Ecological consequences of non-native parasites for native UK fishes

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Abstract

Introductions of non-native species can result in the release of their parasites. Although the majority of parasites are lost during the introduction process, those that do get released can spill over to native species and potentially result in pathological, physiological and ecological impacts. Whilst it is increasingly recognised that native parasites can play important ecological roles, the ecological consequences of non-native parasites remain unclear. Consequently, through study of three host-parasite models, this research investigated the ecological consequences of non-native parasites in UK freshwater fish communities through assessment of their effects on hosts (individuals to populations), and on food web structure.

The three non-native parasite: host systems were *Ergasilus briani* and roach *Rutilus rutilus* and common bream *Abramis brama*, *Bothriocephalus acheilognathi* and common carp *Cyprinus carpio*, and *Anguillicoides crassus* and the European eel *Anguilla anguilla*. These parasites were chosen as they reflect a range of life cycle complexity in parasites. The pathology of each parasite was identified using histology, with *E. briani* having substantial effects on host gill structure, *B. acheilognathi* impacted the intestinal structure of their hosts, and *A. crassus* substantially altered the structure and functioning of the host swimbladder. Whilst infections of *E. briani* and *A. crassus* had minimal effects on the body size, growth and condition of their hosts, chronic infections of *B. acheilognathi* did impact the growth and condition of *C. carpio* when measured over a 12 month period.

Differences in the trophic ecology of the infected and uninfected components of the host populations were identified using stable isotope analysis and associated metrics, and revealed considerable differences in the trophic niche breadth of the infected and uninfected fish. In the component infected with *E. briani*, their trophic niche was constricted, indicating diet specialisation and a shift to feeding on less motile food items. For *C. carpio* infected with *B. acheilognathi*, their niche shifted away that of uninfected fish as they fed on higher proportions of planktonic prey resources. Whilst differences in the trophic ecology of infected and uninfected *A. anguilla* were apparent, this related to differences in their functional morphology that enabled the infected eels to prey upon greater proportions of fish paratenic hosts that resulted in their higher rates of infection.

The wider ecological consequences of the introduced parasite were then investigated using topological and weighted food webs. The topological webs revealed that lifecycle and host specificity were important factors in how each parasite impacted the food web metrics, but in all cases the combined effects of including native parasites in food web structure exceeded that of adding the non-native parasite. However, weighting these food webs by using the dietary data outlined above revealed that these infections were predicted to have greater consequences than predicted topologically, and enabled scenarios of differing parasite prevalence and environmental change to be tested on food web metrics. These revealed that under increasing nutrient enrichment, infected individuals generally benefit via having access to greater food resources, a counter-intuitive resulting from increased algal biomass.

Thus, this research revealed that introductions of non-native parasites have pathological and ecological consequences for their host populations that have measurable effects at the food web level. These outputs have important implications for the management of non-native parasites and their free-living hosts, and should be incorporated into risk-management and policy frameworks.

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Author's declaration

I confirm that the work presented in this thesis is my own work, with the following exceptions:

Chapter 3 and Chapter 4 are based on the following paper published in collaboration with Demetra Andreou, Chris Williams and Robert Britton as:

Pegg, J., Andreou, D., Williams, C. F. and Britton, J. R., 2015. Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite. *Parasitology*. doi:10.1017/S0031182015001237

JP, DA, CW and RB designed the project, JP and DA carried out fieldwork, JP and CW carried out laboratory analysis, JP, DA, RB analysed the data, JP, DA, CW and RB wrote the paper.

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JP, DA, CW and RB designed the project, JP and RB carried out fieldwork, JP, DA and CW carried out laboratory analysis, JP, DA, RB analysed the data, JP, DA, CW and RB wrote the paper.

1. Introduction

This thesis studies how non-native parasites alter food web structure through their interactions with free-living species and their modifications to host foraging behaviour. It uses fish and their parasite fauna as the model species and the UK as the study area. It doing so, the research covers topics including introduced species generally and introduced fishes specifically, their parasite fauna, and the consequences of parasites, including non-native parasites and at individual, population and community levels.

1.1 Introductions of non-native fish

The rate of introductions of species worldwide has more than doubled compared with estimates nearly three decades ago (Gozlan 2008; Gozlan et al. 2010b). These introductions of non-native species have principally been the result of human activity, usually associated with enhancing ecosystem services such as aquaculture, and can be both deliberate or accidental (Vitousek et al. 1996; Koo and Mattson 2004; Gozlan et al. 2010a; Gozlan et al. 2010b). Despite this large volume of introductions, the majority of introduced species fail to establish sustainable populations; of those that do, many only cause minor ecological consequences (Gozlan 2008). However, a small proportion cause more substantial impacts (Manchester and Bullock 2000). These range from genetic consequences through to changes in ecosystem functioning (Cucherousset and Olden 2011). Examples in freshwater fish include habitat alteration, such as increased water turbidity caused by benthic foraging species such as the Common carp *Cyprinus carpio* and goldfish *Carassius auratus* (Richardson and Whoriskey 1992; Britton et al. 2007); genetic

contamination, such as through hybridization between native crucian carp *Carassius carssius* with *C. carpio* and *C. auratus* in England that has resulted in the introgression of gene pools and the loss of pure-strain populations of *C. carassius* (Hanfling et al. 2005), and the introduction of non-native parasites with their free-living hosts, the subject of this research.

Both aquaculture and recreational angling provide important introduction pathways for introduced species, with these responsible for a number of introduced fishes attaining almost global distribution (De Silva et al. 2006; Gozlan et al. 2010b). *Cyprinus carpio*, originally from Southeast Asia, is now commonplace wherever temperatures allow their survival, due to their use in aquaculture and angling (Zambrano et al. 2006; Britton et al. 2007). Nile tilapia *Oreochromis niloticus* has achieved similar distribution levels as a result of intensive pond aquaculture, being prevalent in Asian and South American aquaculture systems (Zambrano et al. 2006; Orsi and Britton 2012). There are, however, a number of other pathways by which fish can be introduced into new ranges, including the ornamental fish trade that is responsible for the introduction of many smaller species of low economic value, with these introductions often being accidental, such as the topmouth gudgeon *Pseudorasbora parva* into Europe from China (Gozlan et al. 2010a).

Introduction pathways for non-native fish parasites tend to echo those of their free-living hosts (Britton 2013). Aquaculture is thus arguably the pathway responsible for the introduction of the majority of non-native fish parasites, with examples including the eel parasite *Anguillicoloides crassus* (Kirk 2003), the Asian tapeworm *Bothriocephalus acheilognathi* (Andrews et al. 1981) and the crustacean copepod

parasite *Ergasilus briani* (Alston and Lewis 1994). These parasites are all now present in the UK following their release into the wild with introduced free-living hosts, and infect fish species which are considered native and naturalised.

1.2 Arrival of parasites with introduced free-living species

When free-living species are moved from their natural range into a new range, they are likely to be host to a number of parasites and other disease causing agents that will be introduced with them. If these pathogens are able to infect new, native hosts in their extended range then the consequences for these hosts are often severe. For example, in the UK, the invasive grey squirrel *Sciurus carolinensis* is the host of the squirrel poxvirus, which is relatively benign in greys, but on transmission to the native red squirrel *Sciurus vulgaris* can cause high mortality rates (Rushton et al. 2006; Bruemmer et al. 2010) and has thus driven the decline of the native squirrel in the UK (Chantrey et al. 2014). The movement of fish around the world for aquaculture purposes has also resulted in the transfer of a number of pathogens that have then gone on to cause considerable issues in the new range. For example, in fish of the Salmonidae family, the pathogen *Yersinia ruckeri*, which causes enteric red mouth disease, has extended its geographic range from North America to Europe with the import of live fathead minnow *Pimephales promelas*. Likewise infectious hematopoietic necrosis virus that causes haematopoietic necrosis was spread along a similar geographic route by the eggs of rainbow trout *Oncorhynchus mykiss*. In the case of both diseases, mortality rates in infected populations can be high (Bovo et al. 1987; Furones et al. 1993)**.**

Moreover, it was the transfer of a fish parasite into a new range that was responsible for one of the most notorious examples of how an introduced pathogen can impact a

naïve host population. *Gyrodactylus salaris* is a monogenean ectoparasite native to the Karelian part of Russia, and the Baltic parts of Finland and Sweden area, where it occurs naturally on fins and skin of Atlantic and Baltic salmon *Salmo salar* when they are in their freshwater phase. It was introduced into Norway through the movement in aquaculture of Rainbow trout *Oncorhynchus mykiss* and was then moved throughout the country via this industry and then through infected fish migrating through rivers and in brackish water in fiords (Hansen et al. 2007). On transmission to wild salmon in Norwegian waters, it subsequently caused disease epidemics that incurred high mortality rates as this strain of salmon had never experienced the pathogen previously (Johnsen 1978; Heggberget and Johnsen 1982; Johnsen and Jensen 1986, 1991). The mortality rates reduced the abundance of juvenile salmon by an average of 86 % and the angler catch of salmon in infected rivers by an average of 87% (Heggberget and Johnsen 1982). Further, these losses of salmon have had cascading effects in the freshwater pearl mussel *Margaritifera margaritifera*, depleting their populations as they depend on juvenile salmon for an important part of their lifecycle (Karlsson et al. 2014). To date, the economic losses to *G. salaris* in Norway are estimated in the region of US $500,000,000 (Hansen et al. 2003).

1.3 How many non-native parasites arrive with free-living non-native hosts?

In Section 1.1 and 1.2, it was outlined that an issue associated with introduced free-living fish is the introduction of their parasite fauna and potentially results in naïve native fish hosts becoming infected and incurring serious consequences. Notwithstanding the potential seriousness of this, a number of studies have suggested that introduced free-living species are host to a much reduced parasite

fauna in their new range compared to their native range (Colautti et al. 2004; Liu and Stiling 2006; Sheath et al. 2015) . This is termed ‘enemy release’ (Colautti et al. 2004). Whilst this is beneficial from the perspective of fewer novel disease causing agents being released with the introduced fish, it is theorised as providing considerable benefit to that fish as it assists its survival and establishment in the new range (hence the term) (Colautti et al. 2004; Sih et al. 2010). This benefit arises from the reduced population regulatory pressures from their natural enemies experienced by the introduced fish in the new range (Torchin et al. 2001; Torchin et al. 2003).

A number of studies on aquatic communities provide strong evidence for enemy release. For example, the invasive European green crab *Carcinus maenas* has significantly reduced parasite diversity and prevalence in its invasive range compared with its natural range, with their greater population biomasses in the invasive range attributed to this (Torchin et al. 2001). Several amphipod species that have invaded British waters host a lower diversity, prevalence and burden of parasites than the native amphipod *Gammarus duebeni celticus* (MacNeil et al. 2003; Prenter et al. 2004b). Of the five parasite species that have been detected, three are shared by both the native and invasive amphipod species, but two are restricted to *Gammarus duebeni celticus* (Dunn and Dick 1998; MacNeil et al. 2003). Torchin et al. (2005), found a similar pattern in mud-snail communities in North America; whilst the native snail *Cerithidea californica* was host to 10 trematode species, the invader *Batillaria cumingi* was host to only one. These specific examples are supported by meta-analyses of native and invasive animals and plants which have revealed a higher-than-average parasite diversity in native populations; for example of 473 plant species naturalized in the United States that had originated from Europe

had, on average, 84% fewer fungal pathogens and 24% fewer virus species than native fauna (Mitchell and Power 2003), whilst introduced fishes in England and Wales had on average less than 9% of the number of macro-parasites they had in their native range (Sheath et al. 2015). Consequently, whilst their impacts are potentially severe in the new range, only a small proportion of non-native parasites are actually likely to be introduced with their hosts (Torchin et al. 2003).

1.4 Infections by non-native parasites in their new range

Despite the reduced number of parasites being present in non-native free-living species in their extended range, it is still likely some will be introduced and it is these which are the focus of this research. These parasites may then persist within the non-native fish population that act as a 'reservoir' of potential disease transmission for the native fish populations as they ensure continual source of infection. This source of infection and subsequent transmission to native hosts is referred to as parasite 'spillover' (Prenter et al. 2004a). For example, in squirrel pox (Section 1.2), the mortality rates of native red squirrels was so high that the virus was predicted to die out through lack of new hosts, but it persists because grey squirrels are asymptomatic and act as a reservoir for 'spillover' opportunities as they arise (Tompkins et al. 2002).

In addition to parasite 'spillover', parasite 'spillback' also occurs in introduced free-living species. This is where the introduced species become infected with native parasites and then act as 'reservoirs' of infection for the subsequent spillback of these parasites to their native hosts (Kelly et al. 2009). For example, in Australia, the invasive Cane toad *Bufo marinus* played an important spillback role in the

emergence of two myxosporean parasites of native frogs, the Green and golden bell frog *Litoria aurea* and the Southern bell frog *Litoria raniformis*, facilitating parasite dispersal and transmission, and the consequent population declines of the frogs (Hartigan et al. 2011). The invasive crayfish *Pacifastacus leniusculus* displays both spillover and spillback. For spillover, it is an asymptomatic host for the introduced fungus *Aphanomyces astaci* - crayfish plague - that is subsequently transmitted to white-clawed crayfish *Austropotamobius pallipes* (Kelly et al. 2009). For spillback, it hosts the native microsporidian *Thelohania contejeani* where it acts as a reservoir of infection for *A. pallipes* which then tends to also cause mortality (Dunn et al. 2009).

1.5 Parasites in infectious food webs

In order to determine how an introduced parasite might impact food webs and their structure, the role of native parasites in food webs needs to be ascertained. In the last decade, there has been a strong focus on how the addition of parasites to food web structure changes web properties (Lafferty et al. 2006). Infectious food webs represent food web structure with parasites included and tend to be compared to their structure when parasites are omitted (the traditional approach). Studies have demonstrated that the infectious food webs tend to have increased chain length, linkage density, nestedness and connectedness (Hudson et al. 2006; Lafferty et al. 2006; Lafferty 2008). These results suggest that food webs are very incomplete unless parasites are included. Thus, just the mere inclusion of parasites in food web topology has had significant effects on understandings of their structure, with the realization that native parasites are integral to the structuring and functioning of ecosystems (Hudson et al. 2006; Lafferty 2008).

Parasites in food webs result in modifications to food web structure in a number of different ways:

1. Parasites contribute significant proportions of the biomass of ecosystems (Johnson et al. 2010). For example, parasites in three estuaries on the Pacific coast of California and Baja California contributed similar amounts of biomass as major free-living groups of animals such small arthropods and polychaetes, and a greater amount of biomass than all the vertebrate apex predators, of fish and birds (Kuris et al. 2008). The Parasite grouped as 'parasitic castrators' contributed the greatest biomass, 1 - 10 kg ha^{-1}, or around 1% of the total biomass of the system. Thus influencing the ecosystems energetics and significantly contributing to the productivity of the system (Kuris et al. 2008).

2. Parasites can induce behavioural changes in their hosts in order to complete their lifecycles, which then modifies the foraging behaviour of the host and so the composition of their diet (Barber et al. 2000). For example, *Ligula intestinalis* infects cyprinid species, altering their swimming behaviour by decreasing the swimming depth of infected individuals (Bean and Winfield 1989; Loot et al. 2001). This benefits the parasite as it increases the chances of the fish being depredated by the final host, a piscivourous bird (Bean and Winfield 1989). The consequence to the fish is that its diet can shift from benthic to pelagic items as a result of its altered swimming behaviour (Bean and Winfield 1989; Loot et al. 2001).

3. Parasites mediate competitive interactions, which will have consequences for the quantitative food web (Hatcher et al. 2006). For example, on St Maarten Island in the Caribbean, two species of Anolis lizard coexist, *Anolis gingivinus* and *Anolis wattsi*. On other Caribbean islands, *A. gingivinus* is larger and more competitive, but on St Maarten, the malarial parasite *Plasmodium azurophilum* is present. This rarely infects *A. wattsi* but is very common in *A. gingivinus*. Wherever infected *A. gingivinus* occur, *A. wattsi* is also present, but wherever uninfected *A. gingivinus* is present then *A. wattsi* is absent (Schall 1992). This has important consequences in terms of lizard community structure, their feeding relationships and competitive interactions, and ultimately, the structure of the topological and quantitative food web.

4. Finally, native parasites often also act as moderators of host populations that will subsequently have important implications for moderating their cascading effects further down the food web. For example, the reproduction of reindeer *Rangifer tarandus* in Svalbard, is regulated by the parasitic nematode *Osteragaia gruehneri* which decreases the fecundity of the reindeer but not their survival (Albon et al. 2002). A feedback loop was detected of a density-dependent parasite-mediated reduction in calf production. As population sizes increased, so the prevalence and abundance of *O. gruehneri* increased in the reindeer and prevented the reindeer populations from achieving very high numbers (Albon et al. 2002). Similarly, the caecal worm *Trichostrongylus tenuis* is a strong regulator of the population cycles of their host the red grouse *Lagopus lagopus scoticus* in northern England (Hudson

1986; Dobson and Hudson 1992). The parasite is transmitted via the heather which is the preferred food of adult birds, whilst young chicks which feed primarily on insects tend to avoid infection. The parasite accumulates in adults and high levels can cause mortality, loss of condition and can reduce the grouse's ability to control its scent, making it vulnerable to predation. Eggs and larvae of *T. tenuis* cannot survive hot dry conditions but thrive in warm humid ones, therefore their abundance and impact is related to prevailing weather patterns (Hudson 1986; Dobson and Hudson 1992; Dobson and Hudson 1995).

1.6 Parasites affect ecosystem structure

Section 1.5 discussed the substantial consequences of parasites on food web topology and the quantitative food web through their actions on individuals and populations. However, parasite-mediated effects on individual hosts can also influence ecosystem structure and function. For example, trematode parasites that infect the foot tissue of the *Austrovenus stutchburyi* cockle modify how the cockle uses its foot to move and burrow after it has been dislodged (Mouritsen and Poulin 2003). The net consequence of this is changes in the structure and functioning of soft-bodied animal communities, as epifauna benefit from the increased surface structure and the infauna are influenced by changes in the hydrodynamics that determine the particle composition in the upper sediment (Mouritsen and Poulin 2003). The herbivourous snail *Littorina littorea* is parasitized by the digenean trematode parasite *Cryptocotyle lingua* in its native European range. Infection by *C.lingua* reduces the consumption rate of individual *L.littorea* by 40 % and this decrease in grazing pressure results in significantly increased abundance of the

macroalgal communities (Wood et al. 2007). The result is that in ecosystems where the parasite has high prevalence in *L. littorea*, ecosystem structure tends to be more dominated by algal communities. Both species have been introduced to North America (Blakeslee et al. 2008), where *L. littorea* has been demonstrated to significantly disrupt native communities by its voracious herbivory (Lubchenco, 1978). Thus in this case the co-introduced parasite appears to moderate the ecological impact of its invasive host.

1.7 Parasites: consequences from individual hosts to ecosystems

Native parasites thus can have substantial consequences for individual hosts that can have additive consequences as levels of biological organisation scale up to population and community levels. 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, all have substantial consequences for food web structure. Nevertheless, it has only been in the last decade that parasites have routinely been considered as integral components of food webs and their structuring role in ecosystems is still often overlooked.

It was also outlined in Sections 1.1 to 1.4 that whilst only a small number of non-native parasites might get introduced with their free-living hosts (enemy release hypothesis), these parasites might then be transmitted to native hosts (parasite spillover). 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 strain that has yet to encounter the parasite) can then have substantial consequences at the individual level (e.g. *G. salaris*). What is less known (certainly compared with native parasites) is how these host consequences of infection by non-native parasites translate into population, community, food web and ecosystem consequences. It is this that is the basis of this research.

1.8 Focal Parasites

This research utilises three non-native fish parasites to test their influences on food web topology and host trophic niche size in wild conditions. The parasites were selected on the basis of the following criteria:

1. They were classed as 'Category 2' parasites by the Environment Agency (EA) (Williams 2013; Environment Agency 2015). This means their natural range does not include England and Wales but they have been introduced, usually with their fish host. This categorisation also means that the EA (who have delegated responsibilities from Department of Environment, Food and Rural Affairs (DEFRA) for regulating the movement of fishes between inland waters in England and Wales) have assessed the parasites as having significant disease potential for native fishes. However, their potential for economic disruption to aquaculture is sufficiently low to not warrant their categorisation as a 'notifiable disease'.
2. The three selected parasites differed in their life cycles, ranging from simple lifecycles (host-to-host) to complex lifecycles involving multiple stages and intermediate hosts (including paratenic hosts). This enabled testing of the

hypothesis that as the parasite life cycle increases in complexity it will increase food web connectivity and linkage density.

Consequently, the three non-native parasites being used are:

- *Ergasilus briani* , a copepod crustacean from South-east Asia with a direct lifecycle, with roach *Rutilus rutilus* and common bream *Abramis brama* being typical fish hosts;
- *Bothriocepahlus acheilognathi*, the 'Asian tapeworm' that has a two stage lifecycle involving a copepod intermediate host and fish final host, usually carp *Cyprinus carpio*; and
- *Anguillicoloides crassus*, a nematode parasite that has as a complex lifecycle with multiple intermediate hosts (copepods and small fish) and the European eel *Anguilla anguilla* as the final host, plus numerous other fish paratenic hosts.

These parasites were introduced into England and Wales via either the fish movement industry for angling (*E. briani*, *B. acheiloganthi*) or the aquaculture industry (*A. crassus*). The following paragraphs outline some of the key characteristics of each parasite.

Ergasilus briani is a crustacean parasite of the family Ergasilidae that can infect a wide range of freshwater fish species, with over 20 recorded fish host species in England and Wales (Alston and Lewis 1994; Williams 2007). The parasite prefers hosts of below 100 mm in length, particularly cyprinid fish (e.g. roach *Rutilus*

rutilus, rudd *Scardinius erythropthalmus* and common bream *Abramis brama*) (Alston and Lewis 1994). *Ergasilus briani* was first recorded in England and Wales in 1982 (Fryer and Andrews 1983). The direct lifecycle means it only requires fish hosts for its completion (Abdelhalim et al. 1991; Figure 1.1). It is the adult female that is parasitic and it attaches to its host via the gill filaments where it feeds on mucus, blood and epithelial cells within the gill tissue. Consequently, a heavy infection on a host can cause respiratory distress through loss of gill function, and decreased tolerance to environmental stressors. This can result in loss of condition, reduced growth, and in extreme cases, death, particularly in juvenile fish (Alston et al. 1996; Dezfuli et al. 2003).

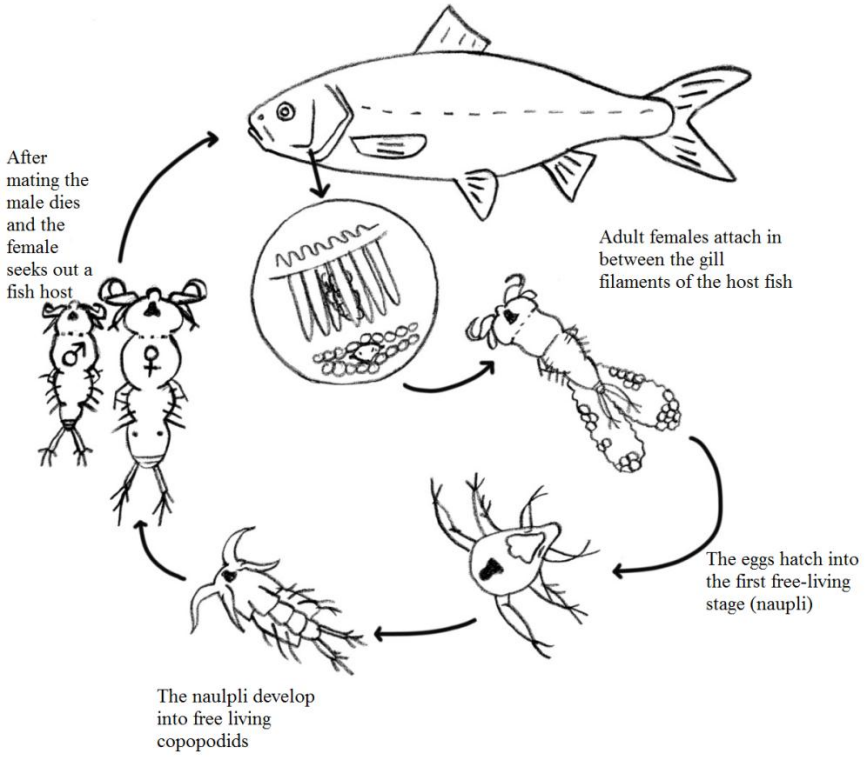

Figure 1.1 Lifecycle of *Ergasilus briani* (adapted from Environment Agency, 2015)

Bothriocephalus acheilognathi is a parasitic flatworm of the class Cestoda. Originally from Asia, it has been spread around the world via the global aquaculture trade in Asian grass carp *Ctenopharyngodon idella*. It is non-host specific, having been recorded in over 200 fish hosts across the world, although its more severe consequences tend to occur in fishes of the Cyprinidae family (Williams et al. 2011; Linder et al. 2012). It has a complex lifecycle (Figure 1.2) involving an intermediate copepod host and one or more definitive fish hosts. In the final fish host, the mature cestodes are within the intestinal tract where they release partially embryonated eggs which then pass out of the fish in their faeces. The eggs settle onto the substrate where they develop into ciliated larvae - coracidium - which then exits the egg shell and swims in the water column until eaten by a copepod. There, it sheds its ciliated outer and burrows into the copepod body cavity where it develops into the proceroid, the first larval stage. A copepod heavily infected with proceroids will move more slowly and be more susceptible to predation by fish (Nie and Kennedy 1993), thus facilitating their transfer to the final fish host. Should a piscivorous fish then consume the final host then this can also result in infection (Linder et al. 2012).

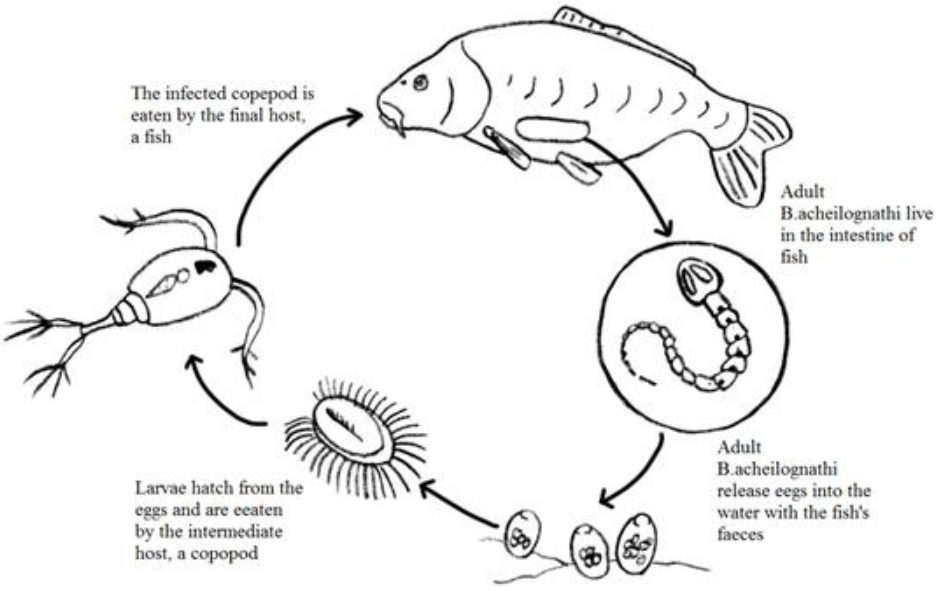

Figure 1.2 Lifecycle of *Bothriocephalus acheilognathi* (adapted from Environment Agency, 2015)

Effects on fish final hosts include damage to the intestinal tract (*cf.* Figure 2.4), physical disturbance, loss of condition, impacts of foraging behaviours and even death (Britton et al. 2011). Reports of 100% mortality in hatchery reared *C. carpio* highlight the pathogenic potential of this parasite (Scholz et al. 2012)

Anguillicoides crassus is a roundworm of the phylum Nematoda that, in its final host *A. anguilla*, infects the swim-bladder. It was introduced into Europe through the importation of infected Japanese eels in the early 1980 and was first recorded in the UK in 1987 (Kirk 2003). Their infections of *A. anguilla* are hypothesised as a contributory factor in their population decline in recent years, as *A. anguilla* make transatlantic spawning migrations, for which it would be expected a functioning

swimbladder is required (Kirk 2003). The lifecycle is complex, involving multiple intermediate and paratenic hosts, plus *A. anguilla* as the final host (Figure 1.3). Whilst juvenile (glass) eels can become infected from feeding on infected copepods, it is the larger eels (> 200 mm) that are more likely to be become infected from their predation of a paratenic host (Kennedy 2007). Indeed, these paratenic hosts are integral to the proliferation of *A. crassus* in European eels, despite there being no record of paratenic hosts in the parasite natural range (Thomas and Ollevier 1992; Kirk 2003).

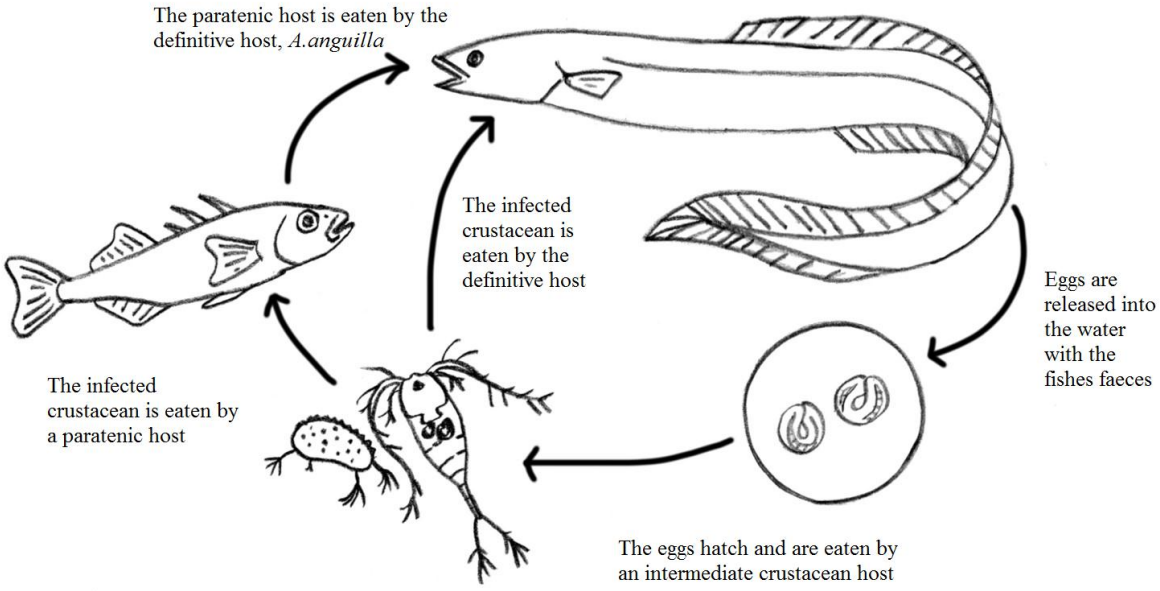

Figure 1.3 Lifecycle of *Anguillicoides crassus* (adapted from Kirk, 2003)

In *A. anguilla*, adult *A. crassus* accumulate in the swim-bladder and as their numbers increase (typically over 50; *cf*. Figure 2.5). The swim bladder becomes thickened as a result of fibrosis (Székely et al. 2009). This damage may remain even after parasites have died or left the eel, with those eels which have experienced high parasite loads previously being left with heavily scarred swim-bladders. The lumen of the swim-bladder is often filled with dead or encapsulated parasites, and in the

most extreme cases, the lumen of the swim-bladder collapses (Székely et al. 2009. Infection has been shown to produce a reduction in swimming speed (Thomas and Ollevier 1992). Nevertheless, the primary cause of *A. crassus* induced mortality is decreased resistance to secondary infections (Szekely 1994). Whilst parasite-induced mortality in wild populations is rare, significant mortalities have occurred in association with adverse environmental stressors (Kirk 2003).

1.9 Definitions of terminology

- 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.
- Non-native invasive species: Any non-native animal or plant that has the ability to spread, causing damage to the environment, the economy, our health and the way we live.
- Parasite: An organism that lives and feeds on or in an organism of a different species and causes harm to its host.
- Host: An organism that harbours a parasite.
- Intermediate host: A host that harbours the parasite only for a short transition period, during which (usually) some developmental stage is completed.
- Definitive host: A host in which the parasite reaches maturity and, if possible, reproduces sexually.
- Paratenic host: A host that is not necessary for the development of a particular species of parasite, but nonetheless may happen to serve to maintain the life cycle of that parasite. In contrast to its development in an intermediate, a parasite

in a paratenic host does not undergo any changes into the following stages of its development

- Naïve host species: A native species having no co-evolutionary history to the non-native parasite.
- Direct lifecycle (of a parasite): Lifecycle is completed on a single host (may have a free-living stage).
- Complex lifecycle (of a parasite): Lifecycle is completed on multiple hosts, including one or more intermediate host in addition to a definitive host.
- Parasite prevalence: The proportion of infected hosts among all the potential hosts examined of a single species.
- Parasite abundance: This is the mean number of parasites found in all the individual infected hosts.

1.10 Research aim and objectives

The research aim is to determine how infection of naïve fish hosts by a non-native parasite impacts individual fish, their populations, their interactions within the community and the food web topology and trophic structure. Using three non-native fish parasites present in the UK, the research objectives are to:

O1. Determine the prevalence and abundance and pathology of *Ergasilus briani* in *Rutilus rutilus* and *Abramis brama* (Chapter 2), *Bothriocephalus acheilognathi* in *Cyprinus carpio* (Chapter 3), and *Anguillicoides crassus* in *Anguilla anguilla* (Chapter 4), and assess the respective impact of each parasite on their host's growth and condition.

O2. Identify how infection by the three focal non-native parasites affects the trophic ecology of their respective host fish populations. Specifically whether parasitism alters their trophic niche size (Chapter 2, 3, 4) and trophic position (Chapters 2, 3, 4); whether there is a temporal component to the ecological impact of parasitism (Chapter 3) and whether trophic ecology can be a predictor to parasitism (Chapter 4)

O3. Assess how infections by native and the three focal non-native parasites modify the topology of aquatic food webs through comparison with the topology when parasites are omitted (Chapter 5);

O4. Identify changes in the functioning of infectious foobwebs caused by the non-native parasites *E. briani* and *B. acheilognathi* (Chapter 6).

1.11 Thesis structure

The structure of the thesis is as follows:

Chapter 1: Introduction. This has provided the rationale for the study and the overall aim and objectives.

Chapter 2: Consistent patterns of trophic niche specialisation in host population infected with a non-native parasite. This chapter provides data on parasite prevalence and abundance of infected with *Ergasilus briani* in *Rutilus rutilus* and *Abramis brama*, the consequences of infection for host fishes and how infection impacts their trophic ecology.

Chapter 3: Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite. This chapter provides data on parasite prevalence and abundance of *Bothriocephalus acheilognathi* in *C. carpio*, the consequences of infection for host fish and how infection impacts their trophic ecology.

Chapter 4: Head morphology and piscivory of European eels, *Anguilla anguilla*, predict their probability of infection by the invasive parasitic nematode *Anguillicoloides crassus*. This chapter provides data on parasite prevalence and abundance of *A. crassus* in *A. anguilla*, the consequences of infection for host fish and the interaction of eel functional morphology and parasite infection.

Chapter 5: Consequences of non-native parasites for topological food webs. This chapter quantifies how infections by native and non-native parasites modify the topology of aquatic food webs.

Chapter 6: Weighted food webs to predict the outcomes of interactions of non-native parasite infection and environmental change. This chapter quantifies how

infections by native and non-native parasites modify the structure and energy flux of aquatic food webs, and uses food web models predictively to determine the outcome of specific scenarios on parasite dynamics and food web structure.

Chapter 7: Discussion: This summarises the outputs of the data chapters (Chapters 2 to 6) and discusses conclusions in relation to the initial aims and objectives.

2. Consistent patterns of trophic niche specialisation in host populations infected with a non-native parasite

2.1 Abstract

Populations of generalist species often comprise smaller sub-sets of relatively specialised individuals whose niches comprise small sub-sets of the overall population niche. Although the ecological drivers of individual trophic specialisation are generally well established, the role of parasitism remains unclear, despite infections potentially altering host foraging behaviours and diet composition. This role was tested here using five wild populations of roach *Rutilus rutilus* and common bream *Abramis brama* infected with the non-native parasite *Ergasilus briani*, a copepod parasite that has a direct lifecycle (i.e. it is not trophically transmitted) that infects gill tissues. Parasite prevalence ranged between 16 and 67 %, with parasite abundances of up to 66 per individual. Pathological impacts included hyperplasia and localised haemorrhaging of gill tissues. There were, however, no differences in the length, weight and condition of infected and uninfected fishes. Stable isotope analyses ($\delta^{13}C$, $\delta^{15}N$) revealed that across all populations, the trophic niche width of infected fishes was consistently and substantially reduced compared to uninfected conspecifics. The trophic niche of infected fishes always sat within that of uninfected fish, revealing trophic specialisation in hosts, with predictions of diet composition indicating this resulted from greater proportions of less motile items in host diets that appeared sufficient to maintain their energetic requirements. The results here suggest trophic specialisation is a potentially important non-lethal consequence of parasite infection that results from impaired functional traits of the host.

2.2 Introduction

Infections by parasites can have considerable consequences for their free-living hosts, including alterations in habitat utilisation, and foraging and anti-predator behaviours (Barber et al. 2000; Lefevre et al. 2009; Dianne et al. 2014). There remains relatively limited knowledge regarding the mechanistic basis of these alterations (Clerc et al. 2015), with this also reflected in aspects of their ecological consequences (Lefevre et al. 2009). It is, however, well established that parasites can have considerable consequences for food web ecology (e.g. Marcogliese and Cone, 1997; Lafferty et al. 2006; Wood et al. 2007), with the trophic consequences of infections resulting from both manipulative parasites affecting the strength of trophic links involved in transmission, and from non-manipulative parasites that impair the functional traits of hosts (Miura et al. 2006; Hernandez and Sukhdeo, 2008). For example, sticklebacks *Gasterosteus aculeatus* infected with *Schistocephalus solidus* preferentially ingest smaller prey items of lower quality compared with uninfected sticklebacks (Milinski 1984; Jakobsen et al. 1988; Cunningham et al. 1994). Thus, parasite infections can restrict the prey handling and ingestion abilities of hosts and/ or reduce the ability of hosts to compete for larger prey items with uninfected individuals due to factors including energetic constraints that result in shifts in competition symmetry between the infected and uninfected individuals (Barber et al. 2000; Britton 2013).

Populations of generalist species are increasingly recognised as comprising smaller sub-sets of relatively specialised individuals whose niches are then small sub-sets of the overall population niche (Bolnick et al. 2003; Bolnick et al. 2007; Quevedo et al. 2009). Empirical studies and foraging models suggest intraspecific competition

increases individual trophic specialisation (Svanback and Persson 2004; Huss et al. 2008). Whilst other drivers of trophic specialisation include increased interspecific competition, the exploitation of new ecological opportunities, and the direct and indirect consequences of predation, there has been little consideration of how natural enemies, such as parasites, affect the magnitude of individual trophic specialisation (Araujo et al. 2011). This is despite the evidence already outlined that infections can alter host foraging behaviours and diet composition. Correspondingly, should parasite infections increase levels of competition for infected individuals then the niche variation hypothesis predicts that their sub-set of the population would become more specialised in their diet (Van Valen 1965). Conversely, under increasing resource competition, a shift to a larger trophic niche by these infected individuals might maintain their energy requirements (Svanback and Bolnick 2007).

Consequently, the aim of this study was to identify how the infection of a model parasite species affects host populations in relation to their trophic niche size and the magnitude of individual trophic specialisation. The objectives were to: (1) quantify the parasite prevalence, abundance, histopathology and energetic consequences of the model parasite on two fish species over five populations; (2) assess the trophic niche size of each fish population, and those of the two sub-sets of each population: uninfected and infected with the parasite; and (3) assess these outcomes in relation to niche theory and individual trophic specialisation. The model species were the copepod parasite *Ergasilus briani* in the host fish species roach *Rutilus rutilus* and common bream *Abramis brama*. Populations in the UK were used; *E. briani* was only introduced in 1982 (Alston and Lewis 1994) and so the parasite and fishes shared little evolutionary history, meaning infections had the potential to produce

pronounced consequences in naïve hosts (Taraschewski 2006). It was predicted that the trophic niche of infected individuals differ from that of uninfected con-specifics due to the consequences of *E. briani* infection, with infected individuals having impaired growth rates and energetics.

2.3 Materials and Methods

2.3.1 Sample collection and initial data collection

Three freshwater study sites were selected in Southern England where *E. briani* infections were known to be present in the fish community. The sites were chosen which best represented the range of habitats occupied by the parasite and it's hosts in the UK, and thus represented the differing conditions that an infected host would be exposed to as well as the different food webs that the parasite could potentially impact.

The Basingstoke canal (Site 1; 51.276414N, 0.820642W) was historically supplemented with cyprinid fish through stocking but now has a self-sustaining fish community; it is of 6 to 10 m in width and maximum depth 2.5 m (Figure 2.1). Henleaze Lake (Site 2; 51.49763N, 2.603867W) is a narrow lake in a former quarry of 450 m in length, and is up to 8 m in width and with depths to 6 m (Figure 2.2). It had been previously stocked with *C. carpio*, *A.brama* and *R. rutilus*, with the latter two species now self-sustaining. Darwell reservoir (Site 3; 50.963617N, 0.440719E) is a water supply reservoir of approximately 63 hectares where the fish community is dominated by *R. rutilus*, perch *Perca fluviatilis* and pike *Esox lucius* (Figure 2.3). It was the stocking activities on each site in the 1980s and 1990s that resulted in *E. briani* introduction.

Figure 2.1 Site 1, Section of the Basingstoke canal. (Photograph by Ronn Strutt).

Figure 2.2 Site 2, Henleaze Lake, October 2013. In the foreground are the swimming platforms and diving boards used by swimmers, the portion of the lake reserved for angling starts beyond the large willow on the right.

Figure 2.3 Site 3, Darwell Reservoir, October 2013.

