Waves of Invaders: Interactions among Invasive Species and their Impacts on Ecosystem Structure and Functioning

Michelle C. Jackson

School of Biological and Chemical Sciences
Queen Mary University of London
Mile End Road, London, E1 4NS

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 with it are the product of my own work. In Chapter Three, I use part of a historical data set collected by my supervisors. In Chapters Four and Seven, I had assistance with data collection from MSc and BSc students. I acknowledge the input of these students at the start of the relevant chapters. The guidance I received from my supervisors is acknowledged in a section dedicated to this purpose. Other authors work is cited and the references listed at the end of the thesis. All other opinions and views given are my own.

Michelle Jackson

February 2012

Abstract

Many freshwater ecosystems sustain several invasive species. Here I examine multiple invasions in two highly invaded and well catalogued catchments; Lake Naivasha, Kenya and River Thames, England. New metrics, derived from stable isotope analysis, are used to provide measures of trophic diversity and to examine dietary interactions among species. I test the hypothesis that functionally similar sympatric species will occupy a smaller niche than their allopatric counterparts. Additionally, I quantify the impact of multiple invasive species on ecosystem structure and functioning in order to address the question; do interactions among species amplify or mitigate one another's impact?

In Lake Naivasha, the stable isotope metrics revealed serial replacement of invasive species due to dietary interactions. Invasive red swamp crayfish were eventually excluded from the lake due to niche restriction in the presence of a more recent invader, the common carp. Now, the crayfish have migrated into the catchment where they overlap with a species of native river crab. Here, I found a novel mechanism of invasion, whereby the crayfish restricted their niche at the invasion front in order to reduce competition with crabs. Crayfish also caused significant changes in invertebrate community structure and increased decomposition rates, which indirectly resulted in displacement of the crabs.

In the Thames catchment, I catalogue the non-indigenous species and show how invasion rates have increased significantly since 1800 due to globalisation. Using the four species of invasive crayfish present (red swamp, signal, Turkish and virile), I demonstrate their extensive diet plasticity using novel measures of niche width and individual specialisation based on stable isotope data. Interactions among the crayfish were examined and this revealed that each species has varying and independent impacts on invertebrate

community structure, algal standing stock and decomposition rates. Hence, interactions among invaders are not expected to amplify or mitigate one another’s impact and instead, the combined impact will be the sum of their allopatric impacts.

Acknowledgments

I would like to thank my principal supervisor, Dr. Jonathan Grey (QMUL), for his invaluable advice and support throughout my time at Queen Mary. Jon's comments have greatly improved all the chapters that comprise this thesis. I am also indebted to Dr. Ian Donohue (Trinity College, Dublin) for his advice and assistance with statistical analysis and to Dr. J. Robert Britton (Bournemouth University) for his support with fieldwork in Kenya. Chapters Three and Four were much improved thanks to the input of Ian and Rob.

This PhD was made possible by funding from the School of Biological and Chemical Sciences at Queen Mary and the UK Environment Agency, for which I am grateful. I would like to thank Dr. Judy England and Adam Ellis of the Environment Agency for their considerable help, particularly Adam, whom without, much of the UK fieldwork would not have been possible.

I am grateful to Adrian Brooker and his colleagues at Hampstead Heath for access to the ponds and assistance with some fieldwork. Thanks also to Claire Frankland from the Museum of London Docklands for access to the museum's collections. Big thanks to Dr Ian Sanders for all his help with the mass spec. I would also like to thank Dr. Andrew Jackson (Trinity College, Dublin) for teaching me how to use 'R' and for his input to Chapter Three.

I had assistance from a number of people with fieldwork; Doris Pichler, Maria Huber, Rebecca Stewart, Phil Sanders, Jessica Hamill, Jessica Jackson, Nikki Jackson, George Gasston, Rick Hayes and Gareth Jenkins. In particular, I would like to thank Paul Fletcher and Reuben Ndolo for all their help in the UK and Kenya, respectively.

I would like to thank Velia Carn, the Campbell-Clause's and the Gasston's for all their hospitability and support in Kenya. I am also indebted to Dr. David Harper and Prof. Kenneth Mavuti, the directors of the Lakes of the Rift Valley Project.

I am thankful to Katie Miller, Tabitha Jones, Jeffrey Taylor, Maaike Milligan and Danny Sheath for their high quality input as part of MSc and BSc degrees. I formally acknowledge their input at the beginning of the chapters to which they contributed.

Finally, I would like to thank my family and friends for their constant support throughout my PhD. Special thanks to Karen Jackson and Chris Spillane for their invaluable encouragement.

Table of Contents

List of tables and figures

Chapter One

Chapter Two

Chapter Three

Chapter Four

Chapter Five

Chapter Six

Chapter Seven

Chapter Eight

Chapter one: Introduction

Global change and biodiversity

Human-mediated global change is accelerating at an unprecedented rate (Pimm *et al.* 1995; Myers & Patz 2009) and the impacts on individuals, populations, species and ecosystems are often unpredictable. The range and abundance of species can shift as a consequence of environmental change, causing alterations in the biotic structure of an ecosystem (Walther *et al.* 2002). Anthropogenic manipulation of the environment, such as global warming, habitat destruction and the spread of non-indigenous species, has resulted in a major loss of diversity in many habitats (Sala *et al.* 2000; Pereira *et al.* 2010). This has endorsed the theory that modern humans have initiated a 6th mass extinction (Leakey & Lewin 1995; Thomas *et al.* 2004b; Wake & Vredenburg 2008) and many models have predicted continued high rates of species loss throughout the next century (e.g. Thomas *et al.* 2004a; Keith *et al.* 2008; Maclean & Wilson 2011). Thomas et al. (2004a) predicted, based on a range of habitats and taxa, that climate change (mid-range projections) and habitat destruction could cause up to 37% and 29% of species to become extinct, respectively by 2050. Systematic threats to biodiversity such as deforestation and the introduction of non-indigenous species can act synergistically with other aspects of global change, including climate change, to accelerate extinction rates (Brook *et al.* 2008).

Aquatic ecosystems are especially susceptible to many aspects of global change (Carpenter *et al.* 1992; Dudgeon *et al.* 2006) and threats such as climate change and the spread of non-indigenous species are likely to act in synergy to alter aquatic ecosystem diversity (Rahel & Olden 2008). Xenopoulos et al. (2005) predicted that 4-22 % of freshwater lotic fish will be extinct by 2070 due to reduced water discharge caused by a combination of climate change and increased anthropogenic withdrawal. Well-publicised extinctions of freshwater species include the extinction of approximately 200 of the >300 endemic haplochromine cichlids in Lake Victoria, East Africa, following the introduction of Nile Perch (*Lates niloticus*) in the 1950s (Witte *et al.* 1992) and the widespread extinctions of many amphibian species due to outbreaks of a chytrid fungus which is promoted by rising temperatures (Pounds *et al.* 2006). In some habitats, global change has increased species diversity and abundance, highlighting the complexity of ecosystem response to change. In Antarctic regions, for example, experiments have shown how warming could increase nematode diversity (Walther *et al.* 2002).

Alterations in species abundance and diversity are caused by a number of mechanisms including adjustments in the phenology, physiology, distribution and interactions of organisms as a response to global change (Walther *et al.* 2002). Rising temperatures can alter the timing of spawning and migration events and newly-introduced species can adjust food web interactions, both of which will ultimately result in major changes in biotic ecosystem structure and dynamics (Petchey *et al.* 1999; Walther *et al.* 2002).

Consequences of changing biodiversity

The responses of individual populations to global change will disrupt their interactions with other species and subsequently have a cascading impact on ecosystem dynamics (Walther *et al.* 2002). Moline et al. (2004) documented a shift in the phytoplankton community in the Antarctic which then altered the zooplankton assemblage by causing major declines in krill and an increase in salps, which are a less preferable food source for higher trophic levels. Consequently, if global change only directly impacts a single trophic level or even a single species within a community, the cascading interactions resulting are likely to have wide-reaching effects through trophic connection. Food web architecture can be altered under global change scenarios, for instance, non-indigenous species can cause trophic shifts in native species (Vander Zanden *et al.* 1999) and habitat loss or over exploitation can reduce food chain length by removing top predators (Dobson *et al.* 2006).

The stability of ecosystems and hence, ecosystem functioning and services are often governed by species diversity (Chapin *et al.* 2000; McCann 2000; Hooper *et al.* 2005). Shifts in assemblage structure may alter the trait diversity of a community when a set of species with particular traits are replaced by species with different traits (Loreau *et al.* 2001). Ecosystem processes that are altered by changes in trait diversity include productivity, decomposition and nutrient cycling (Loreau *et al.* 2001; Hooper *et al.* 2005). Ecosystem functioning is directly and indirectly impacted by global change, for example, climate change can directly alter productivity due to temperature increases (O'Reilly *et al.* 2003; Brander 2007) and non-indigenous species can indirectly alter decomposition rates by altering nutrient dynamics (Wardle *et al.* 2009).

Changes in ecosystem structure and functioning may have economic implications due to altering the services which an ecosystem provides (Schroter *et al.* 2005; Pejchar & Mooney 2009). Climate change and over exploitation alters fishery production (Brander 2007), human global population growth affects water availability (Schroter *et al.* 2005) and non-indigenous species can spread infectious diseases (Pejchar & Mooney 2009).

Invasive species: a significant component of global change

Human-mediated introductions of non-indigenous species are one of the most widespread and damaging aspects of global change (Vitousek *et al.* 1997; Mack *et al.* 2000). Although some introductions have neutral impacts on ecosystems, others have adverse effects on both assemblage composition and ecosystem functioning (Sala *et al.* 2000; Gurevitch & Padilla 2004; Pejchar & Mooney 2009). Invasive species are typically defined as introduced, non-indigenous species that have negative impacts on the native ecosystem or human health (Williamson & Fitter 1996; Lee 2002). Among the species that are introduced, only a small percentage of them will become established and then only a fraction of them again will become invasive (Williamson & Fitter 1996).

Many notorious invasive species now occupy a global distribution and have wide ranging impacts on both the environment and economy, and consequently they are regarded as a significant component of global change (Vitousek *et al.* 1997; MacDougall & Turkington 2005; Crowl *et al.* 2008). The impacts of invasive species are considered to be so damaging, that it has been suggested that they could be the drivers of global change and a major cause of extinctions (Gurevitch & Padilla 2004; Crowl *et al.* 2008). The International Union for Conservation of Nature's Red List of Threatened Species catalogues 2707 species that are vulnerable, endangered or critically endangered and threatened by introduced and/or invasive species (October 2011; http://www.iucnredlist.org). A total of 131 species that are already extinct or at least extinct in the wild were threatened by invasive and/or introduced species, highlighting the severity of the risk that invaders can pose. Anthropogenic activity is blurring biogeographical barriers that have previously separated regions and as a consequence, the spread of invasive species is homogenising the Earth's biota (Vitousek *et al.* 1997; Olden *et* *al.* 2004). International trade and transport networks have facilitated the spread of species outside their natural geographical range and, therefore, the number of non-indigenous species established each year is increasing with globalisation (Hulme 2009). For instance, according to Hulme (2009), the number of invertebrates established per year in Europe increased from less than 1 to approximately 12 between 1800 and 2000 (Figure 1.1).

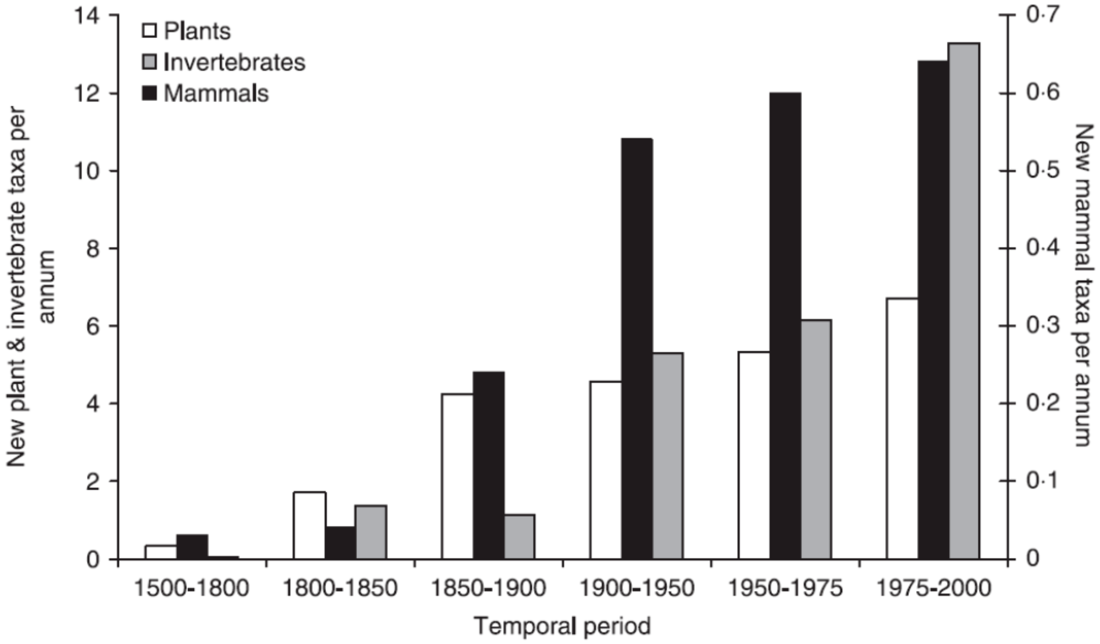

Figure 1.1. Annual rates of increase in the establishment of non-indigenous mammals, invertebrates and plants in Europe since 1500 ad. Taken from Hulme (2009).

Native biodiversity can decrease as a result of both direct and indirect interactions with invasive species. Direct interactions might include interspecific competition for resources, such as food and shelter, or predator-prey interactions. For instance, native red squirrels (*Sciurus vulgaris*) have been eliminated from most of the United Kingdom due to superior competition for resources from invasive grey squirrels (*Sciurus carolinensis*; Gurnell *et al.* 2004) and the globally invasive fire ant (*Solenopsis invicta*) has reduced native ant diversity by up to 70% via competitive exclusion (Porter & Savignano 1990), and even outcompeted and displaced native bluebirds (*Sialia sialis*) in North America due to competition for shared resources (Ligon *et al.* 2011). An example of direct predator-prey interactions is the consumption of endemic cichlids by invasive Nile Perch in Lake Victoria, causing the extinction of 200 cichlid species (Witte *et al.* 1992). Invaders also indirectly displace native species and reduce biodiversity by manipulating the habitat and disrupting natural ecological relationships. Invasive rats (*Rattus spp.*) often severely reduce seabird density on islands by consuming their eggs which may consequently alter invertebrate abundance and diversity due to a reduction in important allochthonous resources that are transferred from the ocean by the seabirds (Towns *et al.* 2009).

Aquatic ecosystems often have high rates of invasion (e.g. Cohen & Carlton 1998; Ricciardi 2006) due to a high degree of intentional introductions for fisheries and the ornamental trade, and accidental introductions such as ballast water transfer (Padilla & Williams 2004). Aquatic invasive species often drastically alter food web structure and hence instigate cascading reactions, which will result in whole community impacts of invasion (Vander Zanden *et al.* 1999). In North America, invasive rainbow smelt (*Osmerus mordax*) has simplified lake food webs by homogenising the fish and zooplankton community by competitive and consumptive interactions, respectively (Beisner *et al.* 2003). Invasive species also have the potential to disrupt the flow of resources between interconnected systems and thus alter food web dynamics (Towns *et al.* 2009). Baxter et al. (2004) showed how invasive rainbow trout (*Oncorhynchus mykiss*) in Japan out-competed the native Dolly Varden char (*Salvelinus malma*) for terrestrial prey that fell into the stream, causing the natives to feed on benthic invertebrates instead. This entirely altered the food web structure and ecosystem linkages by reducing the abundance of emerging insects and therefore reducing the abundance of their natural predators, riparian spiders (Baxter *et al.* 2004). Thus, species invasions have far-reaching implications that can propagate across ecosystem boundaries which often makes their impacts hard to predict. Furthermore, the spread and impact of invasive species can be amplified by other aspects of global change; a change in climate may break down 'filters' that previously acted as barriers to non-indigenous species (Rahel & Olden 2008). Another example, suggested by Byrnes et al. (2007), is that the bias of invasions in marine ecosystems towards lower trophic levels, simultaneously with the extinction of top predators due to over exploitation and habitat alteration, can alter food web structure by reducing food chain length.

Changes in biodiversity and food web structure have implications for ecosystem stability and hence ecosystem functioning (McCann 2000; Hooper *et al.* 2005). Biotic homogenisation can reduce the functional diversity of an ecosystem by removing species with important specialist traits (Olden *et al.* 2004). The loss of certain traits in benthic invertebrate communities can reduce bioturbation (Solan *et al.* 2004), and altered detritivore diversity in streams has implications for decomposition rates (Gessner *et al.* 2010).

Invasive golden apple snails (*Pomacea canaliculata*), a common invader in South East Asia, have caused a shift in ecosystem state and function in many lakes by eliminating aquatic plants due to grazing, and consequently causing changes in nutrient dynamics which in turn causes a shift from clear water to a turbid, algal dominated state (Carlsson *et al.* 2004). A contrasting example comes from the widespread invasive zebra mussel (*Dreissena polymorpha*) which is a efficient filter feeder and hence, increases light penetration and therefore causes lakes to shift to macrophyte-dominated states (Zhu *et al.* 2006). Thus, invaders can also alter ecosystem functioning without necessarily reducing biodiversity.

Invasive species have implications for ecosystem services due to the changes in biodiversity and ecosystem functioning that they can instigate (Pejchar & Mooney 2009). For instance, zebra mussels can have a negative impact on fisheries by competing with fish for plankton prey, they foul boats, docks, piers and water pipes and finally, they bioaccumulate toxins which are passed to higher economically important trophic levels (Pejchar & Mooney 2009). This has severe economic implications, costing millions of pounds in damage to industry each year (Pejchar & Mooney 2009). In fact, the total estimated cost of invasive species in the United States of America alone was almost $120 billion per year in 2005 (Pimentel *et al.* 2005).

Crayfish as invaders

Decapod crustaceans, including shrimps, crabs and crayfish, are common and destructive invaders in aquatic ecosystems (Strayer 2010; Capinha *et al.* 2011). Numerous species of freshwater crayfish in particular, now occupy a global distribution (Capinha *et al.* 2011). In 2008, there were 640 described species of freshwater crayfish and more species are added each year (Figure 1.2; Crandall & Buhay 2008); however, only approximately 20 species boast invasive populations (Hobbs Iii *et al.* 1989; Strayer 2010). Crayfish are important invaders because they are one of the largest and longest lived freshwater invertebrates and they are broadly omnivorous; hence they often act as keystone species (Nyström *et al.* 1996; Crandall & Buhay 2008).

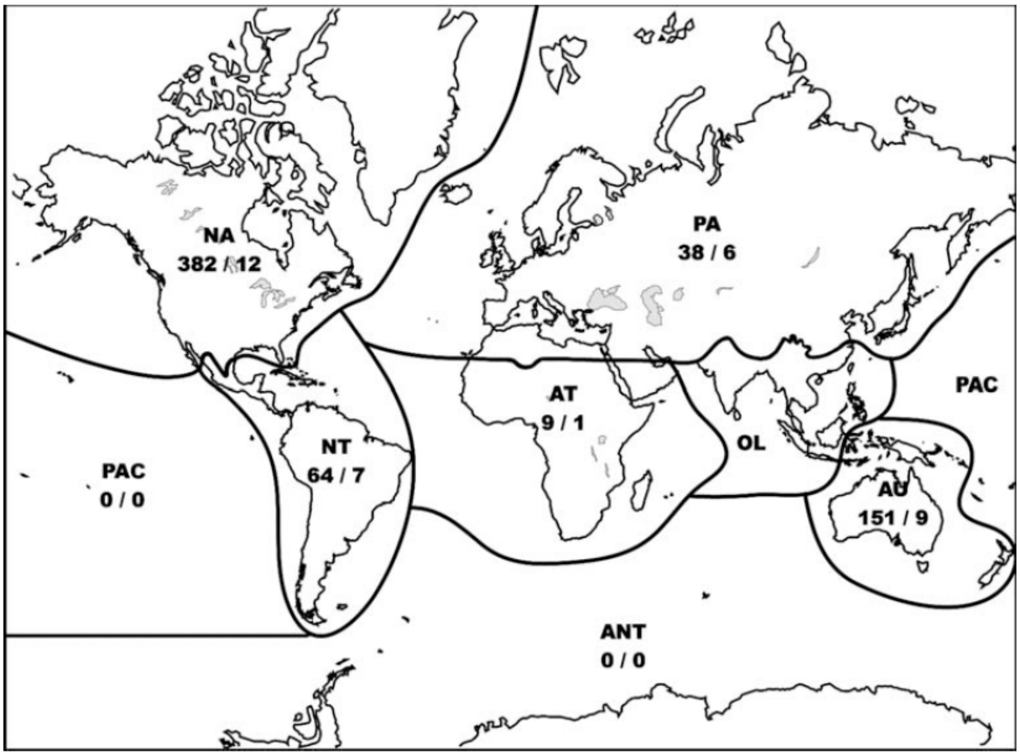

Figure 1.2. The global distribution of freshwater crayfish species in their native ranges. (species number/genus number). PA, Palaearctic; NA, Nearctic; NT, Neotropical; AT, Afrotropical; OL, Oriental; AU, Australasian; PAC, Pacific Oceanic Islands; ANT, Antarctic. Taken from Crandall and Buhay (2008).

Crayfish can cause a number of environmental problems, but this usually only occurs when they are introduced outside of their native geographical range (Holdich 1988). Crayfish have been introduced worldwide for human food, fish forage, the aquarium trade and for bait (Hobbs Iii *et al.* 1989; Strayer 2010). They have negative impacts on both fauna and flora in lentic (e.g. Rodríguez *et al.* 2005; Rosenthal *et al.* 2006) and lotic systems (e.g. Charlebois & Lamberti 1996; Stenroth & Nyström 2003). A prevalent influence of invasive crayfish is the partial or total eradication of aquatic macrophytes due to sediment manipulation and direct consumption of the plants (Nyström & Strand 1996; Gherardi & Acquistapace 2007).

Invasive crayfish regularly out-compete native crayfish because they are not subject to the same factors that control population size such as selective predation and natural enemies (Hill & Lodge 1999). Successfully introduced crayfish commonly exhibit faster growth rates and achieve larger sizes than their native counterparts, which gives them a further advantage by increasing their fecundity and success in shelter competition scenarios (Alonso & Martínez 2006). Additionally, considerable numbers of the UK's only native species, the white clawed crayfish (*Austropotamobius pallipes*), have been wiped out by diseases carried by invasive crayfish, such as crayfish plague, an introduced fungal disease (Alderman *et al.* 1984).

Invasive crayfish have negative impacts on native invertebrate density and diversity, for example, the two most widespread invasive crayfish, Louisiana red swamp crayfish (*Procambarus clarkii*; Figure 1.2) and signal crayfish (*Pacifastacus leniusculus*), which are both native to North America (Capinha *et al.* 2011), significantly reduce invertebrate diversity across Europe due to direct consumption (Stenroth & Nyström 2003; Rodríguez *et al.* 2005; Correia & Anastacio 2008). Invasive crayfish can decrease invertebrate density by more than 70% (e.g. Charlebois & Lamberti 1996) and in the absence of a native crayfish, this efficiency may be partially attributable to the fact prey items have evolved without the introduced predators and therefore have not developed an appropriate escape response (Renai & Gherardi 2004; McCarthy *et al.* 2006). Once established, invasive crayfish may also alter the structure of food webs by replacing other invertebrate predators, such as leeches (Stenroth & Nyström 2003).

Figure 1.3. Invasive Louisiana red swamp crayfish (*Procamabarus clarkii*) from the River Malewa, Kenya in 2008. There are extremely high densites of crayfish in the lower reaches of the river. Carapace length 35 to 55 mm.

Native fish can also be negatively affected, with inverse correlations between signal crayfish and sea trout (*Salmo trutta*), Atlantic salmon (*Salmo salar*), bullhead (*Cottus gobio*) and stone loach (*Noemacheilus barbatulus*) found in England (Guan & Wiles 1997; Peay *et al.* 2009). A change in fish behaviour is the likely consequence of competition for resources such as refuge and food (Guan & Wiles 1997; Light 2005). Reductions in native fish density, even to the extent of extinction, have been reported following a crayfish invasion (Ilhéu *et al.* 2007).

Crayfish invasions are capable of instigating trophic cascades, resulting in changes in ecological functioning. Growth of benthic algae is often promoted in the presence of crayfish and this has been attributed to a release from grazing pressure as crayfish consume invertebrate herbivores (Charlebois & Lamberti 1996) and crayfish activity causing improved light and nutrient conditions (Stenroth & Nyström 2003). Thus, crayfish have complex impacts that often propagate across trophic levels and therefore result in considerable alterations in food web dynamics (Charlebois & Lamberti 1996; Rosenthal *et al.* 2006). Crayfish also alter other aspects of ecosystem functioning, including leaf litter processing (Usio 2000), bioturbation and nutrient dynamics (Stenroth & Nyström 2003; Harvey *et al.* 2011).

Interactions among invaders

Many aquatic environments have been invaded numerous times, including Lake Naivasha in Kenya (Britton *et al.* 2007), San Francisco Bay in North America (Cohen & Carlton 1998), and the Baltic Sea in Northern Europe (Leppäkoski & Olenin 2000). This has given rise to the ‘Invasion Meltdown Model’ which predicts that the disruption caused by the establishment of one invasive species can facilitate the success of further invaders (Simberloff & Von Holle 1999; Ricciardi 2001; Simberloff 2006). However, sympatric invasive species may compete for resources and hence, have a detrimental impact on one another’s success (e.g. Lohrer & Whitlatch 2002). The ‘Biotic Resistance Model’ suggests that species-poor communities are more prone to invasion because they are more likely to have vacant niches (Elton 1958). Yet high diversity suggests that the ecosystem boasts great resource availability and weak species interactions, which implies that multiple invaders will be easily accommodated (McCann *et al.* 1998; Byers & Noonburg 2003).

Empirical evidence supports both facilitative and negative interactions between sympatric invaders in aquatic environments. An example of a negative interaction is the reduction in the density of a historical invader, the European green crab (*Carcinus maenas*) due to predation and shelter competition from a newly-established invader, the Asian shore crab (*Hemigrapsus sanuineus*), on the East coast of North America (Jensen *et al.* 2002; Lohrer & Whitlatch 2002). Facilitative interactions include the beneficial impact of the invasive Asian horn-snail (*Batillaria attramentaria*) in North America by providing habitat for two introduced epibionts (Atlantic slipper shells, *Crepidula convexa* and Asian anemones, *Diadumene lineate*) and causing density increases in introduced mudsnails (*Nassarius fraterculus*) and eelgrass (*Zostera japonica*) due to indirect grazing effects and bioturbation, respectively (Wonham *et al.* 2005).

The occurrence of several invasive species has the potential to moderate or amplify their impacts on native fauna and flora. Griffen et al. (2008) described how the more recent invasion of Asian shore crabs in North America has forced the invasive European green crab to modify its diet to incorporate fewer native mussels. Since the contribution of mussels to the diet of Asian shore crabs does not differ dependent on the presence of European green crabs, this is not due to direct competition for a resource, but results from a rather more complex interaction involving behavioural changes in the initial invader (Griffen & Byers 2008; Griffen *et al.* 2008). On the other hand, the impact of an invasive species can be exacerbated by the presence of other invaders (Ricciardi 2001). Grosholz (2005) illustrated how the invasion of European green crabs facilitated the spread of *Gemma gemma*, a previously established exotic clam, by consuming native bivalves and subsequently reducing interspecific competition.

Experimental data have shown that invasive signal crayfish and invasive rainbow trout in Sweden did not interact to modify one another's impact on benthic invertebrate biomass, tadpoles and macrophytes (Nyström *et al.* 2001). Each species had independent impacts on prey and hence, their combined effects on ecosystem structure reflected responses to the more effective predator (Nyström *et al.* 2001). However, if two sympatric invasive species have similar independent impacts on the ecosystem, their combined effect might be additive. For example, invasive rusty crayfish (*Orconectes rusticus*) and invasive Chinese mystery snails (*Bellamya chinensis*) both independently reduce native snail biomass by consumptive and competitive interactions, respectively (Johnson *et al.* 2009b). The combined presence of the two invaders caused the elimination of one native snail species in experimental conditions because the thick shells of the invasive Chinese mystery snail protected it from crayfish (Johnson *et al.* 2009b). There is also the potential for the negative impacts of one invasive species to be moderated by another such as the predatory invasive pike (*Esox lucius*) which directly consumed invasive Louisiana swamp crayfish in a lake in Spain (Elvira *et al.* 1996), thereby reducing the negative impacts of crayfish reported elsewhere (e.g. Rodríguez *et al.* 2005).

Functionally similar invaders in particular are expected to interact and compete for shared resources. There have been reports of co-existing populations of invasive crayfish species, including signal crayfish and Louisiana red swamp crayfish in Portugal (Bernardo *et al.* 2011) and Japan (Nakata *et al.* 2005), while in other instances there has been serial replacement of invasive crayfish due to superior competition (Hill & Lodge 1999). Hill and Lodge (1999) described that the established invasive Northern clearwater crayfish (*Orconectes propinquus*) in North America was being replaced by the invading rusty crayfish due to competition. Invasive crayfish are unlikely to facilitate one another's establishment; competition is far more probable. However, their interactions may mitigate or amplify one another's impact on ecosystem structure and functioning.

Stable isotope analysis: a tool to examine the mechanisms and consequences of invasive species

Stable isotope analysis is a valuable tool for establishing trophic links and food web structure, particularly in aquatic food webs where observing feeding behaviour is often not feasible. The most commonly used signatures in ecology are those obtained from the naturally occurring ratios of ^{15}N:^{14}N and ^{13}C:^{12}C (Grey 2006). Carbon ratios reflect the consumer's diet with typical enrichment of 0.5-1‰, whereas nitrogen ratios show greater enrichment of 3-4‰ from resource to consumer and as a result they are used as an indication of trophic position (Post 2002). Consequently, using a combination of nitrogen and carbon stable isotope ratios it is possible to establish an animal's food sources and trophic level relative to an isotopic baseline (Grey 2006). The food web consequences of species invasions in aquatic systems (e.g. Vander Zanden *et al.* 1999; Gorokhova *et al.* 2005) and the diet of aquatic invasive species (e.g. Rudnick & Resh 2005; Olsson *et al.* 2009) have been documented using stable isotope analysis. Here, I will use carbon and nitrogen stable isotope analysis as a tool in all the chapters of my thesis to examine the food web consequences of invasion, the diet of invasive species and dietary interactions among invaders. Recently, community metrics derived from stable isotope data have been developed as a tool to examine trophic structure (Layman *et al*. 2007a). I aim to extend this tool box by adapted the metrics to be applicable to populations and developing novel measures of niche width.

General aims and approaches / thesis structure

The broad aim of this thesis was to address how sympatric invasive species in freshwater ecosystems interact with one another, how these interactions impact diet and the subsequent implications for ecosystem structure and functioning. I addressed these aims using two field sites, both chosen for their high rates of invasion, and by using controlled experiments.

Dietary interactions among an invasive fish (common carp; *Cyprinus carpio*) and crayfish (Louisiana red swamp crayfish; *Procambarus clarkii*) in Lake Naivasha, Kenya are examined using newly-developed population metrics, which are derived from stable isotope data (Chapter Three). These metrics are then used as a tool throughout the rest of the thesis. The metrics revealed serial replacement of crayfish by the carp due to crayfish niche restriction in the Lake. My work at Lake Naivasha and surrounding environs then revealed that the crayfish have migrated out of the lake and were heading upstream into the catchment rivers where they co-occur with a native crab (*Potamonautes loveni*). The impact of this dispersal on the ecosystem, and the interactions between the crayfish and crabs, is the subject matter of Chapter Four. I used *in situ* experiments and a field survey to reveal that the crayfish employ a novel mechanism of invasion and alter ecosystem structure and functioning.

The final three data chapters concern freshwater invasive species in the Thames catchment. I investigate the identity of all the non-indigenous freshwater species in the catchment, their vector of introduction, invasion rates and correlations with shipping activity (Chapter Five). The results inform on the history of invaders in the Thames region and have implications for invasive species management.

In Chapter six, I use invasive crayfish in the Thames catchment to demonstrate novel measures of niche width that I have developed during my PhD. There are four species of invasive crayfish present, each with varying ranges. I investigate the role of diet in their invasion success by characterising the food web at four field sites, each harbouring a species in allopatry. I aimed to reveal if niche width, individual specialisation, and/or diet shifting played a role in invasion success.

I have highlighted in the previous sections how invasive crayfish have an impact on invertebrate communities and ecosystem processes in aquatic systems. However, variation in these impacts is yet to be considered when crayfish species occur in sympatry. In Chapter Seven, I use experimental mesocosms to examine dietary interactions among invasive crayfish species and their impacts on ecosystem structure and functioning in order to answer the question: ‘Do interactions among invaders amplify or mitigate one another’s impact on ecosystem structure and functioning?’

Chapter Two: General Methods

Full methodology is given in each data chapter. Here I give more detailed sample collection and stable isotope methods, which are both used throughout the thesis. I also give a detailed description of the two catchments used to examine interactions among invaders.

Sample Collection

Samples were collected from field survey sites for subsequent experimental work or characterisation of the food web using stable isotope analysis (see below). Crayfish and crabs were sampled using Trappy Traps® which were baited with trout pellets when working in England and dead fish when working in Kenya. The traps were left in the water for a known length of time in order to calculate catch per unit effort (CPUE - the number of crayfish or crabs caught per trap per hour), and this was used as a relative measure of density. Benthic invertebrates were collected using pond nets to take kick samples of the benthos and these were hand sorted for later identification. Macrophytes, detritus and biofilm scrapes were also collected from field sites by hand.

Stable isotope analysis

Variation in the preservation techniques of organism tissue prior to stable isotope analyses can cause significant differences in carbon and nitrogen isotopic composition (Feuchtmayr & Grey 2003). In order to remove any potential confounding effect of preservation I routinely froze my samples and thawed them prior to preparation for stable isotope analysis. Stable isotope analysis was carried out on the muscle tissue of fish, crayfish and crabs. White muscle tissue only contains small amounts of lipids and inorganic carbonates compared to the liver and heart, both of which can effect isotopic composition (Pinnegar & Polunin 1999). Hence, white muscle tissue is considered to be the most reliable tissue for use in ecological research since it exhibits the least variability within a tissue sample and it has the lowest fractionation values from resource to consumer (Pinnegar & Polunin 1999). Muscle was removed from under the dorsal fin of fish, the tail of crayfish and the lower abdomen of crabs. Smaller invertebrate species were allowed to gut clear before being analysed whole. It is necessary to perform gut clearance before freezing and subsequent preparation for stable isotope analysis since inclusion of the gut material can result in errors of > 3‰ (Feuchtmayr & Grey 2003).

Once thawed, animal and plant material was oven dried overnight at 60°C to constant weight, ground using an agate pestle and mortar, and 0.5 mg or 0.7 mg, respectively was weighed into 6 x 4 mm tin cups using a ultra microbalance (UMX2 Automated-S, Mettler Toledo^{g}). Samples were then retained in a desiccator until analysis to ensure they remained dry. Carbon and nitrogen isotopic analysis was carried out using an elemental analyser (Flash EA, 1112 series, Thermo-Finnigan^{g}) coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT Delta^{Plus}, Thermo-Finnigan^{g}). Secondary standards (sucrose for carbon; ammonium sulphate for nitrogen) with known relation to international standards (Pee

Dee Belemnite for carbon; nitrogen in air for nitrogen) were used as reference materials. Cyclohaxonone-2, 4-dinitrophemylhydrazone or urea was used as an internal standard and repeat analyses resulted in typical precision of <0.1 ‰ for carbon and <0.3 ‰ for nitrogen. Delta (δ) isotope ratios are expressed as per mille (‰).

Throughout the thesis, Bayesian mixing models are used to quantify the contribution of putative resources to the diet of invaders. The nitrogen and carbon isotopic signatures of both resources and consumers are used to calculate the relative contribution of each resource to the consumer's diet. I use the Bayesian mixing model SIAR (Parnell *et al.* 2010) since it integrates variability in resource and consumer isotope values, providing an advantage over other mixing models. Fractionation factors between resources and the consumers were assumed to be 2.3 ± 0.28 ‰ for δ^{15}N and 0.4 ± 0.17 ‰ for δ^{13}C; these values are derived from freshwater consumers in a meta-analysis by McCutchan et al. (2003).

Study sites

Lake Naivasha catchment, Kenya

The equator runs through the middle of Kenya, East Africa, and parts of the country are extremely arid and prone to drought. Lake Naivasha is, therefore, a very important ecosystem because it is one of only three large freshwater lakes in Kenya, the others being Lakes Victoria and Baringo. All the other large water bodies bound by Kenya are soda lakes such as Lake Elementaita, which is in the catchment of Lake Naivasha. Lake Naivasha dried up completely in the 1890s and since it has naturally refilled, it has been subject to waves of invaders. The first species to be introduced were largemouth bass (*Micropterus salmoides*) and two tilapia species (*Oreochromis leucostictus* and *Tilapia zillii*), all chosen to create a commercial fishery in the lake (Hickley *et al.* 2002). Since then, there have been many more plant and fish introductions to the lake and catchment, including brown trout (*Salmo trutta*) which was introduced into the primary tributary of the Lake, the River Malewa, multiple times (Campbell-Clause, *pers. comms.*). Two more rivers feed the lake, the River Gilgil and River Karati and the source of all three tributaries is the Aberdare mountain range, which is in the highest reaches of the 1750 km^2 catchment (Everard *et al.* 2002).

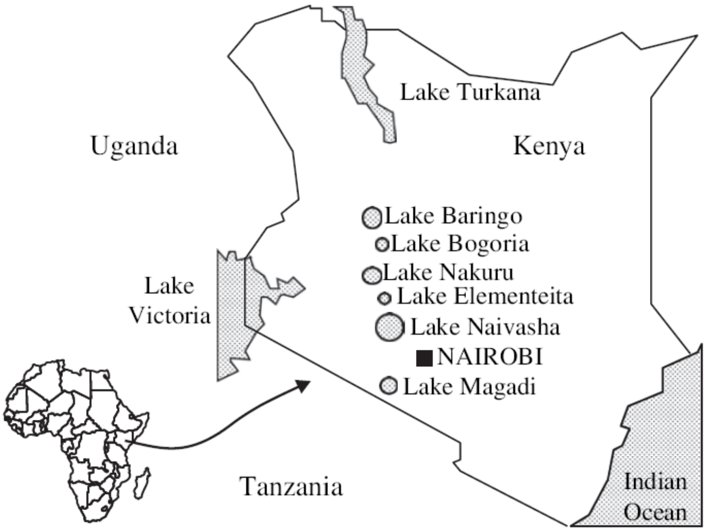

Figure 2.1. Location of Lake Naivasha, Kenya. Taken from Britton et al. (2007)

Lake Naivasha was chosen as a study site to examine interactions among invaders since most of the community assemblage consists of non-indigenous species and the 'Lakes of the Rift Valley Project', led by Dr. David Harper, has been accumulating data on the lake for over 30 years. This long-term data set includes stable isotope data from 2001 when my supervisor, Dr. Jonathan Grey, became involved with the project. Throughout my PhD I have added to the dataset and increased its extent by examining invaders in the tributary rivers.

River Thames catchment, England

The River Thames, which flows through southern England, is the second longest river in the United Kingdom. It flows through Oxford, Reading, Windsor and London before reaching the North Sea near Southend-on-Sea, Essex. The river drains a catchment area of 12930 km^2 and comprises more than 50 inflows. Since the catchment is so large, the total number of invasive species present has not been quantified and will vary greatly within the catchment because it includes still and flowing freshwater and brackish water in the tidal regions. The Thames catchment was chosen as a study area since it has been highly invaded and interactions among the invasive species present have not be considered in management scenarios.

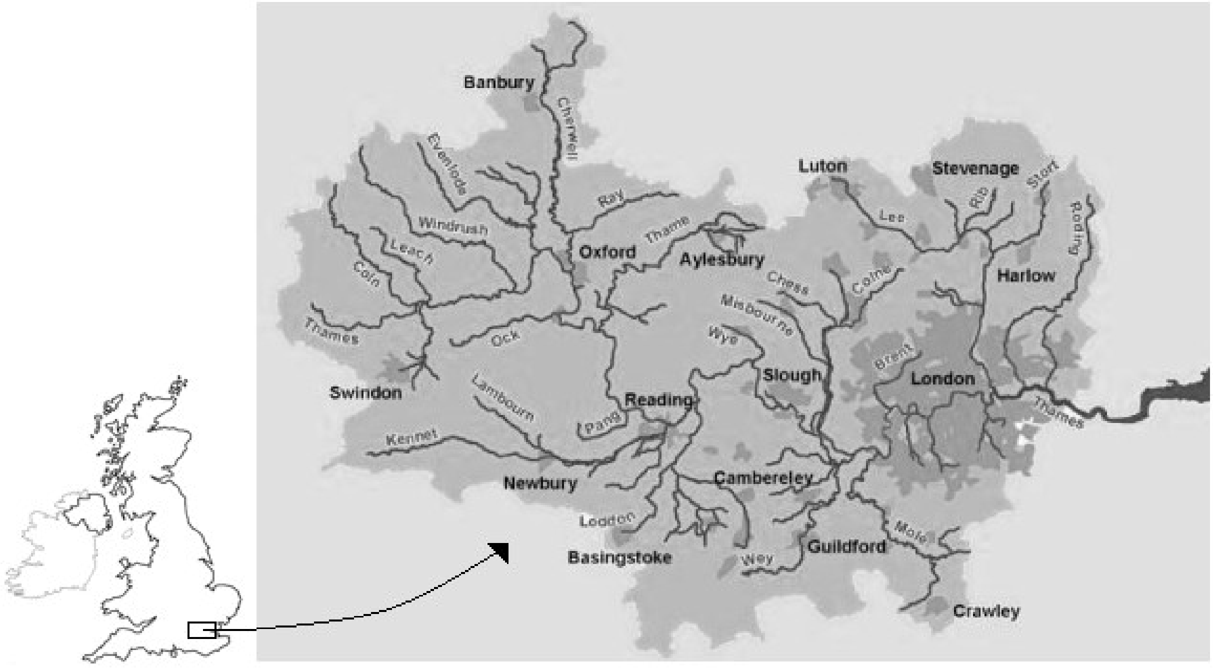

Figure 2.2. Location of the Thames Catchment, England. Edited from an Environment Agency image.

Chapter Three: Population-Level Metrics of Trophic Structure Based on Stable Isotopes and Their Application to Invasion Ecology

This chapter has been published in the journal PLoS One. Michelle C. Jackson, Ian Donohue, Andrew L. Jackson, J. Robert Britton, David M. Harper and Jonathan Grey (2012).
DOI:10.1371/journal.pone.0031757

Declaration on input

The data for this chapter were collected by my supervisor, Dr. Jonathan Grey, and his colleagues, Dr. J. Robert Britton and Dr. David M. Harper, in 2001, 2002, 2003 and 2006; before I started my PhD. I was involved with sampling and data collection in 2007 and 2008, and I consolidated the stable isotope and abundance data for all years in order to produce this chapter.

Abstract

Biological invasions are a significant driver of human-induced global change and many ecosystems sustain sympatric invaders. Interactions occurring among these invaders have important implications for ecosystem structure and functioning, yet they are poorly understood. Here we apply newly developed metrics derived from stable isotope data to provide quantitative measures of trophic diversity within populations or species. We then use these to test the hypothesis that sympatric invaders belonging to the same functional feeding group occupy a smaller isotopic niche than their allopatric counterparts.

Two introduced, globally important, benthic omnivores, Louisiana swamp crayfish (*Procambarus clarkii*) and carp (*Cyprinus carpio*), are sympatric in Lake Naivasha, Kenya. We applied our metrics to an 8-year data set encompassing the establishment of carp in the lake. We found a strong asymmetric interaction between the two invasive populations, as indicated by inverse correlations between carp abundance and measures of crayfish trophic diversity. Lack of isotopic niche overlap between carp and crayfish in the majority of years indicated a predominantly indirect interaction. We suggest that carp-induced habitat alteration reduced the diversity of crayfish prey, resulting in a reduction in the dietary niche of crayfish.

Stable isotopes provide an integrated signal of diet over space and time, offering an appropriate scale for the study of population niches, but few isotope studies have retained the often insightful information revealed by variability among individuals in isotope values. Our population metrics incorporate such variation, are robust to the vagaries of sample size and are a useful additional tool to reveal subtle dietary interactions among species. Although we have demonstrated their applicability specifically using a detailed temporal dataset of species invasion in a lake, they have a wide array of potential ecological applications.

Introduction

The pace of global environmental change has increased substantially in the last hundred years due to new environmental pressures as a result of human activity (Pimm *et al.* 1995). Human-mediated introductions of alien species are one of the most widespread and damaging of these pressures and, although some introductions may have neutral impacts on ecosystems, others have adverse effects on both assemblage composition and ecosystem functioning (Hooper *et al.* 2005). The possibility of waves of successful invasive species facilitating establishment of further invaders by disrupting ecosystem structure and functioning has given rise to the Invasion Meltdown Model (Simberloff & Von Holle 1999; Ricciardi 2001; Simberloff 2006). Many ecosystems now sustain several sympatric invasive species and yet their interspecific interactions are generally poorly understood (Lohrer & Whitlatch 2002). Knowledge of these interactions is key to understanding, and thus predicting, changes in trophic structure and assemblage composition instigated by sympatric and successive invaders. Integral to the Invasion Meltdown Model is that sympatric invaders promote the survival and potentially exacerbate the adverse effects of others (Simberloff & Von Holle 1999). Conversely, sympatric invasive species belonging to the same functional feeding group may exhibit a degree of dietary overlap potentially leading to strong interspecific competition when resources are limiting (Griffen *et al.* 2008).

Stable isotope analysis is a contemporary tool to study the food web consequences of species invasions (Vander Zanden *et al.* 1999; Maguire & Grey 2006; Schmidt *et al.* 2007). In ecological studies, the most commonly used naturally occurring stable isotope ratios are ^{15}N:^{14}N and ^{13}C:^{12}C which can be used to create ‘maps’ of food webs and hence, infer putative energy sources, trophic linkage and trophic position (Grey 2006). Stable isotope metrics enable the quantification of trophic structure at the community-level (Layman *et al.* 2007a) and individual variation of $\delta^{13}C$ and $\delta^{15}N$ within populations can provide useful information on population trophic ecology (Bearhop *et al.* 2004; Layman *et al.* 2007b). Recent developments in isotope ecology have provided statistical frameworks for examining variation among the isotope values of defined groups (Turner *et al.* 2010; Hammerschlag-Peyer *et al.* 2011). Further, Layman et al. (2007b) described how the convex hull area occupied by a species in $\delta^{13}C$-$\delta^{15}N$ isotopic space represents trophic diversity and can, therefore, be used as a quantitative indication of niche space. Jackson et al. (2011) extended these methods and strengthened their ability to cope with disparities in sample size.

Classic theory (Hutchinson 1957; Van Valen 1965) suggests that a given species will occupy a larger realised niche in the absence of interspecific competition and yet sympatric species can only have a limited degree of resource use overlap before competitive exclusion occurs (Pianka 1974). Consequently, there should be an inverse relationship between the isotopic niche space occupied by a species and the degree of interspecific competition it experiences. Thus, sympatric invaders belonging to the same functional feeding group would be expected to exhibit a smaller isotopic niche than their allopatric counterparts. However, a credible alternative hypothesis might be that increased competition for resources results in a more varied diet in order to maintain energy requirements and hence a larger isotopic niche (Svanbäck & Bolnick 2007). We sought to test these hypotheses by applying stable isotope metrics at the population-level, as a logical progression of the metrics proposed by Layman et al. (2007a) which provide quantitative measures of the trophic structure of entire communities.

We chose Lake Naivasha in Kenya to test our hypotheses as it is a large (~150 km^2) natural freshwater ecosystem that has been subject to numerous species introductions over at least an 80 year period (Hickley *et al.* 2002). Britton et al. (2007) reported that five of the six fish species currently present are non-indigenous and the lake also harbours several alien plant species, along with the globally widespread invasive Louisiana red swamp crayfish, *Procambarus clarkii*. Inverse correlations between native submerged macrophyte (*Potamogeton schweinfurthii, P. pectinatus, P. octandrus* and *Najas pectinata*) density and crayfish abundance, resulting in a dynamic, cyclic trend of crayfish and aquatic plant biomass, have led to the suggestion that the crayfish was a keystone species in the lake (Hickley *et al.* 2002). The most recent introduction to the lake in 1998 was of another globally widespread invasive species, carp, *Cyprinus carpio*, which has dominated the commercial fishery since 2003 and can contribute up to 98% of catches (Britton *et al.* 2010). Elsewhere, invasive carp tends to be a keystone species, having profound effects on species composition and trophic linkages (Miller & Crowl 2006). Crayfish and carp belong to the same functional feeding group as both are relatively large benthic omnivores. They might, therefore, be expected to interact strongly as a result of dietary overlap. However, the size discrepancy in adults may influence their preferred food source resulting in distinct dietary niches. Indeed, large carp attain sufficient gape size to ingest crayfish as prey (Britton *et al.* 2007).

We used stable isotope data and concurrent ecological data spanning 8 years (2001 to 2008) to examine the trophic interactions occurring between the most recent invader, carp, and the previously established invader, crayfish. We used new, robust stable isotope metrics applicable to individual populations (Jackson *et al.* 2011) to investigate fluctuations in trophic diversity and quantify shifts in each species' isotopic niche. Specifically, we tested the hypothesis that carp and crayfish would express dietary overlap because they belong to the same functional feeding group and eventually that carp would suppress and/or displace the isotopic niche of crayfish due to competitive superiority.

Methods

Ethics statement

All animal work was conducted in accordance to national and international guidelines to minimise discomfort to animals (Schedule 1 of the Animals [scientific procedures] Act, 1986). Since there were no regulated procedures involved, the Max Planck Institute for Limnology board reviewing the project declared there was no requirement for ethics approval. All necessary permits were obtained for the described field studies from the National Council for Science and Technology, Kenya: NCST 5/002/R/020-D (formerly OP/13/001/12C46).

Sampling and laboratory analyses

Lake Naivasha was sampled annually between 2001 and 2008 over 15-day periods in July. The same ten sites were sampled around the lake each year for carp, crayfish, macrophytes, sediment, plant debris and benthic invertebrates. Adult crayfish abundance was quantified using crayfish traps baited with dead fish. After 1-2 h, traps were lifted, all crayfish were counted and catch per unit effort (CPUE; number of crayfish per trap per hour) calculated. Abundance of carp was estimated using the CPUE from multi-panel gill-nets. Gill nets were lifted after 2-5 h of fishing and all carp were removed for counting, placed in containers and euthanised using an overdose of anaesthetic (MS-222 or benzocaine). Alternative fish sampling techniques to gill netting were not available; seine netting could not be used effectively due to poor shoreline access and the danger of disturbing hippopotami, and electric fishing equipment was not available in that area of Kenya. Submerged plants were sampled by dragging a double-headed rake along the sediment in three 25 m transects at each of the 10 sites, and the quantity of living plant material was estimated on a relative five-point scale. Dominant benthic invertebrates (primarily chironomids and oligochaetes), sediment, plant debris and floating macrophytes (*Eichhornia crassipes*) were also collected from each site for stable isotope analysis (SIA). Concurrent water level data were provided by the Lake Naivasha Riparian Association.

Muscle samples for SIA were taken from crayfish in 2001, 2002, 2003, 2006, 2007 and 2008 and from carp in 2003 (when they first appeared in gill-net samples), 2006, 2007 and 2008. Individuals selected for SIA were sub-sampled from a uniform adult size range (Carp: 200 to 600 mm fork length; Crayfish: 40 to 55 mm carapace length) with a consistent annual mean to ensure inter-annual consistency. Concurrently, we sampled all the abundant basal resources and primary consumers to establish whether inter-annual variation in crayfish and carp isotope values were a result of changes in diet rather than changes in the stable isotope ratios of putative food resources. Ensuring consistency in the isotope values of basal resources over time is especially important when using such a metric approach. All samples were processed on an annual basis to avoid any degradation of tissue. SIA was performed at Queen Mary, University of London following the protocols of Ings et al. (2010). Ratios of ^{15}N:^{14}N and ^{13}C:^{12}C are expressed using conventional delta notations (δ) relative to international standards (sucrose for carbon; ammonium sulphate for nitrogen; see Ings *et al.* 2010).

