Ecological consequences of indigenous and non-indigenous freshwater fish parasites

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A thesis submitted in partial fulfilment of the requirements of Bournemouth University for the degree of Doctor of Philosophy

March 2016

Acknowledgements

I would like to thank my supervisors for their support and advice throughout my PhD. In particular I would like to thank Rob Britton for his tireless efforts in helping and encouraging me over the three years and for his valued friendship. Not only has Rob contributed a great deal of time and effort into my academic output but he has also taken an interest in my health and wellbeing throughout. I would also like to thank Jon Grey for his support; whilst he was not in an official supervisory role he has assisted me with my project on a number of occasions.

My thanks to everyone who has helped me with the large amount of fieldwork my research has involved and thank you to my friends and family who have helped and supported me in a number of other ways.

Thank you to NERC for funding the project and making this research possible and to SUERC and particularly Rona McGill for training me in the analysis of stable isotopes and allowing me to process my samples with you. Further I extend thanks to the Environment Agency for their support with practical elements of my project in the form of field and lab work as well as access to their fish health data bases. Particularly I'd like to thank Chris Williams and Amy Reading for training me to perform fish post-mortem examinations and parasite identification as well as the hours they've spent in the lab with me!

Finally I would like to thank my partner Farah for her love and support during my PhD, always making me laugh and encouraging me on a daily basis.

Abstract

Parasites can have considerable consequences for their freshwater fish hosts, irrespective of whether they are intermediate or final hosts. The ecological consequences of infection arise from processes including parasite manipulation, where the parasite manipulates the host to increase their chance of transmission to the next host in the lifecycle, and parasite-mediated competition, where a consequence of infection is an alteration in the symmetry of competition between hosts and their uninfected conspecifics, or with other species. Whilst there is a great deal of existing knowledge on some of these consequences, there also remain some considerable knowledge gaps. This research covered the role of parasite exposure and water temperature on infection consequences, the foraging responses of fishes to intermediate hosts of the fish parasite *Pomphorhynchus laevis* that has an indigenous and non-indigenous range in Great Britain, the ecological consequences of this parasite for some freshwater fishes across these ranges, the issue of 'enemy release' and 'parasite acquisition' in introduced freshwater fishes, and the ecological consequences of infection by some native parasites for native freshwater fish.

When the freshwater fish chub (*Squalius cephalu*s) was exposed to different levels of intermediate hosts (*Gammarus pulex*) of *P. laevis* under two water temperature treatments, ambient and warmed, it revealed this interaction had considerable consequences for both parasite prevalence and the infection parameters. Whilst parasite prevalence was substantially higher at the elevated temperature, where infections did develop at lower temperatures, they were associated with fewer but larger parasites resulting in significantly higher parasite burdens, indicating complex consequences for host-parasite relationships under conditions of warming.

Studies into parasite manipulation have frequently used the *P. laevis: G. pulex* parasite-intermediate host system for investigating how infections can result in behavioural modifications to the host that then results in their elevated risk of being predated by a fish. Here, comparative behavioural functional response experiments were used to test

differences in the consumption rates of three fishes exposed to either uninfected or infected *G. pulex*, testing the hypothesis that the consumption rate of infected *G. pulex* would be significantly higher. The Type II functional response curves indicated that the results of the experiments were contrary to this hypothesis, with subsequent behavioural and foraging experiments also supporting these results. These counter-intuitive outcomes were also contrary to most other studies that suggested a parasite would manipulate its intermediate host in a way that promotes its transmission to a final host and facilitating the continuation of its life cycle.

The reasons for these outputs were discussed as likely to relate to different selection pressures in this host-parasite system, given this is a generalist parasite with a wide range of potential fish final hosts. This was revealed by studies on this parasite from four fish species from five rivers that demonstrated high parasite prevalence in all species studied and suggested that small-bodied fishes, such as bullhead *Cottus gobio*, might play important roles in the *P. laevis* lifecycle. These prevalences, and the pathological consequences of the *P. laevis* infections, were also consistent across their indigenous and non-indigenous range, suggesting parasite origin had minimal consequences on their virulence and on the susceptibility of hosts to infection.

That parasite origin often has minimal ecological consequences for their ecological impacts was reinforced by work on the 'enemy release hypothesis' in non-native fish in England and Wales. This revealed very few non-native parasites had been introduced with their non-native fish hosts. Those that had been introduced tended to be specialist parasites with direct lifecycles that had little opportunity to be transmitted to native fishes. Instead, the acquisition of native parasites by the non-native fishes was frequently observed, leading to potential concerns these fish would act as reservoir hosts and spill-back the parasites to the native fishes.

Given the low probability of parasite introduction, the ecological consequences of three native parasites with complex lifecycles were then tested on three native fishes, and revealed consistent patterns of trophic niche divergence between infected and uninfected population sub-groups. Whilst the actual mechanism underpinning this, such as parasite-mediated competition, could not be tested, these results did reveal that the

consequences of infection can be far-reaching for hosts and can be measured through a variety of methodologies.

In summary, the research provided some comprehensive insights into many aspects of the pathological and ecological consequences of infection for some freshwater fishes from native/ non-native and indigenous/ non-indigenous parasites. In doing so, it has raised a series of new questions and hypotheses for further investigation, with the host-parasite systems used here capable of answering these.

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Glossary

Throughout the thesis, various terminologies are applied. For consistency of interpretation, the following definitions of some of the specific terms are provided below.

Complex lifecycle: The parasite lifecycle is completed using multiple hosts, including one or more intermediate host, in addition to a definitive host.

Definitive host (and final host and preferred host): A host in which the parasite reaches maturity and, if possible, reproduces sexually.

Direct lifecycle: Lifecycle is completed using a single host (may have a free-living stage).

Host: An organism infected by a parasite, usually the focal parasite in the context of the study.

Introduced species: A species, subspecies or lower taxon, introduced by human action outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce.

Individual trophic niche specialisation: Where the population trophic niche consists of sub-groups of trophically specialised individuals that in entirety comprise the population niche.

Intermediate host: A host of a parasite with a complex lifecycle that is important for an aspect of its development but in which sexual maturity does not occur.

Invasive species: An introduced or translocated species that has successfully survived the introduction process, established a population, dispersed more widely and caused some ecological and/ or economic impacts

Manipulative parasite: A parasite that alters aspects of the phenotypic traits and behaviours of their hosts, such as their morphology, foraging behaviour and habitat use,

that either increase the probability of their transmission from one host to another and/or ensure that their propagules are released in an appropriate location.

Naïve host species: A native (or indigenous) species having no co-evolutionary history to the non-native (or non-indigenous) parasite.

Non-manipulative parasite: A parasite which alters aspects of the phenotypic traits and behaviours of their hosts, such as their morphology, foraging behaviour and habitat use due to, for example, pathological impacts and energetic costs, and is not associated with the parasite manipulating the host to increase its probability of transmission.

Non-native species: A species, subspecies or lower taxon, introduced by human action outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagules of such species that might survive and subsequently reproduce.

Parasite: An organism that lives and feeds on or in an organism of a different species causing harm to its host.

Parasite abundance: This is the mean number of parasites found in all the individual infected hosts.

Parasite-mediated competition: Infection alters the competitive dynamics between interacting species via density and trait effects. Where the otherwise superior competitor species is heavily influenced by the parasite then the process is likely to favour co-existence.

Parasite prevalence: The proportion of infected hosts among all the potential hosts examined of a single species.

Paratenic: A host which is non-essential for the completion of a parasites lifecycle but which still might experience parasite infection

Population trophic niche: Extent of the food resources exploited by a population as set by abiotic and biotic constraints.

Trophic level: the feeding positions within a food chain, such as primary producer, primary consumer and secondary consumer, and where a food chain is the representation of a succession of species that consume another.

Trophic niche constriction: Individuals in the population sub-group consume a restricted range of food items that are also present in the diet of more generalist con-specifics.

Trophic niche divergence: Individuals in the population sub-group consume different food items that are not present in the diet of their conspecifics.

Trophic vacuum: the difference in trophic levels between free-living propagules of the parasite and its final host which, for trophically transmitted parasites, is overcome by use of intermediate hosts within their lifecycle.

Virulence: the severity of the negative consequences a parasite has for a host

Chapter 1

Introduction

This thesis covers a range of research issues associated with the sub-lethal consequences of infection by native and introduced parasites for which freshwater fish act as either the final or intermediate host. The research covers the role of parasite exposure and water temperature on infection consequences, the extent of 'parasite manipulation' for intermediate hosts by a fish parasite with an indigenous and non-indigenous range in Great Britain, the ecological consequences of this parasite for some freshwater fishes across these ranges, the issue of 'enemy release' and 'parasite acquisition' in introduced freshwater fish, and the ecological consequences of infection by some native parasites for native freshwater fish. The rationale for these research approaches are outlined in this Introduction, with the overall research aim and objectives, and the thesis structure, outlined at the end of the Chapter.

1.1 Overview

Freshwater fish are host to a wide range of taxonomically diverse parasites, with this diversity reflected in their lifecycles (Kennedy 1990; Bell and Burt 1991; Poulin and Morand 2000). These lifecycles can be relatively simple, where the parasites are transmitted directly from host to host. For other parasites, the lifecycle can be complex, with the final host usually being a fish or bird at a high trophic position whilst the parasite tends to be of low trophic position (Jansen and Bakke 1991; Britton et al. 2009; Macnab and Barber 2011). Thus, in order to overcome this discrepancy in their respective trophic positions, the parasite must navigate through a series of intermediate hosts before reaching their final host in which they sexually mature (Britton et al. 2009; Macnab and Barber 2011). In doing so, the parasite

overcomes the ‘trophic vacuum’ between their low trophic position and the high trophic position of their final hosts (Parker et al. 2015).

This discrepancy between the trophic positions of many parasites and their hosts, which is then generally reflected in their body sizes, might suggest the consequences of infection for many hosts are minor. This is rarely the case. Infections potentially have marked consequences for the biology and ecology of the host and, therefore, parasites can rarely be considered as relatively passive organisms that are present in or on hosts, but instead should be considered as ‘...a commensal organism (that) must fulfil criteria that include the fact that its presence has a negative effect on its host’ (Begon et al. 1990). Given that parasites usually exploit aspects of their host’s energy resources for their own requirements, then their impacts on the host often have a nutritional basis (Barber et al. 2000). It is, however, unlikely that these are the only impacts of the infection, with pathological consequences usually apparent around the site of attachment to the host, often shown by tissue damage and an associated host reaction to that damage (Britton et al. 2011; Pegg et al. 2015). Pathological impacts are then often accompanied by other consequences that whilst generally being sub-lethal initially, might eventually result in the death of the host, especially through indirect mechanisms (e.g. via predation or secondary infections of damaged tissues). Sub-lethal impacts include physiological imbalances and general malaise that can affect the growth, survival and, ultimately, reproductive fitness of the host (Barber et al. 2000). These are also often accompanied with altered host behaviours, especially in intermediate hosts (Section 1.2).

When the term ‘parasite’ is used, it can refer to a wide diversity of organisms, ranging from viruses to vertebrates such as some lamprey species, especially the sea lamprey *Petromyzon marinus* that can exceed lengths of 1 m (Swink 1991). Here, the focus is on fish macroparasites, an artificial group of metazoan parasites, composed mainly of members of the Platyhelminthes (flatworms, including monogenean and digenean trematodes and cestodes), Nemathelminthes (roundworms and allies, including nematodes and acanthocephalans) and Arthropods (true lice and parasitic copepods) (May and Anderson 1979; Barber et al. 2000). The focus here is on these parasites in a freshwater context, primarily due to the ease of working on these in both a field and laboratory context.

1.2 Parasite consequences for host populations

It was outlined in Section 1.1 that parasites can have substantial consequences for individual fish hosts, with these then potentially having additive consequences as levels of biological organisation scale up to population and community levels (Hatcher et al. 2011; 2012). These tend to be most marked in parasites with complex lifecycles, where individual host consequences include alterations in the symmetry of their competitive interactions, and their habitat utilisation and acquisition of food resources (Hatcher et al. 2006). These could then have substantial consequences for food web structure (Dobson et al. 2006). As the focus here was generally on parasites with complex lifecycles, then their potential consequences for their hosts (both intermediate and final) are discussed in this section.

1.2.1 Parasite lifecycles and the trophic vacuum

It was mentioned briefly in Section 1.1 that parasites have evolved complex lifecycles in which they are trophically transferred up food chains in order for them to overcome the issues of their low trophic position versus the high trophic position of their final host. Recent studies have termed this difference in parasite and host trophic position as the 'trophic vacuum', given that most adult helminth parasites sexually reproduce in vertebrates that have high positions in food chains, with their free-living propagules unable to be transmitted directly to these hosts. This trophic vacuum is thus filled by one or more intermediate hosts (Benesh et al. 2014; Parker et al. 2015).

This raises a number of questions over why the parasite then does not grow and develop further in the intermediate hosts, and instead shows suppressions of growth and reproduction until transmission to the final host, a process that can involve being transmitted through multiple intermediate hosts (Parker et al. 2015). It has been suggested that it relates to selection pressures associated with the increased longevity and higher growth that is possible by the parasite in the final host (due to their relatively large body size) versus intermediate hosts (that are often copepods or gammarid species). The selection pressure is thus for larger parasite body size and higher fecundity at sexual maturity that is most often only possible in the relatively large final host (Parker et al. 2015). Consequently, mechanisms that assist parasite transmission up the food chain, i.e. from intermediate hosts to final hosts, have potentially high ecological and evolutionary significance, such as processes of parasite manipulation (Section 1.2.2).

1.2.2 Parasite manipulation

Host manipulation is a process that assists parasites with complex lifecycles to fill the trophic vacuum (Section 1.2.1). It usually involves the manipulation by the parasite of the intermediate host that alters its behaviour sufficiently to increase the likelihood of that host being predated by the next host in the lifecycle (Loot et al. 2001; Britton et al. 2009; Macnab and Barber 2011).

Amphipods provide strong examples of intermediate hosts that are manipulated by their parasites to facilitate their predation by a fish or bird final host (Britton and Andreou 2016). *Gammarus roeseli* infected with *Polymorphus minutus* exhibit reverse geotaxis (Kennedy 2006), elevating their time spent at the water surface, an area where uninfected conspecifics are rarely found. The reverse geotaxis increases their predation risk by bird final hosts (Bauer et al. 2005; Medoc et al. 2006). Infections by the trematode parasite *Microphallus papillorobustus* divides populations of *Gammarus insensibilis* into two groups: an infected group of individuals that inhabit the surface of salt marshes and an uninfected group of individuals that remain near the bottom (Ponton et al. 2005). This shift in habitat use again relates to the parasite altering the behaviour of the intermediate host in order to promote their predation by bird final hosts (Britton and Andreou 2016). Parasite manipulation has also been detected in fish intermediate hosts. The cestode parasite *Ligula intestinalis* is generally recognised as modifying the behaviour of its intermediate fish hosts (Loot et al. 2001; Britton and Andreou 2016). Where fish are infected, they are increasingly encountered in the littoral zone, increasing their predation risk to the final bird host (Loot et al. 2001; Britton et al. 2009).

1.2.3 Parasite modifications to host phenotype

Parasite infections can also modify the host phenotype through consequences that are not associated with manipulation, such as impaired traits and altered behaviours that result from pathological or physiological impacts (Knudsen et al. 2004) which can then affect other behaviours, such as foraging and prey selectivity (Pegg et al. 2015). An example is the common carp *Cyprinus carpio* when infected, as the final host, with *Bothriocephalus acheilognathi*, a non-native intestinal cestode parasite. Infections impair the foraging ability of hosts through reducing, for example, their consumption rates (Scott and Grizzle 1979; Britton et al. 2011; 2012), causing the infected individuals to increasingly specialise on less motile food sources, which divides their population trophic niche into infected and uninfected sub-groups (Pegg et al. 2015) (Section 1.2.5).

When infection modifies the host phenotype then the modified traits can have a bimodal distribution in the population that results in the development of distinct sub-groups within the populations in which the individuals are grouped by their homogenous traits (Lafferty et al. 2006). Where the modified trait impacts on the habitat utilisation, foraging behaviours and/ or competitive abilities of the host then their diet composition and prey selectivity will be affected (Pegg et al. 2015). An example is provided by *Gammarus pulex* when it feeds on the isopod *Asellus aquaticus*. When *G. pulex* is infected by the acanthocephalan parasite *Echinorhyncus truttae*, it tends to kill significantly fewer *A. aquaticus* than the uninfected individuals, with smaller individuals also preferred (Fielding et al. 2003).

Infections by parasites can also be important through their effects on host foraging time budgets and the associated selectivity in prey items. For example, when *Schistocephalus solidus* infect three-spined sticklebacks *Gasterosteus aculeatus*, the fish increase their foraging time and invest less in anti-predator behaviours (Milinski 1985). Although they have also been shown to select smaller prey than their uninfected conspecifics (Cunningham et al. 1994; Milinski 1984). However Ranta (1995) suggested that larger items were taken by infected individuals compared with uninfected conspecifics, with this being a compensatory mechanism to overcome some of the energy costs caused by the parasite. Thus, irrespective of parasite manipulation, infections can markedly alter the host phenotype and this can have marked consequences for host ecology, such as through parasite mediated competition (Section 1.2.4).

1.2.4 Parasite mediated competition and coexistence

Parasite mediated competition can be extremely complex and occur within and between species, with the potential for important outcomes at the ecosystem level (Holt and Pickering 1985; Holt and Dobson 2006; Hatcher et al. 2012). In its simplest form, a parasite species will have a positive or negative effect on the competitive ability of its host with a clear and specific single outcome - most likely increased or reduced access to a shared resource (Kuris et al. 2008; Hatcher et al. 2012). However, more complex and likely scenarios have been proposed and observed (Hernandez and Sukhdeo 2008; Kuris et al. 2008; Hatcher and Dunn 2011; Hatcher et al. 2012).

At an intraspecific level, the competitive ability of infected hosts may reduce compared to their uninfected conspecifics, potentially leading to intra-specific niche partitioning (Hatcher et al. 2012). For inter-specific competitive interactions, parasites can enhance

the ability of the host to compete if it is more resistant to the parasite than its competitors. This is most evident in introductions of non-native species, such as the grey squirrel (*Sciurus carolinensis*) and red squirrel (*Sciurus vulgaris*) in relation to the parapox virus, and signal crayfish (*Pacifastacus leniusculus*) and white-clawed crayfish (*Austopotamobius pallipes*) with crayfish plague (*Aphanomyces astaci*). In both of these examples, the introduced host species act as a resistant carrier, transmitting the pathogen to the highly susceptible native hosts, reducing the native host's ability to compete due to the infection consequences (Reynolds 1988; Alderman et al. 1990; Naura and Robinson 1998; Daszak 2000; Tompkins et al. 2002, 2003). Conversely, parasites can reverse competitive interactions where one host species outcompetes another in the absence of a parasite, but due to lower resistance to the parasite, they develop infections and subsequently the competitive interaction becomes more symmetrical (Park 1948; Hatcher et al. 2006). Apparent competition occurs when two host species that do not normally compete are infected by the same parasite species that creates a link between them and creates an indirect competitive interaction (Hatcher et al. 2006; 2012). This competition is generally driven by one host species being more resistant to the parasite and acting as a reservoir that feedbacks greater parasite pressure on to the other host species (Hatcher et al. 2006).

Whilst parasites are, by definition, only a negative presence for their hosts, it has been highlighted in many recent reviews that they are actually essential components of ecosystems and can be beneficial in a wider context (Hudson et al. 2006; Hatcher and Dunn 2011; Hatcher et al. 2012). A key component of the positive influence parasites can have on non-host species is through parasite mediated coexistence. This is where infection with a parasite supresses the interspecific competitive ability of its host

sufficiently to enable the host species to coexist with an otherwise inferior competitor species. The effect can even be as extreme as to allow a species to colonise an area when it would otherwise be competitively excluded by the host species. For example, two Caribbean *Alonis* lizard species are only able to coexist when the malarial parasite *Plasmodium azurophilum* reduces the competitive ability of *Alonis gingivinius* (Schall 1992). Parasites can futher influence the coexistence of hosts by mediating intraguild predation (MacNeil et al. 2003; MacNeil and Dick 2011). MacNeil et al. (2003) showed that infection with an acanthocephalan parasite (*Echinorhynchus truttae*) limted the predatory impact of the non-native *G. pulex* on native *Gammarus duebeni*, reducing the impact of the invader and facilitating the coexistence of the two species. In this way, parasite mediated coexistence can be important in maintaining species richness and patterns of biodiversity.

1.2.5 Trophic niche specialisation

Understanding how parasite infections can have consequences beyond the individual hosts can utilise evaluations that assess intra-specific trophic niche sizes and specialization (Britton and Andreou 2016). The trophic niche describes the overall dietary choices of a given population. A larger trophic niche will reflect increased diversity in diet composition; as the trophic niche declines in size it reflects a more restricted diet, i.e. specialisation. It is well known that inter-individual variation in trophic niche can occur within a population due to sex and age class (Shine 1989). However, it is increasingly recognised that more general variability in intra-specific trophic niches occurs, with specialization in the diet of some individuals then resulting in an increased population trophic niche size (Bolnick et al. 2003; Evangelista et al. 2014).

The population trophic niche can thus be thought of as comprising of several sub-sets of smaller trophic niches, in which each sub-set is comprised of individuals exhibiting some differences in their traits that affect their foraging (Araujo et al. 2011). The ecological drivers of these population niche sub-sets cover a range of individual traits and ecological factors (Bolnick et al. 2003), with studies suggesting three major drivers: competitive interactions, predation and ecological opportunity (Araujo et al. 2011). For example, should intraspecific competition increase across the population then some increased intraspecific specialisation might be expected as individuals increasingly compete for the same resource (Araujo et al. 2011). Conversely, elevated interspecific competition can decrease intraspecific specialisation as, due to high exploitation, resources become limiting and individuals expand their diet (i.e. become more general) in order to maintain their energetic requirements (Constantini et al. 2005). This reduction in intraspecific specialisation is also apparent when individuals are under predator pressure (Eklov and Svanback 2006). Both this effect of predation and the respective effects of intra- and inter-specific competition are ultimately a result of a change in ecological opportunity, which effectively describes the potential niche available to an individual (Araujo et al. 2011).

Whilst competitive interactions, predation and ecological opportunity have been recognised as the drivers of trophic niche specialization (Araujo et al. 2011; Evangelista et al. 2014), what is less apparent is how infection by a parasite that modifies host behaviour (e.g. habitat utilisation, foraging rate, time budgets) influences the population trophic niche (Britton and Andreou 2016). Thus, the theory of intra-specific trophic niches provides a framework that can be used to test how infection by a parasite

influences the trophic niche of the host population and any shifts that can then have cascading consequences for inter-specific trophic and competitive relationships. It should also identify whether infection leads to trophic niche constriction**,** where the diet of hosts become more specialised and thus their population trophic niche size remains largely unaltered. Conversely, the infected specialist individuals might be exploiting alternative resources due to either parasite manipulation or modification, resulting in trophic niche divergence that increases the population niche size (Britton and Andreou 2016). Niche divergence has been reported in *C. carpio* populations infected with *B. acheilognathi* (Britton et al. 2011; Pegg et al. 2015), resulting from the mechanisms outlined in Section 1.2.3.

1.2.6 Consequences of climate warming on host: parasite relationships

Current predictions suggest that anthropogenic driven climate change will result in some substantial shifts in both temperature and precipitation patterns, with the extent of the shift dependent upon current and future emission levels, and the region concerned (Trenberth 2011; Anderson and Bows 2011; Chen et al. 2011; Kaufmann et al. 2011). In Great Britain, increases in air temperatures of 1.5 to 2 ^{o}C in the next 50 years are now considered inevitable, i.e. they are likely to occur even if emissions decrease (Murphy et al. 2009). Freshwater parasites and pathogens are strongly linked with climate and, consequently, warming has already significantly impacted disease spread, prevalence and severity across a number of systems in the last 20 years (Altizer et al. 2013).

In addition to the more typical consequences that climate warming may have on parasites, for example facilitating range expansion or accelerating their growth rates, it is likely that there will be many subtle changes in the often highly complex

relationships that exist between host and parasite (Brooks and Hoberg 2007; Macnab and Barber 2011). Such subtle consequences might still have the potential to significantly impact the host species directly and may also result in farther reaching consequences for the wider ecosystem. For example, Macnab and Barber (2011) found that not only did the parasite *S. solidus* grow faster at elevated temperatures but also that it was able to manipulate its stickleback host to select warmer habitats, potentially causing their movements to areas where they might develop novel trophic links (Section 1.2.4). Warmer temperatures also allow some parasitic organisms to complete their life cycles more rapidly and thus attain higher population densities (Marcogliese 2001), with Scott and Nokes (1984) revealing that the highest reproduction rate for *Gyrodactylus bullatarudis* occurred at higher temperatures until their thermal maxima was reached (30 ^{o}C). Similarly, for introduced *Gyrodactylus salaris,* population growth rates and their intensity of infections on Atlantic salmon *Salmo salar* increased as temperatures increased (Jansen and Blake 1991). The virulence of a parasite can also be directly affected by temperature, with higher virulence of Whirling disease associated with temperature increases, which in turn is likely to magnify the impacts of this disease on salmonid fishes (Rahel and Olden 2008).

Due to the negative relationship between dissolved oxygen levels and temperature, warming will also result in decreased oxygen levels in aquatic systems, making aquatic animals particularly vulnerable to climate warming (Ficke et al. 2007). Furthermore, parasites that use ectothermic fish as hosts are not buffered by the thermoregulatory mechanisms that endothermic hosts possess and hence are likely to be more immediately susceptible to environment variation and warming (Macnab and Barber 2012). Due to these factors, and the high global importance and value of healthy capture

fisheries, the effects of aspects of climate change on fish host-parasite relationships could be of high ecological and economic importance.

1.3 Indigenous versus non-indigenous parasites

1.3.1 Non-native versus non-indigenous parasites

In this research, both non-native/ native and indigenous/ non-indigenous parasites are used and referred to in relation to Great Britain. To clarify, when non-native is referred to, it means the parasite or fish is not naturally found anywhere in Great Britain (the converse is true for native, i.e. the species is naturally found in at least some British freshwaters). When the term 'indigenous' is used, it refers to the part of Great Britain where the fish or parasite is naturally found. Where non-indigenous is used, it means the indigenous parasite or fish is not naturally found in that part of Great Britain. For example, *B. acheilognathi* originates from Southeast Asia and so is non-native to Great Britain (Britton et al. 2011), whereas *Pomphorhynchus laevis* is an indigenous parasite to some rivers in Great Britain, specifically rivers flowing eastwards that were formerly linked to the Rhine and Danube river deltas, enabling colonisation at the end of the last glacial period (Kennedy et al. 1996). This parasite is, however, not found naturally in western rivers in Great Britain, such as the River Severn, but it has been translocated via movements of fish, especially European barbel *Barbus barbus* (Kennedy et al. 1996), and thus here it is non-indigenous. Irrespective of whether the parasite is non-native and has been introduced, or is non-indigenous and has been translocated, the mechanisms and processes involved in their survival, establishment and transmission to new host species are likely to be very similar, and thus are treated as such here.

1.3.2 Introduced and translocated parasites

A key feature of the Anthropocene is the translocation and introduction of species outside their native range as a direct result of human activity (Ricciardi 2007). Where these species become established and have a negative effect on one or more species they are considered invasive. The impact of these species, defined by Ricciardi et al. (2013) as "a measurable change in the state of an invaded ecosystem that can be attributed to the alien species" can vary greatly depending on a number of factors. One key factor is the presence of archetypes within the invaded ecosystem (Ricciardi et al. 2013; Kumschick et al; 2015). There are many examples where apex predators have been introduced to islands that had no experience with such species prior to their introduction and the impacts of the alien spcies can be great and alter the entire ecosystem (Wilson et al. 1998; Croll et al. 2005; Dunlop et al. 2015). This is of particular importance when considering impacts of invasive parasite species as a lack of evolutionary history may make hosts particularly vulnerable to heavy infections and severe pathology leading to death and in extreme cases host population crashes (Hansen et al. 2016)

Whilst it might be considered that when a free-living species is introduced into a new range then its parasite fauna will also be introduced, very often the process of introduction filters out many of the parasites that would otherwise have been introduced (Blakeslee 2012), with estimates of approximately only two new parasite species being introduced for every introduced free-living species (Torchin et al. 2003). The hypothesis involved in this is termed 'Enemy release' and it forms an integral component of any study that considers how non-native parasites might influence native species, food webs and ecosystems. It predicts that the parasite loss experienced by introduced species enhances their ability to establish and invade (Keane and Crawley. 2002; Mitchell and

Power 2003; Hatcher and Dunn 2011). Indeed, Torchin and Mitchel (2004) suggested that introduced species escape at least 75 % of their parasites from their native range and thus will gain substantial benefits regarding their fitness and survival in the invasive range (Torchin et al. 2003). The enemy release hypotheses (ERH) has been used as the basis to explain the invasion success of a diverse range of species, including non-native slugs (Ross et al. 2010), mosquitoes (Aliabadi and Juliano 2002) and frogs (Marr et al. 2008).

When a non-native parasite does get introduced into a new ecosystem then as well as infecting their non-native host species, they have the potential to 'spillover' into the native community (*cf.* Chapter 5). If a non-native parasite is able to infect native hosts in this manner, then the consequences can be severe due to the lack of coevolution between the parasite and host (Taraschewski 2006; Blanchet et al. 2010). In general, there are strong examples of how non-native fish pathogens have resulted in severe outcomes, including the non-native monogenean (*Nitzschia sturionis*) introduced to the Aral Sea by the Caspian Sea sturgeon (*Huso huso*) that has resulted in substantial declines in populations of native Aral Sea sturgeon (*Acipenser nudiventris*) (Dogiel et al. 1958). Introductions of non-native monogenean parasites can result in severe outcomes when they spillover in to native fishes (Bauer et al. 2002; Bakke et al. 2007), for example, *G. salaris* caused very high mortalities in Atlantic salmon in over 40 Norwegian rivers and resulted in some severe management practises to control the outbreak (Johnsen and Jensen 1986).

In addition to the non-native free-living species introducing at least some components of their parasite fauna, it can also be the case that native parasites can infect non-native

species. Depending on the severity of infection, this could provide some 'biotic resistance' to the species, inhibiting their ability to survive and establish in their new environment (Mitchell and Power 2003; de Rivera et al. 2005).

1.3.3 Potential trophic consequences of non-native parasites

Whilst only a small number of non-native parasites might get introduced with their free-living hosts, these parasites might be transmitted to native hosts (parasite spill-over) (Section 1.3.2). The non-native fish might then act as a reservoir of native parasites and cause subsequent disease outbreaks in the native hosts (parasite spillback). Transmission of non-native parasites to naïve hosts (including the same species as the introduced host but an inexperienced population that has yet to encounter the parasite) can then have substantial consequences at the individual level (e.g. *G. salaris* in *S. salar*) (Jansen and Blake 1991). What is less known, certainly compared with native parasites, is how these host consequences of infection by non-native parasites translate into population, community and food web consequences (Britton 2013).

1.3.4 Ecological consequences of native parasites

Parasites can have substantial biological and ecological consequences for individual hosts and their populations, irrespective of their origin (Section 1.2). In the last 10 years, there has been an increasing focus on how the incorporation of parasites in food webs affects their structures and properties (Lafferty et al. 2006). 'Infectious food webs' are those food webs in which parasites have been included and they are usually compared to their structure and metrics when parasites are omitted. These infectious food webs tend to have increased chain length, linkage density, nestedness and connectedness (Hudson et al. 2006; Lafferty et al. 2006; 2008). As a result, food webs

with parasites omitted tend to be incomplete and overly simplistic. Moreover, it is increasingly recognised that native parasites are integral to the structuring and functioning of ecosystems (Hudson et al. 2006, Lafferty et al. 2008).

Nevertheless, these studies have tended to focus primarily on food web topology, i.e. the qualitative overview of food web structure. What is less apparent is how parasites modify the quantitative food web. Given that native parasites can have substantial consequences for individual hosts that can be additive as levels of biological organisation scale up to population and community levels (Section 1.2) then there is potential for them to have considerable consequences for the quantitative food web. Indeed, the completion of complex parasite lifecycles, their mediation of population abundance, and alterations in the symmetry of competitive interactions, habitat utilisation and acquisition of food resources, can all have substantial consequences for the competitive relationships between species, the trophic niche size of the host population (Section 1.3) and thus food web structure overall. As these aspects might not be captured easily by topological analyses, then alternative approaches using more quantitative methods, such as stable isotope analysis, should be considered (Pegg et al. 2015; Britton and Andreou 2016; Section 1.4).

1.3.5 Pomphorhynchus laevis as a model parasite to test infection consequences between indigenous and non-indigenous ranges

It was mentioned in Section 1.3.1 that the freshwater parasite *P. laevis* has both an indigenous and non-indigenous range in Great Britain and so provides opportunities to test differences in infection consequences between these ranges. This parasite has a complex lifecycle, utilising *G. pulex* as an intermediate host before reaching its final

host that could be any one of several species of freshwater fish. It is thus considered a generalist parasite (Figure 1.1). Although *P. laevis* has received a great deal of research attention over the last 40 years, there still remains some conflicting findings with regard to the extent of its host manipulation in *G. pulex* (Poulton and Thompson 1987; Bakker et al. 1997; Bollache et al. 2002; Koldonski et al. 2009; Durieux et al. 2012; Perrot-Minnot et al. 2014; Chapter 3). Another area of research on this parasite lacking clarity is the extent to which it is able to utilize different fish species as final hosts. Whilst *B. barbus* and *Squalius cephalus* are known preferred final hosts of *P. laevis* (Hine and Kennedy 1974; Kennedy et al. 1978), adult parasites are also frequently found in smaller bodied, more abundant fish species with evidence of them being able to reproduce and complete their lifecycles in these hosts, as well as them providing a potential paratenic route of infection to a piscivorous host (Kennedy et al. 1978; Kennedy 1996; Medoc et al. 2011). Despite this, there remains limited knowledge on the importance and roles of these small-bodied fishes as hosts, with little consideration given to how this might influence population structuring, adaptive manipulation behaviours, and the overall ecological consequences of *P. laevis* for freshwater food webs.

1.4 Stable isotope analysis to study the ecological consequences of parasite infections

The analysis of ecological parameters that involve fish as secondary and tertiary consumers, such as metrics relating to food web structure and trophic niche size, has traditionally been completed through stomach content analysis. This method is, however, problematic for a number of reasons, including it being incapable of

determining the extent to which a fish is assimilating their energy from their putative food resources - either other fish or invertebrates (Paradis et al. 2008). It also often requires relatively large sample sizes collected over prolonged periods of time. Substantial increases in the understanding of trophic relationships between species and their putative food sources have been gained through stable isotope analyses (SIA) (Vander Zanden et al. 1999; Grey 2006). As the ratios of the stable isotopes of carbon (^{13}C: ^{12}C) and nitrogen (^{15}N: ^{14}N) vary predictably from resource to consumer (Fry 2006), they enable reconstruction of the trophic structure and the analysis of the trophic niche sizes and the overall food web structure (Grey 2006).

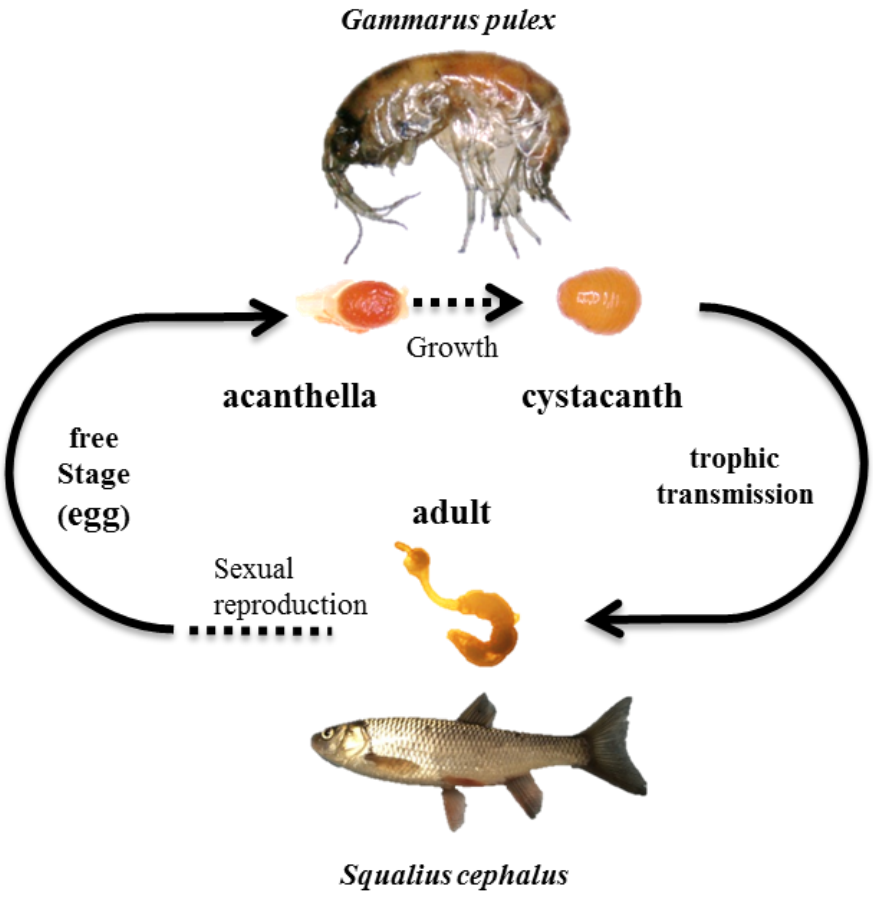

Figure 1.1. Lifecycle of *Pomphorhynchus laevis*. Free living parasite eggs hatch in the water and are consumed by the intermediate host *Gammarus pulex* where the acanthella

grows into an infective cystacanth. When an infected *G. pulex* is consumed by a suitable fish host the cystacanth is activated by the digestive enzymes in the gut of the fish and penetrates the gut wall with its characteristic spiny proboscis attaching itself irreversibly to the gut. Here it matures into an adult worm and reproduces, releasing eggs into the host which are excreted with the host's faeces into the water, thus completing the lifecycle.

The carbon values ($\delta^{13}C$) of a consumer indicate their energy source as animals that feed on the same food source display an isotope composition similar to each other and to the food they assimilate for growth (DeNiro and Epstein 1978; Fry and Sherr 1984). The stable nitrogen isotope ($\delta^{15}N$) typically becomes enriched by 3 to 4 % between prey and predator tissue and so is an indicator of consumer trophic position (Deniro and Epstein 1981; Minagawa and Wada 1984; Figure 1.2). This application of stable isotope techniques, using the predictable relationship between the isotopic composition of consumers and their diet, is sufficiently powerful to detect long-term (e.g. 3 to 6 months) dietary differences between individuals of the same population (Gu et al. 1997; Beaudoin et al. 1999; Fry 2006), such as those that are parasitized with a specific parasite and those that are uninfected (Britton et al. 2012).

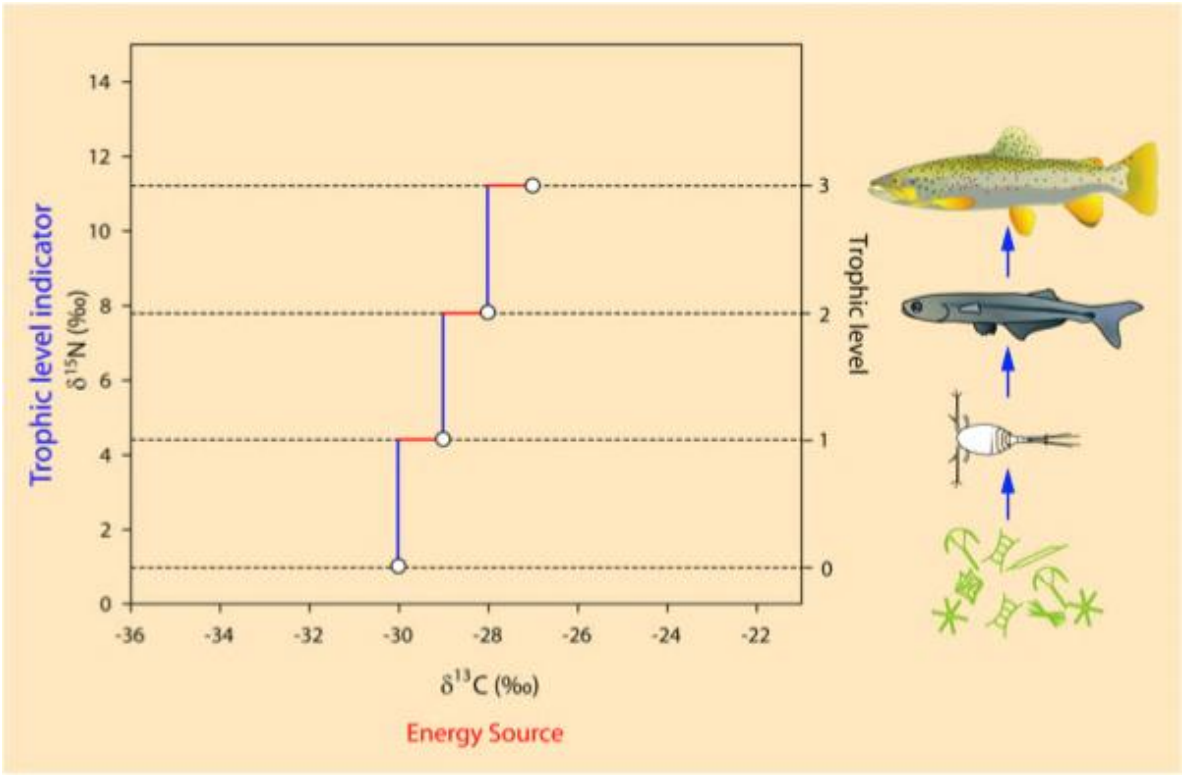

Figure 1.2. Representation of a stable isotope bi-plot showing the increase in trophic level with increases of approximately $3^{0}/_{00}$ in $\delta^{15}N$ and $1^{0}/_{00}$ in $\delta^{13}C$. (Source- Chris Harrod).

1.5 Research aim, objectives and thesis structure

It has been discussed throughout this Chapter that parasites can have considerable consequences for their freshwater fish hosts, whether they are intermediate or final hosts. Whilst there is a great deal of extant knowledge on some of these consequences, there also remains some considerable knowledge gaps, especially in relation to the response of potential fish hosts to intermediate hosts subjected to parasite manipulation and the consequences of infections of the generalist parasite *P. laevis* for small bodied fish final hosts. There are also knowledge gaps on the extent of 'enemy release' for non-native fish in Great Britain, and the quantitative consequences of infection by native

parasites for the trophic ecology of native fishes. Thus, the aim of this research was to assess aspects of the ecological consequences of infection by introduced and native fish parasites for their intermediate and final hosts, using Great Britain as the model region. The research objectives, data chapters and their rationale were:

Objective 1.

Identify the consequences for parasite virulence and host susceptibility to elevated water temperatures expected under climate change projections.

This was completed in Chapter 2 ('*Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species*'). The rationale of the chapter was two-fold. Firstly, using *P. laevis* as the model parasite, it enabled testing of the effects of different levels of parasite exposure levels and water temperatures on the infection levels of a potential host. Secondly, it served as a pilot study to identify how controlled *P. laevis* infections could be produced in laboratory conditions for completing Objective 2.

Objective 2.

Assess fish foraging responses to intermediate gammarid hosts to test hypotheses on parasite manipulation.

This was completed in Chapter 3 ('*Comparative functional responses of fishes reveal differences in the consumption rates of prey populations infected with an acanthocephalan parasite*'). The work tested whether infected and uninfected fish final host species consumed higher numbers and proportions of prey populations when they

were infected with *P. laevis* and determined the underlying mechanisms involved in driving the behaviour of the intermediate and final hosts.

Objective 3.

Quantify the infections of *P. laevis* in wild fish communities across their indigenous and non-indigenous range through investigation of infection consequences in small-bodied fishes.

This was completed in Chapter 4 ('*Infections of Pomphorhynchus laevis in fish final hosts in their indigenous and non-indigenous ranges: prevalences, pathology and trophic consequences*'). The chapter studied five fish communities where *P. laevis* is present, three in the indigenous range and two in the non-indigenous range, and focused primarily on bullhead *Cottus gobio*, minnow *Phoxinus phoxinus* and stone loach *Barbatula barbatula*, as despite their high numerical presence in many rivers where *P. laevis* is present, their potential roles in the lifecycle of the parasite are often overlooked in favour of working on species such as brown trout *Salmo trutta*.

Objective 4.

Evaluate the parasite fauna associated with introduced non-native fishes in Great Britain to determine the extent of enemy release and parasite acquisition in these fishes.

This was completed in Chapter 5 ('*Parasites of non-native freshwater fishes introduced into England and Wales suggest enemy release and parasite acquisition*'**)**. The work was constrained to England and Wales rather than Great Britain, as data were unavailable from Scotland. The research investigated the parasite fauna of a range of

non-native fish to determine whether enemy release was evident (Section 1.3.2) and the extent to which these introduced fish had then acquired native parasites, and the potential consequences of this.

Objective 5.

Assess the infection and trophic consequences of native fish parasites in native fishes to identify whether there is evidence that parasite infections can drive trophic niche specialisation in host populations.

This was completed in Chapter 6 ('*Trophic consequences of infection by native parasites for native fishes: evidence of niche specialisation driven by parasitism?*'). The building argument in this work was that it might be the complexity of the parasite lifecycle that plays the key role in determining the extent of the consequences for the host, rather than the origin of the host (i.e. sub-lethal consequences might be largely independent of whether the parasite is native/ non-native, or indigenous/ non-indigenous). Thus here some sublethal consequences of infection by three native fish parasites were assessed for some native fish hosts. The focus was on trophic niche specialisation (Section 1.2.5).

Chapter 7 was the final chapter ('*Discussion*') and provided an overview and evaluation of the results of each chapter to bring together the main outputs in the context of the wider literature base.

Chapter 2

Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species

This chapter has been published as the following:

Sheath, D.J., Andreou D. and Britton J.R. (2016) Interactions of warming and exposure affect susceptibility to parasite infection in a temperate fish species. *Parasitology* 1-7

2.1 Summary

Predicting how elevated temperatures from climate change will alter host-parasite interactions requires understandings of how warming affects host susceptibility and parasite virulence. Here, the effect of elevated water temperature and parasite exposure level was tested on parasite prevalence, abundance and burden, and on fish growth, using *P. laevis* and its fish host *S. cephalus*. At 60 days post-exposure, prevalence was higher at the elevated temperature (22 ^{o}C) than ambient temperature (18 ^{o}C), with infections achieved at considerably lower levels of exposure. Whilst parasite number was significantly higher in infected fish at 22 ^{o}C, both mean parasite weight and parasite burden was significantly higher at 18 ^{o}C. There were, however, no significant relationships between fish growth rate and temperature, parasite exposure, and the infection parameters. These results reveal that whilst elevated temperature significantly influenced parasite infection rates, it also impacted parasite development rates, suggesting warming could have complex implications for parasite dynamics and host resistance.

2.2 Introduction

Climate change is predicted to alter host-parasite relationships during this century, especially where warming combines with other anthropogenic disturbances (Rohr et al. 2011; Paull et al. 2012; Lõhmus and Björklund 2015). In northern latitudes, where climatic factors are important regulators of host-parasite population dynamics and parasite occurrence, and transmission is regulated by seasonal temperature changes, shortened winter periods could alter host-parasite relationships via alterations in host susceptibility and parasite virulence (Hakalahti et al. 2006; Lõhmus and Björklund 2015). Should growth rates of the hosts and parasites be altered by temperature changes then pathology and transmission rates could also be affected (Raffel et al. 2006; Lafferty 2009). Consequently, predictions tend to be for warming to increase the prevalence of parasites at higher latitudes (e.g. Harvell et al. 2002; Marcogliese 2001; 2008), although there is limited empirical evidence to support this at present (Ward and Lafferty 2004; Bentley and Burgner 2011; Lõhmus and Björklund 2015).

An understanding of how host-parasite interactions will shift under the effects of warming, and the consequences for host populations and their communities, is thus an important aspect of environmental management (Lafferty 2009; Macnab and Barber 2012). Integral to this is developing understanding of how elevated temperatures affect host susceptibility to infection versus their effects on parasite virulence and life cycle completion rates (Harvell et al. 2002; Altizer et al. 2013). The susceptibility of hosts to infection could increase through, for example, thermal stress that leads to reduced immune-competency (Weyts et al. 1999; Nikokelainen et al. 2004) and enhanced consumption rates of prey that leads to increased parasite exposure via intermediate

hosts (Toscano et al. 2014). Parasite fitness and transmission rates could be enhanced by warming through positive effects on their metabolism, resulting in higher numbers of transmission stages being produced, with their rate of development and growth within hosts also accelerated (Paull and Johnson 2011; Callaway et al. 2012). However, should warming result in the temperature optimum for the parasite being exceeded, then their decreased prevalence in host populations might result, with suggestions that increased parasite prevalence due to warming will only occur for a proportion of fish pathogens (Karvonen et al. 2010). Consequently, there is an outstanding requirement to derive enhanced understanding of how warming will affect host-parasite dynamics, particularly the decoupling of the underlying mechanisms involved, i.e. the effects of warming on host susceptibility versus on parasite transmission and virulence.