The sampling methodology used at each site varied according to the physical habitat. At Site 1, samples of *A. brama* were collected in October 2012 and samples of *R. rutilus* in October 2014 using a combination of use of a 25 x 2.7 m micromesh seine net and electric fishing. Samples of *R. rutilus* and *A. brama* were collected from Site 2 in October 2013 using the micromesh seine net. At Site 3, samples of *R. rutilus* were available from a stock assessment exercise completed in October 2013 that captured these fish using a gill net of 30 x 2.5 m and mesh size 33 mm (knot to knot). Logistical constraints meant samples could not be collected from all waters in the same year, although care was taken to ensure sampling took place at the same time at each one (i.e. October) in order to ensure seasonal patterns in the growth and condition of the fishes were similar. The sampling procedure was carried out in such a way as to include all available potential habitats, including marginal and open water environments, to ensure the fish collected were representative of the entire population and any behavioural effect resulting from parasitism that could potentially alter their habitat utilisation did not result in biased samples. Following their capture at all sites, all fish were initially retained in water-filled containers and

for *R. rutilus* and *A.brama*, a random sub-sample of a minimum of 30 individuals per species was taken and transported to the laboratory for processing. Concomitant to the collection of each fish sample, their putative food items were also sampled, including macro-invertebrates (kick-sampling and sweep netting), zooplankton (filtering 10 l of water through a 250 μm filter) and phytoplankton (filtering 10 l of water through a 53 μm filter). Triplicate samples of macro-invertebrate species were taken, where a sample represented between 5 and 20 individuals of that species.

In the laboratory, all fish were euthanized (anaesthetic overdose; MS-222), with weight (W; to 0.01 g), and fork length (L; nearest mm) recorded. A detailed post-mortem was then conducted on each individual *R. rutilus* and *A. brama* for detecting the presence of infections of native and non-native parasites 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. Gill arches from both gill cavities were removed and examined under low power for parasite presence, including *E. briani*. Where *E. briani* was present, their intensity of infection was recorded (number of individual parasites). Hereafter, where an individual *R. rutilus* or *A. brama* is referred to as either infected or non-infected, it refers to the presence/ absence of *E. briani* in that individual during this process. Gill tissue from infected and uninfected individuals was retained and prepared for histopathology. On completion of the post-mortem, a sample of dorsal muscle was taken from a random proportion of the fish samples (sample sizes 6 to 15 per sub-set of fish per population). These, and the macro-invertebrate, zooplankton and phytoplankton samples, were then dried at 60ºC to constant weight before being analysed for their stable isotopes of ^{13}C and ^{15}N at the Cornell Stable 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 $\delta^{15}N/d^{14}N$. 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 $\delta^{13}C$.

2.3.2 Histopathology

Histopathology of gill tissues was completed to assess the pathological changes associated with *E. briani* infection. Sections of gill from infected and uninfected fish were fixed in Bouin's fixative for 24 hours before transferring to 70% Industrial Methylated Spirit. The tissues were trimmed, dehydrated in alcohol series, cleared and then embedded in paraffin wax. Transverse and longitudinal sections of 3 µm were cut on a microtome. These were dried at 50°C, stained using Mayer's haematoxylin and eosin, and examined microscopically for pathological changes and described accordingly.

2.3.3 Data analyses

Infection levels of *E. briani* in *R. rutilus* and *A. brama* were described as their prevalence (number of infected individuals/total number of individuals x 100) and abundance (number of *E. briani* per host). The stable isotope data of *R. rutilus* and *A. brama* were used to assess their trophic niche size and predict their diet composition from the putative food resource data. Trophic niche size was calculated using the metric standard ellipse area (SEAc) in the Stable Isotope Aanalysis in R (SIAR) package (Parnell et al. 2010) in R (R Core Development Team, 2013). SEAc is a bivariate measure of the distribution of individuals in trophic space, where each ellipse encloses ~ 40% of the data and thus represents the core dietary niche of species and so indicates their typical resource use (Jackson et al. 2011; Jackson et al. 2012). It 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), highlighting its utility. The subscript 'c' in SEAc indicated that a small sample size correction was used here due to limited sample sizes. For each population of *R. rutilus* and *A. brama* in each site, SEAc was calculated for two sub-sets of individuals: those infected with *E. briani* and those uninfected. Where SEAc overlapped between the sub-sets, or the SEAc of the sub-set overlapped with another species or sub-set of another species in the community, then the extent of this overlap (as a %) was calculated to identify the extent to which the trophic niches were shared.

To then predict the diet composition of each sub-set of fish, their stable isotope data, plus those of their putative food resources, were applied to Bayesian mixing models that estimated the relative contribution of each putative food resource to the diet of

each individual *R. rutilus* or *A. brama* per site (Moore and Semmens 2008). The models were run using the MixSIAR GUI package in the R computing programme (R Core Development Team 2013). Given that excessive putative food resources can cause mixing models to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species (Phillips et al. 2005). Correspondingly, at Sites 1 and 2, the groups used in the models were Arthropoda, Chironomidae and zooplankton. At Site 3, they were macrophyte, zebra mussel *Dreissena polymorpha*, zooplankton and phytoplankton. Isotopic fractionation factors between resources and consumers in the models were 3.4 ‰ (± 0.98 ‰) for $\delta^{15}N$ and 0.39 ‰ (± 1.3 ‰) for $\delta^{13}C$ (Post, 2002). Outputs were the predicted proportion of each resource to host diet (0 to 1).

2.3.4 Statistical analyses

For each fish species and population infected with *E. briani*, differences between the infected and uninfected hosts were tested for length using ANOVA, and their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ using Mann Whitney U tests. Condition was calculated as Fulton's Condition Factor K, where $K = 100 \times W/L^3$, where L was measured in cm, with differences between infected and uninfected fishes also tested using Mann Whitney U tests. Differences in weight between the infected and uninfected fish per population and species were then tested in a generalized linear model (GLM), where the effect of length on weight was controlled as a co-variate; outputs included estimated marginal means of weight controlled for length for each sub-set of fish and the significance of their differences were identified by pairwise comparisons with Bonferroni correction for multiple comparisons. Differences between the predicted proportions of each putative food source to the diet of infected and uninfected fish

were tested by Mann Whitney U tests. Other than the stable isotope mixing models, all analyses were completed in SPSS v. 22.0. In all analyses, where parametric tests were used, the assumptions of normality of residuals and homoscedasticity were checked, and response variables were log-transformed to meet the assumption if necessary.

2.4 Results

2.4.1 Parasite prevalence and abundance, and effect on fish length and weight

Prevalence and mean parasite abundance was highest at Site 1 for both fishes, with the maximum abundance recorded being 66 *E. briani* in an individual *R. rutilus* (Table 2.1). Other parasites recorded were native species that would be considered as the expected parasite fauna of these fishes in a UK community and were recorded at levels that were considered as not high enough to cause clinical pathology (Hoole et al. 2001) These species are listed in Appendix 2. At Site 1, the non-native parasite *Ergasilus sieboldi* was also detected in the gills of two *A. brama*. Due to the potential for this parasite to confound subsequent analyses, these fish were omitted from the dataset.

Table 2.1 Prevalence and abundance of *Ergasilus briani* per site and species

Site	Species	n	Prevalence (%)	Mean abundance of parasites (± SE)	Range of parasite abundance
1	*A. brama*[1]	45	67	5.71 ± 0.89	0 - 21
1	*R. rutilus*[2]	40	63	6.20 ± 2.09	0 - 66
2	*A. brama*	32	19	1.63 ± 0.85	0 - 16
2	*R. rutilus*	44	16	0.89 ± 0.46	0 - 21
3	*R. rutilus*	64	17	0.40 ± 0.13	0-6

[1]Sampled October 2012

[2]Sampled October 2014

Differences in fish lengths between the infected and uninfected fish were not significant at any site (ANOVA: Site 1: *R. rutilus* $F_{1,19} = 0.11$, $P > 0.05$; *A.brama* $F_{1,29} = 0.01$, $P > 0.05$, Site 2: *R. rutilus* $F_{1,14} = 0.84$, $P > 0.05$; *A.brama* $F_{1,15} = 0.42$, $P > 0.05$, Site 3: *R. rutilus* $F_{1,19} = 0.01$, $P > 0.05$; Table 2.2). Similarly, there were no significant differences between the body weight of infected and uninfected fish at any site when the effect of total length was controlled (GLM: Site 1: *A. brama*: Wald $\chi^2 = 1.27$, $P > 0.05$; *R. rutilus* Wald $\chi^2 = 0.91$, $P > 0.05$; Site 2: *A. brama*: Wald $\chi^2 = 0.001$, $P > 0.05$; *R. rutilus*: Wald $\chi^2 = 0.67$, $P > 0.05$), or in Fulton's condition factor, K (Mann Whitney U tests: Site 1: *A. brama*: $Z = 1.16$, $P > 0.05$; *R. rutilus* $Z = 0.83$, $P > 0.05$; Site 2: *A. brama*: $Z = 0.82$, $P > 0.05$; *R. rutilus*: $Z = 0.48$, $P > 0.05$).

Table 2.2 Sample sizes, mean lengths of subsampled fish and mean stable isotope data of the fish species and putative food resources at each study site.

Site	Species	n	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
1	Uninfected *A. brama*	15	39.6 ± 3.0	-35.25 ± 0.46	16.06 ± 0.93
	Infected *A. brama*	15	39.5 ± 2.4	-35.40 ± 0.67	16.46 ± 0.81
	Arthropoda	3		-32.30 ± 0.56	11.44 ± 0.74
	Chironomidae	3		-34.56 ± 0.86	9.95 ± 0.78
	Zooplankton	3		-32.64 ± 0.76	8.74 ± 0.56
	Uninfected *R. rutilus*	10	64.4 ± 23.9	-35.73 ± 1.66	14.44 ± 0.82
	Infected *R. rutilus*	6	69.0 ± 24.0	-35.54 ± 0.61	13.92 ± 0.35
	Arthropoda	4		-34.65 ± 1.50	11.71 ± 1.17
	Chironomidae	3		-34.52 ± 0.91	10.25 ± 0.30
	Zooplankton	3		-29.15 ± 0.50	6.81 ± 0.49
2	Infected *A. brama*	6	102.7 ± 50.2	-33.08 ± .020	16.09 ± 0.17
	Arthropoda	4		-29.93 ± 2.1	10.67 ± 1.65
	Chironomidae	3		-27.95 ± 0.9	12.52 ± 0.99
	Zooplankton	3		-34.92 ± 1.50	9.32 ± 0.30

(Cont.)

Site	Species	n	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
2	Uninfected *R. rutilus*	10	100.1 ± 22.1	-32.23 ± 1.44	15.37 ± 0 .78
	Infected *R. rutilus*	7	94.3 ± 14.9	- 31.10 ± 1.87	14.64 ± 1.37
	Arthropoda	4		-29.93 ± 2.1	10.67 ± 1.65
	Chironomidae	3		-27.95 ± 0.9	12.52 ± 0.99
	Zooplankton	3		-34.92 ± 1.50	9.32 ± 0.30
3	Infected *R. rutilus*	10	122.7 ± 23.4	-22.43 ± 1.08	12.94 ± 0.34
	Macrophyte	3		-19.17 ± 037	8.72 ± 0.29
	Phytoplankton	3		-29.47 ± 0.89	11.37 ± 0.90
	Zooplankton	3		-30.58 ± 0.90	13.54 ± 0.99
	D. polymorpha	3		-15.30 ± 0.89	7.20 ± 0.40

2.4.2 Histopathology

Histopathological examinations revealed consistent pathological changes associated with *E. briani* infection when infected and uninfected tissues were compared. Parasites attached to the ventral surface of the gill filament, between the hemibranchs, tight to the interbranchial spetum. Whilst dissection of the gill was needed to confirm the presence of *E. briani*, their egg strings were often visible prior to removal of the gills (Figure 1a). During attachment, the parasite's antennae (Figure 1b) were used to engulf the base of the gill filaments, bringing the head of the parasite tight to the gill septum (Figure 1c,d). This frequently led to displacement and distortion of filaments to accommodate the body of the parasite (Figure 1c-e). Parasite attachment led to compression of the gill tissue, with flattening of the

epithelium (Figure 1d,e). This was often accompanied by hyperplasia, localised haemorrhaging, epithelial erosion and compression of blood vessels underlying the body of the parasite (Figure 1e). Although no direct evidence for parasite feeding was observed, localised loss and compression of gill epithelium was often apparent adjacent to the mouth (Figure 1f).

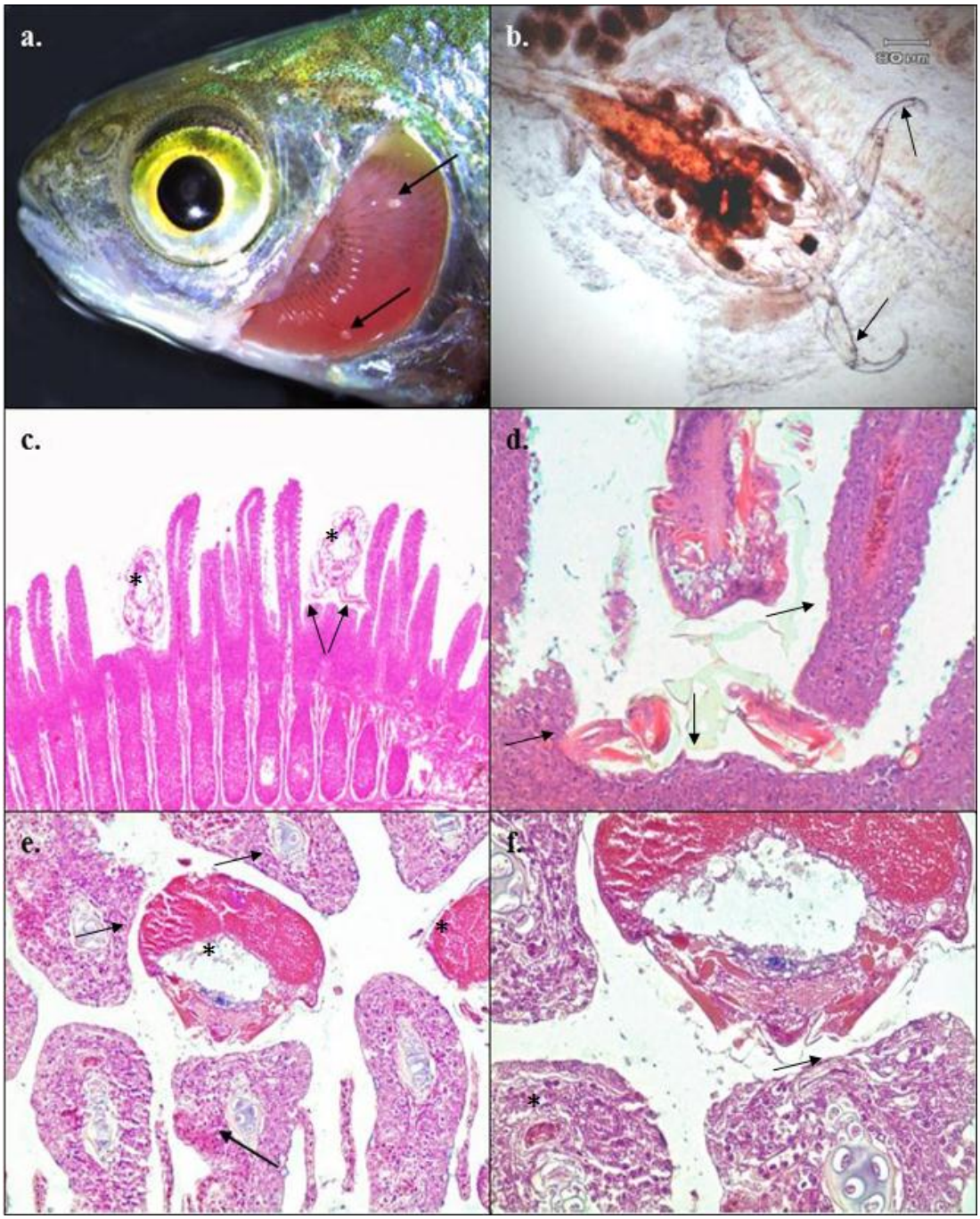

Figure 2.4 Pathology of *Rutilus rutilus* infected with *Ergasilus briani*. a) Presence of two *E. briani* (arrows) attached between the gill filaments following removal of the operculum. b) Whole *E. briani* following dissection of the gill tissue, showing antennae used for attachment (arrows). c) Histopathology of *R. rutilus* gill,

with attachment of two *E. briani* (*) tight to interbranchial septum with displacement of filaments. The antennae can been seen engulfing multiple filaments (arrow). d) Compression and distortion of gill tissue (arrow) adjacent to *E. briani*, indicative of forceful attachment to the base of the gill filaments. e) Transverse section through infected gill arch, with multiple *E. briani* (*) attached between the hemibranchs, with compression and erosion of epithelium, localised haemorrhage (**) and displacement of filaments. f) Gill tissue adjacent to *E. briani*, showing epithelial loss and compression, with constriction of blood vessel underlying the parasite (arrow). Normal vessel shown away from the immediate site of parasite attachment (*).

2.4.3 Stable isotope metrics

The differences in the mean values of $\delta^{13}C$ and $\delta^{15}N$ between the infected and uninfected fish were not significant for any of the species at any site (Mann Whitney: $\delta^{13}C$: Site 1: *A. brama* Z = 0.57, P > 0.05; *R. rutilus* Z = 0.23, P > 0.05 Site 2: *A. brama* Z = 1.19, P > 0.05; *R. rutilus* Z = 1.80, P > 0.05; Site 3: *R. rutilus* Z = 0.01, P > 0.05; δ 15N: Site 1: *A. brama* Z = 0.57, P > 0.05; *R. rutilus* Z = 0.16, P > 0.05; Site 2: *A. brama* Z = 1.30, P > 0.05; *R. rutilus* Z = 1.03, P > 0.05; Site 3: *R. rutilus* Z = 1.48, P > 0.05) (Table 2.2). There was, however, a consistent pattern of trophic niche size (as SEAc) being considerably higher in the uninfected sub-set of fish when compared to their infected conspecifics (Table 2.3), with very few outliers sitting outside of these core niches. The extent of the overlap between the tropic niches of each sub-set of the populations was high, with infected *A.brama* sharing 95 and 100 % of trophic space with uninfected *A. brama* in Sites 1 and 2 respectively, and infected *R. rutilus* shared 91, 69 and 73 % of trophic niche space with uninfected *R. rutilus* in Sites 1, 2 and 3 respectively. Where *R. rutilis* and *A. brama* were present in

sympatry at Site 2, there was minimal overlap in the trophic niches of their uninfected individuals (16.7 %), but this increased between their infected subs-sets of individuals (89.2 %) (Figure 2.5).

Table 2.3 Trophic niche width (as standard ellipse area, SEAc) of the uninfected and infected sub-sets of fish per site, and their relative size and extent of trophic overlap between the infected and uninfected sub-sets of fish.

Site	Species	SEAc uninfected (‰^{2})	SEAc infected (‰^{2})	Trophic overlap (%)
1	*A. brama*	1.63	0.67	94.70
1	*R. rutilus*	4.71	0.47	90.88
2	*A. brama*	1.18	0.12	99.99
2	*R. rutilus*	4.52	3.23	69.31
3	*R. rutilus*	1.99	1.26	73.25

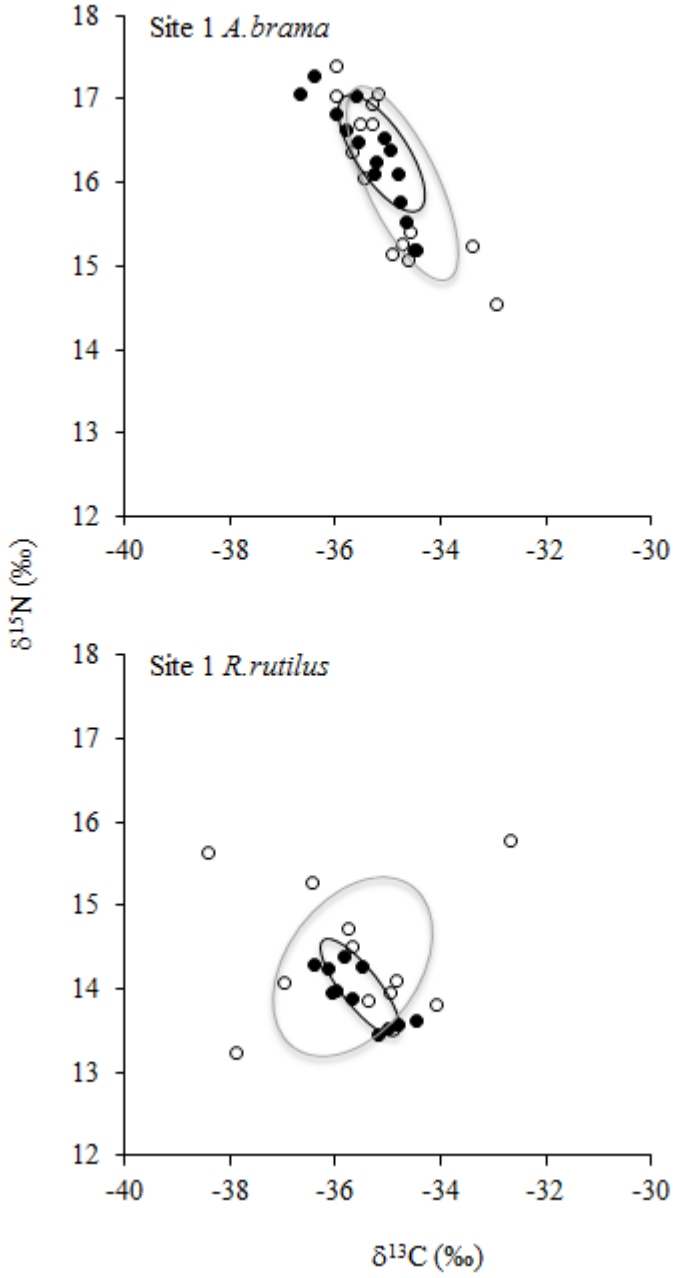

Figure 2.5 Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 1. a) *A. brama* sampled May 2012, b) *R. rutilus* sampled October 2014. The black ellipse represents the infected individuals and the grey ellipse represents uninfected individuals.

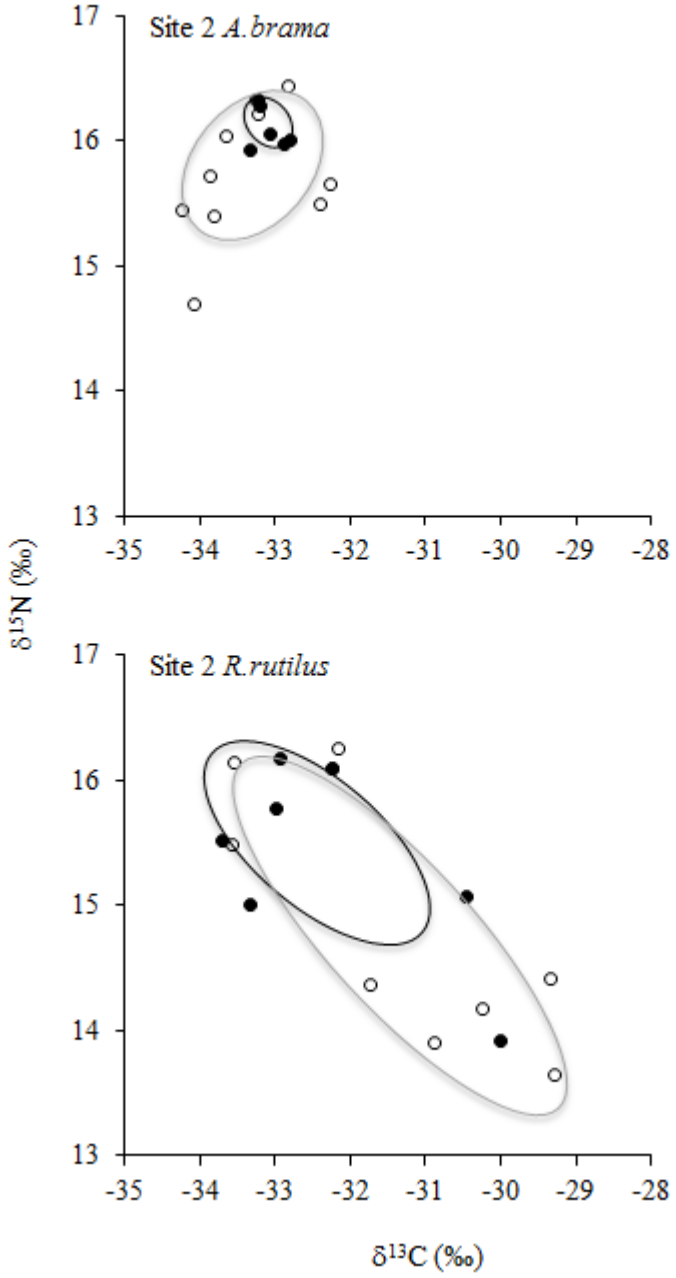

Figure 2.6 Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 2. The black ellipse represents the infected individuals and the grey ellipse represents uninfected individuals.

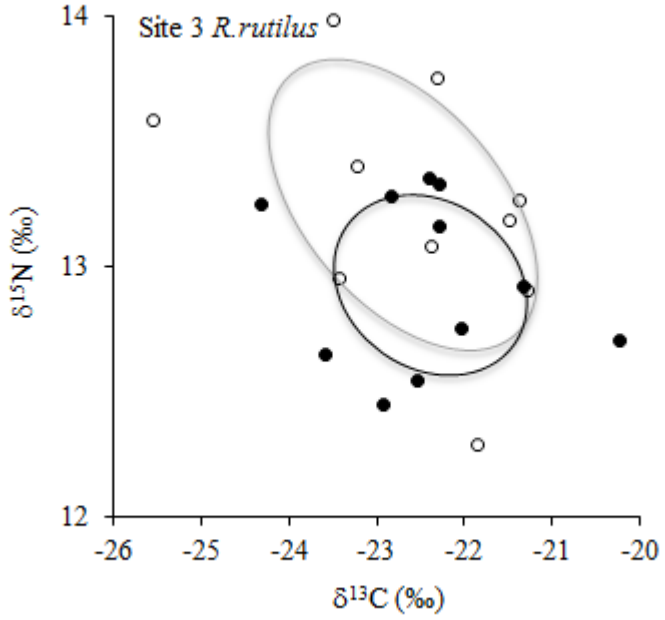

Figure 2.7 Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 3. The black ellipse represents the infected individuals and the grey ellipse represents uninfected individuals.

The outputs of the mixing models predicting the diet compositions of the uninfected and infected fish per species and per site revealed some significant differences between the subsets of fish (Table 2.4). At Site 1, infected fish of both species had significantly higher proportions of chironomid larvae in their diet (*R. rutilus*: $Z = 3.99$, $P < 0.01$, *A. brama* $Z = 4.08$, $P < 0.01$; Table 2.4) than their uninfected conspecifics. This was also apparent in infected *R. rutilus* in Site 2 ($Z = 3.03$, $P < 0.05$), where infected *A. brama* had significantly decreased proportions of zooplankton in their diet ($Z = 3.87$, $P < 0.01$). At Site 3, infected fish consumed greater proportions of macrophyte material ($Z = 3.59$, $P < 0.01$) and reduced proportions of phytoplankton ($Z = 3.87$, $P < 0.01$) than uninfected *R. rutilus* (Table 2.4).

Table 2.4 Summary of the Bayesian mixing models outputs predicting the proportions of each major food item to the diet of infected and uninfected fish per species and sites, and the significance of the differences according to Mann Whitney U Tests (Z), where * P < 0.05; **P < 0.01. Values of the modelled proportions represent their mean and standard error.

			Modelled diet proportion		
Site	Species	Food item	Uninfected	Infected	Z
1	*A. brama*	Arthropoda	0.40 ± 0.14	0.35 ± 0.13	4.59**
		Chironomidae	0.45 ± 0.14	0.51 ± 0.13	4.08**
		Zooplankton	0.15 ± 0.10	0.10 ± 0.08	4.59**
	R. rutilus	Arthropoda	0.59 ± 0.19	0.40 ± 0.19	3.99**
		Chironomidae	0.38 ± 0.19	0.57 ± 0.19	3.99**
		Zooplankton	0.03 ± 0.03	0.03 ± 0.03	0.53
2	*A. brama*	Arthropoda	0.37 ± 0.33	0.40 ± 0.37	0.74
		Chironomidae	0.25 ± 0.17	0.27 ± 0.20	0.35
		Zooplankton	0.38 ± 0.18	0.30 ± 0.19	3.87**
	R. rutilus	Arthropoda	0.51 ± 0.27	0.45 ± 0.31	2.84*
		Chironomidae	0.21 ± 0.16	0.16 ± 0.16	3.03*
		Zooplankton	0.27 ± 0.17	0.31 ± 0.21	3.42**
3	*R. rutilus*	Macrophyte	0.31 ± 0.14	0.36 ± 0.17	3.59**
		Phytoplankton	0.18± 0.01	0.14 ± 0.01	3.87**
		Zooplankton	0.26 ± 0.11	0.28 ± 0.11	0.81
		D. polymorpha	0.24 ± 0.11	0.24 ± 0.12	1.9

2.5 Discussion

Infection of *R. rutilus* and *A. brama* by *E. briani* resulted in gross pathological changes characterised by displacement of gill filaments, loss and compression of epithelium, hyperplasia, and localised haemorrhaging within the filaments as a consequence of parasite attachment. This is consistent with pathological changes associated with other Ergasilid parasites (Alston and Lewis 1994; Dezfuli et al. 2003). When the trophic niche widths of infected and uninfected fishes were compared, these differed as per the prediction and revealed a general and consistent pattern of trophic niche constriction in the infected fishes, suggesting that rather than switching to alternative food items, the infected fishes consumed specific food items that were also within the dietary range of uninfected individuals. Despite this diet specialisation resulting in the trophic niche of infected individuals overlapping with the niche width of the subset of the infected individuals of the other species, this dietary specialisation appeared sufficient to maintain their energetic requirements, given that infection did not adversely affect their individual condition, contrary to the prediction.

Optimum foraging theory models typically assume that individuals rank alternative resources according to their energetic value per unit handling time, with this dependent on the resource traits and phenotypic capacity of individuals to capture, handle and to digest those resources (Araujo et al. 2011). This suggests individuals will feed on the most valuable resources, ignoring lower-value resources when search and handling time could be better spent searching for more valuable ones (Bolnick et al. 2003). Thus, niche variation between individuals is largely dependent on the diversity and abundance of available resources versus the phenotypic traits of

the individual (Crowden and Broom 1980; Stephens and Krebs 1986). The outputs here, revealing that infected fishes had increased specialisation in their trophic niche, were therefore likely to be associated with the phenotypic changes resulting from the infection pathology.

The outputs of this study provided strong evidence from field studies that parasitism can be a driver of trophic niche specialisation. However, in the absence of experimental study, the actual causal mechanisms involved beyond the infections were unable to be tested. Nevertheless, parasites are recognised as impacting host foraging efficiency through a variety of physiological, pathological and behavioural mechanisms, resulting in, for example, altered time budgets through increased time spent foraging (Giles 1983; Barber et al. 1995), and alterations in diet composition compared with non-infected individuals (Milinski 1984). Moreover, in other animals infected with gill parasites, shifts in heart rate and oxygen consumption have been recorded (Schuwerack et al. 2001), along with reduced haemoglobin levels (Montero et al. 2004), which impact swimming efficacy (Duthie and Hughes 1987) and the ability to maintain normal intestinal function while swimming (Thorarensen et al. 1993). In other Ergasilid parasites, gill damage also results in respiratory dysfunction, osmoregulatory failure, and haematological disruption (e.g. Hogans 1989; Abdelhalim et al. 1991; Alston and Lewis 1994; Dezfuli et al. 2003). Consequently, it is speculated that the infected fishes in this study increased their predation of prey that were highly abundant and/ or relatively slow moving, and thus required relatively low energy expenditure to capture and handle during foraging, as a consequence of some energetic costs associated with infection that were not quantified experimentally and thus require further investigation.

Where there are sufficient numbers of predators focusing on specific prey items then this predation pressure can impact these prey populations. Although items such as chironomid larvae tend to ubiquitous and numerous in freshwaters (Cranston et al. 1995), increased predation pressure by infected fishes could result in reduced abundances, potentially invoking cascading effects, particularly if the infected individuals have to increase their food intake to maintain their condition. This is because parasitism can significantly increase predation pressure on prey populations with, for example, *Gammarus pulex* infected with the acanthocephalan parasite *Echinorhynchus truttae* consuming significantly more *Asellus aquaticus* than uninfected conspecifics, enabling them to maintain their condition despite the infection (Dick et al. 2010). For predator populations containing infected individuals, whilst specialisation may be beneficial at the population level as it appears to facilitate the survival of infected individuals despite the pathological impacts incurred (Lomnicki 1988), the sub-set of specialised individuals might be at greater risk from external pressures (Durell 2000). For example, the increased time spent foraging and/ or the utilisation of different habitats to preferentially forage on specific prey items, allied with the potential for their anti-predator behaviours being modified, might result in increased predation risk (Lafferty, 1999; Barber et al. 2000; Ward et al. 2002). Indeed, when infected with *Schistocephalus solidus*, three-spined stickleback *Gasterosteus aculeatus* spend more time foraging as a compensatory mechanism (Giles, 1987), resulting in a trade-off with anti-predator behaviours (Giles, 1983), and thus incurring a greater likelihood of being predated by a piscivorous bird (Milinski, 1985). Similarly, infected banded killifish *Fundulus diaphanous* are more likely to occupy the front of shoals, a position that optimises

feeding opportunities but also carries the greatest risk of predation (Ward et al. 2002).

The focal parasite of this study, *E. briani*, is an introduced parasite to the UK, arriving as a consequence of fish being moved within aquaculture and fisheries (Fryer and Andrews, 1983). It thus represents a parasite that was successfully introduced into the UK, despite such movements often resulting in non-native parasites failing to establish through, for example, enemy release (Sheath et al. 2015). The consequences of introduced parasites within native communities can be varied, but can result in disease outbreaks resulting in high fish losses. For example, the rosette agent *Sphareothecum destruens*, spread via the invasive topmouth gudgeon *Pseudorasbora parva,* can cause high mortality rates in naïve fishes (Andreou et al. 2012) and the impact of the introduced parasitic crustacean *Gyrodactylus salaris* in Norway was the collapse of wild salmon populations in 45 Norwegian rivers (Peeler and Thrush 2004) with an economic cost the in excess of US $500,000,000 (Hansen et al. 2003). Whilst the impact of *E. briani* here was much less dramatic, our outputs suggested that ecological alterations did occur as a potential cost of infection, with modification of host diet composition that constricted the trophic niche of the host component of the population.

Studies on trophic niche specialisation have identified a range of causal factors, particularly inter- and intra-specific competitive processes, predation pressure and impact and the exploitation of new ecological opportunities (Araujo et al. 2011). The role of parasitism in trophic niche specialisation has, conversely, received very little attention. Consequently, our findings that the trophic niches of individuals infected

with *E. briani* were consistently constricted and specialised across five fish populations are important. They strongly suggest that the host consequences of infection, including pathological impacts, could also be an important driver of niche constriction that has been largely overlooked and thus should be incorporated into future studies on the ecological drivers of trophic niche specialisation. They also suggest infection could have some consequences for food web structure (Chapters 5 and 6).

3. Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite

This chapter is based on the published article which is presented in Appendix 6:

Pegg, J., Andreou, D., Williams, C. F. and Britton, J. R., 2015. Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite. *Parasitology*. doi:10.1017/S0031182015001237

3.1 Abstract

In host-parasite relationships, parasite prevalence and abundance can vary over time, potentially impacting how hosts are affected by infection. Here, the pathology, growth, condition and diet of a juvenile *Cyprinus carpio* cohort infected with the non-native cestode *Bothriocephalus acheilognathi* was measured in October 2012 (end of their first summer of their life), April 2013 (end of first winter) and October 2013 (end of second summer). Pathology revealed consistent impacts, including severe compression and architectural modification of the intestine. At the end of the first summer, there was no difference in lengths and condition of the infected and uninfected fish. However, at the end of the winter period, the condition of infected fish was significantly reduced and by the end of their second summer, the infected fish were significantly smaller and remained in significantly reduced condition. Their diets were significantly different over time; infected fish consumed significantly higher proportions of food items <53 μm than uninfected individuals, a likely consequence of impaired functional traits due to infection. Thus, the sub-lethal impacts of this parasite, namely changes in histopathology, growth and trophic niche were dependent on time and/or age of the fish.

3.2 Introduction

Parasite infections often negatively impact the fitness of their hosts, can modulate the dynamics of host populations, and can have consequences for non-host populations through changes in the strength of interspecific competitive relationships (Power & Mitchell 2004). Host responses to infection include altering their life-history traits prior to maturity when individuals allocate more resources to reproduction than growth and survival, as this ensures reproduction before resource

depletion and/or castration (Michalakis & Hochberg 1994; Agnew et al. 2000). This can affect their reproductive effort (Christe et al. 1996; Sorci et al. 1997) and body size (Arnott et al. 2000). Understanding these infection consequences for hosts at the individual level then enables understanding of infection impacts at the population and community levels (Pagan et al. 2008).

In freshwaters, the opportunity for fish parasites to be moved between localities is high due to the introduction pathways of aquaculture, the ornamental fish trade and sport angling (Gozlan et al. 2010; Section 1.1). *Bothriocephalus acheilognathi* is a cestode that is originally from Asia (Xiang-Hua 2007) that has been introduced around the world through the global aquaculture trade in Asian grass carp *Ctenopharyngodon idella* and common carp *Cyprinus carpio* (Salgado-Maldonado & Pineda-López 2003). Whilst the parasite has a broad host range, having been recorded in over 200 fish species, pathological consequences appear to be more severe in fishes of the family Cyprinidae (Williams et al. 2011; Linder et al. 2012; Section 1.8). It has a complex lifecycle involving an intermediate copepod host and a definitive fish host (Linder et al. 2012) (Figure 1.2). While fish are normally infected by consuming infected copepods, there is some evidence that adult worms can additionally be transmitted directly to piscivorous fish that prey on infected fish (Hansen et al. 2007). Consequences for fish hosts include damage to the intestinal tract, loss of condition, impacts on foraging behaviours and mortality (Britton et al. 2011), with high rates of mortality recorded in hatchery reared *C. carpio* (Scholz et al. 2011). Non-lethal consequences of *B. acheilognathi* infection also include changes in trophic ecology. For example, in a population of juvenile *C. carpio,* application of stable isotope analysis on infected and uninfected individuals

suggested infected fish were feeding on items lower in the food web, resulting in energetic consequences (Britton et al. 2011).

To date, studies on the trophic ecology of fish infected with *B. acheilognathi* have focussed on single samples taken during a single growth season (e.g. Britton et al. 2011). This provides limited knowledge on how their trophic niches vary seasonally and in relation to parasite prevalence and abundance, and how this affects metrics such as growth and condition over longer time periods. This is important, as for many host populations, parasite incidence varies seasonally due to factors including the interactions of shifts in the abundance of intermediate hosts, the feeding and/ or reproductive activities of final hosts, the reproductive activity of parasites, and the immune response to infection (Altizer et al. 2006). For example, seasonal changes in levels of *B. achileognathi* infections, stimulated by changes in water temperature, have been recorded in *Gambusia affinis* and *Pimephales promelas* (Granath & Esch 1983; Riggs et al. 1987). Similar seasonal changes have been observed in other parasite/host systems, for example Öztürk and Altunel (2006) observed seasonal and annual changes in *Dactylogyrus* infections across four host species. In chub *Squalius cephalus*, higher condition factors and seasonal variations in gonado-somatic indices (GSI) were associated with decreased immune function and corresponding increases in parasite loads, suggesting differences in the seasonal energy allocation between immune function and somatic and/ or reproductive investment (Lamkova et al. 2007).

Given the recorded trophic consequences of *B. acheilognathi* infection for juvenile *C. carpio* (Britton et al. 2011), the aim of this study was to assess how their sub-

lethal consequences of infection varied over a 12 month period through tracking a single cohort. The objectives were to: (i) quantify temporal changes in parasite prevalence, abundance, histopathology and the energetic consequences of infection of *B. acheilognathi* in juvenile *C. carpio*; and (ii) assess the temporal changes in the trophic ecology and diet of juvenile *C. carpio* infected and uninfected with *B. acheilognathi* through stable isotope analysis.

3.3 Methods

3.3.1 Sample collection and initial data collection

The study population was located in the Greater London area of the UK and where *B. acheilognathi* had been recorded previously. The site was a small pond of 50 m length, 20 m width and maximum depth 1.5 m (Figure 3.1). The sampling programme covered two summer periods and an over-wintering period, with the initial sample collected in early October 2012 (end of the summer period and end of the 2012 growth season), April 2013 (end of the over-wintering period) and October 2013 (end of the summer period and end of the 2013 growth season). The pond contained a mixed population of carp *C. carpio*, rudd *Scardinius erythropthalmus,* and perch *Perca fluviatilis.* Due to fishery management operations, the mature component of the *C. carpio* population was removed from the lake after spawning in 2012, thus all remaining carp were young-of-the-year. Consequently, all fish captured in October 2012 were age 0+ and by October 2013 were 1+ years, i.e. the captured fish throughout the study were of the same cohort, with this verified by age analysis of their scales.

Figure 3.1 The study site, with the Greater London conurbation in the background.

The fish were sampled using traps that had a circle alloy frame of length 107 cm, width and height 27.5 cm, mesh diameter 2 mm and with funnel shaped holes of 6.5 cm diameter at either end to allow fish entry and hence their capture. They were each baited with 5 fishmeal pellets of 21 mm diameter were placed in the trap as an attractant (Dynamite Baits 2010). Alternative sampling methods were trialled initially (seine nets and electric fishing), but were unsuccessful due to the presence of underwater structures (nets) and heavy growth of *Phragmites australis* in the littoral zone (electric fishing). On each sampling occasion, 10 traps were set in the littoral zone at approximately 18.00 hours and lifted at 09.00 hours the next morning. After the traps were lifted, all the juvenile *C. carpio* were removed and transferred to water-filled containers and a random sub-sample of 25 individuals was taken and transported to the laboratory for processing. As the fish were sampled from a private fishery, the numbers were limited in order to minimise the impact on the future

angling stock, as agreed with the fishery managers. In April 2013 and October 2013, samples of the putative food resources of the fish were also taken, covering macro-invertebrates (through kick sampling and sweep netting with a handnet of 0.25 mm mesh), zooplankton (through filtering 10 l of water through a net and filter of 250 μm) and phytoplankton (filtering 10 l of water through a net and filter of 53 μm). For macro-invertebrates, triplicate samples were taken, where a sample represented between 5 and 20 individuals of that species. Putative food resource samples were not able to be collected in October 2012 due to logistical constraints.