Mixing models

We used the Bayesian mixing model SIAR (Parnell *et al.* 2010) to provide an estimate of the relative contribution of various resources assimilated by crayfish and carp. This model integrates variability in resource and consumer isotope values, providing a distinct advantage over other mixing models. Separate mixing models were run for each year for both carp and crayfish using available food resources, including chironomids, oligochaetes, submerged and floating plants, plant debris, benthic fine particulate organic matter (FPOM), and hippo dung. Hippos are pseudo-ruminants and produce large quantities of partially fermented dung; the Naivasha population are conservatively estimated to introduce ~5800 tonnes of dung to the lake per annum (Grey & Harper 2002). In addition, crayfish was included as a resource for carp. These resources were the only ones to be sampled in sufficient abundance for stable isotope analysis and gut content analysis revealed their occurrence in the diet of both study species (Jackson, *pers. obs*). Fractionation factors between resources and the consumers were assumed to be 2.3 ± 0.28 ‰ for $\delta^{15}N$ and 0.4 ± 0.17 ‰ for $\delta^{13}C$, based on a meta-analysis by McCutchan et al. (2003)**.**

Population metrics

We used five quantitative population metrics derived from stable isotope data to reveal key aspects of trophic structure. The metrics were adapted from community–level metrics developed originally by Layman et al. (2007a) based on the mean $\delta^{13}C$ and $\delta^{15}N$ of all species in a community. We used the stable isotope values from all individuals sampled in these calculations, resulting in final metric values encompassing intra-population variation in diet. Additionally, all metrics were bootstrapped (n = 10000; indicated with a subscript ‘$_b$’) based on the minimum sample size in the data set (n = 15) to allow comparison among populations among years because sample size varied. The metric *mean distance to centroid* (CD_b) was used as a measure of population trophic diversity. CD_b is calculated as the mean Euclidean distance of each individual of a population to the $\delta^{15}N$-$\delta^{13}C$ centroid for that population. The population metrics *nitrogen range* (NR_b) and *carbon range* (CR_b) correspond to the distance between the two individuals with the highest and lowest $\delta^{13}C$ and $\delta^{15}N$ values within a population and provide an indication of the total nitrogen and carbon range exploited by a population (Layman *et al.* 2007a). The metric *standard deviation of nearest neighbour distance* ($SDNND_b$) can be used to infer population trophic evenness. $SDNND_b$ is calculated as the standard deviation of Euclidean distances of each individual to its nearest neighbour in stable isotope bi-plot space. The community metric *total area* (TA) can be converted directly to a measure of population niche area (Layman et al. 2007b). However, Layman et al. (2007b) calculated TA from a convex hull drawn around the most extreme data points on an isotope bi-plot. This will give an incomparable measure of niche area when applied to different sample sizes (such as those used in our study; $n = 15\text{-}89$) since the convex hull area will generally increase with sample size even if the underlying population has remained the same (Jackson *et al.* 2011). Consequently, we use standard ellipse area (SEA) as a measure of the mean core population isotopic niche which is robust to variation in sample size, although we acknowledge that a convex hull better emphasises the role of individuals in the overall dispersion within isotope niche space (Jackson *et al.* 2011). Briefly, the standard ellipse is to bivariate data as standard deviation is to univariate data. The standard ellipse of a set of bivariate data is calculated from the variance and covariance of the x and y data and contains approximately 40% of the data (Batschelet 1981; Ricklefs & Nealen 1998) and hence, it reveals the core niche area and is expected to be insensitive to sample size. However, the use of a (n-2) correction on the denominator in place of the standard (n-1) when calculating variances seems appropriate given the loss of an extra degree of freedom involved when dealing with bivariate data. Indeed, as supported by extensive simulation studies (Jackson *et al.* 2011), a sample size corrected version of the standard ellipse area, referred to as SEA_c is employed here to circumvent the bias that arises when sample sizes are small. Explicitly,

$$SEA_c = SEA * (n-1) / (n-2)$$

This correction has the property of increasing SEA_c at small sample sizes in order to correct bias towards underestimation but asymptotes to 1 at infinity. Furthermore, the calculation of SEA_c allows the degree of isotopic niche overlap to be calculated which can be then used as a quantitative measure of dietary similarity among populations. These methods, developed by Jackson et al. (2011), are the first to provide quantitative measures of a population's trophic ecology that account for variation in sample size and correct for small sample sizes. All metrics were calculated using the R statistical computing package (R Development Core Team, 2007), see Jackson et al. (2011) for detailed methodology and Layman et al. (2007a) for original descriptions of the community-level metrics.

Finally, we quantified annual changes in the reliance of crayfish on different resources to elucidate alterations in annual diet following the invasion of carp. We calculated the Euclidean distance between the mean crayfish isotope values for each consecutive year sampled and quantified the angle of change between subsequent mean crayfish isotope signatures. A vector-diagram was used to illustrate the changes (Schmidt *et al.* 2007). Angles of change allow diet shifts to be distinguished from trivial annual fluctuations in species mean isotope values, while the distance of change will indicate the magnitude of any diet shifts.

All data were tested for normality and heteroscedasticity using Kolmogorov-Smirnov and Levene's tests, respectively (in Minitab® 14; Minitab Ltd., Pennsylvania, USA) before further statistical tests. Submerged-plant data failed these assumptions and were $\log_{10}(x+1)$-transformed. We tested for differences in $\delta^{13}C$ and $\delta^{15}N$ between species and among years with permutational analysis of variance (Anderson 2001; Anderson *et al.* 2008) using the PERMANOVA+ add-in to PRIMER® version 6.1 (PRIMER-E Ltd, Plymouth, UK). This was done with Type III sums of squares and was based on a Euclidian distance matrix and 9999 permutations of the residuals under a reduced model.

Results

Temporal changes

Following their establishment, carp CPUE increased rapidly and consistently every year until 2008, when their abundance declined by 50% compared with 2007 (Figure 3.1). There was no correlation between carp and crayfish abundance and water level (carp $r_7 = -0.22$, $P = 0.6$; crayfish $r_7 = 0.38$, $P = 0.4$). Submerged plant relative abundance was correlated inversely with carp abundance ($r_7 = -0.77$, $P = 0.02$) but not with crayfish ($r_7 = 0.35$, $P = 0.44$).

The stable isotope values of basal resources and primary consumers remained consistent throughout the whole period of study (see Figure 3.2) with no significant changes in either $\delta^{13}C$ (permutational ANOVA; $F_{4,81} = 2.10$, $P = 0.10$) or $\delta^{15}N$ (permutational ANOVA; $F_{4,81} = 1.59$, $P = 0.19$; Table 3.1) among years. In contrast, stable isotope values of carp and crayfish varied considerably among years (see below) when compared to the low variability in putative resources (Figure 3.2) and therefore, we attribute any changes in the stable isotope values and population metrics of crayfish and carp to actual diet alteration.

In total, 114 carp and 346 crayfish were analysed for stable isotopes. We found significant interactions between species and year for both $\delta^{13}C$ (permutational ANOVA; $F_{3,449} = 4.38$, $P = 0.006$) and $\delta^{15}N$ ($F_{3,449} = 8.37$, $P = 0.0002$; see Appendix 1 for ANOVA tables). However, $\delta^{13}C$ did not differ between carp and crayfish in 2003 or 2006, but was significantly higher in carp relative to crayfish in both 2007 and 2008 ($p < 0.001$ in both cases; Figure 3.2). Carp $\delta^{15}N$ was consistently significantly higher than that of crayfish ($p \leq 0.0001$ in each case; Figure 3.2).

Mixing models

Native submerged plants were only available as a resource in 2001 due to their cyclic relationship with crayfish abundance in Lake Naivasha (Hickley *et al.* 2002). In 2001, submerged plants were the second most important resource in the diet of crayfish after hippo dung, contributing an average of 30% (Table 3.2). Hippo dung also contributed the most to crayfish diet relative to other resources in 2002, 2006 and 2007. Chironomids were the second most important resource in crayfish diet in 2002 and 2006 and the most important in 2003. The contribution of water hyacinth and benthic FPOM to crayfish diet was negligible in most years (Table 3.2).

There was little variation in the contribution of each resource to crayfish diet between 2001 and 2002 except that because the submerged plants disappeared, the relative proportion of hippo dung increased in 2002. Once carp had appeared in the fishery in 2003, the contribution of each resource to the diet of crayfish varied considerably among years (Table 3.2). There was an increase in the contribution of chironomids until 2007 when there was a large increase in the relative contribution of plant debris (up to 47%; Table 3.2); this was coincident with the highest recorded carp abundance in the lake (Figure 3.1). Crayfish contributed at least 22% to the diet of carp relative to the other resources in all years analysed. Indeed, in 2003 the average contribution of crayfish to the diet of carp was 70%. Chironomids, Oligochaetes and hippo dung also contributed to the diet of carp, whereas the contribution of plant debris, water hyacinth and benthic FPOM was negligible (Table 3.2).

Population metrics

The SEA_c of crayfish and carp did not overlap except in 2006 (Figure 3.3) when crayfish were at very low abundance in the lake (Figure 3.1). The area of overlap comprised 20.4% and 10.9% of total crayfish and carp isotopic niche area, respectively. The SEA_c of carp did not vary notably between years and remained relatively consistent in size, increasing slightly over the duration of the study (Figures 3.3 and 3.4). There was a positive correlation between carp SEA_c and the water level of the lake ($r_5 = 0.99$, $P = 0.04$, Figure 3.1). A higher water level could have improved resource diversity by providing access to terrestrial resources in the inundated zones.

In contrast, the isotopic location of the crayfish SEA_c differed substantially among years and varied significantly in size (Figures 3.3 and 3.4). The SEA_c of crayfish decreased considerably from 2003, when carp were first found in low abundances in the lake, until 2006 and then increased slightly in 2007, by which time carp contributed to 90% of the commercial fishery catch (Figures 3.1, 3.3 and 3.4). In 2008, the crayfish SEA_c increased by approximately three times, coinciding with a 50% reduction in carp CPUE (Figures 3.1 and 3.3). Additionally, crayfish exhibited their lowest NR_b and CR_b when carp catch was highest in 2006 and 2007 (Figures 3.1, 3.3 and Table 3.3). In comparison, carp NR_b and CR_b remained similar in all years (Table 3.3). There was an inverse relationship between carp catch and both crayfish CR_b ($r_5 = -0.89$, $P = 0.02$; Figure 5A) and crayfish SEA_c ($r_5 = -0.89$, $P = 0.02$; Figure 3.5B).

The angle of change between each consecutive mean crayfish isotope value indicated an increasingly ^{13}C-depleted diet (Figure 3.6) with one exception (2003 to 2006). This was in parallel to the decline in availability of submerged plants which had high $\delta^{13}C$ values (-5.6 ± 0.1‰). Once carp had become established as the dominant fish species, crayfish exhibited angles of change reflecting a shift in diet towards lower trophic levels (*i.e.* reduced $\delta^{15}N$, Figure 3.6). The only exception to this pattern occurred between 2007 and 2008 when the mean crayfish $\delta^{15}N$ increased, coinciding with a 50% decline in carp CPUE (Figure 3.1). The magnitude of change in crayfish diet was greatest between 2002 and 2003 (Figure 3.6), coincident with carp first appearing in the fishery (Figure 3.1).

The diversity of crayfish diet, measured as the *mean distance to centroid* (CD_b) was lowest in 2006 and 2007 (Table 3.3) coinciding with the highest relative abundance of carp (Fig. 3.1). Further, there was a significant inverse correlation between crayfish CD_b and carp abundance (CPUE: $r_5 = -0.95$, $P = 0.004$; Figure 3.5C), and a significant inverse correlation between carp abundance and crayfish standard deviation of mean nearest neighbour distance ($SDNND_b$; $r_5 = -0.95$, $P = 0.003$; Figure 3.5D), a measure of the spread of individuals within isotopic space. Crayfish abundance was not, however, correlated significantly with any carp isotopic population metrics (NR_b: $r_5 = -0.42$, $P = 0.40$; CR_b: $r_5 = -0.08$, $P = 0.87$; CD_b: $r_5 = -0.26$, $P = 0.62$; $SDNND_b$: $r_5 = -0.24$, $P = 0.65$; SEA_c: $r_5 = -0.20$; $P = 0.7$).

Tables and figures

Table 3.1. $\delta^{13}C$ and $\delta^{15}N$ values for basal resources and dominant benthic primary consumers. Each value (mean ± standard error) is based on multiple sampling efforts (n) each year. Chironomid and oligochaete samples comprised >20 individuals each time.

Resource	Year	$\delta^{13}C$	$\delta^{15}N$	n
Chironomids	2001	-18.7±0.7	5.7±0.4	3
	2002	-18.1±0.6	4.9±1.2	5
	2003	-17.5±0.2	6.0±0.5	5
	2006	-16.3±0.1	6.1±0.2	5
	2007	-17.5±1.2	5.9±0.7	5
Oligochaetes	2001	-16.0±0.3	3.5±0.5	3
	2002	-17.5±0.4	4.5±0.7	3
	2007	-16.1±1.0	5.1±0.1	2
Sediments	2001	-18.0±0.1	2.8±0.1	3
	2002	-17.5±0.6	2.5±1	4
	2003	-18.2±0.5	2.7±0.4	3
	2006	-17.6±0.4	2.8±1.1	3
	2007	-18.3±0.3	2.7±0.7	3
Plant debris	2001	-16.8±1.0	3.1±1.1	3
	2002	-16.7±0.4	1.4±0.7	3
	2003	-17.3±0.8	1.9±1.2	3
	2006	-16.8±0.4	2.1±0.6	3
	2007	-17.3±0.7	2.0±1.1	3
Water hyacinth	2001	-24.5±0.2	3.3±0.2	3
	2003	-26.6±0.5	4.0±0.2	3
	2007	-24.9±0.3	3.9±1.0	3
Hippo dung	2001	-13.4±0.4	4.1±0.2	6
	2002	-12.8±0.6	4.9±0.2	4
	2003	-13.3±0.6	4.3±0.1	5
	2006	-13.4±0.4	4.9±0.2	7
	2007	14.2±0.5	4.6±0.04	6
Submerged plants	2001	-5.6±0.1	-0.4±0.2	9

Table 3.2. The relative contribution of putative resources to the diet of crayfish and carp from 2001 to 2007. Estimated using Bayesian mixing models. Contributions are designated as estimated low 95% highest density region (hdr), mean contribution, and high 95% hdr.

Species	Year	Resource	Low 95% hdr	Mean % contribution	High 95% hdr
Crayfish	2001	Chironomids	0.00	0.10	0.25
		Oligochaetes	0.00	0.07	0.19
		Plant debris	0.00	0.05	0.13
		Benthic FPOM	0.00	0.03	0.09
		Water hyacinth	0.00	0.02	0.05
		Hippo dung	0.09	0.43	0.77
		Submerged plants	0.02	0.30	0.58
	2002	Chironomids	0.00	0.10	0.21
		Oligochaetes	0.00	0.09	0.21
		Plant debris	0.00	0.02	0.06
		Benthic FPOM	0.00	0.03	0.09
		Hippo dung	0.65	0.75	0.85
	2003	Chironomids	0.68	0.77	0.87
		Plant debris	0.00	0.02	0.04
		Benthic FPOM	0.00	0.02	0.05
		Water hyacinth	0.00	0.01	0.03
		Hippo dung	0.10	0.18	0.26
	2006	Chironomids	0.34	0.40	0.46
		Plant debris	0.00	0.04	0.08
		Benthic FPOM	0.00	0.04	0.09
		Hippo dung	0.46	0.52	0.58
	2007	Chironomids	0.00	0.05	0.13
		Oligochaetes	0.00	0.11	0.26
		Plant debris	0.12	0.30	0.47
		Benthic FPOM	0.00	0.12	0.30
		Water hyacinth	0.00	0.02	0.06
		Hippo dung	0.23	0.39	0.53
Carp	2003	Chironomids	0.00	0.12	0.32
		Plant debris	0.00	0.04	0.12
		Benthic FPOM	0.00	0.04	0.13
		Water hyacinth	0.00	0.05	0.10
		Hippo dung	0.00	0.05	0.15
		Crayfish	0.41	0.70	0.92
	2006	Chironomids	0.00	0.12	0.25
		Plant debris	0.00	0.04	0.11
		Benthic FPOM	0.00	0.04	0.10
		Hippo dung	0.05	0.19	0.33
		Crayfish	0.40	0.61	0.81
	2007	Chironomids	0.00	0.08	0.20
		Oligochaetes	0.00	0.10	0.26
		Plant debris	0.00	0.02	0.04
		Benthic FPOM	0.00	0.02	0.04
		Water hyacinth	0.00	0.01	0.03
		Hippo dung	0.03	0.26	0.48
		Crayfish	0.22	0.52	0.83

Table 3.3. Population metrics for carp and crayfish in Lake Naivasha, grouped by year caught. NR_b= $\delta^{15}N$ range; CR_b= $\delta^{13}C$ range; CD_b= mean distance to centroid; $SDNND_b$=standard deviation of mean nearest neighbor distance; SEA_c=standard ellipse area. The number of individuals used to calculate the metrics is shown in parentheses.

	NR_b	CR_b	CD_b	$SDNND_b$	SEA_c
Carp 2003 (*n* = 15)	3.06	2.59	1.05	0.39	2.30
Carp 2006 (*n* = 37)	4.18	1.94	1.26	0.30	2.57
Carp 2007 (*n* = 31)	2.88	3.46	1.22	0.37	3.23
Carp 2008 (*n* = 30)	3.64	3.74	1.26	0.47	3.58
Crayfish 2001 (*n* = 30)	2.91	6.98	1.94	0.54	5.56
Crayfish 2002 (*n* = 89)	4.07	8.70	2.26	0.66	9.24
Crayfish 2003 (*n* = 85)	5.53	5.04	1.82	0.59	6.68
Crayfish 2006 (*n* = 84)	1.93	2.75	0.83	0.27	1.37
Crayfish 2007 (*n* = 30)	2.22	2.70	0.92	0.29	1.86
Crayfish 2008 (*n* = 28)	3.16	5.69	1.74	0.49	5.75

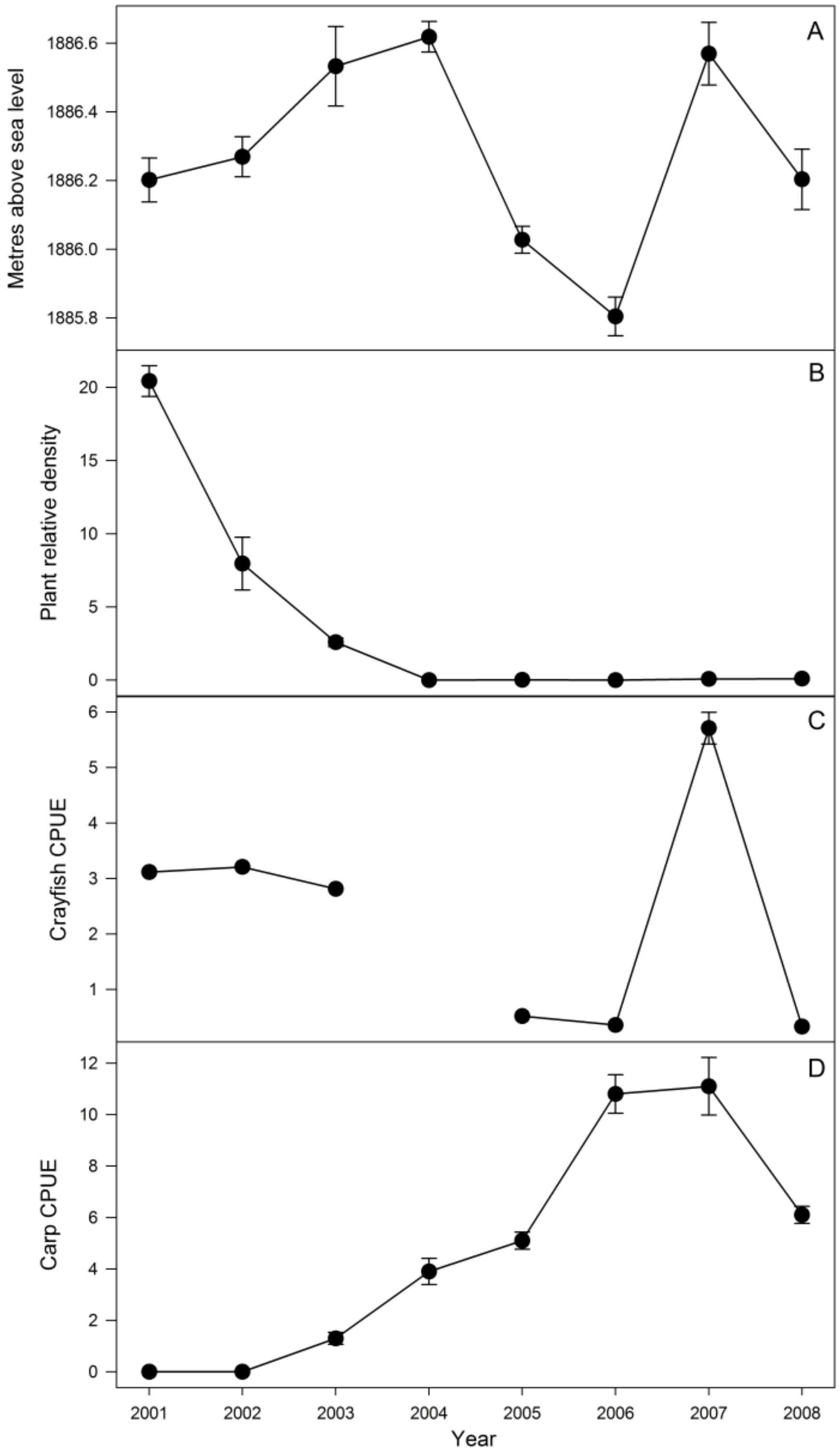

Figure 3.1. Mean (± standard error) annual water level (A), submerged plant relative abundance (B), crayfish CPUE (C) and carp CPUE (D) from 2001 to 2008.

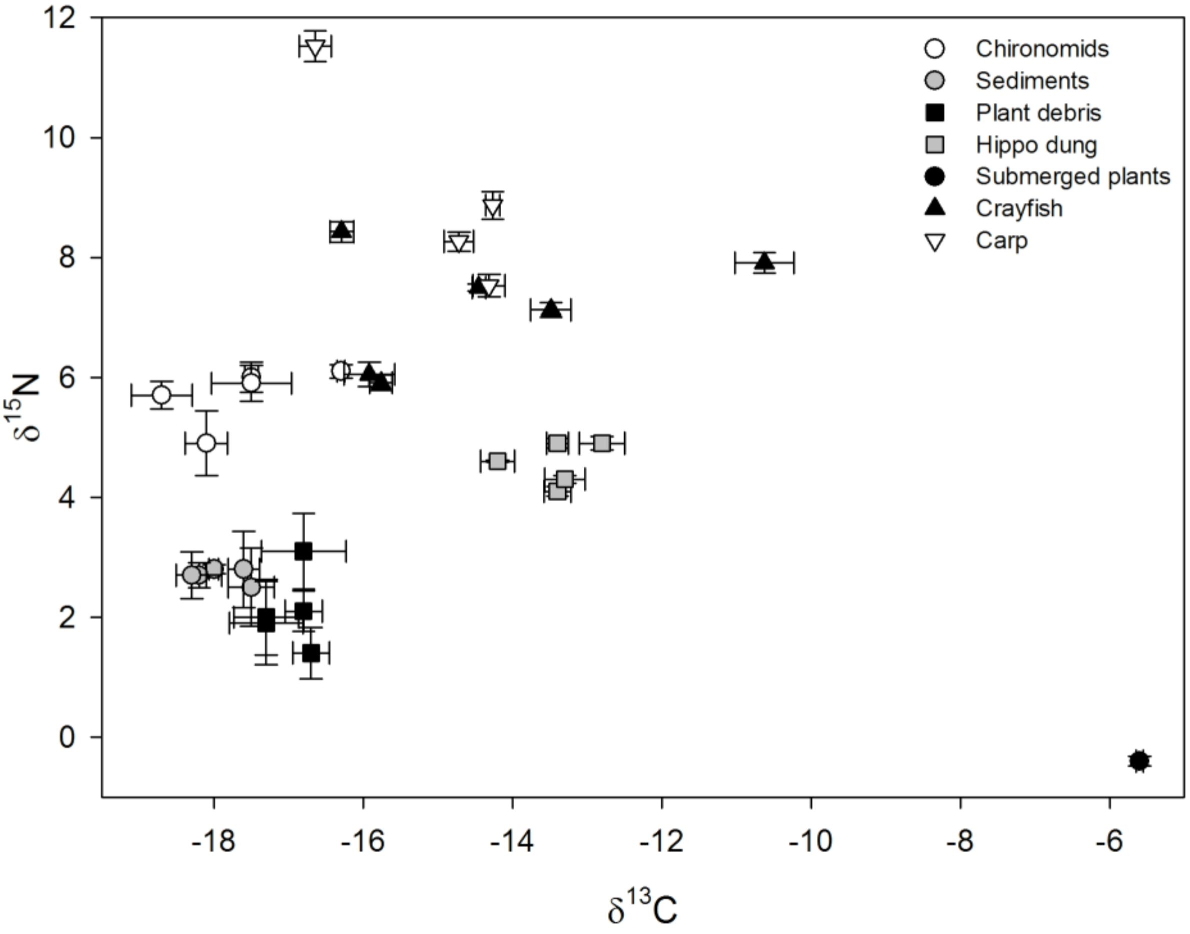

Figure 3.2. Stable isotope bi-plot showing the intra- and inter-annual variation in isotope values of resources, carp and crayfish. Each data point represents an annual mean and the error bars represent the intra-annual standard error.

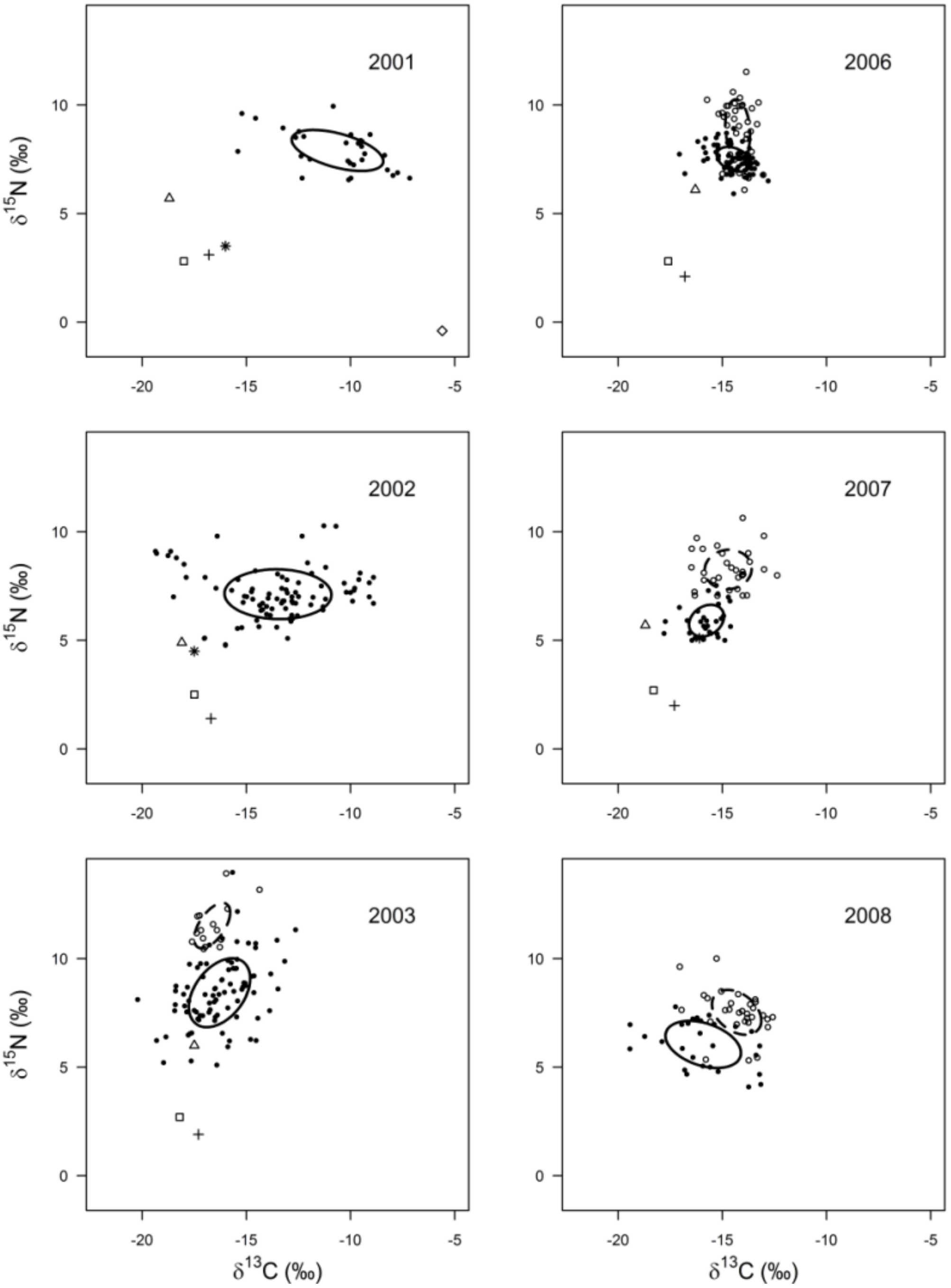

Figure 3.3. Stable isotope bi-plots for each year, illustrating the isotopic niche of carp and crayfish. The black circles represent individual crayfish and the open circles represent individual carp. The lines enclose the standard ellipse area (SEA_c) for each year for both crayfish (solid) and carp (dashed). Mean values of resource points are also shown; benthic FPOM (open square), plant debris (cross), submerged plants (open diamond), chironomids (open triangle) and oligochaetes (asterisk).

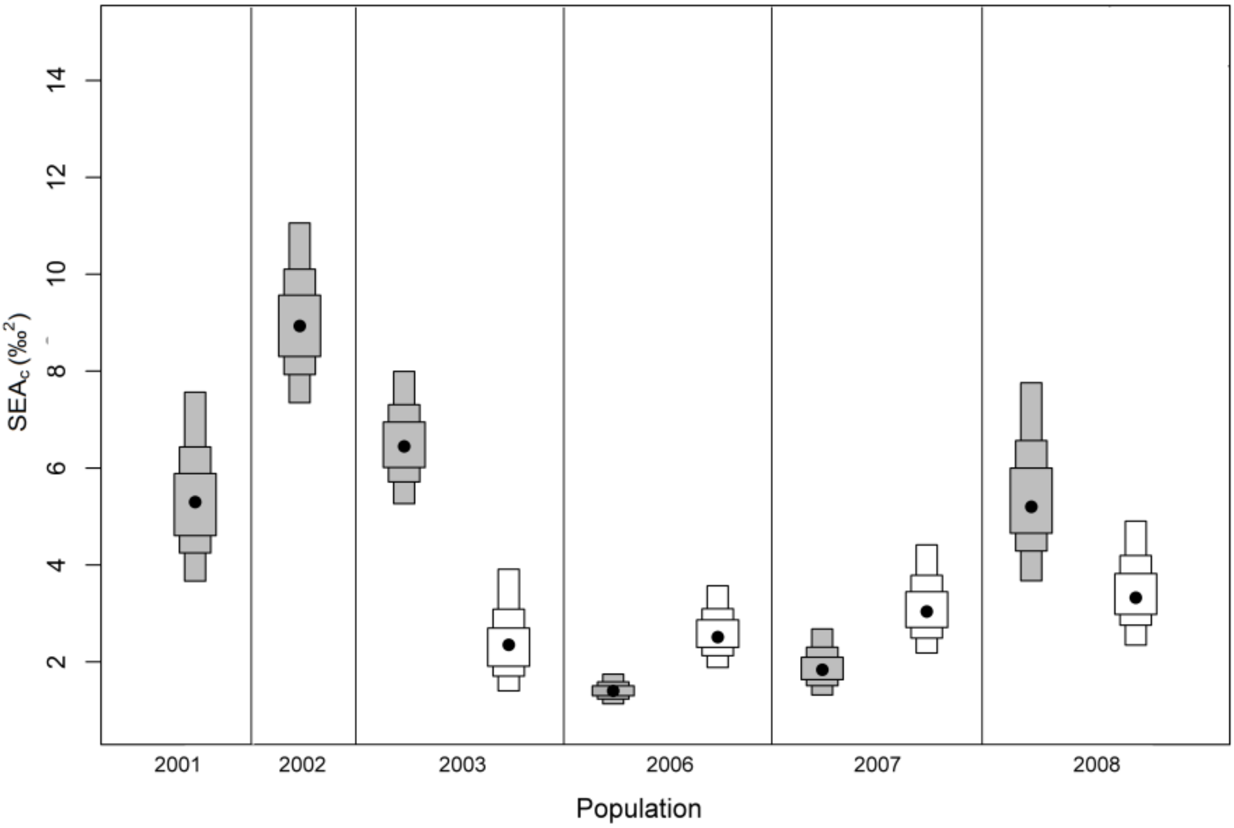

Figure 3.4. Density plot showing the confidence intervals of the standard ellipse areas. The black points correspond to the mean standard ellipse area for each group while the grey and white boxed areas reflect the 95, 75 and 50% confidence intervals for crayfish and carp, respectively.

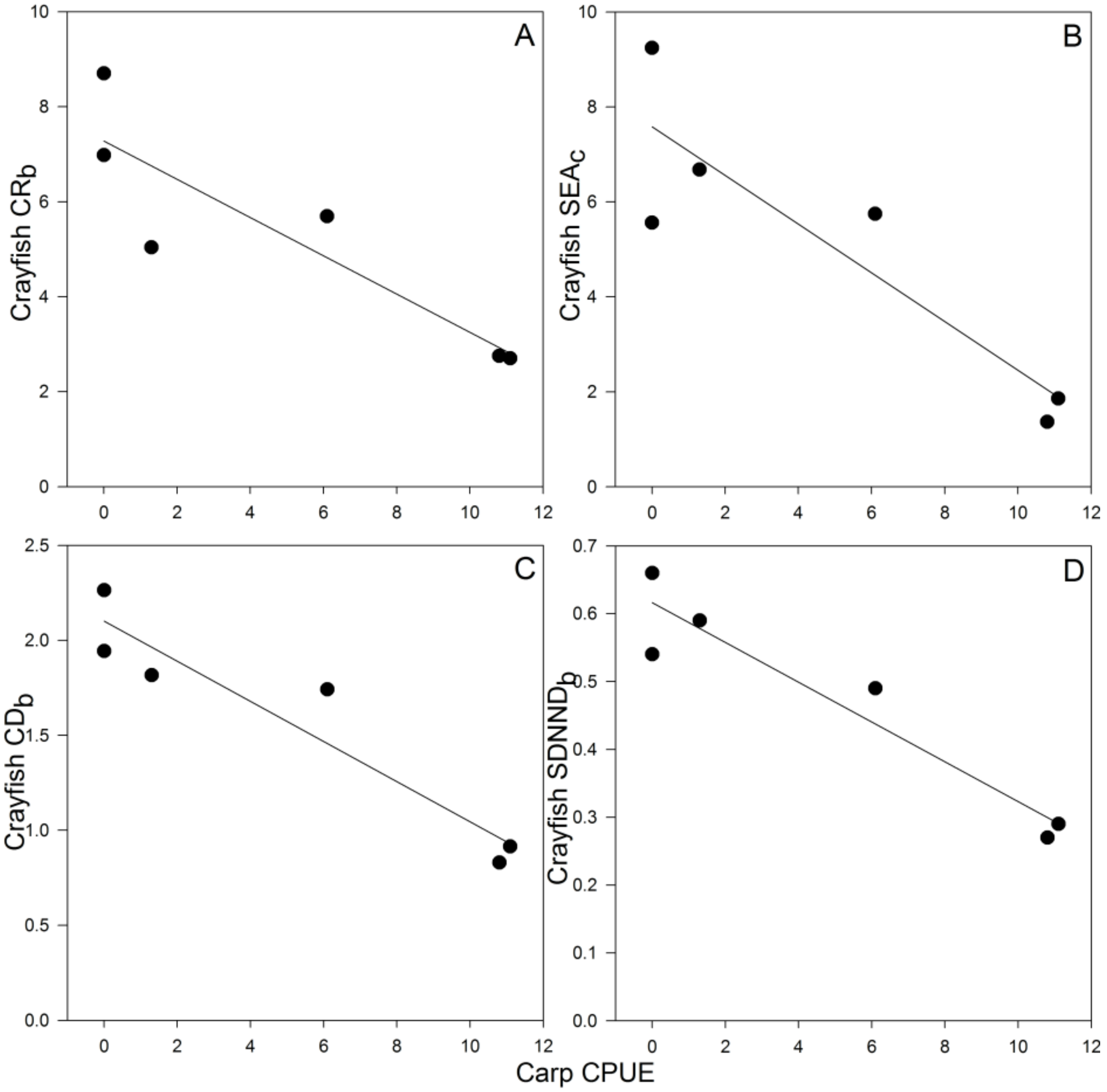

Figure 3.5. Relationships between carp CPUE and crayfish population metrics. A. carbon range (CR_b), B. standard ellipse area (SEA_c), C. mean distance to centroid (CD_b) and D. standard deviation of nearest neighbour distance ($SDNND_b$). Least-square regression lines are included for illustrative purposes only.

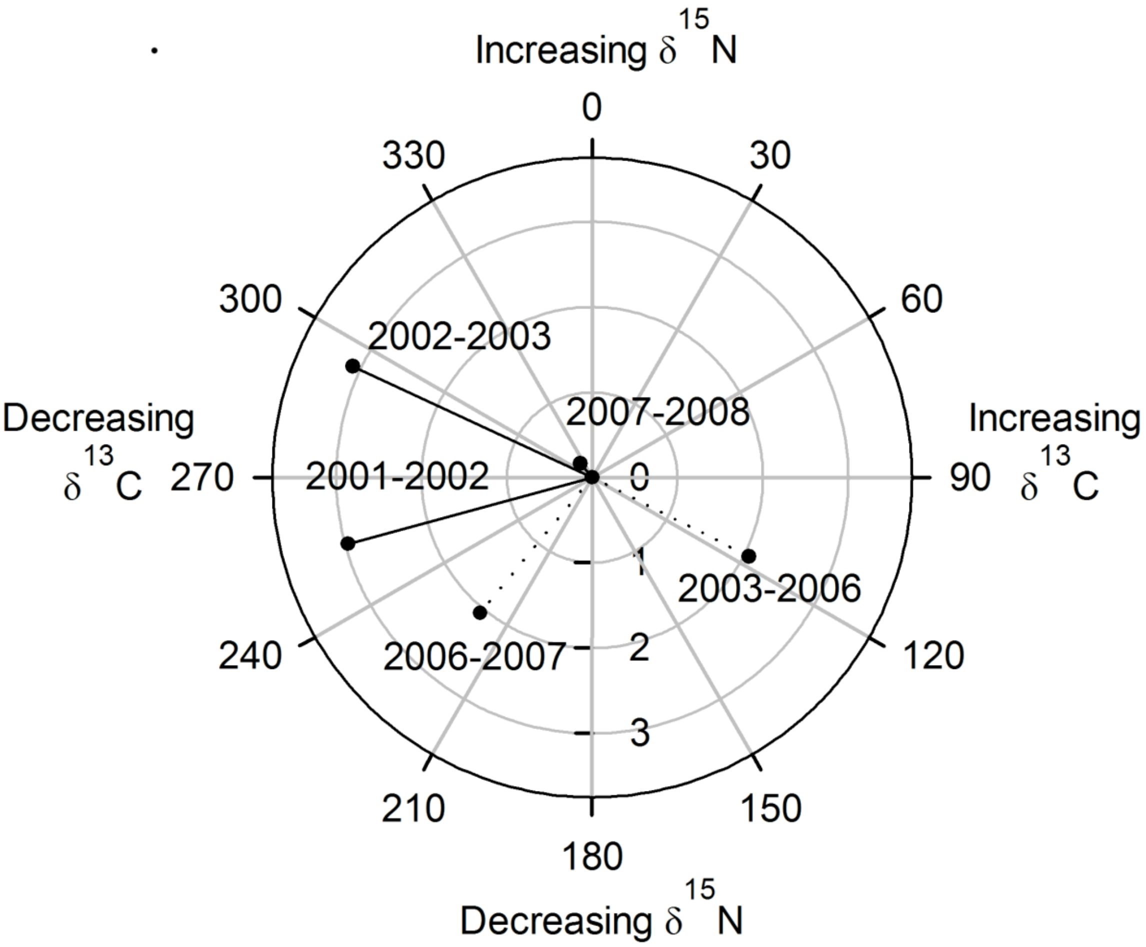

Figure 3.6. Arrow-diagrams showing the change in mean crayfish isotopic composition before and after carp dominance. Lines show the change before (solid lines) and after (dashed lines) carp dominance. Each arrow displays the mean isotopic change of crayfish carbon and nitrogen values compared to the previous sampling period. The length of each arrow represents the magnitude of change and the direction illustrates the angle of change.

Discussion

Our stable isotope-derived metrics of trophic structure provide novel ways of quantifying interactions among populations and/or species and their application revealed new insights into interactions between two globally widespread sympatric invasive species. There was a considerable reduction in crayfish isotopic niche (measured as SEA_c) following establishment of carp, another benthic omnivore, thus supporting our hypothesis that carp would suppress (and/or displace) the isotopic niche of crayfish. The isotopic niche of crayfish subsequently increased in size when they appear to have been largely released from interspecific competition in 2008 as carp abundance declined sharply, presumed to be due to heavy fishing pressure in the commercial fishery. The angle and magnitude of annual change in mean crayfish isotope values indicated that there was also displacement of the crayfish niche following carp establishment. This isotopic niche shift throughout the study was directed away from a previously important resource; native submerged plants (Hickley *et al.* 2002).

Following the carp population expansion (2003 to 2006), crayfish also exhibited an isotopic change toward a lower trophic position, suggesting a shift in diet to avoid dietary overlap and subsequent competition with carp. The only deviation from this trend was between 2007 and 2008 when crayfish shifted toward a slightly higher trophic position, coincident with a 50% reduction in carp CPUE. The relative contribution (shown by Bayesian mixing models) of putative food resources to the diet of crayfish altered after carp establishment, supporting our hypothesis that the location of the dietary niche of crayfish would change following the carp invasion. Chironomids and hippo dung contributed to the diet of crayfish in all years, whereas the relative contribution of plant debris was below 10% except in 2007. This exception coincided with the highest measure of carp abundance, when crayfish may have been forced to feed on a lower quality resource as a result of competition or carp habitat alteration. Furthermore, our stable isotope-derived population metrics suggest that there was an important interaction occurring between the two invaders; high carp abundance reduced the diversity of crayfish diet (measured as CD_b), increased packing of individuals in isotopic space (measured as $SDNND_b$), limited the total range of exploited resources (measured as CR_b), and reduced the number of trophic levels utilised (measured as NR_b). In contrast, the abundance of crayfish did not affect any isotopic metrics of carp, indicating that crayfish presence had little impact on carp trophic ecology. Hence, the interaction among crayfish and carp was asymmetric, with carp altering crayfish trophic ecology and not vice versa.

Asymmetric competition may have arisen due to the larger size carp attain, which can provide competitive superiority (Young 2004). Carp and crayfish were isotopically distinct in all years except 2006 when the core dietary niche of carp and crayfish overlapped and when carp abundance was reduced by the commercial fishery. The majority of niche partitioning in all other years was due to higher $\delta^{15}N$ values of carp, implying a higher trophic position. Crayfish was the most important assimilated resource for carp relative to the other resources in all years analysed (shown using Bayesian mixing models) and this is supported by the analyses of carp gut contents (Britton *et al.* 2007). This suggests that intraguild predation, whereby a superior predator (i.e. carp) both feeds on and competes for resources with another species (i.e. crayfish), played a role in the decline in crayfish abundance (Holt & Huxel 2007). We observed a reduction in crayfish trophic diversity at times of high carp abundance when there was no isotopic niche overlap (measured using SEA_c) in the majority of years, suggesting that the two invaders also interacted indirectly, possibly due to behavioural modifications or competition for non-food resources.

On three recent sampling occasions (Jul and Nov 2009, March 2010), we failed to trap any crayfish and, on the basis of our isotope data, it may imply that the interaction with carp has led to the virtual elimination of crayfish in Lake Naivasha. Serial replacement of invasive species is an alternative theory to that of the Invasion Meltdown scenario (Simberloff & Von Holle 1999). Indeed, invasive carp also reduce crayfish abundance in their native habitat, which is attributed to carp-induced habitat depletion (Hinojosa-Garro & Zambrano 2004). Common carp feed in the benthic zone which can uproot macrophytes (Petr 2000). An inverse correlation between carp CPUE and the relative abundance of native submerged plants, an important food source for crayfish in Lake Naivasha (Hickley *et al.* 2002), indicates possible habitat alteration by carp in the lake. Furthermore, a decline in plant abundance will also reduce the number of macroinvertebrates associated with those plants and may thus be responsible for the decline in the dietary niche area of crayfish. The mean annual change of crayfish isotope values was directed away from exploitation of submerged plants, reflecting the decline in their availability.

A diverse diet range and/or variation in resource use among individual crayfish from 2001 to 2003 (prior to carp dominating the fishery) was illustrated by substantial variability in isotope values and hence a high trophic diversity (measured as CD_b) and large spread over isotopic space (measured as $SDNND_b$). We infer, therefore, that the variety of available resources declined as a result of carp-induced habitat alteration from 2006 onwards, thus forcing the crayfish to exploit less diverse prey items which resulted in reduced isotopic variability and significantly reduced CD_b, SEA_c, CR_b and $SDNND_b$ values (Layman *et al.* 2007b). The predation risk posed by carp may have altered crayfish foraging behaviour and use of refuge and, hence, caused a change in resource choice (Gherardi *et al.* 2011). The alternative scenario, that increased competition for resources would result in a more varied diet to maintain energy requirements, was rejected. Despite the reduction in crayfish abundance, Lake Naivasha has shown no measurable ecological recovery (primarily in terms of submerged plants), suggesting that carp has replaced crayfish as a keystone species. Our study therefore highlights the dynamic nature of highly invaded ecosystems and indicates that the virtual elimination of crayfish from the lake will likely force carp to utilise other resources. This, in turn, suggests that there will soon be another significant shift in the food web structure of Lake Naivasha.

A combination of stable isotope derived-population metrics and Bayesian mixing models revealed a complex interaction between invasive carp and crayfish in Lake Naivasha. The detrimental impact carp had on the crayfish population appears to have been due to a number of dietary interactions, including predation (Britton *et al.* 2007) and indirect dietary interactions mediated via habitat depletion. We were able to draw these conclusions from the population metrics due to the consistency in the isotopic composition of each resource throughout the study, signifying that the changes in crayfish niche size and position were a consequence of changes in the proportion and/or identity of assimilated resources. This conclusion is supported further by the results of the Bayesian mixing model, SIAR. It is important to consider fluctuations in resource isotopic composition, since the isotopic area occupied by putative resources will directly influence the isotopic area occupied by consumers. It is also important to consider that the metric SEA_c quantifies the core comparable isotopic niche of a species or population and hence, the community metric TA (Layman *et al.* 2007a) may be more applicable to some analyses if the full isotopic area occupied by the species/population is required.

The population metrics calculated from stable isotope data proved a useful tool to reveal subtle dietary interactions between species and demonstrated potential for application to a wide range of fields in ecology. The increasing pace of global environmental change has had substantial impacts on local biodiversity and it is imperative to understand those ecological interactions ultimately responsible for the patterns observed. The community metrics developed by Layman et al. (2007a) caused some controversy and discussion when first published (Hoeinghaus & Zeug 2008) but ultimately added another ecological tool-set to help unravel the complexity of food webs. Through providing a logical extension to their approach, such population-level metrics can now be used widely in conjunction with appropriate measures of ecosystem structure and functioning to reveal the direct and indirect consequences of local environmental change on populations.

Chapter Four: Playing to one's strengths; dietary niche compression aids invasion in the presence of functionally similar natives

This chapter is formatted as submitted to the journal *Proceedings of the Royal Society B: Biological Sciences.* Michelle C. Jackson, Ian Donohue, Katie Miller, J. Robert Britton and Jonathan Grey.

Declaration on input

I am indebted to Katie Miller for her invaluable assistance with the fieldwork in 2009.

Abstract

Invasive species are a key component of global change with implications for biodiversity and ecosystem services. Theory, supported by empirical studies, suggests that invasive species gain an advantage by occupying a broader niche when competing with established natives. Here, we use field experiments and surveys to examine the dietary mechanisms leading to an invader's success (the globally widespread invasive crayfish, *Procambarus clarkii*) in the presence of a functionally similar native species (a crab, *Potamonautes loveni*). Contrary to expectation, we show that the invasive crayfish population exhibited dietary niche constriction in the presence of native crabs and only extended their niche breadth in the absence of crabs. Despite this rather surprising finding, native crab abundance declined at those sites invaded by the crayfish over our three year study, becoming locally extinct at one. Our data therefore indicate a novel mechanism facilitating successful

invasion, whereby invasive species reduce their population niche breadth by diet homogenisation at the invasion front in order to reduce competition with well-established natives. Moreover, our results demonstrate that replacement of the native crabs by crayfish also has considerable implications for ecosystem structure and functioning; the presence of the invasive crayfish increased rates of leaf litter breakdown and reduced densities of benthic invertebrates significantly compared with the native crabs. Our study highlights that the mechanisms of invasion are not fixed across species and ecosystems, and that it is important to consider variation in the mechanisms of invasions both to optimise management strategies and to predict impacts of successful invaders on the structure and functioning of ecosystems.

Introduction

The increasingly rapid spread of biological invaders in recent decades comprises a key driver of global environmental change with major implications for biodiversity and ecosystem functioning (Chapin *et al.* 2000; Gurevitch & Padilla 2004; Pejchar & Mooney 2009). Invasive species can alter fundamental ecological processes from the level of the individual through to the whole ecosystem (Peltzer *et al.* 2009), frequently inducing change in the distribution of populations of native species via a range of competitive interactions, predation pressure and/or habitat depletion (Mack *et al.* 2000; Simon & Townsend 2003; Hooper *et al.* 2005). Classic invasion theory predicts that a successful invader can establish themselves in an unoccupied niche or displace similar native species by overlapping their niche and eventually out-competing them for limited resources (Elton 1958). A generalist diet is a common trait of successful invaders allowing them to exploit numerous resources in an unfamiliar environment opportunistically, eventually leading to the depletion of resource pools and the exclusion of competing native species (Snyder & Evans 2006). Hence, the decline of native species following an invasion is often a consequence of direct interspecific competition for resources (e.g. Porter & Savignano 1990; Olsson *et al.* 2009).

Classic theory (Van Valen 1965) suggests that a species will occupy a larger niche area in the absence of interspecific competition and, hence, competing invasive and native species would be expected to occupy smaller niches than their allopatric counterparts. Alternatively, increased competition for resources might necessitate an increase in dietary niche breadth in order to maintain energy requirements (Svanbäck and Bolnick 2007). The dietary niche breadth of invasive species and their native competitors has, however, rarely been examined, even though it is likely to be a key factor in determining invasion success and predicting the impact of invaders on biodiversity.

Invasive species can have dramatic and often irreversible effects on ecosystem services, frequently with considerable economic implications (Pejchar & Mooney 2009), particularly in aquatic ecosystems which tend to be especially susceptible to invasion (Rahel & Olden 2008). Some of the most destructive invaders in freshwater habitats are crayfish, which can alter the structure of ecosystems and instigate marked changes in ecosystem processes, for example, by homogenising biotic assemblages and altering rates of both primary production and decomposition (Stenroth & Nyström 2003; Gherardi & Acquistapace 2007). Food webs can, therefore, change profoundly under their influence because they are relatively large, opportunistic omnivores that can attain high densities rapidly (Renai & Gherardi 2004). However, the full range of ecological mechanisms underpinning crayfish impacts on ecosystem structure and functioning has yet to be examined fully.

Amongst invasive crayfish species, the Louisiana swamp crayfish (*Procambarus clarkii*) is a particularly important invader of freshwater ecosystems due to its global distribution (Capinha *et al.* 2011). In the 1960s, the species was introduced to multiple locations throughout East Africa and its effects there remain largely unknown. Nevertheless, Louisiana swamp crayfish continues to be introduced to new water bodies both to enhance fisheries and in an attempt to control schistosomiasis (a parasitic disease) as crayfish feed on the intermediate host (snails) that carry schistosome larvae (Mkoji *et al.* 1999). The widespread introduction of Louisiana swamp crayfish has led to encroachment on the range of many native freshwater crab species, several of which are highly endangered (Cumberlidge *et al.* 2009). Decapods often substitute for smaller macroinvertebrate taxa as shredders in the tropics (Dudgeon 1999) and, therefore, crabs and crayfish may occupy the same functional role. They are both benthic omnivores with similar feeding mechanisms, and both are key drivers of detrital processing (Nyström *et al.* 1996; Dobson *et al.* 2002). Consequently, only minor differences in ecosystem structure and functioning might be expected were crayfish to invade ecosystems containing native crabs due to the high potential for functional redundancy (Naeem 1998). However, O'Connor and Bruno (2007) demonstrated that different species from the same functional feeding group can vary significantly in their effects on ecosystems. Furthermore, invasive species tend to be more voracious than their native counterparts (e.g. Bubb *et al.* 2006), implying that Louisiana swamp crayfish have the potential to cause considerable alteration to ecosystem structure and processes were they to replace native crabs.

