been suggested; abundance correlated negatively with increasing crayfish density (Guan and Wiles, 1997, Bubb et al., 2009). Intriguingly, the presence of invasive signal crayfish might also benefit bullhead, as suggested by a field experiment in Sweden, where juvenile signal crayfish were the third most numerous prey in the gut content of bullhead (Dahl, 1998).

The impacts of signal crayfish on fish have not been entirely limited to research focused on small benthic species. Occupation of refugia by salmon parr (*Salmo salar* Linnaeus) was reduced by signal crayfish presence in arenas within an artificial channel (Griffiths et al., 2004). Other findings relating to larger fish taxa have shown neutral as well as potentially beneficial effects; in experimental trials trout fry survival was unaffected by signal crayfish (Stenroth and Nystrom, 2003),

whilst signal crayfish in Swedish lakes were the dominant prey item of large perch (46 % by occurrence) (Nystrom et al., 2006).

Invasive species are sometimes closely related to the native species with which they interact, but despite this may not be functionally equivalent. For example, exotic predators tend to have a greater impact on prey populations than native predators (Salo et al., 2007). This has been demonstrated in crayfish, whereby signal crayfish had a greater impact on gastropods and consumed more aquatic macrophytes than indigenous noble crayfish (Nystrom and Strand, 1996, Nystrom et al., 1999). Furthermore, competition for shelter has been demonstrated to be more intense between bullhead and signal crayfish than with the indigenous white-clawed crayfish (Bubb et al., 2009).

Within the UK, the influence of signal crayfish on aquatic communities might be greater than that of the native white-clawed crayfish. Furthermore, signal crayfish are likely to show greater adaptability in their environmental requirements than white-clawed crayfish. This has been shown for signal crayfish in relation to noble crayfish in Sweden (Olsson et al., 2009). Therefore, 'crayfish naïve' localities are likely to see the establishment of signal crayfish populations, where their impacts are likely to be heightened, as compared with localities that have a recent history of white-clawed crayfish presence.

General aims and approaches / thesis structure:

In the broadest sense, the remit of this thesis was to investigate impacts resulting from signal crayfish presence in lowland, lotic water bodies in the UK. As no previous knowledge of the population genetics of signal crayfish existed, in addition

to directly studying impacts I made the first population genetics study of this species in the UK. This is the subject matter of Chapter Two. I sought to use both mitochondrial and microsatellite markers in order to measure the genetic diversity of crayfish populations and to test for the presence or absence of spatial patterns of diversity within and among these populations. Results inform on the history of signal crayfish introductions, provide some context to the PhD and have implications for the management of crayfish.

Signal crayfish are known to have impacts on the macroinvertebrate communities of freshwater ecosystems. However these impacts have generally been investigated in experimental setups or within single water bodies. In Chapter Three I present the results of field work conducted to investigate whether the published effects of signal crayfish on the macroinvertebrate community are detectable at broader spatial scales. In order to achieve this aim I used sites on eight broadly similar streams, proximate to one another by location and geography.

The significance of signal crayfish ontogeny in relation to their impacts is poorly understood; surprisingly few studies have considered both life stage and biomass. Furthermore, although it has been noted that signal crayfish are likely to have significant impacts on lotic ecosystems through their role as ecosystem engineers (Harvey et al., 2011), no study has of yet demonstrated how signal crayfish can impact invertebrate communities through such a role. Simultaneous effects of signal crayfish on macroinvertebrates, measures of ecosystem functioning and the composition of sediment were investigated in a field experiment, presented in Chapter Four. The importance of crayfish body size and biomass were considered in

relation to all measurements taken and the consequences of engineering effects for various macroinvertebrate taxa were also observed.

Evidence suggests that signal crayfish have negative impacts on small benthic fish populations. Comparable to the situation as regards effects on macroinvertebrates, these impacts have not been shown at scales above single stretches of individual rivers. The mechanisms by which these fish populations might be negatively affected are unclear. Furthermore, how interactions between benthic fish and signal crayfish affect the macroinvertebrate community are unknown. In Chapter Five I addressed these knowledge gaps through survey work and implementation of recent advances in stable isotope ecology to study diet at the population level. The consequences of interactions between signal crayfish and bullhead for the benthos were studied in artificial channel experiments.

Whilst published work on the impacts of signal crayfish on small fish is scant, even less is known about the effect of signal crayfish introduction on populations of larger fish species. In the final data chapter I approach this topic using the European chub (*Squalius cephalus* Linnaeus), a potential competitor and reciprocal predator of signal crayfish. In addition to stable isotope mixing models, recently developed stable isotope metrics were used in order to study the diet of chub. Scalimetry was used in combination with the stable isotope approach in order to determine whether chub growth rates were affected by signal crayfish.

The theoretical background of the ecological studies that comprise chapters three through to six is combined into a framework in Figure 1.6. This figure is not

exhaustive, but highlights those interactions involving signal crayfish which I investigated within the PhD.
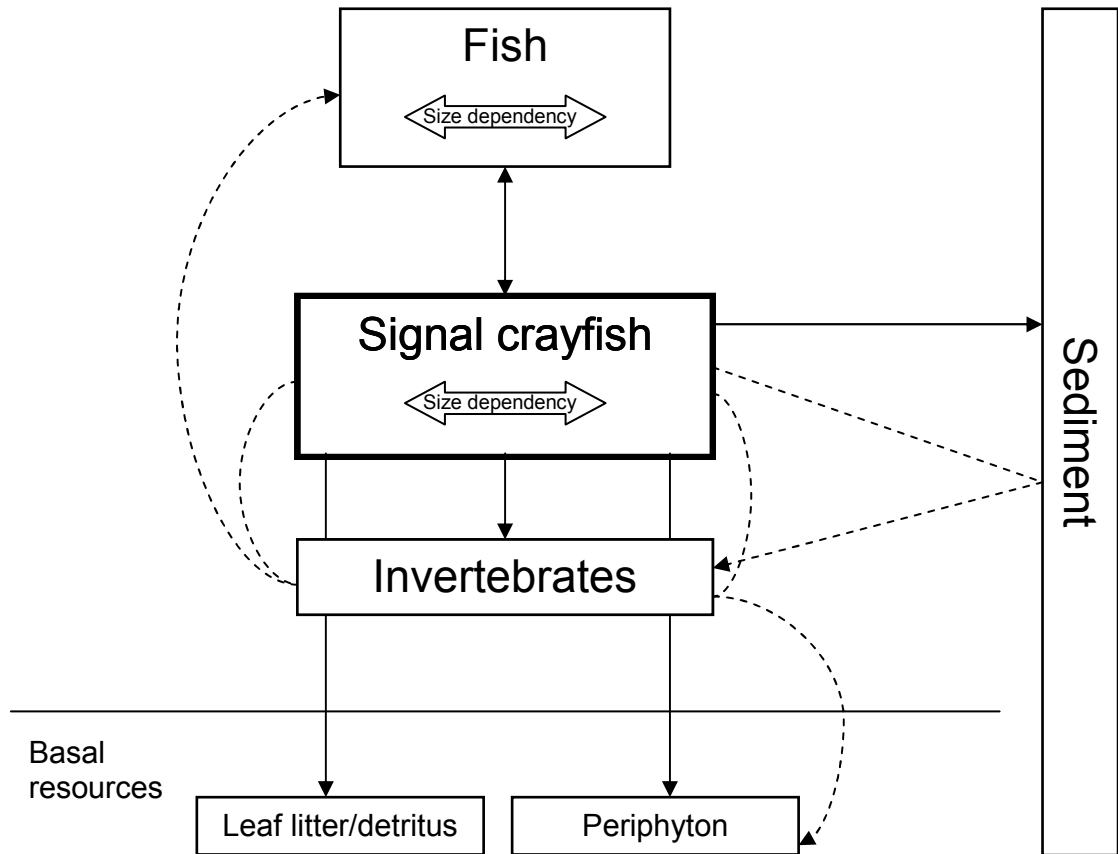
Figure 1. 6. A schematic representing the interactions between crayfish and various elements of the communities of which they are a part, both biotic and abiotic. In reality, both the number of components and connections is far greater than that displayed, however those that are relevant to the original work contained in this PhD are included. Solid line arrows represent direct interactions, while dashed line arrows represent indirect interactions, making connections via intermediaries with which a direct interaction does occur.

As explained above in the introduction, signal crayfish can have top-down regulatory effects on a community and direct trophic interactions are represented by

the solid arrows extending from crayfish to invertebrates, leaf litter and periphyton. In the case of fish, reciprocal predation is represented by a double ended arrow, and the direction of predation will largely be dependent on both crayfish and fish size. Through predation of grazers, in particular Gastropods, grazing of periphyton is likely to be suppressed by crayfish. This is represented by the dashed arrow between crayfish and periphyton. The importance of invertebrates in the diet of signal crayfish is likely to be of significance to those fish species that also depend on this dietary group. This is represented by the indirect arrow between crayfish and fish. Finally, signal crayfish can also directly affect the composition of sediments. This is likely to have indirect effects on the macroinvertebrate community associated with the substrate and is represented by the dashed arrow between crayfish and invertebrates. All these interactions may be dependant on crayfish life stage / size.

Chapter Two: The population genetics of signal crayfish in the UK, with particular reference to the Thames basin

Introduction

Molecular techniques provide an invaluable tool in both the fundamental and applied study of invasive species. At the most basic level, protein or DNA molecular markers can be used to measure genetic diversity within populations of invasive species, which is often reduced owing to founder effects in colonising populations (Tsutsui et al., 2000, Lindholm et al., 2005). Not only can comparisons with populations of an invasive species in their native range reveal a loss of diversity, but the identification of source populations is also possible (Novak and Mack 2001, Lindholm et al., 2005). Use of microsatellite markers can even enable temporal and spatial patterns of invasions to be inferred (Estoup et al., 2004, Guillemaud et al., 2010). For example, a combination of microsatellite markers and historical records provided support for a stepwise migration-foundation model with founder events in the cane toad, *Bufo marinus* (Linnaeus) in Australia (Estoup et al., 2004).

The freshwater habitat represents a special case in the application of molecular ecology, owing to the inherent network-type connectivity seen in river and stream systems and, contrastingly, the relative lack of connectivity in pond and lake systems. For example, within a stream network, trout populations that were relatively more connected displayed greater genetic variability and less differentiation than those that were less connected (Neville et al., 2006). Furthermore, severe founder effects were detected in recently established populations, and in streams that regularly dried out (Neville et al., 2006). Genetic structure can be

dependent on the scale of river networks, as in the case of the Idaho giant salamander (*Dicamptodon aterrimus* Cope), which was significantly structured at hierarchical scales of streams, catchments and basins (Mullen et al., 2010). However, the life history of many freshwater species results in structuring not based solely on the dendritic patterns formed by watercourses. Whilst genetic differentiation was found at the catchment scale in the damsel fly *Calopteryx splendens* (Harris), at finer scales both isolation by distance (IBD) following watercourses and Euclidean straight line distances between pairs of sites were supported, demonstrating the importance of overland dispersal in the adult life stage (Chaput-Bardy et al., 2008).

Crayfish are not likely to be constrained entirely by river networks, as over land migration has been reported (Lodge et al., 2000). Haplotypes unique to a drainage basin or groups of adjacent drainage basins appear common in crayfish, with examples in North America, Australia and New Zealand (Fetzner and Crandall, 2003, Hughes and Hillyer, 2003, Smith and Smith, 2009). These studies indicated dispersal among populations within catchments, but little migration by individuals between catchments, reflecting the relatively low levels of dispersal by crayfish over land.

In general, the application of molecular techniques to the study of crayfish has tended to be driven by concern for the conservation of declining native species, rather than exploring the invasion process of non-native species. In particular, the genetic diversity of native crayfish in Europe has received much attention (eg Grandjean et al., 1997, Grandjean et al., 2000, Zaccara et al., 2005). There are, however, some exceptions, where molecular techniques have been applied to invasive crayfish taxa. Comparative work using a Cytochrome c Oxidase subunit I (COI) mtDNA region revealed unique haplotypes of *Orconectes virilis* (Hagen)

introduced in Europe, not yet recorded from within the native range in North America (Filipová et al., 2010). The authors extended this work by identifying various North American crayfish species in Europe through the sequencing of the COI marker (Filipová, 2011). Supported with historical records, microsatellite analyses of North American red swamp crayfish indicated a source and likely sink populations in China (Yue et al., 2010). Evidence of founder effects were found in all populations studied and no support for IBD based on geographical distance was demonstrated, which the authors argued indicated translocation by humans.

There are currently no published studies concerning the population genetics of signal crayfish. What is known of the history of signal crayfish introduction into Europe and the UK is described in Chapter One (pages 32 - 33). To recap, crayfish were officially introduced into hundreds of localities from the late 1970s until around 1990. Since this time signal crayfish populations have become established nation-wide, extending from Cornwall to the Highlands region of Scotland. Although signal crayfish can migrate substantial distances along rivers (Bubb et al., 2004), given the low levels of dispersal between catchments revealed in previous studies of other crayfish species, it is almost certain that the large majority of the spread of the signal crayfish between catchments is attributable to human translocation.

Aims and hypotheses:

This study aimed to investigate for the first time the genetic structure of invasive signal crayfish in the UK, with emphasis on populations found within the Thames river basin. Lotic habitats that formed part of a river network were used, in order to test for network connectivity between populations. However it should be noted that signal crayfish are also found in lentic habitats in the UK.

The secondary nature of the introduction of signal crayfish to the UK, via Sweden, superficially represents the possibility of two founder events having occurred. However, given the scale of introduction to both Sweden from North America and to the UK from Sweden, and the evident success of this species, I hypothesised that signal crayfish in the UK would not show low levels of genetic variability commonly seen in founder events.

Secondly, owing to high levels of stocking and human translocation I hypothesised that signal crayfish populations of the Thames basin would not show evidence of IBD, whether tested using river network distances or Euclidean distances.

In order to test these hypotheses, I sought to employ both mtDNA and microsatellite markers. A combination of the two techniques is desirable, as mtDNA markers confer relatively more ancestral information pertaining to the relationships between individuals and populations, whilst microsatellites allow for a finer, landscape scale analysis to be made and might therefore elucidate patterns between populations within catchments.

Methods

Collection of crayfish samples:

Crayfish were sampled in 2008 and 2009 from a total of 11 populations, 9 of which were located within the Thames basin. Populations were defined at the catchment level (details are given in Table 2.1). Within the Thames basin, sampling was designed to be as even as possible; however, selection was constrained as the majority of samples were provided through routine Environment Agency crayfish surveys. Where possible, a minimum of five adult males and females were collected

from each population. Muscle tissue was either taken from crayfish immediately following capture, or otherwise crayfish were kept whole at -20ºC to await processing. For each individual sampled, a small section of muscle tissue was cut away with a scalpel and stored in ethanol. Tissue samples were taken from either the tail or a cheliped or other walking leg. Size class was categorised as either <20, 20 – 29, 30 – 39 or >40 mm carapace length and sex was recorded. Prepared samples were then frozen at -20ºC until DNA extraction.

Extraction of DNA:

Total DNA was extracted using a Qiagen DNeasy 96 Blood & Tissue kit according to the protocol provided. A small block of tissue approximately 3 x 1 x 1 mm was cut and rinsed thoroughly through four washes of distilled water. Excess water was blotted away and the tissue striated with a scalpel multiple times before addition to the digestion mixture. Upon completion of extraction DNA was stored at -20ºC.

Description of mtDNA markers and associated methods:

Published 'global' primer pairs were employed to amplify two mitochondrial markers. These were a 500- 650 base pair (bp) fragment of the 16S RNA coding region and a 710 bp region of the cytochrome c oxidase subunit I gene (COI). The 16S region primers used were 16Sar (5'-CGCCTGTTTATCAAAAACAT-3') and 16Sbr (5'-CCGGTCTGAACTCAGATCACGT-3'), taken from Palumbi *et. al.* (1991). For the COI region HCO 2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') and LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') were used, following Folmer *et. al.*, (1994).

The Polymerase Chain Reaction (PCR) was used to amplify mtDNA markers. For each individual sample, the reaction mixture for the 16Sar / br primer pair comprised 4.7 µl ddH$_2$O, 1.5 µl 10x PCR reaction buffer (Roche), 1.2 µl MgCl$_2$ stock solution (25mM, Roche), 1 µl of each primer, 0.5 µl dNTPs and 0.1 µl FastStart Taq DNA Polymerase (Roche). The reaction mixture was premixed and added to 5 µl of 1: 10 diluted stock DNA, giving a total reaction volume of 15 µl. The following PCR conditions were found to be optimal: 95 ºC for 15 minutes, proceeded by 35 cycles each containing a denaturing step of 95 ºC for 30s, a primer binding step of 48 ºC for 30s and an extension step of 72 ºC for 30s. This was followed by a final extension step at 72 ºC for 10 minutes before a final 10 minutes at 12 ºC. PCR reactions were carried out in either a Bio-Rad C1000 or S1000 thermal cycler. The protocol for the primer pair HCO 2198 / LCO 1490 was identical to that of 16Sar / br, except for the following modifications. In the reaction mixture 4.4 ml ddH$_2$O and 1.5 ml MgCl$_2$ were used and PCR conditions were set with an annealing temperature of 61 ºC.

In order to check whether PCRs were successful, and to quantify the concentration of product in each reaction-well, all samples were run on a 2 % agarose gel, stained with SYBR® Safe DNA gel stain. Fermentas GeneRuler^{TM} 1kb DNA Ladder was added to the peripheral wells of each row to allow the quantification of PCR product. Gels were run at 70 V for one hour before being visualised in a UV transilluminator. Samples that underwent successful amplification were prepared for commercial sequencing. Excess deoxynucleotides and primers were removed from PCR product using ExoSAP-IT (GE Healthcare) in accordance with the manufacturer's guidelines. The final product was diluted to an approximate concentration of 5 ng µl^{-1}, as specified by Eurofins MWG Operon, who

carried out all Sanger sequencing. For the 16S fragment, the primer 16Sar was supplied for sequencing, and for the COI marker HCO 2198 was used. However, when HCO 2198 reads were problematic, the reverse primer, LCO 1490 was used in its place.

Microsatellite primer design and optimisations:

High quality signal crayfish muscle tissue of an adult female and an adult male was preserved in ethanol. This tissue was sent to Genetic Identification Services (GIS, 9552 Topanga Canyon Blvd, Chatsworth, CA 91311) and enriched microsatellite libraries of di- and tri-nucleotide motifs were produced. Microsatellite repeats within the received libraries were screened by eye and an initial 14 pairs of primers were selected. Selection was based on primer suitability and a preference for long repeat regions. In most cases chosen primers followed those suggested by GIS, whilst the remainder were designed online using the package Primer3 (Version 0.4.0). Primers were synthesised with either TAMRA, HEX or FAM fluorescent dyes to allow multiplex sequencing of microsatellites. After initial attempts at optimisation, a further nine primer pairs were synthesised. The second round of selection was based on the most promising results seen in optimisation, which were all microsatellites of relatively short lengths. Therefore the shortest repeats were chosen.

Qiagen Type-it® Microsatellite PCR kits were used for amplification of microsatellites using the same DNA for which the extraction process was explained in the mitochondrial marker methods. For PCRs a wide range of conditions were tested, which included varying the concentration of DNA, the number of cycles within the PCR, the annealing temperature, whether the reaction was multiplex and whether or not Q Solution (provided by Qiagen to aid problematic PCRs) was added

to the reaction mixture. Furthermore, the concentration at which the product was sequenced varied from neat to 1: 150.

Sequencing was carried out in a 3730 DNA analyzer and output was processed in Genemapper (Version 4). Owing to an inability to score microsatellites in the output data (see results), no statistical analyses were conducted.

mtDNA analyses:

All sequencing reads were checked visually and, where peak data were messy, the results were rejected. BioEdit sequence alignment editor (Version 7.0.5.3) was used for alignment of sequences and creation of reverse complements of LCO 1490 reads when this primer had been used in place of HCO 2198. Aligned and curtailed 16S and COI results were then concatenated to create sequences to be used in subsequent analysis. However, as reported in the results section, the concatenated sequence was not used in all analyses. To aid comparisons with the literature, diversity indices were calculated for the COI sequence only.

Estimation of the genetic diversity of signal crayfish was undertaken based on calculation of sequence diversity (h) and nucleotide diversity (π), both using Arelquin (Version 3.5.1.2). Based on the concatenated sequence the relationship between haplotypes was visualised through construction of a haplotype network in Network and Network Publisher (Versions 4.6.0.0 and 1.1.0.7 respectively), using the median joining option. Median joining networks are able to rapidly process large data sets and multi-state characters are incorporated (Posada and Crandall, 2001). Furthermore, they have been shown to perform well in comparative tests among a range of alternative models (Woolley et al., 2008).

In order to test for isolation by distance among populations, the software IBD 1.52 (Bohonak, 2002) was used. Genetic distance was first calculated in GenAlEx (Version 6.41). I used straight line Euclidean distance as well as the shortest distance along the river network to measure distances among populations of the Thames basin. Furthermore, straight line Euclidean distance was used for analysis among all 11 populations. 10,000 randomisations were used in each case. For populations that were made up of more than one site within a catchment, distances were either measured from the site furthest downstream, where sites were spread along a single river, or from the first common downstream confluence, where sites were located on separate river branches within a catchment. To investigate whether male and female crayfish showed differing levels of gene flow between populations a Mann-Whitney test was used to compare the genetic distance of male and female crayfish among all populations. Finally, to test for partitioning of genetic variance in and among populations at catchment and regional scales, Analysis of Molecular Variance (AMOVA) was carried out in GenAlEx using 9,999 permutations. Genetic variance was tested at three hierarchical scales: between individuals within populations, between individuals among populations, and between individuals of populations belonging to three regions. Regions were assigned based on drainage basin identity, with all populations of the Thames basin considered as belonging to a single region.

Results

mtDNA:

One hundred and nine individuals were successfully sequenced for 16S and COI mitochondrial markers. The 16S and the COI regions gave sequences 262 and 596 bp in length respectively following editing. Thus, a concatenated total length of 858 bp was available for analysis. A total of 16 haplotypes was recorded among the 11 populations (Figure 2.1). The 16S region was effectively redundant in the detection of haplotypes, with all 16 being represented by the COI region alone. In order to allow comparison with published studies, molecular diversity indices were calculated for the COI region, and not the fully concatenated data inclusive of the 16S marker. Genetic diversity was variable among populations, with a range of one to seven haplotypes (Table 2.2). However, average gene diversity (h) was generally high: values varied from 0.000 to 0.944, with a mean of 0.509; and nucleotide diversity (π) was also high, with values of 0.000 to 0.025, mean 0.009. Five of the 11 populations contained one or more unique haplotypes, with a maximum of four in the Loddon population.

Published haplotypes that match COI haplotype sequences recorded in this study were identified using nucleotide BLAST searches online. Five matches revealed identical sequences to existing signal crayfish haplotypes, while 11 sequences were found to be novel. A summary of the BLAST results is given in Table 2.3. Identical matches for haplotypes were identified from across the invasive range in Scotland, the Czech Republic and Hungary and within the native range in both California and Oregon of the USA. Haplotype 8 was a direct match of a haplotype of *Pacifastacus leniusculus klamathensis* (Assession number JF437999.1).

The haplotype network for all samples based on the concatenated sequence data is shown in Figure 2.2. Owing to the evident mixing of haplotypes that has occurred with the introduction of crayfish into the UK, the network is not arranged with any reference to localities where individuals were collected from. The network supported the result that haplotype 8 was distinct from all others, with a minimum of 33 mutations required to reach an extant observed haplotype.

Irrespective of whether Euclidean or river network distance was used, there was no indication of IBD among signal crayfish populations of the Thames basin (Table 2.4, Figure 2.3). Equally, IBD based on Euclidean distance among all 11 populations was not supported. Furthermore no difference in genetic distance was found between male and female crayfish (Mann-Whitney $W = 2997.0$, $n_1 = n_2 = 55$, $P = 0.72$). Crayfish populations did however show significant structuring, both within the Thames basin and at a regional scale (Table 2.5), with 5% of variation distributed among regions, 37 % among populations and 58% within populations.

Microsatellites:

Despite the testing of a total of 23 candidate primer pairs under a range of PCR conditions, satisfactory sequence data could not be obtained. Many primers produced no results. Others gave clear evidence of microsatellite alleles; however the stutter of output data was such that confident decisions on allele size could not be made. Consequently data were not suitable for further analysis.

The reasons behind the atypical microsatellite results are unclear. In contrast to the wealth of publications based on mitochondrial markers in crayfish, published work reporting the use of microsatellites is markedly rare. Doctorate students of Keith Crandall (a leader in crayfish genetics) attempted microsatellite work in

crayfish, but encountered difficulties and subsequently did not publish their results (Crandall, personal communication). Signal crayfish have a diploid number of chromosomes of 186; a very large number for an animal (Komagata and Komagata, 1992) (Incidentally, *Pacifastacus leniusculus trowbridgii* has perhaps the highest chromosome number within the animal kingdom, with a diploid number of 376 (Niyama, 1962)). This unusually high chromosome number may be a result of historic polyploidy events (Lecher et al., 1995), and this might help to explain the difficulties encountered. Whole genome sequencing of crayfish is required, for a better understanding of the difficulties experienced when attempting microsatellite work.

Table 2. 1. Location and composition of each population of signal crayfish, grouped at catchment level. The number (n) of samples per population, sites comprising each population (with number of samples per site in parentheses) and their longitude and latitude are given.

Population / Catchment		n	Sites included	Latitude and Longitude	
1	Wey	10	Ock (10)	51° 11' 2.367" N	0° 37' 12.098" W
2	Loddon	9	Whitewater (6)	51° 21' 0.531" N	0° 56' 20.348" W
			Hart (3)	51° 17' 10.413" N	0° 52' 32.471" W
3	Darent	9	Darent (9)	51° 18' 24.49" N	0° 12' 41.97" E
4	Roding	9	Roding (5)	51° 41' 10.530" N	0° 14' 37.433" E
			Roding (4)	51° 39' 9.345" N	0° 7' 13.79" E
5	Lee	10	Lee (4)	51° 48' 14.403" N	0° 3' 59.791" W
			Lee (4)	51° 48' 9.413" N	0° 4' 21.204" W
			Lee (2)	51° 48' 29.940" N	0° 1' 14.312" W

Continued overleaf.

Table 2. 1 continued.

	Population / Catchment	n	Sites included	Latitude and Longitude	
6	Colne	10	Colne (4)	51° 41' 58.031" N	0° 20' 59.835" W
			Colne (1)	51° 40' 46.378"N	0° 22' 8.258" W
			Gade (4)	51° 39' 24.351"N	0° 25' 29.966" W
			Gade (1)	51° 42' 39.399"N	0° 26' 43.804" W
7	Thame	10	Thame (10)	51° 45' 5.27"N	1° 1' 59.59" W
8	Evenlode	10	Glyme (10)	51° 50' 29.86"N	1° 21' 40.36" W
9	Cherwell	9	Cherwell (10)	52° 10' 18.608"N	1° 12' 32.952" W
Non Thames basin:					
10	Tern	9	Tern (9)	52° 51' 13.69"N	2° 34' 33.14" W
11	Rother (West)	9	Rother (9)	51° 0' 13.522"N	0° 53' 2.433" W

Table 2. 2. Nucleotide diversity indices of signal crayfish COI sequences by population. *k*, number of haplotypes (number of which were unique in parentheses), *h*, sequence diversity (±1SD), *π*, nucleotide diversity (±1SD) within a population. The number (n) of individuals per haplotype for a given site are also represented in brackets following sequence codes.

Population	*k*	*h*	*π*	n
Wey	1 (0)	0.000 ± 0.000	0.000 ± 0.000	2 (10)
Loddon	5 (4)	0.806 ± 0.120	0.016 ± 0.009	3 (4), 7 (2), 9 (1), 11 (1), 16 (1)
Darent	3 (1)	0.556 ± 0.165	0.012 ± 0.007	3 (6), 5 (2), 15 (1)
Roding	7 (3)	0.944 ± 0.070	0.025 ± 0.014	1 (1), 3 (1), 4 (2), 5 (2), 8 (1), 12 (1), 14 (1)
Lee	3 (0)	0.378 ± 0.181	0.002 ± 0.002	1 (1), 2 (1), 4 (8)
Colne	4 (0)	0.533 ± 0.180	0.006 ± 0.004	1 (7), 2 (1), 3 (1), 4 (1)
Thame	4 (2)	0.711 ± 0.118	0.018 ± 0.010	1 (1), 3 (5), 6 (3), 13 (1)

Continued overleaf.

Table 2. 2 continued.

Population	k	h	π	n
Evenlode	4 (1)	0.778 ± 0.091	0.011 ± 0.006	1 (4), 2 (3), 5 (2), 10 (1)
Cherwell	2 (0)	0.389 ± 0.164	0.008 ± 0.005	2 (7), 5 (2)
Non Thames basin:				
Tern	2 (0)	0.556 ± 0.090	0.004 ± 0.003	1 (5), 2 (4)
Rother (West)	1 (0)	0.000 ± 0.000	0.000 ± 0.000	1 (9)
Total	16	0.821 ± 0.019	0.010 ± 0.005	

Table 2. 3. Results of BLAST searches for individual haplotype sequences. Codes in bold represent haplotypes not exactly matched. Maximum identity scores of the closest matches are given along with the region of origin. * Filipová, 2011, [†] Soroka, 2002, [‡] Toon et al., 2010.

Haplotype code	Closest match	Max ID	Origin of closest match
1	JF437997.1*	100	Scotland, UK
2	JF437995.1*	100	Czech Republic
3	JF437997.1*	98	Scotland, UK
4	JF437998.1*	100	Oregon, USA
5	JF437996.1*	100	Hungary
6	AF525226.1[†]	98	Poland
7	EU921148.1[‡]	99	California, USA
8	JF437999.1*	100	California, USA
9	JF437998.1*	99	Oregon, USA
10	EU921148.1[‡]	99	California, USA
11	EU921148.1[‡]	99	California, USA
12	AF525226.1[†]	99	Poland
13	AF525226.1[†]	98	Poland
14	JF437997.1*	97	Scotland, UK
15	JF437997.1*	98	Scotland, UK
16	JF437997.1*	97	Scotland, UK

Table 2. 4. Mantel test output for IBD among the sampled populations of crayfish. 10,000 randomisations were made in each case.

Measure	Z	r^2	P
Thames basin			
Geographic distance	370660.8	0.003	0.618
River network distance*	436234.8	0.043	0.903
All populations			
Geographic distance[†]	641944.7	0.012	0.726

Table 2. 5. Hierarchical AMOVA results for crayfish populations. The two populations in catchments separate from the Thames basin, the Rother and Tern, were designated as distinct regions. * = $P \leq 0.05$, *** = $P \leq 0.001$.

Source of Variation	d.f.	Sum of squares	% of variation	Statistic	P
Among regions	2	4.211	5	Φ_{RT}	**0.047***
Among populations	8	14.585	37	Φ_{PR}	**<0.001***
Within populations	93	23.800	58	Φ_{PT}	**<0.001***

Figure 2. 1. Haplotype frequencies for the 11 populations of signal crayfish included in the study. Populations are numbered following Table 2.1. Each colour represents a distinct haplotype and wedge size is proportional to haplotype frequency within a population. Colours refer to the following haplotype codes: yellow, 1; red, 2; light blue, 3; green, 4; purple, 5; salmon, 6; white, 7; grey with dots, 8; navy, 9; pink, 10; grey blue, 11; dark green, 12; orange, 13; brown, 14; turquoise, 15 and blue, 16. Small scale map drawn from Edina Digimap (© Crown Copyright Ordinance Survey. An EDINA Digimap/JISC supplied service).
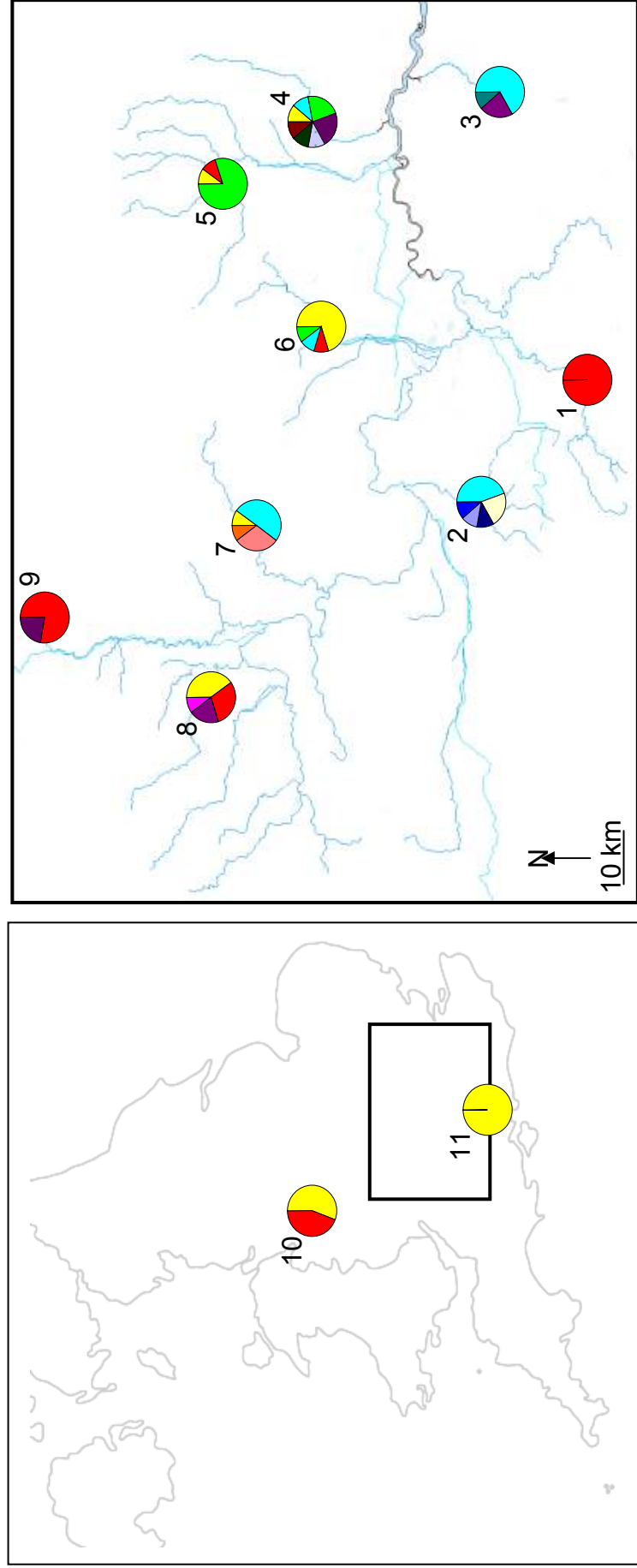
Figure 2. 2. Haplotype network for *Pacifastacus leniusculus* of 11 UK populations. Numbers in bold refer to haplotype code (see Table 2.3), numbers in red represent the number of mutations between nodes and size of a given circle is proportional to the frequency of a haplotype.
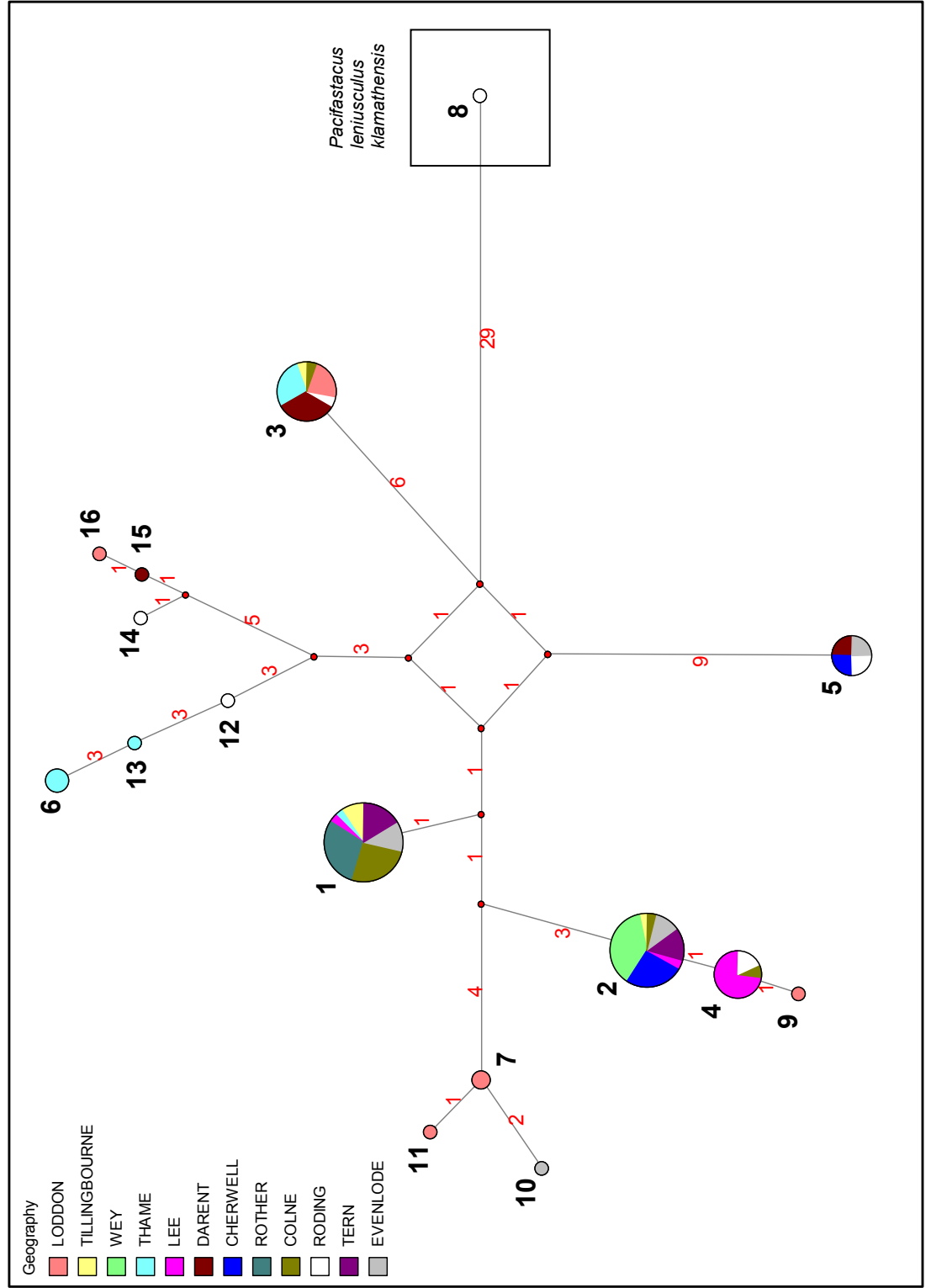
Figure 2. 3. Scatter plots of the geographic and genetic distances between pairs of populations. a) Genetic distance by Euclidean distance for populations of the Thames basin. b) Genetic distance by network distance for populations of the Thames basin. c) Genetic distance by Euclidean distance for all populations.
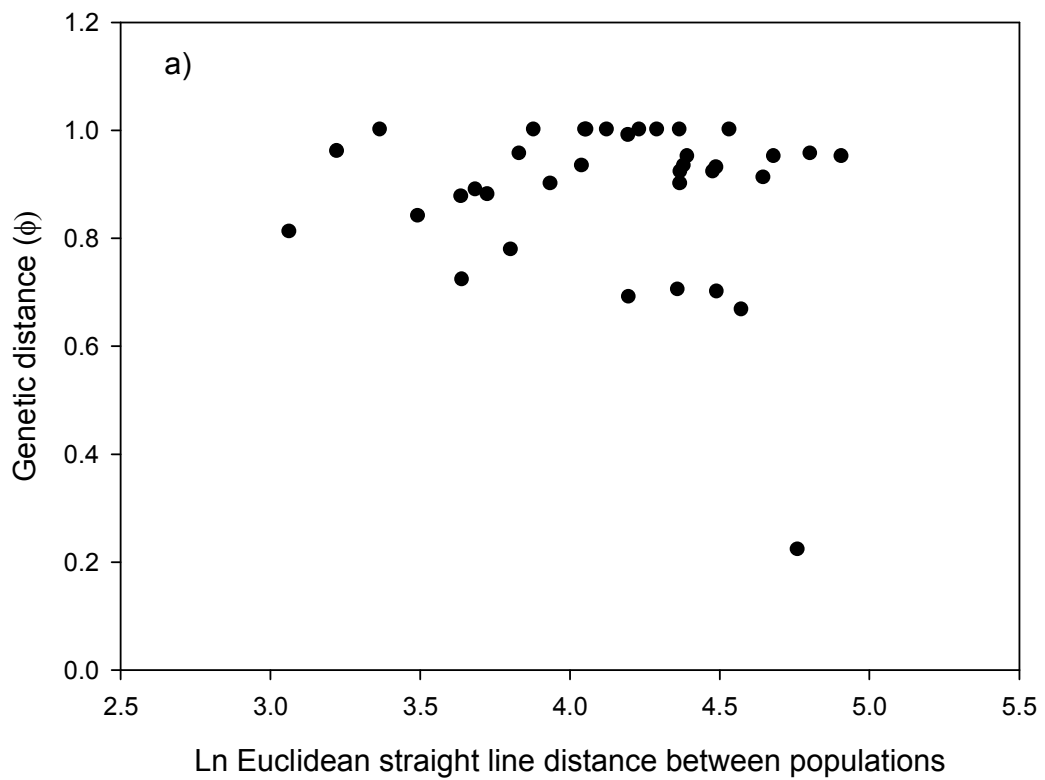
Figure 2. 3 continued.
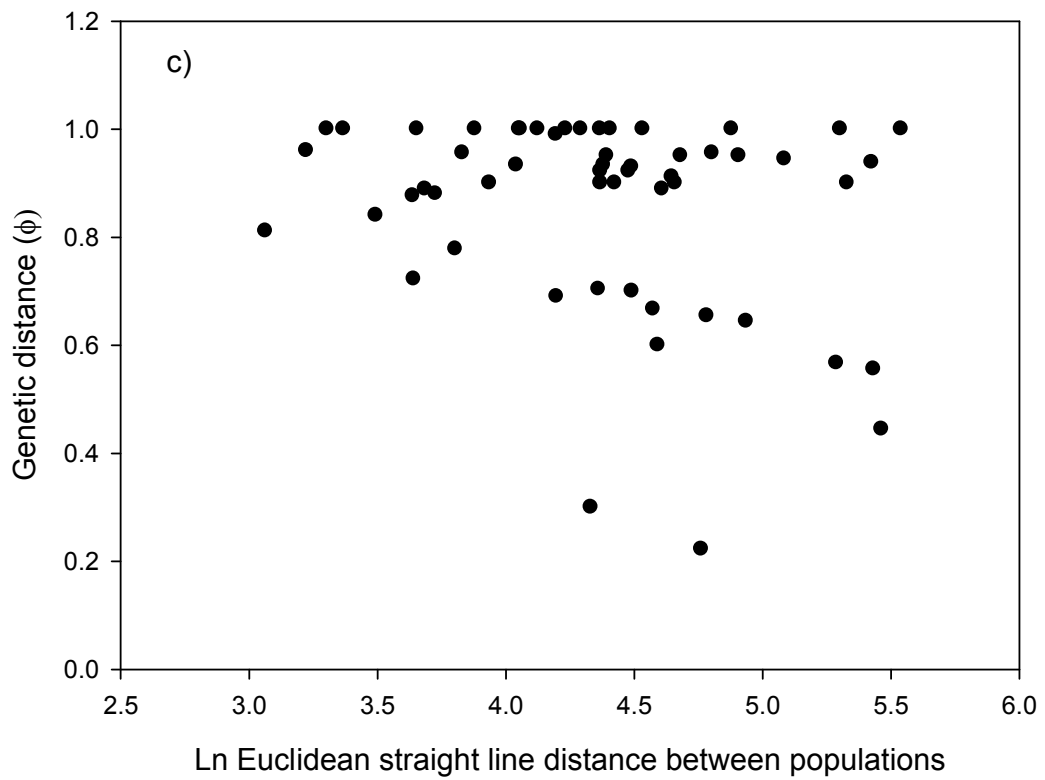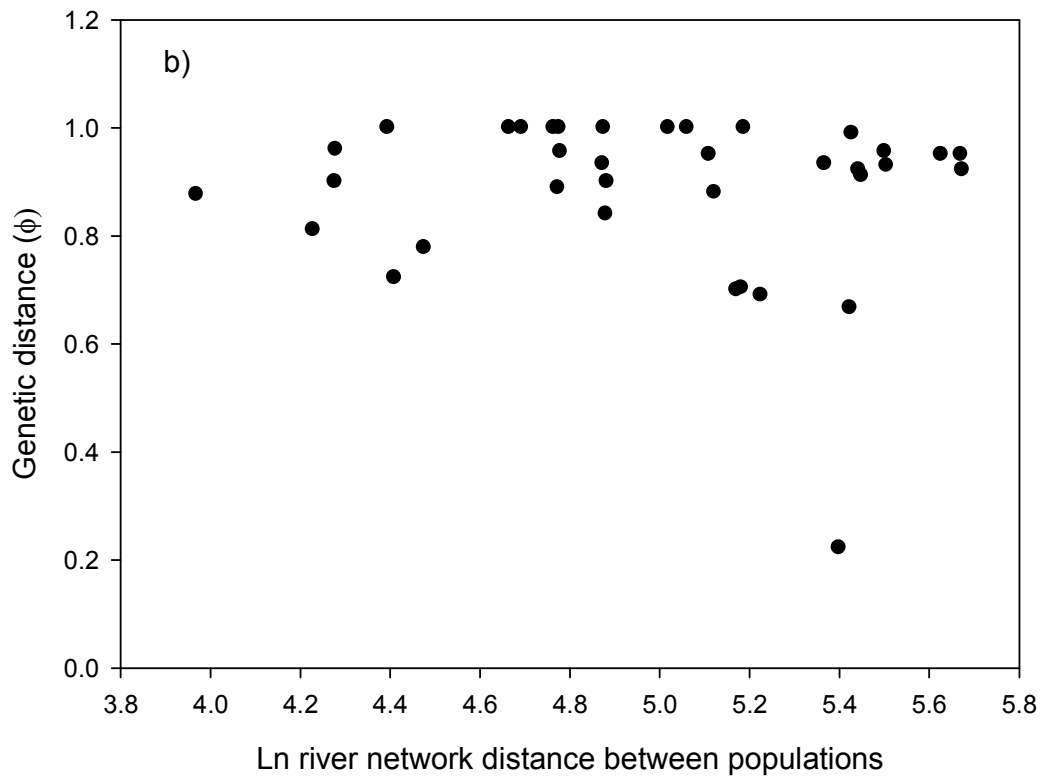
Discussion

The results confirmed the first hypothesis; signal crayfish showed high overall genetic diversity, in line with expectations based on their deliberate and extensive introduction into the UK. Not only was overall diversity high, but some individual populations exhibited surprisingly high levels of genetic diversity. The mean COI sequence diversity of 0.514 was approximately twice that seen in native white clawed crayfish in Italy (0.270; Zaccara et al, 2005) and the mean nucleotide diversity of 0.009 a third greater than that of native *Paranephrops planifrons* (White) in New Zealand (0.006; Smith and Smith, 2009). Although mean sequence diversity of *P. planifrons* (0.616) was greater than that of signal crayfish, the highest sequence diversity of a single population of signal crayfish was 0.944, a value apparently not matched in any published crayfish genetic work. Therefore, not only do the results prove that a founder effect did not occur, but in fact it is quite possible that, through admixture, some signal crayfish populations in the UK might show greater diversity than populations within the natural range. Whilst this is speculation and population genetic work in the natural range is required, it is clear that the normal characteristic of crayfish populations in their native ranges, where catchment watersheds represent barriers to dispersal (Fetzner and Crandall, 2003, Hughes and Hillyer, 2003, Smith and Smith, 2009), was overcome through the human mediated nature of signal crayfish introduction into the UK.

The haplotype network did not provide evidence that any of the observed haplotypes evolved since the introduction of signal crayfish into the UK. Five haplotypes gave identical matches to haplotypes seen across Europe and North America and were distributed throughout the network. These represent ancestral

haplotypes which evolved in North America rather than having derived in the UK. Furthermore, barring the case of haplotype 11, individuals with haplotypes peripheral in the network (that were different by a single mutation to the extant haplotype from which they evolved) did not show geographic patterns congruent with the haplotypes having evolved in British waters, i.e. no phylogeographic signature was found.

Comparisons with published sequences and the haplotype network revealed one haplotype belonging to *Pacifastacus leniusculus klamathensis*, which was distinct from all other haplotypes that represented the nominate subspecies *Pacifastacus leniusculus leniusculus*. The individual exhibiting this haplotype therefore represents the first genetic evidence of a second subspecies of *Pacifastacus leniusculus* having been introduced into Europe from North America. It is highly likely that this haplotype has introgressed into the *Pacifastacus leniusculus leniusculus* subspecies, as all other individuals within the population from which it was taken displayed haplotypes of *Pacifastacus leniusculus leniusculus*. The distinctness of this haplotype provides support for subspecies classification, in contrast to doubts previously raised owing to morphological overlap within introduced ranges, both in North America and in Japan (Riegel, 1959, Kawai et al., 2004). Introgression will almost certainly have occurred within Europe, as the provenance of the *P. l. klamathensis* haplotype was Oregon, whilst the remainder of haplotypes belonged to a group seemingly derived in California. Intriguingly, the only available record of signal crayfish introduction into Europe from Oregon is that of illegal stocking into France (Souty-Grosset, 2006). It is therefore likely that illegal stockings were also made into Sweden or the UK from Oregon.