The aim of this Chapter was thus to test how elevated temperature affected host susceptibility to infection under different parasite exposure levels, and how this affected parasite prevalence and intensity. The objectives were to quantify how temperature and parasite challenges affected: (i) infection outcomes (as parasite prevalence), (ii) host infection parameters (as parasite abundance, mean individual weight and burden); and (iii) host somatic growth rates. Outcomes were assessed in relation to the effects of temperature elevation on the host-parasite relationship and the potential mechanisms involved. The model parasite was *P. laevis*, an acanthocephalan with a complex lifecycle whose final hosts are a wide range of fishes (Neveda et al. 2003). The model final host was *S. cephalus*, a preferred freshwater fish host of *P. laevis* (Hine and Kennedy 1974). This parasite uses the freshwater shrimp *G. pulex* as its intermediate host. It is a conspicuous orange-yellow parasite that is visible through the transparent cuticle of *G. pulex* (Bakker et al. 1997) enabling external examination of individual live

G. pulex for identification of their parasite status (infected/ uninfected), the number of parasites it is infected by and the infectious status of those parasites (Section 1.3.5; Figure 1.1). Transmission to fish hosts is via consumption of infected *G. pulex*, with some evidence that the parasite manipulates the behaviour of this intermediate host to increase their probability of being preyed upon and so assisting their transmission to the final host (e.g. Franceschi et al. 2008; Dianne et al. 2011; Labaude et al. 2015).

2.3 Materials and methods

2.3.1 Experimental design and pre-experiment data collection

The *S. cephalus* used in the experiment were all between 69 and 89 mm starting length (mean 80.8 ± 0.8 mm) and age 1+ years. They were sourced from an aquaculture site in Southern England in August 2014. Although they had not been exposed to the parasite during their lifetime, they were produced from broodstock that had originally been collected from a river where *P. laevis* was present naturally (River Kennet, Berkshire, England). On the aquaculture site, the fish were reared in outdoor ponds (approximate water temperatures at the time of collection: 15 to 19 ^{o}C), with some supplementary feeding with pelletized fishmeal. On arrival to the laboratory, the fish were tagged with passive integrated transponder tags (PIT tags) so that individual fish could be tracked through the experiment. Concomitantly, they were measured (fork length, nearest mm) and weighed (*W*, nearest 0.1 g). They were then allowed to recover and acclimate to laboratory conditions by being held in tanks held at 18 ^{o}C for 14 days on a 16:8 hour light: dark cycle. In addition, a sub-sample of 5 fish were removed from the sample on arrival to the laboratory. These were euthanized and dissected to check for the presence of *P. laevis*. None of these fish were infected. Infections of other parasites were very

light and considered part of the natural parasite fauna of the fish in Southern England. Recorded levels were not considered high enough to cause clinical pathology (Hoole et al. 2001).

2.3.2 Parasite exposure

The *S. cephalus* were challenged by *P. laevis* by exposing individuals to known numbers of infected *G. pulex*. These were collected from a local river, the Hampshire Avon (latitude: 50.8865, longitude: -1.7883), when water temperatures were approximately 18 ^{o}C. They were then held in laboratory conditions at 18 ^{o}C for 96 hours, with infectious individuals then identified visually (Bakker et al. 1997; Bauer and Rigaud 2015), with a subset confirmed by dissection (N = 30, all infected). As multiple infections were identifiable in the *G. pulex* (Bakker et al. 1997), then individuals were only used here that were host to one parasite. Exposure of the fish to the parasite was done individually, with the fish transferred to 10 L tanks containing dechlorinated water with supplementary oxygenation provided via an air stone and pump, and at a water temperature of 18 ^{o}C. Prior to parasite exposure, the fish were held in the tanks for 24 hours with no feeding to ensure standardised levels of hunger.

Each individual fish was then exposed to a specific number of infected *G. pulex* from the following options: 0 (as a control), 5, 10, 20, 40 and 60. There were 10 fish used at each level of exposure. After 24 hours, the fish were removed from the tanks, with confirmation that all the *G. pulex* had been consumed. For each exposure level, the fish were then split randomly into 2 groups of 5 and transferred into 45 L tank aquaria at either 18 or 22 ^{o}C. These tank aquaria were arranged on a flow-through system using recirculated water (originally dechlorinated tap water), with a different system used for

each temperature. Across the two flow-through systems used, the tanks were identical in dimensions, the water was taken from the same original source, and the tanks contained identical environmental enrichment for the fish in terms of refugia (lengths of plastic pipe of 65 mm diameter) and cover (artificial macrophytes).

2.3.3 Post-experiment data collection and analysis

Following their exposure to *P. laevis*, the fish were held in their tanks for 60 days under a 16:8 hour light: dark regime, with feeding daily using crushed pelletized fishmeal (approximately 2 % mean starting body mass/ day). At the end of this period, the fish were removed from their tanks, euthanized, identified by scanning their PIT tag, re-measured and weighed. They were then dissected, with intestinal examinations to identify individuals in which infections by *P. laevis* had developed. For infected fish, parasites were removed, counted and weighed (mg).

These data enabled parasite prevalence to be assessed as the proportion of infected fish per temperature/ exposure treatment. The effects of temperature (T) and parasite exposure (PE; as the number of consumed intermediate hosts) on parasite prevalence were then tested using a probability of infection (PoI) model using binary logistic regression in: $\text{PoI} = e^{(a+b\text{T}+c\text{PE})} / 1+ e^{(a+b\text{T}+c\text{PE})}$, where a, b and c were binary logistic regression coefficients (Equation 2.1). This also provided the significance of both variables on parasite prevalence. As the tank conditions were identical across the individual fish, with only water temperature and levels of exposure to the parasite via intermediate hosts being different, then the model did not take account of the fish being within different tanks per temperature treatment; i.e. the individual fish were being treated as the replicate unit in the model.

The following infection parameters were then calculated from the data of the infected fish. Parasite abundance was determined as the total number of parasites per host and the total mass of parasites per host, and enabled calculation of the mean parasite weight per host. Parasite burden was calculated as the proportion of the body weight of each host comprising *P. laevis* (Pegg et al. 2015). Differences in these infection parameters, plus parasite prevalence, between temperatures were tested using generalized linear models (GLM), with parasite exposure level as the covariate. In all models, data on uninfected fish were not included as their inclusion in the models would introduce a bias in outputs, given the higher numbers of uninfected fish at the lower temperature/ levels of parasite exposure. For parasite number, a Poisson log-linear model was used as the data represented parasite counts. As with the binary logistic regression model, the data for individual fish in these models were used as the replicate units due to the identical conditions the fish were in, i.e. this was not considered as artificially inflating the number of degrees of freedom in the models that would otherwise result in pseudo-replication. The reported model outputs then included the mean value of the infection parameters per temperature treatment (as estimated marginal means, with the effects of parasite exposure as the covariate controlled in the model) and their standard error. To identify if differences between these mean values were significant, linearly independent pairwise comparisons were used with Bonferroni adjustment for multiple comparisons. Differences in infection parameters were then tested between the parasite exposure levels using the same process, except temperature was used as the covariate in these models.

Finally, to determine if infection influenced the growth rate of the fish, specific growth rate (*SGR*) was calculated as the change in body mass of the fish over the experimental

period, from $[\ln W_{t+1} - \ln W_t] / t] \times 100$, where W_t = starting weight, W_{t+1}= finishing weight, and t = number of days between W_t and W_{t+1}. Differences in specific growth rates of fish between temperatures and parasite exposure levels were then tested using GLMs as described above, with multiple linear regression analysis then used to test the influence of the infection parameters, temperature and parasite exposure on SGR. This provided the significance of the predictor variables and their standardized beta coefficients (β). Variables with the highest β value had the strongest singular contribution to the model.

2.4 Results

2.4.1 Probability of infection

At the conclusion of the 60 days after parasite exposure, there were considerable differences in infection levels apparent between temperatures and exposure levels (Figure 2.1). The logistic regression model revealed both temperature and exposure level had significant effects on parasite prevalence (Figure 2.1; Table 2.1). At 18 ^{o}C, infection required higher parasite exposure levels compared with 22 ^{o}C, with 50 % prevalence requiring exposure to 6 intermediate hosts at 22 ^{o}C, but 26 at 18 ^{o}C (Figure 2.1).

Table 2.1**.** Binary logistic regression coefficients (*cf.* Equation 2.1), and their statistical significance, for the probability of infection of *Squalius cephalus* by *Pomphorhynchus laevis* according to temperature and parasite exposure.

Parameter	Symbol in Equation 2.1	Coefficient	Standard error	P
Constant	a	-18.97	6.21	0.02
Temperature	b	0.82	0.28	<0.01
Parasite exposure	c	0.16	0.05	<0.01

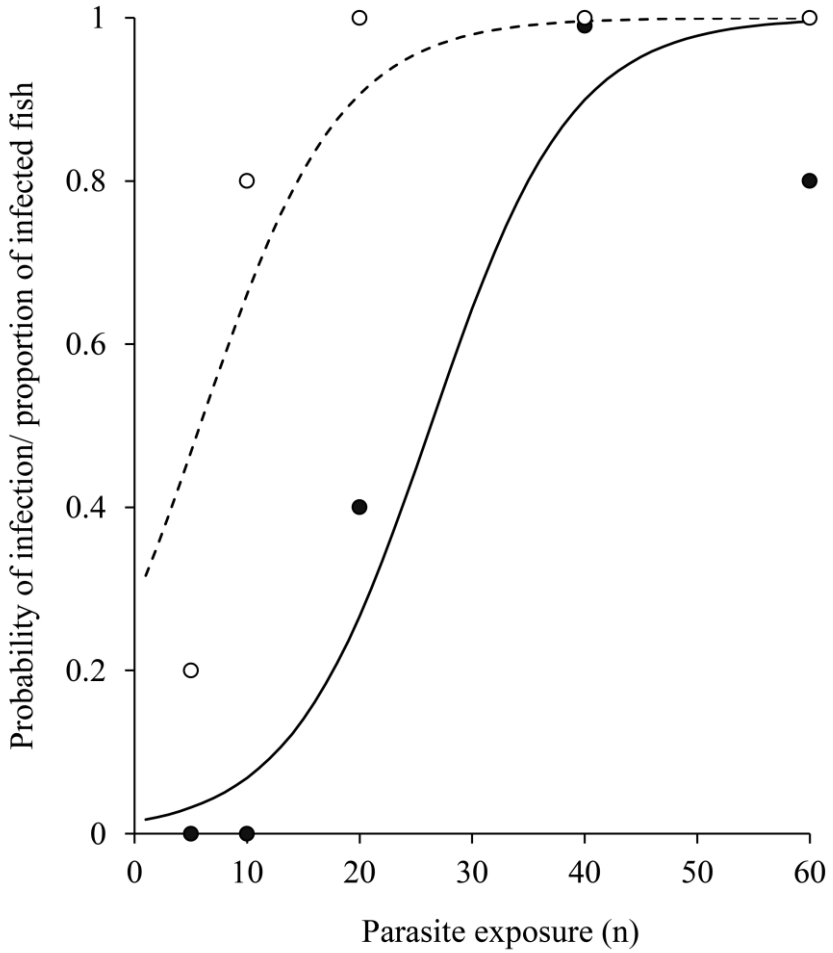

Figure 2.1. Relationship between parasite exposure and (i) proportion (0-1) of infected fish at 18 ^{o}C (filled circles) and 22 ^{o}C (open circles), and (ii) probability of infection (0 to 1 scale) according to binary logistic regression (*cf.* Table 2.1; Equation 2.1) at 18 ^{o}C (solid line) and 22 ^{o}C (dashed line).

2.4.2 Infection parameters

The GLM testing the effect of temperature on the parasite abundance in the infected fish revealed that there were significant differences in the mean numbers of parasites between the two treatments (Wald $\lambda^2 = 4.23$, $P = 0.04$), with mean parasite number significantly higher at 22 ^{o}C than 18 ^{o}C ($P < 0.01$; Figure 2.2a). The effect of exposure on parasite abundance also revealed significant differences in mean number (Wald $\lambda^2 = 20.46$, $P < 0.01$), with significantly higher numbers of parasites per infected fish at exposure to 40 intermediate hosts (mean number: 7.80 ± 0.98) than at all than other exposure levels (mean numbers: 2.42 to 3.46; $P < 0.01$ in all cases; Figure 2.2b). In both GLMs, the effect of the covariate was also significant ($P < 0.05$).

Temperature was not a significant predictor of parasite abundance when it was measured as the total parasite mass in the infected fish (Wald $\lambda^2 = 0.01$, $P = 0.92$; Figure 2.2c), but parasite exposure was (Wald $\lambda^2 = 13.10$, $P = 0.01$). Mean total parasite mass was higher at 40 intermediate hosts (mean parasite mass: 24.23 ± 3.06 mg) than all other exposure levels (mean parasite mass range: 9.02 to 17.12 mg), although the differences were only significant between 40 and 60 hosts (difference 15.20 ± 4.35 mg; $P < 0.01$) (Figure 2.2d).

The mean weight of individual parasites in the infected fish was significantly influenced by temperature (Wald $\lambda^2 = 9.48$, $P < 0.01$), being higher at 18 than 22 ^{o}C ($P < 0.01$; Figure 2.2e). The effect of parasite exposure on the mean weight of individual parasites was also significant (Wald $\lambda^2 = 13.29$, $P < 0.01$), with higher means at lower exposure levels (Figure 2.2f). The effect of temperature on parasite burden was significant (Wald

$\lambda^2 = 15.37$, $P < 0.01$), with significantly higher burdens at 18 (0.23 ± 0.03 %) than 22 ^{o}C (0.06 ± 0.03 %) ($P < 0.01$). The effect of exposure on parasite burden was, however, not significant (Wald $\lambda^2 = 7.63$, $P = 0.11$).

2.4.3 Fish growth

Mean fish weight at the start of the experiment was 5.20 ± 0.16 g and at the end was 7.89 ± 0.31 g. The effect of temperature and parasite exposure on fish growth (as SGR) was not significant in either GLM (Wald $\lambda^2 = 0.01$, $P = 0.91$; Wald $\lambda^2 = 5.01$, $P = 0.28$ respectively). Multiple regression revealed the effects on SGR of all infection parameters, exposure and temperature were not significant ($R^2 = 0.11$; $F_{5,23} = 0.77$, $P = 0.56$), with no significant predictors (all $P > 0.05$).

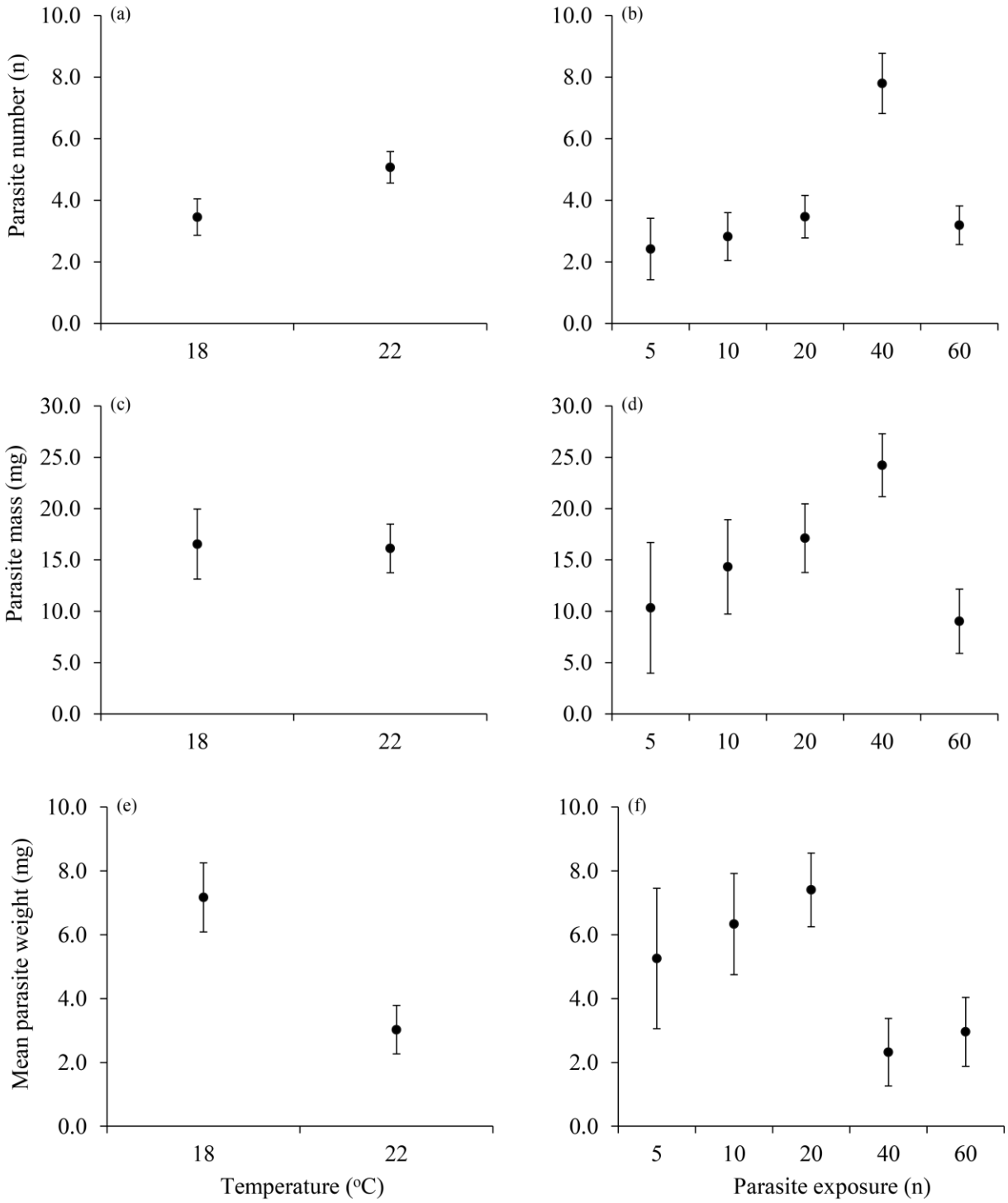

Figure 2.2. Mean adjusted parasite number and mass, and mean parasite weight per fish (from generalised linear models) according to temperature (a, c, e), where parasite exposure was the model covariate, and parasite exposure (b, d, f), where temperature was the covariate. Error bars represent standard error.

2.5 Discussion

Elevated water temperature had a significant and positive effect on parasite prevalence, with parasite infections developing from exposure to lower numbers of intermediate hosts in the warmer water. Despite these clear differences in prevalences, the effects of temperature and parasite exposure on the infection parameters of the individual hosts were relatively complex. Although elevated temperature resulted in increased parasite number in hosts, this involved a trade-off with their mass, with significantly smaller parasites present in hosts held at higher temperatures and resulting in significantly lower parasite burdens. These outputs on the infection parameters are a contrast to Macnab and Barber (2012), who revealed that elevated temperature increased the growth rates of the parasite *S. solidus* (Müller) in three-spined stickleback *G. aculeatus* Linnaeus.

A major challenge in understanding how warming will affect host-parasite interactions is decoupling the individual effects of warming on the susceptibility of hosts to infection from the effects on parasite virulence. Here, the collection and holding of the parasite intermediate hosts, and the holding of the fish and their exposure to the parasite, was all completed at 18 ^{o}C, an ambient temperature representative of temperate freshwaters in the late summer period (Britton 2007). The exposed fish were then held at either this ambient temperature or an elevated temperature (+4 ^{o}C) for the experimental period. With the initial parasite exposure all being completed at ambient temperature, it is suggested that the effect of the sudden temperature elevation in the treatment altered the susceptibility of the fish hosts to infection (Hakalahti et al. 2006), rather than it affecting the parasite virulence (Lõhmus and Björklund 2015). The sudden increase in temperature for this fish meant it was not possible to decouple the effect of

temperature on susceptibility *per se* from the specific effect of the rapid temperature increase. Nevertheless, that the net effect of the elevated temperature increased host susceptibility to infection was supported by evidence from other studies that suggest it often results in substantial negative consequences for fish immuno-competence (Dittmar et al. 2014), as it potentially shifts energy allocation from immunological processes (Poisot et al. 2009) and/ or acts as an additional stressor that compromises the immune response (Cramp et al. 2014).

The complex effects of both temperature and parasite exposure on the infection parameters within the hosts were related to either temperature impacting the development rate of parasites or the increased parasite number in hosts at elevated temperatures resulting in marked density-dependent effects, resulting in relatively high densities of parasites with relatively small body sizes (Luong et al. 2011). It is suggested that the latter explanation was more consistent with the outcomes of the experiment, given that these revealed fish exposed to high numbers of intermediate hosts at the ambient temperature resulted in low parasite numbers compared with the elevated temperature, but with these parasites being substantially larger, resulting in significantly higher parasite burdens.

Notwithstanding, as elevated temperatures can have both marked effects on the development rates of parasites in temperate regions (Tinsley et al. 2011) and on fish immune function, disease resistance and fitness (Cramp et al. 2014), then it remains difficult to definitively decouple the effects of warming on these aspects of the infection dynamics from these data. It is thus recommended that these outputs serve as an initial assessment of the effects of warming temperatures and parasite exposure levels on these

host-parasite dynamics, enabling the design of subsequent experiments of greater complexity that should enable, for example, greater assessment of how warming affects the development rate of the parasite within hosts, such as their maturity (e.g. Altizer et al. 2013), how temperature affects the immune response of hosts (e.g. Nikokelainen et al. 2004), and how parasite virulence is affected by the interactions of warming with other environmental variables, and the influence of this on selection (e.g. Wolinska and King 2009). Given the ease at which it was demonstrated that fish final hosts, such as *S. cephalus*, can be infected experimentally with known numbers of *P. laevis* via *G. pulex* intermediate hosts, then this host-parasite model would provide a strong model host-parasite system to answer these questions in both controlled and semi-controlled conditions. For example, to decouple the effects of host susceptibility from parasite virulence across different temperatures could utilise experiments where the fish and intermediate hosts are held at the different temperatures prior to exposure (unlike here, where they were all initially held at 18 ^{o}C) and then used in the experimental design used here. Parasites from these initial experiments could then be harvested and used to produce laboratory grown parasites in *G. pulex* that are raised across the different temperatures. Their subsequent exposure to the fish would then be completed in a fully-factorial experimental design that enables quantification of differences in virulence and hosts susceptibility across the different generations and rearing temperatures of both *G. pulex* and the host fish. However, another factor that must be considered here is the effect of different temperatures on the rates of intermediate host breakdown within the fish host's stomach. MacNeil et al. (2001) demonstrated that amphipods persist in the stromach of trout for twice as long as 9.5°C as they do at 19.5°C. Hence infection success of *P. laevis* may be linked to breakdown times of the intermediate host body and the subsequent release of the cystacanth within the final host.

Despite the strong effect of temperature on parasite prevalence and development, there were no measureable consequences for the hosts, with no differences in the specific growth rates of the fish between the controls, temperature and exposure treatments. Studies have suggested that *P. laevis* is a relatively benign parasite in temperate European fluvial fishes (Hine and Kennedy 1974)**,** with the effects of acanthocephalan parasites generally being more related to the consequences of their pathology rather than their loading (Latham and Poulin 2002). Thus, it is suggested that the effect of elevated temperature on this host-parasite system was primarily in relation to altering host susceptibility to infection, with this then influencing parasite development and dynamics via density-dependent mechanisms within hosts. Consequently, the importance of these findings are that they indicate that warming could result in substantial shifts in disease progression via altered host susceptibility, but potentially with concomitant changes in parasite infectivity and development.

Chapter 3

Comparative functional responses of fishes reveal differences in the consumption rates of prey populations infected with an acanthocephalan parasite

3.1 Summary

Trophically transmitted parasites with complex lifecycles can manipulate the behaviour of their intermediate hosts to increase their likelihood of transmission to the final host. The acanthocephalan parasite *P. laevis* is suggested as manipulating the behaviour of its intermediate host *G. pulex* by reducing their anti-predator responses. However, responses of fish to this change in behaviour have not been tested. Comparative functional responses were used here to test the hypothesis that fish foraging on infected *G. pulex* would have higher consumption rates and foraging parameters than when feeding on uninfected conspecifics. It was tested using three model fishes, the preferred final hosts *S. cephalus* and *B. barbus*, and the naïve *Carassius auratus*. Contrary to the hypothesis, the functional response models revealed all fishes had significantly higher consumption rates of uninfected than infected *G. pulex*, especially *S. cephalus* and *B. barbus*. For *C. auratus*, significant differences were only apparent at high prey densities. A foraging experiment exposing *S. cephalus* and *C. auratus* to 20 *G. pulex* of varying proportions of infected and uninfected individuals revealed their consumption of uninfected *G. pulex* increased with the proportion available. For infected *G. pulex*, this pattern was also apparent for *C. auratus*, but was not for *S. cephalus*. Behavioural trials then revealed that predator cues from *S. cephalus* significantly decreased the activity of infected *G. pulex*, whereas their activity levels in the presence of cues from *C. auratus* were higher than uninfected conspecifics but similar to dechlorinated tap water. These outputs suggest this is a complex host-parasite relationship where a range of intrinsic and extrinsic factors influences the behaviour of the intermediate host and the foraging responses of fish final hosts. Contrary to the parasite manipulation

hypothesis, potential final hosts might not always select and consume more infected intermediate hosts.

3.2 Introduction

Parasite infections have varying sub-lethal effects on host species, including altering the symmetry of competition between infected and uninfected individuals and modifying the host phenotype through differences in behaviour and habitat utilisation (Barber et al. 2000; Hatcher et al. 2006; 2012). These consequences can affect their interactions with prey populations, and non-host conspecifics and populations (Hatcher et al. 2012; Pegg et al. 2015). Thus, understanding the consequences of parasitism for hosts and the behavioural changes invoked in infected individuals is important for understanding their ecological and evolutionary relationships (Parker et al. 2003; Perrot-Minnot et al. 2007).

Parasites with complex life-cycles involving trophic transmission often have marked phenotypic effects on their intermediate hosts that generally lead to increased transmission rates to the final host (Barber 2013; Lélu et al. 2013). There are several theories and mechanisms that underpin this aspect of the host-parasite relationship (Lafferty 1999). These include the behaviour modification hypothesis, where acquired or modified behaviours of the intermediate host assists transmission to the final host (Fredensborg 2014), trait mediated effects, where parasite-induced changes in the host phenotype can indirectly result in increased predation rates to final hosts (Dunn et al. 2012) and parasite manipulation, where the parasite directly manipulates host behaviour to increase their probability of being predated upon by the final host (Poulin 2013). These all attempt to explain the mechanisms by which the parasite is directly or

indirectly increasing their probability of being transmitted to the final host (Lefevre et al. 2009; Amundsen et al. 2013; Dianne et al. 2014).

Whilst the parasite manipulation hypothesis has received a great deal of research attention, (Nickol 2005; Perrot-Minnot et al. 2007), these studies have tended to focus on how infection suppresses the anti-predator responses and behaviours of intermediate hosts (Kamiya and Poulin 2012; Weinreich et al. 2013; Swartz et al. 2015). The general pattern is that infection alters the anti-predator responses of intermediate hosts, a process that should increase their vulnerability to predation and thus potentially increases parasite transmission to the final host (Perrot-Minnot et al. 2007; Dianne et al. 2012). Experimental research approaches involve use of olfactory cues and/ or predator presence/ absence, with marked differences in responses of infected and uninfected individuals (e.g. Perrot-Minnot et al. 2007; Franceschi et al. 2008; Dianne et al. 2011**).**

The acanthocephalan parasite *P. laevis* and its intermediate host *G. pulex* is frequently used as a model system to test for parasite manipulation, with it increasingly accepted that manipulation increases the probability of the parasite being transmitted to a fish final host (e.g. Lagrue et al. 2007; Franceschi et al. 2008; Dianne et al. 2011; Labaude et al. 2015). Manipulation is primarily in *G. pulex* behaviour (Table 3.1), including reversal of their anti-predator responses (e.g. Perrot-Minnot et al. 2007**)**. Modification of host behaviour only occurs when the parasite reaches its infectious cystacanth stage (Franceschi et al. 2008), and generally results in infected individuals decreasing their use of refugia and being attracted to predatory cues, increasing their predation risk (Cézilly et al. 2000; Kaldonski et al. 2007; Franceschi et al. 2008; Dianne et al. 2011; Table 3.1). Nevertheless, not all studies report evidence of manipulation, with

variability in host behaviours and manipulation apparent between populations, age and sibship (Franceschi et al. 2008; 2010a,b; Table 3.1). Moreover, no studies had then tested the response of the fish to the manipulated behaviours of *G. pulex*, such as alterations in their foraging behaviour (e.g. increased consumption rate of infected individuals) and prey selectivity (e.g. higher preferences for infected individuals).

Behavioural functional responses describe the resource uptake rate of a predator as a function of their resource density (Murray et al. 2016). As they provide strong insights into predator-prey relationships (Dick et al. 2014), they can be applied to testing the foraging responses of predatory final host species to their prey populations that comprise intermediate hosts, such as *G. pulex* infected and uninfected with *P. laevis*. They can also reveal whether there are energetic costs associated with infection of the final host that inhibits their foraging success, as observed in juvenile carp *C. carpio* infected with the non-native parasite *B. acheilognathi* (Britton et al. 2012). Comparative functional response approaches have been developed recently that enable testing of differences in functional response parameters and curves between different predator and prey species, especially when these involve Type II functional responses (e.g. Alexander et al. 2013; Barrios-O'Neill et al. 2014).

Given the potential for *P. laevis* to manipulate the behaviour of *G. pulex* in the presence of a predator (Table 3.1), this host-parasite system was used with three model fish species to test the following hypotheses: (1) the consumption rate and functional response parameters of the fishes will be significantly higher when feeding on infected versus uninfected *G. pulex* due to parasite manipulation; (2) specific activities of infected *G. pulex* will be elevated in the presence of fish predator cues compared with

their activity in clean water and uninfected conspecifics, explaining the patterns in hypothesis 1; and (3) the consumption rate and functional response parameters of infected fishes will be significantly reduced compared to uninfected fishes, irrespective of the infection status of the prey.

Table 3.1. Summary of behavioural manipulation of *Pomphorhynchus laevis* on its intermediate host *Gammarus pulex*. 'Alteration' is relative to uninfected individuals, where – reduced, + increased, / no change; 'Infection' is either natural or in the laboratory (experimental, where eggs were taken from infected individuals of the fish species named); 'Prevalence' is parasite prevalence in *G. pulex*; 'Predator' is the fish species used in the experiments; and '*G. pulex*', '*P. laevis*' and 'Fish' is the river where they were sourced from and where Sz = Switzerland and Fr = France. *Stickleback *Gasterosteus aculeatus*; brown trout *Salmo trutta*; perch *Perca fluviatilis*; bullhead *Cottus gobio*; chub *Squalius cephalus*; dace *Leuciscus leuciscus*; grayling *Thymallus thymallus*.

Phenotypic trait	Alteration	Infection	Prevalence	Fish predator*	*G. pulex*	*P. laevis*	Fish	Reference
Photophobia	-	Natural	16.5 %		Wohlensee, (Sz)			Bakker et al. 1997
	-	Natural	8.8 %		Ouche (Fr)			Bauer et al. 2000
	-	Natural			Ouche (Fr)			Cezilly et al. 2000
	-	Experimental			Suzon (Fr)	Vogue (Fr)		Durieux et al. 2012
	-	Experimental			Suzon (Fr)	Vogue (Fr)		Perrot-Minnot et al. 2014
	-	Natural			Ouche (Fr)			Tain et al. 2006
	-	Natural	0-80 %		Avon (UK)			Kennedy et al. 1978
Appearance	+ predation	Natural	16.5 %	Stickleback	Wohlensee (Sz)		Wohlensee (Sz)	Bakker et al. 1997
	/ predation	Natural		Brown trout	Ouche (Fr)			Koldonski et al. 2009

Predator cue avoidance	-	Natural	-	Perch	Waldibach (Sz)		Lake Lucerne (Sz)	Baldauf et al. 2007
	-	Experimental (chub)		Brown trout	Suzon (Fr)	Vogue (Fr)	Aquaculture	Diane et al. 2011
	-	Natural	30 % (drift)	Bullhead				Kaldonski et al. 2007
Visual predator avoidance	/	Natural	-	Perch	Waldibach (Sz)		Lake Lucerne (Sz)	Baldauf et al. 2007
Pairing success	-	Natural	10.9 %		Tille (Fr)			Bollache et al. 2001
	-	Natural	7.4 %		Tille, France			Bollache et al. 2002
	/	Natural	20 %		Tille (Fr)			Poulton and Thompson 1987
Competitive performance	+	Natural	10.9 %		Tille (Fr)			Ballache et al. 2001
Fecundity	-	Natural	7.4 %		Tille (Fr)			Ballache et al. 2002
	-	Natural	20 %		Severn, UK			Poulton and Thompson 1987
Refuge use	-	Experimental (chub)		Brown trout	Suzon (Fr)	Vogue (Fr)	Aquaculture	Dianne et al. 2011
	-	Experimental (chub)		Chub	Suzon (Fr)	Vogue (Fr)	Not specified	Dianne et al. 2014

	-	Natural	30 % (in drift)	Bullhead	Ouche (Fr)		Ouche (Fr)	Kaldonski et al. 2007
	-	Experimental (chub)			Suzon (Fr)	Vogue (Fr)		Perrot-Minnot et al. 2014
Foraging rate	+	Experimental (chub)			Suzon (Fr)	Vogue (Fr)	Aquaculture	Dianne et al. 2011
	+	Experimental (chub)		Chub	Suzon (Fr)	Vogue (Fr)	Not specified	Dianne et al. 2014
Grouping	-	Experimental (chub)		Brown trout	Suzon (Fr)	Vogue (Fr)	Aquaculture	Durieux et al. 2012
	/	Experimental (chub)			Suzon (Fr)	Vogue (Fr)		Perrot-Minnot et al. 2014
Activity	/	Experimental (chub)		Brown trout	Suzon (Fr)	Vogue (Fr)	Aquaculture	Durieux et al. 2012
	-	Experimental (chub)			Suzon (Fr)	Vogue (Fr)		Perrot-Minnot et al. 2014
Drifting	+	Natural	0.38 % benthos 6.46 % drift		Ouche (Fr)			Lagrue et al. 2007
	+	Natural	84 % in drift 33.5 % in benthos		Teme (UK)			McCahon et al. 1991

Vulnerability to predation	+	Natural	0.38 % benthos 6.46 % drift	Bullhead	Ouche (Fr)	Ouche (Fr)	Lagrue et al. 2007
	/	Natural	0.38 % benthos 6.46 % drift	Frog	Ouche (Fr)	Ouche (Fr)	Lagrue et al. 2007
	+	Natural	30 % (in drift)	Bullhead	Ouche (Fr)	Ouche (Fr)	Kaldonski et al. 2007
	+	Natural		Brown trout,	Ouche (Fr)	Aquaculture	Kaldonski et al. 2009
	+	Natural		Stickleback	Wohlensee (Sz)	Wohlensee (Sz)	Mazzi and Bakker 2003
	+	Natural	0-80 %	Dace, grayling	Avon (UK)	Not specified	Kennedy et al. 1978

3.3 Methods

3.3.1 Animal collection and maintenance

Samples of the intermediate host, *G. pulex*, were collected for experimental use from the River Avon in Hampshire, Southern England (latitude: 50.8865, longitude: -1.7883) when water temperatures were approximately 12 to 18 ^{o}C. They were then held in tank aquaria (10 L) in a mix of river and de-chlorinated tap water at 18 ^{o}C for at least 96 hours before being used. The infected and uninfected individuals were initially identified visually (Bauer and Rigaud 2015), with this validated by the dissection of 30 individuals. The infected individuals were all used at the cystacanth stage when the parasite is infectious to fish hosts (Section 1.3.5; Figure 1.1). The range of parasite prevalence in these samples was 22 to 29 %. Hereafter, where a fish or *G. pulex* is referred to as either infected or uninfected, it refers to its infection status by *P. laevis*.

The three model fishes used to test the hypotheses were *B. barbus*, *S. cephalus* and *C. auratus*. In the Hampshire Avon, both *S. cephalus* and *B. barbus* are final hosts of *P. laevis* (e.g. Hine and Kennedy 1974a; Kennedy 1996), whereas *C. auratus* is not present. The fishes were all sourced from the same aquaculture site in Southern England where *P. laevis* was not present and so all individuals used in experiments had no previous direct exposure. However, the broodstock of *S. cephalus* and *B. barbus* were from the River Kennet, Berkshire, Southern England, where *P. laevis* is naturally present (Kennedy et al. 1989) and hence were used as experienced 'host fishes'. To the best of the candidate's knowledge, the previous generations of the *C. auratus* had never been exposed to *P. laevis*. As the species is, however, capable of developing *P. laevis* infections (e.g. Sures and Siddel 2001), they were used here as a 'naïve host'. Note that

for *B. barbus*, only uninfected fish were used in the initial functional response experiments and the species was then not used further. This was because, compared to *S. cephalus* and *C. auratus*, they tended to require considerably longer time to acclimate to aquaria conditions and to demonstrate natural foraging behaviours (personal observations by candidate).

All fish used were of age 1+ years and of 70 mm minimum length. On their arrival to laboratory aquaria, they were all initially measured (fork length, nearest mm) and tagged with a 12 mm passive integrated transponder (PIT) tag to enable their individual identification during subsequent experiments. The fish were then allowed to acclimate to aquaria conditions for 20 days prior to their use. Throughout their holding, they were maintained at a constant water temperature of 18 ^{o}C and on a 16:8 h light: dark cycle and, other than when they were being used experimentally, when they were starved to 24 hours before use, feeding was daily using a formulated pelletized feed, based on fish-meal.

To test Hypothesis 2, *S. cephalus* infected with *P. laevis* were used. *B. barbus* were not used for reasons mentioned above. Infected *C. auratus* were also not used for their propensity for developing heavy *P. laevis* infections (*cf.* 3.4 Results) and so tended to be close to their end-points as outlined in the ethical review process. The infected *S. cephalus* were produced by individually exposing 30 fish to 35 infected *G. pulex* in 10 L tanks for 24 hours (all were consumed). They were then held for 60 days at 18 ^{o}C to enable the infections to develop as an initial trial indicated this period was sufficient to produce high parasite prevalence and abundance (Chapter 2). At the conclusion of all experiments, all fish were euthanized (over-dose of anaesthetic, MS-222), and the body

cavity and intestine dissected to confirm their infection status. Where an individual *S. cephalus* had been used experimentally as an infected fish but dissection suggested that an infection had not developed then it was removed from the dataset via its PIT tag code.

3.3.2 Comparative functional response experiments

The functional responses of uninfected *S. cephalus*, *B. barbus* and *C. auratus*, and infected *S. cephalus*, were determined for infected and uninfected *G. pulex.* The experiments were completed in 10 L tanks of oxygenated de-chlorinated tap water at 18 ^{o}C. Fish were identified by their PIT tag code before being placed into tanks individually where they were left for 24 hours to acclimate during which time they received no food to standardise hunger levels.

The fish were then exposed to a pre-defined, but randomly selected, number of prey items, where the prey item was always either infected *G. pulex* or uninfected *G. pulex*. Numbers of prey items were across six prey densities, 2, 4, 8, 16, 32, 64, with each completed using between three and five replicates (Alexander et al. 2013; Barrios-O'Neill et al. 2014). The exception was for *C. auratus*, where 128 items also had to be used to reach the asymptote of their consumption rate (*cf.* 3.4 Results). In addition to the *G. pulex*, dead chironomid larvae were also tested at the same densities for the uninfected and infected fishes for comparative purposes. Exposure to the prey items was for one hour, after which the fish were removed from the tanks and the numbers of remaining prey items counted to enable calculation of the number consumed in that hour.

For each fish species and food item, their number of prey items consumed versus initial prey density were analysed for their functional response parameters of attack rate (*a*) and handling time (*h*) using the frair package (Pritchard 2014) in R (Team R 2014). Logistic regression defined the shape the shape of the relationship between prey density and prey consumed, allowing identification of functional response type. A Type II functional response was identified by decreasing prey consumption with increasing prey density and a significant negative first order term (Juliano 2001). Functional responses were then modelled using maximum likelihood estimation (MLE:Bolker 2012) with the Random Predator Equation (Rogers 1972), which assumes a Type II response and non-replacement of prey: $N_e = N_0 (1 – \exp (a (N_e h\text{-}t)))$, where N_e is the number of prey eaten, N_0 is the initial density of prey, *a* is the attack rate, *h* is the handling time and t is the total time available. In Frair, the 95 % confidence intervals were calculated with 2000 non-parametric bootstraps for the functional response parameters, producing Type II curves that allow comparison of functional responses. Where the 95 % confidence intervals of the curves overlapped then they were interpreted as not being significantly different and vice-versa (Paterson et al. 2015). These enabled comparative functional responses to be assessed between the fish species where exposed to uninfected and infected *G. pulex*, and chironomid larvae, plus between uninfected and infected *S. cephalus* exposed to the same prey items.

3.3.3 Feeding trials using uninfected and infected intermediate hosts

Differences in the feeding rate of the model fishes in relation to the number of infected and uninfected *G. pulex* were then completed in a series of feeding trials using *S. cephalus* and *C. auratus* only. These were completed in the same equipment as per the functional response experiments, using the same design in terms of time of exposure.

Here, however, all trials exposed the fish to 20 *G. pulex*, either as all infected or uninfected, and in proportions of 5:15, 10:10 and 15:5 infected: uninfected, with three replicates each. At the end of each trial, the total number of consumed *G. pulex* was determined, along with the number of these that were infected and uninfected. Differences in the total number consumed were tested between the *G. pulex* groups using t-tests. The numbers of infected and uninfected *G. pulex* consumed versus their initial number were then tested using linear regression, with the 95 % confidence limits of the regression coefficient *b* stored. For each fish species, two regression tests were completed: number of uninfected or infected *G. pulex* consumed versus their initial numbers. Where there were overlaps in their 95 % confidence intervals of *b*, then the slopes of the regression lines were interpreted as not being significantly different, i.e. for that fish species, there was no significant difference in in the relationship of their consumption rate with starting number of infected and uninfected *G. pulex* (Chapter 5; Sheath et al. 2015).

3.3.4 Trait mediated activity of Gammarus pulex

To quantify the effect of predator presence and absence on *G. pulex* behaviour, this experiment measured the individual activity of the uninfected and infected *G. pulex* in the presence and absence of fish predator cues. The experiments measured the individual activity of *G. pulex* by placing them into a circular experimental arena of 12 cm diameter and height 10 cm, and on which one black line was drawn across its base and another one was drawn around the circumference at 5 cm from the base. It was lit from above with a constant brightness of 350 lux. The arena was filled with water and an individual *G. pulex* of known infection status was then released. Its activity was then measured for three periods of 2 minutes, each separated by intervals of 2 minutes where

no behavioural responses were recorded. Individual activity was measured as the number of occasions on which the individual *G. pulex* crossed the line on the base (i.e. to measure horizontal movement) and the number of occasions it crossed the line around the circumference (i.e. to measure vertical movements). Individuals were used only once and all were dissected to confirm infection status with the cystacanth stage (Section 1.3.5; Figure 1.1).

Conditions of predator presence and absence in the arenas were achieved via manipulation of the water in which the individual *G. pulex* were exposed to. Conditions of predator absence (i.e. the control) were achieved by the water in the experimental arena comprising only of de-chlorinated tap water (18 ^{o}C). Predator presence was achieved by using fish-conditioned water which was produced by holding an individual *S. cephalus* (121 mm fork length) (for natural host cues) or *C. auratus* (108 mm fork length) (for naïve host cues) in a 10 L aquarium for 14 days. Both fish were fed daily *ad libitum* with live unparasitised *G. pulex* in order to strengthen the predation signal (Wudkevich et al. 1997; Dianne et al. 2014). No *G. pulex* were fed to the fish on the day of the activity experiments. For each experimental combination (uninfected, infected *G. pulex*; *S. cephalus* predator presence, *C. auratus* predator presence, predator absence), 30 replicates were completed.

The initial test determined the consistency in the activity of the individual *G. pulex* between each measurement period (paired t-tests). Differences in the activity levels between each treatment were then tested using poisson log-linear generalized linear models (GLM), as the activity data represented count data. Separate models were calculated for horizontal and vertical activity. Model outputs were the significance of

treatment, infection status and their interaction on activity levels, plus the estimated marginal means of the number of horizontal or vertical movements made by the *G. pulex* in each treatment and their 95 % confidence intervals. The significance of differences in the estimated marginal means per treatment was indicated by linearly independent pairwise comparisons, with Bonferroni adjustment for multiple comparisons.

3.4 Results

3.4.1 Comparative functional response experiments

The functional response analyses all reported significant, negative first order terms, indicating Type II functional responses (Table 3.2). For the host fishes *S. cephalus* and *B. barbus*, there were significant differences in their handling times between uninfected and infected *G. pulex* (Table 3.3), with the 95 % confidence limits of their Type II curves only overlapping at prey densities of below 20 and 6 respectively (Figure 3.1). In all cases, their consumption rates were higher for uninfected versus infected *G. pulex* (Figure 3.1, Table 3.3). For *C. auratus*, the naïve host species, there was no significant difference in attack rate but there was a significant difference in handling time (Table 3.3), and in combination, this resulted in considerable overlap in their Type II curves, particularly at food densities of 64 and below (Figure 3.1).

For *S. cephalus* that were infected and uninfected with *P. laevis*, there were no significant differences in their functional response parameters when they were exposed to infected *G. pulex* (Table 3.3). Although there were significant differences apparent for both uninfected *G. pulex* and chironomid larvae (Table 3.3), their Type II functional

response curves showed considerable overlap in the consumption rates of the uninfected and infected fish for all food types, suggesting that there was no overall difference in their consumption rates of these prey (Figure 3.2). Following the exposure in the experiments to the infected *G. pulex*, parasite prevalence was 100 % in all fishes. In the two host species, *S. cephalus* were infected at abundances of between 1 and 5 adult *P. laevis*, and *B. barbus* had consistent infections of only 1 adult. In the naïve host, *C. auratus*, infections of between 8 and 27 adult *P. laevis* developed, some of which had migrated straight through the intestine and become embedded in muscle tissue. In all cases, fishes that were only exposed to uninfected *G. pulex* did not develop infections.

Table 3.2. First order linear coefficient results from logistic regressions for the predator and prey combinations. All values indicate a Type II functional response.

Predator	Prey	First order term	P
Uninfected *B. barbus*	Infected *G. pulex*	-0.045	<0.001
Uninfected *B. barbus*	Uninfected *G. pulex*	-0.032	<0.001
Uninfected *S. cephalus*	Chironomid larvae	-0.012	<0.001
Uninfected *S. cephalus*	Infected *G. pulex*	-0.052	<0.001
Uninfected *S. cephalus*	Uninfected *G. pulex*	-0.037	<0.001
Infected *S. cephalus*	Chironomid larvae	-0.012	<0.001
Infected *S. cephalus*	Infected *G. pulex*	-0.056	<0.001
Infected *S. cephalus*	Uninfected *G. pulex*	-0.072	<0.001

Table 3.3. Test results of the comparative functional responses across the different fishes and according to infection status of both fish and *Gammarus pulex* (I = infected; U = uninfected). The parameter estimates were calculated using the Random Predator Equation (Rogers 1972), with statistically significant differences in the parameters of attack rate (a) and handling time (h) between species ($\alpha = 0.05$) shown in bold.