Here, through field experiments and surveys, we examine the individual and combined effects of the Louisiana swamp crayfish and a key native crab species, *Potamonautes loveni,* on ecosystem structure and functioning. The study site was the River Malewa, an important inflow river of Lake Naivasha in Kenya (East Africa). We hypothesise that the invasive crayfish will have a significantly different impact on ecosystem structure and functioning compared to the native crabs due to their invasive status. To examine the mechanisms that might facilitate successful invasion by the crayfish in the presence of the native crab, we quantified the dietary niche width of both species in both allopatric and sympatric conditions. We hypothesise that the invasive crayfish will occupy a wider niche than the native crabs. Spatial overlap with a functionally similar species could lead to resource depletion and therefore impact the diet of either or both species, potentially altering their relative competitive abilities. We quantified this through the analysis of carbon and nitrogen stable isotopes from muscle tissue which provides information on dietary preferences integrated over time. We then used novel methods that incorporate individual variation of stable isotope ratios to provide quantitative measures of total foraging niche space at the population level (Layman *et al.* 2007b; Newsome *et al.* 2007; Jackson *et al.* 2011). Specifically, we examined whether alteration of the dietary niche width of either of these functionally similar species could facilitate competitive exclusion of the crabs and successful invasion by the crayfish.

Methods

Study system

The River Malewa in central Kenya is the primary tributary of Lake Naivasha, an important freshwater lake in Kenya's Rift Valley. The catchment of the lake (1750 km^2) is dominated by dry savannah and bush, with original dense forest now occurring only in the highest parts of the Aberdare mountain range (the source of the Malewa). Louisiana swamp crayfish were introduced to Lake Naivasha in 1970 and spread subsequently throughout the catchment as a result of further introductions and natural dispersal, leading to their intrusion upon the range of native river crabs, *Potamonautes loveni* (Foster & Harper 2007).

Experimental design

We conducted two field experiments in the River Malewa near Gilgil (0°31 5' 5.42" S, 36° 24' 3.33" E; Figure 4.1); the first in April 2009 and the second in March 2010. Both experiments were of 28 days duration and comprised the same two fixed factors in a fully-factorial experimental design: the presence of the native crab *P. loveni* (two levels: present and absent) and the presence of Louisiana swamp crayfish (two levels: present and absent). The experiments differed, however, in both the level of replication (experiment 1: $n = 4$; experiment 2: $n = 6$) and the range of response variables that were quantified (Table 4.1). Results from experiments that manipulate density and biomass, such as we use here, can be sensitive to the selected total mixture density. They can, however, still effectively quantify the extent to which the measured outcome is a consequence of the mixture components (presence or absence of crayfish and crabs). We manipulated the presence of the experimental organisms within steel-framed cages, which had a 3150 cm^2 solid base surrounded by stainless steel mesh (5 mm aperture, to enclose/exclude crayfish and crabs but allow the drift or dispersal of other invertebrates) on the sides and lids, and were 30 cm in height. The entire base of each cage was covered with pebble and gravel substrata from the adjacent river bed and a native macrophyte (from Family Scrophulariaceae, ~5 g fresh weight) was added to recreate natural conditions. A 20 cm^2 tray was also filled with river substratum and seeded with invertebrates from a uniform kick sample taken from the experimental site. Crayfish and crabs were collected from allopatric populations in the River Malewa. All treatments containing crabs or crayfish comprised four individuals, with a combined biomass of ~30 g fresh weight. The weight (g) and carapace length (mm) of all individuals was recorded before they were marked for later identification. Where both crabs and crayfish were present together, treatments contained two individuals of both species, all of similar biomass. Our four treatments (crayfish only; crabs only; crayfish and crabs; neither crayfish nor crabs) were assigned to cages, which were placed along a 40 m stretch of the river (Fig. 4.1), following a randomised block design. The mesh sides of the cages were cleaned every four days to ensure build up of debris did not affect water movement. Two enclosures in the second experiment were omitted from analysis as they were largely destroyed by hippos (Table 4.1).

Experiment sampling protocol

We quantified leaf litter breakdown using plastic mesh bags (aperture 5 mm), which contained a known mass (~ 2.5 g) of air-dried leaf litter (from a native riparian shrub; *Dovyalis abyssinica*) fastened to the side of each cage at the commencement of the experiments. The leaf litter remaining in each mesh bag after 72 hours (to avoid total decomposition of material) was washed and then dried at 60ºC to constant weight. The exponential decay rate coefficient (*k*) was calculated for each treatment as a measure of the rate of leaf litter breakdown as:

$$k = \frac{\log\left(\frac{M_t}{M_0}\right)}{t}$$

where t is the duration of exposure (in days), M_0 is the initial dry mass (in grams) and M_t is the dry mass at time t (Hieber & Gessner 2002).

We measured epilithic algal standing stock by placing a terracotta tile (10 x 10 cm) in each cage at the start of the experiment. Tiles were removed on the final day of the experiment and all biofilm was washed off and filtered through GF/C filters (Whatman®, Maidstone, UK) before adding 15 ml of 90% acetone. After 24 hours the samples were centrifuged and the supernatant was used for spectrophotometry. We then used chlorophyll *a* concentration, quantified following Jeffery and Humphrey (1975) and expressed as mg cm^{-2}, as a measure of epilithic algae standing stock. We also quantified the benthic invertebrates in the cages at the end of the experiment by removing, counting and identifying all organisms in 20 cm^2 trays that were filled with substrata from the experimental site and placed in each cage at the start of the experiment. All invertebrates were stored in 70% ethanol prior to identification (to genus level) and enumeration.

On the final day of each experiment, crayfish and crabs were removed from the enclosures and their carapace length (mm) and total weight (g) recorded. Measurements of carapace length and total fresh weight were then used to calculate growth rates of individual crab and crayfish over the duration of the experiment. They were then frozen overnight and thawed before dissecting a portion of muscle from the tail of crayfish and the abdomen of crabs for the analysis of stable isotopes. The duration of the experiment (28 days) was chosen to allow time for crayfish and crab muscle tissue to equilibrate with their diet in experimental conditions due to fast growth rates at warm river temperatures (15 to 20 ºC; e.g. Fantle *et al.* 1999; Fry *et al.* 2003; McIntyre & Flecker 2006). All samples were oven-dried at 60°C, ground using an agate pestle and mortar and 0.6 to 1.0 mg weighed into 6 x 4 mm tin cups using a microbalance. Stable carbon and nitrogen isotope analyses were carried out using an elemental analyser (Flash EA 1112 series, Thermo-Finnigan, Waltham, Massachusetts, USA) coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT $Delta^{Plus}$, Thermo-Finnigan, Waltham, Massachusetts, USA). Secondary standards (sucrose for carbon; ammonium sulphate for nitrogen) with known relation to international standards (Pee Dee Belemnite for carbon; nitrogen in air for nitrogen) were used as reference materials. Delta (δ) isotope ratios are given in units of per mille (‰). Cyclohaxonone-2,4-Dinitrophemylhydrazone was used as an internal standard and resulted in typical precision of <0.1‰ for carbon and <0.3% for nitrogen.

Field surveys

We examined the distribution of both crabs and crayfish at four sites in the River Malewa, located approximately 10, 30, 35 and 40 km upstream from Lake Naivasha (Figure 4.1), in 2008, 2009 and 2010. We quantified the abundance of adult crayfish and crabs on multiple occasions at each site in each year, using a series of crayfish traps of 5 mm mesh baited with dead fish and left for between 1 - 2 hours. On lifting the traps, all crayfish and/or crabs were removed, counted, weighed and their carapace measured to the nearest millimetre. We used the catch per unit effort (CPUE; number of individuals per trap per hour) to quantify relative abundance among sites. Subsamples (*n* = 6 to 18) of a selected common size range of crabs (26 - 35 mm carapace width) and crayfish (31 - 45 mm carapace length) from all sites on all sampling occasions were prepared for stable isotope analysis as described previously. Epilithic algae, macrophytes, detritus and dominant benthic invertebrate species were also collected from all the sites for subsequent stable isotope analysis to ensure that basal resource signatures did not change and hence, any changes in consumer isotope signatures were not simply a result of fluctuating signatures in putative resources.

Data analyses

We used analysis of variance (ANOVA) to test for effects of the presence of crayfish and crab, both separately and together, on algal biomass, rates of leaf litter breakdown, trophic niche widths (see below), total invertebrate abundance and invertebrate taxon richness. We measured leaf litter breakdown in both field experiments (Table 4.1) and therefore tested for differences between experiments by incorporating experiment number as an additional random factor. No main or interactive effect of experiment number was found and this factor was then removed to maximise the power of the subsequent analysis. The Student-Newman-Keuls procedure was used to make post-hoc comparisons among levels of significant terms. We used permutational multivariate analysis of variance (PERMANOVA; Anderson et al., 2008) to test for effects of our experimental manipulations on the structure of invertebrate assemblages using the PERMANOVA+ add-in to PRIMER® version 6.1 (PRIMER-E Ltd, Plymouth, UK). These analyses were done with 9999 permutations of the residuals under a reduced model (McArdle & Anderson 2001; Anderson *et al.* 2008) and were based on Bray-Curtis similarity matrices calculated from log (X + 1)-transformed abundance data. Similarity percentages (SIMPER; Clark & Warwick 2001) analysis was used to determine the contribution of each benthic invertebrate taxon to the pairwise Bray-Curtis dissimilarities between experimental treatments. This method determines which taxa were affected most strongly by the presence or absence of crayfish and/or crabs. All analyses were balanced and variables were transformed where necessary prior to analysis to homogenise variances.

The stable isotope-derived population metrics of carbon range (CR_b) and nitrogen range (NR_b) were used as measures of the trophic niche width of crabs and crayfish (Layman *et al.* 2007a) in both the field experiments and field surveys. CR_b and NR_b were calculated as the

Euclidean distance between the individuals with the lowest and highest $\delta^{13}C$ and $\delta^{15}N$ respectively and therefore reflect the total range of isotopic space used by a group of organisms. To enable comparison among variable sample sizes in both the field experiments and field surveys, the metrics were bootstrapped (n = 10000; indicated with a subscript '$_b$') based on the minimum sample sizes (2 individuals in the field experiment [in the enclosures containing 2 crabs and 2 crayfish] and 6 individuals in the field surveys [at field sites with declining crab numbers]). We then used ANOVA to test for differences in the trophic niche width of crayfish and crabs in sympatric and allopatric conditions in the field experiment. However, given that one of our experimental treatments contained no crayfish or crabs, and could not therefore provide measurements of trophic niche widths of either species, our statistical model testing for differences in trophic niche width comprised two fixed factors: species (two levels: crabs and crayfish) and the presence of interspecific competition with a functionally similar species (two levels: competition and no competition). For the field survey data, we tested for correlations between our metrics of trophic niche width of each species and the corresponding CPUE of its functionally similar competitor. To ensure comparability in the isotopic data among survey sites and years, we used PERMANOVA to test for any effect of site or year on the stable isotopic signature of putative resources (biofilm, macrophytes, detritus and dominant invertebrate taxa). We found no differences in stable isotope values of putative resources either among survey sites or years (see Appendix 2), indicating that any differences in trophic niche width between crayfish and crabs were attributable to actual dietary variation.

Finally, we used the Bayesian mixing model Stable Isotope Analysis in R (SIAR; Parnell *et al.* 2010) to estimate the contribution of various putative food resources to the diets of crab and crayfish in the field experiment. The model integrates variability in resource isotope values explicitly, providing a distinct advantage over other mixing models. Fractionation factors used between resources and consumers were 0.4 ± 0.17 ‰ for δ^{13}C and 2.3 ± 0.28 ‰ for δ^{15}N (McCutchan Jr *et al.* 2003). We ran separate mixing models for each individual using leaf litter, macrophytes, biofilm and dominant invertebrate species (from the families Hydropsychidae, Oligochaeta, Leptophlebiidae, Heptageniidae, Chironomidae, Baetidae and Simuliidae) as potential resources for both species. We then used PERMANOVA to test for variation in the diet of crayfish and crabs in sympatric and allopatric conditions, following the statistical model outlined previously, and SIMPER to elucidate the contribution of each putative food resource to the mean Bray-Curtis dissimilarity between the diets of the two species.

Results

Field experiments

There was a significant interaction between crayfish and crab presence on rates of leaf litter breakdown (ANOVA; $F_{1,36} = 12.66$, $P = 0.001$; all ANOVA and PERMANOVA tables are shown in Appendix 2). Allopatric populations of crayfish elevated breakdown rates significantly compared to the exclosures, whereas allopatric populations of crabs had no effect (Figure 4.2). Correspondingly, sympatric populations had an intermediate effect on breakdown rates (Figure 4.2). However, neither crabs nor crayfish affected the standing stock of epilithic algae.

The presence of crayfish altered the structure of benthic macroinvertebrate assemblages significantly (PERMANOVA; Pseudo-$F_{1,17} = 6.05$, $P = 0.004$), driven primarily by reductions in the abundance of hydropsychid caddis flies (Trichoptera) together with an increase in the abundance of oligochaete worms (SIMPER analysis; Table 4.2)). Further, the presence of crayfish reduced the density of benthic invertebrates (ANOVA, $F_{1,16} = 9.39$, $P = 0.007$, Figure 4.3). In contrast, crab presence had no effect on the structure or density of benthic invertebrate assemblages. However, benthic invertebrate taxon richness was reduced significantly in the presence of crayfish or crabs in both allopatry and sympatry (ANOVA; crayfish: $F_{1,16} = 6.76$, $P = 0.019$; crabs: $F_{1,16} = 4.84$, $P = 0.043$; Figure 4.3).

The diet of crayfish as inferred from stable isotope data differed from those of crabs (PERMANOVA; Pseudo-$F_{1,57} = 2.72$, $P = 0.048$) but the presence of a functionally similar competitor caused no shift in the inferred dietary preferences of either species. The differences in diet were driven by a greater preference by crayfish for benthic invertebrates, particularly Hydropsychidae, whereas crabs incorporated a greater proportion of biofilm into their diet (see diet SIMPER analysis in the Appendix). In spite of this, the nitrogen range (NR_b) of both crabs and crayfish was reduced significantly in the presence of the other functionally similar species (ANOVA; $F_{1,17} = 4.64$, P = 0.046; Figure 4.4) but there was no effect of species or competition on carbon range (CR_b).

The presence of a functionally similar competitor reduced growth rates of both crabs and crayfish significantly (as evinced by changes in both their fresh weight [ANOVA; $F_{1,44} = 10.09$, P = 0.003] and carapace length [ANOVA; $F_{1,81} = 4.02$, P = 0.048]; Table 4.3). However, we found no evidence that competition affected growth rates of either species disproportionately.

Field surveys

Crabs and crayfish occurred in sympatry at the two middle sampling sites (Sites 2 and 3) of the survey for at least two of the three study years (Figure 4.5). Both sites show a similar trend from 2008 to 2010 of crayfish CPUE increasing simultaneously with a decline in crab CPUE. In fact, crabs appeared to be locally extinct at Site 2 by 2010, while the crayfish population was seven times larger compared with the previous year. Allopatric populations of crayfish and crabs were maintained throughout the study period at, respectively, the most downstream (Site 1) and upstream (Site 4) of our survey locations (Figure 4.5). The decrease in crayfish CPUE at the most downstream site adjacent to Lake Naivasha (Site 1; Figure 4.1) is consistent with concurrent patterns in the lake, and is likely a consequence of competition with a more recent invader, the common carp (*Cyprinus carpio*), to the lake ecosystem (see Chapter Three).

We found significant inverse correlations between the abundance of crabs and both the carbon range (CR_b; $r_{11} = -0.80$, $P = 0.002$; Figure 4.6b) and nitrogen range (NR_b; $r_{11} = -0.67$, $P = 0.018$; Figure 4.6a) of crayfish over the four sites. However, neither measure of crab trophic niche width correlated significantly with the abundance of crayfish (CR_b: $r_{11} = -0.54$, $P = 0.071$; NR_b: $r_{11} = -0.53$, $P = 0.074$).

Tables and figures

Table 4.1. Response variables quantified during the two field experiments. The number of replicated enclosures/exclosures is shown in parentheses.

Experiment 1	**Experiment 2**
Litter breakdown rates (*n* = 4)	Litter breakdown rates (*n* = 6)
Algal biomass (*n* = 4)	Benthic invertebrate assemblage structure (*n* = 5)
	Stable isotope analysis (allopatric crayfish: *n* = 5 [24 individuals]; allopatric crabs: *n* = 5 [20 individuals]; sympatric crayfish: *n* = 5 [10 individuals]; sympatric crabs: *n* = 5 [10 individuals])

Table 4.2. Results of SIMPER analysis indicating the five benthic invertebrate taxa affected most strongly by the presence of crayfish.

Taxon	**Mean abundance in crayfish absence**	**Mean abundance in crayfish presence**	**Contribution to dissimilarity (%)**	**Cumulative contribution (%)**
Hydropsychidae	19.27	9.82	63.61	63.61
Oligochaeta	1.18	1.64	10.84	74.45
Heptageniidae	1.82	0.45	8.15	82.6
Baetidae	1.09	0.09	6.42	89.02
Heptageniidae	0.91	0.18	5.70	94.72

Table 4.3. Average growth of crayfish and crabs in the field experiments in allopatric and sympatric conditions.

	Total weight gain (g)		Carapace length growth (mm)	
	Experiment 1	**Experiment 2**	**Experiment 1**	**Experiment 2**
Allopatric Crayfish	2.10 ± 0.44	0.91 ± 0.30	3.50 ± 0.69	1.14 ± 0.24
Sympatric Crayfish	1.62 ± 0.65	0.16 ± 0.07	1.60 ± 0.51	1.06 ± 0.31
Allopatric Crabs	0.60 ± 0.40	0.41 ± 0.19	2.29 ± 1.02	0.57 ± 0.16
Sympatric Crabs	0.20 ± 0.03	- 0.32 ± 0.26	0.50 ± 0.29	0.28 ± 0.22

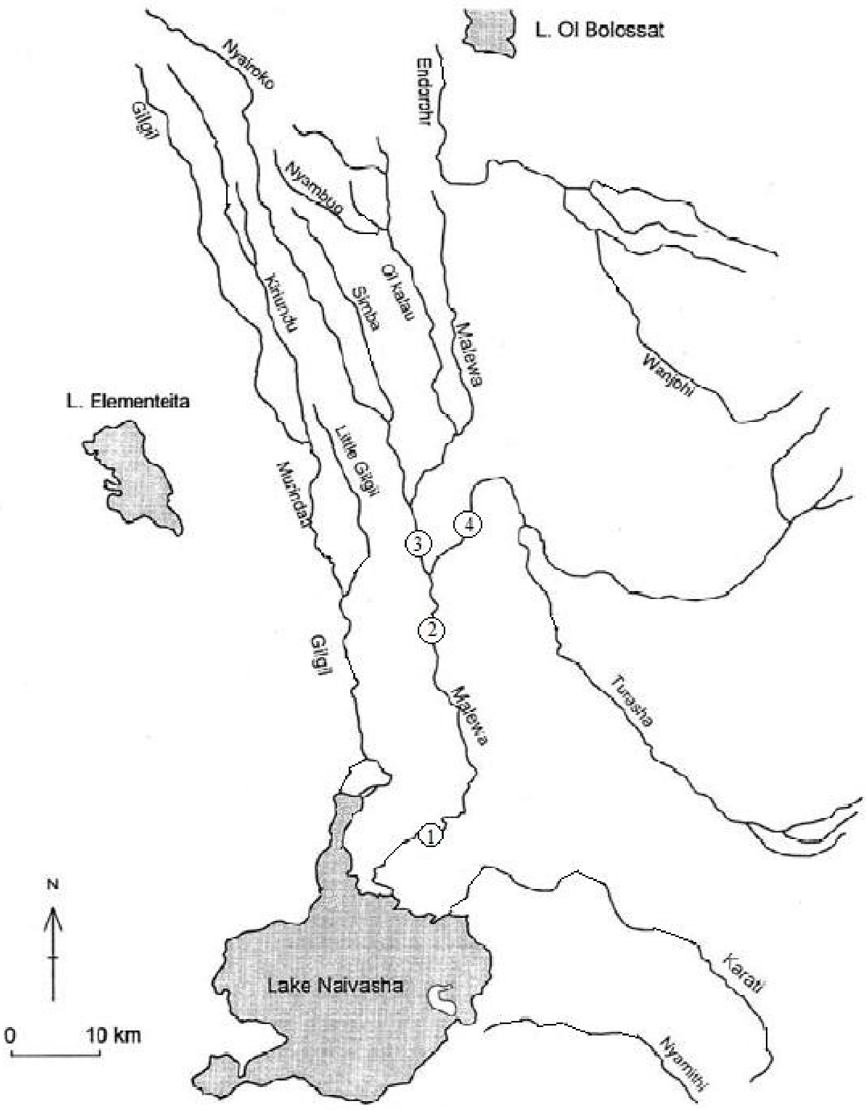

Figure 4.1. Lake Naivasha and its main inflows, showing the locations of the four field survey sites on the River Malewa. The two field experiments were located at Site 2.

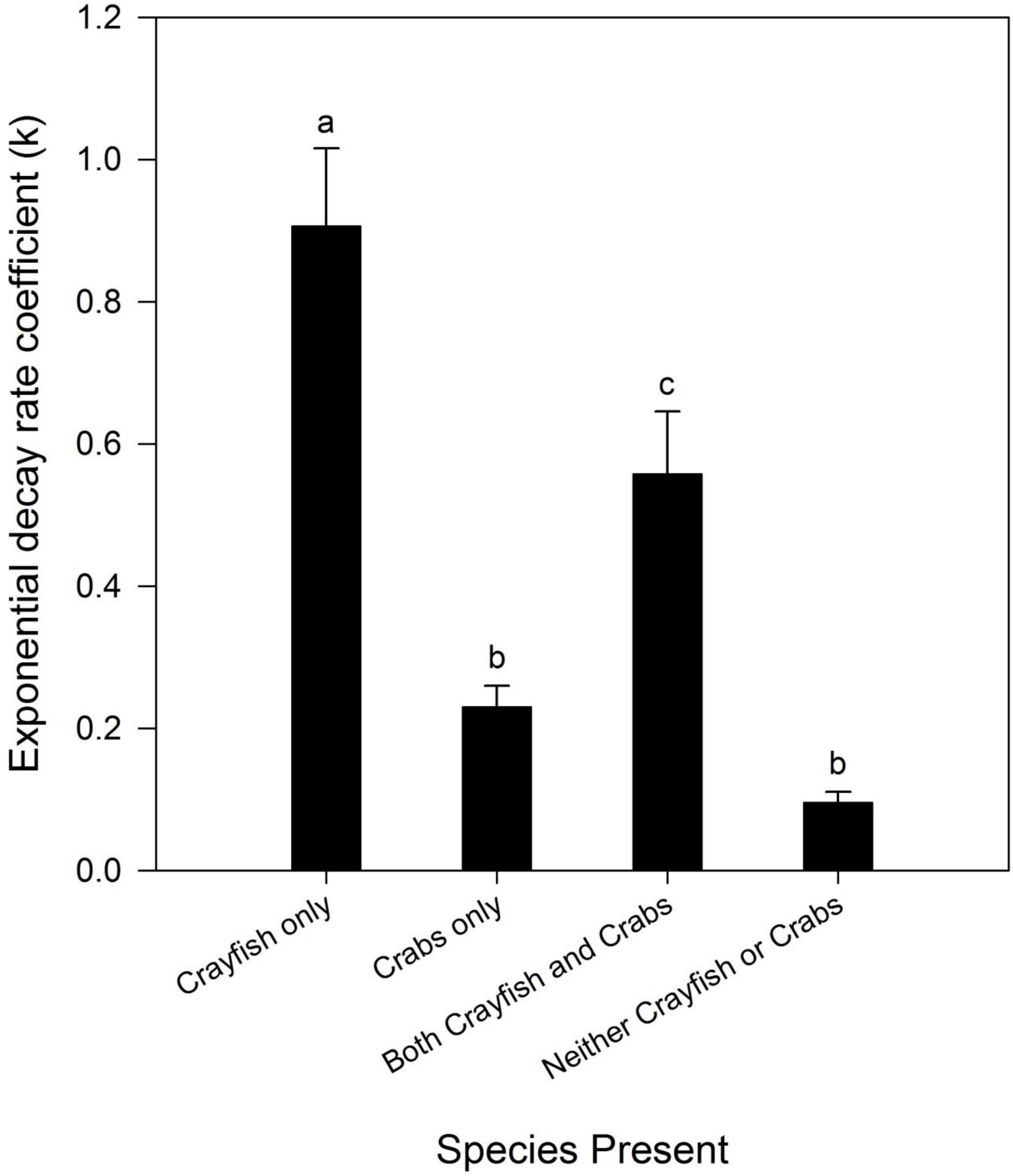

Figure 4.2. Leaf litter breakdown rates (mean ± s.e.) in the experimental cages, measured as exponential decay rate coefficients (*k*). Letters (a, b) indicate groups of treatments that are statistically indistinguishable from each other (P > 0.05).

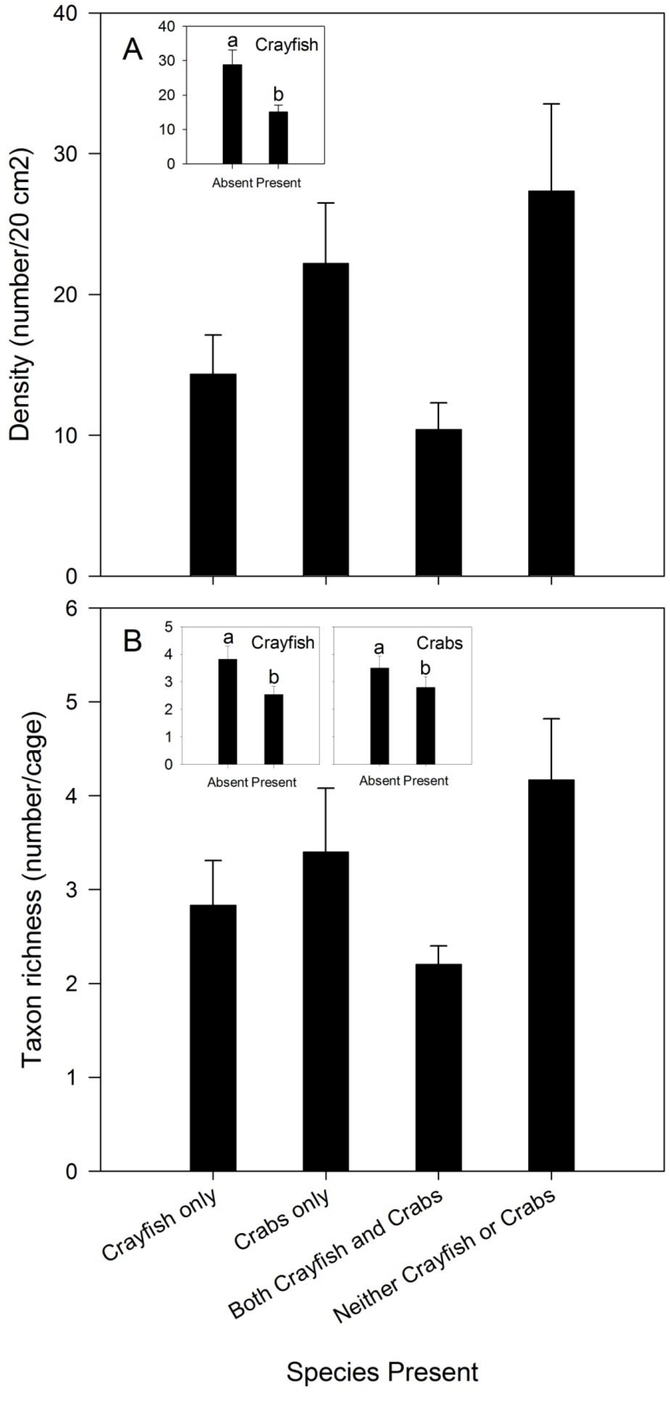

Figure 4.3. Total abundance (A) and taxon richness (B) of benthic invertebrate assemblages in the experimental enclosures (mean ± s.e.). Figure insets show effects of crayfish and/or crab presence and absence. Letters (a, b) indicate groups that are statistically indistinguishable from each other on each graph ($P > 0.05$).

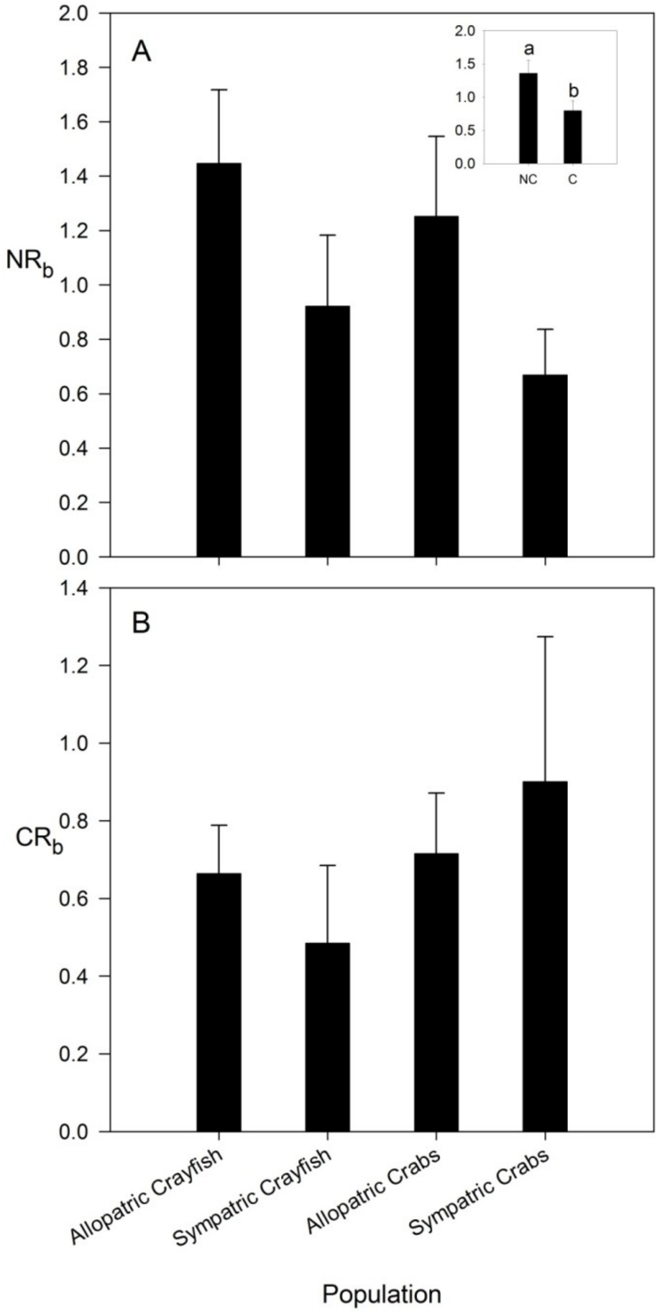

Figure 4.4. The nitrogen (A) and carbon range (B) of crayfish and crabs in both allopatric and sympatric conditions in the experimental enclosures (mean ± s.e.). Figure insets show effects of no competition (NC) and competition (C) on crabs and crayfish. Letters (a, b) indicate groups that are statistically indistinguishable from each other ($P > 0.05$).

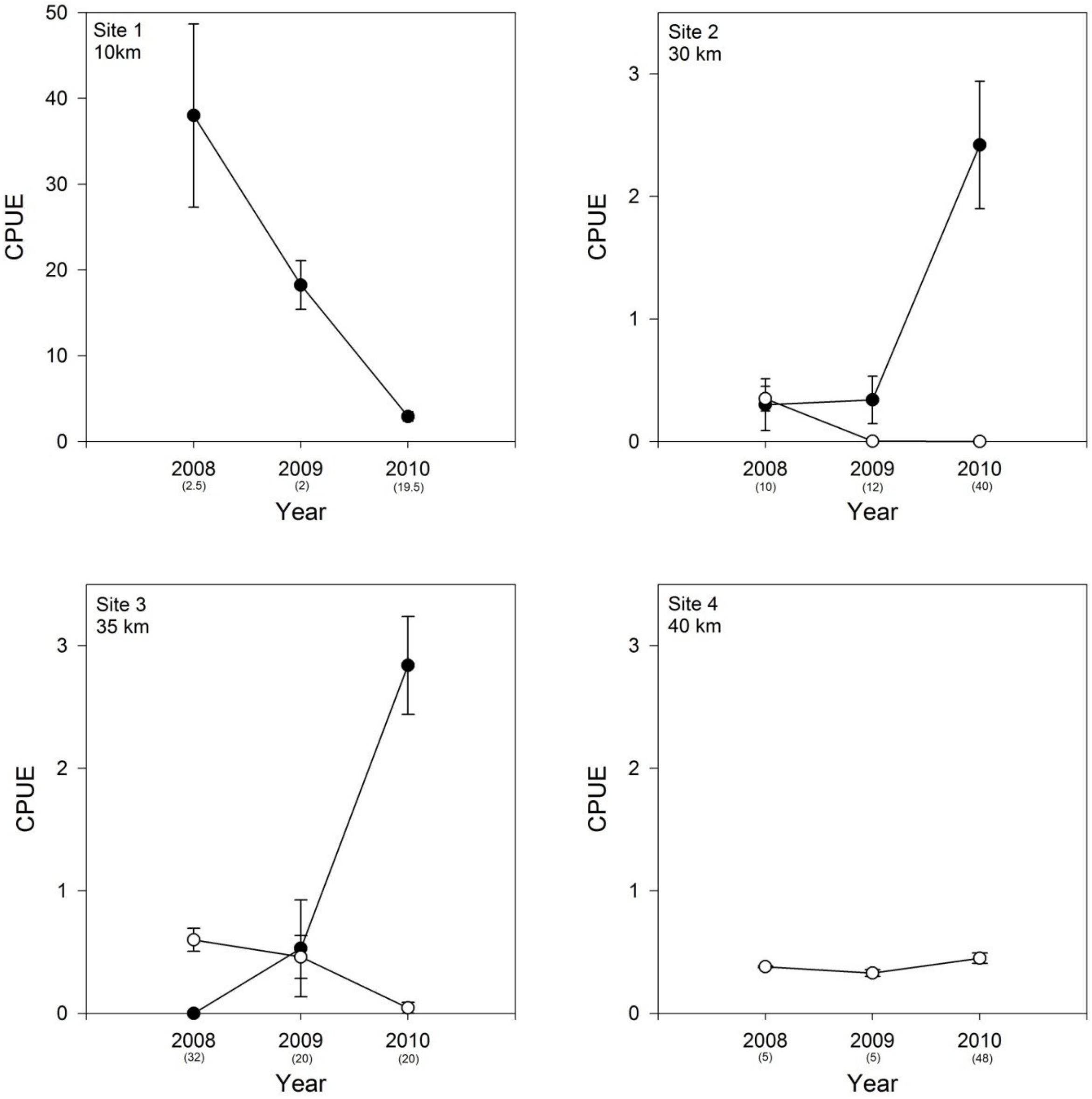

Figure 4.5. Catch per unit effort (CPUE) of crayfish (black circles) and crabs (open circles) from 2008-2010 based on multiple trapping sessions at four sites (mean ± s.e.). Total number of trapping hours is shown in parentheses on the x-axis. The distance indicated on the graphs refers to the total distance upstream from the lake. Note the different scale on the Y-axis for Site 1.

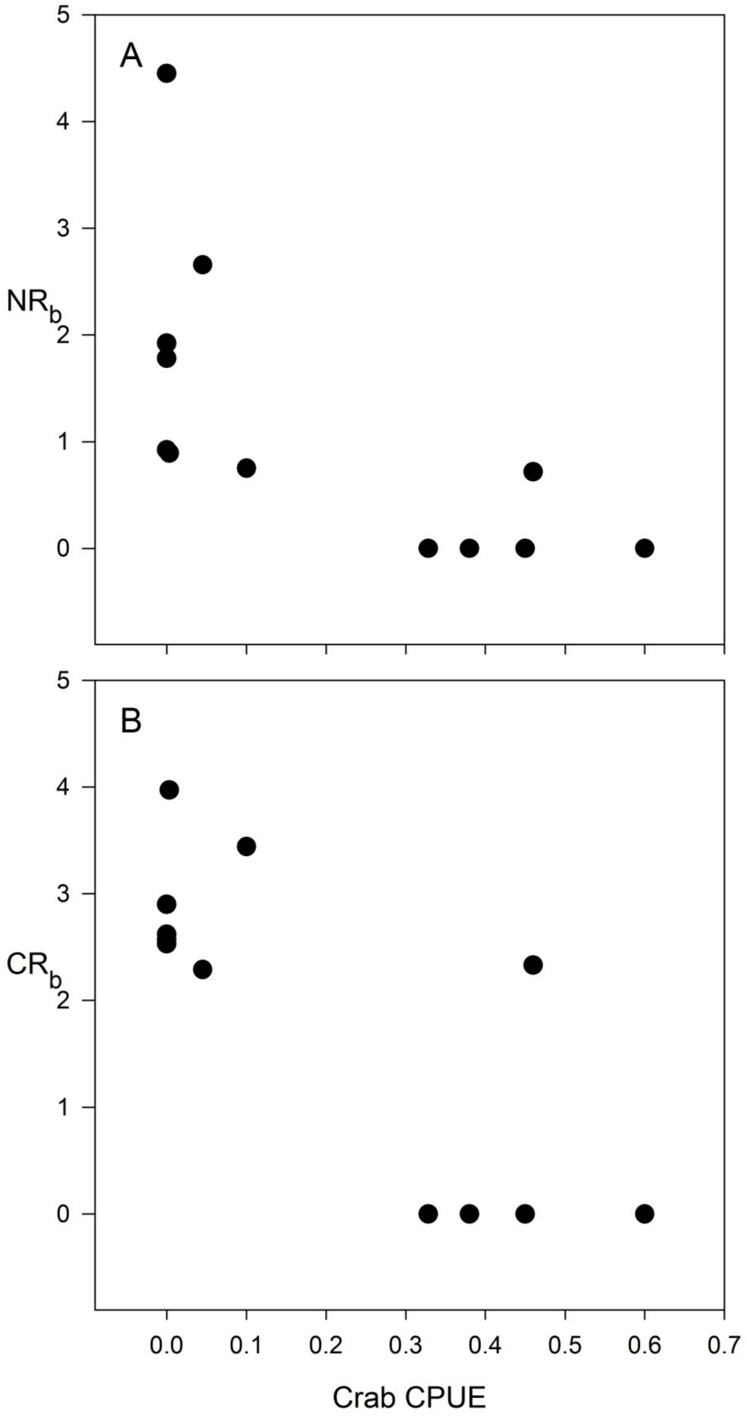

Figure 4.6. Relationships between crab CPUE and the nitrogen range (A) and carbon range (B) of crayfish in the field surveys.

Discussion

Biological invasions are a crucial aspect of global change and the diet of invaders will have ramifications for their impact on the ecosystem. In 1958, Elton proposed that invasive species can out-compete similar native competitors by occupying a broader niche. This classic mechanism of invasion was exemplified recently by Olsson et al. (2009), who showed that the trophic niche breadth of an invasive crayfish species (*Pacifastacus leniusculus*) was double the size of that of the native (*Astacus astacus*). Indeed, it is typically reported that invasive species occupy a broad niche and suppress the niche of similar natives (e.g. Thomson 2004). Invaders have been shown, for example, to reduce the foraging range of native competitors (e.g. Human & Gordon 1996; Thomson 2004) and displace them from refuge (Dunn *et al.* 2009). In marked contrast with this established mechanism of invasion, our findings demonstrate that constriction of niche breadth can also be integral to a successful invasion strategy. In our field experiments, competition between crayfish and crabs caused a decline in the utilised nitrogen range of both species. However, our field surveys revealed that the carbon and nitrogen range of the invasive crayfish was reduced significantly in the presence of crabs while we found no effect of crayfish on the niche width of crabs. This indicates strongly that the invasive crayfish population exhibited dietary niche constriction in order to reduce the intensity of competition with the functionally similar native crabs.

Although it has been shown recently that invasive species can alter their feeding behaviour in the presence of native species (e.g. Harrington *et al.* 2009), as far as we are aware this study provides the first example of the constriction of the dietary niche of a successfully invasive population in the presence of a functionally similar native species. Native crab abundance declined at those sites invaded by the crayfish over the three years of our study, becoming locally extinct at one. This finding was unexpected, given that niche constriction or partitioning usually promotes species coexistence (Finke & Snyder 2008; Gilbert *et al.* 2008).

Our study indicates a novel mechanism supporting successful invasion, whereby invasive species reduce their population niche breadth at the invasion front in order to reduce competition with well-established natives. We nonetheless found reduced growth rates of both crab and crayfish individuals in sympatric conditions, indicating that both species were still being affected negatively by the presence of the other. While a broad diet breadth may not be a reliable predictor of invasion success (e.g. Kolar & Lodge 2002), our results support the idea that diet flexibility through diet shifting and resource use efficiency could be a common trait of successful invaders (Tillberg *et al.* 2007; Zhang *et al.* 2010). Crayfish are omnivorous which allows them to survive when resources are limited by shifting their diet to alternative resources to compensate. This gives them an advantage as an invasive species when exploiting niches with novel resources, which may explain the widespread and global distribution of many invasive crayfish species (Capinha *et al.* 2011). Such flexibility in diet choice does not necessary imply a wide niche breadth because in some scenarios it may benefit the consumer to focus on one abundant resource (Popa-Lisseanu *et al.* 2007). However, examination of stable isotope-inferred dietary preferences, the results of which were supported strongly by direct analysis of gut contents (Jackson, *pers. obs.*), revealed no significant overall shift in the diets of individual crayfish or crabs in the presence of a functionally similar competitor. Therefore, the fact that we found significant reductions in the trophic niche breadth of crayfish populations in the presence of crabs in both the field experiments and field surveys indicates strongly that, although the overall dietary preferences of crayfish were not altered in the presence of crabs, the individuals within the crayfish population were tending to feed in a more similar manner, i.e. playing to their strengths, when in the presence of crabs. In other words, the presence of a functionally similar competitor triggered dietary homogenisation within the crayfish population.

Since the crayfish and crab species in our study ecosystem are both relatively large decapod Crustacea, an *a priori* assumption of our study was that individuals of each, of the same biomass, would have similar effects on measures of ecosystem structure and functioning in our experimental enclosures. However, invasive crayfish had a considerably greater influence on detrital processing as measured by markedly increased leaf litter breakdown compared to native crabs. Gut content analysis demonstrated that this was due to direct consumption (Jackson, *pers. obs.*); indeed, leaf litter is considered to be an important part of crayfish diet when available (Bobeldyk & Lamberti 2008). Previous studies have found that functionally similar sympatric crab species can interact to moderate one another's impact due to consumer redundancy (Griffen & Byers 2008). However, we found intermediate leaf litter breakdown rates (i.e. the sum of the allopatric impact of two individual crabs and two individual crayfish) in the sympatric treatment in our field experiment suggesting there was no consumer interaction. We also found that invasive crayfish can severely alter benthic invertebrate assemblage structure and reduce their densities considerably, consistent with previous research (Stenroth & Nyström 2003; Lodge *et al.* 2005). However, native crabs had no effect on benthic assemblages. The most abundant benthic invertebrate taxon at the experimental site, larvae of the hydropsychid caddis fly, was reduced most heavily in the presence of crayfish. Gut content analyses supported strongly the stable isotope-inferred diets and confirmed this taxon as a prominent dietary item indicating that direct consumption was the likely cause of their decline (Jackson, *pers. obs.*). Thus, in spite of the strong similarities between these decapod crustaceans, replacement of the native crabs by the invasive crayfish would likely alter both ecosystem structure and functioning considerably. Moreover, these shifts in ecosystem structure and functioning may even have facilitated indirectly the replacement of the native crabs by the crayfish (Parker *et al.* 1999).

The mechanisms and consequences relating to replacement of native crayfish by invasive crayfish have been well documented from around the world (e.g. Hill & Lodge 1999; Nakata & Goshima 2006), while interactions among native river crabs and invasive crayfish have been mostly overlooked with a few exceptions (e.g. Foster & Harper 2007). Our results suggest that invasive crayfish are replacing native crabs in the River Malewa, with important structural and functional consequences at the ecosystem level. Such changes have the potential to alter considerably ecosystem services such as fishery production and water quality, both extremely important in this arid region of East Africa. Additionally, many river crabs in East Africa are already highly endangered (Cumberlidge *et al.* 2009) and local biodiversity will only become increasingly impaired as crayfish spread or are introduced further in both rivers and lakes (Lodge *et al.* 2005). Therefore, this study has important implications for invasive species management throughout the African continent and beyond.

Our study highlights that the mechanisms of invasion are not fixed across species and ecosystems. We have shown that a globally widespread invasive crayfish can reduce its trophic niche breadth in the presence of a native competitor in order to facilitate invasion. It is important to consider variation in the mechanisms of invasions both to enhance management strategies on global change and to predict impacts of successful invaders on ecosystems.

Chapter Five: Accelerating invasion rates in a highly disturbed catchment

This chapter is formatted as submitted to the journal *Biological Invasions*. Michelle C. Jackson and Jonathan Grey.

Abstract

Invasive species are a significant component of global change with major implications for biodiversity and ecosystem processes. Although sympatric invaders are expected to interact and alter one another's impact, most studies focus on individual conspicuous invaders. Global invasion rates are accelerating and homogenising the world's fauna and flora, and so it is important to understand multiple invasions. Here we present data on the freshwater non-indigenous species established in the catchment of the River Thames, England. We identified a total of 96 non-indigenous species, 55% of which were introduced intentionally to England. Our analysis shows that 53% of the species became established in the last 50 years and invasion rates have significantly increased since 1800. Analysis of shipping records revealed a positive correlation with non-indigenous species establishment, suggesting the global transport network has facilitated many species invasions. Our calculated modern (post 1961) invasion rates predict that one non-indigenous species will become established every 50 weeks, despite legislation aiming to prevent their establishment, making the Thames catchment one of the most highly invaded freshwater systems in the world.

Introduction

Increased international trade has fostered the spread of species outside their native ranges (Cohen & Carlton 1998) and other environmental disturbances, such as habitat destruction, have facilitated the establishment of non-indigenous species (Marvier *et al.* 2004). Although some introductions may have neutral impacts on ecosystems, other species become invasive and have adverse effects on native assemblage composition and ecosystem functioning (Hooper *et al.* 2005). The spread of invasive species is homogenising the world's biota (Smart *et al.* 2006) and invaders can have dramatic and often irreversible effects on diversity and ecosystem services, frequently with considerable economic implications (Crowl *et al.* 2008; Pejchar & Mooney 2009). Hence, it is within human interest to recognize the invasive distribution of species.

Freshwater ecosystems are particularly susceptible to invasions by non-indigenous species because they are highly manipulated by man (Dudgeon *et al.* 2006; Strayer 2010). Species are often introduced intentionally for culinary or sport purposes or inadvertently, such as in the ballast water of boats (Ruiz & Carlton 2003). Furthermore, water-ways such as rivers and canals are convenient highways, allowing swift dispersal rates and acting as links between different habitats (Rahel & Olden 2008). Many aquatic ecosystems sustain numerous non-indigenous species (e.g. Cohen & Carlton 1998; Ricciardi 2006) and yet interactions among them are rarely examined. Sympatric invaders have the potential to interact and alter one another's impact on ecosystem structure and functioning (e.g. Grosholz 2005; Griffen *et al.* 2008; Johnson *et al.* 2009b) and hence it is important to catalogue multiple invasions.

The spread of non-indigenous species is a global problem and several international, European and domestic policies exist that aim to reduce the spread and impact of non-indigenous species including the updated UK Wildlife and Countryside Act of 1981. This states that it is illegal to allow any animal “which is not ordinarily resident in and is not a regular visitor to Great Britain in a wild state” to escape into the wild. The European Union’s Water Framework Directive (2000) requires that all water bodies achieve “good ecological status” by 2015. Water bodies containing detrimental invasive species may not meet this requirement which highlights the importance of developing knowledge on the range and vectors of invaders.

The River Thames and its catchment has been heavily disturbed in the past as a result of a dense human population in the surrounding area (Francis *et al.* 2008). Furthermore, there are high levels of river traffic and the estuary is a busy international port, endorsing the possibility of high rates of invasion. Here we aim to catalogue the freshwater invasive species present in the Thames and its catchment and to quantify invasion rates in relation to accelerating globalisation. We use available literature, databases and our own field data to compile a list of the aquatic invasive species present in order to examine vectors of establishment, native range and estimate invasion rates.

Methods

Available literature and databases (see Appendix 3) on established non-indigenous species were used alongside our own field data to obtain a list of non-indigenous species in the Thames catchment. The list includes established aquatic species that spend all or part of their life cycle in freshwater ecosystems. Established species were defined as those which have maintained a natural breeding population for at least one generation.

We assigned each species to a category of *Vector of introduction*; stocking (of sports fisheries), aquaculture/farming (food and fur), ornamental species (ponds and aquaria), contaminants of stocking, aquaculture/farming and ornamental stocks, and species that were accidently transported by other means (e.g. in the ballast water of boats). We also calculated the number of species that were native to each continent, counting them more than once if their native range covered multiple continents. Finally, *Year of establishment* is defined as the first record of a species within the catchment. If the literature stated a range of years, we used the latest year. A reliable year of discovery was available for 83 species and these were grouped by decade to analyse invasion rates using correlation analysis. A significant correlation between decade of establishment and number of invasive species discovered a decade would indicate that the rate of establishment has changed. Stepwise regression was used to explore the relationship between invasion rate and decade.

The amount of cargo passing through the Port of London was used as a measure of shipping activity; the data were sourced from the Port of London Authority (PLA) box file on trade statistics and PLA annual reports held by the Museum of London Docklands. The number of tonnes of cargo passing through the port was available for at least one year a decade from 1830. We tested for a correlation between the annual average cargo passing through the port and the number of species established each decade. All statistical analyses were carried out in Minitab-14$^{®}$ and images were created in SigmaPlot-10$^{®}$.

Results and discussion

In total, 96 non-indigenous freshwater species have been recorded as established in the Thames catchment (Table 5.1; see Appendix 3 for the full list), 3 of which are now extinct in the wild in the UK. This total comprises 72% of all freshwater non-indigenous species established in the UK (Keller *et al.* 2009) highlighting the high levels of invasion in the London area and the possibility that the Thames catchment is the original source of many non-indigenous species in the UK. A long history of human activity in the region has probably facilitated many of the established non-indigenous species since disturbances, such as habitat destruction, can favour invaders (Marvier *et al.* 2004).

The most common non-indigenous taxonomic groups in the Thames catchment were plants, followed by fish (Table 5.1). Platyhelminthes and non-decapod crustaceans also made noticeable contributions to the total, largely due to the accidental introduction of parasites which comprise 9/10 of the established platyhelminthes and 6/10 of the non-decapod crustaceans. The majority of non-indigenous species recorded in the Thames catchment were native to North America (n=38), followed by Asia (n=29) and Europe (n=24). Other European countries, including France and Germany, also receive many non-indigenous species from North America via independent pathways (García-Berthou *et al.* 2005). A total of 53 species were introduced intentionally for farming, fisheries or in the ornamental trade (Table 5.1). The remaining species were either introduced as contaminants of the above trades or accidently transported by other means, such as in the ballast water of boats (Table 5.1). The relative abundance of species from each taxonomic group established each decade has changed over time, simultaneous with changes in the prominent vector of introduction (Figure 5.1). For instance, since 1920 there was an increase in the number of discovered plants and fish in concordance with an increase in species originating from the ornamental and aquarium trades (Figure 5.1). There was also a noticeable increase in species arriving accidently as contaminants of other intentionally introduced species (Figure 5.1).