The second hypothesis of the study was supported - crayfish populations did not show evidence of IBD. Current haplotypes evolved within the native range of

signal crayfish. As a consequence of the mixing of crayfish of various provenances within the introduced range, any evidence for IBD based on mitochondrial DNA that may have existed in North American populations was lost. The result is a distribution of haplotypes among populations that reflects mixed origins. Based on the historical record, a wide-scale mixing of crayfish of at least four US localities occurred in Sweden (Souty-Grosset, 2006). This figure may be higher owing to possible illegal introductions.

Significant genetic structuring was seen within and among populations, and furthermore, among regions. This may simply reflect the high level of recent seeding of crayfish into the UK and insufficient time for haplotypes to have reached equilibrium. However it also indicates genetic separation of populations, supported by the fact that five populations contained one or more private haplotypes. As these private haplotypes were unlikely to have evolved in Britain, genetic structuring reflects the random distribution in the UK of what must have been relatively rare haplotypes within incoming groups of crayfish originating from Sweden and any other source populations.

Although possibly a result of bias in sampling, the two populations that comprised crayfish exhibiting a single haplotype might represent expanding haplotypes which are experiencing particular success in these areas. Presumably these represent either small-scale human mediated introductions or cases of 'natural' colonisation. Illegal stocking or translocation of crayfish by humans is likely to lead to founder effects, as such introductions are likely to involve a small number of individuals, unlike the scale seen in official introductions, where hundreds and in some cases thousands of crayfish were introduced (Holdich, 1995). Alternatively,

colonisation by a small number of individuals from nearby populations could have led to founder effects such as these occurring.

This study represents the first population level genetic characterisation of an invasive crayfish species in the UK. The results confirmed expectations of genetic structure. The scale of introduction has been such that signal crayfish did not show depauperate diversity and as their distribution throughout the UK has in general been attributable to human translocation, no evidence of IBD was discovered. Diversity within some populations surpassed expectations and displayed markedly high levels, suggesting that non-native species might in some circumstances see increased diversity in populations within their introduced ranges. Considering the high levels of genetic diversity seen at multiple localities, from a management perspective the outlook is bleak. Not only are signal crayfish physically well established in the UK, but clearly they are also well established in a genetic sense.

Chapter Three: Signal crayfish and the benthic macroinvertebrate community of chalk streams

Introduction

Recap from the introductory chapter:

Chapter One outlined a diverse range of impacts attributable to invasive species. Not only are they capable of extirpating or even bringing native taxa to extinction, but invasive species are known to cause shifts in community composition, mediate cascading effects and modify ecosystems across trophic levels through direct and indirect means. Owing to continental separation across millennia, freshwater ecosystems are particularly vulnerable to invasion by non-indigenous species. One such invasive group is crayfish.

Because of their wide distribution, their role in extirpations of indigenous crayfish and possible status as keystone species, invasive crayfish have been the focus of much research. Introduced crayfish can reduce both the diversity and densities of various flora and fauna and furthermore influence all trophic levels in freshwater food webs, both directly and indirectly. Signal crayfish are the most widely distributed non-indigenous crayfish and exhibit stronger interactions with native biota than the native crayfish they replace. They appear to play a key predatory role; favouring less mobile, benthic macroinvertebrate taxa in particular. Evidence suggests that the predatory status of signal crayfish shows ontogenetic shift, however the direction of such shifts are not consistent.

Rationale:

Despite the above findings, to my knowledge no published study has investigated the relationship between crayfish and the macroinvertebrate community across separate, naturally occurring water bodies (be they lentic or lotic), using natural variation in the density / biomass of crayfish. This approach represents a natural progression from previous work dominated by small scale enclosure / exclosure and artificial pond and channel experiments. The exceptions, where natural populations of crayfish and the invertebrate community have been studied, involve either single lake surveys with a temporal aspect (Wilson et al., 2004, McCarthy et al., 2006) or crayfish density or presence / absence along a single stream or river (Charlebois and Lamberti, 1996, Crawford et al., 2006). Therefore, the wider scale generality of patterns, so far described, is largely unknown.

Are the effects of invasive crayfish strong enough to be distinguished from all other variables that may influence aquatic communities among separate water bodies? In communities like streams, where the dispersal of invertebrates is high and disturbances frequent and unpredictable, then effects of predation may be obscured owing to rapid recolonisation (Palmer et al., 1996). Streams can also show diverse environmental conditions, even at a small scale, and macroinvertebrate diversity may be quite different in adjacent streams or even within streams, owing to local factors (Townsend et al., 1983, Sponseller et al., 2001). These considerations might partly explain the discrepancy between the large volume of published experimental work outlining impacts of crayfish, and the relative paucity of literature based on surveys supporting the experimental data.

The study sites used in this study were all chalk streams; characterised by year-round relative hydrological stability, and diverse, productive aquatic communities (Berrie, 1992). Chalk streams are a rare habitat type of conservation importance. In Europe they are protected under the Habitats Directive (92/43/EEC); highlighting the significance of any impacts of invasive crayfish. However, crayfish should not be considered in isolation when evaluating the effect of predation on the macroinvertebrate communities of these ecosystems. At shallow riffle-type sites, bullhead can be significant predators (Mann, 1971, Woodward et al., 2008). Abundances of seven macroinvertebrate taxa were reduced in bullhead treatments of a stream enclosure / exclosure experiment (Dahl, 1998). There are numerous similarities between these two organisms, and interactions between them are the basis of Chapter Five of this thesis (for detailed background see pages 146 -147). Their diet is likely to overlap considerably, with Chironomidae and Ephemoptera being important prey items of both species (Dahl, 1998, Stenroth and Nystrom, 2003). Furthermore, invasive signal crayfish themselves have been shown to be an important prey item of bullhead, being the third most numerous prey item in a stream enclosure experiment (Dahl, 1998). The importance of crayfish ontogeny is apparent, with only smaller crayfish being potential prey items of bullhead owing to gape limitation.

I hypothesised that across geographically proximate chalk streams in the south of England, increasing densities of signal crayfish would correlate with decreasing invertebrate abundance and total biomass. I expected relatively more exposed (i.e. non-sediment dwelling) and slow moving taxa in particular to show such negative associations. As taxon richness has been shown to be reduced by signal crayfish, I

also predicted a reduction in macroinvertebrate diversity with increasing abundances of crayfish.

Some evidence suggests that younger crayfish are more predatory than older conspecifics (page 25 of the introductory chapter), therefore sites with relatively higher abundances of smaller individuals should reveal stronger negative relationships between crayfish and the macroinvertebrate community.

Finally, I expected measures of the macroinvertebrate community not only to relate to the abundance and biomass of crayfish, but also to that of bullhead. Owing to interactions with bullhead the foraging behaviour of smaller and larger crayfish may differ, with resulting differences in crayfish impacts on invertebrate groups.

Methods

Eight sites were selected on eight chalk streams and rivers in the Thames basin in the south east of England (Figure 3.1). The sites were chosen for their proximity to one another, in order to minimise variation in water chemistry or other variables that might explain macroinvertebrate diversity and abundances and to ensure the biota of all sites belonged to the same regional pool of species.

Site selection was conducted in late August / early September, 2008. The intended survey required a natural gradient of crayfish densities, so that regression-based statistics could be used. In order to verify that a gradient would be achieved, two field workers carried out timed searches of half an hour at candidate sites. Timed searches involved manual searching of likely refugia of crayfish with hand-nets. Crayfish were collected for the duration of the search, after which they were measured and then returned to the water. An approximate measure of the stream wetted width, taken to the nearest metre, was made using a tape measure. The

degree of shading at sites was estimated as a percentage of canopy cover attributable to trees or shrubs. A crude measure of the dominant substrate was made using a simplified version of the Wentworth Scale (Wentworth, 1922), taken from standard crayfish monitoring protocol (Peay, 2003): sand (<2 mm), gravel (2mm – 1.5 cm), pebble (>1.5 cm – 6.5 cm) and cobble (>6.5 cm). The site characteristics are outlined in Table 3.1.

In order to quantitatively sample signal crayfish and bullhead, a modified Hess sampler was used (see below). As dense stands of emergent riparian vegetation impeded the use of the modified Hess sampler, sites were selected with minimal emergent riparian vegetation and sampling was conducted in October, when riparian vegetation had died back from its summer maximum. Sites were also excluded if signs of extensive burrowing by crayfish were present. Following these guiding principles the accuracy of measuring crayfish densities was maximised. This study aimed to investigate impacts of signal crayfish on the benthos within the river channel, and not the invertebrate community associated with riparian vegetation.

A modified Hess sampler was used to quantify crayfish density rather than use of trapping and catch per unit effort data. Trapping of crayfish can give inaccurate relative density values and furthermore a misleading impression of the demographic structure of crayfish populations. Temperature affects the activity levels of crayfish (Bubb et al., 2004) and is therefore likely to bias catch. Futhermore, trapping has been shown to be biased towards the capture of large individuals (Usio et al., 2009). As an express aim of this study was to consider the role of life stage of crayfish in determining their impacts, trapping was rejected as a viable method for sampling crayfish.

Based on the measured wetted width, the sampling area of each site was standardised to approximately 50 m^2. For example, a site with a wetted width of 5 metres would have a length of 10 metres. This area was divided into a grid of metre units. 25 points within this grid were selected at random using the random data feature in Minitab (Version 14.1). 20 points were generated to assign sampling locations for the modified Hess sampler and five for Surber sample positions. The two were mutually exclusive as sampling using either piece of equipment disturbs the sediment, precluding use of the other.

The modified Hess sampler (Figure 3.2) was constructed out of a metal bin, with a basal area of 0.13 m^2. The base of the bin was removed and windows 17 cm in height and 30 cm wide were cut into opposite sides, 10 cm from the base. Plastic mesh (mesh size 4 x 12 mm) was used to cover one of the windows. A four sided net, approximately 27 cm long, was fashioned from the same mesh, the end of which was attached to the screw top lid of a plastic pot. The sampler was used in the field as follows. Firstly it was placed down onto the substrate with the netted window pointing upstream. On placement, the sampler was rotated back and forth, to embed the sampler so that gaps between the streambed and the edges were kept to a minimum. Once securely in place, any large stones were carefully removed from inside the sampler. The remaining substrate was scooped with the current back through the net and into the pot until all loose substrate had been removed. The basal area was therefore destructively sampled to ensure that all crayfish and bullhead present were sampled. Although small 0 + crayfish may have been able to pass through the mesh, this was minimised by the rapid flushing into the pot. The contents of the plastic pot was emptied into a bucket and processed so that any crayfish and / or bullhead present were accounted for. Quantified sampling of the

macroinvertebrate community was achieved with a Surber sampler (sampling area 0.0625 m^2, mesh size 330 µm). For each Surber sample taken, the stream substrate was agitated for one minute.

Sampling was carried out across 12 consecutive days in October, 2008 to reduce any confounding effect of temporal variation in the macroinvertebrate assemblage. In order to minimise disturbance of the reach, samples were collected from downstream to upstream. Modified Hess and Surber samples were collected concurrently, dependent on the order the sample locations appeared in, as sampling progressed upstream. Surber samples were stored in plastic bags and frozen at – 20 °C on return to the laboratory. Crayfish and bullhead were measured (carapace length and total length respectively) using vernier callipers prior to being returned to the water. Finally, stream depth and flow readings were made adjacent to the five points where Surber samples were taken.

In the laboratory Surber samples were thawed and washed through a 500 micron sieve before sorting. All invertebrates were removed and identified to the lowest taxonomic level possible. The invertebrates were then photographed on a background of graph paper divided into 1 mm units. These images were used to measure invertebrates in the open source software ImageJ and length-mass regression equations from the literature were used to derive biomass (Calow, 1975, Benke et al., 1999, Baumgartner and Rothhaupt, 2003, Hall et al., 2006, Miyasaka et al., 2008, Edwards et al., 2009). Individual crayfish biomass was calculated using an allometric equation derived from my own data: $y = 3.215x - 0.5913$, where y equals the $\log_{10}$ wet mass (g) and x the $\log_{10}$ carapace length (cm) of crayfish (r^2 0.97; $F_8 = 272.17$; $P < 0.001$). Bullhead wet weight was derived using a published regression (Edwards et al., 2008).

Analyses:

Before investigating how measures of crayfish and bullhead correlated with measures of the benthos, individual-based rarefaction curves were generated based on total macroinvertebrate counts (Figure 3.3) in order to determine whether the communities of the sites had been sufficiently sampled. Total individual counts were generated by pooling all individuals from the five Surber samples taken.

As well as species richness, the Simpson diversity index was used as a measure of diversity. It is expressed as a reciprocal so that larger values represent greater diversity. The Simpson index was chosen for its emphasis on dominance over richness; appropriate owing to the highly uneven nature of abundances of individual taxa observed within the benthos. Furthermore it is less sensitive to sample size than the commonly used alternative, the Shannon index (Magurran, 1988). Crayfish were not included in these measures of the benthos.

To meet assumptions of normality and homogeneity of variance, $\log_e(x)$, $\log_e(x + 0.1)$ and $\log_e(x + 1)$ transformations were used. Best subset regressions were carried out in Minitab (Version 14.1). Variance inflation factors were included in output to ensure predictor variables were not co-linear.

Results

The chosen sites provided a range of crayfish densities, closely following in relative terms the absolute abundances produced by timed manual searches. Where no crayfish are found by manual search, it is highly likely that no crayfish will be found through quantitative measurement. Indeed, three sites yielded no crayfish by either

manual search or use of the modified Hess sampler. For the five sites where crayfish were present, relative densities as determined by timed manual searches correlated strongly with absolute densities ($F_{1,3}$ = 51.85, P = 0.006, $r_{(adj.)}^2$ = 92.7). At sites where crayfish were present, densities ranged from 0.8 to 3.5 m^{-2}, with a mean density of 1.8 m^{-2}; a full table of total abundance and biomass values for crayfish and bullhead of each site is given in Table 3.2. In order to assign a functionally meaningful size threshold representing smaller and larger crayfish, carapace length was pooled for all sites and a histogram plotted (Figure 3.4). Based on this histogram a carapace length of 20 mm was set as the threshold to divide crayfish into small and large size classes. Although confounded by site, this point likely represents a division between 0+ and 1+ crayfish. Similar size-divisions between first and second year cohorts have been seen for signal crayfish in Swedish ponds, an English river and a Japanese marsh (Abrahamsson, 1971, Guan and Wiles, 1999, Usio et al. 2009).

No relationship was seen between measures of crayfish density or biomass and the taxon richness of the sites. However, there was a strong positive relationship between site width and taxon richness (Figure 3.5; $F_{1,7}$ = 39.30, P = 0.001, $r_{(adj.)}^2$ = 84.5). Crayfish and bullhead density correlated positively with the reciprocal Simpson diversity index (Table 3.2). No significant relationships were found between crayfish and bullhead. As bullhead density appeared to positively correlate with width ($F_{1,7}$ = 4.40, P = 0.081, $r_{(adj.)}^2$ = 32.7), this multiple linear regression was repeated with width substituted for bullhead density. The result was non-significant (P = 0.161). Log Chironomidae abundance was negatively correlated with crayfish; this was non-significant for crayfish density, but significant for crayfish biomass (Table 3.2, Figure 3.6). In contrast log Chironomidae abundance was positively

correlated with bullhead, both when bullhead were represented by density and biomass (Table 3.2, Figure 3.6). When crayfish life stage was taken into account, only the large size-class showed a significant relationship with log Chironomidae abundance (Table 3.2, Figure 3.7). The greatest amount of variance in Chironomidae abundance was explained when both crayfish biomass and bullhead biomass were included in a multiple linear regression (Table 3.2). The relationships between crayfish and both log *Asellus aquaticus* (Linnaeus) and log *Baetis sp* abundance were similar to that seen for Chironomidae; crayfish biomass showed a stronger negative relationship than did crayfish density and the relationship held for large crayfish but not for small crayfish size classes (Table 3.2, Figures 3.7, 3.8, 3.9 and 3.10). Log Oligochaeta abundance was positively related to measures of crayfish and bullhead; borderline in significance for density, but strongly significant for measures of biomass (Table 3.2). This relationship was strengthened by considering only log large crayfish biomass (Table 3.2). Table 3.3 gives a summary of results by crayfish measure (density or biomass) and size class.

Invertebrate biomass was overwhelmingly dominated by *Gammarus pulex* (Linnaeus), with a mean percentage of the total invertebrate biomass of 66%. No other taxon saw a mean percentage of total biomass in double figures. Measures of crayfish density and biomass displayed no relationship with total invertebrate biomass, however when *G. pulex* was excluded from the measure, a different picture emerged. Removal of *G. pulex* led to a reduction in the dominance of the total invertebrate biomass by any single taxon. The resulting five most dominant taxa, in order, were Hydropsychidae, 27%, *Baetis sp*, 14%, Oligochaeta, 10%, Chironomidae, 9 % and Hirudinea, 8%. Log total macroinvertebrate biomass exclusive of *G. pulex* correlated positively with site width and negatively with crayfish density and

biomass (Table 3.4). These relationships held where measures of 1+ crayfish density and biomass were used, however whilst regressions incorporating 0+ crayfish where significant overall, 0+ crayfish density and biomass were not themselves significant factors within the regressions. Regarding individual taxa, biomass results reflected those for abundance, with two exceptions – no relationships between crayfish and Chironomidae or Oligochaeta biomass were found to be significant.

Table 3. 1. Basic site information for the sites used in the survey. Values of depth and flow are given with error (± 1 SE).

Site	Approximate width (m)	Depth (cm)	Flow (m s⁻¹)	Shading (%)	Dominant substrate
Ash	8	10.3 (±1.2)	0.11 (±0.02)	70	pebble
Beane	4	11.2 (±0.6)	0.13 (±0.04)	50	pebble
Mimram	10	15.8 (±1.6)	0.31 (±0.04)	75	gravel
Princey Brook	4	31.1 (±1.6)	0.04 (±0.01)	95	gravel*
Rib (Wadesmill)	9	16.1 (±2.6)	0.22 (±0.08)	80	gravel
Stanstead Brook	3	approx 10	0.13 (±0.04)	95	gravel
Stort	7	14.4 (±1.4)	0.12 (±0.02)	70	gravel
Ver	6	11.0 (±1.2)	0.37 (±0.03)	0	gravel

*layer of silt covered substrate

Table 3. 2. Signal crayfish and bullhead total abundance and biomass (wet weight) for each of the sites sampled. Values of crayfish biomass were derived using the equation given on page 74, while bullhead biomass was calculated using the regression of Edwards et al. (2008).

Site	Crayfish density ($\#\ m^{-2}$)	Crayfish biomass ($g\ m^{-2}$)	Bullhead density ($\#\ m^{-2}$)	Bullhead biomass ($g\ m^{-2}$)
Ash	0.00	0.00	4.67	16.22
Beane	0.78	1.54	0.00	0.00
Mimram	0.00	0.00	1.95	5.98
Princey Brook	1.95	49.04	0.39	0.44
Rib (Wadesmill)	3.46	48.74	1.30	1.84
Stanstead Brook	1.17	22.67	0.00	0.00
Stort	1.56	1.78	2.34	13.89
Ver	0.00	0.00	0.78	5.98

Table 3. 3. Regression equations for simple linear regressions (SLR) and multiple linear regressions (MLR) of various measures of the macroinvertebrate community for the eight sites surveyed. Predictor variables used were crayfish density (x_1), log crayfish biomass (x_2), small crayfish density (x_3), small crayfish biomass (x_4), log large crayfish density (x_5), log large crayfish biomass (x_6), bullhead density (x_7), log bullhead biomass (x_8) and width (x_9) for the eight sites. $* = P \leq 0.05$, $** = P \leq 0.01$ $*** = P \leq 0.001$.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Taxon richness						
SLR	$y = -0.86 + 1.76x_9$	1,6	0.85	39.30	**0.001*****	n.a.
1/D						
MLR	$y = 0.38 + 1.06x_1 + 0.90x_7$	2,5	0.67	8.08	**0.027***	1.1
Log Chrionomidae						
SLR	$y = 2.21 - 0.37x_1$	1,6	0.31	4.12	0.089	n.a.
SLR	$y = 1.33 + 0.33x_7$	1,6	0.46	6.98	**0.038***	n.a.

Continued overleaf.

Table 3. 3 continued.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log Chrionomidae						
SLR	$y = 2.34 - 0.33x_2$	1,6	0.66	14.79	**0.009****	n.a.
SLR	$y = 1.13 + 0.51x_8$	1,6	0.65	13.71	**0.010****	n.a.
MLR	$y = 1.74 - 0.21x_2 + 0.31x_8$	2,5	0.82	16.74	**0.006****	1.6
By crayfish size class:						
SLR	$y = 2.09 - 0.74x_3$	1,6	0.06	1.44	0.275	n.a.
SLR	$y = 2.04 - 0.95x_4$	1,6	0.00	0.82	0.400	n.a.
SLR	$y = 1.42 - 0.43x_5$	1,6	0.59	11.09	**0.016***	n.a.
SLR	$y = 1.92 - 0.22x_6$	1,6	0.71	17.88	**0.006****	n.a.
Log _Asellus aquaticus_						
SLR	$y = 1.58 - 0.51x_1$	1,6	0.31	4.17	0.087	n.a.
SLR	$y = 1.78 - 0.48x_2$	1,6	0.72	18.61	**0.005****	n.a.

Continued overleaf.

Table 3. 3 continued.

Dependent variable	Regression equation		d.f.	r^2_{adj}	F	P	VIF
Log *Asellus aquaticus*							
By crayfish size class:							
SLR	$y = 1.17 - 0.40x_3$		1,6	0.00	0.19	0.681	n.a.
SLR	$y = 1.16 - 0.61x_4$		1,6	0.00	0.16	0.702	n.a.
SLR	$y = 0.49 - 0.59x_5$		1,6	0.57	10.43	**0.018***	n.a.
SLR	$y = 1.17 - 0.30x_6$		1,6	0.70	17.66	**0.006****	n.a.
Log *Baetis sp*							
SLR	$y = 2.91 - 0.88x_1$		1,6	0.40	5.57	0.056	n.a.
SLR	$y = 3.00 - 0.66x_2$		1,6	0.51	8.27	**0.028***	n.a.
By crayfish size class:							
SLR	$y = 2.69 - 1.92x_3$		1,6	0.15	2.28	0.182	n.a.
SLR	$y = 2.71 - 3.09x_4$		1,6	0.14	2.18	0.190	n.a.

Continued overleaf.

Table 3. 3 continued.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log *Baetis sp*						
SLR	$y = 1.09 - 0.95x_5$	1,6	0.61	11.81	**0.014***	n.a.
SLR	$y = 2.17 - 0.44x_6$	1,6	0.59	10.93	**0.016***	n.a.
Log Oligochaeta						
MLR	$y = 0.13 + 0.98x_1 + 0.46x_7$	2,5	0.55	5.20	0.060	1.1
MLR	$y = -1.18 + 0.95x_2 + 1.16x_8$	2,5	0.79	14.38	**0.008****	1.1
By crayfish size class:						
MLR	$y = 0.26 + 2.70x_3 + 1.29x_7$	2,5	0.49	4.40	0.079	1.0
MLR	$y = 1.86 + 1.05x_4 + 0.67x_7$	2,5	0.64	7.18	**0.034***	1.3
MLR	$y = 0.623 + 3.15x_5 + 0.35x_8$	2,5	0.08	1.29	0.353	1.0
MLR	$y = -0.167 + 0.66x_6 + 1.29x_8$	2,5	0.91	34.63	**0.001*****	1.7

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Table 3. 4. Matrix table summarising measures of crayfish that were statistically significant in Table 3.2, represented by their *P* value. * = *P* ≤0.05, ** = *P* ≤0.01 *** = *P* ≤0.001.

Predictor	1/D	Log Chironomidae	Log *Asellus aquaticus*	Log *Baetis sp*	Log Oligochaeta
Crayfish density	**0.028***[†]	n.s.	n.s.	n.s.	**0.028***[†]
Log crayfish biomass	n.s.	**0.009****	**0.005***	**0.028***	**0.003****[†]
Small crayfish density	n.s.	n.s.	n.s.	n.s.	**0.038***[†]
Small crayfish biomass	n.s.	n.s.	n.s.	n.s.	n.s.
Log large crayfish density	n.s.	**0.016***	**0.018***	**0.014***	**0.015***[†]
Log large crayfish biomass	n.s.	**0.006****	**0.006***	**0.016***	**<0.001*****[†]

[†]*P* value taken from MLR (see Table 3.2).

Table 3. 5. Regression equations for simple linear regressions (SLR) and multiple linear regressions (MLR) of measures of macroinvertebrate biomass. Predictor variables used were crayfish density (x_1), log crayfish biomass (x_2), small crayfish density (x_3), small crayfish biomass (x_4), log large crayfish density (x_5), log large crayfish biomass (x_6) and width (x_7) for the eight sites. $* = P \leq 0.05$, $** = P \leq 0.01$.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log total biomass excluding G. pulex:						
MLR	$y = 1.50 - 0.55x_1 + 0.52x_7$	2,5	0.86	21.56	**0.003****	1.0
MLR	$y = 2.18 - 0.42x_2 + 0.43x_7$	2,5	0.86	23.17	**0.003****	1.1
By crayfish size class:						
MLR	$y = 1.57 - 1.02x_3 + 0.48x_7$	2,5	0.70	9.13	**0.021***	1.1
MLR	$y = 1.34 - 1.25x_4 + 0.50x_7$	2,5	0.66	7.82	**0.029***	1.0
MLR	$y = 0.92 - 0.54x_5 + 0.44x_7$	2,5	0.86	22.68	**0.003****	1.1
MLR	$y = 1.81 - 0.27x_6 + 0.40x_7$	2,5	0.87	24.03	**0.003****	1.2

Continued overleaf.

Table 3. 5 continued.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log *Asellus aquaticus* biomass:						
SLR	$y = 1.71 - 0.51x_1$	1,6	0.18	2.58	0.160	n.a.
SLR	$y = 1.97 - 0.51x_2$	1,6	0.58	10.78	**0.017***	n.a.
By crayfish size class:						
SLR	$y = 1.18 - 0.07x_3$	1,6	0.00	0.00	0.950	n.a.
SLR	$y = 1.11 - 0.15x_4$	1,6	0.00	0.01	0.937	n.a.
SLR	$y = 0.64 - 0.57x_5$	1,6	0.36	4.88	0.069	n.a.
SLR	$y = 1.31 - 0.31x_6$	1,6	0.52	8.44	**0.027***	n.a.
Log *Baetis sp* biomass:						
SLR	$y = 2.79 - 0.66x_1$	1,6	0.21	2.91	0.14	n.a.
SLR	$y = 3.01 - 0.59x_2$	1,6	0.47	7.24	**0.036***	n.a.

Continued overleaf.

Table 3. 5 continued.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log *Baetis sp* biomass:						
By crayfish size class:						
SLR	$y = 2.74 - 1.72x_3$	1,6	0.14	2.17	0.191	n.a.
SLR	$y = 2.66 - 2.37x_4$	1,6	0.05	1.40	0.282	n.a.
SLR	$y = 1.37 - 0.79x_5$	1,6	0.47	7.32	**0.035***	n.a.
SLR	$y = 2.27 - 0.39x_6$	1,6	0.55	9.44	**0.022***	n.a.

Table 3. 6. Pearson's *r* values for comparison of the strength of relationship between selected taxa and large crayfish density and large crayfish biomass.

Predictor	Log Chironomidae	Log *Asellus aquaticus*	Log *Baetis sp*
Log large crayfish density	-0.806	-0.797	-0.814
Log large crayfish biomass	-0.865	-0.864	-0.804

Figure 3. 1. Location of the eight sites used in the current study. Small scale map drawn from Edina Digimap (© Crown Copyright Ordinance Survey. An EDINA Digimap/JISC supplied service).
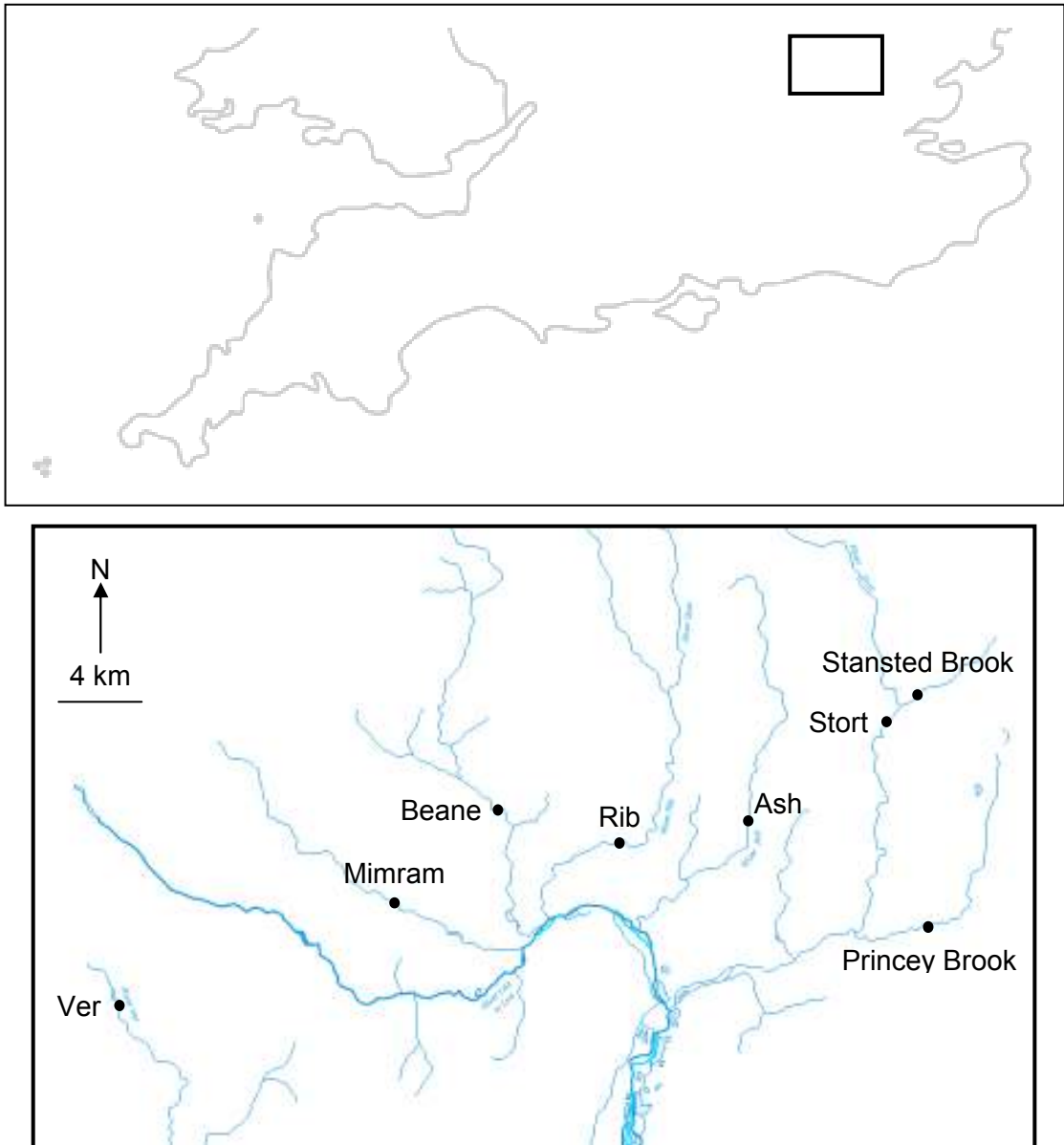
Figure 3. 2. The modified Hess sampler used for the quantification of crayfish and bullhead densities.

Figure 3. 3 (following page). Individual-based rarefaction curves for total macroinvertebrate counts of the Ash (a), Beane (b), Mimram (c), Princey Brook (d), Rib (Wadesmill) (e), Stanstead Brook (f), Stort (g) and Ver (h). Total individual counts were generated by pooling all individuals from the five Surber samples taken at each site. Cumulative counts of taxa were then made after randomising the order of the total sample.

Cumulative taxon richness

Inividual number
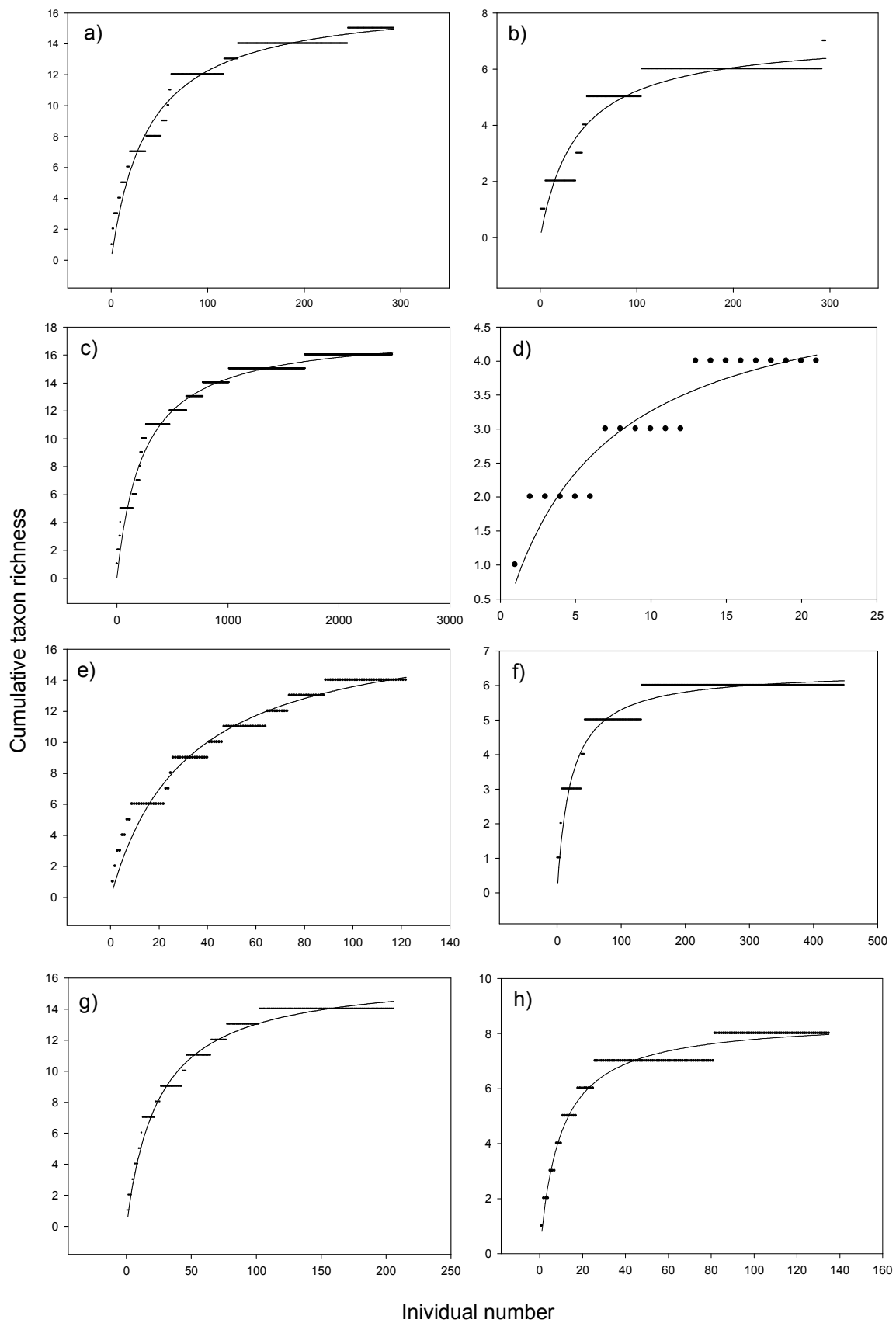
Figure 3. 4. A histogram of crayfish carapace length for all sites combined.
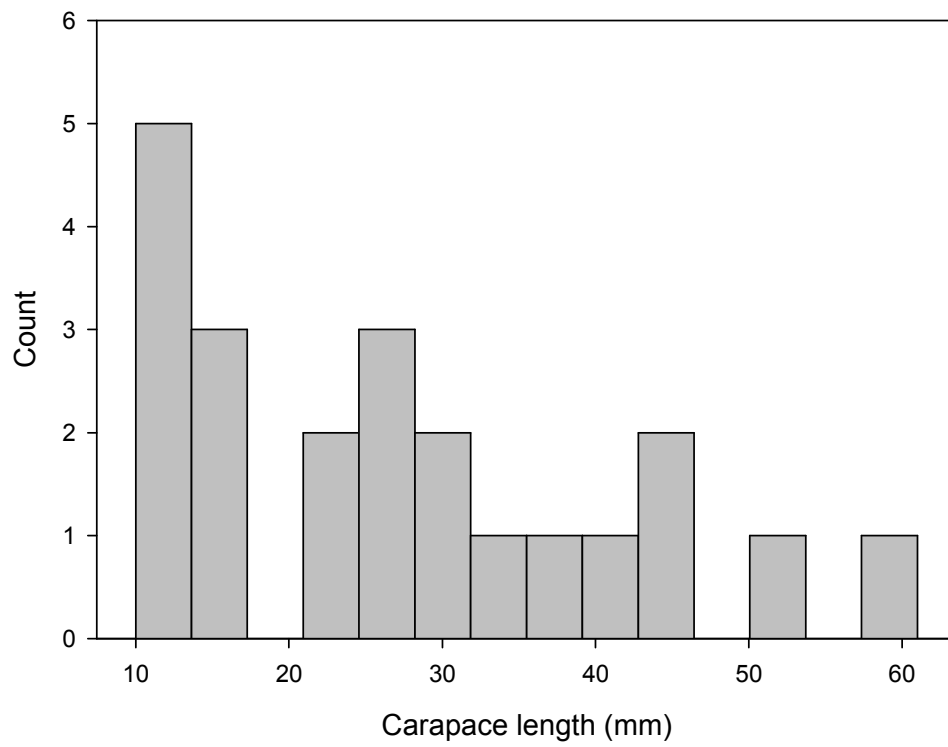
Figure 3. 5. The relationship between site wetted width and the total number of taxa

present.