In the laboratory, all fish were euthanized (anaesthetic overdose; MS-222), with weight (W; to 0.01 g), and fork length (L; nearest mm) recorded. A detailed post-mortem was then conducted on each individual for detecting the presence of infections of native and non-native parasites 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. The entire digestive tract was removed and examined under low power for detecting the presence of intestinal parasites, including *B. acheilognathi*. When *B. acheilognathi* was recorded, their abundance was recorded (by number, and mass to nearest 0.001 g). Hereafter, where an individual *C. carpio* is referred to as either infected or non-infected, it refers to the presence/absence of *B. acheilognathi* in that individual during this process. Intestinal tissue from infected and uninfected individuals was retained and prepared for histopathology.

On completion of the post-mortem, a sample of dorsal muscle was taken from a proportion of the fish samples (sample sizes 6 to 15 per sub-set of fish per

population). These, and the macro-invertebrate, zooplankton and phytoplankton samples, were then dried at 60ºC to constant weight before being analysed for their stable isotopes of ^{13}C and ^{15}N at the Cornell Stable Isotope Laboratory (New York, USA) (Section 2.3.1). The initial stable isotope data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰).

3.3.2 Histopathology

Histopathology of the intestinal tract was completed to assess the pathological changes associated with *B. acheilognathi* infection. Sections of intestine were sampled from infected as well as uninfected fish. These sections were fixed in Bouin's fixative for 24 hours before transferring to 70% Industrial Methylated Spirit. The tissues were trimmed, dehydrated in alcohol series, cleared and then embedded in paraffin wax. Transverse and longitudinal sections of 3 µm were cut using a microtome and dried at 50°C. These sections were stained using Mayer's haematoxylin and eosin, and examined microscopically for pathological changes and described accordingly.

3.3.3 Data analyses

Infection levels of *B. acheilognathi* in *C. carpio* were described as their prevalence (number of infected individuals/total number of individuals × 100) and abundance (number of *B. acheilognathi* per host). The mass of parasite was also expressed as a proportion of host weight to represent the parasite burden. The stable isotope data of *C. carpio* were used to assess their trophic niche size and predict their diet composition from the putative food resource data. Trophic niche size was calculated using the metric standard ellipse area (SEA_c) in the SIAR package (Parnell et al.

2010) in R (R Core Development Team, 2013). SEAc is a bivariate measure of the distribution of individuals in trophic space, where each ellipse encloses ~ 40% of the data and thus represents the core dietary niche of species and so indicates their typical resource use (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 limited sample sizes (< 30). For each population of *C. carpio* on each survey date, SEAc was calculated for two sub-sets of individuals: those infected with *B. acheilognathi* and those uninfected, and the extent of the overlap of their niches determined (%).

To then predict the diet composition of each sub-set of fish, their stable isotope data, plus those of their putative food resources, were applied to Bayesian mixing models that estimated the relative contribution of each putative food resource to the diet of each individual *C. carpio* (Moore & Semmens 2008). The models were run using the MixSIAR GUI package in the R computing programme (R Core Development Team 2013; Stock & Semmens 2013). Given that excessive putative food resources can cause mixing models to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species (Phillips et al. 2005). The groups used in the models were arthropods, zooplankton (i.e. samples captured in the net of mesh size 250 μm) and phytoplankton (i.e. samples captured in the net of mesh size 53 μm). Isotopic fractionation factors between resources and consumers in the models were 3.4 ‰ (± 0.98 ‰) for $\delta^{15}N$ and 0.39 ‰ (± 1.3 ‰) for $\delta^{13}C$ (Post 2002). Outputs were the predicted proportion of each resource to host diet (0 to 1).

3.3.4 Statistical analyses

For each fish species and population infected with *B. acheilognathi*, differences between the infected and uninfected hosts were tested using ANOVA for length, and their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$. Condition was calculated as Fulton's Condition Factor (K, $100 \times W/L^3$) where L was measured in cm, with differences between infected and uninfected fishes also tested using ANOVA. Differences between the predicted proportions of each putative food source to the diet of infected and uninfected fish were also tested using ANOVA. Other than the stable isotope mixing models, all analyses were completed in SPSS v. 22.0. In all analyses, the assumptions of normality of residuals and homoscedasticity were checked prior to use. Where error is expressed around the mean, it represents standard error.

3.4 Results

3.4.1 Parasite prevalence and abundance

Across the three sampling periods, parasite prevalence remained relatively constant (61, 58 and 60 % in October 2012, April 2013 and October 2013, respectively; Table 1). Parasite abundance was greatest in October 2012 (mean 10.7 ± 2.3) and lowest in April 2013 (mean 5.4 ± 1.5) (Table 3.1). Parasite abundance was significantly different between October 2012 and April 2013 (ANOVA: $F_{1,45} = 9.38$, $P < 0.01$) but not between April 2013 and October 2013 (ANOVA: $F_{1,45} = 1.22$, $P > 0.05$), and October 2012 and October 2013 (ANOVA: $F_{1,45} = 4.05$, $P > 0.05$). Mean parasite burden was greatest in October 2012 (3.9 ± 0.8 %) and lowest in October 2013 (1.7 ± 0.5 %). There was a significant difference between the parasite burden in October 2012 and October 2013 (ANOVA: $F_{1,45} = 5.85$, $P < 0.05$), but not between October 2012 and April 2013 (ANOVA: $F_{1,45} = 1.92$, $P > 0.05$), and April 2013 and October

2013 (ANOVA: $F_{1,45} = 0.22$, $P > 0.05$) (Table 1). Of other parasites recorded, these were all native species that would be considered as the expected parasite fauna of these fishes in a UK community and were recorded at levels that were considered as not high enough to cause clinical pathology (Hoole et al. 2001).

Table 3.1 Prevalence and abundance of *Bothriocephalus acheilognathi* in *Cyprinus carpio* by sampling date

Date	n	Prevalence (%)	Mean abundance of parasites (± SE)	Range	Mean weight of parasite burden (percentage of hosts weight ± SE)	Range (%)
Oct 12	23	61	10.7 ± 2.3	0 - 35	3.9 ± 0.8	0 - 9.5
Apr 13	24	58	3.4 ± 0.9	0 - 14	2.2 ± 0.9	0 - 19.4
Oct 13	25	60	5.4 ± 1.5	0 - 26	1.7 ± 0.5	0 - 8.8

3.4.2 Histopathology

Histopathological examinations revealed consistent pathological changes associated with *B. acheilognathi* infection. The presence of *B. acheilognathi* within the gut of infected carp was usually evident prior to dissection of the intestine, with the mass of pale tapeworms visible through the distended gut wall (Figure 3.2a). Dissection of the intestinal tract revealed attachment sites of *B. acheilognathi* within the anterior region of the tract with mass of proglottids filling a large proportion of the gut lumen (Figure 3.2b, c). Heavy infections caused near complete occlusion of the intestinal tract. Histopathological observations confirmed thinning and compression of the gut wall with displacement of internal organs, including the swim bladder (Figure 3.2c). During attachment, the scoleces of *B. acheliognathi* engulfed the intestinal folds, leading to marked compression of the epithelium (Figure 3.2d). At the point of attachment, the intestine was severely compressed, with loss of normal gut architecture, loss of epithelium and near exposure of the basement membrane (Figure 3.2e, f). Infection was frequently accompanied by an increase in lymphocytes throughout the epithelium and lamina propria (Figure 3.2e) compared to uninfected fish. In very heavy infections, pressure exerted by the mass of parasites within the intestine caused thinning of the musculature and forced the gut wall against the inside of the body cavity (Figure 3.2f).

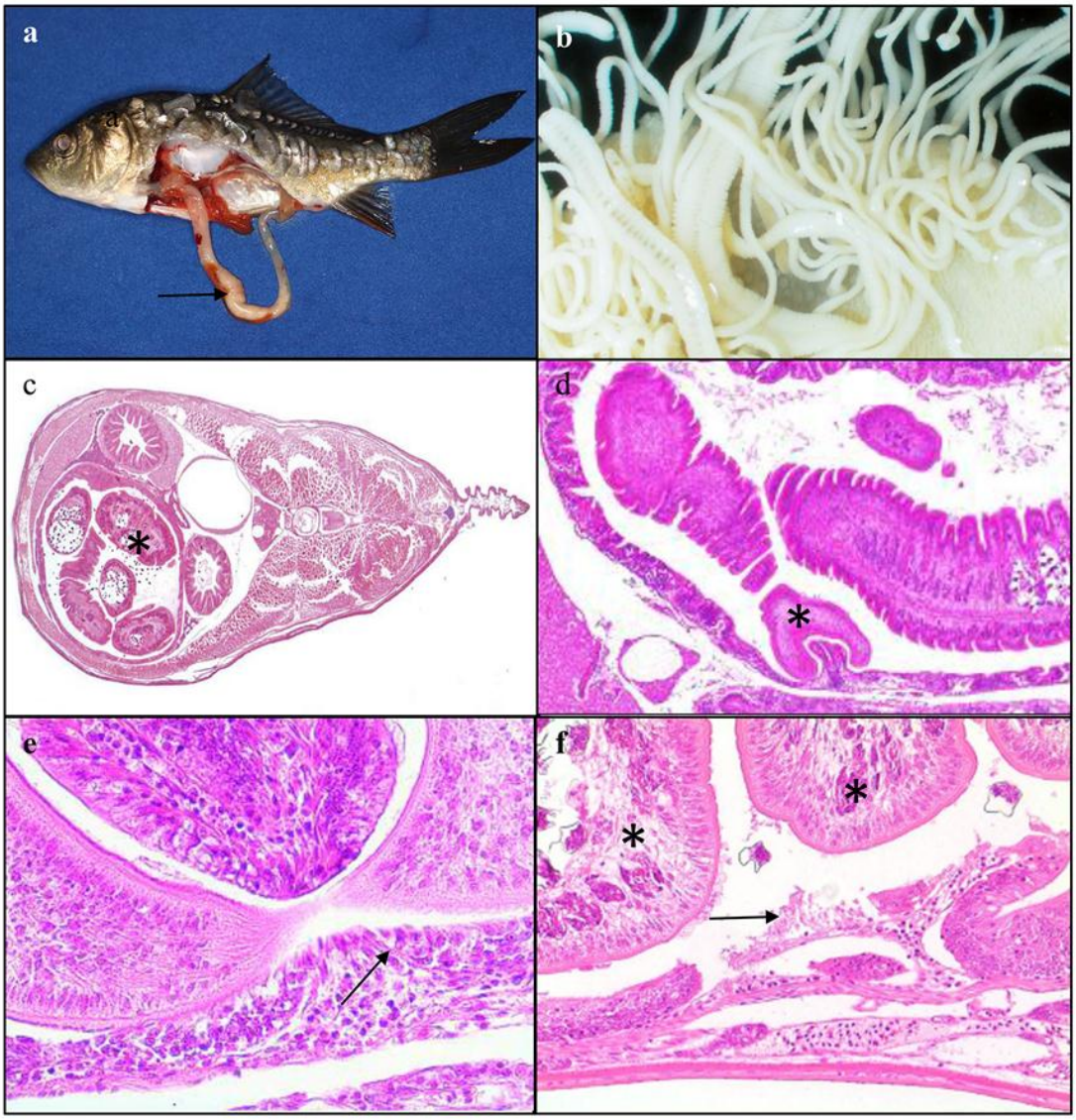

Figure 3.2 Pathology of *Cyprinus carpio* infected with *Bothriocephalus acheilognathi*.

a) *B. acheilognathi* infection in juvenile common carp, with resulting pale distended intestine. b) Attachment of multiple *B. acheilognathi* within the intestine, many with mature proglottids. c) Transverse section through juvenile carp showing B. acheilognathi occupying the anterior intestine (*), with compression of the gut wall and displacement of internal organs, including the swim bladder. d) B. acheilognathi attachment site showing the scolex (*) pinching the gut wall and flattening of normal intestinal folds throughout infected regions of the gut. e) Pronounced compression of epithelium at the apex of scolex attachment, with loss of epithelium, thinning of

musculature and near exposure of the basement membrane (arrow)> Lymphocytes may be seen within the lamina propria f) Flattening of intestinal folds with epithelial erosion (arrow) as a consequence of pressure exerted by the body of tapeworms (*) within the intestine.

3.4.3 Effect of infection on fish length and condition

There was no significant difference in lengths of the uninfected and infected fish sampled in October 2012 and April 2013 (ANOVA: Oct 12: $F_{1,21} = 1.04$, $P > 0.05$; April 13: $F_{1,22} = 2.31$, $P > 0.05$; Fig. 3.3). In October 2013, however, the uninfected fish were significantly larger than infected fish (ANOVA: Oct 13: $F_{1,23} = 14.38$, $P < 0.01$; Figure 3.3). Whilst there were no significant differences in the condition (K) of infected and uninfected *C. carpio* in October 2012 (ANOVA: $F_{1,21} = 0.00$, $P > 0.05$), there was in April 2013 (ANOVA: $F_{1,22} = 11.68$, $P < 0.01$) and this significant difference remained in October 2013 (ANOVA: $F_{1,23} = 6.57$, $P < 0.05$) (Figure 3.4).

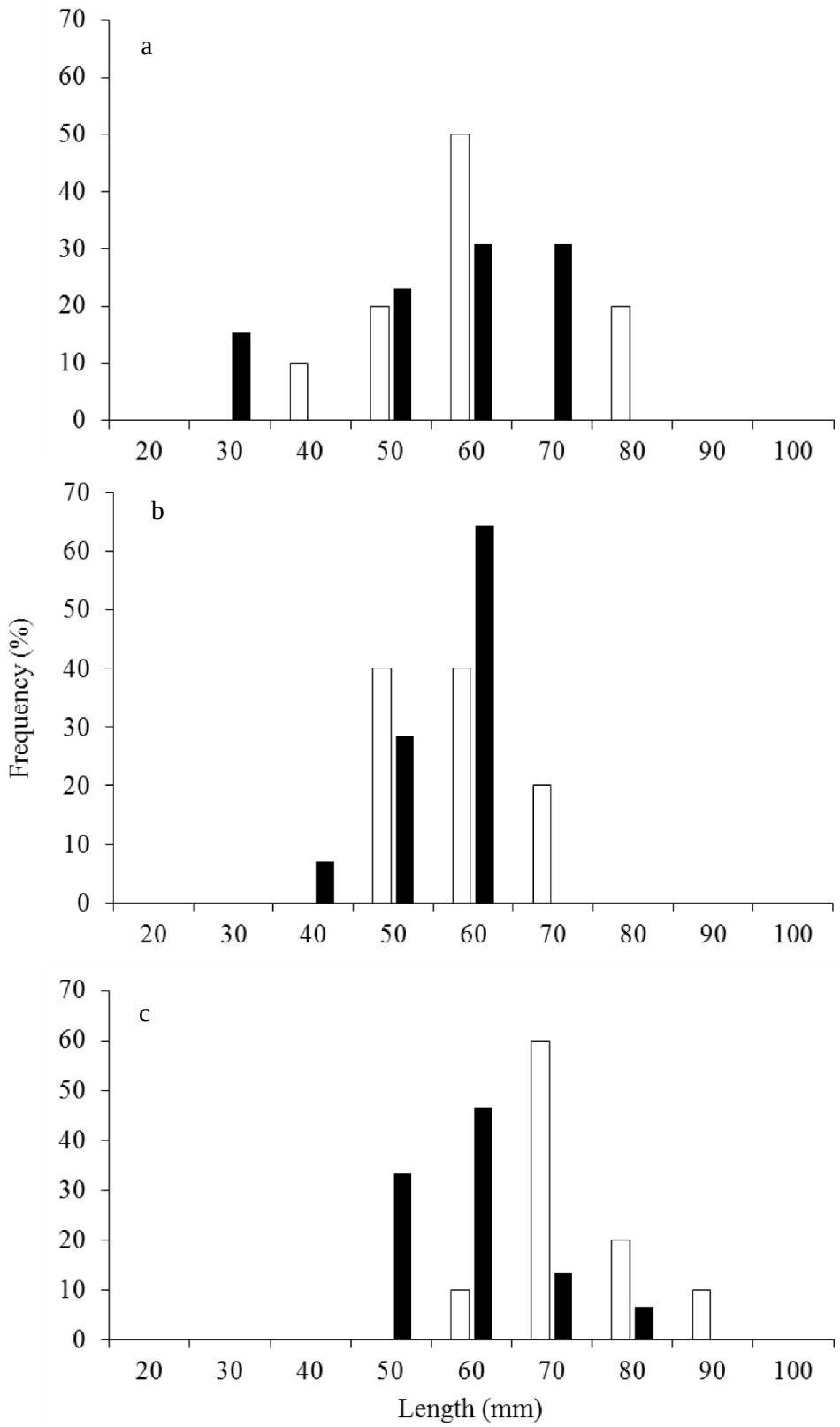

Figure 3.3 Length frequency histograms of infected (black) and uninfected (white) *Cyprinus carpio*, in: (a) October 2012, n = 23; (b) April 2013, n = 24; and (c) October 2013, n = 25.

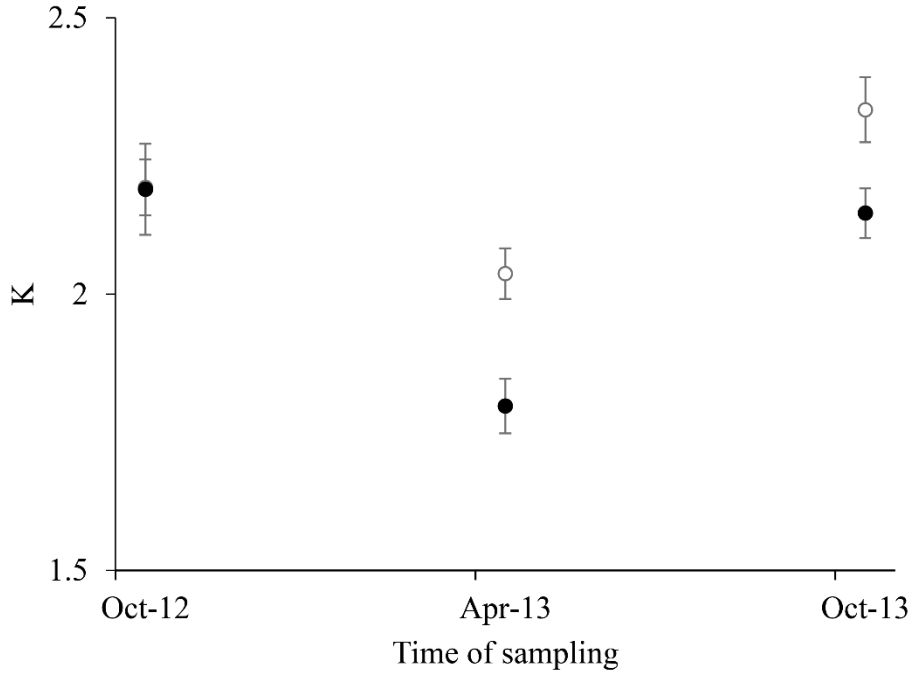

Figure 3.4 Fulton's condition factor (K) of infected (black circles) and uninfected (white circles) *Cyprinus carpio* over the study period. Error bars represent standard error.

3.4.4 Stable isotope metrics

The mean values of $\delta^{13}C$ and $\delta^{15}N$ of the infected and uninfected fish were significantly different in April 2013 (ANOVA $\delta^{13}C$: $F_{1,22}$ =10.62, $P < 0.01$, $\delta^{15}N$: $F_{1,22}$ =10.94, $P < 0.01$) and October 2013 (ANOVA $\delta^{13}C$: $F_{1,23}$ =20.88, $P < 0.01$, $\delta^{15}N$: $F_{1,23}$ =21.77, $P < 0.01$) (Table 3.2). By contrast, in October 2012, only $\delta^{13}C$ was significantly different between the groups (ANOVA $\delta^{13}C$: $F_{1,21}$ =13.83, $P < 0.01$, $\delta^{15}N$: $F_{1,21}$ = 3.39, $P > 0.05$) (Figure 4). In all cases where differences between the isotopes of the groups were significant, the infected fish had enriched $\delta^{15}N$ and depleted $\delta^{13}C$.

Table 3.2 Sample size, mean lengths of sub-sampled fish and mean stable isotope data.

Date	Species	n	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
	Uninfected *C. carpio*	9	66.1 ± 3.32	-32.31 ± 0.59	17.79 ± 1.19
Oct-12	Infected *C. carpio*	14	58.7 ± 4.57	-33.14 ± 0.48	18.60 ± 0.90
	Uninfected *C. carpio*	6	64.6 ± 1.92	-32.44 ± 0.67	18.00 ± 1.11
Apr-13	Infected *C. carpio*	10	60.4 ± 1.91	-33.69 ± 0.78	19.61 ± 0.84
	Arthropoda	11		-33.65 ± 1.39	13.42 ± 0.37
	Plankton < 250µm	3		-36.54 ± 0.76	18.68 ± 1.24
	Plankton > 250µm	3		-30.63 ± 1.25	17.42 ± 0.47
	Uninfected *C. carpio*	9	78.7 ± 2.84	-32.07 ± 0.94	17.93 ± 1.31
Oct-13	Infected *C. carpio*	14	64.67 ± 2.35	-34.03 ± 1.12	20.02 ± 0.93
	Arthropoda	8		-34.33 ± 0.99	10.13 ± 0.41
	Plankton < 250µm	2		-36.37 ± 0.15	19.38 ± 0.74
	Plankton > 250µm	2		-30.09 ± 0.97	17.16 ± 1.16

The outputs of the mixing models predicting the diet composition of the uninfected and infected fish revealed some significant differences between the two groups (Table 3). In both April and October 2013, infected fish were predicted to have a significantly higher proportion of plankton less than 250 μm in their diet compared with uninfected fish (mean 41 ± 6% in April and 57 ± 2% in October; ANOVA April: $F_{1,22}$ = 863.33, P < 0.01, October: $F_{1,23}$ =372.70, P < 0.01). Arthropoda were predicted to comprise a significantly higher proportion of the diets of uninfected fish on both sampling dates (mean 50 ± 4% in April and 32 ± 3% in October; ANOVA April: $F_{1,22}$ = 874.04, P < 0.01, October: $F_{1,23}$ = 173.33, P < 0.01). Plankton greater than 250 μm made up a smaller proportion of the diet of uninfected fish than infected fish in April (29 ± 4% vs 33± 6%; ANOVA $F_{1,22}$ = 143.43, P < 0.01) and a larger proportion in October (45± 2% vs 24± 2%; ANOVA $F_{1,23}$ = 448.76, P < 0.01) (Table 3.3).

Table 3.3 Summary of the Bayesian mixing models outputs predicting the proportions of each major food item to the diet of infected and uninfected fish on each sample occasion, and the F value from ANOVA, where **P < 0.01. Values of the predicted proportions represent their mean and standard error. Sample sizes as Table 3.2

		Modelled diet proportion (± SE)		
Date	Food item	Uninfected	Infected	F
Apr-13	Arthropoda	0.50 ± 0.04	0.26 ± 0.04	874.0**
	Plankton < 250µm	0.21 ± 0.03	0.41 ± 0.06	863.3**
	Plankton > 250µm	0.29 ± 0.04	0.33 ± 0.06	143.4**
Oct-13	Arthropoda	0.32 ± 0.03	0.18 ± 0.02	173.3**
	Plankton < 250µm	0.23 ± 0.03	0.57 ± 0.02	372.7**
	Plankton > 250µm	0.45 ± 0.02	0.24 ± 0.02	448.7**

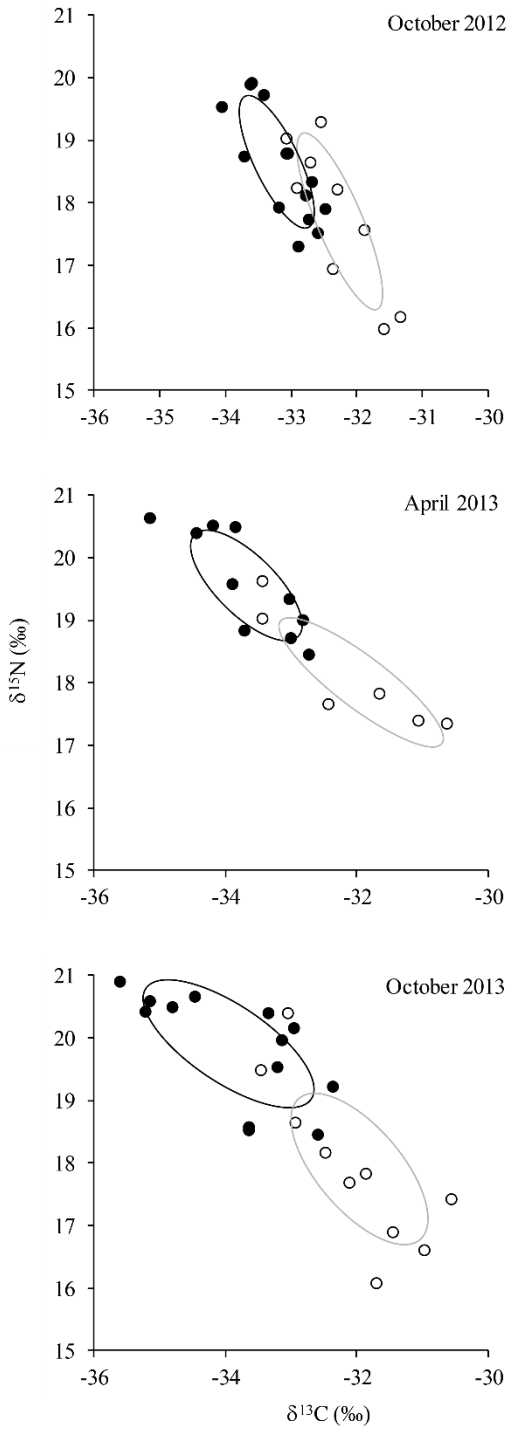

Figure 3.5 Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Cyprinus carpio* sampled in a) October 2012, b) April 2013 and c) October 2013. The black circles mark the infected individuals and the black line the SEAc of infected individuals. The white circles represent data from uninfected individuals and the grey line represents the SEAc of uninfected individuals.

3.5 Discussion

Sampling of the juvenile fish over the 12 month period revealed that infection by *B. acheilognathi* resulted in the development of long-term pathological and ecological consequences. Although the hosts sampled at the end of their first summer revealed little difference in lengths and condition compared with their uninfected conspecifics, the outputs of stable isotope analysis revealed they already had a significantly different diet composition. The condition of infected fish was significantly reduced after their first winter and by the end of their second summer, they were significantly smaller than uninfected fish and remained in significantly reduced condition. The diet of these two sub-sets of fish also remained significantly different over this time.

Other studies on *B. acheilognathi* have also suggested that infection causes a range of foraging consequences for hosts, including impairment of their ability to capture prey (Scott & Grizzle 1979; Britton et al. 2011; Britton et al. 2012; Scholz et al. 2012). The shift towards foraging on less motile, more easily available food sources by hosts has also been observed in other parasitized populations. For example, the freshwater amphipod *Gammarus roeseli* infected with the acanthocephalan *Polymorphus minutus* (as an intermediate host) consumed equivalent numbers of dead isopods as uninfected conspecifics, but fewer live isopods (Medoc et al. 2011). In stickleback *Gasterosteus aculeatus*, parasitism by the cestode *Schistocephalus solidus* tends to lead to selection of smaller prey items (Barber et al. 1995). Shifts in host feeding behaviours arise through a variety of mechanisms; for example, parasites utilise energy reserves of their hosts, infection may increase metabolic costs or be associated with increases in energetically demanding immune functions

(Barber et al. 2000). Hosts infected with strongly debilitating parasites may also exhibit reduced activity levels that impact foraging behaviours (Britton et al. 2011; Britton 2013). Thus, infection consequences frequently manifest as changes in energy budgets expenditure and, subsequently, appetite, foraging and diet composition (Barber et al. 2000). Moreover, in fish populations, the frequency distribution of phenotypic trait values often follows a normal distribution, reflecting genotypic differences and environmental noise, but parasitic infection can shift the mean value of traits, increasing their variance at the population level (Poulin & Thomas 1999). This was apparent in the *C. carpio* of this study where the increase in the trophic niche size of the host population was related to it comprising two, almost discrete niches that corresponded with uninfected and infected carp.

Over the study period, temporal changes were also detected in parasite burden. These tended to reduce over time, despite being sufficient to incur pathological and ecological consequences. Although this reduction might relate to the mortality of hosts with high parasite abundances, seasonal shifts in aspects of fish parasite infections are often apparent in temperate regions due to its influence on the behaviours, habitat utilisation and immune responses of potential hosts (Bromage et al. 2001; Bowden et al. 2007). Given these can vary between host species then parasite prevalence and abundance can show considerable variability across species within communities. For example, in reservoirs in North Carolina, USA, *B. acheilognathi* abundance was highest in fathead minnow *Pimephales promelas* and red shiner *Notropis lutrensis* in autumn, whereas it was highest in winter in mosquito fish *Gambusia affinis* (Riggs et al. 1987). For parasites whose transmission to final hosts is through trophic links, the phenology of intermediate hosts is also important,

with seasonal changes in copepod communities identified as a driver of the different infection levels of *B. acheilognathi* observed in fish host communities (Riggs et al. 1987). Temporal and spatial changes in definitive host infection level that result from varying transmission success due to shifts in the dynamics of intermediate host populations have also been recorded across a range of fishes and their parasites (Amundsen et al. 2003; Jiménez-Garcia & Vidal-Martínez 2005).

The divergence in the lengths of the infected and uninfected fish that developed over time has the potential to restrict host fitness, as in most fish species, maturation is associated with size and thus faster growing individuals will mature earlier in life (Scott 1962; Bagenal 1969; Ali & Wootton 1999). Furthermore, larger fish are more fecund, and thus contribute more to the population (Hislop 1988; Beldade et al. 2012;). Whilst a reduction in growth associated with parasitism has been recorded in a variety of species, such as the rainbow smelt *Osmerus mordax* infected by protocephalid parasites (Sirois & Dodson 2000), and farmed and wild salmonids infected with sea lice (e.g. *Lepeophtheirus salmonis*) (Costello 2006), it is not the universal response to parasitism (Loot et al. 2001). Indeed, rapid growth aligned with parasitic castration in hosts is the response recorded in other cestode parasites, such as *Ligula intestinalis* (Thompson & Kavaliers 1994; Loot et al. 2001) and *Schistocephalus solidus* (Arnott et al. 2000; Barber et al. 2000).

In summary, significant differences in the condition and body lengths of infected and uninfected populations developed over the course of the study, with histopathology revealing substantial local damage in the intestine of hosts. Analyses then revealed the diet composition of the infected fish was predicted to comprise of a significantly

higher proportion of smaller items (< 53 μm) than uninfected fish. Thus, it was demonstrated that in this cohort of juvenile *C. carpio*, sub-lethal impacts of parasitism included substantial histopathological consequences that resulted in significant growth and trophic impacts whose development could have been overlooked had the temporal context of the study been lacking. It is thus especially important to investigate the temporal influence of parasitism in any evaluation of potential parasite impacts on trophic niche and condition of the host. These outputs also suggest some modifications to food webs infected with *B.acheilognathi*, as their hosts forage on different prey taxa (Chapters 5 and 6).

4. Head morphology and piscivory of European eels, *Anguilla anguilla*, predict their probability of infection by the invasive parasite parasitic nematode *Anguillicoloides crassus*

This chapter is based on the published article which is presented in Appendix 6:

Pegg, J., Andreou, D., Williams, C. F. and Britton, J. R., 2015, Head morphology and piscivory of European eels, *Anguilla anguilla*, predict their probability of infection by the invasive parasitic nematode *Anguillicoloides crassus*. *Freshwater Biology*, 60: 1977–1987.

4.1 Abstract

The morphology of animal body structures influences their function; intra-population plasticity in diet composition can occur where head morphology limits gape size. The European eel, *Anguilla anguilla*, a critically endangered catadromous fish, shows significant intra-population variations in head width, with broader-headed individuals being more piscivorous. Infection of eels during their freshwater phase by *Anguillicoloides crassus*, an invasive nematode parasite, involves paratenic fish hosts. Here, the relationship between their infection status and head functional morphology (as head width/total length ratio; HW:TL) was tested across three populations and the proportion of fish in diet (estimated by stable isotope mixing models) across three populations.

In all populations the extent of piscivory in the diets of individual eels increased significantly as their HW:TL ratios increased. There were no significant differences between infected and uninfected eels in their total lengths and hepatic-somatic indices. However, the HW:TL ratios of infected eels were significantly higher than those of uninfected eels and, correspondingly, their diet comprised a higher proportion of fish. Logistic regression revealed head morphology and diet were significant predictors of infection status, with models correctly assigning up to 78 % of eels to their infection status. Thus, eel head functional morphology significantly influenced their probability of being infected by invasive *A. crassus*, most likely through increased exposure to fish paratenic hosts. Accordingly, the detrimental consequences of infections are likely to be focused on those individuals in freshwater populations whose functional morphology enables greater specialisation in piscivory.

4.2 Introduction

Phenotypic differences in morphology, physiology and behaviour are frequently observed between parasitized and non-parasitized individuals (Lafferty 1999; Krist 2000; Miura et al. 2006). Although often considered in the context of parasite-induced changes to the host post-infection (Blanchet et al. 2009), some traits increase the susceptibility of individuals to infection, resulting in a small number of hosts harbouring the majority of parasites (Viljoen et al. 2011). These traits include host body size, where increased size favours the development of larger parasite loads (Lindenfors et al. 2007); social behaviours, where increased social interactions increase parasite transmission (Viljoen et al. 2011); and sex, as oestrogens can stimulate immunity whereas testosterone can act as an immuno-suppressant (Folstad and Karter 1992), so that males often have higher parasite loads (Schalk and Forbes 1997; Moore and Wilson 2002). Functional traits that enable the development of specialized feeding behaviours in individuals can also increase the risk of infection by trophically transmitted parasites through increased exposure to intermediate hosts (Bolnick et al. 2003). For example, different feeding specializations of individuals within Arctic charr (*Salvelinus alpinus*) populations result in aggregations of helminth parasites in those individuals that persistently forage on the pelagic copepods that act as intermediate hosts (Knudsen et al.2004).

Paratenic hosts can play important roles in the transmission of trophically transmitted parasites (Ewald 1995; Galaktionov 1996), as they increase parasite fitness and ensure that larvae that would otherwise be ‘lost’ in unsuitable hosts are recovered (Morand et al. 1995). They can assist transmission when obligate intermediate hosts are not represented strongly in the diet of final hosts (Medoc et al.

2011; Benesh et al. 2014; Moehl et al. 2009), and thus facilitate parasite transfer along food chains and across trophic levels (Marcogliese 2007). For example, *Alaria* trematode parasites, whose obligate amphibian intermediate hosts are rarely consumed by their canine final host, also have mammalian and bird paratenic hosts that substantially increase their transmission rates (Moehl et al. 2009). Paratenic hosts also increase the time over which potential hosts are vulnerable to infection. For example, because the obligate intermediate hosts of *Bothriocephalus barbatus* and *Bothriocephalus gregarious* are copepods, their flatfish final hosts are vulnerable to infection during their planktonic juvenile stages (Robert et al. 1988). However, as *B. gregarious* also has a gobiid fish paratenic host, the predaceous adult stages of potential hosts continue to be exposed to the parasite, resulting in higher prevalence rates than for *B. barbatus* (Robert et al. 1988; Morand et al. 1995).

The nematode parasite *Anguillicoloides crassus* was introduced from Asia into Europe in the 1980s, where it infects the freshwater lifestages of the European eel, *A. anguilla*, (Kirk 2003), now a critically-endangered species (Jacoby and Gollock 2014). A number of factors have been suggested as contributing to the decline of European eel populations, including *A. crassus* infections as these affect swim-bladder function (Lefebvre et al. 2013). This parasite has a complex life cycle; in the native range, infection of Japanese eel is via ingestion of crustacean intermediate hosts (Nagasawa at al. 1994), but in Europe a wide range of species, primarily fishes, also act as paratenic hosts (Szekely 1994; Kennedy 2007). Although not evident in the native range (Thomas and Ollevier 1992), studies suggest that the consumption of paratenic fish hosts has contributed to increased transmission rates and prevalence

in *A. anguilla* (Szekely 1994; Sures and Streit 2001; Kirk 2003; Knopf and Mahnke 2004).

Within populations, *A. anguilla* exhibits considerable variation in head width, with 'broad-headed individuals' and 'narrow-headed individuals' (Lammens and Visser 1989; Proman and Reynolds 2000; Tesch 1977; Tesch 2003), although a recent study suggests that there is continuous morphological variation rather than a dichotomy (Cucherousset et al. 2011). As with other species where head morphology limits energy acquisition (Smith and Skulason 1996; Bulte, Irschick and Blouin-Demers 2008), these differences in head morphology have been related to individual specialisation, with broader-headed *A. anguilla* individuals being more piscivorous (Cucherousset et al. 2011). This chapter investigated how *A. anguilla* head morphology, diet and trophic ecology influence the infection status and parasite load with *A. crassus* over three river populations. It was predicted that variation in the functional head morphology of *A. anguilla* leads to significant differences in individual diet composition and trophic niche, significantly influencing the probability of infection by *A. crassus* in broader-headed individuals through their increased parasite exposure via fish paratenic hosts.

4.3 Methods

4.3.1 Sample collection and initial data collection

The three study sites were all lowland rivers in England where *A. anguilla* was known to be infected with *A. crassus*, and the eel population was abundant and thus destructive sampling would not be detrimental to their status. The sites were the River Huntspill (Site 1; 8 to 12 m width, maximum depth 3 m; Lat: 51.198440N Long: 2.993181W), the St. Ives Chub stream (Site 2; 4 to 8 m width, maximum depth 1.5 m; 52.331143N Long: 0.061219E), and a side channel of the River Frome (Site 3; 4 to 8 m width, maximum depth 1.5 m; Lat: 50.679668N Long: 2.181917W).

Figure 4.1 River Huntspill study site: a typical section showing the river's uniform channel.

Figure 4.2 The survey site on the St Ives chub stream.

Figure 4.3 The study section of the River Frome (Photograph by Phil Williams).

Sampling was completed in August 2013 (Sites 1 and 2) and August 2014 (Site 3), and methods were dependent on site characteristics. At Site 1, a series of fyke nets (6.5mm mesh, 50cm D front hoop, 3m leader) was placed across the width of the river and all captured eels removed after 24 hours. At Sites 2 and 3, sampling was by electric fishing, using a back-mounted Smith-Root LR-24 Backpack (50 MHz pulsed DC at approximately 2 Amps). At all sites, silver eels (sexually mature, pre-spawning eels) were returned without processing. Yellow eels were retained in water-filled containers and a maximum of 24 individuals were selected randomly and taken back to the laboratory for processing. This sample size avoided removal from small river populations of excessive numbers of a critically endangered apex predator. Samples of putative food items were also collected from each site, including samples of small prey fishes (*Phoxinus phoxinus*, *Cottus gobio* and *Gymnocephalus cernua*, presence dependent on site, maximum 10 individuals per species) and macro-invertebrates, collected using a combination of electric fishing, kick-sampling with a hand net of 6 mm mesh and a 40 m micro-mesh seine net. Triplicate samples were taken of each macro-invertebrate species where possible. Thus, these samples comprised either a single individual (fish) or were pooled samples of single species (macro-invertebrates; n = 5 to 20 individuals per sample).

In the laboratory, all fish were euthanized through an anaesthetic overdose (MS-222), with weight, total length and head width of the eels measured (Cucherousset et al. 2011). A detailed post-mortem was then conducted on the eels and other fishes using a standard protocol (Hoole et al. 2001; Appendix 1) to detect infections by native and non-native parasites. Skin scrapes and internal organs were examined with the aid of low and high power microscopy to enable parasite identification. Eel

swim bladders were removed and the numbers of male, female and juvenile *A. crassus* counted. As *A. crassus* exhibits marked sexual dimorphism, with females at least 10 times larger than males and it is the female parasites that primarily cause the gross pathological damage of the swim bladder (Figure 4.4; Lefebvre et al. 2013), only counts of the large, female nematodes were used in subsequent analyses as the measure of parasite abundance. These female parasites were also the dominant form of *A. crassus* encountered in the swim bladders. In addition, as the lifecycle of the parasite is relatively short (a few months) compared with the duration of the freshwater life phase of eels (minimum 3 years), then the absence of *A. crassus* at post-mortem does not preclude that an eel has been repeatedly infected and severely affected in the past. Consequently, uninfected eels were identified by both an absence of *A. crassus* in combination with a swimbladder wall of transparent-yellowish colouration (i.e. undamaged, indicating no previous infection), as per Lefebvre et al. (2002). The liver was also removed and weighed, and a sample of dorsal muscle taken for stable isotope analysis. The muscle samples, along with samples from other fishes and the putative food resources, were then oven dried at 60ºC until they achieved constant weight, before processing and analysis at the Cornell Isotope Laboratory New York, USA. Note that due to financial constraints, only 60 of the 86 eels were analysed. The initial stable isotope data were in the format of delta (δ) isotope ratios expressed per mille (‰).

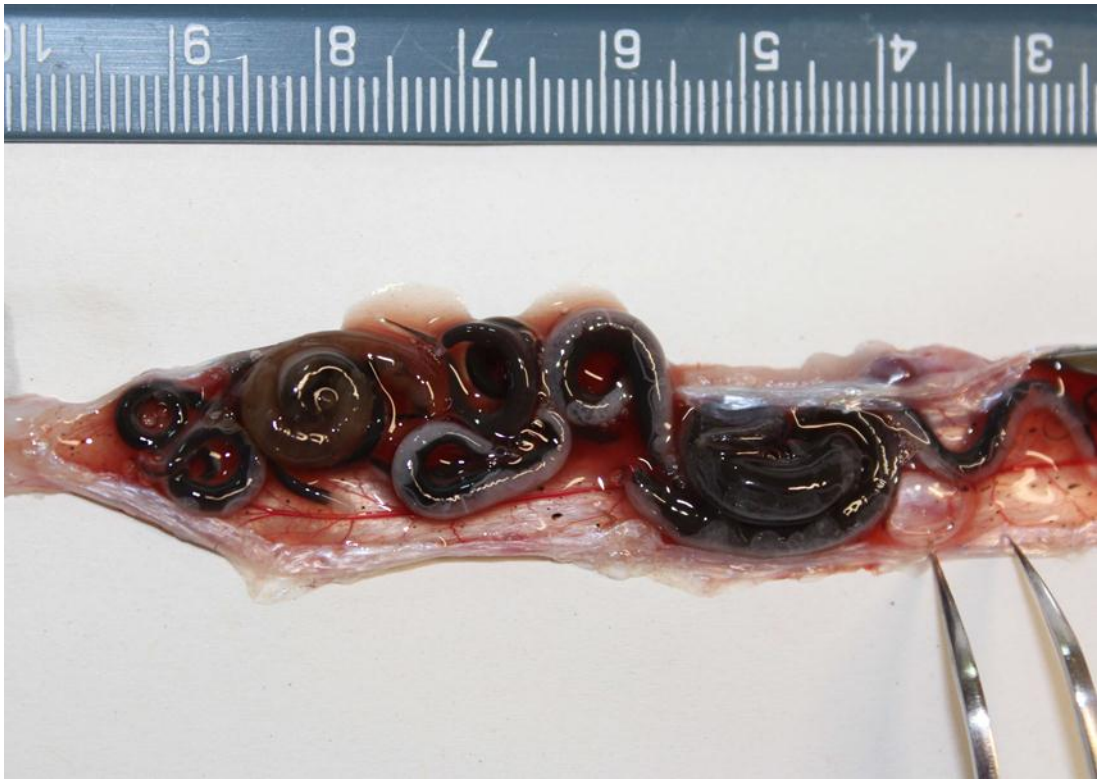

Figure 4.4 Adult female *Anguillicoides crassus* in a swim bladder. The white patches on the parasite's body are gonads. (Photograph by Chris Williams, Environment Agency).