Our analysis shows that 53% of the non-indigenous species in the Thames catchment were recorded after 1961. Hence, half of the established non-indigenous species in the region have been introduced in the last 50 years. Between 1800 and 2010, the rate of establishment accelerated for both intentionally and accidentally introduced non-indigenous species (Figures 5.2 and 5.3). Invasion rates significantly increased over time (Intentional: $n = 21$, Pearson correlation = 0.78, $P<0.001$; Accidental: $n = 21$, Pearson correlation = 0.69, $P<0.001$), reaching 1.3 species per year between 1980 and 1989 (Figure 5.3). The Wildlife and Countryside act of 1981 aimed to eradicate the establishment of invasive species in Great Britain. Since then, 15 accidentally introduced species and 11 species introduced intentionally to Great Britain have become established in the Thames catchment. Invasion rates appear to have fallen after 1990 and between 2001 and 2010 the rate was 0.6 species per year (Figure 5.3), suggesting that the legislation may have been effective. However, it often takes a number of years for novel non-indigenous species to be discovered and hence, we consider that this rate is conservative (Keller *et al.* 2009).

Between 1800 and 2010 the average rate of invasion was 0.43 species per year which is slightly less than the overall invasion rate of UK freshwaters during the same period (0.67 species per year; Keller *et al.* 2009). The highest recorded rates of invasion in aquatic ecosystems were 3.7 species per year between 1961 and 1995 in San Francisco Bay (Cohen & Carlton 1998), 1.8 species per year between 1961 and 2006 in the Great Lakes (Ricciardi 2006) and 1.2 species per year between 1961 and 1999 in the Baltic Sea (Leppäkoski & Olenin 2000). In the Thames catchment, the invasion rate between 1961 and 2010 was 1.04

species per year, similar to modern (post 1961) invasion rates in the Hudson River Basin (1 species per year; Mills *et al.* 1996), making the Thames catchment one of the most highly invaded freshwater systems in the world. Moreover, high invasion rates appear to be sustained, despite the efforts of the Water framework directive to achieve “good ecological status” of all water bodies by 2015.

Shipping activity in the Port of London (measured in tonnes of cargo passing though the port from 1830) was positively correlated with non-indigenous species establishment (n = 17, Pearson correlation = 0.81, $P < 0.001$) suggesting that the spread of non-indigenous species is facilitated by the global transport network. Up to 90% of the worlds trade is carried by sea (Kaluza *et al.* 2010) and therefore it is likely to be a major contributor to both accidental and intentional biological invasions. Furthermore, an analysis of global cargo ship movements indicated that ports in Western Europe are highly connected to ports on the East coast of North American (Kaluza *et al.* 2010) and we found that nearly 40% of the non-indigenous species in the Thames catchment were native to North America. The Peak of shipping activity in the Port Of London was in the 1960s (60 million tonnes) and the following decade saw the highest recorded invasion rate in the catchment (1.3 species per year). We suggest that this is due to a lag time between the invasion of a species and its discovery.

Tables and figures

Table 5.1. Non-indigenous species organised by taxonomic group and vector of introduction. Vector of introduction abbreviations are as follows; stocking (S), aquaculture/farming (A), ornamental trade (O), contaminant (C), contaminant of stocking (C-S), contaminant of aquaculture/farming (C-A), contaminant of ornamental trade (C-O) and accidental transportation (T).

Group	S	A	O	C	C-S	C-A	C-O	T	Total
Viruses							1		1
Fungi						1			1
Plants		2	20				1		23
Nematodes						1	1		2
Oligochaetes							2	1	3
Platyhelminthes					1	4	4	1	10
Insects				1	1				2
Cnidarians							1		1
Molluscs								8	8
Crustacean Decapods		2	2					1	5
Crustacean non-decapods				3	1		2	4	10
Fish	5	1	8		1				15
Amphibians		1	2				1		4
Birds			7						7
Mammals		3						1	4
Total	5	9	39	4	4	6	13	16	96

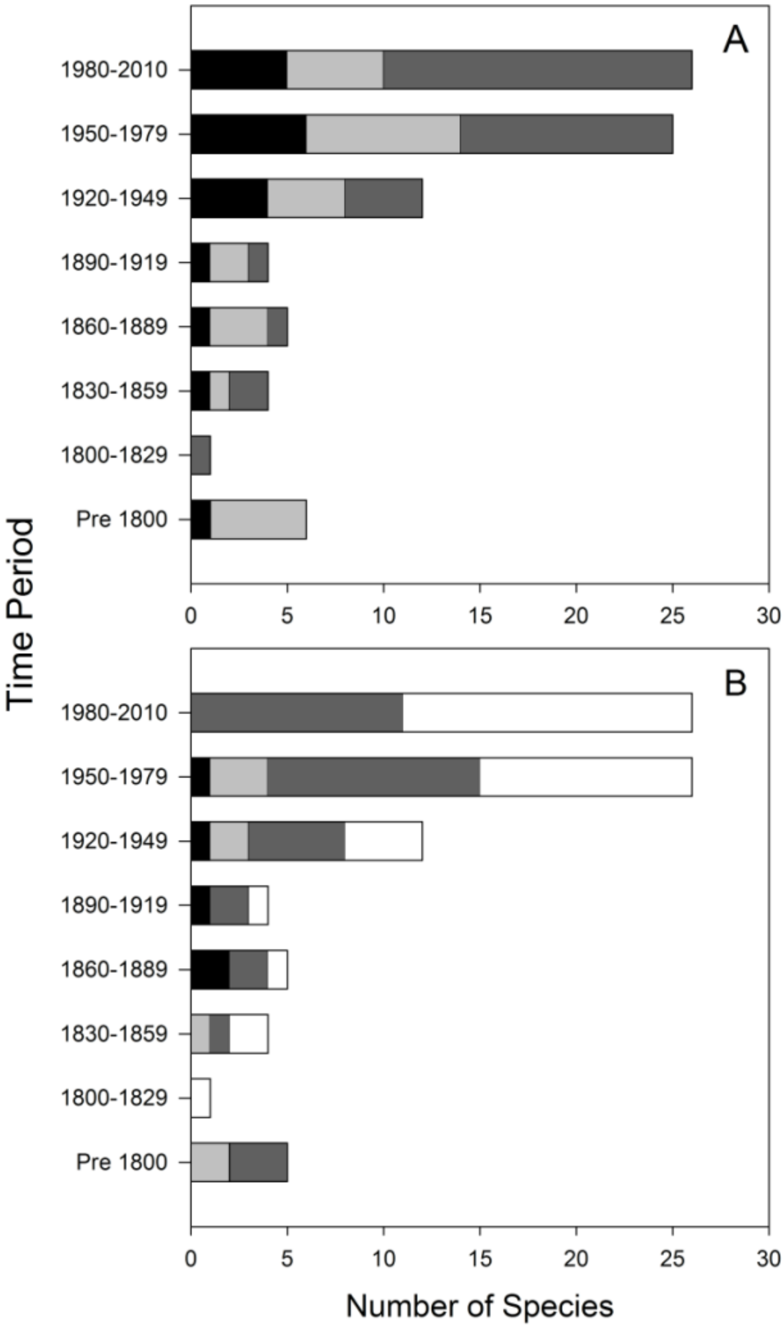

Figure 5.1. The non-indigenous species established every 30 years organised by taxonomic group (A) and vector of introduction (B). In A, black represents plants, light grey represents vertebrates and dark grey represents invertebrates. In B, black represents stocking, light grey represents ornamental trades, dark grey represents aquaculture/farming and white represents contamination or accidently transported.

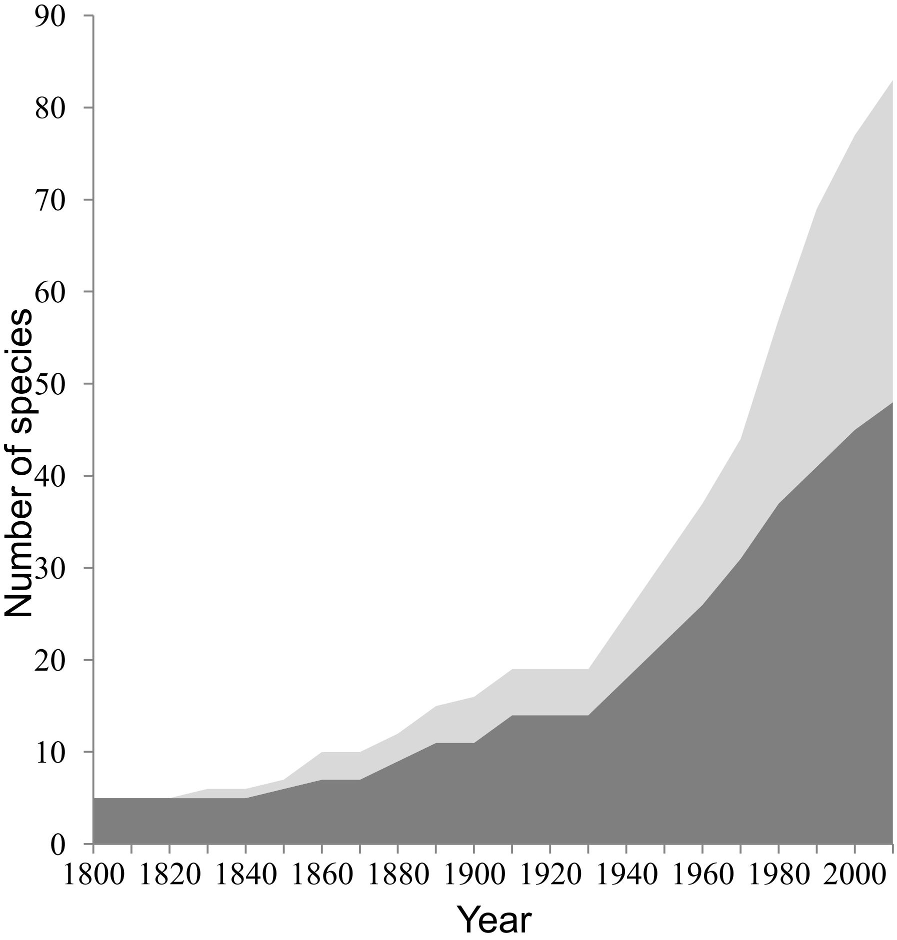

Figure 5.2. Cumulative total of intentionally (dark grey) and accidently (light great) introduced non-indigenous freshwater species.

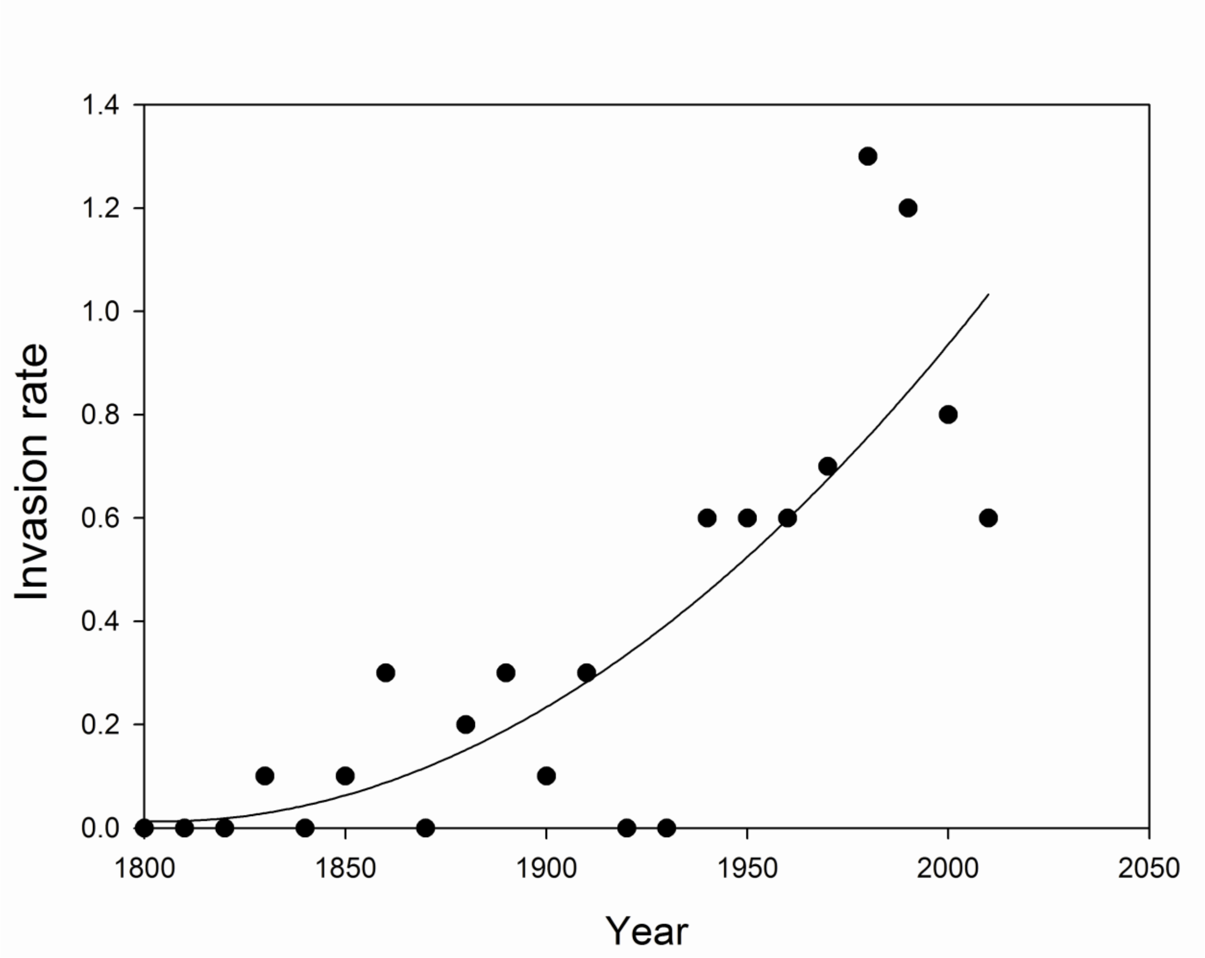

Figure 5.3. Average annual invasion rate calculated for each decade to overcome lags in reports of established non-indigenous species. Stepwise regression: *R-sq* = 63.7, $P < 0.001$.

Conclusions

Habitat manipulation (Francis *et al.* 2008) and climate change (Johnson *et al.* 2009a) are clearly having marked effects within the Thames catchment and it is now clear that non-indigenous species are also a significant component of global change in this region as a result of human activity. Invasion rates in the Thames catchment match those of other highly invaded aquatic systems despite covering a smaller area, yet the ecological effects of these invasions are not well-studied. It appears that human mediated invasions will continue to be a problem in the Thames, and hence a focal point for UK introductions, since invasions rates are still on an accelerating trajectory with no real sign of a plateau, regardless of recent legislation.

Chapter six: Incorporating resource heterogeneity and individual specialisation into measures of isotopic niche width; application to invasion ecology

Declaration on input

Adam Ellis, from the Environment Agency in Hertfordshire, provided me with the data on crayfish distribution in the Thames catchment and assisted with crayfish trapping in the Lee Navigation.

Abstract

Stable isotope ecology is a growing field and recent studies have shown how variation in isotope values among individuals of the same population can be used as a quantitative measure of dietary niche width. These measures, including ellipse areas and convex hulls have their applications, however they do not incorporate resource heterogeneity and individual specialisation which could result in flawed estimates of niche size. Here I show how resource variation can be incorporated into the ellipse area measure of niche width and describe a novel method to estimate niche size based on stable isotope Bayesian mixing models. This method is the first measure of dietary niche width that considers both resource heterogeneity and individual specialisation. I demonstrate the application of these novel methods using the four species of invasive crayfish (Louisiana red swamp, signal, Turkish and virile) that are found in the Thames Catchment. The diet of an invasive species has implications for its success and impact on the ecosystem and therefore it is an important aspect of invasion ecology. Stable isotope metrics revealed a high degree of dietary plasticity in all four species of crayfish and low levels of individual specialisation reveal that the invaders are true generalists. Some niche partitioning between the four invasive species and evidence of niche shifts to readily available resources suggests that they could coexist in the UK.

Introduction

The ecological niche was originally described by Hutchinson (1957) as a hypervolume in n-dimensional space with environmental variables as axes. A multivariate combination of the environmental variables utilised by a species, including resource and habitat use, and the influence of predators and competitors will all contribute to the ecological niche of an organism (Van Valen 1965; Newsome *et al.* 2007). Niche dimensions relating to diet are an important aspect of the ecological niche since food web interactions, including predator-prey relationships and interspecific competition for resources, are fundamental in structuring communities (Hutchinson 1957; Semmens *et al.* 2009).

The total niche of a species is influenced by lower levels of biological organisation, including populations and individuals (Semmens *et al.* 2009). In some instances, individual variation in diet can account for most of the total population niche width (Bolnick *et al.* 2003). The possibility of heterogeneous individuals should therefore be considered when measuring niche width (Bolnick *et al.* 2010). The total trophic niche width of a population (TNW) can be defined as the total variance in the resources used by all members of that population (Roughgarden 1972; Bolnick *et al.* 2010). Hence, TNW has a within individual component (WIC) and a between individual component (BIC; Roughgarden 1972; Bolnick *et al.* 2002; Bolnick *et al.* 2010). The WIC is the variance in the use of different resources by a typical individual; does the individual use all the available resources equally or specialise in one resource? The BIC is the variation in the use of a particular resource by all individuals; do all individuals use the resource in equal or unequal proportions? Hence, TNW = WIC + BIC (Roughgarden 1972; Bolnick *et al.* 2002). If total niche width can be explained by variation between individuals (BIC; Figure 6.1 A), the population comprises many dietary specialists, each utilising a fraction of the whole populations dietary niche (Bolnick *et al.* 2002; Svanbäck & Persson 2004). On the other hand, if total niche width can be explained by resource use variation within individuals (WIC; Figure 6.1 B), the population comprises many dietary generalists, all utilising the entire populations dietary niche (Bolnick *et al.* 2002; Svanbäck & Persson 2004). Hence, a wide niche breadth at the population level can be achieved by high levels of individual specialisation or by high levels of generalism within individuals (Figure 6.1). In contrast, a true specialist population would consist of all individuals feeding on the same resource (Figure 6.1 C).

Recently, stable isotope data have been used to quantify the niche width of an organism (Layman *et al.* 2007b; Newsome *et al.* 2007; Bolnick *et al.* 2010). In Chapters Three and Four I showed how intra-population variability in δ^{15}N and δ^{13}C can be used to calculate a Standard Ellipse Area (SEA_c) as a measure of isotopic niche that is robust to variations in sample size (Jackson *et al.* 2011). However, the WIC of a population's total niche width is not incorporated into SEA_c. Despite this, the application of SEA_c is a useful tool to examine the niche width of populations providing that the isotope variability of putative resources remains consistent across the spatial and/or temporal scale of the study (i.e. no significant differences in resource isotope variability, as in Chapters Three and Four). However, resource heterogeneity will result in un-comparable SEA_cs since the isotopic area covered by putative resources will influence consumer SEA_c regardless of actual niche width (Figure 6.2). Thus, it is important to consider variability in the isotopic composition of resources when calculating niche width using a stable isotope approach (Semmens *et al.* 2009). In this chapter I firstly aim to adapt SEA_cs to incorporate resource isotopic variability so that SEA_c can be used as a measure of isotopic niche width when the isotopic composition of resources across the study scale is statistically distinguishable. Secondly, I describe a novel method of calculating a population's trophic niche from stable isotope data that incorporates both the WIC and BIC of a population's TNW. My approach uses the Bayesian mixing model SIAR and thus the isotope composition of putative resources to estimate the proportional contribution of resources to individuals or populations (Parnell *et al.* 2010). This measure of TNW (called SIAR-TNW for the purpose of this paper) accounts for resource variability and hence, it is the first measure of isotopic TNW that can confidently be compared across spatial and temporal scales. Finally, I show how SIAR can be used to quantify the degree of individual specialisation in a population by adapting equations first developed by Roughgarden (1972). I apply these novel measures of trophic diversity to a neglected aspect of invasion ecology; the role of diet in invasion success.

In Chapter Five, I illustrated how human activities have promoted the worldwide spread of many aquatic non-indigenous species (e.g. Cohen & Carlton 1998; Ricciardi 2006). The trophic ecology of an invasive animal plays a key role in the invasion process and the capability of individuals to exhibit plasticity in resource choice can be fundamental in determining invasion success (Sakai *et al.* 2001; Sol *et al.* 2002; Zhang *et al.* 2010). Classic theory suggests that invasive species have a wider niche than their native counterparts (Elton 1958) and this is supported by empirical data (e.g. Human & Gordon 1996; Olsson *et al.* 2009). Furthermore, Jeschke and Strayer (2006) showed how diet breadth is positively correlated with invasion success in mammals and birds. However, in Chapter Four, I showed how Louisiana red swamp crayfish exhibited a novel mechanism of invasion in rivers in Kenya to invade an occupied niche. Niche restriction in the presence of a native crab allowed the crayfish to establish a population by reducing niche overlap, and therefore potential competition, with the crabs. Hence, a wide niche breadth may not always be a successful mechanism of invasion and instead, flexibility in diet choice can be more important. I propose that plasticity in resource choice and resource use efficiency are vital to the success of crayfish invasions. Plasticity in resource choice, or diet flexibility, allows the fitness of a species to remain constant across a range of environments and thus, promotes invasion success (Tillberg *et al.* 2007).

Here I use invasive crayfish from across a spatial and temporal scale to demonstrate the application of new measures of TNW. I also demonstrate how stable isotope analysis can be used to examine trophic diversity, diet shifting, and the degree of individual specialisation. Crayfish are omnivores and therefore the impact they have on food web structure is typically profound (e.g. Lodge *et al.* 1994). Omnivory can decouple trophic cascades and alter the energy flow in an ecosystem and therefore crayfish have a disproportional impact on food web dynamics (Nyström *et al.* 1999). Consequently, it is important to understand the trophic structure of invasive crayfish populations, particularly in ecosystems with multiple species of invasive crayfish, such as the Thames catchment. The generalist and omnivorous nature of crayfish diet is thought to facilitate invasion. However, the role of diet has yet to be explicitly linked to crayfish invasion success. Incomplete dietary information can hinder the understanding of the community wide effects of an invasion (Tillberg *et al.* 2007) and therefore it is important to understand the trophic ecology of invaders.

There are seven species of crayfish in England, six of which are invasive. In Chapter Five, I showed that four of these invasive species are present in the Thames catchment, but little is known of their ecology here. The four species are Louisiana red swamp crayfish (*Procambarus clarkii*), Turkish crayfish (*Astacus leptodactylus*), signal crayfish (*Pacifastacus leniusculus*), and virile crayfish (*Orconectes virilise*). They have highly variable distributions and densities which could indicate different degrees of invasion success. Here, I investigate the diet of these crayfish using stable isotope methods in order to elucidate any variability between species and to reveal the role of diet in crayfish invasion success.

Calculation of novel stable isotope metrics

Corrected Standard Ellipse Areas

The population metric SEA_c is the area of an ellipse in isotopic space that encompasses the population's mean core isotopic niche (Jackson *et al.* 2011). The subscript '$_c$' indicates that a small sample size correction factor was used (see Chapter Three). Stable isotope-derived metrics, such as SEA_c, are affected by the isotopic signatures of putative resources because the isotopic composition of consumers reflects that of their diet after fractionation (Grey 2006). This means that a larger SEA_c or isotopic area occupied by the resource assemblage may result in a larger consumer isotopic niche. Hence, two populations with a SEA_c of the same area may not actually have the same niche size unless the resources available to both populations also have the same SEA_c (Figure 6.2). If resources across a study scale are statistically distinguishable, SEA_cs are therefore not comparable. In order to overcome this hurdle, the SEA_c of the principal resource community must be considered in the calculation of niche width. This measure can then be used to correct the SEA_c of the consumer population:

$$\left(\frac{\text{consumer population SEA}_c}{\text{resource community SEA}_c}\right) \text{x } 100$$

The corrected SEA_c is expressed as a percentage of the resource community SEA_c. This gives a comparable corrected metric which is robust to variation in the resource assemblages and which can confidently be compared over spatial and temporal scales which encompass resource heterogeneity (see Figure 6.2).

SIAR derived measure of TNW (SIAR-TNW)

The Bayesian mixing model SIAR (Parnell *et al.* 2010) is used to provide an estimate of the proportional contribution of various resources assimilated by a consumer population. Fractionation factors (between resources and consumers) and concentration dependence can be incorporated into the model. Separate mixing models must be run for each individual sampled from a population (SIAR SOLO; Parnell *et al.* 2010), using all putative resources, SIAR-TNW is then calculated as:

$$WIC = \frac{\sigma\ (Pj|i)}{N}$$

$$BIC = \frac{\sigma\ (Pi|j)}{n}$$

$$SIAR\,TNW = WIC + BIC$$

Where p_{ij} is the proportion of resource j in the diet of the individual i, N is the number of individuals sampled and n is the number of putative resources (adapted from Roughgarden 1972). This gives a measure of niche width (SIAR-TNW) by calculating the typical population variance in resource use within an individual (WIC) and the typical variance between individuals in the use of each available resource (BIC).

Prior information of diet, based on observation, gut content and/or the literature is essential in order to select the resources included in the mixing model since SIAR-TNW is sensitive to how resource categories are defined. For instance, omitting a utilised resource from the model will result in a deflated measure of niche breadth (De Caceres *et al.* 2011).

SIAR derived measure of individual specialisation (SIAR-PS$_i$)

Separate mixing models must be run for each individual within a population (SIAR SOLO) and for the population as a whole (SIAR; Parnell *et al.* 2010) using all putative resources. The mean estimated proportional contribution of each resource to the diet of each individual and each population is then used to calculate diet specialisation using a proportional similarity index (PS$_i$), which quantifies the resource overlap between an individual and its population:

$$\mathrm{PS}_i = \sum_j \min(\mathrm{p}_{ij}, \mathrm{q}_j)$$

Where p_{ij} is the proportion of resource j in the diet of the individual i, and q_i is the proportion of resource j in the population as a whole (Roughgarden 1972). The level of individual specialisation in the population was then measured as the average of the PS_i values. A PS_i of 1.0 which indicate that individuals have exactly the same diet as the population average and a PS_i of 0.00 would indicate that individuals have a completely different diet from the population average.

Methods

Invasion success

The non-native range of an invasive species will give an indication of its success as an invader, therefore I used Environment Agency records to calculate a success rating (SR_i) for each species of crayfish within the Thames catchment:

$$\mathrm{SR}_i = \frac{\sum \mathrm{C}_i}{\mathrm{N}_i}$$

Where C_i is the total number of Thames sub-catchments that species *i* had been recorded in (from a total of 55) by 2009 and N_i is the number of years since the first record of species *i* in the Thames catchment.

Study sites

Our sampling sites were Hampstead Heath and the Lee Navigation in North London (Figure 6.3). Hampstead Heath comprises 18 man-made ponds, some of which are interconnected, with populations of Louisiana red swamp crayfish and Turkish crayfish. The Lee Navigation runs from Hertford to the River Thames and contains populations of both signal crayfish and virile crayfish. At Hampstead Heath I sampled the 'Bird Sanctuary Pond' for Louisiana red swamp crayfish and 'Highgate Pond 1' for Turkish crayfish (Figure 6.3). On the Lee Navigation I sampled near Carthagena Lock in Broxbourne for signal crayfish and near Picketts Lock in Edmonton for virile crayfish (Figure 6.3).

Sampling

Sampling took place in the summer of 2009 and 2010 at each site on multiple occasions. Adult crayfish abundance was quantified using crayfish traps with 5 mm mesh and baited with trout pellets. After 24 hours the traps were lifted and all crayfish were removed, identified, counted and the carapace length measured to the nearest millimetre using callipers before being frozen overnight. After thawing, a portion of muscle tissue was removed from the tail of a subsample of crayfish (n = 8 to 16) from a uniform size range (35-45mm carapace) for stable isotope analysis. A uniform size range was used to ensure that samples taken across a spatial and temporal scale were comparable. Catch per unit effort (number of crayfish per trap per hour; CPUE) was used to quantify relative abundance. Invertebrates were sampled using a pond net and dominant species were collected for stable isotope analysis as well as detritus and macrophytes to enable the characterisation of the food web at each site. In 2009, I also sampled the local fish population using seine nets in the littoral zones.

Data analysis

Corrected SEA_c were calculated as described above for each population of crayfish at each of the four sites in both years using macrophytes, detritus and the five most abundant invertebrate taxa as the principal resource community. I used four additional quantitative population metrics derived from stable isotope data to reveal key aspects of trophic structure in each crayfish population (for more details on calculation of metrics see Chapter Three; Layman *et al.* 2007a; Jackson *et al.* 2011). The metrics *nitrogen range* (NR_b) and *carbon range* (CR_b) indicate the total isotopic nitrogen and carbon range exploited by each population (Layman *et al.* 2007a). *Standard deviation of distance to centroid* ($SDCD_b$) is a measure of population trophic diversity and *standard deviation of nearest neighbour distance* ($SDNND_b$) infers population trophic evenness (spread of individuals over isotopic space). The subscript '$_b$' indicates that the metric was bootstrapped (n=10000) on the minimum sample size in the data set (n=8).

The Bayesian mixing models (SIAR and SIAR SOLO) were run using detritus, macrophytes, dominant invertebrate species and fish as potential resources for each crayfish population at each site, in each year. Fractionation factors between resources and consumers were assumed to be 2.3 ± 0.28 ‰ for $\delta^{15}N$ and 0.4 ± 0.17 ‰ for $\delta^{13}C$ based on a meta-analysis (McCutchan Jr *et al.* 2003). The results were used to illustrate the composition of each population's diet and to calculate SIAR-TNW and SIAR-PS, as described above.

Finally, I calculated the Euclidian distance between the mean isotopic values in 2009 and 2010 for each species of crayfish as a measure of diet shifting between years. This gives a quantitative measure of the magnitude of diet change between years (Schmidt *et al.* 2007) and therefore I propose it can be used as a measure of diet flexibility.

Results

Invasion success

The four species of crayfish were ranked in the following order of invasion success; signal, Turkish, virile and finally, Louisiana red swamp (Table 6.1). Signal crayfish were widespread, occurring in 61.8% of the sub-catchments whereas Louisiana red swamp and virile crayfish only occupied isolated populations within a single sub-catchment. At the selected study sites, all crayfish populations occurred in allopatry (Figure 6.1) and the abundance of each species was equivalent to catching approximately one crayfish per trap every 15 hours in both years, except at Carthagena Lock, where the CPUE was higher and equal to catching one signal crayfish every 2 – 2.5 hours (Table 6.2).

Stable isotope analysis

At all four sites, the isotopic signature of invasive crayfish species was slighter higher than or similar to the other dominant invertebrate species (Figures 6.4 and 6.5). At Hampstead Heath, Louisiana red swamp crayfish were more distinct from the invertebrate resources than Turkish crayfish were in neighbouring ponds due to a slightly higher δ^{15}N and thus a higher trophic position (Figure 6.4) suggesting consumption of more animal material. Similarly, in the Lee Navigation in 2009, virile crayfish were more isotopically distinct (also in δ^{15}N) from the invertebrate resources than signal crayfish were in the community upstream at Carthagena Lock (Figure 6.3; Figure 6.5).

Population metrics

The stable isotope derived population metrics revealed that virile crayfish had the widest NR_b in both years, suggesting a broad diet encompassing numerous trophic levels (Table 6.2). A wide niche breadth and a diet incorporating a number of resources varying in carbon composition was indicated by the highest CR_b in signal and Turkish crayfish in 2009 and 2010, respectively (Table 6.2). Louisiana red swamp crayfish had some of the lowest measures of CR_b and NR_b in both years (Table 6.2), indicating that they rely on a small range of resources. Trophic diversity ($SDCD_b$) and evenness ($SDNND_b$) were highest in signal crayfish in 2009 and virile crayfish in 2010 (Table 6.2), indicating an even spread of individuals over a wide isotopic area. Of the crayfish populations at Hampstead Heath, Turkish crayfish had the largest CR_b, $SDCD_b$, $SDNND_b$ and corrected SEA_c (Table 6.2) which suggests they had a more variable diet than the neighbouring populations of Louisiana red swamp crayfish. The corrected SEA_c was largest in the Lee Navigation populations of crayfish (Table 6.2) and hence, signal and virile crayfish utilised the highest percentage of resource SEA_c. Virile crayfish had the largest annual average corrected SEA_c (mean ± standard error; 23.7 ± 8.8) followed by signal crayfish (19.2 ± 0.6), Turkish crayfish (8.7 ± 1.6) and finally Louisiana red swamp crayfish (4.2 ± 1.1).

Bayesian mixing models

The Bayesian mixing models revealed that the diet of each crayfish species at the population level included both animal and plant material (Figures 6.6 and 6.7). Figures 6.6 and 6.7 show how all the putative resources contributed fairly evenly to assimilated diet, which highlights the generalist nature of invasive crayfish. The submerged plant, *Elodea sp.*

was the most important resource in the diet of Louisiana red swamp crayfish, contributing an average of 35% and 26% in 2009 and 2010, respectively (Figure 6.6). In 2009, Gastropoda (aquatic snails) were the second most important resource (13%) followed by the crustacean, *Gammarus pulex* (9%;) and in 2010 Chironomidae larvae (Diptera) were the second most important (16%) followed by Zygoptera (Odonata; 14%; Figure 6.6).

The most important resource in the diet of Turkish crayfish was Gastropoda in 2009 (18%) and *G.pulex* in 2010 when it contributed 31% on average. Other important resources in both years were *Elodea* (13% in both years) and the crustacean, *Asellus aquaticus* (11% in 2009 and 17% in 2010; Figure 6.6).

The two most important resources to signal crayfish in 2009 were detritus (30%) and Elodea (21%) followed by small clams, *Pisidium sp.* (11%; Figure 6.7). In the Lee Navigation, a new resource was made available in 2010 due to range expansion (or a large increase in density) of the invasive zebra mussel, *Dreissena polymorpha*. Signal crayfish took advantage of this new resource and hence *D. polymorpha* contributed an average of 19% to assimilated diet in 2010 (Figure 6.7). *Elodea* and *Pisidium sp.* were also important in 2010, contributing 17% and 10%, respectively (Figure 6.7).

In 2009, *Pisidium sp., A. aquaticus* and *G. pulex* all contributed equally (14% each) to the diet of virile crayfish, whereas in 2010 detritus became more important, contributing 25%, followed by *A. aquaticus* (15%) and *G. pulex* (11%; Figure 6.7). Unlike signal crayfish, virile crayfish did not switch their diet to rely on the new abundant resource of *D. polymorpha* in 2010, suggesting they have a lower level of diet flexibility despite a wide niche breadth (Table 6.2; Figure 6.7).

SIAR-TNW and SIAR-PS$_i$

The WIC was consistently higher than the BIC, indicating that the TNW of all the crayfish populations was explained primarily by resource use variation within individuals and thus, the crayfish in the Thames catchment are true generalists (Figure 6.1 B; Table 6.3). SIAR-TNW values (Table 6.3) were generally consistent with corrected SEA$_c$ values (Table 6.2), demonstrating the strength of these independent measures of niche width. The only deviation from this trend was the niche width of Louisiana red swamp crayfish in 2009 and signal crayfish in 2010 which expressed inconsistent corrected SEA$_c$ and SIAR-TNW values. Virile crayfish had the largest annual average SIAR-TNW (mean ± standard error; 0.18 ± 0.02) followed by Louisiana red swamp crayfish (0.15 ± 0.2), signal crayfish (0.12 ± 0.02) and finally, Turkish crayfish (0.10 ± 0.03).

Of all the crayfish populations, the average diet of individual Turkish crayfish in 2009 (calculated using Bayesian mixing models) had the highest proportional similarity to the entire population's average diet (Table 6.3). A PS$_i$ value of 1.0 would indicate that the individual crayfish all consume the same diet and Turkish crayfish scored 0.9 ± 0.02, indicating that the population in Hampstead Pond 1 is made up of many dietary generalists (i.e. true generalists; Figure 6.1 B; Table 6.3). Signal crayfish also had high PS$_i$ values (0.82 ± 0.04 in 2009 and 0.84 ± 0.04 in 2010; Table 6.3) suggesting that the populations are also made up of true generalists. A Ps$_i$ of 0.0 would indicate that the individual crayfish all specialise in different resources and hence the population has a generalist diet because it is made up of many different dietary specialists (i.e. individual specialisation; Figure 6.1 A). The lowest PS$_i$ was 0.68 ± 0.03 in virile crayfish in 2010, indicating a degree of individual specialisation but also a diet which included some shared and common resources (Table 6.3). As expected, low PS$_i$ values (indicating some individual specialisation) were consistent with high measures of BIC (indicating variation between individuals in resource choice) which demonstrates the strength of the metrics.

Dietary plasticity

Figure 6.8 illustrates the variability among the isotopic values of the four crayfish populations; the species were distinct in isotopic space. The Euclidian distance between the mean isotopic values in 2009 and 2010 was highest in Turkish crayfish (14.93 ‰), indicating that they exhibited the largest diet shift and hence they are flexible in diet choice (Figure 6.5). The Euclidian distance between the mean Louisiana red swamp crayfish isotope value in 2009 and 2010 was just 1.52 ‰ suggesting consistency in diet choice (Figure 6.8). Of the Lee Navigation populations, virile crayfish had the largest diet shift (7.97 ‰), closely followed by signal crayfish (5.63 ‰; Figure 6.8).

Tables and figures

Table 6.1. The invasion success of each species of crayfish in the Thames catchment based on distribution among the sub-catchments and years since first recorded.

Species	Year of first record (number of years until 2009)	Number of Sub-catchments established in	Success rating
Red swamp	1991 (18)	1	0.06
Turkish	1988 (21)	6	0.29
Signal	1976 (33)	34	1.03
Virile	2004 (5)	1	0.20

Table 6.2. Mean population metrics (2.5% and 97.5% quantile), corrected standard ellipse areas and CPUE (mean ± standard error) for each crayfish population in 2009 and 2010.

Population	Year	CR_b (‰)	NR_b (‰)	$SDCD_b$ (‰)	$SDNND_b$ (‰)	Corrected SEA_c (%)	CPUE
Bird Sanctuary RSC	2009	2.07 (0.95-2.98)	2.07 (0.64-2.88)	0.46 (0.23-0.69)	0.38 (0.13-0.68)	5.30	0.06 ± 0.04
	2010	1.43 (0.48-1.84)	1.33 (0.68-1.60)	0.30 (0.15-0.44)	0.26 (0.10-0.44)	3.09	0.04 ± 0.05
Hampstead 1 TC	2009	2.87 (1.41-3.64)	1.33 (0.56-1.85)	0.51 (0.23-0.75)	0.35 (0.12-0.64)	7.10	0.06 ± 0.04
	2010	3.68 (2.25-4.43)	1.15 (0.54-1.54)	0.60 (0.28-0.89)	0.37 (0.14-0.76)	10.28	0.10 ± 0.07
Carthagena SC	2009	3.29 (0.91-5.73)	1.31 (0.62-1.88)	0.70 (0.17-1.33)	0.60 (0.10-1.47)	18.55	0.46 ± 0.15
	2010	1.31 (0.74-1.52)	2.52 (0.63-4.33)	0.52 (0.16-0.98)	0.46 (0.10-1.09)	19.81	0.39 ± 0.10
Picketts VC	2009	2.08 (1.09-1.75)	3.37 (1.53-5.06)	0.62 (0.27-1.00)	0.49 (0.18-0.91)	14.96	0.09 ± 0.06
	2010	2.04 (0.98-2.68)	3.31 (1.15-3.79)	0.66 (0.23-0.93)	0.53 (0.15-0.97)	32.50	0.07 ± 0.05

Table 6.3. The within individual (WIC) and between individual (BIC; mean ± standard error) of each population's total niche width (SIAR-TNW) and the proportional similarity indices (SIAR-PS_i; mean ± standard error) for each crayfish population in 2009 and 2010.

Population	Year	WIC	BIC	SIAR-TNW	SIAR-PS_i
Bird Sanctuary RSC	2009	0.125 ± 0.01	0.041 ± 0.02	0.166	0.71 ± 0.04
	2010	0.098 ± 0.01	0.028 ± 0.01	0.126	0.82 ± 0.04
Hampstead 1 TC	2009	0.047 ± 0.01	0.026 ± 0.01	0.072	0.90 ± 0.02
	2010	0.095 ± 0.01	0.036 ± 0.01	0.131	0.73 ± 0.03
Carthagena SC	2009	0.087 ± 0.01	0.051 ± 0.01	0.138	0.82 ± 0.04
	2010	0.061 ± 0.01	0.047 ± 0.01	0.108	0.84 ± 0.04
Picketts VC	2009	0.093 ± 0.01	0.067 ± 0.02	0.16	0.75 ± 0.04
	2010	0.141 ± 0.01	0.065 ± 0.02	0.206	0.68 ± 0.03

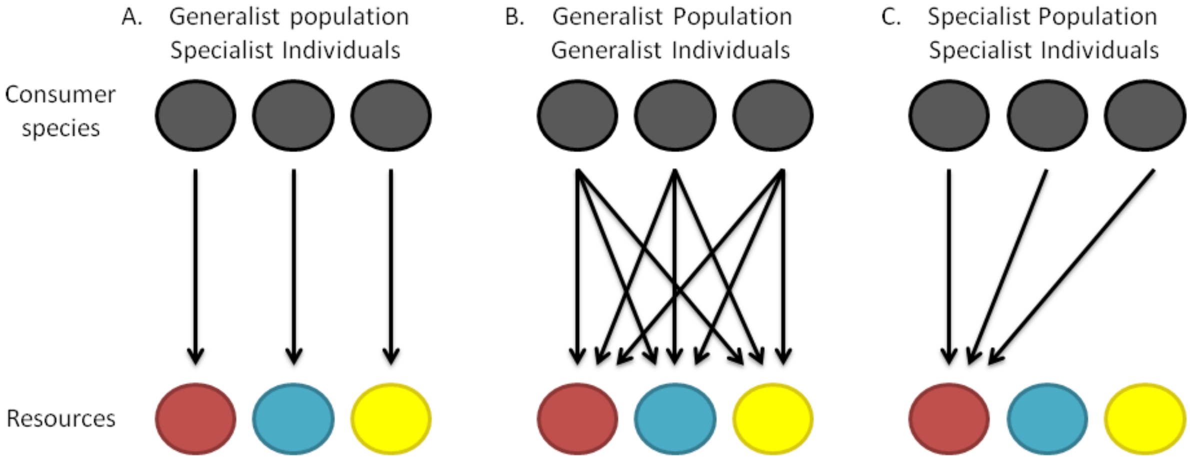

Figure 6.1. A schematic diagram showing three populations of consumers exhibiting three different feeding behaviours; a generalist population caused by individual specialisation (A), true generalists (B) and true specialists (C). Coloured circles represent different resources and black circles represent individual consumers. Reproduced from Bearhop et al. (2004).

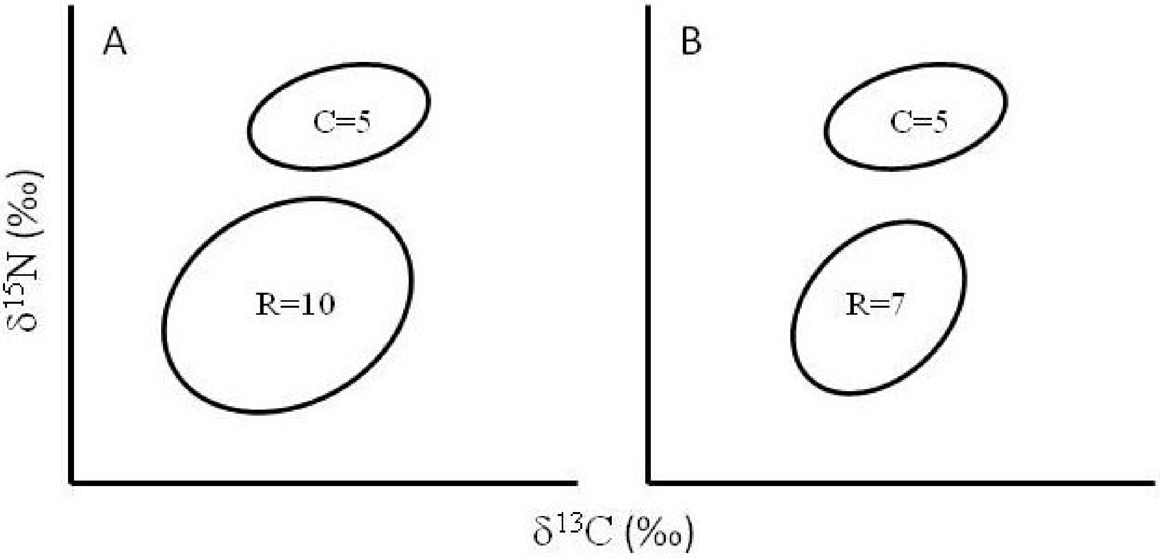

Figure 6.2. Stable isotope bi-plots of simulated data showing the SEA_c of consumers (C) and the available resources (R) in two populations (A and B). In both population A and B, the consumers have a SEA_c of 5 ‰^{2}. However the actual niche area of population B is larger because the resources available to the consumers are less variable in isotopic composition. The available resources in population A occupy a SEA_c of 10‰^{2} and therefore the consumers have a corrected SEA_c metric values of (100*(C÷R)) 50% whereas the available resources in population B occupy a SEA_c of 7‰^{2} and therefore the consumers have a corrected SEA_c metric value of 71.4%. These corrected values, expressed as a percentage, give a more comparable measure of niche width since they are adjusted to account for resource variation.

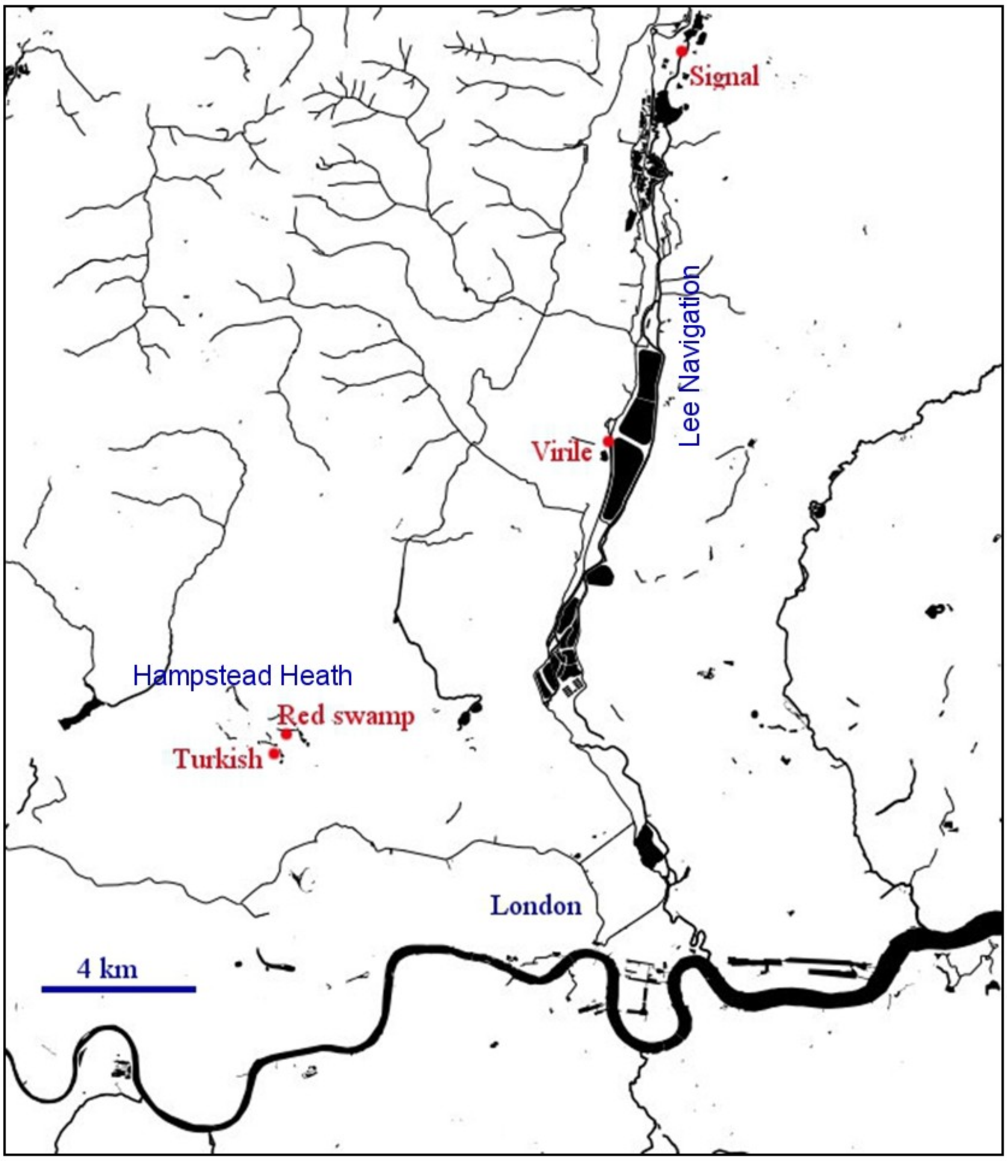

Figure 6.3. Map of the water bodies in the London area of the Thames catchment (black) showing the location of the four sites, each harbouring one species of crayfish (red).

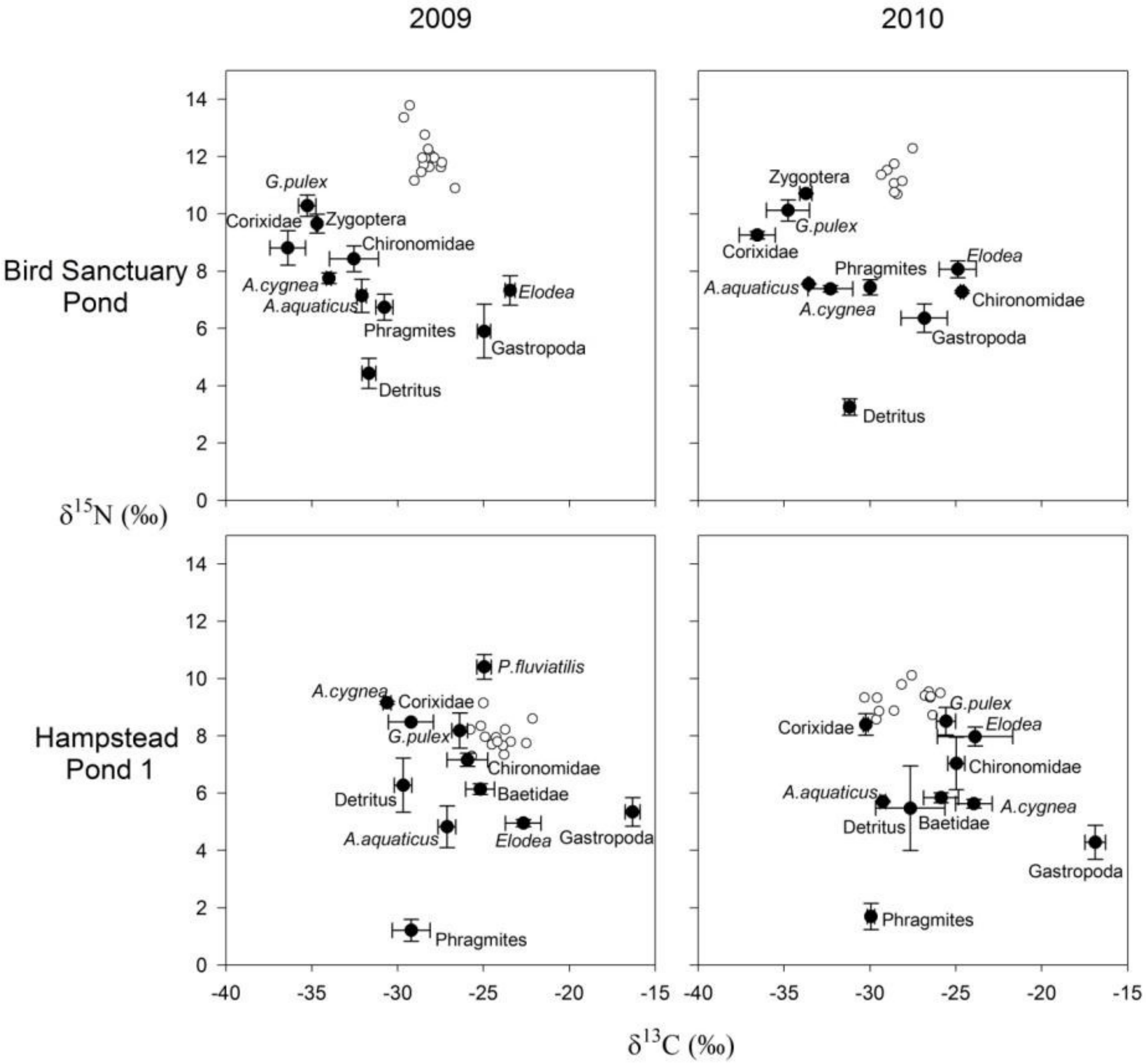

Figure 6.4. Stable isotope bi-plots of the Hampstead Heath pond food webs derived from data collected from the Bird Sanctuary Pond and Hampstead Pond 1 in summer 2009 and 2010. Open symbols represent individual crayfish; Louisiana red swamp at the Bird Sanctuary Pond and Turkish at Hampstead Pond 1. Closed symbols represent putative resources (means ± standard errors, *n* = 3 to 10) which were present and sufficiently abundant during collection to be analysed for stable isotopes.