$$y = -0.86 + 1.76x$$
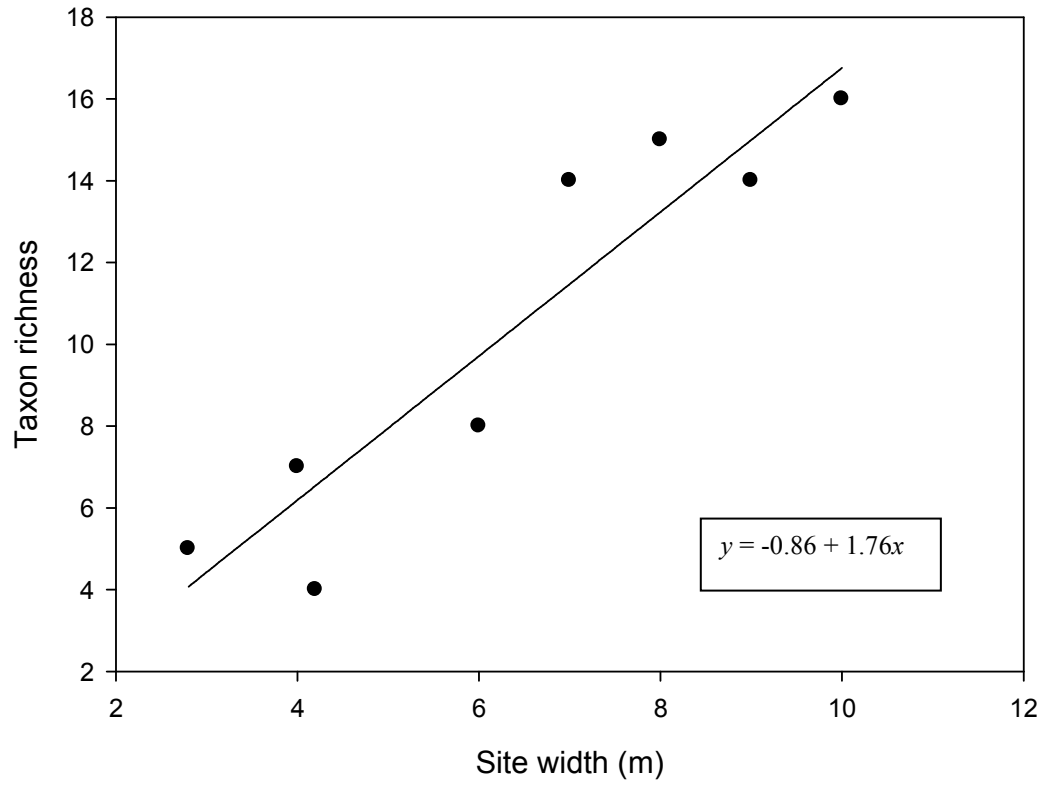
Figure 3. 6. The relationship between log Chironomidae abundance and crayfish
density and biomass as well as bullhead abundance and biomass.
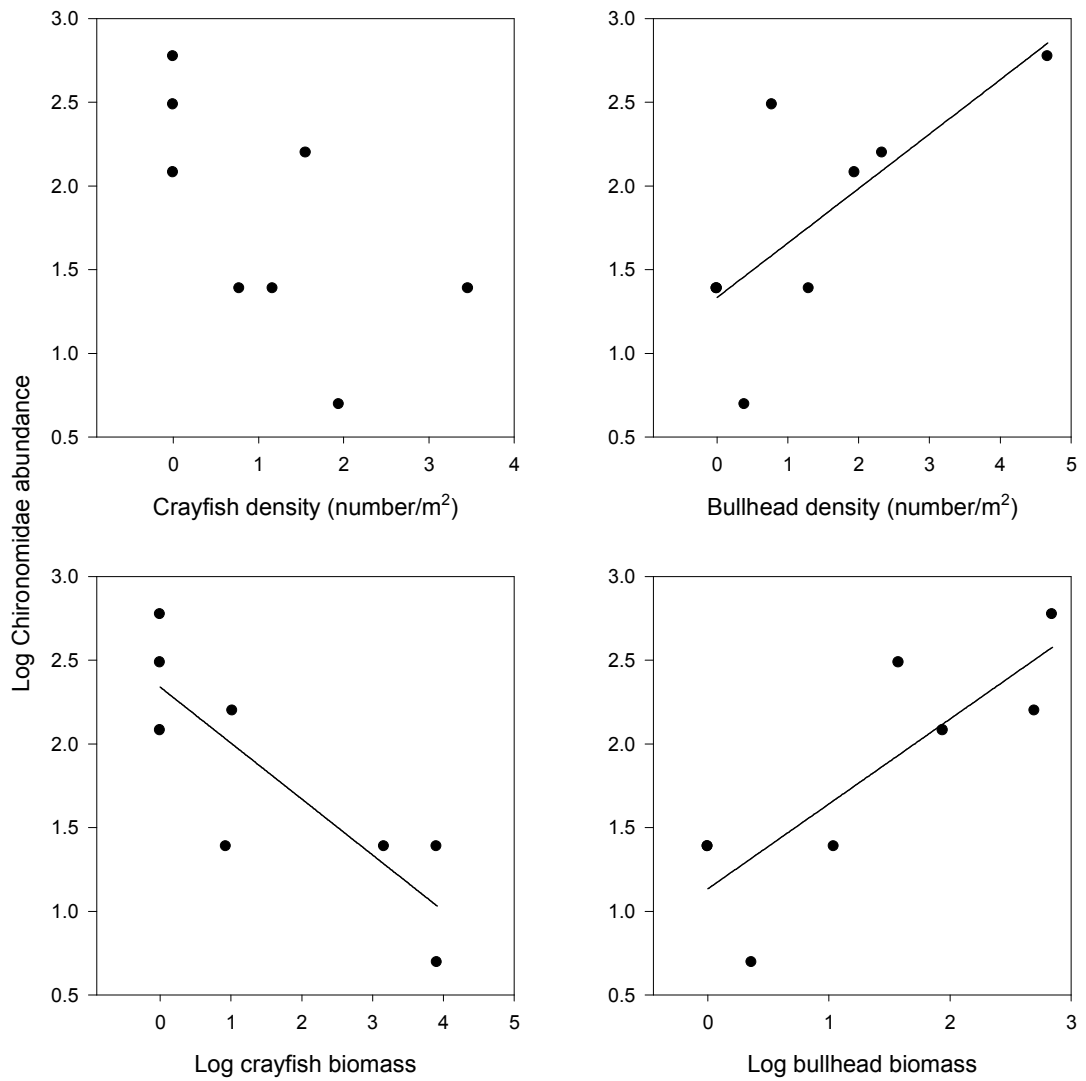
Figure 3. 7. The relationship between crayfish and Chironomidae abundance, with crayfish density and biomass both split by size class.
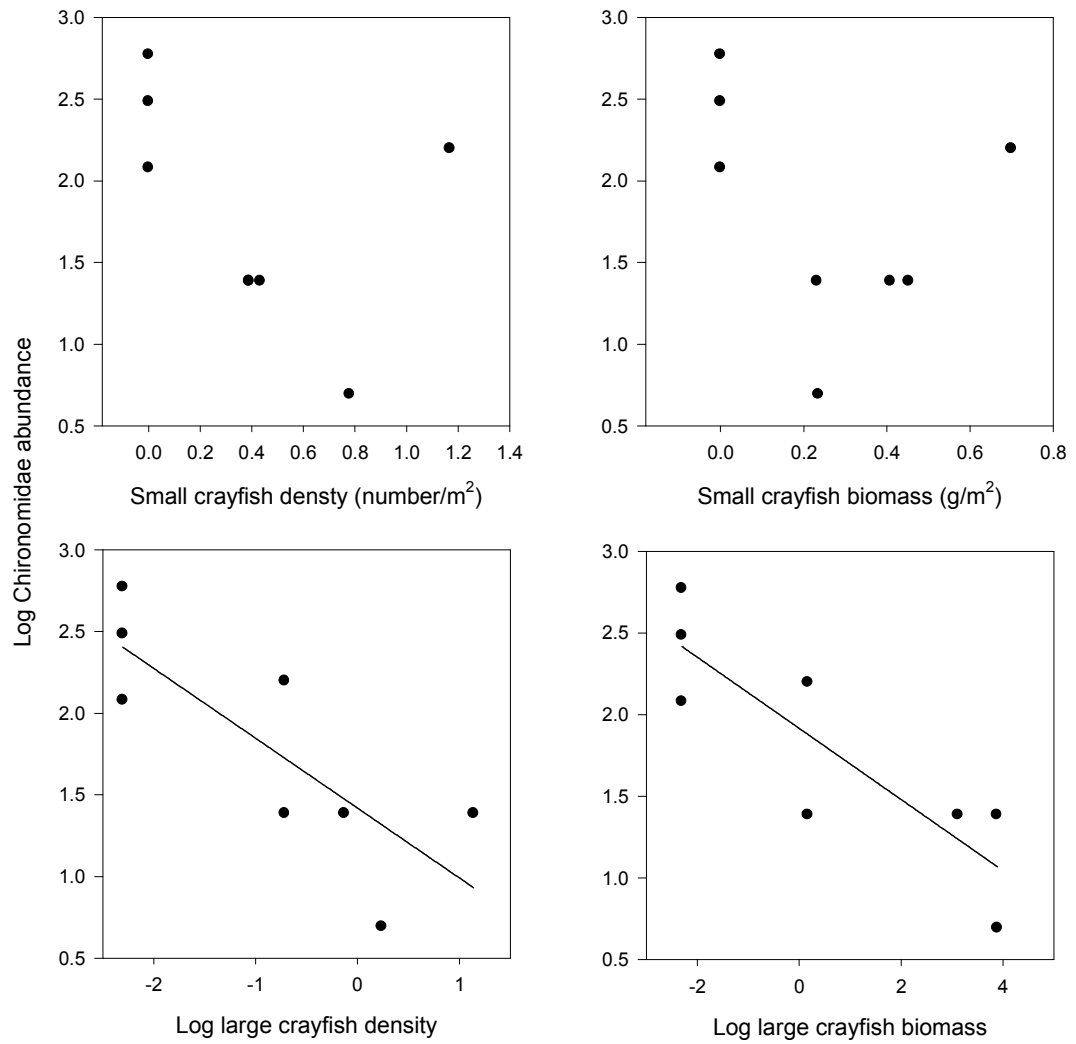
Figure 3. 8. The relationship between log *Asellus aquaticus* abundance and crayfish density and abundance.
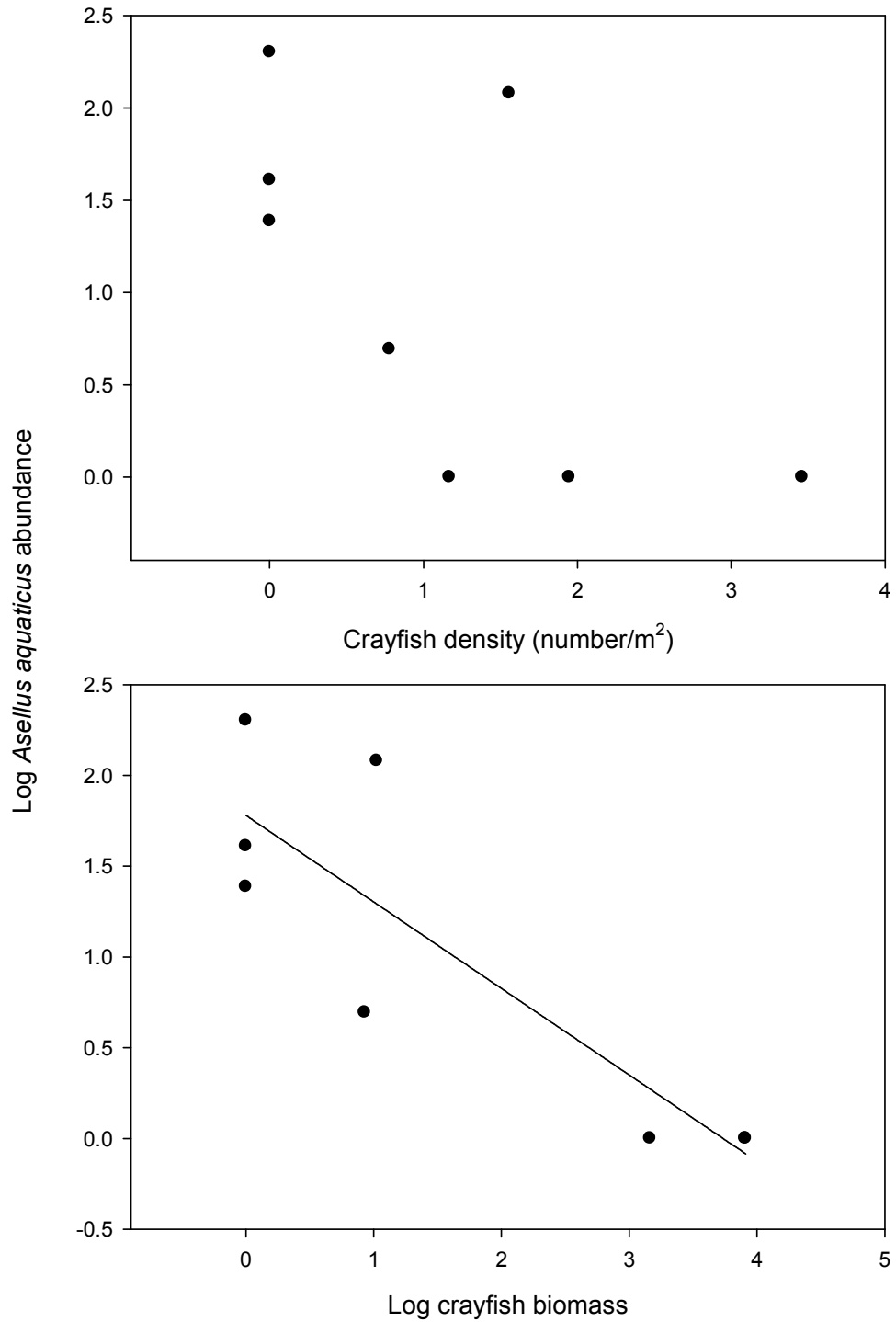
Figure 3. 9. The relationship between crayfish and *Asellus aquaticus* abundance, with crayfish density and biomass both split by size class.
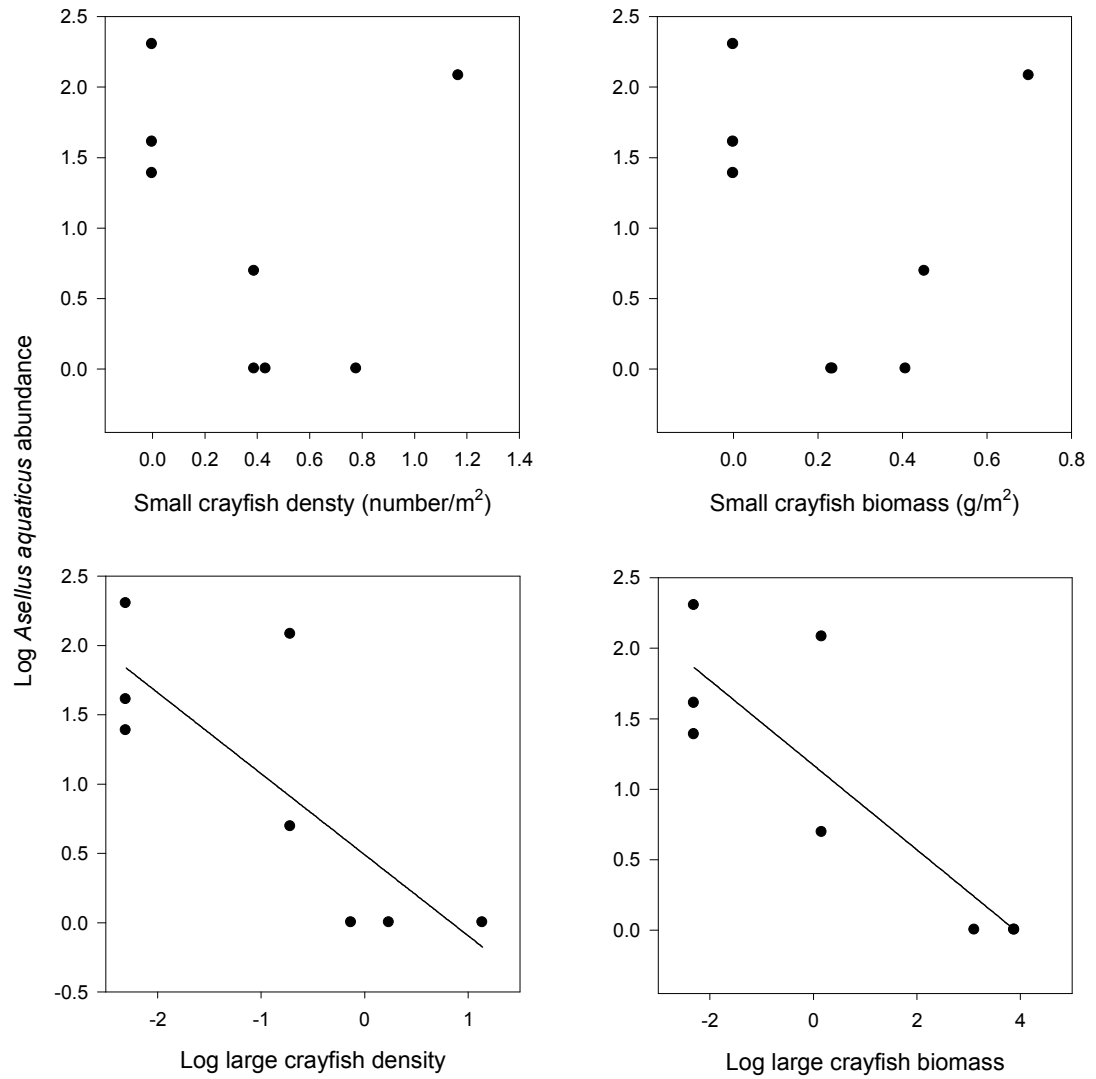
Figure 3. 10. The relationship between log *Baetis sp* abundance and crayfish density and abundance.
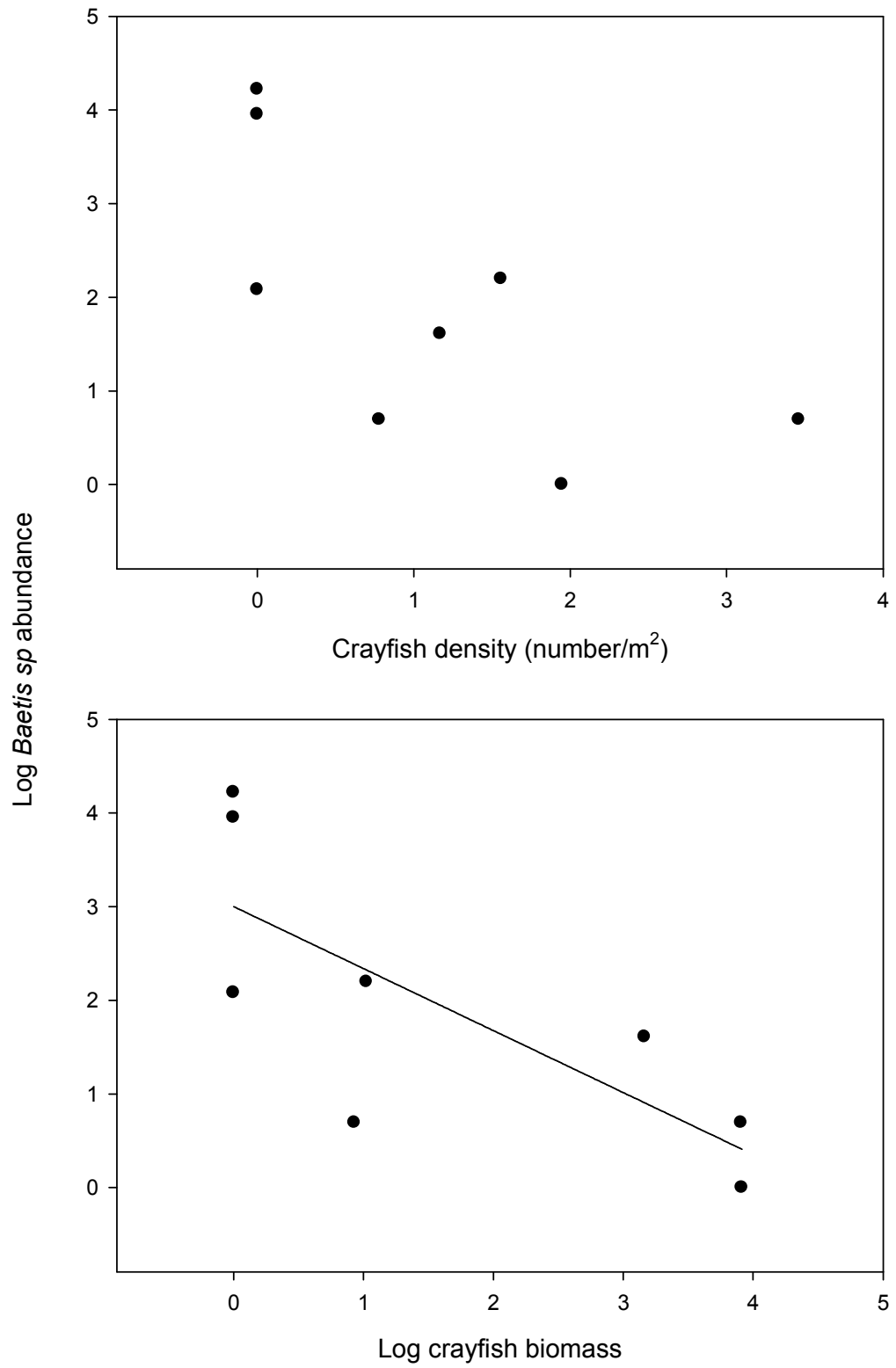
Figure 3. 11. The relationship between crayfish and *Baetis sp* abundance, with
crayfish density and biomass both split by size class.
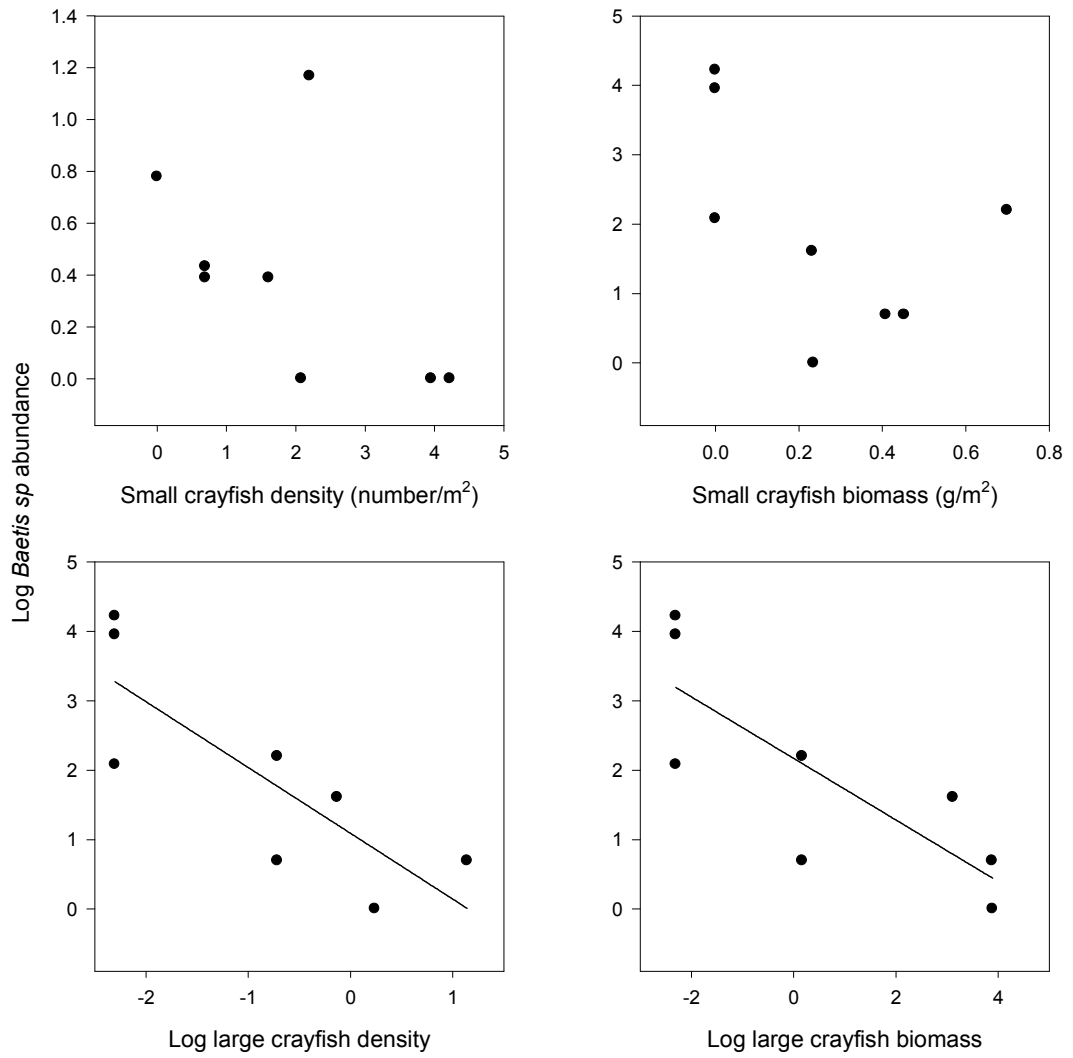
Discussion

As far as I am aware, this is the first study to demonstrate significant relationships between natural crayfish populations and macroinvertebrate communities across multiple water bodies. It provides evidence that commonly observed patterns between crayfish and benthic insects seen in a substantial volume of published experimental work might manifest at the population level of these taxa in natural populations, even when crayfish densities are low. As a correlational study, the results of this survey cannot be used to infer causal relationships between crayfish and the macroinvertebrate community. Therefore, the discussion of results will suggest possible explanations for the observed relationships.

The accurate quantification of crayfish populations is difficult to achieve. The comparison of crayfish abundances as measured by timed manual searches in the preliminary work and use of the modified Hess sampler suggests that timed manual searches are a viable option for the quantification of the relative abundance of crayfish in stream habitats. While it is preferable to obtain absolute densities to better compare between studies, owing to the time-consuming and laborious nature of the use of the modified Hess sampler, timed manual searches represent an acceptable alternative.

At sites where crayfish were present, densities ranged from 0.8 to 3.5 signal crayfish m^{-2}. These values are low relative to measures made in rivers of the UK, where densities have ranged from 3.7 to 21.7 (Guan and Wiles, 1997), and from 9.1 to 23.6 m^{-2} (Bubb et al., 2009). However, they are comparable to other studies conducted in stream habitats, with recorded densities of 0.89 (Light, 2005) and 0.74 m^{-2} (Usio et al., 2006). Relatively few larger crayfish were recorded. It has

previously been shown that the distribution of young-of-the-year and adult crayfish can be spatially distinct, with densities of large crayfish 3 – 20 times higher in deeper waters, as compared to shallow waters of a stream (Creed, 1994). As the present study focused on relatively shallow habitats it is therefore unsurprising that larger individuals were low in abundance.

Judging by the rarefaction curves (Figure 3.3), it appears that not all taxa were detected at some of the sites. Therefore, some caution is recommended in the interpretation of the results. While the most common taxa were recorded, rarer taxa may have been present but not sampled effectively. However, as the surface area of the stream channel sampled was consistent among sites, differences in the rarefaction curves likely reflect genuine differences between the sites.

The absence of any significant relationship between crayfish and macroinvertebrate taxon richness cannot be interpreted as proof against the existence of such a relationship. However, as taxon richness was strongly positively correlated with site width, with approximately 85% of variance explained, it seems likely that if any relationship between crayfish and taxon richness did exist, its effect was small. Neither crayfish abundance nor biomass was correlated with site width and so whatever explanatory variable (or variables) that correlated with width to determine taxon richness was of much greater importance than any net effect of invasive crayfish. Species richness of benthic insects has previously been shown to positively correlate with channel width (Malmqvist and Eriksson, 1995, Malmqvist and Hoffsten, 2000, Heino et al., 2003), although this is not a ubiquitous trend (Townsend et al., 1997). Channel width increases with distance downstream, and with increasing distance downstream, stream systems can show increases in microhabitat availability and production (Vannote et al., 1980), i.e. habitat

complexity increases. According to theory, species richness will increase in more heterogeneous habitats (Townsend and Hildrew, 1994), and although empirical tests have yielded mixed results in stream systems (Vinson and Hawkins, 1998), macroinvertebrate richness and evenness have been shown to increase with increased substrate diversity (Minshall, 1984, Boyero, 2003). A field based experimental study revealed increasing species richness with substrate heterogeneity, whilst no response was seen to crayfish treatment (Brown and Lawson, 2010). As I did not determine habitat complexity, I cannot assess whether this was an important determinant of the benthic community.

Diversity represented by the reciprocal Simpson's index was positively correlated with the density of the two dominant predators of the streams. As no relationship was found between crayfish and taxon richness, it is likely that this trend reflects a positive relationship with the evenness of taxa. A positive relationship between crayfish and macroinvertebrate evenness might result from an environmental factor to which both crayfish density and macroinvertebrate evenness are positively correlated. For example, crayfish densities are dependent on the availability of suitable refugia (Lodge, 1994, Nystrom et al., 2006, Olsson et al., 2009). A high abundance of refugia will reflect increased substrate heterogeneity and as mentioned above, increased substrate diversity has been linked to increased macroinvertebrate richness and evenness in streams (Minshall, 1984, Boyero, 2003, Brown and Lawson, 2010). However, the relationship between crayfish and the evenness of the benthos may also result from direct interactions. This is predicted by the compensatory mortality hypothesis, where mortality is dependent on the dominance of a species (Connell, 1978). A precedent for such a relationship is provided by work of Thorp and Cothran, where species evenness increased in a

linear fashion with increasing density of predatory Odonata nymphs in a mesocosm experiment (Thorp and Cothran, 1984). The authors suggested that preferential consumption of dominant prey; prey switching on the basis of their relative abundance; or non-selective predation combined with patch switching could give rise to such patterns. Returning to the present study, when crayfish biomass was used in place of density, the multiple linear regression for the reciprocal Simpson's index was not significant. Therefore it appears that if crayfish did indeed have a direct effect on the evenness of the macroinvertebrate community, per capita effect was more important than a consideration of crayfish biomass.

It is possible that the negative relationships between crayfish and Chironomidae, Baetidae and Asellidae abundance, as well as Baetidae and Asellidae biomass, resulted directly from predation. Chironomidae and Ephemeroptera made up approximately 45 % and 20 % respectively of the stomach content of signal crayfish in a stream enclosure experiment in Sweden (Stenroth and Nystrom, 2003). Furthermore, Chironomidae and Ephemeroptera were amongst the five most common food items of signal crayfish gut content in wild caught individuals from the River Great Ouse in England (Guan and Wiles, 1998). Isopoda have also been found in the gut content of crayfish, although in low numbers (Stenroth and Nystrom, 2003). These predatory effects can manifest at the population level of prey. Ephemeroptera and Isopoda abundance within crayfish treated enclosures were significantly reduced, providing strong evidence for an impact of crayfish on these groups (Stenroth and Nystrom, 2003). Although Diptera abundance was unaffected by crayfish treatment in the Stenroth and Nyström 2003 paper, data in the following chapter of this thesis shows that crayfish can reduce Chironomidae abundance within stream substrate (page 118).

Crayfish have been shown to reduce total invertebrate biomass experimentally (Nystrom et al., 1996, Stenroth and Nystrom, 2003); however this effect has not been demonstrated in natural benthic communities. Total invertebrate density was reduced in two longitudinal studies of single water courses (Charlebois and Lamberti, 1996, Crawford et al., 2006), however total invertebrate biomass was either not recorded (Charlebois and Lamberti, 1996) or did not show any relationship with crayfish (Crawford et al., 2006). I found that the ubiquitous and highly dominant *G. pulex* obscured a significant negative relationship with the biomass of the benthos. Considering the magnitude of crayfish body mass relative to that of the benthos (at sites where crayfish were present, their mean dry weight biomass was over two times that of the remainder of the benthos) and their clear predatory nature, such a negative relationship is hardly surprising and might be attributable to direct impacts, as revealed by experimental work.

As significant relationships between crayfish and the abundance of Chironomidae and Oligochaeta were not reflected in the biomass results of these taxa, it would suggest a relationship between their body size distributions and crayfish density / biomass. However, no correlations were seen with mean body mass. The taxonomic resolution of data did not allow discrimination between whether the species composition within these taxa altered, or whether the size distribution within populations of single species changed.

The size-threshold used to separate crayfish by life-stage appeared to be functionally significant (Table 3.3). In contrast to the hypothesis that smaller crayfish would show relatively stronger relationships with the benthos, negative relationships between crayfish and measures of Chironomidae, Baetidae and Asellidae were significant for the large size-class, but not the small size-class. A

preference for Chironomidae and Ephemeroptera in larger crayfish has previously been shown using gut content analysis (Whitledge and Rabeni, 1997, Guan and Wiles, 1998) implying that the per capita effect of crayfish increases with body-mass. My data for Chironomidae and Asellidae abundance tends to concur, whereby large crayfish biomass explained more variation and significance was increased, compared with large crayfish density (Table 3.2). Furthermore, Pearson correlation co-efficients were more strongly negative for the large biomass measure (Table 3.5). While it seems intuitive that the per capita impacts of a taxon which spans such a large range of body sizes should vary, very little work has been published on the subject of per capita effects of crayfish. There are however a couple of exceptions, where this theme has been investigated. For example, grazing of *Cladophora glomerata* (Linnaeus) was twelve times greater for large, than for young-of-year crayfish (Creed, 1994). Also, large signal crayfish (>30 mm orbital carapace length) were shown to have a greater negative effect on submerged macrophytes, benthic algal biomass and invertebrate taxon richness than small crayfish (<30 mm orbital carapace length) (Usio et al., 2009).

These results emphasise the importance of consideration of biomass in species interactions. This is particularly significant in the case of crayfish, as relatively few previous studies have considered the effects of biomass and ontogeny. However, a further possible explanation for the difference in the relationships between the two measures of crayfish life-stage and invertebrate abundances might be the time taken for crayfish effects to manifest. 0+ crayfish have by definition had a relatively short time to have any influence on their environment. Excluding consideration of size class, in a long-term lake study the influence of crayfish on the invertebrate community was found to lag by one year (McCarthy et al., 2006).

The positive relationship between crayfish and Oligochaeta abundance is likely to be indirect, rather than direct. Oligochaeta can be abundant when other macroinvertebrates are absent, especially where low oxygen tension results from large quantities of decomposing organic matter (Giller and Malmqvist, 1998). Total invertebrate densities increased in crayfish treatments of an enclosure / exclosure experiment, owing to an increase in abundance of small size taxa, including Oligochaeta (Usio et al., 2009). Where direct impacts and engineering effects of crayfish were disentangled, Oligochaeta responded negatively to sediment removal rather than crayfish predation (Usio and Townsend, 2004). As crayfish act as ecosystem engineers (see page 25 and results of the following chapter), it seems they are more likely to have indirect effects, if any, through modification of the substrate, rather than direct consumptive effects. Oligochaeta do not appear to be a common prey item of crayfish, although this is difficult to prove owing to the soft tissue of Oligochaeta being largely unrecognisable in gut content. Even when efforts were made to overcome this problem, Oligochaeta were not identified from gut content of red swamp crayfish; no remains were found in slides prepared specifically with the aim of identifying chaetae (Smart et al., 2002).

My results indicate that signal crayfish can have a significant impact on the macroinvertebrate communities of streams and that crayfish life-stage and / or biomass is important in determining such impacts. No published work I am aware of has demonstrated similar patterns derived from multiple water bodies. Although causation cannot be determined, these results are supported by experimental evidence within the literature. Whilst also tackling other questions, the experimental approaches used in Chapters Four and Five also tested whether signal crayfish had

direct impacts on the benthos. A synthesis of the results from these separate

approaches is given in the concluding chapter.

Chapter Four: The functional significance of invasive crayfish as omnivores and ecosystem engineers in a stream food web

Introduction

Invasive species are often viewed as keystone species, as they can exert disproportionately strong effects on recipient ecosystems, usually via trophic interactions and / or habitat modification (Ricciardi et al., 1997, Letnic et al., 2009). A well-established concept in community ecology is that of the trophic cascade, whereby consumers have indirect effects on non-adjacent trophic levels (Hairston, 1960, Threlkeld, 1988), and this provides a useful, simplified view of a particular type of food web interaction. However, often the picture is more complicated in reality, especially in complex food webs with omnivorous consumers (Nystrom et al., 1996, Usio, 2000, Bruno and O'Connor, 2005, Ho and Pennings, 2008). Omnivory may decouple a potential trophic cascade by acting upon multiple trophic levels simultaneously (Nystrom et al., 1996, Usio, 2000). A non-trophic factor which further complicates the influence of a species (especially some aquatic species) on their local environment is ecosystem engineering, which can alter the physical habitat of multiple species that occupy different positions in the food web.

Trophic cascades have been widely reported in many aquatic ecosystems, although this might reflect a bias in the literature, rather than a ubiquitous phenomenon (Woodward, 2009). Certain taxa and ecosystems have characteristics that seem to favour the manifestation of cascades; for example, crayfish have

indirectly increased algal standing stock in streams via predation on grazing macroinvertebrates and have facilitated epilithic diatoms and sessile, grazing insects via exclusion of filamentous algae (Creed, 1994, Lodge et al., 1994). Although almost all studies to date have focused on cascading interactions associated with autochthonous algal pathways in the food web, cascades affecting the other major energy subsidy of aquatic ecosystems, allochthonous leaf litter, have recently been demonstrated in a chalk stream system, where a predatory fish, the bullhead, indirectly suppressed leaf-litter breakdown via reduced consumption by a dominant shredder, *Gammarus pulex* (Woodward et al., 2008).

In contrast to many predatory fish, crayfish are true omnivores that have the potential to decouple trophic cascades. In a small pond study, crayfish catch per unit effort correlated negatively with the biomass of predatory invertebrates, herbivorous / detritivorous invertebrates, macrophytes and detritus, while periphyton was unaffected: i.e., the cascade did not ramify to the algal resources at the base of the web (Nystrom et al., 1996). This goes against the expected pattern of alternating impacts on successively lower trophic levels predicted by the classical trophic cascade model, and is congruent with omnivorous feeding dissipating the effects of strong pairwise feeding interactions (McCann, 2000). Crayfish in New Zealand stimulated the breakdown rates of leaf-litter, which led to the reduction in abundance of invertebrates associated with the leaf packs as resources were depleted (Usio, 2000), thereby obscuring any potential direct impacts on invertebrate abundance.

Benthic fishes in tropical ecosystems can disturb stream sediments to such an extent that this ecosystem engineering has impacts on the benthic community, independent of those of direct consumption (Flecker, 1997, Flecker and Taylor, 2004, Taylor et al., 2006). Similarly, as outlined within Chapter One, crayfish can act as

ecosystem engineers (Creed and Reed, 2004, Statzner et al., 2000, Statzner et al., 2003, Usio and Townsend, 2004, Zhang et al., 2004); with crayfish bioturbation influencing invertebrate communities within leaf packs (Usio and Townsend, 2004). However, no study, to date, has considered the effect of crayfish on the sediment and invertebrates associated with the bulk gravel of the stream channel.

Furthermore, there is scant published work on how the ontogeny of a focal species may influence either associated trophic cascades or engineering effects. While the importance of prey ontogeny for trophic cascades has been demonstrated through both modelling and empirical work (Chase, 1999, Mumby et al., 2006), examples of predator ontogeny modifying cascades are difficult to find (but see Persson et al., 2003). There is also little evidence of how ecosystem engineer characteristics might change with ontogeny, although life-stage in two disparate ecosystem engineer taxa, a salt marsh cordgrass and a marine bivalve, has been shown to have consequences for their associated faunal communities (Neira et al., 2007, Koivisto and Westerbom, 2010). As described in the introduction of Chapter One (page 25), crayfish ontogeny has been shown both to be important (Whitledge and Rabeni, 1997, Guan and Wiles, 1998, Correia and Anastacio, 2008, Stenroth et al., 2008) as well as unimportant (Whitledge and Rabeni, 1997, Bondar and Richardson, 2009, Stenroth et al., 2008) in determining their diet. Where evidence suggests that younger crayfish are more predatory, such crayfish-grazer-periphyton cascades as were outlined above might be expected to be stronger in magnitude where these life-stages are better represented.

To address this apparent knowledge gap, I aimed to measure the simultaneous impacts of an aquatic omnivore on community structure and multiple ecosystem process rates (i.e., detrital breakdown and algal standing stock), whilst also taking

into account potential ecosystem engineering effects on the influence of sediment on the macroinvertebrate assemblage. Using the invasive signal crayfish in an enclosure / exclosure cage experiment, I measured the impact of this large omnivore on macroinvertebrates associated with both leaf packs and the streambed sediment, and any resultant trophic cascades affecting both herbivorous and detrital-based food chains. Furthermore, I quantified ecosystem engineering effects of the crayfish on sediment particle distribution, and investigated its importance for macroinvertebrate assemblages. Differences in crayfish size class and body mass were also taken into account, in order to examine whether their functional role in the food web changes with growth.

I hypothesised that, through a reduction of the abundance / biomass of grazer macroinvertebrates, crayfish would give rise to a trophic cascade, whereby algal standing stock is increased owing to diminished consumption by grazers. I expected this effect to be greater where crayfish biomass is made up of smaller individuals, as they are likely to be more carnivorous than larger individuals.

Secondly, I hypothesised that any potential detritivore-detritus trophic cascade was likely to be obscured by omnivorous feeding, especially by the larger, more detritivorous crayfish.

Finally, through engineering effects, I expected crayfish to reduce fine sediment in gravel interstices; this change in microhabitat composition is likely to alter the macroinvertebrate assemblage associated with the stream sediment.

Methods

Study Site:

The River Ver is a chalk stream in Hertfordshire, southern England (51°45'55"N, 0° 22'06"W). Three hundred Signal Crayfish juveniles were legally introduced approximately 30 years ago (local resident, personal communication), and presumably signal crayfish have been present ever since, with the possibility of secondary invasions from the River Colne, of which the Ver is a tributary (Ellis (Environment Agency), personal communication). Water temperature was 7.3°C and pH 7.69, recorded at the end of the study period.

Experimental set up:

An enclosure / exclosure cage experiment was carried out using crayfish caught from the Ver. In order to make up numbers of the larger size class of crayfish, two individuals were donated by a colleague at Queen Mary University. The cages (basal area of 0.04 m^2) were identical to those used to assess the impacts of predatory invertebrates (Woodward and Hildrew, 2002) and fish (Woodward et al., 2008) in other stream systems. As in these earlier studies, each cage was clad in 4 mm aperture plastic mesh, to allow free movement of invertebrate species except crayfish, and seeded with gravel from the stream bed.

Within each cage a 10 cm x 10 cm unglazed ceramic tile was added to quantify algal standing stock and a mesh pack of 10mm aperture was filled with 3 g of dried oak (*Quercus robur* Linnaeus) leaf-litter to measure breakdown rates (after Woodward et al. 2008).

Experimental treatments were assigned thus to each cage: no crayfish (control); one large crayfish (carapace length 41 – 46 mm); one small crayfish (carapace length 21 – 32 mm); six small crayfish (carapace length 21 – 32 mm). The six small crayfish were approximately equal in biomass to one large crayfish (~30 g wet weight). The body mass of crayfish was calculated with the following allometric equation, derived from original data: $y = 3.215x - 0.5913$, where both y and $x = \log_{10}$ wet mass (g) of crayfish (r^2 0.97; $F_8 = 272.17$; $P < 0.001$). Considering the basal area of the cages, these treatments represented densities and biomasses as follows: one large crayfish, density 25 m^{-2}, wet weight biomass ~700 g m^{-2}; one small crayfish, 25 m^{-2}, ~60 g m^{-2}; six small crayfish, 150 m^{-2}, ~700 g m^{-2}. Densities of 25 m^{-2} and a total biomass of ~60 g m^{-2} are comparable with values seen in the literature for rivers in the UK (Guan and Wiles, 1997, Bubb et al., 2009). Although values of 150 m^{-2} and ~700 g m^{-2} are artificially high, the experiment was constrained by the size of the pre-existing cages used, and the size classes of crayfish available at the field site. However, the differences seen between these treatments in the results section demonstrate that these comparisons were of value.

The 40 cages were set up in the centre of the stream channel in 10 transects of blocks of four cages, positioned side-by-side, with positions of each treatment within a block assigned at random. Each treatment was replicated 10 times, giving 10 blocks, which were spaced out along an 80m stretch of stream.

The experiment ran from 27th February until 27th March 2008. At the end of the experiment, one cage treated with six small individuals was missing a crayfish; and an individual from a further cage (single large crayfish treatment), whilst alive, was almost completely unresponsive to stimulus. These two cages were removed from all subsequent analysis. Algal colonisation tiles were scrubbed with a

toothbrush and the biofilms were washed into 30 ml darkened plastic bottles and frozen immediately at -20 °C. Leaf litter was removed from the mesh packs and then placed immediately into individually labelled bags. The gravel from each cage was washed through a 500 μm sieve with the <500 μm fraction subsequently transferred to a separate zip-lock bag.

In the laboratory the sediment of each cage was sifted and all invertebrates above 500 μm in size were retained. Macroinvertebrates were separated and identified to the lowest taxonomic level possible, then photographed on a sheet of graph paper divided into 1 mm units. Invertebrate lengths were measured using the software ImageJ and published length-mass regressions were used to determine their biomass. The remaining sediment was then oven dried at 60°C to constant mass, passed through a series of sieves of 0.25, 0.5, 1, 2 and 4 mm apertures, and the mass of each fraction recorded. Four sediment samples were lost before they could be measured.

The macroinvertebrates from the mesh packs were separated from the litter and identified as per the samples from the cages. The litter was dried to a constant mass at 60°C and then weighed. Breakdown rates were calculated as percentage dry mass loss per day. Chlorophyll *a* concentrations were determined spectrophotometrically following acetone extraction, and used as a proxy measure of algal standing stock (expressed as mg ml^{-1}). Each sample was filtered on a 47 mm glass fibre filter paper (GF/C Whatman), before being added to a 10 ml solution of 90% acetone and left for 24 hours in a lightless refrigerator at 5 ºC for chlorophyll *a* extraction to occur. The solution was then centrifuged at 2530 rpm for five minutes. A subsample was poured into a 5 ml cuvette and absorbance was measured at 630, 647 and 664 nm in a spectrophotometer (WPA Biomave II, Cambridge UK). The

spectrophotometer was calibrated with a 90% acetone solution. Chlorophyll *a*

concentration was calculated as per Jeffrey and Humphrey (Jeffrey and Humphrey,

1975).

As the turnover rate of tissues can be slow, the influence of previous diet on

stable isotope ratios can be long lasting (McCutchan et al., 2003). Therefore the two

large individuals that were not collected from the Ver were excluded from stable

isotope analysis. For all other individuals, the abdomen was removed and the end

gut extracted. All exoskeleton was removed, leaving muscle tissue only. This was

macerated in a glass vial and then oven dried at 60 °C for 48 hours before being

homogenised with an agate pestle and mortar. For each sample 0.6 ± 0.03 mg was

weighed into a tin cup and run in an elemental analyser (Flash EA, 1112 series,

Thermo-Finnigan) coupled to a continuous flow isotope ratio mass spectrometer

(Finnigan MAT DeltaPlus, Thermo-Finnigan). Owing to the unequal sample size

resulting from the exclusion of two large crayfish of different provenance and the

moribund large crayfish, 10 subsets of seven individuals were randomly generated

for small crayfish treatments and mean $\delta^{13}C$ ranges were generated. In this way the

$\delta^{13}C$ range of crayfish grouped by size class could be compared.

Data were analysed using Minitab Version 14.1, following $\log_e(x)$, $\sqrt{x}$ or x^3

transformation to meet the assumptions of normality and homogeneity of variance,

where necessary. General Linear Models (GLMs) were used to analyse the data,

however where data were non-normal, Spearman's rank was used for simple

correlations of ranked data. Treatment and block were treated as fixed factors, while

measures of the sediment were included as co-variables. In order to test for any

engineering effects of crayfish on the sediment, the skew of the sediment particle

size distribution and the percentage fine sediment (<0.5 mm) were tested against

treatment. These measures of particle size, seen within the granulometry literature,

were calculated as outlined in Bunte and Abt (2001). The equation of Warren (1974)

was used to calculate skew. They are used here to explore relationships between

sediment and its resident fauna. For taxa whose data could not be normalised,

relationships between abundance / biomass and measures of the sediment were tested

using Spearman's rank.

Species richness and the Simpson diversity index were used as a measure of

diversity. The latter is expressed as the reciprocal in order for a more positive

number to represent greater diversity.

Results

Ecosystem engineering:

Crayfish promoted the removal of finer sediment, as revealed by the sediment size

distributions of the cages, which were skewed towards coarser particle sizes in the

two treatments containing the small size class of crayfish (Figure 4.1). When only

treatment was taken into account significance was borderline (GLM, $F_{3.33} = 2.83$, P

$= 0.055$), however inclusion of block identity increased the significance of treatment

in the model (GLM, treatment, $F_{3.33} = 3.68$, $P = 0.028$; block, $F_{9.33} = 3.26$, $P =$

0.012).

Community structure:

Mean taxon richness of macroinvertebrates from within the sediment of cages was

lower in all three crayfish treatments compared with the control, but non significant.

(Figure 4.2). Treatment and the log proportion of sediment particles under 0.5 mm

were significant factors for the diversity (log reciprocal Simpson index) of macroinvertebrates of the sediment (Figure 4.3; GLM, $F_{3.33} = 5.30$; $P = 0.005$ and $F_{1.33} = 9.91$; $P = 0.004$ respectively). Diversity was reduced in the six small crayfish treatment compared with all other treatments (Tukey's, control, $P = 0.015$; one large crayfish, $P = 0.012$; one small crayfish, $P = 0.010$).

Few taxa, of the 29 found, were present in sufficient numbers to justify separate statistical analysis. Chironomidae were the second most common taxon of the sediment, representing 26% of total abundance and 4% of total biomass overall. Log Chironomidae abundance and biomass were reduced in the six small crayfish treatment, relative to all other treatments (Table 4.1, Figure 4.4). The abundance and biomass of *Gammarus pulex* and *Simulium* spp. were lower in all crayfish treatments compared to the control; however these differences were not statistically significant (Figure 4.5).

G. pulex was by far the most dominant macroinvertebrate, both in terms of abundance (mean 2218 m^{-2}) and biomass (mean 4.12 g m^{-2}), representing 67% of total macroinvertebrate abundance and 84% of total biomass respectively (excluding crayfish). The proportion of total macroinvertebrate abundance made up by *G. pulex* was greater in the six small crayfish treatment relative to all other treatments and increased with higher values for the proportion of sediment particles under 0.5 mm (Figure 4.6a; GLM, treatment, $F_{3.33} = 5.39$; $P = 0.005$, sediment <0.5mm, $F_{1.33} = 8.61$; $P = 0.006$); however no difference was observed for the proportion of total macroinvertebrate biomass (cubed for normality) made up by *G. pulex* (Figure 4.6b). In contrast, the proportion of both total macroinvertebrate abundance and biomass made up by Chironomidae was significantly affected by treatment (Figure 4.6a and

b; GLM, proportion of total abundance, $F_{3.37} = 3.98$; $P = 0.016$; log proportion of total biomass, $F_{3.37} = 4.11$; $P = 0.014$).

After *G. pulex* and Chironomidae, the next most abundant taxon was *Silo nigricornis*, representing 2% of total macroinvertebrate abundance. This and all taxa less abundant were combined into one group that shall be referred to as 'rare taxa'. Although borderline in significance, the abundance of rare taxa (normalised by $\sqrt{x}$ transformation) was reduced in all crayfish treatments relative to the control (Figure 4.7; GLM, $F_{3.37} = 2.83$; $P = 0.053$).

In order to remove confounding effects of crayfish, relationships between the skew of the particle size distribution and resident fauna were explored using only the data from control cages. Chironomidae abundance and biomass were positively correlated with the skew of the sediment (Figures 4.8a and b. Linear regression, log abundance, $F_{1,8} = 14.37$, $P = 0.007$; log biomass, $F_{1,8} = 5.93$, $P = 0.045$). The mean biomass of *G. pulex* was strongly negatively correlated with the skew of the sediment (Figure 4.9; linear regression, $F_{1,7} = 11.32$, $P = 0.015$). Ceratopogonidae (Diptera) abundance and biomass positively correlated with the skew of sediment (Figure 4.10a and b; abundance, $n = 34$, $r_S = 0.420$, $P < 0.050$; biomass, $n = 34$, $r_S = 0.366$, $P < 0.050$); however owing to the paucity of this taxon, data for all treatments had to be used in this analysis. It is therefore possible that this relationship was confounded by crayfish treatment. *Silo nigricornis* (Pictet) was the dominant caddis larvae, comprising 2% of total macroinvertebrate abundance and 8% of total biomass. Its abundance and biomass negatively correlated with increasing proportions of fine particles <0.5 mm within the sediment (Figure 4.11a and b; abundance, $n = 34$, $r_S = -0.657$, $P < 0.010$; biomass, $n = 34$, $r_S = -0.620$, $P < 0.010$).

Treatment had no apparent effect on the colonisation of leaf packs by macroinvertebrates; however, the proportion of fine sediment within cages correlated with the abundances of both *G. pulex* and Chironomidae within leaf packs. Log *G. pulex* abundance correlated positively with the log proportion of sediment particles <0.5 mm (Figure 4.12a; $n = 31$, Pearson's $R = 0.516$, $P = 0.003$), while the square root of Chironomidae abundance negatively correlated with the same measure (Figure 4.12b; $n = 31$, Pearson's $R = -0.453$, $P = 0.010$).

Ecosystem process rates: algal production and litter breakdown:

Log Chlorophyll *a* concentrations on the colonisation tiles differed among treatments (Figure 4.13a; GLM, $F_{3.35} = 3.05$, $P = 0.042$). A Tukey's post hoc test revealed a significant difference between the six small crayfish treatment and the control ($P = 0.033$). Breakdown rates of oak leaves were not significantly affected by treatment (Figure 4.13b). Evidence of crayfish contributing to increased litter breakdown, as compared with breakdown rates where no crayfish were present, was seen only in the case of larger crayfish.

Stable isotopes:

Using $\delta^{15}N$ as a proxy for trophic height, stable isotopes revealed that the large crayfish occupied a higher trophic position than the smaller crayfish (Figure 4.14a; GLM, $F_{2.26} = 3.86$; $P = 0.035$). This was significant against crayfish of the six small treatment (Tukey's, $P = 0.040$), and close to significance against those of the single small treatment (Tukey's, $P = 0.074$). The $\delta^{13}C$ range of large crayfish was narrower than that observed for small crayfish. Large crayfish had a range of 0.7‰,

individuals of the single small crayfish treatment 1.7‰ and those of the six small

crayfish treatment 1.4‰. Figure 4.13b represents those subsets closest to these mean

values, with values of 1.7‰ and 1.4‰ for the single and six small crayfish

treatments, respectively.