4.3.2 Data analysis

Infection levels of *A. crassus* in *A. anguilla* were described as their prevalence (number of infected individuals/total number of female *A. crassus* x 100) and abundance (number of mature female *A. crassus* per eel). Hereafter, where an *A. anguilla* individual is referred to as either infected or non-infected, it refers to the presence/ absence of *A. crassus* in that individual during the post-mortem. Ratios of head width to total length (HW:TL) in the *A. anguilla* populations were determined (Proman and Reynolds 2000), and were used as a morphological index (Cucherousset et al. 2011). To standardise HW:TL ratios across the sites, their values within each site were expressed as their standardized residual values from their

population mean. Hepato-somatic index (HSI), a measure of energy storage, was then calculated for each individual *A. anguilla* using the formula: HSI = liver weight (g)/ total bodyweight (g). Note this could not be completed for *A. anguilla* from Site 3.

Anguilla anguilla diet composition and trophic niche size was investigated at each site using the stable isotope data. Diet composition was assessed using Bayesian mixing models that estimated the relative contribution of each putative food resource to the diet of each individual *A. anguilla* per site (Moore and Semmens 2008). The models were run using the MixSIAR GUI package in the R computing programme (Stock and Semmens 2013; R Development Core Team 2013). Given that excessive putative food resources can cause mixing models to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species, as per Phillips et al. (2005). Accordingly, models at each site always included 'prey fishes'. At Site 1, they also included one macro-invertebrate group, 'Arthropoda' (*Gammarus pulex*, Hydropsychidae and Simuliidae spp.). At Site 2, differences in stable isotope data within the Arthropoda enabled inclusion of two groups in the mixing model (1: *Gammarus pulex* and *Asellus aquaticus*, 2: other Arthropoda), and at Site 3, two groups of Arthropoda (as Site 2), plus Lymnaea sp. Isotopic fractionation factors between resources and consumers in the models were 3.4 ‰ (± 0.98 ‰) for $\delta^{15}N$ and 0.39 ‰ (± 1.3 ‰) for $\delta^{13}C$ (Post 2002). Outputs were the predicted proportion of each resource to eel diet (0 to 1), with the predicted proportion of fish used as a measure of the extent of piscivory in each individual *A. anguilla*. The stable isotope data were then used to calculate the standard ellipse area (SEA_c) for the infected and

uninfected eels at each site using the SIAR package (Parnell et al. 2010) in the R computing program (R Development Core Team 2013) (as per Section 2.3.3).

4.3.3 Statistical analysis

Differences in $\delta^{13}C$ and $\delta^{15}N$ between infected and uninfected *A. anguilla* at each site were tested using generalized linear models (GLM); the stable isotope data were dependent variables and infection status was the independent variable. The effect of total *A. anguilla* length was included in initial models but removed if its effect was not significant. In subsequent analyses, as the data used were standard for all sites, they were combined and used in linear mixed models. 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 *A. anguilla* were used as true replicates, models were fitted with site as a random effect on the intercept. Thus, the model testing for difference in *A. anguilla* weight according to *A. crassus* infection used weight as the dependent variable, infection status as the independent variable, site as the random effect and total length as the covariate (Garcia-Berthou 2001). The significance of the difference in weight between the groups was determined by pairwise comparisons of estimated marginal means, adjusted for multiple comparisons (Bonferroni). Differences in hepatic-somatic index, mean HW:TL ratios, total lengths and the extent of piscivory in diet between infected and uninfected *A. anguilla* were then tested using the same model structure, but without length as a covariate. Finally, the effect of HW:TL ratios on the extent of piscivory in eel diet was tested across the sites using linear regression.

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 data of each individual eel on their (i) HW:TL ratio, and (ii) estimated proportion of fish in their diet, using equation 1: $e^{(a+bx)}/ 1+e^{(a+bx)}$, where *a* and *b* were the regression coefficients, and x either HW:TL ratio or proportion of fish in diet. A final PoI model used both HW:TL ratios and estimated proportion of fish in their diet (D) in equation 2: $e^{(a+b\text{HW:TL}+cD)} / 1+ e^{(a+b\text{HW:TL}+cD)}$, where *a*, *b* and *c* were the regression coefficients. Predicted group membership and its probability (infected or uninfected) were stored as model outputs, with differences in probabilities tested between groups using Mann Whitney U tests. Predicted group membership was compared with the actual data set and expressed as the proportion that were correctly assigned.

The relationships of parasite abundance (as number of mature female *A. crassus*) with total length, body mass, hepatic-somatic index, HW:TL ratios and extent of piscivory were then tested in two ways. Firstly, the abundances were grouped by the number of mature female parasites present in the swim bladder, where low = 1 to 3 parasites, medium = 4 to 6 and high > 7. These groups were then used in linear mixed models using the same model structures as already described for infected and uninfected eels. The abundance data were then used as the continuous variable in multiple regression, where total length, body mass, hepatic-somatic index, HW:TL ratios and extent of piscivory were used as explanatory variables. Outputs were assessed according to the values of the standardised β coefficients (higher values indicate a greater contribution to the variance of the data) and the significance of the explanatory variables.

Other than the stable isotope mixing models, all analyses were completed in SPSS v. 21.0. In all analyses, the assumptions of normality of residuals and homoscedasticity were checked, and response variables were log-transformed to meet the assumption if necessary.

4.4 Results

Across the three *A. anguilla* populations, prevalence of *A. crassus* ranged between 58 and 70 % per population, with abundance between 1 and 13 mature female parasites per infected individual (Table 4.1). Of the 86 eels sampled across all the sites, 54 were infected with *A. crassus* (63 %). Nine native parasites were also recorded on the eels across the sites, all at minor levels of infection, and thus were considered inconsequential (Hoole et al. 2001). *Gymnocephalus cernua* was recorded as a paratenic host of *A. crassus* at Sites 1 and 2. The application of stable isotope mixing models to the stable isotope data (Table 4.2) revealed a significant increase in the proportion of fish in diet as HW:TL ratio increased ($R^2 = 0.28$, $F_{1,58} = 4.82$, $P = 0.03$; Figure 4.4).

Table 4.1 Prevalence and abundance of *Anguillicoloides crassus* in the *Anguilla anguilla* populations

Site	n	Prevalence (%)	Mean abundance of female parasites (± SE)	Range
1	30	70	2.61 ± 0.52	0 - 8
2	30	63	2.05 ±0.54	0 - 5
3	26	58	2.66 ± 0.70	0 - 13

Table 4.2 Sample sizes and mean total lengths, and $\delta^{13}C$ and $\delta^{15}N$, of infected and uninfected *Anguilla anguilla* at each site, plus the mean $\delta^{13}C$ and $\delta^{15}N$ values of their putative food resources used in mixing models. Error around the mean is standard error.

Site	Species	n	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
1	Infected *A. anguilla*	9	467 ± 73	-31.14 ± 0.29	21.48 ± 0.23
	Uninfected *A. anguilla*	9	460 ± 81	-32.28 ± 0.36	20.54 ± 0.74
	Prey fishes			-32.33 ± 0.10	22.72 ± 0.66
	Arthropoda			-30.66 ± 0.18	19.88 ± 0.22
2	Infected *A. anguilla*	10	422 ± 143	-29.22 ± 0.16	21.00 ± 0.28
	Uninfected *A. anguilla*	9	433 ± 152	-30.27 ± 0.41	20.68 ± 0.21
	Prey fishes			-29.93 ± 0.30	20.00 ± 0.45
	Arthropoda 1			-31.61 ± 0.43	14.94 ± 0.14
	Arthropoda 2			-31.62 ± 0.13	16.33 ± 0.17
3	Infected *A. anguilla*	9	363 ± 86	-30.18 ± 0.53	13.65 ± 0.20
	Uninfected *A. anguilla*	14	321 ± 102	-29.48 ± 0.28	13.06 ± 0.08
	Prey fish			-30.53 ± 0.31	12.30 ± 0.24
	Arthropoda 1			-32.44 ± 0.09	8.34 ± 0.18
	Arthropoda 2			-29.92 ± 0.33	8.72 ± 0.23
	Lymnaea			-21.96 ± 0.11	7.73 ± 0.01

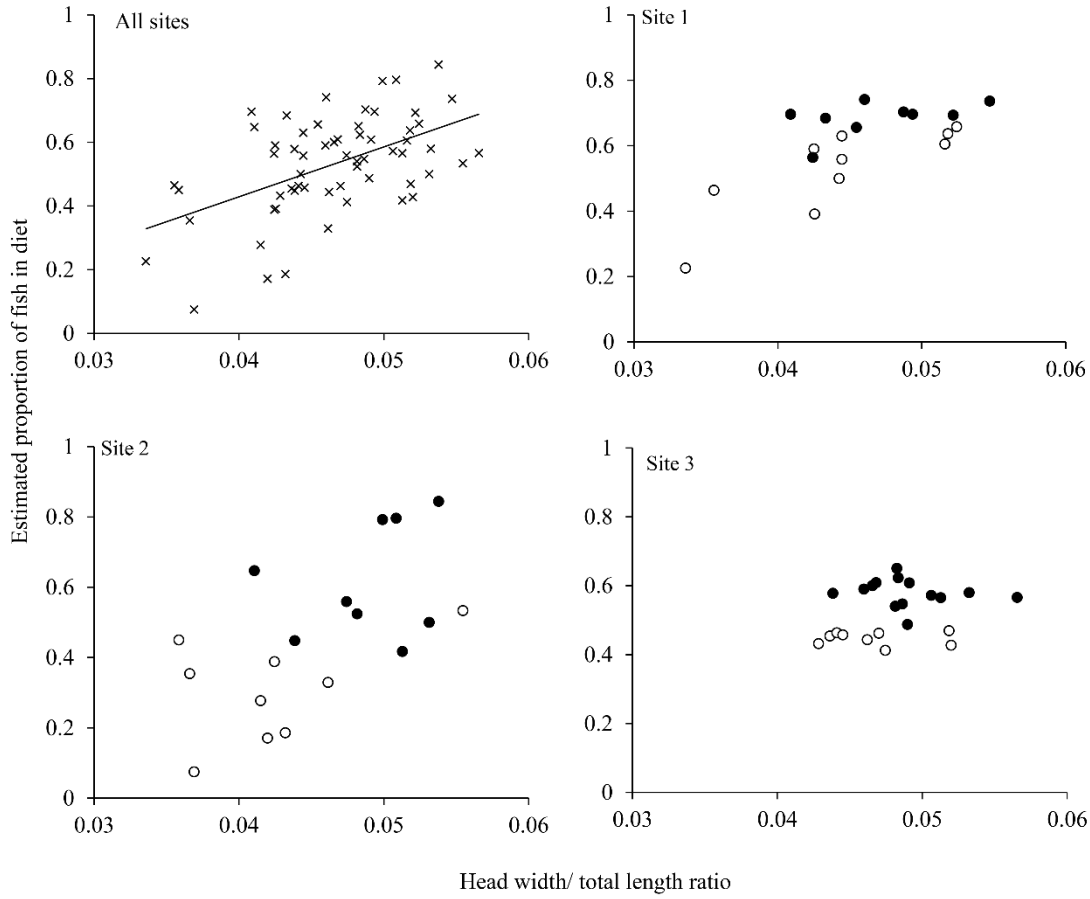

Figure 4.5 Relationship between head width and total length (HW:TL) ratio and estimated extent of piscivory in the diet of *Anguilla anguilla* in all sites (×), where the solid line represents the significant relationship between the variables according to linear regression, and for Sites 1 to 3 according to their infection status by *Anguillicoloides crassus* (infected: ●; uninfected: ○).

Differences in the stable isotope values for infected and uninfected *A. anguilla* were significant for $\delta^{13}C$ from Sites 1 and 2 (GLM: Site 1: Wald χ^2 = 6.84, mean difference 1.14 ± 0.30 ‰, $P < 0.01$; Site 2: Wald χ^2 = 6.13, mean difference 1.05 ± 0.42 ‰, $P < 0.01$) and for $\delta^{15}N$ from Site 3 (GLM: Wald χ^2 = 8.49, mean difference 0.59 ± 0.21 ‰, $P < 0.01$) (Table 4.2; Fig. 4.5). Across all sites, infected eels had significantly larger HW:TL ratios and higher estimated proportions of fish in their

diet compared with uninfected eels ($P < 0.01$; Table 4.3, 4.4; Fig. 4.5). There were, however, no significant differences between infected and uninfected eels in their total lengths, body mass and hepatic somatic index ($P > 0.05$; Table 4.4). Trophic niche size, as SEAc, was higher in infected *A. anguilla* than uninfected *A. anguilla* from Site 1 (3.11 vs. 2.61 ‰^{2}) and 3 (3.10 vs. 1.10 ‰^{2}), with the converse for Site 2 (2.65 vs. 1.63 ‰^{2}). The amount of overlap in the trophic niches of the uninfected and infected *A. anguilla* was relatively low, with infected *A. anguilla* sharing 34.8, 15.4 and 9.2 % of trophic niche space with uninfected *A. anguilla* in Sites 1, 2 and 3 respectively (Fig. 4.6).

Table 4.3 Mean head width/ total length ratios (HW:TL) and mean proportion of fish in the diet of *Anguilla anguilla* uninfected and infected with *Anguillicoloides crassus* in the three study sites. Error around the mean is standard error.

Site	*A. anguilla* infection status	HW:TL	Proportion of fish in diet
1	Uninfected	0.042 ± 0.002	0.31 ± 0.05
	Infected	0.049 ± 0.001	0.61 ± 0.06
2	Uninfected	0.044 ± 0.002	0.53 ± 0.04
	Infected	0.048 ± 0.001	0.69 ± 0.02
3	Uninfected	0.046 ± 0.001	0.45 ± 0.01
	Infected	0.049 ± 0.001	0.58 ± 0.01

Table 4.4 Outputs of linear mixed models testing the significance of (a) *Anguilla anguilla* total length, (b) *A. anguilla* body mass, (c) hepatic-somatic index (HSI), (d) standardised ratio of head width to total length, and (e) extent of piscivory in diet on the infection status of *A. anguilla* from three populations. Site was the random effect on the y intercept.

(a) Infection status ~ total length: AIC = 721.0; log likelihood = 717.0	
Pairwise comparison	Mean difference (estimated marginal means)
Infected vs. uninfected	11.2 ± 28.1 mm, P > 0.05
(b) Infection status ~ body mass: AIC = 634.7; log likelihood = 630.7	
Pairwise comparison	Mean difference (estimated marginal means)
Infected vs. uninfected	1.5 ± 13.1 g, P > 0.05
(c) Infection status ~ HSI: AIC = -138.6; log likelihood = -142.6	
Pairwise comparison	Mean difference (estimated marginal means)
Infected vs. uninfected	0.01 ± 0.01, P > 0.05
(d) Model: Infection status ~ HW:TL: AIC = -447.1; log likelihood = -451.1	
Pairwise comparison	Mean difference (estimated marginal means)
Infected vs. uninfected	0.002 ± 0.001, P = 0.003
(e) Model: Infection status ~ Extent of piscivory: AIC = -57.8; log likelihood = -61.8.	
Pairwise comparison	Mean difference (estimated marginal means)
Infected vs. uninfected	0.18 ± 0.04, P < 0.001

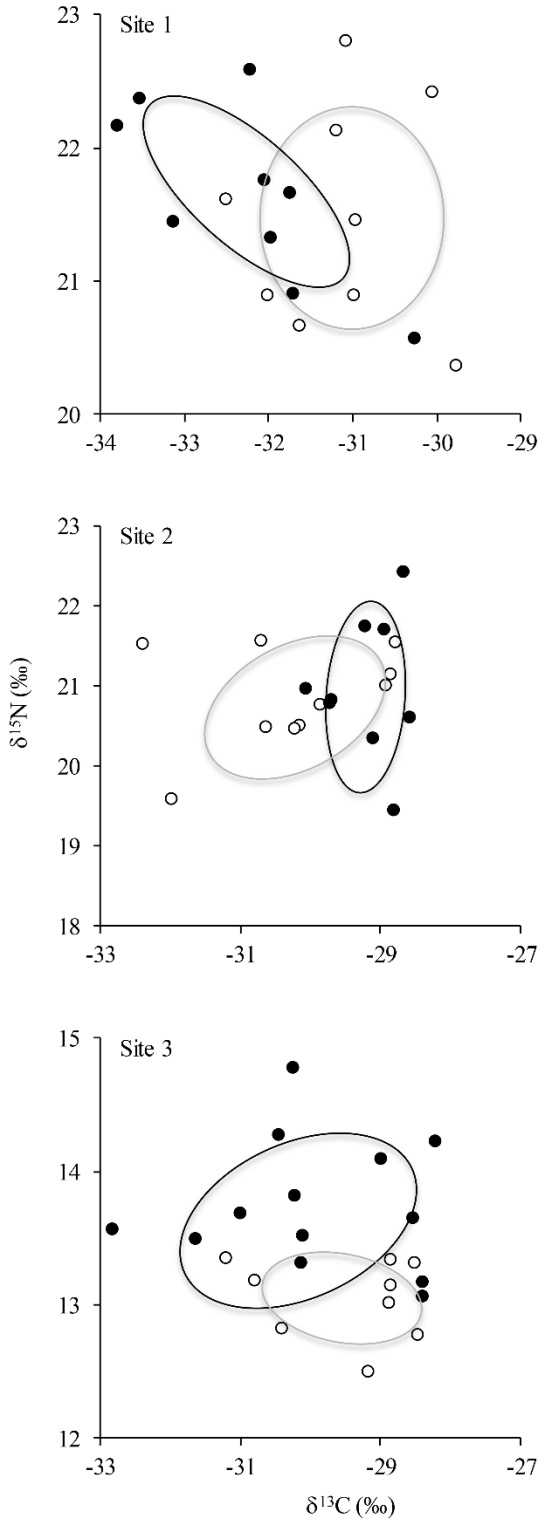

Figure 4.6 Stable isotope bi-plots of infected (●) and uninfected *Anguilla anguilla* (○) at each site. Black ellipses represent the trophic niche size (as standard ellipse area) of infected eels and grey ellipses represent those of uninfected eel. Note different X and Y axes values for the sites.

The binary logistic regression models were all significant, revealing both HW:TL ratios and the extent of piscivory had significant effects on *A. crassus* infection (Table 4.5). Comparison of predicted group membership revealed that HW:TL ratio correctly assigned 72 % of *A. anguilla* to their observed infection status, HW:TL ratio and extent of piscivory correctly assigned 76 %, and extent of piscivory 78 %. In the latter model, the difference in the mean probability of infection between uninfected and infected *A. anguilla* was significant (uninfected: 0.34 ± 0.05; infected: 0.71 ± 0.04; Mann Whitney U test $Z = -4.72$, $P < 0.01$) (Table 4.5).

Table 4.5 Binary logistic regression coefficients (Equation 1) and their statistical significance for the probability of infection of *Anguilla anguilla* by *Anguillicoloides crassus* according to (a) ratio of head width to total length (HW:TL), (b) predicted proportion of fish in *A. anguilla* diet and (c) both variables.

(a)

Parameter	Symbol in equation 1	Coefficient	Standard error	*P*
Constant	a	0.15	0.28	0.58
HW:TL	x	176.10	7.37	<0.01

(b)

Parameter	Symbol in equation 1	Coefficient	Standard error	*P*
Constant	a	-8.49	2.47	<0.01
Diet	x	18.61	.33	<0.01

(c)

Parameter	Symbol in equation 2	Coefficient	Standard error	*P*
Constant	a	-8.50	2.60	<0.01
HW:TL	b	169.85	81.57	0.03
Diet	c	18.57	5.54	<0.01

The linear mixed models testing the significance of differences in biometrics according to light, medium and heavy *A. crassus* infections across the 32 infected *A. anguilla* revealed some significant differences in lengths between these groups (Table 4.6). However, there were no significant differences in HW:TL ratios, extent of piscivory in diet, hepatic-somatic index and weight (Table 6), where the effect of length as a covariate was significant in the latter model ($P < 0.01$). When these variables were used in a multiple regression with parasite abundance used as a continuous variable, the overall model was not significant ($R^2 = 0.17$; $F_{4,27} = 1.19$, $P > 0.05$), and none of the variables had significant effects on parasite abundance ($P > 0.05$ in all cases). Total length had the highest standardised β coefficient ($\beta = 0.39$, $P > 0.05$)

Table 4.6 Outputs of linear mixed models testing the significance of *Anguillicoloides crassus* abundance (low, medium, heavy infections) on (a) total length, (b) body mass, (c) hepatic-somatic index (HSI), (d) standardised ratios of head width to total length and (e) extent of piscivory. Site was the random effect on the y intercept.

(a) Parasite abundance ~ total length: AIC = 355.5; log likelihood = 351.5, $P = 0.01$	
Pairwise comparison	Mean difference (estimated marginal means)
Low/ medium	121.9 ± 37.7 mm, $P = 0.01$
Low/ high	87.8 ± 45.6 mm, $P > 0.05$
Medium/ high	34.0 ± 46.0 mm, $P > 0.05$
(b) Parasite abundance ~ body mass: AIC = 315.2; log likelihood = 311.2, $P > 0.05$	
Pairwise comparison	Mean difference (estimated marginal means)
Low/ medium	15.3 ± 21.1 g, $P > 0.05$
Low/ high	7.9 ± 23.5 g, $P > 0.05$
Medium/ high	7.4 ± 22.0 g, $P > 0.05$
(c) Parasite abundance ~ HSI: AIC = -102.9; log likelihood = -106.9, $P > 0.05$	
Pairwise comparison	Mean difference (estimated marginal means)
Low/ medium	0.01 ± 0.01, $P > 0.05$
Low/ high	0.01 ± 0.01, $P > 0.05$
Medium/ high	0.01 ± 0.01, P > 0.05

(Cont.)

(d) Model: Parasite abundance ~ HW:TL: AIC = -229.0; log likelihood = -233.0, $P > 0.05$	
Pairwise comparison	Mean difference (estimated marginal means)
Low/ medium	0.01 ± 0.01, $P > 0.05$
Low/ high	0.01 ± 0.01, $P > 0.05$
Medium/ high	0.01 ± 0.01, $P > 0.05$
(e) Model: Parasite abundance ~ piscivory: AIC = -59.89; log likelihood = -63.86; $P > 0.05$	
Pairwise comparison	Mean difference (estimated marginal means)
Low/ medium	0.03 ± 0.03, $P > 0.05$
Low/ high	0.03 ± 0.03, $P > 0.05$
Medium/ high	0.06 ± 0.04, $P > 0.05$

4.5 Discussion

Anguilla anguilla head morphology is related to intra-population diet specialisation whereby broader-headed fish are more piscivorous (Cucherousset et al. 2011). Consequently, that head width: total length ratios were significantly higher in eel infected by *A. crassus* in the three populations suggests this was associated with their increased piscivory. This then infers that the consumption of paratenic fish hosts by *A. anguilla* was important for *A. crassus* transmission in these populations. This inference was also supported by the outputs of the stable isotope mixing models. Whilst these indicated that all of the eels were facultative piscivores, individuals with higher estimated proportions of fish in their diet had greater probabilities of

being infected with *A. crassus*. Thus, both head width: total length ratios and the estimated proportion of fish in diet were significant predictors of infection status, with up to 78 % of eels correctly assigned by the models.

The trophic fractionation between the eels and their prey fishes was often low and highly variable, but generally below the 3.4 ‰ $\delta^{15}N$ that would be expected had their diet been based entirely on fish, i.e. one trophic level (Grey 2006). This variability in fractionation was then reflected in the predictions from the mixing models of the proportions of fish in the diet of individual eels, where the mean for all eels was 0.53 (± 0.02 SE) and range 0.08 to 0.84. It should be noted that the mixing models provided estimates of diet composition based on standard isotopic fractionation factors and given that mixing models are sensitive to the fractionation factors used (Phillips et al. 2014) then these might have influenced their outputs. Had species-specific fractionation factors been available then some absolute differences in the dietary proportions might have resulted (Bond and Diamond 2011; Phillips et al. 2014). Whilst this suggests some uncertainty in the extent of the actual differences in piscivory between the infected and infected eels, it remains that broader headed eels tend to be more piscivorous (e.g. Cucherousset et al. 2011) and the study outputs revealed that the probability of infection increased significantly as head width increased, irrespective of diet predictions. An alternative approach to providing robust estimates of the extent of piscivory in *A. anguilla* diet would have been stomach contents analysis, although this was not feasible with the low *A. anguilla* sample numbers available. Indeed, the sample sizes used per population in the study were relatively low compared with other recent studies on *A. crassus* (e.g. Lefebvre et al. 2013), but this was unavoidable given the endangered status of eel populations

generally allied with the sampled populations being from small rivers. Consequently, although the study outputs were unambiguous across the sites with consistent infection patterns apparent, the use of small sample sizes and the diet estimates being derived from mixing models does introduce some inherent uncertainties in the overall output.

The recent study in Southern France of Lefebvre et al. (2013) revealed that *A. anguilla* with severe swim bladder damage due to *A. crassus* infections had greater body lengths and mass compared to non-infected individuals of the same age. The authors postulated that their findings were most likely due to the most active foragers growing faster and having a greater probability of becoming repeatedly infected via trophic-transmission and with infection having a low energetic burden. Here, the research did not reveal a similar significant difference in body length and mass between infected and non-infected individuals, or any effect of parasite abundance on biometrics, although the mean infection levels we recorded (< 3.0) were lower than those (4.1 ± 4.4) reported by Lefebvre et al. (2013). Whilst it cannot be discounted this being a potential effect of a smaller sample size used here, these findings are consistent with other studies (Koops and Hartmann 1989; Wuertz et al. 1998). Irrespective, it can be argued argue that these outputs provide empirical support for the interpretations of Lefebvre et al. (2013). However, rather that the most active foragers are most vulnerable to the parasite, as the more piscivorous individuals that are repeatedly exposed to the parasite, most likely via increased consumption of paratenic fish hosts, facilitated by their head functional morphology. It is speculated that the consequent greater energetic intake associated with piscivory would then facilitate the faster growth rates observed by Lefebvre et al. (2013).

Notwithstanding these significant relationships between functional morphology, diet and *A. crassus* infections, it is acknowledged that the extent of piscivory of individual *A. anguilla* at the time of infection could not be determined. Consequently, it cannot definitively be concluded that infection was a causal consequence of head functional morphology. Moreover, in some fishes, parasitism causes shifts in feeding behaviour and trophic position through mechanical processes and/ or changes in energy demand (Barber et al. 2000; Britton et al. 2011), and can induce changes in habitat utilisation that can influence foraging behaviours (Blanchet et al. 2009; Britton et al. 2009). Thus, it cannot be discounted that the shift to piscivory in *A. anguilla* occurred post-infection. However, this scenario was considered unlikely, as *A. anguilla* head morphology is a well-recognised functional trait known to enable greater individual specialisation in piscivory (Proman and Reynolds 2000; Cucherousset et al. 2011), and was documented in their populations prior to the introduction of *A. crassus* into Europe (Moriarty 1974; Tesch 1977). In addition, the development of the trait of 'broad-headedness' is apparent throughout the life of individual eels (from glass eel to maturity; Proman and Reynolds 2000) and thus is unlikely to be a parasite-induced trait (Decharleroy et al. 1990; Moravec et al. 1994). As such, it is proposed that the higher extent of piscivory that was apparent through this functional morphology in infected *A. anguilla* at the time of sampling was most likely a causal factor in their infection, with their increased consumption of fish paratenic hosts at least partially responsible. However, we also recognise that other factors, such as individual differences in MHC genes and differences in cytokine regulation, might have also influenced the host qualities of these eels, so that vulnerability to *A. crassus* infection is likely to depend on more

complex factors than diet and functional morphology alone (Knopf and Lucius 2008).

Several studies of *A. crassus* in *A. anguilla* have suggested that body size is a strong predictor of infection, with larger *A. anguilla* having higher levels of prevalence and abundance than smaller *A. anguilla* (Barus and Prokes 1996; Schabuss et al. 2005; Lefebvre et al. 2013). In German populations, however, there was no correlation between infection status and *A. anguilla* length and weight (Wuertz et al. 1998), as with here. Overall, it is suggested that body length and mass are relatively crude metrics to test against *A. crassus* infection, as *A. anguilla* growth rates in their freshwater life-stage can be extremely variable (e.g. 14 to 152 mm per year (Aprahamian 2000)), and the duration of the freshwater lifestage can be as low as 3 to 5 years (Camargue Lagoon, France; Melia et al. 2006) and as high as 33 to 57 years (Burrishole, Ireland; Poole and Reynolds 1996). Therefore, assessing infection levels using a metric that is subject to such variability over time and space might be limited in its utility for understanding infection dynamics. We suggest that measurements that incorporate head functional morphology are a more appropriate metric due to its influence on diet composition and the apparent importance of paratenic hosts in *A. crassus* transmission.

Whilst the actual role of *A. crassus* in the decline of *A. anguilla* populations remains unclear, the pathology associated with infections has been related to increased freshwater mortality in populations exposed to additional environmental stressors (Kirk 2003). Additionally, the damage to the swim bladder severely impacts on swimming performance (Palstra et al. 2007), and can thus potentially disrupt

spawning migrations (Barry et al. 2014; Pelster 2015). Thus, in conclusion, it is suggested these consequences of parasitism in *A. anguilla* are focused on those individuals in populations whose functional morphology enables greater specialisation in piscivory, through a mechanism of greater parasite exposure via higher consumption of paratenic fish hosts. It also means that the effect of the parasite, whilst potentially important for food web topology (Chapter 5) is less likely to result in food web alterations when weighting is applied. Thus *A.crassus* is only assessed in food web topology (Chapter 5) and is not considered thereafter.

5. Consequences of non-native parasites for topological food webs

5.1 Abstract

Infectious food webs (food webs where parasites are included) tend to have distinct properties from those where parasites are excluded, having increased chain length, linkage density, nestedness and connectedness. Parasite inclusion in topological food webs has highlighted that parasites are integral to the structuring and functioning of ecosystems. However, how non-native parasites alter food web topology and metrics remains uncertain. Here, topological food webs were built for each focal non-native parasite to test their influence on food web structure and metrics. The metrics used were food chain length, connectance and nestedness, the latter two being measures of the web stability and robustness. At all sites, food web connectance was greatest in the free-living species web, and chain length was highest in the fully infected web. Two main factors were identified as important in determining the extent of alteration when the addition of a non-native parasite to a topological web was completed: the complexity of the extant food web and the complexity of the lifecycle of the non-native parasite. When a non-native parasite with a complex lifecycle was added to a complex web (*Anguillicoides crassus*), it had less effect on food web connectivity and nestedness than when than a complex non-native parasite was added to a simpler extant food web (*Bothriocephalus acheilognathi*). Thus, whilst the consequences of non-native parasites for food web topology and associated metrics appeared context dependent, all had less effect on food web topology than the addition of the native parasite fauna.

5. 2 Introduction

5.2.1 Topological food webs and parasites

Food webs represent ecological communities via networks of trophic relationships, and the structure and complexity of these networks influence community dynamics and stability (Bascompte et al. 2003; Dunne et al. 2005). Analysis of food webs can be used to investigate ecosystem changes and address general ecological questions. For example, food-web analyses of species additions and deletions can be used to understand the impact of invasions and extinctions (Dunne et al. 2002a; Petchey et al. 2008a). In particular, species introductions - in addition to increasing species richness - can alter food-web topology because a new species might act as a consumer of, or a new resource for, existing species, or provide the critical resource needed for other consumers to invade the web (Amundsen et al. 2013).

The case for including parasites in food webs has been well established in recent years (Lafferty et al. 2006b; Marcogliese 2007; Hatcher and Dunn 2011; Hatcher et al. 2012). The inclusion of parasites in topological food webs affects network structure (Amundsen et al. 2003; Hudson et al. 2006; Lafferty et al. 2006a; Lafferty et al. 2006b; Hernandez and Sukhdeo 2008; Lafferty 2008; Amundsen et al. 2009; Amundsen et al. 2013), increases food-web complexity (Hudson et al. 2006) and alters ecosystem stability (Dobson et al. 2006; Wood et al. 2007). Thus, it has been realized through these studies that including parasites in food webs, i.e. building infectious food webs, is fundamental to understanding food web structure and energy flux. For example, along the Pacific coast of North America, the invasive Japanese mud snail *Batillaria cumingi* has competitively excluded the native mud snail *Cerithidea californica* (Torchin et al. 2005). This replacement would appear to have

minimal consequences for the topology of the food-web as one species is being replaced directly with another with similar functional traits. However, once parasites are considered then the topology of the food web is altered substantially, as *B. cumingi* is host to only one trematode parasite whilst *C. californica* hosted eleven (Lafferty and Kuris 2009) Thus, this loss of 10 species from the food web has repercussions reflected in a range of food web metrics, including reduced complexity, robustness and connectedness which occurred with the arrival of the invasive snail.

When introduced species do not extirpate native species then parasite diversity could increase as for every introduced free-living species, two parasite species are, on average, also introduced (Torchin et al. 2003). Direct empirical evidence for shifts in food web topology arising from the introduction of free living species with their parasites is provided by invasive fishes in the pelagic food web of Lake Takvatn, Norway (Amundsen et al. 2013). Introductions into this subarctic lake of Arctic charr *Salvelinus alpinus* and three-spined stickleback, and their co-introduced parasites, strongly altered the pelagic food web structure through increasing species richness from 39 to 50 species (the two fishes plus nine parasites). This increased the number of nodes and trophic links in the topological food web, the food-chain length and the total number of trophic levels in the food web (Amundsen et al. 2013). Food web complexity also increased, as revealed through increased linkage density, degree distribution, vulnerability to natural enemies, and nestedness, all of which may have consequences for network functioning and stability (Dunne et al. 2002a; Hatcher and Dunn 2011; Amundsen et al. 2013).

When parasites are co-introduced with their free-living hosts, substantial alterations in the structure of the qualitative food web can thus result, highlighting the importance of accounting for native and introduced hosts and parasites in food-web studies (Britton 2013). Furthermore, these changes in structure result not simply from increases in diversity and complexity when parasites are included, but are instead attributable to the unique roles that parasites play in food webs (Dunne et al. 2013). In their roles as resources, parasites have close physical intimacy with their hosts, and thus are concomitant resources for the same predators. In their roles as consumers, they can have complex life cycles and inverse consumer–resource body-size ratios, different from many free-living consumers (Dunne et al. 2013). These unique roles of parasites in food webs result in differing patterns of connection compared to free-living species in the case of their roles as resources, and differences in the breadth and contiguity of trophic niches between parasites and free-living species in the case of their roles as consumers (Dunne et al. 2013).

Nevertheless, there remains a lack of studies examining how non-native parasites affect food web topology in relation to different parasite lifecycles and assessing how the additive effect of firstly native parasites and then the non-native parasite modify food web structure. It is this that is being addressed here.

5.2.2 Food web metrics to measure ecological parameters

Food webs have long been used to visualise and describe ecological communities through analysis of their networks. A number of metrics, of which some of the most important and widely used are described below, describe aspects of food web topology that can be calculated in order to explore the relationship of community

properties. These methods complement more conventional dynamical modelling, experimental and comparative approaches that are traditionally used to explore questions in stability-diversity and species richness-ecosystem function research (Dunne et al. 2002a). Consequently, the utility of food web models is not just their visual representation of food web structure but also their ability to determine food web metrics that allow comparison between the food web in the presence or absence of certain species. Note, however, that differences in values will not be associated with a significance value; instead they are designed to reveal the scale of modification through their numerical output. Theoretical work has demonstrated how these measures relate to community stability properties such as robustness and vulnerability to extinction and/ or invasion (Hatcher and Dunn 2011). The most useful metrics are used in this chapter and are described below:

Food chain length. Food-chain length is an important food web property as it affects a variety of ecosystem functions, such as primary and secondary production, rates and stability of material cycling, and persistence of higher-order predators under human-exploitation (Post 2002b). Food chain length indicates the number of times chemical energy is transformed from a consumer's diet into a consumer's biomass along the food chains that lead to the species. Maximum food chain length is the maximum number of links between basal resources and top predator species (Hatcher and Dunn 2011), whereas characteristic chain length is the mean chain length for the web (Dunne et al. 2002a). Mean chain length is the metric used here. As a general rule, parasites tend to considerably increase food chain length (Thompson et al. 2012) with, for example, the addition of parasite species increasing the maximum chain length (or height) of the food webs of the Ythan Estuary, Wales,

from 9 to 10, and for Loch Leven, Scotland, from 4 to 5, with parallels increase in mean chain length (Huxham and Raffaelli 1995).

Connectance. Connectance of a food web (also called web density) is the percentage of the possible links that are realized, i.e. it is the ratio of observed links to the total number of possible links. Traditionally for a web of F species, the possible links comprise a matrix of size F^2 (Martinez 1991; Warren 1994). Here, however, the modification developed by (Lafferty et al. 2006b) is used that was specifically designed for parasitized webs. In this modified version, connectance (C) is calculated as $C = Lo/[(F + P)^2]$, where Lo is number of observed links, F the number of free-living species, and P the number of parasites. Including parasite species in a food web increases both the numerator and the denominator, i.e. number of observed and possible links (Lafferty et al. 2006b), however both need not change the same amount. For example the addition of a single parasite species with multiple hosts, would increase the numerator more than it would increase the denominator, thus connectance is a valuable metric as altered not just by the addition of parasite numbers but by the properties of those added species. A full description of connectance in the context of parasites is provided in lafferty at al (2006b). Overall inclusion of parasites tends to increase connectance (Lafferty et al. 2006b), for example, analysis of seven food webs with and without parasites revealed that including parasites always increased connectance (Dunne et al. 2013).

Nestedness. Nestedness, also termed clustering coefficient when referring to webs in general, describes an aspect of how links are organised in a network. In a perfectly nested network, each species interacts with a strict subset of other species in order of

increasing generality. Nestedness has implications for community robustness (Hatcher and Dunn 2011) and is a relative measure of the cohesiveness of a network. This pattern of interactions occurs because both generalists (species with many interactions in the network) and specialists (species with few interactions in the network) tend to interact with generalists, whereas specialist-to-specialist interactions are rare (Bascompte et al. 2003). If perturbed, a highly nested community is predicted to recover because species are less likely to be isolated after the loss of other species (Bascompte et al. 2003). Previous studies have produced conflicting results when considering the addition of parasites in food webs. For example, relative nestedness increased in the Carpinteria salt marsh food web (USA) with the addition of parasites (Lafferty et al. 2006b), whilst adding parasites decreased nestedness in the food web of Muskingum Brook, New Jersey, USA (Hernandez and Sukhdeo 2008).

5.2.3 Aims and objectives

The aim of the chapter was thus to determine how the inclusion of native and introduced non-native macro-parasites modifies food web topology and associated metrics. The objectives were to:

(i) assess the extent of topological food web modification caused by parasites by analysing food web topology under three states: (1) free-living species only; (2) free-living species and their native macro-parasites; and (3) free-living species, their native macro-parasites and the non-native parasite. This objective was completed for each non-native parasite, i.e. *E. briani, A. crassus* and *B. acheilognathi* within a modelled environment; and

(ii) determine how parasite life-cycle (i.e. direct or complex) affects food-web topology, irrespective of its native or non-native status. This objective was completed using a simplified, theoretical food web.

5. 3 Materials and methods

5.3.1 Modelling the topological food web: data used to build food web

The basis of the food webs was data on the fish community and their parasite fauna. These data were derived as per Chapters 2, 3 and 4. One series of food web models was constructed per non-native parasite species, using one of each study sites as the modelled environment. For the latter, the site selected was considered the most representative of the parasite's invaded habitat (subjective of the author) and where the most information was available on the food web components. Consequently, the sites were:

- *Ergasilus briani*, Site 1: Basingstoke canal (Section 2.3, Figure 2.1);
- *Bothriocephalus acheilognathi*, Site 2: Greater London fishery (Section 3.3, Figure 3.1)
- *Anguillicoides crassus*, Site 3: River Huntspill (Section 4.3, Figure 4.1).

It was then necessary to include parasites for species at lower trophic levels than fish in order to provide a more comprehensive infectious food web model. However, logistical constraints had prevented the detailed analysis of the parasites of macro-invertebrates from field samples. Consequently, a heuristic approach was adopted for parasites of macro-invertebrates, a common approach for topological food web

studies (Srinivasan et al. 2007; Petchey et al. 2008b; Amundsen et al. 2013). Data on the parasite fauna of the macro-invertebrate fauna were collated from a combination of literature review and from the Natural History Museum Host Parasite Database (Gibson et al. 2005). The actual macro-invertebrate species included in each food web were, however, determined from field survey data as described in Sections 2.3, 3.3 and 4.3, with supplementary data also provided by the Environment Agency at Sites 1 and 3. For the trophically transmitted parasites that were detected in a fish species, their known intermediate and final (e.g. bird or mammal) host species were included in the food web model irrespective of their detection in field samples, on the assumption that their absence in samples was a false-negative recording due to their requirement for completion of the parasite lifecycle (Cooper and Cooper 2008).

To avoid the construction of highly complex food webs involving substantial aquatic: terrestrial links then logical limits were placed on the models that constrained them to each focal aquatic system per non-native parasite species. This meant that birds and mammals were the end point of the aquatic food web and did not continue by including the terrestrial links associated with these species. This is standard convention in building topological food webs for aquatic systems and enables them to be of manageable size and of relevance to the ecological question(s) they address (Polis et al. 1997; Trebilcol et al. 2013).

The parasite fauna of fish and macro-invertebrates was recorded from field data. Additionally samples were collected in the field of phyto- and zooplankton (Sections 2.3, 3.3, 4.3), however data on species identifications were often relatively limited and not necessarily representative of species present on a seasonal basis. Where data were limited then functional groups of taxa were used instead and which shared the

same set of predators and prey within a food web. Again, this heuristic approach is a widely accepted convention in structural food-web studies that aims to reduce methodological biases related to uneven resolution of taxa within and among food webs (Dunne et al. 2002a). Full lists of species/functional species for each network are available in Appendix 2.