	S. cephalus: I vs. U *G. pulex*	*B. barbus*: I vs. U *G. pulex*	*C. auratus*: I vs. U *G. pulex*	Uninfected *G. pulex*: I vs. U *S. cephalus*	Infected *G. pulex*: I vs. U *S. cephalus*	Chironomid larvae: I vs. U *S. cephalus*
a	3.92/ 2.09	0.95/ 1.18	2.89/ 2.69	6.32/ 2.09	5.48/ 3.92	5.35/ 1.37
Z	1.75	-0.139	-0.37	2.98	0.79	4.78
P	0.08	0.90	0.70	**<0.01**	0.43	**<0.01**
h	0.89/0.03	0.76/ 0.12	0.03/ 0.02	0.05/0.03	0.08/ 0.09	0.02/ 0.03
Z	6.10	2.85	-2.91	2.65	-0.76	-0.68
P	**<0.01**	**<0.01**	**<0.01**	**<0.01**	0.45	0.50

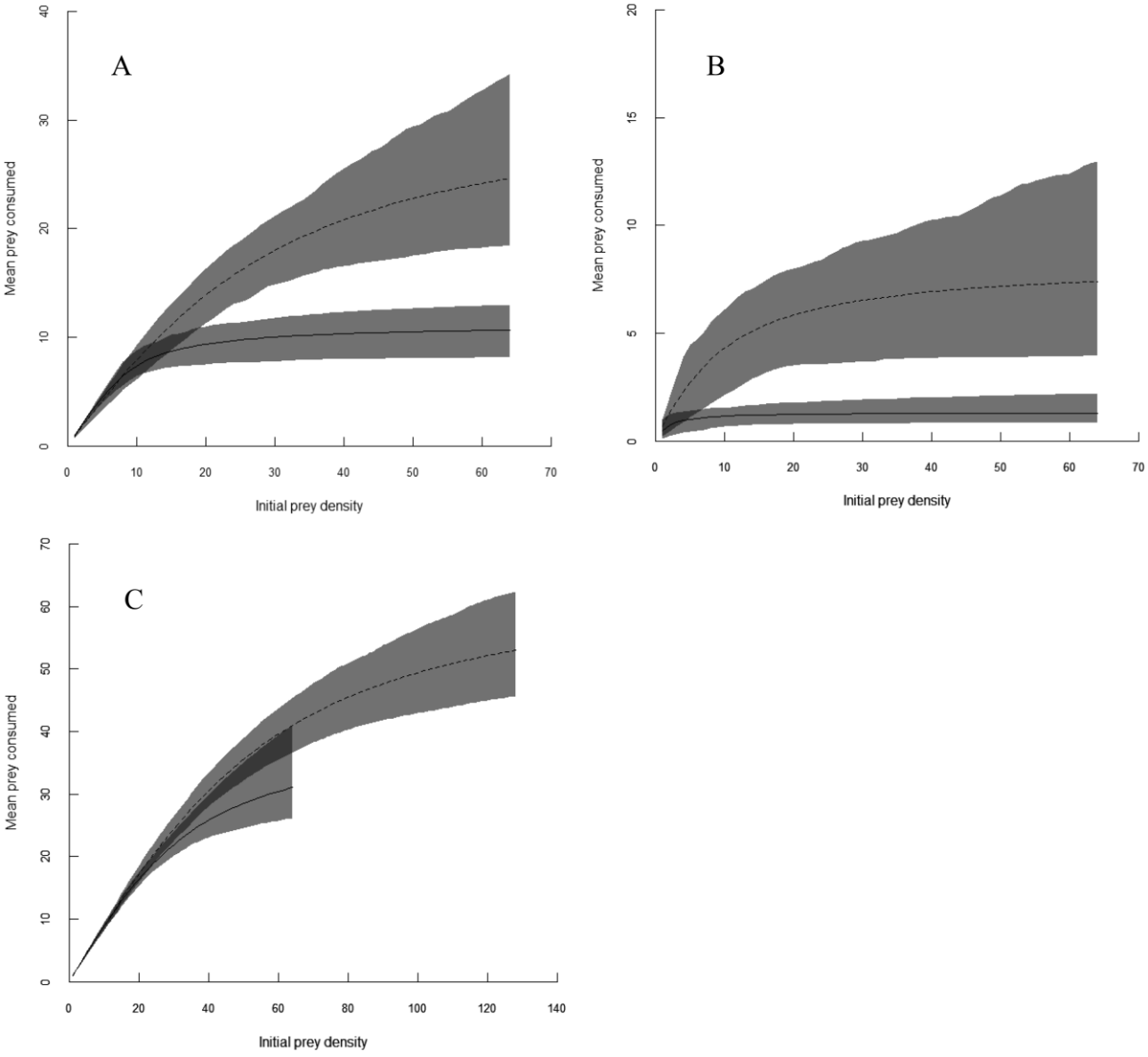

Figure 3.1. Type II functional response curves for (A) *Squalius cephalus*, (B) *Barbus barbus* and (C) *Carassius auratus* fed *Gammarus pulex* infected with *Pomphorhynchus laevis* (solid line) and uninfected *G. pulex* (dashed line). Lines indicate mean functional response. Light grey shading represents 95 % equi-tailed confidence intervals (CI) for each species, with dark grey shading representing the overlap in the CIs of the species. Note differences on the x and y axes for clarity of presentation.

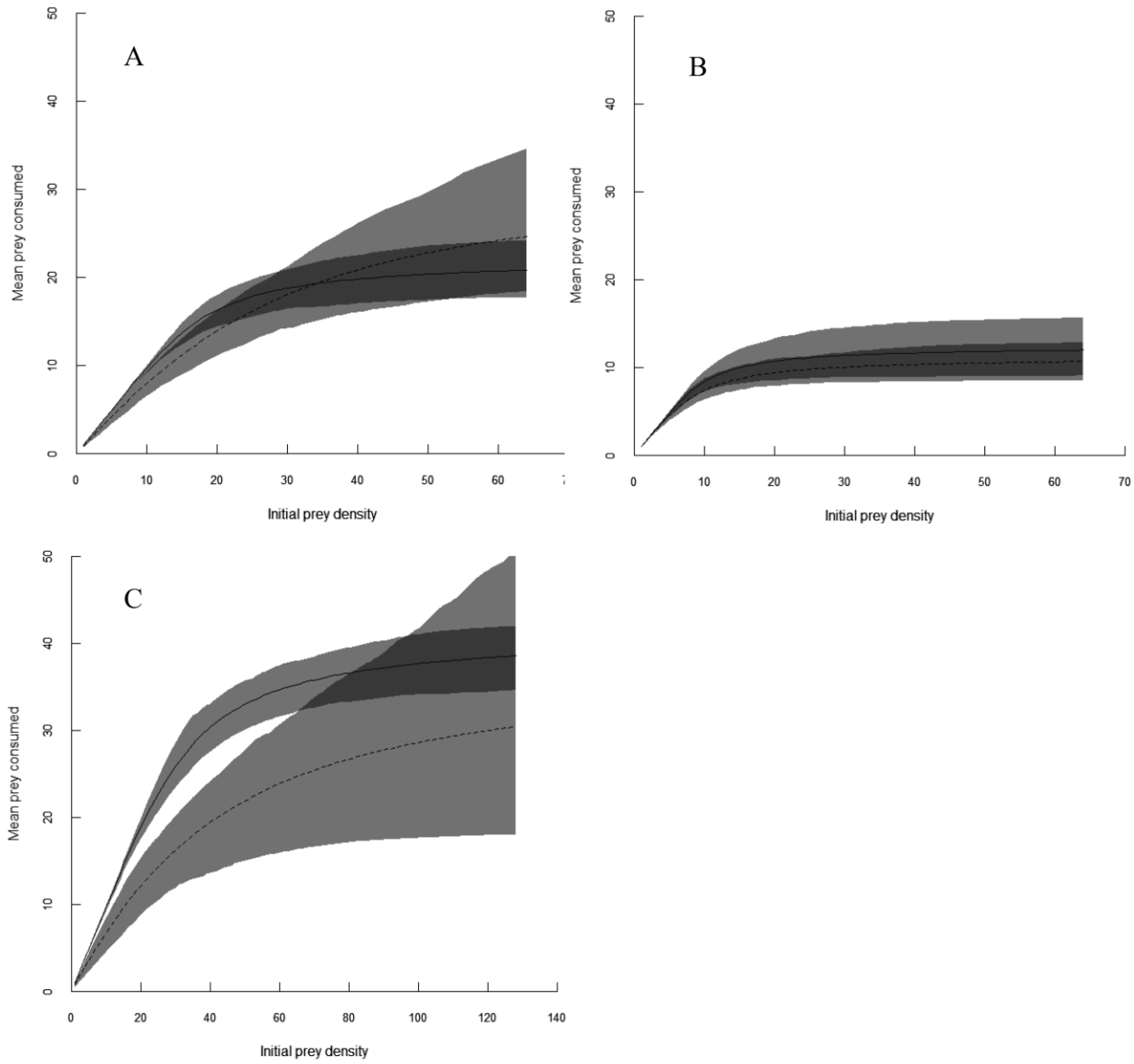

Figure 3.2. Type II functional response curves for *Squalius cephalus* infected and uninfected with *Pomphorhynchus laevis*, and exposed to (A) uninfected *Gammarus pulex*, (B) *G. pulex* infected with *Pomphorhynchus laevis* and (C) chironomid larvae. Lines indicate mean functional response with solid lines representing infected *S. cephalus* and dashed lines represent uninfected *S. cephalus*. Light grey shading represents 95 % equi-tailed confidence intervals (CI) for each species, with dark grey shading representing the overlap in the CIs of the species. Note differences on the x and y axes for clarity of presentation.

3.4.2 Feeding trials using a mixture of uninfected and infected intermediate hosts

The feeding trials exposing *S. cephalus* and *C. auratus* to different numbers of uninfected and infected prey revealed that there were no significant differences in the mean total number of *G. pulex* consumed between treatments (t-test: t = 1.96, P = 0.23; t = -0.11, P = 0.66 respectively; Figure 3.3). For both fishes, there was a significant increase in the number of uninfected *G. pulex* consumed as the number available increased per treatment (*S. cephalus*: $R^2 = 0.80$; $F_{1,10} = 40.1$, $P < 0.01$; *C. auratus*: $R^2 = 0.38$; $F_{1,10} = 6.2$, $P = 0.03$ respectively; Figure 3.4). This significant relationship was also apparent for *C. auratus* and infected *G. pulex*, ($R^2 = 0.65$; $F_{1,10} = 18.8$, $P < 0.01$), but was not for *S. cephalus* ($R^2 = 0.11$; $F_{1,10} = 1.22$, $P = 0.29$) (Figure 3.4).

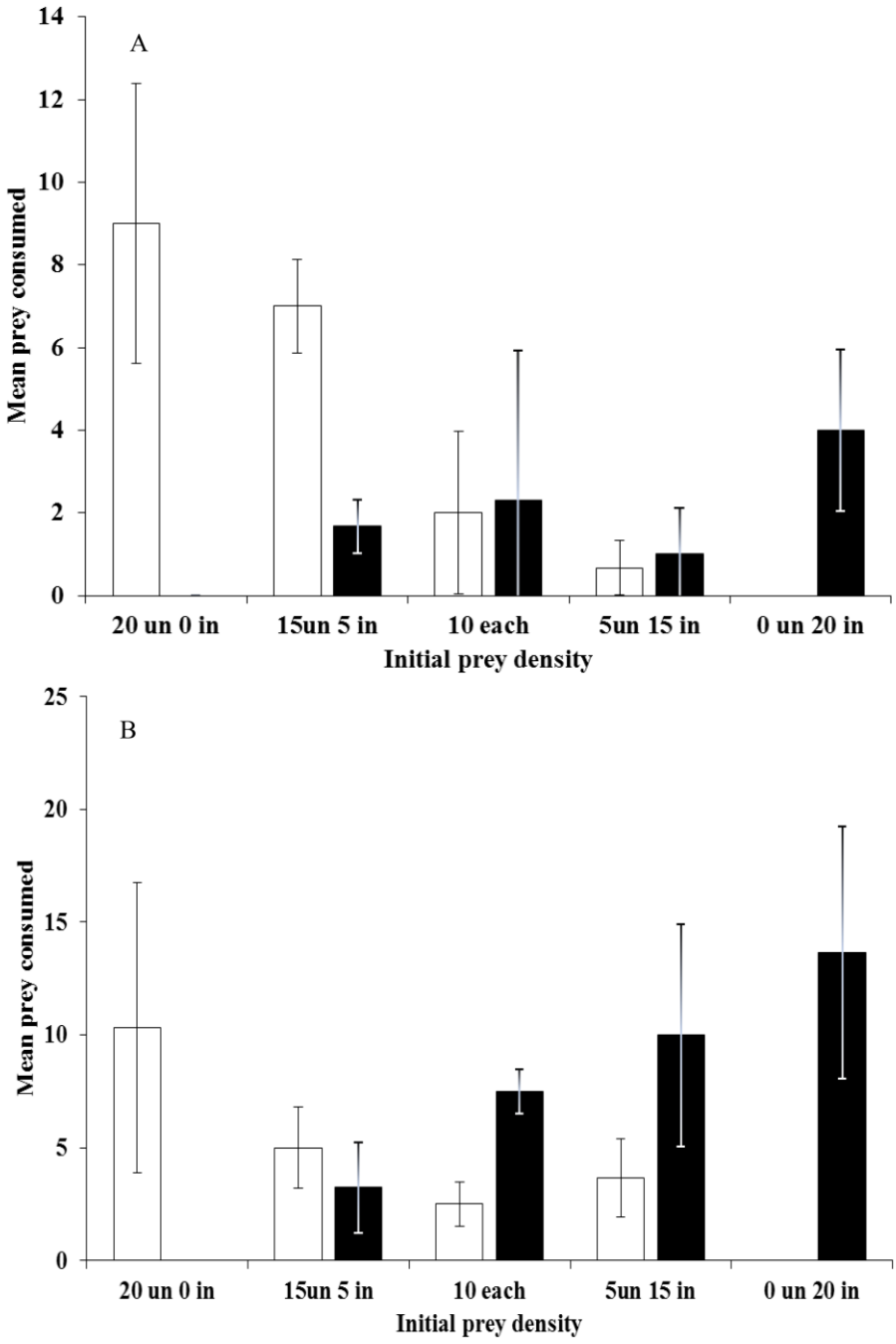

Figure 3.3. (A) Initial number of uninfected (un) and infected (in) *Gammarus pulex* versus number of uninfected (hollow bars) and infected (solid bars) consumed by *Squalius cephalus* in the mixed feeding trial. Error bars are 95 % confidence limits. (B) Initial number of uninfected (un) and infected (in) *Gammarus pulex* versus number of uninfected (hollow bars) and infected (solid bars) consumed by *Carassius auratus* in the

mixed feeding trial. Error bars are 95 % confidence limits. Note differences on the y axis for clarity of presentation.

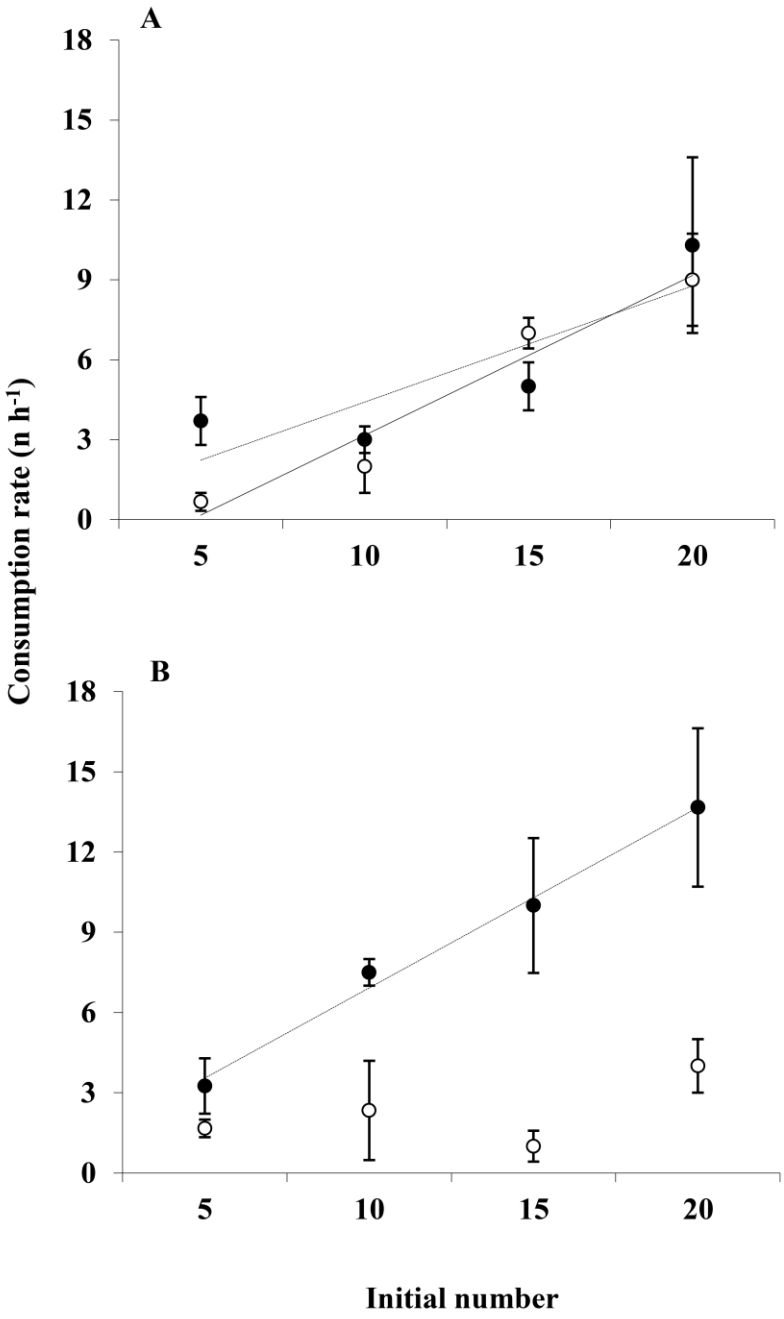

Figure 3.4. (A) Relationship of initial number of released uninfected *Gammarus pulex* versus the consumption rate of *G. pulex* in the feeding trials, where clear circles: *Squalius cephalus*, filled circles: *Carassius auratus*, solid line: significant relationship for *S. cephalus* according to linear regression; dashed line: significant relationship for

C. auratus according to linear regression. (B) shows the same relationships but for the number and consumption rate of infected *G. pulex*.

3.4.3 Trait mediated activity of Gammarus pulex

There were no significant differences in the activity levels between the three time intervals of the uninfected and infected *G. pulex* in water with and without predator cues (Table 3.4). Thus, for subsequent tests, activity levels were summed for the three periods. The effects of treatment, infection and their interaction on *G. pulex* movements in the horizontal plane were all significant in the GLM (Table 3.5). Outputs revealed that horizontal movements of *G. pulex* were significantly reduced for infected versus uninfected *G. pulex* in each treatment (Table 3.5). Whilst the activity of infected *G. pulex* in the *S. cephalus* treatment was significantly reduced from the control, this was not apparent in the *C. auratus* treatment (Table 3.5). In the vertical plane, the effect of treatment, infection and their interaction were also all significant in the GLM (Table 3.5). Movements of infected *G. pulex* were significantly reduced in the *S. cephalus* treatment compared with uninfected conspecifics and the control (Table 3.5). In the presence of *C. auratus* cues, movements of infected *G. pulex* were significantly increased versus uninfected conspecifics, but were not significantly different to those for both uninfected and infected conspecifics in the control (Table 3.5).

Table 3.4. Significance of differences (pairwise t-test) in activity levels between temporal replicates (time 2, 6 and 10 minutes) in *Gammarus pulex* infected and uninfected with *Pomphorhynchus laevis*, as movements in the horizontal and vertical planes. Clean: predator absence; chub: *Squalius cephalus* predator cue presence; goldfish: *Carassius auratus* predator cue presence.

Treatment	T	df	P
Horizontal, clean, uninfected			
2 and 6	0.26	29	0.80
2 and 10	0.40	29	0.69
6 and 10	0.10	29	0.92
Horizontal clean infected			
2 and 6	0.83	29	0.41
2 and 10	0.46	29	0.65
6 and 10	0.50	29	0.62
Horizontal, chub, uninfected			
2 and 6	0.34	29	0.74
2 and 10	0.35	29	0.73
6 and 10	1.25	29	0.22
Horizontal, chub, infected			
2 and 6	0.83	29	0.42
2 and 10	0.14	29	0.89
6 and 10	0.76	29	0.46
Vertical, clean, uninfected			
2 and 6	1.32	29	0.20
2 and 10	0.12	29	0.90
6 and 10	2.00	29	0.55
Vertical, clean, infected			
2 and 6	0.95	29	0.35
2 and 10	0.70	29	0.49
6 and 10	0.65	29	0.52

Vertical, chub, uninfected			
2 and 6	1.65	29	0.11
2 and 10	2.70	29	0.06
6 and 10	1.39	29	0.18
Treatment	T	df	P
Vertical, chub, infected			
2 and 6	0.14	29	0.89
2 and 10	1.46	29	0.16
6 and 10	1.83	29	0.06
Horizontal goldfish, uninfected			
2 and 6	0.40	29	0.69
2and 10	0.65	29	0.52
6 and 10	0.33	29	0.75
Horizontal, goldfish, infected			
2 and 6	0.94	29	0.36
2 and 10	1.67	29	0.11
6 and 10	0.22	29	0.83
Vertical, goldfish, uninfected			
2 and 6	0.34	29	0.74
2 and 10	0.71	29	0.48
6 and 10	1.09	29	0.28
Vertical, goldfish, infected			
2 and 6	0.80	29	0.43
2 and 10	0.56	29	0.58
6 and 10	1.02	29	0.32

Table 3.5. (A) Mean activity in horizontal and vertical plane of *Gammarus pulex* uninfected (U) and infected (I) with *Pomphorhynchus laevis* in the control (Cn) and predator cue treatments using *Squalius cephalus* (Sc) and *Carassius auratus* (Ca); (B) Outputs of factorial Generalized linear models (GLM) for each activity plane; (C) Significance in the difference of the mean activity levels between treatments and infections according to each linearly independent pairwise comparisons with Bonferroni adjustment for multiple comparisons from the GLM.

(A)

Horizontal				
Treatment	*P. laevis* status	Mean activity	Lower 95 %	Upper 95 %
Cn	U	52.7	50.1	55.3
	I	24.0	22.3	25.8
Sc	U	54.7	52.2	57.5
	I	16.5	15.1	18.1
Ca	U	38.4	36.3	40.7
	I	23.9	22.3	25.8
Vertical				
Cn	U	30.1	28.2	32.1
	I	36.4	34.3	38.6
Sc	U	17.0	15.6	18.5
	I	10.9	9.8	12.2
Ca	U	21.6	20.0	23.4
	I	33.7	31.7	35.8

(B)

	Horizontal plane GLM		Vertical plane GLM	
Model term	Wald χ^2	P	Wald χ^2	P
Intercept	62456.1	< 0.01	36957.9	< 0.01
Treatment	32.5	< 0.01	450.5	< 0.01
Infection	874.5	< 0.01	3.7	0.05
Interaction (Treatment x infection)	107.3	< 0.01	104.7	< 0.01

(C)

Horizontal	Significance of pairwise comparisons (Bonferroni adjusted for multiple comparisons)				
	Cn (U)	Cn (I)	Sc (U)	Sc (I)	Ca (U)
Cn (U)					
Cn (I)	< 0.01				
Sc (U)	1.0	< 0.01			
Sc (I)	< 0.01	< 0.01	< 0.01		
Ca (U)	< 0.01	< 0.01	< 0.01	< 0.01	
Ca (I)	< 0.01	1.0	< 0.01	< 0.01	< 0.01

Vertical					
	Cn (U)	Cn (I)	Sc (U)	Sc (I)	Ca (U)
Cn (U)	-				
Cn (I)	< 0.01	-			
Sc (U)	< 0.01	< 0.01	-		
Sc (I)	< 0.01	< 0.01	< 0.01	-	
Ca (U)	< 0.01	< 0.01	< 0.01	< 0.01	-
Ca (I)	0.20	1.0	< 0.01	< 0.01	< 0.01

3.5 Discussion

All fishes consumed significantly more uninfected than infected *G. pulex* in the functional response and foraging experiments, although this was only apparent for *C. auratus* at the highest prey densities. This was generally contrary to Hypothesis 1, developed from published behavioural manipulation studies, which predicted increased predation rates of infected *G. pulex* should result as a consequence of behavioural manipulation by *P. laevis*. The *G. pulex* behavioural trials then indicated that the activity of infected individuals was significantly reduced in water containing their predator cues versus clean water, and when compared with uninfected conspecifics. This was contrary to Hypothesis 2. In cues from the naïve host *C. auratus*, however, activity of infected *G. pulex* was elevated in the vertical plane compared with uninfected conspecifics but was similar to clean water in both planes, suggesting a negligible response overall. Finally, there was no difference in the consumption rates of uninfected and infected *S. cephalus* across the three prey types, contrary to Hypothesis 3. The associated issues related with these findings are discussed in the following sub-sections.

3.5.1 Does absence of intermediate host-manipulation explain the reduced consumption of infected Gammarus pulex?

The consistent demonstration that infected *G. pulex* were consumed at significantly lower rates and proportions compared to uninfected conspecifics by the two host fishes suggested an absence of parasite manipulated behaviours. This is despite a number of parasite manipulation experiments using this host-parasite model that have generally shown strong evidence of manipulated behaviours that ought to increase their susceptibility to predation (*cf.* Table 3.1). These differences in outcomes between the

functional response data here and these studies might relate to differences in the experimental designs of the experiments. For example, the functional response experiments provided only a basic environment for *G. pulex* with no refugia, as this would have impacted prey availability and thus prey density, invalidating the functional responses. Reduced use of refugia by infected *G. pulex* in the presence of fish predator cues has been measured experimentally, with this likely to result in increased probability of predation (e.g. Kaldonski et al. 2007; Dianne et al. 2011; 2014; Perrot-Minnot et al. 2014). Thus, it is not necessarily known how the infected and uninfected *G. pulex* would react to predator cues in the absence of refugia. However, whilst reduced use of refugia by infected *G. pulex* has been demonstrated and interpreted as a reduction in anti-predator response, this does not automatically mean it will result in an increased predation rate by specific fish species. Another driver of lower consumption of infected *G. pulex* may well be fish prefence of unifected individuals. If fish are avaible to differentiate between infected and uninfected individuals, then they they may choose to avoid infected individuals to avoid parasitism. However, the current experimental design did not allow for testing of this.

3.5.2 Drivers of variability in parasite manipulation

Experimental evidence suggests that the extent of parasite manipulation of *G. pulex* can vary according to the ages of both *G. pulex* and *P. laevis*, and genetic variance of the parasites resulting from aspects such as sibship (Franceschi et al. 2008; 2010a, b). For example, manipulation tends to increase with *P. laevis* cystacanth age (Franceschi et al. 2008), including increased photophilia (Franceschi et al. 2010a). These studies also highlight that the extent of manipulation varies across different origins of *P. laevis*. For example, when six different wild *P. laevis* populations, all derived from *S. cephalus*,

were experimentally exposed to *G. pulex*, variability in the extent of manipulation was subsequently apparent (Franceschi et al. 2010b). In our experiments, *G. pulex* were consistently collected from the same stretch of river, so avoiding confounding factors associated with using different source populations of *P. laevis* in experiments.

This variability in the manipulation of *G. pulex* by *P. laevis* collected from different sources is potentially important (Franceschi et al. 2008; 2010a, b). It suggests that there could be some selection issues in this host-parasite system that are being overlooked in parasite manipulation studies. For example, where a final fish host species, such as *C. gobio*, is highly abundant in the source river, then the majority of *P. laevis* might complete their lifecycles in this species, rather than species such as *S. cephalus* and *B. barbus* that are usually present in much lower abundance. Thus, the selection pressures in the host-parasite system would be for manipulative behaviours that increase the probability of consumption by the common final host (i.e. *C. gobio*) and these could differ from behaviours that favour consumption by other fishes due to inherently different habitat utilisation and foraging behaviours (Andreasson 1971; Hellawell 1971; Noble et al. 2007a.b). This might ultimately lead to the development of some host specialisation in this generalist parasite (Farrell et al. 2015). Correspondingly, these issues potentially provide some explanation of some of the variability apparent in the extent of manipulation observed between studies, given many of these used *P. laevis*, *G. pulex* and final fish hosts from across a range of sites and rivers (e.g. Baldauf et al. 2007; Kaldonski et al. 2009; Dianne et al. 2011; Durieux et al. 2012; Dianne et al.; 2014; Perrot-Minnot et al. 2014; Table 3.1).

3.5.3 Alternative modes of optimising parasite transmission rates to final hosts

The apparent lack of *P. laevis* manipulation in *G. pulex* detected in our study population could also be potentially explained by a lack of positive selection for manipulation due to the high abundance of infected *G. pulex* in the river. Both infected and uninfected *G. pulex* were sufficiently abundant to enable the collection of large sample sizes (total numbers > 8000 individuals) from relatively low sampling efforts on small areas (e.g. 9 m^2) of much larger gravel riffles and in which up to 29 % of individuals were infected. In addition, previous studies on the river revealed parasite prevalence in *G. pulex* of between 3 and 31 % through the year (Hine and Kennedy 1974b), where the fish final hosts included *C. gobio* and *S. cephalus*, with infections acquired in all months (Hine and Kennedy 1974a). Thus, given the high abundances of *G. pulex* and fish final hosts, and the relatively high parasite prevalences in both, there could be little selection pressure for developing manipulation of intermediate host behaviours, i.e. the potential final hosts are continuously exposed to infected *G. pulex*, irrespective of their behaviours in this system. Finally, Franceschi et al. (2010b) suggested that naturally infected *G. pulex* intermediate hosts were less sensitive to manipulation than naïve hosts, suggesting some evolved resistance to parasite manipulation and could provide at least a partial explanation to the patterns observed here, given that naturally infected *G. pulex* were used throughout.

3.5.4 Are the lower consumption rates of infected G. pulex driven by final host differences in foraging behaviour?

An alternative hypothesis to explain the difference in consumption rates of the infected and uninfected *G. pulex*, and particularly between the host and naïve final host species, could relate to differences in foraging styles, whereby the two host species were inefficient in their foraging performances compared with *C. auratus* due to poor prey

detection. However, all three species are capable of foraging on the benthos and so were likely to have relatively similar foraging styles and behaviours in the functional responses experiments, and indeed all ate at least some prey (Krause 1993; Richardson et al. 1995; Britton and Pegg 2011).

Alternatively, the generally similar consumption rates of *C. auratus* of infected and uninfected *G. pulex,* at least at lower prey densities, might relate to their status as a naïve host. In the presence of *C. auratus* predator cues, the vertical movements of the infected *G. pulex* were significantly elevated compared to the uninfected, although they were still similar to the clean water. This suggests that rather than this being evidence for manipulation, the infected *G. pulex* were not perceiving the *C. auratus* cues as a threat, a contrast to *S. cephalus* cues. This seems reasonable, as no *C. auratus* are present in the river where *G. pulex* were collected. This would then at least partially explain the similar consumption rates of uninfected and infected *G. pulex* by *C. auratus* across most prey densities. Indeed, it might be unexpected that parasite manipulation of an intermediate host would occur in the presence of a new fish species as this would be maladaptive, given the new species could be a non-suitable final host.

To summarise, previous studies indicated that *P. laevis* generally exhibits strong manipulation of its intermediate host that is hypothesised as leading to increased consumption of infected individuals by a range of fish final host species. The experiments here, however, demonstrated that in a highly simplified habitat, two preferred final host fishes had a strong preference for consuming uninfected *G. pulex*. Further, behavioural experimental results suggested that infection suppressed *G. pulex* activity and this might have been a causal factor in this pattern. In entirety, these outputs

emphasize that whilst *P. laevis* can manipulate the behaviour of *G. pulex*, this is a complex host-parasite relationship influenced by a range of extrinsic and intrinsic factors that do not necessarily result in the increased consumption of infected intermediate hosts by fish final hosts. It also raises questions on the use of multiple final fish hosts in the lifecycle, including the importance of highly abundant fish species, such as *C. gobio*, in the host-parasite dynamics and thus in selection terms, the importance of these fishes for developing parasite manipulation in *G. pulex*. Some of these aspects are thus explored in Chapter 4 where the infections by *P. laevis* are investigated in three highly abundant small-bodied fishes, plus *S. cephalus*, across a number of rivers in Southern and Western England.

Chapter 4

Infections of *Pomphorhynchus laevis* in fish final hosts in their indigenous and non-indigenous ranges: prevalences, pathology and trophic consequences

Some of the Results from this chapter are being published in:

Medoc V, Sheath DJ, Andreou D., Firmat C & Britton JR (submitted). Parasitism, biological invasions and network analyses: predicting shifts in food-web structure following introductions of free-living hosts and their parasites. Advances in Ecological Research.

4.1 Summary

P. laevis is a generalist acanthocephalan parasite with a complex lifecycle. Although well studied in some fish final hosts, for small-bodied species of negligible fishery interest, such as bullhead *C. gobio*, minnow *P. phoxinus* and stone loach *B. barbatula*, there is limited information on infections and their ecological consequences, especially in Great Britain, where it is suggested the parasite has an indigenous and non-indigenous range. Thus, parasite infection metrics, the severity of pathological consequences and the trophic consequences of infection were assessed for each fish species, plus, the preferred final host, chub *S. cephalus*, over five rivers that covered their indigenous and non-indigenous range. Infections were apparent in all species from lengths of 41 mm, with prevalences up to 96 % in *C. gobio.* Infection probability tended to increase with fish length. Pathological consequences included penetration of the intestine and embedding into surrounding tissues, including the muscle of the peritoneal cavity. Some significant impacts on condition were recorded, although these varied by species and river. There were general patterns of trophic niche specialisation between the infected and uninfected sub-groups of fish of each host population, with strong trophic niche constriction of hosts in some populations, but with niche divergence in others, suggesting strong context dependency at both population and river levels. There were no differences detected in host infection consequences between the parasite's indigenous and non-indigenous range. Overall, these outputs suggested that these small-bodied fishes could play important roles in the population dynamics of *P. laevis*, with infections then resulting in some important ecological consequences.

4.2 Introduction

The life history traits of parasites, such as their mode of transmission, life-cycle complexity and host specificity, all influence aspects of their population dynamics (Barrett et al. 2008; Archie and Ezenwa 2011). These also affect the consequences for host populations, such as the extent of parasite manipulation (Britton and Andreou 2016; Chapter 3). Host specificity can influence patterns of parasite gene flow between host species (Poulin and Keeney 2008); parasites with high host specificity might have limited gene flow between different host species, leading to strong differentiation between conspecifics infecting different hosts (Archie and Ezenwa 2011). Selection for host-specific parasite manipulation could even result in the development of specificity, even where the parasite is normally a generalist (Section 3.5). However, to understand patterns of parasite population dynamics and potential structuring across host ranges, especially within the same community, firstly requires fundamental knowledge to be developed on the parasite-host relationships and the host consequences of infections at the individual, population and community level.

The consequences of parasitism for individual hosts can include manipulated behaviours, altered foraging performance and modified phenotypic traits (Barber et al. 2000; Hatcher et al. 2006; 2012; Chapter 3). In modifying aspects of the host phenotype, there is also potential for altering their access to food resources via, for example, parasite mediated competition, resulting in shifts in their trophic niche (Krichbaum et al. 2010; Pegg et al. 2015). It has recently been postulated that these alterations to the host phenotype caused by both manipulative and non-manipulative parasites could be an important driver of trophic niche specialisation within host

populations (Britton and Andreou 2016; Section 1.2.5). Trophic niche specialisation is where the population niche comprises of a series of sub-groups of smaller niches formed by individuals that specialise on specific food items (Bolnick et al. 2007; Quevedo et al. 2009). This specialisation could thus develop from the phenotypic alterations that occur as sub-lethal consequences of infection (Britton et al. 2011; Pegg et al. 2015). This could then result in trophic niche constriction, where the infected individuals specialise on a subset of the prey items already being consumed by uninfected individuals, or trophic niche divergence, where the infected individuals consume alternative prey items to uninfected conspecifics due to, for example, habitat partitioning caused by manipulation, or altered foraging success caused by a modified functional trait (Britton et al. 2011; Pegg et al. 2015). This divergence would then result in an increased population trophic niche (Britton and Andreou 2016; Section 1.2.5).

The generalist parasite *P. laevis* has a complex lifecycle that uses *G. pulex* as its intermediate host, but a number of different final fish host species (Figure 1.1). In Great Britain, its distribution is discontinuous, with a number of different strains apparently having developed due to its initial biogeography and subsequent spread (Kennedy et al. 1989; O'Mahony et al. 2004). Kennedy et al. (1989) suggested that as the continental freshwater cyprinid fishes colonized post-glacial mainland Britain via the eastward-flowing rivers and the Thames-Rhine link, they also brought *P. laevis*. They then argued that the more extensive distribution of *P. laevis* in Britain today results from: (1) the early formation of a marine strain that colonized the Baltic and North Sea and estuaries of North Sea rivers, (2) later deliberate transfers of infected *B. barbus* to western flowing English rivers from the River Thames (e.g. Antognazza et al. 2015), and (3) anthropogenic transfers to Ireland of infected cyprinids from England **(**Kennedy et al.

1989). This suggests that within mainland Great Britain, there will be indigenous *P. laevis* populations in eastern flowing rivers, such as the Thames catchment, and non-indigenous populations, in more western flowing rivers that resulted from fish translocations, such as in the River Severn catchment. This then provides opportunities for testing host infection consequences in the *P. laevis* indigenous and non-indigenous ranges. In addition, studies on *P. laevis* infections have tended to focus on specific fishes that are relatively large-bodied and usually have some fishery interests, such as *S. cephalus*, *A. anguilla* and *S. trutta* (Kennedy et al. 1978; Bates and Kennedy 1991; Dezfuli 1991). This means that for populations of fish species which the parasite can infect and are present in large numbers in many British lowland rivers, such as *C. gobio, B. barbatula* and *P. phoxinus,* there is limited knowledge on the parasite dynamics and consequences, despite the possibility of these fishes playing key roles in maintaining the populations of this parasite and perhaps affecting selective pressures for parasite manipulation in *G. pulex* (Section 3.5).

Consequently, the aim of this chapter was to investigate the pathological and ecological consequences of *P. laevis* infections for four fish species, of which three tend to be numerically dominant but rarely studied in Great Britain (*C. gobio*, *B. barbatula*, *P. phoxinus*). This was completed across five fish communities; three from the *P. laevis* indigenous range in the River Thames catchment, and two in their non-indigenous range where their presence in the two rivers was likely to be due to introductions of *B. barbus* **(**Kennedy et al. 1989; Antognazza et al. 2015; Table 4.1). Objectives were to: (1) assess parasite infection metrics and the association with fish length for each fish species and river; (2) assess the severity of pathological consequences of infection by *P. laevis* across the host fishes; (3) determine the trophic consequences of *P. laevis* infection for

each fish species and river, including assessment of whether infection is a driver of trophic niche specialisation; and (4) determine any differences in the parasite-host relationship between the *P. laevis* indigenous and non-indigenous range.

4.3 Materials and Methods

4.3.1 Sample collection and initial data collection

Five rivers were investigated for their infections of *P. laevis* in the fish community. All were lowland rivers in England where infections were known to be apparent in at least one fish species (Table 4.1).

Table 4.1. Rivers, locations, characteristics, sampling dates and species studied for investigating the host-parasite dynamics of *Pomphorhynchus laevis***,** where (NI): non-indigenous *P. laevis* population; (I) indigenous *P. laevis* population; Cg: *Cottus gobio*; Bb: *Barbatula barbatula*; Pp: *Phoxinus phoxinus*; Sc: *Squalius cephalus*. Note Avon was the Hampshire Avon. NGR = national grid reference.

River	Location (NGR)	Mean width (m)	Mean depth (m)	Sampling dates	Species sampled
Avon (NI)	SU148095	15	1.6	May 2015	Cg, Bb, Pp
Darent (I)	TQ517605	4	1.5	Jul 2014 - Sep 2015	Cg, Bb, Pp, Sc
Kennet (I)	SU528661	3	1	June 2015	Cg, Bb, Pp
Loddon (I)	SU747680	7	1.4	June 2015	Cg, Bb, Pp
Teme (NI)	SO734558	10	1.5	October 2015	Cg, Bb, Pp

Fish samples were collected by electric fishing using a back-mounted Smith-Root LR-24 Backpack (50 MHz pulsed DC at approximately 2 Amps). The exception was the Hampshire Avon, where the fish samples were contaminants of invertebrate kick samples in a sweep net, collected during sampling for infected *G. pulex*. With the exception of the River Darent, only samples of bullhead *C. gobio*, stone loach *B. barbatula* and minnow *P. phoxinus* were removed from each river on each sampling occasion to avoid conflict with fishery interests and to enable focus on the numerically dominant species in the fish communities. The River Darent was the only river where repeat sampling was completed, with samples collected in July 2014, January 2015, April 2015 and September 2015. Following sampling, fish were sorted in water-filled aerated containers, with a maximum of 30 individuals per species selected randomly and taken back to the laboratory for processing. Samples of putative food items were also collected from each site, focusing on *G. pulex* as these were the dominant macro-invertebrate in all cases.

In the laboratory, the fish were euthanized through an anaesthetic overdose (MS-222), with fork length (or total length if the species has no fork in the tail) and weight of each fish recorded. A detailed post-mortem was then conducted on each fish for specifically detecting infections of *P. laevis* using a standard protocol adapted from Hoole et al. (2001) (Appendix 1). Skin scrapes and internal organs were examined with aid of low and high power microscopy to enable parasite identification. Fish intestinal tracts were removed and where infections were noted then the numbers of individual *P. laevis* were counted. At the completion of this process, a sample of dorsal muscle was then taken for stable isotope analysis (Section 1.4). The muscle samples, along with samples from other fishes and the putative food resources, were then oven dried at 60 ºC until they

achieved constant weight, before processing and analysis at the Cornell Isotope Laboratory New York, USA. At this laboratory, each sample was prepared by grinding and then weighing approximately 0.5 mg into a tin cup, with the actual weight recorded accurately using a Sartorius MC5 microbalance. The samples were then analysed for their carbon and nitrogen isotopes using a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer. The outputs from the spectrometer included data on the carbon and nitrogen stable isotope ratios that could be then be expressed relative to conventional standards as $\delta^{13}C$ and $\delta^{15}N$, respectively (Section 1.4), where $\delta^{13}C$ or $\delta^{15}N$ = [Rsample/Rstandard-1] x 1000, and R is $\delta^{13}C$ / $\delta^{12}C$ or $\delta^{15}N$ / $\delta^{14}N$. Standards references were Vienna Pee Dee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. A standard of animal (mink) was run every 10 samples to calculate an overall standard deviation for both $\delta^{15}N$ and $\delta^{13}C$ to ascertain the reliability of the analyses. The overall standard deviation of the animal standard was not more than 0.23 ‰ for $\delta^{15}N$ and 0.14 ‰ for $\delta^{13}C$. The initial stable isotope data outputs were then in the format of delta (δ) isotope ratios expressed per mille (‰).

To account for any variations in the isotopic baseline between rivers that would affect trophic comparisons of species, $\delta^{15}N$ and $\delta^{13}C$ ratios were corrected for each species based on the isotopic signatures of benthic invertebrate primary consumers (Jackson and Britton 2014). Trophic position (TP) of the fish was calculated using the following equation: $TPi = [(\delta^{15}N_i - \delta^{15}N_{base})/3.4]+2$, where TP_i is the trophic position of the fish, $\delta^{15}N_i$ is the isotopic ratio of the fish, $\delta^{15}N_{base}$ is the isotopic ratio of primary consumers, 3.4 is the fractionation between trophic levels and 2 is the trophic position of the baseline organism (Post 2002). The mean $\delta^{15}N$ value of several individuals of *G. pulex* was used as the baseline for each river. Fish $\delta^{13}C$ was also corrected, based on isotopic

data of *G. pulex* in the rivers, following Olsson et al. (2009): $\delta^{13}C_{corr} = (\delta^{13}C_i - \delta^{13}C_{mean})/$ CR, where $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of the fish, $\delta^{13}C_i$ is the uncorrected isotope ratio of the fish, $\delta^{13}C_{mean}$ is the mean invertebrate isotope ratio and CR is the invertebrate carbon range (δ^{13}Cmax - δ^{13}Cmin).

4.3.2 Histopathology

Histopathology of the intestinal tract and, occasionally, the surrounding tissues, was completed to assess the pathological changes associated with *P. laevis* infection. These tissue sections were fixed in Bouin's fixative for 24 hours before transferring to 70 % Industrial Methylated Spirit (Pegg et al. 2015). The tissues were trimmed, dehydrated in alcohol series, cleared and then embedded in paraffin wax. Transverse and longitudinal sections of 3 µm were dried at 50 °C, stained using Mayer's haematoxylin and eosin, and examined microscopically for pathological changes and described accordingly (Pegg et al. 2015).

4.3.3 Data analysis

For *C. gobio*, *B. barbatula* and *P. phoxinus*, all analyses for the River Darent samples (other than stable isotope analyses) were performed on the mean values taken from across the seasonal samples due to low sample size and low variation between seasons for these species. For all species and rivers, infection levels of *P. laevis* per fish species were described as their prevalence (number of infected individuals/ total number processed x 100) and abundance (number of *P. laevis* per infected individual per fish species). Hereafter, where an individual fish is referred to as either infected or non-infected, it refers to the presence/ absence of *P. laevis* in that individual during the post-mortem. Condition was calculated as Fulton's condition factor (K), using the equation:

$K = W/L^3$, where K = Fulton's condition factor, W = the weight of the fish, and L is the length (Nash et al. 2006).

The trophic ecology of the fish populations per river was analysed using the stable isotope data. Comparison of the trophic niches of the population, and the infected and uninfected sub-groups, were completed using standard ellipse areas (SEA_c) using the SIBER package (Jackson et al. 2011) in the R computing program (R Development Core Team, 2013). This is a bivariate measure of the distribution of individuals in trophic space; each ellipse encloses ~ 40 % of the data and, therefore, represents the core dietary niche, indicating typical resource use within the analysed group of individuals (Jackson et al. 2011; Jackson et al. 2012). The subscript 'c' in SEA_c indicated that a small sample size correction was used due to the limited number of fish sampled was from some populations. Where SEA_c overlapped between the infected and uninfected fish, the percentage of overlap was calculated to indicate the extent to which they shared food resources. This metric of SEA_c has been widely applied to describing the dietary niche of a wide range of species in recent years (e.g. Grey and Jackson 2012; Guzzo et al. 2013; Abrantes et al. 2014). Note for the River Darent, samples were analysed for stable isotopes from the seasonal samples collected in summer, winter and spring, but not autumn. Samples from the latter period were not included due to their likely high similarity to those collected in summer due to the relatively small time interval between sampling that would have given insufficient time for isotopic turnover to equilibrium in tissues, especially for the larger bodied *S. cephalus*, as turnover is a function of time, temperature and body mass (e.g. Vander Zanden et al. 2015).

4.3.4 Statistical analysis

Differences in body weight and condition between infected and uninfected fish were assessed in general linear models, combining data from each river for each species. The model structure thus had weight or condition as the dependent variable, infection status as the independent variable, and, for weight, length as the covariate to control for its effect on weight (Garcia-Berthou 2001). In all cases, to correct for the inflated number of residual degrees of freedom that would have occurred in the model if the data of individual fish were used as true replicates, models were fitted with river as a random effect on the intercept. The significance of the difference in weight and condition between the groups was determined by linearly independent pairwise comparisons of estimated marginal means, adjusted for multiple comparisons (Bonferroni). In all these models, the dependent variables were all log-transformed to meet assumptions of normality of residuals and homoscedasticity. As infection status was binomial (0 = uninfected, 1 = infected), binary logistic regression was used to build probability of infection (PoI) models that determined PoI from the length data of each individual fish per species from all rivers (for *C. gobio*, *B. barbatula* and *P. phoxinus*) or seasons (for *S. cephalus*) using Equation 4.1: $e^{(a+bx)}/ 1+e^{(a+bx)}$, where *a* and *b* were the regression coefficients, and x was fish length. Other than the stable isotope analyses, all analyses were completed in SPSS v. 21.0.

4.4 Results

4.4.1 Bullhead Cottus gobio

Parasite prevalence across the populations ranged between 50 and 96 % (mean: 71.2 % ± 9.1 %) and abundance between 1 and 38 (mean= 5.2 ± 0.76) (Table 4.2). Combining these data across the populations into the binary logistic regression model revealed that

the influence of length on infection status of individuals was significant, with larger individuals having a higher probability of being infected (Table 4.3; Figure 4.1). When comparing infected versus uninfected fish, differences were significant in both their condition (GLM: $F_{1,107} = 6.00$; $P = 0.02$) and weight when controlled for length (GLM: $F_{1,107} = 3.87$; $P = 0.05$), with infected individuals having higher condition factors and weight. In the latter model, the effect of length as the covariate was also significant ($P < 0.01$).

The pathology of infection in the intestine itself indicated little host response, with the site of penetration looking unremarkable; despite penetration of the epithelium and intestinal wall, the surrounding tissue appeared relatively unaffected (Figure 4.2A). In some cases (n=8), the parasite had penetrated straight through the intestine and into surrounding tissues, including the muscle of the peritoneal cavity (Figure 4.3), with the proboscis extending through the dermis up toward the epidermis as far as the basement membrane (Figure 4.3B). There was then resultant inflammation, degeneration and localised necrosis of the muscle (Figure 4.3C, D).

For the stable isotope data analysis, data for *C. gobio* from the Hampshire Avon were not compared between the uninfected and infected sub-groups due to the low proportion of uninfected fish in samples (Table 4.2). For the other four populations, whilst there was some variability in the patterns of their trophic niches, they all indicated some trophic niche specialisation (the infected and uninfected sub-groups of fish) (Table 4.4; Figure 5.5). For the fish from the River Kennet and Loddon, the trophic niches of the infected sub-group were substantially smaller than the uninfected, and generally sat within the same isotopic space (Figure 5.5). This indicated their trophic response to

infection was niche constriction and thus had little influence on the size of the overall population trophic niche. In contrast, for fish from the Rivers Teme and Darent, the infected sub-group had substantially larger niche sizes than the uninfected fish (Table 4.4), with their niches also showing substantial divergence (Table 4.4; Figure 4.5). The consequence was an increased population trophic niche size due to the infections (Table 4.4; Figure 4.5).