Table 4. 1. GLM output for Chironomidae data. ** = $P \leq 0.01$ *** = $P \leq 0.001$.

| | Factor | | | | Tukey's post hoc test | | |
| | Treatment | | Block | | Control | 1 large | 1 small |
$R^2(adj)$ %	P value	F	P value	F	P value	P value	P value
Log Chironomidae abundance	64.31	**<0.001*****	8.51	**<0.001*****	6.02	**<0.001*****	**0.005****	**0.002****
Log Chironomidae biomass	61.46	**<0.001*****	13.20	**0.004****	3.75	**<0.001*****	**<0.001*****	**<0.001*****

Figure 4. 1. The skew of the particle size distributions for the sediment of cages of each treatment (+ 1 SE).
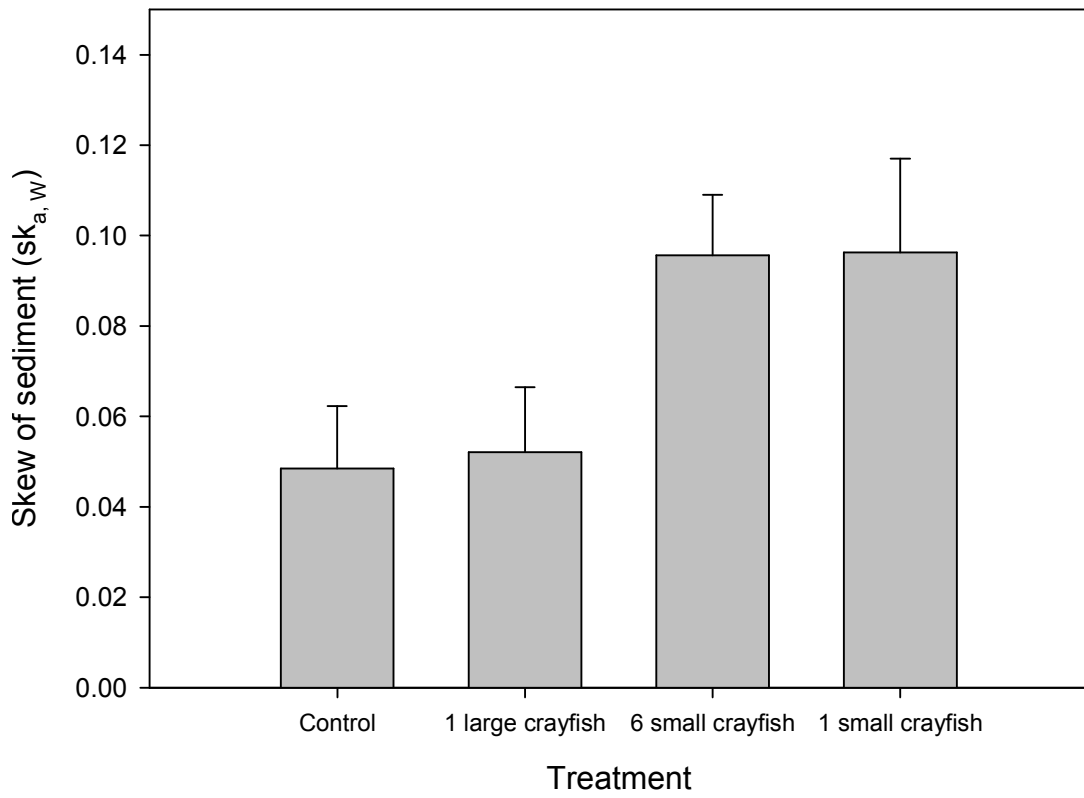
Figure 4. 2. Macroinvertebrate taxon richness (+ 1 SE) of each treatment.
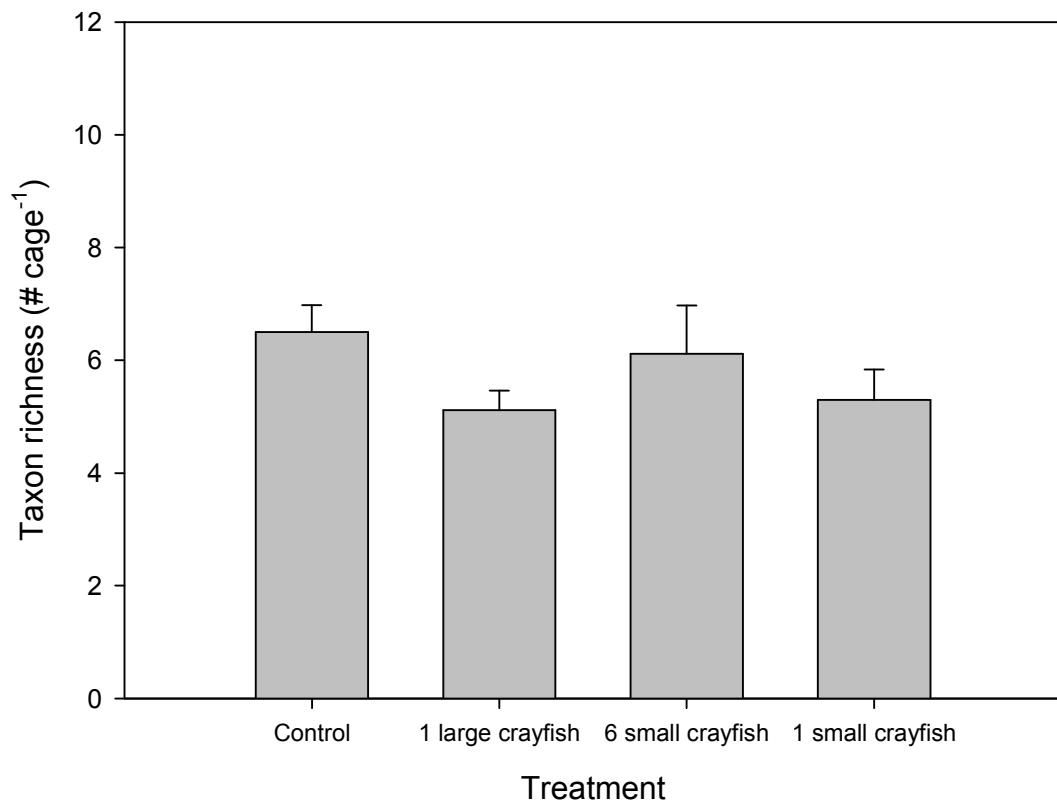
Figure 4. 3. The natural log of the reciprocal of Simpson diversity (+ 1 SE) for each treatment.
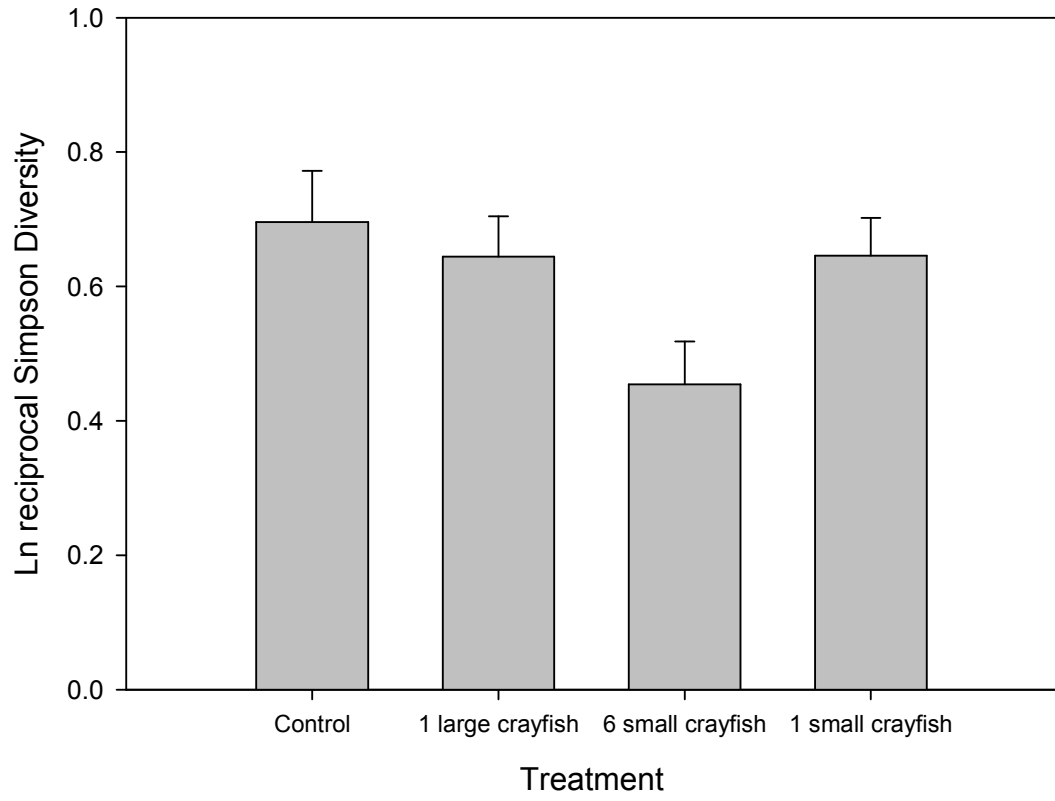
Figure 4. 4. Abundance (a) and biomass (b) of Chironomidae for each treatment (+ 1 SE).
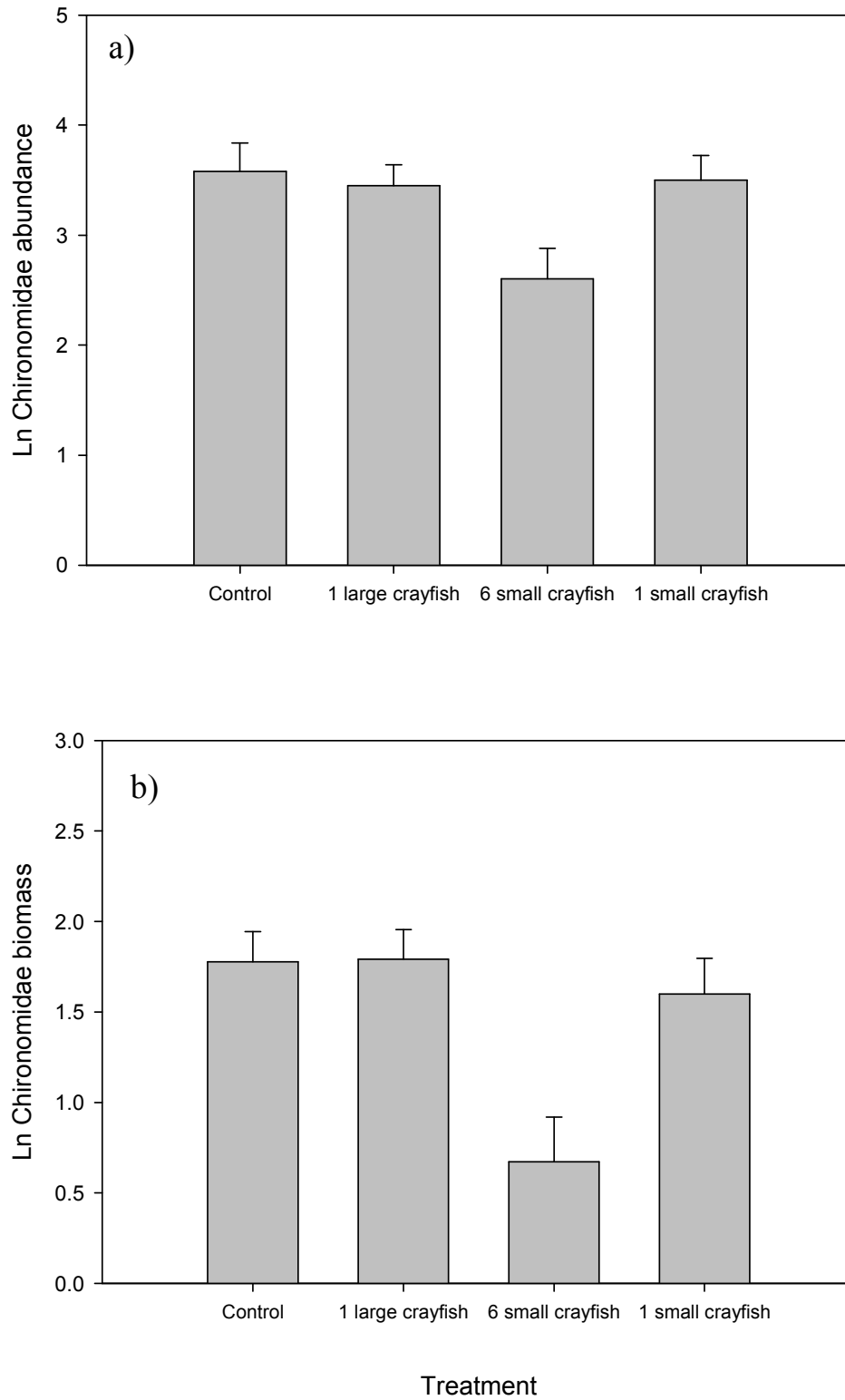
Figure 4. 5. Abundance (a) and biomass (b) of *Gammarus pulex* and *Simulium* spp. for each treatment (+ 1 SE).
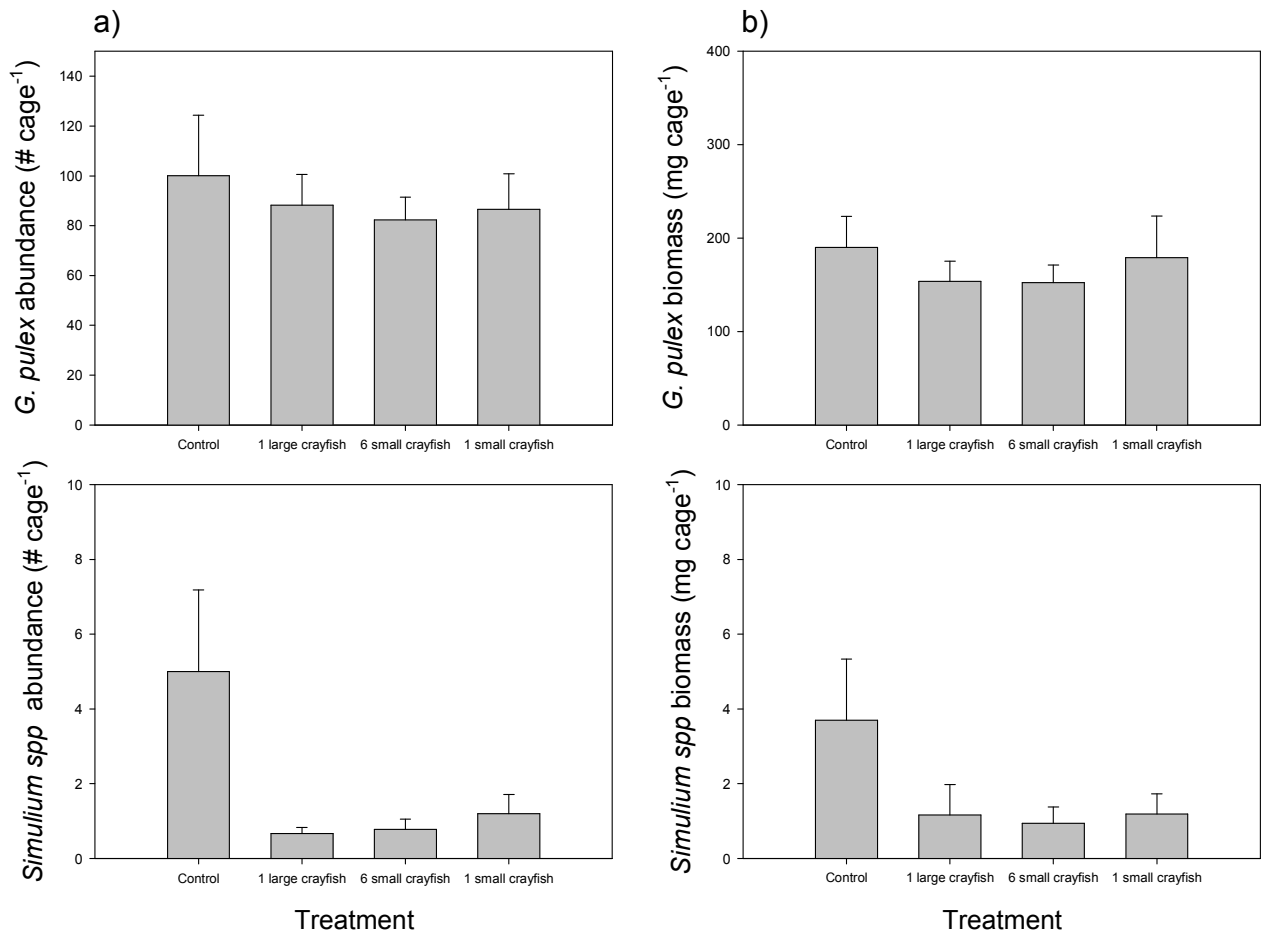
Figure 4. 6. The proportion of total macroinvertebrate abundance (a) and biomass
(b) made up by *Gammarus pulex* and Chironomidae for each treatment (+ 1 SE).
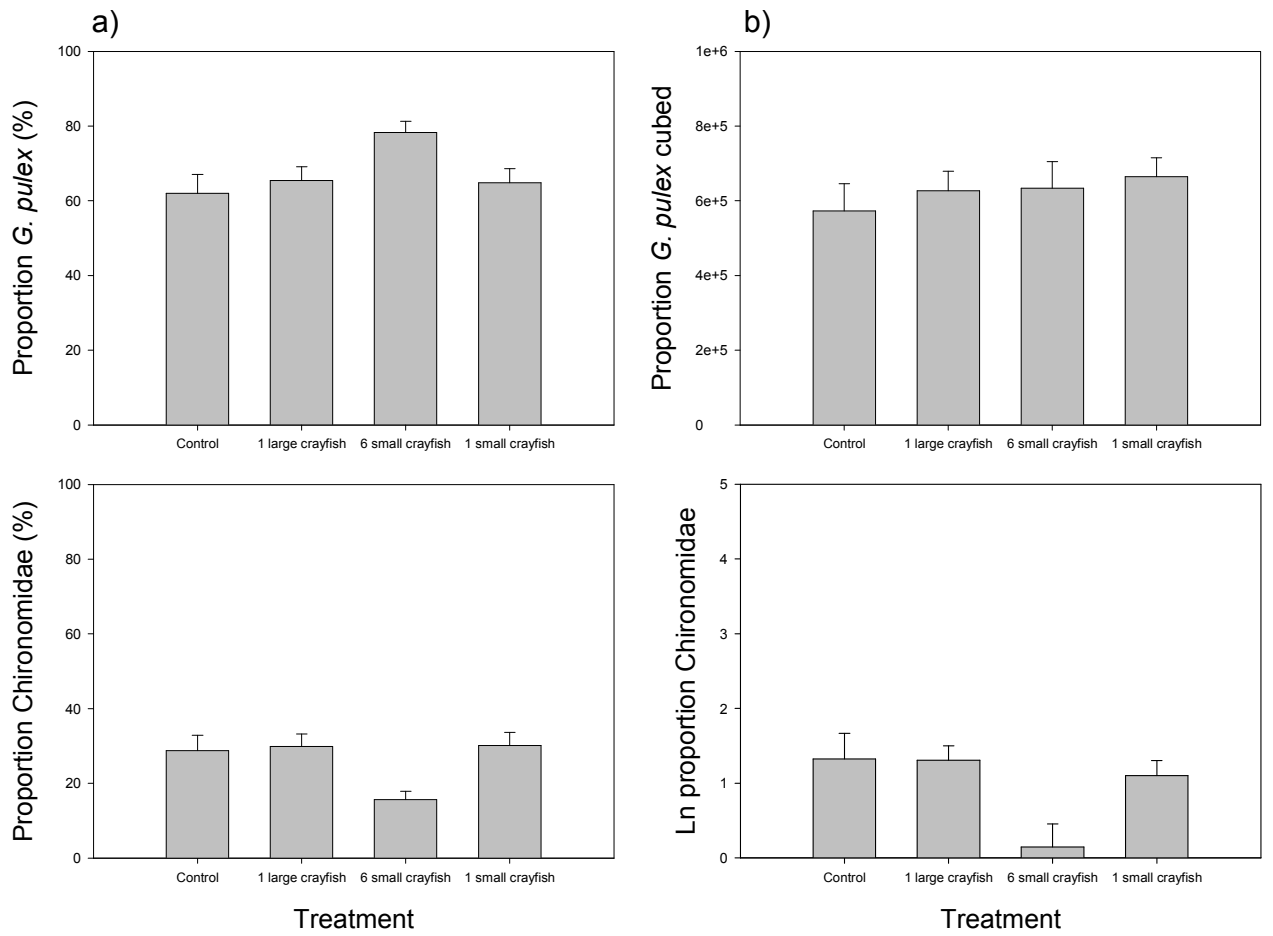
Figure 4. 7. The square root abundance of rare taxa (+ 1 SE), by treatment.
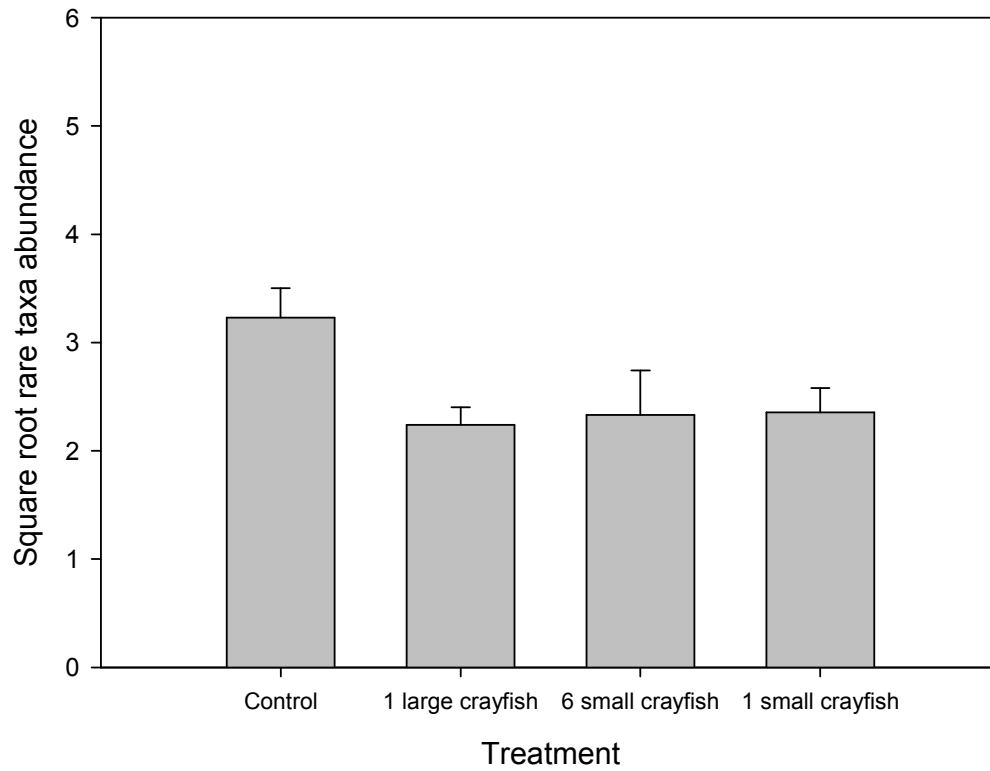
Figure 4. 8. The relationship between the skew of the sediment particle size distribution and Chironomidae abundance (a) and total biomass (b).
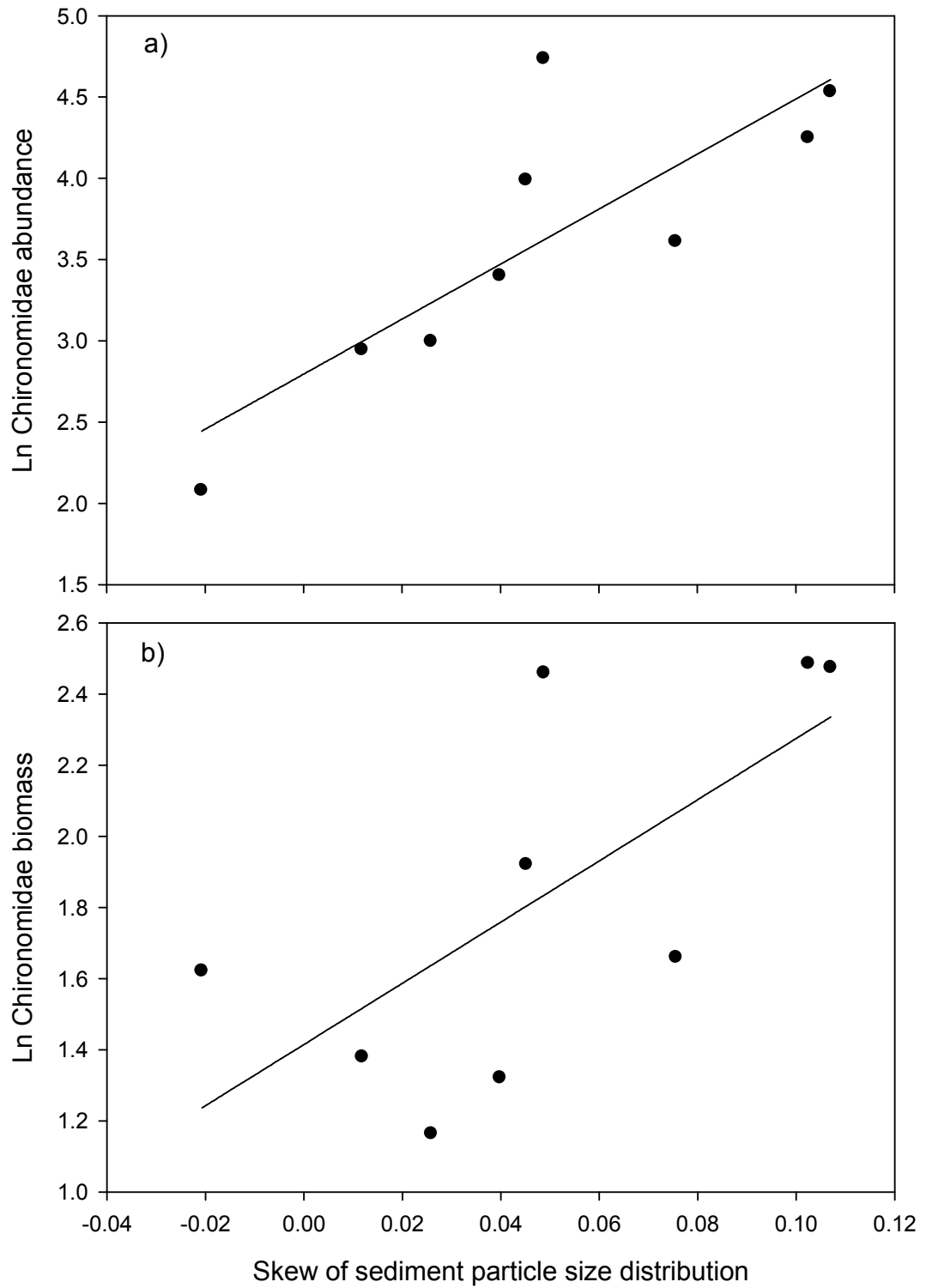
Figure 4. 9. The relationship between the skew of the sediment particle size

distribution and *G. pulex* mean biomass.
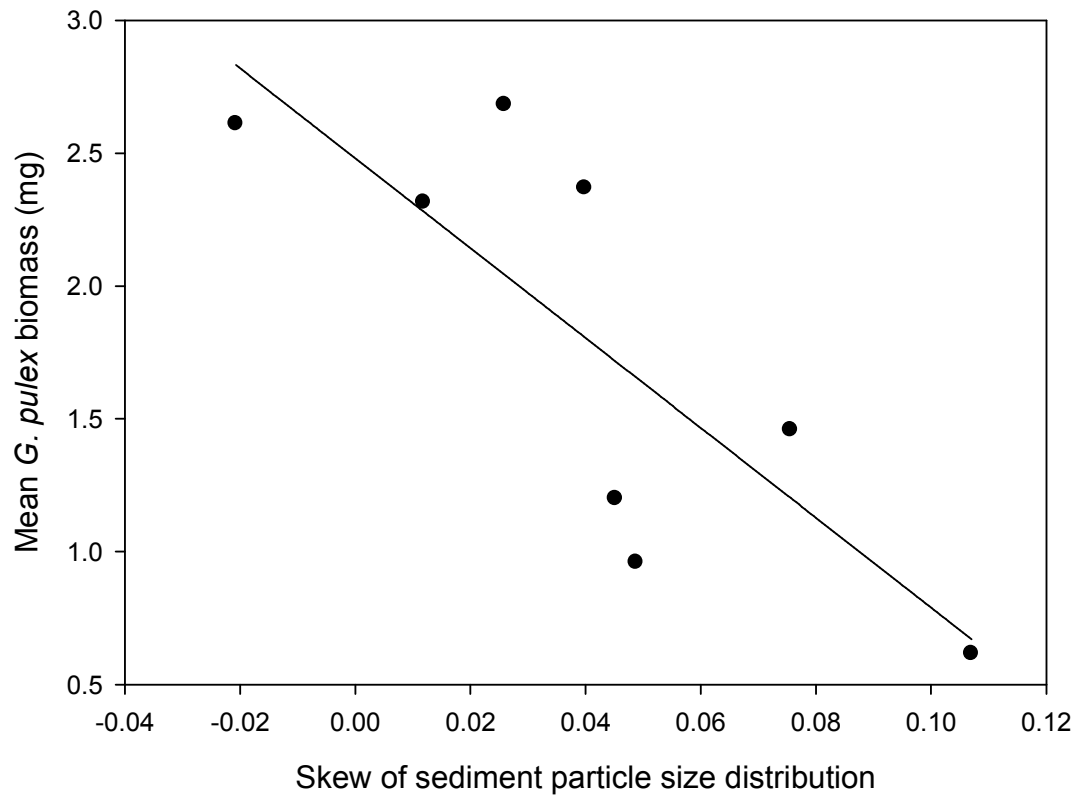
Figure 4. 10. The relationship between the skew of the sediment particle size distribution and Ceratopogonidae abundance (a) and biomass (b).
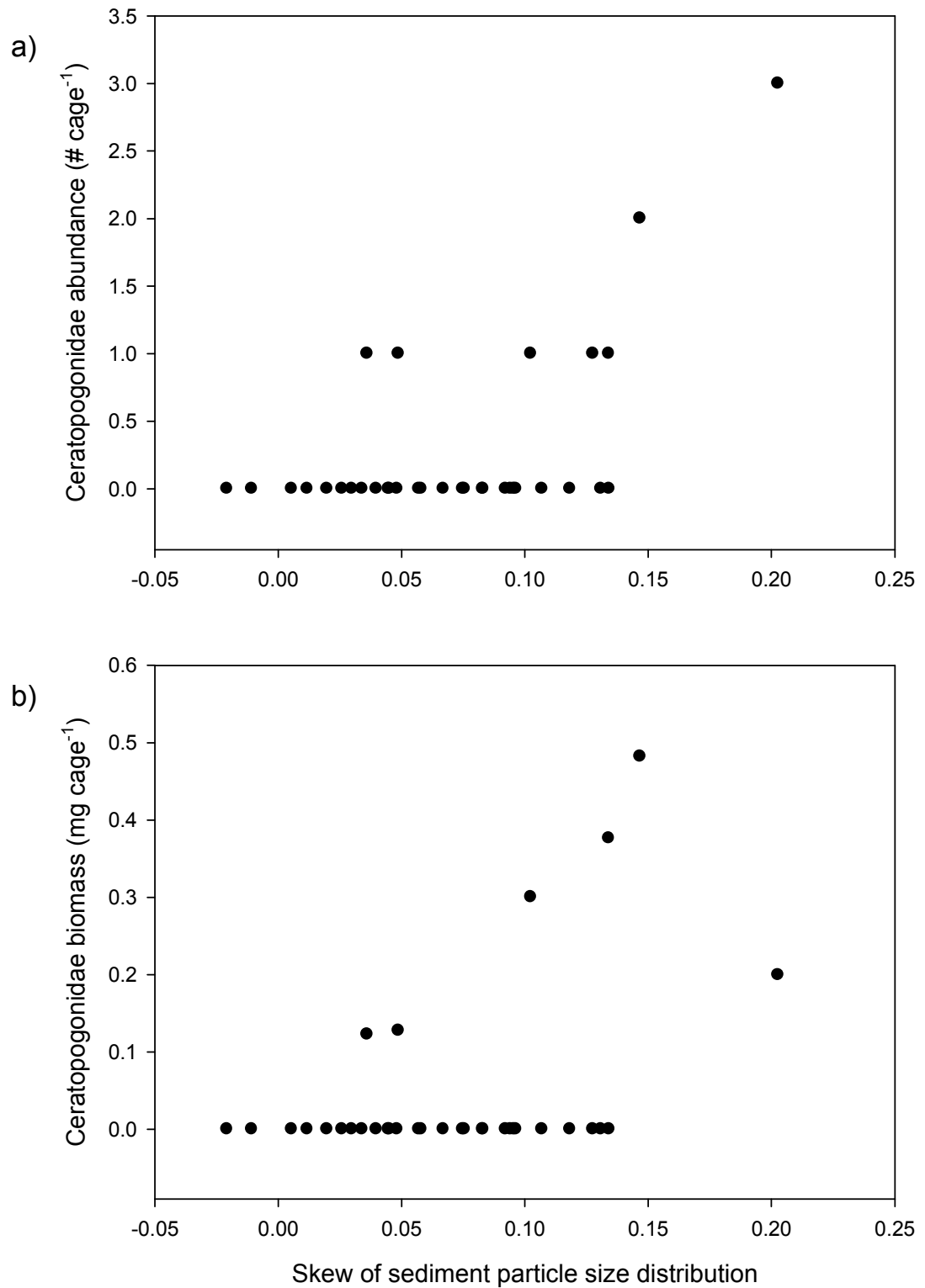
Figure 4. 11. The relationship between the proportion of sediment under 0.5mm in size and the abundance of *Silo nigricornis*.
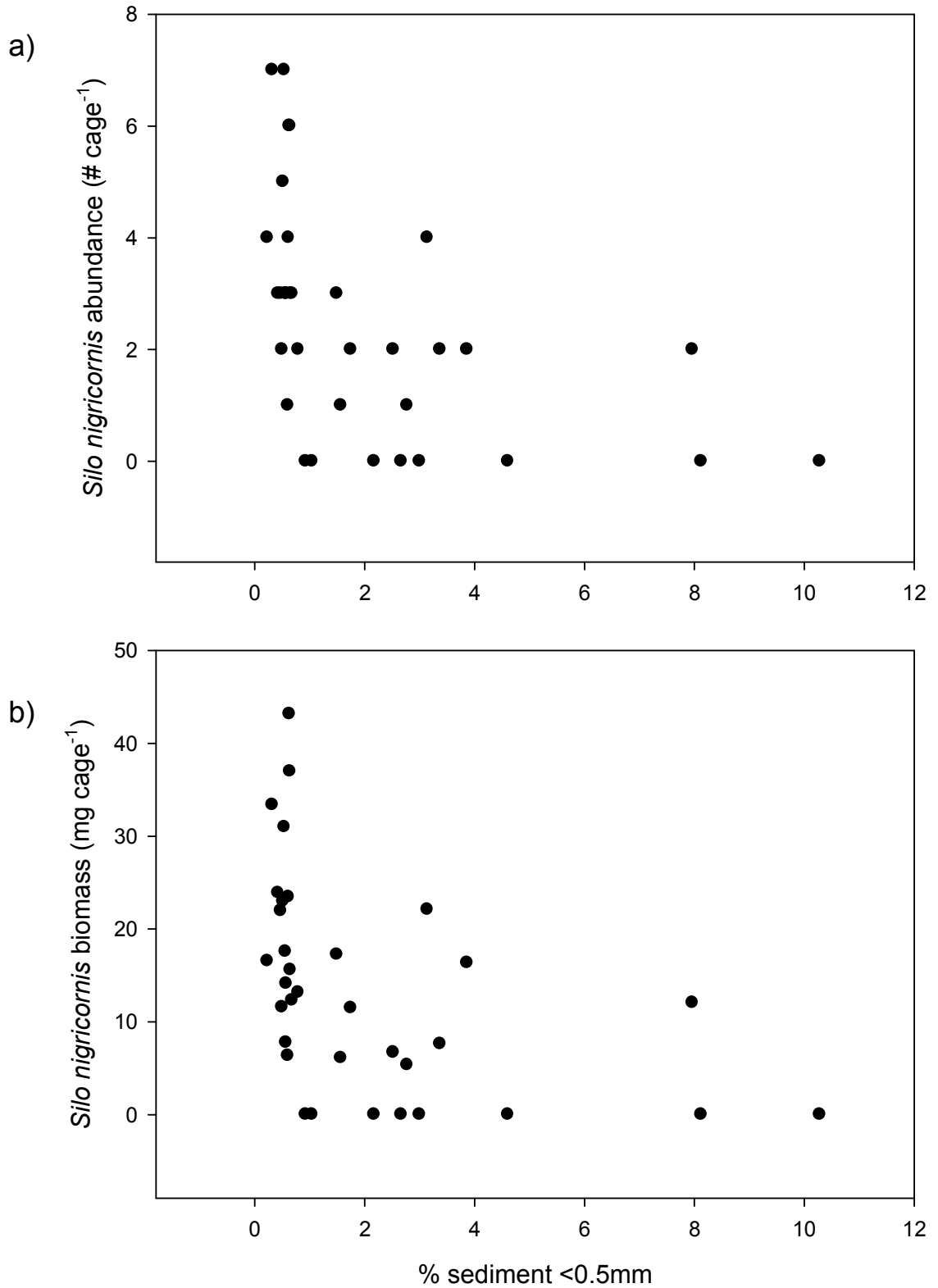
Figure 4. 12. The relationship between the proportion of cage sediment under 0.5

mm in size and the abundance of *G. pulex* (a) and Chironomidae (b) of the leaf packs.
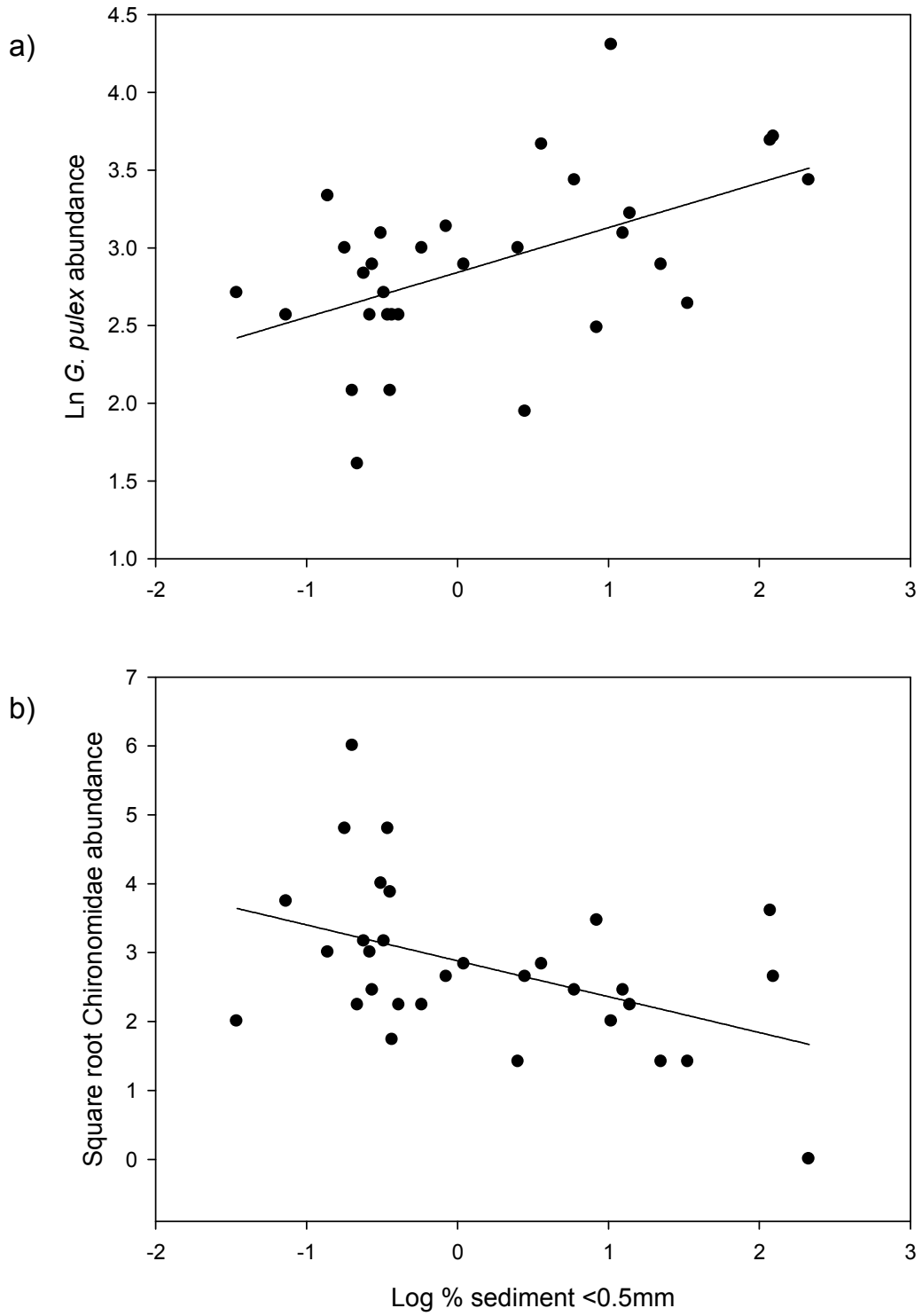
Figure 4. 13. a) Log chlorophyll *a* (+ 1 SE) removed from colonisation tiles at the end of the experiment. b) Percentage mass loss (+ 1 SE) of leaf litter packs.
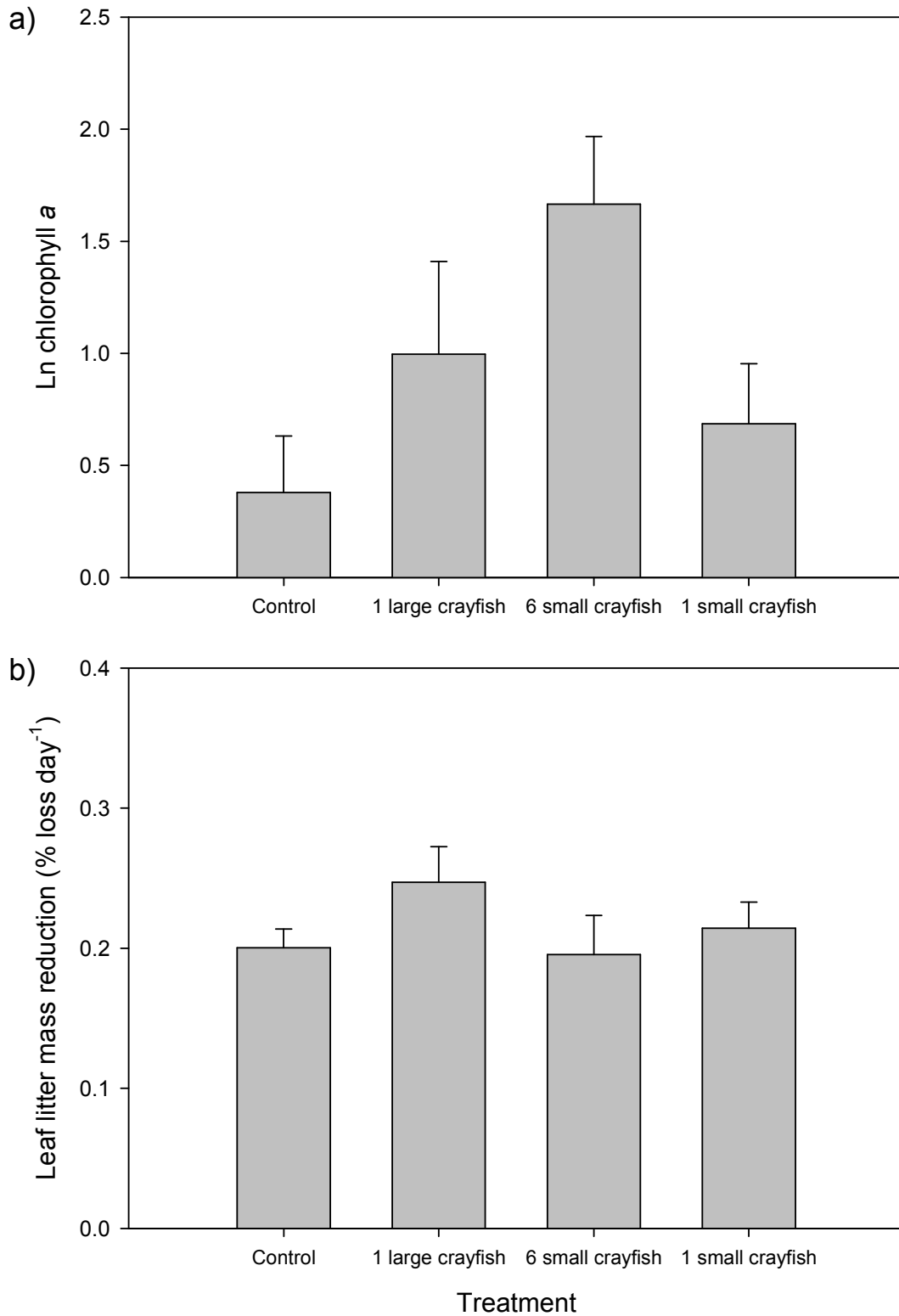
Figure 4. 14. a) δ^{15}N represents the relative trophic positions (means + 1 SE) of crayfish of each treatment. b) Horizontal scatter plots demonstrate the δ^{13}C ranges for crayfish of each treatment. The points for the single and six small crayfish treatments are subsets with a range closest to the mean range derived from 10 random subsamples.
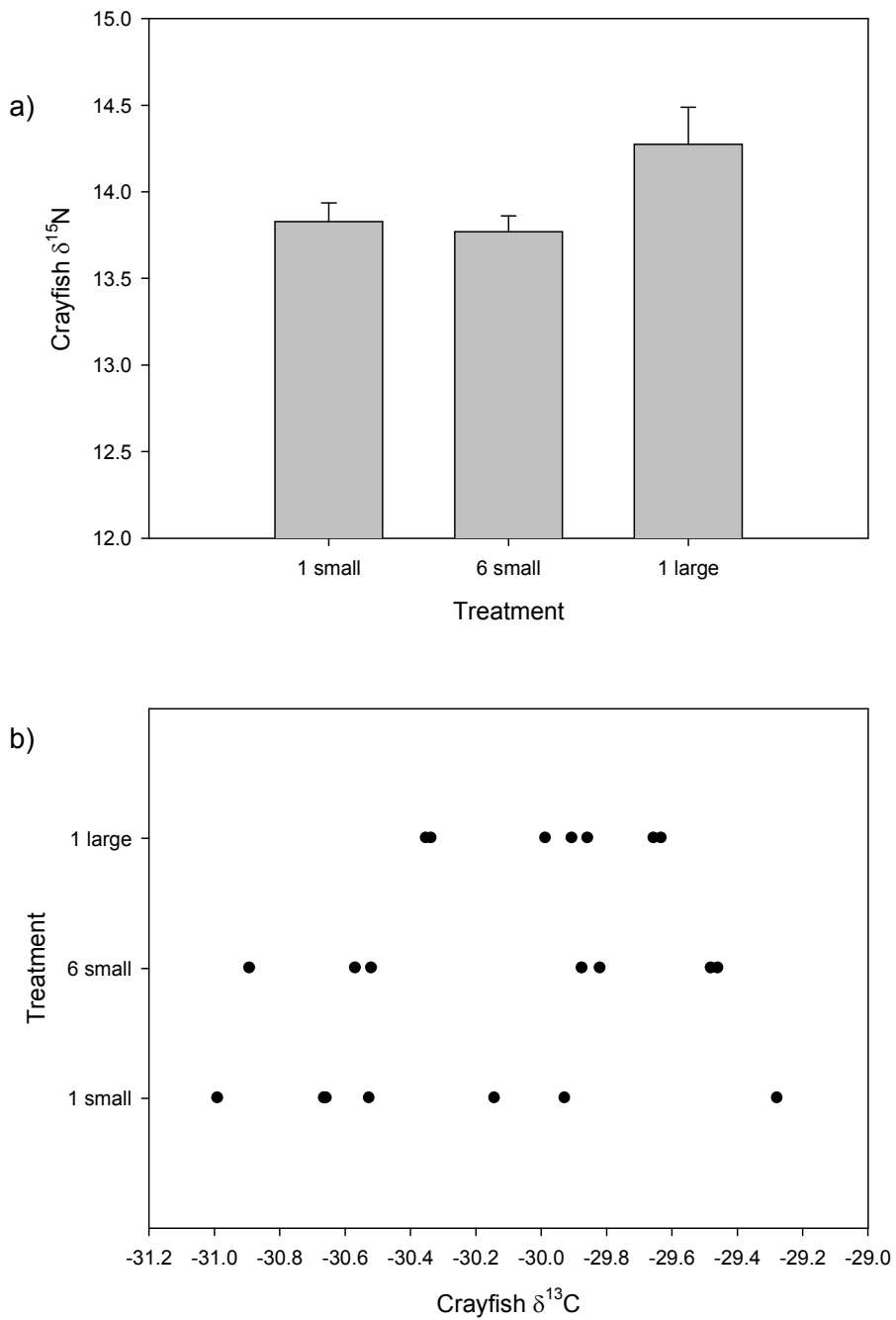
Discussion

Ecosystem engineering:

Crayfish did act as ecosystem engineers but there was a body size effect: small crayfish led to increased suspension of fine sediment whereas large crayfish did not. Presumably sediment was suspended via the foraging behaviour of crayfish or during intraspecific interactions. As the skew of sediment distributions of single small crayfish and six small crayfish treated cages were near identical, it seems that foraging behaviour alone might account for the increased suspension of fine material – as solitary crayfish cannot have been engaged in intraspecific interactions. Consequently, this discrepancy between size class effects likely reflects ontogenetic behavioural and dietary differences. Chironomidae abundance was significantly reduced in the sediment of cages treated with six small crayfish, but not in those of large crayfish or a single small crayfish. As the impact on the sediment was similar for a single small crayfish and six small crayfish, the reduction in Chironomidae abundance was almost certainly attributable to predation by crayfish. This clear preference for sediment dwelling chironomids might explain the influence of small crayfish on the sediment distribution. However, activity levels might simply be different between crayfish life stages.

The results suggest that small crayfish are capable of modifying the substrate they inhabit, while crayfish six times greater in body mass make no noticeable difference to the distribution of the sediment. It is also interesting that no difference in the distribution of sediment was revealed between the single small crayfish treatment and the six small crayfish treatment. It might be expected that the foraging effects on sediment would be additive, however it is possible that the activity of a single 'smaller' crayfish is sufficient to re-suspend fine sediment at the rate at which

it is being deposited, in this case within the cage. Therefore any extra crayfish activity would have little further effect.

Not only did crayfish directly influence the composition of sediment, but I can provide evidence of how this direct 'engineering' effect is likely to have indirect consequences for invertebrates of the sediment. Chironomidae and Certaopogonidae were found to correlate positively with the skew of the sediment particle distribution; the same measure of the sediment that small crayfish influenced. Furthermore, the mean biomass of *G. pulex* correlated with this skew. Therefore, it is likely that crayfish have size dependent indirect effects on macroinvertebrates of the sediment. However this did not extend to macroinvertebrates of the leaf packs, some of which responded to the proportion of surrounding sediment comprised of fine particles, but not to the measure of skew.

There exist very few examples of how engineering effects are determined by ontogeny and yet such effects might be common across ecosystems. As a marine example, the benthic carnivorous fish, *Parupeneus barberinus* (Lacépède) is a likely candidate for displaying engineering characteristics dependent on ontogenetic dietary shift. Smaller individuals have been shown to forage mostly within the top 2 cm of sediment, while larger fish often reach depths of 10 cm (Lukoschek and McCormick, 2001). It seems likely that such differences would result in varying changes to the structure of the sediment. The results emphasise how it can be problematic to view food webs as being made up by species with fixed functional roles. Recent years have seen growing support for trait-based measures of individuals in food webs, rather than simply classifying species by identity alone (Reiss et al., 2009). This has mainly been considered through size-structuring of communities and feeding

relationships (Woodward et al., 2005). The findings highlight how a non-trophic trait can change with body size.

If crayfish can have simultaneous direct and indirect effects on the macroinvertebrate community, then it is important to determine the net consequences. Clearly at the higher density treatment of small crayfish, the net effect on Chironomidae abundance and biomass was negative (Figure 4.4). Ceratopogonidae were too scarce for any effects, net or otherwise, to be tested. No significant effects of treatment on either mean Chironomidae or *G. pulex* biomass were found.

Community structure:

Crayfish have previously been shown to reduce macroinvertebrate diversity, in both standing and running waters (see pages 28 & 34 - 35 of Chapter One), but this study is the first, I am aware of, to show that this effect is dependant on the life stage of the crayfish, when accounting for biomass. As taxon richness showed no reduction in the six small crayfish treatment, the clear reduction in diversity (as represented by the Simpson diversity index) may be attributable to a decrease in the evenness of the invertebrate community. This appears to be the case, as the second most abundant taxon, Chironomidae, was heavily reduced. The already highly dominant *G. pulex* therefore skews the evenness further; with the next most abundant invertebrate after the Chironomidae, *Silo nigricornis*, making up just 2% of the total abundance. The corresponding results for *G. pulex* and Chironomidae percentage dominance of the total number of invertebrates followed this pattern – a decrease in Chironomidae abundance meant *G. pulex* made up a greater proportion of the resulting total number remaining (Figure 4.6). However, for dominance of total benthos biomass, *G. pulex* results did not follow this pattern. Despite a significant reduction in the proportion

of the biomass made up by Chironomidae, there was not a concurrent increase in the

contribution of *G. pulex* to the total biomass, indicating a shift in the size distribution

of *G. pulex* in the six small crayfish treatments.

Further support for crayfish reducing the evenness of the benthos is the

reduction seen in the abundance of rare taxa, which was similar in all the crayfish

treatments (Figure 4.7). This result is surprising when considered in contrast to the

Chironomidae results. The relatively abundant Chironomidae were only reduced in

the six small crayfish treatment, while rare taxa, of which individual taxa comprised

2% or less of the benthos, were reduced in all treatments. Many of the taxa within

the rare taxa grouping were much larger in size than the Chironomidae and might

therefore have been more readily consumed than the Chironomidae. Free-living taxa

may also have been more accessible to crayfish relative to the typically tubicolous

Chironomidae. The results for rare taxa abundance suggest that, overall, any

influence of sediment composition is insignificant in comparison to the direct effects

of crayfish. Furthermore, across the size-range used in this study, crayfish appear to

act as generalist predators. Alternatively, it may be the case that some of the rarer

taxa displayed predator avoidance.