Following collation of all of the species (or functional groups) of the piscivorous birds and mammals, fish, macro-invertebrates, zooplankton and phytoplankton, and their parasites, their feeding relationships were determined. For those involving the fish species, these were constructed through analysis of their stomach contents and the outputs of mixing models in stable isotope analysis (Section 2.4, 3.4, 4.4). For the other species being modelled, their feeding relationships were derived heuristically from literature reviews based on their typical diet composition. This latter method is again the standard methodology used to reconstruct trophic relationships in similar food web studies (Amundsen et al. 2013).

5.3.2 Preparing data for modelling

Following collation of the species lists to be modelled and derivation of their feeding relationships, these data were then prepared for inputting into the food web models. This involved the construction of a binary matrix (completed in MS Excel 2010), where the relationship between each species included in the food web model was recorded as 0 (no feeding interaction) or 1 (feeding interaction). The direction of that relationship (i.e. which was the predator and which was the prey) was determined by their direction within the matrix, whereby the x-axis of the matrix listed all the species as predators and the y-axis of the matrix listed all the species as prey/

producers. Thus, in Figure 5.1, Species A is a producer, species B predates A only, species C predates both species A and B and is cannibalistic. Species D predates species B and C. Species D may be a free-living predator or a parasite, as both would be represented in the same way. The food web matrices used for model construction are provided in Appendix 3.

	A	B	C	D
A	0	0	0	0
B	1	0	0	0
C	1	1	1	0
D	0	1	1	0

Figure 5.1 Example of the structure of a network matrix as used in this study, where 0 represents no feeding interaction and 1 represents a feeding interaction.

On their completion in MS Excel, the matrices were then transferred into R using the package gdata (Warnes et al. 2015). This package comprises of various tools for data manipulation, including the transformation of Excel spreadsheets into R readable formats.

5.3.3 Food web modelling using igraph

Following conversion into R of the matrices being used as the basis of the food web models, they were then converted into food webs (networks) using the network analysis package igraph (Csardi and Nepusz 2006). This is an open source software package that is used to create, manipulate and analyse the properties of graphs and networks. It has the capability of specifying whole graph properties as well as those

of individual nodes (here, the species in the food web) and links (here, their feeding relationships). These properties represented the food web metrics outlined in Section 5.2, i.e. connectedness, nestedness and food chain length.

5.3.4 Model finalisation

The parameterised food web models that were constructed in igraph, as outlined above, were only considered as final (i.e. complete) when the tests revealed they had small-world properties (Montoya and Sole 2002). This 'small world' attribute refers to a food web that has many loosely connected nodes, non-random dense clustering of a few nodes (i.e. keystone species), and small path length compared to a regular lattice (Montoya and Sole 2002; Williams et al. 2002; Montoya et al. 2006). As the webs were constructed heuristically (at least in part) then the small world test was applied as a quantitative step to assess whether the food web could be considered to have realistic structure and were comparable to other published food webs (Montoya and Sole 2002; Proulx et al. 2005; Montoya et al. 2006).

This 'small world' procedure for model finalisation involved generating networks with equivalent numbers of nodes (species) and links using the random graph generator function in igraph. The connectance (C), number of links (L) and number of Nodes (species) (N) of the modelled food web were then compared with those of the random equivalent network (rand), i.e. C_{rand}, L_{rand}, N_{rand}. As the networks in this study are small (<100 species) and small networks, whilst displaying small world properties fail to meet traditional mathematical criteria (Dunne et al. 2002a), a small network correction was applied (Humphries and Gurney 2008). This recognises the fact that small networks sit on a continuum of small world network attributes, whilst

having somewhat different mathematical properties and compensates for this. Thus, for a food web to be a small world network and this considered final, then:

$$(C / C_{rand}) / (L / L_{rand}) \geq 0.012N^{1.11}$$

At their completion, no webs failed this test.

5.3.5 Modelled scenarios

For each modelled non-native parasite system, three food webs were created, (1) free-living species, native parasites and the focal non-native parasite; (2) free-living species and native parasites only, derived by deletion of the foci non-native parasite species from the data matrix prior to its running in i-graph; and (3) free-living species only, derived by deletion of the native parasites from the matrices prior to their running in igraph. This sequential method of deleting species to create new food webs follows the procedure of (Amundsen et al. 2013). For each of these food web scenarios, the graph metrics relating to the major ecological metrics of connectance, nestedness and mean shortest chain length were obtained using igraph functions and compared between them.

5.3.6 Parasite life-history testing using a simple model

To address the second objective of the Chapter regarding the consequences of parasites with differing lifecycle properties, a basic model based on a simple pyramid of free living species was constructed (Odum and Barrett 2005) that was equivalent to a highly simplified version of the real food web. The properties of this web were established as described in the steps above and then two directly- or two trophically-transmitted parasites were added, and the metrics recalculated to assess differences between parasite's life-history on network metrics. The theoretical direct parasites were modelled as if they parasitised only one species of fish in one case,

and two species in the other case, whilst the trophically transmitted parasites were modelled to each infect multiple fish and invertebrate hosts and the single bird included in the model food web.

5. 4 Results

5.4.1 Site 1, Ergasilus briani

The food web comprised of 42 species, of which 28 were free-living species, 13 were native parasites and *E. briani* (Table 5.1). The removal of native parasites from the food web resulted in web properties that differed substantially from that in which they were included (Table 5.1; Figure 5.2b, c). The number of species decreased from 41 to 28, with 58 links removed from the web. Nestedness was reduced in the web containing only free-living species, as was mean chain length, whilst connectance was greater. Differences between metrics of the web containing *E. briani* and all native parasites and free-living species were minor, with *E. briani* removal slightly increasing connectance and nestedness but reducing mean chain length (Table 5.1; Figures 5.2a and b).

Table 5.1 Summary of food web metrics for Site 1: (1) free-living species, native parasites and the *Ergasilus briani*; (2) free-living species and native parasites only; and (3) free-living species only.

	1	2	3
Species	42	41	28
Links	241	239	181
Nestedness	0.578	0.592	0.537
Connectance	0.205	0.208	0.231
Mean chain length	1.632	1.618	1.18

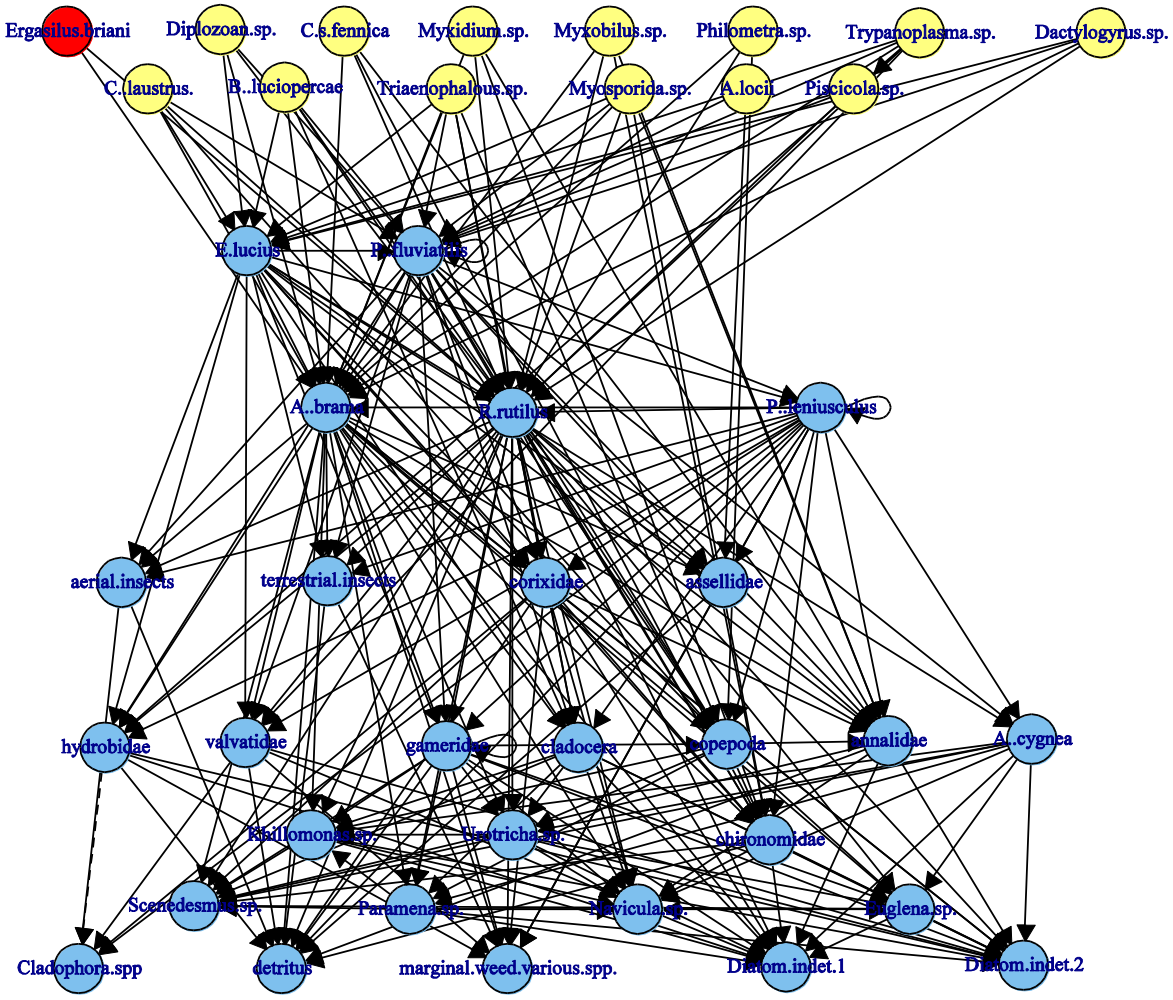

Figure 5.2a Food web of Site 1 free-living species (blue circles), native parasites (yellow circles) and the non-native parasite *Ergasilus briani* (red circle)

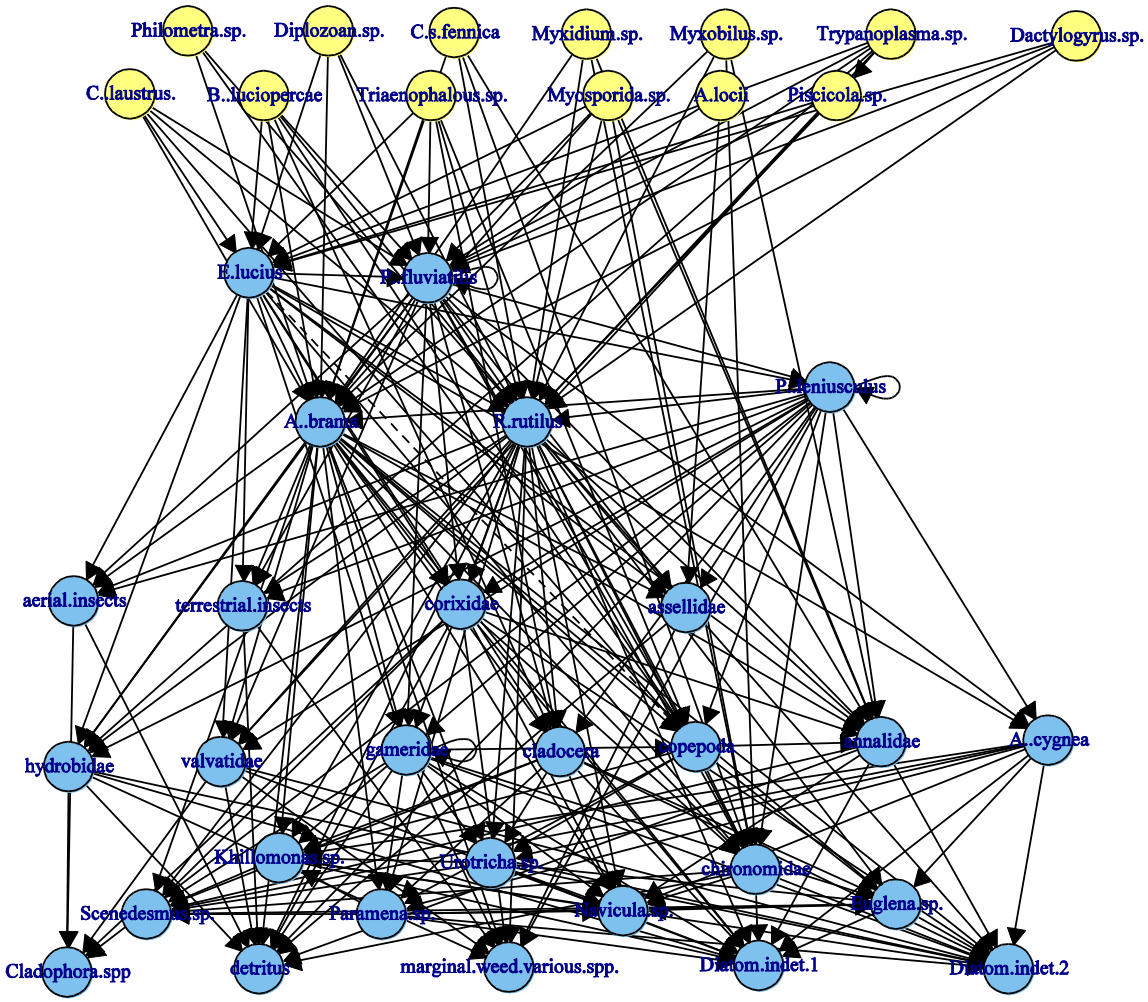

Figure 5.2b Food web of Site 1 free-living species (blue circles) and native parasites (yellow circles)

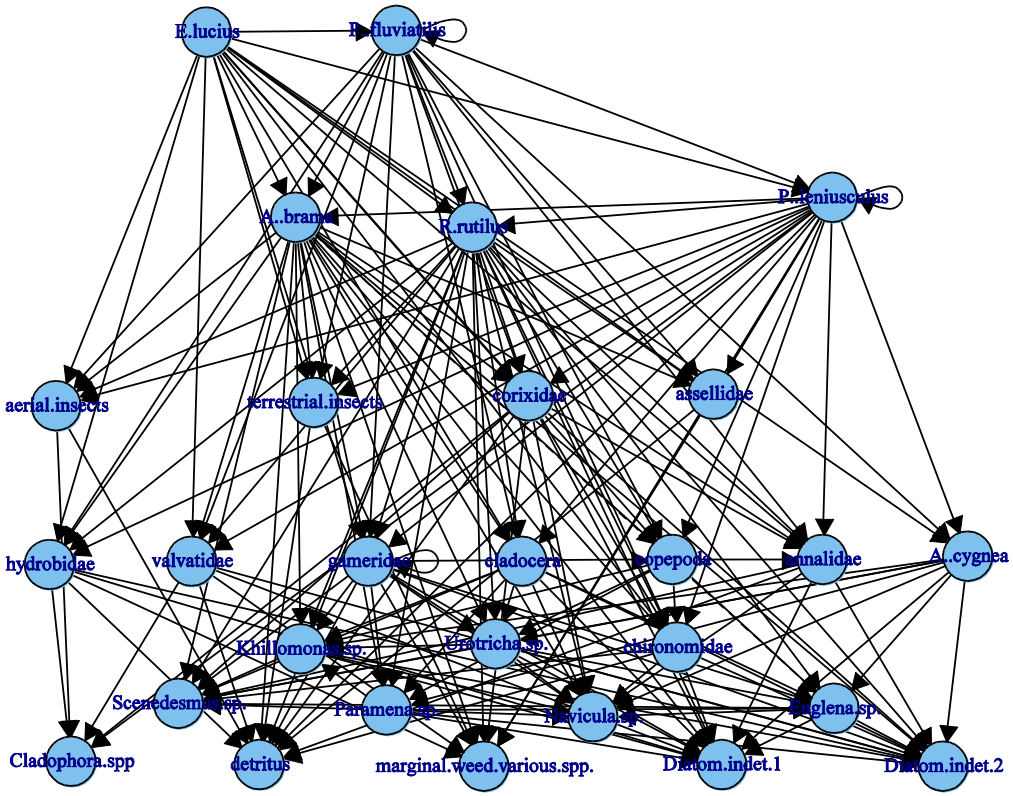

Figure 5.2c Food web of Site 1 free-living species.

5.4.2 Site 2, Bothriocephalus acheilognathi

Site 2 had a relatively species-poor network when compared to the other sites, with only five native parasites and one non-native parasite used in the model. The removal of all native parasites resulted in the loss of twelve links, whilst the removal of *B. acheilognathi* removed eight links, thus its impact on the network metrics was relatively large compared to that of the native species (Figure 5.3a, b and c, Table 5.2). The removal of both native parasites and *B. acheilognathi* resulted in a decrease mean chain length and an increase in nestedness. The removal of *B. acheilognathi* decreased connectance but increased nestedness (Table 5.2).

Table 5.2 Summary of web metrics for Site 2. (1) free-living species, native parasites and *Bothriocephalus acheilognathi*; (2) free-living species and native parasites only; and (3) free-living species only

	1	2	3
Species	38	37	32
Links	215	207	195
Nestedness	0.37	0.394	0.417
Connectance	0.183	0.175	0.19
Mean chain length	1.852	1.649	1.415

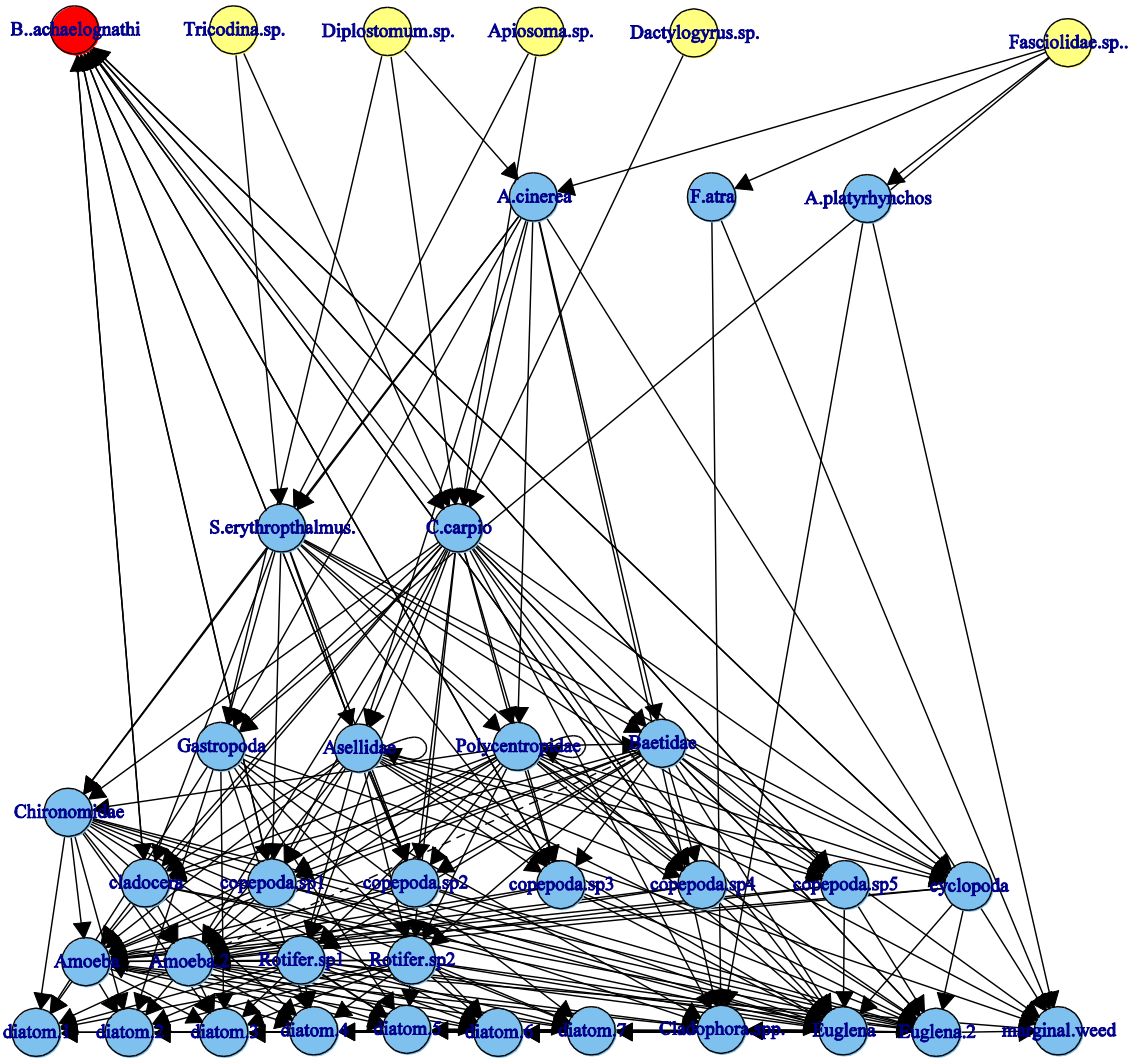

Figure 5.3a Food web of Site 2 free-living species (blue circles), native parasites (yellow circles) and the non-native parasite *Bothriocephalus acheilognathi* (red circle)

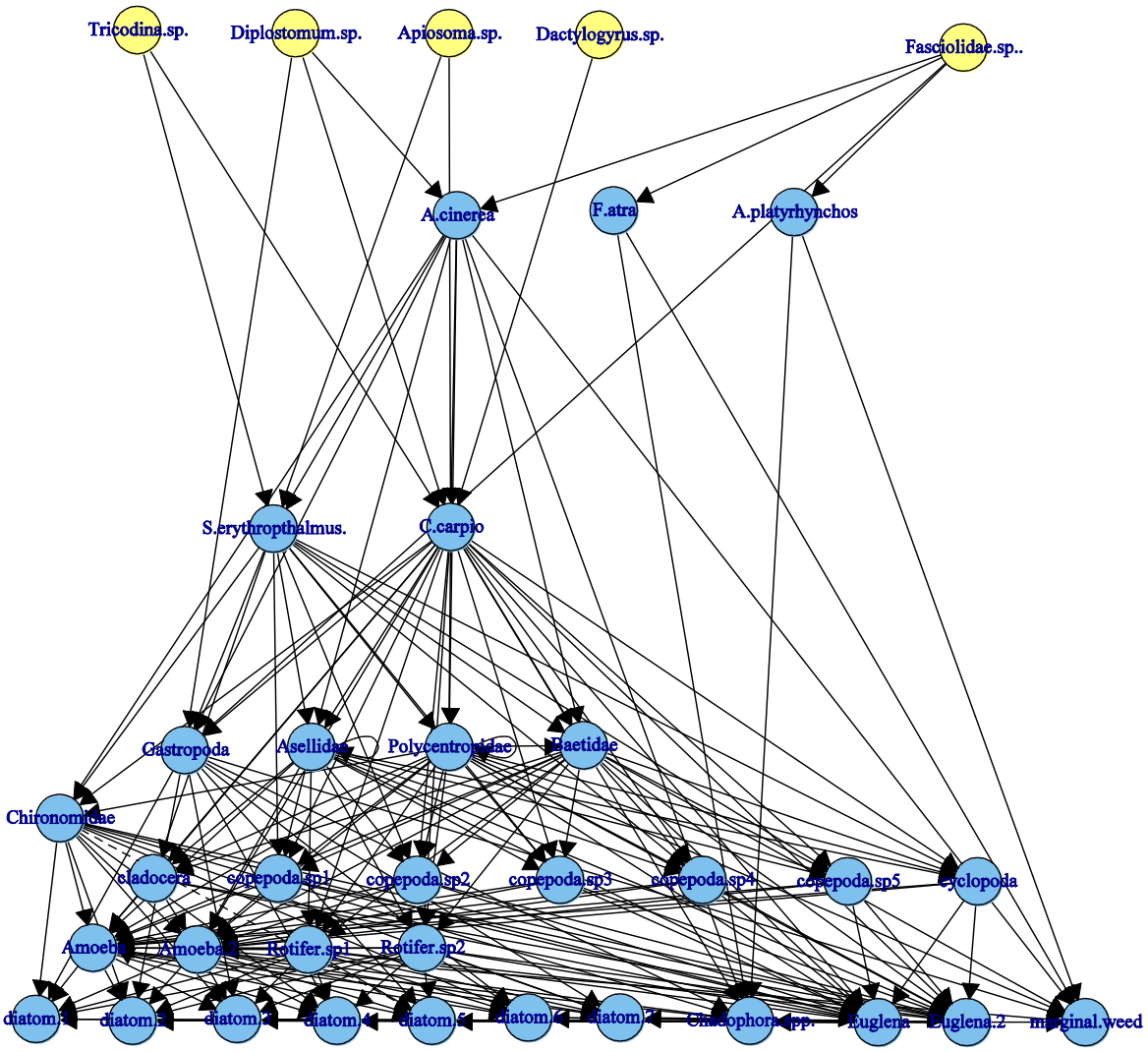

Figure 5.3b Food web of Site 2 free-living species (blue circles) and native parasites (yellow circles)

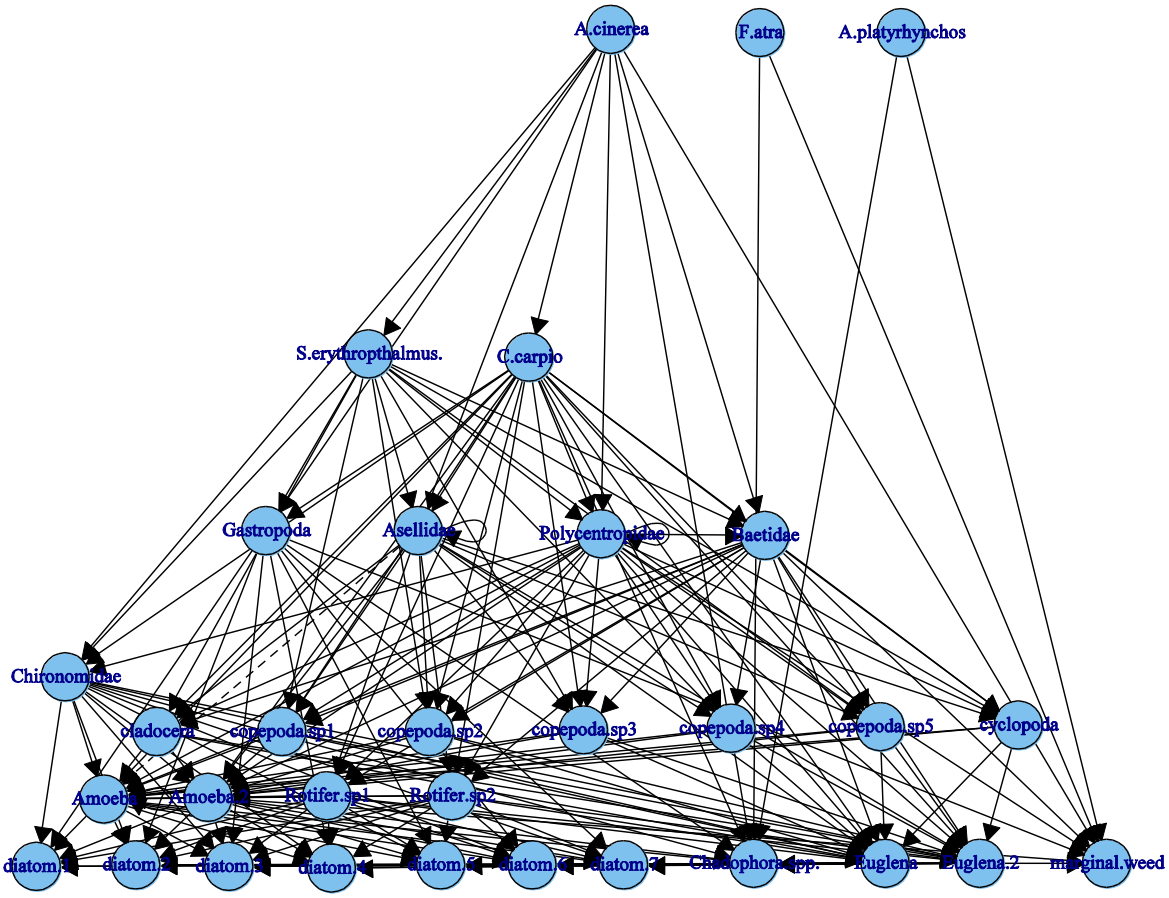

Figure 5.3c Food web of Site 2 free-living species

5.4.3 Site 3, Anguillicoides crassus

Site 3 had the highest number of species used in the food web models, with 55 free-living species, 19 native parasites and *A. crassus* (Table 5.3). The removal of native parasites from the food web increased nestedness and connectance, but decreased mean chain length (Table 5.3; Figures 5.4a,b). The number of links decreased by 166, with each native parasite contributing, on average, less than 9 of those links. By contrast, removal of *A. crassus* decreased the number of links by 25 and resulted in a decrease of all three metrics (nestedness, connectance and mean chain length) (Table 5.3; Figures 5.4a and b). However, in all metrics, as the network was relatively complex then the extent of the change was small when compared to the combined impact of the native parasite species, and the overall values for the metrics of nestedness and connectance were still lower in the infected web than in the free-living species web (Table 5.3).

Table 5.3 Summary of web metrics for site 3. (1) free-living species, native parasites and the *Anguillicoides crassus;* (2) free-living species and native parasites only; and (3) free-living species only

	1	2	3
Species	75	74	55
Links	772	747	581
Nestedness	0.408	0.403	0.438
Connectance	0.187	0.184	0.192
Mean chain length	1.743	1.737	1.415

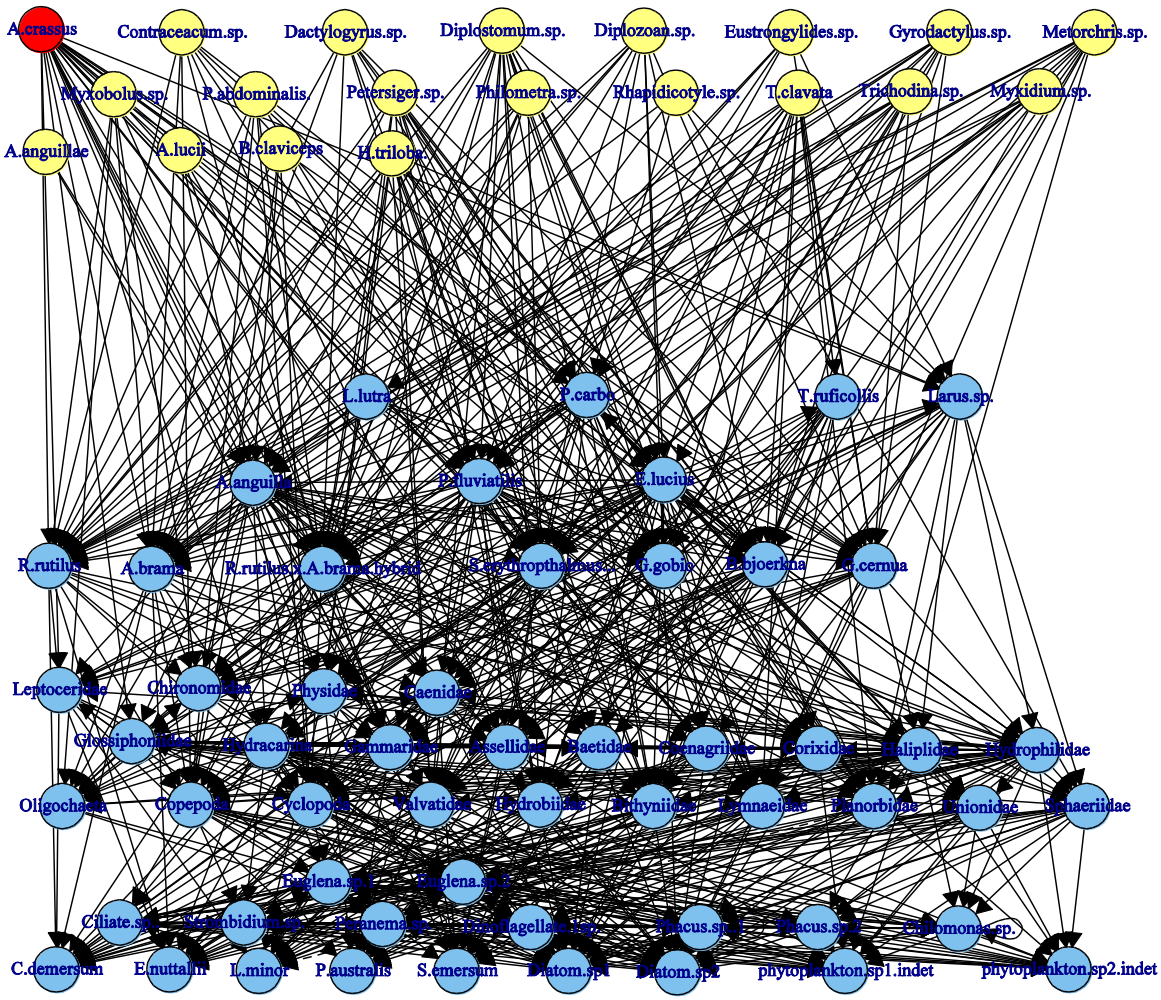

Figure 5.4a Food web of Site 3 free-living species (blue circles), native parasites (yellow circles) and the non-native parasite *Anguillicoides crassus* (red circle)

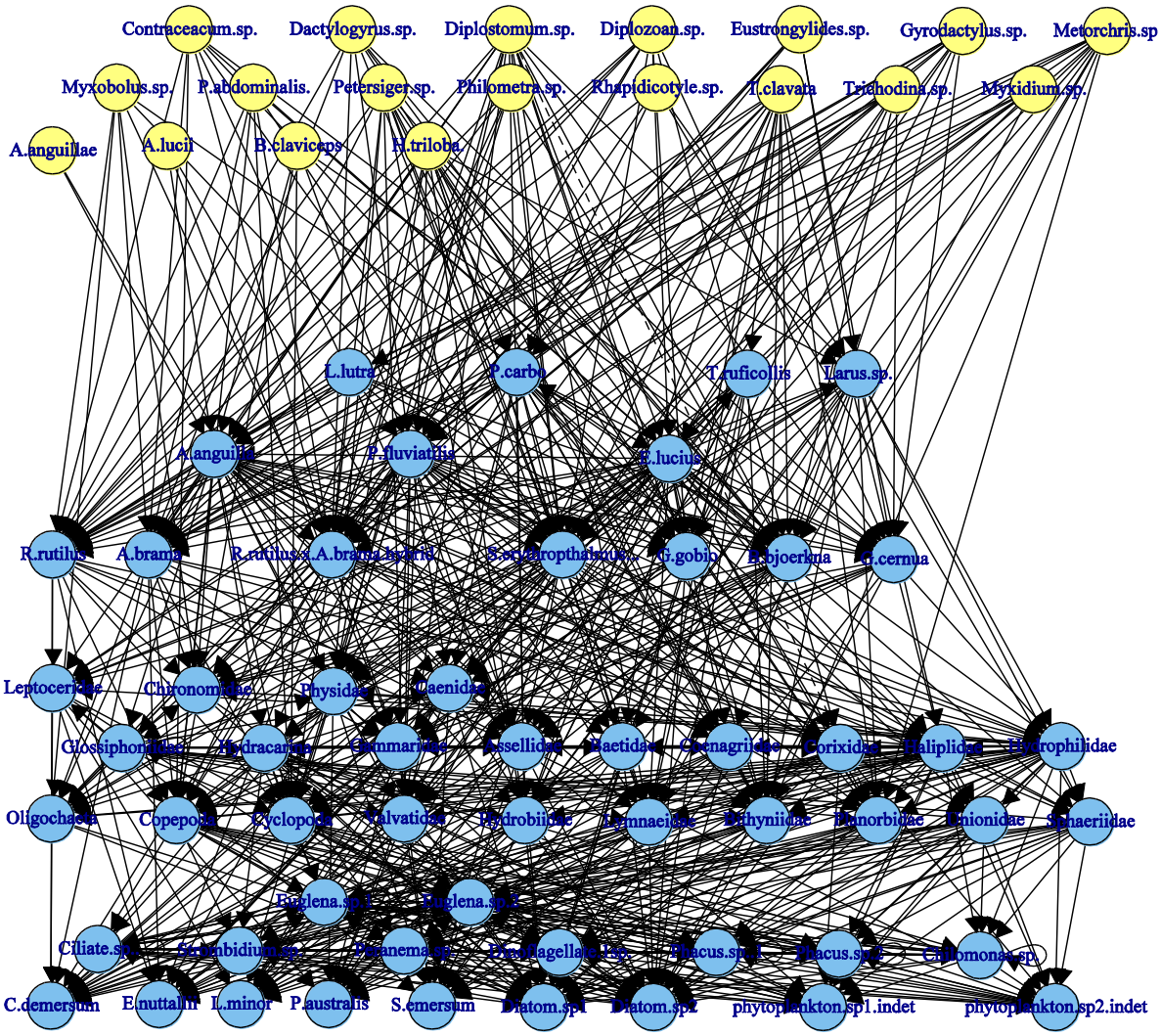

Figure 5.4b Food web of Site 3 free-living species (blue circles) and native parasites (yellow circles)

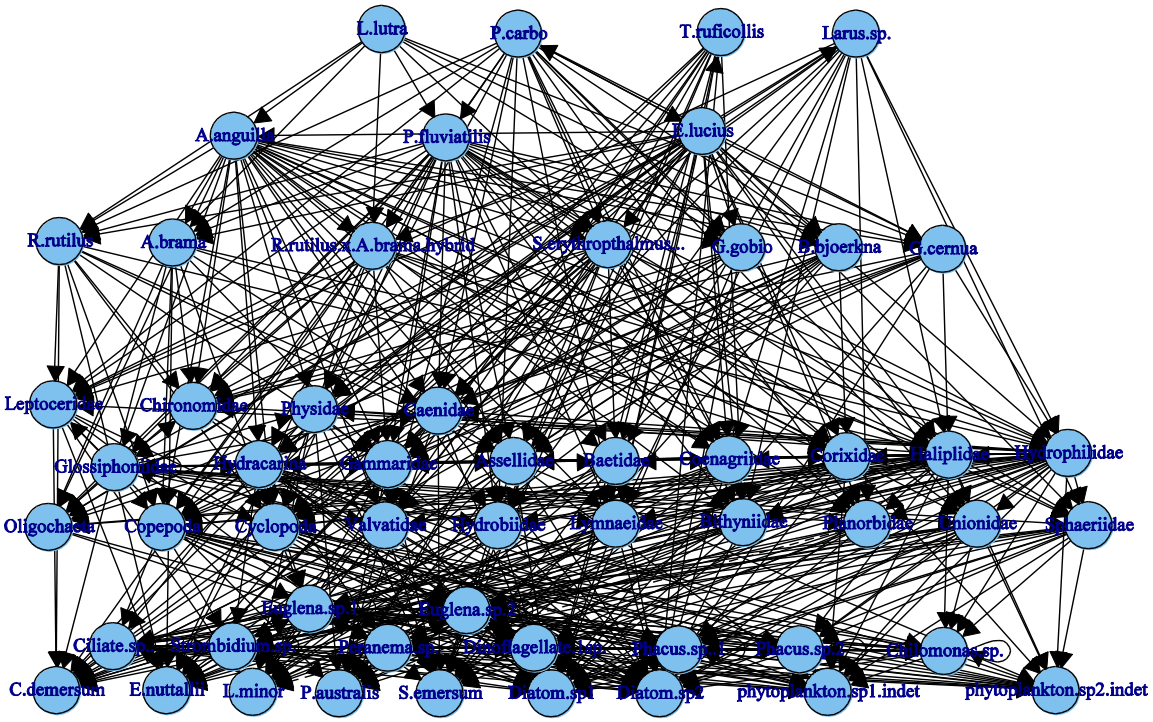

Figure 5.4c Food web of Site 3 free-living species

5.4.4 Model web with theoretical parasites

Comparison of the theoretical food webs revealed marked differences when two directly transmitted parasites were added versus two trophically-transmitted parasites. The metrics nestedness and connectance were lower in the network containing the directly transmitted parasites when compared with free-living species only (Table 5.4). Conversely these metrics were then greater in the network with two trophically transmitted parasites added (Table 5.4). Both the infected webs had a greater mean chain length than the food web of only free-living species, although the magnitude of this difference was greater in the web containing the directly transmitted parasites (Table 5.4, Figures 5.5 a,b,c).

Table 5.4 Summary of the simple model web metrics, where A: free-living species only, B: free-living species plus two directly transmitted parasites; and C: free-living species plus two trophically-transmitted parasites

	Species	Links	Nestedness	Connectance	Mean chain length
A	14	24	0.255	0.122	1.475
B	16	27	0.234	0.121	1.725
C	16	35	0.383	0.156	1.558

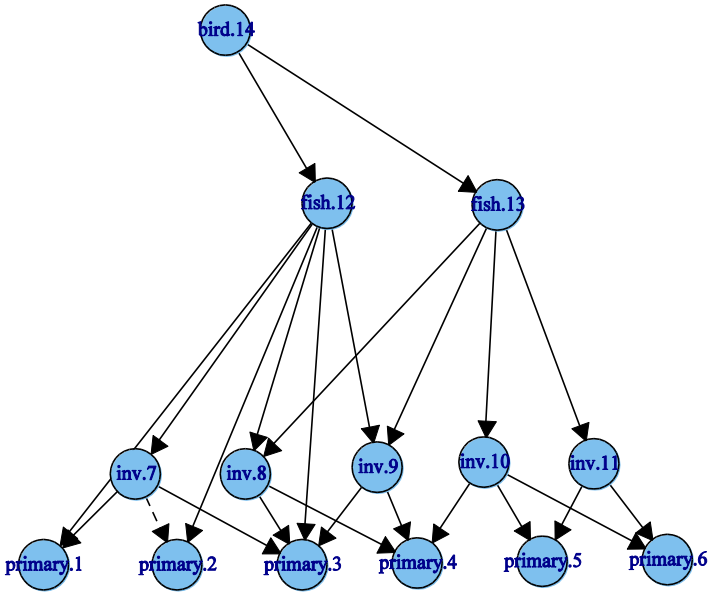

Figure 5.5a Basic theoretical model web of free-living species

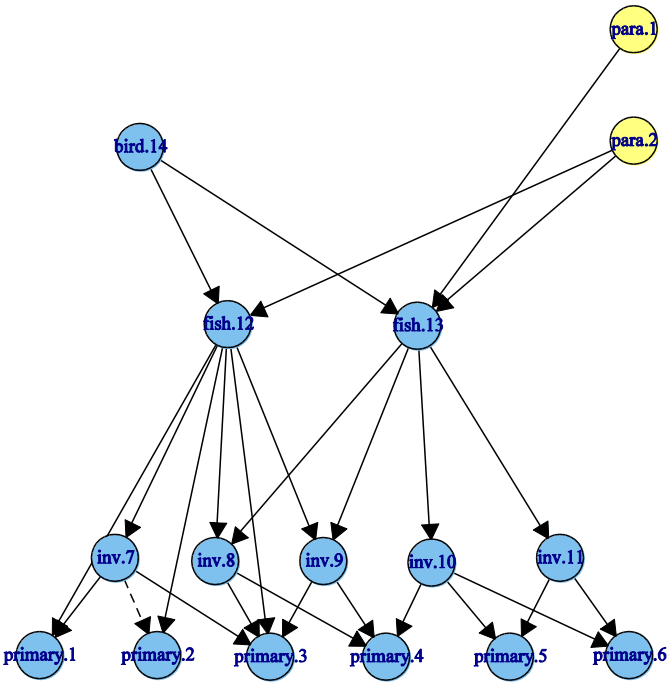

Figure 5.5b Basic model web with the addition of two parasites with direct lifecycles and high host specificity.