Table 4.2. Mean length and length ranges of all, infected (I) and uninfected (U) *Cottus gobio* per river, and the number sampled (n), parasite prevalences ('Prevalence') and abundances (of those infected) ('Abundance').

	Mean length (mm)			Length range (mm)			n	Prevalence (%)	Abundance	
River	All	I	U	All	I	U			Mean	Range
Teme	49.0 ± 1.2	50.6 ± 1.2	47.4 ± 2.0	37-55	44-55	37-55	20	50	3.9 ± 0.9	1-11
Kennet	72.3 ± 2.3	74.0 ± 2.6	65.4 ± 4.2	57-99	57-99	58-82	26	81	4.1 ± 1.5	1-27
Loddon	55.8 ± 1.6	56.5 ± 2.7	55.0 ± 1.9	41-80	41-80	46-68	28	50	2.5 ± 0.7	1-8
Avon	58.0 ±1.4	57.8 ± 1.4	64.0 ± 0.0	48-73	48-73	64	26	96	5.8 ± 1.0	1-26
Darent	68.1 ± 2.6	69.0 ± 3.2	65.0 ± 3.4	49-93	49-93	55-69	18	77	9.2 ± 3.3	1-38

Table 4.3. Binary logistic regression coefficients (Equation 4.1), and their statistical significance, for the probability of infection of *Cottus gobio* by *Pomphorhynchus laevis* according to fish length.

Parameter	Symbol in equation 4.1	Coefficient	Standard error	*P*
Constant	a	-2.69	1.38	0.05
Fish length	x	0.06	0.02	0.01

Table 4.4. Mean stable isotope data per *Cottus gobio* population and their trophic niche size according to standard ellipse area (SEA_c, after correction to trophic position and Ccorr) of the sampled population ('Population'), and the uninfected (U) and infected (I) sub-groups, and the extent of the trophic niche overlap between the two sub-groups.

River	Mean $\delta^{13}C$ (‰)		Mean $\delta^{15}N$ (‰)		SEA_c			
	U	I	U	I	Population	U	I	Overlap (%)
Teme	-30.0 ± 0.1	-30.5 ± 0.1	13.7 ± 0.1	13.4 ± 0.1	0.02	0.01	0.02	0.0
Kennet	-31.1 ± 0.8	-31.0 ± 0.2	13.0 ± 0.4	13.1 ± 0.1	0.04	0.10	0.02	1.7
Loddon	-29.7 ± 0.4	-30.0 ± 0.3	19.1 ± 0.2	19.3 ± 0.1	0.08	0.08	0.06	4.2
Avon	-31.4 ± 0.0	-31.4 ± 0.4	13.7 ± 0.0	12.0 ± 0.2	-	-	-	-
Darent	-28.8 ± 0.2	-28.6 ± 0.1	16.0 ± 0.1	15.5 ± 0.1	0.02	0.01	0.02	0.0

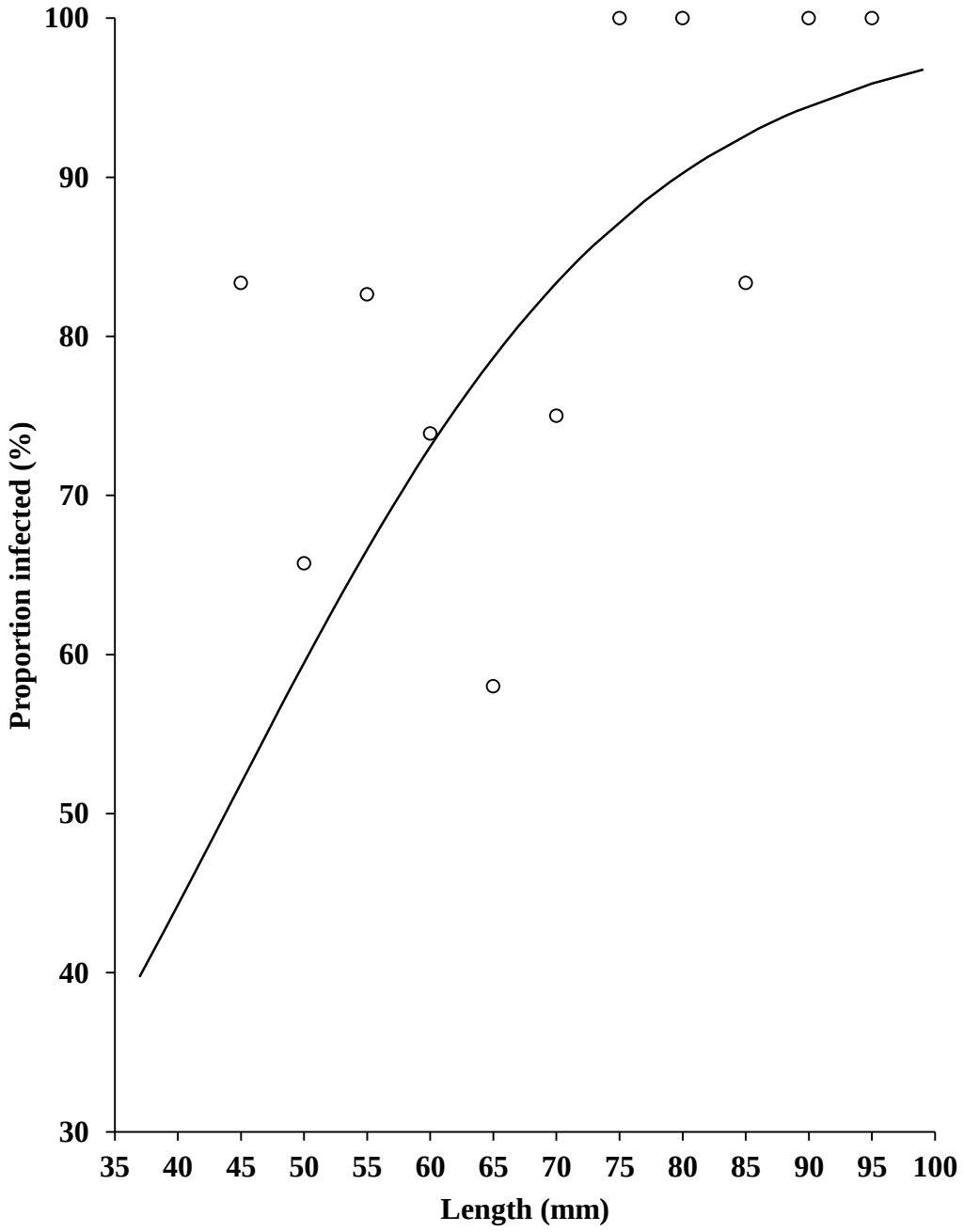

Figure 4.1. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Cottus gobio* by *Pomphorhynchus laevis* according to length (as 5 mm increments), where hollow circles represents the proportion of infected individuals in that size class and the solid line is the relationship between fish length and the probability of infection according to binary logistic regression (cf. Table 4.2).

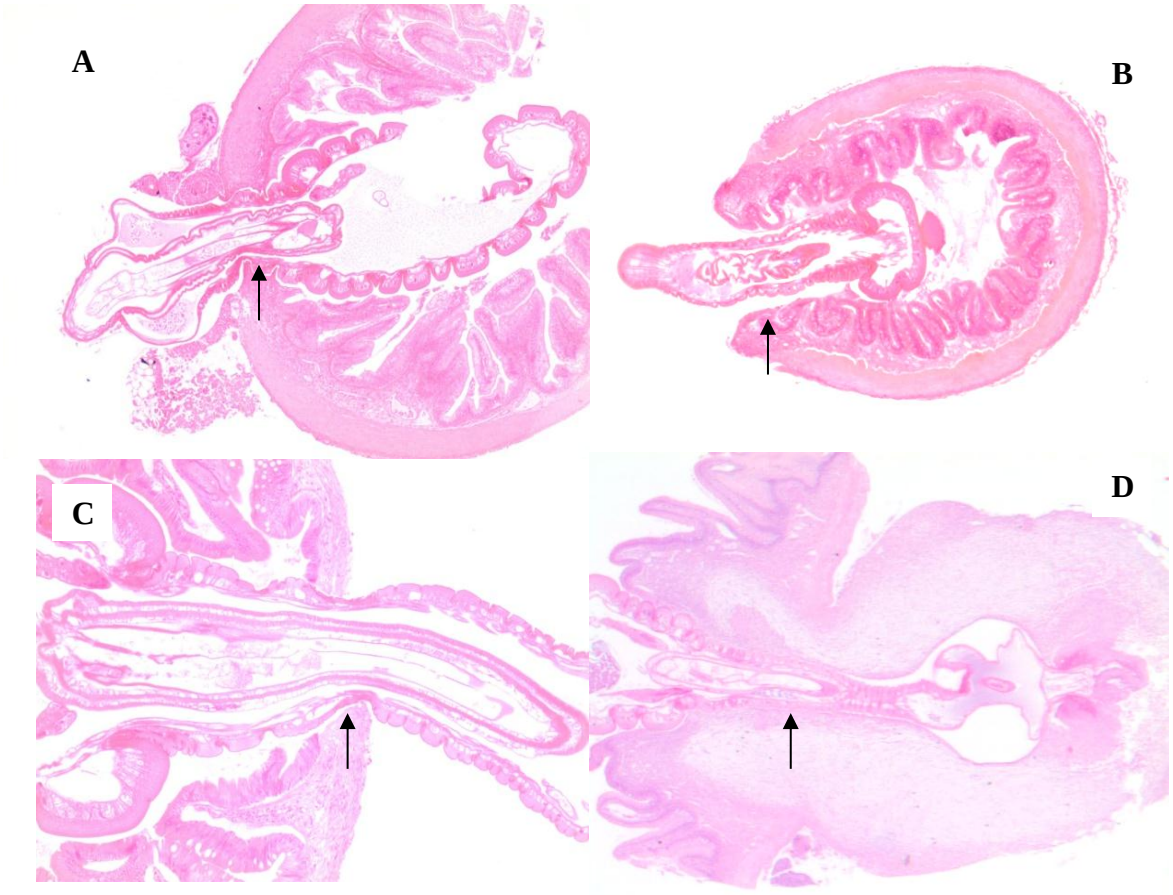

Figure 4.2. Transverse gut sections showing the resulting pathology of *Pomphorhynchus laevis* infection in A) Bullhead *Cottus gobio* with some evidence of inflammation surrounding the bulb of the parasite, with leaking of necrotic cells into the peritoneum. Sections of the intestine beyond the immediate site of attachment remain relatively normal. B) Stoneloach *Barbatula barbatula*, showing complete penetration of the gut, but limited host response to infection with absence of inflammatory response. This may be indicative of very recent infection, or potentially differences in immunological and cellular responses of difference fish species. C) Minnow *Phoxinus phoxinus* showing similar reaction to B; complete penetration of all layers of the gut, with mechanical compression and localised loss of epithelium adjacent to the neck of the parasite, but very little in the way of host response. D) Chub *Squalius cephalus*, showing massive fibrogranulomatous lesion surrounding the parasite and extending

through all layers of the gut. The neck, bulb and proboscis have been engulfed by host tissue. This comprises loose connective tissue with eosinophillic granular cells and lymphocytes, increasing in severity around the proboscis of the parasite. In all cases the worm has penetrated the gut wall with the site of penetration indicated by the black arrows.

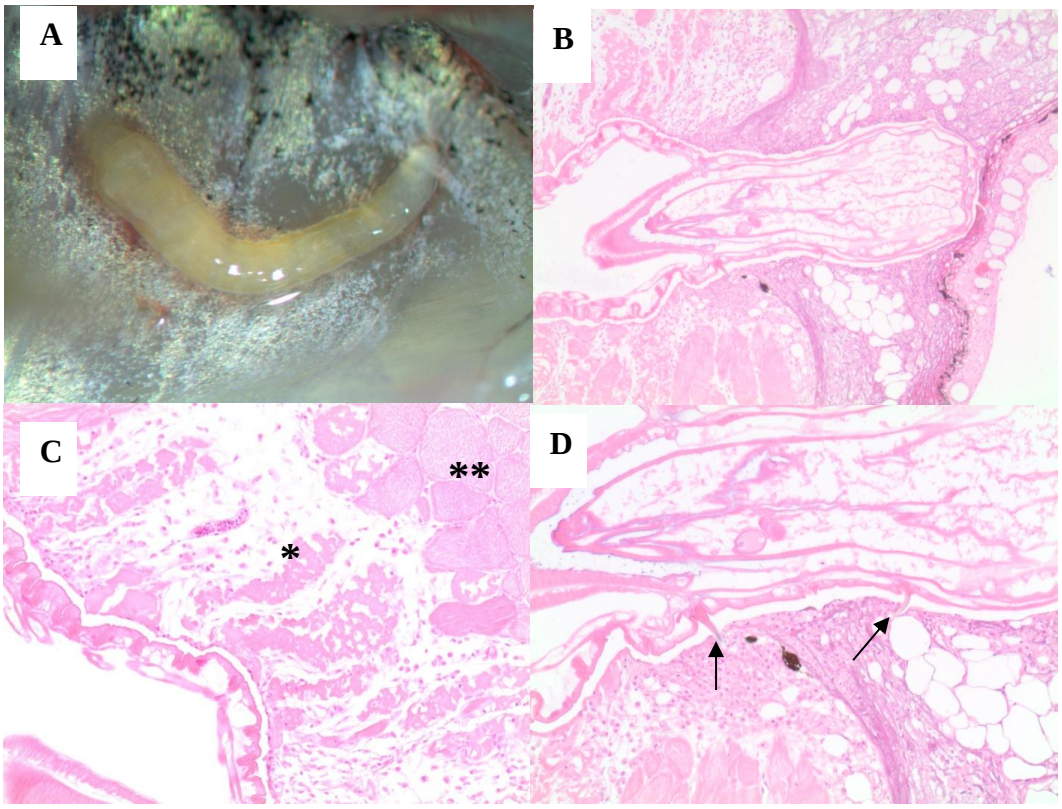

Figure 4.3. Pathology of infection with *Pomphorhynchus laevis* in the peritoneal muscle of *Cottus gobio*, showing: (A) *P.laevis* attached in body wall of 'host species' following total penetration of intestinal tract. The neck, bulb and proboscis of the parasite is deeply embedded within the musculature; (B) Transverse section through body wall, showing deep attachment of the parasite through the body muscle, extending into the dermis as far as the basement membrane of the epidermis, approaching full thickness pathology and perforation of the body (pigment cells of the fish skin can be seen in black); (C) Transverse section through dermis of 'host' showing degeneration of muscle (*) surrounding the neck and body of the parasite. Muscle fibre disruption,

inflammation and myophagia were evident within these regions. Normal muscle can be seen beyond the site of infection (**); (D) High power magnification of proboscis showing spines (arrows) anchoring the parasites firmly in place.

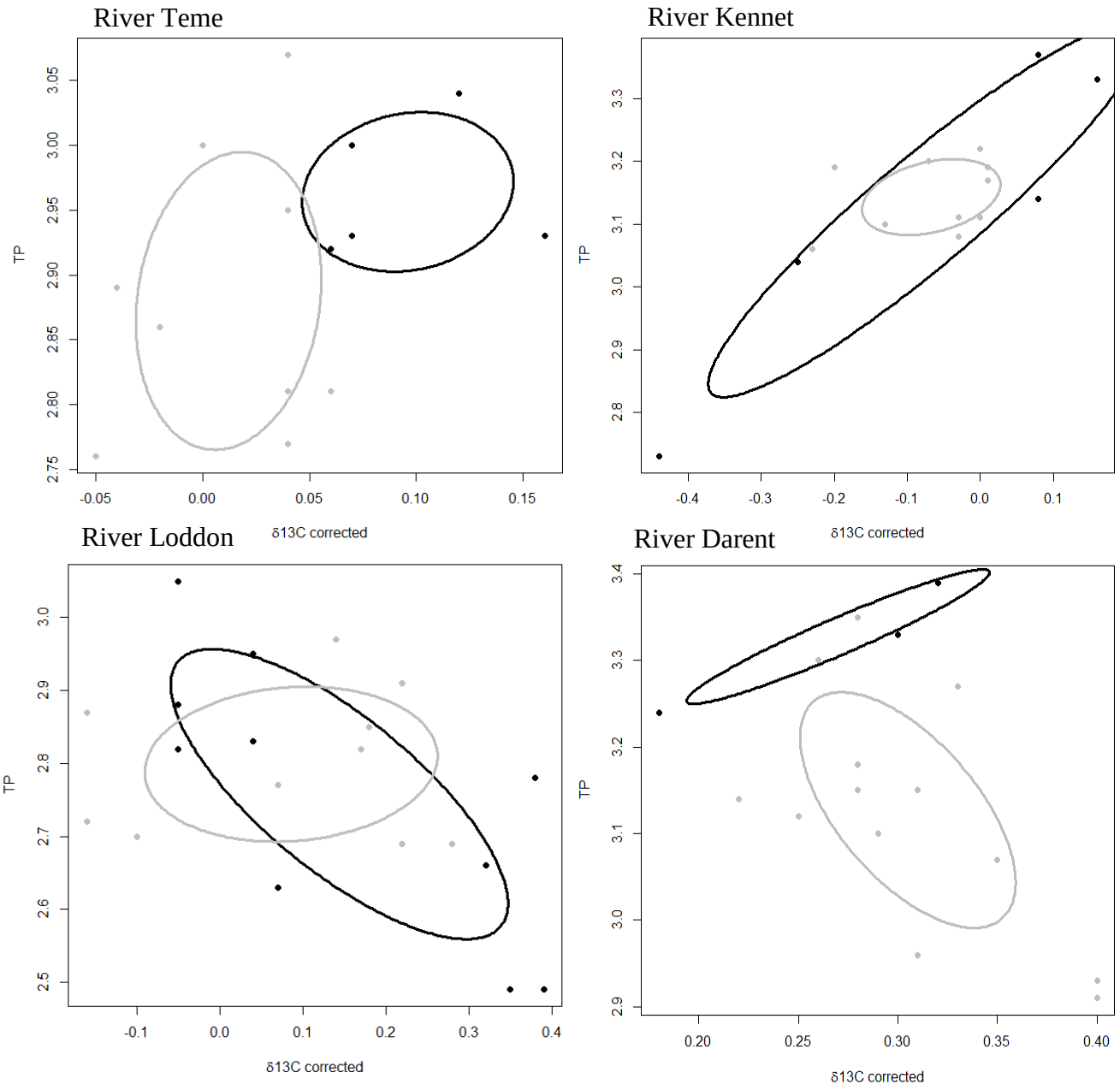

Figure 4.4. Stable isotope biplots for *Cottus gobio* infected (grey circles) and uninfected (black circles) with *Pomphorhynchus laevis* per river, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from TP and Ccorr. Note differences in scales on axes.

4.4.2 Minnow Phoxinus phoxinus

Parasite prevalence across the populations ranged between 29 and 43 % (mean: 35.3 ± 3.1 %) and abundance between 1 and 9 (mean: 2.1 ± 0.2) (Table 4.5). Combining these data across the populations into the binary logistic regression model revealed that the influence of length on infection status of individuals was significant, with larger individuals having a significantly higher probability of being infected (Table 4.6; Figure 4.5). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1, 136} = 3.93$; $P = 0.06$) or weight when controlled for length (GLM: $F_{1, 136} = 0.82$; $P = 0.37$). The effect of length on weight as a covariate was significant in the model ($P < 0.01$).

The pathology of infection in the intestine indicated little host response at the site of intestine penetration and despite penetration of epithelium and intestine wall, the surrounding intestine tissue appeared relatively unaffected (Figure 4.2C). The stable isotope data for *P. phoxinus* for the four populations revealed whilst there was some variability in the patterns of their trophic niches between the infected and uninfected sub-groups, they all indicated some trophic niche specialisation (Table 4.7; Figure 4.6). For the fish from the River Darent and Loddon, the trophic niches of the infected sub-group were substantially smaller than the uninfected, and generally sat within the same isotopic space (Figure 4.6). This indicated their trophic response to infection was niche constriction and thus had little influence on the size of the population trophic niche. In contrast, for fish from the River Avon, the infected sub-group had a larger niche size than the uninfected fish (Table 4.7), with their niche also showing substantial divergence (Table 4.7; Figure 4.6). The consequence was an increased population trophic niche size due to the infection (Table 4.7; Figure 4.6).

Table 4.5. Mean length and length ranges of all, infected (I) and uninfected (U) *Phoxinus phoxinus* per river, and the number sampled (n), parasite prevalences ('Prevalence') and abundances (of those infected) ('Abundance').

	Mean length (mm)			Length range (mm)			n	Prevalence (%)	Abundance	
River	All	I	U	All	I	U			Mean	Range
Avon	49.0 ± 2.9	59.8 ± 3.1	44.7 ± 2.9	31-68	53-68	31-60	14	28.6	1.0 ± 0.0	1
Kennet	53.8 ± 1.2	56.3 ± 2.2	52.4 ± 1.4	44-74	47-74	44-62	30	36.7	2.91 ± 0.8	1-9
Loddon	60.3 ± 1.1	62.5 ± 2.7	59.2 ± 1.1	47-82	47-82	47-69	40	32.5	1.5 ± 0.3	1-5
Darent	60.2 ± 0.8	61.5 ± 0.9	59.2 ± 1.2	42-76	51-70	42-76	60	43.3	2.15 ± 0.3	1-6

Table 4.6. Binary logistic regression coefficients (Equation 4.1), and their statistical significance, for the probability of infection of *Phoxinus phoxinus* by *Pomphorhynchus laevis* according to fish length

Parameter	Symbol in equation 4.1	Coefficient	Standard error	*P*
Constant	a	-5.21	1.85	0.05
Fish length	x	0.08	0.03	0.01

Table 4.7. Mean stable isotope data per *Phoxinus phoxinus* population and their trophic niche size according to standard ellipse area (SEA_c, after correction to trophic position and Ccorr) of the sampled population ('Population'), and the uninfected (U) and infected (I) sub-groups, and the extent of the trophic niche overlap between the two sub-groups.

River	Mean $\delta^{13}C$ (‰)		Mean $\delta^{15}N$ (‰)		SEA_c			
	U	I	U	I	Population	U	I	Overlap (%)
Kennet	-31.9 ± 0.3	-31.2 ± 0.1	12.3 ± 0.2	12.7 ± 0.1	0.04	0.04	0.03	1.0
Loddon	-28.5 ± 0.3	-28.4 ± 0.2	17.3 ± 0.5	17.0 ± 0.4	0.18	0.27	0.12	12.1
Avon	-30.7 ± 0.2	-30.1 ± 0.2	12.1 ± 0.2	13.5 ± 0.2	0.06	0.04	0.04	0.0
Darent	-28.8 ± 0.1	-28.8 ± 0.1	15.1 ± 0.2	15.3 ± 0.1	0.05	0.08	0.02	2.4

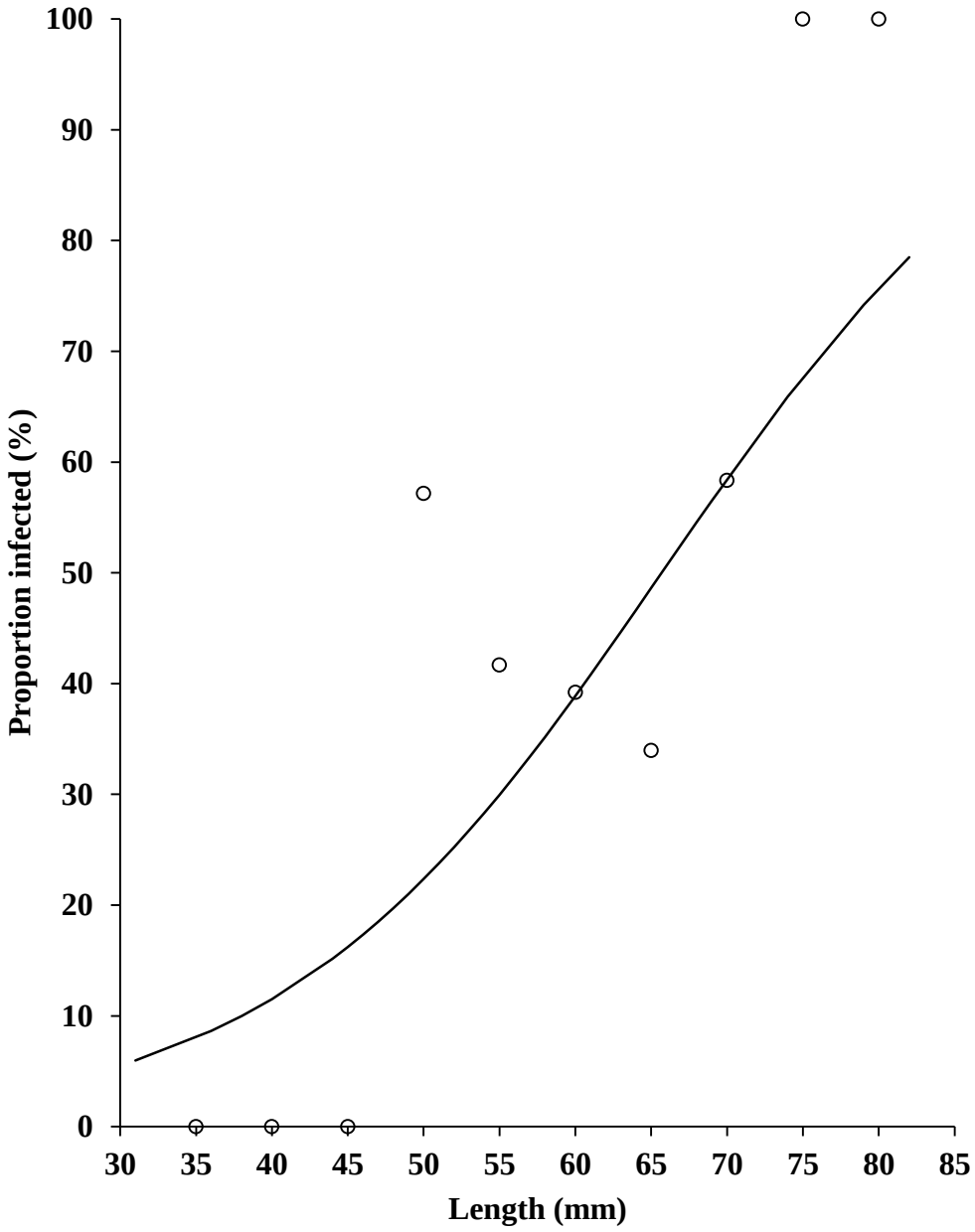

Figure 4.5. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Phoxinus phoxinus* by *Pomphorhynchus laevis* according to length (as 5 mm increments), where hollow circles represents the proportion of infected individuals in that size class and the solid line is the relationship between fish length and the probability of infection according to binary logistic regression (cf. Table 4.6).

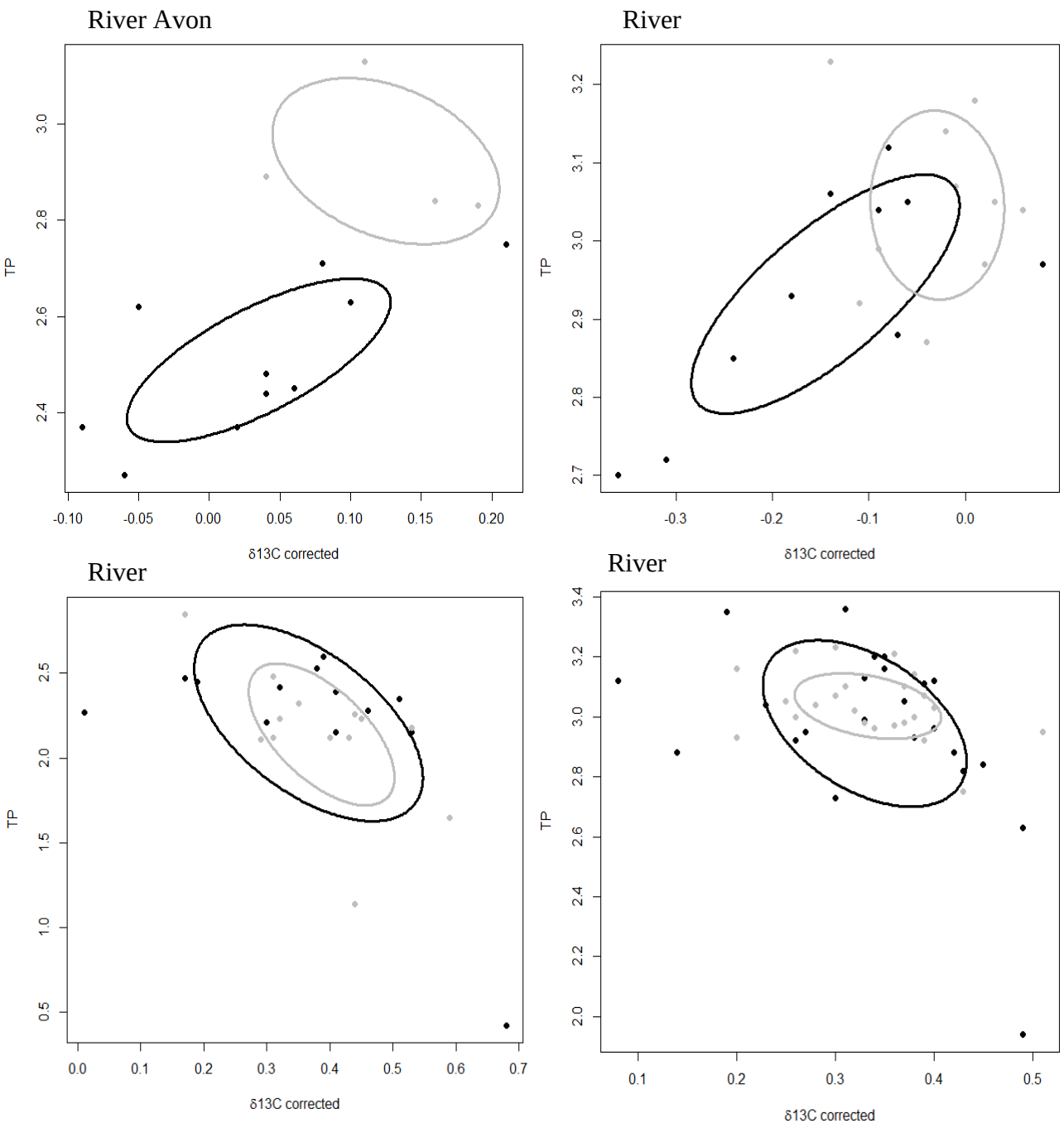

Figure 4.6. Stable isotope biplots for *Phoxinus phoxinus* infected (grey circles) and uninfected (black circles) with *Pomphorhynchus laevis* per river, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from TP and Ccorr. Note differences in scales on axes.

4.4.3 Stone loach Barbatula barbatula

Parasite prevalence across the populations ranged between 50 and 80 % (mean: 68.3 % ± 9.3 %) and abundance between 1 and 6 (mean= 2.0 ± 0.2) (Table 4.8). Combining these data across the populations into the binary logistic regression model revealed that the influence of length on infection status of individuals was not significant (Table 4.9; Figure 4.7). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1, 32} = 0.63$; $P = 0.43$) or weight when controlled for length (GLM: $F_{1, 32} = 1.67$; $P = 0.21$), although length was significant as the covariate ($P < 0.01$).

The pathology of infection in the intestine indicated a limited host immune response with a lack of cellular responses; however mechanical changes were significant with complete penetration of the intestine. (Figure 4.2B). Due to relatively limited sample sizes per river, especially of uninfected fish (Table 4.8), only stable isotope data for *B. barbatula* from the River Teme were analysed. Despite the low sample size, these data indicated some trophic niche specialisation (Table 4.10; Figure 4.7). The trophic niche of the infected sub-group was substantially larger than the uninfected and also showed substantial divergence, resulting in an increased population trophic niche size due to *P. laevis* infection (Table 4.10; Figure 4.7).

Table 4.8. Mean length and length ranges of all, infected (I) and uninfected (U) *Barbatula barbatula* per river, and the number sampled (n), parasite prevalences ('Prevalence') and abundances (of those infected) ('Abundance').

	Mean length (mm)			Length range (mm)			n	Prevalence (%)	Abundance	
River	All	I	U	All	I	U			Mean	Range
Teme	75.1 ± 3.7	75.0 ± 6.0	75.2 ± 5.2	56-89	56-89	56-85	10	50.0	0.8 ± 0.3	1-3
Avon	70.3 ± 9.0	76.0 ± 9.8	53.0 ± 0.0	53-90	57-90	53	4	75.0	2.0 ± 0.6	1-3
Darent	90.4 ± 1.8	89.5 ± 2.3	93.8 ± 2.3	71-110	71-110	89-110	25	80.0	2.2 ± 0.3	1-6

Table 4.9. Binary logistic regression coefficients (Equation 4.1), and their statistical significance, for the probability of infection of *Barbatula barbatula* by *Pomphorhynchus laevis* according to fish length.

Parameter	Symbol in equation 4.1	Coefficient	Standard error	*P*
Constant	a	-1.45	3.63	0.65
Fish length	x	0.02	0.04	0.58

Table 4.10. Mean stable isotope data of *Barbatula barbatula* population in the River Teme and their trophic niche size according to standard ellipse area (SEA_c, after correction to trophic position and Ccorr) of the sampled population ('Population'), and the uninfected (U) and infected (I) sub-groups, and the extent of the trophic niche overlap between the two sub-groups.

Mean $\delta^{13}C$ (‰)		Mean $\delta^{15}N$ (‰)			SEA_c		
U	I	U	I	Population	U	I	Overlap (%)
-30.6 ± 0.1	-29.8 ± 0.2	15.5 ± 0.4	15.3 ± 0.1	0.14	0.07	0.10	1.0

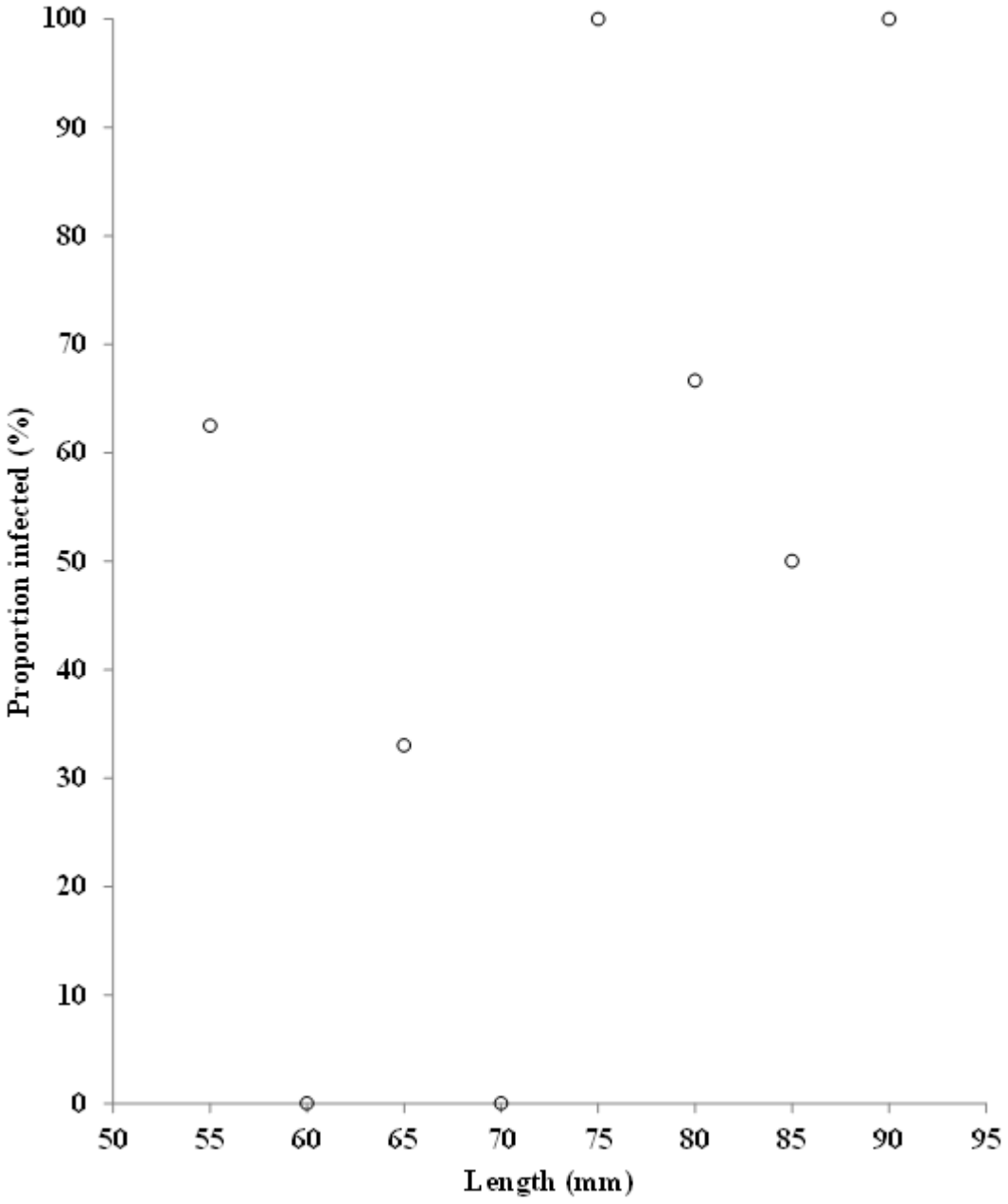

Figure 4.7. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Barbatula barbatula* by *Pomphorhynchus laevis* according to length (as 5 mm increments), where hollow circles represents the proportion of infected individuals in that size class.

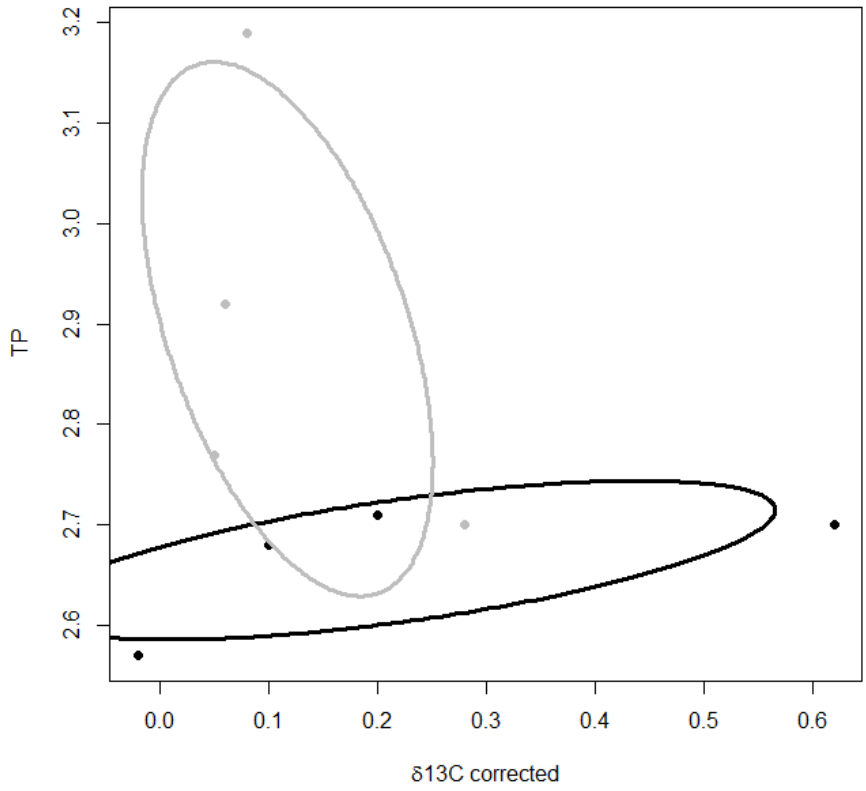

Figure 4.8. Stable isotope biplot for *Barbatula barbatula* infected (grey circles) and uninfected (black circles) with *Pomphorhynchus laevis***,** where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from TP and Ccorr.

4.4.4 Chub, Squalius cephalus

Only fish from the River Darent were assessed here, with samples of *S. cephalus* from the other rivers being unable to be collected. In the Darent population, parasite prevalence across the seasons ranged between 60 and 79 % (mean: 75.5 % ± 4.6 %) and abundance between 1 and 30 (mean: 4.8 ± 0.7) (Table 4.11). Combining these data across the seasons into the binary logistic regression model revealed that the influence of length on infection status of individuals was significant, with larger individuals having a higher probability of being infected (Table 4.12; Figure 4.9). When comparing infected versus uninfected fish, differences were not significant in their weight when controlled for length (GLM: $F_{1,89} = 1.68$; $P = 0.20$). However, infected fish did have significantly lower condition factors (GLM: $F_{1,89} = 10.82$; $P < 0.01$).

The pathology of infection in the intestine of *S. cephalus* indicated marked differences to that observed in the other fishes (Figure 4.2). There was a strong host response in all in infected individuals, with the site of penetration and the immediate surrounding tissue displaying a number of changes (Figure 4.2D). A granulomatous lesion engulfed the neck, bulb and proboscis of the parasite, leading to encapsulation (Figure 4.2D). Looking in greater detail (Figure 4.10), there was loose connective tissue present, with eosinophillic granular cells and lymphocytes (Figure 4.10D). The epithelium, stratum granulosum and muscle layers were replaced by fibrogranulomatous tissue, with increased numbers of lymphocytes throughout all layers with a marked loss of normal intestine architecture (Figure 4.10C, D). The presence of a range of cell types, including firbroblasts and inflammatory cells, indicated a fibrogranulomatous response (Figure 4.10D). Localised changes included flattening of the epithelium, with loss, erosion and necrosis of the mucosa adjacent to the neck (Figure 4.10C). The intestine was

essentially non-functional in these regions. Whilst these are severe changes, they nevertheless remained localised, with adjacent regions of intestine being unaffected (Figure 4.10).

The stable isotope data revealed that across the seasons, there was some variability in the patterns of their trophic niches between the infected and uninfected sub-groups; however, they all suggested some degree of infection-driven trophic niche specialisation (Table 4.13; Figure 4.11). For the fish from the winter and spring samples, the trophic niches of the infected sub-group were substantially smaller than the uninfected, and generally sat within a similar isotopic space (Figure 4.11). This indicated their trophic response to infection was niche constriction and thus had little influence on the size of the population trophic niche. In contrast, for fish from the summer sample, the infected sub-group had a larger niche size than the uninfected fish (Table 4.13), with their niche also showing some divergence, increasing the population trophic niche size (Table 4.13; Figure 4.11).

Table 4.11. Mean length and length ranges of all, infected (I) and uninfected (U) *Squalius cephalus* per season, and the number sampled (n), parasite prevalences ('Prevalence') and abundances (of those infected) ('Abundance').

	Mean length (mm)			Length range (mm)			n	Prevalence (%)	Abundance	
Season	All	I	U	All	I	U			Mean	Range
Autumn 2015	116.8 ± 8.3	131.0 ± 9.8	95.5 ± 10.1	66-170	80-170	66-136	15	60.0	2.2 ± 0.6	1-7
Winter 2015	88.1 ± 11.8	94.5 ± 14.0	64.3 ± 15.6	38-211	42-211	38-92	14	78.6	2.8 ± 0.7	1-8
Spring 2015	117.1 ± 7.5	124.7 ± 9.2	92.4 ± 4.7	64-220	64-220	78-111	30	76.7	5.3 ± 1.2	1-23
Summer 2014	142.6 ± 9.0	149.5 ± 10.5	116.0 ± 13.5	52-253	52-253	60-181	39	79.5	6.0 ± 1.3	1-30

Table 4.12 Binary logistic regression coefficients (Equation 4.1), and their statistical significance, for the probability of infection of *Squalius cephalus* by *Pomphorhynchus laevis* according to fish length.

Parameter	Symbol in equation 4.1	Coefficient	Standard error	*P*
Constant	a	-0.91	0.73	0.21
Fish length	x	0.02	0.01	0.01

Table 4.13. Mean stable isotope data per *Squalius cephalus* sample and their trophic niche size according to standard ellipse area (SEA_c, after correction to trophic position and Ccorr) of the sampled population ('Population'), and the uninfected (U) and infected (I) sub-groups, and the extent of the trophic niche overlap between the two sub-groups.

Season	Mean $\delta^{13}C$ (‰)		Mean $\delta^{15}N$ (‰)		SEA_c			
	U	I	U	I	Population	U	I	Overlap (%)
Winter 2015	-29.1 ± 0.5	-27.9 ± 0.2	14.5 ± 0.7	13.6 ± 0.3	0.12	0.23	0.09	2.7
Spring 2015	-28.3 ± 0.4	-28.0 ± 0.3	14.0 ± 0.3	14.0 ± 0.3	0.09	0.13	0.06	4.9
Summer 2014	-28.4 ± 0.2	-27.9 ± 0.1	13.7 ± 0.2	13.6 ± 0.1	0.08	0.05	0.09	2.8

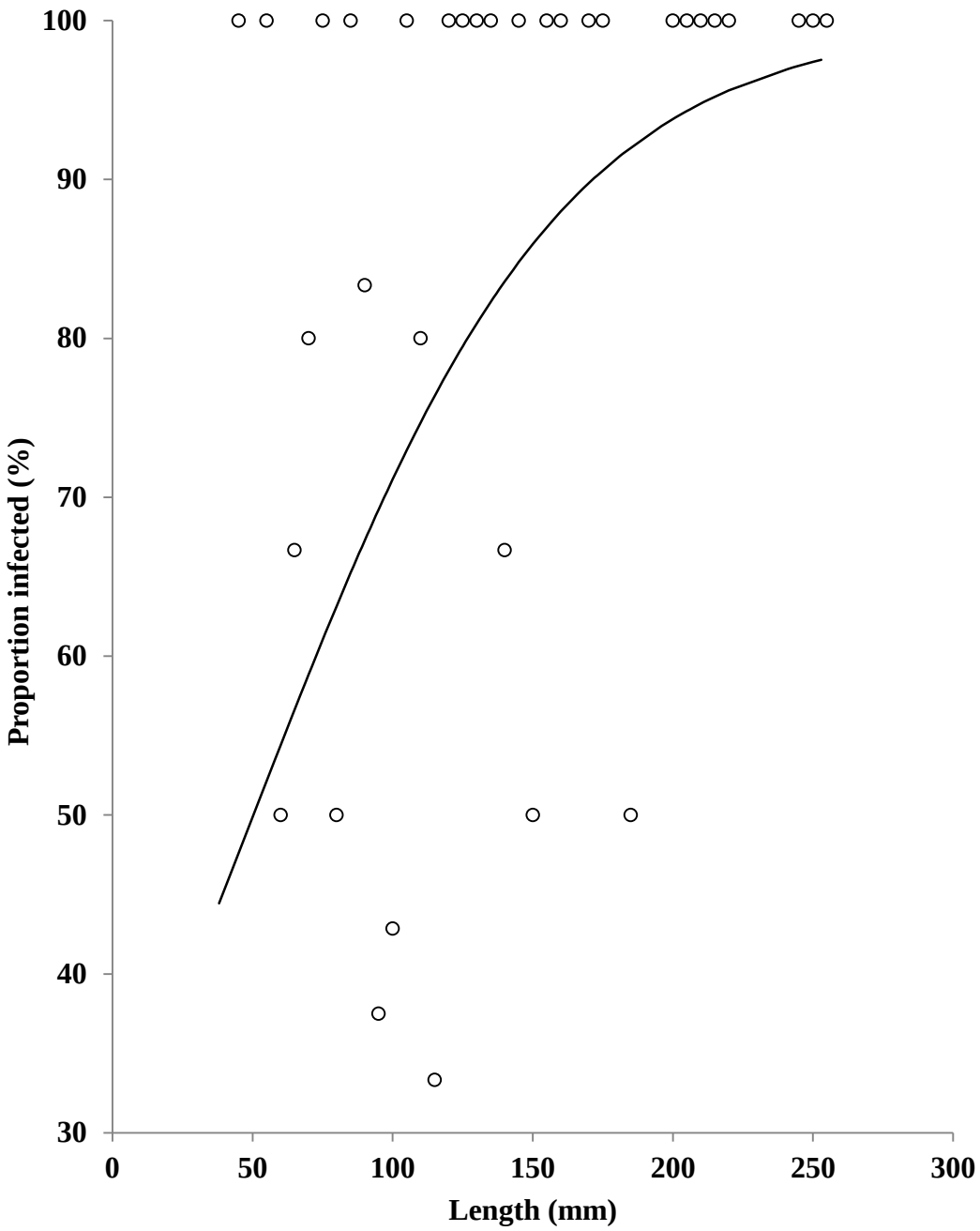

Figure 4.9. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Squalius cephalus* by *Pomphorhynchus laevis* according to length (as 5 mm increments), where hollow circles represents the proportion of infected individuals in that size class and the solid line is the relationship between fish length and the probability of infection according to binary logistic regression (cf. Table 4.12).