Sedentary and soft-bodied macroinvertebrates are favoured by crayfish as

prey (Whitledge and Rabeni, 1997). Gut content analysis of signal crayfish in

Swedish streams revealed that, after detritus, the most frequently occurring food

items, in order of frequency of occurrence, were Simuliidae and Chironomidae

(Stenroth and Nystrom, 2003). Similarly, the gut contents of crayfish in an English

lowland river revealed that, excluding crayfish fragments, Chironomidae were

volumetrically the most important animal food item (Guan and Wiles, 1998). The

results presented here provide evidence that the preference of crayfish for these items

suppresses prey abundance at a fine spatial scale and that these impacts can vary with crayfish life stage.

Ecosystem process rates: algal standing stock and litter breakdown:

Crayfish had a clear positive effect on algal standing stock, but it was ambiguous as to whether this was attributable to a trophic cascade or non-trophic engineering effects, where crayfish re-suspend fine sediment / organic matter from the surface on which periphyton grows – thus improving light conditions for algal growth. However, owing to the following points, I believe the evidence indicates trophic cascading effects having contributed to the periphyton results. The only significant increase in the concentration of chlorophyll *a* was seen in the six small crayfish treatment relative to the control. The single measure of the sediment composition, found to be affected by crayfish, was the skew of the sediment particle size distribution. Skew was equally affected in both the small crayfish treatments, yet results for chlorophyll *a* are dissimilar. Furthermore, no measures of the composition of the sediment were found to correlate with chlorophyll *a*.

The distinction of the two alternative explanations for the increased algal biomass is important, as in the trophic cascade scenario energy flow from producers to primary consumers is reduced, whereas in the engineering argument, it is algal standing stock that is enhanced in the presence of crayfish. In the first case the energetic input into the ecosystem is unchanged but follows an altered pathway, whereas in the second case this energetic input is increased.

Ontogeny was important in determining community level effects of the crayfish: gram for gram; large and small crayfish did not have the same effect on algal standing stock. This re-emphasises the point made above, that there is a need

for quantitative measures of specific traits when considering an organism's role within an ecosystem. Trophic cascades, dependent on predator ontogeny, are likely to be common in aquatic ecosystems; as demonstrated with theoretical modelling, where consumer cohort size structure can lead to a catastrophic collapse of predator populations (De Roos and Persson, 2002). Empirical data has demonstrated how large cannibalistic perch can drive dynamic, whole-lake trophic cascades (Persson et al., 2003).

No crayfish-detritivore-detritus cascade was observed. The main reason for this did not appear to be a decoupling of a potential cascade, as predicted, but the fact that the predominant shredder found within leaf packs, *G. pulex*, was unaffected by crayfish presence. This is in marked contrast to the trophic cascade attributable to bullhead predation on *G. pulex* (Woodward et al., 2008), which is of particular relevance owing to the co-occurrence of these two species and potential competition between them (see Chapter Five).

It is important to note that for the invertebrate diversity and chlorophyll *a* data, where the six small crayfish treatment was significantly different to the single large crayfish treatment, the per capita effect of the larger crayfish is likely to have been greater. Furthermore, contrary to expectations, and despite the gram-for-gram increased impact of smaller crayfish on Chironomidae and the diversity of macroinvertebrates, on a per capita basis, stable isotope data revealed that large crayfish occupied a higher trophic position, relative to the small crayfish, at this locality. It should be noted, however, that owing to the slow turnover time of stable isotopes in muscle tissue (McCutchan et al., 2003), differences in stable isotope ratios between the two size classes most likely represented the diet of crayfish prior to the cage experiment. In the instances of invertebrate diversity and chlorophyll *a*

therefore, the results demonstrate how, per unit biomass, smaller individuals can have a greater impact than their larger conspecifics.

It has previously been suggested that crayfish often act as keystone species – inducing disproportionate effects in the ecosystems of which they are part (Creed, 1994, Charlebois and Lamberti, 1996, Nystrom et al., 1996, Olsson et al., 2006). While this study has shown that the impacts of invasive signal crayfish may be broad – influencing the macroinvertebrate community, the physical environment and a component of ecosystem functioning – these effects were relatively weak. In some cases, sediment characteristics or block effects were equally or more important than crayfish treatment, suggesting that the term 'keystone species' might not justifiably be applied to signal crayfish, at least in the context of this study.   Furthermore, it is important to restate that the density of the six small crayfish treatment was artificially high, and therefore 'keystone effects', such as suppression of prey abundance / trophic cascades, are unlikely to be observed at lower, more natural densities. The presence of invasive crayfish typically promotes anxiety from a biodiversity perspective, and thus, these results may seem encouraging. However, it should be noted that the study was conducted at a site where signal crayfish have been present for some time. We cannot be sure whether the crayfish have historically led to extirpation of species from the site, or whether their effects would be more pronounced at a naïve site. However, as streams are open systems, extirpation seems unlikely.

As the relative impacts of crayfish varied with life stage, the results of this experiment are of relevance for the management of invasive signal crayfish, as the widespread trapping of signal crayfish, that currently occurs, selectively removes

larger individuals (Usio et al., 2009).   There is a distinct possibility that the

demography of wild populations is skewed towards smaller individuals, which

appear to have different functional roles within invaded ecosystems than their larger

conspecifics. More work is required to determine whether trapping actually leads to

net benefits.

Chapter Five: Interactions between invasive crayfish and a native benthic fish and implications for the benthic assemblage

Declaration on input

I am indebted to Adrian Hards, an MSc student previously based at Queen Mary, for his input to this chapter. Adrian constructed the channels used in the experimental section of this study. Furthermore, Adrian conducted the day-to-day maintenance and sampling of the channels and carried out the initial processing of samples.

Introduction

The previous two chapters have focused on the direct trophic impacts of signal crayfish and their role as ecosystem engineers. In this chapter potential competitive effects of signal crayfish invasion are considered, using the native bullhead as a competitor. Furthermore, the consequences of competitive interactions on the benthos are investigated.

A recap on invasive species and competitive interactions and the ecological relationships between fish and crayfish:

As discussed in the introductory chapter, competition for resources, and non-trophic interactions between native and invasive species, can lead to declines in indigenous

taxa. These relationships can have indirect consequences elsewhere within a food web, particularly for prey where interactions occur between native and non-native predators. Competitive and predatory interactions between crayfish and fish are potentially complex and they are poorly understood.

Crayfish and bullhead:

The dominant predators of the stream ecosystems under investigation are the invasive signal crayfish and a native fish species, the bullhead. The diets of the two species can be inferred to overlap, with Chironomidae and Ephemoptera being important prey items of both taxa (Dahl, 1998, Stenroth and Nystrom, 2003). However, interactions between these predators extend beyond competition for prey alone. Both bullhead and crayfish are crepuscular / nocturnal foragers and seek refuge during daylight hours (Smyly, 1957, Hill and Lodge, 1994). It has been shown that signal crayfish generally out-compete bullhead for shelter (Guan and Wiles, 1997, Bubb et al., 2009) which is likely to have consequences for bullhead mortality through increased exposure to predators at sites where refugia are limited. It is intuitive that the combined effects of increased competition for both prey and shelter would result in negative impacts of signal crayfish on bullhead populations. Indeed this has been shown in two separate studies; bullhead abundance correlated negatively with increasing crayfish density (Guan and Wiles, 1997, Bubb et al., 2009). However, while an impact has been demonstrated locally, i.e. two cases within single rivers; it has not been shown at a greater scale, for example across streams / rivers of a catchment.

The presence of invasive signal crayfish can also benefit bullhead, as was shown in a field experiment in Sweden, where juvenile signal crayfish were the third

most numerous prey item in the gut content of bullhead (Dahl, 1998). However, reciprocal predation between the two species is likely, as observations of signal crayfish preying upon bullhead have been made in aquaria (Guan and Wiles, 1997). Therefore, the ontogeny of both species is important in determining the impact of invasive crayfish on bullhead; smaller bullhead will be at greater risk of predation and competition for shelter from larger crayfish, whereas large bullhead will experience reduced predation pressure and find a novel prey item in smaller crayfish.

Crayfish mediated reductions in invertebrate abundance have already been outlined (pages 34 - 35), and, furthermore, support for such relationships was demonstrated in Chapters Three and Four. Sculpin (fish belonging to the superfamily Cottoidea, which includes bullhead) have been shown to have powerful top-down effects in lotic systems (Cheever and Simon, 2009). Abundances of mayfly nymphs, *Gammarus pulex*, signal crayfish, caddis larvae, stonefly larvae and *Potamopyrgus antipodarum* (Gray) were all reduced by bullhead in enclosure / exclosure experiments (Dahl, 1998, Woodward et al., 2008). Bullhead are capable of reducing the abundance of a dominant shredder, *G. pulex* to such a degree that a trophic cascade can result, where the breakdown of leaf litter is suppressed (Woodward et al., 2008). Despite clear overlap in bullhead and crayfish prey, some degree of complementary predatory effects might be expected, as sculpin have been shown to forage selectively for motile invertebrate prey, while crayfish consume more sessile prey (Cuker et al., 1992, Nystrom and Strand, 1996, Nystrom et al., 1999).

Stable isotope methods:

The use of stable isotopes in ecology has become increasingly common in recent decades as a tool for dietary analysis. However their use has tended towards making comparisons between taxa, focusing on their mean stable isotopic values, while intraspecific variation has largely been ignored (Grey, 2006). There are exceptions, and shifts in diet following an invasion have previously been demonstrated with the use of stable isotopes (Vander Zanden et al., 1999, Maguire and Grey, 2006). However the stable isotope approaches employed have generally been limited to independent measures such as mean $\delta^{13}C$ or $\delta^{15}N$ values. The intraspecific variation in stable isotope values of individuals within a population is of interest, as these differences can reflect the variation in diet among the individuals making up the population.

The simplest measures proposed for testing intraspecific variation using stable isotopes are ranges or variance in $\delta^{13}C$ and $\delta^{15}N$ (Bearhop et al., 2004). The $\delta^{13}C$ range of individuals within a population can yield information on the dietary breadth among individuals; it is a measure of differing dependence on shared food items and / or consumption of a different pool of food items. The $\delta^{15}N$ range can represent at how many trophic levels a population is feeding. Variance in $\delta^{13}C$ and $\delta^{15}N$ has previously been used to demonstrate release from competition and dietary niche shift in populations of perch and roach (*Rutilus rutilus*, Linnaeus) after large scale fish removal in a lake (Syvaranta and Jones, 2008).

Metrics of somewhat greater complexity, derived from stable isotope data, have been proposed for analysing interspecific variation of isotopic niche space in whole communities (Layman et al., 2007). These include the convex hull total area (TA) of a community and the centroid distance (CD) of species within it. TA is the

value obtained when straight lines are drawn around the peripheral taxa of a community in $\delta^{13}C$ and $\delta^{15}N$ bivariate space, encompassing all species (Figure 5.1). This measure therefore combines $\delta^{13}C$ and $\delta^{15}N$ information and is a representation of the total niche space of the community. CD is the mean Euclidean distance of all taxa in the community from the centroid, the centroid being the mean $\delta^{13}C$ and $\delta^{15}N$ value of all taxa combined. Therefore CD is a measure of the mean degree of trophic diversity within the community.

While these metrics were proposed principally for whole community analysis, TA has been applied to great effect in single taxon populations in order for comparisons of intraspecific variation to be made among spatially distinct populations (Darimont et al., 2009, Olsson et al., 2009, Quevedo et al., 2009). For example, niche partitioning was shown in perch, with distinct intrapopulation niches of pelagic and littoral foraging individuals (Quevedo et al., 2009). However a shortcoming of TA is that it is highly sensitive to sample size (Jackson et al., 2011). An alternative has been developed based on the standard ellipse area (SEA), which effectively is to bivariate data what standard deviation is to univariate data. Standard ellipses have been combined with corrections to allow for robust analyses to be made, even when sample sizes are low (Jackson et al., 2011). Throughout this and the following chapter, corrected SEA (SEA$_c$) is used. A visual representation is shown in Figure 5.1.

Stable isotope metrics representing intraspecific variation of individuals among populations must be used with some caution. Variation among individuals of a population will be determined in large part by the identity and proportion of food items in their diet. Individual differences in physiology and diet-tissue fractionation will add some noise to the metrics, but this will be small, with variation being

predominantly ecological in origin (Bearhop et al., 2004). More important in the context of the work presented here is the possible variation among populations attributable to variation in prey isotopic values. As δ^{13}C and δ^{15}N in basal resources can vary with geographic location and season (Yoshioka et al., 1994, Post, 2002), for the same suite of prey items, eaten in identical proportions (identical for the same individuals between location and season – not identical diet among individuals), the isotopic niche space of a population of predator x might vary substantially between location / time point (Figure 5.2). Despite this caveat, these metrics represent a powerful ecological tool where the sampling protocol is appropriate (Layman and Post, 2008). As the sites used in the current study were spatially and temporally proximate, and all of approximately the same ecotype, confounding variation in resources was kept to a minimum. Furthermore, variables which may contribute to differences in producer δ^{13}C, including water velocity (Finlay et al., 1999) and shading (Finlay, 2001) were measured for inclusion in analyses.

The distance between the centroids of sympatric populations of competing taxa is of interest, as with decreasing distance there is an implication that more of the diet is shared. If competition with crayfish for prey items results in a shift in bullhead diet away from prey items shared with crayfish, I expect the distance between centroids of these populations will increase with increasing crayfish densities. All these measures can be produced for each site's bullhead and crayfish populations and analysed in relation to the abundance of crayfish.

Aims and hypotheses:

The aims of this study can broadly be divided into two parts. The first aim was to address the question of whether invasive crayfish negatively affect populations of a

native competitor, the bullhead. While experimental work and limited field data suggest such negative relationships exist, it has not been shown at a regional scale and furthermore the mechanisms by which bullhead populations might show declines are poorly understood. To address these issues a field survey was carried out across multiple stream sites to observe and compare natural densities of crayfish and bullhead where they co-occur. Further, the two taxa were sampled for stable isotope analyses and population metrics based on stable isotopes were used to explore dietary trends of the sympatric species. Channel experiments were also conducted, in part to determine if the isotope results obtained from the field data would be reproducible with experimental treatments under controlled conditions.

I hypothesised that bullhead abundance would correlate negatively with increasing abundance of crayfish across sites on chalk streams of the north and north east of the Thames catchment, where bullhead and crayfish co-occur. Furthermore, this would be driven by the abundance of larger size classes of crayfish. I also expected to see a narrowing in the breadth of bullhead diet with increasing abundance of crayfish, despite smaller crayfish potentially becoming prey items. The negative correlation between crayfish and the abundance of shared prey items found in these streams (reported in Chapter Three) supports this hypothesis.

There is a paucity of publications on the effects of introduced (predatory) competitors and the consequence of interactions for lower trophic levels. Therefore, the second aim of this study was to investigate competitive interactions between signal crayfish and bullhead, and how interaction, if it occurred, would affect the top-down regulation of the stream benthos. This aim was addressed with an artificial channel experiment.

I hypothesised that owing to the antagonistic interactions between crayfish and bullhead, foraging efficiency of these competitors would be reduced when in sympatry, as compared with top-down effects of either taxon in allopatry.

Methods

Survey work:

In order to compare the densities and diets of bullhead and crayfish in sympatry, six sites on proximate chalk streams in the south east of England were selected (Table 5.1, Figure 5.3). Sites were selected in order to give a gradient of natural bullhead and crayfish densities. Site selection was limited to localities where the two species co-occurred to remove the influence of whatever factors might have excluded one or other of the taxon completely, i.e. all sites were suitable for both bullhead and crayfish. Unfortunately, the difficulties in selecting sites suitable for use of the modified Hess sampler (as outlined on page 72) meant that the sample size was low. Furthermore, two sites were located on the same river. These two sites had crayfish populations with different size distributions and therefore represent distinct data.

Fieldwork was carried out between the 19th and 30th of October, 2009. Quantification of bullhead and crayfish populations followed the protocol described in Chapter Three (page 73). In this instance bullhead and crayfish were not returned to the stream but taken for stable isotope analysis and frozen on return to the laboratory. Once quantification of bullhead and crayfish density was complete, additional individuals were collected by manual search with pond nets. The search continued until 10 individuals of both taxa had been collected or alternatively until the entire 50 m^2 of the site had been thoroughly searched.

Crayfish were prepared for stable isotope analysis in the same way as described in Chapter Four (page 116). In the case of bullhead, muscle tissue was cut away from both flanks of the tail and the skin removed, leaving a sample of pure muscle. Samples were dried to constant mass at 60 ºC and the subsequent process was the same as in the case of crayfish samples. Both bullhead fork length and crayfish carapace length were measured with vernier callipers. Crayfish sex was also recorded.

The free-to-download packages SIAR and Stable Isotope Bayesian Ellipses in R (SIBER) were used for calculation of metrics *sensu* Layman and SEA$_c$ respectively (Parnell et al., 2010, Jackson et al., 2011). The distance equation,

$$d = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}$$

was used to calculate distance between centroids. Where *d* equals the distance between centroids and *x* and *y* are paired coordinates of bullhead and crayfish population centroids. Data was analysed using Minitab v14.1, following $\log_e(x)$ transformation to meet the assumptions of normality and homogeneity of variance, where necessary. Best subset regressions were carried out and variance inflation factors were included in output to ensure predictor variables were not co-linear.

Experimental work:

Artificial channels were constructed out of PVC troughs 1.8 m long, 0.185 m wide and with an average depth of 0.14 m (Figure 5.4). Constant flow was provided at a rate of 0.08 L s^{-1} from a recirculating supply. The outflow to each channel was covered with 4 mm mesh; allowing passage of almost all invertebrate drift, but preventing escape of bullhead or crayfish. In order to prevent accumulation of carbonic acid a filter containing limestone (calcium carbonate and calcium

magnesium carbonate) was connected to the second holding tank. Weekly measurements of water temperature, pH and velocity were made for each channel. The light treatment in the aquarium was 12 hour cycles of light and dark. Lights came on at 10:00 and went out at 22:00.

Fauna, substrate and water used in the experiment were all collected from the River Darent (Figure 5.5). The Darent is a chalk stream tributary of the river Thames approximately 40 km in length. Bullhead were collected at a site at Farningham (Figure 5.5; OS: TQ 546 670) and crayfish at a site at Otford (Figure 5.5; OS: TQ 524 593). Both were collected by manual search using hand nets. As no crayfish were observed at the Farningham site, it is unlikely that the bullhead had had previous interaction with crayfish.

For logistical reasons, water, substrate and invertebrates associated with the substrate were collected at a third site, located at Eynsford (Figure 5.5; OS: TQ 540 655). Capacity was approximately 600 L, made up by approximately 420 L of river water and 180 L of distilled water and alkaline solution (Smart and Barko, 1985). Substrate was dominated by pebbles and cobbles, on a bed of sand and gravel (substrate types as defined by Wentworth, (Wentworth, 1922)). Approximately 8.3 L of mixed sediment were added to each channel. Owing to the importance of substrate particle size for the benthic community (Boulton et al., 1998, Rempel et al., 2000) the stream bed at the Farningham site was sampled for sand and fine grain substrate and approximately 1.7 L of this was added to each channel.

Further to the invertebrates collected in the original sediment collection, haphazard kick samples were taken on the 16th of June to ensure that the channels were seeded with sufficient numbers of invertebrates to represent natural abundances. Samples were thoroughly mixed before being distributed equally among channels.

The remaining invertebrates were kept for further weekly additions in order to simulate invertebrate in-migration. Owing to an apparent lack of snails at the beginning of the experiment, *Radix peregra* (Müller) were taken from Regents Canal on the 4th June, and 30 individuals added per channel. Five more were added per channel at weekly intervals, together with the additions of kick sampled invertebrates.

Experimental treatments were assigned randomly. These were bullhead only, crayfish only and a mixed treatment of both bullhead and crayfish (see Figure 5.4 for assignments). No predator-free control was included; however, as bullhead are a common native predator in chalk streams, the bullhead treatment represents a control to the invasive predator treatment. In order for population based stable isotope metrics to be derived and tested, and to ensure realistic densities of crayfish and bullhead, a total of eight individuals were added to each channel. This represented densities of approximately 20 individuals m^{-2}, which is within the range of naturally occurring densities of both bullhead and signal crayfish (Woodward et al., 2008, Bubb et al., 2009). Bullhead and crayfish only treatments comprised of eight individuals, while the mixed treatment consisted of four bullhead and four crayfish, thus controlling for density. Bullhead and crayfish size distributions reflected natural size distributions of populations in the Darent. Bullhead fork length measured from 41 to 79 mm and in each treatment an even distribution of sizes was used. Mean size was approximately equal in all (eight individuals and four individuals) treatments. Crayfish only treatments were made up of one 'large' (carapace length 48.0 ± 2.0 mm) and seven 'medium' (carapace length 24.6 ± 2.1 mm) sized individuals and the mixed treatment one 'large' and three 'medium' sized individuals. Individuals were selected to keep the mean size approximately equal between the eight and four individual groupings.

Whilst density of bullhead and crayfish was controlled for, and individuals of approximately equal sizes were used, biomass of crayfish was greater than that of bullhead. However, owing to their differing foraging strategies (bullhead are likely to pursue more motile prey than crayfish (Cuker et al., 1992, Nystrom et al., 1999) and the distinctly different body-types of the two taxa (a vertebrate fish with a relatively light endoskeleton and an invertebrate crustacean with a heavy exoskeleton), it is not clear that equal biomass would equate with equal functionality / energy requirements. As the densities used reflect densities seen in the field and the size distributions were determined by natural size distributions, the experimental setup made worthwhile comparisons.

Consultation was made with the Home Office to ensure that the work carried out complied with British Law. Licensing under the Animals (Scientific Procedures) Act 1986 was deemed not necessary. The 52 day experiment ran from the 1st June to the 23rd July. In addition to bullhead and crayfish, algal colonisation tiles and leaf packs were added to the channels (as per Figure 5.4). Eight algal colonisation tiles (5 x 5 cm) were placed four apiece in a chequered pattern on two larger tiles (15 x 15 cm) (see Figure 5.4). A smaller tile was taken from each channel at 17, 31, 45 and 52 days for measurement of chlorophyll *a*. The remainder were sampled at the end of the experiment for stable isotope analysis. Each tile removed for quantification of chlorophyll *a* was scraped with a scalpel, brushed with a nylon toothbrush and rinsed with distilled water. Samples were processed as per methods described in Chapter Four (pages 115 - 116).

Tetrahedral leaf packs were constructed from 9 mm mesh and contained 3.5 ± 0.02 g of air dried alder leaves (*Alnus* spp.). Leaf packs were placed in channels and kept in place on the surface of the channel bed by the surrounding substrate. Owing

to the extensive processing of the first set of leaf packs (left for two weeks),

subsequent sets were replaced weekly. Leaves removed for processing were rinsed

of detritus and invertebrates in plastic trays. All invertebrates were returned to their

respective channels alive. Rinsed leaves were dried at 60 ºC to constant mass then

weighed. Reduction in leaf mass was then expressed as % mass loss day^{-1}. A

sample of leaf litter was kept for stable isotope analysis.

Whilst pebbles and cobbles of the substrate provided some refugia, each

channel contained a single standardised refuge (transected length of drainpipe, 100 x

105 x 55 mm in height) that provided complete cover (see Figure 5.4). Day (11:00)

and night time (23:00) shelter occupancy were recorded for the final 22 days of the

experiment. It was noted whether the shelter was occupied and if so by which

species. Data were expressed as percentage occupancy on a species by species basis.

Each channel was checked daily for mortality of bullhead or crayfish. If

mortalities were found they were replaced with size matched individuals. For

identification purposes, all replacements were given a distinctive marking. Twice

daily checks were made of invertebrate drift, which collected at the end of each

individual outlet pipe (250 μm mesh). Nocturnal (09:00, an hour prior to lights on)

and diurnal (21:00, an hour before lights off) drift were collected. Invertebrates were

identified and counted by eye before being returned to the upstream end of the

channel to which they belonged.

At the end of the experiment, four replicate Hess samples (sample area:

0.0087 m^{-2}; mesh: 60 μm) were taken from each channel to quantify the benthos.

Samples were taken every 40 cm from the downstream end to minimise disturbance

of the benthos. Samples were frozen immediately for subsequent processing.

Defrosted samples were processed as per Surber samples of Chapter Three (page 74).

Once identification and measurements had been made, invertebrates were either preserved in ethanol or dried at 60 ºC for stable isotope analysis. Bullhead and crayfish were removed from the channels following collection of the Hess samples and frozen for stable isotope analysis. Processing followed the same procedure as described above for stable isotope analysis of the survey work (page 153). Rare taxa were classified as per Chapter Four (page 119).

For analysis, $\log_e(x)$ and $\sqrt{x}$ transformations were used where necessary to ensure normal distributions of data. Effects of treatment were tested using General Linear Models in Minitab (Version 14.1). Where data could not be normalised comparisons were made using Kruskal-Wallis tests.

Results

Survey work:

No relationship was observed between the density or biomass of crayfish and the abundance or biomass of bullhead (Table 5.2). Although lacking baseline correction, there was a general separation in isotopic niche space between bullhead and crayfish populations among sites (Figure 5.6). When considered on a per site basis (for which baseline correction is unnecessary), the isotopic niche spaces of co-occuring crayfish and bullhead populations, as represented by SEA_c, were found to be consistently distinct (Figure 5.7). Individual bullhead and crayfish showed isotopic overlap at three of the six sites; however in general the degree of overlap was marginal.

Significant relationships were found between the log SEA_c values of bullhead and both crayfish biomass and the width of the sites (Table 5.2). The relationship with crayfish biomass was negative. When crayfish were split into two size classes,

as per the threshold used in Chapter Three (page 76), the relationship strengthened considerably when large crayfish density was substituted into the model (Table 5.2). Log bullhead CD showed a similar relationship (Table 5.2). Bullhead population δ^{15}N range was found to correlate negatively with the density of larger crayfish, but positively with small crayfish density (Table 5.2). A negative relationship was observed between site width and the distance between the centroids of bullhead and crayfish populations (Table 5.2, Figure 5.8).

Crayfish sample sizes did not allow for stable isotope metrics to be generated for crayfish split by size class, therefore metrics represented all crayfish of each population. Population metrics did not co-vary with those of bullhead populations and no significant relationships were found between crayfish population metrics and measures of bullhead or site characteristics.

Channel experiment:

The physical characteristics of the water used in the experiment are given in Table 5.3.

Benthic invertebrates:

Combining all Hess samples at the end of the experiment yielded a total of 12 macroinvertebrate taxa, found at densities ranging from approximately 10 to 1,500 m^{-2} (Table 5.4). Taxon richness and Simpson diversity of the benthos were reduced in both the treatments which included crayfish, relative to the bullhead only treatment (Figures 5.9 and 5.10, Table 5.5); the magnitude of reduction was similar in both the crayfish only and bullhead and crayfish treatments. The most common

taxon, *Gammarus pulex*, was reduced to a similar degree in the bullhead and mixed treatments, relative to the crayfish only treatment and approached significance (Table 5.5, Figure 5.11). Chironomidae (the second most numerous taxon), *Potamopyrgus antipodarum*, Elmidae and *Radix peregra* suggested trends of depletion in the two treatments including crayfish, but were not significant except for *Radix peregra* (Kruskal-Wallis, $H = 7.62$, d.f. $= 2$, $P = 0.022$) (Table 5.5, Figure 5.11). Oligochaeta abundance appeared to be reduced in the mixed treatment (Figure 5.11); however the relationship was not significant (Table 5.5).

As algal standing stock was significantly affected in crayfish treatments (see below), the dominant grazing taxa, the Gastropoda, were combined for further analysis. Abundance was heavily reduced to a similar magnitude in both crayfish treatments (Table 5.5, Figure 5.12). Rare taxa were reduced in treatments with crayfish relative to the bullhead treatment, close to significance (Figure 5.13; Kruskal-Wallis, $H = 5.50$, d.f. $= 2$, $P = 0.064$). No rare taxa were sampled in the mixed treatment channels.

The total biomass of macroinvertebrates was lower in the mixed treatment relative to both single species treatments (Table 5.5, Figure 5.14). The total biomass of individual taxa generally followed the abundance results, with a few differences worth highlighting (Table 5.5, Figure 5.15). The apparent reductions in Chironomidae abundance in crayfish treatments and Oligochaeta abundance in the mixed treatment were greatly diminished when these taxa were measured by their biomass. In contrast, whilst Elmidae abundance showed no significant pattern by treatment, Elmidae biomass was significantly reduced in the mixed treatment in comparison with the bullhead treatment. Combined Gastropoda biomass showed the same relationship to treatment as for abundance, however as biomass could not be

satisfactorily transformed, non-parametric output did not yield significance (Figure 5.16; Kruskal-Wallis, $H = 5.61$, d.f. $= 2$, $P = 0.061$). Reduction in the biomass of rare taxa, in treatments including crayfish, was closer to significance than for absolute abundance data (Figure 5.17; Kruskal-Wallis, $H = 5.84$, d.f. $= 2$, $P = 0.054$).

Ecosystem processes:

Barring the final set, all leaf packs from channels where crayfish were present experienced a substantial increase in breakdown rate relative to the bullhead treatment (Table 5.6, Figure 5.18). Overall, mean leaf litter breakdown per channel was increased by over a third in treatments where crayfish were present and breakdown rates were similar irrespective of crayfish density (Figure 5.19). However, as the data for the mean breakdown rates could not be normalised, non-parametric analysis failed to show significance (Kruskal-Wallis, $H = 5.42$, d.f. $= 2$, $P = 0.066$).

Algal accumulation on tiles was found to increase exponentially in treatments including crayfish, whereas algal standing stock in channels containing only bullhead remained relatively constant (Figure 5.20). A comparison of chlorophyll *a* concentrations (mg cm^{-2}) for the final time point at seven weeks demonstrated clearly that standing stock was reduced in the bullhead only treatment relative to treatments with crayfish (GLM; $r^2_{(adj)} = 58.50\%$; treatment, $F_{(1,8)} = 6.64$, $P = 0.030$). Tukey post-hoc tests revealed values for both crayfish treatments to be different to those of the bullhead only treatment (crayfish only, $P = 0.062$; mixed, $P = 0.035$). Not only was the effect greater in the mixed treatment by the end of the experiment, but the effect was seen earlier in this treatment than in the allopatric crayfish treatment. At week six of the experiment, periphyton standing stock was greater in the mixed

treatment compared with the bullhead treatment but this was not the case for allopatric crayfish (GLM; $r^2_{(adj)}$ = 59.75%; treatment, $F_{(1,8)}$ = 6.94, P = 0.028; Tukey's comparison with the bullhead treatment; crayfish only, P = 0.653; mixed, P = 0.027).

Shelter use:

Sharing of the drainpipe refugia was rare; this occurred on two occasions in the bullhead treatment, where two individuals were found sharing the refuge. Crayfish in allopatry and bullhead and crayfish in sympatry never shared refugia. Allopatric shelter use was remarkably similar (Figure 5.21a), with no statistical difference for day or night time occupancy. In sympatry, day-time occupancy of the shelter was higher for crayfish than for bullhead (Figure 5.21b; GLM; $r^2_{(adj)}$ = 95.19%; species identity, F = 100.00, P = 0.001). A direct comparison between allopatric and sympatric data was made by ignoring species identity. Night time occupancy was increased in the mixed treatment relative to both bullhead and crayfish treatments (Figure 5.22; GLM; $r^2_{(adj)}$ = 80.37%; treatment, F = 17.38, P = 0.003, Tukey's; P = 0.03 and P = 0.012 respectively). There was no apparent disproportionate representation in night time shelter use by either bullhead or crayfish.

Stable isotopes:

The minimum number of bullhead and crayfish which survived the duration of the experiment was three in each case. Therefore, three individuals of approximately the same sizes were compared among channels. Whilst bullhead and crayfish showed

separation in isotopic space, the stable isotope metrics describing bullhead and

crayfish populations showed no differences between treatments (Figure 5.23).

Table 5. 1. Basic site information of the six sites used where bullhead and crayfish were found in sympatry. Depth and flow (± 1SE) were measured on the day of sampling. Substrate categories as defined by Wentworth (1992).

Site	Approximate width (m)	Depth (cm)	Flow (m s^{-1})	Shading (%)	Dominant substrate
Chess	4	11.7 (±1.41)	0.17 (±0.06)	65	pebble
Misbourne	2	18.4 (±1.28)	0.06 (±0.01)	95	gravel
Princey Brook	4	17.3 (±3.26)	0.03 (±0.01)	95	gravel*
Rib (Paynes Mill)	8	12.5 (±1.91)	0.13 (±0.02)	75	gravel
Rib (Wadesmill)	9	17.0 (±1.38)	0.13 (±0.03)	80	gravel
Stort	7	11.2 (±1.43)	0.14 (±0.02)	70	gravel

*layer of silt covered substrate

Table 5. 2 Signal crayfish and bullhead total abundance and biomass (wet weight) for each of the sites sampled. Values of crayfish biomass were derived using the equation given on page 72, while bullhead biomass was calculated using the regression of Edwards et al. (2008).

Site	Crayfish density (# m^{-2})	Crayfish biomass (g m^{-2})	Bullhead density (# m^{-2})	Bullhead biomass (g m^{-2})
Chess	0.78	9.19	3.89	19.13
Misbourne	0.39	0.66	2.34	13.46
Princey Brook	1.17	14.29	0.78	2.62
Rib (Paynes Mill)	1.94	1.49	1.95	3.67
Rib (Wadesmill)	6.23	12.76	3.50	8.77
Stort	5.06	4.17	4.28	29.77

Table 5. 3. Regression equations for simple linear (SLR) and multiple linear regressions (MLR) of various stable isotope measures of bullhead populations for the six sites surveyed. Predictor variables used were crayfish biomass (x_1), site width (x_2), large crayfish density (x_3) and small crayfish density (x_4) for the six sites. Distance between bullhead and crayfish centroids is abbreviated to BCC. $* = P \leq 0.05$, $** = P \leq 0.01$.

Dependent variable	Regression equation	d.f.	r^2_{adj}	F	P	VIF
Log SEA$_c$	$y = -0.56 - 0.09x_1 + 0.17x_2$	1,3	0.87	18.03	**0.021***	1.0
Log SEA$_c$	$y = -0.78 - 1.24x_3 + 0.20x_2$	1,3	0.93	34.63	**0.008****	1.1
Δ^{15}N range	$y = 1.09 - 0.54x_3 + 0.17x_4$	1,3	0.93	31.90	**0.010****	1.1
Log CD	$y = -0.70 - 0.69x_3 + 0.10x_2$	1,3	0.77	9.54	**0.050***	1.1
BCC	$y = 2.81 - 0.17x_2$	1,4	0.73	14.56	**0.019***	n.a.

Table 5. 4. Characteristics of water in the artificial channels.

Variable	Mean ± SD
Discharge (L s^{-1})	0.08 ± 0.01
pH	8.03 ± 0.07
Temperature (ºC)	19.4 ± 0.04
Velocity (m s^{-1})	0.019 ± 0.003

Table 5. 5. List of all macroinvertebrate taxon recorded in Hess samples at the end of the channel experiment. Taxa are listed in order of decreasing total combined abundance and mean densities per treatment are given. Treatments are represented by B, bullhead only; C, crayfish only; and B + C, bullhead and crayfish.

Taxon	Total abundance	Mean density by treatment (# m^{-2})		
		B	C	B + C
Gammarus pulex	318	782.25	1532.57	727.97
Chironomidae	48	245.85	105.36	105.36
Oligochaeta	46	223.50	181.99	38.31
Potamopyrgus antipodarum	39	287.36	38.31	47.89
Elmidae	36	181.99	95.79	67.05
Radix peregra	13	127.71	0.00	0.00
Sphaeriidae	7	41.51	28.74	0.00
Sericostomatidae	4	38.31	0.00	0.00
Diptera (other)	2	12.77	9.58	0.00
Ephemerella danica	1	9.58	0.00	0.00
Ephemera ignita	1	9.58	0.00	0.00
Hirudinea	1	12.77	0.00	0.00

Table 5. 6. GLM output for measures of the benthos with treatment as the predictor variable. Treatments are represented as B, bullhead; C, crayfish; and B + C, bullhead and crayfish. Log reciprocal Simpsons diversity is abbreviated to log 1/D. * = $P \leq 0.05$, ** = $P \leq 0.01$.

	$R^2(adj)$ %	P value	F	Tukey's post hoc tests		
				B : C	B : B + C	C : B + C
				P value	P value	P value
Taxon richness	66.29	**0.016***	8.87	**0.031***	**0.021***	0.947
Log 1/D	77.87	**0.005****	15.07	**0.005****	**0.012***	0.698
Abundances						
G. pulex	46.73	0.064	4.51	0.102	0.980	0.080
Chironomidae	42.42	0.081	3.95	0.111	0.111	1.000
Log Oligochaeta	36.67	0.107	3.32	0.981	0.129	0.163

Continued overleaf.

Table 5. 6 continued.

| | R^2(adj) % | P value | F | Tukey's post hoc tests | | |
| | | | | B : C | B : B + C | C : B + C |
				P value	P value	P value
Log *P. antipodarum*	29.15	0.150	2.65	0.164	0.242	0.948
Elmidae	33.33	0.125	3.00	0.259	0.123	0.831
Square root Gastropoda	59.63	**0.028***	6.91	**0.034***	0.053	0.926
Biomass						
Log total biomass	72.63	**0.009****	11.61	0.571	**0.009****	**0.028***
G. pulex	47.47	0.061	4.61	0.141	0.801	0.062

Continued overleaf.

Table 5. 6 continued.

	$R^2(adj)$ %	P value	F	Tukey's post hoc tests		
				B : C	B : B + C	C : B + C
				P value	P value	P value
Square root Chironomidae	0.00	0.520	0.73	0.580	0.574	0.999
Oligochaeta	0.00	0.537	0.69	0.894	0.767	0.513
Log *P. antipodarum*	25.27	0.176	2.35	0.197	0.263	0.970
Log Elmidae	52.29	**0.046***	5.38	0.628	**0.043***	0.142

Table 5. 7. GLM output for leaf litter % dry mass loss day^{-1} of each set of leaf packs. Treatments are represented as B, bullhead; C, crayfish; and B + C, bullhead and crayfish. ** = $P \leq 0.01$ *** = $P \leq 0.001$.

Leaf pack set	$R^2(adj)$ %	P value	F	Tukey's post hoc tests		
				B : C	B : B + C	C : B + C
				P value	P value	P value
Week 3	78.61	0.004**	15.70	0.007**	0.007**	0.995
Week 4	87.55	0.001***	29.13	0.001***	0.001***	0.998
Week 5	85.34	0.001***	24.29	0.002**	0.003**	0.847
Week 6	79.52	0.004**	16.54	0.004**	0.009**	0.760
Week 7	48.37	0.058	4.75	0.119	0.063	0.874

Figure 5. 1. A visual representation of the difference between the convex hull total area (TA) and the corrected standard ellipse area SEA$_c$ for sample data in δ^{13}C and δ^{15}N bivariate space. Crosses represent individuals within a population. The solid line is the convex hull of the population and the dotted line the SEA$_c$.
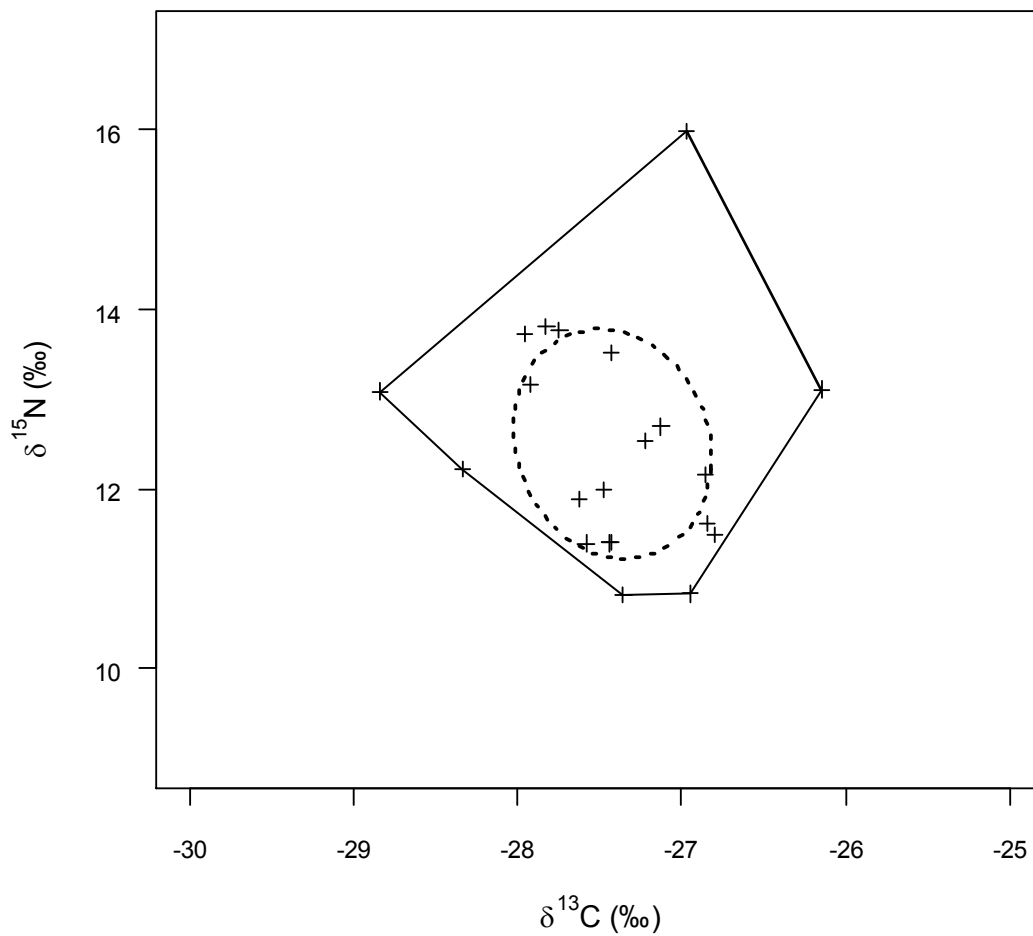
Figure 5. 2. A graphic representation of how stable isotope metrics may be affected by variation in basal resource isotope values. The figures are schematic and do not represent real data. Furthermore, values for SEA_c are fictitious and the ellipses themselves are not drawn with accuracy or to scale. The green triangle and brown circle represent two basal resources. These are consumed by individuals of a single population, each individual represented by a cross. Among the three figures the diet of each individual of the population contains the same proportions of the two resources. In figure b) these resources happen to be relatively proximate in their $\delta^{13}C$, whilst in figure c) they happen to be relatively distinct; this results in population-wide narrowing and expanding of isotopic niche, respectively.
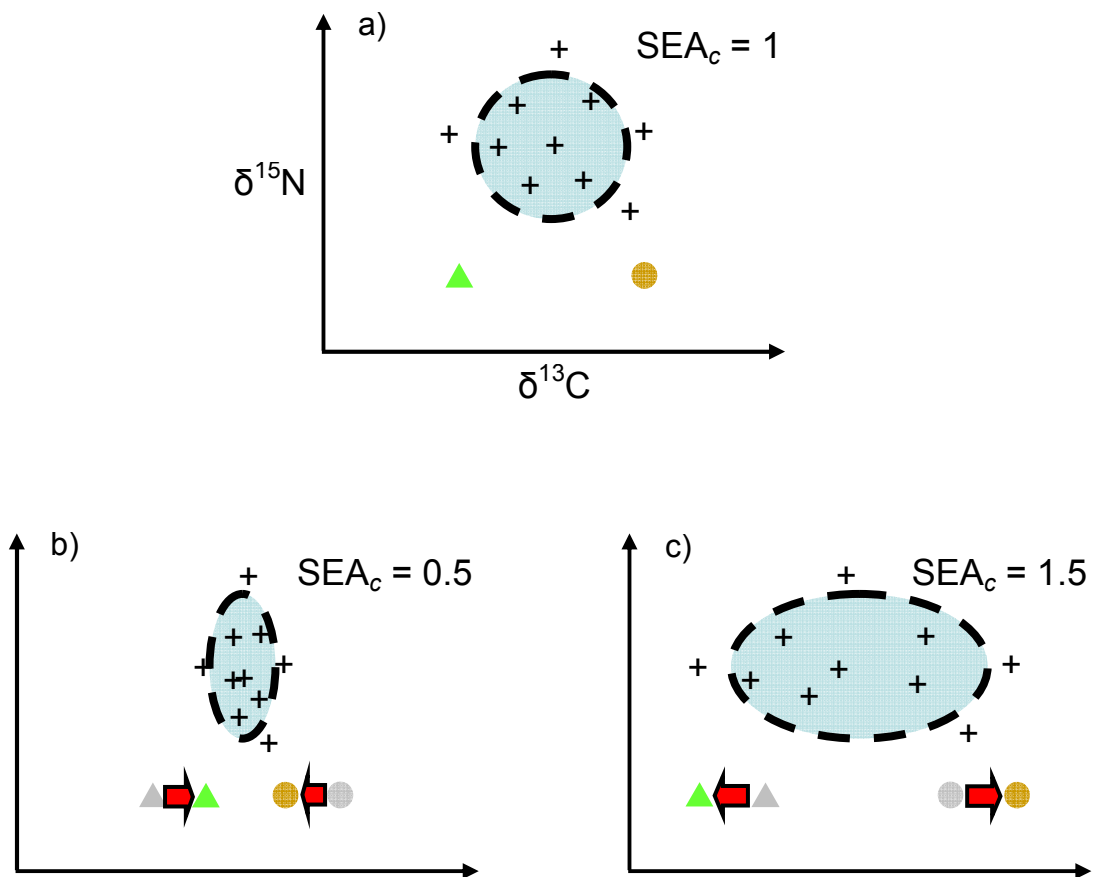
Figure 5. 3. Location of the six sites of south east England used for the field survey where bullhead and signal crayfish co-occur. Small scale map drawn from Edina Digimap (© Crown Copyright Ordinance Survey. An EDINA Digimap/JISC supplied service).
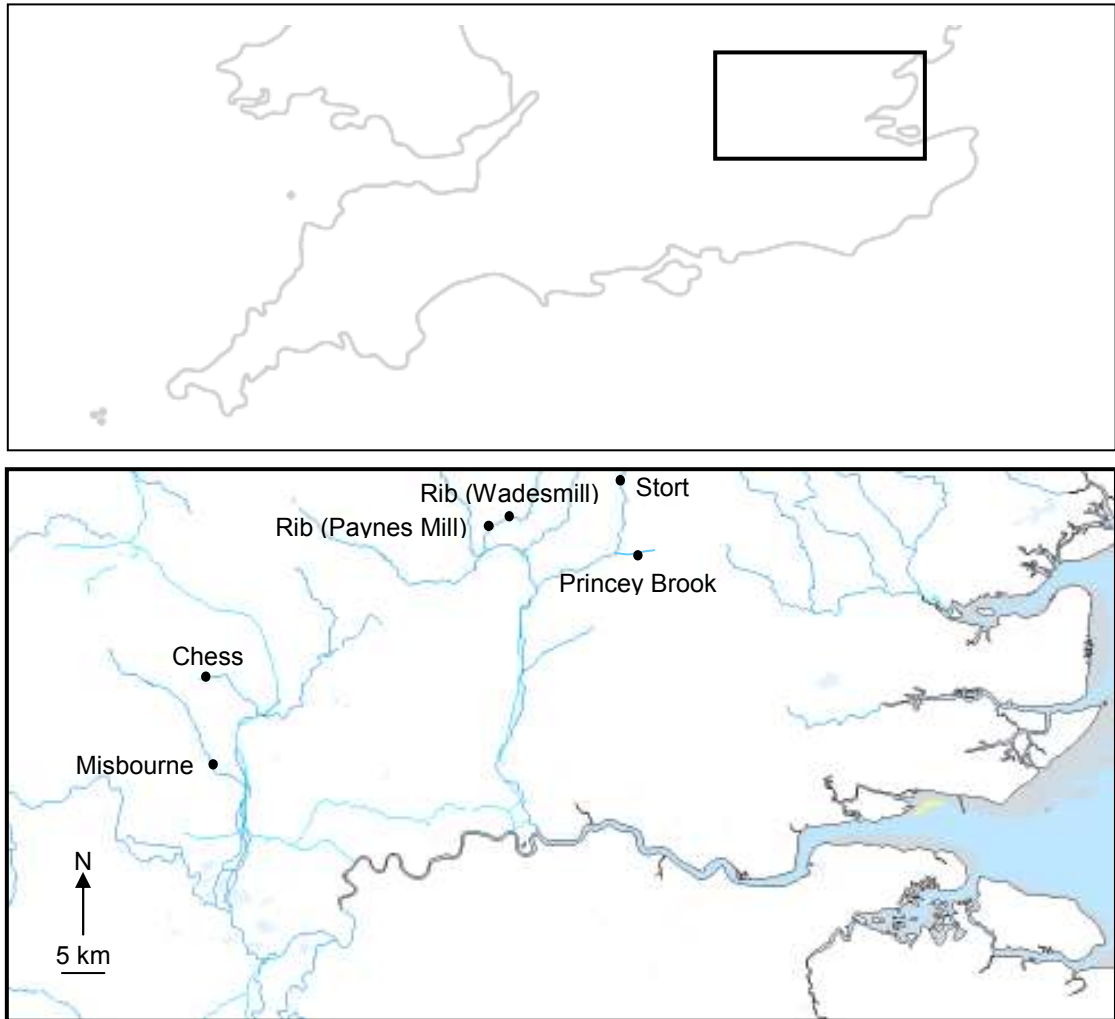
Figure 5. 4. a) Schematic of the channel set up with randomly assigned treatments labelled: bullhead only (B), crayfish only (C) and bullhead and crayfish (B + C). b) An individual channel with approximate placing of algal colonisation tiles, shelter and leaf pack. c) Following page, a photograph showing part of the setup.