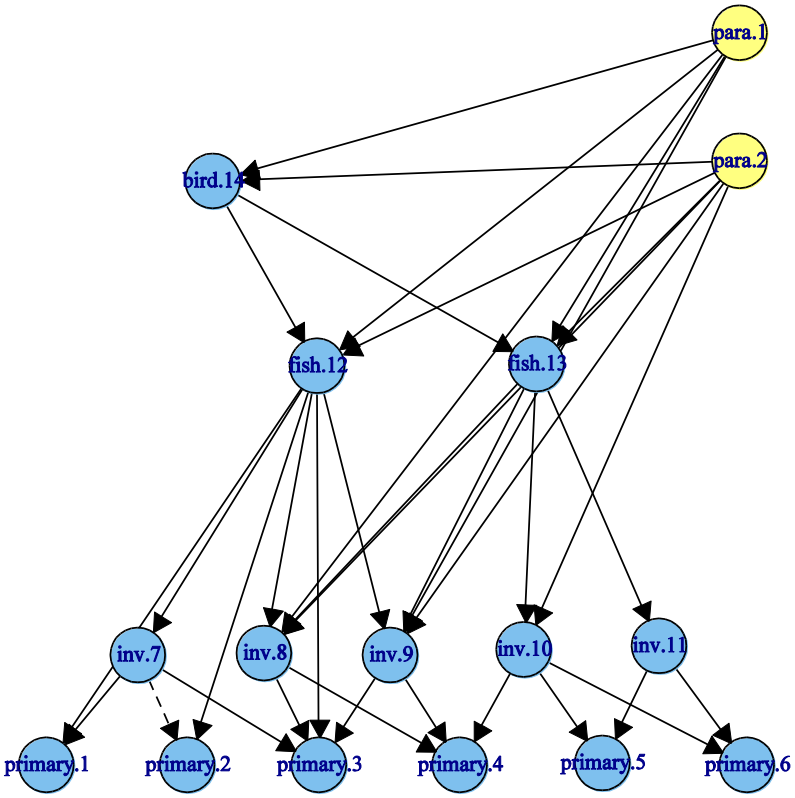

Figure 5.5c Basic model web with the addition of two trophically-transmitted parasites with complex lifecycles and multiple hosts

5. 5 Discussion

The effects on the topological food web of adding native parasites to the free-living species followed by the addition of a non-native parasite were successfully modelled. Their effects varied at each site according to the complexity of the extant free-living communities and their parasite fauna. At Site 3, the most complex site, the inclusion of native parasites substantially altered the number of species and links in the food web, and impacted the food web metrics as a result, with only minor changes then caused by the inclusion of *A. crassus*. At Site 1, the effect of *E. briani* on the food web was minimal, primarily because it is a directly-transmitted parasite that, consequently, only created two new links. Whilst Site 2 was the least complex, involving the lowest number of species and links, as the focal non-native parasite, *B. acheilognathi*, was trophically-transmitted then when compared with *E. briani*, it had a relatively large effect on the food web metrics, with the creation of 8 new links and markedly reduced nestedness. This comparison of topological changes incurred in the food web by directly-transmitted and trophically-transmitted parasites was also supported by the theoretical models that revealed similar patterns.

The characteristics of the parasites used in the food web models were thus a large influence on the food web topology. This indicates that it is the ecology and biology of a parasite that will determine its influence on food web structure rather than, for example, its native/ non-native status. This also means there is likely to be considerable variability in the influences of different parasites on food web models due to issues including:

Host specificity: Many parasite species are specific to only a single host, whereas others have multiple hosts, with examples of extreme generalists such as the

amphibian parasite *Batrachochytrium dendrobatidis* that infects over 500 species (Bielby et al. 2015). From a food web perspective, the larger the number of links that a parasite has potential to make, the stronger its effect on the food web metrics. From an invasion perspective, a generalist parasite has an increased chance of successful establishment due to a greater number of potential host species (Taraschewski 2006; Douda et al. 2012). The destabilization in the food web models incurred by the addition of direct, specific parasites is also consistent with empirical data, as parasites with high host specificity are particularly vulnerable to secondary extinctions (Lafferty and Kuris 2009).

Lifecycle and strategy: Parasites differ widely in their life history strategy, and this variability is key both to their mode of life as well as their impact on a food web (Thompson et al. 2005). A direct lifecycle can be advantageous in that it only requires the definitive host for completion, whereas a parasite with a complex lifecycle might require a series of intermediate hosts prior to transmission to the final host. In the case of the latter, the use of paratenic hosts can increase the probability of transmission, as observed with *A. crassus* (Chapter 4).

***Parasite detection*:** A problem with infectious food web studies such as this is that discrepancies in parasite detection rates can have significant effects on the outcomes of food web construction and analysis. As Poulin (1992) notes, parasite species that have been observed more frequently are more likely to have a more complete record of their hosts and ecology simply as a result of chance. For example, there is a much higher prevalence of records for copepod parasites than monogeneans, despite these two groups of ecto-parasites sharing similar direct lifecycles (Poulin 1992), a result of copepods having received a greater amount of research effort. Another related factor likely to skew structure of any food web model incorporating parasites is the

extent of the scrutiny that the hosts have been subjected to. For example, parasites of commercially- and recreationally-important species are far more likely to have been identified and studied than those of other species (Henderson et al. 2003). This was reflected here where there was extensive literature on the fish parasites but with substantially less available for macro-invertebrate species, other than those involved in parasite trophic-transmission.

Comparison of the effects of parasites in food web metrics of this study with other studies revealed the following similarities and differences.

***Connectance*:** Here, in all three sites, connectance was reduced in the food webs with parasites compared with only free-living organisms. Whilst this is contrary to the majority of parasite-based food web studies (e.g. Martinez 1991; Huxham et al. 1996; Memmott et al. 2000), it is in agreement with the recent study of Amundsen et al. (2013) of Lake Takvatn. This is of particular interest as this study considered the impact of non-native fish and their associated parasites on web characteristics, as opposed to the majority of other studies, which consider only native parasites. Connectance is important in biological systems as robustness, the ability of a system to resist cascading extinctions, increases with food-web connectance. In particular, food webs experience `rivet-like' thresholds past which they display extreme sensitivity to removal of highly connected species. Higher connectance delays the onset of this threshold (Dunne et al. 2002b). Thus, an observed reduction in connectance may signal an increase in the vulnerability of a system to extinctions.

Nestedness: The nestedness of the food web increased with the addition of parasites at Site 1, as also shown in the Carpinteria salt marsh food web (Lafferty et al. 2006b), but decreased at Sites 2 and 3, as seen in the infectious food webs of

Muskingum Brook, New Jersey, USA (Hernandez and Sukhdeo 2008). Similar to connectance, nestedness is considered to increase ecological stability, as a nested system should recover better from perturbation as species are not isolated (Bascompte et al. 2003). Reciprocal specialisation is the process that results in non-nested patterns in networks and occurs, for example, when a parasite specialises on a particular host through co-evolutionary processes (Joppa et al. 2010). Whilst reciprocal specialisation is relatively rare in ecological networks (Joppa et al. 2009), it is more frequent in parasites (Pedersen et al. 2005). Thus, the reduced nestedness in this Chapter was the result of the inclusion of highly specialised parasites in the food webs.

Mean chain length: At all sites, the mean chain length increased with the addition of parasites, a trend consistent with all the studies cited above. Food chain length is of interest in that it can be an indicator of limiting factors to a system, such as resource availability and productive space, and it can modify key ecosystem functions such as nutrient cycling, primary productivity and atmospheric carbon exchange (Post 2002a). Furthermore, food chain length can influence the concentration of contaminants in top predators (Kidd et al. 1998), and indeed parasites have been shown to play a role of sink to pollutants, for example *Pomphorhynchus laevis* has been shown to act as a bioaccumulator of the heavy metals, lead and cadmium (Sures and Siddall 1999; Thomas et al. 2000).

Determining the sub-lethal and ecological consequences of parasites can be inherently difficult, and here a topological food web model approach was used in order to identify the wider ecological implications of parasite introductions. The use of network modelling was shown to provide a valuable analytical tool for

understanding how parasites can modify food web structure over multiple trophic levels, and highlighted how the unique properties of parasites may alter networks in a manner that differs from free-living species. From single host species to the case of *B. dendrobatidis*, with the ability to infect over 500 species (Bielby et al. 2015), there can be considerable variability in the parasite impact. Similarly, the properties of the receiving system are critical in mitigating or exacerbating their effect, as shown in comparisons of the effects of *A. crassus*, *E. briani* and *B. acheilognathi* in the selected freshwater food webs of this chapter.

6. Weighted food webs to predict the outcomes of interactions of non-native parasite infection and environmental change

6.1 Abstract

Weighted topological food webs incorporate the strength of the predator-prey relationships into their network and thus have greater complexity and realism than unweighted webs, and can provide a strong predictive tool. Weighting can be completed via incorporating energy transfer between predators and prey that reflect their measured trophic interactions. Here, the stable isotope data (Chapters 2 and 3) and topological food webs (Chapter 5) were integrated to provide weighted food web models for *E. briani* and *B. acheilognathi* that were then used to test scenarios of environmental change on food web structure using (i) the relative proportions of producers and primary consumers that contribute to diets of higher consumers (i.e. fish); and (ii) biomass of fish species that models of fixed biomass would be predicted to support. Models predicted that increasing parasite prevalence in host populations of *E. briani* would have little impact on food web structure, whereas increasing parasite prevalence in host *C. carpio* populations of *B. acheilognathi* would alter the overall structure of the food web and ratio of trophic levels to each other, with higher consumers directly consuming more primary producers and a lower biomass of primary consumers. Models then simulated how environmental disturbance affected the weighted food webs and suggested that shifts to more eutrophic conditions provided some net benefits for infected fishes via facilitating their increased biomass through the provision of increased food resources based on primary producers. Thus, where infection consequences of non-native parasites are

sub-lethal and include some constraints on host foraging performance, then eutrophication could provide these fishes with greater food availability and thus resilience to both the adverse effects of parasitism and environmental change.

6.2 Introduction

6.2.1 Weighted food webs

The food webs developed in Chapter 5 provided a topological description of the complexity of the networks in the presence and absence of parasites, including the focal non-native parasites. They produced descriptive statistics from the networks that enabled, for example, comparison in food web metrics between infected and uninfected webs, and between webs constructed from different systems involving different parasites.

A short-coming of the topological approach is, however, that all links are treated as equal, giving no indication of the strength of each relationship, such as whether a prey item was major food component of a predator, consuming it regularly, or rather just a minor component, preying upon it infrequently (Bersier et al. 2002). Consequently, when food webs can be 'weighted' by including a measure of the strengths of predator-prey relationships in the network, then the resultant food web model has greater complexity allied with more realism (Zhang and Guo 2010), thus improving its utility as a predictive tool (Thompson et al. 2012).

Different metrics, such as strength of the trophic interaction (Emmerson and Raffaelli 2004), or the amount of energy flow (Amundsen et al. 2013), can be incorporated into ecological networks in order to weight the network dependent on

the question the network analysis aims to answer. In the case of the former, body size has been used in a number of studies (Woodward et al. 2005), such as the study of Emmerson and Raffaelli (2004) examining dynamics of food web stability in the Ythan estuary, whilst Dorresteijn et al. (2015) used the frequency of interaction to weight a terrestrial food web and investigate human impact on large mammal behaviours and predation patterns in Transylvania. More frequently, energy is incorporated into webs to create realistic simulations of trophic interactions in food webs, and where these steps have been taken to incorporate trophic data into weighted networks then important ecological attributes have been determined, for example, estimating food chain length from basal energy (Thompson and Townsend 2005; Arim et al. 2007), or determining the importance of terrestrial input in aquatic systems (Kawaguchi et al. 2003).

6.2.2 Stable isotopes as a means of gathering food web information

Stable isotope analysis increasingly represents an effective ecological tool for elucidating trophic relationships in food webs (Peterson et al. 1985; Grey 2006; Semmens et al. 2009). The application of $\delta^{13}C$ and $\delta^{15}N$ to food web structure has enabled reconstructions of the trophic relationships between species (Sections 2.4, 3.4, 4.4) and identified the basis of production, such as allochthonous versus autochthonous energy inputs (McCutchan et al. 2005; Grey 2006). They can be used to determine trophic niche sizes and associated relationships between species (Sections 2.4, 3.4, 4.4; (Layman et al. 2007; Jackson et al. 2011; Jackson et al. 2012)), and estimate diet composition (Sections 2.4, 3.4, 4.4, Jackson et al. 2011). Thus, through stable isotope analysis, it is possible to establish not only if predator prey relationships exist between species, but also estimate the relative proportions of

each food item in the diet of each consumer species. In doing so, it provides a methodology that can be used as the basis for 'weighting' topological food webs.

6.2.3 Maintaining food web equilibrium and impact of introducing non-native species

Food webs are driven by a combination of bottom-up (from primary producers) and top-down (from consumers) processes (Reid et al. 2000). Shifts in this balance can have significant impacts on the web community. An example of a bottom-up process impacting food web structure is the shift from eelgrass (*Zostera marina*) to sea lettuce (*Ulva lactuca*) as a dominant producer in Canadian estuaries, the result of anthropogenic eutrophication that caused major shifts in the composition of major faunal and floral communities, and reduced fish species richness and abundance (Schein et al. 2012). There are multiple examples of trophic cascades resulting from top-down processes, where changes in predator-prey relationships alter the food web beyond the immediate prey populations. For example, experimental manipulations of fish in a Northern California river revealed removal of predatory fish, which consume predatory insects and fish fry, increased the survival of these species that in turn fed on chironomid larvae. In the presence of fish, filamentous green algae were very limited and were infested with chironomids. When the larger fish were absent, this released the predation pressure on the smaller predators that previously suppressed chironomids, resulting in substantially reduced algal grazing and increased algal biomass (Power 1990).

Non-native invasive species can also have significant impacts when they invade food webs (Vitousek et al. 1996), for example, invasive zebra mussels *Dreissena*

polymorpha in the Hudson River estuary reduced phytoplankton densities by up to 85%, with associated declines in planktonic grazers that drastically transformed the food web (Caraco et al. 1997; Strayer et al. 2014). The invasion of *Pseudorasbora parva* into ponds in the UK, induced multiple changes in the foodweb, with shifts to a cyanobacteria dominated phytoplankton community, and increased trophic overlap between cohabiting fish species, that reduced somatic growth in *R. rutilus* (Britton et al. 2010). In Topanga Creek, California, benthic macroinvertebrate abundance and species richness was lower in the presence of the invasive red swamp crayfish, *Procambarus clarkii*. This change in the structure of the web impacted the California newt *Taricha torosa* (endemic species) and the California steelhead trout *Oncorhynchus mykiss irideus* (endangered), which are predators of the depleted macroinvertebrate community (Garcia et al. 2015). Adding an additional species to a food web is, therefore, more than a simple topological addition, as it can potentially have multiple cascading trophic consequences throughout the entire foodweb.

Due to their small size, parasites are rarely considered in a trophic context except when their total biomass is such that they represent a significant food resource (Kuris et al. 2008). Yet in Chapters 2 and 3, two ways were identified in which parasitism can alter trophic niche of hosts by causing them to become more specialised in their diet (as in the case of *Ergasilus briani* infected *R. rutilus* and *A. brama;* Chapter 2) or to shift their trophic niche, preying on different resources (as in *Bothriochephalus acheilognathi* infected *C. carpio,* Chapter 3). Although some examples of significant dietary changes induced by native parasites exist, for example cyprinids infected with *Ligula intestinalis* shift to exploiting prey items for which competition is less (Loot et al. 2001), the impacts of non-native parasites on naïve hosts are often more

severe as their hosts lack any co-evolved mechanisms of resistance or tolerance (Johnsen and Jensen 1986), and thus can provide excellent model species to study the whole foodweb consequences of opportunistic parasitism.

Numerous factors, such as host density (Jansen et al. 2012), co-existence of other parasites (Cox 2001) or environmental abiotic variables (Sures 2008), affect parasite prevalence and abundance, yet levels of infection are critical to the impact of the parasite on its host population (MacKenzie and Abaunza 1998). Application of weighted models allows variability in infection level to be incorporated into the food web, and the scale of infection consequences to be investigated, which is very difficult to achieve empirically.

6.2.4 Non-native parasites in a disturbed system

Invasive species can cause habitat degradation with, for example, burrowing and foraging by the invasive crayfish *Procambarus clarkii* causing structural damage to river banks and increasing erosion (Angeler et al. 2001). However, many invasive species are opportunistic, taking advantage of other forms of ecosystem change, such as habitat disturbance, rather than being the drivers of change themselves (Gurevitch and Padilla 2004; MacDougall and Turkington 2005). Anthropogenic eutrophication is a major cause of degradation of freshwater systems (Carpenter et al. 1998), as the increase in biologically available nitrate and phosphate impacts aspects of the water chemistry and biota, and alters ecosystem structure and functioning (Smith et al. 1999; Dodds et al. 2009). The effect is a shift from a system in which macrophytes are significant primary producers to ones in which phytoplankton are dominant, reducing water clarity and resulting in further declines in macrophyte biomass,

further releasing nutrients to drive the phytoplankton dynamics (Hough et al. 1989; Smith et al. 1999). Moreover, eutrophication often increases parasite prevalence in host populations (Lafferty 2008), especially those parasites that are generalists with local recruitment and short life cycles (Marcogliese 2001). Correspondingly, the consequences of the interactions of anthropogenic eutrophication and parasite prevalence on host populations are a key focus of this Chapter.

6.2.5 Aim and objectives

The aim here was to develop weighted food web models for each food web of Chapter 5 in order to provide an analysis of how food web structure was altered by parasites when the feeding relationships of the consumer species were accounted for. Objectives (O) were to:

O1. Develop the method of weighting the topological food webs from Chapter 5 using the outputs of stable isotope analysis outlined in previous data chapters;

O2. Apply the weighting to the topological food web models to develop final models capable of predicting the impacts of the parasites on food web structure and energy flux; and

O3. Use the final weighted webs to quantify the ecological consequences of parasites on infected fishes under scenarios of altered parasite prevalence and anthropogenic eutrophication, where the latter is represented by shifts in the proportions of phytoplankton and macrophytes.

6.3 Materials and Methods

6.3.1 Data used to build food web

Data from the stable isotope analyses from Sites 1 and 2 (*cf*. Chapter 5) were used in which the following non-native parasites were present:

- *Ergasilus briani*, Site 1: Basingstoke canal (Section 2.3, Figure 2.1);
- *Bothriocephalus acheilognathi*, Site 2: Greater London fishery (Section 3.3, Figure 3.1)

The effect of *Anguillicoides crassus* on *Anguilla anguilla* was not included in this chapter as although significant differences in the trophic niche of infected and uninfected *A.anguilla* were observed, the differences were not necessarily due to infection by *A. crassus* but were instead related to eel functional morphology (Chapter 4).

Data collection at each site was as per Sections 2.3 and 3.3. This provided data on the stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ for the infected and uninfected fish in the host populations, the other fish species present, and their putative food resources. Bayesian mixing models were used to estimate the proportions of these food resources in the diets of all fish species, including the infected and uninfected components of the host populations (Sections 2.4 and 3.4). For the other species present in the food web but for which these dietary data were not collected and analysed (primarily the piscivorous birds and the macro-invertebrates), a heuristic approach was used, applying published information on their diet compositions to the food web calculations, as per (Vander Zanden et al. 1997; Vadeboncoeur et al. 2002).

6.3.2 Preparing the data for modelling

The basis of the weighted food web modelling was the topological webs used in Chapter 5. However, as here they were being combined with the outputs of the stable isotope mixing models, the topological models were modified so they matched the way in which fish putative food resources were combined in the mixing models. For example, rather than including a number of arthropod species in the web, these were now combined into a single node as the mixing models had combined their data due to minimal differences in stable isotope values (Phillips et al. 2005). An advantage of weighting the foodweb in this manner was that as well as adding content, it eliminated an issue generally encountered in topological webs, whereby the level of taxonomic sensitivity of the data can skew their metrics (Williams and Martinez 2000)

The next step was to construct a matrix that described the feeding relationships between each species (or species grouping) in the food web. As per Chapter 5, this was completed in MS Excel 2010 but whereas there it was based on binary relations (0 and 1), here they were based on the dietary proportions (scale of 0 to 100) that were estimated from the mixing models and the heuristic analysis that quantified the strengths of the relationships between the consumers and prey species/ groups; 95% confidence intervals were calculated from the standard error of the mixing models and also incorporated into the matrix. As per Section 5.3, the direction of that relationship (i.e. which was the predator and which was the prey) was determined by their direction within the matrix, whereby the y-axis of the matrix listed all the species as predators and the x-axis of the matrix listed all the species as prey/ producers. Thus, in the example of Figure 6.1, Species A is a producer, species B

predates A only, species C's diet comprises 20-30% of species A and 70-80% of B. The diet of species D comprises approximately equal amounts of species B and C. The diets of infected and uninfected fish were estimated in Sections 2.4 and 3.4, and diets of the total population with varying infection prevalences were calculated by combining appropriate proportions of these (Section 6.2.5). The food web matrices used for model construction are provided in the results section.

	A	B	C	D
A	0	0	0	0
B	100	0	0	0
C	20-30	70-80	0	0
D	0	45-55	40-60	0

Figure 6.1 ***Example of the structure of a proportional network matrix***

Note that whilst the purpose of this Chapter was to investigate the quantitative changes in energy flow and trophic interactions caused by parasite infections, only the free-living species were presented in the weighted webs. This was because the contribution of the parasites to the diet of any consumer was always < 1 %. Therefore, it was the impact of the parasite on the hosts that was modelled through modelling the effects on host diet according to different prevalence levels, rather than including the parasite itself in the weighted models. On their completion in MS Excel, the matrices were then transferred into R using the package gdata (Warnes et al. 2015).

6.3.3 Food web modelling using igraph

Following conversion of the matrices into R, they were then converted into food webs (networks) using the network analysis package igraph (Csardi and Nepusz 2006), as described in Section 5.3. The model networks had the following two simple rules:

- The total diet of all consumers had to equal 100% of any other food source at the start of the model. Unless this was met, then consumers were unable to switch diets during predictions of environmental change.
- If the proportion of an item being consumed by an organism or group increased, then it was assumed the consumer eats proportionally more of that item, and as a consequence, the biomass of that consumer will increase.

For example, a consumer with a diet comprising items x, y and z, and where n is the starting proportion of diet at time $_t$, then:

$n_t = n_x + n_y + n_z = 100$

If the biomass of x is doubled the diet of the consumer would be

$n_t = 2n_x + n_y + n_z > 100$

and the biomass (b) of that consumer would increase proportionally and where overall biomass per trophic level is determined as diminishing to 10 % of the previous trophic level at each trophic level (Pauly and Christensen 1995).

When ‘top-down’ changes occur, if the proportion of an item consumed increases then it is assumed that that item must exist in that proportion, and thus its prey must increase proportionally also, i.e. there is a cascading effect in the model. For

comparison, the web can then be recalculated with fixed starting quantities, using the new proportions.

6.3.4 Metrics measured

This study measured two primary metrics:

1. The relative proportions that producers and primary consumers contributed to the diets of the focal higher consumers (i.e. fish).
2. The biomass of fish species that a model of fixed biomass but differing weighting and topology (i.e. different proportions of producers or differing diets of consumers) would be predicted to support.

These are measured as proportional changes on a scale of 0 to 1 from an original web, i.e. one that contains no fish infected with the focal parasite (*E. briani* or *B. acheilognathi*) and with a community of primary producers at their proportions originally measured at the study sites (Chapters 2 and 3).

6.3.5 Predictive modelling of scenarios

The development of the initial food web was based on the dietary proportions of the host fish population according to the stable isotope analysis, i.e. they reflected the differences measured between the infected and uninfected individuals. Thus, the modelled diet of the infected fish was initially as per their observed parasite prevalence, with the relative proportions of the remainder of the food web calculated accordingly. This final model was then used as the basis for predicting the consequences of scenarios on the food web structure according to the following scenarios (S):

S1. Shifts in parasite prevalence of the host populations (using 0, 25, 50, 75, 100 %).

S2. Shifts in the proportion of primary producers, with increasing proportions of phytoplankton to macrophyte, simulating the outcomes of increasing anthropogenic eutrophication.

S3. The interaction of (1) and (2) above.

For S1 at Site 1 where two fishes (*R. rutilus* and *A. brama)* were present that were host to *E*. *briani*, the infection level was kept the same for both species in the model, as this generally reflected the observed similarity in their infection levels (Table 2.1) and is consistent with the preferred size of fish that the parasite infects, which does not differ significantly between these two host species (Alston and Lewis 1994).

For S2, the scenario of anthropogenic eutrophication centred on the resultant shift that tends to occur in eutrophic freshwaters, i.e. from macrophyte to phytoplankton domination (Hough et al. 1989). The infected and uninfected food webs were adjusted by decreasing the proportion of macrophyte in the foodweb, to 75%, 50%, 25% and 0% of the starting macrophyte biomass and increasing the phytoplankton by the same amount so the total biomass remained constant.

For S3, the scenarios combined all those completed in S1 with those completed in S2. All tested scenarios are summarised in Table 6.1 and 6.2.

Table 6.1 Scenarios modelled, to test the combined impact of disturbance (removal of macrophyte and replacement with phytoplankton) and differing levels of infection with *Ergasilus briani*.

		E.briani infection level in host population.				
		0	25%	50%	75%	100%
Percentage of Macrophyte depleted	0	✓	✓	✓	✓	✓
	25%	✓	✓	✓	✓	✓
	50%	✓	✓	✓	✓	✓
	75%	✓	✓	✓	✓	✓
	100%	✓	✓	✓	✓	✓

Table 6.2 Scenarios modelled, to test the combined impact of disturbance (removal of macrophyte and replacement with phytoplankton) and infection differing levels of with *B. acheilognathi***.**

		B.acheilognathi infection level in host population.				
		0	25%	50%	75%	100%
Percentage of Macrophyte depleted	0	✓	✓	✓	✓	✓
	25%	✓	✓	✓	✓	✓
	50%	✓	✓	✓	✓	✓
	75%	✓	✓	✓	✓	✓
	100%	✓	✓	✓	✓	✓

6.4 Results

6.4.1 Site 1: Ergasilus briani

Creating the weighted web

The simplified food web comprised of 10 nodes and 19 weighted links (Figure. 6.2). Of these 19 links, the majority were weighted empirically and the remaining links were weighted heuristically. Table 6.3 summarises the mixing model outputs used in the completion of the initial food web model, with the additional data supplied in Appendix 4.

Table 6.3 Summary of the Bayesian mixing models outputs predicting the proportions of each major food item to the diet of infected and uninfected *A. brama* and *R. rutilus*.

Species	Food item	Modelled diet proportion (± SE)	
		Uninfected	Infected
A. brama	Arthropoda	0.40 ± 0.14	0.35 ± 0.13
	Chironomidae	0.45 ± 0.14	0.51 ± 0.13
	Zooplankton	0.15 ± 0.10	0.14 ± 0.08
R. rutilus	Arthropoda	0.59 ± 0.19	0.40 ± 0.19
	Chironomidae	0.38 ± 0.19	0.57 ± 0.19
	Zooplankton	0.03 ± 0.03	0.03 ± 0.03

The matrices created using the stable isotope proportions are supplied in Appendix 5. These were used to construct simple weighted models (e.g. Figure 6.2) in which each link in the model represents 1% of the organism/ group's diet. Thus, in Figure 6.2, the shift between the diet of both infected and uninfected *R. rutilus and A. brama*

from one favouring arthropods to one favouring Chironomidae can be observed though the change in the link density.

Scenario 1: Changing parasite prevalence under constant environmental conditions

The scenario modelled here was maintaining the biomass of all fish at the original level whilst differing the levels of parasite prevalence in the host populations, specifically 0%, 25%, 50%, 75% and 100% prevalence. The major component of the diet of uninfected *R. rutilus* was arthropods, whilst chironomid larvae were the major component of the diet of *A. brama*, with arthropods comprising lower dietary proportions (Table 6.3). In infected individuals of both species, the diet shifted to having chironomid larvae as the major constituent. So whilst some changes occurred within trophic levels, as neither species fed (in a measurable quantity) upon primary producers, no structural changes were observed in the food web as regards the relative contribution of producers and consumers to the higher trophic levels, and there were negligible changes in the biomass of the two fish species (Figure 6.3).

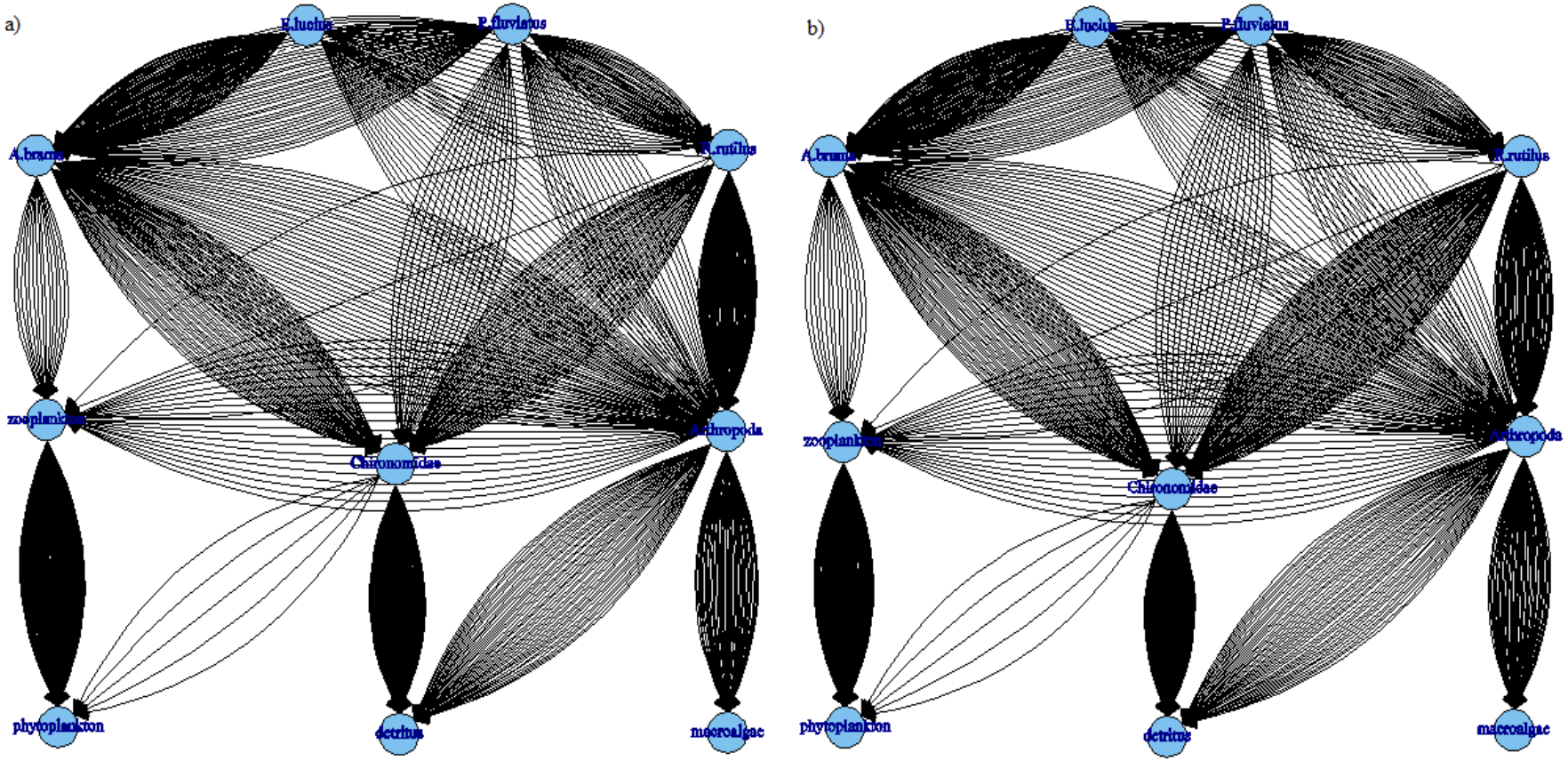

Figure 6.2 Example of weighted food webs created based on stable isotope feeding niche data. a) is a food web in which no *Rutilus rutilus* and *Abramis brama,* are infected with *Ergasilus briani* b) is a food web in which 100% of both *R. rutilus* and *A. brama* are infected with *E. briani*. Each line represents 1% of the species' or group's diet.

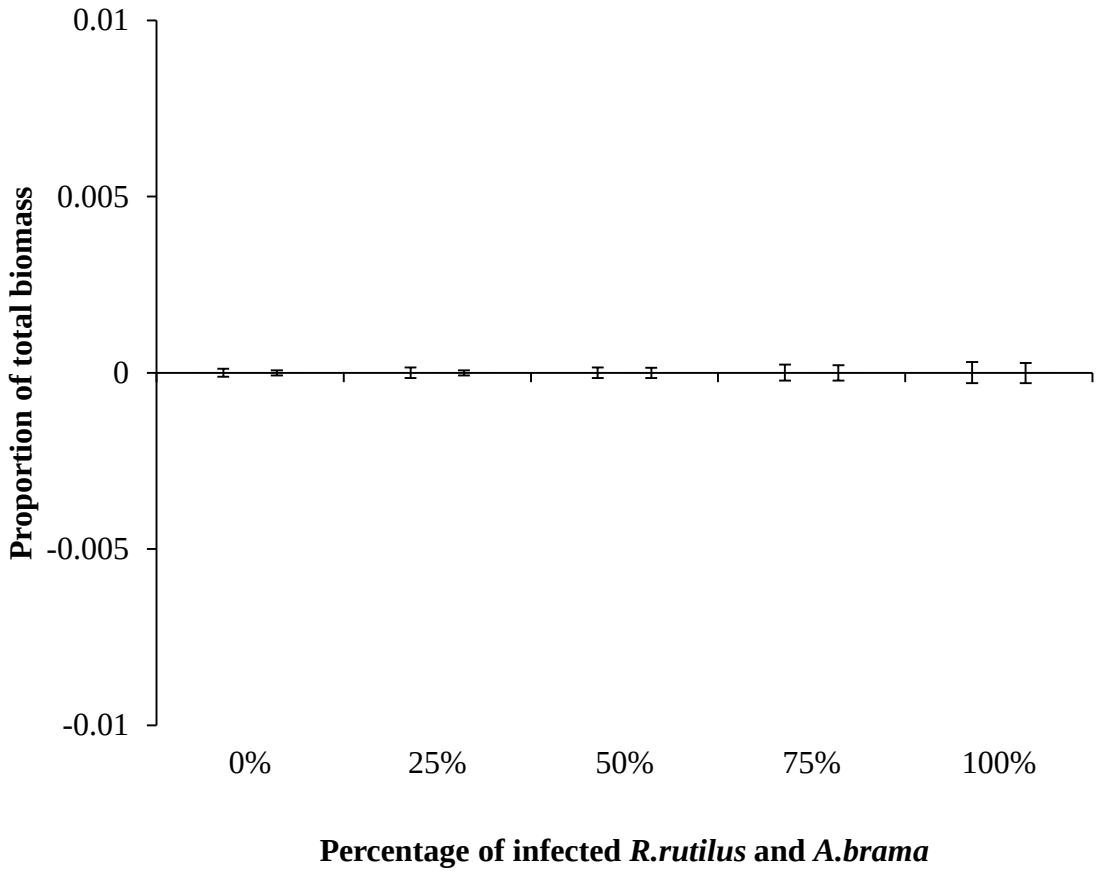

Figure 6.3 Changes in the proportion of the total biomass of the food web contributed by the first (producers) (dark grey bars) and second (primary consumers) (pale grey bars) trophic levels. Error bars represent 95% confidence intervals.

Scenario 2 environmental change with fixed numbers of parasites.

The scenario modelled here was a shift from the original system where macrophytes contributed 14% of the primary production, to one dominated by phytoplankton. This was achieved by deleting 25%, 50%, 75% and 100% of the macrophyte biomass from the original model web (i.e. the modelled system comprised 14%, 10.5%, 7%, 3.5% and 0% of the biomass provided by macrophyte, with this lost biomass replaced by phytoplankton biomass, ensuring the total biomass of the system remained constant).

Two initial food webs were developed, one in which no fish were infected with *E. briani* (i.e. 0% parasite prevalence) and a second with both *A. brama* and *R. rutilus* infected at the levels recorded in the field, 67% and 63% respectively (Section 2.4). The biomass of uninfected and infected fish of both species decreased with decreasing proportions of macrophytes (Figure 6.4 and, b), with a proportionally greater decline in *R. rutilus* biomass than *A. brama* biomass, and the reduction in both species being less in the infected populations than in the uninfected populations (Figure 6.4). This biomass reduction occurred due to a bottom-up change in the proportion of arthropods available to the fish, as their availability reduced as the macrophytes proportion reduced. As the uninfected fish consumed proportionally more arthropoda than the infected fishes, then their biomass was more impacted by the arthropod reduction. The infected fish fed more on chironomid larvae that fed upon detritus, and thus was less impacted by changes in macrophyte proportions.

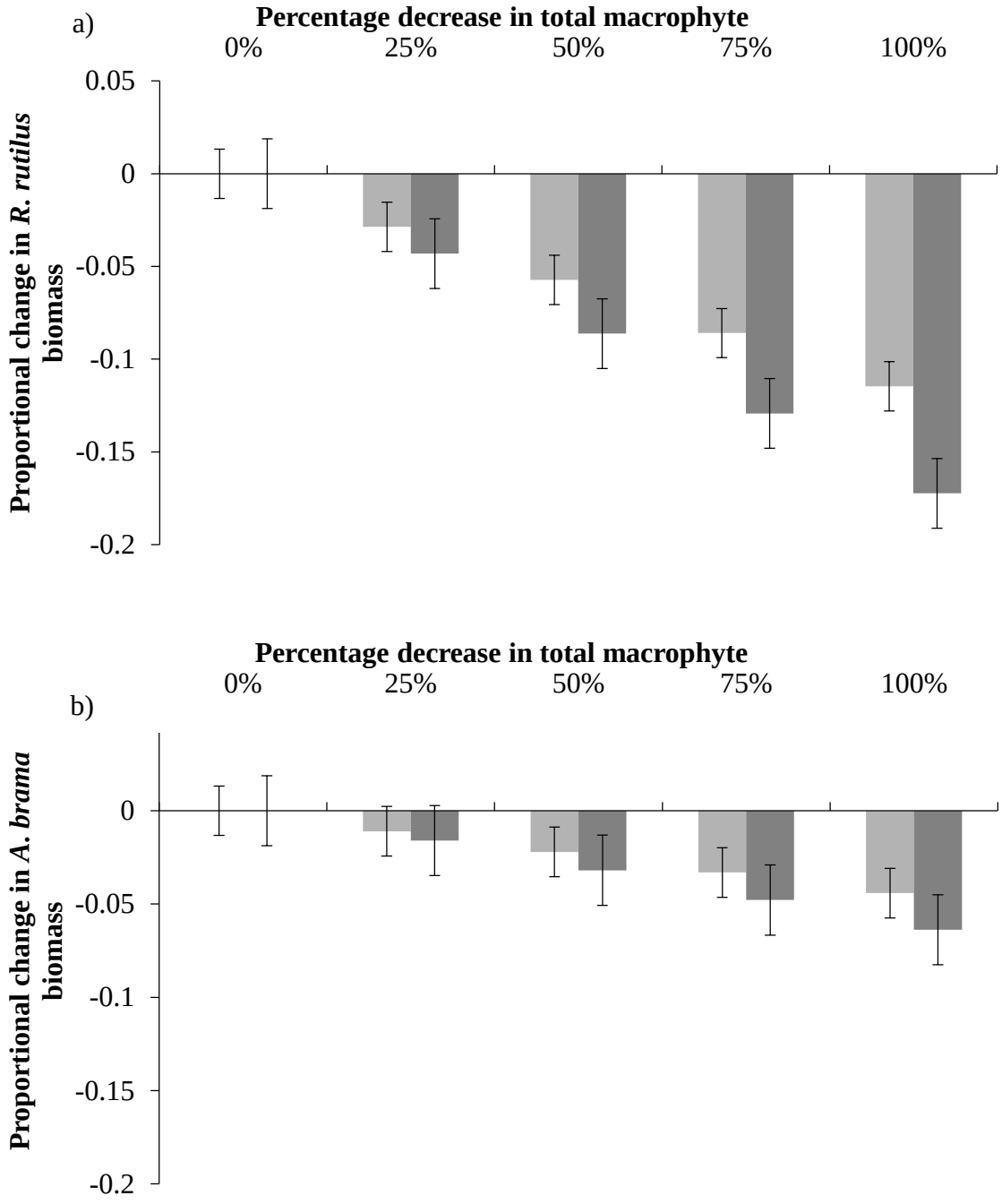

Figure 6.4 Proportional change (0-1) in species' biomass of a) uninfected *Abramis brama* (dark grey) and infected with levels of *Ergasilus briani* encountered in the study site on which the model is based (light grey); and b) uninfected *R. rutilus* (dark bars) and nfected with observed levels of *E. briani* encountered (light

grey) with changing macrophyte proportions. Error bars are 95% confidence intervals.

Scenario 3 - effects of changing environmental conditions versus changing parasite prevalence.

The scenario modelled here was a combination of Scenario 1 and Scenario 2, with reductions in macrophyte allied with changes in parasite prevalence, resulting in 25 modelled permutations (Table 6.2).

The predictions resulting from the scenario testing are similar to the pattern observed in the outputs of Scenario 2 (Figure 6.5). The eventual elimination of the macrophyte biomass results in declines in the *A. brama* and *R. rutilus* populations, but for both species the decline in biomass was less in parasitised fish, due to their greater reliance on chironomid larvae that were less affected by the changes in the primary producers.