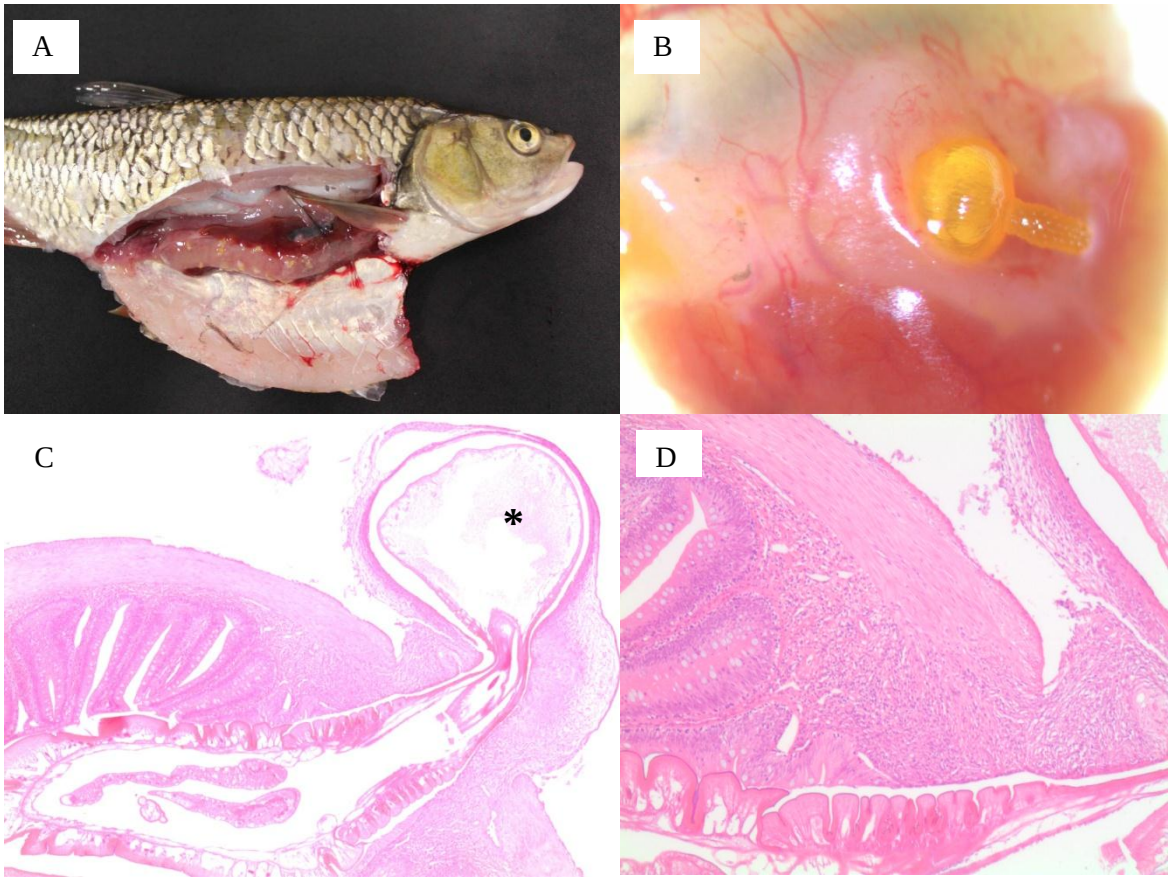

Figure 4.10. A) *Squalius cephalus* infected with several adult *Pomphorhynchus laevis* worms. The worms are visible by their orange coloured proboscis and bulb which can be seen protruding from the gut wall. B) Low power micrograph of a single *P. laevis* showing complete penetration by the bulb and proboscis through the intestine. The raised collar of tissue surrounding the parasite is indicative of a host response. C) Histological transverse section through intestine of *S. cephalus* with a single adult *P. laevis* penetrating through the gut wall, with the proboscis bulb (*) providing firm attachment. The neck can be seen passing through all layers of the gut, allowing the body to extend into the gut lumen. D) Transverse section through gut of *S. cephalus* immediately surrounding the neck of the worm showing loss of normal gut architecture in the vicinity of the parasite. There is compression and loss of epithelium adjacent to

the neck of the parasite, with fibrogranulomatous tissue extending through the stratum compactum and replacing normal muscle layers.

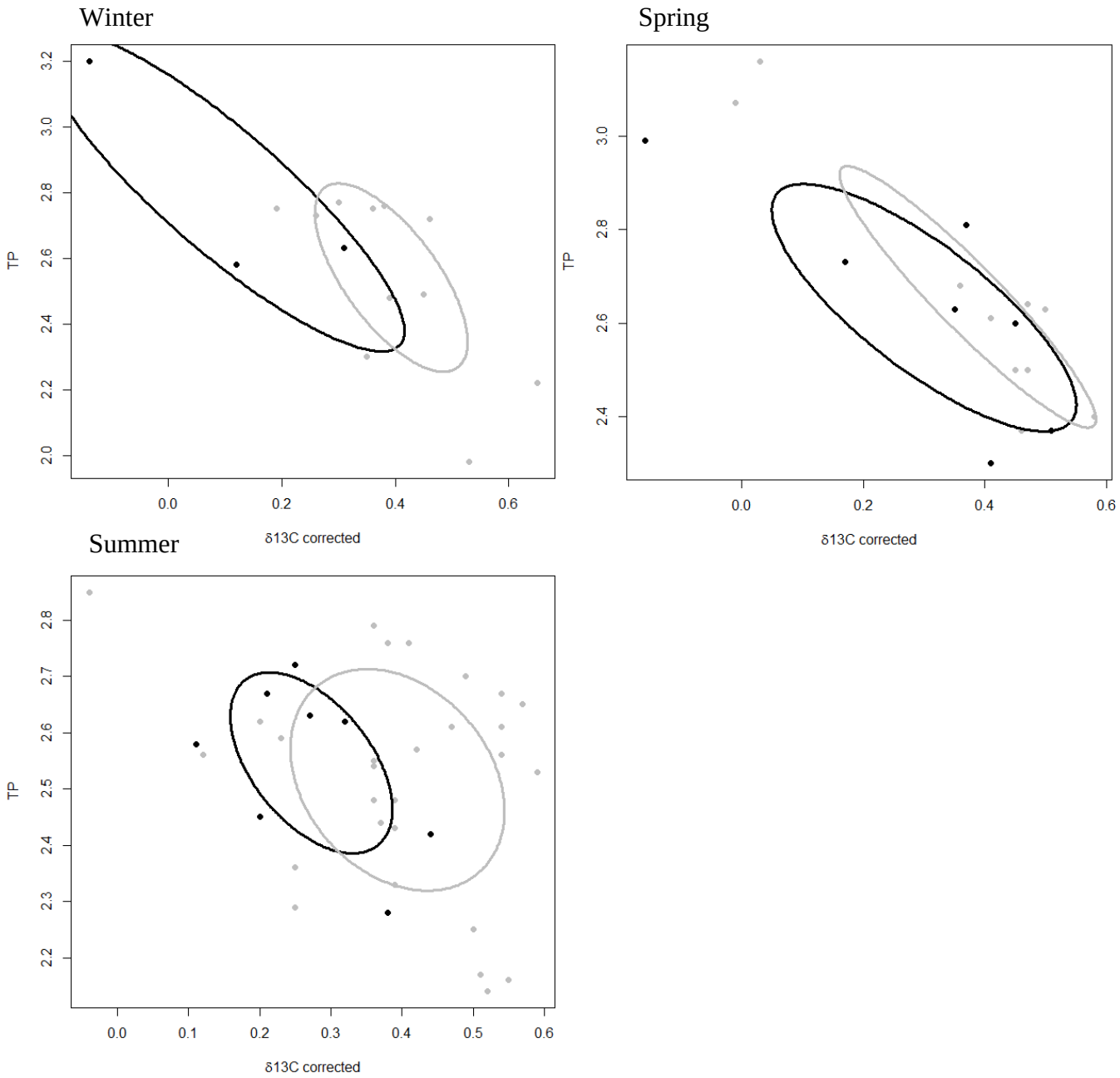

Figure 4.11. Stable isotope biplots for *Squalius cephalus* infected (grey circles) and uninfected (black circles) with *Pomphorhynchus laevis* per season, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses

denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from TP and Ccorr.

4.5 Discussion

In each river, the analysed host populations were fishes within multi-species communities. With the exception of three-spined stickleback *G. aculeatus*, for which fish were sampled but infections were not detected and thus were not reported here, infections by *P. laevis* were present in all analysed species. Also present in samples from the River Darent were species including gudgeon *Gobio gobio* where sample sizes were insufficient to warrant further analysis but where prevalences exceeded 50 % in summer samples. In addition, evidence from literature suggests that for species not analysed here for fishery reasons, such as *L. leuciscus*, *S. trutta* and *B. barbus*, also act as final hosts (Harris 1972; Kennedy et al. 1978; Dezfuli et al. 2001; Laimgruber et al. 2005; Djikanovic 2010). Moreover, fish from 41 to 253 mm were infected, with no larger fish included in the study, suggesting that there was low host and size-specificity in this parasite across the rivers, with recent data on juvenile fish from the River Teme also suggesting that *B. barbus* as small as 20 mm standard length can develop infections (C. Gutmann-Roberts, personal communication). Thus, these outputs indicate the use of a wide range of fish final host species and across a very broad length range.

Given this apparent low host and size-specificity, it is important to derive more understandings of aspects of the parasite population dynamics. Numerically at least, observations suggested that *C. gobio* and *P. phoxinus* were the dominant fishes in these rivers, being regularly captured in macro-invertebrate kick-samples as well as electric fishing, with *B. barbatula* also present in particularly high abundance in the River Teme at the time of sampling (JR Britton, personal communication). It should be noted, however, that the size range of these fishes and their habitat preferences make

population estimates difficult, with specialist point abundance electric fishing usually required (Carter et al. 2004) which was not possible here for logistical reasons. Irrespective, parasite prevalence tended to be high in these small-bodied fishes, with up to 96 % of all sampled *C. gobio*, up to 43 % of *P. phoxinus* and up to 80 % of *B. barbatula* being infected, with these being relatively high levels of prevalence compared to previous studies on these species from similar rivers and other *P. laevis* populations, although there was considerable variability in these data with prevalences recorded between 0 and 89 % across the species and studies (Table 4.14). This combination of generally high prevalence and abundance of these fishes, at least in the rivers in this study, suggest that these small-bodied species could play key roles in the population dynamics of *P. laevis* by providing large abundances of highly susceptible final hosts that will forage on prey populations that include a high proportion of intermediate hosts. These infected small fishes can also then also infect larger fishes through post-cyclic transmission via their predation (Kennedy 1999; Medoc et al. 2011), resulting in larger-bodied species, such as adult *S. cephalus*, *A. anguilla*, pike *Esox lucius* and brown trout *S. trutta* becoming vulnerable to infection at size ranges when they would be least likely to predate upon *G. pulex*. Given this high numerical abundance of available final hosts, it could be argued that selection pressures were likely to be low for species-specific manipulation behaviours by the parasite on the intermediate host, as suggested in Section 3.5.

Nevertheless, Hine and Kennedy (1974) suggested that in the River Avon, only *S. cephalus* and *B. barbus* were preferred hosts of *P. laevis*, with parasites occasionally maturing in *S. trutta* and *L. leuciscus*, but with the parasite not growing or maturing in other host species. Conversely, other studies have indicated that *C. gobio* are a suitable

final host in which *P. laevis* can complete its life cycle (Rumpus 1975; Kennedy 1996; 1999, Lagrue et al. 2007) and there were also mature *P. laevis* found in *C. gobio* from all rivers in this study, albeit in relatively low proportion. Rumpus (1975) also reported mature *P. laevis* in very low numbers in *B. barbatula* from the River Avon. In combination, these suggest that these small fishes are likely to play at least a partial role in the successful completion of the parasite lifecycle in these rivers, potentially leading to some selective pressures for their infection via intermediate hosts, given that these fishes are present in relatively high numbers when compared with species such as *S. cephalus*. Plus, these fishes also facilitate completion of the *P. laevis* lifecycle through post-cyclic transmission (Kennedy 1999; Medoc et al. 2011), with these species likely to comprise a proportion of the diet of larger cyprinids such as *B. barbus* (Basic et al. 2015). Thus, it can be argued that these small bodied, highly abundant fishes might actually play a major role in the maintenance of *P. laevis* populations in these rivers through providing large numbers of hosts of vulnerable size ranges to infection due to their frequent feeding on macro-invertebrates such as *G. pulex* as soon as they achieve gape sizes when these can be prey upon and ingested (e.g. Andersson et al. 1986; Davey et al. 2006). The ecological consequences of infection then become important to understand, such as their trophic and food web impacts. In addition, they suggest that there is potential for the development of specificity in this generalist parasite, such as via selection for host-specific parasite manipulation (Section 3.5). It is thus recommended that further work on this is completed in due course to understand whether there is the development of host-specificity between the different fishes of the community, and how this affects parasite population dynamics and genetic structuring and how this affects gene flow in their overall population.

Table 4.14. Summary of *Pomphorhynchus laevis* prevalence data in *Cottus gobio*, *Phoxinus phoxinus* and *Barbatula barbatula* from studies completed across European freshwaters. Prevalence represents parasite prevalence GB = Great Britain, Fr = France, Sl = Slovakia; Note that abundance has not been included due to variation in the method of calculation across the studies.

Host species	**River**	**Sampling date(s)**	**Number (N)**	**Prevalence (%)**	**Source**
Cottus gobio	Otter (GB)	June 1994	32	50.0	Kennedy 1996
C. gobio	Ouche (Fr)	Spring 2005	8	0.0	Medoc 2011
C. gobio	Vingeanne (Fr)	Spring 2005	1	0.0	Medoc 2011
C. gobio	Culm (GB)		8	62.5	Kennedy 1999
C. gobio	Teme (GB)	Autumn 2015	20	50.0	This study
C. gobio	Kennet (GB)	Summer 2015	26	81.0	This study
C. gobio	Loddon (GB)	Summer 2015	28	50.0	This study
C. gobio	Avon (GB)	Spring 2015	26	96.0	This study
Barbatula barbatula	Otter (GB)	June 1994	32	0.0	Kennedy 1996
B. barbatula	Ouche (Fr)	Spring 2005	52	3.8	Medoc 2011

Host species	River	Sampling date(s)	Number (N)	Prevalence (%)	Source
B. barbatula	Vingeanne (Fr)	Spring 2005	3	0.0	Medoc 2011
B. barbatula	Vogue (Fr)	Spring 2009	3	0.0	Medoc 2011
B. barbatula	Culm (GB)		30	27.5	Kennedy 1999
B. barbatula	Avon (GB)	Spring 2015	14	28.6	This study
B. barbatula	Kennet (GB)	Summer 2015	30	36.7	This study
B. barbatula	Loddon (GB)	Summer 2015	40	32.5	This study
B. barbatula	Darent (GB)	Spring 2015	60	43.3	This study
Phoxinus phoxinus	Ouche (Fr)	Spring 2005	54	57.4	Medoc 2011
P. phoxinus	Ouche (Fr)	Spring 2009	92	13.0	Medoc 2011
P. phoxinus	Vogue (Fr)	Spring 2009	125	83.2	Medoc 2011
P. phoxinus	Culm (GB)		33	9.1	Kennedy 1999
P phoxinus	Lake Vihorlat (Sl)	Winter 2001	129	88.9	Dudinak et al 2003
P phoxinus	Teme (GB)	Autumn 2015	10	50.0	This study
P phoxinus	Avon (GB)	Spring 2015	4	75.0	This study
P phoxinus	Darent (GB)	Spring 2015	25	80.0	This study

For most of the studied fishes here, there was a significant relationship between body size and prevalence, with increased body size increasing the probability of infection. Previous studies support this pattern, with strong correlations between host length and parasite prevalence also reported for *C. gobio* and *B. barbatula* (Rumpus 1975). Nevertheless, juvenile fish data from the River Teme suggested *B. barbus* can develop infections from size ranges as low as 20 mm standard length, presumably at sizes when their gape size becomes sufficiently large to enable ingestion of *G. pulex* (C. Gutmann-Roberts personal communication). Increased abundance and prevalence of acanthocephalans with increasing host size has been reported in many studies (Hine and Kennedy 1974; Muzzall 1980; Diamant 1989, Hassanine and Al-Jahdali 2007) and is considered to be a result not only of increased gape size but other factors including feeding preferences, habitat use and increased exposure time to infected intermediate hosts and/ or free living parasite stages (Hooper 1983; Diamant 1989). Correspondingly, the interaction between host body size and increased prevalence will vary between species due to species-specific ontogenetic changes that will also affect vulnerability to infection via determining the importance of intermediate hosts to the diet of the potential final host (Diamant 1989; Martins et al. 2001).

The sub-lethal consequences of infections for hosts included some alterations in their condition and weight, and pathological consequences, including penetration of the epithelium and intestinal wall, although this was not accompanied by a host response for *C. gobio* and *P. phoxinus*. Such a lack of response could have significant implications for gut function, risk or peritonitis, energetics and subsequent establishment of other parasites, however further investigation would be needed to conclude whether the lack of immune response is typical in these hosts. These sub-

lethal consequences are potentially important in infections of parasites such as *P. laevis*, as the phenotypic alterations to the traits of final hosts may be relatively subtle in comparison to fishes that act as intermediate hosts, such as cyprinid fishes infected with *L. intestinalis* (Loot et al. 2001; Britton et al. 2008; Chapter 6). Here, despite the apparent paucity of marked changes in aspects of their phenotype (at least externally), there were some marked differences in the trophic niches of the infected and uninfected sub-groups per species and river. For example, in *C. gobio*, there was strong niche constriction and specialisation evident in the Rivers Kennet and Loddon, with a similar pattern evident for *P. phoxinus* in the Darent and Loddon. In the Teme, infected *C. gobio* and *B. barbatula* both had trophic niches that were larger than their uninfected sub-groups and showed substantial divergence. Consequently, whilst there appeared to be some context dependency in the trophic responses at the population level, there were some relatively consistent patterns evident that suggested some parasite-driven trophic niche specialisation (Britton and Andreou 2016). In the absence of clear changes to the host phenotype by infection then the processes involved in this specialisation were not apparent. It might have related to parasite mediated competition, where the energetics involved in maintaining body condition in the face of pathological challenges by the parasite could have resulted in reduced foraging performances (Krichbaum et al. 2010; Pegg et al. 2015). Logistical constraints prevented further study on this here, although it should be noted that in Chapter 3, there were no significance differences in the functional response parameters between infected and uninfected *S. cephalus* when held individually and in controlled conditions, weakening the argument that parasite mediated competition might have been a key process in driving these differences in trophic niches. Irrespective, the effects of infections at a food web level included some substantial modifications in trophic structure, with alterations in aspects such as energy

pathways, suggesting a need for further investigation into these patterns and processes (Britton and Andreou 2016).

Introduced parasites can pose a greater threat to their new hosts than co-evolved parasites as the hosts tend to lack anti-parasite behaviours, and strong immune responses, resistance and resilience to infections (Anderson et al. 2004; Rosenblum et al. 2010; Britton 2013). This host-switching to naïve fishes can thus result in higher parasite prevalences and abundances, increased pathological consequences and greater consequences arising from sub-lethal impacts (Taraschewski 2006; Britton et al. 2011). Consequently, understanding how *P. laevis* infections affect the host fishes between their indigenous and non-indigenous range was important in the context of understanding the individual host responses in populations that had originally been naïve. Comparisons of parasite prevalence and abundance per species across the rivers provided no evidence for higher infection levels in the non-indigenous range, with the highest levels for *B. barbatula* recorded in the River Darent, for *P. phoxinus* in the Darent and Kennet, and for *C. gobio*, prevalence was variable across the two ranges but with abundance highest in the Darent. Whilst data were only available for *S. cephalus* from the Darent, prevalence and abundances were also generally high.

This lack of difference in these parasite metrics between the ranges suggested that either parasite pathogenicity was sufficiently high in the indigenous range to make comparisons with more naïve fishes superfluous, or the parasite has been present in the non-indigenous range for a sufficiently long period that over successive generations of fish host populations, some evolved responses to infection have developed. Although the more commonly reported pattern is for large differences in host response in a

parasites introduced and natural range (e.g. Ebert 2005; Kalbe and Kurtz 2006; Franceschi et al. 2010), a lack of difference has been shown in other studies (Kaltz and Shykoff 2002). Whilst there is strong evidence for local adaptation and evolution of host response of over time (Franceschi et al. 2010), there is also presence of innate immunity in many vertebrate and invertebrate species that may account for these results (Kalbe and Kurtz 2006). Furthermore, it has been found that generalist parasites tend to exhibit less local adaptation than those with a very specific host range (Lajeunesse and Forbes 2002), which again might account for the similarity between sites in *P. laevis* population dynamics and responses of their respective hosts.

In summary, infections of *P. laevis* were apparent across a range of species in the fish communities studied, with a range of sub-lethal consequences evident, albeit with some context dependency between populations. Some evidence of parasite maturation was evident in the small-bodied fishes, suggesting they were acting as preferred final hosts. Consequently, the combination of their high numerical abundances and parasite prevalences suggest they play important roles in maintaining populations of *P. laevis*, with their sub-lethal consequences resulting in some important ecological alterations in the host population and wider freshwater community. The work presented here also suggests further work is necessary on this host-parasite model, on both the processes leading to parasite-driven trophic specialisation and how host-specific infections might drive parasite population structuring in the final fish host community. The outputs also suggested that differences in host responses to parasite infections might depend more on the pathogenicity of the parasite than on their status as native/ non-native, or indigenous/ non-indigenous. Consequently, these aspects will be explored next, in

Chapters 5 (enemy release of parasites from non-native fish) and 6 (ecological consequences of native fish parasites).

Chapter 5

Parasites of non-native freshwater fishes introduced into England and Wales suggest enemy release and parasite acquisition

This chapter is published in full as:

Sheath, D.J., Williams, C.F., Reading, A.J. and Britton, J.R., 2015. Parasites of non-native freshwater fishes introduced into England and Wales suggest enemy release and parasite acquisition. *Biological Invasions*, 17, 2235-2246.

5.1 Summary

When non-native species are introduced into a new range, their parasites can also be introduced, with these potentially spilling-over into native hosts. However, in general, evidence suggests that a high proportion of their native parasites are lost during introduction and infections by some new parasites from the native range might occur, potentially resulting in parasite spill-back to native species. These processes were investigated here using parasite surveys and literature review on seven non-native freshwater fishes introduced into England and Wales. Comparison of the mean numbers of parasite species and genera per population for each fish species in England and Wales compared to their native ranges revealed less than 9 % of the native parasite fauna were present in their populations in England and Wales. There was no evidence suggesting these introduced parasites had spilled over into sympatric native fishes. The non-native fishes did acquire parasites following their introduction, providing potential for parasite spill-back to sympatric fishes, and this resulted in no significant differences in overall mean numbers of parasites per population between the native and introduced ranges. Through this acquisition, the non-native fishes also had mean numbers of parasite species and genera per population that were not significantly different to sympatric native fishes. Thus, the non-native fishes in England and Wales showed evidence of enemy release, but acquired new parasites following introduction (showing potential for spill-back), but there was no evidence of parasite spill-over into native fishes.

5.2 Introduction

Introductions of non-native species raise concerns over the impacts they can have on native biodiversity, including predation pressure, increased competition and disruptions to ecosystem functioning (Hulme et al. 2009; Pysek et al. 2010). When free-living species are introduced then their parasite fauna can also be introduced (Williams et al. 2013). These parasites could then potentially spill-over into native species, since resistance and tolerance of these new hosts to infection will be low (Torchin et al. 2003; Kelly et al. 2006). Both lethal and sub-lethal host consequences might be incurred, with the latter including pathological, physiological, and/or behavioural changes, with likely adverse consequences for growth, survival, and fitness (Tompkins et al. 2001; Hewlett et al. 2009; Britton 2013). However, the introduction process might filter out many of these parasites through, for example, only a small sub-set of free-living individuals of low parasite diversity being removed from the native range and/ or their parasites having high host specificity, with these hosts absent in the new range (Torchin et al. 2003). Of those parasites that are introduced, consequences for the receiving ecosystem will vary according to factors including the complexity of their lifecycle, their ability to spill-over to native species, and the extent of the natural resistance and resilience to infection in these new hosts (Kelly et al. 2009).

Interactions between introduced species and parasites have raised a number of hypotheses in invasion biology. The ‘Enemy Release Hypothesis’ (ERH) predicts that the parasite loss experienced by non-native species will enhance their ability to establish and invade (Section 1.3.2) (Keane and Crawley 2002; Mitchell and Power 2003; Hatcher and Dunn 2011). Torchin and Mitchell (2004) suggested that when a species is

introduced, it ‘escapes’ at least 75 % of its parasites from the native range and thus will gain substantial benefits regarding their fitness and survival in the invasive range (Torchin et al. 2003). The ERH has been used as the basis to explain the invasion success of a diverse range of species, including non-native slugs (Ross et al. 2010), mosquitoes (Aliabadi and Juliano 2002) and frogs (Marr et al. 2008). In fish, for two introduced fish species (*Apollonia melanostoma* and *Proterorhinus semilunaris*) in the North American Great Lakes, parasite diversity in both species was considerably lower than their native range, despite them also being present for approximately 100 years (Kvach and Stephien 2008). Many relevant studies have focused on invasive plants that show enemy release processes (e.g. Keane and Crawley 2002; Mitchell and Power 2003; Liu and Stiling 2006), but support is also present in other taxa, including fish (e.g. Poulin et al. 2010).

The ‘Parasite spill-over’ (PSO) hypothesis suggests that those parasites that have been introduced might now ‘spill-over’ to native species (Prenter et al. 2004; Kelly et al. 2009; Britton 2013). This is a concern, as the lack of co-evolution between the parasite and its new host potentially results in low resistance and resilience to infection (Taraschewski 2006). In addition, some native parasites might be transmitted from the native species to the non-native species; if the non-native species is a competent host that acts a reservoir of infection, it can result in parasite spillback (PSB) to native species, increasing their disease impacts at individual and population levels (Section 1.3.2) (Kelly et al. 2009).

An issue with these hypotheses in non-plant taxa is the lack of empirical data available for the parasite fauna of many introduced species. Consequently, the aim here was to

use these hypotheses as the basis for investigating the parasite fauna of non-native freshwater fish and the native freshwater fish communities in which they reside. The rationale for using freshwaters was that drainage basins tend to act as biogeographic islands and thus present obstacles to natural fish migration (and so barriers to their parasites also) between basins (Gozlan et al. 2010). The study area was England and Wales, hereafter referred to as the 'introduced range'. The study objectives were to: (1) compare the diversity and characteristics (internal/ external attachment; specialist/ generalist) of the parasite fauna of non-native fishes between freshwaters in the introduced range and their native ranges; and (2) assess the diversity and characteristics of parasites in non-native fish populations in the introduced range, and compare them to the diversity and characteristics of the parasites present in the native fish of the host communities. Specialist parasites were those where their literature suggested very high host specificity, whereas generalists were those of lower host specificity. These outputs were then discussed in relation to enemy release, parasite spillover and parasite spillback processes.

5.3 Methods

5.3.1 Non-native fish species

The non-native fish present in the introduced range that were used in the study were European catfish *Silurus glanis,* pumpkinseed *Lepomis gibbosus,* topmouth gudgeon *Pseudorasbora parva,* sunbleak *Leucaspius delineatus,* black bullhead *Ameiurus melas,* bitterling *Rhodeus amarus* and fathead minnow *Pimephlaes promelas*. The justification for their use was that data on their parasite fauna were available for at least one population and these species are not used in aquaculture in England and Wales and so

any fish present in the wild were unlikely to have been previously exposed to any anti-parasite treatments (Table 5.1). In general, their distributions in England and Wales are very restricted due to fish movement legislation and regulations; indeed, the *A. melas* and *P. promelas* populations used in the study were the only populations present in the countries and both have since been eradicated. By contrast, the following non-native fishes were omitted from the study to avoid confounding issues as their heavy use in aquaculture would have potentially exposed them to a range of anti-parasite treatments: common carp *C. carpio*, goldfish *C. auratus*, rainbow trout *Oncorhynchus mykiss* and ide *Leuciscus idus*. Indeed, should any of these species be sampled in the wild in England and Wales then there is high probability they originated from a fish-farm as there are, for example, few naturally recruiting populations of *C. carpio* and *O. mykiss* present (Fausch 2007; Britton et al. 2010). In addition, the regulations on their releases into the wild in the countries are comparatively light compared with the species included in this study, with regulations concerning *C. carpio* and *O. mykiss* broadly similar to some native fishes.

5.3.2 Data collection

Data on the parasite fauna of the selected non-native fish were collated from two sources. Firstly, data on the parasite fauna of the non-native fish in freshwaters in the introduced range were collated from parasite surveys completed between 2005 and 2013 initially by the Environment Agency as part of routine monitoring of non-native species, parasites and disease in wild fish populations, and latterly by the author for purpose of this research. With the exception of *R. amarus* from the River Great Ouse in Eastern England, the waters were all lentic sites located in lowland areas below 200 m altitude; their precise locations cannot be revealed due to business confidentiality reasons. The

predominance of lentic sites in the study is because the study species are rarely recorded in rivers in England and Wales. After the populations were sampled by either seine netting or fish traps (method dependent on the species and habitat being sampled), the captured fish were removed from the gears, identified to species level and the non-native fish removed and transported alive to the laboratory. At the same time, samples of any native fish captured were also taken to the laboratory with the maximum sample size taken of a total of 30 native fishes. For this study, data were only included where the minimum sample sizes per native fish species was 10 individuals. Once at the laboratory, the fish were euthanized through an anaesthetic overdose (benzocaine solution 5 % w/v) and a detailed post-mortem conducted for the detection of non-native parasites, adapted from Hoole et al. (2001) (Appendix 1). Skin scrapes and internal organs were examined with aid of low and high power microscopy to enable parasite identification. Note that the data recorded in these surveys was the presence of the parasites, but not their prevalence (proportion of fish per species infected with that parasite) or parasite abundance (number or weight of parasites per fish). As such, no data were tested on parasite prevalence or abundance in subsequent analyses.

Secondly, data on the parasite fauna of the non-native fishes in their native ranges, and supplementary data for the fishes in the introduced range, were collated from literature using searches completed in Web of Science, and supplemented by Google Scholar, using Boolean logic search terms including the host fish species and terms including all of their hosts countries (taken from www.Fishbase.org), ‘parasite’, ‘pathogen’, ‘native’, ‘fauna’, ‘health check’ and combinations of these. Data collated from the available papers were lists of parasites hosted by each fish species; in the majority of cases, data were not available on parasite prevalence or abundance and so are not presented here.

Also, in a minority of the parasite recordings, the parasite genus was provided but not the species (e.g. Diplostomum sp.). As such, some subsequent analyses used counts of parasite number based on both species and genera; where species were used, the assumption was used that these recordings represented one species. Also, given that mxyosporidia are seldom reported in studies, their data were removed from the data set entirely to standardise the datasets for both ranges. At the conclusion of the data collection from both the laboratory work and literature reviews, further reviews were then completed for each parasite species to determine their site of attachment (i.e. whether they were internal or external parasites) and host specificity (generalist/ specialist).

5.3.3 Data analyses

Luque and Poulin (2007) outlined that host sample size is often an important correlate of detected parasite species richness and so the effect of study effort should be controlled in parasite richness studies to eliminate spurious sampling effects. Consequently, our data were initially tested for the relationship between study effort and parasite number (species and genera), and where this was significant then the data were corrected by dividing the number of parasite species (and genera) in each range by the number of studies or populations used to collate these data.

To compare parasite diversity between the ranges, and between the non-native fish and sympatric native fish in waters in the introduced range, the methodology was based on linear regression. To compare parasite diversity between the ranges, the first test compared the mean number of parasite species and genera per population for the non-native fishes in their native range versus the number of these parasite species and genera

detected in their populations in the introduced range. The gradient of the regression line (*b*) that described the relationship of the mean parasite species/ genera per population between the ranges tested the null hypothesis that there were equal numbers of the parasite species/ genera per population in both ranges. The null hypothesis was rejected when *b* was significantly different to 1.0 and vice-versa, based on its 95 % confidence limits (Keith et al. 2009). The regression output also indicated if the gradient of *b* was significantly different to zero. To then compare the mean number of parasites per population between both ranges, irrespective of parasite origin, the same test was used, except the data for the introduced range used the mean number of all parasite species and genera recorded per population and fish species.

The numbers of parasite species in the non-native fish and their sympatric native fish species within the invaded fish communities of the introduced range were tested using the same methodology as described. The null hypothesis was the sympatric native and non-native fish species had equal numbers of parasites per population. In this test, genus data were not included as the species level data were largely complete. The values for the native fish were calculated for the community and as such were corrected for higher number of native fish species present versus one non-native fish species.

To compare differences in the parasite characteristics between the ranges, the species level data were used only, as the genera data were not appropriate for identifying differences in host specificity and site of attachment. For each of the three datasets described above, the mean numbers of internal, external, specialist and generalist parasite species per population were tested between the ranges and groups using Mann

Whitney U tests, as transformation did not normalise the data. All statistics were completed in SPSS v. 21.

5.4 Results

The number of parasite species and genera present in the non-native fish in their native range that were also present in these fish species in the introduced range was low, with only 8.5 % of the native parasite species recorded in both ranges (Table 5.1). The relationship between the number of parasite species/ genera and study effort was significant (species: $R^2 = 0.52$, $F_{1,12} = 12.79$, $P < 0.01$; genera: $R^2 = 0.53$, $F_{1,12} = 13.41$, $P < 0.01$; Table 5.1). Comparing the mean number of native parasite species and genera per population between the ranges using linear regression revealed that the gradient of both regression lines were not significantly different to zero (species: $b = 0.22$, $P = 0.27$; genera: $b = 0.26$, $P = 0.36$; Figure 5.1a) but were significantly different to 1.0 (species: 95% confidence intervals: -0.24 to 0.67; genera: 95 % confidence intervals -0.40 to 0.91; Figure 5.1a), rejecting the null hypothesis. There was a significant difference in the mean number of specialist parasite species per population between the ranges (Mann Whitney U Test: $Z = -2.86$, $P < 0.01$), but not in the mean numbers of internal, external and generalist parasite species per population (Mann Whitney: $P > 0.05$ in all cases). Of these parasites recorded in the introduced range, the following were new additions to the British freshwater fish parasite fauna (Kirk 2004): *Thaparocleidus vistulensis* and *Ergasilus sieboldi* in *S. glanis* (Reading et al. 2011), *Onchoceleidus dispar* from *L. gibbosus* (Hockley et al. 2011) and *Ancyrocephalus pricei* from *A. melas*. The cestode parasite *Proteocephalus ocellatus* was also detected in the intestinal tract of *S. glanis*; although it has previously been recorded in imported

fish on an aquaculture site, it was thought to have been eradicated (Andrews and Chubb 1984). Its detection here suggests it might actually have established in England and Wales.

When all the parasite species and genera (irrespective of their origin) that were recorded in the non-native fishes in both ranges were tested against study effort, the relationships were also significant (species: $R^2 = 0.47$, $F_{1,12} = 10.53$, $P< 0.01$; genera: $R^2 = 0.46$, $F_{1,12} = 10.56$, $P< 0.01$; Table 5.2). In these data, *L. delineatus* were an extreme outlier due to their high number of parasites per population in the introduced range (6.0; Table 5.2). Comparing the mean number of parasite species and genera per population between the ranges using linear regression revealed that with *L. delineatus* omitted as an outlier the gradients of the regression lines were not significantly different to zero (species: $b = 0.21$, $P = 0.34$; genera: ($b = 0.57$, $P = 0.19$) or 1.0 (species: 95 % confidence intervals: -0.81 to 1.22; genera: 95 % confidence intervals -0.45 to 1.60; Figure 5.1b), with this also the case for both regression lines with *L. delineatus* included ($P > 0.05$). Thus, the null hypothesis was not rejected. There were no significant differences in the mean number of internal, external, specialist or generalist parasites per population between the ranges (Mann Whitney U Test, $P >0.05$ in all cases).

In waters in the introduced range where the non-native fish were present, the numbers of parasite species were compared between the non-native and sympatric native fishes (Table 5.3). For *L. delineatus* and *P. promelas*, there were no comparative data for sympatric fish and so these were omitted from the data. The relationship between number of populations and parasite number was significant ($R^2 = 0.51$, $F_{1,8}= 8.35$; $P = 0.02$) and comparing the mean number of parasite species per population between the

native and non-native fishes using linear regression revealed no significant difference between them (b = 0.51, P = 0.51) and 1.0 (95 % confidence intervals: -2.56 to 3.58) (Figure 5.2). There were also no significant differences in the number of internal, external and generalist parasites between the groups (Mann Whitney U Test, $P > 0.05$ in all cases). Too few specialist parasites were present in data to warrant their testing.

Table 5.1. Number of studies, and species and genera of parasites recorded in the native range of the non-native fishes, the number of these native parasites recorded in the 'Introduced' range (England and Wales), and the characteristics of these parasite species in both ranges (site of attachment and host specificity).

					Parasite species characteristics (%)				
Species	Range	Studies	Native genera	Native species	Internal	External	Specialist	Generalist	References
Silurus glanis	Native	20	41	54	69	31	19	81	1-8
	Introduced	6	6	6	50	50	17	83	This study
Lepomis	Native	10	25	34	29	71	18	82	9-19
gibbosus	Introduced	1	3	3	0	100	0	100	20
Pseudorasbora	Native	10	13	13	62	38	8	92	21-22
parva	Introduced	4	1	0	-	-	-	-	This study
Leucaspius	Native	12	9	11	55	45	9	91	23-28
delineatus	Introduced	1	2	2	50	50	0	100	29
Ameiurus melas	Native	25	12	15	80	20	20	80	30-40
	Introduced	1	0	0	-	-	-	-	This study
Rhodeus amarus	Native	16	33	42	45	55	10	90	41
	Introduced	4	4	4	75	25	0	100	This study
Pimephales	Native	13	14	19	47	53	16	84	42-50
promelas	Introduced	1	1	1	100	0	0	100	This study

1 Copp et al. (2009); 2 Barzegar and Jalali (2010); 3 Soylu (2005); 4 Mancheva et al. (2014) ;5 Zdarska and Nebesarova (2005); 6 Sattari et al. (2002); 7 Roohi et al. (2014); 8 Pazooki and Masoumian (2012); 9 Hanek and Fernando (1978); 10 Esch (1971); 11 Cone and Anderson (1977); 12 Rye and Baker (1984); 13 Piasecki and Falandysz (1994); 14 Hudson and Bowen (2002); 15 Grupcheva and Nedeva (2000); 16 Osborn (1911); 18 Aho et al. (1976); 18 Wilson and Ronald (1967); 19 Taylor et al. (1994); 20 Hockley et al. 2011; 21 Gozlan et al. (2010); 22 Zhang et al. (2007); 23 Avdul et al. (2011); 24 Skenderovic et al. (2011); 25 Molnar (1976); 26 Kirjušina and Vismanis (2007); 27 Davydov et al. (2003); 28 Galationov (1980); 29 Beyer et al. (2005); 30 Bangham (1941); 31 Lincicome and Van Cleave (1949); 32 Van Cleave (1921); 33 Steelman (1938); 34 Wallace (1935); 34 McAllister and Bursey (2011); 35 Seamster (1948); 36 Hugghins (1954); 37 Davidova et al. (2008); 38 Mizelle and Cronin (1943); 39 Dronen and Underwood (1980); 40 Tkach and Mills (2011); 41 Held and Peterka (1974); 42 Wilmer and Rogers (1969); 43 Olsen (1986); 44 McDowell et al. (1992); 45 Radabaugh (1980); 46 Knipes and Janovy (2009); 47 Mitchell et al. (1982); 48 Samuel et al. (1976); 49 Merrit and Pratt (1964); 50 Voth and Larson (1968).

Table 5.2. Number of studies, and species and genera of parasites recorded in the non-native fishes in their native range, the total number of parasites recorded in these fishes in England Wales ('Introduced'), and the characteristics of the parasite species (site of attachment and host specificity). Refs as per table 5.1.

					Parasite species characteristics (%)				
Species	Range	Studies	Genera	Species	Internal	External	Specialist	Generalist	References
Silurus glanis	Native	20	41	54	72	28	26	74	1-8
	Introduced	6	7	7	57	43	14	86	This study
Lepomis gibbosus	Native	10	25	34	33	67	17	83	9-19
	Introduced	1	7	7	57	43	0	100	20
Pseudorasbora	Native	10	13	13	54	31	8	92	21-22
parva	Introduced	4	2	2	100	0	0	100	This study
Leucaspius	Native	12	9	11	54	46	18	82	23-28
delineatus	Introduced	1	6	6	66	34	0	100	29
Ameiurus melas	Native	25	12	15	80	20	20	80	30-40
	Introduced	1	1	2	0	100	50	50	This study
Rhodeus amarus	Native	16	33	42	45	55	10	90	41
	Introduced	4	10	11	45	55	0	100	This study
Pimephales	Native	13	14	19	42	58	11	89	42-50
promelas	Introduced	1	1	1	0	100	0	100	This study

Table 5.3. Comparison of the numbers of parasite species of the non-native and sympatric fish present in fish communities in the UK, where N: native fish community, NN non-native fish populations, and the characteristics of the parasite species (site of attachment and host specificity).

					Parasite species characteristics (%)			
Species	Fish communities studied (n)	Species group	Fish species	Parasite species recorded (n)	Internal	External	Specialist	Generalist
Silurus glanis	3	N	1-13	17	29	71	0	100
		NN		6	66	34	17	83
Lepomis gibbosus	1	N	1, 9, 13	3	0	100	0	100
		NN		7	57	43	0	100
Pseudorasbora parva	2	N	1, 9, 14	5	42	58	0	100
		NN		2	100	0	0	100
Ameiurus melas	1	N	2, 3, 9	7	29	71	0	100
		NN		2	0	100	50	50
Rhodeus amarus	3	N	3, 6, 9, 12, 13, 15	26	53	47	0	100
		NN		11	45	55	0	100

1 *Scardinius erythropthalmus*; 2 *Cyprinus carpio*; 3 *Perca fluviatilis*; 4 *Barbus barbus*; 5 *Anguilla anguilla*; 6 *Abramis Brama*; 7 *Squalius cephalus*; 8 *Leuciscus leuciscus*; 9 *Rutilus rutilus*; 10 *Tinca tinca*; 11 *Carassius carassius*; 12 *Esox lucius*; 13 *Gobio gobio*; 14 *Gasterosteus aculeatus*; 15 *Gymnocephalus cernus*

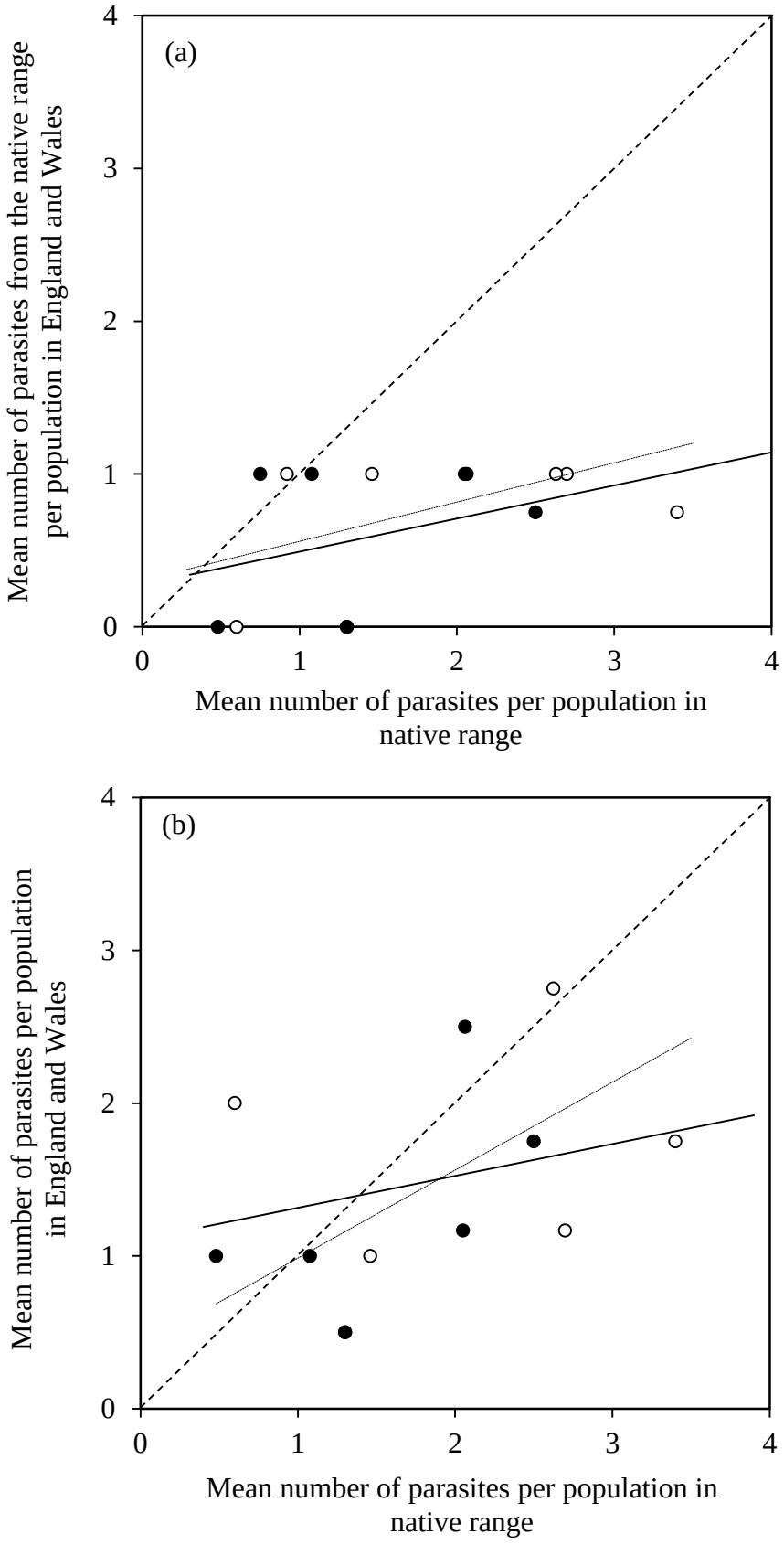

Figure 5.1. (a) Comparison of the mean number of parasites per population in the native range of the non-native fish in Table 1 versus the mean number of these native parasites

recorded per population in the introduced range, and their relationships according to linear regression (species: $R^2 = 0.23$, $F_{1,5} = 1.50$, $P > 0.05$); genus: $R^2 = 0.16$, $F_{1,5} = 1.01$, $P > 0.05$).

(b) Comparison of the mean number of parasites per population in the native range of the non-native fish in Table 1 versus their mean number of parasites recorded per population in The introduced range, and their relationships according to linear regression (species: $R^2 = 0.08$, $F_{1,4} = 0.33$, $P > 0.05$); genus: $R^2 = 0.38$, $F_{1,4} = 2.45$, $P > 0.05$).

Open circles are species data, filled circles are genus data, solid lines represent fitted relationships (linear regression) for the species level data and the dotted line for genus level data, and dashed lines represent the null hypothesis that there are equal numbers of parasites per population between the native ranges and the introduced range.