a)

b)

Colonisation tiles   Shelter   Leaf Pack

Outlet

Inlet
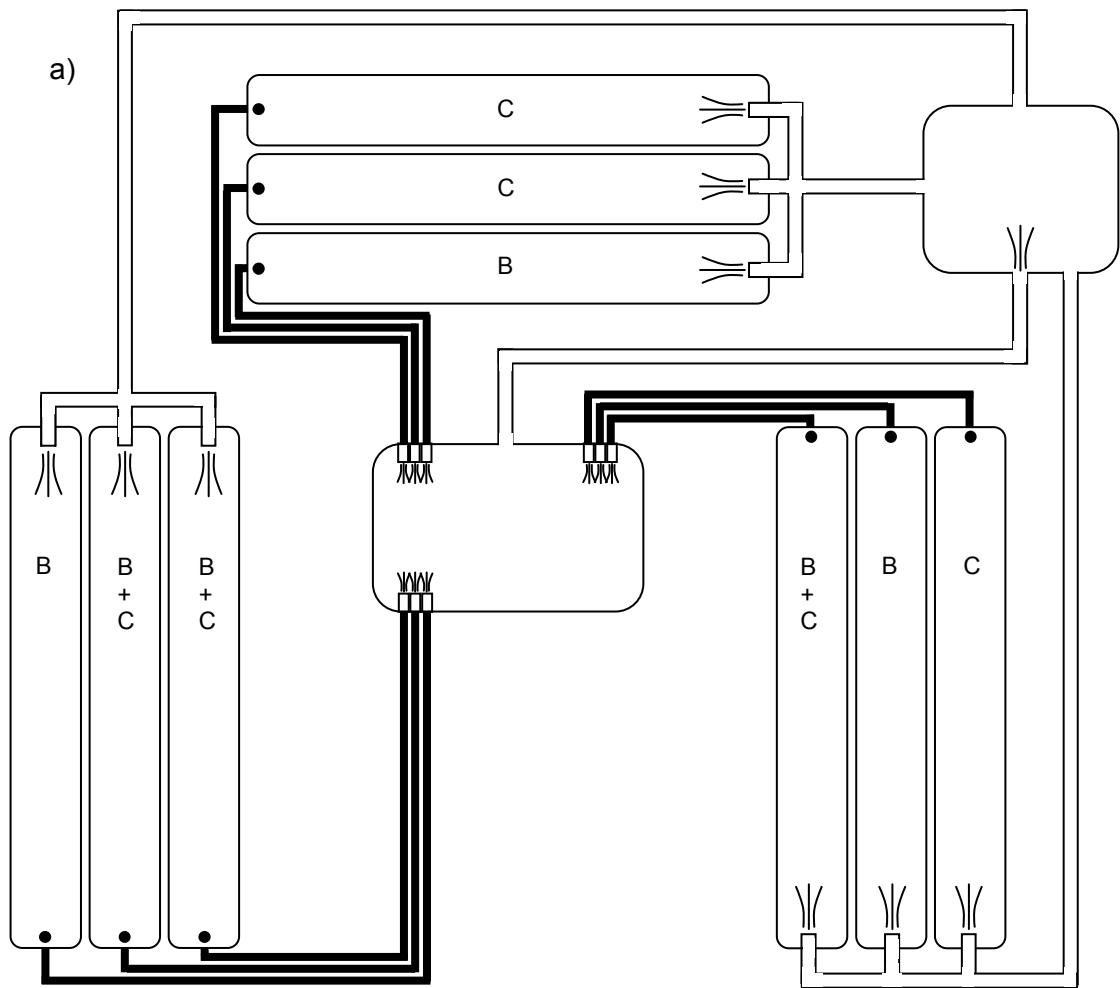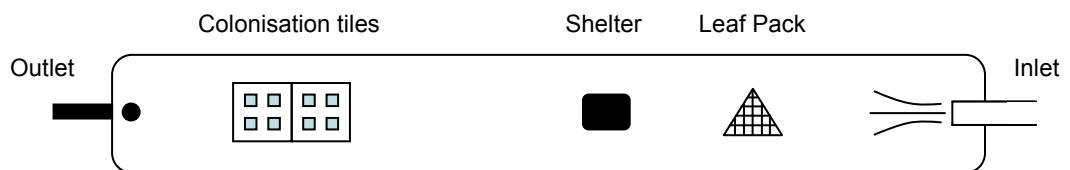
c)

Figure 5. 5. Location of sites on the river Darent used for collection of fauna, substrate and water used in the channel experiment. Small scale map drawn from Edina Digimap (© Crown Copyright Ordinance Survey. An EDINA Digimap/JISC supplied service).
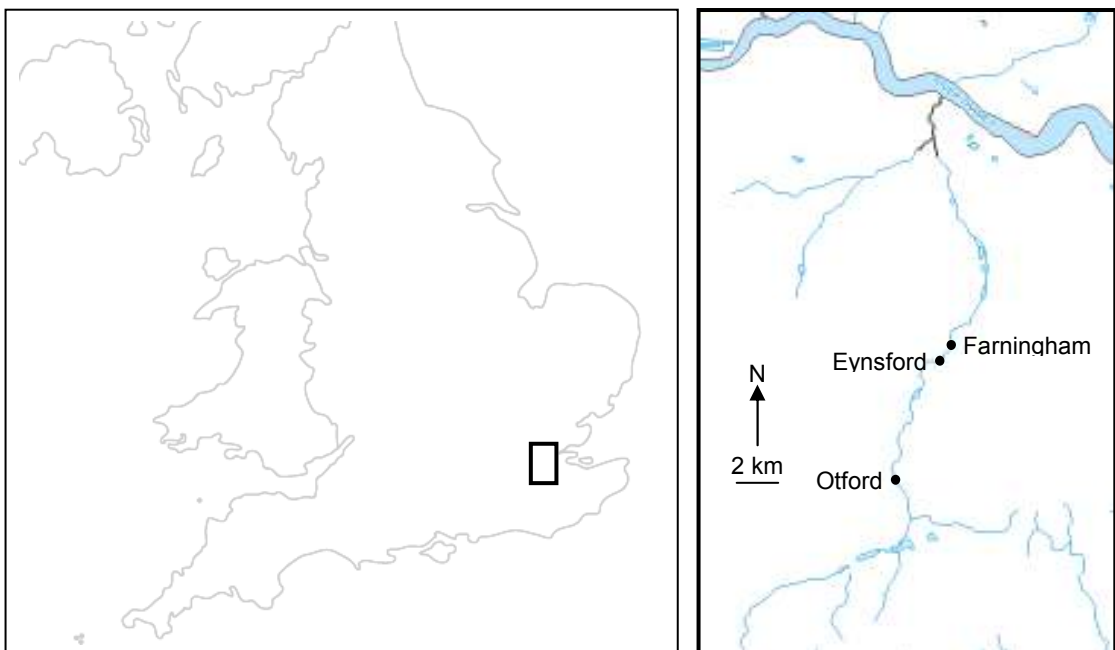
Figure 5. 6. Bi-variate plot of the SEA_c of bullhead (black open triangles) and crayfish (grey closed circles) populations for all sites.
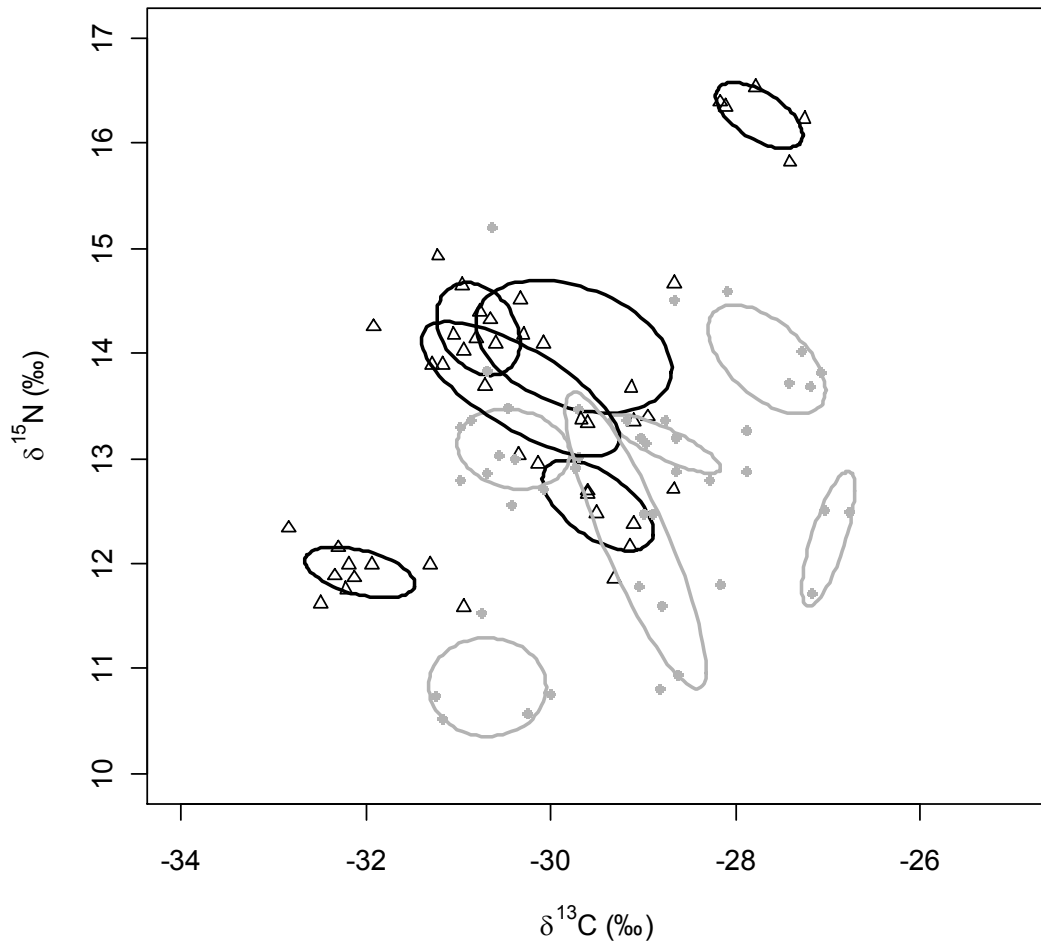
Figure 5. 7. Bi-variate plots of the δ^{13}C and δ^{15}N values of individual bullhead and crayfish for each site. Standard ellipse areas are plotted around each population. Black open triangles represent bullhead, while grey closed circles represent crayfish.

Chess
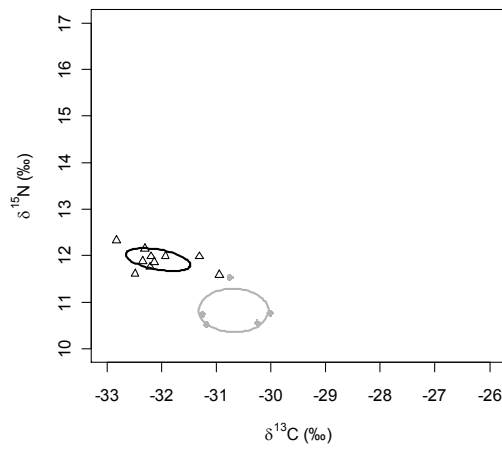
Misbourne
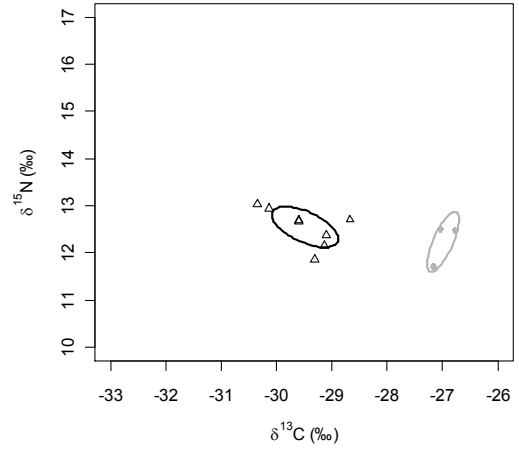
Princey Brook
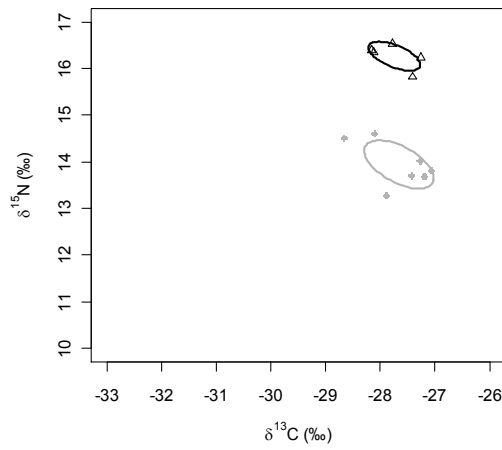
Rib (mid)
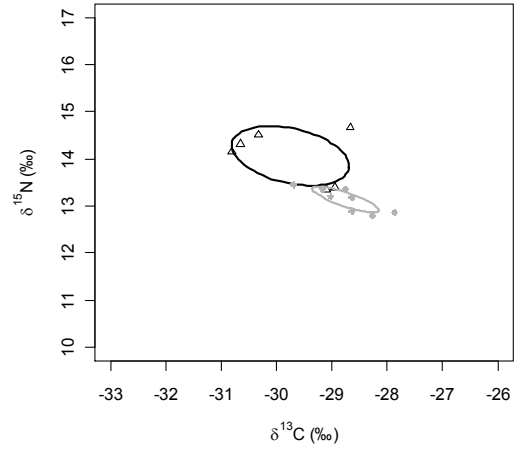
Figure 5. 7 continued.

Rib (up)   Stort
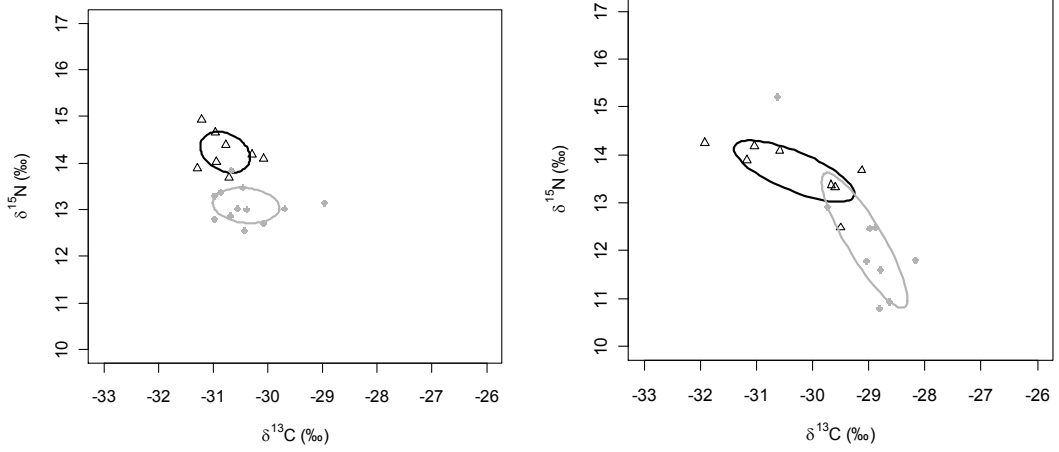
Figure 5. 8. Regression plot of site width and the distance between crayfish and

bullhead population centroids.

$y = 2.810 - 0.169x$
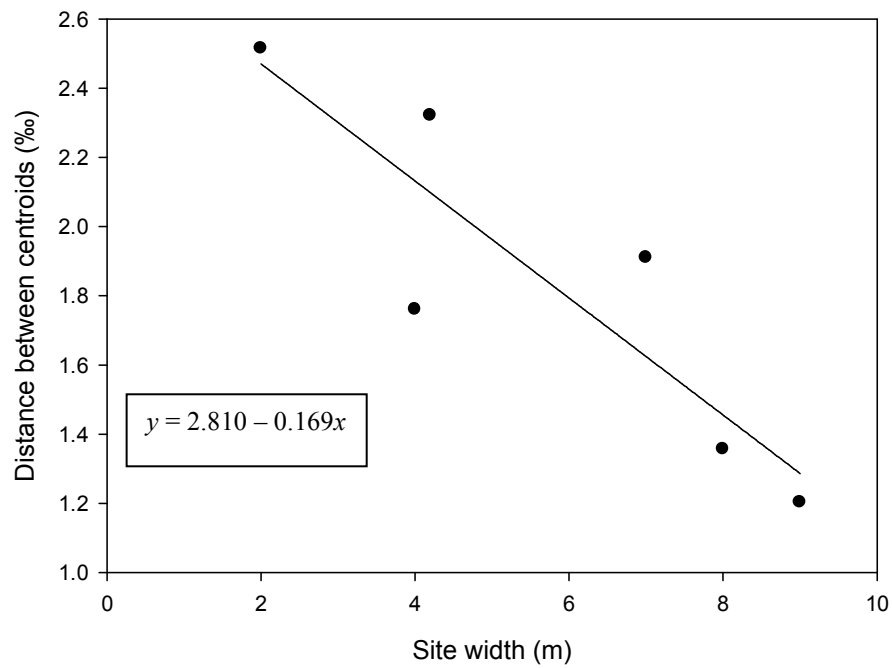
Figure 5. 9. Taxon richness of the benthos of channels (+ 1 SE) for each treatment.

The bullhead and crayfish treatment is represented by B + C.
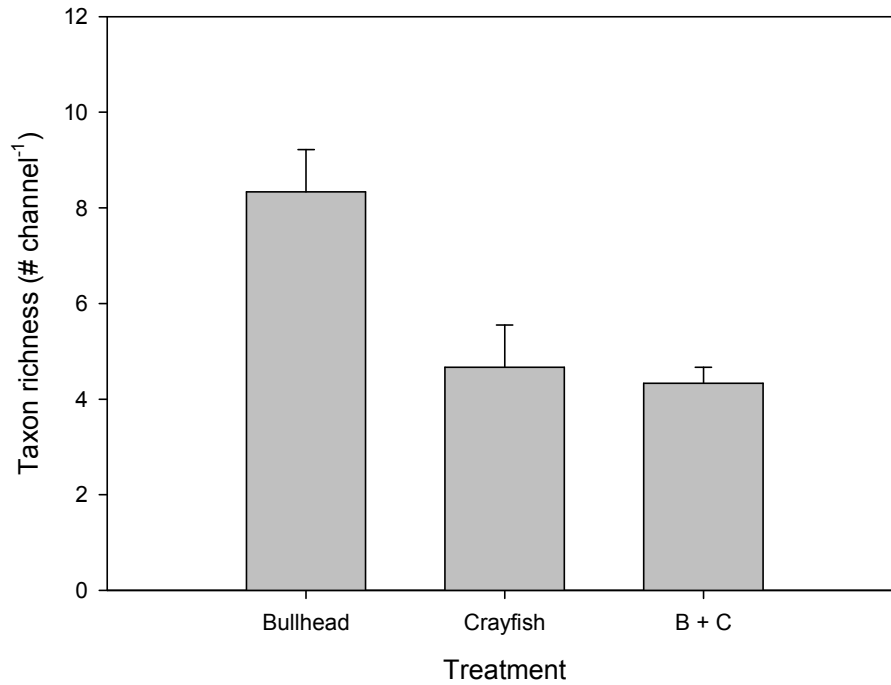
Figure 5. 10. Log reciprocal Simpson's diversity of the benthos of channels (+ 1 SE)

for each treatment.

181
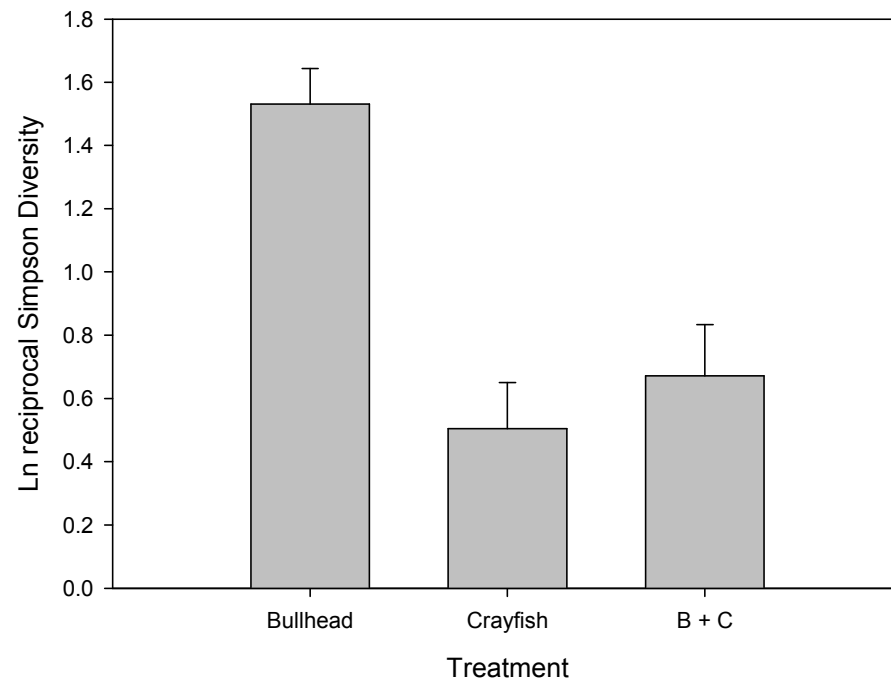
Figure 5. 11. The abundances of the six most numerous taxa (+ 1 SE) for each treatment.
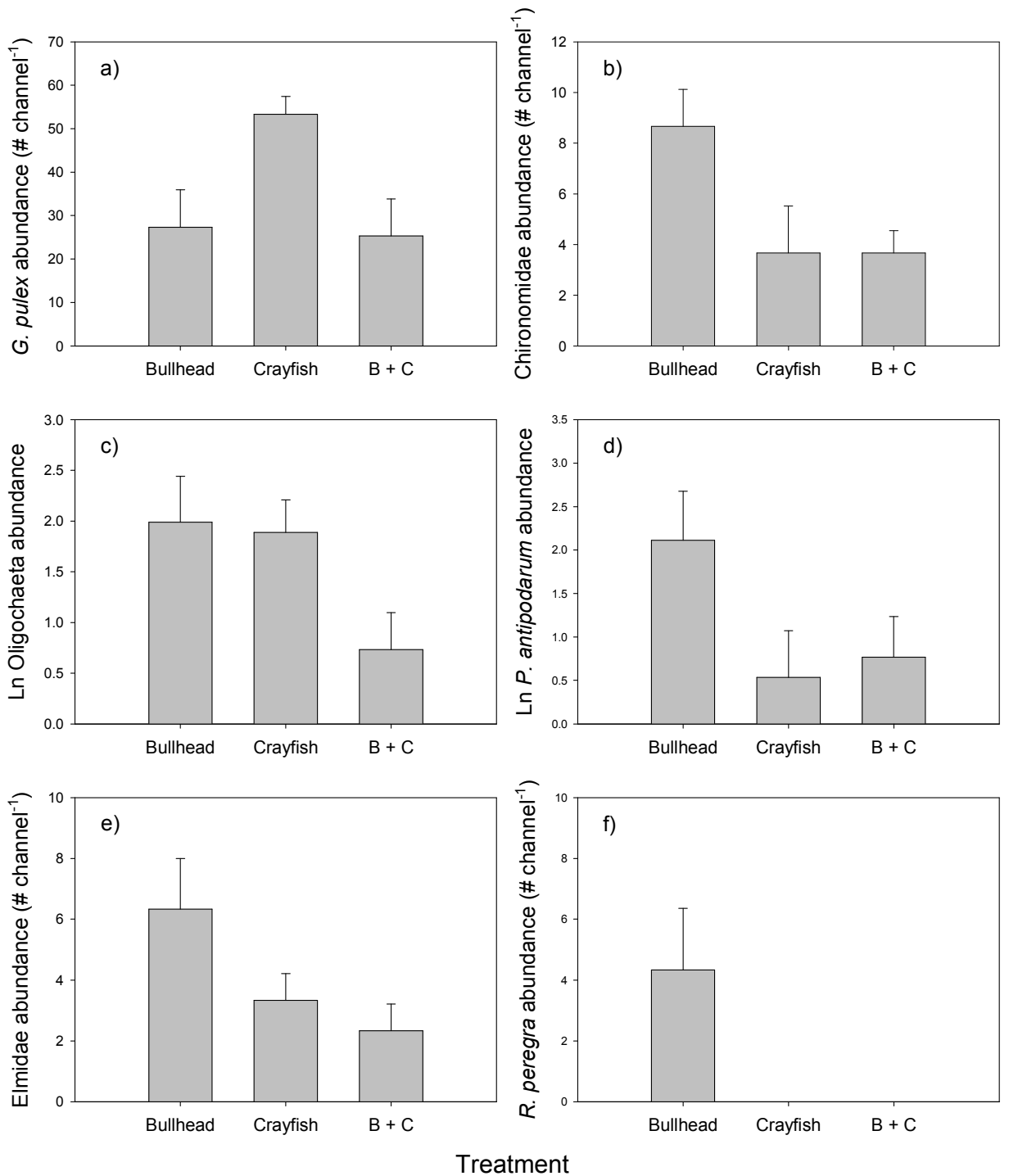
Figure 5. 12. The combined abundance of Gastropoda (+ 1 SE) for each treatment.
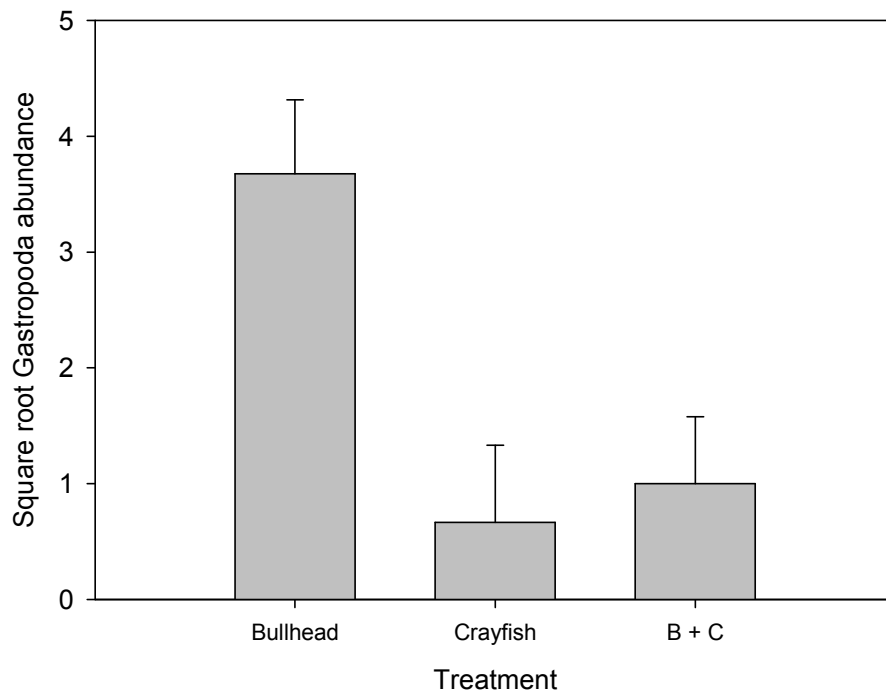
Figure 5. 13. The abundance of rare taxa (+ 1 SE) for each treatment.
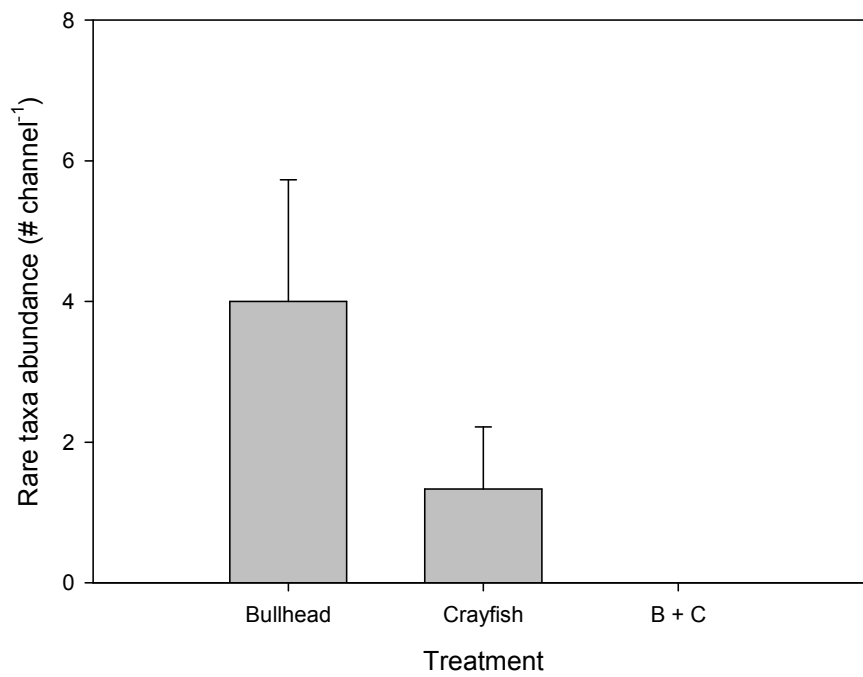
Figure 5. 14. The total dry weight biomass (+ 1 SE) of benthic macroinvertebrates collected from the experimental channels.
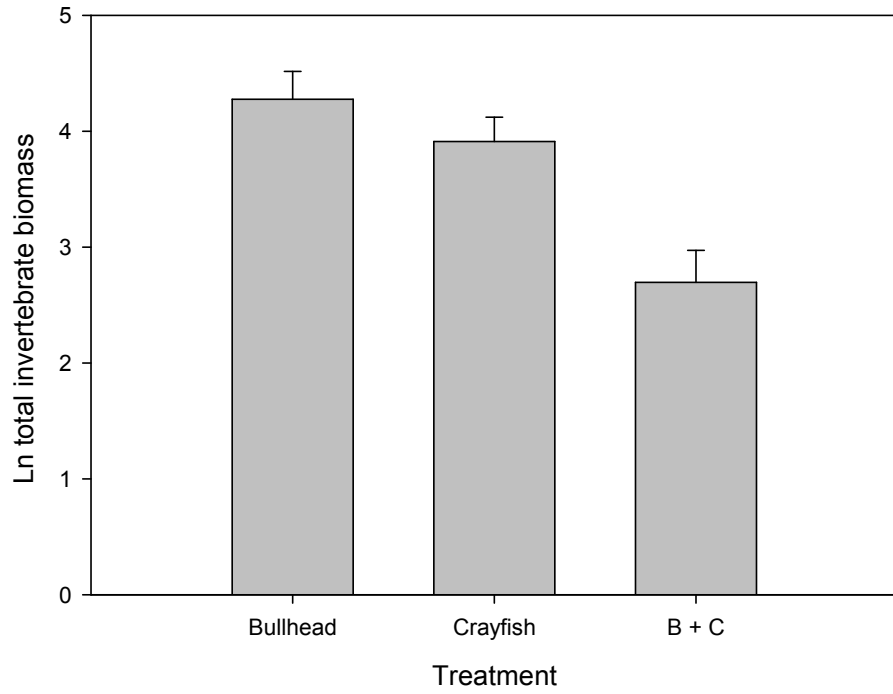
Figure 5. 15. Dry weight biomass of the six most numerous taxa (+ 1 SE) for each
treatment.
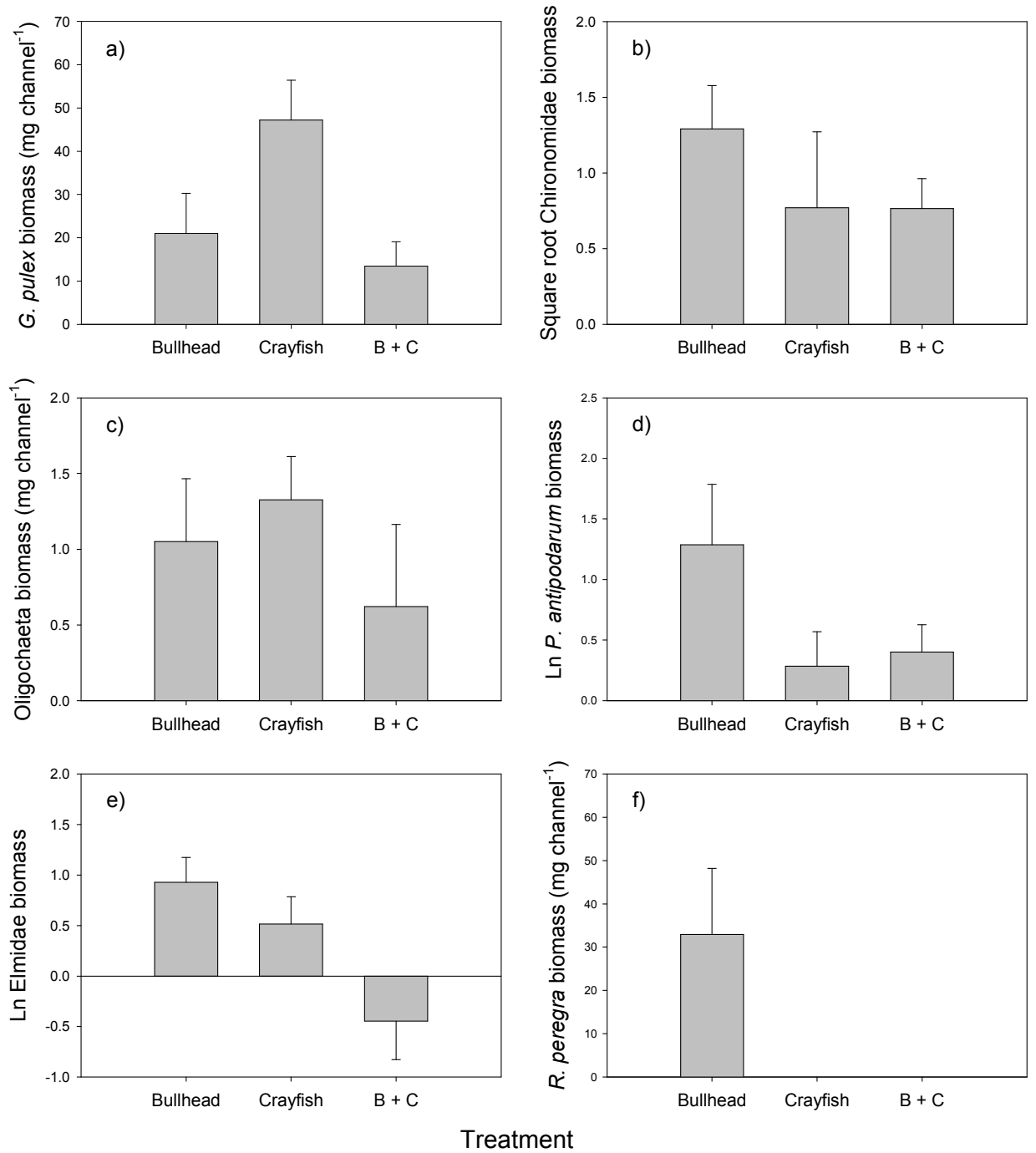
Figure 5. 16. Gastropoda combined dry weight biomass (+ 1 SE) for each treatment.
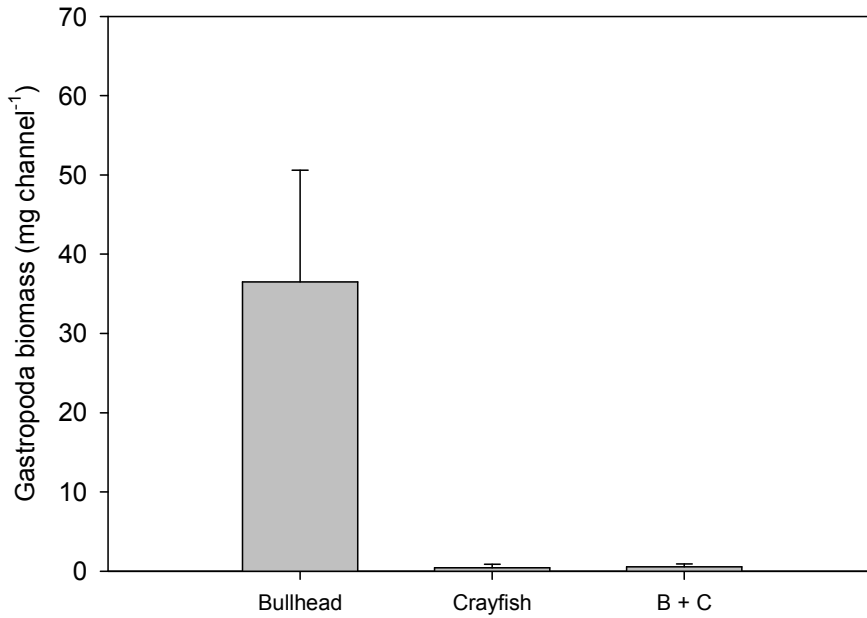
Figure 5. 17. Rare taxa combined dry weight biomass (+ 1 SE) for each treatment.
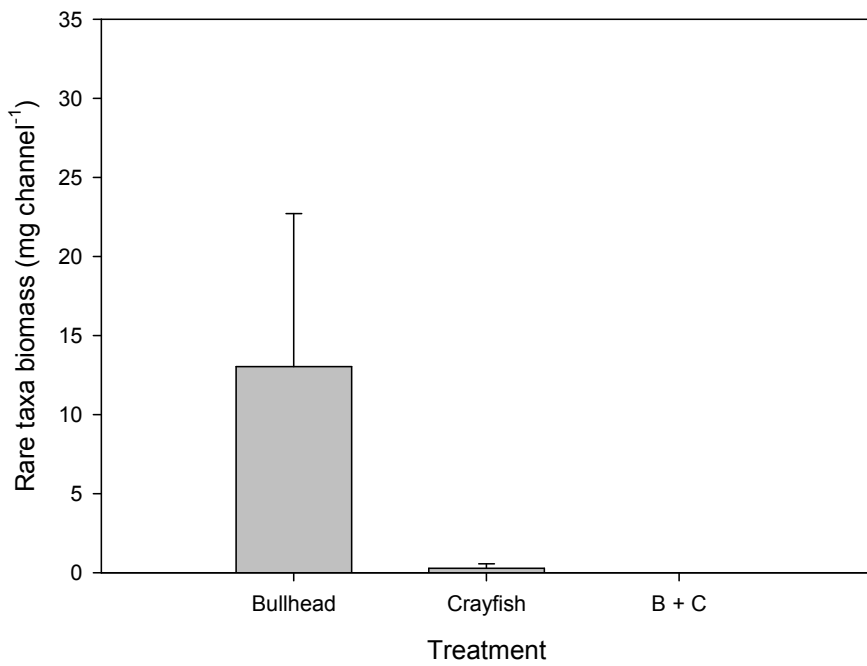
Figure 5. 18. Leaf litter breakdown rates (± 1 SE) for each set of leaf packs left in the channels for one week. Time represents the day on which leaf packs were removed from the channels. Treatments are bullhead (open triangles), crayfish (grey circles) and the mixed treatment (black circles).
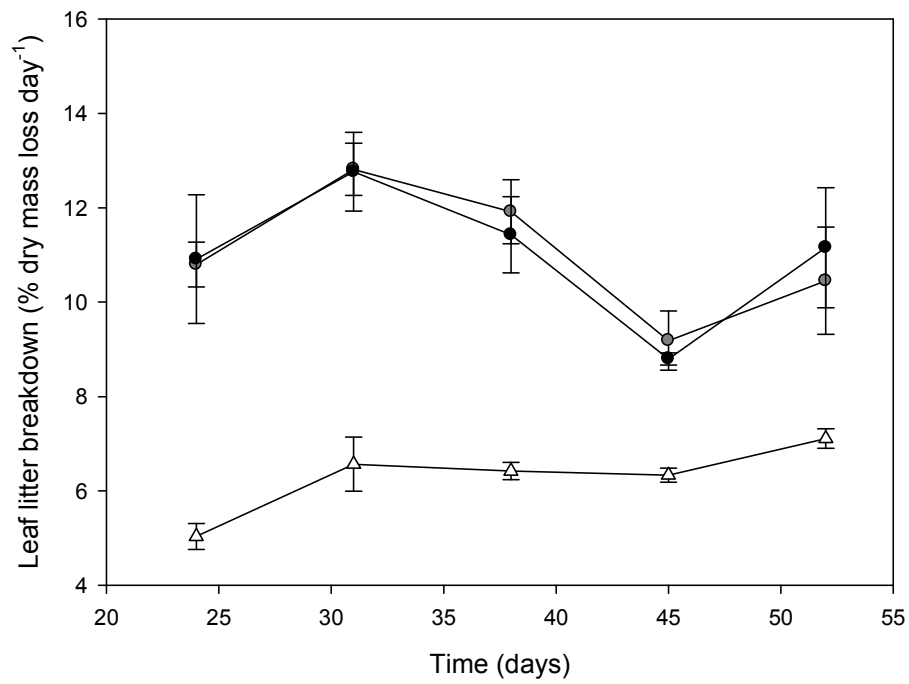
Figure 5. 19. Mean values of leaf litter breakdown (+SE) among treatments.
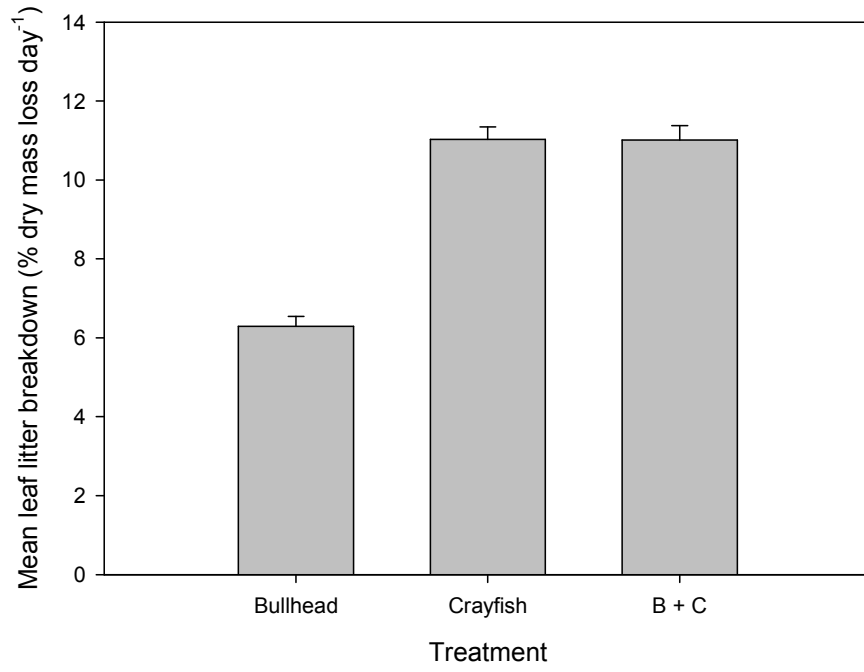
Figure 5. 20. Mean algal standing stock (±SE) (as measured by chlorophyll *a* concentration) for colonisation tiles at intervals spanning the duration of the experiment for raw (a) and log (b) values. Treatments are bullhead (open triangles), crayfish (grey circles) and the mixed treatment (black circles).
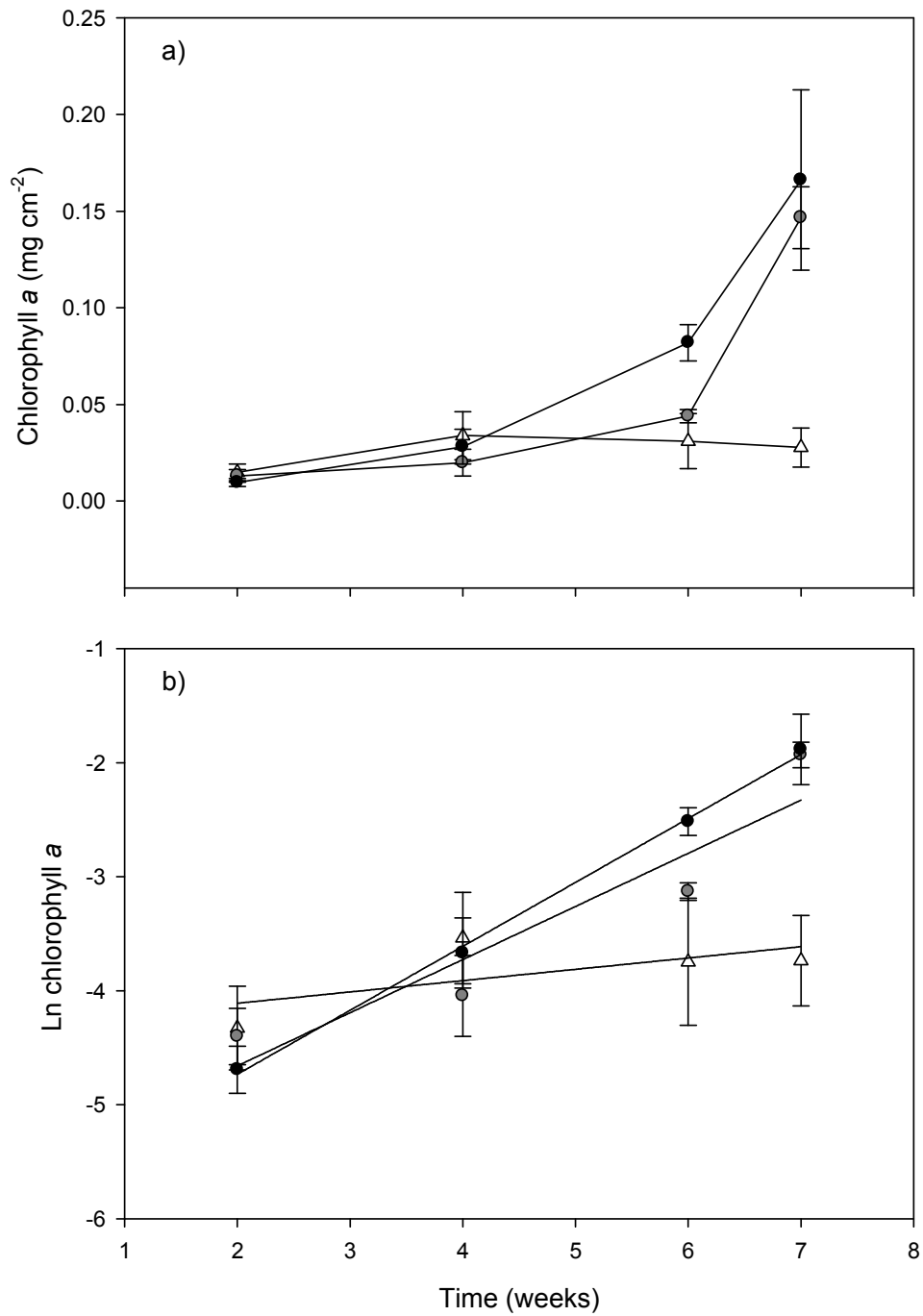
Figure 5. 21. a) Shelter occupancy by bullhead and crayfish in allopatry. Black bars represent night time measurements (23:00) and, grey bars day time measurements (11:00). b) Shelter occupancy by bullhead and crayfish in sympatry.
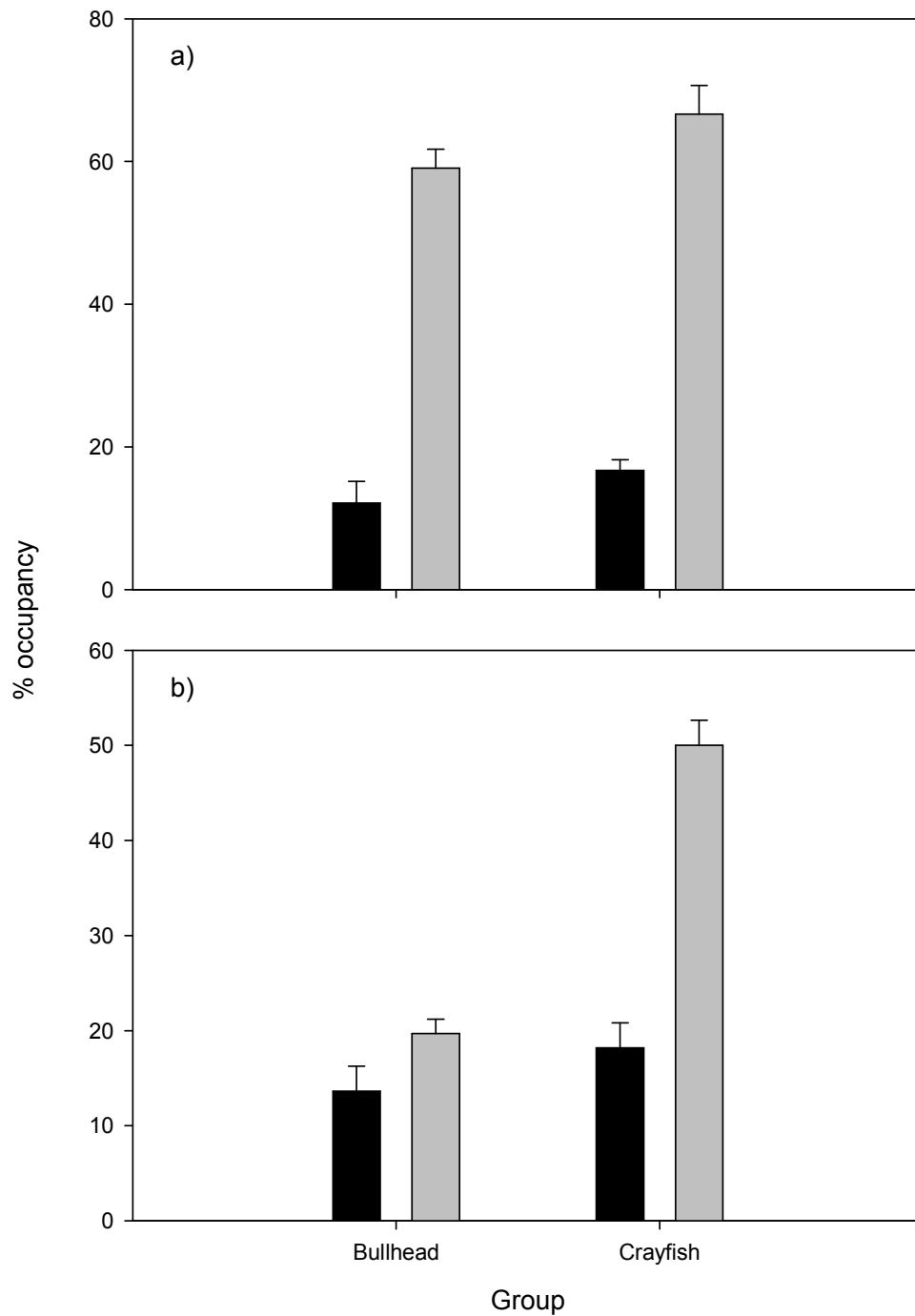
Figure 5. 22. Mean percentage occupancy (+SE) of the shelter. In the mixed treatment these values are for both species combined. Black bars represent night time occupancy and grey bars day time occupancy.
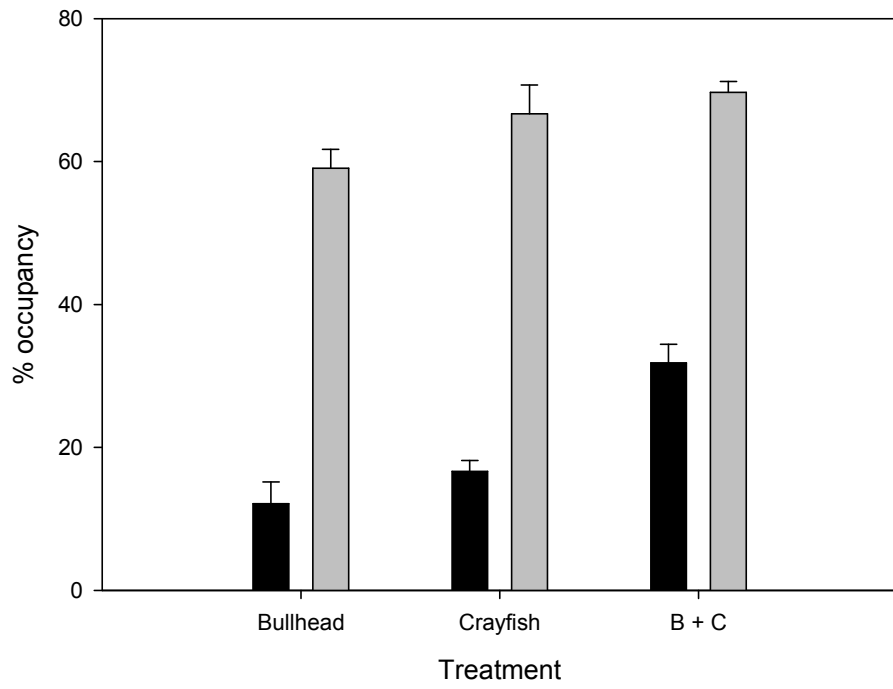
Figure 5. 23. SEA$_c$ of bullhead populations in allopatry and in sympatry with crayfish populations. Bullhead individuals are represented by open triangles and crayfish by grey circles. Solid line SEA$_c$ represent allopatric populations while dotted line SEA$_c$ represent sympatric populations.
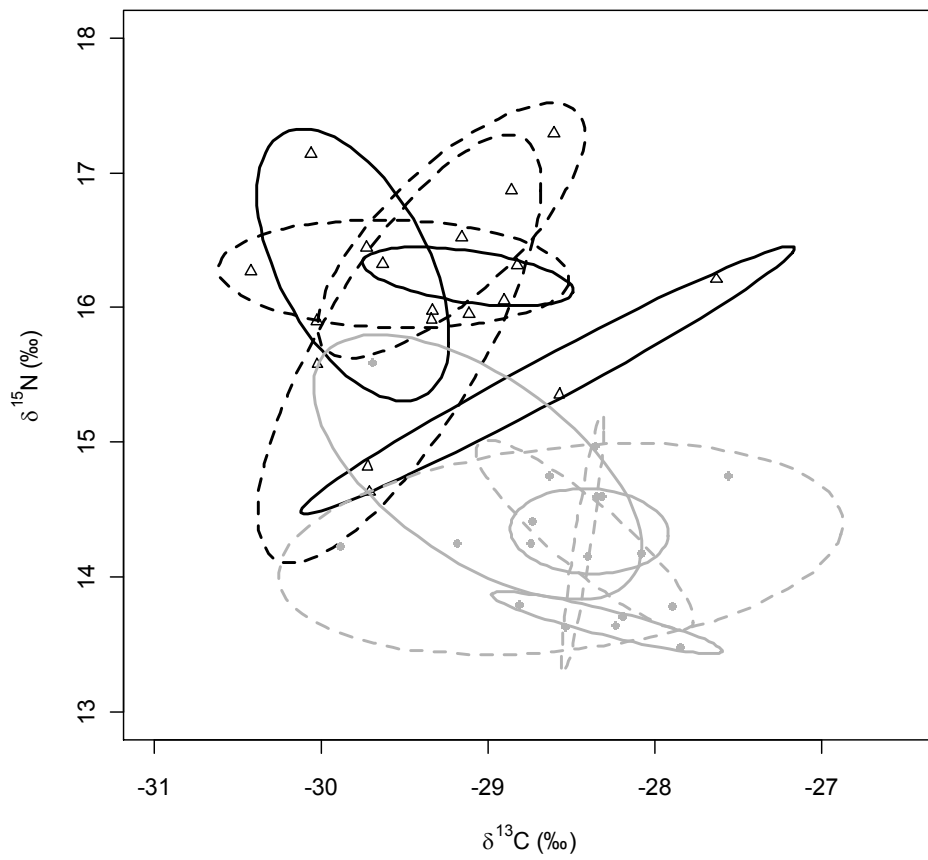
Discussion

The results of both the field and experimental work gave no direct indication that invasive crayfish were a causal factor for an increase in bullhead mortality. However, field results did suggest important consequences of crayfish presence for bullhead diet, and these appeared to be positive as well as negative, depending on the size class of crayfish considered. While the stable isotope results obtained from field data were not reproduced in the channel experiment, impacts of crayfish on macroinvertebrates in the channels and results of the survey work reported in Chapter Three provided mechanisms which might partly explain the field results. Furthermore, the predatory impact of sympatric bullhead and crayfish of the channel experiment revealed patterns implying synergism / complementary predatory effects.