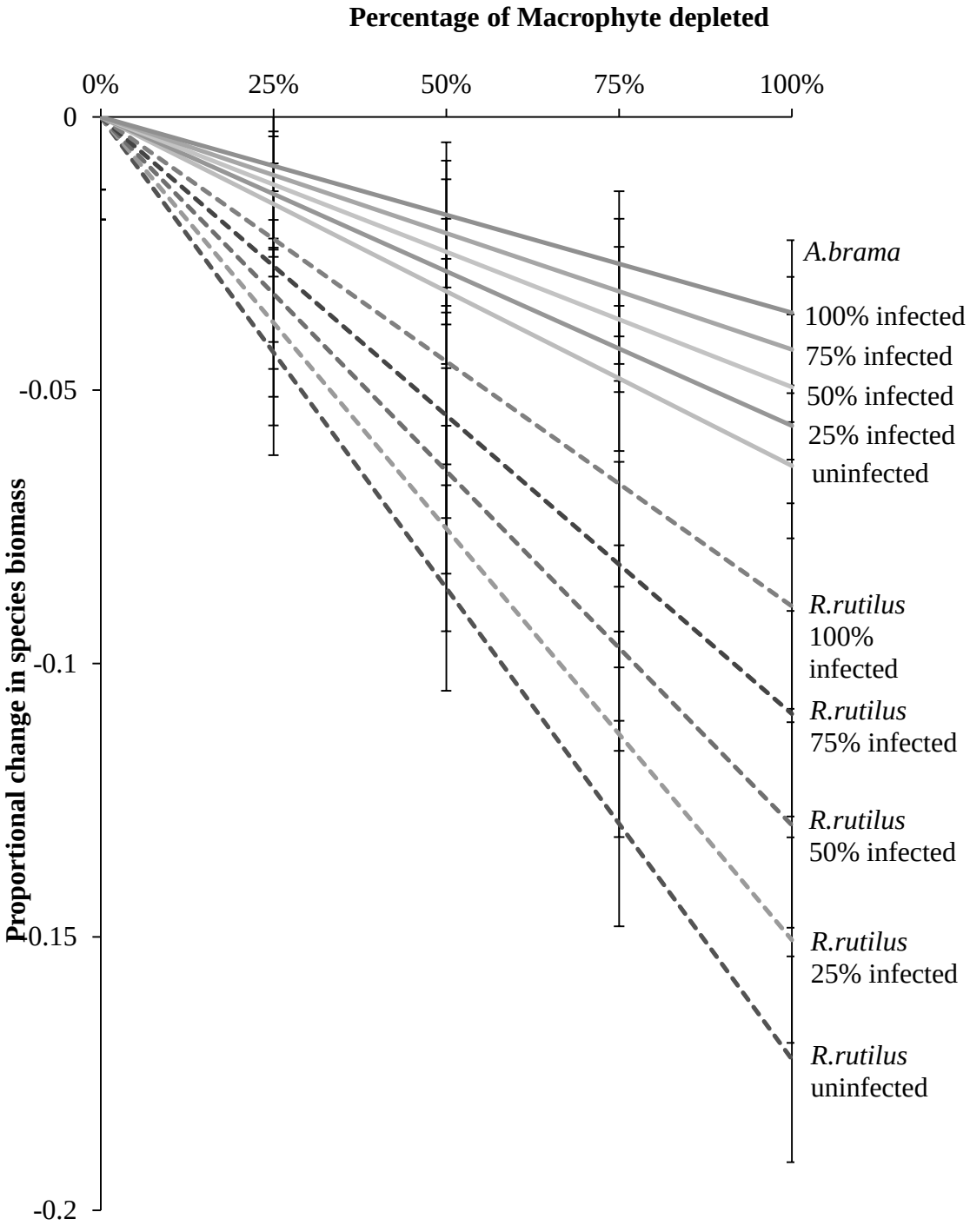

Figure 6.5 Proportional changes (0 to 1) of species' biomass, for *Abramis brama* and *Rutilus rutilus* populations with differing parasite prevalences and increasing proportions of macrophytes removed from the model. Error bars represent 95% confidence intervals.

6.4.2 Site 2: Bothriocephalus acheilognathi

Creating the weighted web

The simplified food web comprised of 8 nodes and 13 weighted links (Figure 6.6), of which the majority of links were weighted empirically, using the outputs of the stable isotope mixing models (Chapter 3). The remaining links were developed heuristically from published data. Table 6.4 summarises the mixing model outputs used in the completion of this web, with additional data supplied in Appendix 4.

Table 6.4 Summary of the Bayesian mixing models outputs predicting the proportions of each major food item to the diet of *Scardinius erythrophthalmus,* and infected and uninfected *Cyprinus carpio.*

Species	Food item	Modelled diet proportion (± SE)	
S. erythrophthalmus	Arthropoda	0.46 ± 0.04	
	Plankton < 250μm	0.24 ± 0.04	
	Plankton > 250μm	0.11 ± 0.03	
	Macrophyte	0.19 ± 0.02	
C. carpio		Uninfected	Infected
	Arthropoda	0.50 ± 0.04	0.26 ± 0.04
	Plankton <250μm	0.21 ± 0.03	0.41 ± 0.06
	Plankton > 250μm	0.29 ± 0.04	0.33 ± 0.06

The matrices created using the stable isotope proportions are supplied in Appendix 5. These were used to construct simple weighted models (e.g. Figure 6.6) in which each link in the model represents 1% of the organism/ group's diet. Thus, in Figure 6.6,

the shift between the diet of an infected and uninfected *C. carpio* from one favouring arthropods, to one favouring phytoplankton, can be observed via the change in link density.

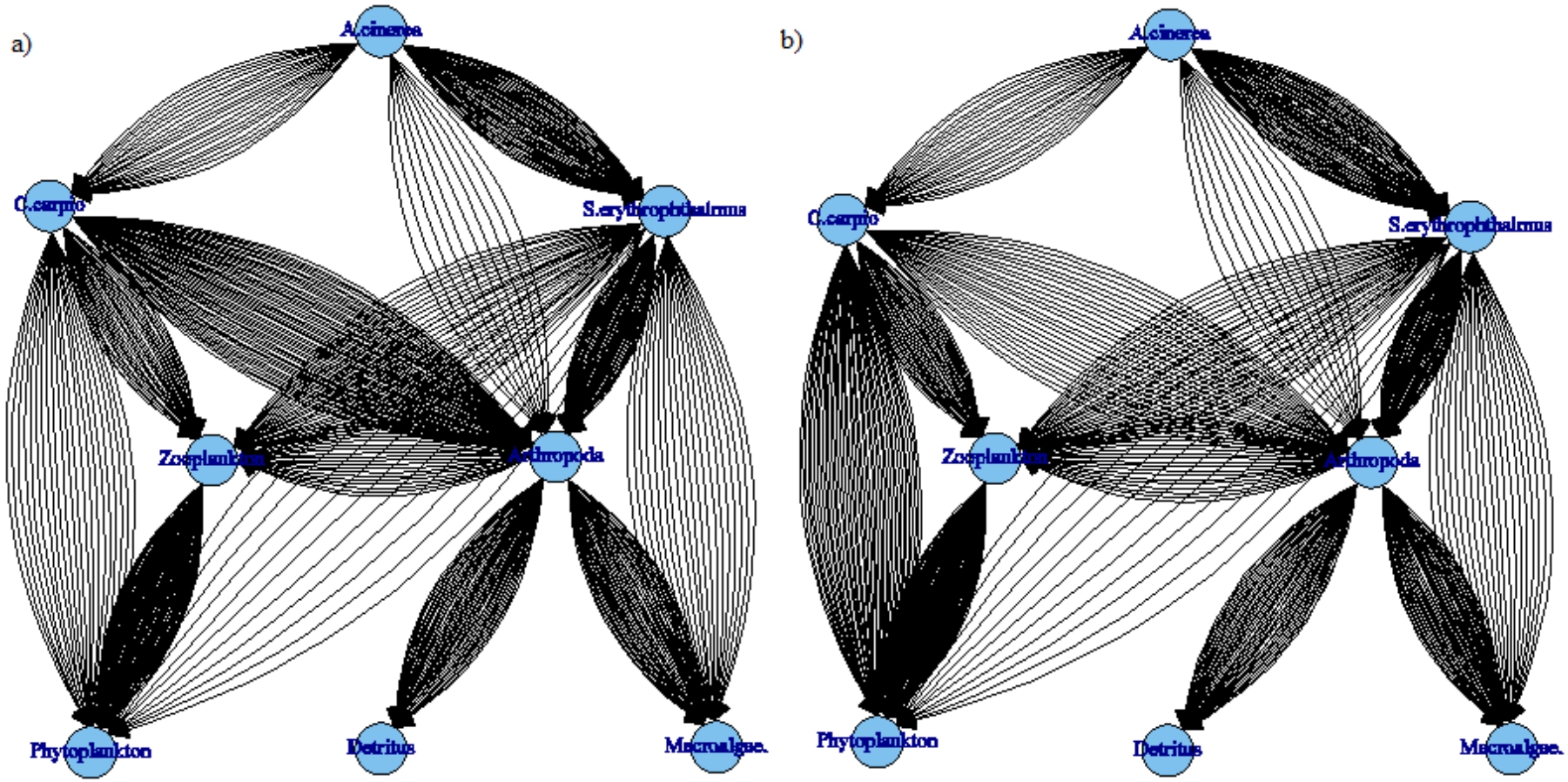

Figure 6.6 Example of weighted food webs created based on stable isotope feeding niche data. a) is a food web in which no *Cyprinus carpio*, are infected with *Bothriocephalus acheilognathi* b) is a food web in which 100% of *C. carpio* are infected with *B. acheilognathi*. Each line represents 1% of the species' or group's diet.

Scenario 1: Changing parasite prevalence under constant environmental conditions

In this scenario, fish biomass was maintained at the original level whilst differing the levels of parasite prevalence in the population of *C. carpio*, specifically at 0%, 25%, 50%, 75% and 100% prevalence. No changes were made to other higher consumers *S. erythrophthalmus* and *Ardea cinerea*, thus the biomass of fish and birds remained constant in the modelled scenarios. As empirical data had suggested infection by *B. acheilognathi* resulted in a dietary shift from arthropod dominated diet to phytoplankton being the most consumed item then, assuming a closed system, from a food web perspective this meant the structure shifted, with the first trophic level contributing an incrementally greater proportion of the total biomass as parasite prevalence increased (Figure 6.7). Concomitantly, the proportion contributed to total biomass by the second trophic level decreased.

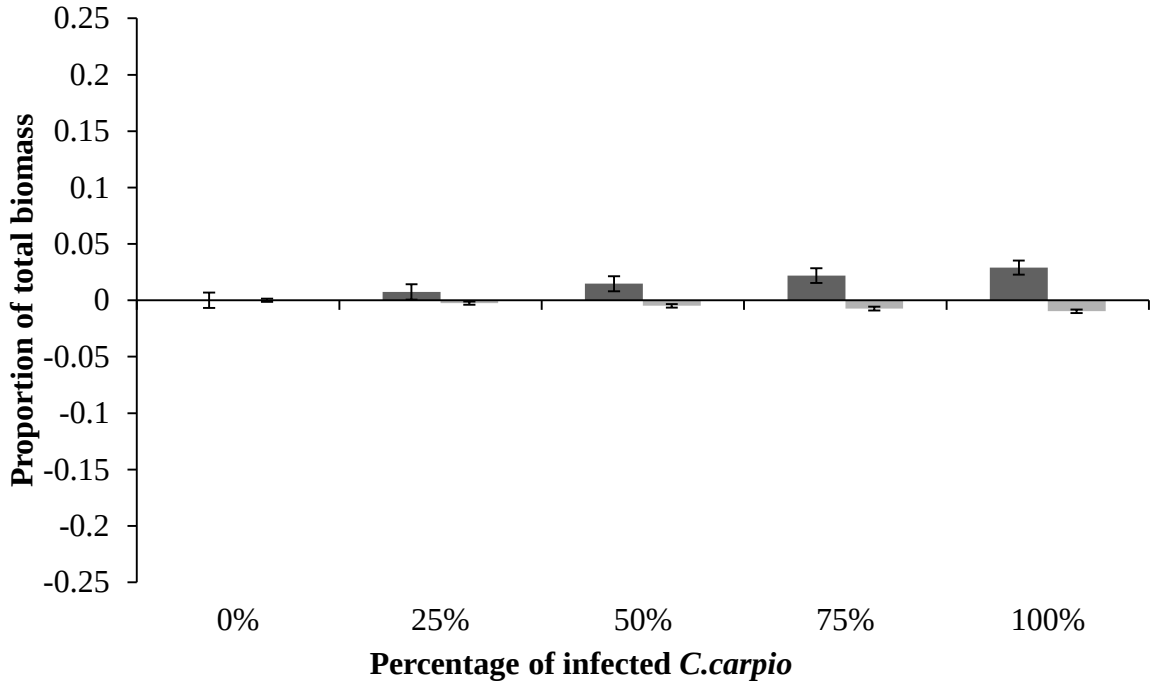

Figure 6.7 Changes in the proportion of the total biomass of the food web contributed by the first (producers) (dark grey bars) and second (primary consumers) (pale grey bars) trophic levels. Error bars represent 95% confidence intervals.

Scenario 2 environmental change with fixed numbers of parasites.

The scenario modelled here was a shift in from the original system where macrophytes contributed 26% of the primary production, to one dominated by phytoplankton. This was achieved by deleting 25%, 50%, 75% and 100% of the macrophyte biomass from the original model web (i.e. 26%, 19.5%, 13%, 6.5% and 0% of the biomass was provided by macrophyte). The impact these changes had on the rest of the food chain was calculated.

Two initial food webs were developed, one in which no *C. carpio* were infected with *B. acheilognathi* (i.e. 0% parasite prevalence) and a second with 61% of *C. carpio* infected - the level recorded in the field (Section 3.4). The biomass of both uninfected and infected *C. carpio* increased with decreasing levels of macrophytes (Figure 6.8a), but increased more in infected fish than uninfected fish. This was because the infected fish fed to a greater extent on phytoplankton, which increased as macrophyte decreased, whilst the diet of uninfected fish had a smaller portion of macrophyte, and a larger portion of arthropods – a group which fed on macrophyte, and therefore declined as a consequence of the decline in macrophyte biomass. As arthropods comprised the majority of the diet of *S. erythropthalmus* then their population biomass decreased as macrophyte decreased.

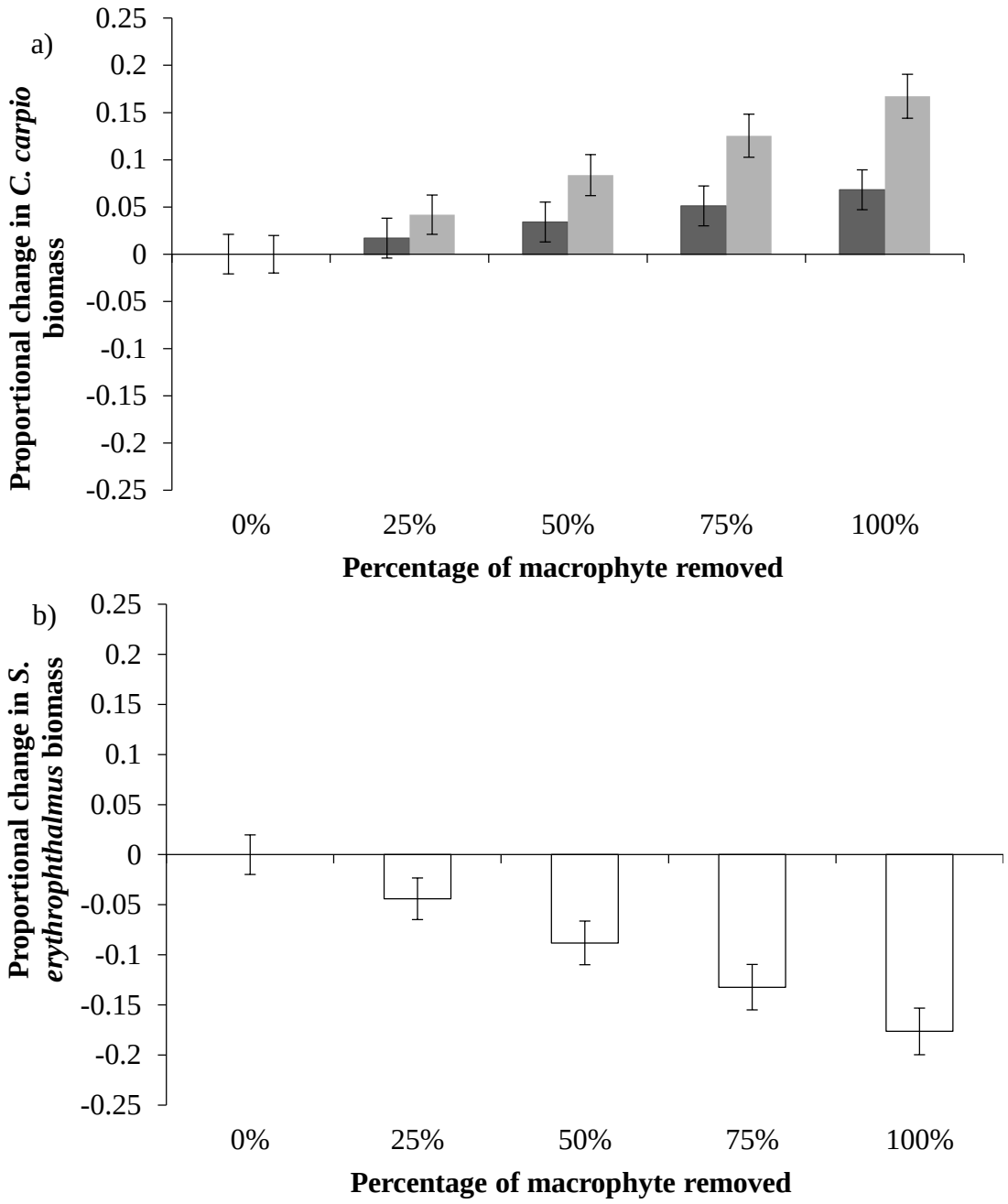

Figure 6.8 Proportional changes in a) uninfected *Cyprinus carpio* population biomass (dark grey bars) and *C. carpio* population biomass where with 61% of fish were infected with *Bothriocephalus acheilognathi* (light grey bars), and b) *S. erythropthalmus* (clear bars), with increasing percentage of macrophyte removed from the model. Equal biomass of phytoplankton was added so total biomass of producers remained constant. Error bars represent 95% confidence intervals.

Scenario 3 - effects of changing environmental conditions versus changing parasite prevalence.

The scenario modelled here combined Scenario 1 and Scenario 2, with reductions in macrophyte allied with changes in parasite prevalence, resulting in 25 modelled permutations (Table 6.2).

Two distinct patterns were clear. Firstly, in all cases, reducing macrophyte and proportionally increasing phytoplankton increased the overall biomass of *C. carpio* (Figure 6.9). Secondly, this increase was proportionally greater in the infected populations. Thus, the scenario in which the highest biomass of *C. carpio* was predicted was one in which all fish were infected and all macrophyte was removed. In this case, the predicted total biomass of *C. carpio* was approximately 24% higher than that of the original system due to the higher reliance of the infected fish on phytoplankton in their diet (Table 6.4; Figure 6.9).

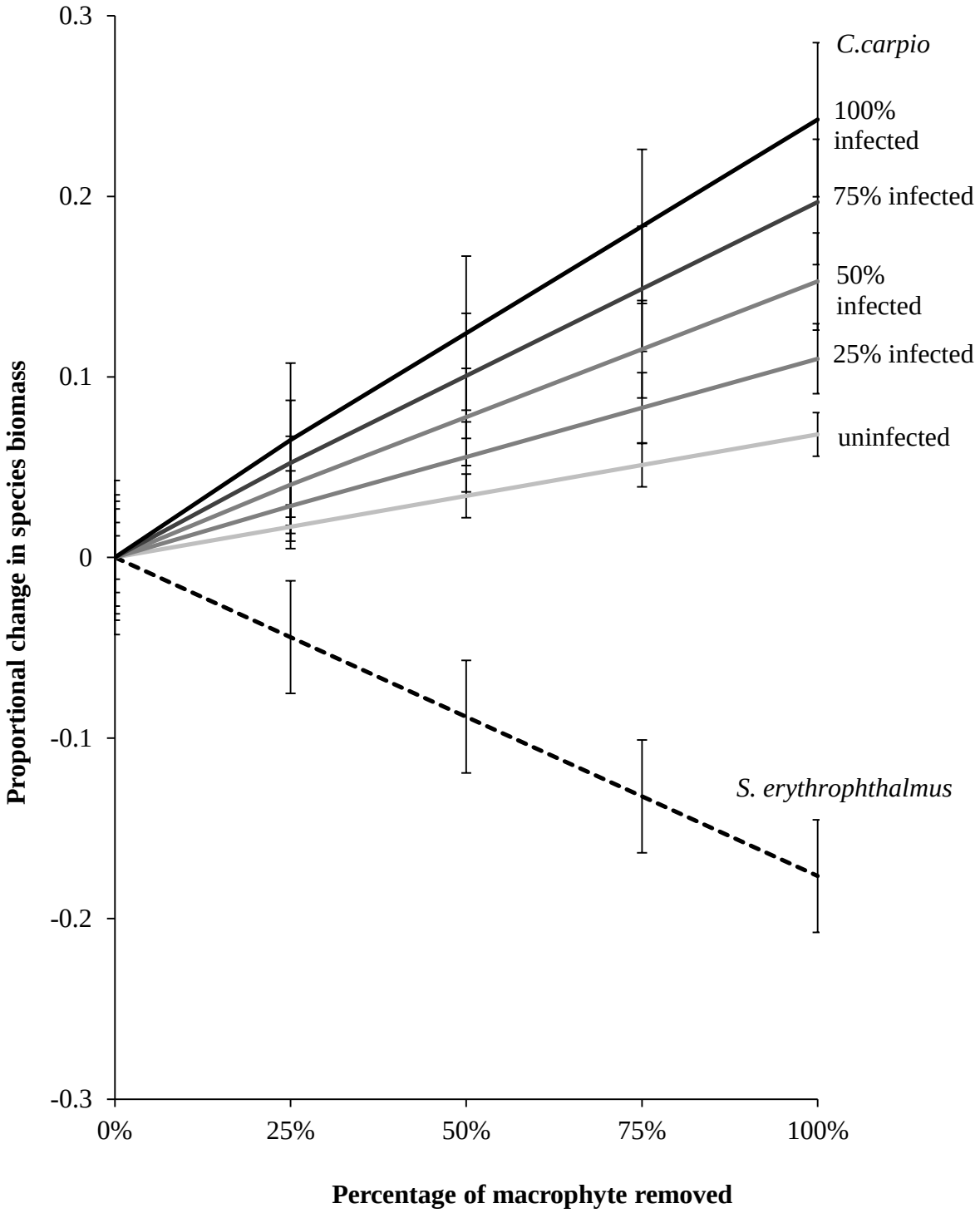

Figure 6.9 Proportional changes of species biomass, for *Cyprinus carpio* populations with differing infection levels and *Scardinius erythrophthalmus*, with increasing percentage of macrophyte removed from the model. Error bars represent 95% confidence intervals.

6.5 Discussion

Chapter 5 revealed that whilst the addition of native parasites to food webs greatly altered the food web topology, the addition of non-native parasites had a relatively minor effect, including when the non-native parasite was trophically transmitted. This, however, did not consider the effect of the non-native parasites on the trophic ecology of the host. Through incorporation of the information on how infection altered the trophic niche of the infected fishes, it was demonstrated here that the infections with non-native parasites can have more substantial consequences for the food web than demonstrated topologically. Building weighted food webs that utilised data on the parasite-mediated modified trophic niches of the host fishes demonstrated that parasites can have a substantial influence on how the fish population and community responds to environmental changes. Thus, the weighted models suggest that the host population and food web consequences of infection, that already include contributing biomass (Johnson et al. 2010), mediating competitive interactions (Hatcher et al. 2006) and moderating host populations (Dobson and Hudson 1995), also includes altering how hosts could respond to environmental changes.

Altering the parasite prevalence in the host populations had relatively minor consequences for their population biomass, with the consequences of environmental changes through eutrophication (modelled as decreased proportions of macrophytes) being more pronounced on both the uninfected and infected individuals. Indeed, alterations in water quality can have pronounced implications for parasite ecology (Lafferty and Kuris 1999), often resulting in improved conditions for parasites should their host density increase, with generalist fish species such as *R. rutilus*

usually being favoured by eutrophic conditions (Beardsley and Britton 2012; Elliott et al. 2015). Eutrophic conditions also influence parasite prevalence through the associated increased productivity that increase the abundance of intermediate hosts. For example, Beer and German (1993) outlined that eutrophication improved conditions for snails (intermediate host) that, when combined with escapee farmed ducks (final host), accelerated the life cycle of the digenean *Trichobilharzia ocellata*. Valtonen et al. (1997) also discussed how increasing eutrophication in lakes over time was associated with greater overall parasite species richness in two fish species, including *R. rutilus*. Consequently, the scenario of increased parasite prevalence and anthropogenic eutrophication is realistic in the context of the host populations used and thus the model outputs should have relatively wide application to freshwaters and their fish communities.

The interaction of environmental change with increased parasite prevalence for *C. carpio* infected with *B. acheilognathi* resulted in significantly increased biomass in infected individuals. Although counter-intuitive, the energetic effects of *B. acheilognathi* can be relatively minor to host fishes, particularly once they attain lengths at which they only act as reservoirs of infection (e.g. >100 mm (Britton et al. 2012), with mortality incurred by the parasite being primarily in fishes < 50 mm (Britton et al. 2011). Moreover, whilst reduced condition in infected individuals was observed over time in this research, this only developed over a 12 month time frame. For *E. briani*, although all scenarios of eutrophication resulted in reduced biomass of the fish populations, this was reduced in the infected individuals. Again, whilst this parasite can cause mortality in hosts, this can be size-selective, with smaller hosts being more susceptible (Dezfuli et al. 2003; Linder et al. 2012), and thereafter, the

consequences of infection appeared relatively minor in this study, with no differences detected in condition between infected and uninfected fish. Consequently, this suggests that providing the infection by these parasites for host fishes is not at a life stage that results in their mortality, then the sub-lethal consequences of infection can actually result in increased biomass of infected individuals due to an increase in the availability of their preferred food types due to environmental changes.

That infection was predicted to increase population biomass as environmental conditions degraded was a consequence of their parasite-mediated altered trophic niche, with fish infected with *E. briani* tending to consume food items of lower motility and those infected with *B. acheilognathi* selecting items small enough to either easily consume or to pass through their partially blocked intestine. The increased biomass of phytoplankton that occurs in lakes through eutrophication (Smith et al. 1999) thus provides the *C. carpio* infected with *B. acheilognathi* with a food resource that is likely to be unlimiting. For *R. rutilus* and *A. brama* infected with *E. briani*, their principal feeding on chironomid larvae, a food resource that was not impacted directly by the altered conditions and would most likely thrive in the eutrophic conditions (Langdon et al. 2006), resulted in their increased biomass. Thus, providing the hosts were able to survive and tolerate the parasite infections, they were able to then have some resilience to this aspect of environmental change.

The models predicted that the uninfected fishes would either have increased declines in their biomass in eutrophic conditions (*R. rutilus* and *A. brama*) or increase in biomass but at a lesser rate than infected fish (*C. carpio*). The model could not,

however, incorporate diet switching in the uninfected fishes and so could not reflect any changes in their diet that would most likely occur as their food resources changed. Indeed, eutrophication is frequently associated with alterations in fish diet, such as through changes in prey size structure (Hayward and Margraf 1987) and prey species (Winfield et al. 2012). Consequently, it is likely that considerable alterations in the diet composition of the uninfected fish would occur with the onset of eutrophic conditions and it is likely that this would ensure that their responses to the altered conditions were as equal, if not higher, than for the infected fishes. Thus, whilst it can be argued that the predictions for the infected fishes were robust and ensured their survival in the face of the changes, some caution is needed when comparing their output to the uninfected fishes, especially given the plasticity in diet observed in generalist cyprinid species such as *R. rutilus* and *C. carpio* (Kahl and Radke 2006; Britton et al. 2007; Estlander et al. 2010). Nevertheless, these outcomes reveal the high utility in developing weighted models to predict the outcomes of changes in parasite prevalence and environmental change on fish populations and communities that are affected by introduced parasites, and indicate that their outcomes can be counter-intuitive, with the altered trophic niches of hosts caused by infection providing some subsequent benefits that ensure they are able to take advantage of the new conditions.

7. Discussion

7.1 Introduced parasites (Chapter 1)

Introductions of free-living species are often accompanied with the release of their parasites (De Silva et al. 2006; Gozlan et al. 2006; Gozlan et al. 2010). In fisheries, this often occurs most commonly via the movement of fish or eggs for aquaculture purposes (De Silva et al. 2006; Peeler et al. 2011). Whilst many non-native parasites are lost during the introduction process (Colautti et al. 2004), those that are released into new environments have the potential to cause significant harm to their hosts (Poulin et al. 2011). Whilst infections are known to cause mortality and high morbidity in their hosts (Bovo et al. 1987; Gozlan et al. 2005; Johnson et al. 2010), there has been less attention paid to their sub-lethal ecological consequences, despite the important roles that native parasites are known to play as ecosystem engineers and within food webs (Mouritsen and Poulin 2003; Dobson et al. 2006; Hatcher et al. 2006). Thus here, through use of three host-parasite models, with those three parasites having differing lifecycles from simple direct transmission to complex multi-host lifecycles, the ecological consequences of parasites introduced into the UK was investigated through their effects on hosts (from individual to population effects) and food web structure, the results are summarised in Table 7.1.

Table 7.1 Summary of impacts revealed in this study in infected hosts, and infected communities for the three focal parasites, related to the thesis's research objectives (Section 1.10).

	Objective					
Host/Parasite system	O1 Pathology	O1 Host growth and condition	O2 Trophic niche width	O2 Trophic position	O3 Topological web impact	O4 Weighted web impact
E. briani in *R.rutilus* and						
A. brama	✓	×	✓	×	×	✓
B. acheilognathi in *C. carpio*	✓	✓*	×	✓	✓	✓
A. crassus in *A. anguilla*	✓	×	×	✓	✓	-

* Significant difference observed only after extended period of infection

7.2 Individual host consequences of non-native fish parasites (Chapters 2, 3 and 4)

7.2.1 Pathology

Infections of all three parasites resulted in noticeable pathological effects on host fishes. In Chapter 2, *R. rutilus* and *A. brama* infected with *E. briani* were examined and the gross pathological changes included displacement of gill filaments, hyperplasia and localised haemorrhaging within the filaments as a consequence of parasite attachment, as well as localised loss and compression of gill epithelium attributed to parasite feeding. These findings were consistent with pathological changes associated with other Ergasilid parasites (Alston and Lewis 1994; Dezfuli et al. 2003). In Chapter 3, the pathology of *B. acheliognathi* infection in juvenile *C. carpio* was described. During dissections, the parasite was often visible as a large solid mass in the intestine prior to its opening. Within the intestine, at the point of attachment, the scolesces of the parasites pinched the intestinal folds, compressing the epithelium and, in places, almost exposing the basement membrane. Heavy infections caused near complete occlusion of the intestinal tract, thinning and compressing the gut wall, and displacing internal organs, including the swim bladder. These outcomes were consistent with reported impacts of *B. acheliognathi* (Britton et al. 2011b). In Chapter 4, the pathology of *A. crassus* in *A. anguilla*, two specific stages of infection were observed. The initial stage was where parasites were present, often in large numbers and occupying the swimbladder. The second stage was following the departure of the parasite when the swimbladder walls were left scarred and opaque, as also noted from other studies (Lefebvre et al. 2002; Kirk 2003).

7.2.2 Host growth and condition

Infection by the non-native parasites appeared to have only minor consequences for the growth (as differences in 0-group fish length) and condition of individual hosts in two of the three focal host/parasite systems. For *R. rutilus* and *A. brama* infected with *E. briani* (Chapter 2), and *A. anguilla* infected with *A. crassus* (Chapter 4), there were no significant differences in body length and condition, and hepatosomatic index (*A. anguilla* only), between the infected and uninfected individuals. In Chapter 4, by monitoring a cohort of juvenile *C.carpio* infected with. *B. acheliognathi* over a 12 month period, substantial and significant changes were, however, detected that developed over time. Whilst there were no significant differences in length of infected and uninfected fish on initial sampling, this altered after 12 months, with lengths of infected individuals now being significantly smaller than uninfected. Similarly, in initial samples, differences in condition (as Fulton's condition factor, K) between infected and uninfected fish were not significantly different, but were by month 12. This highlights the potential requirement to measure infection consequences over long-time periods, and suggests that the lack of differentiation observed in the other parasite: host systems might have been related to only taking samples on discrete occasions.

7.3 Trophic consequences of infection at the population level (Chapters 2 and 3)

Chapters 2 and 3 demonstrated how infection by non-native parasites could induce significant but differing changes in the trophic niche of the infected component of a host population. In Chapter 2, niche constriction was apparent in the infected components of the *R. rutilus* and *A. brama* populations, with this being consistent

across the different sites studied. Chapter 3 revealed that the trophic niche of *C. carpio* infected with *B. acheilognathi* differed significantly from that of uninfected fish, with a distinct shift in resource utilisation that increased the trophic niche of the overall population. Stable isotope mixing models predicted these changes occurred through the diet of infected *R. rutilus* and *A. brama* becoming less diverse and more focused on less motile food items, whilst for infected *C. carpio*, their diet shifted from one with a high arthropod content to one more dependent on phytoplankton.

Optimum foraging theory predicts that animals will feed on the most valuable resources, ignoring lower-value resources when search and handling time could be better spent searching for more valuable resources (Bolnick et al. 2003). The factors acting in this process are the resource traits and phenotypic capacity of individuals to capture, handle and to digest those resources (Araujo et al. 2011). Thus, niche variation between individuals is largely dependent on the diversity and abundance of available resources versus the phenotypic traits of the individual (Crowden and Broom 1980; Stephens and Krebs 1986). Here, it was suggested that the parasite infection was acting as a trait that exerted a strong influence on their niche variation. Moreover, the functional response of a consumer is the relationship between prey density and prey consumption (Holling 1959), thus is a useful descriptor of predator behaviour and their impacts on prey populations (Dick et al. 2010), with a previous study on young-of-year *C. carpio* detecting a reduced functional response in individuals infected with *B. acheilognathi* compared with uninfected individuals (Britton et al. 2011b). Infected fish had higher handling times and longer searching times for food, potentially providing some explanation for the patterns observed here. The determinants of these remain uncertain, but potentially relate to the

parasite blocking the intestine and in doing so, reducing feeding motivation, and food and energy intake (Scott and Grizzle 1979; Britton et al. 2011a).

Although the causal mechanisms behind the niche constriction measured in *R. rutilus* and *A. brama* infected with *E. briani* can only be speculated as they were unable to be tested here, other studies suggest that infections by other Ergasilid parasites that result in similar gill damage have consequences of respiratory dysfunction, osmoregulatory failure and haematological disruption (Hogans 1989; Abdelhalim et al. 1991; Alston and Lewis 1994; Dezfuli et al. 2003). Thus, the reduced ability of infected fishes to access the same resources as uninfected ones might relate to their reduced foraging abilities caused by such issues. Irrespective of their underlying mechanisms, in both cases it was apparent that infected fishes increased their predation of prey items that were highly abundant and/ or relatively slow moving, and thus presumably required relatively lower energy expenditure to capture and handle during foraging.

7.4 Does trophic niche impact the probability of infection? (Chapter 4)

Phenotypic differences in behaviours are frequently reported between individual fish uninfected and infected with specific parasites (Barber et al. 2000; Loot et al. 2001). This, however, tends to be more in the context of parasite-induced changes to the host post-infection (Blanchet et al. 2009). Chapter 4 demonstrated an alternate scenario, whereby the host phenotype influenced their probability of infection. Within populations of *A. anguilla*, *v*ariation in head morphology is common, with individuals on a spectrum between broad-headed and narrow-headed (Lammens and Visser 1989; Proman and Reynolds 2000; Tesch 2003). These differences in head

morphology have been related to individual specialisation, with broader-headed *A. anguilla* individuals being more piscivorous (Cucherousset et al. 2011). The parasite *A. crassus* has multiple paratenic hosts in its invasive range resulting in elevated parasite exposure in piscivorous animals, including *A. anguilla*, the definitive European host. Thus, the eels with broader head widths have increased probability of infection by *A. crassus*, as they have greater exposure to the parasite through consuming higher proportions of paratenic fish hosts. Indeed, the logistic regression model revealed head morphology and diet were significant predictors of infection status, with up to 78 % of eels correctly assigned to their infection status in models (Section 4.4).

7.5 Infectious food webs (Chapters 5 and 6)

Chapter 5 and 6 illustrated the utility of food web structure to investigate the consequences of additions of new parasites into aquatic communities (Dunne et al. 2002; Petchey et al. 2008; Amundsen et al. 2013). These chapters also illustrated how data derived for food webs can be applied in different ways with consequent contrasting outcomes, i.e. the topological versus weighting approaches. In Chapter 5, topological changes were modelled going from food webs including all parasites (including non-native) and free-living species, to ones where only free-living species were modelled. Several factors were identified as critical to the scale of impact caused by introduced parasites to web topology, including host specificity, complexity of lifecycle and the extant diversity of the communities being invaded. When *A. crassus*, a parasite with a complex lifecycle, was present in a relatively diverse fish and native parasite community, their effect on topological metrics were reduced compared to *B. acheilognathi* when their host population was within a

relatively simple fish community, despite a similarly complex lifecycle. Whilst the connectance, nestedness and chain length of the food webs were all altered by the addition of the non-native parasites, the magnitude of that change was, in all cases, far less than the change caused by the addition of native parasites to a non-parasitised food web.

In Chapter 6, the dietary data produced in Chapters 2 and 3 were incorporated into simplified versions of the topological webs from Chapter 5 to create weighted webs, and those weighted webs were then applied to test the outcomes of a series of scenarios that tested outcomes of changes in parasite prevalence and nutrient enrichment. In contrast to the food web topology, the weighted models revealed how even a single introduced parasite with a simple direct lifecycle can have substantial food web level effects. Where infection resulted in its host feeding at a lower trophic level, the entire structure of the web shifted, with the biomass of the first trophic level increasing proportionally to the second. Chapter 6 further demonstrated how the conditions of eutrophic systems could be beneficial to infected hosts, which tended to feed on abundant food items of lower nutritional status. Thus, providing that the hosts were able to survive and tolerate infections, they then had some resilience to this aspect of environmental disturbance. This interaction suggests that the effects of global changes, such as anthropogenic eutrophication and introduced species, could have counter-intuitive consequences for fish communities via their interactions that result in additive or synergistic outcomes.

7.6 Management of non-native parasites

Freshwater fish in the UK are a valuable resource. Considering specifically the species studied in this research, export figures from Britain for elvers and mature *A.anguilla* are £3.5 and £2.75 million per annum, respectively (Peirson et al. 2001). Meanwhile the value of recreational sport fishing, for species including *C.carpio*, *R.rutilus* and *A.brama* in the UK is valued in the region of £1 billion (Hickley and Chare 2004). In addition, inland fisheries have great value in terms of existence value, rural economics and the social benefit of urban fisheries (Peirson et al. 2001). Thus there is considerable need to protect stocks against potentially harmful novel parasites. In practice this is balanced against the benefits of stock movement and enhancement, and the practicalities of management and enforcement of any restrictions (Hickley and Chare 2004). Whilst predicting the impact of a non-native species is difficult (Manchester and Bullock 2000), the findings of this study add new information to the body of evidence available for decision makers governing UK fisheries management. Previous to this study, risk assessments for non-native parasites considered the potential impact that parasite may have on its host (Williams 2007; Williams et al. 2013). However this study has demonstrated that even in scenarios where infection may not appear to have marked consequences for the growth or condition of a host, and thus the parasite appears benign, this can be a superficial assessment, as there might be trophic consequences apparent that subsequently manifest as wider consequences at the food web or even ecosystem level. Thus, this research has highlighted that in considering the issues of non-native parasites, looking beyond immediate host pathological and energetic consequences and looking at wider ecological perspectives can provide contrasting evaluations of impact.

These aspects are important to consider in a management context given that controlling the distribution and spread of introduced parasites is inherently difficult in wild situations (Hoole et al. 2001). Unlike in aquaculture systems, treatment via medical interventions is not feasible (Ward 2007) and, irrespective, there would be a high risk of potentially serious side effects on native invertebrate fauna (Kolodziejska et al. 2013). Thus, in lentic situations at least, available options are limited to either dewatering and removing all fish to eliminate all the parasite life stages, or accepting a degree of parasitism and managing the infected stock (Simberloff 2009; Davies and Britton 2015). In lotic situations, arguably only the latter option is available in a disease context (Williams et al. 2013), although introduced *G. salaris* has been managed successfully in Norwegian rivers using a biocide approach (Johnsen and Jensen 1991; Cable et al. 2000). Under present legislation, the movement of fish infected with the three parasites used in this research to online waters in England and Wales is prohibited (Agency 2015). Any such prohibition has financial implications for the fish movement industry (Williams et al. 2013) and, therefore, ought not to be taken lightly. However, the results of this study tend to support the continued control of *E.briani, B.acheilognathi* and *A.crassus*. Furthermore, these results suggest that the consideration of wider, non-lethal consequences of non-native parasites that move beyond individual pathology and condition assessments should be incorporated into the decision-making and risk-assessment processes.

For fishery managers, knowledge of parasite behaviour is already used in a disease management context, when spread of trophically transmitted parasites is controlled

by elimination of intermediate hosts. For example, infections of diplostomatid eye-flukes are controlled in aquaculture situations by controlling snail populations (Chappell 1995) and prevention of contact between gulls and farmed fish can reduce the spread of the digenean *Cryptocotyle lingua* (Kristoffersen 1991). The research presented here provides evidence on how manipulation of the physical habitat and food resources could be manipulated in a way as to limit parasite transmission. For example, Chapter 6 highlighted how a eutrophic system suited the diet of infected hosts, thus it could be construed that a relatively undisturbed system would be less favourable, thus a simple measure of maintaining relatively high macrophyte abundances with a diverse macroinvertebrate fauna could create an environment that could potentially support a greater proportion of fish that remain uninfected by the non-native parasites.