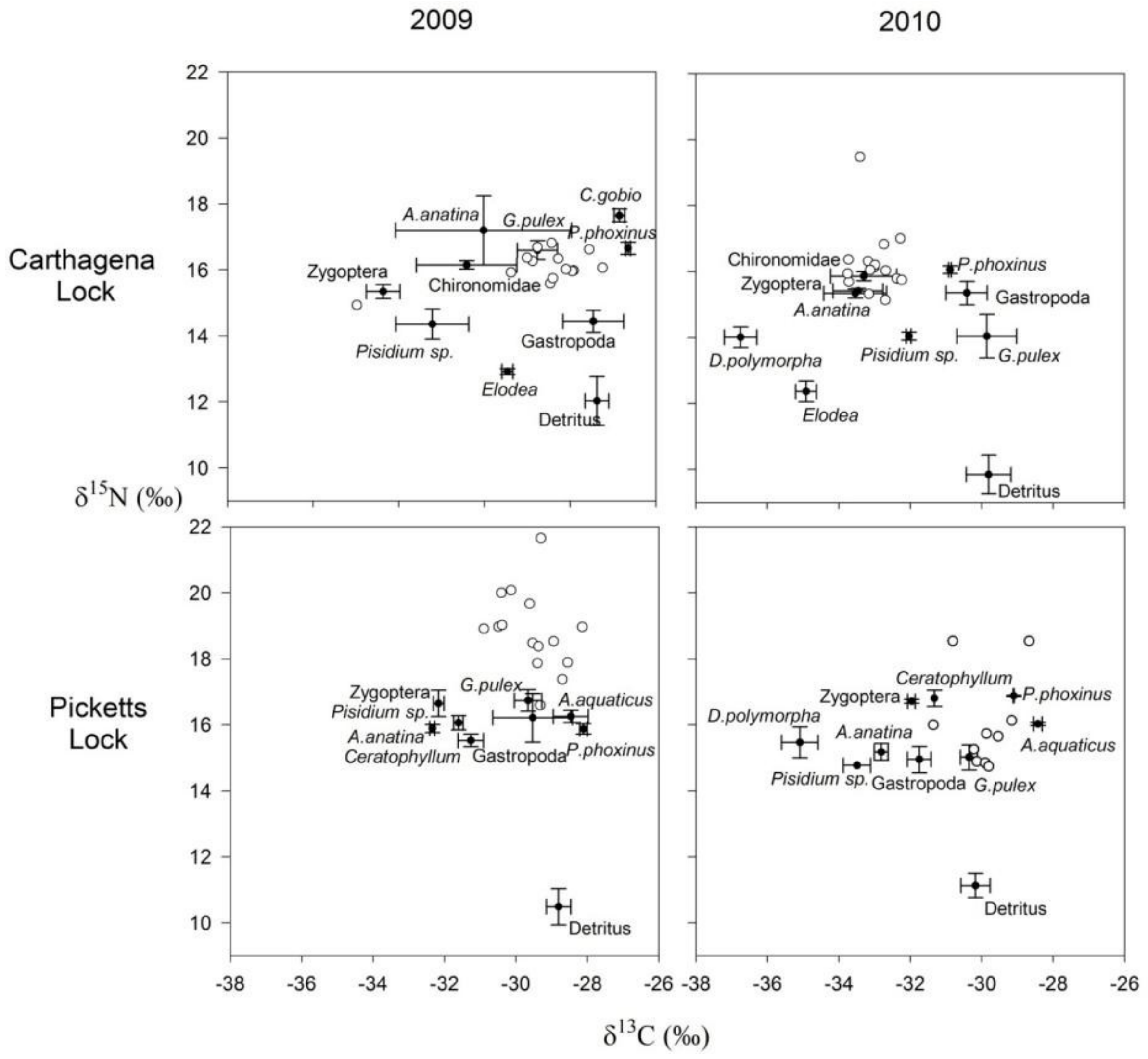

Figure 6.5. Stable isotope bi-plots of the Lee Navigation food web derived from data collected from Carthagena and Picketts Locks in summer 2009 and 2010. Open symbols represent individual crayfish; signal at Carthagena Lock and virile at Picketts Lock. Closed symbols represent putative resources (means ± standard errors, *n* = 3 to 10) which were present and sufficiently abundant during collection to be analysed for stable isotopes.

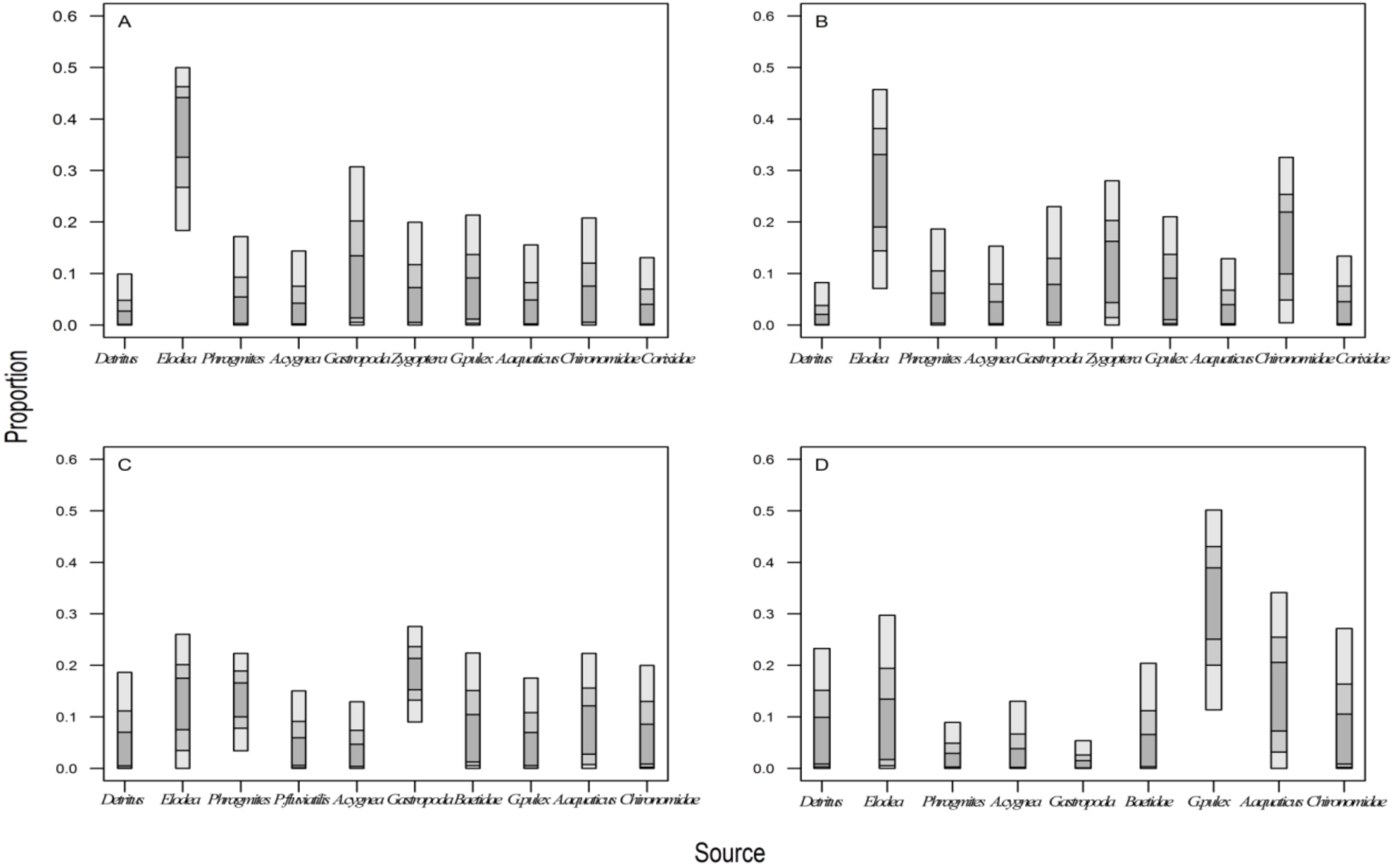

Figure 6.6. Posterior estimates of the proportions of diet sources assimilated by red swamp crayfish at the Bird Sanctuary in 2009 (A) and 2010 (B) and Turkish crayfish at Hampstead 1 in 2009 (C) and 2010 (D). Estimates are based on the SIAR mixing model and are represented as follows: 50% shown by the central dark grey area of the bar, 75% probability include the medium grey area, 95% are shown by the entire bar.

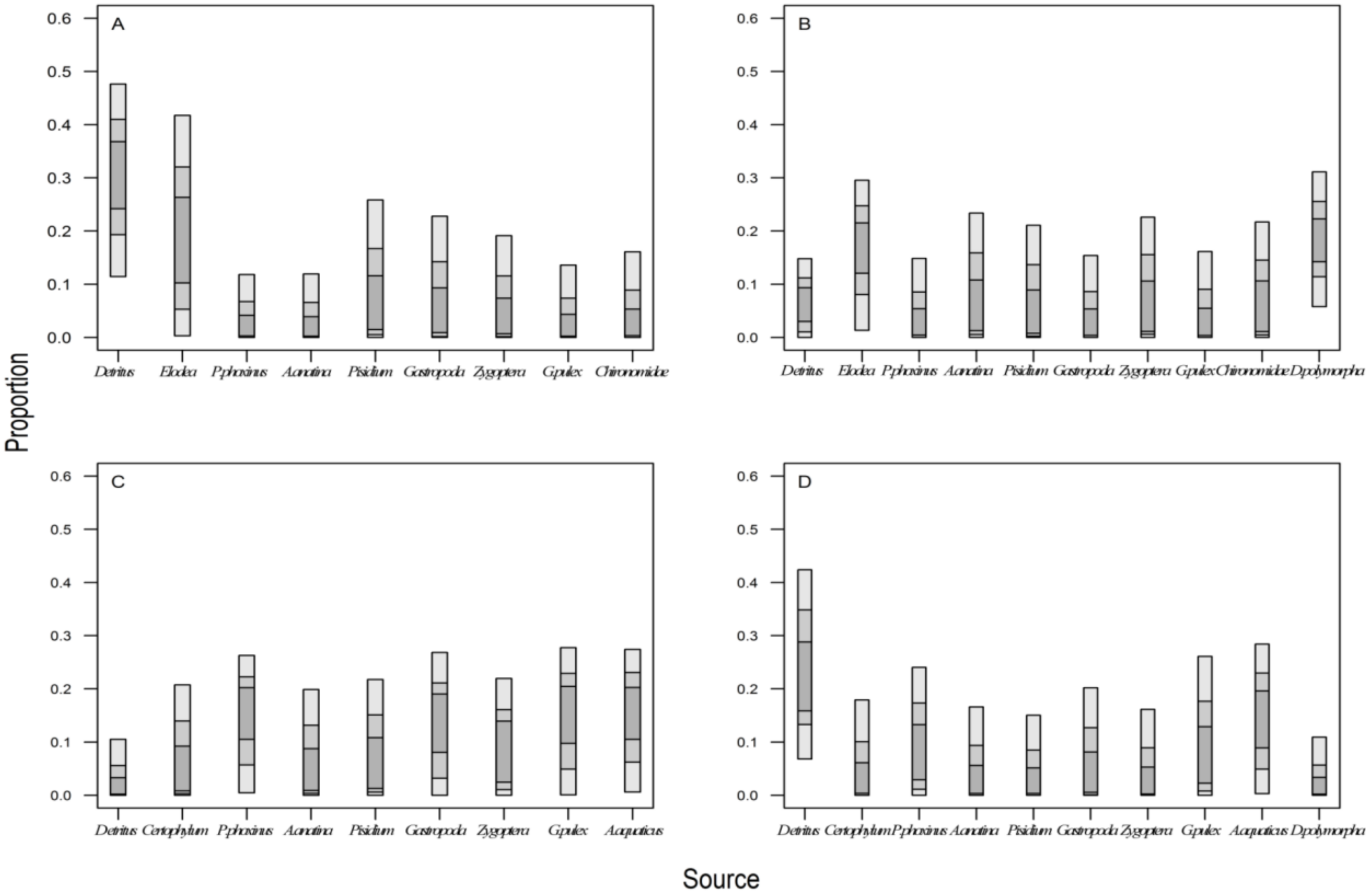

Figure 6.7. Posterior estimates of the proportions of diet sources assimilated by signal crayfish at Carthagena Lock in 2009 (A) and 2010 (B) and virile crayfish at Picketts Lock in 2009 (C) and 2010 (D). Estimates are based on the SIAR mixing model and are represented as follows: 50% shown by the central dark grey area of the bar, 75% probability include the medium grey area, 95% are shown by the entire bar.

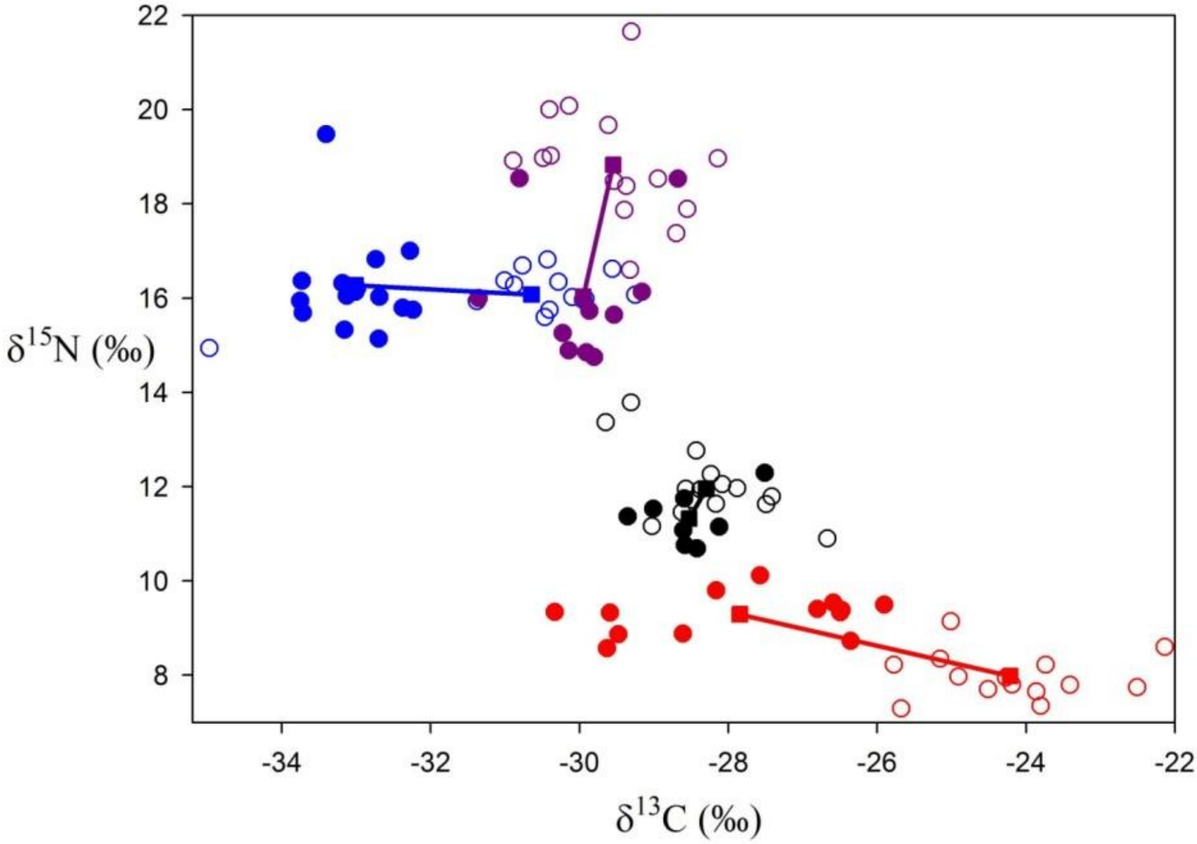

Figure 6.8. Stable isotope bi-plot showing individual Louisiana red swamp (black), Turkish (red), signal (blue) and virile (purple) crayfish in 2009 (open circles) and 2010 (closed circles) at the study sites. Lines represent the mean change in isotopic composition for each species between 2009 and 2010.

Discussion

Application of novel stable isotope metrics

Past studies have shown how consumer isotope variability can be used to measure niche width and individual specialisation of populations (Bolnick *et al.* 2002; Layman *et al.* 2007b; Jackson *et al.* 2011). However, consumer isotope variability is not only influenced by individual variation in consumer diet, but also by variation in the isotopic composition of the putative resources (Semmens *et al.* 2009). If the identity of resources varies across the study scale (temporal or spatial), or there are significant differences in the isotopic composition of the same resources across the study scale, such measures of niche width will be confounded by this variability. Thus, it is important to incorporate both consumer and resource isotopic variability into measures of niche width.

SEA_c is a robust measure of consumer isotopic variability which is not influenced by sample size (Jackson *et al.* 2011), unlike other measures of niche width using the convex hull method (e.g. Layman *et al.* 2007a). My corrected SEA_c accounts for the isotopic composition of the putative resources and thus increases the strength and scope of this metric.

Newsome et al. (2007) and Semmens et al. (2009) suggest the use of mixing models to measure niche width. In comparison to these studies, SIAR-TNW is a particularly strong measure of niche width since it uses the Bayesian mixing model SIAR which, unlike other mixing models, incorporates intra-resource variability (i.e. variability in resource isotope composition in samples taken at the same time, from the same site). SIAR-TNW is likely to reveal a more accurate measure of TNW than corrected SEA_c because it calculates WIC and BIC at the lowest possible point of detail (explicit estimates of the proportion of each resource in the diet of each individual). However, it is important to ensure that appropriate fractionation factors are used as these can significantly alter the output of the mixing model.

Stable isotope derived metrics are limited by a number of assumptions including: that isotope signatures have been estimated over an appropriate spatial and temporal scale; that all putative resources have been sampled; and that appropriate fractionation factors have been used. If these assumptions are met then corrected SEA_c, SIAR-TNW and SIAR-PS_i ultimately add another ecological tool-set to help unravel the trophic ecology of populations and the complexity of food webs.

My framework provides quantitative measures of niche width and individual specialisation based on actual resource use. However, resource use is not the same as resource preference since resource availability will influence the diet of an individual, population or species (De Caceres *et al.* 2011). Future work should, therefore, involve incorporating resource abundance and/or density into the models.

The role of diet in invasion success

A common generalisation in invasion ecology is that niche breadth is positively correlated with invasion success (e.g. Jeschke & Strayer 2006) and this can be referred to as the ‘niche breadth – invasion success hypothesis’ (Vazquez 2006). The position of the four invasive crayfish species in isotopic space suggested that they were all generalist omnivores at the population level, which commonly results in a wide niche (Vazquez 2006). The Bayesian mixing models also indicated that all four crayfish populations consumed multiple resources, including both plants (the macrophytes, *Elodea* and *Ceratophyllum*, and detritus) and animals (e.g. crustaceans such as *G. pulex* and *A. aquaticus,* and molluscs including snails and freshwater clams). The estimates of the relative proportion of resources assimilated varied between species and therefore crayfish species can have distinct diets, despite being generalist omnivores.

Recently, it has been suggested that a broad diet at the population level does not necessarily indicate that individuals have equally broad diets (Bolnick *et al.* 2002; Svanbäck & Persson 2004; Araujo *et al.* 2011). Instead, a generalist population may consist of many individuals specialising in different resources (Bolnick *et al.* 2002; Svanbäck & Persson 2004). Bayesian mixing models on individual crayfish demonstrated that there was some variability in resource use among individuals of the same population. However, the variability was generally low in all species and the WIC of TNW was consistently higher than the BIC suggesting that most resources were shared and hence, crayfish in the Thames catchment are true generalists. In 2010, virile crayfish demonstrated the highest degree of individual specialisation of the four crayfish species. Nonetheless, the diet of all individuals still included many shared resources since the average similarity of individuals to the population diet was 68%. Individual red swamp, Turkish and signal crayfish had a diet that, on average, was at least 71%, 73% and 82% similar to their population diets, respectively. This indicates that all four species are generalists at the individual and the population level in the Thames catchment.

I have ruled out individual specialisation as a major contributor to dietary niche breadth and therefore variation within individuals (WIC) explains the majority of each species niche (Bolnick *et al.* 2002). Niche size, measured as corrected SEA_c and SIAR-TNW varied among species and years. Virile crayfish had the largest corrected SEA_c and SIAR-TNW indicating a broad generalist diet (Layman *et al.* 2007b; Jackson *et al.* 2011). A diet that spanned numerous trophic levels was also signified by the highest recorded NR_b. Virile crayfish had the second lowest success rating in the catchment after Louisiana red swamp crayfish, suggesting that a broad diet does not always facilitate a successful invasion. However, virile crayfish were only discovered in the Thames catchment in 2004 and they have extended their range throughout the Lee Valley considerably since then. Low propagule pressure may have hindered their range expansion since it appears that they have only been introduced to one site in the catchment which is in stark contrast to the more successful signal crayfish (Lockwood *et al.* 2005; Ahern *et al.* 2008). In the late 1970s, Signal crayfish were introduced from Sweden to around 300 sites in the United Kingdom, 17 of which were in the Thames catchment (Hayes 2012). There was a large increase in range and population density of virile crayfish in the Lee Navigation in 2011 (Adam Ellis, *pers. comm.*) and hence it may have been too early to assess the success of their invasion in 2009, just five years after their discovery in the catchment.

Signal crayfish was the most successful species by a substantial margin and this corresponded with some of the highest measures of niche diversity. Of the three remaining species, they had the highest corrected SEA_c, incorporating a wide range of carbon resources (measured as CR_b) and trophic levels (measured as NR_b). If resources were limited, the smaller isotopic niche of signal crayfish compared to virile crayfish could be due to higher levels of intraspecific competition in the signal population at Carthagena Lock since crayfish density was much greater there than in the virile crayfish populations at Picketts Lock.

Of the Hampstead Heath populations, Turkish crayfish generally had the largest measures of niche width (CR_b, NR_b and corrected SEA_c), which is consistent with the 'niche breadth – invasion success hypothesis' since they have been more successful than Louisiana red swamp crayfish in the Thames catchment. Louisiana red swamp crayfish are more successful than Turkish crayfish on a global scale, however they are generally more successful at lower latitudes since their native range is sub-tropical (Capinha *et al.* 2011). In contrast to the measures of corrected SEA_c, Louisiana red swamp crayfish had the second highest annual average SIAR-TNW. The inconsistency in these measures of niche width is probably due to the different way the resource community heterogeneity is incorporated into the metrics; corrected SEA_c simply quantifies the isotopic area of the principal resource community while SIAR-TNW estimates the proportional contribution of each resource to each individual.

Louisiana red swamp and Turkish crayfish had lower trophic diversity ($SDCD_b$) and evenness (of individuals in their spread over isotopic space; $SDNND_b$) than the Lee Navigation populations, suggesting some clustering of individuals in isotopic space and therefore a smaller population niche width. The Lee Navigation crayfish (virile and signal) generally had higher measures of niche width and trophic diversity, suggesting that crayfish in lotic ecosystems may occupy broader niches than those in lentic ecosystems.

Successful invasive species often exhibit diet shifts once preferred resources become limited, exhibiting resource use efficiency (Shochat *et al.* 2006; Tillberg *et al.* 2007; Ruffino *et al.* 2011). Turkish crayfish had the most dissimilar diets between years, suggesting a diet shift and hence dietary plasticity (Tillberg *et al.* 2007; Zhang *et al.* 2010). Signal crayfish had the second largest diet shift and Bayesian mixing models indicated that was due to the consumption of a new resource, the invasive zebra mussel, illustrating the opportunistic nature of signal crayfish. Greater opportunism and hence, flexibility in resource choice will have facilitated the spread of signal crayfish in the Thames catchment (Tillberg *et al.* 2007). In aquatic environments such as the Lee Navigation and Hampstead Heath, resources are often spatially and temporally patchy and hence, the ability to use available resources efficiently and switch diets is a fundamental trait for invaders (Ruffino *et al.* 2011). As generalist foragers, Turkish, signal and virile crayfish all exhibited a noticeable shift in isotopic space between years, suggesting resource use efficiency and therefore dietary plasticity (Zhang *et al.* 2010; Ruffino *et al.* 2011). The Louisiana red swamp crayfish population had the most similar diet between years. Consistency in resource choice could be due to consistency in environmental variables however, in neighbouring ponds, Turkish crayfish exhibited the largest diet shift. This difference could be due to disparity in resource isotopic shifts between the ponds in Hampstead Heath and hence, future work could involve incorporating resource isotopic shifts into this model. Nonetheless, the small diet shift measured in Louisiana red swamp crayfish may signify low levels of dietary plasticity, which in turn could explain their low invasion success in the Thames catchment. Since Louisiana red swamp crayfish have successfully invaded all continents except Australia and Antarctica (Holdich & Sibley 2003), it is more likely that their failure to expand their range is due to the lack of optimal temperature conditions.

Conclusions

Successful invaders are characterised by a number of traits associated with a wide niche, including rapid growth, production and dispersal rates, high tolerances of environmental conditions and a flexible diet. Here, I have shown how the latter contributes to crayfish invasion success, although the other traits will also play an important role. It is important to understand the trophic ecology of an invader as it is often closely related to the magnitude of the impact (Rehage *et al.* 2005) and in Chapter Seven, I will investigate variation in the ecosystem level impact of the four crayfish species.

Stable isotope derived metrics demonstrated that different methods of diet flexibility, including diet shifting and a wide niche, have facilitated successful invasions by the four invasive crayfish species in the Thames catchment. There are high degrees of diet flexibility and resource use efficiency in the crayfish and hence the actual assimilated resources will vary spatially and temporally according to availability, especially since the results revealed that crayfish are true generalists with limited individual specialisation. Thus, measures of niche breadth, trophic diversity, diet plasticity and individual specialisation are likely to vary between sites and therefore, this study is not meant to be a generalisation.

These sophisticated analytical tools, applicable to stable isotope data, proved useful in examining the trophic ecology of invasive species. My novel measures of niche width (corrected SEA_c and SIAR-TNW) and individual specialisation (SIAR-PS_i) are the first quantitative measures of trophic diversity, derived from stable isotopes, which consider individual variation in resource use. They are applicable to a wide range of fields in ecology and hence, a valuable tool to reveal the detailed trophic ecology of populations.

Chapter Seven: Dietary interactions among invaders: impact on ecosystem structure and functioning

Declaration on input

I am indebted to the following Queen Mary graduates; Tabitha Jones, Jeffrey Taylor, Maaike Milligan and Danny Sheath, for their input to this chapter. Maaike and Danny assisted with the first mesocosm experiment as part of their BSc, and Tabitha and Jeffery assisted with the second mesocosm experiment as part of their MSc. They all contributed towards the sampling of the mesocosms and the initial processing of samples.

Abstract

Many aquatic ecosystems sustain multiple invasive species and the interactions among them will have implications for food web dynamics and ecosystem structure and functioning. Here I examine interactions among invasive crayfish species that are present in the Thames catchment (Louisiana red swamp and Turkish in Hampstead Heath and signal and virile in the Lee Navigation) using experimental mesocosms in order to address the questions: 1) Do sympatric invasive crayfish occupy a smaller niche than their allopatric counterparts due to resource competition? And 2) Do interactions among invasive crayfish species amplify or mitigate one another's impact on the ecosystem? Niche width did not vary

significantly between allopatric and sympatric populations of all species. Niche partitioning between sympatric species suggests they could coexist in the wild. All four species of crayfish altered invertebrate community structure but in subtly different ways, relating to their feeding habits. A trophic cascade whereby the crayfish fed on invertebrate shredders, such as *Gammarus pulex*, reducing net leaf litter decomposition was decoupled by Louisiana red swamp and signal crayfish by their direct consumption of leaf litter. There was evidence of a further trophic cascade whereby benthic algal standing stock was promoted in the presence of crayfish due to their consumption of invertebrate grazers, such as *Lymnaea* sp.. There was only one case of an amplified or mitigated impact of coexisting invaders; invertebrate predators, such as Hirudinea, were significantly reduced in density in the presence of sympatric Louisiana red swamp and Turkish crayfish and not when each species was in allopatry. Subsequently, the impact of sympatric invasive crayfish species is independent and will equal the sum of their allopatric impacts.

Introduction

Biological invasions are recognised as a significant driver of global environmental change with consequences from the individual, through to the whole ecosystem level of organisation (Sala *et al.* 2000; Simon & Townsend 2003; Ricciardi 2007). In Chapter Four, I discussed how invasive crayfish severely disrupt the organisation of native communities by displacing native species or by reducing their abundance (Gurevitch & Padilla 2004). Alterations in species assemblage and biodiversity can have major implications for both ecosystem processes (Olden *et al.* 2004; Hector & Bagchi 2007) and ecosystem services

(Pejchar & Mooney 2009). Freshwaters ecosystems are commonly invaded due to a high degree of anthropogenic manipulation (Rahel 2007); for instance, in Chapter Five I showed that the Thames catchment is one of the most highly invaded aquatic systems in the world. Here, I examine how interactions among invaders, specifically those among invasive crayfish species in the Thames catchment, can alter ecosystem structure and functioning using experimental mesocosms.

As the pace of global change accelerates, many ecosystems sustain multiple invaders (e.g. Cohen & Carlton 1998; Ricciardi 2006) and the interactions among them will have important consequences. The Invasion Meltdown model predicts that the disturbance caused by an invasive species will facilitate the establishment of further invaders and thus amplify one another's impact on the ecosystem (Simberloff & Von Holle 1999; Simberloff 2006). For instance, Grosholz (2005) illustrated how the invasion of European green crab (*Carcinus maenas*) on the East coast of North America facilitated the spread of the amethyst gem clam (*Gemma gemma*), a previously established invader, by consuming native bivalves and subsequently reducing competition pressure. Furthermore, functionally-similar invaders can act in synergy to magnify the impacts they have individually on ecosystem structure and functioning (Simberloff & Von Holle 1999; Simberloff 2006). Rudnick and Resh (2005) demonstrated that the non-indigenous Chinese mitten crab (*Eriocheir sinensis*) and Louisiana red swamp crayfish (*Procambarus clarkii*) share a similar omnivorous diet in the San Francisco Bay delta, which could result in amplification of the negative effects they have on the ecosystem.

On the contrary, there is the possibility that sympatric invasive species will moderate one another's impact by each controlling the abundance of the other via competitive or predator-prey interactions. For instance Lohrer and Whitlach (2002) found that the invasive

Asian shore crab (*Hemigrapsus sanuineus*) reduced the abundance of another established invader, the European green crab (*Carcinus maenas*) in North America via direct consumption. Functionally similar sympatric invasive species will compete for resources including shelter (e.g. Alonso & Martínez 2006) and food (Griffen *et al.* 2008), and this also might mitigate their adverse impacts. However, in Chapter Three I showed how competition from a new invader, common carp, has displaced invasive Louisiana red swamp crayfish in Lake Naivasha in Kenya with no apparent recovery in the lake from the detrimental impact of the crayfish and hence, serial replacement of invasive species might not alter the overall impact on the ecosystem.

Crayfish are widespread conspicuous invaders with a range of impacts on ecosystem structure and functioning (Capinha *et al.* 2011). Invasive species of crayfish regularly out-compete and replace native crayfish (e.g. Hill & Lodge 1999; Alonso & Martínez 2006; Dunn *et al.* 2009) but interactions among sympatric invasive crayfish are rarely examined (Johnson *et al.* 2009b) despite the increasing likelihood of them occurring in sympatry. There are seven species of crayfish in the United Kingdom, only one of which is native (Holdich *et al.* 1999; Keller *et al.* 2009). In Chapter Five, I found that four of the invaders occur in the Thames catchment and yet little, if anything is known of how they might interact. Populations of signal (*Pacifastacus leniusculus*) and virile (*Orconectes virilis*) crayfish co-occur in the Lee Navigation, North East London (Ahern *et al.* 2008) and populations of Louisiana red swamp (*Procambarus clarkii*) and Turkish (*Astacus leptodactylus*) crayfish co-occur in Hampstead Heath, North London. Here I use controlled mesocosm experiments to examine interactions between signal crayfish and virile crayfish and between Louisiana red swamp and Turkish crayfish to address the question: do interactions among invaders amplify or mitigate one another's impact on ecosystem structure and functioning? In Chapter Six I showed that there is variation in diet between the four species and here I also use stable isotope approaches to examine dietary interactions between the invaders in order to answer the question: do sympatric invasive species occupy a smaller niche than their allopatric counterparts due to resource competition? The results of each experiment are reported and analysed separately with a shared conclusion section.

Methods

Experimental design

Two outdoor mesocosm experiments were conducted in 2010 using twenty fibreglass ponds, measuring 168 by 104 cm and with a depth of 38 cm (238 l). Each experiment ran for a total of six weeks. The first experiment examined interactions between signal crayfish and virile crayfish and the second, interactions between Louisiana red swamp crayfish and Turkish crayfish. Prior to initiating the experiments, each mesocosm had 2 cm of clean rock and gravel substrata added and was filled with rainwater which was continually aerated by aquaria pumps with air-stones. A half section of drainpipe (20 cm length) was added as a crayfish refuge and a stack of three terracotta tiles (10 x 10 cm), each separated by 5mm, were added as invertebrate refuge. Each mesocosm was seeded with equal densities of benthic invertebrates, zooplankton and macrophytes from the same sites from which the crayfish were sourced (Lee Navigation in experiment one; Hampstead Heath in experiment two). Live *Daphnia* and chironomids were also purchased from a pet shop and an equal amount added to each mesocosm prior to the experiments. Each experiment had 5 replicates of 4 treatments and each crayfish replicate had an equal density and biomass of crayfish of the same sex based on field densities (see Table 7.1).

Table 7.1. The replicated ($n = 5$) treatments ($n = 4$) used in each experiment ($n = 2$). The numbers in parentheses represent the number of individual crayfish used from each species in each replicate followed by the total biomass of those individuals. Crayfish abbreviations are as follows: Louisiana red swamp crayfish, RSC; Turkish crayfish, TC; signal crayfish, SC and virile crayfish, VC.

Experiment 1	**Experiment 2**
SC (6; 171 ± 10g)	RSC (6; 224 ± 10g)
VC (6; 171 ± 10g)	TC (6; 224 ± 10g)
SC (3; 85 ± 5g) and VC (3; 85 ± 5g)	RSC (3; 112 ± 5g) and TC (3; 112 ± 5g)
Neither species (0; 0)	Neither species (0; 0)

Experimental sampling protocol – Dietary analysis

On the final day of each experiment, crayfish were frozen overnight and then thawed before dissecting a portion of muscle from the tail for the analysis of stable isotopes. Samples of weed, leaf litter, macrophytes and invertebrates form each pond were also taken for stable isotope analysis.

Experimental sampling protocol – Ecosystem structure and functioning

Leaf litter of *Alnus glutinosa* was collected and dried before being used in the experiments to examine decomposition rates. Three plastic mesh bags (aperture 1, 5 and 10 mm) containing a known mass (~ 3 g) of the air-dried leaf litter were fastened to the bottom of each mesocosm at the start of the experiments. Only the 10 mm bags were accessible to crayfish, the 5 mm were accessible to other smaller benthic invertebrates and the 1 mm bags only accessible to microbial organisms. This allowed the direct and indirect impacts of crayfish on leaf litter breakdown to be measured. The leaf litter remaining in each mesh bag after 6 weeks was washed and then dried at 60 ºC to constant weight. The exponential decay rate coefficient (k) was calculated for each treatment as a measure of the rate of leaf litter decomposition as:

$$k = \frac{\log_e\left(\frac{M_t}{M_0}\right)}{t}$$

Where t is the duration of exposure (in days), M_0 is the initial dry mass (in grams) and M_t is the dry mass at time t (Hieber & Gessner 2002).

I measured epilithic algal standing stock by placing a terracotta tile (5 x 5 cm) in each cage at the start of the experiment. Tiles were removed on the final day of the experiment and all biofilm was washed off and filtered through GF/C filters (Whatman®, Maidstone, UK) before adding 15 ml of 90% acetone. Similarly, pelagic algal standing stock was measured by filtering water samples at the end of the experiment through GF/C filters (Whatman®, Maidstone, UK) before adding 15 ml of 90% acetone. After 24 hours in the dark, the samples were centrifuged and the supernatant was used for spectrophotometry. I then used chlorophyll *a* concentration, quantified following Jeffery and Humphrey (1975) and expressed as mg cm^{-2} or mg ml^{-2}, as a measure of epilithic algae and pelagic algae standing stock, respectively.

The structure of the zooplankton community was studied at the end of the experiment by filtering 5 l of pond water through a 250-µm sieve. Samples were preserved in 70% IMS (Industrial Methylated Spirit) and subsequently sub-sampled (10ml) before identification and counting. I quantified the benthic invertebrates in the mesocosms at the end of the experiment by removing, counting and identifying all organisms in water samples, sediment cores, leaf packs and invertebrate refugia. All invertebrates were stored in 70% IMS prior to identification and counting. After identification, benthic invertebrates were assigned to a functional feeding group for further analysis.

Water samples (50 ml) were taken at the end of the experiment and filtered through a 0.45 µm filter before the nitrate and phosphate concentration of the water column was analysed using a continuous flow auto-analyser (Skalar San, De-Breda, Netherlands) with standard colorimetric techniques (Kirkwood 1996).

Data analyses

Analysis of variance (ANOVA; all carried out in Minitab 14®) was used to test for differences in $\delta^{13}C$ and $\delta^{15}N$ between populations of crayfish in each experiment. Discrimination analysis was also used on the raw stable isotope data of crayfish to investigate if the species classification could correctly predict the species of each individual. This tested for differences in isotope signatures, and therefore diet, between species.

I used the stable isotope-derived population metrics carbon range (CR_b) and nitrogen range (NR_b) as measures of the trophic niche width of crayfish (See the following for methods: Chapter Three; Layman *et al.* 2007a; Jackson *et al.* 2011). CR_b and NR_b were calculated in the statistical package 'R' as the Euclidean distance between the individuals with the most enriched and most depleted $\delta^{13}C$ and $\delta^{15}N$ respectively. To enable comparison among variable sample sizes (3 individuals in the treatments with 2 species of crayfish and 6 individuals in the treatments with allopatric crayfish), the metrics were bootstrapped (n = 10000; indicated with a subscript '$_b$') based on the minimum sample sizes of 3. ANOVA was then used to test for difference in CR_b and NR_b between populations of crayfish.

The Bayesian mixing model, Stable Isotope Analysis in 'R' (SIAR; Parnell *et al.* 2010), was used to estimate the contribution of various abundant putative food resources to the diets of each crayfish species in the mesocosms. Fractionation factors between resources and consumers were assumed to be 2.3 ± 0.28 ‰ for $\delta^{15}N$ and 0.4 ± 0.17 ‰ for $\delta^{13}C$ based on a meta-analysis (McCutchan Jr *et al.* 2003). I ran separate mixing models for each population in each pond using leaf litter, macrophytes, weeds and the three dominant invertebrate species as potential resources. I then used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for variation in the diet of crayfish species in sympatric and allopatric conditions, using the PERMANOVA+ add-in to PRIMER®

version 6.1 (PRIMER-E Ltd, Plymouth, UK). These analyses were done with 9999 permutations of the residuals under a reduced model (Anderson *et al.* 2008) and were based on Bray-Curtis similarity matrices calculated from log (X + 1)-transformed data. If significant differences were detected, similarity percentages analysis (SIMPER in PRIMER®; Clark & Warwick 2001) was then used to elucidate the contribution of each assimilated resource to the mean Bray-Curtis dissimilarity between the diets of the crayfish populations.

I also used permutational multivariate analysis of variance (PERMANOVA; Anderson 2001) to test for effects of the experimental manipulations on the structure of zooplankton and benthic invertebrate assemblages using the statistical model outlined above. Similarity percentages analysis (Clark & Warwick 2001) was then used to determine the contribution of benthic invertebrate taxa to the mean Bray-Curtis dissimilarities between treatments using presence or absence of each crayfish species as factors. This method determines which taxa were affected most strongly by the presence of each species of crayfish in each experiment.

Shannon's diversity index was calculated for the zooplankton and benthic invertebrate community following Shannon (1948). I used ANOVA to test for an effect of treatment on nutrient concentration, leaf litter breakdown, algal standing stock, zooplankton abundance and diversity, benthic invertebrate diversity and the abundance of each benthic invertebrate functional feeding group. The Student-Newman-Keuls procedure was used following all ANOVA tests to make *post-hoc* comparisons among levels of significant terms. All analyses were balanced and variables were transformed where necessary prior to analysis to homogenise variances.

Results – signal and virile crayfish

Dietary interactions

The $\delta^{15}N$ was significantly lower in populations of allopatric and sympatric signal crayfish compared to allopatric and sympatric virile crayfish ($F_{3,80} = 31.93$, $P < 0.001$; Figure 7.1; All signal and virile crayfish experiment ANOVA tables are in Appendix 4A). Sympatric virile crayfish also had a significantly higher $\delta^{15}N$ than their allopatric counterparts ($F_{3,80} = 31.93$, $P < 0.001$; Figure 7.1). This difference, between two populations of the same species under experimental conditions, confirms that the duration of both experiments was sufficient for isotopic turnover in crayfish muscle tissue.

The $\delta^{13}C$ was significantly lower in populations of allopatric signal crayfish compared to allopatric and sympatric virile crayfish ($F_{3,80} = 6.06$, $P = 0.001$; Figure 7.1). Discrimination analysis correctly classified 88.1 % of signal crayfish (37 of 42) and 83.3 % of virile crayfish (35 of 42) suggesting that each species occupied a distinct area of isotopic space.

Carbon range and nitrogen range, both measures of isotopic niche width, were significantly higher in populations of allopatric virile crayfish than sympatric signal crayfish (CR_b: $F_{3,16} = 3.87$, $P = 0.029$; NR_b: $F_{3,16} = 3.70$, $P = 0.034$; Figure 7.2).

The proportional contribution of six putative resources to the diet of crayfish varied significantly between populations (Pseudo-$F_{3,16} = 2.74$, $P = 0.044$; Figure 7.3). SIMPER analysis revealed that the largest difference in population diet was between allopatric signal crayfish and both allopatric and sympatric populations of virile crayfish, primarily due to less leaf litter (which contributed up to 28.5 % to dissimilarity) and more Chironomidae (contributing up to 23.0% to dissimilarity) in the diet of allopatric signal crayfish (Table 7.2;

Figure 7.3). Interestingly, the two populations with the most similar diets were sympatric signal crayfish and sympatric virile crayfish and this was largely due to similar contributions of silk weed and Baetidae (Table 7.2; Figure 7.3). Analysis of the ratio of plant to animal material in the diet of each population in each pond revealed that, on average, signal crayfish consumed slightly more animal material and virile crayfish were more herbivorous (Figure 7.3).

Assemblage composition

The benthic invertebrate community structure differed significantly between treatments (Pseudo-$F_{3,16} = 2.30$, $P = 0.01$). The assemblage was the most similar in treatments with crayfish (average similarity between 75.0% and 76.6%) and hence, the control was the most dissimilar. The same five taxa were affected the most by signal or virile crayfish and, in total they contributed almost 50% to the dissimilarity in the community assemblage between the presence or absence of both species of crayfish (Table 7.3). Chironomidae were reduced by crayfish presence, however, Corophiidae and Limnephilidae were reduced in the presence of virile crayfish but increased by signal crayfish (Table 7.3).

The total abundance of grazers and scrapers appeared lower in the treatment with neither species compared to all crayfish treatments but it was not significant ($F_{3,16} = 2.72$, $P = 0.079$; Figure 7.4A). The number of gatherers and shredders did not differ significantly between treatments (Figure 7.4B). All crayfish treatments had significantly lower numbers of predators and carnivorous scavengers ($F_{3,16} = 8.78$, $P = 0.001$; Figure 7.4C) and Diptera ($F_{3,16} = 8.78$, $P = 0.001$; Figure 7.4D) compared to the treatment with neither species.

The zooplankton assemblage consisted primarily of *Daphnia* and Cyclopoida across all treatments and there was no difference in the community assemblage structure or density between treatments (Figure 7.5). Diversity, measured as Shannon's diversity index, of benthic invertebrates was significantly higher in the signal crayfish treatment compared to the virile crayfish treatment and the neither species treatment ($F_{3,16} = 8.54$, $P = 0.001$; Figure 7.6A). However, the treatment with no crayfish had a species richness of 14.8 ± 1.5, which was the highest observed in all of the treatments, including the signal treatment which had an average species richness of just 9.2 ± 0.6. There was no difference in zooplankton diversity between treatments (Figure 7.6B).

Algal standing stock

Benthic and pelagic algal standing stocks were highest in the signal crayfish and neither species treatments, respectively. There was a near significant difference between the signal crayfish and neither species treatment in the chlorophyll-*a* concentration in the benthos ($F_{3,16} = 2.51$, $P = 0.095$; Figure 7.7A) and between the both species and neither species treatment in the chlorophyll-*a* concentration in the water column ($F_{3,16} = 2.97$, $P = 0.063$; Figure 7.7B).

Leaf litter decomposition

In bags with a mesh size of $1mm^2$, there was no significant difference in leaf decay rates between treatments (Figure 7.8A). In bags with a mesh size of $5mm^2$, leaf litter decomposition was significantly higher in the signal crayfish treatment compared with the neither species and virile crayfish treatments ($F_{3,16} = 9.24$, $P = 0.001$; Figure 7.8B). For bags of mesh size 10mm^2, leaf litter decomposition was significantly lower in the virile crayfish treatment compared to the neither species and signal crayfish treatment; the latter was significantly higher than all other treatments ($F_{3,16} = 16.95$, $P < 0.001$; Figure 7.8C).

Nutrient analysis

The nitrate and phosphate concentrations did not differ between treatments after 6 weeks.

Tables and Figures - signal and virile crayfish

Table 7.2. SIMPER analysis indicating how variation in the proportional contribution of resources to crayfish diet contributes to diet dissimilarity between populations of allopatric and sympatric signal (SC) and virile crayfish (VC).

Resource	Average contribution to Allopatric VC diet	Average contribution to Sympatric SC diet	Contribution to dissimilarity (%)	Cumulative contribution (%)
Macrophyte	0.26	0.2	26.14	26.14
Chironomidae	0.09	0.16	25.23	51.37
Asellidae	0.08	0.13	18.54	69.9
Silk weed	0.2	0.17	13.16	83.07
Baetidae	0.13	0.13	10.19	93.26
			Average dissimilarity =	*13.81*

Resource	Average contribution to Allopatric SC diet	Average contribution to Sympatric VC diet	Contribution to dissimilarity (%)	Cumulative contribution (%)
Leaf litter	0.09	0.17	28.5	28.5
Chironomidae	0.16	0.12	22.97	51.46
Macrophyte	0.26	0.21	21.2	72.67
Asellidae	0.1	0.11	12.73	85.4
Silk weed	0.17	0.18	8.47	93.87
			Average dissimilarity =	*14.89*

Resource	Average contribution to Allopatric VC diet	Average contribution to Sympatric VC diet	Contribution to dissimilarity (%)	Cumulative contribution (%)
Macrophyte	0.26	0.21	27.45	27.45
Chironomidae	0.09	0.12	21.62	49.07
Asellidae	0.08	0.11	16.72	65.79
Leaf litter	0.14	0.17	13.2	78.99
Silk weed	0.2	0.18	10.64	89.63
Baetidae	0.13	0.13	10.37	100
			Average dissimilarity =	*11.42*

Resource	Average contribution to Sympatric SC diet	Average contribution to Sympatric VC diet	Contribution to dissimilarity (%)	Cumulative contribution (%)
Chironomidae	0.16	0.12	26.42	26.42
Macrophyte	0.2	0.21	17.3	43.72
Leaf litter	0.14	0.17	16.75	60.47
Asellidae	0.13	0.11	16.27	76.74
Baetidae	0.13	0.13	12.71	89.45
Silk weed	0.17	0.18	10.55	100
			Average dissimilarity =	*9.48*

Table 7.3. Results of SIMPER analysis indicating the primary benthic invertebrate taxa affected by the presence of signal (SC) and virile crayfish (VC). Mean abundance (± standard error) is displayed using the raw data for clarity however, the analysis was performed on Log(x+1)-transformed data. Please note, standard errors will be high since each mean covers two treatments; for instance. abundances in SC absence will be an average of the neither species treatment and the VC only treatment.

Taxon	Mean abundance in SC absence	Mean abundance in SC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Chironomidae	664.0 ± 125.0	193.0 ± 26.9	10.0	10
Limnephilidae	4.9 ± 1.1	12.5 ± 4.8	8.97	18.97
Corophiidae	10.1 ± 1.8	14.1 ± 4.2	8.78	27.75
Lymnaeidae	7.8 ± 3.2	3.0 ± 0.6	7.67	35.42
Gammaridae	5.0 ± 1.3	7.1 ± 2.7	7.43	42.86

Taxon	Mean abundance in VC absence	Mean abundance in VC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Corophiidae	543.0 ± 149.0	314.0 ± 57.8	12.22	12.22
Limnephilidae	9.6 ± 3.6	7.0 ± 3.5	9.40	21.62
Gammaridae	16.0 ± 3.7	6.3 ± 1.3	8.98	30.60
Lymnaeidae	7.3 ± 3.2	5.8 ± 1.0	7.38	37.98
Chironomidae	7.7 ± 2.5	3.5 ± 0.6	6.49	44.47

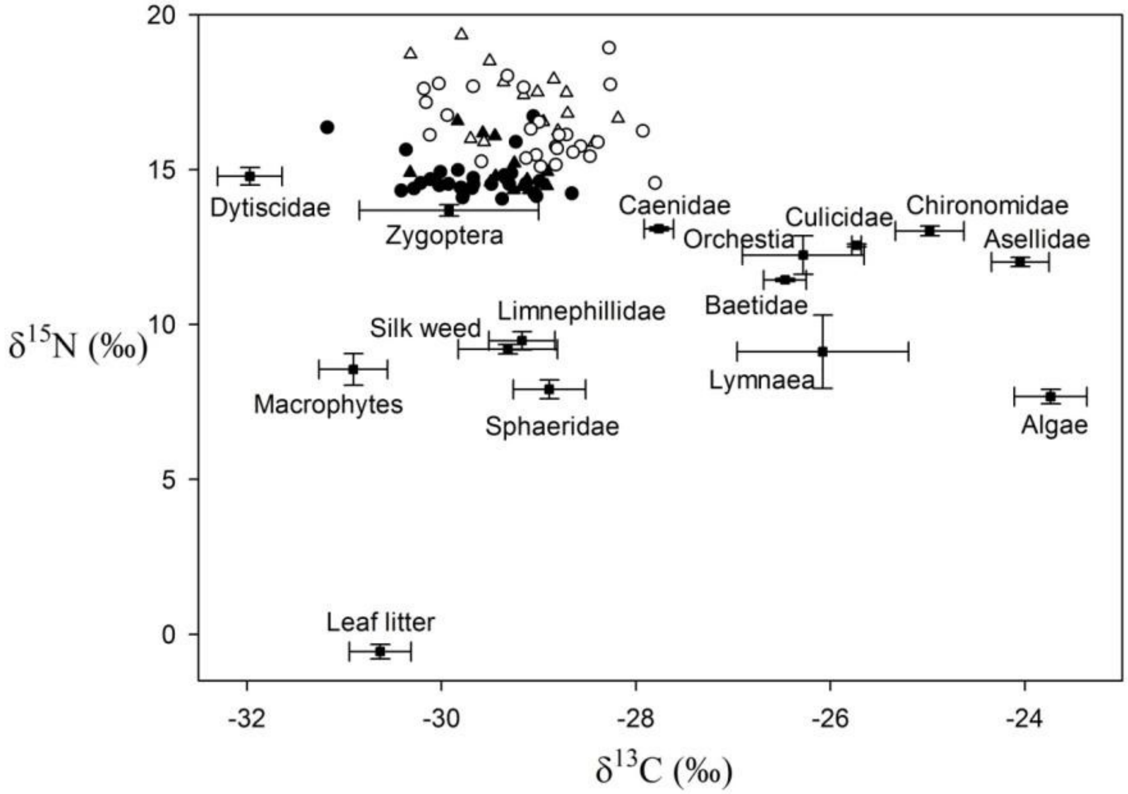

Figure 7.1. Stable isotope bi-plot of the mesocosm food webs. Squares present the average isotopic signature of resources from all ponds (mean ± standard error), filled symbols represent individual signal crayfish and open symbols represent individual virile crayfish in allopatric (circles) and sympatric (triangles) populations.