Stable isotope results:

Using isotopic niche space as a proxy, it appears that the dietary niche of sympatric bullhead and crayfish populations are distinct. This does not mean that dietary items are not shared, but indicates differing proportional reliance on food items. Considering the importance of vascular matter in the diet of crayfish, which is not seen in bullhead (Dahl, 1998, Guan and Wiles, 1998), the diets of the two taxa would not be expected to overlap entirely. Differences between the trophic fractionation rates of the two species are unlikely to account for the difference in stable isotope ratios. While specific estimates of trophic fractionation rates for these species do not exist, studies on related taxa show that it is reasonable to assume rates will be similar (McCutchan et al., 2003 and references therein, Rudnick and Resh, 2005).

The negative correlation between bullhead SEA$_c$ and increasing densities of larger crayfish may result directly from an increasing impact of crayfish on the diet of bullhead. Previous work indicates dietary overlap between bullhead and crayfish, with Chironomidae and Ephemoptera being preferred prey items of each (Dahl, 1998, Stenroth and Nystrom, 2003). Survey work presented in Chapter Three revealed negative correlations between large crayfish and the abundance of both Chironomidae and Baetidae. Both the cage experiment of Chapter Four and the channel experiment of this chapter revealed reduced abundances of rare taxa attributable to crayfish and in the case of the channel experiment reduced taxon richness also. Furthermore, presence of signal crayfish has been associated with reduced gut fullness and growth rates of sculpin (Light 2002, in Light, 2005). Therefore reduced abundances and diversity of prey taxa may lead to reduced dietary niches of bullhead. Additionally, evidence for a crayfish mediated grazer-algae trophic cascade seen in the cage experiment was confirmed in the channel experiment (see discussion below). Increased standing stock of periphyton owing to a reduction in grazing means that the quantity of autochthonous matter entering the stream food web is reduced. As autochthonous and allochthonous materials, in aquatic systems, typically have distinct stable isotope values (Rounick et al., 1982), a reduction in autochthonous input is likely to constrict the isotopic niche of the consumer and predator community.

A narrowing of bullhead diet at the population level with increasing crayfish density could also be interpreted as a consequence of bullhead specialisation on crayfish as prey. There are several reasons why this is unlikely to be the case. While larger bullhead of a population might specialise on young of the year crayfish, smaller individuals will be less able to exploit this resource owing to gape-limitation.

Secondly, in the analysis, when large crayfish density was replaced with small crayfish density in the multiple regression, no variation was explained. Finally, the finding that the $\delta^{15}N$ range of bullhead populations correlated negatively with large crayfish density, but positively with small crayfish density, would not only refute the idea, but suggest the opposite. The negative relationship with large crayfish density is in agreement with the relationship seen for bullhead SEA_c. The positive relationship with small crayfish density however could result directly from the consumption of small crayfish by bullhead. This is supported by gut content analysis where signal crayfish were the third most common prey item in previous experimental work (Dahl, 1998). In Chapter Six it is shown that crayfish occupy a trophic level approximately 3.5‰ above basal consumers. Assuming an equivalent trophic position for the crayfish of this study, addition of crayfish to the diet of bullhead, which is likely to be otherwise comprised mainly of primary consumers, would increase the trophic level of bullhead. As mentioned above, owing to gape limitation larger bullhead will consume a greater proportion of crayfish. The consequence of this will be a bullhead population with an increased range of trophic position among individuals.

The inclusion of site width in the analyses was important; this is clearly demonstrated in the results by the positive relationships with both bullhead population SEA_c and CD. This might relate to the findings of the survey work in Chapter Three, where taxon diversity positively correlated with stream width. Assuming prey taxa do not have identical stable isotope ratio compositions, an increase in the diversity of prey items is likely to give rise to a greater range of $\delta^{13}C$ and $\delta^{15}N$ values (Bearhop et al., 2004). Furthermore, although generally associated with greater spatial scales than seen within this study, with increasing site width the

relative contribution of autochthonous production generally increases from low order to mid-order streams (Vannote et al., 1980). In-stream production based on autochthonous and allochthonous inputs will give rise to prey items with distinct δ^{13}C stable isotopic signatures (Rounick et al., 1982). Therefore, in general, wider sites allow invertebrate populations to encompass a greater δ^{13}C range than narrower streams.

A probable explanation for the decreasing distance between the centroids of bullhead and crayfish, with increasing site width, is that this relates to an increasing contribution of autochthonus input with increasing width. With greater availability of autochthonously derived prey, crayfish are likely to consume proportionally more of these taxa and less detritus, therefore increasing diet overlap with bullhead.

The above discussion of the field based stable isotope results is based on correlations and therefore no conclusions regarding the mechanisms behind the observed patterns can be made. The explanations given represent plausible contributing factors, however it is also possible that correlations between isotopic measures and the crayfish community correlate with another, unmeasured, variable or variables. Therefore the discussion of the field isotope results has been made with caution. The stable isotope results of the channel experiment did not reproduce those of the field survey, which would have provided a direct link between crayfish and the isotopic metrics of bullhead populations.

There are several possible reasons why the isotope data from the channel experiment did not provide supporting evidence for the field data. Of primary importance is whether the experiment was long enough in duration for the muscle tissue of bullhead to come to reflect their diet whilst in the channels; i.e. was the

turnover of muscle tissue rapid enough for a change in stable isotope ratios to be measureable within a seven week period? For example, it can take longer for a consumer to reach equilibrium in $\delta^{13}C$ than it does for that consumer to quadruple in mass (McCutchan et al., 2003). While comparable data for $\delta^{15}N$ does not exist, the assumption is that consumer $\delta^{15}N$ will not necessarily reflect recent diet (McCutchan et al., 2003). Therefore, the duration of the experiment may simply have been too short for change in bullhead diet to be observed.

A further potential problem is that the channel experiment setup was too simplistic for realistic community wide variation in $\delta^{13}C$ and $\delta^{15}N$. The channels effectively were made up of a single microhabitat, whereas various microhabitats are inevitably found in the field. Microhabitat can be an important determinant of stable isotope baseline values (Grey et al., 2004) and therefore baseline variation in the experiment may have been artificially low. Invertebrate diversity was also low and therefore a large pool of isotopically distinct invertebrates might not have been achieved.

Further work is required to determine the correct explanation of the stable isotope results of the field work. Repetition of the field work with the addition of gut content analysis of the bullhead would be a valuable addition. Experiments to specifically test the turnover rates of bullhead tissues are required in order to explore the feasibility of experimental use of stable isotope population metrics. An alternative to muscle would be liver, which is known to show higher turnover rates (Tieszen et al., 1983) and would therefore reflect more recent diet in its stable isotope composition (Bearhop et al., 2004).

Benthic invertebrates and ecosystem processes:

Synergistic impacts of bullhead and crayfish were found in the combined treatment. Total invertebrate biomass was similar in the two allopatric treatments, but significantly reduced when the two competing predators were found in allopatry. Invertebrate abundance data provided evidence that crayfish and bullhead induced complementary predatory effects, arising from different prey preference. The most abundant invertebrate both in terms of abundance and biomass was *G. pulex* (57% of the total invertebrate biomass), which was reduced in bullhead treatments relative to the crayfish treatment. Contrastingly, crayfish reduced the biomass of Gastropda, the second most dominant invertebrate in terms of biomass (26%), relative to the bullhead treatment. Combined with a lack of density dependence of impacts, these results apparently explain how the total biomass of the invertebrate community was reduced in the mixed predator treatment relative to predators in allopatry. Increased reduction in elmid larval biomass in the mixed treatment provided further evidence for synergism, suggesting that the impact of crayfish on this group was increased in the presence of a competitor. As a significant trend was not seen for Elmidae abundance, it can be inferred that crayfish selectively preyed on larger elmid larvae only.

For all results that showed a relative impact of crayfish or bullhead on a measure of the benthos, or on ecosystem processes, there was no evidence of density dependent effects. Intermediate values for measures in the mixed treatment, as compared with bullhead and crayfish allopatric values, were not found. Therefore, not only did impacts of crayfish appear density independent, but there was no apparent mediation of impacts on the benthos or on ecosystem processes through interference between the competing predators. This is despite clear evidence, based

on shelter usage, of interference having occurred. Theoretically, an impact of crayfish on bullhead diet could be manifested through a change in bullhead foraging behaviour owing to the dominance of signal crayfish over bullhead. Not only did crayfish strongly out-compete bullhead for shelter during daylight hours, but shelter use was increased during the night by both species. Therefore it seems that while some degree of interference occurred throughout the 24 hour cycle, it was not of a sufficient magnitude to have significant consequences for foraging. There was however a lack of predatory risk in the experimental setup. Dependent on shelter limitation, dominance of crayfish over bullhead in the field might increase bullhead exposure to other vertebrate predators (Bubb et al., 2009). In which case not only may bullhead mortality be increased, but increased time occupied in seeking refugia and predator evasion may decrease time spent foraging.

A combination of results provided strong support for a crayfish-grazer-algal trophic cascade having occurred. The large reduction in Gastropoda abundance, attributable to crayfish, provided a mechanism by which the cascade manifested. The time series demonstrated that the difference in algal standing stock between treatments was likely to increase in the long term. At the termination of the experiment, algal standing stock was still in the process of accruing in crayfish treated channels, whilst standing stock in the bullhead treatment was essentially constant (Figure 5.19a).

Evidence of crayfish-snail-periphyton cascades has previously been found (Lodge et al., 1994, Nystrom et al., 1999). There is some disagreement in the literature as to whether increases in algal accrual are attributable to direct predatory effects of crayfish on grazers, or whether crayfish themselves promote algal accrual through removal of deposits upon a substrate (Lodge et al., 1994, Stenroth and

Nystrom, 2003). As no deposition of fine sediment / detritus on colonisation tiles was found in any of the treatments of this study, the results support a trophic cascading impact of crayfish. It should also be noted that *P. antipodarum* have been shown to be significantly depleted by bullhead (Woodward et al., 2008). Therefore the increased algal standing stock in crayfish treatments likely represented an increased impact of crayfish on *P. antipodarum* as compared to that of bullhead on *P. antipodarum*; i.e. a no-predator control might have displayed reduced algal standing stock relative to the bullhead treatment.

The trophic cascade both manifested sooner, and showed greater significance at the end of the experiment, in the mixed treatment as compared with the allopatric crayfish treatment. The reason for this is unclear, as gastropod abundance was reduced to a similar degree in both treatments with crayfish. One possible explanation is the greater reduction of Elmidae biomass observed in the mixed treatment; as elmid larvae have been assigned to the scraper functional feeding group (Merritt and Cummins, 2006).

The leaf litter breakdown rates confirm the importance of crayfish as shredders (Usio and Townsend, 2001) and are of particular interest when considering the combined influence of crayfish and bullhead on breakdown rates. Although they also act as predators, *G. pulex* are important shredders (Kelly et al., 2002) and are the dominant native macroinvertebrate in the chalk streams that comprise the field-based work throughout this thesis, often comprising more than 90% of total invertebrate number / biomass. Furthermore, *G. pulex* was the dominant native macroinvertebrate in this experimental study, making up a mean of 57% of invertebrate numbers across all channels. However, the importance of *G. pulex* as shredders in the experiment was low relative to crayfish. As the densities of *G. pulex*

represented in the channels were comparable to those found in natural systems, with a mean density exceeding 1000 m^{-2} (Woodward et al., 2008), it is likely that the introduction of signal crayfish has significant implications for this important ecosystem process. Sites where signal crayfish are present are likely to have greatly increased rates of detrital breakdown and therefore increased allochthonous input. Furthermore, crayfish are likely to mitigate cascading effects of bullhead. Bullhead are capable of inducing bullhead-*G. pulex*-leaf litter breakdown cascades (Woodward et al., 2008). Despite a reduction in *G. pulex* abundance / biomass in the current study, overall leaf litter breakdown was comparable between the crayfish only and mixed treatments.

Crayfish mediated reductions in macroinvertebrate biomass and taxon richness have previously been shown experimentally (Nystrom et al., 1996, Stenroth and Nystrom, 2003). My results confirm these patterns, but also demonstrate that crayfish can significantly reduce diversity as represented by Simpson's index. Contrastingly, one instance of a positive effect on invertebrate evenness has previously been shown in juvenile crayfish (Correia and Anastacio, 2008). This discrepancy might be explained by the conflicting effects crayfish can have on the Simpson index. In the current study, lower Simpson values likely reflect the reduced taxon richness attributable to crayfish predation; whereas it is possible, in Correia and Anastácio's study, that increased values represented increased evenness of the invertebrate community. This is supported by the results seen in Chapter Three of this thesis.

The richness of rare taxa has previously been shown to be reduced by crayfish (Usio et al., 2009) ('rare taxa' was defined as taxon not making up more than five percent

of the total invertebrate abundance or density). Results indicated that crayfish preferentially preyed upon larger invertebrates. This was supported by the increased significance of both Elmidae and rare taxa reductions when measured by their biomass, rather than by their abundance. Out of a total of 12 taxa, the third, fifth and sixth taxa with the largest individuals were taxa that belonged to the rare taxa group. This is counter to the findings of some studies which have demonstrated that larger invertebrate taxa tend to be relatively less affected by crayfish than smaller taxa (Nystrom et al., 1996, Nystrom and Perez, 1998).

Shelter use:

Shelter use results support previous work that has shown that crayfish can outcompete bullhead for refugia (Guan and Wiles, 1997, Bubb et al., 2009). In the study of Guan and Wiles only day-time measurements were made and crayfish were introduced for three day periods on two occasions separated by a three day interval. For Bubb *et. al.*, the experimental time scale was under 24 hours and tests were conducted in a circular glass bowl, 30cm in diameter. The results presented here represent a longer time scale (one month acclimatisation followed by 22 days of measurements) in a channel system and it therefore seems increasingly likely these results would be reflected in the field. An increase in night-time shelter use, in the mixed treatment, suggests that both bullhead and crayfish behaviour may be modified when in sympatry.

Summary:

As the results suggest both a positive and a negative impact of crayfish on bullhead (crayfish as a prey item, but also as a competitor), it is perhaps unsurprising that no patterns were observed regarding crayfish presence and bullhead abundance. This is not to say that crayfish do not cause reductions in bullhead numbers in these ecosystems, as has been shown in two separate studies along single rivers (Guan and Wiles, 1997, Bubb et al., 2009). However, the evidence presented here suggests that across rivers / streams there is no simple linear pattern between the abundances of the two species once crayfish have established. Owing to the dominance of crayfish over bullhead, the relationship is likely to also be dependant on refugia availability. Furthermore, the field densities of crayfish in this study ranged from 0.39 to 6.23 m^{-2}, whereas in those surveys where a negative relationship between signal crayfish and bullhead density has been found, densities ranged from 3.67 to 21.67 (Guan and Wiles, 1997) and from 9.1 to 23.6 m^{-2} (Bubb et al., 2009). Therefore, it might be that crayfish densities at the stream sites I sampled are not high enough for a negative relationship with crayfish to be observed.

   The results do, however, demonstrate the significance of a shift in communities containing a single dominant benthic predator, the bullhead, to communities containing two functionally distinct predators, bullhead and signal crayfish. Not only do direct predatory effects of crayfish and bullhead appear capable of resulting in synergistic impacts on the benthos, but their influences on two major energy inputs to aquatic ecosystems are quite different. Bullhead have previously been shown to induce detritivore-detritus cascades, whereas crayfish appear to mediate grazer-algal cascades, and furthermore appear capable of greatly increasing levels of detrital breakdown, in comparison with native shredders.

Chapter Six: Interactions between signal crayfish and a large native fish

Declaration on input:

I am indebted to Kevin Wood, an MSc student previously based at Queen Mary, for his input to this chapter. In order to sample chub, Kevin carried out all rod and line fishing. Furthermore, Kevin carried out the measurement and analysis of chub scales and prepared samples for stable isotope analysis.

Introduction

Only a limited number of studies investigating the possible impacts of crayfish on small fish species have been published (e.g. Guan and Wiles, 1997, Bubb et al., 2009), but even less work has attempted to study potential effects of crayfish on larger fish taxa. There are studies where effects of crayfish on eggs or juvenile stages of larger fish species were investigated (Savino and Miller, 1991, Griffiths et al., 2004); however, potential impacts of crayfish on large fish in their adult stages are poorly understood.

Two of the principles which applied to interactions between crayfish and small benthic fish are likely to also apply to interactions with larger fish; these are competition for resources and reciprocal predation. Competition for prey, whether intra- or interspecific, is a negative type of interaction that can give rise to decreased

growth rates of competitors (Dunham, 1980, Smith, 1983, Persson and Greenberg, 1990). Contrastingly, reciprocal predation can be viewed as being negative or positive, depending on the life stage considered. As ingestion of prey by predatory fish is gape limited (Hambright et al., 1991), and owing to a general increase in gape size with increasing fish length (Ray and Corkum, 1997, Nilsson and Bronmark, 2000, Lima-Junior and Goitein, 2003), the potential for crayfish consumption is relatively greater for larger fish species. As outlined in the introductory chapter, crayfish can be an important prey item of relatively large fish taxa (Didonato and Lodge, 1993, Garvey et al., 1994, Fortino and Creed, 2007) and consumption of crayfish has been shown to increase, intraspecifically, with fish length (Hellawell, 1971a, Weidel et al., 2000). Therefore, what might be considered a positive impact of crayfish on native fish is likely to be greater for larger fish taxa, as compared with smaller taxa.

The relative importance of competition between large fish and crayfish will depend on the degree to which the species of fish in question is reliant on benthic invertebrates, macrophytes and detritus, i.e. to what extent dietary overlap occurs with crayfish. Dietary shift is a common consequence of competition between fish species (Werner and Hall, 1976, Persson and Greenberg, 1990, Persson and Hansson, 1999) and similar effects might be expected where competition between crayfish and fish occurs. A degree of niche partitioning between crayfish and fish is likely to occur, owing to the obligate benthic nature of crayfish. In lotic systems terrestrial invertebrates are an important energy source for fish (Kawaguchi and Nakano, 2001, Allan et al., 2003), but are largely unavailable to crayfish, and consequently are not reported as an important prey item of crayfish.

The European chub was chosen as a focal species to test for impacts of signal crayfish on a native large fish. As true omnivores, chub feed on macrophytes, algae, terrestrial and aquatic invertebrates (including crayfish), small fish, and amphibians (Hellawell, 1971a). They might therefore encounter considerable competition when in sympatry with crayfish. In addition to competition for food items, it seems likely that juvenile chub would be more affected by the presence of crayfish than larger individuals, as smaller fish are more likely to be negatively affected by antagonistic interactions with crayfish (Rahel and Stein, 1988). Finally, as mentioned above, increasingly large chub will be able to ingest a greater range of size-classes of crayfish. A positive relationship between chub size and consumption of native crayfish has previously been demonstrated (Hellawell, 1971a).

Aims and hypotheses:

The first aim of this study was to test whether the growth rates of chub were reduced when in sympatry with signal crayfish. It was predicted that this effect would be greater for smaller chub than for larger chub, owing to a greater occurrence of antagonistic interactions and a reduced ability to utilise the crayfish as a prey resource. The second aim was to compare the diet of chub when in allopatry and when in sympatry with crayfish. It was hypothesised that in response to competition with crayfish, chub would consume proportionately more terrestrial invertebrates – a resource relatively unavailable to crayfish. It was predicted that the inclusion of crayfish into chub diet would result in an increase in dietary niche at the population level, with larger chub consuming relatively more crayfish than smaller chub.

Methods

Four small clay rivers in southern England were used in the study (Figure 6.1) and two distinct yet comparable approaches to studying crayfish invasion, using scalimetry, were made. The Cherwell and Evenlode are tributaries of the river Thames and signal crayfish were first recorded in these rivers in 1995 and 2000 respectively. In both cases signal crayfish were found throughout the entire lengths of the rivers within a year of the first recordings of their presence (Environment Agency staff, personal communication). Archived chub scales for these rivers were provided by the EA and fish caught prior to 1995 (Cherwell) or 2000 (Evenlode) provided pre-invasion growth data, while chub that spawned after these dates were used for post-invasion growth rates. In the Rother and Chad Brook, rather than using pre and post invasion data for single sites, the halted progression of signal crayfish invasion upstream enabled the comparison of proximate invaded and non-invaded sites within rivers. In the case of the Rother, signal crayfish invasion occurred between 1973 and 1975 (EA 2008). A road bridge and weir marked the limit of upstream invasion (51°00'13.32"N, 00°53'08.86"W, Figure 6.1). The invaded site comprised of a length of river beginning at the weir, finishing downstream at the approximate location 51°00'16.51"N, 00°52'30.90"W. The non-invaded site ran upstream of the weir, extending to a point at approximately 51°00'11.73"N, 00°53'35.68"W. In Chad Brook, signal crayfish invaded from the confluence with the river Stour within the last decade (Environment Agency staff, personal communication). The extent of invasion upstream was again prevented by a weir (52°04'42.20"N, 00°42'56.06"E, Figure 6.1). The invaded site was demarked by the weir and a location downstream at 52°04'35.52"N, 00°42'32.02"E; the non-invaded site by the weir and an approximate location upstream at 52°04'48.10"N,

00°43'35.28"E. Scales from chub sampled in the Rother and Chad Brook were collected as described below.

Whilst growth rate analysis using scalimetry was carried out for all four rivers, dietary and stable isotope analyses were conducted on two rivers only; the Rother and Chad Brook. The invaded and non-invaded sites of the Rother and Chad Brook were sampled for crayfish and other potential food items, of chub, in May 2008. Crayfish were sampled by manual searching with a pond net. 18 individuals were taken at the Rother and 19 at the Chad Brook invaded site. Aquatic invertebrates, macrophytes and small fish were collected by kick sampling and manual search. A minimum of five individuals were pooled for each invertebrate taxon collected, five or more individuals were collected per fish species and for each macrophyte species sampled, five leaves from different individual plants were pooled. In the Rother small fish were 1+ cyprinids, stone loach, bullhead and the common minnow (*Phoxinus phoxinus* Linnaeus). In Chad Brook small fish were stone loach, bullhead, minnow and the three-spined stickleback (*Gasterosteus aculeatus* Linnaeus). Approximately 250 g of detritus was taken from the channel substrate. In order to collect terrestrial invertebrates, riparian vegetation was sampled with a sweep net and five individuals were pooled per taxon. All samples were frozen on return to the laboratory to await processing for stable isotope analysis. Crayfish were processed as per page 116 and small fish as per bullhead, described on page 153. The remaining sampled prey items were pooled in their entirety. All samples were homogenised in glass vials before drying at 60 °C for 48 hours, then ground to a fine powder using an agate pestle and mortar, weighed to 0.6 mg and run for stable isotope analysis as per methods on page 116.

Chub were sampled by angling in June and July, 2008. For all chub caught, fork length and mass were measured and three scales were taken from the flank, between the dorsal fin and the lateral line. As there was no option to destructively sample, sex could not be recorded and chub were returned unharmed to the water. In the laboratory, the area comprising the outermost two annuli of each scale was cut away for stable isotope analysis. The resulting samples were finely chopped and then processed for stable isotope analysis as outlined above. To enable the derivation of a regression, that would describe the relationship between scale and muscle tissue stable isotope values, five individuals each of three year classes of chub, fed on a constant, artificial diet, were sacrificed. The year classes were 0+, 1+ and 2+ and were provided by Calverton Fish Farm (Nottingham, UK). Pure muscle tissue, taken from above the lateral line on the flank, was used for stable isotope analysis.

Analyses:

Growth rates were calculated using scalimetry, which has previously been shown to be a viable technique using the scales of chub (Hellawell, 1971b, Mann, 1976). A fork length of 15.9 mm was assumed for the onset of scale formation (Economou et al., 1991), and chub length-at-age was back-calculated according to the Fraser-Lee formula (Lee, 1920).

Baseline corrections were made to allow the measurement of trophic position of individual chub and crayfish. For each river six basal consumers were used for this purpose, three aquatic and three terrestrial. For the Rother these were made up by aquatic taxa of the orders Trichoptera, Amphipoda and Ephemeroptera, and

terrestrial taxa of the orders Coleoptera, Hemiptera and Hymenoptera. For Chad

Brook aquatic taxa belonged to Gastropoda, Amphipoda and Heteroptera, and

terrestrial taxa to Coleoptera, Hemiptera and Diptera. Regressions were made

through the six basal consumers. The perpendicular distance from this generated

baseline, to individual chub and crayfish (as measured by change along the $\delta^{15}N$

axis), gave their trophic position.

Owing to documented ontogenetic shifts, stable isotope based metrics and

mixing models were calculated for two size classes of chub. Chub aged $\leq 5+$ have

been shown to consume higher proportions of terrestrial and aquatic inverts

(excepting crayfish) and less vascular material and small fish than $\geq 6+$ chub

(Hellawell, 1971a). Data from this study combined with that of Mann (1976) gave a

mean fork length of 231.7 mm for 5+ chub. Therefore 'small' chub were defined as

< 232 mm and 'large' chub as ≥ 232. As mentioned in the previous chapter, SEA_c is

robust against small sample sizes (Jackson et al., 2011). However, owing to the

difficulty of catching chub, some sample sizes in this study were particularly small.

In view of this, to control for sample size among populations, 10 random subsets of

three individuals were generated whenever $n > 3$; a sample size of three being the

minimum for SEA calculation. SEA_c was calculated for each subset and the mean

SEA_c value was used for further analysis. The free-to-download package, SIBER,

was used for calculation of SEA_c (Jackson et al., 2011). Estimation of population

trophic range was made as per the individual calculations, however the perpendicular

distance from the baseline to the lower and upper bounds of the SEA_c was measured,

rather than to points represented by individuals.

The software IsoSource (free-to-download) was used for mixing models

(Phillips and Gregg, 2003). Values for trophic fractionation ($\Delta^{13}C$ and $\Delta^{15}N$) in chub

muscle tissue were estimated by averaging the results of four controlled feeding experiments. Values for carbon fractionation in large freshwater fish were +2.0 ‰ (*Coregonus nasus* (Pallas), Hesslein et al., 1993), +1.3 ‰ (*Oncorhynchus mykiss* (Walbaum), Rounick and Hicks, 1985), +1.9 ‰ and +3.3 ‰ (*Oncorhynchus mykiss* and *Salvelinus fontinalis* (Mitchill), McCutchan et al., 2003), giving a mean value of 2.1 ‰. As chub are omnivorous and nitrogen fractionation has been shown to be dependent on nitrogen content of food (Adams and Sterner, 2000), a mean value of +2.3 ‰ was used, as per McCutchan *et al.* (2003). When running IsoSource an increment of 1 % and a tolerance of 0.01 were used. Cannibalism was excluded from the chub mixing models as within this study few of the sampled individuals were physically large enough to ingest conspecifics sampled. Furthermore cannibalism does not appear to be a common occurrence in chub (Hellawell, 1971a, Mann, 1976).

Whilst ontogenetic shift in the diet of crayfish is well reported in the literature (outlined in the introductory chapter, page 25) and the importance of crayfish life-stage has been a recurring theme within this thesis, separating crayfish by size class had negligible effects on IsoSorce output. Therefore, in this instance crayfish are considered as a single group. $\Delta^{13}C$ was set at +2 ‰, based on a feeding experiment with red swamp crayfish (Rudnick and Resh, 2005). As in the case for chub, owing to the omnivorous habit of crayfish, $\Delta^{15}N$ was set at +2.3 ‰. Cannibalism in signal crayfish has been widely reported (Guan and Wiles, 1997, Stenroth and Nystrom, 2003), and crayfish were therefore included as a potential prey item when running IsoSource.

All statistical analyses were carried out in Minitab Version 14.1. Data were first tested for normality and equality of variance and $\log_e(x)$ transformed to meet these

assumptions where necessary. General Linear Models were used to test for differences in the growth rates of each size class of chub between invaded and non-invaded sites of each river, with invasion status set as a fixed factor. A linear regression was used to test the relationship between chub scale and muscle stable isotope values. A further GLM was used to test the relationship between chub length and baseline corrected trophic position; trophic position was set as a co-variable and invasion status and site as fixed factors.

Results

Complementary results for chub growth rates were found for comparisons made pre and post signal crayfish invasion (Cherwell and Evenlode) and between non-invaded and invaded sites (Rother and Chad Brook). For all four rivers 0+ chub growth rates were significantly lower when in sympatry with crayfish as compared to chub in allopatry (Table 6.1, Figure 6.2). This difference was not seen in subsequent year classes, except in the Evenlode, where a significant difference remained in 1+ and 2+ chub. In contrast to the results for the youngest chub, increased growth rates were seen in older year classes when in sympatry with crayfish, although the age at which this transition occurred was variable (Table 6.1, Figure 6.2). Chub from the Cherwell did not show this pattern; however, this may be owing to insufficient data, with 6+ and 7+ fish lacking from the dataset for the non-invaded period.

Stable Isotopes:

A linear relationship was found between the $\delta^{13}C$ and $\delta^{15}N$ values of chub scale and muscle tissue ($F_{1,13} = 40.17$, $p < 0.001$ and $F_{1,13} = 60.51$, $p < 0.001$ respectively).

Muscle $\delta^{13}C$ was depleted relative to scale $\delta^{13}C$ (mean ± SD: -2.2 ± 0.5 ‰), whilst $\delta^{15}N$ values were enriched (0.8 ± 0.3 ‰). Regression equations describing these relationships were used to convert scale isotope values of wild caught chub for all subsequent analysis (Figure 6.3).

With increasing fork length, chub occupied an increasingly higher trophic position as represented by baseline corrected $\delta^{15}N$ (Table 6.2, Figure 6.4). Although not significant, across the size range sampled, there was a trend for chub of a given size of the invaded sites to occupy a higher trophic position than chub of non-invaded sites. Furthermore, there was an indication that increasing trophic position with size showed a steeper relationship for chub in sympatry with crayfish. Site was not important in determining the trophic position of chub. Crayfish in the Rother shared a similar trophic position to chub, however crayfish in Chad Brook occupied a significantly higher level than sympatric chub and crayfish in the Rother (GLM, $F_{2,49}$= 30.31, $p<$ 0.001, Tukey's $p<$ 0.0001 and $p<$ 0.0001). In general, the isotopic niche space occupied by chub and crayfish was distinct (Figure 6.6).

For chub of the non-invaded Rother, at the population level, both size classes shared a similar isotopic niche space, as represented by SEA_c. At the invaded site, there was clear evidence of individual specialisation within the larger size class of chub (Figure 6.6); isotopic niche space of the large size class showed a profound enlargement in sympatry with crayfish (Table 6.3). The trophic range of this group encompassed by the SEA_c increased from 2.05 to 4.72 ‰. Furthermore, the maximum trophic position of large chub from the invaded site was 2.5 ‰ higher than that of crayfish, representing almost one discrete trophic level difference between the maximum chub and maximum crayfish $\delta^{15}N$ values. In Chad Brook an enlargement

of isotopic niche was not seen for large chub, although their niche space shifted towards that of crayfish.

Crayfish potentially constituted a large part of chub diet, for both size classes, at the invaded sites of both rivers (Table 6.4). For the Rother, crayfish comprised 23 to 40 % of the diet of small chub, and 0 to 66 % of large chub. However, as the three large chub of this site showed such marked individual specialisation (see Figure 6.6), IsoSource models were run separately for each. One specialised on crayfish, with a minimum contribution of 60 %; a second on terrestrial invertebrates, with a minimum contribution of 47 % (while crayfish made up 0 to 35 %); and the third was a generalist, with similar ranges for all potential prey (crayfish 0 to 31 %). For Chad Brook, crayfish contributed 0 to 45 % and 0 to 60 % of chub diet for small and large size classes respectively. At the non-invaded sites on the Rother, chub diet comprised almost entirely of terrestrial invertebrates and small fish (Table 6.4). However, it is interesting to note that for large chub, the minimum contribution of terrestrial invertebrates decreased from 61 % at the non-invaded site to 7 % at the invaded site. For small chub, the drop was less extreme, from 67 to 55 %. The same pattern was seen in Chad Brook, where the minimum contribution from terrestrial invertebrates dropped from 17 to 0 % for the large chub. Furthermore, the maximum contribution of terrestrial invertebrates to large chub was reduced at the invaded site in both rivers; from 61 to 46 % (Rother) and 61 to 47 % (Chad Brook). Therefore, the mixing model output suggested a change in the diet of large chub in the Rother, with a minimum contribution of terrestrial invertebrates of 61 % at the non-invaded site and a maximum of 46 % at the invaded site. This pattern was driven by the individual that specialised on crayfish, with a maximum terrestrial invertebrate contribution of 25 %, but was tempered by the individual that specialised on

terrestrial invertebrates. No dietary items were confirmed for crayfish in the Rother, with almost identical contribution ranges for all potential food sources (Table 6.4). Output for crayfish in Chad Brook revealed some dietary preference, with a minimum contribution of aquatic invertebrates of 24 %.

Table 6. 1. GLM output for chub growth rates at invaded sites as compared with non-invaded sites for each size class of chub.   * = $P \leq 0.05$, ** = $P \leq 0.01$ *** = $P \leq 0.001$.

Site	Size class	n	d.f.	r^2_{adj}	F	P
Rother	0+	19	1,17	27.39	7.79	**0.013***
	1+	19	1,17	0.00	0.28	0.601
	2+	19	1,17	0.00	0.22	0.642
	3+	17	1,15	0.00	0.75	0.399
	4+	8	1,6	69.76	17.15	**0.006****
	5+	8	1,6	52.99	8.89	**0.025***
Chad Brook	0+	26	1,24	36.55	15.40	**0.001*****
	1+	26	1,24	0.10	1.02	0.322
	2+	26	1,24	0.00	0.56	0.462
	3+	25	1,23	7.02	2.81	0.107
	4+	21	1,19	0.00	0.37	0.553
	5+	11	1,9	0.00	0.13	0.725
	6+	8	1,6	71.28	18.37	**0.005****

Continued overleaf.

Table 6. 1 continued.

Site	Size class	n	d.f.	r^2_{adj}	F	P
Cherwell	0+	58	1,56	16.94	12.63	**0.001*****
	1+	51	1,49	0.00	0.36	0.552
	2+	37	1,35	1.15	1.42	0.241
	3+	26	1,24	0.00	0.34	0.565
	4+	17	1,15	0.00	0.55	0.470
	5+	15	1,13	0.00	0.54	0.476
Evenlode	0+	68	1,66	48.04	62.95	**0.001*****
	1+	66	1,64	25.66	23.43	**0.001*****
	2+	64	1,62	10.65	8.51	**0.005****
	3+	53	1,51	0.00	0.50	0.483
	4+	43	1,41	0.00	0.53	0.473
	5+	37	1,35	12.85	6.31	**0.017***
	6+	35	1,33	1.43	1.49	0.230

Table 6. 2. GLM output for the relationship between chub length and baseline corrected trophic position, including site and invasion status. ** $= P \leq 0.01$.

n	r^2_{adj}	Variable / factor	d.f.	F	P
44	17.39	Log length	1,41	11.27	**0.002****
		Invasion status	1,41	1.69	0.201
		Site	1,41	1.58	0.216

Table 6. 3. The trophic position (and SEA$_c$ values) of chub and crayfish in the rivers Rother and Chad Brook, as measured by the perpendicular distance from a linear regression fitted to six primary consumers (sloping isotope baseline) to the lower and upper boundaries of the SEA$_c$ ellipses. Chub SEA$_c$ values in brackets represent the mean value of 10 randomly generated subsets of 3, where more than three individuals were available.

River and invasion status	Group	baseline corrected δ^{15}N (‰)			SEA$_c$ value
		lower limit	upper limit	range	
Rother					
Non-invaded	small chub	3.09	3.96	0.87	0.93 (n.a.)
	large chub	2.76	4.81	2.05	2.32 (2.85)
Invaded	small chub	3.19	4.62	1.43	1.38 (1.46)
	large chub	2.04	6.76	4.72	22.90 (n.a.)
	crayfish	2.35	4.31	1.86	1.58

Continued overleaf.

Table 6. 3 continued.

River and invasion status	Group	baseline corrected $\delta^{15}N$ (‰)			SEA$_c$ value
		lower limit	upper limit	range	
Chad Brook					
Non-invaded	small chub	n.a.	n.a.	n.a.	n.a
	large chub	2.93	3.74	0.81	2.28 (1.52)
Invaded	small chub	2.77	3.57	0.80	0.91 (0.70)
	large chub	3.57	4.41	0.84	1.63 (n.a)
	crayfish	3.35	4.24	0.89	2.02

Table 6. 4 (following page). IsoSource output for chub and crayfish in the rivers Rother and Chad Brook, indicating the relative contribution of each food source to chub and crayfish diets. For each potential food source a minimum and maximum possible contribution is given, expressed as a percentage of the diet. The term n.a. indicates a food source was unavailable to chub.

	crayfish		terrestrial invertebrates		aquatic invertebrates		small fish		detritus		macrophytes		chub	
	min	max	min	max	min	max	min	max	min	max	min	max	min	max
non-invaded Rother														
small chub	n.a.	n.a.	67	68	0	2	27	28	0	1	2	5	n.a.	n.a.
large chub	n.a.	n.a.	61	61	1	6	32	32	0	1	1	5	n.a.	n.a.
invaded Rother														
small chub	23	40	55	63	0	3	0	14	0	3	0	3	n.a.	n.a.
large chub	0	66	7	46	0	24	0	45	0	26	0	25	n.a.	n.a.
crayfish	0	55	0	44	0	49	0	42	0	46	0	47	0	42

Continued overleaf.