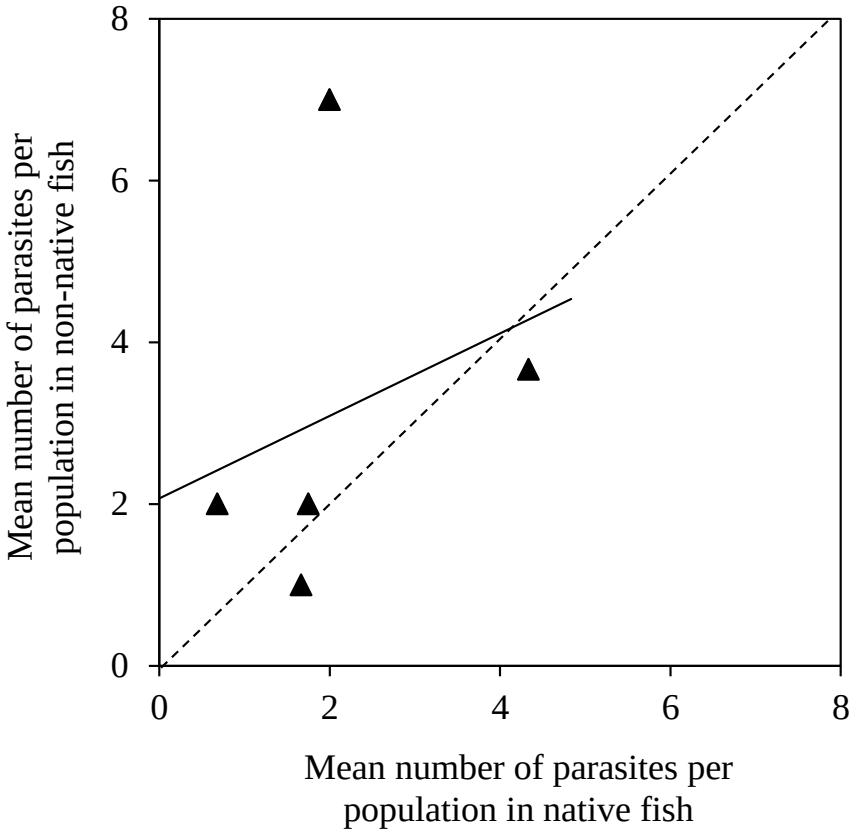

Figure 5.2. Comparison of the mean number of parasite species per population of the native and non-native fish when in sympatry in the introduced range, where non-native fish were *Silurus glanis*, *Lepomis gibbosus*, *Pseudorasbora*. *parva*, *Ameiurus melas* and *Rhodeus amarus*. The solid line represents the fitted relationship for data (linear regression; and their relationships according to linear regression (R^2 = 0.51, $F_{1,3}$= 0.28, P > 0.05) and the dashed line represents the null hypothesis that there are equal numbers of parasites per population in the non-native and native fish.

5.5 Discussion

The study outputs suggested when the non-native fishes were introduced into England Wales, they had undergone aspects of enemy release as only 8.5 % of their native parasite fauna remained. Of those that had been introduced with the fish, the majority were members of the Monogenea class of parasites. The relatively high host specificity of these parasites has so far limited disease risks to native fish populations (e.g. Hockley et al. 2011; Reading et al. 2011). However, examples of serious disease outbreaks following the translocation of monogenean parasites, such as *G. salaris* (Bauer et al. 2002; Bakke et al. 2007), highlights the importance of continued monitoring and prompt risk assessment to inform management (Williams et al. 2013).

There was no evidence that parasite spillover had occurred in any of the studied fish communities in the introduced range, with no recordings of the introduced parasites in the sympatric native fishes. Whilst there were seven parasites recorded in both the non-native fish and sympatric native fish communities, these were all generalist parasites native to England and Wales that had been acquired by the non-native fish. This does indicate that there was potentially some biotic resistance to these fishes (Mitchell and Power 2003). In the absence of parasite prevalence and abundance data, however, it could not be assessed whether these infections were likely to be having sufficient sub-lethal consequences in the non-native fish to prevent their long-term survival and establishment.

In general, the loss of natural parasite fauna is often used as an explanatory variable in the invasion success of many non-native species (e.g. MacLeod et al. 2010; Mitchell

and Power 2010; Ross et al. 2010). In evaluating enemy release, it is important to understand why reductions in parasite fauna are occurring and why some parasites do manage to survive the introduction process. Here, some (but not all) of the parasites that survived the introduction process into England and Wales were monogenean parasites with direct lifecycles and no intermediate hosts (Jimenez-Garcia et al. 2001). The persistence of these parasites in the introduced range was likely to have been assisted by their simple lifecycle, providing there were sufficient numbers of hosts available. Indeed, MacLeod et al. (2010) concluded that life cycle complexity and transmission efficiency were the more likely causes of introduced parasites failing to establish in the new range rather than the parasites being lost during the introduction process. Nevertheless, there are a number of studies that do not support the ERH (e.g. Ramalho et al. 2009; Lacardo et al. 2013; Poulin and Mouillot 2003). For example, Lacerda et al. (2013) suggested that as important as the number and diversity of parasites present in the non-native species are the effects of the parasites on the hosts, with parasite prevalence and abundance often being greater in the introduced range. As this aspect could not be assessed here due to the absence of data on parasite prevalence and abundance, it is an aspect of the parasite fauna of non-native fish in England and Wales that should be studied subsequently.

Comparisons between the parasite fauna of the native and non-native fish in the study provided no evidence of parasite spill-over, perhaps due to the low number of introduced parasites generally that made this an unlikely process. Nevertheless, other studies suggest it remains an important process due to the potential for damaging outcomes occurring in infected native hosts (e.g. Taraschewski 2006; Prenter et al. 2004; Liu and Stiling 2006). In some cases, spill-over occurs at relatively high levels, as

the majority of introduced parasites spill over into native hosts (Jimenez-Garcia 2001), such as the helminth parasite fauna from the non-native lizard, *Tupinambis merianae*, into the native reptile fauna (Ramalho et al. 2009). By contrast, there was greater evidence in the current study of native parasites infecting the non-native fishes and this process is generally well reported (e.g. Jimenez-Garcia 2001; Krakau et al. 2006). For example, the two salmonid fish species *O. mykiss* and *S. trutta* accumulate parasite communities in their introduced ranges at similar abundances to their host range, negating any beneficial consequences they might have gained from enemy release (Poulin and Mouillot 2003). In the current study, the acquired parasites resulted in similar mean parasite numbers per population in England and Wales to the native range of the fishes. These mean number of parasite species per population were also not significantly different to those in the sympatric fishes present in the invaded communities of England and Wales. Although some caution in these conclusions is warranted due to the relatively low numbers of populations that could be studied in England and Wales that limited the power of statistical tests. The observed patterns in the data, however, were also very supportive of these conclusions.

The importance of parasite dynamics in the establishment and invasion processes of non-native species is through the advantages provided to those species in terms of their traits and fitness when their parasite fauna is reduced (Torchin et al. 2003). It can enable increased resource allocation for somatic growth and reproduction, and increase immune responses to infections of native parasites (Joshi and Vrieling 2005). In combination, these serve to increase the probability of the establishment and invasion, thus subsequently altering interactions in the host fish community (Keane and Crawley 2002). Whilst fish introductions in England Wales are routinely screened for certain

‘notifiable’ diseases before release (at least where introduction is intentional and approved; Davies et al. 2013), there is arguably a requirement for increased parasite screening for introductions of non-native fish. However, with differing legislative, economic and political drivers, managing the introduction and spread of non-native pathogens represents a complex global challenge (Williams et al. 2013). Although few examples were found of non-native fish pathogens being imported directly on the seven non-native fishes studied here, Phillips et al. (2010) found that, whilst initially an invasive species may experience parasite release, those that do remain - even in low abundance - might re-establish, having consequences on both the non-native host and the wider fish community. Moreover, the detection tools used in screening are important. Here, only those parasites were considered that could be detected through routine health screening which included low and high-power microscopy (Hoole et al. 2001). However, these are unlikely to detect intra-cellular pathogens such as *Sphaerothecum destruens* that is hosted by *P. parva* (Gozlan et al. 2005). This pathogen has proved difficult to detect in wild populations, due to its size and the absence of disease or gross tissue damage, but is now increasingly being detected as molecular methodologies improve, with this resulting in the recent detection of its presence and distribution in countries such as the Netherlands (Spikmans et al. 2013). This is important, given that this pathogen is associated with potentially substantial mortality rates in salmonid and cyprinid fishes (Andreou et al. 2012). In addition, it suggests that whilst the recorded numbers of non-native parasites were low in this study, with negligible spill-over, this might not cover all pathogens being hosted by the non-native fishes. Others might have been introduced but were not detected using the methodologies employed.

In summary, it was revealed that the introduction of these seven non-native fishes in England and Wales was not concomitant with the introduction of a high diversity of non-native parasites. Whilst there was some evidence of native parasites infecting these non-native fishes (and so, potentially, leading to parasite spill-back), there was negligible evidence of parasite spill-over from the non-native to the native fishes. Whilst some caution is needed on this given the case study of *S. destruens* in *P. parva*, overall it suggests that enemy release could provide some partial explanations for the survival and establishment success of some non-native fishes in England and Wales. It also suggests that from the perspective of the impact of a parasite on both an individual host and their population, it is aspects of the parasite virulence and specificity that could be most important, rather than its origin.

Chapter 6

Trophic consequences of infection by native parasites for native fishes: evidence of niche specialisation driven by parasitism?

6.1 Summary

The consequences of parasitism can include alterations to host pathology, physiology and behaviour, potentially resulting in alterations to diet that lead to the development of trophic niche specialisation between the infected and uninfected population sub-groups. To test whether parasitism by native parasites can result in trophic niche specialisation, three parasites with complex lifecycles were studied across three fish hosts using two populations per host-parasite system. For roach *Rutilus rutilus* infected with the cestode parasite *L. intestinalis*, trophic niche specialisation was strongly evident in parasitized individuals from one of the sites, with individual infected fish occupying elevated areas of trophic space, indicating they had higher trophic positions than uninfected con-specifics. For three-spined stickleback *G. aculeatus* infected with the cestode *S. solidus*, samples collected from a site in autumn revealed some shifts in individual niches, with infected fish feeding significantly lower in the food web than uninfected conspecifics, increasing the population trophic niche size. For perch *Perca fluviatilis* infected with *Triaenophorus nodulosus* the population trophic niche size was substantially larger with the parasite in one site, but this was less apparent in the other. These findings provide some evidence that these native parasites result in some trophic niche specialisations of infected fishes, but further work is needed to explore the ecological processes that produce these patterns in niche divergence.

6.2 Introduction

Parasite infections can often result in significant consequences for the biology of their hosts (Barber et al. 2000), involving shifts in host pathology, physiology and behaviour (Barnard and Behnke 1990; Barber and Huntingford 1995; Loot et al. 2008). These changes, affecting foraging and anti-predator behaviours, and habitat preferences, tend to then have adverse consequences for host condition, growth and fitness (Fenton and Brockhurst 2008; Horky et al. 2014). Thus, parasites have the potential to profoundly shape the dynamics of their host populations and communities, and alter the symmetry of intra- and inter-specific competitive relationships (Perrin and Christie 1996; Hudson et al. 2006; Luque and Poulin 2007).

Ecological studies generally tend to focus on analysing parameters at the population level, using mean values for measured parameters in order to make comparisons between, for example, populations of the same species under different levels of disturbance. This is despite the high variation of these parameters that can be present within each population, i.e. high intra-specific trait variability (Bolnick et al. 2003). Whilst it is recognised that both sex and age will lead to trait variations in individuals within populations (Polis 1984; Shine 1989), it is increasingly apparent that considerable variation can occur within individuals of the same age and sex class. For example, red-ear sunfish *Lepomis microlophus* in a Florida lake had very high variation in trophic niche between individuals, as indicated by significant differences in their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$, with this resulting from individual dietary differences that arose from differences in habitat utilisation (Fry et al. 1999).

The drivers of variability in individual trophic niches can be complex and diverse, as a wide range of individual traits and ecological factors have the potential to lead to high levels of variation (Bolnick et al. 2003). Nevertheless, studies on intra-specific trophic specialization tend to focus on three main drivers: inter- and intra-specific competition, ecological opportunity and predation (Araujo et al. 2011; Evangelista et al. 2014). Increased intraspecific competition tends to increase individual specialization (Svanbäck and Bolnick 2008; Araujo et al. 2011; Svanback et al. 2011). For example, Evangelista et al. (2014) found that brown trout *S. trutta* exhibited higher variability in their trophic niche in streams where their population density was highest. The importance of variability in individual niches within a population niche was highlighted by Svanbäck and Bolnick (2007) and Huss et al. (2008) who studied stickleback *G. aculeatus* and perch *P. fluviatilis* respectively. They both revealed that as the population densities of the species increased, the strength of intra-specific competition increased and this led to higher variability in individual trophic niches that, in entirety, produced a larger population trophic niche than in low density populations. Conversely, increased interspecific competition generally reduces individual trophic specialization (Constantini et al. 2005; Araujo et al. 2011) likely to be due to the niche variation hypothesis that suggests that ecological release from other competing species leads to increased generalisation and, therefore, individual niches converge rather than diverge (Van Valen 1965).

Whilst the importance of intra-specific variation in trophic niche size is increasingly recognised, Araujo et al. (2011) argued that whilst infection by parasites could be a driver of variability, studies have tended to focus only on competition, ecological opportunity and predation. This is important, as the consequences of parasite infection

for hosts that have already been outlined, such as shifts in foraging behaviour and habitat utilisation (Barber et al. 2000), have the potential to have substantial consequences for the trophic niche of the host and thus, if parasite prevalence is high in the population, could potentially result in greater individual variability in niche size (Section 1.2.5). Indeed, Britton and Andreou (2016) suggested that infections by some parasites could result in considerable trophic niche specialisation between the sub-groups in a population that are infected and uninfected by specific parasites due to the infection-induced phenotypic modifications of hosts. Supporting evidence for how parasite infection could potentially modify trophic niche size was provided by Britton et al. (2011) who revealed that common carp *C. carpio* infected with the Asian tapeworm *B. acheilognathi* had significantly lower trophic positions than uninfected conspecifics, i.e. the infected fish had a different diet composition and so a different trophic niche than the uninfected fish. Pegg et al. (2015) suggested similar patterns apparent in other populations, with this related to the sub-lethal consequences of infection that altered their diet through changes in foraging behaviours (Section 1.2.5).

It was discussed in Chapter 4 that there were minimal differences apparent between the indigenous and non-indigenous range of *P. laevis* in parasite prevalences and trophic consequences of infection for three fish species. Chapter 5 revealed that although non-native parasites can be introduced with non-native fishes, this rarely occurs in Great Britain, with the acquisition of native parasites by the non-native fish being the more common process. Given these outcomes, here the trophic consequences of infection of native fishes by three native fish parasites with complex lifecycles were investigated in Great Britain to identify whether parasite-driven phenotypic changes result in trophic niche divergence between the infected and uninfected fishes that are independent of

parasite origin and their co-evolution with their host species. The objectives were: (1) for three native fish: native parasite systems, assess parasite prevalence, parasite abundance and differences in the mean lengths and weights of the infected versus uninfected conspecific fish; and (2) using the populations from objective 1, identify how intra-specific trophic niche size was modified by infection through assessment of trophic niche size of the uninfected and infected sub-groups in the population. It was predicted that for each focal host population, infected fish would be of lower condition than uninfected fish in the same population and have differences in their trophic niches to uninfected as a consequence of infection. Unlike Chapter 4, histopathology was not completed as two of the parasites occupy the body cavity of the fish and in the other, the histopathology of infections has already been described in the literature (*cf*. Section 6.3.1).

6.3 Materials and methods

To determine how parasite infection modified the trophic niche of hosts, metrics relating to the trophic niche size of the host population was determined using the analysis of their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ (Section 1.4; 4.3). Consequently, this section describes the host: parasite models used and the waters from which they were sampled, how the samples were collected and how the subsequent data were analysed, including by stable isotope analysis (Section 1.4; 4.3). As the majority of samples were collected by the Environment Agency on behalf of the research project, the focus was on the collection of fish samples and this resulted in samples of macro-invertebrates being collected that were not suitable for including in stable isotope analyses, due to low sample sizes and diversity. Consequently, the focus in the chapter is on identifying differences in trophic niche breadths and positions between the infected and uninfected

fish per population, with additional analyses on their diet not completed, i.e. there was no complementary use of Bayesian mixing models to quantity differences in diet compositions (Philips et al. 2014). This had a detrimental consequence for understanding aspects of the trophic patterns observed and the implications of this are discussed further in Section 6.5.

6.3.1 Host: parasite models

Three host parasite models were chosen for this study, all of which included a common native fish species as a host to a common cestode parasite.

Rutilus rutilus: Ligula intestinalis

Roach (*R. rutilus*) are an abundant and widespread freshwater fish species found in a variety of habitats, including lakes and rivers, across Great Britain. They are a common intermediate host to the plerocercoid larvae of *L. intestinalis*, an intestinal tapeworm that has a three stage life cycle. This involves infection of copepods (primary intermediate host) cyprinid fishes such as *R. rutilus* (secondary intermediate host) and a fish-eating bird (final host). Infection with *L. intestinalis* can cause severe pathology in cyprinid fishes as they occupy space in the body cavity and can comprise over 20 % of the fish body mass (Figure 6.1) (Sweeting 1976; Taylor and Hoole 1989). They can then affect the habitat utilisation, growth, behaviour and reproduction of the hosts (Loot et al. 2001; Loot et al. 2002; Carter et al. 2005). In particular, infected fish tend to be encountered in more littoral areas than uninfected conspecifics (Loot et al. 2002; Britton et al. 2008), this is associated with increasing the opportunity for the infected fish to be predated by a bird, so completing the parasite lifecycle (Loot et al. 2001).

Figure 6.1. The parasite load of *Ligula intestinalis* from an individual *Rutilus rutilus* host (this study)

Gasterosteus aculeatus: Schistocephalus solidus

The three-spined stickleback *G. aculeatus* is a small (< 80 mm) species of freshwater fish native to the UK and is found in a multitude of habitats including rivers, streams, ponds and brackish waters. They are the second intermediate host to the plerocercoid larvae of *S. solidus*. Similar to *L. intestinalis*, *S. solidus* has a three-stage lifecycle involving infection of copepods (primary host), cyprinid fishes (secondary host), and a fish eating bird (final host). As an intestinal tapeworm in *G. aculeatus*, it can cause severe pathology and affect anti-predator behaviours (Giles 1983), as well as inhibiting spawning in infected individuals (Arme and Owen 1967; Schultz et al. 2006). The parasite can attain large sizes relative to their hosts (Figure 6.2); up to 40 % of host

body weight can comprise of *S. solidus* (Hopkins and Smyth 1951). Infection thus can physically inhibit feeding on some prey items (Wright et al. 2006). The impact of *S. solidus* on host habitat choice and foraging behaviour is likely to be modified to differing extents throughout the parasite life cycle as host behavioural changes that make it more susceptible to avian predators are usually only encountered once *S. solidus* reaches reproductive size (Barber et al. 2004).

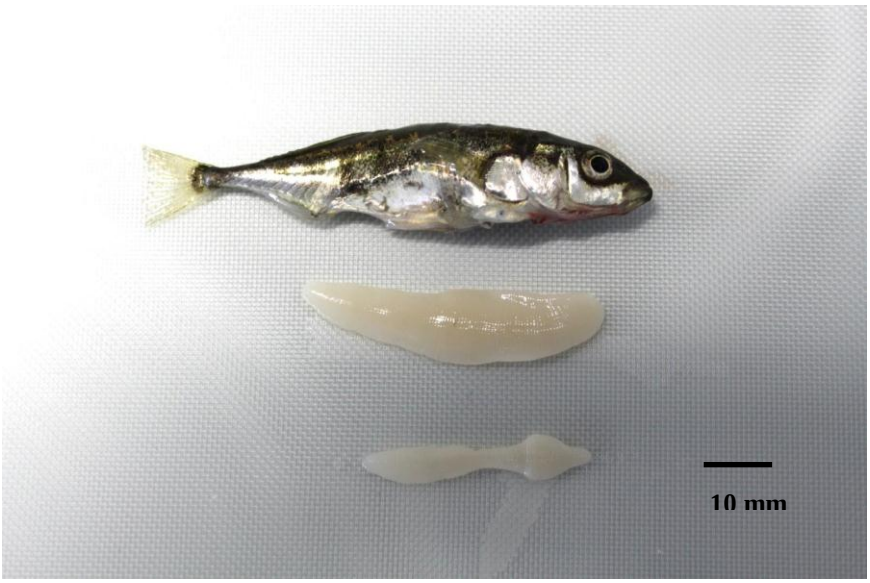

Figure 6.2. Parasite load of *Schistocephalus solidus from* a single *Gasterosteus aculeatus* host

Perca fluviatilis: *Triaenophorus nodulosus*

European perch *P. fluviatilis* is a common facultative predatory fish encountered in many lowland fish communities in Great Britain and they are a secondary intermediate or final host to the plerocercoid larvae of *T. nodulosus*. This parasite also has a three-stage lifecycle involving infection of copepods (primary intermediate host) and variety of fish species as secondary intermediate hosts. The final host is an obligate predatory fish, usually pike *Esox lucius* but also *P. fluviatilis*, a facultative predator. *T. nodulosus*

infects the liver of *P. fluviatilis* (Figure 6.3) and severe infections can result in acute liver pathology that can slow host growth rate (Brinker and Hamers 2007). The liver is an important glycogen store in *P. fluviatilis*, hence a heavy infection of *T. nodulosus* may drive a higher feeding rate in hosts as they try to maintain sufficient energy levels and become less reliant on their glycogen reserves (Mehner and Wieser 1994).

Figure 6.3. Parasite load of *Triaenophorus nodulosus* on the liver of its second intermediate host European perch (*Perca fluviatilis*).

6.3.2 Study sites

The study sites used for each host: parasite system are provided in Table 6.1. Note that for *G. aculeatus*: *S. solidus*, only one study site was able to be studied but was sampled twice (Spring [S1] and Autumn 2013 [S2]) to account for seasonal differences in parasite prevalence and impact. This population was then extirpated by management

activities concerning *P. parva* that were independent of this project and so could not be studied further.

Table 6.1 Overview of the study sites

Site reference	Site	Latitude	Longitude	Host	Parasite	Water type
R1	River Ash	51.3981	-0.4295	*R. rutilus*	*L. intestinalis*	Stream
R2	Cotton Moss	53.2343	-2.3767	*R. rutilus*	*L. intestinalis*	Lake
S1	Crampmoor	51.0001	-1.4499	*G. aculeatus*	*S. solidus*	Pond
S2	Crampmoor	51.0001	-1.4499	*G. aculeatus*	*S. solidus*	Pond
P1	Bedwell fishery	51.7534	-0.1499	*P. fluviatilis*	*T. nodulosus*	Lake
P2	Limes fishery	51.5949	-0.6819	*P. fluviatilis*	*T. nodulosus*	Lake

Fish were sampled using a variety of fishing gears in order to provide a representative sample of fish suitable for parasitological analyses. More specifically:

- R2, P1, P2: All fish were sampled using a seine net (length = 40 m, depth = 4.5 m, mesh size 12 mm) (Templeton 1995).
- R1: A 100 m section of the stream was electric fished (using an Electro-catch generator powered (250 kVA) unit set at 50 MHz pulsed DC at approximately 2 Amps) between fixed stop nets, to collect all species. (Cowx and Lararque 1991).
- S1, S2: Fish were sampled using a combination of a 25 m micromesh seine net (depth = 1.5 m) and baited minnow traps (1 m x 350 mm x 350 mm with a 60 mm diameter opening (Britton et al. 2011).

6.3.3 Fish data collection and stable isotope analyses

The fish species composition of each sample at each site on each sampling occasion was recorded with the samples taken back to the laboratory being for parasitological

analyses. Following Hoole et al. (2001), this comprised of 30 fish per focal host species where sample size made this possible, with 10 individuals of other fish also taken for all other species where available to examine the whole fish parasite community. These numbers provided representative fish samples that would provide information on their parasite fauna and their biological metrics. The fish were transferred to water held in aerated tanks before being transported to the laboratory for subsequent analysis. There, they were euthanized with an overdose of anaesthetic (MS-222), measured (fork length, nearest mm) and weighed (to 0.001 g). A parasitological survey was then completed on each fish (Appendix 1). During this process in *P. fluviatilis*, the liver was removed and weighed (to 0.001 g). Samples of dorsal muscle were then taken for stable isotope analysis (Section 4.3). This muscle sample was immediately transferred to a drying oven at 60 ^{o}C and dried to constant weight (approximately 48 hours).

The samples were then processed at the Life Sciences mass Spectrometry Facility of the Natural Environment Research Council at East Kilbride, Scotland. The dried samples were ground into a homogenous powder and approximately 0.5 mg weighed out into a tin cup, with the actual weight recorded using a Satorius MC5 microbalance. The nitrogen and carbon isotopes were then analysed using a Costech elemental analyser coupled to a Delta V mass spectrometer (Thermo scientific, Milan, Italy). Ratios of ^{15}N:^{14}N and ^{13}C:^{12}C were expressed in parts per mille ($^{0}/_{00}$), relative to international standards (gelatine, glycine, alanine and glutamic acid of known isotopic composition in relation to atmospheric nitrogen in air (N) and Pee Dee Belemnite (C)). The outputs of the stable isotope analysis were determined as as δ^{13}C and δ^{15}N, as described in Section 4.3.1. They were not corrected as each host population and site were analysed separately, with no direct comparisons made between them.

6.3.4 Data analysis and statistical analyses

As per the fish populations infected with *P. laevis* (Section 4.3), the infection levels of each host population were described here as their prevalence (number of infected individuals/ total number process x 100) and abundance (number or mass of parasites per infected individual per fish species). Hereafter, where an individual fish is referred to as either infected or non-infected, it refers to the presence/ absence of the focal parasite in that individual during the post-mortem (Section 6.3.1). Condition was calculated as Fulton's condition factor (K), using the equation: $K = W/L^3$, where K = Fulton's condition factor, W = the weight of the fish, and L is the length (Section 4.3). Differences in weight and condition between the infected and uninfected fish per population and the relationship between fish length and the probability of infection were completed as per Section 4.3. As infection status was binomial (0 = uninfected, 1 = infected), binary logistic regression was used to build probability of infection (PoI) models that determined PoI from the length data of each individual fish using Equation 6.1: $e^{(a+bx)}/\ 1+e^{(a+bx)}$, where a and b were the regression coefficients, and x was fish length.

As with Chapter 4, the primary aim of the stable isotope analysis was to identify whether infection by the focal parasite had sufficiently impacted their hosts to reduce their ability to exploit the same food resources as uninfected conspecifics and later their trophic niche. Correspondingly, this was completed as described in Section 4.3, with calculation of standard ellipse areas in the SIBER package in R for the infected and

uninfected sub-groups of fish in the population. Other than the stable isotope analyses, all analyses were completed in SPSS v. 21.0.

6.4 Results

6.4.1 *Rutilus rutilus: Ligula intestinalis*

Site R1: River Ash

Parasite prevalence was 33 %, and the abundance in infected individuals ranged from 1 to 7 (mean: 3.1 ± 1.0) and weight 1.6 to 6.8 g (mean: 3.5 ± 0.7), equivalent to 9 to 26 % of total host body weight (Table 6.2). The binary logistic regression model revealed that the influence of length on infection status of individuals was not significant, although all fish were above 70 mm in length anyway, restricting the power of the test regarding length range (Table 6.1, 6.3; Figure 6.4). When comparing infected versus uninfected fish, significant differences were detected in their condition (GLM: $F_{1,19} = 6.32$; $P = 0.02$) with infected individuals having significantly lower condition. Corrected weight when controlled for length did not differ significantly between the two groups (GLM: $F_{1,18} = 1.43$; $P = 0.25$)

The infected *R. rutilus* had significantly increased values of $\delta^{15}N$ compared with uninfected conspecifics (Wald $\chi^2 = 19.16$, $P < 0.01$) (Table 6.4). There was no significant effect of infection on $\delta^{13}C$ (Wald $\chi^2 = 0.13$, $P = 0.65$). The SEA_c plot revealed that the trophic response to infection was an elevated trophic position occupying different isotopic space than the uninfected group, with very little overlap and thus increasing size of the population trophic niche (Table 6.4, Figure 6.5).

Table 6.2. Mean length and length ranges of all, infected (I) and uninfected (U) fish hosts per site where R1 and R2 are the River Ash and Cotton Moss lake respectively and the host is *Rutilus rutilus* infected/uninfected with *Ligula intestinalis;* S1 and S2 are Crampmoor fish farm in the spring and autumn respectively, and the host is *Gasterosteus aculeatus* infected/ uninfected with *Schistocephalus solidus*; and P1 and P2 are Bedwell fishery and Limes green fishery respectively and the host is *Perca fluviatilis* infected/uninfected with *Triaenopherous nodulosus.* Also shown are the number of fish sampled (n), and the parasite prevalences ('Prevalence'), abundances (of those infected) ('Abundance') and parasite mass (P mass).

		Mean length (mm)			**Length range (mm)**			**Prevalence (%)**	**Abundance**		**P mass (g)**
Site	n	All	I	U	All	I	U		Mean	Range	Mean
R1	21	119.9 ± 4.4	120.9 ± 4.9	119.4 ± 5.3	70-165	95-148	70-165	33	3.1 ± 1.0	1-7	3.5 ± 0.7
R2	22	100.3 ± 1.5	101.8 ± 1.7	97 ± 2.5	89-119	90-119	89-108	68	1.8 ± 0.3	1-4	1.6 ± 0.2
S1	22	43.0 ± 2.9	48.4 ± 2.8	31.4 ± 4.2	23-64	23-64	24-51	68	2.2 ± 0.5	1-7	0.2 ± 0.1
S2	20	27.4 ± 0.5	27.0 ± 0.5	27.8 ± 0.8	23-33	25-29	23-33	57	1.7 ± 0.4	1-5	0.1 ±0.01
P1	24	127.8 ± 4.5	179.7 ± 5.6	167.8 ± 6.6	100-210	150-210	100-196	42	1.0 ± 0.6	1-6	0.1 ± 0.01
P2	19	133.8 ± 5.9	133.1 ± 6.8	137.3 ± 11.9	110-221	110-221	120-160	84	4.8 ± 1.0	1-12	0.1 ± 0.02

Table 6.3. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Rutilus rutilus* by *Ligula intestinalis* according to fish length at site R1.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	A	-1.16	2.89	0.69
Fish length	x	<0.01	0.02	0.87

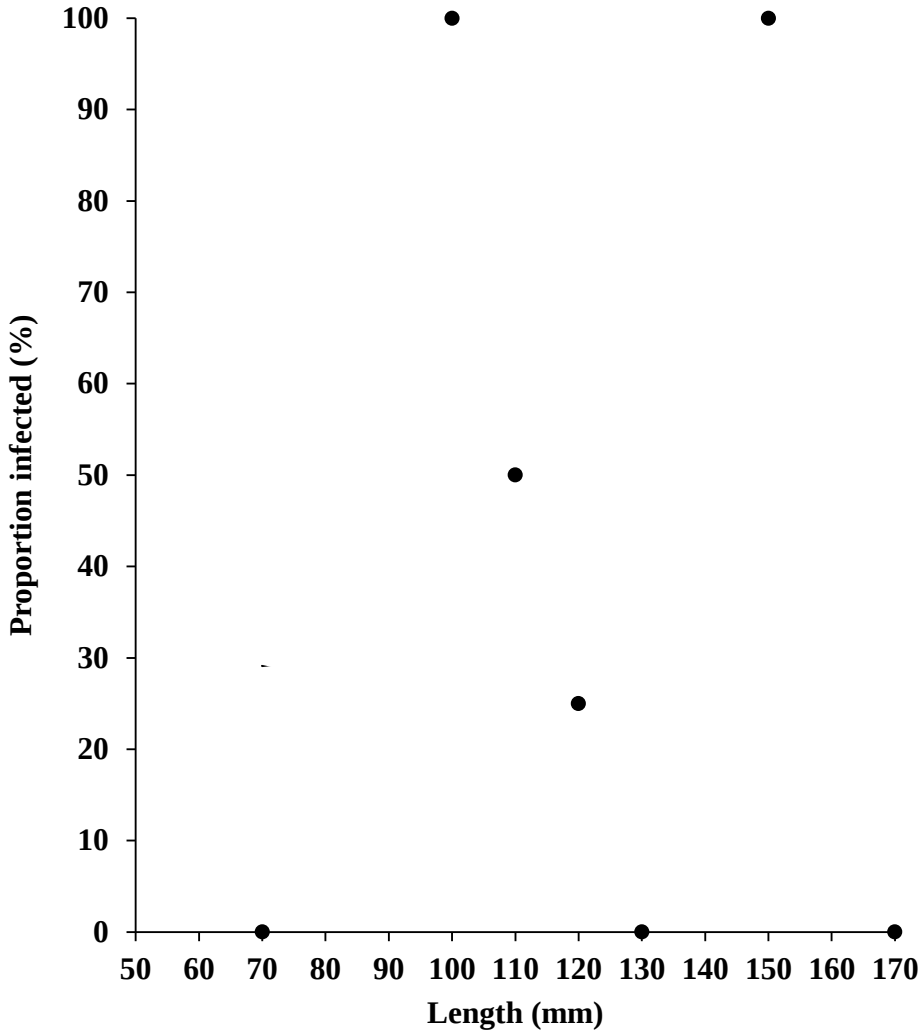

Figure 6.4. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Rutilus rutilus* by *Ligula intestinalis* according to length (as 10 mm increments) where solid circles represents the proportion of infected individuals in that size class.

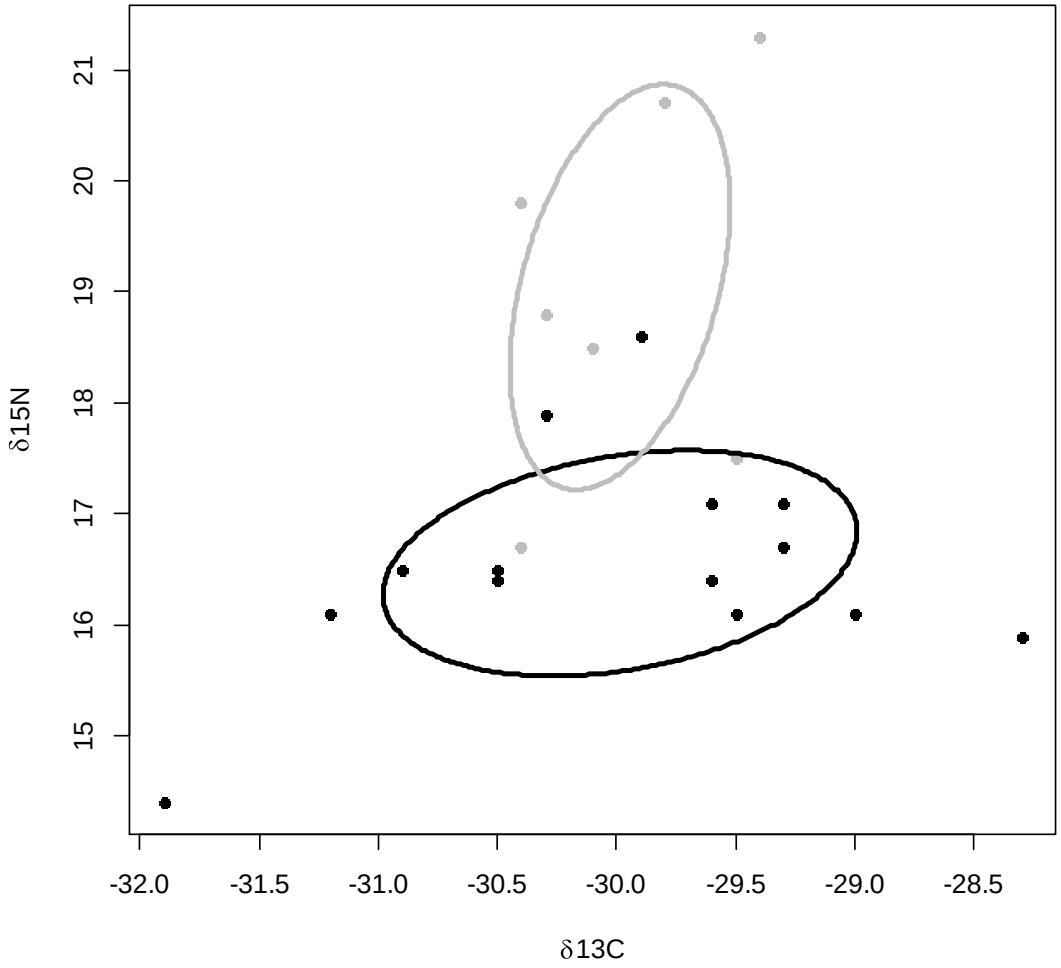

Figure 6.5. Stable isotope biplot for *Rutilus rutilus* infected (grey circles) and uninfected (black circles) with *Ligula intestinalis* from the River Ash, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$**.**

Table 6.4. Mean stable isotope data of hosts per site and their trophic niche size according to standard ellipse area (SEA_c, after correction to trophic position and Ccorr) of the sampled population ('population'), and the uninfected (U) and infected (I) sub-groups, and the extent of the trophic niche overlap between the two sub-groups. Where R1 and R2 are the River Ash and Cotton Moss lake respectively and the host is *Rutilus rutilus* infected/uninfected with *Ligula intestinalis;* S1 and S2 are Crampmoor fish farm in the spring and summer respectively, and the host is *Gasterosteus aculeatus* infected/ uninfected with *Schistocephalus solidus*; and P1 and P2 are Bedwell fishery and Limes green fishery respectively and the host is *Perca fluviatilis* infected/uninfected with *Triaenopherous nodulosus.*

Site	Mean $\delta^{13}C$ (‰)		Mean $\delta^{15}N$ (‰)		SEA_c			
	U	I	U	I	Population	U	I	Overlap (%)
R1	-29.99 ± 0.3	-29.99 ± 0.2	16.56 ± 0.3	19.0 ± 0.6	4.45	3.04	2.43	7.4
R2	-29.2 ± 0.3	-29.5 ± 0.1	16.8 ± 0.2	16.7 ± 0.2	1.31	1.37	1.10	67.0
S1	-35.4 ± 0.4	-34.4 ± 0.7	7.7 ± 0.3	7.6 ± 0.2	5.73	3.13	6.30	78.0
S2	-33.9 ± 0.7	-33.0 ± 0.7	7.6 ± 0.1	7.0 ± 0.1	3.08	1.53	3.09	12.2
P1	-29.5 ± 0.1	-28.3 ± 0.4	17.1 ± 0.1	16.6 ± 0.3	1.94	0.47	3.52	23.8
P2	-34.9 ± 0.3	-34.7 ± 0.3	18.7 ± 0.7	18.4 ± 0.3	4.35	1.73	4.95	88.2

Site R2: Cotton Moss Lake

Parasite prevalence was 68 %, and the abundance in infected individuals ranged from 1 to 4 (mean: 1.8 ± 0.3) and weight 0.5 to 2.9 g (mean: 1.6 ± 0.2), equivalent to 4 to 16 % of total host body weight (Table 6.2). The binary logistic regression model revealed that the influence of length on infection status of individuals was not significant (Table 6.5; Figure 6.6). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1,20} = 0.44$; $P = 0.52$) or corrected weight when controlled for length (GLM: $F_{1,19} = 0.96$; $P = 0.34$).

There was no significant effect of *L. intestinalis* infection on $\delta^{13}C$ (Wald $\chi^2 = 2.02$, $P > 0.05$) or $\delta^{15}N$ (Wald $\chi^2 < 0.01$, $P > 0.05$) (Table 6.4). The SEA_c plot revealed that the trophic niche of infected fish was slightly constricted but occupied a similar isotopic space with a high level of overlap and therefore had little impact on the overall size of the population trophic niche (Table 6.4, Figure 6.7).

Table 6.5. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Rutilus rutilus* by *Ligula intestinalis* according to fish length at site R2.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	a	-12.55	2.05	0.15
Fish length	x	0.13	2.28	0.13

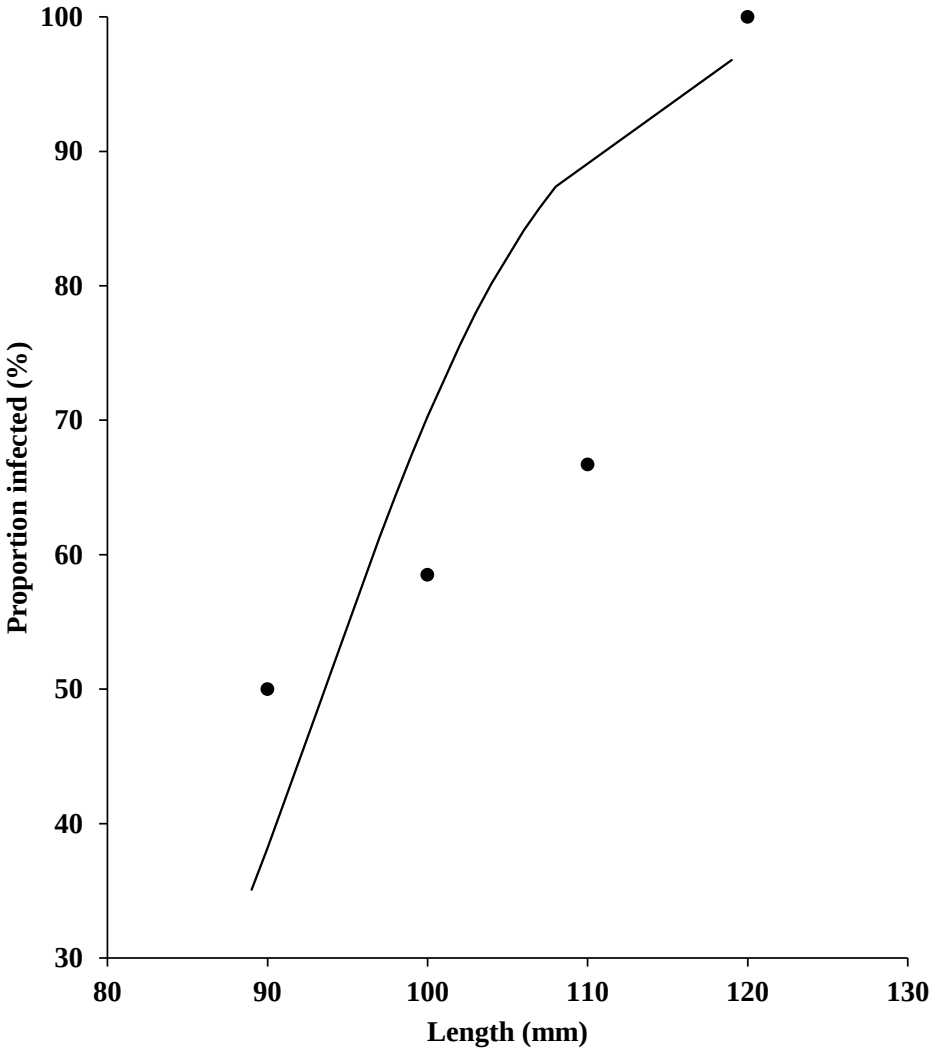

Figure 6.6. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Rutilus rutilus* by *Ligula intestinalis* according to length (as 10 mm increments) where solid circles represents the proportion of infected individuals in that size class and the solid line is the relationship between

fish length and the probability of infection according to binary logistic regression (*cf.* Table 6.5).

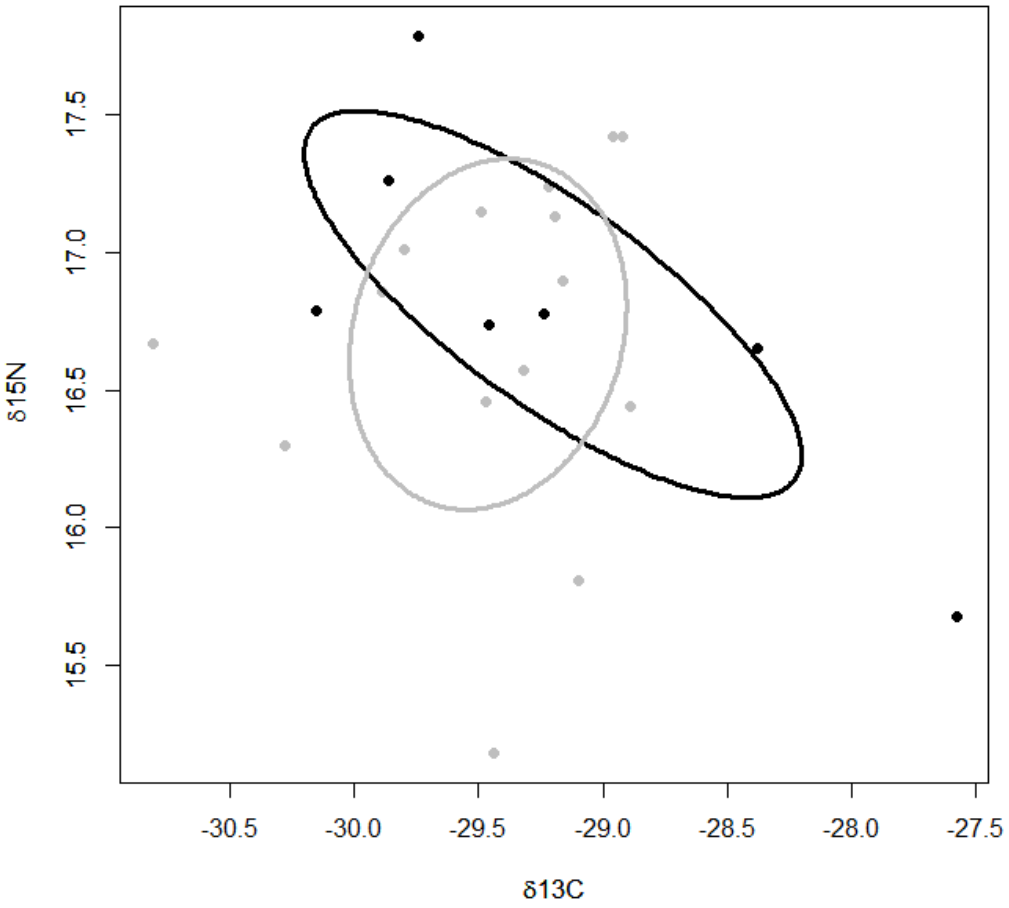

Figure 6.7. Stable isotope biplot for *Rutilus rutilus* infected (grey circles) and uninfected (black circles) with *Ligula intestinalis* from site R2, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$.

6.4.2 Gasterosteus aculeatus: Schistocephalus solidus

S1: Crampmoor, Spring 2013

Parasite prevalence of *S. solidus* in this *G. aculeatus* population in spring 2013 was 68 %, and the parasite abundance in infected individuals ranged in number from 1 to 7 (mean: 2.2 ± 0.5) and weight 0.002 to 0.08 g (mean: 0.2 ± 0.1), equivalent to 0.4 to 1.6 % of total host body weight (Table 6.2). The binary logistic regression model revealed that the influence of length on infection status of individuals was significant, with larger individuals having a significantly higher probability of being infected (Table 6.6; Figure 6.8). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1,20} = 0.01$; $P = 0.97$) or corrected weight when controlled for length (GLM: $F_{1,19} = 0.27$; $P = 0.61$).

Infection with *S. solidus* did not significantly affect $\delta^{13}C$ (Wald $\chi^2 = 2.11$, $P > 0.05$) or $\delta^{15}N$ (Wald $\chi^2 = 3.61$, $P > 0.05$) (Table 6.4). However, the SEA_c plot revealed that the trophic response to infection was an expanded trophic niche, although it did broadly occupy the same isotopic space as the uninfected group, with high trophic overlap (Table 6.4, Figure 6.9).

Table 6.6. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Gasterosteus aculeatus* by *Schistocephalus solidus* according to fish length at site S1.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	A	-4.00	1.90	0.04
Fish length	X	0.12	0.05	0.01

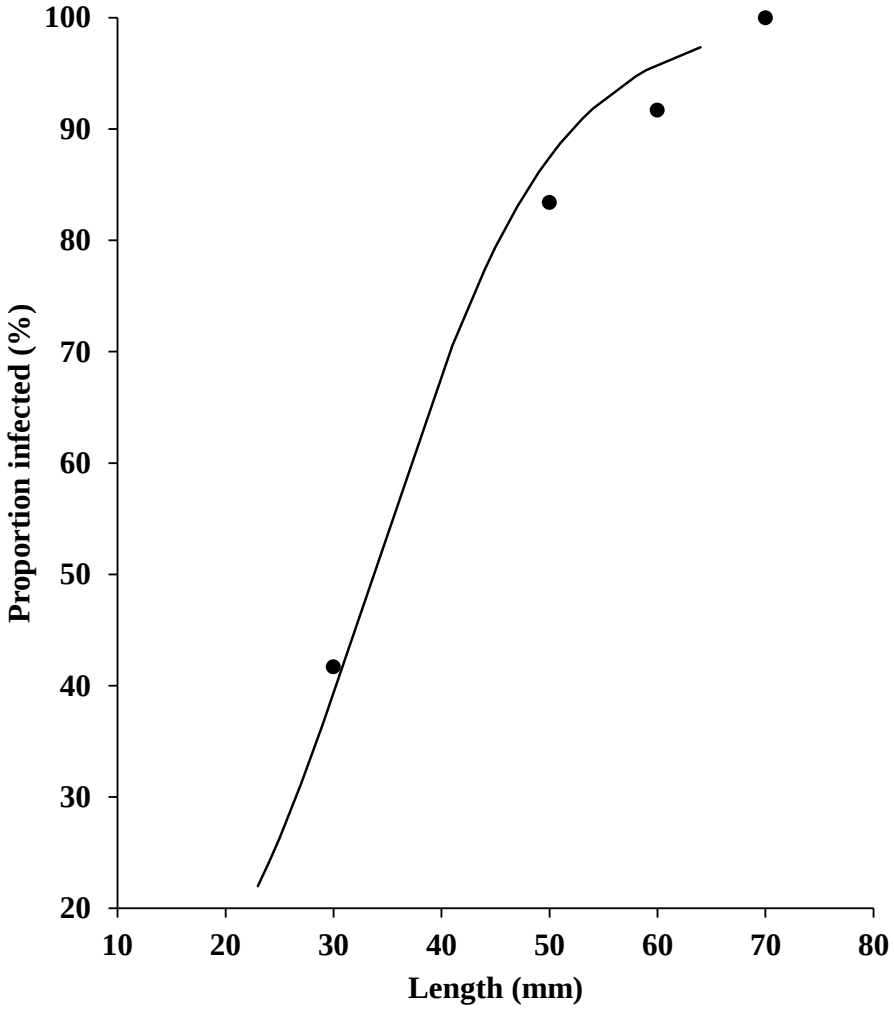

Figure 6.8. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Gasterosteus aculeatus* by *Schistocephalus solidus* according to length (as 10 mm increments) where solid circles represents the proportion of infected individuals in that size class and the solid line is the relationship

between fish length and the probability of infection according to binary logistic regression (*cf.* Table 6.6).