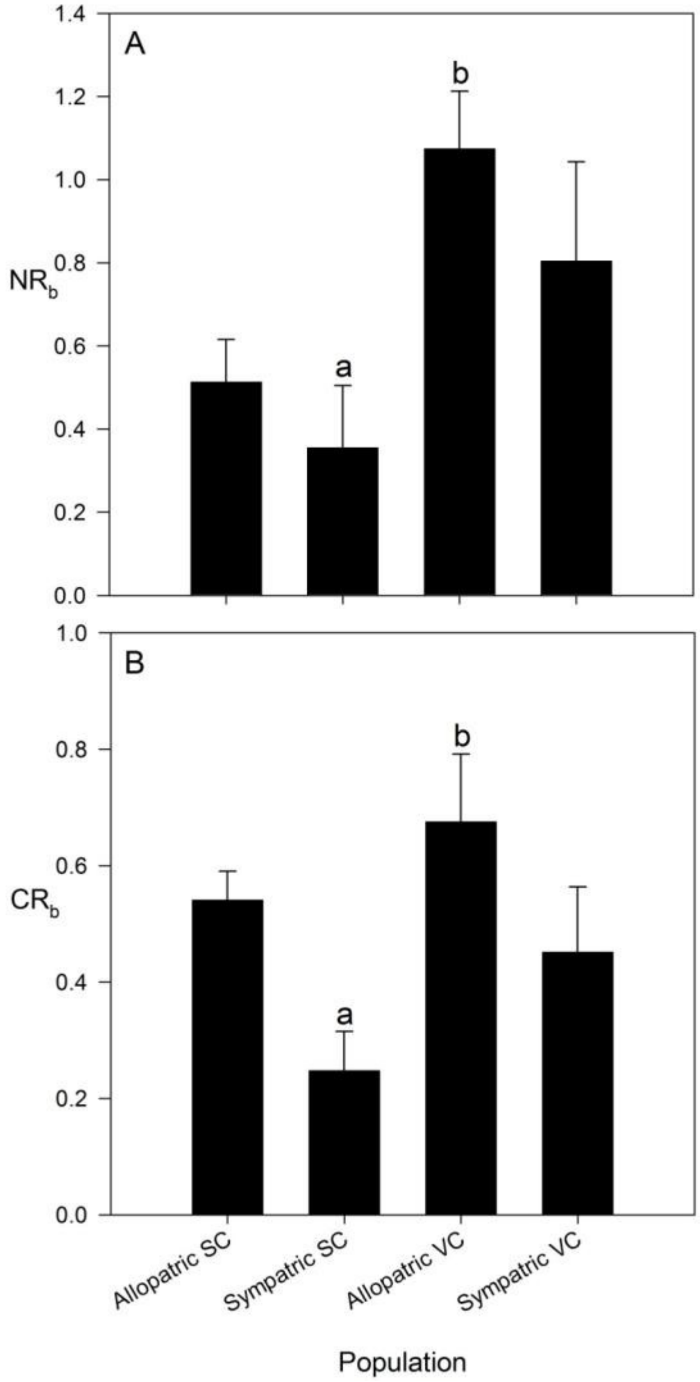

Figure 7.2. The nitrogen range (A) and carbon range (B) of allopatric and sympatric populations of signal (SC) and virile (VC) crayfish (*n* = 5; mean ± standard error). Lower case letters indicate groups that are statistically distinguishable.

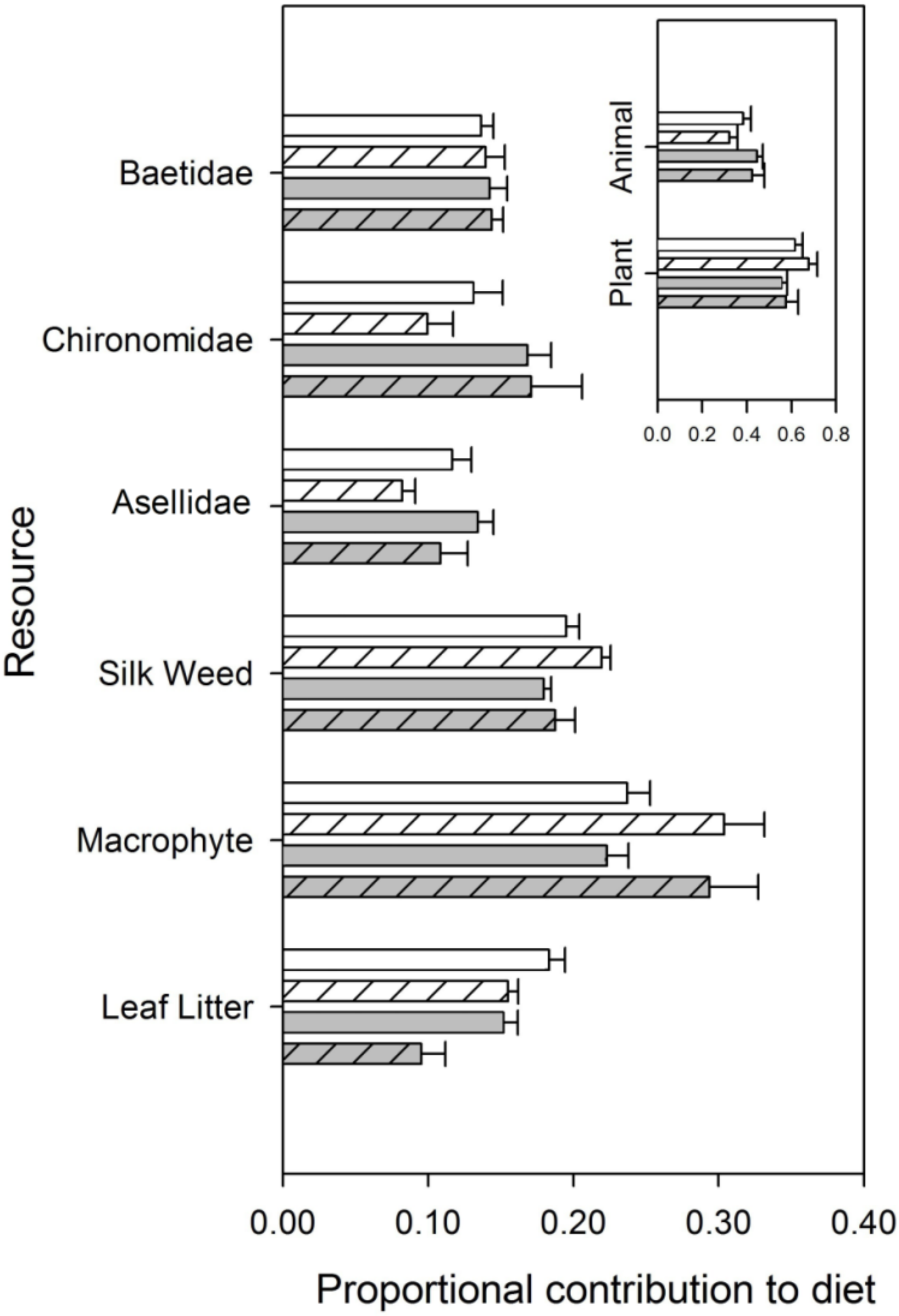

Figure 7.3. Proportional contribution of putative resources (mean ± standard error) to the diet of signal (grey) and virile crayfish (white) in allopatric (line filled) and sympatric (unfilled) populations. The inset graph shows the sum of the proportional contributions of animal and plant material (mean ± standard error).

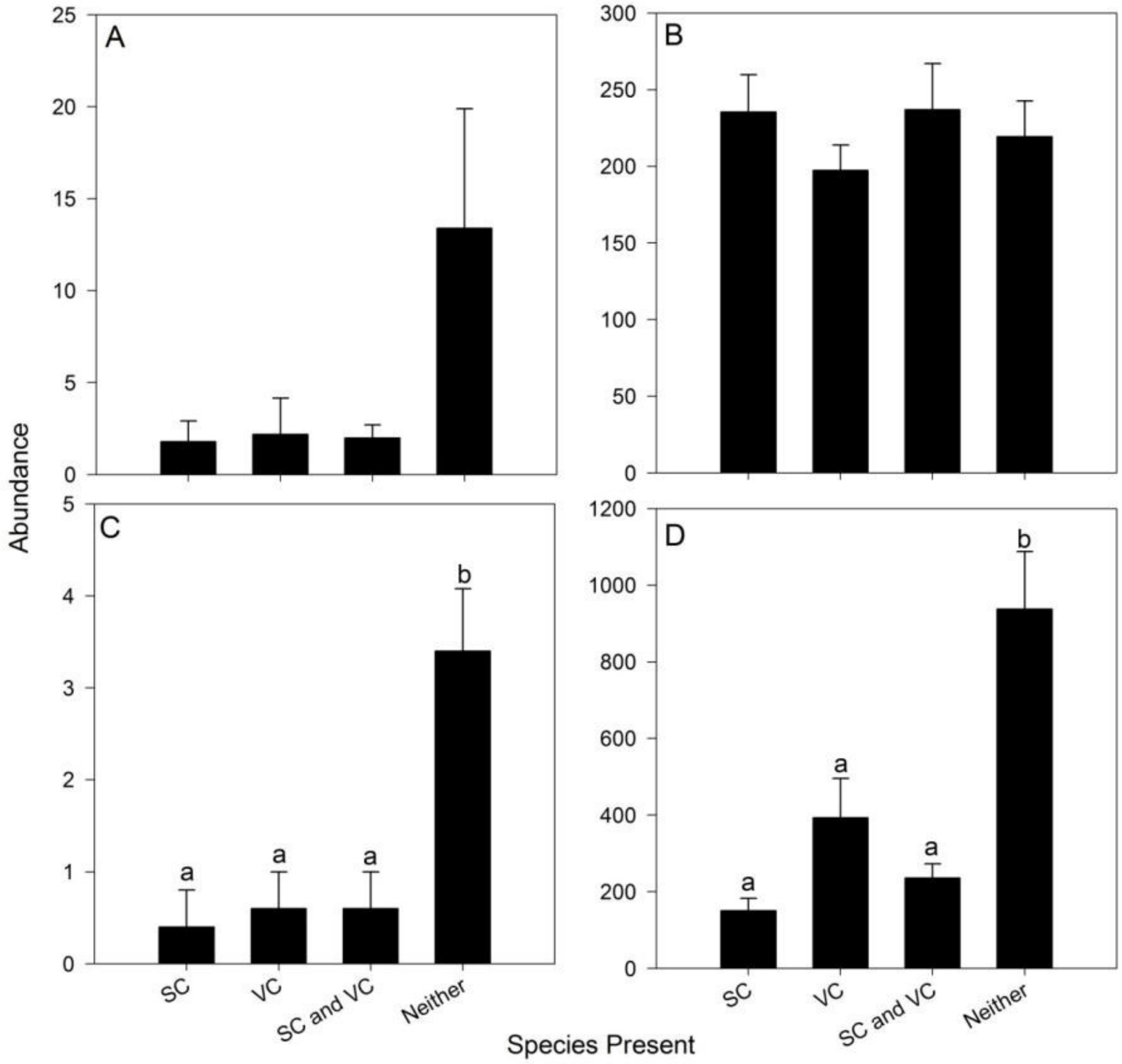

Figure 7.4. The number of grazers and scrapers (A); gatherers and shredders (B); predators and carnivorous scavengers (C) and Diptera (D) in each treatment (*n* = 5; mean ± standard error) where SC is signal crayfish and VC is virile crayfish. Lower case letters indicate groups that are statistically distinguishable.

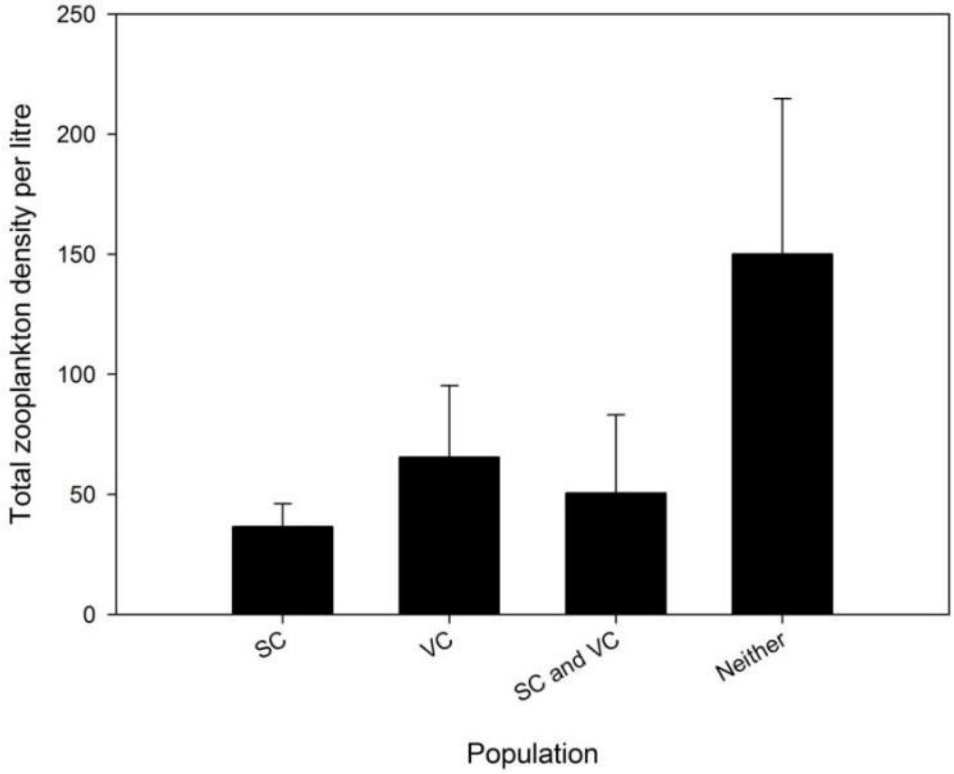

Figure 7.5. The total increase in zooplankton density (per litre) between the start and end of the 6 week experiment (*n* = 5; mean ± standard error) where SC is signal crayfish and VC is virile crayfish.

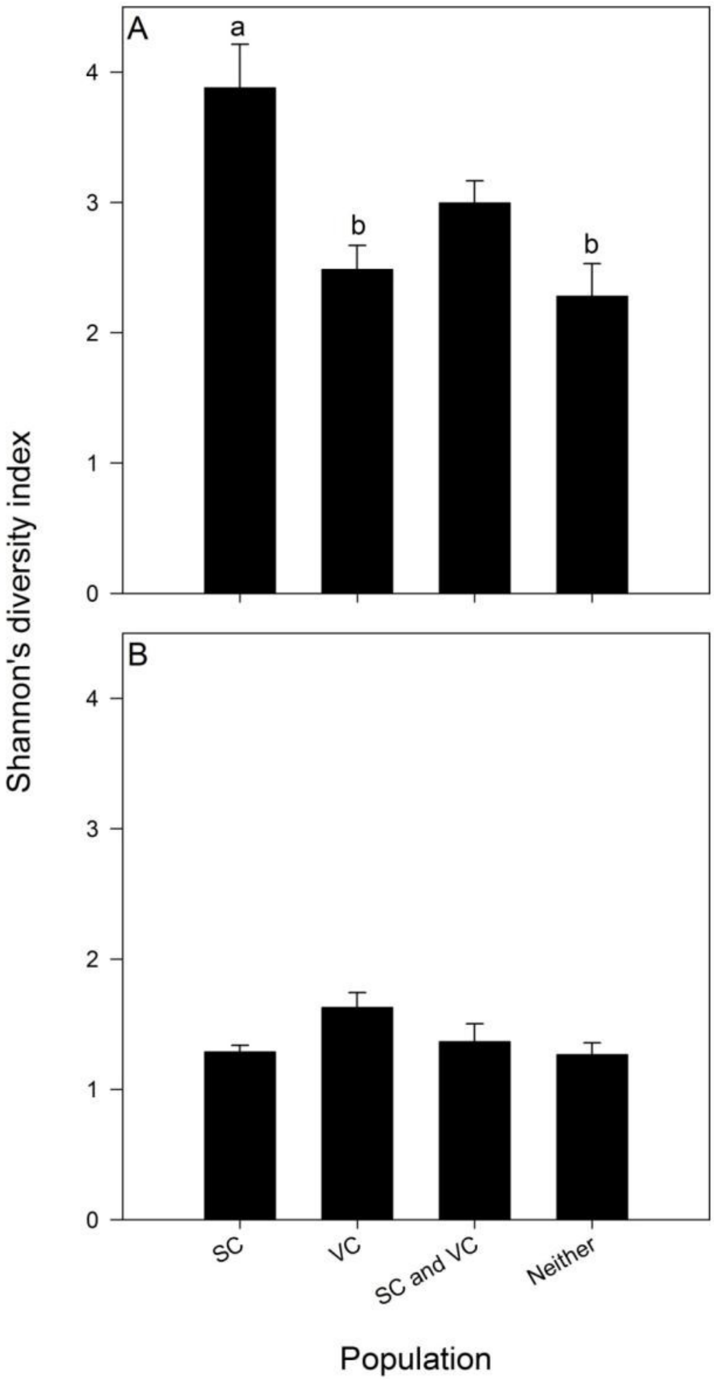

Figure 7.6. Shannon's diversity index of benthic invertebrates (A) and zooplankton (B) in each treatment (*n* = 5; mean ± standard error) where SC is signal crayfish and VC is virile crayfish. Lower case letters indicate groups that are statistically distinguishable.

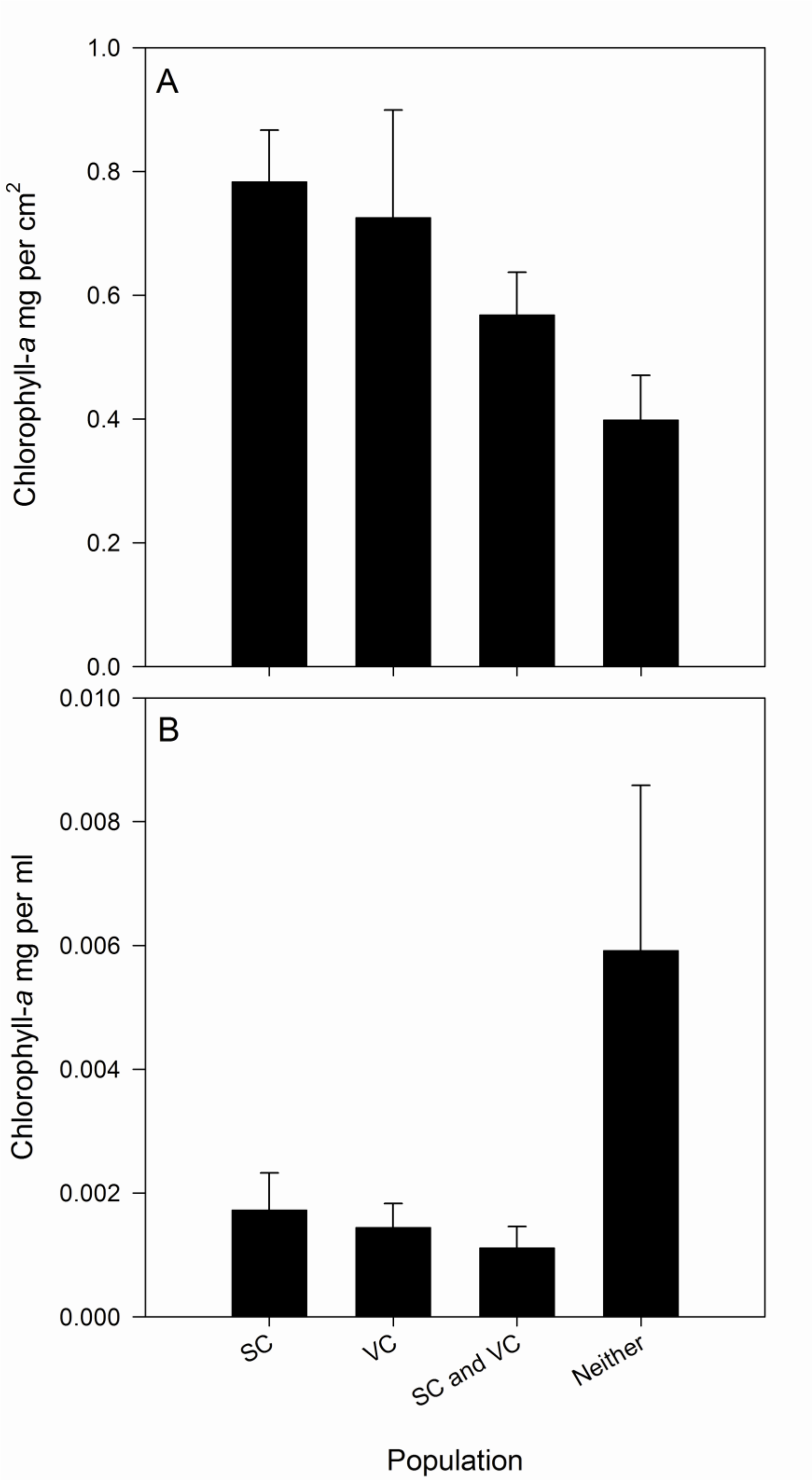

Figure 7.7. Algal standing stock measured as chlorophyll-*a* concentration on standardised tile surfaces on the benthos (A) and in the water column (B) after 4 weeks (*n* = 5; mean ± standard error) where SC is signal crayfish and VC is virile crayfish.

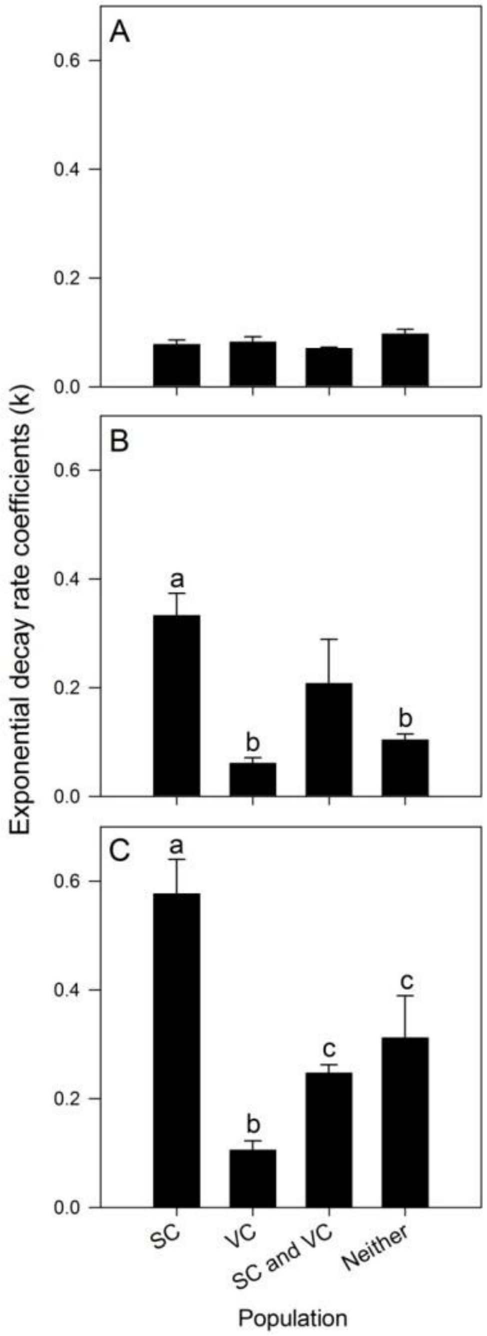

Figure 7.8. Leaf litter decomposition rates (*n* = 5; mean ± standard error) measured as exponential decay rate coefficients (*k*) in bags with a mesh of 1mm^2 (A), 5mm^2 (B) and 10mm^2 (C) where SC is signal crayfish and VC is virile crayfish. Lower case letters indicate groups that are statistically distinguishable.

Analysis – signal and virile crayfish

There was significant variation between signal and virile crayfish diet and this had some implications for the impact of each species on ecosystem structure and functioning. The virile crayfish populations had higher nitrogen signatures, implying a higher trophic level than signal crayfish. However Bayesian mixing models showed that the dissimilarity between each species diet was primarily caused by a higher ratio of animal to plant material in the diet of signal crayfish, suggesting the possibility that fractionation factors between resource and consumer may have differed between crayfish species. There was also a discrepancy in carbon values between populations; signal crayfish had lower carbon signatures than virile crayfish due to differences in preferred resource choice. Interestingly, Bayesian mixing models showed that resource use was more similar between species when they occurred in sympatric populations, suggesting that interspecific competition was not the cause of diet variation.

The benthic invertebrate community was different in the three crayfish treatments when compared to the treatment with neither species, largely due to less Chironomidae, Gammaridae and Lymnaeidae and this was probably due to direct consumption by the crayfish. Additionally, all of the crayfish treatments had lower taxon richness than the treatment with no crayfish, which contained up to 17 benthic invertebrate species. Other studies have also found that signal crayfish can reduce native invertebrate species richness in Sweden (e.g. Stenroth & Nyström 2003), whilst the impact of invasive virile crayfish in Britain and in other invaded areas, such as Canada, is largely unknown. Despite lower taxon richness, Shannon's diversity index was highest in the signal crayfish treatment and this was due to higher species evenness since the rarer taxa, which were only found in the neither species treatment, such as Zygoptera and Elmidae, were only present in small numbers.

The abundance of grazers and scrapers were reduced in all crayfish treatments and gut content analysis suggests this was due to direct predation on Gastropods (Jackson *pers. obs*.). Predators and carnivorous scavengers were also reduced in all crayfish treatments compared to the neither species treatment which is consistent with an *in situ* experiment by Stenroth and Nyström (2003) who found that invasive signal crayfish had stronger impacts on invertebrate predators than non-predatory taxa due to competition. Diptera larvae (Chironomidae and Culicidae) were reduced in the presence of both crayfish species, suggesting direct consumption had a larger effect than the positive effect of sediment sorting by crayfish (Usio & Townsend 2004).

Benthic algal standing stock was highest in the signal crayfish treatment and lowest in the neither species treatment and, although the effect was not significant, I suggest this was a result of a trophic cascade involving a reduction in grazing pressure due to consumption of the grazer *Lymnaea* by crayfish (Charlebois & Lamberti 1996; Nyström *et al.* 1999). Nyström et al. (1999) found a that a smilar trophic cascade was initiated by noble (*Astacus astacus*) and signal crayfish.

Signal and virile crayfish had opposite effects on leaf litter breakdown. Signal crayfish increased breakdown and virile crayfish decreased breakdown compared to the neither species treatment and the opposite allopatric crayfish treatment. Signal crayfish probably increased breakdown of leaf litter due to direct consumption and higher feeding rates than virile crayfish (Usio 2000). Despite Bayesian mixing models suggesting that plant material was more important in the diet of virile crayfish, signal crayfish occupied a lower trophic position, nearer the leaf litter isotopic signature, and virile crayfish had a stronger negative impact on benthic invertebrate abundances. Therefore, I suggest that virile crayfish indirectly reduced breakdown rates via a trophic cascade whereby their consumption of invertebrates reduced the number of species shredding leaf litter. Signal crayfish decoupled this trophic cascade by feeding directly on the leaf litter (Usio 2000) and therefore fulfilling the functional role of the shredders it was simultaneously removing.

Niche width, measured as carbon range and nitrogen range, was higher in allopatric populations of virile crayfish compared to populations of sympatric signal crayfish, suggesting that virile crayfish expressed greater resource choice plasticity when compared to signal crayfish. In Chapter Six I also found that virile crayfish had a wider niche than signal crayfish in the Lee Navigation populations.

Results – Louisiana red swamp and Turkish crayfish

Dietary interactions

The $\delta^{15}N$ was significantly lower in populations of allopatric and sympatric Turkish crayfish compared to allopatric and sympatric Louisiana red swamp crayfish ($F_{3,77} = 42.07$, $P < 0.001$; Figure 7.9; All Louisiana red swamp and Turkish crayfish experiment ANOVA tables are in Appendix 4B). The $\delta^{13}C$ was significantly lower in populations of allopatric and sympatric Louisiana red swamp crayfish compared to allopatric and sympatric Turkish crayfish ($F_{3,77} = 19.76$, $P < 0.001$; Figure 7.9). Discrimination analysis correctly classified 90.48 % of Louisiana red swamp crayfish (38 of 42) and 93.31% of Turkish crayfish (36 of 39) suggesting each species occupied a distinct area in isotopic space.

The nitrogen range did not differ significantly between crayfish populations (Figure 7.10A), however, the carbon range was significantly wider in populations of allopatric Louisiana red swamp crayfish compared to populations of sympatric Turkish crayfish ($F_{3,16} = 6$, $P = 0.006$; Figure 7.10B).

The proportional contribution of six putative resources (leaf litter, macrophyte, silk weed, *Asellus* sp., Chironomidae and *Lymnaea* sp.) to the diet of allopatric and sympatric populations of Louisiana red swamp and Turkish crayfish did not vary significantly (Figure 7.11). All crayfish populations had an omnivorous diet which included approximately equal proportions of animal to plant material; however, on average Turkish crayfish had a slightly more carnivorous diet than Louisiana red swamp crayfish (Figure 7.11).

Assemblage composition

There was no overall difference in the benthic invertebrate community structure between treatments. The assemblage was most similar in the treatments containing crayfish (average similarity between 65.2% and 69.4%) and the Louisiana red swamp crayfish and neither species treatment were the most dissimilar (average similarity of 51.3%). Variation in just three taxa explained more than 50% of the dissimilarity in the community assemblage between both Louisiana red swamp and Turkish crayfish absence or presence (Table 7.4); there were less Chironomidae and more Lymnaeidae and Asellidae in the absence of both species (Table 7.4).

The total abundance of grazers and scrapers was significantly lower in all treatments with crayfish present compared to the treatment with neither species ($F_{3,16} = 4.75$, $P = 0.015$; Figure 7.12A). The number of gatherers and shredders (Figure 7.12B) and Chironomidae (Figure 7.12D) did not differ significantly between treatments. Predator and carnivorous scavenger abundance was significantly lower in the treatment with both Louisiana red swamp and Turkish crayfish compared to the treatment with neither species ($F_{3,16} = 3.06$, $P = 0.05$; Figure 7.12C).

The zooplankton assemblage consisted primarily of *Daphnia* and *Ceriodaphnia* across all treatments and there was no difference in the community assemblage structure or density between treatments (Figure 7.13). Diversity, measured as Shannon's diversity index, of benthic invertebrates (Figure 7.14A) and zooplankton (Figure 7.14B) did not differ significantly between treatments.

Algal standing stock

Chlorophyll-*a* concentrations were significantly higher on standardised tile surfaces, left for 6 weeks on the benthos, in the treatment with Louisiana red swamp crayfish compared to the treatment with neither species ($F_{3,16} = 4.5$, $P = 0.018$; Figure 7.15A). There was no difference between treatments in the chlorophyll-*a* concentration in the water column (Figure 7.15B).

Leaf litter decomposition

In bags with a mesh size of 1mm^2 leaf litter decomposition was significantly higher in the treatment with neither crayfish species compared to both treatments with Turkish crayfish present ($F_{3,16} = 6.86$, $P = 0.003$; Figure 7.16A). For bags of mesh size 5mm^2, leaf litter decomposition was also significantly higher in the treatment with neither crayfish species compared to the allopatric Turkish crayfish treatment ($F_{3,16} = 3.75$, $P = 0.033$; Figure 7.16B). Those of mesh size 10mm^2, which allowed access by crayfish, had leaf litter decomposition rates significantly lower in the Turkish crayfish treatment compared to the Louisiana red swamp crayfish treatment ($F_{3,16} = 3.68$, $P = 0.034$; Figure 7.16C).

Nutrient analysis

The nitrate and phosphate concentration in the water column did not vary between treatments after 6 weeks.

Tables and Figures – Louisiana red swamp and Turkish crayfish

Table 7.4. Results of SIMPER analysis indicating the primary benthic invertebrate taxa affected by the presence of Louisiana red swamp (RSC) and Turkish crayfish (TC). Mean abundance (± standard error) is displayed using the raw data for clarity however, the analysis was performed on Log(x+1)-transformed data. Please note, standard errors will be high since each mean covers two treatments, for instance abundances in RSC absence will be an average of the neither species treatment and the TC only treatment.

Taxon	Mean abundance in RSC absence	Mean abundance in RSC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Lymnaeidae	62.0 ± 90.7	11.7 ± 13.3	20.07	20.07
Chironomidae	157.0 ± 95.4	196.3 ± 106.1	19.82	39.89
Asellidae	17.6 ± 21.7	14.5 ± 14.3	11.85	51.74
Turbellaria	9.9 ± 13.0	2.8 ± 6.1	9.30	61.04
Oligochaeta	2.8 ± 4.21	1.6 ± 1.2	5.37	66.41

Taxon	Mean abundance in TC absence	Mean abundance in TC presence	Contribution to dissimilarity (%)	Cumulative contribution (%)
Chironomidae	175.1 ± 121.7	165.8 ± 79.9	20.55	20.55
Lymnaeidae	64.2 ± 89.8	11.0 ± 9.3	20.23	40.07
Asellidae	19.6 ± 22.2	15.5 ± 12.7	12.09	52.86
Turbellaria	10.2 ± 13.23	3.7 ± 5.2	9.68	62.54
Dytiscidae	2.1 ± 2.7	2.5 ± 3.5	5.25	67.80

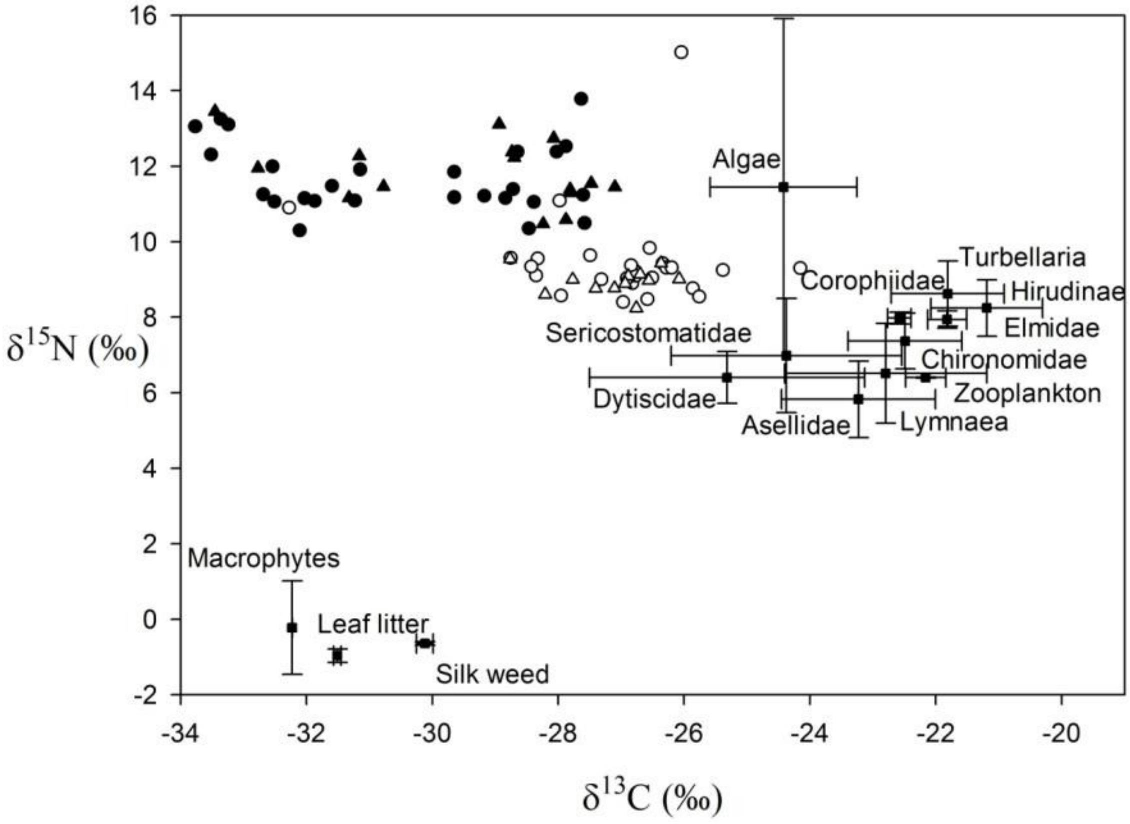

Figure 7.9. Stable isotope bi-plot of the mesocosm food webs. Squares present the average isotopic signature of resources from all ponds (mean ± standard error), filled symbols represent individual Louisiana red swamp crayfish and open symbols represent individual Turkish crayfish in allopatric (circles) and sympatric (triangles) populations.

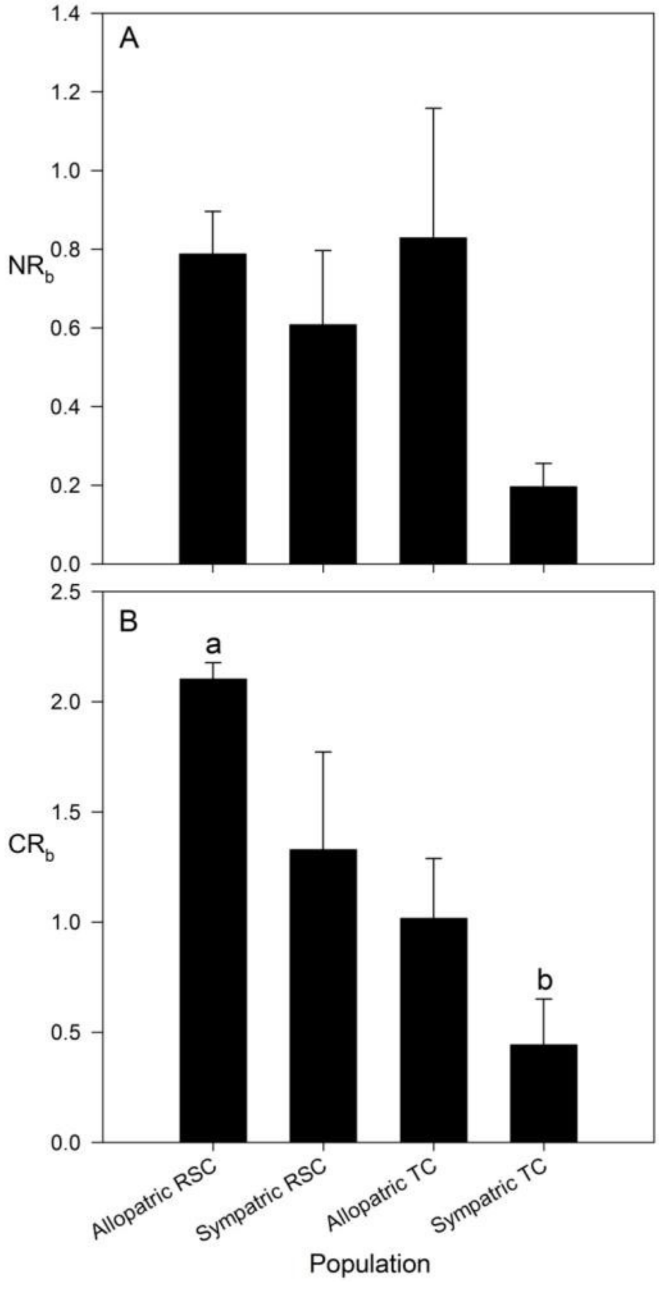

Figure 7.10. The nitrogen range (A) and carbon range (B) of allopatric and sympatric populations of Louisiana red swamp (RSC) and Turkish (TC) crayfish (*n* = 5; mean ± standard error). Lower case letters indicate groups that are statistically distinguishable.

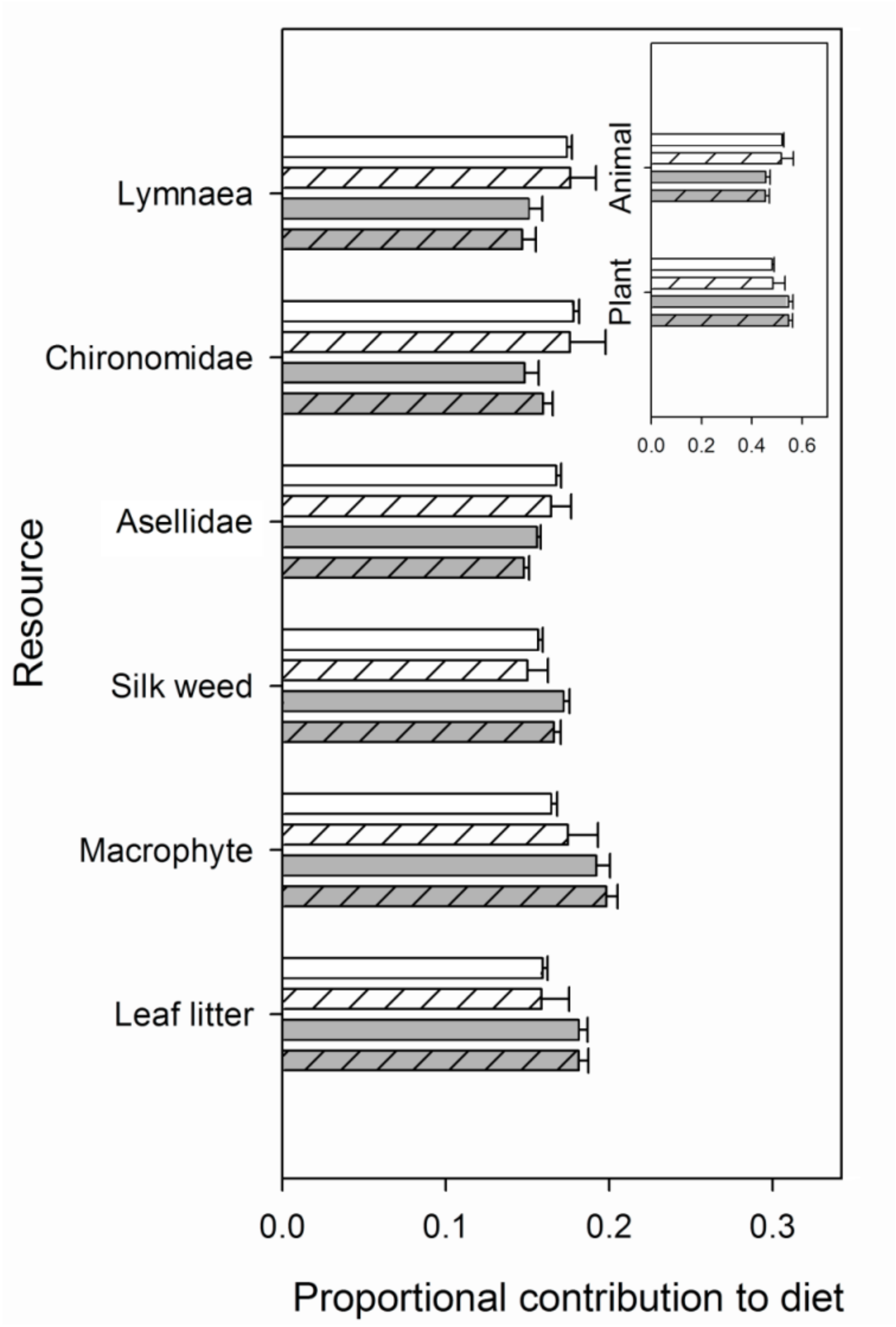

Figure 7.11. Proportional contribution of putative resources (mean ± standard error) to the diet of Louisiana red swamp crayfish (grey) and Turkish crayfish (white) in allopatric (line filled) and sympatric (unfilled) populations. The inset graph shows the sum of the proportional contributions of animal and plant material (mean ± standard error).

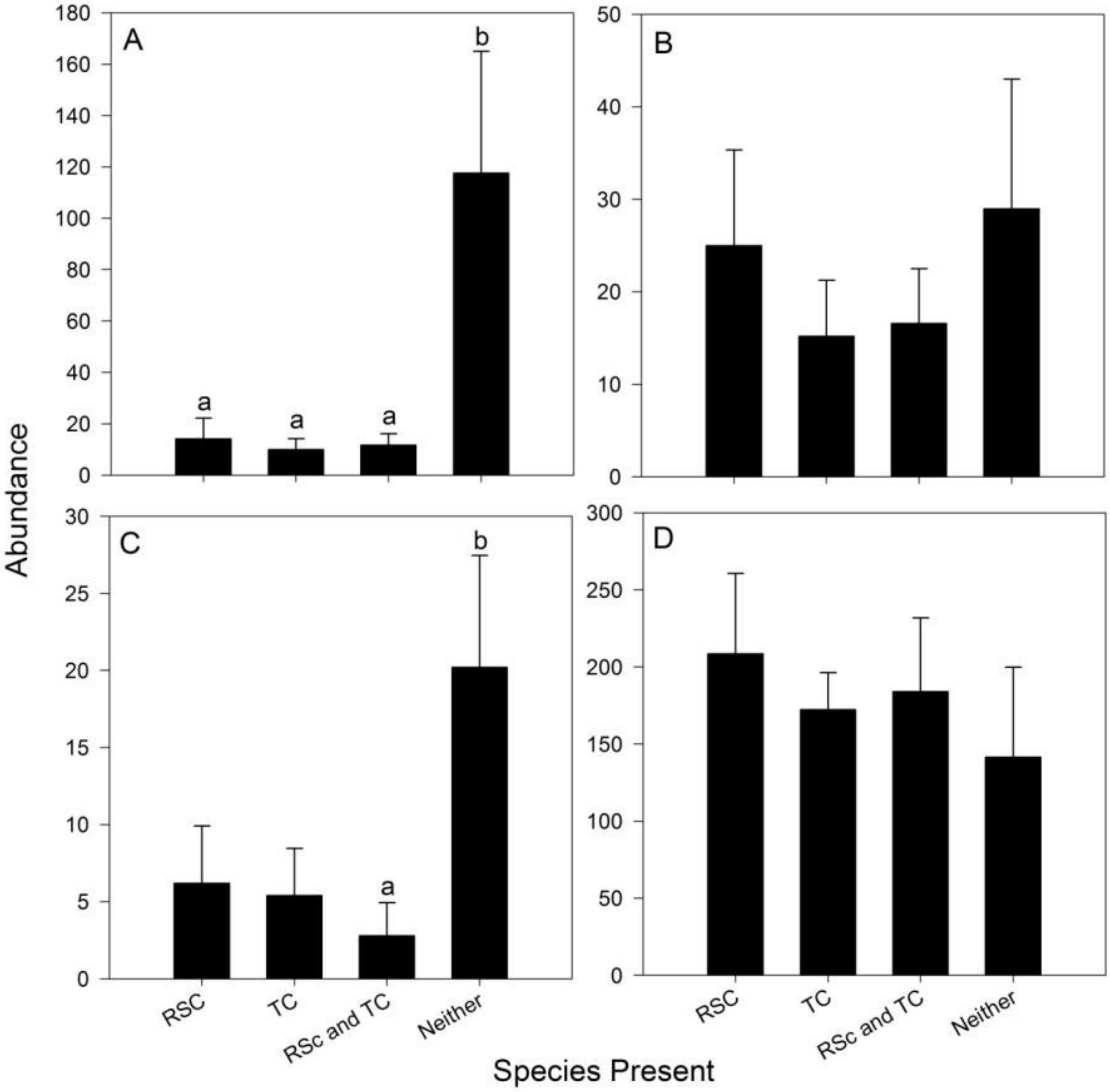

Figure 7.12. The number of grazers and scrapers (A); gatherers and shredders (B); predators and carnivorous scavengers (C) and chironomids (D) in each treatment (*n* = 5; mean ± standard error) where RSC is Louisiana red swamp crayfish and TC is Turkish crayfish. Lower case letters indicate groups that are statistically distinguishable.

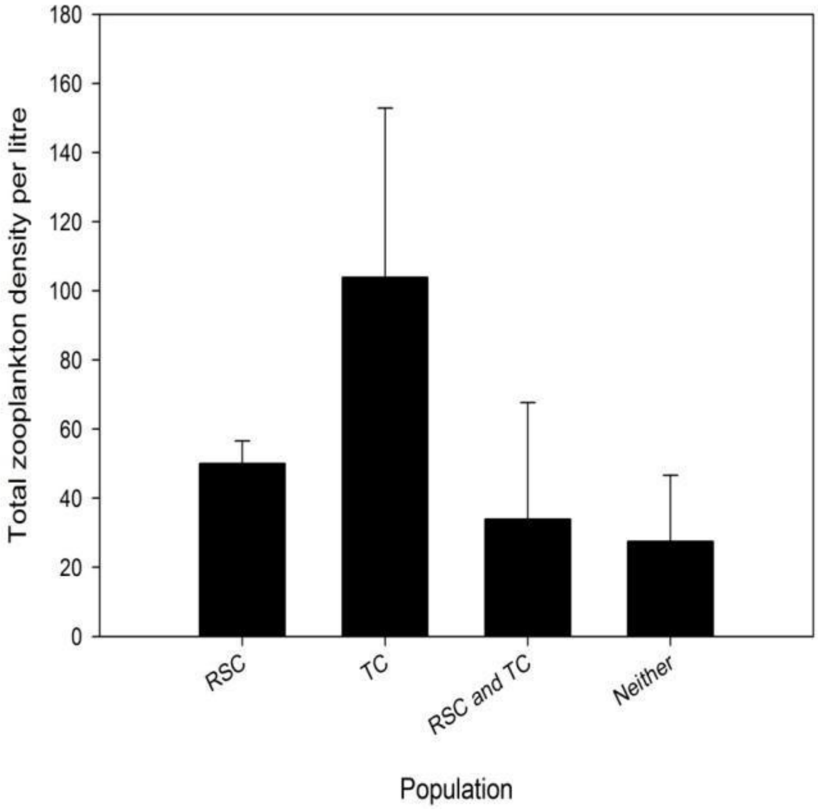

Figure 7.13. The total increase in zooplankton density (per litre) between the start and end of the 6 week experiment (*n* = 5; mean ± standard error) where RSC is Louisiana red swamp crayfish and TC is Turkish crayfish.

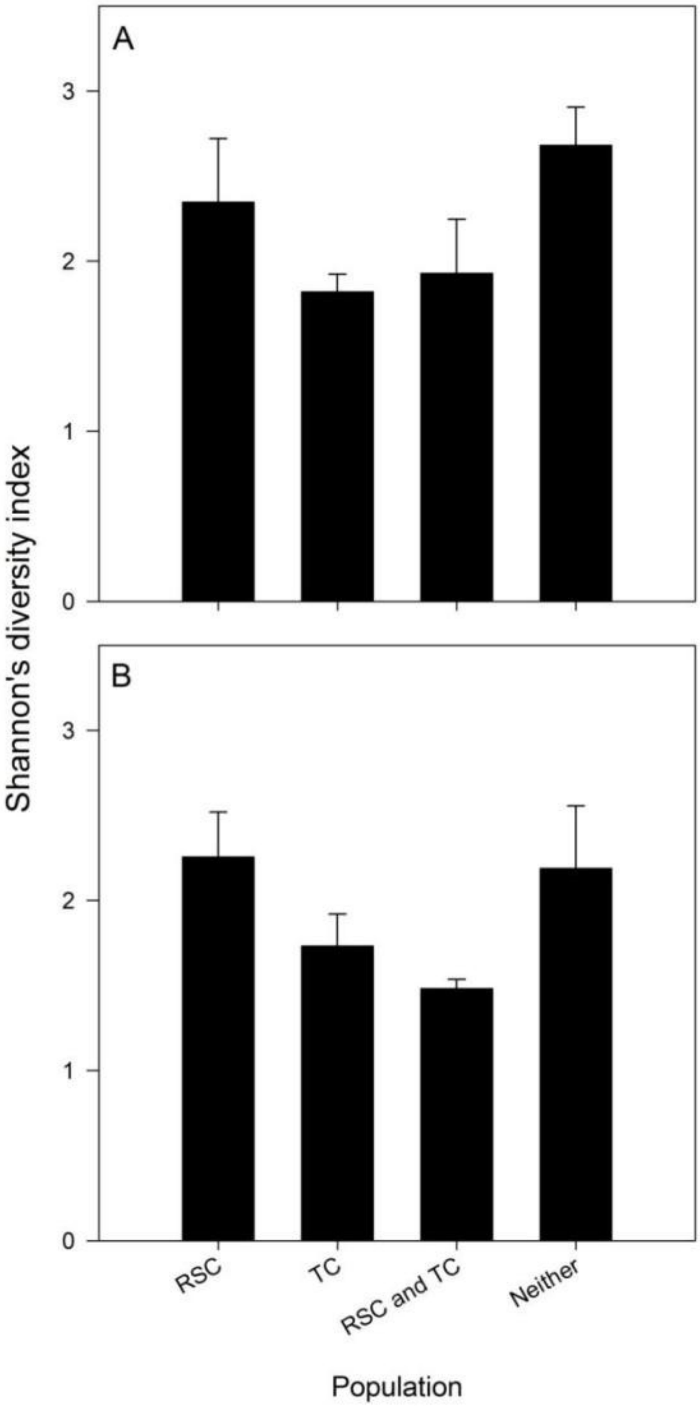

Figure 7.14. Shannon's diversity index of benthic invertebrates (A) and zooplankton (B) in each treatment (*n* = 5; mean ± standard error) where RSC is Louisiana red swamp crayfish and TC is Turkish crayfish.