Table 6. 4 continued.

	crayfish		terrestrial invertebrates		aquatic invertebrates		small fish		detritus		macrophytes		chub	
	min	max	min	max	min	max	min	max	min	max	min	max	min	max
non-invaded CB														
small chub	n.a.	n.a.	0	55	0	22	1	39	0	22	0	85	n.a.	n.a.
large chub	n.a.	n.a.	17	61	0	7	7	34	0	7	0	25	n.a.	n.a.
invaded CB														
small chub	0	45	0	57	0	24	0	36	0	28	0	86	n.a.	n.a.
large chub	0	60	0	47	0	24	0	48	0	28	0	77	n.a.	n.a.
crayfish	0	43	0	17	24	62	0	50	0	24	0	30	0	29

Figure 6. 1. The locations of the four clay rivers used in the study. For Chad Brook and the Rother, asterisks mark the leading front of crayfish invasion. Small scale map drawn from Edina Digimap (© Crown Copyright Ordinance Survey. An EDINA Digimap/JISC supplied service).
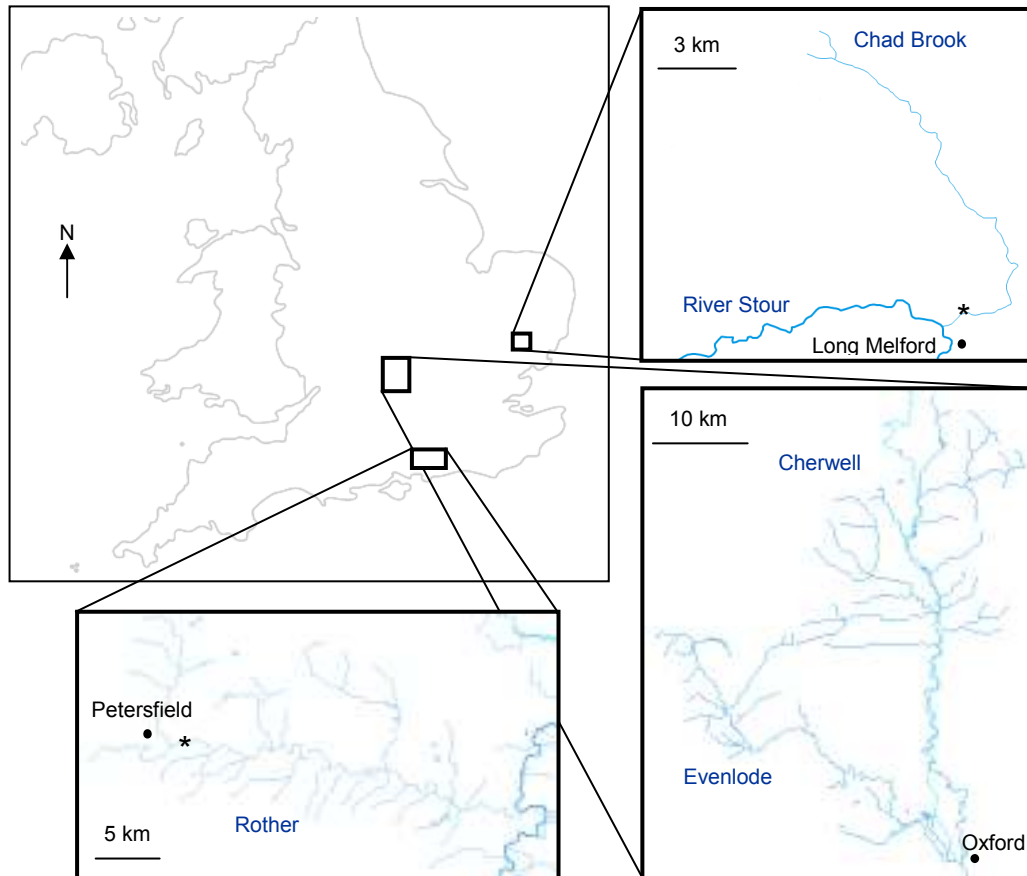
Figure 6. 2. Mean growth rates (± SE) of chub per year-class for non-invaded (closed circles) and invaded (open circles) sites of a) the Rother, b) Chad Brook, c) the Cherwell, and d) the Evenlode.
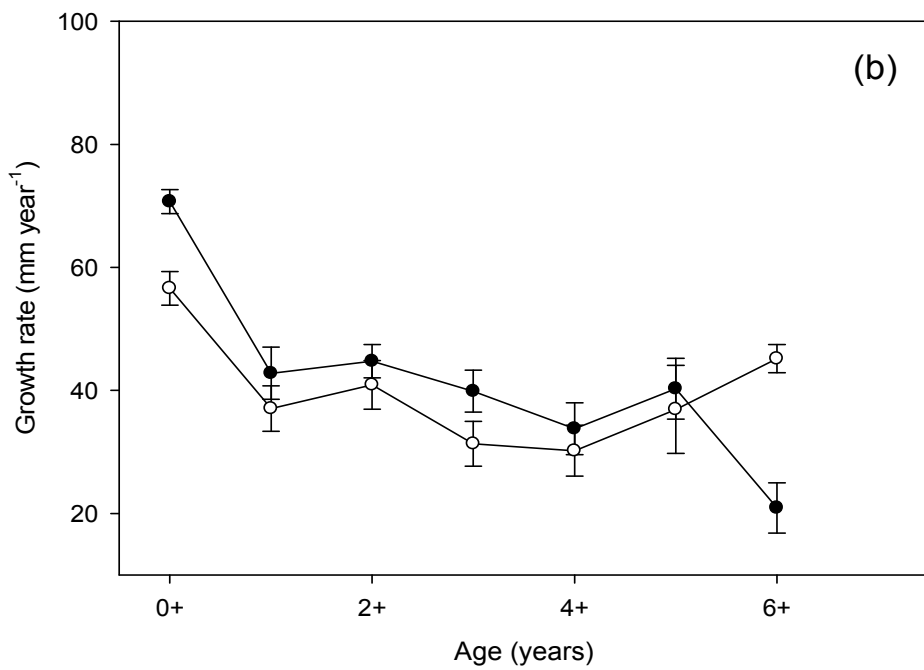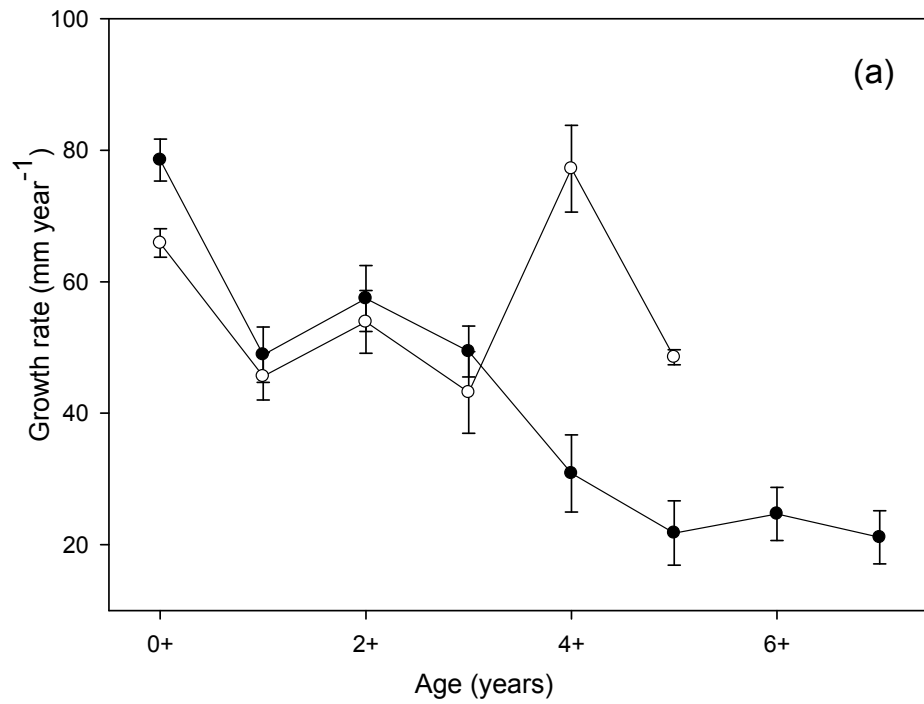
Figure 6. 2 continued.
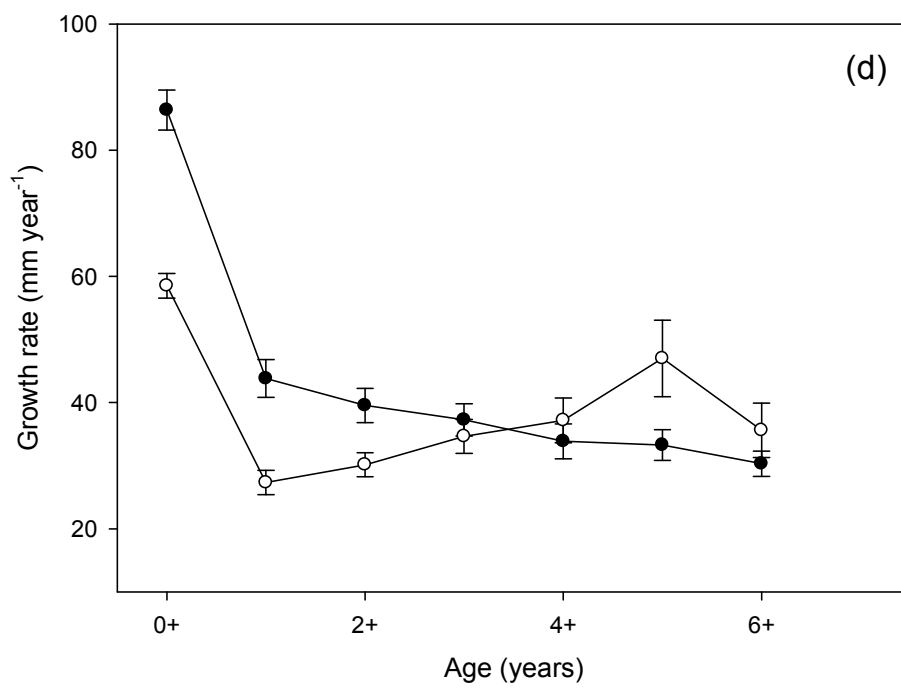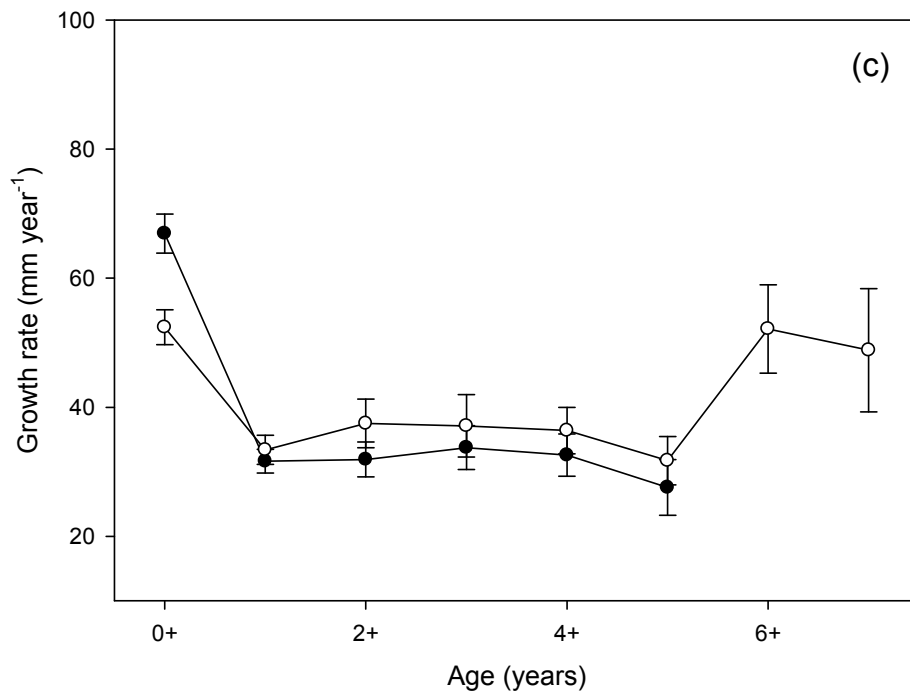
Figure 6. 3. The relationship between $\delta^{13}C$ and $\delta^{15}N$ values in scale and in muscle tissue of chub. For the relationship of $\delta^{13}C$, r^2_{adj} was 75.6 % and for that of $\delta^{15}N$ r^2_{adj} was 82.3 %.
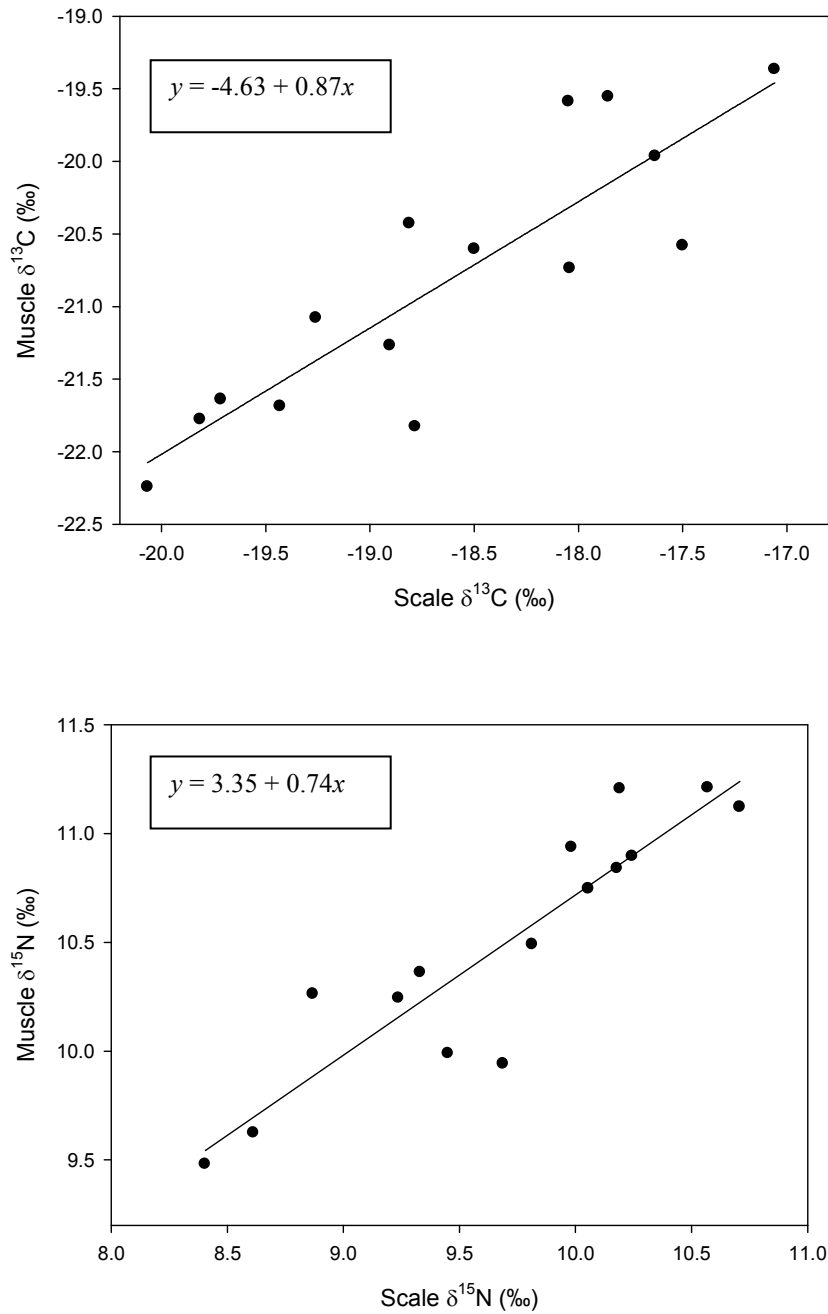
Figure 6. 4. Chub trophic position, as represented by baseline corrected δ ^{15}N, by
fork length, for individuals of non-invaded (open triangles) and invaded sites (closed
circles).
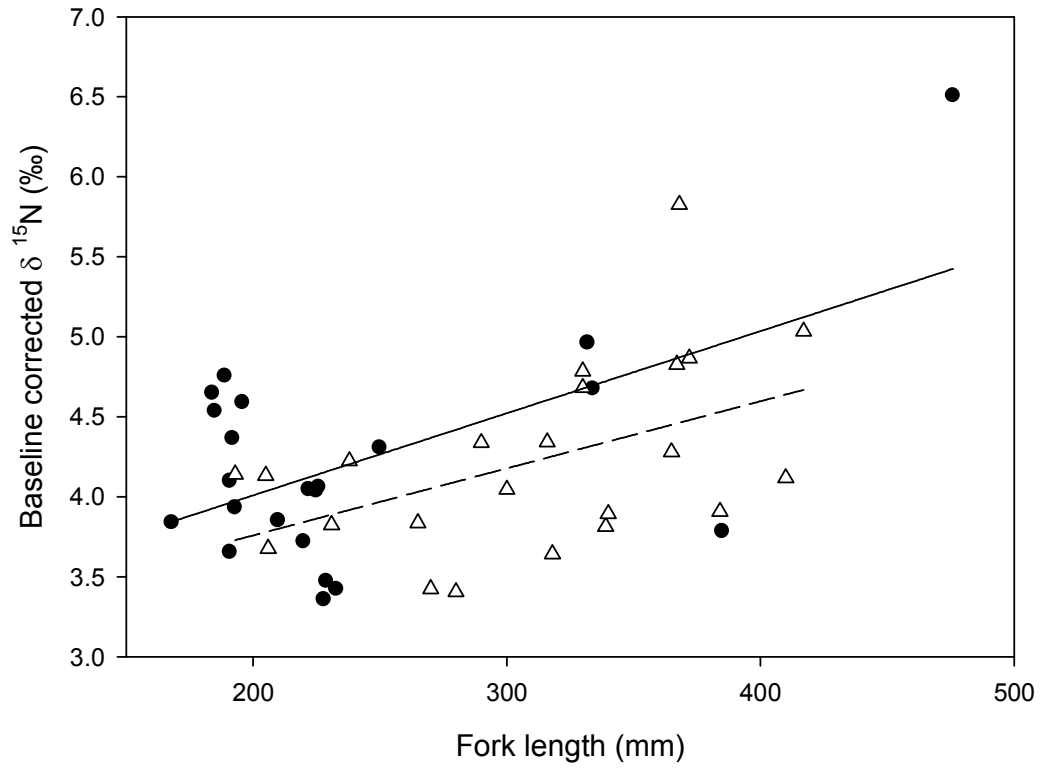
Figure 6. 5. Stable isotope bi-plots of a) the Rother and b) Chad Brook, with mean values (± SE) for chub in invaded and non invaded sites, crayfish and small fish. Regressions through primary consumers represent baselines from which trophic status was measured.

(a)

(b)

● Chub (non-invaded)
○ Chub (invaded)
▼ Crayfish
✖ Small fish
◆ Macrophytes
◇ Detritus
△ Terrestrial grass seeds
■ Aquatic basal consumers
□ Terrestrial basal consumers
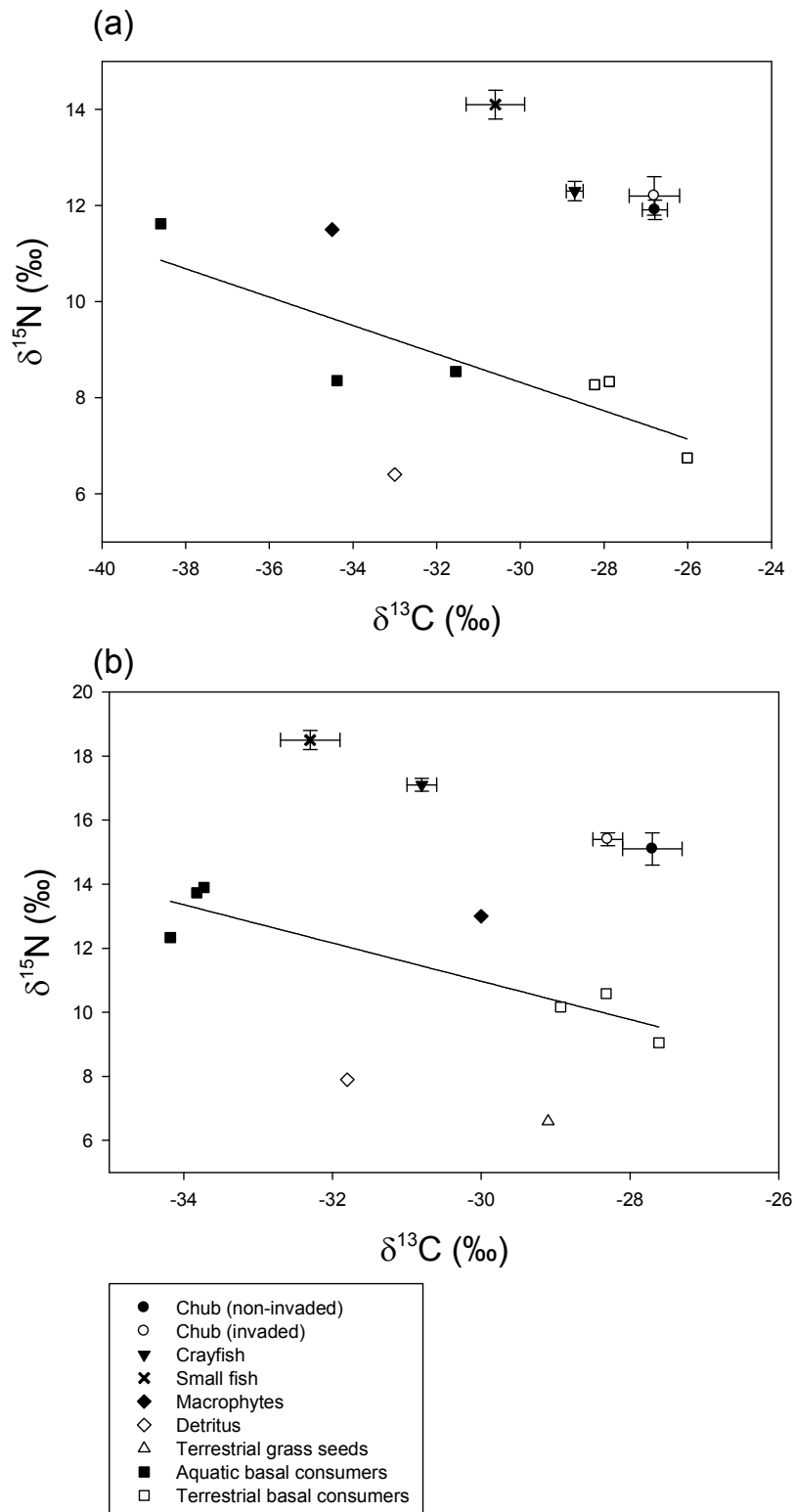
Figure 6. 6. Isotope bi-plots and associated corrected Standard Ellipse Areas (SEAc)

for chub and crayfish from a) the Rother and b) Chad Brook. Black represents large

chub and dark grey small chub. Closed circles are chub of non-invaded sites and

open circles chub of invaded sites. Solid line ellipses are the SEAc of uninvaded

sites whereas dashed line ellipses are those of invaded sites. The light grey crosses

and dotted ellipse represent crayfish.
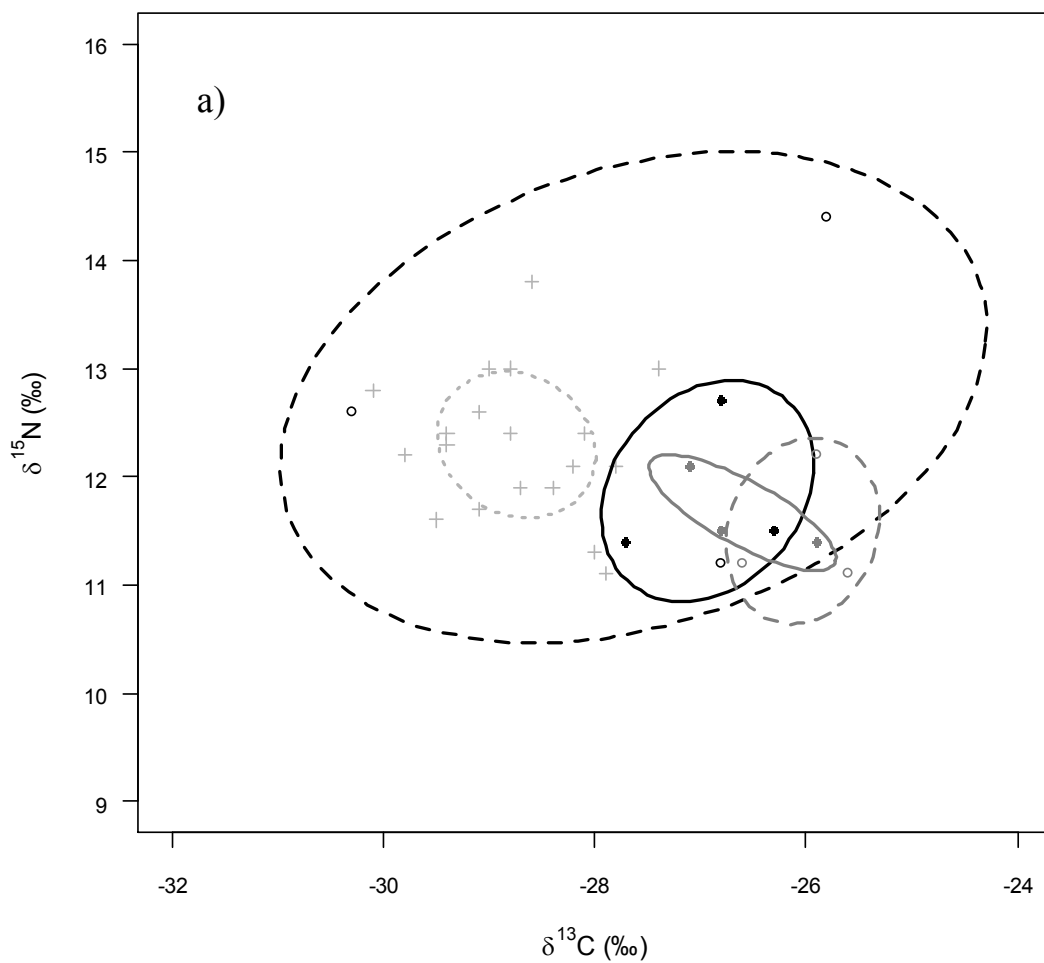
Figure 6. 6 continued.
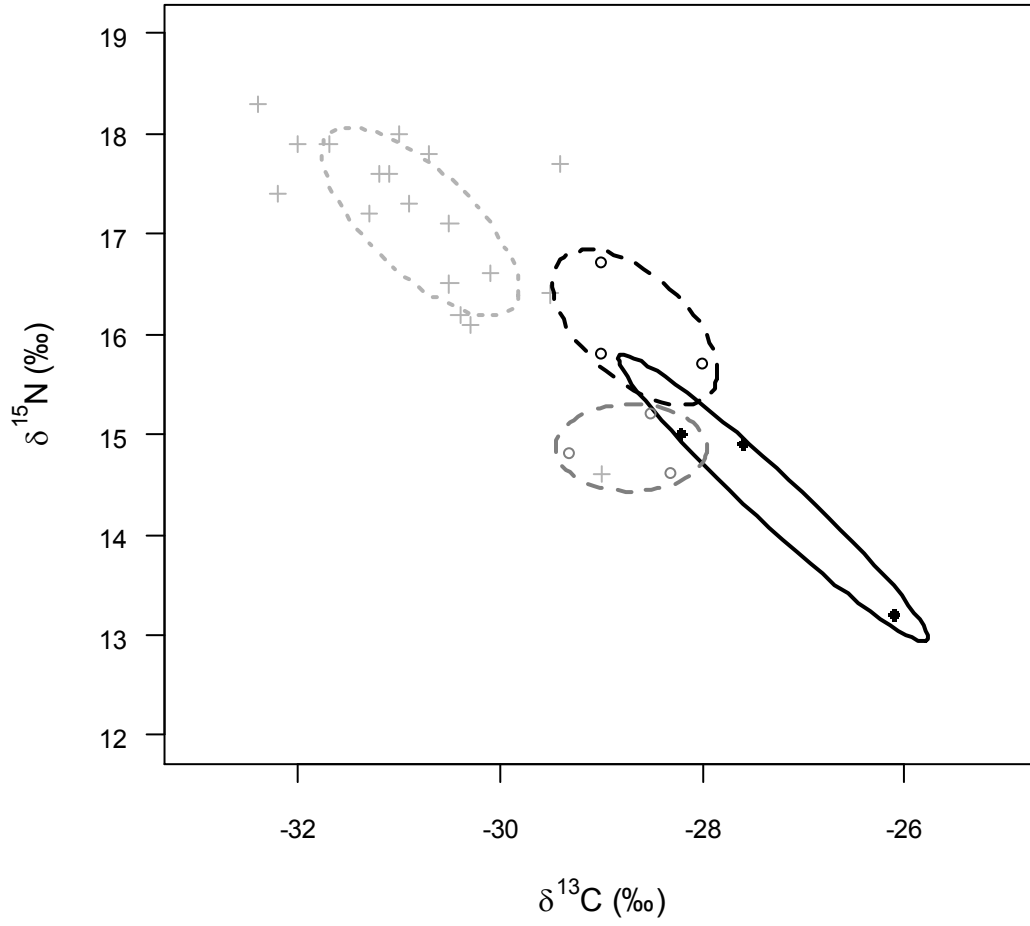
Discussion

The vast majority of literature regarding invasion biology focuses on the negative effects of non-native species. In fact the term 'invasive species' is often purposely chosen as an alternative to 'non-native species' in order to convey a sense of negative impacts attributable to an organism. There are however examples of non-native species having positive effects on recipient ecosystems (Letnic et al., 2009, Straube et al., 2009). The results of this study suggest that a non-native species can have positive as well as negative effects on single species within a recipient community. A clear negative impact was manifested in reduced growth rates of 0+ chub, whilst a positive effect was seen in later age classes, when growth rates increased. It is feasible that over the course of a lifetime an individual chub might be subject to both negative and positive consequences of crayfish presence.

The reduction in the growth rate of 0+ chub was in agreement with the first hypothesis. Although the growth rate results were based on correlational field data, a consistent effect was seen for the two complementary methods used, making a strong argument for an impact attributable to crayfish. Crayfish are unlikely to be a successful predator of juvenile chub, but predation on small fish by signal crayfish has been observed (Guan and Wiles, 1997) and non-lethal, indirect effects of predators are known to reduce prey growth rates (Werner and Anholt, 1996, Nakaoka, 2000, Stoks, 2001). Two possible explanations for such results are a change in foraging behaviour and / or disruption of feeding caused by the predator (Nakaoka, 2000). The former results from an increase in time engaged in predator avoidance and therefore a reduction in time spent foraging (Werner and Anholt, 1993, Anholt and Werner, 1995). Juvenile chub have been shown to reduce foraging behaviour in riffles as a response to simulated avian predation threat, resulting in reduced growth

rates (Allouche and Gaudin, 2001). It is also likely that 0+ chub face strong competition for prey, with crayfish. Aquatic invertebrates can dominate the diet of 0+ chub, comprising over 90 % of diet by occurrence (Mann, 1976). In particular Chironomidae are favoured, making up to 40 % of dietary items in some cases (Mann, 1976, Garner, 1996). As crayfish feed heavily on this group, and have been shown experimentally to reduce their abundance (e.g. Guan and Wiles, 1998, Stenroth and Nystrom, 2003), competition with 0+ chub is likely.

The prediction that growth rates of larger chub would not be as strongly affected as those of smaller chub was validated. However, it had not been predicted that larger chub would display increased growth rates when in sympatry with crayfish, which was revealed at three of the four sites. The increased growth rates of larger chub might owe to consumption of crayfish. This is supported by the stable isotope results which revealed both consumption of crayfish and a trend of increased trophic position, among chub of given length, at invaded sites. Although various studies have shown consumption of non-native prey by native predators (Gorokhova et al., 2004, Garcia and Protogino, 2005, Carlsson and Strayer, 2009), I have only been able to find a single example where such predation apparently resulted in the increased growth rate of a native predator (King et al., 2006).

Where older chub showed significant increases in growth rates, it was generally of much greater magnitude than the decreases seen in 0+ individuals. This should not, however, be interpreted as an indication that the net effect of crayfish is positive for chub. 0+ chub are likely to be less able to afford reduced growth rates as compared with later life-stages, owing to the increased predation risk in fish associated with being of a smaller size, and the importance of fitness at the end of the feeding season for over-winter survival (Sogard, 1997).

An increase in the trophic position of chub, with increasing size, has previously been demonstrated on the basis of gut content analysis (Hellawell, 1971a). The results of this study represent the first quantitative confirmation of this relationship, in chub, using stable isotopes. Although there was a general trend for trophic position to be increased for a given size of chub at the invaded sites, considerable individual variation was seen, indicating individual specialisation of chub across their size range. A few smaller individuals of invaded sites occupied some of the lowest trophic levels seen in chub across the sites; however, numerous small individuals occupied a considerably higher trophic position when in sympatry with crayfish. The stable isotope mixing model results provided evidence that this variation among smaller chub was attributable to the consumption of crayfish. Therefore it appears that even within relatively smaller size classes of chub, utilisation of crayfish and individual specialisation was seen.

Supporting the second hypothesis, stable isotope data provided evidence that larger chub consumed proportionately more crayfish than smaller chub. In the Rother, individual specialisation of large chub greatly increased the population level isotopic niche. This was partly owing to the incorporation of crayfish into the diet of one individual. Along with the individual that specialised on terrestrial invertebrates, the stable isotope results reveal distinct individual specialisation which has not previously been reported in chub. Considering the nature of stable isotope ratios in tissues, the search images used by these chub must have remained fairly constant for considerable periods of time.

A second observed dietary shift added further support to the second hypothesis. It had been predicted that chub would rely to a greater extent on

terrestrial invertebrates following invasion by signal crayfish. However, contrary to expectations, a reduction in the contribution of terrestrial invertebrates to large chub was found. A reduction in contribution of one food source must be compensated for by increased consumption of an alternative source. Owing to the evidence that crayfish were consumed by chub and the published negative effects of crayfish on invertebrates and macrophytes, it seems most likely that compensation was made through predation on crayfish. Hellawell (1971a) documented that aerial insects are eaten readily by younger chub, but little by older individuals. Gut content analysis, in the same study, showed increased consumption of fish, frogs and white-clawed crayfish by larger chub.

It seems clear that for chub, invasion by signal crayfish can have both negative and positive effects. The key question, that the present study cannot answer, is whether the net effect of crayfish invasion for chub is negative or positive. Examples of non-native species having net positive effects are extremely rare (but see Letnic et al., 2009), however this is most likely a result of a bias in research focus and publication towards negative impacts of invasive species (Thieltges et al., 2006). Complicating the issue is the difficulty of defining positive and negative effects, which is dependent on the organism or level of ecological organisation being considered. In the case of chub and signal crayfish, long term study of population dynamics would be required in order to come to an unambiguous and objective conclusion as to whether crayfish invasion is negative or positive.

Chapter Seven: Final discussion and conclusions

The findings of this PhD have been wide-ranging. The population genetic work was the first conducted on invasive crayfish in the UK, and demonstrated high levels of genetic diversity in signal crayfish. This was not true for all populations; however it is clear that the introduction of this species was highly successful in terms of creating genetically viable populations. From an ecological point of view, crayfish had significant impacts at all levels of the food webs in which they had established. Direct and indirect effects were made at the very base of food webs, where autochthonous and allochthonous inputs were affected. Macroinvertebrate communities exhibited various responses to crayfish in experimental studies, some of which appeared to have manifested at larger spatial scales in natural communities. At the top end of the trophic scale, fish populations appeared to both suffer costs, and gain benefits, as a result of crayfish invasion.

Auto Critique:

This thesis employed multiple approaches in the study of signal crayfish invasion. Community analysis and stable isotopes were repeatedly used in conjunction in order to make inferences pertaining to crayfish diet. Although these methods were used with some success, the thesis might have benefited from complementary gut content analysis to confirm feeding links. This is particularly true in the case of bullhead, however, gut content analysis of crayfish is notoriously difficult. Amorphous material, which sometimes represents a large proportion of the gut content, precludes identification of various prey taxa (Momot, 1995).

Direct effects:

Signal crayfish were shown to have wide ranging impacts on biodiversity and ecosystem functioning. Effects were both direct and indirect - the two not necessarily mutually exclusive. Across studies, direct effects were observed in macroinvertebrate communities in particular. On balance, signal crayfish did not appear to be important in determining absolute taxon richness, perhaps the most obvious measure of biodiversity. Whilst a reduction in taxon richness, attributable to crayfish, was found in the channel experiments, this was not reflected within the *in situ* cage experiment or field survey. This is not surprising when considering that streams are open systems and extirpation of taxa is therefore unlikely. However, crayfish clearly modified macroinvertebrate communities in terms of the relative abundances of taxa. These effects were evidently mediated by crayfish within the two experimental pieces of work and suggest that the patterns revealed in natural communities were also attributable to crayfish.

The diversity of the benthos, as measured by the Simpson diversity index, consistently showed relationships with crayfish. However, the direction of these relationships was not consistent; reductions in diversity were seen within the experimental work, whilst a positive relationship was discovered in the field, when a co-dominant predator, the bullhead, was also considered. Two explanatory factors help to clarify this apparent paradox; these are the intensity of predation and the degree of openness of the systems studied. Barring the six small crayfish treatment of the cage experiment, densities of crayfish fell within natural bounds. Therefore the degree of predation within the experimental setups can largely be considered realistic. Crayfish densities from sites comprising the field survey also fell within

the known range; however, they were toward the lower end of the scale, whilst those of the experimental work were relatively high. The discrepancy in the intensity of predation is likely to have had consequences for prey communities and might explain the contrasting results. Not only was the level of predation high in the experimental systems, but some level of realism will inevitably have been lost by their inherent artificial nature. In contrast to the open nature of streams, the channel experiment was a completely closed system. Cages used in the field experiment were open; however the spatial scale of measurement was small, and samples were spatially closely associated with crayfish, when present. Therefore predation pressure was more intense in experimental setups not only in terms of density, but also in terms of the nature and scale at which measurements were made.

Direct impacts at the taxon level were entirely consistent for the ubiquitous and abundant Chironomidae, which were reduced by crayfish in both experimental approaches and displayed negative correlations with crayfish in natural populations, in terms of abundance - suggesting the manifestation of direct impacts. Reduction of rare taxa by crayfish represented another consistent result of experimental work. Whether this was reflected in natural communities, or not, cannot be determined from the field survey. The definition of rare taxa is rendered meaningless by the extreme variation in the total invertebrate number that was observed between sites, driven by the abundance of *G. pulex*. Variables that do not pertain to crayfish presence will certainly have been important in producing this variation in *G. pulex* abundance and therefore a single threshold to measure rare taxa between sites would not have been appropriate.

The total biomass of the macroinvertebrate community was reduced in the mixed predator treatment of the channel experiment, relative to treatments where

predators were found in allopatry. This resulted from complementary prey preferences of crayfish and bullhead; bullhead reduced the biomass of the dominant *G. pulex* whilst crayfish reduced the biomass of various other taxa. The density of crayfish and bullhead was also found to correlate significantly with the Simpson diversity of macroinvertebrate communities measured in the survey work, implying possible top-down regulation of these communities by their dominant predators. Complementing the channel experiment results, when *G. pulex* was excluded, total biomass of the benthos was negatively correlated with crayfish.

The negative relationship between crayfish and Chironomidae abundance was lost when Chironoimdae were measured in terms of their biomass both in the channel experiment and survey work, whereas in the cage experiment the relationship was consistent. These results suggest that impacts of crayfish on invertebrate taxa can be more complicated than straightforward reductions in number, and that size distributions can also be affected. Whilst this could be attributable to selective predation based on invertebrate size, indirect effects of crayfish are also likely to have played a role.

Not all direct effects of crayfish pertained to their impact on macroinvertebrate communities. Crayfish were themselves exploited as a prey item. This was clearly demonstrated in the chub study, where chub of various sizes were shown to consume crayfish. There was also an indication that bullhead, a relatively small fish species, consumed crayfish. Whilst this was not confirmed, evidence taken from the literature and preliminary experimental work as part of a parallel undergraduate study supports such a claim. Crayfish also had direct effects at the lowest trophic level. Crayfish in the channel experiment were shown to break down

leaf litter at a high rate. Although not significant, there was also a trend of increased

breakdown of leaf litter by larger crayfish in the cage experiment.

Indirect effects:

Signal crayfish were found to have indirect effects that were both trophically and

non-trophically mediated. A crayfish-grazer-periphyton trophic cascade was

indicated by the results of the cage experiment and was confirmed by the results of

the channel experiment. In the case of the cage experiment, measurements of

chlorophyll *a* were taken at a single time point and it cannot therefore be said

whether the difference between treatments would have been sustained over a longer

time period. However, measurements were taken at intervals throughout the channel

experiment and the difference between treatments was found to increase with time.

These fairly consistent results have implications for the whole aquatic community, as

the quantity of autochthonous energy that entered higher trophic levels in these

systems presumably was reduced. Admittedly speculative, such a suppression of

autochthonous energy might have led to the negative relationship between the

isotope niche of bullhead populations and crayfish presence.

An indirect impact of crayfish, that was mediated through abiotic means, was

the effect of crayfish ecosystem engineering on resident fauna in the sediment within

cages of the field experiment. Although further investigation into such effects were

not made in other areas of the thesis, this represented a novel finding as previous

work has not regarded crayfish engineering and the bulk sediment of lotic systems.

Positive and negative effects:

Although caution is required in the use of the terms 'positive' and 'negative' to describe the impacts of an invader, they help to highlight the point that not all consequences of crayfish presence appeared to be negative for native fauna, in particular fish. As regards the effects summarised above, the vast majority can be argued to be negative effects; numbers and biomass of native invertebrates were reduced and autochthonous input to higher trophic levels was suppressed. Whilst indirect effects resulting from engineering are likely to benefit some taxa, but not others, when predation on invertebrates is also considered, it is clear that the net impact will generally be negative, as seen for Chironomidae.

Positive effects as well as negative effects were seen in both of the studies that investigated the impact of signal crayfish on fish. Intriguingly, it was not clear what the *net* impacts were likely to have been. In terms of negative effects, although it must be emphasised that this work was correlative, there was evidence of competition having occurred between crayfish and both bullhead and chub. Bullhead isotopic niche correlated negatively with increasing densities of large crayfish, whilst chub stable isotopes indicated dietary overlap. Furthermore growth rates of $0 +$ chub were reduced at invaded sites; possibly a consequence of competition. Conversely, it appears that both bullhead and chub utilised signal crayfish as a novel prey item. This was supported by an increased isotopic niche space of bullhead populations with increasing densities of $0 +$ crayfish, and by stable isotope mixing model output, which gave clear evidence that chub preyed upon crayfish. Whilst the data did not allow an evaluation to be made for chub, there was no indication that net impacts were positive or negative for bullhead, as no relationship between densities of bullhead and crayfish were found.

The importance of crayfish body size:

A recurrent theme throughout much of the thesis was the importance of crayfish body size. A search of the literature reveals no consistent patterns regarding ontogenetic dietary shifts in crayfish, and examples of studies where per capita and / or biomass effects were considered are rare. In the cage experiment, despite an increased gram-for-gram impact of smaller crayfish on several community measures, larger crayfish were more predatory at this locality. Furthermore, the smaller size class of crayfish only, was found to have significant engineering effects, and therefore only the smaller size class was likely to have had indirect impacts on invertebrates in the sediment.

The survey work also suggested negative impacts driven primarily by larger crayfish, both for various measures of the macroinvertebrate community and for the isotopic niche size of populations of bullhead. Whilst in these cases there may not have been sufficient time for the impacts of 0 + crayfish to manifest, if crayfish were indeed driving the correlations, the results appear consistent with an increased per capita predatory role of larger crayfish, as suggested by the cage experiment results. Finally, as a prey item for fish, crayfish size class is inevitably important. Both bullhead and chub are gape limited and crayfish must therefore fall under a particular size threshold, determined by an individual's gape, in order to be ingested.

A summary of the interactions and factors discussed under the above four subheadings is given in Figure 7.1, taken from the introductory chapter (page 39).
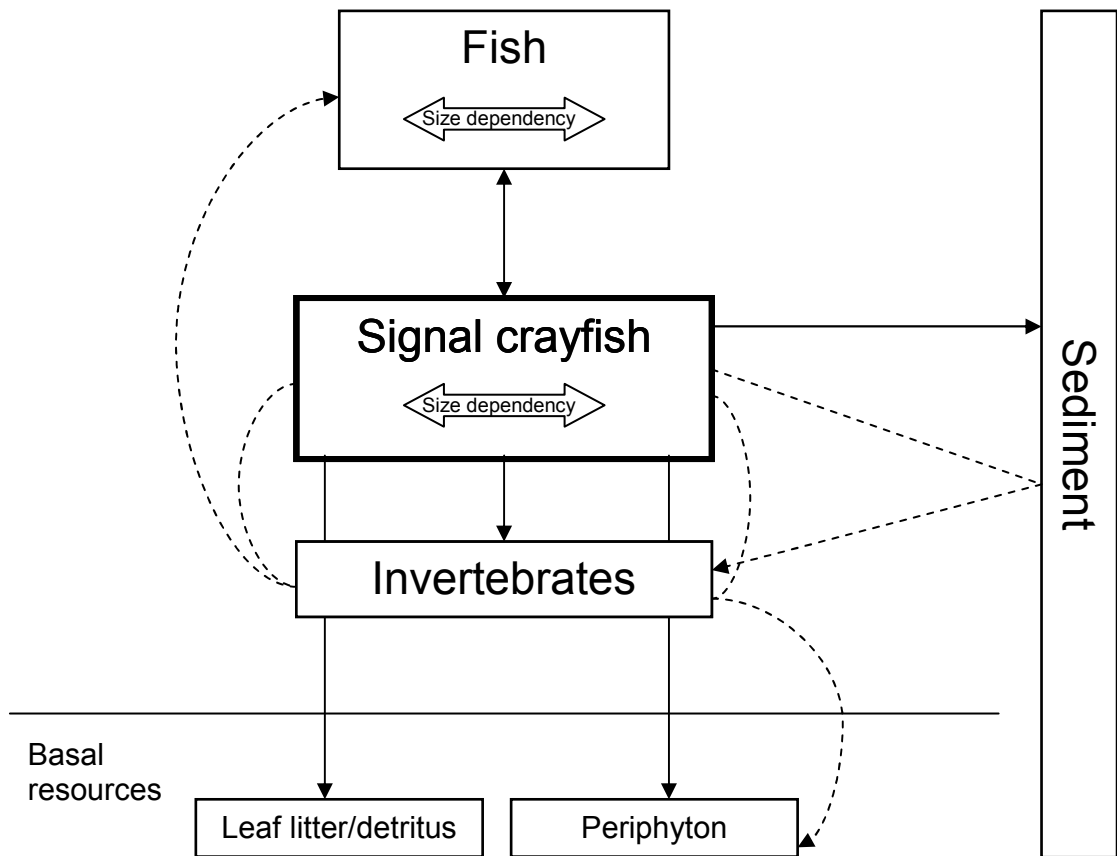
Figure 7. 1. A schematic representing the interactions between crayfish and various elements of the communities of which they are a part, both biotic and abiotic. See page 39 for original legend.

The results of the PhD have gone some way in confirming the links outlined in Figure 7.1. A direct impact on invertebrates was found and was of sufficient magnitude as to result in the suppression of their communities in experiments. Such relationships appeared to be reflected in field data. A strong direct trophic link between crayfish and leaf litter was found in the channel experiment. As a result, any reduction in the shredding attributable to other macroinvertebrates (through predation by crayfish on such macroinvertebrates) was clearly irrelevant.

Support for both the direct and indirect relationships between crayfish and fish was provided by the work with bullhead and chub. Furthermore the relative

sizes of crayfish and fish did appear to be of importance in determining the strength / direction of such relationships. However, the relative importance of direct and indirect effects for fish was not addressed within the PhD and is an area that requires further work. This is of particular importance if the correct management decisions are to be made in order to conserve and sustain freshwater fisheries. Long term mesocosm experiments would be an appropriate approach to determine net impacts of signal crayfish on fish.

An increase in periphyton standing stock was found in both experiments, supporting an indirect effect of crayfish via a trophic cascade. This was supported by the heavy reduction in grazers in the channel experiment.

In the field experiment crayfish were found to influence the composition of the sediment. Understanding how this might influence the invertebrate community associated with this microhabitat will require further work in order to disentangle the direct impact of crayfish on the invertebrate community from potential indirect effects through modifying the sediment. This is of applied significance, as the ecosystem engineering effect was dependent on crayfish body size. Modification of crayfish population structure through trapping may therefore alter the abiotic role of this species and should be considered in any management programme directed at signal crayfish.

Signal crayfish as an invasive keystone species?

Signal crayfish demonstrated two attributes that are regularly associated with keystone species; a role as a top predator (with resultant trophic cascades) (examples: Fritts and Rodda, 1998, Letnic et al., 2009) and ecosystem engineering effects (Anderson et al., 2006, Brown and Lawson, 2010). Crayfish have previously been

assigned keystone species status, both as native and invasive species. However, a consideration of the magnitude of effects is important in determining keystone status, keystone species being defined as those which have a disproportionate impact on communities relative to their abundance / biomass (Power et al., 1996). Unfortunately it is not possible to put a value on the relative importance of the effects crayfish had in the studies comprising this thesis. Despite this, some context of the magnitude of their impacts can be provided. For example, native predator identity (bullhead) and site width were significant explanatory factors in the invertebrate field study, often explaining as much or more variation than crayfish *per se*. In the cage experiment, whilst crayfish were a significant factor in GLMs describing various responses measured, in several cases, random block effects and sediment characteristics unaffected by crayfish were found to be as important, if not more important, than crayfish themselves. Furthermore, density and biomass in the six small crayfish treatment, where most significant results were observed, were higher than natural values seen in the literature. No overall effect of crayfish at the population level was observable for bullhead, despite indication of dietary shift in bullhead attributable to crayfish. Considering the relative magnitude of the effects of crayfish I believe it would be inappropriate to assign keystone species status on the basis of the results.

Implications:

From a management perspective and a practical point of view, the seeming importance of crayfish size class has implications for management decisions. As trapping is known to be biased towards removing larger individuals, it is certain to lead to altered size distributions of crayfish populations. The results of this thesis

suggest that this may alter both direct and indirect impacts of crayfish on the recipient community. More work is required to test the effect of crayfish removal on demographics and subsequent functional responses within their communities.

It is accepted that, realistically, there is no hope of removing signal crayfish from UK waters. They are effectively a naturalised species in the UK. The results of this thesis in one sense confirm concerns that the presence of this non-native species is having detrimental effects. However, as far as the results of this study went, impacts were perhaps not of a sufficient magnitude to claim that invaded ecosystems are under threat, owing to signal crayfish presence. Having said this, although the severity of effects caused by crayfish do not appear worrying in isolation, they represent an additional stressor for aquatic ecosystems, in combination with the existing, multiple stressors that they already face (Ormerod et al., 2010). It should be noted that the lowland streams and rivers used for the work presented in this thesis belong to catchments that are intensively farmed, with relatively high levels of urbanisation. It may be that impacts of crayfish were obscured against a background of stressors associated with these land uses. Impacts of signal crayfish may, therefore, be more dramatic in pristine ecosystems.

It is also possible that, depending on the native organism in question, the presence of signal crayfish is not an entirely negative phenomenon. As exemplified by the chub work, more research is required to say whether crayfish might overall be beneficial to large fish species. In addition to large fish taxa, there are likely to be predators of crayfish that see only positive, and not negative effects. For example, various terrestrial vertebrate predators, grey heron (*Ardea cinera*) and otters (*Lutra lutra*) are all known predators of invasive red swamp crayfish (Delibes and Adrian, 1987, Peris et al., 1994, Beja, 1996). Signal crayfish can also be said to benefit

mankind, through their use as a food source. This is of course the very reason that they were introduced into the UK in the first instance.

Signal crayfish are just one of six established non-indigenous crayfish in the UK. While signal crayfish are widely abundant, the other five species currently display limited distributions. The prospect of climate change brings with it the possibility that future conditions may shift in favour of non-native crayfish other than signal crayfish. Furthermore, strict control over the human mediated movement of freshwater crayfish is lacking. It is conceivable, therefore, that a succession of increases in the distribution and abundance of various invasive crayfish taxa could occur in Britain. This scenario represents the possibility of a series of stressor events and it seems intuitive that such a scenario would lead to greater instability of ecosystems relative to long-term establishment of a single non-indigenous crayfish species. Management of these less common non-native crayfish should be prioritised, not least because, with their restricted ranges, control is feasible.

The use of reference sites is a well known practice in conservation and restoration biology (Primack, 2002). In its reference state, an ecosystem might be said to be 'healthy', or to be suffering a minimum of anthropogenic disturbance. What reference should be used when considering the impact of signal crayfish? Prior to the introduction of the crayfish plague, white-clawed crayfish appear to have been abundant and widespread (Holdich and Reeve, 1991). At localities where white-clawed crayfish extirpated before signal crayfish introduction, does establishment by signal crayfish promote the return of the ecosystem towards something more similar to that of the reference state? Is it even possible to know what the reference status of

ecosystems would have been when white-clawed crayfish were abundant? These issues are difficult to approach and philosophically become more complicated when longer time scales are considered.

As in the case of signal crayfish introduction, it is likely that white-clawed crayfish were brought to England by humans for culinary purposes (Holdich, 2009). There is scant evidence to support the presence of white-clawed crayfish in the UK prior to much more than five hundred years ago (Holdich, 2009). This raises the question of whether the UK strictly has a native crayfish and therefore whether the reference state of aquatic systems should in fact be crayfish free. If this were the case, the arguably increased impact of signal crayfish relative to white-clawed crayfish becomes incidental – a shift from crayfish-free water bodies to those with crayfish, clearly being of greater ecological significance.

For obvious practical reasons, most ecological studies lack long term temporal data. This is unfortunate as regards research in invasion ecology, owing to the possibility that the impacts of invasive species subside with time, as ecosystems 'adjust' to the presence of an introduced organism (Strayer et al., 2006). The introduction of signal crayfish is relatively recent and impacts therefore might appear significant, but could diminish with time.

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