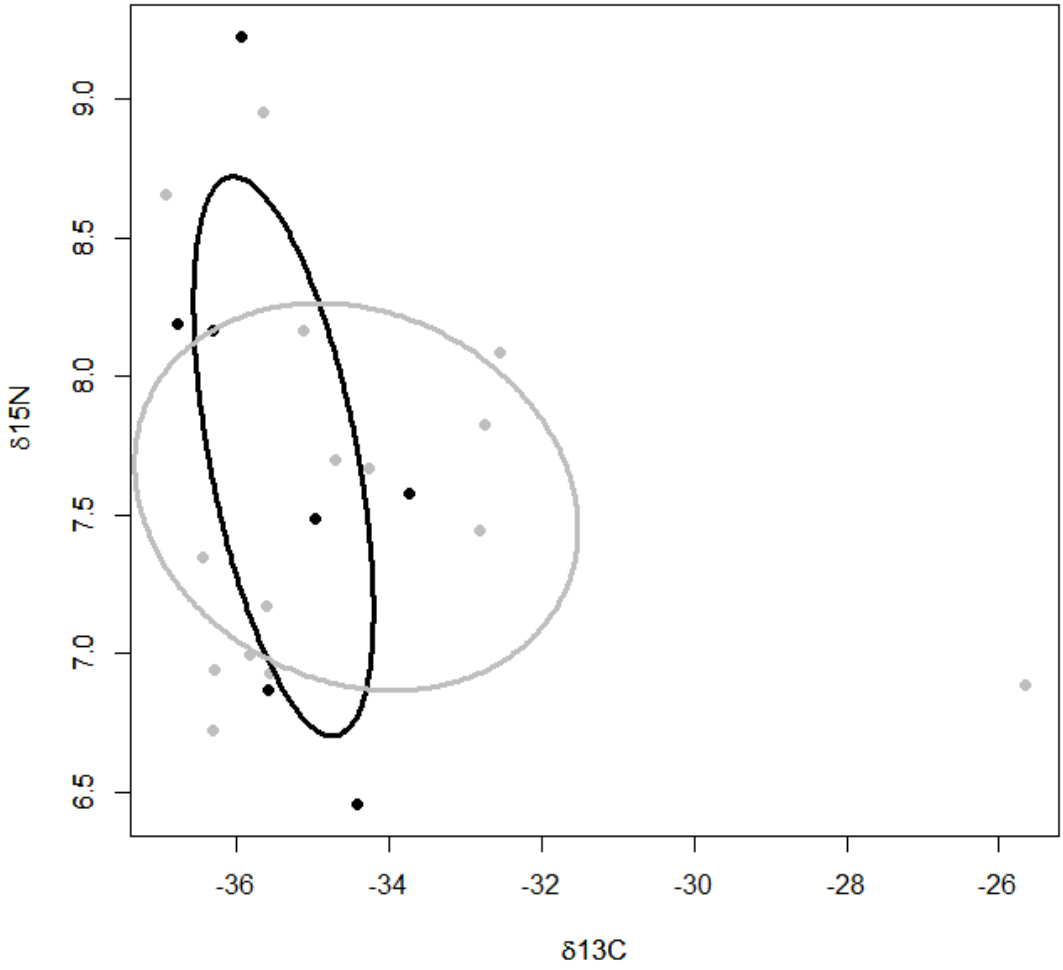

Figure 6.9. Stable isotope biplot for *Gasterosteus aculeatus* infected (grey circles) and uninfected (black circles) with *Schistocephalus solidus* from site S1, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$.

S2: Crampmoor, Autumn 2013

Parasite prevalence of *S. solidus* in this *G. aculeatus* population was 57 % in autumn 2013, and the parasite abundance of infected individuals ranged in number from 1 to 5 (mean: 1.7 ± 0.4) and weight 0.01 to 0.08 g (mean: 0.1 ± 0.01), equivalent to 11 to 48 % of total host body weight (Table 6.2). The binary logistic regression model revealed that the influence of length on infection status of individuals was not significant, although the length range of sampled fish was very limited that inhibited the comparisons made in the spring sample (Table 6.7; Figure 6.10). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1,18} = 2.27$; $P = 0.15$) or corrected weight when controlled for length (GLM: $F_{1,17} = 3.75$; $P = 0.07$).

Infection with *S. solidus* did not significantly affect $\delta^{13}C$ (Wald $\chi^2 = 0.0.86$, $P > 0.05$). It did, however, significantly lower $\delta^{15}N$ (Wald $\chi^2 = 23.35$, $P < 0.01$) (Table 6.4). The SEA_c plot revealed that the result of infection was expansion of the host trophic niche with infected individual occupying a lower trophic position than the uninfected population with a small amount of overlap between the two groups resulting in a larger trophic niche of the whole population (Table 6.4, Figure 6.11).

Table 6.7. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Gasterosteus aculeatus* by *Schistocephalus solidus* according to fish length at site S2.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	A	5.94	6.81	0.38
Fish length	X	-0.22	0.25	0.38

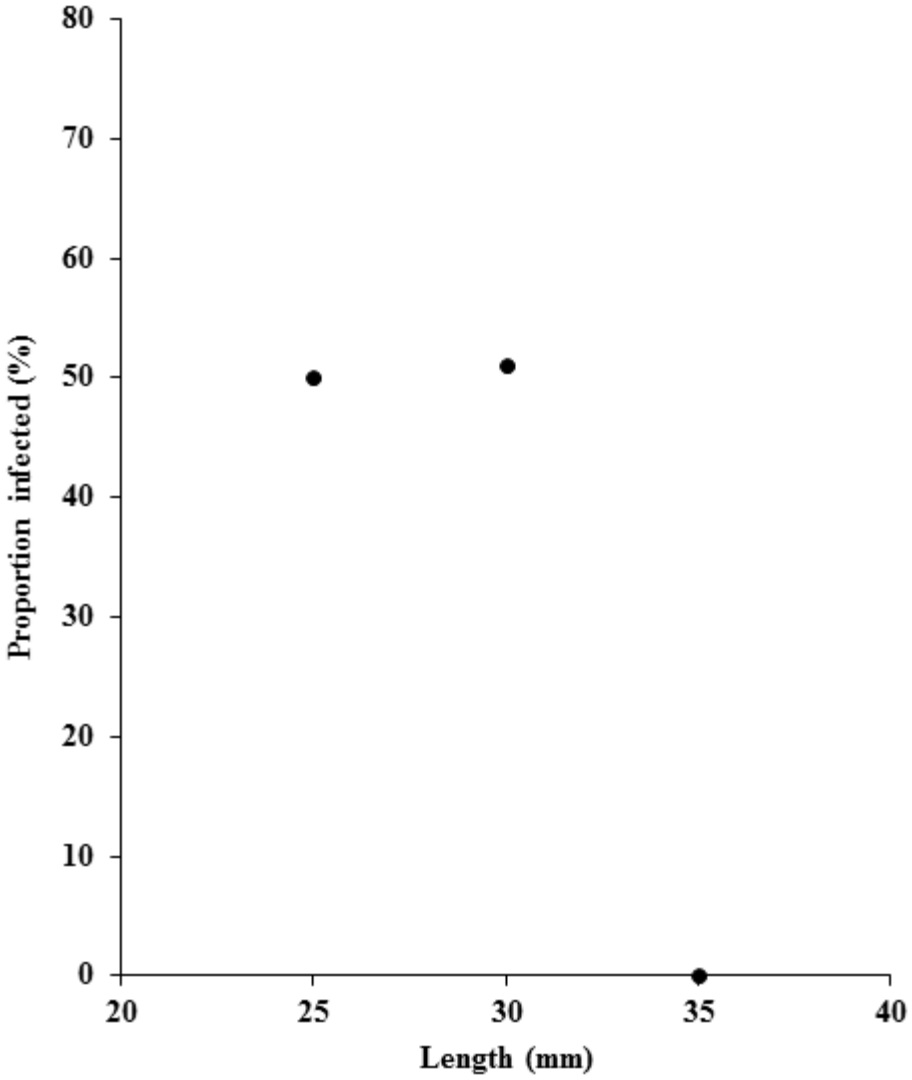

Figure 6.10. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Gasterosteus aculeatus* by *Schistocephalus*

solidus according to length (as 5 mm increments) where solid circles represents the proportion of infected individuals in that size class.

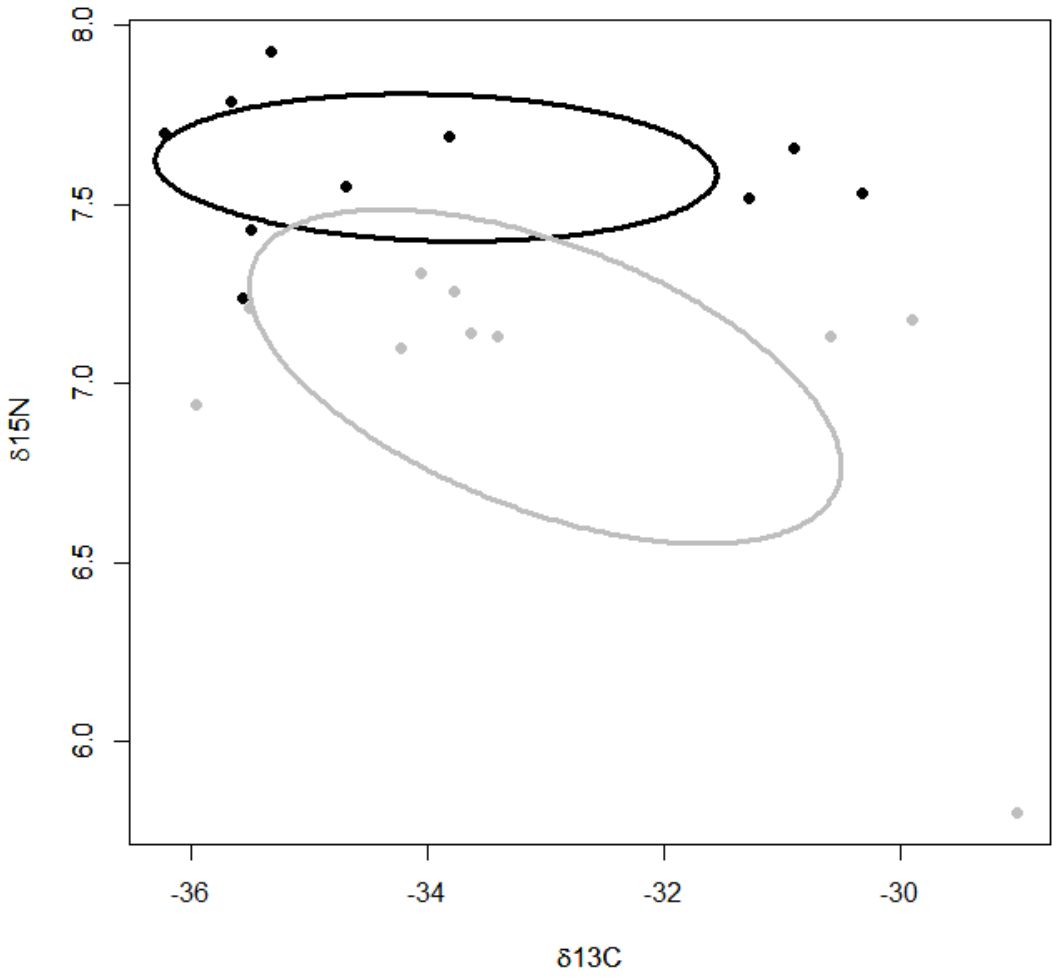

Figure 6.11. Stable isotope biplot for *Gasterosteus aculeatus* infected (grey circles) and uninfected (black circles) with *Schistocephalus solidus* from site S2, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$.

6.4.3 Perca fluviatilis: Triaenophorus nodulosus

Site P1: Bedwell fish farm

Parasite prevalence of *T. nodulosus* in this *P. fluviatilis* population was 42 %, and the parasite abundance of infected individuals ranged in number from 1 to 6 (mean: 1.0 ± 0.6) and weight 0.01 to 0.06 g (mean: 0.1 ± 0.01), equivalent to 0.7 to 2.3 % of total host liver weight (Table 6.2). The binary logistic regression model revealed that the influence of length on infection status of individuals was not significant. (Table 6.8; Figure 6.12). When comparing infected versus uninfected fish, there were significant differences in their condition (GLM: $F_{1,22}$ = 5.44; *P* = 0.03), but with infected individuals having increased condition. The effect on infection on corrected weight when controlled for length was not significant (GLM: $F_{1,21}$ = 1.01; *P* = 0.33).

Perca fluviatilis infected with *T. nodulosus* had significantly increased values of $\delta^{13}C$ compared with uninfected conspecifics (Wald χ^2 = 8.97, P < 0.01), but there was no significant effect on $\delta^{15}N$ (Wald χ^2 = 1.14, *P* >0.05). The SEA_c plot revealed that the trophic response to infection was an expanded trophic niche that greatly increased the size of the population trophic niche (Table 6.4, Figure 6.13).

Table 6.8. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Perca fluviatilis* by *Triaenophorus nodulosus* according to fish length at site P1.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	a	-5.59	4.28	0.19
Fish length	x	0.03	0.02	0.21

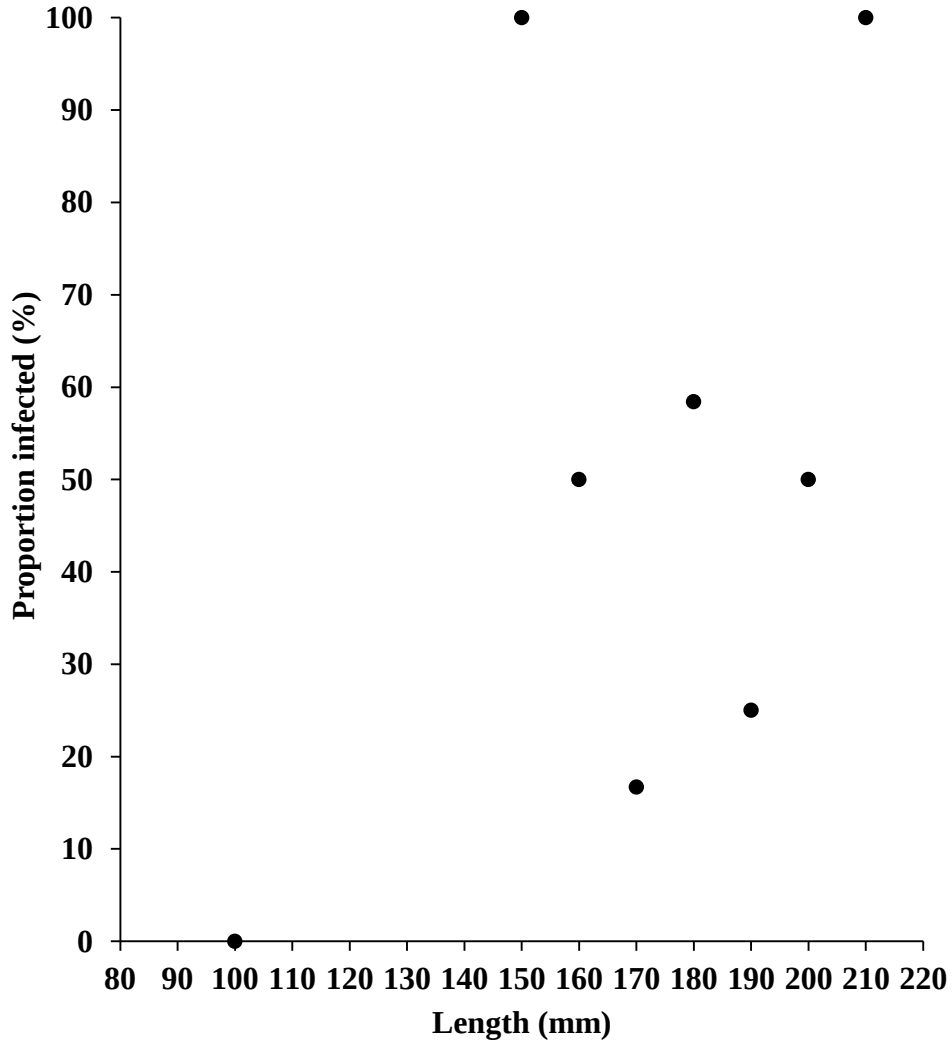

Figure 6.12. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Perca fluviatilis* by *Triaenophorus nodulosus* according to length (as 10 mm increments) where solid circles represents the proportion of infected individuals in that size class.

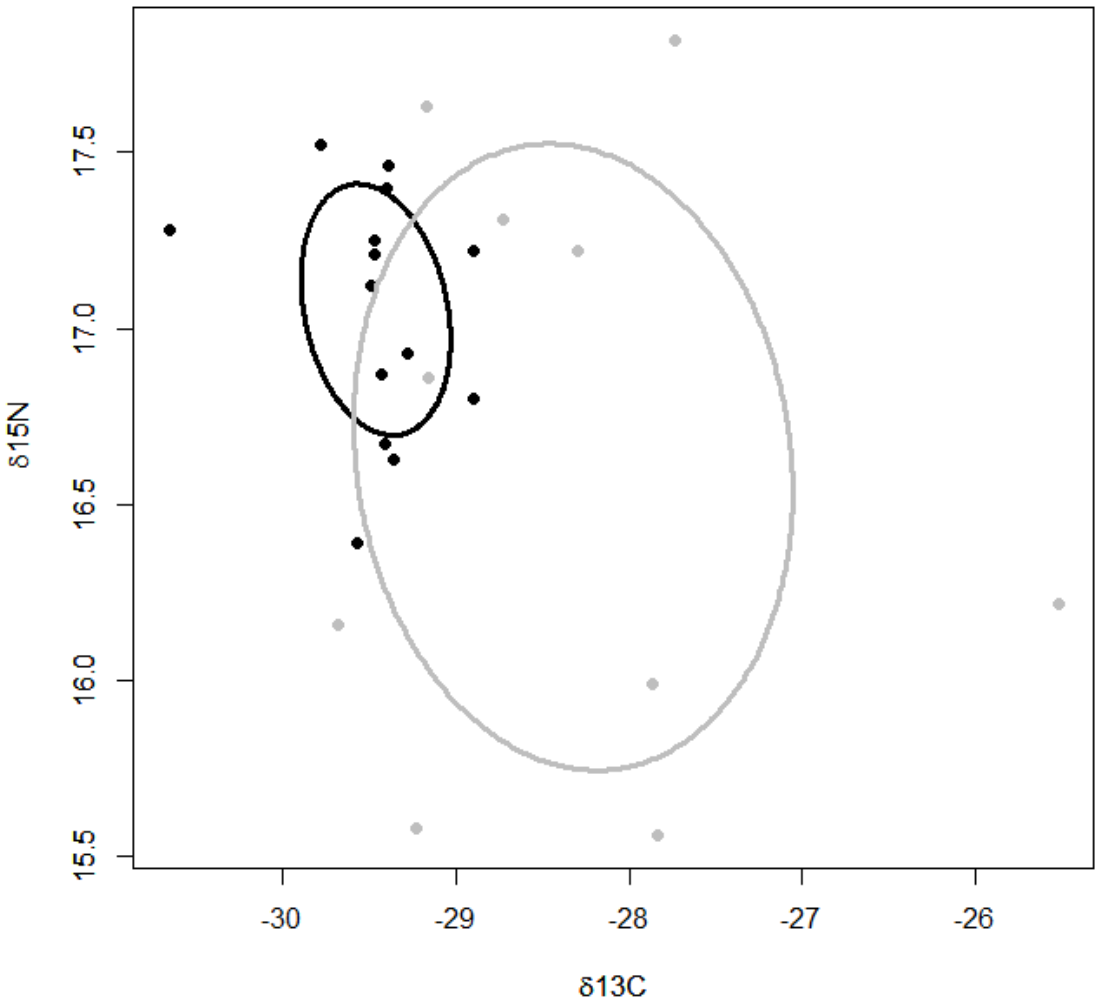

Figure 6.13. Stable isotope biplot for *Perca fluviatilis* infected (grey circles) and uninfected (black circles) with *Triaenophorus nodulosus* from site P1, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$.

Site P2: Limes fishery

Parasite prevalence of *T. nodulosus* in this *P. fluviatilis* population was 84 %, and the parasite abundance of infected individuals ranged in number from 1 to 12 (mean: 4.8 ± 1.0) and weight 0.01 to 0.25 g (mean: 0.1 ± 0.02), equivalent to 1 to 60 % of total host liver weight (Table 6.2). The influence of length on infection status of individuals was not significant (Table 6.9; Figure 6.14). When comparing infected versus uninfected fish, differences were not significant in their condition (GLM: $F_{1,17} = 0.02$; $P = 0.90$) or for weight when controlled for length (GLM: $F_{1,16} = 1.05$; $P = 0.32$).

At this site, infection with *T. nodulosus* did not have a significant effect on $\delta^{13}C$ (Wald $\chi^2 = 0.07$, $P > 0.05$) or $\delta^{15}N$ (Wald $\chi^2 = 0.02$, $P > 0.05$) (Table 6.4). The SEA_c plot revealed that the trophic response to infection was an expanded trophic niche that substantially increased the size of the population trophic niche, despite their occupation of similar isotopic space (Table 6.4, Figure 6.15).

Table 6.9. Binary logistic regression coefficients (Equation 6.1), and their statistical significance, for the probability of infection of *Perca fluviatilis* by *Triaenophorus nodulosus* according to fish length at site P2.

Parameter	Symbol in equation 6.1	Coefficient	Standard error	*P*
Constant	a	2.49	3.16	0.43
Fish length	x	-0.01	0.02	0.79

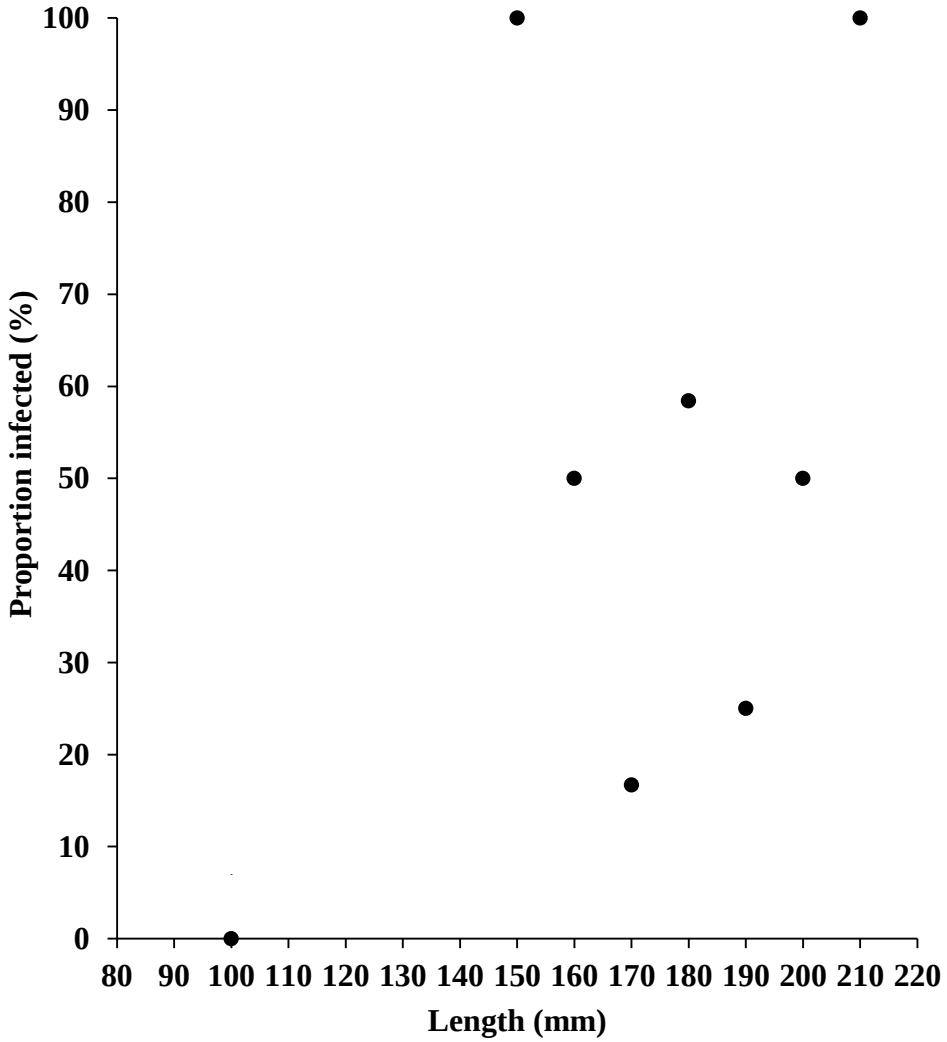

Figure 6.14. Probability of infection (expressed as between 0 and 100, where 100 represents all individuals being infected) of *Perca fluviatilis* by *Triaenophorus nodulosus* according to length (as 10 mm increments) where solid circles represents the proportion of infected individuals in that size class.

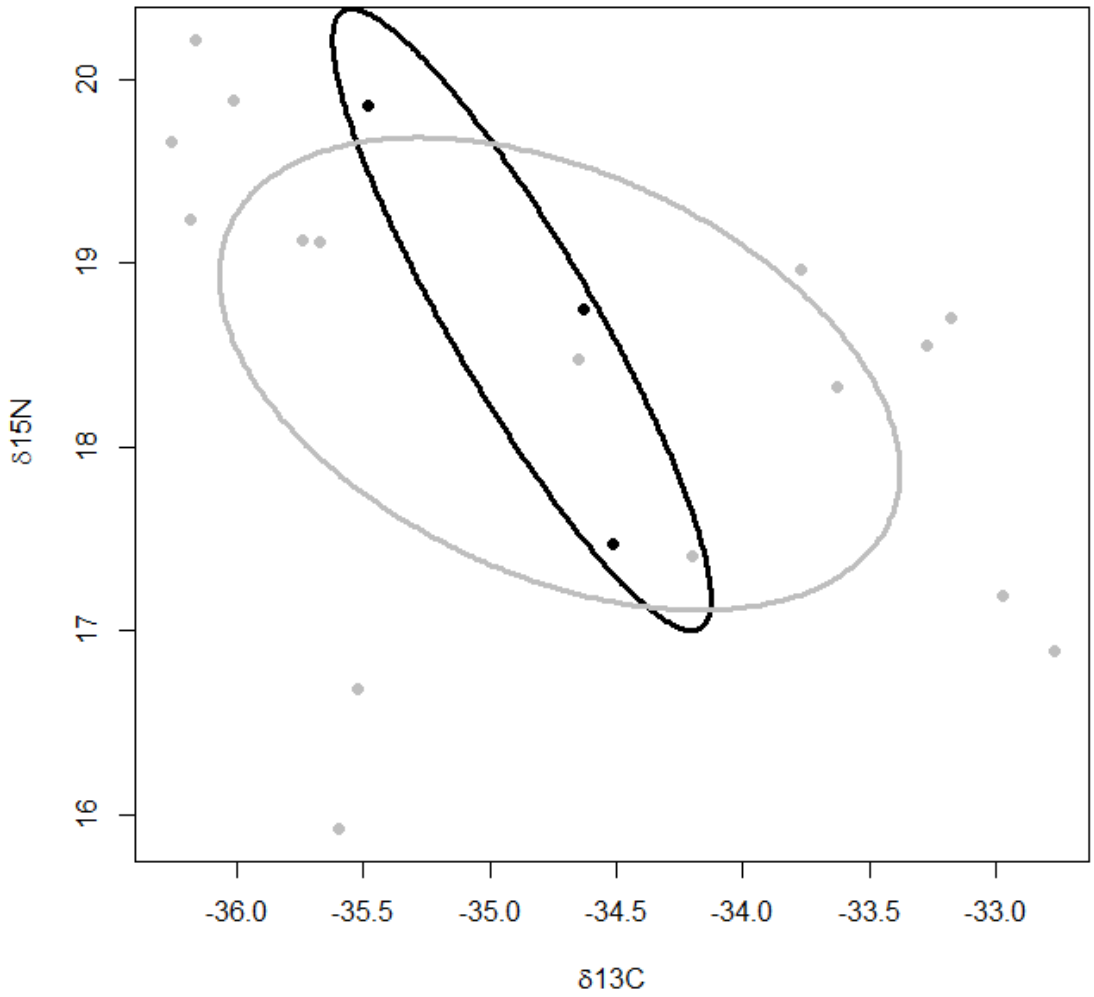

Figure 6.15. Stable isotope biplot for *Perca fluviatilis* infected (grey circles) and uninfected (black circles) with *Triaenophorus nodulosus* from site P2, where the grey ellipses denote the trophic niche size of the infected sub-group and the black ellipses denote the trophic niche size of the uninfected sub-group, and where trophic niche size represents SEA_c calculated from $\delta^{15}N$ and $\delta^{13}C$.

6.5 Discussion

In each of the host-parasite systems, at least one of the study sites showed that parasite infection had a substantial influence on the trophic niche size of the host population, with infection resulting in a larger population trophic niche than if the parasite was absent. For *R. rutilus*: *L. intestinalis*, this was apparent at site R1, where individual infected fish occupied elevated areas of trophic space, indicating they had higher trophic positions than uninfected con-specifics. For *G. aculeatus*: *S. solidus*, shifts in individual niches were apparent in S2, the sample collected in autumn, where infected fish were feeding significantly lower in the food web than uninfected conspecifics, increasing the population trophic niche size, potentially increasing interspcific resource competiton. For *P. fluviatilis*: *T. nodulosus* at site P1, the population trophic niche size was substantially larger with the parasite and whilst this shift was also alluded to in P2, the relatively low number of uninfected fish reduced the ability to detect this. Thus, these findings align strongly to the hypothesis that infection by these native parasites result in some trophic niche specialisations of infected fishes. Notwithstanding, at site R2, and in the sample of *G. aculeatus*: *S. solidus* collected in spring (S1), the infection consequences for the population trophic niche was low, indicating an element of context dependency in the mechanisms involved. However, due to the absence of robust macro-invertebrate samples, the dietary reasons for these patterns were unable to be explored here and that remains a short-coming. Consequently, the potential processes underpinning these patterns are discussed in this section but must remain speculative.

At site R1, *R. rutilus* infected with *L. intestinalis* were feeding at significantly higher trophic levels than uninfected conspecifics (as indicated by $\delta^{15}N$), indicating that the

population trophic niche size was influenced by infection through it driving intra-specific trophic specialization. Although the actual mechanism incurring this within the fish and within the food web could not be tested here, it seems likely through the influence of the parasite on the foraging behaviour and habitat utilisation of the infected fish. For example, Adamek et al. (1996) revealed that the diet composition of infected *R. rutilus* was significantly different to uninfected conspecifics with the infected fish consuming higher quality animal food items compared with the more plant based diets of the uninfected roach (Adamek et al. 1996). This aligns with the findings in site R1 which showed a similar pattern, albeit at R1 the use of stable isotope analysis allowed a more quantitative evaluation of how this influenced trophic niche size. This change in foraging range and shift towards higher quality food items may be due to the physical cost of parasitism leading to increased nutrient demand and so consequently enhanced feeding motivation for taking high quality food items in the infected fish (Pascoe and Mattey 1977). Notwithstanding, infection by *L. intestinalis* also affects habitat choice, with Loot et al. (2001) revealing that infected *R. rutilus* in a small river were encountered more frequently in littoral habitats than uninfected counterparts. This was hypothesised as being the consequence of the parasite directly modifying the behaviour of the host in order to increase the likelihood of it being predated by a fish-eating bird and thus completing the parasite lifecycle ('Parasite increased trophic transmission'; Loot et al. 2001; 2002; Britton et al. 2008). This shift in habitat would also be likely to promote a shift in diet composition due to the differences in food availability between the littoral and open water habitats, and this would potentially be a key driver of the differences observed here in the trophic niches of the two population sub-sets.

At site R2, however, the infected fish did not show differences in their trophic niche when compared to their uninfected conspecifics, despite parasite prevalence and abundance being similar at both sites. Although not tested, this was believed to relate to the habitat typologies of the two sites. Site R1, where infection strongly influenced intra-specific trophic specialization, was a river with a heterogeneous and highly complex habitat that was likely to have provided high diversity in the invertebrate communities that enabled the fish to diverge in their diet and trophic niche according to infection and the action of the parasite on host behaviour. By contrast, site R2 was a man-made lake used for recreational angling in which the habitat features that would add complexity and increase the diversity of food items available had largely been removed as these impeded access to angler. Consequently, this simplification of the habitat structure was likely to have resulted in invertebrate communities with reduced diversity, so limiting opportunities for trophic niche divergence between the infected and uninfected conspecifics. This contrast highlights the level of site specificity and context dependency within complex host parasite interactions.

Analysis of *G. aculeatus* infected with *S. solidus* in spring 2013 (S1) revealed they were feeding at similar trophic levels to uninfected conspecifics (as indicated by $\delta^{15}N$). There was, however, some evidence of infected individuals feeding on a slightly wider range of resources. By contrast, analyses completed in autumn 2013 (S2) revealed *G. aculeatus* infected with *S. solidus* were feeding at significantly lower trophic levels than uninfected conspecifics (as indicated by $\delta^{15}N$), indicating that the population trophic niche size was being influenced by the parasite. Whilst the actual mechanism incurring this consequence in S2 within the fish and within the food web was unable to be tested, it is probable that this was through the influence of the parasite on the foraging

behaviour, anti-predator behaviours and habitat utilisation of the infected fish. There is considerable empirical evidence for these in this host-parasite system, with Barber and Huntingford (1995) suggesting that *G. aculeatus* infected with *S. solidus* had increased feeding motivation due to higher energetic demands and this modified their foraging behaviour. The presence of *S. solidus* plerocercoids in the visceral cavity restricts prey choice by increasing the handling time of large prey items, reducing the nutritional advantages of these prey items (Wright et al. 2006). This can lead to a switch in prey choice from larger to smaller prey items (Barber and Huntingford 1995) and so this could be the potential driver behind the difference in trophic level found here.

The difference in output between spring (S1) and autumn (S2) was likely to have resulted from seasonality and the life cycle of *S. solidus.* Warmer temperatures significantly increase the growth rates of *S. solidus* plerocercoids (Macnab and Barber 2012) and so the plerocercoids in late summer and autumn tend to be larger, as found here also. This is important, as plerocercoid size is important in determining the impact of *S. solidus* on *G. aculeatus* behaviour, with larger parasites having more marked impacts (Barber et al. 2004). It has been shown experimentally that when presented with a simulated avian threat, *G. aculeatus* hosts infected with immature *S. solidus* (< 50 mg) did not differ significantly in their response time to uninfected fish. However, *G. aculeatus* with infections of *S. solidus* over 50 mg, the size at which they are mature and ready to move their avian final stage host (Barber and Svensson 2003), were significantly slower to respond (Barber et al. 2004). Furthermore, uninfected *G. aculeatus* rarely left cover after disturbance; however those infected with *S. solidus* plerocercoids > 50 mg regularly left cover (Barber et al. 2004). These behavioural manipulations result in increased ecological opportunities for infected *G. aculeatus*,

including foraging time budgets and foraging habitats, which can all influence diet and so the trophic niche of the individual (Araujo et al. 2011; Evangelista et al. 2014). Sticklebacks infected with large *S. solidus* plerocercoids are, however, also physically restricted in their choice of prey items (Wright et al. 2006) and so the combination of reduced anti-predator behaviours and an increase in nutrient demand in infected fish in autumn could have resulted in more time feeding on items in lower trophic positions than the uninfected fish.

At site P1, *P. fluviatilis* infected with *T. nodulosus* were utilising a significantly wider trophic range than uninfected conspecifics (as indicated by $\delta^{13}C$), indicating that the population trophic niche size was influenced by infection. It is likely this was through the influence of the parasite on the host physiology, cellular pathology and, in turn, these impacting the foraging behaviour and habitat utilisation of the infected fish. The parasite was encountered at high abundance within the liver and resulted in visual discolouration and deterioration of the remaining liver tissue. The liver is an important glycogen store in *P. fluviatilis* and so its pathology is likely to impair its function and lead to reduced energy storage. Therefore, a heavy infection of *P. fluviatilis* may drive *P. fluviatilis* to have increased energy demands and consequently increase their feeding rate as they try to maintain sufficient energy levels and become less reliant on their glycogen reserves (Mehner and Wieser 1994). This increased energy demand could be causing the increased trophic range found here, as *P. fluviatilis* infected with *T. nodulosus* attempt to meet the demand by expanding their choice of prey items. Whilst a similar result to P1 was suggested at P2, with *P. fluviatilis* infected with *T. nodulosus* utilising a wider trophic range than uninfected conspecifics (as indicated by $\delta^{13}C$), however this result was not significant. This might have been due to the low number of

uninfected *P. fluviatilis* available for analysis. Alternatively, it is also possible that site differences including food and habitat availability, as well as environmental parameters, affected the consequences of parasitism.

In summary, across all of the host-parasite systems, there was consistent evidence that infection had some substantial influences on intra-specific trophic specialization and resulted in a larger population trophic niche than when the parasite was absent. These findings have potentially large implications on food web dynamics and community structuring and further highlight the importance of parasites in food webs. Although parasites are now a well-documented aspect of food web ecology (Marcogliese et al. 1997; Byers 2009; Lafferty and Kuris 2009) there are still knowledge gaps relating to their role in shaping population trophic niches. Despite the generally consistent outputs of the present study it is clear from the variation in results between sites and seasons that there is a certain amount of site specificity and context dependency present in the effects of parasite infection on intraspecific specialisation and this has to be taken into consideration when drawing general conclusions and making comparisons with existing and future studies. These future studies will need greater focus on the processes leading to these patterns and should include experimental approaches as well as field approaches.

Chapter 7:

Discussion

7.1 Overview

The subject of parasite introductions and their subsequent consequences has received a great deal of research attention (e.g. Johnsen and Jensen 1988; Alderman et al. 1990; Kennedy 1994; Blanc 1997; Naura and Robinson 1998; Gozlan et al. 2009; Pegg et al. 2011; Williams et al. 2013; Pegg et al. 2015). Studies often suggest that when introduced parasites infect new hosts then the consequences for native fauna can be considerable, either through disease emergence or through alterations in host biology and ecology that accumulate over time (Hatcher et al. 2011; 2012; Britton 2013; Britton and Andreou 2016). Nevertheless, processes such as enemy release suggest these adverse effects might be limited to a relatively low number of parasite species, with few non-native parasites actually being introduced with their free-living hosts (Chapter 5).

Movements of fish within biogeographic regions and countries can also result in parasites that are indigenous to some river catchments being translocated to others where they are non-indigenous (Byers 2002; Chapter 4). Given that the fauna in the non-indigenous range might not have experienced the parasite previously then their effects are likely to be similar to an introduced parasite from elsewhere, i.e. the lack of recent experience and co-evolution between the parasite and resident fishes might result in poor anti-parasite behaviours, and low resistance and resilience in the naïve hosts (Simberloff and Spilling 1996; Ruiz et al. 1999). For both non-indigenous and non-native parasites, understanding their potential and realised consequences in wild situations can be challenging, ideally requiring some knowledge of the infection consequences in both ranges and also information on initial host responses, whilst accepting that impacts are often only assessed after the host-parasite interactions have

been present over several generations when some resistance, resilience and adaptation might have already developed (Ridenhour and Nuismer 2007).

Developing understandings of parasite consequences and processes for multiple host species within the same parasite lifecycle also poses considerable challenges with, for example, the lifecycle of *P. laevis* involving transmission to a relatively large number of potential final hosts via a single intermediate host species, *G. pulex*. Despite a large volume of work completed on parasite manipulation of *G. pulex* infected with *P. laevis* (Section 3.1, 3.2), there remains some limited understandings of how different final fish hosts respond to that manipulation. Work in Chapter 3 and 4 suggested it results in complex interactions in both controlled and wild environments. Moreover, given that their wild environments are being subjected to increasing global changes, such as climate change, which could result in some marked changes on host-parasite relationships (Brooks and Hoberg 2007), then these interactions could be subject to considerable alterations in parasite virulence and host susceptibilities, as explored in Chapter 2.

The sub-lethal consequences of parasite infections, including changes to host biology and ecology via alterations to behaviour and physiology (Barber et al. 2000), have the potential for causing marked changes in the ecology of host populations (Hatcher and Dunn 2011; Hatcher et al. 2012). Processes such as parasite-mediated competition and parasite manipulation can result in substantial shifts in the habitat utilisation and diet of individual hosts, potentially incurring considerable alterations in population trophic niches and in food web structure (Britton 2013; Britton and Andreou 2016). Understanding these consequences for both native and non-native parasites, and

indigenous parasites in non-indigenous ranges, should provide insight into the ecological consequences of parasitism that can determine the relative importance of lifecycle complexity and parasite origin in its impact assessment. Indeed, work completed in Chapters 4 and 6 indicated that trophic consequences in host populations are apparent across a range of parasites, including in non-indigenous and indigenous ranges, with this explored further in Section 7.3.4.

Overall, this study aimed to assess a range of foraging and trophic consequences for fish hosts by infections of introduced and native parasites. Initially, *P. laevis* and a range of native fishes were used as the host-parasite model (Chapters 2 to 4), followed by investigations into the extent of enemy release from non-native fish using Great Britain as the model area (Chapter 5). As this work revealed a high extent of release from their native parasite fauna but acquisition of a range of native parasites, Chapter 6 studied the trophic consequences of infection by some native parasites for some native fishes. In the remainder of this chapter, aspects of these results are thus explored further in order to highlight synergies between the different approaches, consistencies with existing knowledge and to emphasise the novel perspectives.

7.2 Experimental studies on *Pomphorhynchus laevis*

7.2.1 Experimental infections and temperature effects

A primary outcome of Chapter 2 (in terms of the Ph.D. research at least) was the demonstration that fish held under laboratory conditions could be experimentally infected with *P. laevis* via infected *G. pulex*, with the conditions required to achieve infections revealed. This was a critical step, as it then enabled the work designed for

completion in Chapter 3 to proceed. In doing so, this work also indicated the effect on a host species of water temperature increases and differences in parasite exposure levels on parasite prevalence as well as the infection parameters of parasite abundance, mean parasite weight and parasite burden. The interaction of temperature and parasite exposure had considerable consequences for both parasite prevalence and the infection parameters; whilst prevalence was substantially higher at the elevated temperature, where infections did develop at lower temperatures, they were associated with fewer but larger parasites, resulting in significantly higher parasite burdens. Despite this, there was no effect of any parameter detected on host growth rates when they were tested against control fish. They highlighted that elevated temperatures can result in some complex interactions between parasite virulence and host susceptibility, and these will require subsequent experimental decoupling in order to derive more advanced understandings.

These outputs were consistent with other studies in this area that also suggested temperature plays a significant role in parasite dynamics (Harvell et al. 2002; Tinsley et al. 2011; Zamora-Vilchis et al. 2012). There is, however, variability in the extent and direction of these effects. For example, some studies reveal warmer temperatures have a negative effect on parasite development and life cycle completion (Karvonen et al. 2010; Lohmus and Bjorklund 2015), whilst others suggest warming could be strongly advantageous to the parasite (Tinsley et al. 2011; Macnab and Barber 2012; Zamora-Vilchis et al. 2012; Lohmus and Bjorklund 2015). This demonstrates that there is a level of system and host-parasite context dependency in these relationships. In addition, it remains unclear what the impact of warming will be for non-indigenous parasites and in particular how warming could potentially facilitate their spread (Harvell

et al. 2002; Tinsley et al. 2011; Zamora-Vilchis et al. 2012). For parasites introduced from other climatic regions, warming will also potentially enhance parasite growth and maturation rates, and this could also increase their virulence (Brooks and Hoburg 2007). Coupled with the potential for decreased host immunity with increased temperature and increased susceptibility to infection (Hakalahti et al. 2006; Poisot et al. 2009; Cramp et al. 2014; Dittmar et al. 2014), elevated water temperatures could therefore have considerable consequences for host-parasite relationships.

7.2.2 Behavioural functional responses in the context of parasite manipulation

Studies into parasite manipulation have frequently used the *P. laevis*: *G. pulex* parasite-intermediate host system for investigating how infections can result in behavioural modifications to the host that then results in their elevated risk of being predated by a fish (Table 3.1). Despite many of these studies showing high consistency in their outcomes, primarily via reduced anti-predator responses in infected *G. pulex*, they often lack consistency in many aspects of their sample collection (Table 3.1). For example, in Baldauf et al. (2007), investigating responses to predator cues, the source of *G. pulex* differed from the source of the fish (*P. fluviatilis*) from which the cues were derived. This is despite some experimental evidence indicating that the extent of manipulation of the intermediate host can vary across different origins of *P. laevis*. This was highlighted by Franceschi et al. (2010b), who demonstrated that when *P. laevis* was collected from six different *S. cephalus* populations and exposed experimentally to *G. pulex*, there was considerable variation in the extent of the *G. pulex* manipulated behaviours that developed across the different source populations. The extent of manipulation can also vary with the ages of both parasite and the host, and through relatively subtle genetic variance in the parasites, such as through maternal effects and sibship (Franceschi et al.

2008; 2010a, b). Moreover, given that *P. laevis* is capable of infecting a wide range of final fish hosts that have a range of foraging behaviours, and these hosts can have varying abundances across river fish assemblages, then whilst manipulation might be demonstrated, it might not be clear whether the behaviour is being selected to promote consumption by a particular fish species or is being selected more generally. This is potentially important because, for example, a manipulated behaviour that promotes the predation of *G. pulex* by *C. gobio* might differ greatly from one promoting their predation by *S. cephalus*, given the potentially marked differences in foraging behaviours, patch fidelity and habitat utilisation between these fishes (Noble et al. 2007a, b). Given that the changes in foraging behaviours of fish hosts due to manipulation appears not to have been tested previously, this suggested that there remained considerable uncertainty in many aspects relating to transmission of *P. laevis* lifecycle through the trophic vacuum.

Consequently, the initial objective of Chapter 3 was to utilise comparative behavioural functional response experiments to test differences in the consumption rates of three fishes exposed to either uninfected or infected *G. pulex*, testing the hypothesis that the consumption rate of infected *G. pulex* would be significantly higher. The Type II functional response curves and their 95 % confidence intervals contrary to this hypothesis, especially in *S. cephalus* and *B. barbus*, which are both final hosts of *P. laevis* in the river from which the *G. pulex* were collected. In *C. auratus*, used here as a naïve host, consumption rates were similar for both infected and uninfected *G. pulex* until high food densities were reached, at which point the consumption rates were again elevated for the uninfected *G. pulex*. These counter-intuitive outcomes were also contrary to most other studies that suggested a parasite would manipulate its

intermediate host in a way that promotes its transmission to a final host and facilitate the continuation of its life cycle (e.g. Kennedy et al. 1978; Bakker et al. 1997; Bauer et al. 2000; Cezilly et al. 2000; Baldauf et al. 2007; Diane et al. 2011; 2014).

In Sections 3.5 and 4.5, a number of factors were outlined and discussed that could have produced these patterns in the foraging responses of the fishes to the infected and uninfected *G. pulex*. There was particular emphasis on the potential for low selection pressures for manipulation in the host-parasite system, given the very high abundance of *G. pulex* in many lowland rivers, their parasite prevalences throughout the year (up to 29 % in this study) and the high abundance of potential fish final hosts present in the community. In combination, it could thus be argued that there would be relatively low selection pressure for parasite manipulation in *G. pulex*, given that the abundant small-bodied fishes in the river were likely to be almost continuously exposed to infected *G. pulex*. Moreover, Chapter 2 predicted that at water temperatures of 18 ^{o}C, a typical ambient summer water temperature in Southern England (Britton 2007), 50 % prevalence of *P. laevis* was achieved when *S. cephalus* consumed only 26 infected individual *G. pulex*, with this threshold exposure reducing substantially at higher temperature, i.e. potential hosts only require exposure to a relatively low number of intermediate hosts in order to develop an infection. Thus, the patterns detected in the remainder of Chapter 3 in relation to *G. pulex* behaviours in the presence of difference predator cues might ultimately be explained by a lack of manipulated behaviours in this intermediate host that result from a lack of selection pressure in this aspect of the parasite lifecycle. This potentially represents an important novel outcome for both this host-parasite system and studies on parasite manipulation and the trophic vacuum more generally. It also suggests greater emphasis is required on the experimental design of

future studies (e.g. sources, ages and genetic variability of the animals used) that should also incorporate measurements of the response of fish hosts to the behaviours of the intermediate hosts.