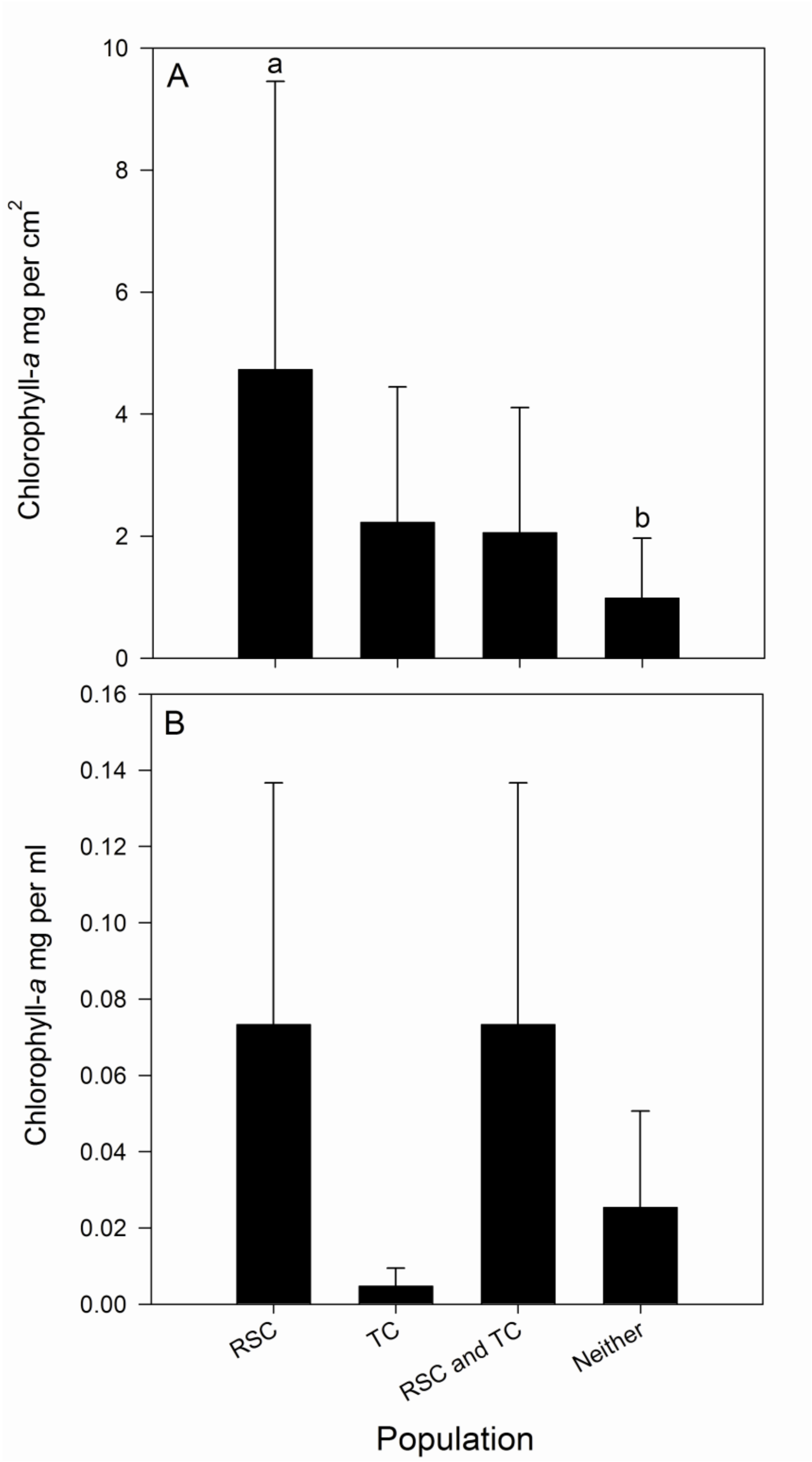

Figure 7.15. Algal standing stock measured as chlorophyll-*a* concentration on standardised tile surfaces on the benthos (A) and in the water column (B) after 6 weeks (*n* = 5; mean ± standard error) where RSC is Louisiana red swamp crayfish and TC is Turkish crayfish. Lower case letters indicate groups that are statistically distinguishable.

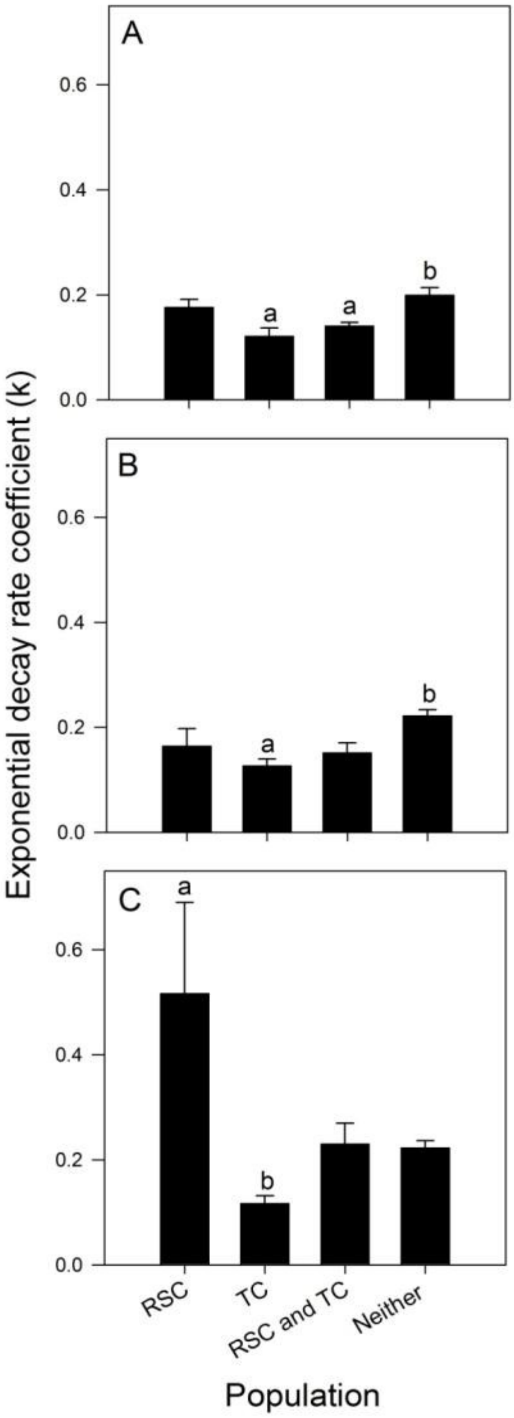

Figure 7.16. Leaf litter decomposition rates (*n* = 5; mean ± standard error) measured as exponential decay rate coefficients (*k*) in bags with a mesh of 1mm^2 (A), 5mm^2 (B) and 10mm^2 (C) where RSC is Louisiana red swamp crayfish and TC is Turkish crayfish. Lower case letters indicate groups that are statistically distinguishable.

Analysis – Louisiana red swamp and Turkish crayfish

Bayesian mixing models showed that both Louisiana red swamp and Turkish crayfish had similar omnivorous diets; however, the species were isotopically distinct suggesting some variation in the relative contribution of resource. Louisiana red swamp crayfish had lower carbon and higher nitrogen signatures than Turkish crayfish, suggesting discrepancy in resource choice and a higher trophic position. Importantly, these differences in diet resulted in variation in the impact each species had on the ecosystem.

Both species of crayfish had similar impacts on ecosystem structure. The strongest impact was on the abundance of grazers and scrapers which were reduced in all crayfish treatments and gut content analysis suggests this was due to direct predation on the dominant grazer in the ponds; snails from the genus *Lymnaea* (Jackson *pers. obs*.). The results revealed that more than 50% dissimilarity in the invertebrate assemblage, between the presence and absence of both crayfish species, could be attributed to variation in the abundance of just three taxa; Lymnaeidae, Chironomidae (Diptera) and Asellidae. The abundance of Asellidae was reduced which, similar to Lymnaeidae, was probably due to direct consumption, yet in contrast to the signal and virile crayfish experiment, the abundance of Chironomidae was actually elevated by the presence of both crayfish species. This was an unexpected result because Chironomidae are often considered to be a component of crayfish diet (e.g. Stenroth & Nyström 2003) and I found chironomid remains in the guts of both Louisiana red swamp and Turkish crayfish (Jackson *pers. obs*.). I suggest that bioturbation by crayfish (Parkyn *et al.* 1997; Harvey *et al.* 2011) may have improved habitat and food availability for chironomids by exposing surfaces for colonisation (Usio & Townsend 2004).

Stenroth and Nyström (2003) found that invasive crayfish had stronger impacts on native invertebrate predators than non-predatory taxa. The number of predators and carnivorous scavengers were only reduced in the sympatric Louisiana red swamp and Turkish crayfish treatment compared to the treatment with no species. This indicates an amplified impact whereby the presence of both invasive crayfish species synergistically increases competition pressure with native benthic invertebrates and hence, causes a decline in their abundance.

Benthic algae standing stock was higher in the Louisiana red swamp crayfish treatment compared to the neither species treatment and this was probably caused by the same trophic cascade described above, whereby the consumption of grazers by crayfish reduces grazing pressure on algae and hence, promotes algal growth (Charlebois & Lamberti 1996). Benthic algae standing stock was also higher in the Turkish crayfish and both species treatments compared to the neither species treatment and although the effect was not significant, I suggest that this trophic cascade mechanism is occurring in all crayfish treatments but to different degrees.

As in the signal and virile crayfish experiment, Louisiana red swamp and Turkish crayfish had opposite effects on leaf litter breakdown; Louisiana red swamp crayfish directly increased breakdown and Turkish crayfish indirectly decreased breakdown when compared to the neither species treatment. I propose that Louisiana red swamp crayfish accelerated breakdown rates by direct consumption based on gut content analysis (Jackson *pers. obs.*) and on our stable isotope results. Previous studies have also found that detritus is a significant component of crayfish diet (e.g. Stenroth & Nyström 2003) and that decomposition can be elevated in the presence of crayfish (Usio 2000). Despite appearing to occupy a higher trophic level, the isotopic carbon signature of Louisiana red swamp crayfish was closer to plant based resources suggesting a more herbivorous diet than Turkish crayfish, which sat closer to invertebrate resources in isotopic space. Turkish crayfish had a slightly stronger impact on benthic invertebrate abundance than Louisiana red swamp crayfish and stable isotope analysis suggests that they had a slightly more carnivorous diet. Turkish crayfish initiated the same trophic cascade as virile crayfish in the first experiment, indirectly reducing decomposition by consuming the other invertebrates responsible for shredding leaf litter. Signal and Louisiana red swamp crayfish decouple this trophic cascade by feeding directly on the leaf litter (Usio 2000).

Niche width, measured as carbon range, was higher in allopatric populations of Louisiana red swamp crayfish compared to populations of sympatric Turkish crayfish. Olsson et al. (2009) measured niche width using the convex hull method (Layman *et al.* 2007a) and found that invasive signal crayfish had a wider niche than native noble crayfish due to greater plasticity in resource choice, allowing considerable variation in the position of their niche between habitats. Our results suggest that Louisiana red swamp crayfish expressed greater resource choice plasticity than Turkish crayfish and that the latter were more affected by a functionally similar competitor.

Conclusions

The mesocosm experiments confirmed that there is variation in invasive crayfish species diet, consistent with the field survey results in Chapter Six. The diet variation had important implications for the structure and functioning of the mesocosm ecosystem; each species of invasive crayfish in the Thames catchment had subtly different impacts.

In the second experiment, Louisiana red swamp and Turkish crayfish caused increased Chironomidae abundance due to their sediment sorting activity which exposed surfaces for colonisation and hence increased habitat and food availability (Usio & Townsend 2004; Harvey *et al.* 2011). In contrast, signal and virile crayfish reduced Chironomidae abundance, suggesting the negative effects of direct consumption outweighed the positive sediment sorting effect (Stenroth & Nyström 2003). Chironomidae larvae were found in nearly 50% of the signal crayfish guts examined by Stenroth and Nyström (2003), indicating that they can be an important part of crayfish diet. Furthermore, my Bayesian mixing models indicated that Chironomidae contributed to the diet of all four invasive crayfish species. The contrasting effects of invasive crayfish species can have implications for food web structure and ecosystem functioning, especially since different species of Chironomidae can have varying functional roles as the group encompasses many feeding groups.

All four species of crayfish, in both allopatric and sympatric populations, reduced the abundance of grazers and scrapers, which consisted primarily of Gastropoda. Stable isotope and gut content analysis indicated that this was by direct consumption. Slower moving organisms, such as snails, are common in the diet of invasive crayfish because they are easily caught and therefore profitable prey (Parkyn *et al.* 1997; Nyström *et al.* 1999). The observed reduction in the abundance of grazers initiated a tropic cascade by reducing grazing pressure on benthic algae and hence increasing algal standing stock in the benthos, although the effect was only significant when Louisiana red swamp crayfish were present in allopatric populations. A similar trophic cascade has been recorded in both lentic and lotic ecosystems after crayfish invasions (Charlebois & Lamberti 1996).

All four crayfish species altered leaf litter decomposition rates compared to the treatments with no crayfish, but by two different mechanisms. Louisiana red swamp and signal crayfish increased leaf litter breakdown and since this occurred in mesh bags that were accessible to crayfish, this was due to direct consumption (Usio 2000). In contrast, Turkish and virile crayfish reduced leaf litter breakdown and given that this was in bags that were not directly accessible to crayfish, was most likely caused by a trophic cascade whereby the crayfish consumed the invertebrates that would normally shred leaf litter. This indirectly reduced net decomposition, despite the crayfish utilising leaf litter as a resource. Crayfish simultaneously affect intermediate consumers and their basal resources because they are omnivores (Usio 2000). Louisiana red swamp and signal crayfish decouple the potential trophic cascade by having a stronger direct impact on leaf litter, probably due to higher feeding rates than Turkish and virile crayfish. Feeding rates can vary among similar aquatic invaders, including shore crabs (DeGraaf & Tyrrell 2004) and freshwater fish (Rehage *et al.* 2005) but to my knowledge, feeding rates have not been compared among crayfish. Gatherers and shredders were present in the lowest abundances in the Turkish and virile crayfish allopatric treatments in the second and first experiment, respectively. This suggests that their effect on the intermediate consumers (invertebrate shredders) was stronger than the effect of Louisiana red swamp and signal crayfish. Consequently, Turkish and virile crayfish preserved the trophic cascade and Louisiana red swamp and signal crayfish decoupled the trophic cascade.

Signal and virile crayfish both had negative impacts on other predators and carnivorous scavengers in the experiments and this could be due to direct competition for invertebrate prey (McCarthy *et al.* 2006). Alternatively, crayfish may have consumed the invertebrate predators because the assemblage was dominated by slow-moving species such as Odonata and Hirudinea which can easily be caught by crayfish (Stenroth & Nyström 2003).

One aim of these experiments was to address the question; do interactions among invasive crayfish amplify or mitigate one another’s impact on the ecosystem. Of all the aspects of ecosystem structure and function I measured, the only evidence of a synergistic impact was the combined negative effect of Louisiana red swamp and Turkish crayfish on invertebrate predators and carnivorous scavengers. In allopatric conditions, neither crayfish species had a significant effect, but in the sympatric Louisiana red swamp and Turkish crayfish treatment, predators and carnivorous scavengers were reduced in numbers. This suggests a synergistic negative impact which may be due to predator-prey links (Stenroth & Nyström 2003) and/or competition pressure (McCarthy *et al.* 2006). In both experiments, all the other measures of ecosystem structure and functioning showed either an intermediate or an insignificant effect in the sympatric treatments. This suggests that interactions among invasive crayfish populations do not modify their allopatric impacts on ecosystem structure and functioning and instead, there is an effect which is intermediate to the two allopatric population's impacts.

The second question I aimed to address was; do sympatric invaders occupy a smaller niche than their allopatric counterparts? All four crayfish species occupied a distinct area in isotopic space despite sharing some resources. A distinct dietary niche suggests that interspecific competition will be low and hence the niche width should be consistent between allopatric and sympatric populations. There was no difference in niche width between populations of the same species, suggesting that the presence of a functionally similar rival had no effect on any of the crayfish species.

All four crayfish species shared similar resources but preferences towards certain resources varied between species which suggests that they can co-exist in the Thames catchment. My results indicate that interactions among the invaders will not amplify one another's impact when they occur in the same densities as they would in allopatry. Nonetheless, if there is no competition between species for resources due to a degree of resource partitioning, sympatric populations of two crayfish species will have the potential to reach a higher total crayfish density than a single crayfish species in a given area (Siepielski *et al.* 2011). The density of a crayfish is positively correlated with the magnitude of its impact on ecosystem structure and functioning (e.g. Parkyn *et al.* 1997). Thus, the synergistic impact of co-occurring invasive crayfish is likely to be worse (i.e. amplified; see Figure 8.1, Chapter Eight) than the allopatric impact of a single species, especially since each species has subtly different effects on the ecosystem.

The controlled mesocosm experiments confirmed my findings in Chapter Six; the four species of invasive crayfish in the Thames catchment have different trophic ecology and some resource partitioning. There was little evidence of interactions between the invaders since niche width was not affected by the presence of a rival species and sympatric populations showed an ecosystem level impact which was intermediate to the impact of the two allopatric populations. Despite this, resource partitioning between the invaders will allow coexistence in the Thames catchment and promote higher densities than if the species were sharing the same niche (Finke & Snyder 2008; Siepielski *et al.* 2011). Therefore, sympatric invasive crayfish are likely to have amplified negative impacts in the Thames catchment (Figure 8.1, Chapter Eight).

Chapter Eight: General Conclusions

Originality

This PhD has demonstrated the application of stable isotope analysis to invasion ecology, explored dietary interactions among invasive species in freshwaters and finally, revealed the negative impact invaders can have on native communities and ecosystem processes. New quantitative metrics, derived from stable isotope data, were developed to investigate the trophic ecology of invasive species and, in conjunction with measures of ecosystem structure and functioning, have been used to unravel the complex mechanisms of invasion and the subsequent impact on the host ecosystem. The majority of published studies have considered the ecology or impact of a single invasive species; this PhD was progressive by considering interactions between invaders and examining both the ecology and impact of each species in allopatric and sympatric conditions. This is especially important given that there can be facilitative interactions among invaders and multiple invasions occurring simultaneously (Simberloff & Von Holle 1999; Simberloff 2006), with ramifications for the entire ecosystem.

Critique

A number of complementary techniques were employed to examine the trophic ecology of invasive species and their impact on the recipient ecosystem. Despite the evident success of these techniques, there are a number of other methods that could have further improved the PhD, such as quantitative gut content analysis and metagenomic measures of species diversity, given time and funding. Detailed gut content analysis would have been useful in all chapters which examined species diet to support the results from stable isotope mixing models. Metagenomics is used to produce a profile of diversity from a biological sample and could have been used in the *in situ* experiments in Chapter Four or mesocosm experiments in Chapter Seven to provide quantitative measures of diversity for algal or meiofauna samples.

Stable isotope analysis as a tool in invasion ecology

Stable isotope analysis is a contemporary tool to examine food web structure and recently, studies have being using stable isotopes to calculate quantitative metrics that reveal the trophic ecology of a population (Layman *et al.* 2007a; Semmens *et al.* 2009; Jackson *et al.* 2011). Here, I demonstrated the application of these techniques to invasion ecology and developed novel measures of niche width, diet plasticity and the degree of individual specialisation, which ultimately expand this tool box. Although any studies that employ stable isotope methods are subject to a number of assumptions (Grey 2006; Semmens *et al.* 2009), the application of these techniques proved valuable to reveal mechanisms of invasion and interactions among species. For instance, measures of niche width illustrated how invasive Louisiana swamp crayfish had a less diverse diet in the presence of native crabs to avoid interspecific competition and Bayesian mixing models revealed niche partitioning between sympatric species of invasive crayfish which explained the disparity in their impacts on ecosystem structure and functioning.

Mechanisms of invasion

In Lake Naivasha, Kenya, I found evidence of serial replacement of invasive species with the most recent invader, common carp, replacing the previously successful Louisiana red swamp crayfish. Carp abundance increased simultaneously with a decline in crayfish catch over the eight year study and stable isotope-derived population metrics revealed that this was due to crayfish niche constriction in the presence of carp. This is in contrast to the Invasion Meltdown Model (Simberloff & Von Holle 1999; Simberloff 2006) which suggests that invaders facilitate one another. Despite this, I found evidence of Invasion Meltdown in the River Thames Catchment, England, where invasion rates have been accelerating since 1800. The catchment harbours 96 non-indigenous species in the freshwater sections alone and analysis of shipping activity revealed a positive correlation with species discovery dates, suggesting that anthropogenic activity has promoted the establishment and dispersal of invaders.

Diet plays a key role in the invasion process and in the subsequent success of invaders, yet it is rarely examined. Louisiana red swamp crayfish abundance declined in Lake Naivasha in response to the invasion by common carp and although it appears conditions in the lake were then untenable for them, they still persevered by migrating into the River Malewa, the primary tributary to the lake. Here, I found a novel mechanism of invasion whereby the Louisiana red swamp crayfish actually restricted their dietary niche at the invasion front, contrary to expectations. A wide niche breadth is thought to be a common trait of successful invaders however, in the presence of a well established native crab species; the crayfish were able to expand their range by converging on fewer resources to avoid dietary overlap and interspecific competition with the crabs. On the contrary, dietary analysis of the four invasive crayfish species in the Thames catchment revealed wide measures of niche breadth and hence, I suggest that resource use efficiency, dietary flexibility and plasticity in resource choice are more important traits for invaders than a wide niche breadth per se. Invasive crayfish in the Thames exhibited all these traits, including shifts in diet choice which demonstrates ecological plasticity and the ability to use resources efficiently. Indeed, invasive signal crayfish switched their diet to rely on a newly available resource, the invasive zebra mussel.

Interactions among invaders

As discussed above, I found evidence for both facilitative and inhibitive interactions among invaders. The inhibitive interaction between carp and crayfish in Lake Naivasha was largely indirect, through which carp activity in the benthos altered the habitat structure of the lake and thus, reduced the diversity of resources available to crayfish. There was also evidence of a predator-prey interaction, with the invasive carp incorporating crayfish into their diet. Thus, interactions among invaders can be both direct and indirect.

The invasion meltdown scenario exposed in the Thames catchment was explored further by using controlled experiments and these revealed little variation in niche breadth between allopatric and sympatric populations of invasive crayfish species, suggesting that they can coexist. Field surveys revealed some variation in resource choice (niche partitioning) between species. If resources are not limited or there is a degree of niche partitioning between invaders, they will be able to coexist with no interspecific competition. Consequently, interactions among invaders can be both facilitative and inhibitive and this will depend partly on the degree of niche overlap and resource availability.

Impacts of invasive crayfish species

Invasive crayfish in both the River Malewa and the Thames catchment had detrimental impacts on aspects of ecosystem structure and functioning. In the River Malewa, Louisiana red swamp crayfish altered native community structure by displacing a native species of crab and causing a severe decline in the abundance of smaller invertebrate prey species, primarily caddisflies. Similarly, controlled experiments, using Louisiana red swamp crayfish from the Thames catchment, showed a negative impact on native community structure by reducing the abundance of snails. This instigated a trophic cascade by reducing grazing pressure on epiphytes and subsequently promoting their growth (Charlebois & Lamberti 1996). There was evidence to suggest that a similar trophic cascade was also caused by another three species of invasive crayfish in the Thames catchment (signal, virile and Turkish crayfish) although the impact was not as strong. Signal, virile and Turkish crayfish also had negative impacts on community structure, by causing declines in various smaller invertebrate prey species such as caddisflies, snails and *Asellus* sp.

Decomposition rates in the River Malewa were accelerated in the presence of invasive Louisiana red swamp crayfish. Likewise, rates were increased in the presence of Louisiana red swamp or signal crayfish in the Thames catchment experiments. In contrast, virile and Turkish crayfish decreased decomposition rates by initiating a trophic cascade in which obligate invertebrate shredder species were consumed by crayfish and thus, net decomposition was reduced. Louisiana red swamp and signal crayfish decoupled this trophic cascade by directly consuming leaf litter to a greater degree than Turkish and virile crayfish (Usio 2000).

There was little evidence to suggest that interactions among the invasive crayfish species in the Thames catchment would alter their independent impacts on ecosystem structure and functioning. I hypothesised that competitive interactions could mitigate or amplify their effect but instead, their impacts were independent. Since stable isotope data showed that the four species are likely to coexist if resources are not limited, the impact the multiple invaders will have on the ecosystem will be the total sum of their allopatric impacts (see Figure 8.1). Hence, it is of vital importance to stop any further dispersal of invasive crayfish in the Thames catchment.

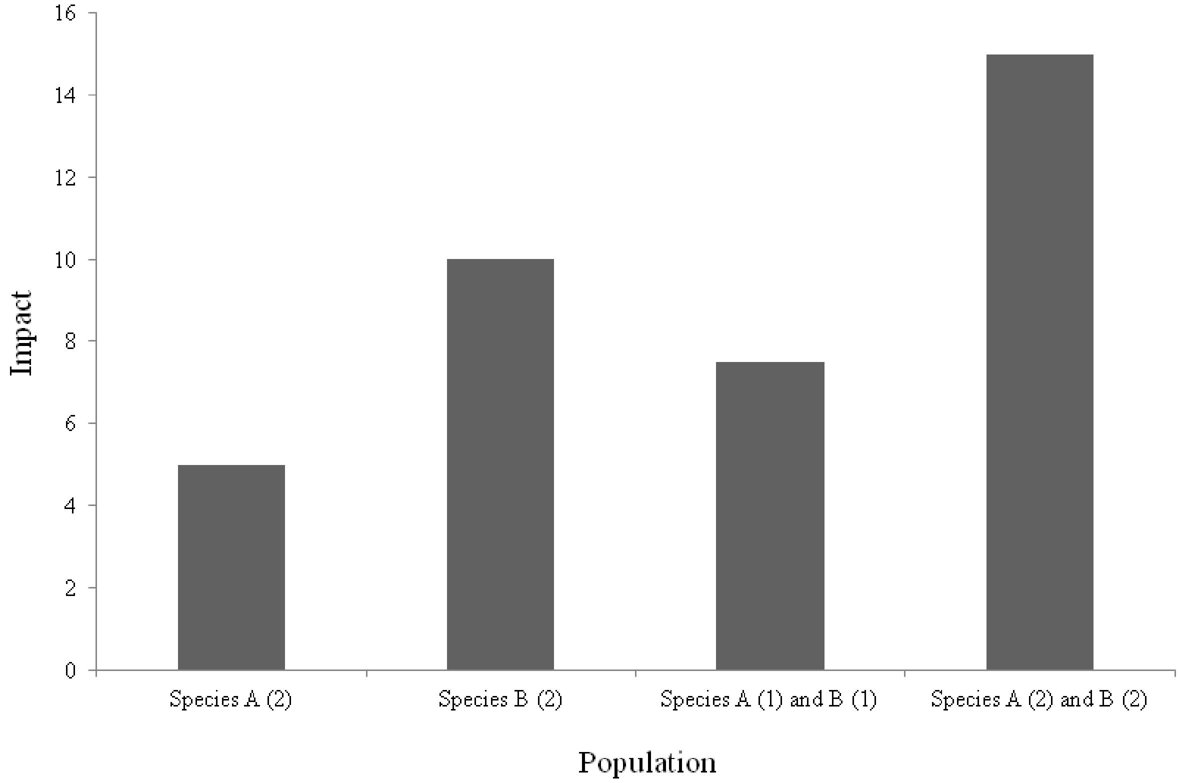

Figure 8.1. A schematic graph showing the impact on the ecosystem of crayfish species A and B in allopatric and sympatric populations; densities are shown in parentheses. A population of 2 individuals of species A has an impact of 5 and a population of 2 individuals of species B has an impact of 10 and since their interactions do not alter one another's impact, a population of 1 individual of each species will have an intermediate impact of 7.5. However, since there is a degree of niche partitioning, species A and B can coexist in the same densities as the allopatric populations. Hence, the impact of species A and B will be 15; the sum of their independent impacts.

Implications

Many of the results presented in my thesis have implications for invasive species management and control. Lake Naivasha has been subject to waves of invasion over the last century and many of the invaders benefit fishery production, an important form of revenue for local communities (Hickley *et al.* 2002). However, the impact of Louisiana red swamp crayfish in the catchment will have negative implications for the local communities by reducing water quality and promoting erosion (Statzner *et al.* 2000). From an ecological perspective, there are further negative implications since many of the crab species in East Africa are endemic and critically endangered (Cumberlidge *et al.* 2009) and the rapid dispersal rates of crayfish will result in encroachment on their ranges in the near future (Capinha *et al.* 2011). Thus, stronger enforcement and education of local people on the subject are required to curb further dispersal of crayfish in East Africa.

I found that the accelerating rates of biological invasion in the Thames catchment were positively correlated with shipping activity and this should be considered in invasive species management strategies in the UK. Tighter enforcement on ship licences and ballast water transfer will help prevent the dispersal and establishment of further invasive species in the Thames catchment. Above, I suggested that due to a degree of niche partitioning, the four invasive crayfish species in the Thames area will be able to coexist, and in recent months (Autumn 2011) sympatric populations of signal and virile crayfish, and signal and Louisiana red swamp crayfish have been found (A. Ellis, Environment Agency, unpublished data). The independent impacts of each species in sympatric conditions will result in an overall elevated impact on the ecosystem (Figure 8.1), including homogenisation of native biota, and therefore, further legislation to help prevent the spread of these species in the catchment is essential.

My research in both Kenya and England revealed many detrimental effects of invasive freshwater species, including declines in native species abundance, changes in the functioning of ecosystems and elevated impacts of multiple invaders. Many of the impacts caused by the invaders will, in turn, alter ecosystem services and therefore have economic implications, further stressing the necessity for more robust control.

The stable isotope-derived metrics that were used throughout this thesis have useful applications for a wide range of ecological questions. Used together, the metrics provided important insights into the trophic ecology of populations and the food web structure of communities. In the face of accelerating global change, it is important to understand the impact humans are having on biodiversity, ecosystem processes and fundamentally, ecosystem services (Hooper *et al.* 2005; Pejchar & Mooney 2009). The metrics provide quantitative response variables that can be compared among populations, species and communities, over temporal and spatial scales and hence, they should prove useful in assessing the impact of anthropogenic global change on ecosystems.

This thesis has highlighted the impact that multiple biological invasions can have at different levels of biological organisation, from the individual to the whole ecosystem. Realistically, aspects of global change will interact, either synergistically and potentially amplifying their impact on the environment, or antagonistically, which may counter-balance their independent outcomes. For instance, a change in climate may break down ‘filters’ that previously acted as barriers to invasive species (Rahel & Olden 2008). As a result, multiple species invasions need to be considered alongside other aspects of global change, including habitat destruction and climate change, in order to fully understand the impact that anthropogenic activity is having on freshwater ecosystems (Rahel *et al.* 2008).

References

Ahern D., England J. & Ellis A. (2008). The virile crayfish, Orconectes virilis (Hagen, 1870; Crustacea: Decapoda: Cambaridae), identified in the UK. *Aquatic Invasions*, 3, 102-104.

Alderman D.J., Polglase J.L., Frayling M. & Hogger J. (1984). Crayfish plague in Britain. *Journal of Fish Diseases*, 7, 401-405.

Alonso F. & Martínez R. (2006). Shelter competition between two invasive crayfish species: a laboratory study. *Bull. Fr. Pêche Piscic*, 380, 1121-1132.

Anderson M.J. (2001). Permutational test for univariate or multivariate analysis of variance and regression. *Canadian Journal of Fisheries and Aquatic Sciences*, 58, 626-639.

Anderson M.J., Gorley R.N. & Clarke K.R. (2008). PERMANOVA+ for PRIMER: A guide to software and statistical methods. *PRIMER-E, Plymouth, U.K.*

Araujo M.S., Bolnick D.I. & Layman C.A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, 14, 948-958.

Batschelet E. (1981). *Circular Statistics in Biology*. Academic Press, London.

Baxter C.V., Fausch K.D., Murakami M. & Chapman P.L. (2004). Fish invasion restructures stream and forest food webs by interrupting reciprocal prey subsidies. *Ecology*, 85, 2656-2663.

Bearhop S., Waldron S., Fuller R.A., Macleod H. & Adams C.E. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *Journal of Animal Ecology*, 73, 1007-1012.

Beisner B.E., Ives A.R. & Carpenter S.R. (2003). The effects of an exotic fish invasion on the prey communities of two lakes. *Journal of Animal Ecology*, 72, 331-342.

Bernardo J.M., Costa A.M., Bruxelas S. & Teixeira A. (2011). Dispersal and coexistence of two non-native crayfish species (*Pacifastacus leniusculus* and *Procambarus clarkii*) in NE Portugal over a 10-year period. *Knowledge and Management of Aquatic Ecosystems*, 401.

Bobeldyk A.M. & Lamberti G.A. (2008). A Decade after Invasion: Evaluating the Continuing Effects of Rusty Crayfish on a Michigan River. *Journal of Great Lakes Research*, 34, 265-275.

Bolnick D.I., Ingram T., Stutz W.E., Snowberg L.K., Lau O.L. & Paull J.S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B-Biological Sciences*, 277, 1789-1797.

Bolnick D.I., Svanbäck R., Fordyce J.A., Yang L.H., Davis J.M., Hulsey C.D. & Forister M.L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, 161, 1-28.

Bolnick D.I., Yang L.H., Fordyce J.A., Davis J.M. & Svanbäck R. (2002). Measuring individual-level resource specialization. *Ecology*, 83, 2936-2941.

Brander K.M. (2007). Global fish production and climate change. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 19709-19714.

Britton J.R., Boar R.R., Grey J., Foster J., Lugonzo J. & Harper D.M. (2007). From introduction to fishery dominance: the initial impacts of the invasive carp *Cyprinus carpio* in Lake Naivasha, Kenya, 1999 to 2006. *Journal of Fish Biology*, 71, 239-257.

Britton J.R., Harper D.M., Oyugi D.O. & Grey J. (2010). The introduced *Micropterus salmoides* in an equatorial lake: a paradoxical loser in an invasion meltdown scenario? *Biological Invasions*, 12, 3439-3448.

Brook B.W., Sodhi N.S. & Bradshaw C.J.A. (2008). Synergies among extinction drivers under global change. *Trends in Ecology & Evolution*, 23, 453-460.

Bubb D.H., Thom T.J. & Lucas M.C. (2006). Movement, dispersal and refuge use of co-occurring introduced and native crayfish. *Freshwater Biology*, 51, 1359–1368.

Byers J.E. & Noonburg E.G. (2003). Scale dependent effects of biotic resistance to biological invasion. *Ecology*, 84, 1428-1433.

Byrnes J.E., Reynolds P.L. & Stachowicz J.J. (2007). Invasions and Extinctions Reshape Coastal Marine Food Webs. *Plos One*, 2(3): e295.

Capinha C., Leung B. & Anastacio P. (2011). Predicting worldwide invasiveness for four major problematic decapods: an evaluation of using different calibration sets. *Ecography*, 34, 448-459.

Carlsson N.O.L., Bronmark C. & Hansson L.A. (2004). Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology*, 85, 1575-1580.

Carpenter S.R., Fisher S.G., Grimm N.B. & Kitchell J.F. (1992). Global change and freshwater ecosystems. *Annual Review of Ecology and Systematics*, 23, 119-139.

Chapin F.S., Zavaleta E.S., Eviner V.T., Naylor R.L., Vitousek P.M., Reynolds H.L., Hooper D.U., Lavorel S., Sala O.E., Hobbie S.E. & others (2000). Consequences of changing biodiversity. *Nature*, 405, 234–242.

Charlebois P.M. & Lamberti G.A. (1996). Invading crayfish in a Michigan stream: Direct and indirect effects on periphyton and macroinvertebrates. *Journal of the North American Benthological Society*, 15, 551-563.

Clark K.R. & Warwick R.M. (2001). *Changes in marine communites: an approach to statistical analysis and interpretation.* Primer-E, Plymouth, UK.

Cohen A.N. & Carlton J.T. (1998). Accelerating Invasion Rate in a Highly Invaded Estuary. *Science*, 279, 555-555.

Correia A.M. & Anastacio P.M. (2008). Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecological Research*, 23, 729-734.

Crandall K.A. & Buhay J.E. (2008). Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae-Decapoda) in freshwater. *Hydrobiologia*, 595, 295-301.

Crowl T.A., Crist T.O., Parmenter R.R., Belovsky G. & Lugo A.E. (2008). The spread of invasive species and infectious disease as drivers of ecosystem change. *Frontiers in Ecology and the Environment*, 6, 238-246.

Cumberlidge N., Ng P.K.L., Yeo D.C.J., Magalhães C., Campos M.R., Alvarez F., Naruse T., Daniels S.R. & Esser L.J. (2009). Freshwater crabs and the biodiversity crisis: importance, threats, status, and conservation challenges. *Biological Conservation*, 142, 1665–1673.

De Caceres M., Sol D., Lapiedra O. & Legendre P. (2011). A framework for estimating niche metrics using the resemblance between qualitative resources. *Oikos*, 120, 1341-1350.

DeGraaf J.D. & Tyrrell M.C. (2004). Comparison of the Feeding Rates of Two Introduced Crab Species, *Carcinus maenas* and *Hemigrapsus sanguineus*, on the Blue Mussel, *Mytilus edulis*. *Northeastern Naturalist*, 11, 163-167.

Dobson A., Lodge D., Alder J., Cumming G.S., Keymer J., McGlade J., Mooney H., Rusak J.A., Sala O., Wolters V., Wall D., Winfree R. & Xenopoulos M.A. (2006). Habitat loss, trophic collapse, and the decline of ecosystem services. *Ecology*, 87, 1915-1924.

Dobson M., Magana A., Mathooko J.M. & Ndegwa F.K. (2002). Detritivores in Kenyan highland streams: more evidence for the paucity of shredders in the tropics? *Freshwater Biology*, 47, 909–919.

Dudgeon D. (1999). *Tropical Asian streams: Zoobenthos, ecology and conservation*. Hong Kong University Press, Hong Kong.

Dudgeon D., Arthington A.H., Gessner M.O., Kawabata Z.I., Knowler D.J., Lévêque C., Naiman R.J., Prieur-Richard A.H., Soto D., Stiassny M.L.J. & others (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81, 163-182.

Dunn J.C., McClymont H.E., Christmas M. & Dunn A.M. (2009). Competition and parasitism in the native White Clawed Crayfish *Austropotamobius pallipes* and the invasive Signal Crayfish *Pacifastacus leniusculus* in the UK. *Biological Invasions*, 11, 315-324.

Elton C.S. (1958). *The ecology of invasions by plants and animals*. Methuen, London.

Elvira B., Gnicola G. & Almodovar A. (1996). Pike and red swamp crayfish: a new case on predator-prey relationship between aliens in central Spain. *Journal of Fish Biology*, 48, 437-446.

Everard M., Vale J.A., Harper D.M. & Tarras-Wahlberg H. (2002). The physical attributes of the Lake Naivasha catchment rivers. *Hydrobiologia*, 488, 13-25.

Fantle M.S., Dittel A.I., Schwalm S.M., Epifanio C.E. & Fogel M.L. (1999). A food web analysis of the juvenile blue crab, Callinectes sapidus, using stable isotopes in whole animals and individual amino acids. *Oecologia*, 120, 416-426.

Feuchtmayr H. & Grey J. (2003). Effect of preparation and preservation procedures on carbon and nitrogen stable isotope determinations from zooplankton. *Rapid Communications in Mass Spectrometry*, 17, 2605-2610.

Finke D.L. & Snyder W.E. (2008). Niche partitioning increases resource exploitation by diverse communities. *Science*, 321, 1488-1490.

Foster J. & Harper D. (2007). Status and ecosystem interactions of the invasive Louisianan red swamp crayfish *Procambarus clarkii* in East Africa. In: *Biological Invaders in Inland Waters: Profiles, Distributions and Threats.* (ed. Gherardi F), pp. 91-101.

Francis R.A., Hoggart S.P.G., Gurnell A.M. & Coode C. (2008). Meeting the challenges of urban river habitat restoration: developing a methodology for the River Thames through central London. *Area*, 40, 435-445.

Fry B., Baltz D.M., Benfield M.C., Fleeger J.W., Gace A., Haas H.L. & Quinones-Rivera Z.J. (2003). Stable isotope indicators of movement and residency for brown shrimp (*Farfantepenaeus aztecus*) in coastal Louisiana marshscapes. *Estuaries*, 26, 82-97.

García-Berthou E., Alcaraz C., Pou-Rovira Q., Zamora L., Coenders G. & Feo C. (2005). Introduction pathways and establishment rates of invasive aquatic species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences*, 62, 453-463.

Gessner M.O., Swan C.M., Dang C.K., McKie B.G., Bardgett R.D., Wall D.H. & Haettenschwiler S. (2010). Diversity meets decomposition. *Trends in Ecology & Evolution*, 25, 372-380.

Gherardi F. & Acquistapace P. (2007). Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology*, 52, 1249-1259.

Gherardi F., Mavuti K.M., Pacini N., Tricarico E. & Harper D.M. (2011). The smell of danger: chemical recognition of fish predators by the invasive crayfish *Procambarus clarkii*. *Freshwater Biology*, 56, 1567-1578.

Gilbert B., Srivastava D.S. & Kirby K.R. (2008). Niche partitioning at multiple scales facilitates coexistence among mosquito larvae. *Oikos*, 117, 944-950.

Gorokhova E., Hansson S., Höglander H. & Andersen C.M. (2005). Stable isotopes show food web changes after invasion by the predatory cladoceran *Cercopagis pengoi* in a Baltic Sea bay. *Oecologia*, 143, 251-259.

Grey J. (2006). The use of stable isotope analyses in freshwater ecology: Current awareness. *Polish Journal of Ecology*, 54, 563-584.

Grey J. & Harper D.M. (2002). Using stable isotope analyses to identify allochthonous inputs to Lake Naivasha mediated via the hippopotamus gut. *Isotopes in Environmental and Health Studies*, 38, 245-250.

Griffen B.D. & Byers J.E. (2008). Community impacts of two invasive crabs: the interactive roles of density, prey recruitment and indirect effects. *Biological Invasions*, 11, 927-940.

Griffen B.D., Guy T. & Buck J.C. (2008). Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology*, 77, 32-40.

Grosholz E.D. (2005). Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. *Proceedings of the National Academy of Sciences*, 102, 1088-1091.

Guan R.Z. & Wiles P.R. (1997). Ecological impact of introduced crayfish on benthic fishes in a British lowland river. *Conservation biology*, 11, 641-647.

Gurevitch J. & Padilla D.K. (2004). Are invasive species a major cause of extinctions? *Trends in Ecology & Evolution*, 19, 470-474.

Gurnell J., Wauters L.A., Lurz P.W.W. & Tosi G. (2004). Alien species and interspecific competition: effects of introduced eastern grey squirrels on red squirrel population dynamics. *Journal of Animal Ecology*, 73, 26-35.

Hammerschlag-Peyer C.M., Yeager L.A., Araujo M.S. & Layman C.A. (2011). A hypothesis-testing framework for studies investigating ontogenetic niche shifts using stable isotope ratios. *PloS one*, 6, e27104.

Harrington L.A., Harrington A.L., Yamaguchi N., Thom M.D., Ferreras P., Windham T.R. & Macdonald D.W. (2009). The impact of native competitors on an alien invasive: temporal niche shifts to avoid interspecific aggression? *Ecology*, 90, 1207-1216.

Harvey G.L., Moorhouse T.P., Clifford N.J., Henshaw A.J., Johnson M.F., Macdonald D.W., Reid I. & Rice S. (2011). Evaluating the role of invasive aquatic species as drivers of fine sediment-related river management problems: The case of the signal crayfish (*Pacifastacus leniusculus*). *Progress in Physical Geography*.

Hayes R.B. (2011). Consequences for lotic ecosystems of invasion by signal crayfish. PhD Thesis, *School of Biological and Chemical Sciences*, Queen Mary University of London.

Hector A. & Bagchi R. (2007). Biodiversity and ecosystem multifunctionality. *Nature*, 448, 188-190.

Hickley P., Bailey R., Harper D.M., Kundu R., Muchiri M., North R. & Taylor A. (2002). The status and future of the Lake Naivasha fishery, Kenya. *Hydrobiologia*, 488, 181-190.

Hieber M. & Gessner M.O. (2002). Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83, 1026-1038.

Hill A.M. & Lodge D.M. (1999). Replacement of resident crayfishes by an exotic crayfish: the roles of competition and predation. *Ecological Applications*, 9, 678-690.

Hinojosa-Garro D. & Zambrano L. (2004). Interactions of common carp (*Cyprinus carpio*) with benthic crayfish decapods in shallow ponds. *Hydrobiologia*, 515, 115-122.

Hobbs Iii H.H., Jass J.P. & Huner J.V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana*, 299-316.

Hoeinghaus D., J. & Zeug S., C. (2008). Can stable isotope ratios provide for community-wide measures of trophic structure? Comment. *Ecology*, 89, 2353-2357.

Holdich D.M. (1988). The dangers of introducing alien animals with particular reference to crayfish. *Freshwater Crayfish*, 7, 15-30.

Holdich D.M., Rogers W.D. & Reynolds J.D. (1999). Native and alien crayfish in the British Isles. *Crayfish in Europe as alien species. How to make the best of a bad situation*, 221-235.

Holdich D.M. & Sibley P.J. (2003). Crayfish in Europe–an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. *Management & Conservation of Crayfish Conference Nottingham*, 15-34.

Holt R.D. & Huxel G.R. (2007). Alternative prey and the dynamics of intraguild predation: Theoretical perspectives. *Ecology*, 88, 2706-2712.

Hooper D.U., Chapin F.S., Ewel J.J., Hector A., Inchausti P., Lavorel S., Lawton J.H., Lodge D.M., Loreau M., Naeem S., Schmid B., Setälä H., Symstad A.J., Vandermeer J. & Wardle D.A. (2005). Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecological Monographs*, 75, 3-35.

Hulme P.E. (2009). Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology*, 46, 10-18.

Human K.G. & Gordon D.M. (1996). Exploitation and interference competition between the invasive Argentine ant, *Linepithema humile*, and native ant species. *Oecologia*, 105, 405-412.

Hutchinson G.E. (1957). The multivariate niche. In: *Cold Spr. Harb. Symp. Quant. Biol*, pp. 415-421.

Ilhéu M., Bernardo J.M. & Fernandes S. (2007). Predation of invasive crayfish on aquatic vertebrates: the effect of *Procambarus clarkii* on fish assemblages in Mediterranean temporary streams. In: *Biological Invaders in Inland Waters: Profiles, Distributions and Threats.* (ed. Gherardi F), pp. 543-558.

Ings N.L., Hildrew A.G. & Grey J. (2010). Gardening by the psychomyiid caddisfly *Tinodes waeneri*: evidence from stable isotopes. *Oecologia*, 163, 127-139.

Jackson A.L., Inger R., Parnell A.C. & Bearhop S. (2011). Comparing isotopic niche widths among and within communities: Bayesian analysis of stable isotope data. *Journal of Animal Ecology*, 80, 595-602.

Jeffrey S.W. & Humphrey G.F. (1975). New spectrophotometric equations for determining chlorophylls a, b, c 1 and c 2 in algae, phytoplankton and higher plants. *Biochem. Physiol. Pflanz*, 167, 191-194.

Jensen G.C., McDonald P.S. & Armstrong D.A. (2002). East meets west: competitive interactions between green crab *Carcinus maenas*, and native and introduced shore crab *Hemigrapsus spp*. *Marine Ecology Progress Series*, 225, 251-262.

Jeschke J.M. & Strayer D.L. (2006). Determinants of vertebrate invasion success in Europe and North America. *Global Change Biology*, 12, 1608-1619.

Johnson A.C., Acreman M.C., Dunbar M.J., Feist S.W., Giacomello A.M., Gozlan R.E., Hinsley S.A., Ibbotson A.T., Jarvie H.P. & Jones J.I. (2009a). The British river of the future: How climate change and human activity might affect two contrasting river ecosystems in England. *Science of The Total Environment*, 407, 4787-4798.

Johnson P., Olden J., Solomon C. & Vander Zanden M. (2009b). Interactions among invaders: community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia*, 159, 161-170.

Kaluza P., Koelzsch A., Gastner M.T. & Blasius B. (2010). The complex network of global cargo ship movements. *Journal of the Royal Society Interface*, 7, 1093-1103.

Keith D.A., Akcakaya H.R., Thuiller W., Midgley G.F., Pearson R.G., Phillips S.J., Regan H.M., Araujo M.B. & Rebelo T.G. (2008). Predicting extinction risks under climate change: coupling stochastic population models with dynamic bioclimatic habitat models. *Biology Letters*, 4, 560-563.

Keller R.P., Zu Ermgassen P.S.E. & Aldridge D.C. (2009). Vectors and Timing of Freshwater Invasions in Great Britain. *Conservation Biology*, 23, 1526-1534.

Kirkwood D.S. (1996). Nutrients: practical notes on their determination in seawater. *Techniques in Marine Environmental Science, No. 17. ICES. Copenhagen, Denmark.*

Kolar C.S. & Lodge D.M. (2002). Ecological predictions and risk assessment for alien fishes in North America. *Science*, 298, 1233-1236.

Layman C.A., Arrington D.A., Montaña C.G. & Post D.M. (2007a). Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology*, 88, 42-48.

Layman C.A., Quattrochi J.P., Peyer C.M. & Allgeier J.E. (2007b). Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecology Letters*, 10, 937-944.

Leakey R. & Lewin R. (1995). *The Sixth Extinction: Biodiversity and Its Survival.* Doubleday, New York.

Lee C.E. (2002). Evolutionary genetics of invasive species. *Trends in Ecology & Evolution*, 17, 386-391.

Leppäkoski E. & Olenin S. (2000). Non-native species and rates of spread: lessons from the brackish Baltic Sea. *Biological Invasions*, 2, 151-163.

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

Ligon R.A., Siefferman L. & Hill G.E. (2011). Invasive Fire Ants Reduce Reproductive Success and Alter the Reproductive Strategies of a Native Vertebrate Insectivore. *Plos One*, 6(7): e22578.

Lockwood J.L., Cassey P. & Blackburn T. (2005). The role of propagule pressure in explaining species invasions. *Trends in Ecology & Evolution*, 20, 223-228.

Lodge D.M., Kershner M.W., Aloi J.A. & Covich A.P. (1994). Effects of an omnivorous crayfish (*Orconectes rusticus*) on a freshwater littoral food web. *Ecology*, 75, 1265-1281.

Lodge D.M., Rosenthal S.K., Mavuti K.M., Muohi W., Ochieng P., Stevens S.S., Mungai B.N. & Mkoji G.M. (2005). Louisiana crayfish (*Procambarus clarkii*; Crustacea: Cambaridae) in Kenyan ponds: non-target effects of a potential biological control agent for schistosomiasis. *African Journal of Aquatic Science*, 30, 119-124.

Lohrer A.M. & Whitlatch R.B. (2002). Interactions among aliens: Apparent replacement of one exotic species by another. *Ecology*, 83, 719-732.

Loreau M., Naeem S., Inchausti P., Bengtsson J., Grime J.P., Hector A., Hooper D.U., Huston M.A., Raffaelli D., Schmid B., Tilman D. & Wardle D.A. (2001). Ecology - Biodiversity and ecosystem functioning: Current knowledge and future challenges. *Science*, 294, 804-808.

MacDougall A.S. & Turkington R. (2005). Are invasive species the drivers or passengers of change in degraded ecosystems? *Ecology*, 86, 42-55.

Mack R.N., Simberloff D., Mark Lonsdale W., Evans H., Clout M. & Bazzaz F.A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications*, 10, 689–710.

Maclean I.M.D. & Wilson R.J. (2011). Recent ecological responses to climate change support predictions of high extinction risk. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 12337-12342.

Maguire C.M. & Grey J. (2006). Determination of zooplankton dietary shift following a zebra mussel invasion, as indicated by stable isotope analysis. *Freshwater Biology*, 51, 1310-1319.

Marvier M., Kareiva P. & Neubert M.G. (2004). Habitat destruction, fragmentation, and disturbance promote invasion by habitat generalists in a multispecies metapopulation. *Risk analysis*, 24, 869-878.

McArdle B.H. & Anderson M.J. (2001). Fitting multivariate models to community data: A comment on distance-based redundancy analysis. *Ecology*, 82, 290-297.