7.3 Indigenous and non-indigenous parasites in field studies

7.3.1 Evidence of enemy release and parasite acquisition?

The enemy release hypothesis is important in terms of predicting the (low) numbers of parasites that are likely to be introduced with a non-native host (Keane and Crawley 2002; Mitchell and Power 2003; Hatcher and Dunn 2011). Chapter 5 revealed that very few parasites have been introduced into Great Britain with their non-native fish hosts, with this outcome consistent with other studies around the world that suggest it is a relatively common phenomenon associated with non-native species introductions (Torchin et al. 2003). Indeed, parasite acquisition was by far the more common process in the fishes examined in Chapter 5, with several examples of native parasites infecting non-native fish. These findings suggest that the risk of introducing a parasite with a non-native fish host is low and, moreover, even when this does occur then these are often highly specialised parasites that pose minimal ecological threat to native fishes, such as *T. vistulensis* in *S. glanis* (Reading et al. 2012) and *O. dispar* in *L. gibbosus* (Hockley et al. 2011). It is acknowledged that there remains a requirement for authorities to maintain regulatory processes and procedures to prevent non-native fish parasites from being introduced, given there remains some potential for disease emergence and associated adverse economic and ecological consequences (Williams et al. 2013). However, it also suggests that from an ecological perspective, greater focus could be given to the complexity of parasite lifecycles and parasite-driven host

phenotypic alterations as the driver of ecological consequences for infected fish hosts, rather than focusing on the origin of that parasite.

7.3.2 Parasite prevalence, abundance and relationships with fish length

In the case of *P. laevis*, parasite prevalence and abundance differed between sites and host species (Chapter 4). However, in all species and sites, prevalence was relatively high and reached up to 96 % in *C. gobio*. There were also minimal differences in parasite prevalences and abundances between the indigenous and non-indigenous ranges of *P. laevis*, again suggesting that the origin of the parasite was of limited importance to its virulence in the host populations. Whilst other studies show greater variability in prevalences of *P. laevis* (Table 3.1), there was again no consistent pattern between rivers across the two ranges. It was argued that the high parasite prevalences in small bodied, abundant fishes like *C. gobio* and *P. phoxinus* in lowland rivers in England was potentially important as they provide *P. laevis* with an alternative final host species to less abundant fishes such as *S. cephalus* and *B. barbus* (Section 4.5). In each river studied in Chapter 4, it appeared that there were multiple preferred hosts in the fish communities, raising further questions in relation to both the development of host manipulation in the intermediate host but also the development of final host specificity in an apparently generalist parasite.

Chapter 6 indicated that there were few significant relationships between the probability of being infected with the three native parasites and fish length, although this might have been related to some low size ranges of some of the sampled fishes. For *P. laevis* in Chapter 4, there was a strong pattern of significantly higher probability of infection with fish length for most fish species, with this likely to be related to the gape

size of the fishes, i.e. once the gape size has developed sufficiently to enable that species to consume *G. pulex* then there is a relatively high probability of developing infections. The smallest infected fish detected here was a 41 mm *C. gobio*, although in the River Teme, one of the study rivers, *B. barbus* have since been found to be infected at standard lengths of only 20 mm (Section 4.5). That these relatively small fishes have *P. laevis* infections is important given that the most severe consequences of parasitism are often seen in juvenile fish (Britton and Pegg 2011), with resilience and competence to infection usually only being more apparent in larger fishes.

7.3.3. Pathological consequences of parasite infections

Pathological consequences were only assessed for *P. laevis* in Chapter 4, but these revealed interesting comparisons between the 'preferred host' of *S. cephalus* and the small bodied hosts (*C. gobio*, *P. phoxinus*, *B. barbatula*). In *S. cephalus*, infection caused a classic host response, whereby the proboscis and bulb of the parasite were encapsulated in a fibrous response at the site of penetration on the intestine, limiting the pathology of the infection to a localised area of tissue. In the three small-bodied fishes, there was little or no host response to infection, with the parasites often penetrating right through the intestinal wall and occasionally into surrounding tissues (e.g. muscle and ovary). This lack of response and subsequent penetration of the surrounding tissues has the potential to increase the extent of negative consequences of infection for these hosts relative to *S. cephalus.* For example, if the parasite was to penetrate right through the muscle of the peritoneal cavity it would allow water to enter this, which would likely be fatal. Sub-lethal consequences could take the form of reduced fitness as a result of the parasite penetrating the testis or ovaries, causing necrosis in the gonads. Whilst *S. cephalus* appeared to be investing considerable resources into their immune

responses, the lack of immune response in the small-bodied hosts are likely to ultimately result in more adverse energetic and fitness consequences. This further highlighted the importance of considering these small-bodied fishes as important host species and, in the case of pathology, it raises the question as to why they were not exhibiting the kind of infection responses observed in *S. cephalus*.

7.3.4 Trophic consequences of parasite infections at the population level

It was outlined in Section 1.2.5 that parasites potentially incur trophic consequences for their hosts through altering the host phenotype, resulting in the infected population sub-group consuming different prey items to the uninfected sub-group, or specialising on certain items present in the diet of both sub-groups (Britton and Andreou 2016). This potentially results from processes such as parasite-mediated competition in which the altered host phenotype modifies their interactions with their prey communities, uninfected conspecifics and non-host species, altering their diet composition and trophic niche (Hatcher and Dunn 2011; Hatcher et al. 2012). Parasite driven trophic niche specialisation was investigated in both Chapter 4 and 6. The underlying processes were unable to be investigated further, such as parasite mediated competition and whether infection resulted in reduced consumption rates and ability to forage on certain prey items as per *C. carpio* infected with *B. acheilognathi* (Britton et al. 2011; 2012). However, the outputs of both chapters did reveal patterns of trophic niche specialisation in all of the studied fish host populations, with strong divergence evident in some populations, whilst niche constriction was more evident in others. There was some context dependency in the extent of the niche specialisation, with differences apparent from infections of *P. laevis* in both time (e.g. for *S. cephalus* in the River Darent) and space (e.g. for *C. gobio* across all rivers). Although unable to be investigated here, other

than through body condition with inconclusive results across the tested species, the possibility remains that these dietary changes might also lead to alterations in energy acquisition that could then manifest as a further adverse consequence of infection.

For the three native fish parasites studied in Chapter 6, there was also considerable variation in some of the patterns of the trophic consequences. Where *P. fluviatilis* were infected with *T. nodulosus*, they occupied a substantially larger trophic niche than uninfected conspecifics at one site, although this was less evident at the other. Unlike *P. laevis* and *T. nodulosus*, where the infected fish were final hosts (or at least are capable of being final hosts), the other two parasite species investigated were intermediate hosts; both *L. intestinalis* and *S. solidus* used fish-eating birds as final hosts and utilised *R. rutilus* and *G. aculeatus* to navigate through the trophic vacuum (Section 1.2.1). The parasite infections in both of these fishes tended to fill the body cavity (personal observation) and this, along with potential behavioural changes via parasite manipulation, usually results in marked differences in habitat utilisation between the infected and uninfected conspecifics (Loot et al. 2001; 2002; Britton et al. 2008), potentially leading to resource partitioning (Milinski 1985; Loot et al. 2001). Indeed, evidence of parasite-driven trophic niche divergence was apparent for both parasites, although for *S. solidus*, the effects were seasonal. This again highlights the importance of context dependency in the ecological consequences of parasites at population levels. In the case of *S. solidus*, the habitat partitioning, and thus the resource partitioning and niche divergence, was likely to relate to the period when the parasites start to develop within the fish (Milinski 1985).

7.4 Conclusions and future directions

7.4.1 Main findings

The main findings of this Ph.D. research were that:

- Effects of warming could be marked for host: parasite relationships, altering parasite prevalence and abundance as well as parasite development and maturation.
- Existing parasite manipulation studies might be too simplistic and need to take greater cognisance of differences in manipulation between different fish final hosts and across different populations. Conflicting results from this research and the majority of the existing literature highlight the need for consistency and full understanding of the complexities of host manipulation.
- Roles of small-bodied and abundant fish species in the parasite population dynamics of *P. laevis* might be more important than assumed at present, raising questions on host-specificity and manipulation.
- Enemy release is evident in a number of non-native fishes introduced to England and Wales, and these hosts are experiencing parasite acquisition.
- Major parasite infections appear to incur trophic niche specialisations, although the processes by which this might occur are not yet clear, but are likely to be related to phenotypic changes in aspects of the foraging behaviours and habitat utilisation of the hosts.
- The biogeographic origin of the parasite might have only a minor role in determining its host consequences, with the ecological consequences more related to the complexity of the parasite lifecycle, its infection pathways and their potential to alter the physiology and behaviour of its host.

7.4.2 Research approaches

Much of the research completed represented important initial steps into understanding aspects of the host-parasite relationship and so it was not always possible to draw definitive conclusions. With the exception of Chapter 5 on enemy release, all of the data chapters had a tendency to raise a series of questions for further study in relation to the ecological consequences of parasites for fish hosts in ambient and future environmental conditions. This was especially the case for Chapters 4 and 6 that were field based and so were not able to test the processes that lead to the patterns observed. Consequently, it is recommended that research in the following areas is completed in order to take these outcomes forward into future studies.

7.4.3 Role of warming on host: parasite relationships

There are a number ways in which the role of warming in the *P.laevis: S. cephalus* host: parasite relationship, and indeed that of other species, can be further investigated experimentally. Two very simple ways to do this are to use different temperatures and different host species; this could add width to the level of understanding but offers little in the way of clarifying exactly what impact warming could be having. Varying the temperature at different stages throughout the experiments should identify when warming impacts on parasite virulence. Similarly, developing larger sample sizes of infected individuals would enable more temporal perspectives to be investigated on the role of temperature in parasite maturation in the host, and its effect on parasite fecundity. A further area of interest would be to test the thermal preferences of infected and uninfected hosts in a similar way to the experiments performed by Macnab and Barber (2010), where they investigated the thermal preferences of *G. aculeatus* infected with *S. solidus*. For example, should the completion of the parasite lifecycle be shortened in warmer conditions would the host behaviour be manipulated to move

towards these areas of thermal elevation? This has potential implications for population sub-group dietary specialisation due to habitat partitioning according to temperature.

7.4.4 Parasite manipulation

The existing body of literature on *P. laevis* suggests that there is some experimental inconsistency in the source of parasites and the model host used. In order to draw more robust conclusions on host manipulation, it is recommended that parasites are taken from the same source as their hosts with these then used to produce generations of laboratory infected intermediate hosts that are used across specific host fishes in order to produce parasites where the potential for developing maternal and species-specific effects is strong. This has been successfully achieved in other models such as *G. aculeatus* and *S. solidus*; in doing so, it has enabled more precise outcomes to be produced with high repeatability and consistency (Foster and Baker 2004; Gibson 2005; Heins and Baker 2008; Barber 2010; Macnab and Barber 2012). Following production of generations of fish host-specific lineages raised at specific temperatures experiments can be designed that cross infect other species across different temperatures, and measure the consequences for manipulation of the intermediate hosts, such as testing for different forms of manipulation when the parasites and intermediate host is exposed to different fish hosts. Experimental designs can then become more complex with, for example, the addition of lights to create brighter patches in the experimental arena to assess the potential consequences of photophilia exhibited by infected *G. pulex* on their probability of predations. Similarly, the addition of refugia and its utilisation by *G .pulex* could be investigated in terms of vulnerability to predation in order to identify the

forms of behavioural manipulation that actually do result in increased consumption rates of infected *G. pulex*.

7.4.5 Generalist, complex fish parasites in fish communities of multiple species

In order to fully understand the generalist nature of parasites like *P. laevis* and the role of small-bodied fish hosts within their life cycle, further investigations are required. Firstly, a comprehensive understanding on the viability of each species as a definitive host is required to ascertain whether *P. laevis* can mature within them and thus complete its lifecycle. From there, field studies should continue to assess the extent to which the hosts are actually used in the wild and, coupling field studies with laboratory experiments, should also more accurately quantify the roles these fishes have as paratenic hosts for larger bodied fish such as *S. cephalus* and *P. fluviatilis* (Medoc et al. 2011) Future studies should also aim to investigate whether there is genetic structuring in the parasite population across the different fish host species to reveal whether there is the development of host specificity within this generalist parasite and the role this could play in relation to manipulation of the intermediate host.

7.5.6 Trophic niche specialisation due to parasitism

Future research should focus on identifying the drivers of trophic niche specialisation of population subgroups of parasitized host communities. There are a number of potential drivers that require further work, with the direct effect of host manipulation on the trophic niche of the infected population sub-group being the most straight forward to study via aquaria based experiments that tests foraging behaviours and prey selectivity between the infected and uninfected sub-groups. These can utilise experimentally infected fish as was completed in Chapter 3. A further aspect to consider is the role of

parasite mediated competition in relation to how this modifies the competitive interactions of the hosts with uninfected conspecifics and other fishes in the community (Hatcher et al. 2011; 2012) and how this then influences the trophic niche of each species involved and thus food web structure. Allied to this is whether these competitive interactions are forming novel competitive interactions in the communities, as this could have important implications for energy flux through the food web. Indeed, parasites that manipulate their intermediate hosts in order to increase their chances of being trophically transmitted to the next host might also be making their hosts more vulnerable to predation by a non-host species. For example, salmonid fishes are presented with a novel feeding opportunity when crickets and grasshoppers (e.g. *Nemobius sylvestris*) are parasitized by hairworm parasites (e.g. *Paragordius tricuspidatus*). The hosts are manipulated to enter rivers in order that the lifecycles of the hairworm can be completed but in doing, can result in their predation by the fishes (Sato et al. 2008). Consequently, such novel and cross-ecosystem feeding links could be created via parasitism and so have important implications for food web structure.

Thus, this research provided some comprehensive insights into many aspects of the pathological and ecological consequences of infection for some freshwater fishes from native/ non-native and indigenous/ non-indigenous parasites. The research also raised a series of new questions and hypotheses for investigation, with the approaches of Section 7.5 being suggested as appropriate for answering and testing these.

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Appendices

Appendix 1- Post-mortem examination methodology

Detailed breakdown of the parasitological checks carried out on fish in chapters 4, 5 and 6. Adapted from:

Hoole, D., Bucke, D., Burgess, P. and Wellby, I., 2001. *Diseases of carp and other cyprinid fishes*. Oxford: Fishing News Books.

External check

Gross examination of the external features of the fish to identify any abnormalities that could influence their parasite fauna, such as wounds or lesions that could be resulting in secondary infections. These were recorded in the laboratory notes in case so subsequent analysis in case they were acting as a confounding factor (note: this was never the case). The external parasite check comprised of a ‘skin scrape’ (a cover slip was taken and run

along the body of the fish, then the mucus collected was examined under a microscope (x 10 to x 400) to reveal any external parasites living on the skin) and10 scales from the lateral line (as parasites can be encountered within the lateral line canal).

Detailed internal examination

The skin and body wall musculature are cut away to reveal the internal organs. The first incision was made parallel to the operculum from just dorsal to the lateral line, to below the pectoral fin-joint and round to the mid-line of the fish. Holding the pectoral fin with forceps, a second incision is made along the midline of the fish to a point between the opercula. Pulling the pectoral fin up and away from the body exposes the pericardial cavity and the heart.

Heart removal and examination

The heart is removed using forceps just in front of the bulbus arteriosus, and pulling the whole heart gently out of the pericardial cavity. The heart is then placed on a petri dish with phosphate buffered saline (PBS) and examined under a low power dissecting microscope. The organ is then cut longitudinally to reveal the interior; this procedure is done at x10 magnification.

After removal of the heart a ventrolateral opening in the body of the fish is made by using blunt ended scissors from the top of the first incision along the flank just ventral to the lateral line, curving the cut ventrally to the vent. Remove the resulting flap from the fish, making sure that all internal organs remain intact. To gain access to the kidneys in cyprinids, the swimbladder is gently removed.

Visceral organs

The spleen, liver and kidney are examined *in situ*, and any parasites noted. Small pieces of organ (approximately 2 mm size) are taken, placed on slide with a small amount of saline, squashed using the coverslip and examined under a compound phase contrast microscope at x100 and x400 magnification.

Intestine

The gastro-intestinal tract is carefully removed from the body cavity and the intestine is opened using a longitudinal cut and examined in PBS under a low power microscope, noting any abnormalities and parasites.

Gills

Gills are removed intact, by cutting each end of the branchial arches. Examination of the gills is carried out in PBS under a low power dissection microscope, teasing out the connective tissue between the gill filaments and examining for parasites. Squashes of gill tissue are made from a number of filaments and examined at magnification x100 and x400 in phase contrast, for parasites.

Eyes and nasal cavity

Following a general external examination of the eye in which any abnormalities, e.g. lens opacity, are noted, the organ is removed by slipping a pair of curved forceps under the eyeball, and cutting the connective tissue below and around it. The lens and humour

of the eye are examined in a petri dish containing PBS under a low power light microscope, taking care not to damage the lens during removal. Following removal of the nasal flaps, an examination of the nasal cavity can be made under low power dissection microscope, and any parasites noted.

Brain

A transverse cut is made vertically into the head of the fish, dorsal to the top of the operculum. The brain, which is located posterior-dorsally to the eyes, can be removed intact and examined for any obvious signs of disease, e.g. tumours, haemorrhaging and necrosis.

Appendix 2

Supplementary information for Chapter 5.

2a) Search terms for literature search

***Silurus glanis* :** Afghanistan, Armenia, Azerbaijan, Georgia, Iran, Kazakhstan, Turkey, Turkmenistan, Uzbekistan, Albania, Austria, Belarus, Bulgaria, Czechia, Estonia, Germany, Greece, Hungary, Latvia, Lithuania, Moldova, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Sweden, Switzerland, Ukraine

***Lepomis gibbosus*:** Albania, USA, Canada, America

***Pseudorasbora parva*:** China, Japan, Korea, Mongolia, Russia

***Leucaspius delineates*:** Armenia, Azerbaijan, Georgia, Iran, Kazakhstan, Turkey, Austria, Belarus, Bosnia Herzegovina, Bulgaria, Croatia, Czechia, Denmark, Estonia,

France, Germany, Greece, Hungary, Latvia, Lithuania, Moldova, Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Ukraine

***Ameiurus melas*:** Canada, Mexico, USA, America

***Rhodeus amarus*:** Iran, Turkey, Albania, Austria, Belarus, Belgium, Bosnia Herzegovina, Bulgaria, Croatia, Czechia, France, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Macedonia, Montenegro, Netherlands, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Switzerland, Ukraine.

***Pimephales promelas*:** USA, Canada, Mexico, America

2b) Supplementary table 1- List of the parasites of non-native fish in their natural range

Fish species	**Class**	**Genus**	**Species**	**Range**	**Lifecycle**	**Host Specificity**	**Internal/external**	**Reference**
Siluris glanis	Conoidasida	*Eimeria*	*siluri*	Uzbekistan	Simple	Specialist	Internal	1
Siluris glanis	Oligohymenophorea	*Trichodina*	*acuta*	Widespread	Simple	Generalist	External	1
Siluris glanis	Oligohymenophorea	*Trichodina*	*nigra*	Widespread	Simple	Generalist	External	1
Siluris glanis	Oligohymenophorea	*Trichodinella*	*epizootica*	Widespread	Simple	Generalist	External	1
Siluris glanis	kinetoplastids	*Trypanoplasma*	*ninaekohljakimovi*	/	Complex	\	Internal	1
Siluris glanis	Acanthocephala	*Acanthocephalus*	*anguillae*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Acanthocephala	*Acanthocephalus*	*lucii*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Acanthocephala	*Acanthocephalus*	*clavula*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Acanthocephala	*Leptorhynchoides*	*plagicephalus*	Eurasia	Complex	Specialist	Internal	1
Siluris glanis	Acanthocephala	*Pomphorhynchus*	*laevis*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Crustacea	*Argulus*	*coregoni*	Eurasia	Simple	Generalist	External	1
Siluris glanis	Crustacea	*Argulus*	*foliaceus*	Eurasia	Simple	Generalist	External	1
Siluris glanis	Crustacea	*Ergasilus*	*sieboldi*	Widespread	Simple	Generalist	External	1
Siluris glanis	Crustacea	*Lamproglena*	*pulchella*	Eurasia	Simple	Generalist	External	1
Siluris glanis	Crustacea	*Pseudotracheliastes*	*stellifer*	Eurasia	Simple	Specialist	External	1
Siluris glanis	Myxosporea	*Myxobolus*	*exiguus*	Widespread	Complex	Generalist	Both	1
Siluris glanis	Myxosporea	*Myxobolus*	*muelleri*	Widespread	Complex	Generalist	Both	1
Siluris glanis	Myxosporea	*Sphaerospora*	*schulmani*	\	Complex	\	Internal	1
Siluris glanis	Nematoda	*Camallanus*	*lacustris*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Nematoda	*Camallanus*	*truncatus*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Nematoda	*Cucullanus*	*sphaerocephalus*	Eurasia	Complex	Specialist	Internal	1
Siluris glanis	Nematoda	*Eustrongylides*	*excisus*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Nematoda	*Raphidascaris*	*acus*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Nematoda	*Schulmanela*	*petruschewskii*	Eurasia	Simple	Generalist	Internal	1
Siluris glanis	Cestoda	*Bothriocephalus*	*acheilognathi*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Cestoda	*Glanitaenia*	*oculata*	Eurasia		Specialist		1
Siluris glanis	Cestoda	*Silurotaenia*	*siluri*	Eurasia		Specialist	Internal	1
Siluris glanis	Cestoda	*Triaenophorus*	*crassus*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Monogenea	*Thaparocleidus*	*magnus*	Eurasia	Simple	Specialist	External	1
Siluris glanis	Monogenea	*Thaparocleidus*	*siluri*	Eurasia	Simple	Specialist	External	1

Siluris glanis	Monogenea	*Thaparocleidus*	*vistulensis*	Eurasia	Simple	Specialist	External	1
Siluris glanis	Trematoda	*Aphanurus*	*stossichi*	Eurasia	Complex	Specialist	Internal	1
Siluris glanis	Trematoda	*Azygia*	*lucii*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Bunocotyle*	*cingulata*	Eurasia	Simple	Generalist		1
Siluris glanis	Trematoda	*Bucephalus*	*polymorphus*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Bunodera*	*luciopercae*	Eurasia	Complex	Generalist	External	1
Siluris glanis	Trematoda	*Cephalogonimus*	*retusus*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Cotylurus*	*pileatus*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Diplostomum*	*spathaceum*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Metagonimus*	*yokogawai*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Nicolla*	*skrjabini*	Widespread	Complex	Generalist	Internal	1
Siluris glanis	Trematoda	*Orientocreadium*	*siluri*	Eurasia	Complex	Specialist	Internal	1
Siluris glanis	Trematoda	*Spaerostomum*	*bramae*	Eurasia		Generalist		1
Siluris glanis	Trematoda	*Tylodelphys*	*clavata*	Eurasia	Complex	Generalist	Internal	1
Siluris glanis	Crustacea	*Lernaea*	*spp*	Azerbaijan	Simple	Generalist	External	2
Siluris glanis	Monogenea	*Ancylodiscoides*	*siluri*	Turkey	Simple	Specialist	External	3, 4
Siluris glanis	Monogenea	*Ancylodiscoides*	*vistulensis*	Turkey	Simple	Generalist	External	3, 4
Siluris glanis	Cestoda	*Siluritaenia*	*siluri*	Turkey	Complex	Specialist	Internal	3, 5
Siluris glanis	Nematoda	*Eustrongylides*	*excisus*	Turkey	Complex	Generalist	Internal	3, 6
Siluris glanis	Cestoda	*Proteocephalus*	*osculatus*	Iran	Complex		Internal	7
Siluris glanis	Cestoda	*Bothriocephalus*	*spp*	Iran	Complex	Generalist	Internal	7
Siluris glanis	Trematoda	*Aphanurus*	*stossichi*	Iran	Complex	Generalist	Internal	8
Siluris glanis	Trematoda	*Bunocotyle*	*cingulata*	Iran	Complex	Generalist	Internal	8
Siluris glanis	Nematoda	*Anisakis*	*schupakovi*	Iran	Complex	Generalist	Internal	8
Lepomis gibbosus	Bivalvia	*Glochidia*	*sp.*	N. America	Complex	Generalist	Internal	13
Lepomis gibbosus	trematoda	*Ichthyocotylurus*	*platycephalus*	N. America	Complex	Generalist	External	13
Lepomis gibbosus	trematoda	*Tylodelphys*	*clavata*	N. America	Complex	Generalist	Internal	13
Lepomis gibbosus	Nematoda	*Schulmanela*	*petruschewskii*	N. America	Complex	Generalist	Internal	13
Lepomis gibbosus	Acanthocephala	*Acanthocephalus*	*lucii*	N. America	Complex	Generalist	Internal	13

Lepomis gibbosus	Acanthocephala	*Paracanthocephalus*	*sp.*	N. America	Complex	Generalist	Internal	13
Lepomis gibbosus	Crustacea	*Ergasilus*	*sieboldi*	N. America	Simple	Generalist	External	13
Lepomis gibbosus	Crustacea	*Caligus*	*lacustris*	N. America	Simple	Generalist	External	13
Lepomis gibbosus	Crustacea	*Neoergasilus*	*japonicus*	N. America	Simple	Generalist	External	14
Lepomis gibbosus	Crustacea	*Lernaea*	*cruciata*	N. America	Simple	Generalist	External	14
Lepomis gibbosus	trematoda	*Urocleidus*	*similis*	Canada	Simple	Generalist	External	11, 15
Lepomis gibbosus	trematoda	*Tetracotyle*	*sp*	Canada	Complex	Generalist	Internal	11
Lepomis gibbosus	trematoda	*Uvulifer*	*ambloplitis*	Canada	Complex	Generalist	External	11
Lepomis gibbosus	trematoda	*Posthodiplostomum*	*minimum*	Canada	Complex	Generalist	Internal	11
Lepomis gibbosus	trematoda	*Clinostomum*	*marginatum*	Canada	Complex	Generalist	Internal	11, 16
Lepomis gibbosus	trematoda	*Diplostomum*	*scheuringi*	Canada	Complex	Generalist	Internal	11, 17
Lepomis gibbosus	trematoda	*Diplostomum*	*huronense*	Canada	Complex	Generalist	Internal	11, 18
Lepomis gibbosus	trematoda	*Apophallus*	*brevis*	Canada	Complex	Generalist	Internal	11, 19
Lepomis gibbosus	Nematoda	*Spiroxys*	*sp.*	Canada	Complex	Generalist	Internal	11
Lepomis gibbosus	nematoda	*Eustrongylides*	*sp.*	Canada	Complex	Generalist	Internal	11
Lepomis gibbosus	Monogenea	*Actinocleidus*	*gibbosus*	N. America	Simple	Specialist	External	20-23
Lepomis gibbosus	Monogenea	*Actinocleidus*	*recurvatus*	N. America	Simple	Specialist	External	20-23
Lepomis gibbosus	Monogenea	*Cleidodicus*	*robustus*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Monogenea	*Urocleidus*	*acer*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Monogenea	*Urocleidus*	*attenuatus*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Monogenea	*Urocleidus*	*dispar*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Monogenea	*Urocleidus*	*ferox*	N. America	Simple	Specialist	External	20-23
Lepomis gibbosus	Copepoda	*Achtheres*	*ambloplitis*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Copepoda	*Ergasilus*	*caeruleus*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Copepoda	*Ergasilus*	*centrarchidarum*	N. America	Simple	Generalist	External	20-23
Lepomis gibbosus	Mollusca	*Lampsilis*	*radiata*	N. America	Complex	Generalist	External	20-23
Lepomis gibbosus	Nematoda	*Spinitectus*	*sp.*	N. America	Complex	Generalist	Internal	20-23
Lepomis gibbosus	Myxosporidia	*Myxobolus*	*osburni*	N. America	Complex	Generalist	Internal	20-23
Lepomis gibbosus	Myxosporidia	*Myxobolus*	*uvuliferis*	N. America	Complex	Generalist	Internal	20-23
Lepomis gibbosus	Myxosporidia	*Myxobolus*	*magnaspherus*	N. America	Complex		Internal	20-23
Lepomis gibbosus	Myxosporidia	*Myxobolus*	*dechtiari*	N. America	Complex		External	20-23

Lepomis gibbosus	Myxosporidia	*Myxobilatis*	*ohioensis*	N. America	Complex		Internal	20-23
Lepomis gibbosus	Nematoda	*Hysterothylacium*	*analarum*	N. America	Complex	Generalist	Internal	20-23
Pseusorasbora parva	Trematoda	*Clinostomum*	*complantatum*	Japan	Complex	generalist	internal	24
Pseusorasbora parva	Trematoda	*Parabucephalopsis*	*parasiluri*	Japan	Complex	generalist	internal	24
Pseusorasbora parva	Trematoda	*Pararhynchoides*	*ozakii*	Japan	Complex	generalist	internal	24
Pseusorasbora parva	Trematoda	*Holostephanus*	*metorchis*	Korea	Complex	generalist	internal	24
Pseusorasbora parva	Cestoda	*Digramma*	*sp.*	China	Complex	generalist	internal	24
Pseusorasbora parva	Cestoda	*Ligula*	*sp.*	China	Complex	generalist	internal	24
Pseusorasbora parva	Acanthocephala	*Acanthocephalus*	*opsariichthydis*	Japan	Complex	generalist	internal	24
Pseusorasbora parva	Trematoda	*Echinochasmus*	*japonicus*	Korea	Complex	generalist	External	24
Pseusorasbora parva	Monogenea	*Gyrodactylus*	*parvae*	China	Simple	Specialist	External	24
Pseusorasbora parva	Digenea	*Centrocestus*	*armatus*	Korea	Complex	generalist	External	24
Pseusorasbora parva	Digenea	*Monorchotrema*	*taihokui*	Taiwan	Complex	generalist	External	24
Pseusorasbora parva	Digenea	*Clonorchis*	*sinensis*	Widespread	Complex	generalist	Both	24
Leucaspius delineatus	Trematoda	*Posthodiplostomum*	*cuticula*	Poland	Complex	generalist	Internal	25-29
Leucaspius delineatus	Trematoda	*Dactylogyrus*	*sp.*	Bosnia	Simple	generalist	External	25-29
Leucaspius delineatus	Monogenea	*Dactylogyrus*	*minor*	Hungary	Simple	Specialist	External	25-29
Leucaspius delineatus	Monogenea	*Dactylogyrus*	*fraternis*	Hungary	Simple	Specialist	External	25-29
Leucaspius delineatus	Oligoohymenophorea	*Apiosoma*	*sp.*	Latvia	Simple	generalist	External	25-29

Leucaspius delineatus	Sporozoa	*Eimeria*	*sp.*	Latvia	Complex	generalist		25-29
Leucaspius delineatus	Myxosporea	*Myxobolus*	*ellipsoides*	Latvia	Complex	generalist	Internal	25-29
Leucaspius delineatus	Nematoda	*Rhabdochona*	*denudata*	Latvia	Complex	generalist	Internal	25-29
Leucaspius delineatus	Maxillopoda	*Argulus*	*foliaceus*	Latvia	Simple	generalist	External	25-29
Leucaspius delineatus	Maxillopoda	*Ergasilus*	*sieboldi*	Latvia	Simple	generalist	External	25-29
Leucaspius delineatus	Cestoda	*Ligula*	*intestinalis*	Ukraine	Complex	generalist	Internal	25-29
Leucaspius delineatus	Trematoda	*Mesostephanus*	*appendiculatus*	Georgia	Complex	generalist	Internal	28
Ameirus melas	Cestoda	*Proteocephalus*	*ambloplitis*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Copepoda	*Ergasilus*	*versicolor*	N. America	Simple	Generalist	External	30-37
Ameirus melas	Cestoda	*Corallobothrium*	*fimbriatum*	N. America	Complex	Specialist	Internal	30-37
Ameirus melas	Nematoda	*Spiroxys*	*sp.*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Acanthocephala	*Leptorhynchoides*	*thecatus*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Monogenea	*Gyrodactylus*	*fairporti*	N. America	Simple	Generalist	External	30-37
Ameirus melas	Trematoda	*Phyllodistomum*	*caudatum*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Trematoda	*Sellacotyle*	*mustelae*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Cestoda	*Corallotaenia*	*parva*	N. America	Complex	Specialist	Internal	30-37
Ameirus melas	Trematoda	*Cleidodiscus*	*pricei*	N. America	Simple	Generalist	Internal	30-37
Ameirus melas	Trematoda	*Cleidodiscus*	*sp.*	N. America	Simple	Generalist	Internal	30-37
Ameirus melas	Trematoda	*Hysteromorpha*	*tribola*	N. America	Complex	Generalist	Internal	30-37
Ameirus melas	Monogenea	*Cleidodiscus*	*mirabilis*	N. America	Simple	Generalist	External	38
Ameirus melas	Digenea	*Pseudomagnivitellinum*	*ictalurum*	N. America	Simple	specialist	Internal	39
Ameirus melas	Digenea	*Alloglossidium*	*fonti*	N. America	Simple	specialist	Internal	40
Rhodeus amarus	Monogenea	*Dactylogyrus*	*bicornis*	Europe	Simple	Specialist	External	41
Rhodeus amarus	Monogenea	*Dactylogyrus*	*rarissimus*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Dactylogyrus*	*suecicus*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Dactylogyrus*	*yinwenyingae*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Gyrodactylus*	*laevis*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Gyrodactylus*	*rhodei*	Europe	Simple	Specialist	External	41

Rhodeus amarus	Monogenea	*Gyrodactylus*	*vimbi*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Paradiplozoon*	*homoion*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Monogenea	*Digenea*	*sp. 1*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Digenea*	*sp. 2*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Digenea*	*spp.*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Bucephalus*	*polymorphus*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Rhipidocotyle*	*illense*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Sphaerostomum*	*bramae*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Sphaerostomum*	*globiporum*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Paryphostomum*	*radiatum*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Petasiger*	*sp.*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Tylodelphys*	*clavata*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Posthodiplostomum*	*brevicaudatum*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Posthodiplostomum*	*cuticola*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Apharyngostrigea*	*cornu*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Ichthyocotylurus*	*platycephalus*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Ichthyocotylurus*	*variegatus*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Monogenea	*Holostephanus*	*spp.*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Clinostomum*	*complanatum*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Metorchis*	*xanthosomus*	Europe	Complex	Generalist	Both	41
Rhodeus amarus	Monogenea	*Apophallus*	*muehlingi*	Europe	Complex	Generalist	External	41
Rhodeus amarus	Cestoda	*Cestoda*	*sp.*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Cestoda	*Ligula*	*intestinalis*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Cestoda	*Neogryporhynchus*	*cheilancristrotus*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Nematoda	*Nematoda*	*sp.*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Nematoda	*Pseudocapillaria*	*tomentosa*	Europe	Simple	Generalist	Internal	41
Rhodeus amarus	Nematoda	*Philometra*	*sp.*	Europe	Complex	Generalist	Internal	41
Rhodeus amarus	Nematoda	*Cosmocerca*	*sp.*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Mollusca	*Anodonta*	*sp.*	Europe	Complex	Generalist	External	41
Rhodeus amarus	Mollusca	*Unio*	*sp.*	Europe	Complex	Generalist	External	41
Rhodeus amarus	Annelida	*Piscicola*	*geometra*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Crustacea	*Crustacea*	*sp.*	Europe	Simple	Generalist	External	41

Rhodeus amarus	Crustacea	*Ergasilus*	*sieboldi*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Crustacea	*Lernaea*	*cyprinacea*	Europe	Simple	Generalist	Both	41
Rhodeus amarus	Crustacea	*Caligus*	*sp.*	Europe	Simple	Generalist	External	41
Rhodeus amarus	Crustacea	*Argulus*	*foliaceus*	Europe	Simple	Generalist	External	41
Pimephlaes promelas	Cestoda	*Ligula*	*intestinalis*	widespread	Complex	generalist	Internal	42-44
Pimephlaes promelas	Trematoda	*Uvulifer*	*ambloplitis*	Americas	Complex	generalist	Internal	42-44
Pimephlaes promelas	Copepoda	*Ergasilus*	*cyprinaceus*	N. America	Simple	generalist	External	42-44
Pimephlaes promelas	Oligohymenophorea	*Trichodina*	*sp.*	N. America	Simple	generalist	External	42-44
Pimephlaes promelas	Myxosporea	*Myxosoma*	*funduli*	N. America		generalist	External	42-44
Pimephlaes promelas	Monogenea	*Dactylogyrus*	*bychowski*	N. America	Simple	generalist	External	42-44
Pimephlaes promelas	Monogenea	*Gyrodactylus*	*hoffmani*	N. America	Simple		External	42-44
Pimephlaes promelas	Trematoda	*Gyrodactylus*	*sp.*	N. America	Simple	generalist	External	42-44
Pimephlaes promelas	Trematoda	*Neascus*	*sp.*	N. America	Complex	generalist	Internal	42-44
Pimephlaes promelas	Trematoda	*Neascus*	*sp.*	N. America	Complex	generalist	Internal	42-44
Pimephlaes promelas	Trematoda	*Ornithodiplostomum*	*ptychocheilus*	N. America	Complex	specialist	Internal	45
Pimephlaes promelas	Trematoda	*Dactylogyrus*	*spp.*	N. America	Simple	generalist	Internal	46
Pimephlaes promelas	Trematoda	*Posthodiplostomum*	*minimum*	N. America	Complex	generalist	Internal	47
Pimephlaes promelas	Acanthocephala	*Neoechinorhynchus*	*rutili*	N. America	Complex	generalist	Internal	48, 49
Pimephlaes promelas	Trematoda	*Crassiphiala*	*bulboglossa*	N. America	Complex	generalist	External	50
Pimephlaes promelas	Trematoda	*Neascus*	*pyriformis*	N. America	Complex	generalist	External	50
Pimephlaes promelas	Trematoda	*Centrovarium*	*lobotes*	N. America	Complex	generalist	Internal	50

Pimephlaes promelas	Cestoda	*Proteocephalus*	*spp.*	N. America	Complex	generalist	Internal	50
Pimephlaes promelas	crustacea	*Ergasilus*	*confusus*	N. America	Simple	generalist	External	50
Pimephlaes promelas	crustacea	*Ergasilus*	*spp.*	N. America	Simple	generalist	External	50
Pimephlaes promelas	crustacea	*Lernaea*	*cyprinacea*	N. America	Simple	generalist	External	50

1 Copp et al. (2009); 2 Barzegar & Jalali (2010); 3 Soylu (2005); 4 Mancheva et al. (2014) ;5 Zdarska and Nebesarova (2005); 6 Sattari et al. (2002); 7 Roohi et al. (2014); 8 Pazooki and Masoumian (2012); 9 Hanek and Fernando (1978); 10 Esch (1971); 11 Cone and Anderson (1977); 12 Rye and Baker (1984); 13 Piasecki and Falandysz (1994); 14 Hudson and Bowen (2002); 15 Grupcheva and Nedeva (2000); 16 Osborn (1911); 18 Aho et al. (1976); 18 Wilson and Ronald (1967); 19 Taylor et al. (1994); 20 Hockley et al. 2011; 21 Gozlan et al. (2010); 22 Zhang et al. (2007); 23 Avdul et al. (2011); 24 Skenderovic et al. (2011); 25 Molnar (1976); 26 Kirjušina and Vismanis (2007); 27 Davydov et al. (2003); 28 Galationov (1980); 29 Beyer et al. (2005); 30 Bangham (1941); 31 Lincicome and Van Cleave (1949); 32 Van Cleave (1921); 33 Steelman (1938); 34 Wallace (1935); 34 McAllister and Bursey (2011); 35 Seamster (1948); 36 Hugghins (1954); 37 Davidova et al. (2008); 38 Mizelle and Cronin (1943); 39 Dronen and Underwood (1980); 40 Tkach and Mills (2011); 41 Held and Peterka (1974); 42 Wilmer and Rogers (1969); 43 Olsen (1986); 44 McDowell et al. (1992); 45 Radabaugh (1980); 46 Knipes and Janovy (2009); 47 Mitchell et al. (1982); 48 Samuel et al. (1976); 49 Merrit and Pratt (1964); 50 Voth and Larson (1968).

2c)

Supplementary table 2 list of parasite species detected in native fishes where the non-native fish were present. The non-native fish species in the first column enables cross-referencing of these parasite lists with the data presented in table 5.3.

Non-native fish species	**Genus**	**Species**	**Lifecycle**	**Host Specificity**	**Internal/external**
Siluris glanis	*Echinostomatidae*		Complex	generalist	internal
Siluris glanis	*Dactylogyrus*		Simple	generalist	external
Siluris glanis	*Trichodina*		Simple	Generalist	External
Siluris glanis	*Diplostomum*		Complex	generalist	internal
Siluris glanis	*Tylodelphys*		Complex	Generalist	Internal
Siluris glanis	*Skijabillanus*	*scardinii*			
Siluris glanis	*Bothriocephalus*		Complex	Generalist	Internal
Siluris glanis	*contracaecum*		Complex	Generalist	Internal
Siluris glanis					
Siluris glanis	*Ichthyophthirius*	*multifiliis*	simple	generalist	External
Siluris glanis	*Trichodina*		Simple	Generalist	External
Siluris glanis					
Siluris glanis	*Epistylis*	*sp.*	Simple	Generalist	External
Siluris glanis	*Argulus*	*foliaceus*	Simple	Generalist	External
Siluris glanis	*Trypanosoma*		Complex	Generalist	Internal
Siluris glanis	*Trichodina*		Simple	Generalist	External
Siluris glanis	*Icthyoboda*	*necator*			
Siluris glanis	*Echinostamatidae*		Complex	Generalist	External
Siluris glanis	*Piscola*	*geometra*	Simple	Generalist	External
Siluris glanis	*Dactylogyrus*		Simple	generalist	external
Siluris glanis	*Diplozoidae*		Simple	Generalist	External
Siluris glanis	*Khawia*	*sinensis*	Complex	Generalist	internal

Lepomis gibbosus	*Argulus*	*sp.*	simple	generalist	external
Lepomis gibbosus	*Dactylogyrus*	*sp.*	simple	generalist	external
Lepomis gibbosus	*Diplozoan*	*sp.*	simple	generalist	external
Pseusorasbora parva	*Trichodina*		Simple	Generalist	External
Pseusorasbora parva	*Dactylogyrus*		Simple	Generalist	External
Pseusorasbora parva	*Diplostomum*		Complex	Generalist	Internal
Pseusorasbora parva	*Argulus*	*foliaceus*	Simple	Generalist	External
Pseusorasbora parva	*Schistocephalus*	*solidus*	Complex	Generalist	internal
Ameirus melas	*Diplozoan*	*paradoxeum*	simple	generalist	External
Ameirus melas	*Dactylogyrus*	*sp.*	simple	generalist	External
Ameirus melas	*Ergasilus*	*briani*	simple	generalist	External
Ameirus melas	*Neoergasilus*	*japonicus*	simple	generalist	External
Ameirus melas	*Argulus*	*foliaceus*	simple	generalist	External
Ameirus melas	*Contracaecum*		complex	generalist	internal
Ameirus melas	*Ligula*	*intestinalis*	complex	generalist	internal
Rhodeus amarus	*Tylodelphis*		Complex	Generalist	Internal
Rhodeus amarus	*Anguillocola*	*crassus*	Complex	Generalist	Internal
Rhodeus amarus	*Acanthocephalus*	*lucii*	Complex	Generalist	Internal
Rhodeus amarus	*Camallanus*	*lacustris*	Complex	Generalist	Internal
Rhodeus amarus	*Bunodera*	*luciopercae*	Complex	Generalist	external
Rhodeus amarus	*Apiosoma*		Simple	generalist	External
Rhodeus amarus	*Trichodina*		Simple	Generalist	External

Rhodeus amarus	*Paraergasilus*	*longidigitus*	Simple	generalist	External
Rhodeus amarus	*Raphidascaris*	*acus*	Complex	Generalist	Internal
Rhodeus amarus	*Triaenophorous*	*nodulosus*	Complex	generalist	Internal
Rhodeus amarus	*Neoechinorhynchus*	*rutili*	Complex	generalist	Internal
Rhodeus amarus	*Acanthocephalus*	*lucii*	Complex	generalist	Internal
Rhodeus amarus	*Piscicola*	*geometra*	Simple	Generalist	External
Rhodeus amarus	*Tetraonchus*	*monenteron*	Simple	generalist	External
Rhodeus amarus	*Allocredium*	*isoporum*			
Rhodeus amarus	*Crepidostomum*	*sp.*	Complex	generalist	Internal
Rhodeus amarus	*Philometra*	*sp.*	Complex	Generalist	Internal
Rhodeus amarus	*Echinochasmus*		Complex	Generalist	Internal
Rhodeus amarus	*Glochidia*		Complex	Generalist	External
Rhodeus amarus	*Trichodina*		Simple	Generalist	External
Rhodeus amarus	*black spot*		Complex	Generalist	External
Rhodeus amarus	*Trypanosoma*		Complex	Generalist	Internal
Rhodeus amarus	*Myxobolus*		Complex	Generalist	Both
Rhodeus amarus	*Philometra*	*sp.*	Complex	Generalist	Internal
Rhodeus amarus	*Hysteromorpha*	*tribola*	Complex	Generalist	Internal
Rhodeus amarus	*Tylodelphys*		Complex	Generalist	Internal
Rhodeus amarus	*Caryophyllaeus*	*laticeps*	Complex	Generalist	Internal
Rhodeus amarus	*myxidium*				
Rhodeus amarus	*Diplostomum*		Complex	Generalist	Internal

2d)

Supplementary table 3- List of the parasites of non-native fish in the UK for table 3

Fish species	Class	Genus	Species	Life cycle	Host Specificity	Internal/external	Reference
Siluris glanis	Digenea	*Diplostomum*	*sp.*	Complex	Generalist	Internal	This study
Siluris glanis	Maxillopoda	*Ergasilus*	*sieboldi*	Simple	Generalist	External	This study
Siluris glanis	Cestoda	*Proteocephalus*	*osculatus*	Complex	Generalist	Internal	This study
Siluris glanis	Kinetoplastida	*Trypanosoma*	*sp.*	Complex	Generalist	Internal	This study
Siluris glanis	Nematoda	*Camallanus*	*lacustris*	Complex	Generalist	Internal	This study
Siluris glanis	Monogenea	*Thaparocleidus*	*vistulensis*	Simple	Specialist	External	This study
Lepomis gibbosus	Nematoda	*Contracaecum*	*rudolphii*	Complex	Generalist	Internal	Hockley et al 2011
Lepomis gibbosus	Monogenea	*Onchocleidus*	*dispar*	Simple	Generalist	Internal	Hockley et al 2011
Lepomis gibbosus	Nematoda	*Nematode*	*sp.*			Internal	Hockley et al 2011
Lepomis gibbosus	Acanthocephala	*Acanthocephalus*	*sp.*			Internal	Hockley et al 2011
Lepomis gibbosus	Bivalvia	*Glochidia*	*sp.*			External	Hockley et al 2011
Lepomis gibbosus	Oligoohymenophorea	*Trichodina*	*sp.*			External	Hockley et al 2011
Lepomis gibbosus	Oligoohymenophorea	*Apiosoma*	*sp.*			External	Hockley et al 2011
Ameirus melas	Mongenea	*Ancyrocephalus*	*pricei*	Simple	Specific	External	This study
Ameirus melas	Maxillopoda	*Argulus*	*sp*	Simple	Generalist	External	This study
Rhodeus amarus	Digenea	*Diplostomum*	*sp.*	Complex	Generalist	Internal	This study

Rhodeus amarus	Digenea	*Tylodelphis*	*clavata*	Complex	Generalist	Internal	This study
Rhodeus amarus	Nematoda	*Anguillocola*	*crassus*	Complex	Generalist	Internal	This study
Rhodeus amarus	Monogenea	*Ichthyocotylurus*	*variegatus*	Complex	Generalist	Internal	This study
Rhodeus amarus	Nematoda	*Raphidascaris*	*sp.*	Complex	Generalist	Internal	This study
Rhodeus amarus	Digenea	*Paracoenogonimus*	*ovatus*	Complex	Generalist	External	This study
Rhodeus amarus	Trematoda	*Echinochasmus*	*spp.*	Complex	Generalist	External	This study
Rhodeus amarus	Myxosporea	*Myxosporidean*	*sp.*		Generalist	Internal	This study
Rhodeus amarus	Oligoohymenophorea	*Ichthyophthirius*	*multifiliis*	Simple	Generalist	External	This study
Rhodeus amarus	Digenea	*Bucephalus*	*polymorphus*	Complex	Generalist	External	This study
Rhodeus amarus	Digenea	*Rhipidocotyle*	*campanula*	Complex	Generalist	External	This study
Rhodeus amarus	Digenea	*Neascus*	*sp.*	Complex	Generalist	External	This study
Pimephlaes promelas	Oligoohymenophorea	*Trichodina*	*sp.*	Simple	Generalist	External	This study