McCann K., Hastings A. & Huxel G.R. (1998). Weak trophic interactions and the balance of nature. *Nature*, 395, 794-798.

McCann K.S. (2000). The diversity-stability debate. *Nature*, 405, 228-233.

McCarthy J.M., Hein C.L., Olden J.D. & Vander Zaden M.J. (2006). Coupling long-term studies with meta-analysis to investigate impacts of non-native crayfish on zoobenthic communities. *Freshwater Biology*, 51, 224-235.

McCutchan Jr J.H., Lewis Jr W.M., Kendall C. & McGrath C.C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, 102, 378-390.

McIntyre P.B. & Flecker A.S. (2006). Rapid turnover of tissue nitrogen of primary consumers in tropical freshwaters. *Oecologia*, 148, 12-21.

Miller S.A. & Crowl T.A. (2006). Effects of common carp (*Cyprinus carpio*) on macrophytes and invertebrate communities in a shallow lake. *Freshwater Biology*, 51, 85-94.

Mills E.L., Strayer D.L., Scheuerell M.D. & Carlton J.T. (1996). Exotic species in the Hudson River Basin: A history of invasions and introductions. *Estuaries and Coasts*, 19, 814-823.

Mkoji G.M., Hofkin B.V., Kuris A.M., Stewart-Oaten A., Mungai B.N., Kihara J.H., Mungai F., Yundu J., Mbui J., Rashid J.R., Kariuki C.H., Ouma J.H., Koech D.K. & Loker E.S. (1999). Impact of the crayfish *Procambarus clarkii* on *Schistosoma haematobium* transmission in Kenya. *The American journal of tropical medicine and hygiene*, 61, 751-751.

Moline M.A., Claustre H., Frazer T.K., Schofield O. & Vernet M. (2004). Alteration of the food web along the Antarctic Peninsula in response to a regional warming trend. *Global Change Biology*, 10, 1973-1980.

Myers S.S. & Patz J.A. (2009). Emerging Threats to Human Health from Global Environmental Change. *Annual Review of Environment and Resources*, 34, 223-252.

Naeem S. (1998). Species Redundancy and Ecosystem Reliability. *Conservation Biology*, 12, 39-45.

Nakata K. & Goshima S. (2006). Asymmetry in mutual predation between the endangered japanese native crayfish *Cambaroides japonicus* and the north american invasive crayfish *Pacifastacus leniusculus*: a possible reason for species replacement. *Journal of Crustacean Biology*, 26, 134-140.

Nakata K., Tsutsumi K., Kawai T. & Goshima S. (2005). Coexistence of two North American invasive crayfish species, *Pacifastacus leniusculus* (Dana, 1852) and *Procambarus clarkii* (Girard, 1852) in Japan. *Crustaceana*, 78, 1389-1394.

Newsome S.D., Martinez del Rio C., Bearhop S. & Phillips D.L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, 5, 429-436.

Nyström P. & Strand J.A. (1996). Grazing by a native and an exotic crayfish on aquatic macrophytes. *Freshwater biology. Oxford*, 36, 673-682.

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

Nyström P., Brönmark C. & Granéli W. (1999). Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos*, 545-553.

Nyström P.E.R., Bronmark C. & Graneli W. (1996). Patterns in benthic food webs: a role for omnivorous crayfish? *Freshwater Biology*, 36, 631-646.

O'Connor N.E. & Bruno J.F. (2007). Predatory fish loss affects the structure and functioning of a model marine food web. *Oikos*, 116, 2027–2038.

O'Reilly C.M., Alin S.R., Plisnier P.D., Cohen A.S. & McKee B.A. (2003). Climate change decreases aquatic ecosystem productivity of Lake Tanganyika, Africa. *Nature*, 424, 766-768.

Olden J.D., LeRoy Poff N., Douglas M.R., Douglas M.E. & Fausch K.D. (2004). Ecological and evolutionary consequences of biotic homogenization. *Trends in Ecology & Evolution*, 19, 18-24.

Olsson K., Stenroth P., Nyström P. & Granéli W. (2009). Invasions and niche width: does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology*, 54, 1731-1740.

Padilla D.K. & Williams S.L. (2004). Beyond ballast water: aquarium and ornamental trades as sources of invasive species in aquatic ecosystems. *Frontiers in Ecology and the Environment*, 2, 131-138.

Parker I.M., Simberloff D., Lonsdale W.M., Goodell K., Wonham M., Kareiva P.M., Williamson M.H., Von Holle B., Moyle P.B. & Byers J.E. (1999). Impact: Toward a Framework for Understanding the Ecological Effects of Invaders. *Biological Invasions*, 1, 3-19.

Parkyn S.M., Rabeni C.F. & Collier K.J. (1997). Effects of crayfish (*Paranephrops planifrons* : Parastacidae) on in-stream processes and benthic faunas: a density manipulation experiment. *New Zealand Journal of Marine and Freshwater Research*, 31, 685-692.

Parnell A.C., Inger R., Bearhop S. & Jackson A.L. (2010). Source partitioning using stable isotopes: Coping with too much variation. *PLoS One*, 5, e9672.

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

Pejchar L. & Mooney H.A. (2009). Invasive species, ecosystem services and human well-being. *Trends in Ecology & Evolution*, 24, 497-504.

Peltzer D.A., Allen, R.B., Lovett, G.M., Whitehead, D. and Wardle, D.A. (2009). Effects of biological invasions on forest carbon sequestration. *Global Change Biology*, 16, 732-746.

Pereira H.M., Leadley P.W., Proenca V., Alkemade R., Scharlemann J.P.W., Fernandez-Manjarres J.F., Araujo M.B., Balvanera P., Biggs R., Cheung W.W.L., Chini L., Cooper H.D., Gilman E.L., Guenette S., Hurtt G.C., Huntington H.P., Mace G.M., Oberdorff T., Revenga C., Rodrigues P., Scholes R.J., Sumaila U.R. & Walpole M. (2010). Scenarios for Global Biodiversity in the 21st Century. *Science*, 330, 1496-1501.

Petchey O.L., McPhearson P.T., Casey T.M. & Morin P.J. (1999). Environmental warming alters food-web structure and ecosystem function. *Nature*, 402, 69-72.

Petr T. (2000). Interactions between fish and aquatic macrophytes in inland waters. A review. *FAO Fisheries Technical Paper 396*.

Pianka E.R. (1974). Niche overlap and diffuse competition. *Proceedings of the National Academy of Sciences*, 71, 2141-2145.

Pimentel D., Zuniga R. & Morrison D. (2005). Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics*, 52, 273-288.

Pimm S.L., Russell G.J., Gittleman J.L. & Brooks T.M. (1995). The future of biodiversity. *Science*, 269, 347-350.

Pinnegar J.K. & Polunin N.V.C. (1999). Differential fractionation of delta C-13 and delta N-15 among fish tissues: implications for the study of trophic interactions. *Functional Ecology*, 13, 225-231.

Popa-Lisseanu A.G., Delgado-Huertas A., Forero M.G., Rodriguez A., Arlettaz R. & Ibanez C. (2007). Bats' Conquest of a Formidable Foraging Niche: The Myriads of Nocturnally Migrating Songbirds. *Plos One*, 2, e205.

Porter S.D. & Savignano D.A. (1990). Invasion of Polygyne Fire Ants Decimates Native Ants and Disrupts Arthropod Community. *Ecology*, 71, 2095-2095.

Post D.M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83, 703-718.

Pounds J.A., Bustamante M.R., Coloma L.A., Consuegra J.A., Fogden M.P.L., Foster P.N., La Marca E., Masters K.L., Merino-Viteri A., Puschendorf R., Ron S.R., Sanchez-Azofeifa G.A., Still C.J. & Young B.E. (2006). Widespread amphibian extinctions from epidemic disease driven by global warming. *Nature*, 439, 161-167.

Rahel F.J. (2007). Biogeographic barriers, connectivity and homogenization of freshwater faunas: it's a small world after all. *Freshwater Biology*, 52, 696-710.

Rahel F.J., Bierwagen B. & Taniguchi Y. (2008). Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. 22, 551-561.

Rahel F.J. & Olden J.D. (2008). Assessing the Effects of Climate Change on Aquatic Invasive Species. *Conservation Biology*, 22, 521-533.

Rehage J.S., Barnett B.K. & Sih A. (2005). Foraging behaviour and invasiveness: do invasive Gambusia exhibit higher feeding rates and broader diets than their noninvasive relatives? *Ecology of Freshwater Fish*, 14, 352-360.

Renai B. & Gherardi F. (2004). Predatory Efficiency of Crayfish: Comparison Between Indigenous and Non-Indigenous Species. *Biological Invasions*, 6, 89-99.

Ricciardi A. (2001). Facilitative interactions among aquatic invaders: is an "invasional meltdown" occurring in the Great Lakes? *Can. J. Fish. Aquat. Sci*, 58, 2513-2525.

Ricciardi A. (2006). Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. *Diversity and Distributions*, 12, 425-433.

Ricciardi A. (2007). Are Modern Biological Invasions an Unprecedented Form of Global Change? *Conservation Biology*, 21, 329-336.

Ricklefs R.E. & Nealen P. (1998). Lineage-dependent rates of evolutionary diversification: analysis of bivariate ellipses. *Functional Ecology*, 12, 871-885.

Rodríguez C.F., Bécares E., Fernández-aláez M. & Fernández-aláez C. (2005). Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions*, 7, 75-85.

Rosenthal S.K., Stevens S.S. & Lodge D.M. (2006). Whole-lake effects of invasive crayfish (*Orconectes spp*.) and the potential for restoration. *Canadian journal of fisheries and aquatic sciences/Journal canadien des sciences halieutiques et aquatiques*, 63, 1276-1285.

Roughgarden J. (1972). Evolution of niche width. *American Naturalist*, 683-718.

Rudnick D. & Resh V. (2005). Stable isotopes, mesocosms and gut content analysis demonstrate trophic differences in two invasive decapod crustacea. *Freshwater Biology*, 50, 1323-1336.

Ruffino L., Russell J.C., Pisanu B., Caut S. & Vidal E. (2011). Low individual-level dietary plasticity in an island-invasive generalist forager. *Population Ecology*, 53, 535-548.

Ruiz G.M. & Carlton J.T. (2003). *Invasive species: vectors and management strategies*. Island Press, Washington, D.C.

Sakai A.K., Allendorf F.W., Holt J.S., Lodge D.M., Molofsky J., With K.A., Baughman S., Cabin R.J., Cohen J.E., Ellstrand N.C. & others (2001). The Population Biology of Invasive Species. *Annual Review of Ecology and Systematics*, 305-332.

Sala O.E., Chapin Iii F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sanwald E., Huenneke L.F., Jackson R.B. & Kinzig A. (2000). Global Biodiversity Scenarios for the Year 2100. *Science*, 287, 1770-1770.

Schmidt S.N., Olden J.D., Solomon C.T. & Vander Zanden M.J. (2007). Quantitative approaches to the analysis of stable isotope food web data. *Ecology*, 88, 2793-2802.

Schroter D., Cramer W., Leemans R., Prentice I.C., Araujo M.B., Arnell N.W., Bondeau A., Bugmann H., Carter T.R., Gracia C.A., de la Vega-Leinert A.C., Erhard M., Ewert F., Glendining M., House J.I., Kankaanpaa S., Klein R.J.T., Lavorel S., Lindner M., Metzger M.J., Meyer J., Mitchell T.D., Reginster I., Rounsevell M., Sabate S., Sitch S., Smith B., Smith J., Smith P., Sykes M.T., Thonicke K., Thuiller W., Tuck G., Zaehle S. & Zierl B. (2005). Ecosystem service supply and vulnerability to global change in Europe. *Science*, 310, 1333-1337.

Semmens B.X., Ward E.J., Moore J.W. & Darimont C.T. (2009). Quantifying Inter- and Intra-Population Niche Variability Using Hierarchical Bayesian Stable Isotope Mixing Models. *PLoS One.*, 4, e6187.

Shannon C.E. (1948). The mathematical theory of communication. *Bell System Tech. J.*, 27, 379–423, 623–656.

Shochat E., Warren P.S., Faeth S.H., McIntyre N.E. & Hope D. (2006). From patterns to emerging processes in mechanistic urban ecology. *Trends in Ecology & Evolution*, 21, 186-191.

Siepielski A.M., Mertens A.N., Wilkinson B.L. & McPeek M.A. (2011). Signature of ecological partitioning in the maintenance of damselfly diversity. *The Journal of Animal Ecology*, 80, 1163-73.

Simberloff D. (2006). Invasional meltdown 6 years later: important phenomenon, unfortunate metaphor, or both? *Ecology Letters*, 9, 912-919.

Simberloff D. & Von Holle B. (1999). Positive interactions of nonindigenous species: Invasional Meltdown? *Biological Invasions*, 1, 21-32.

Simon K.S. & Townsend C.R. (2003). Impacts of freshwater invaders at different levels of ecological organisation, with emphasis on salmonids and ecosystem consequences. *Freshwater Biology*, 48, 982-994.

Smart S.M., Thompson K., Marrs R.H., Le Duc M.G., Maskell L.C. & Firbank L.G. (2006). Biotic homogenization and changes in species diversity across human-modified ecosystems. *Proceedings of the Royal Society B: Biological Sciences*, 273, 2659-2659.

Snyder W.E. & Evans E.W. (2006). Ecological effects of invasive arthropod generalist predators. *Annual review of ecology, evolution, and systematics*, 37, 95-95.

Sol D., Timmermans S. & Lefebvre L. (2002). Behavioural flexibility and invasion success in birds. *Animal Behaviour*, 64, 516-516.

Solan M., Cardinale B.J., Downing A.L., Engelhardt K.A.M., Ruesink J.L. & Srivastava D.S. (2004). Extinction and ecosystem function in the marine benthos. *Science*, 306, 1177-1180.

Statzner B., Fievet E., Champagne J.Y., Morel R. & Herouin E. (2000). Crayfish as geomorphic agents and ecosystem engineers: Biological behavior affects sand and gravel erosion in experimental streams. *Limnology and Oceanography*, 45, 1030-1040.

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

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

Svanbäck R. & Bolnick D.I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, 274, 839-844.

Svanbäck R. & Persson L. (2004). Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. *Journal of Animal Ecology*, 73, 973-982.

Thomas C.D., Cameron A., Green R.E., Bakkenes M., Beaumont L.J., Collingham Y.C., Erasmus B.F.N., de Siqueira M.F., Grainger A., Hannah L., Hughes L., Huntley B., van Jaarsveld A.S., Midgley G.F., Miles L., Ortega-Huerta M.A., Peterson A.T., Phillips O.L. & Williams S.E. (2004a). Extinction risk from climate change. *Nature*, 427, 145-148.

Thomas J.A., Telfer M.G., Roy D.B., Preston C.D., Greenwood J.J.D., Asher J., Fox R., Clarke R.T. & Lawton J.H. (2004b). Comparative losses of British butterflies, birds, and plants and the global extinction crisis. *Science*, 303, 1879-1881.

Thomson D. (2004). Competitive interactions between the invasive European honey bee and native bumble bees. *Ecology*, 85, 458-470.

Tillberg C.V., Holway D.A., LeBrun E.G. & Suarez A.V. (2007). Trophic ecology of invasive Argentine ants in their native and introduced ranges. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 20856-20861.

Towns D.R., Wardle D.A., Mulder C.P.H., Yeates G.W., Fitzgerald B.M., Parrish G.R., Bellingham P.J. & Bonner K.I. (2009). Predation of seabirds by invasive rats: multiple indirect consequences for invertebrate communities. *Oikos*, 118, 420-430.

Turner T.F., Collyer M.L. & Krabbenhoft T.J. (2010). A general hypothesis-testing framework for stable isotope ratios in ecological studies. *Ecology*, 91, 2227-2233.

Usio N. (2000). Effects of crayfish on leaf processing and invertebrate colonisation of leaves in a headwater stream: decoupling of a trophic cascade. *Oecologia*, 124, 608-614.

Usio N. & Townsend C.R. (2004). Roles of crayfish: Consequences of predation and bioturbation for stream invertebrates. *Ecology*, 85, 807-822.

Van Valen L. (1965). Morphological variation and width of ecological niche. *The American Naturalist*, 99, 377-377.

Vander Zanden M.J., Casselman J.M. & Rasmussen J.B. (1999). Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, 401, 464-467.

Vazquez D.P. (2006). Exploring the relationship between niche breadth and invasion success. *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature*, 307-322.

Vitousek P.M., D'Antonio C.M., Loope L.L., Rejmanek M. & Westbrooks R. (1997). Introduced species: a significant component of human-caused global change. *New Zealand Journal of Ecology*, 21, 1-16.

Wake D.B. & Vredenburg V.T. (2008). Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 11466-11473.

Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-Guldberg O. & Bairlein F. (2002). Ecological responses to recent climate change. *Nature*, 416, 389-395.

Wardle D.A., Bellingham P.J., Bonner K.I. & Mulder C.P.H. (2009). Indirect effects of invasive predators on litter decomposition and nutrient resorption on seabird-dominated islands. *Ecology*, 90, 452-464.

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

Witte F., Goldschmidt T., Wanink J., Oijen M., Goudswaard K., Witte-Maas E. & Bouton N. (1992). The destruction of an endemic species flock: quantitative data on the decline of the haplochromine cichlids of Lake Victoria. *Environmental Biology of Fishes*, 34, 1-28.

Wonham M.J., O'Connor M. & Harley C.D.G. (2005). Positive effects of a dominant invader on introduced and native mudflat species. *Marine Ecology-Progress Series*, 289, 109-116.

Xenopoulos M.A., Lodge D.M., Alcamo J., Marker M., Schulze K. & Van Vuuren D.P. (2005). Scenarios of freshwater fish extinctions from climate change and water withdrawal. *Global Change Biology*, 11, 1557-1564.

Young K.A. (2004). Asymmetric competition, habitat selection, and niche overlap in juvenile salmonids. *Ecology*, 85, 134-149.

Zhang W., Hendrix P.F., Snyder B.A., Molina M., Li J., Rao X., Siemann E. & Fu S. (2010). Dietary flexibility aids Asian earthworm invasion in North American forests. *Ecology*, 91, 2070-2079.

Zhu B., Fitzgerald D.G., Mayer C.M., Rudstam L.G. & Mills E.L. (2006). Alteration of ecosystem function by zebra mussels in Oneida Lake: Impacts on submerged macrophytes. *Ecosystems*, 9, 1017-1028.

Appendices

Appendix 1. ANOVA tables from Chapter Three.

Table 1. Results of PERMANOVA testing for an effect of species (crayfish or carp) and year on $\delta^{13}C$

Source of variation	df.	SS	MS	*F*	*P*
Species	1	24.41	24.41	2.25	0.23
Year	5	918.37	183.67	71.95	0.0001
Species*Year	3	33.54	11.18	4.38	0.005
Residual	449	1146.2	2.55		

Table 2. Results of PERMANOVA testing for an effect of species (crayfish or carp) and year on $\delta^{15}N$

Source of variation	df.	SS	MS	*F*	*P*
Species	1	272.94	272.94	27.29	0.015
Year	5	351.73	70.35	56.79	0.0001
Species*Year	3	31.10	10.37	8.37	0.0001
Residual	449	556.22	1.24		

Appendix 2. ANOVA tables from Chapter Four

Table 1. Results of ANOVA testing for an effect of crayfish and crab presence on rates of leaf decomposition. The dependent variable was square-root transformed prior to analysis to stabilize heterogeneous variances.

Source of variation	df.	SS	MS	*F*	*P*
Crayfish	1	0.54	0.54	72.68	≤0.0001
Crabs	1	0.01	0.01	1.68	0.2
Crayfish*Crabs	1	0.09	0.09	12.66	0.0011
Residual	36	0.27	0.01		

Table 2. Results of ANOVA testing for an effect of crayfish and crab presence on epilithic algal chlorophyll *a* concentrations.

Source of variation	df.	SS	MS	*F*	*P*
Crayfish	1	0.03	0.03	0.92	0.36
Crabs	1	0.00	0.00	0.17	0.69
Crayfish*Crabs	1	0.01	0.01	0.33	0.57
Residual	12	0.34	0.03		

Table 3. Results of PERMANOVA testing for an effect of crayfish and crab presence on the structure of benthic invertebrate assemblages.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Crayfish	1	5047	5047	6.05	0.004
Crabs	1	1030.6	1030.6	1.23	0.3
Crayfish*Crabs	1	241.5	241.5	0.29	0.85
Residual	17	15028			

Table 4. Results of ANOVA testing for an effect of crayfish and crab presence on the abundance of benthic invertebrates.

Source of variation	df.	SS	MS	*F*	*P*
Crayfish	1	832.05	832.05	9.39	0.007
Crabs	1	252.05	252.05	2.84	0.11
Crayfish*Crabs	1	6.05	6.05	0.07	0.8
Residual	16	1418.4	88.65		

Table 5. Results of ANOVA testing for an effect of crayfish and crab presence on the taxon richness of benthic invertebrate assemblages.

Source of variation	df.	SS	MS	*F*	*P*
Crayfish	1	8.45	8.45	6.76	0.019
Crabs	1	6.05	6.05	4.84	0.043
Crayfish*Crabs	1	0.05	0.05	0.04	0.84
Residual	16	20	1.25		

Table 6. Results of PERMANOVA testing for differences in the stable isotope-inferred dietary preferences of crabs and crayfish in both allopatric and sympatric conditions.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Species	1	3938.7	3938.7	2.72	0.048
Competition	1	639.84	639.84	0.44	0.74
Species*Competition	1	1631.6	1631.6	1.13	0.31
Residual	57	82549	1448.2		

Table 7. Results of SIMPER analysis indicating the five food resources that contributed most to the dissimilarity between the stable isotope-inferred diets of crayfish and crabs.

Taxon	Mean proportional abundance in crayfish diets	Mean proportional abundance in crab diets	Contribution to dissimilarity (%)	Cumulative contribution (%)
Hydropsychidae	0.25	0.15	26.41	26.41
Biofilm	0.12	0.18	15.84	42.25
Simuliidae	0.17	0.12	13.6	55.85
Chironomidae	0.12	0.10	8.46	64.31
Oligochaeta	0.09	0.08	8.11	72.42

Table 8. Results of ANOVA testing for an effect of species and competition on dietary niche width (measured as NR_b).

Source of variation	df.	SS	MS	*F*	*P*
Species	1	0.26	0.26	0.76	0.4
Competition	1	1.61	1.61	4.64	0.046
Species*Competition	1	0.00	0.00	0.01	0.91
Residual	17	5.88	0.35		

Table 9. Results of ANOVA testing for an effect of species and competition on dietary niche width (measured as CR_b).

Source of variation	df.	SS	MS	*F*	*P*
Species	1	0.28	0.28	1.06	0.32
Competition	1	0.00	0.00	0.00	0.99
Species*Competition	1	0.17	0.17	0.65	0.43
Residual	17	4.54	0.27		

Table 10. Results of ANOVA testing for an effect of experiment, species and competition on weight gain of crayfish and crabs over 6 weeks.

Source of variation	df.	SS	MS	*F*	*P*
Species	1	30.03	30.03	10.35	0.002
Competition	1	29.27	29.27	10.09	0.003
Species*Competition	1	0.26	0.26	0.09	0.77
Experiment	1	0.30	0.30	0.10	0.75
Species*Experiment	1	0.80	0.80	0.27	0.60
Competition*Experiment	1	15.79	15.79	5.44	0.02
Species*Competition*Experiment	1	0.21	0.21	0.07	0.79
Residual	44	127.7	2.90		

Table 11. Results of ANOVA testing for an effect of experiment, species and competition on carapace growth of crayfish and crabs over 6 weeks.

Source of variation	df.	SS	MS	*F*	*P*
Species	1	13.04	13.04	3.29	0.07
Competition	1	15.96	15.96	4.02	0.048
Species*Competition	1	0.01	0.01	0.003	0.96
Experiment	1	22.74	22.74	5.73	0.02
Species*Experiment	1	0.90	0.90	0.23	0.63
Competition*Experiment	1	10.70	10.70	2.70	0.10
Species*Competition*Experiment	1	0.11	0.11	0.03	0.87
Residual	81	321.35	3.97		

Table 12. Results of PERMANOVA testing for an effect of survey site and year on the stable isotope signature of putative resources of crabs and crayfish.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Species	5	222.1	44.42	2.04	0.1
Site	3	55.98	18.66	1.44	0.3
Year	1	0.14	0.14	0.02	0.97
Species*Site	4	50.78	12.7	3.31	0.06
Species*Year	5	54.79	10.96	2.85	0.09
Site*Year	3	11.77	3.92	1.07	0.45
Species*Site*Year	4	15.35	3.84	1.82	0.1
Residual	42	88.63	2.11		

Appendix 3. List of freshwater non-indigenous species recorded as established in the Thames Catchment. Data used in Chapter Five.

Key for vector of introduction: Stocking (S), Aquaculture/farming (A), Ornamental trade (O), Unknown contaminant (C), Contaminant of stocking (C-S), Contaminant of aquaculture/farming (C-A), Contaminant of ornamental trade (C-O) and Accidental transportation (T).

Group	Common name	Species	Native range	To Britain	To Thames catchment	Vector	References
Virus	Spring viraemia of carp	*Rhabdovirus carpio*	Europe, Russia and the Middle East	1976	1976	C-O	1,2
Fungi	Crayfish Plague	*Aphanomyces astaci*	North America	1981	1981	C-A	3
Plants	Sweet-flag	*Acorus calamus*	North America and Asia	1666	1666	A	4,5
	Slender Sweet-flag	*Acorus gramineus*	West Asia	1986	1986	O	2,6
	Cape Pondweed	*Aponogeton distachyos*	Southern Africa	1889	1931	A	2,7
	Water Fren	*Azzolla filiculoides*	South America	1883	1905	O	4,5,7
	Fanwort	*Cabomba caroliniana*	Americas	1969	1991	O	2,6
	Bog Arum	*Calla palustris*	Europe	1861	1873	O	2,5,8
	New Zealand Pigmyweed	*Crassula helmsii*	Australasia	1911	1956	O	4,7
	Brazilian Waterweed	*Egeria densa*	South America	1950	?	O	2,8
	South American Waterweed	*Elodea callitrichoides*	South America	1948	1955	O	2,7
	Canadian Waterweed	*Elodea canadensis*	North America	1842	1852	O	4,7
	Nuttall's Waterweed	*Elodea nuttallii*	North America	1966	1975	O	4,7
	Floating Pennywort	*Hydocotyle ranunculoides*	North America	1991	1994	O	9

	Curly Waterweed	*Lagarosiphon major*	Southern Africa	1944	1970	O	2,7
	Least Duckweed	*Lemna minuta*	Americas	1977	?	O	2,4
	Water Primrose	*Ludwigia Grandiflora*	South America	1999	?	O	2
	American Skunk-cabbage	*Lysichiton americanus*	North America	1947	1947	O	4,7
	Parrots Feather	*Myriophyllum aquaticum*	South America	1945	1945	O	2,7,8
	Spatter-dock	*Nuphar advena*	Europe and North America	1963	1986	O	2,6
	Pickerelweed	*Pontederia cordata*	Americas	1949	1986	O	2,4
	Pond Crystalwort	*Riccia rhenana*	Europe, Africa, Americas	1952	?	C-O	2,10
	Broadleaf Arrowhead	*Sagittaria Latifolia*	Americas	1941	1941	O	4,7
	Narrow leaved arrowhead	*Sagittaria subulata*	North America	1962	1962	O	4,8
	Tapegrass	*Vallisneria spiralis*	Europe	1868	1970	O	2,4
Nematodes	Eel parasite	*Anguillicola crassus*	East Asia	1987	1987	C-A	11,12
	Parasitic worm in fish	*Philometroides sanguinea*	East Europe and Russia	1982	1982	C-O	4,13
Oligochaetae	Oligochaete	*Branchiura sowerbyi*	Asia	1892	1958	C-O	2,14
	Oligochaete	*Limnodrilus Cervix*	North America	1965	1965	C-O	2,15
	Oligochaete	*Sparganophilus tamesis*	North America	1892	1892	T	16,17
Platyhelminthes	Tapeworm (fish parasite)	*Atractolytocestus huronensis*	Asia	1993	1993	C-A	4,18
	Tapeworm (fish	*Bothriocephalus*	West Asia	1970	1979	C-A	19,20

	parasite)	*acheilognathi*					
	Trematode (fish parasite)	*Dactylogyrus anchoratus*	Asia	1972	1972	C-O	[4,20]
	Triclad	*Dugesia tigrina*	North America	1940	1952	C-O	[21,22]
	Tapeworm (fish parasite)	*Khawia sinensis*	West Asia	1986	1990	C-S	[4,23]
	Tapeworm (fish parasite)	*Monobothrium wageneri*	Europe	1992	1992	C-O	[24]
	Triclad	*Planaria torva*	Europe	1935	1981	T	[21]
	Trematode (fish parasite)	*Pseudodactylogyrus anguillae*	Australasia and East Asia	1987	1987	C-A	[2,20]
	Trematode (fish parasite)	*Pseudodactylogyrus bini*	Australasia and East Asia	1988	1988	C-A	[2,20]
	Digenean	*Sanguinicola inermis*	Russia	1977	1977	C-O	[20,25]
Insecta	Water Beetle	*Cryptopleurum subtile*	East Asia	1958	1994	C	[4,26]
	Weevil	*Stenopelmus rufinasus*	North America	1929	?	C-S	[2]
Cnidarians	Freshwater jellyfish	*Craspedacusta sowerbyi*	West Asia	1880	1880	C-O	[27]
Molluscs	Asiatic Clam	*Corbicula fluminea*	East Asia	1998	2004	T	[28]
	Zebra Mussel	*Dreissena polymorpha*	East Europe and Middle East	1820	1820	T	[29]
	Limpet	*Ferrissia wautieri*	North Africa	1976	1976	T	[30]
	Trumpet Ram's-horn	*Menetus dilatatus*	North America	1869	1974	T	[29]
	Oblong Orb Mussel	*Musculium transversum*	North America	1856	1856	T	[29]

	False Dark Mussel	*Mytilopsis leucophaeata*	North America	2002	2002	T	31
	Snail	*Physa spp. (P. gyrina widespread, P. acuta and P. heterostropha possibly present)*	North America	1800s	1981	T	29
	Jenkins's Spire Snail	*Potamopyrgus antipodarum*	Australasia	1852	1852	T	29,32
Crustacean Decapods	Turkish Crayfish	*Astacus leptodactylus*	East Europe	1975	1975	A	33
	Chinese Mitten Crab	*Eriocheir sinensis*	West Asia	1935	1935	T	34
	Virile crayfish	*Orconectes virilis*	North America	2004	2004	O	35
	Signal crayfish	*Pacifastacus leniusculus*	North America	1975	1975	A	33
	Red Swamp Crayfish	*Procambarus clarkii*	North and Central America	1991	1991	O	33
Crustacean non-decapods	Carp lice	*Argulus japonicus*	West Asia	1990	?	C-O	36
	Amphipod	*Chelicorophium curvispinum*	East Europe and Middle East	1935	1982	T	2,37
	Amphipod	*Crangonyx pseudogracilis*	North America	1935	1935	T	33
	Water flea	*Daphnia ambigua*	Americas	1948	1948	T	4,38
	Parasitic Copepod	*Ergasilus briani*	Europe	1982	?	C-S	4,20,39
	Parasitic Copepod	*Ergasilus sieboldi*	Europe	1967	?	C	4,20
	Parasitic Copepod	*Lernaea cyprinacae*	Asia	1966	1966	C-O	20,40
	Parasitic Copepod	*Neoergasilus*	East Europe and	1980	?	C	4,20

		japonicus	Middle East				
	Amphipod	*Orchestia cavimana*	East Europe	1942	1942	T	2,41
	Parasitic Copepod	*Paraergasilus longidigitus*	West Asia	1994	?	C	4
Fish	Sterlet	*Acipenser ruthenus*	Europe	1986	1986	O	4,42
	Rock Bass	*Ambloplites rupestris*	North America	1937	1937	S	2,43
	Black Bullhead	*Ameiurus melas*	North America	1880	?	O	4,44
	Goldfish	*Carassius auratus*	Asia	1694	1746	O	44,45
	Common Carp	*Cyprinus carpio*	Europe and Asia	1490's	~1500	A	4,44,46
	Pumpkinseed	*Lepomis gibbosus*	North America	1890	1938	O	43,47
	Orfe	*Leuciscus idus*	Europe	1875	1959	O	43,48
	Rainbow Trout	*Oncorhynchus mykiss*	North and Central America	1884	1884	S	2,43,44
	Fathead Minnow	*Pimepales promelas*	North America	2002	2002	O	4
	Guppy	*Poecilia reticulata**	South America	1963	1963	O	4,43
	Topmouth Gudgeon	*Pseudorasbora parva*	West Asia	1985	2003	C-S	49,50
	Bitterling	*Rhodeus amarus*	Europe	1920	1956	O	2,43
	Brook trout	*Salvelinus fontinalis*	North America	1869	1876	S	2,43,44
	Pikeperch	*Sander lucioperca*	Europe	1878	1970s	S	44,51
	European Catfish	*Silurus glanis*	East Europe	1864	1906	S	4,43
Amphibia	American bullfrog	*Rana catesbeiana*	North America	1999	1999	O	52
	Edible Frog	*Rana esculenta*	Europe	1837	1846	A	44,53
	Marsh Frog	*Rana ridibunda*	Europe	1884	1884	O	44,53
	Alpine Newt	*Triturus alpestris*	Europe	1970	1970	C-O	44,53
Birds	Mandarin Duck	*Aix galericulata*	East Asia	1866	1932	O	2,48
	Wood Duck	*Aix sponsa*	North America	1873	1960	O	2,44
	Egyptian Goose	*Alopochen*	Africa	1795	1795	O	4,44,48,54

		aegyptiacus					
	Bar-headed goose	*Anser indicus*	Asia	1960	2004	O	4,55
	Canada Goose	*Branta canadensis*	North America	1725	1785	O	4,48
	Black Swan	*Cygnus atratus*	Australia	1902	1902	O	48,56
	Ruddy Duck	*Oxyura jamaicensis*	North America	1952	1965	O	4,57
Mammals	American Mink	*Mustela vison*	North America	1956	1961	A	2,44,48
	Coypu	*Myocastor coypus**	South America	1932	1943	A	2,48
	Muskrat	*Ondatra zibethicus**	North America	1927	?	A	2
	Brown Rat	*Rattus norvegicus*	Central Asia	1720	?	T	2,48

Appendix 3 References

1 Bucke, D. & Finlay, J. Identification of spring viraemia in carp (Cyprinus carpio L) in Great Britain. *Veterinary Record* **104**, 69-69 (1979).

2 Keller, R. P., Zu Ermgassen, P. S. E. & Aldridge, D. C. Vectors and Timing of Freshwater Invasions in Great Britain. *Conservation Biology* **23**, 1526-1534 (2009).

3 Holdich, D. M. & Sibley, P. J. Crayfish in Europe–an overview of taxonomy, legislation, distribution, and crayfish plague outbreaks. *Management & Conservation of Crayfish Conference Nottingham*, 15-34 (2003).

4 Hill, M. O. *Audit of non-native species in England*. (English Nature, 2005).

5 Salmon, C. E., Pearsall, W. H., Taylor, W. F. & Wolley-Dod, A. H. *Flora of Surrey: being an account of the flowering plants, ferns and Characeae, with notes on the topography, climate and geology, and a history of the botanical investigation of the county*. (G. Bell & sons, ltd., 1931).

6 Preston, C. D., Pearman, D. A. & Dines, T. D. *New atlas of the British and Irish flora. An atlas of the vascular plants of Britain, Ireland, the Isle of Man and the Channel Islands*. (Oxford University Press, 2002).

7 Burton, R. M. *Flora of the London area*. (London Natural History Society, 1983).

8 Clapham, A. R., Tutin, T. G. & Moore, D. M. *Flora of the British Isles. 3rd edition*. (Cambridge University Press, 1987).

9 Newman, J. R. & Dawson, F. H. Ecology, distribution and chemical control ofHydrocotyle ranunculoidesin the UK. *Hydrobiologia* **415**, 295-298 (1999).

10 Hill, M. O., Preston, C. D. & Smith, A. J. E. *Atlas of the Bryophytes of Britain and Ireland, vols 1-3*. (Harley, 1991).

11 Hickley, P. & Chare, S. Fisheries for non-native species in England and Wales: angling or the environment? *Fisheries Management and Ecology* **11**, 203-212 (2004).

12 Kennedy, C. R. & Fitch, D. J. Colonization, larval survival and epidemiology of the nematode Anguillicola crassus, parasitic in the eel, Anguilla anguilla, in Britain. *Journal of Fish Biology* **36**, 117-131 (1990).

13 Williams, C. F. Impact assessment of non-native parasites in freshwater fisheries in England and Wales. (2007).

14 Mann, K. H. Occurrence of an Exotic Oligochaete Branchiura sowerbyi Beddard, 1892, in the River Thames. *Nature* **182**, 732-732 (1958).

15 Kennedy, C. R. The distribution and habitat of Limnodrilus Claparede (Oligochaeta: Tubificidae). *Oikos* **16**, 26-38 (1965).

16 Brinkhurst, R. O. *A Guide for the Identification of British Aquatic Oligochaeta*. (Freshwater Biological Association, 1971).

17 Sherlock, E. & Carpenter, D. An updated earthworm list for the British Isles and two new [] exotic'species to Britain from Kew Gardens. *European Journal of Soil Biology* **45**, 431-435 (2009).

18 Bazsalovicsová, E. *et al.* Population study of Atractolytocestus huronensis (Cestoda: Caryophyllidea), an invasive parasite of common carp introduced to Europe: mitochondrial cox1 haplotypes and intragenomic ribosomal ITS2 variants. *Parasitology Research* (2011).

19 Andrews, C., Chubb, J. C., Coles, T. & Dearsley, A. The occurrence of Bothriocephalus acheilognathi Yamaguti, 1934 (B. gowkongensis) (Cestoda: Pseudophyllidea) in the British Isles. *Journal of Fish Diseases* **4**, 89-93 (1981).

20 Kennedy, C. R. Introductions, spread and colonization of new localities by fish helminth and crustacean parasites in the British Isles: a perspective and appraisal. *Journal of Fish Biology* **43**, 287-301 (1993).

21 Young, J. O. & Reynoldson, T. B. Continuing dispersal of freshwater triclads (Platyhelminthes; Turbellaria) in Britain with particular reference to lakes. *Freshwater Biology* **42**, 247-262 (1999).

22 Wright, J. F. Colonization of rivers and canals in Great Britain by Dugesia tigrina (Girard)(Platyhelminthes: Tricladida). *Freshwater Biology* **17**, 69-78 (1987).

23 Yeomans, W. E., Chubb, J. C. & Sweeting, R. A. *Khawia sinensis* (Cestoda: Caryophyllidea)—;an indicator of legislative failure to protect freshwater habitats in the British Isles? *Journal of Fish Biology* **51**, 880-885 (1997).

24 Gibson, D. I. *Monobothrium-wageneri* - another imported tapewormestablished in wild british fresh-water fishes. *Journal of fish biology* **43**, 281-285 (1993).

25 Kirk, R. S. & Lewis, J. W. The Distribution and Host Range of Species of the Blood Fluke Sanguinicola in British Freshwater Fish. *Journal of Helminthology* **68**, 315-318 (1994).

26 Duff, A. G. & Buckland, P. I. *Checklist of beetles of the British Isles*. (AG Duff, 2008).

27 Green, J. in *Freshwater Forum.* 69-76.

28 Elliot, P. & zu Ermgassen, P. S. E. The Asian clam (Corbicula fluminea) in the River Thames, London, England. *Aquatic Invasions* **3**, 54-60 (2008).

29 Kerney, M. P. *Atlas of the land and freshwater molluscs of Britain and Ireland.* (Harley, 1999).

30 Holyoak, D. T. Ferissia wautieri (Mirolli)(Pulmonata: Ancylidae) naturalized in Sussex, England. *Journal of conchology* **29**, 349-350 (1978).

31 Bamber, R. N. & Taylor, J. D. The brackish water mussel Mytilopsis leucophaeta (Conrad, 1831)(Bivalvia: Dreissenidae) in the River Thames. *Journal of Conchology* **37**, 403-404 (2002).

32 Ponder, W. F. Potamopyrgus antipodarum - a molluscan coloniser of Europe and Australia. *Journal of Molluscan Studies* **54**, 271-271 (1988).

33 Holdich, D. M., Rogers, W. D. & Reynolds, J. D. in *Crayfish in Europe as alien species. How to make the best of a bad situation* (eds F. Gherardi & D. M. Holdich) 221-235 (A.A. Balkema, Rotterdam and Brookfield., 1999).

34 Clark, P. F. *et al.* The alien Chinese mitten crab, Eriocheir sinensis (Crustacea : Decapoda : Brachyura), in the Thames catchment. *Journal of the Marine Biological Association of the United Kingdom* **78**, 1215-1221 (1998).

35 Ahern, D., England, J. & Ellis, A. The virile crayfish, Orconectes virilis (Hagen, 1870)(Crustacea: Decapoda: Cambaridae), identified in the UK. *Aquatic Invasions* **3**, 102-104 (2008).

36 Rushton-Mellor, S. K. Discovery of the fish louse, Argulus japonicus Thiele (Crustacea: Branchiura). *Britain.–Aquaculture and Fisheries Management* **23**, 269-271 (1992).

37 Bratton, J. H. Corophium curvispinum Sars (Crustacea: Amphipoda) recorded in the London area. *The London Naturalist* **61**, 63-63 (1982).

38 Maier, G. Daphnia invasion: population dynamics of Daphnia assemblages in two eutrophic lakes with particular reference to the introduced alien Daphnia ambigua. *Journal of Plankton Research* **18**, 2001-2015 (1996).

39 Kennedy, C. R. A checklist of British and Irish freshwater fish parasites with notes on their distribution. *Journal of Fish Biology* **6**, 613-644 (1974).

40 Fryer, G. The parasitic copepod Lernaea cyprinacea L. *Britain. J. nat. Hist* **2**, 531-533 (1968).

41 Curry, A., Grayson, R. F. & Milligan, T. D. New British records of the semi-terrestrial amphipod Orchestia cavimana. *Freshwater Biology* **2**, 55-56 (1972).

42 Zięba, G. *et al.* Recent releases and dispersal of non-native fishes in England and Wales, with emphasis on sunbleak Leucaspius delineatus (Heckel, 1843). *Aquatic Invasions* **5**, 155-161 (2010).

43 Wheeler, A. & Maitland, P. S. The scarcer freshwater fishes of the British Isles. *Journal of Fish Biology* **5**, 49-68 (1973).

44 Lever, C. *The Naturalised Animals of the British isles*. (Paladin, 1977).

45 Copp, G. H., Wesley, K. J. & Vilizzi, L. Pathways of ornamental and aquarium fish introductions into urban ponds of Epping Forest (London, England): the human vector. *Journal of Applied Ichthyology* **21**, 263-274 (2005).

46 Araujo, F. G., Bailey, R. G. & Williams, W. P. Spatial and temporal variations in fish populations in the upper Thames estuary. *Journal of Fish Biology* **55**, 836-853 (1999).

47 Klaar, M., Copp, G. H. & Horsfield, R. Autumnal habitat use of non-native pumpkinseed Lepomis gibbosus and associations with native fish species in small English streams. *Folia Zool* **53**, 189-202 (2004).

48 Fitter, R. S. R. *The ark in our midst*. (1959).

49 Gozlan, R. E., Pinder, A. C. & Shelley, J. Occurrence of the Asiatic cyprinid Pseudorasbora parva in England. *Journal of Fish Biology* **61**, 298-300 (2002).

50 Pinder, A. C., Gozlan, R. E. & Britton, J. R. Dispersal of the invasive topmouth gudgeon, *Pseudorasbora parva* in the UK: a vector for an emergent infectious disease. *Fisheries Management and Ecology* **12**, **411–414** (*2005*).

51 Copp, G. H., Wesley, K. J., Kovacˇ, V., Ives, M. J. & Carter, M. G. Introduction and establishment of the pikeperch Stizostedion lucioperca (L.) in Stanborough Lake (Hertfordshire) and its dispersal in the Thames catchment. *The London Naturalist* **82**, 139-153 (2003).

52 Banks, B., Foster, J., Langton, T. & Morgan, K. British bullfrogs? *British Wildlife*, 327-330 (2000).

53 Arnold, H. R. *Atlas of amphibians and reptiles in Britain*. (HMSO, 1995).

54 Crick, H. *et al.* Non-native birds breeding in the UK in 2002. *British Birds* **97**, 633-637 (2004).

55 Holling, M. Non-native breeding birds in the United Kingdom in 2003, 2004 and 2005. *BRITISH BIRDS* **100**, 638-638 (2007).

56 Blair, M. J., McKay, H., Musgrove, A. J. & Rehfisch, M. M. Review of the status of introduced non-native waterbird species in the agreement area of the African-Eurasian Waterbird Agreement Research contract CR0219. (2002).

57 Snow, D. W. *The status of birds in Britain and Ireland*. (Blackwell Scientific, 1971).

Appendix 4A. ANOVA tables from Chapter Seven; signal and virile crayfish experiment.

Table 1. Results of ANOVA testing for an effect of population on $\delta^{15}N$.

Source of variation	df.	SS	MS	*F*	*P*
Population	3	79.96	26.65	31.93	0.000
Residual	80	66.79	0.84		

Table 2. Results of ANOVA testing for an effect of population on $\delta^{13}C$.

Source of variation	df.	SS	MS	*F*	*P*
Population	3	6.02	2.01	6.06	0.001
Residual	80	26.47	0.33		

Table 3. Results of ANOVA testing for an effect of population on nitrogen range (NR_b).

Source of variation	df.	SS	MS	*F*	*P*
Population	3	1.52	0.51	3.70	0.034
Residual	16	2.20	0.14		

Table 4. Results of ANOVA testing for an effect of population on carbon range (CR_b).

Source of variation	df.	SS	MS	*F*	*P*
Population	3	0.48	0.16	3.87	0.029
Residual	16	0.67	0.04		

Table 5. Results of PERMANOVA testing for differences in the proportional contribution of six putative resources to the diet of populations of allopatric SC, sympatric SC, allopatric VC and sympatric VC.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Population	3	644.1	214.7	2.74	0.044
Residual	16	1255.1	78.44		

Table 6. Results of ANOVA testing for an effect of treatment on the abundance of grazers and scrapers.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	487.8	162.6	2.72	0.079
Residual	16	956.8	59.8		

Table 7. Results of ANOVA testing for an effect of treatment on the abundance of gatherers and shredders.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	5136	1712	0.60	0.63
Residual	16	45968	2873		

Table 8. Results of ANOVA testing for an effect of treatment on the abundance of predators and carnivorous scavengers.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	30.95	10.32	8.78	0.001
Residual	16	18.80	1.18		

Table 9. Results of ANOVA testing for an effect of treatment on the abundance of Diptera larvae.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	1875200	625067	14.16	0.00
Residual	16	706136	44134		

Table 10. Results of PERMANOVA testing for an effect of treatment on the structure of zooplankton assemblages.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Treatment	3	1251.5	417.16	1.583	0.1381
Residual	16	4216.8	263.55		

Table 11. Results of ANOVA testing for an effect of treatment on the increase in total zooplankton density (square-root transformed).

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	102.9	34.3	1.93	0.166
Residual	16	284.4	17.8		

Table 12. Results of ANOVA testing for an effect of treatment on the benthic invertebrate diversity.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	7.61	2.537	8.54	0.001
Residual	16	4.75	0.297		

Table 13. Results of ANOVA testing for an effect of treatment on zooplankton diversity.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.41	0.138	2.56	0.091
Residual	16	0.86	0.054		

Table 14. Results of ANOVA testing for an effect of treatment on chlorophyll-*a* concentration on standardised tile surfaces in the benthos after 4 weeks.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.4478	0.1493	2.51	0.095
Residual	16	0.9498	0.0594		

Table 15. Results of ANOVA testing for an effect of treatment on chlorophyll-*a* concentration in the water column after 4 weeks (square-root transformed).

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.0053	0.0017	2.97	0.063
Residual	16	0.0096	0.0006		

Table 16. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 1mm^2.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.001854	0.000618	2.11	0.139
Residual	16	0.004686	0.000293		

Table 17. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 5mm^2 (square-root transformed).

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.3043	0.1014	9.24	0.001
Residual	16	0.1757	0.0110		

Table 18. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 10mm^2 (square-root transformed).

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.47885	0.15962	16.95	0.000
Residual	16	0.15067	0.00942		

Table 19. Results of ANOVA testing for an effect of treatment on nitrate concentration.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	76249	25416	0.79	0.521
Residual	16	418340	32180		

Table 20. Results of ANOVA testing for an effect of treatment on phosphate concentration.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	11.02	3.67	1.20	0.349
Residual	16	39.84	3.06		

Appendix 4B. ANOVA tables from Chapter Seven; Louisiana red swamp and Turkish crayfish experiment.

Table 1. Results of ANOVA testing for an effect of population on $\delta^{15}N$.

Source of variation	df.	SS	MS	*F*	*P*
Population	3	122.906	40.969	42.07	0.00
Residual	77	74.983	0.974		

Table 2. Results of ANOVA testing for an effect of population on $\delta^{13}C$.

Source of variation	df.	SS	MS	*F*	*P*
Population	3	199.13	66.38	19.76	0.00
Residual	77	258.61	3.36		

Table 3. Results of ANOVA testing for an effect of population on nitrogen range (NR_b).

Source of variation	df.	SS	MS	*F*	*P*
Population	3	1.25	0.42	2.10	0.14
Residual	16	3.19	0.20		

Table 4. Results of ANOVA testing for an effect of population on carbon range (CR_b).

Source of variation	df.	SS	MS	*F*	*P*
Population	3	7.18	2.40	6.0	0.006
Residual	16	6.39	0.40		

Table 5. Results of PERMANOVA testing for differences in the proportional contribution of six putative resources to the diet of populations of allopatric RSC, sympatric RSC, allopatric TC and sympatric TC.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Population	3	179.9	59.967	1.558	0.23
Residual	16	615.82	38.489		

Table 6. Results of PERMANOVA testing for an effect of treatment on the total benthic invertebrate community structure.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Treatment	3	3032.6	1010.9	1.18	0.31
Residual	16	13754	859.64		

Table 7. Results of ANOVA testing for an effect of treatment on the abundance of grazers and scrapers.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	41862	13954	4.75	0.015
Residual	16	47031	2939		

Table 8. Results of ANOVA testing for an effect of treatment on the abundance of gatherers and shredders.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	661	220	0.47	0.71
Residual	16	7464	467		

Table 9. Results of ANOVA testing for an effect of treatment on the abundance of predators and carnivorous scavengers.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	921	307	4.06	0.05
Residual	16	1608	100		

Table 10. Results of ANOVA testing for an effect of treatment on the abundance of chironomids.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	11607	3869	0.35	0.79
Residual	16	179312	11207		

Table 11. Results of PERMANOVA testing for an effect of treatment on the structure of zooplankton assemblages.

Source of variation	df.	SS	MS	Pseudo-*F*	*P*
Treatment	3	2013	671.02	0.99	0.44
Residual	16	10797	674.8		

Table 12. Results of ANOVA testing for an effect of treatment on the increase in total zooplankton density.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	16665	5555	1.11	0.38
Residual	16	80337	5021		

Table 13. Results of ANOVA testing for an effect of treatment on the benthic invertebrate diversity.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	2.357	0.786	2.08	0.143
Residual	16	6.049	0.378		

Table 14. Results of ANOVA testing for an effect of treatment on zooplankton diversity.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	2.052	0.684	2.24	0.112
Residual	16	4.876	0.305		

Table 15. Results of ANOVA testing for an effect of treatment on chlorophyll-*a* concentration on standardised tile surfaces in the benthos after 6 weeks.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	37.69	12.56	4.5	0.018
Residual	16	44.70	2.79		

Table 16. Results of ANOVA testing for an effect of treatment on chlorophyll-*a* concentration in the water column after 6 weeks.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.04214	0.01405	1.55	0.24
Residual	16	0.14543	0.00909		

Table 17. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 1mm^2.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.018572	0.006191	6.86	0.003
Residual	16	0.014430	0.000902		

Table 18. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 5mm^2.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.02452	0.00817	3.75	0.033
Residual	16	0.03488	0.00218		

Table 19. Results of ANOVA testing for an effect of treatment on decomposition rates in bags with a mesh of 10mm^2.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.4411	0.1470	3.68	0.034
Residual	16	0.6386	0.0399		

Table 20. Results of ANOVA testing for an effect of treatment on nitrate concentration.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	0.02786	0.00929	0.14	0.936
Residual	16	1.08354	0.06772		

Table 21. Results of ANOVA testing for an effect of treatment on phosphate concentration.

Source of variation	df.	SS	MS	*F*	*P*
Treatment	3	7.256	2.419	0.95	0.442
Residual	16	40.90	2.556		