7.7 Potential short-comings of the research approach

In all cases in this research, the number of fish populations studied per non-native parasite was limited and the sample sizes often relatively limited. This was the result of logistical and financial constraints, low numbers of known fish populations infected with some of the parasites, and problems in obtaining permissions to remove large sample sizes of fishes of unknown infection status at the time of collection, especially *A. anguilla* as these have recently been assessed in the IUCN Red List as critically endangered (Jacoby and Gollock 2014). The three model parasites were chosen as they were all introduced into the UK and have differing complexities in their lifecycles (Section 1.9), yet the fact that they all occupied different hosts and those hosts occupied different habitats could confound the ability to make strong comparisons between them. Furthermore, in terms of data collection,

only the consequences of *B. acheilognathi* on their hosts were able to be measured over an extended time period. Nevertheless, it can also be argued that this approach still provided some extremely insightful outcomes that were then used as the basis for modelling approaches that resulted in substantially increasing the extant knowledge on these parasite-host systems and their consequences for freshwater food webs.

In this study, stable isotope analysis was used as the method to determine dietary differences in the fishes rather than more traditional dietary analytical tools, such as stomach contents analysis. The benefits of using stable isotope analysis are through its provision of a much longer temporal perspective on diet composition, with the timescale dependent on the tissues analysed (e.g. 4 to 6 months for muscle and fin-tissue; Jackson et al. 2012). It avoids the requirement for completing stomach contents analysis on cyprinind fishes that are agastric, thus have relatively long intestinal tracts that are often full of material whose contents are sufficiently masticated by the action of the pharyngeal teeth to make their accurate identification extremely difficult (Grey 2006). Had stomach contents analysis been used, then it would also have meant much larger sample sizes would have required collecting over much longer timeframes and at different times of day in order to ensure that dietary comparisons between infected and uninfected fish reflect their actual differences and are not biased due to sampling issues. Notwithstanding these issues, it is acknowledged that the diet composition of the fishes were estimated from mixing models rather than from direct observations, and mixing model performance is dependent upon the quality of data and knowledge used to build them (Phillips et al. 2014; Busst et al. 2015).

In addition, whilst infections by both *B. acheilognathi* and *E. briani* both resulted in differences in trophic niche between infected and uninfected fish, the mechanism by which these changes occurred were suggested but not tested further, and this remains an outstanding research requirement.

7.8 Future directions

As with any study based on wild population sampling, increasing the spatial and temporal replication of samples should ultimatly increase understandings of the results and identify where these have inherent context dependency versus general patterns that are ecologically relevant (Eberhardt and Thomas 1991; Kratzer and Warren 2012; Hadfield et al. 2014). In a regulatory context, there are currently seven 'Category 2' non-native parasites (those considered harmful) and seven novel parasite species (introduced and of un-assessed impact) in England (summarised in Table 7.2), providing many options to expand the scope of the research in terms of model parasite: host systems. These parasites include species that parasitise different hosts, have different host specificity and different lifecycle complexities (Table 7.2). Whether these factors lead to any overarching themes in terms of parasite impact is unlikely, but from a risk management perspective attempting to establish if this is the case could lead to better management of non-native parasites in the UK.

Table 7.2 Non-native Category 2 and Novel fish parasites in England, the complexity of their lifecyles, and specificity of their final hosts (adapted from Environment Agency 2015).

Fish host	Complexity of life cycle		Specificity of final host	
Pomphorhynchus laevis	Complex	Intermediate amphipod host	Non-specific	Salmonids and riverine cyprinid fish species
Anguillicoloides crassus	Complex	Intermediate crustacean hosts, multiple paratenic hosts	Specific	*Anguilla anguilla*
Monobothrium wagneri	Complex	Intermediate copepod hosts	Specific	*Tinca tinca*
Bothriocephalus acheilognathi	Complex	Intermediate copepod hosts	Specific	*Cyprinus carpio* and variants
Philometroides sanguineus	Complex	Intermediate copepod hosts	Specific	*Carassius carassius* and *Carassius auratus*

(Cont.)

Fish host	Complexity of life cycle	Specificity of final host	
Ergasilus sieboldi	Direct	Non-specific	Multiple salmonid and cyprinid fish species
Ergasilus briani	Direct	Non-specific	Multiple salmonid and cyprinid fish species
Lernea cyprinacea	Direct	Non-specific	Cyprinid species
Tracheliastes polycolpus	Direct	Non-specific	Multiple salmonid and cyprinid fish species
Tracheliastes maculates	Direct	Non-specific	Multiple salmonid and cyprinid fish species
Ergasilus gibbus	Direct	Specific	*Anguilla anguilla*
Pellucidhaptor pricei	Direct	Specific	*Abramis brama*
carp edema virus (CEV)	Direct	Specific	*Cyprinus carpio* and variants
Herpesvirus anguillae (HVA)	Direct	Specific	*Anguilla anguilla*

Additionally, a major finding of Chapter 3 was the importance of repeated observation of parasite impact over an extended timescale, a feature which could be incorporated into future studies but one that has distinct resource and logistical implications.

The decision to base the dietary analyses on stable isotope analysis was deliberate due to the reasons outlined in Section 7.8. When applied appropriately, it provides a powerful ecological tool that has been applied to a wide range of ecological questions, such as assessing the ecological impacts of non-native fishes (Cucherousset et al. 2012). Nevertheless, future studies could also incorporate some stomach content analyses to verify the outcomes. It should, however, be noted that studies that rely on both stable isotope analysis and stomach contents analysis often show contrasting outcomes, for example food items found in high abundance in stomach contents may in fact only be briefly temporally abundant therefore their overall contribution to the fishes diet may be over–represented, so due to the different timescales the results of the two methods can be contradictory rather than complementary (Locke et al. 2013).

As previous experimental studies have shown changes in functional response as a result of parasitism (Dick et al. 2010; Britton et al. 2012), then behavioural functional response models could be applied further to parasites, such as *E. briani*, in order to derive greater mechanistic understandings of the processes underlying the development of differences in trophic niche. This could then be supplemented by studies examining the physiological impacts of the parasite, for example by measuring haematocrit levels (e.g. Jones and Grutter 2005), or experimentally testing

the comparative excretion metabolites associated with stress such as ammonia (Buttle et al. 1996) and steroids (Pankhurst 2011).

Finally, the weighted models have much potential for refinement, addition and expansion. For example, the survival of all infected fish is assumed, yet both of the parasites used in the weighted models are known to result in some host mortality (Alston and Lewis 1994; Scholz et al. 2012). Consequently, models could be developed that build in mortality rates, although this would require further information on how the parasite results in host death, e.g. directly via pathological damage and/ or indirectly via energetic consequences that result from heavy infections. Similarly, modelling reactive changes into the diet of uninfected fish, to capitalise on increased abundances of non-preferred items would enhance the realism of the model, and provide a more representative insight into the competitive interactions of infected and uninfected conspecifics. Furthermore, the model outcomes have yet to be validated by empirical study, with controlled experiments in mesocosm contexts potentially providing systems where this could be completed. An example is provided by (Buck et al. 2015) who successfully used mesocosm experiments to demonstrate the community impacts of an amphibian parasite, revealing that contrary to their predictions the effects of nutrient supplementation and infection were additive rather than interactive. Thus, testing the impacts of the focal parasites in their fish hosts in a similar fashion could corroborate the model outputs or suggest areas of improvement, such that the model could have ultimately have greater research and management value as a predictive tool for assessing the potential impact of these parasites in future scenarios of environmental change.

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Appendix 1. Post-mortem examination methodology

Adapted from:

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

Detailed internal examination

The skin and body wall musculature is cut away to reveal the internal organs. The first incision is 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 discolouration, haemorrhaging, tumours, abnormalities, parasites etc., noted. Small pieces of each organ (approximately 2mm 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 should be carefully removed from the body cavity, noting any discoloration, haemorrhaging, fluid retention, necrosis, tumours, fat deposition, etc. The intestine is opened using a longitudinal cut and examined in PBS under a low power microscope, noting the contents and any abnormalities and parasites.

Gills

Gills are removed intact, by cutting each end of the branchial arches separately, and their general appearance and any abnormalities, e.g. Necrosis clubbing or haemorrhaging, noted. 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, a brief examination of the nasal cavity can be made under low power dissection microscope, and any abnormalities and 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. Lists of species and functional species used in topological food webs in Chapter 5

Table A2.1 Site 1 Species list

Free-living species	**Parasites**
Navicula sp.	*Ergasilus briani*
Scenedesmus sp.	*Diplozoan* sp.
Diatom spp.	*C. fennica*
Cladophora spp.	*Myxobilus* sp.
Marginal weed various spp.	*Myxidium* sp.
Detritus	*Philometra* sp.
Urotricha sp.	*Dactylogyrus* sp.
Paramena sp.	*Trypanoplasma* sp.
Khillomonas sp.	*C. laustrus*
Euglena sp.	*B. luciopercae*
Corixidae	*Triaenophalous* sp.
Annelida	*Myosporida* sp.
Chironomidae	*A.locii*
Cladocera	*Piscicola* sp.
Copepoda	
Assellidae	
Gammaridae	
Hydrobidae	
Valvatidae	
P. leniusculus	
A. cygnea	
Aerial insects	
Terrestrial insects	
A. brama	
R.rutilus	
P. fluviatilis	
E.lucius	

Table A2.2 Site 2 Species list

Free-living species	**Parasites**
Diatom spp	*A.platyrhynchos*
Marginal weed	*F.atra*
Cladophora spp.	*Apiosoma* sp.
Euglena spp	*Dactylogyrus* sp.
Amoeba	*Tricodina* sp.
Rotifer	*B. achaelognathi*
Cladocera	*Diplostomum* sp.
Cyclopoda	*Fasciolidae* sp.
Copepoda	
Gastropoda	
Chironomidae	
Baetidae	
Polycentropidae	
Asellidae	
C.carpio	
S.erythropthalmus	
A.cinerea	

Table A2.3 Site 3 Species list

Free-living Species	**Parasites**
C.demersum	*A. crassus*
E.nuttallii	*Contraceacum* sp.
L.minor	*Dactylogyrus* sp.
P.australis	*Diplostomum* sp.
S.emersum	*Diplozoan* sp.
Diatom spp.	*Eustrongylides* sp.
Phytoplankton spp.	*Gyrodactylus* sp.
Ciliate sp.	*Metorchris* sp.
Strombidium sp.	*Myxobolus* sp.
Peranema sp.	*P. abdominalis*
Dinoflagellate sp.	*Petersiger* sp.
Phacus spp.	*Philometra* sp.
Chilomonas sp.	*Rhapidicotyle* sp.
Euglena spp.	*T.clavata*
Copepoda	*Trichodina* sp.
Cyclopoda	*Myxidium* sp.
Valvatidae	*A.anguillae*
Hydrobiidae	*A.lucii*
Bithyniidae	*B.claviceps*
Physidae	*H.triloba*
Lymnaeidae	
Planorbidae	
Unionidae	
Sphaeriidae	
Oligochaeta	
Glossiphoniidae	
Hydracarina	
Gammaridae	
Assellidae	
Baetidae	

Caenidae

Coenagriidae

Corixidae

Haliplidae

Hydrophilidae

Leptoceridae

Chironomidae

A. anguilla

P. fluviatilis

E. lucius

A. brama

R. rutilus

R. rutilus x *A.brama* hybrids

S. erythropthalmus

B. bjoerkna

G. cernua

G. gobio

P. carbo

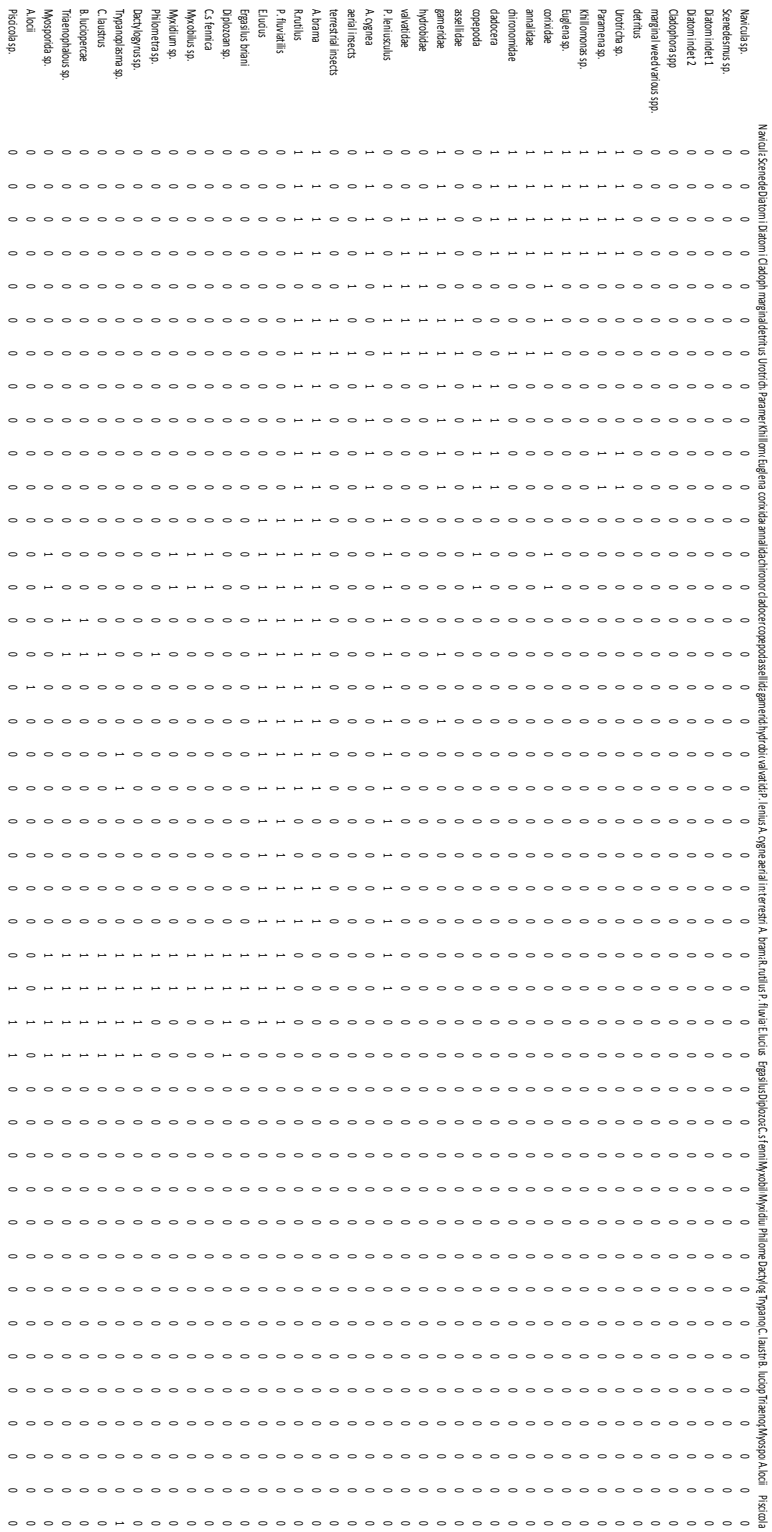
Larus sp.

T. ruficollis

L. lutra

Appendix 3. Food web matrices for topological webs in Chapter 5

Table A3.1 Site 1 Binary matrix



	Navicula sp.	Scenedesmus sp.	Diatom indet 1	Diatom indet 2	Cladophora spp	marginal weed various spp.	detritus	Urotricha sp.	Paramecia sp.	Khillomonas sp.	Euglena sp.	cornixidae	annalidae	chironomidae	cladocera	copepoda	asellidae	gammeridae	hydrobiidae	valvatidae	P. lenisculus	A. cygnea	aerial insects	terrestrial insects	A. brama	R.rutilus	P. fluviatilis	E.lucius	Ergasilus briani	Diplozoan sp.	C.s fennica	Myxobilus sp.	Myxidium sp.	Philometra sp.	Dactylogyrus sp.	Tryparnaga brama sp.	C.laustrus	B. luciopercae	Triaenophalous sp.	Myxosporida sp.	A.locii	Piscicola sp.
Navicula Scenedes	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom Diatom	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom Cladoph	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
marginal detritus	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urotricha Paramec	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Khillomon Euglena	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cornixidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
annalidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
chironomidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cladocera	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
asellidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gammeridae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
hydrobiidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
valvatidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. lenisculus	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. cygnea	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
aerial insects	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
terrestrial insects	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A. brama	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R.rutilus	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P. fluviatilis	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.lucius	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ergasilus briani	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplozoan sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.s fennica	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxobilus sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxidium sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philometra sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactylogyrus sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tryparnaga brama sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C.laustrus	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. luciopercae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Triaenophalous sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxosporida sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.locii	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Piscicola sp.	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A3.2 Site 2 Binary Matrix

	diator	diator	diator	diator	diator	diator	margi	Cladoj	Euglei	Amoe	Euglei	Amoe	Rotife	Rotife	cladocc	cyclop	copep	copep	copep	copep	copep	Gastrc	Chiror	Baetic	Polyce	Aselli	C.carp	S.eryt	A.cine	A.plat	F.atra	Apios	Dactyl	Tricod	B. ach	Diplos	Fascio
diatom 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
diatom 7	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
marginal weed	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cladophora spp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena 2	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amoeba 2	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer sp1	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rotifer sp2	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cladocera	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
cyclopoda	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda sp1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda sp2	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda sp3	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda sp4	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
copepoda sp5	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gastropoda	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	1	1	1	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Polycentropidae	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aselidae	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
C.carpio	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.erythropthalmus	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.cinerea	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.platyrhynchos	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
F.atra	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aposoma sp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactylogyrus sp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tricodina sp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. achaelognathi	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplostomum sp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fasciolidae sp.	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table A3.3 Site 3 Binary Matrix

	C.der	E.nut	L.mir	P.aus	S.em	Diator	Diator	phyto	phyto	Ciliate	Strom	Peran	Dinofl	Phacu	Phacu	Chilor	Eugle	Eugle	Cope	Cyclo	Valva	Hydro	Bithy	Physi	Lymn	Planc	Unior	Spha	Oligo	Gloss	Hydra	Gam	Asse	Baeti	Caer	Coer	Corix	Halip
C.demersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.nuttallii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L.minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.australis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.emersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom sp2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phytoplankton sp1.indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phytoplankton sp2.indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ciliate sp.	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strombidium sp.	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peranema sp.	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinoflagellate 1sp.	0	0	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus sp. 1	0	0	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus sp.2	0	0	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilomonas sp.	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena sp.1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena sp.2	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclopoda	0	0	0	0	0	1	1	1	1	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Valvatidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bithyniidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaeidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unionidae	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hydracarina	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gammaridae	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asellidae	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagriidae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Haliplidae	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
Hydrophilidae	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Leptoceridae	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.anguilla	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
P.fluviatilis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
E.lucius	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
A.brama	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0
R.rutilus	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	1
R.rutilus x A.brama hybrid	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1
S.erythropthalmus	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0
B.bjoerkna	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	1	0
G.cernua	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	1
G.gobio	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0
P.carbo	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	1	1	0	1
Larus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	1	1	0	1	0	0	1	1	1	0	1
T.ruficollis	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
L.lutra	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.crassus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	0	1	0	0	1	1	1	0	1	1	0	1	0
Contraceacum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1		0	0	0	0
Dactylogyrus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplostomum sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Diplozoan sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eustrongylides sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Gyrodactylus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metorchris sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Myxobolus sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
P.abdominalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petersiger sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Philometra sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhapidicotyle sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
T.clavata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0
Trichodina sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxidium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
A.anguillae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
A.lucii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0
B.claviceps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H.triloba	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0

	Hydro	Lepto	Chiro	A.ang	P.fluv	E.luciu	A.brar	R.rutil	R.rutil	S.eryt	B.bjoe	G.cerr	G.gobi	P.car	Larus	T.rufic	L.lutra	A.cras	Contra	Dactyl	Diplos	Diploz	Eustro	Gyrod	Metor	Myxob	P.abd	Peters	Philor	Rhapi	T.clav	Tricho	Myxid	A.ang	A.lucii	B.clav	H.trilo
C.demersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.nuttallii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L.minor	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.australis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.emersum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom sp1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diatom sp2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phytoplankton sp1.indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
phytoplankton sp2.indet	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ciliate sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Strombidium sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Peranema sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dinoflagellate 1sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus sp. 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Phacus sp.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chilomonas sp.	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena sp.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euglena sp.2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Copepoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cyclopoda	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Valvatidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrobiidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Bithyniidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Physidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lymnaeidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Planorbidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unionidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sphaeriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oligochaeta	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glossiphoniidae	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydracarina	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gammaridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asellidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Baetidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Caenidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coenagriidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Corixidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Haliplidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Hydrophilidae	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Leptoceridae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chironomidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.anguilla	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.fluviatilis	1	1	1	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
E.lucius	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.brama	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R.rutilus	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
R.rutilus x A.brama hybrid	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
S.erythropthalmus	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.bjoerkna	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G.cernua	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
G.gobio	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.carbo	0	1	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larus sp.	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T.ruficollis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L.lutra	0	0	0	1	1		1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.crassus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Contraceacum sp.	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Dactylogyrus sp.	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplostomum sp.	0	0	0	1	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Diplozoan sp.	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eustrongylides sp.	0	0	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gyrodactylus sp.	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Metorchris sp.	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxobolus sp.	0	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
P.abdominalis	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Petersiger sp.	0	0	0	0	0	0	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Philometra sp.	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhapidicotyle sp.	0	0	0	0	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
T.clavata	0	0	0	0	1		1	1	1	1	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trichodina sp.	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Myxidium sp.	0	0	1	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.anguillae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A.lucii	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B.claviceps	0	0	0	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
H.triloba	0	0	0	0	0	0	1	1	1	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix 4. Additional data used to construct diet niches in Chapter 6

Table A4.1 Summary of proportions of the proportion of major food items in the diet of consumers based on Bayesian mixing model outputs (this study) and published literature.

Species	Food item	Diet proportion	Data source
Chironomidae	Detritus	0.95 ± 0.05	Armitage et al. 2012
	Phytoplankton	0.05 ± 0.05	
Arthropoda	Macroalgae	0.40 ± 0.05	Williams and Feltmate 1992
	Detritus	0.40 ± 0.05	
	Zooplankton	0.20 ± 0.05	
Esox lucius	Arthropoda	0.22 ± 0.04	This study
	A.brama	0.60 ± 0.05	
	R.rutilus	0.18 ± 0.02	
Perca fluviatus	Chironomidae	0.15 ± 0.03	This study
	Arthropoda	0.19 ± 0.03	
	A.brama	0.36 ± 0.04	
	R.rutilus	0.30 ± 0.03	
Ardea cinerea	Arthropoda	0.10 ± 0.05	Draulans 1988
	C.carpio	0.20 ± 0.05	
	S.erythrophthalmus	0.50 ± 0.05	

Appendix 5. Weighted start matrices used in Chapter 6

Table A5.1 Site 1: Weighted matrices

Infection: uninfected — Low 95% confidence interval

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	85	-5	0	0	0	0	0	0	0
arthropoda	30	30	0	10	0	0	0	0	0	0
A.brama	0	0	0	-12	18	24	0	0	0	0
R.rutilus	0	0	0	-34	1	53	0	0	0	0
E.lucius	0	0	0	0	0	12	50	8	0	0
P.fluviatus	0	0	0	0	5	9	26	20	0	0

low — High 95% confidence interval

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	105	15	0	0	0	0	0	0	0
arthropoda	50	50	0	30	0	0	0	0	0	0
A.brama	0	0	0	42	72	56	0	0	0	0
R.rutilus	0	0	0	40	75	65	0	0	0	0
E.lucius	0	0	0	0	0	32	70	28	0	0
P.fluviatus	0	0	0	0	25	29	46	40	0	0

Infection: 25%

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	85	-5	0	0	0	0	0	0	0
arthropoda	30	30	0	20	0	0	0	0	0	0
A.brama	0	0	0	-13	19	23	0	0	0	0
R.rutilus	0	0	0	-34	6	48	0	0	0	0
E.lucius	0	0	0	0	0	12	50	8	0	0
P.fluviatus	0	0	0	0	5	9	26	20	0	0

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	95	5	0	0	0	0	0	0	0
arthropoda	40	40	0	20	0	0	0	0	0	0
A.brama	0	0	0	22	53	43	0	0	0	0
R.rutilus	0	0	0	12	52	56	0	0	0	0
E.lucius	0	0	0	0	0	22	60	18	0	0
P.fluviatus	0	0	0	0	15	19	36	30	0	0

Infection: 50%

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	85	-5	0	0	0	0	0	0	0
arthropoda	30	30	0	20	0	0	0	0	0	0
A.brama	0	0	0	-13	19	23	0	0	0	0
R.rutilus	0	0	0	-34	6	48	0	0	0	0
E.lucius	0	0	0	0	0	12	50	8	0	0
P.fluviatus	0	0	0	0	5	9	26	20	0	0

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	95	5	0	0	0	0	0	0	0
arthropoda	40	40	0	20	0	0	0	0	0	0
A.brama	0	0	0	1	34	30	0	0	0	0
R.rutilus	0	0	0	-16	29	47	0	0	0	0
E.lucius	0	0	0	0	0	22	60	18	0	0
P.fluviatus	0	0	0	0	15	19	36	30	0	0

Infection: 75%

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	85	-5	0	0	0	0	0	0	0
arthropoda	30	30	0	20	0	0	0	0	0	0
A.brama	0	0	0	-13	19	23	0	0	0	0
R.rutilus	0	0	0	-34	6	48	0	0	0	0
E.lucius	0	0	0	0	0	12	50	8	0	0
P.fluviatus	0	0	0	0	5	9	26	20	0	0

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	95	5	0	0	0	0	0	0	0
arthropoda	40	40	0	20	0	0	0	0	0	0
A.brama	0	0	0	-20	16	16	0	0	0	0
R.rutilus	0	0	0	-44	6	37	0	0	0	0
E.lucius	0	0	0	0	0	22	60	18	0	0
P.fluviatus	0	0	0	0	15	19	36	30	0	0

Infection: 100%

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	85	-5	0	0	0	0	0	0	0
arthropoda	30	30	0	10	0	0	0	0	0	0
A.brama	0	0	0	-13	24	19	0	0	0	0
R.rutilus	0	0	0	-34	20	34	0	0	0	0
E.lucius	0	0	0	0	0	12	50	8	0	0
P.fluviatus	0	0	0	0	5	9	26	20	0	0

	macroalgae	detritus	phytoplankton	zooplankton	chironomidae	arthropoda	A.brama	R.rutilus	E.lucius	P.fluviatus
macroalgae and	0	0	0	0	0	0	0	0	0	0
phytoplankton	0	0	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0	0	0
zooplankton	0	0	100	0	0	0	0	0	0	0
chironomidae	0	75	-15	0	0	0	0	0	0	0
arthropoda	20	20	0	0	0	0	0	0	0	0
A.brama	0	0	0	-41	-4	4	0	0	0	0
R.rutilus	0	0	0	-71	-17	28	0	0	0	0
E.lucius	0	0	0	0	0	2	40	-2	0	0
P.fluviatus	0	0	0	0	-5	-1	16	10	0	0

Table A5.2 Site 2: Weighted matrices

Infection: Uninfected — Low 95% confidence interval

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	30	30	0	20	0	0	0	0
C.carpio	0	0	15	21	42	0	0	0
S.erythroc	8	0	0	13	35	0	0	0
A.cinerea	0	0	0	0	0	10	40	0

Infection: Uninfected — High 95%confidence interval

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	50	50	0	30	0	0	0	0
C.carpio	0	0	27	37	58	0	0	0
S.erythroc	30	0	22	35	57	0	0	0
A.cinerea	0	0	0	0	20	30	60	0

Infection: 25%

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	30	30	0	20	0	0	0	0
C.carpio	0	0	18	21	36	0	0	0
S.erythroc	8	0	0	13	35	0	0	0
A.cinerea	0	0	0	0	0	10	40	0

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	50	50	0	30	0	0	0	0
C.carpio	0	0	34	39	52	0	0	0
S.erythroc	30	0	22	35	57	0	0	0
A.cinerea	0	0	0	0	20	30	60	0

Infection: 50%

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	30	30	0	20	0	0	0	0
C.carpio	0	0	22	21	30	0	0	0
S.erythroc	8	0	0	13	35	0	0	0
A.cinerea	0	0	0	0	0	10	40	0

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	50	50	0	30	0	0	0	0
C.carpio	0	0	40	41	46	0	0	0
S.erythroc	30	0	22	35	57	0	0	0
A.cinerea	0	0	0	0	20	30	60	0

Infection: 75%

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	30	30	0	20	0	0	0	0
C.carpio	0	0	25	21	24	0	0	0
S.erythroc	8	0	0	13	35	0	0	0
A.cinerea	0	0	0	0	0	10	40	0

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	50	50	0	30	0	0	0	0
C.carpio	0	0	47	43	40	0	0	0
S.erythroc	30	0	22	35	57	0	0	0
A.cinerea	0	0	0	0	20	30	60	0

Infection: 100%

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	30	30	0	20	0	0	0	0
C.carpio	0	0	29	21	18	0	0	0
S.erythroc	8	0	0	13	35	0	0	0
A.cinerea	0	0	0	0	0	10	40	0

	macroalga	detritus	phytoplan	zooplankt	arthropod	C.carpio	S.erythroc	A.cinerea
macroalga	0	0	0	0	0	0	0	0
detritus	0	0	0	0	0	0	0	0
phytoplan	0	0	0	0	0	0	0	0
zooplankt	0	0	100	0	0	0	0	0
arthropod	50	50	0	30	0	0	0	0
C.carpio	0	0	53	45	34	0	0	0
S.erythroc	30	0	22	35	57	0	0	0
A.cinerea	0	0	0	0	20	30	60	0

Appendix 6. Published papers

Chapter 3:

Pegg, J., Andreou, D., Williams, C. F. and Britton, J. R., 2015. Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite. *Parasitology*. doi:10.1017/S0031182015001237

Chapter 4:

Pegg, J., Andreou, D., Williams, C. F. and Britton, J. R., 2015, Head morphology and piscivory of European eels, *Anguilla anguilla*, predict their probability of infection by the invasive parasitic nematode *Anguillicoloides crassus*. *Freshwater Biology*, 60: 1977–1987.

Temporal changes in growth, condition and trophic niche in juvenile *Cyprinus carpio* infected with a non-native parasite

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SUMMARY

In host–parasite relationships, parasite prevalence and abundance can vary over time, potentially impacting how hosts are affected by infection. Here, the pathology, growth, condition and diet of a juvenile *Cyprinus carpio* cohort infected with the non-native cestode *Bothriocephalus acheilognathi* was measured in October 2012 (end of their first summer of life), April 2013 (end of first winter) and October 2013 (end of second summer). Pathology revealed consistent impacts, including severe compression and architectural modification of the intestine. At the end of the first summer, there was no difference in lengths and condition of the infected and uninfected fish. However, at the end of the winter period, the condition of infected fish was significantly reduced and by the end of their second summer, the infected fish were significantly smaller and remained in significantly reduced condition. Their diets were significantly different over time; infected fish consumed significantly higher proportions of food items <53 μm than uninfected individuals, a likely consequence of impaired functional traits due to infection. Thus, the sub-lethal impacts of this parasite, namely changes in histopathology, growth and trophic niche were dependent on time and/or age of the fish.

Key words: Trophically transmitted parasite, cestode, stable isotope analysis, *Bothriocephalus acheilognathi*, diet, fitness.

INTRODUCTION

Parasite infections often negatively impact the fitness of their hosts, can modulate the dynamics of host populations, and can have consequences for non-host populations through changes in the strength of interspecific competitive relationships (Power and Mitchell, 2004). Host responses to infection include altering their life-history traits prior to maturity when individuals allocate more resources to reproduction than growth and survival, as this ensures reproduction before resource depletion and/or castration (Michalakis and Hochberg, 1994; Agnew *et al*. 2000). This can affect their reproductive effort (Christe *et al*. 1996; Sorci *et al*. 1997) and body size (Arnott *et al*. 2000). Understanding these infection consequences for hosts at the individual level then enables understanding of infection impacts at the population and community levels (Pagan *et al*. 2008).

In freshwaters, the opportunity for fish parasites to be moved between localities is high due to the introduction pathways of aquaculture, the ornamental fish trade and sport angling (Gozlan *et al*. 2010). *Bothriocephalus acheilognathi* is a cestode that is originally from Asia (Xiang-Hua, 2007) that has been introduced around the world through the global aquaculture trade in Asian grass carp *Ctenopharyngodon idella* and common carp *Cyprinus carpio* (Salgado-Maldonado and Pineda-López, 2003). Whilst the parasite has a broad host range, having been recorded in over 200 fish species, pathological consequences appear to be more severe in fishes of the family Cyprinidae (Williams *et al*. 2011; Linder *et al*. 2012). It has a complex lifecycle involving an intermediate copepod host and a definitive fish host (Linder *et al*. 2012). While fish are normally infected by consuming infected copepods, there is some evidence that adult worms can additionally be transmitted directly to piscivorous fish that prey on infected fish (Hansen *et al*. 2007). Consequences for fish hosts include damage to the intestinal tract, loss of condition, impacts on foraging behaviours and mortality (Britton *et al*. 2011), with high rates of mortality recorded in hatchery-reared common carp *C. carpio* (Scholz *et al*. 2012). Non-lethal consequences of *B. acheilognathi* infection also include changes in trophic ecology. For example, in a population of juvenile *C. carpio*, application of stable isotope analysis to infected and uninfected individuals suggested infected fish were feeding on items lower in the food web, resulting in energetic consequences (Britton *et al*. 2011).

To date, studies on the trophic ecology of fish infected with *B. acheilognathi* have focused on single samples taken during a single growth season (e.g. Britton *et al*. 2011). This provides limited knowledge on how their trophic niches vary seasonally and in

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relation to parasite prevalence and abundance, and how this affects metrics such as growth and condition over longer time periods. This is important, as for many host populations, parasite incidence varies seasonally due to factors, including the interactions of shifts in the abundance of intermediate hosts, the feeding and/ or reproductive activities of final hosts, the reproductive activity of parasites and the immune response to infection (Altizer *et al*. 2006). For example, seasonal changes in levels of *B. achileognathi* infections, stimulated by changes in water temperature, have been recorded in *Gambusia affinis* and *Pimephales promelas* (Granath and Esch, 1983; Riggs *et al*. 1987). Similar seasonal changes have been observed in other parasite/ host systems, for example Öztürk and Altunel (2006) observed seasonal and annual changes in *Dactylogyrus* infections across four host species. In chub *Squalius cephalus*, higher condition factors and seasonal variations in gonado-somatic indices were associated with decreased immune function and corresponding increases in parasite loads, suggesting differences in the seasonal energy allocation between immune function and somatic and/or reproductive investment (Lamkova *et al*. 2007).

Given the recorded trophic consequences of *B. acheilognathi* infection for juvenile *C. carpio* (Britton *et al*. 2011), the aim of this study was to assess how their non-lethal consequences of infection varied over a 12-month period through tracking a single cohort. The objectives were to: (i) quantify temporal changes in parasite prevalence, abundance, histopathology and the energetic consequences of infection of *B. acheilognathi* in juvenile *C. carpio*; and (ii) assess the temporal changes in the trophic ecology and diet of juvenile *C. carpio* infected and uninfected with *B. acheilognathi* through stable isotope analysis.

METHODS

Sample collection and initial data collection

The study population was located in the Greater London area of the UK and where *B. acheilognathi* had been recorded previously. The site was a small pond of 50 m length, 20 m width and maximum depth 1·5 m. The sampling programme covered two summer periods and an over-wintering period, with the initial sample collected in early October 2012 (end of the summer period and end of the 2012 growth season), April 2013 (end of the over-wintering period) and October 2013 (end of the summer period and end of the 2013 growth season). Due to fishery management operations, the mature component of the *C. carpio* population was removed from the lake after spawning in 2012, thus all remaining carp were young-of-the-year. Consequently, all fish captured in October 2012 were age 0+ and by October 2013 were 1+ years, i.e. the captured fish throughout the study were of the same cohort, with this verified by age analysis of their scales.

The fish were sampled using traps that had a circle alloy frame of length 107 cm, width and height 27·5 cm, mesh diameter 2 mm and with funnel shaped holes of 6·5 cm diameter at either end to allow fish entry and hence their capture. They were each baited with five fishmeal pellets of 21 mm diameter were placed in the trap as an attractant (Dynamite Baits 2015). Alternative sampling methods were trialled initially (seine nets and electric fishing), but were unsuccessful due to the presence of underwater structures (nets) and heavy growth of *Phragmites australis* in the littoral zone (electric fishing). On each sampling occasion, 10 traps were set in the littoral zone at approximately 18·00 h and lifted at 09·00 h the next morning.

After the traps were lifted, all the juvenile *C. carpio* were removed and transferred to water-filled containers and a random sub-sample of 25 individuals was taken and transported to the laboratory for processing. As the fish were sampled from a private fishery, the numbers were limited in order to minimize the impact on the future angling stock, as agreed with the fishery managers. In April 2013 and October 2013, samples of the putative food resources of the fish were also taken, covering macro-invertebrates (through kick sampling and sweep netting with a handnet of 0·25 mm mesh), zooplankton (through filtering 10 litres of water through a net and filter of 250 μm) and phytoplankton (filtering 10 litres of water through a net and filter of 53 μm). For macro-invertebrates, triplicate samples were taken, where a sample represented between 5 and 20 individuals of that species. Putative food resource samples were not able to be collected in October 2012 due to logistical constraints.

In the laboratory, all fish were euthanized (anaesthetic overdose; MS-222), with weight (W; to 0·01 g), and fork length (L; nearest mm) recorded. A detailed post-mortem was then conducted on each individual for detecting the presence of infections of native and non-native parasites using a standard protocol adapted from Hoole *et al*. (2001). Skin scrapes and internal organs were examined with aid of low- and high-power microscopy to enable parasite identification. The entire digestive tract was removed and examined under low power for detecting the presence of intestinal parasites, including *B. acheilognathi*. When *B. acheilognathi* was recorded, their abundance was recorded (by number and mass to nearest 0·001 g). Hereafter, where an individual *C. carpio* is referred to as either infected or non-infected, it refers to the presence/absence of *B. acheilognathi* in that individual during this process. Intestinal tissue from infected and uninfected individuals was retained and prepared for histopathology.

On completion of the post-mortem, a sample of dorsal muscle was taken from a proportion of the fish samples (sample sizes 6–15 per sub-set of fish per population). These, and the macro-invertebrate, zooplankton and phytoplankton samples, were then dried at 60 °C to constant weight before being analysed for their stable isotopes of ^{13}C and ^{15}N at the Cornell Stable Isotope Laboratory (New York, USA). The initial stable isotope data outputs were in the format of delta (δ) isotope ratios expressed per mille (‰).

Histopathology

Histopathology of the intestinal tract was completed to assess the pathological changes associated with *B. acheilognathi* infection. Sections of intestine were sampled from infected as well as uninfected fish. These sections were fixed in Bouin's fixative for 24 h before transferring to 70% Industrial Methylated Spirit. 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.

Data analyses

Infection levels of *B. acheilognathi* in *C. carpio* were described as their prevalence (number of infected individuals/total number of individuals × 100) and abundance (number of *B. acheilognathi* per host). The mass of parasite was also expressed as a proportion of host weight to represent the parasite burden. The stable isotope data of *C. carpio* were used to assess their trophic niche size and predict their diet composition from the putative food resource data. Trophic niche size was calculated using the metric standard ellipse area (SEA_c) in the SIAR package (Parnell *et al.* 2010) in R (R Core Development Team, 2013). SEAc is a bivariate measure of the distribution of individuals in trophic space, where each ellipse encloses ~40% of the data and thus represents the core dietary niche of species and so indicates their typical resource use (Jackson *et al.* 2011, 2012). The subscript 'c' in SEA_c indicated that a small sample size correction was used due to limited sample sizes (<30). For each population of *C. carpio* on each survey date, SEAc was calculated for two sub-sets of individuals: those infected with *B. acheilognathi* and those uninfected, and the extent of the overlap of their niches determined (%).

To then predict the diet composition of each sub-set of fish, their stable isotope data, plus those of their putative food resources, were applied to Bayesian mixing models that estimated the relative contribution of each putative food resource to the diet of each individual *C. carpio* (Moore and Semmens, 2008). The models were run using the MixSIAR GUI package in the R computing program (R Core Development Team, 2013; Stock and Semmens, 2013). Given that excessive putative food resources can cause mixing models to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species (Phillips *et al.* 2005). The groups used in the models were arthropods, zooplankton (i.e. samples captured in the net of mesh size 250 μm) and phytoplankton (i.e. samples captured in the net of mesh size 53 μm). Isotopic fractionation factors between resources and consumers in the models were 3·4‰ (±0·98‰) for $\delta^{15}N$ and 0·39‰ (±1·3‰) for $\delta^{13}C$ (Post, 2002). Outputs were the predicted proportion of each resource to host diet (0–1).

Statistical analyses

For each fish species and population infected with *B. acheilognathi*, differences between the infected and uninfected hosts were tested for length, and their stable isotopes of $\delta^{13}C$ and $\delta^{15}N$ using analysis of variance (ANOVA). Condition was calculated as Fulton's Condition Factor (K, $100 \times W/L^3$) where L was measured in cm, with differences between infected and uninfected fishes also tested using ANOVA. Differences between the predicted proportions of each putative food source to the diet of infected and uninfected fish were also tested using ANOVA. Other than the stable isotope mixing models, all analyses were completed in SPSS v. 22·0. In all analyses, the assumptions of normality of residuals and homoscedasticity were checked prior to use. Where error is expressed around the mean, it represents standard error.

RESULTS

Parasite prevalence and abundance

Across the three sampling periods, parasite prevalence remained relatively constant (61, 58 and 60% in October 2012, April 2013 and October 2013, respectively; Table 1). Parasite abundance was greatest in October 2012 (mean 10·7 ± 2·3) and lowest in April 2013 (mean 5·4 ± 1·5) (Table 1). Parasite abundance was significantly different between October 2012 and April 2013 (ANOVA: $F_{1,45} = 9{\cdot}38$, $P < 0{\cdot}01$) but not between April 2013 and October 2013 (ANOVA: $F_{1,45} = 1{\cdot}22$, $P > 0{\cdot}05$), and October 2012 and October 2013 (ANOVA: $F_{1,45} = 4{\cdot}05$, $P > 0{\cdot}05$). Mean parasite burden was greatest in October 2012 (3·9 ± 0·8%) and lowest in October 2013 (1·7 ± 0·5%). There was a significant difference between the parasite burdens in October 2012 and October 2013 (ANOVA: $F_{1,45} = 5{\cdot}85$, $P < 0{\cdot}05$), but not between October 2012 and April 2013 (ANOVA: $F_{1,45} = 1{\cdot}92$, $P > 0{\cdot}05$) and April

Table 1. Prevalence and abundance of *Bothriocephalus acheilognathi* by sampling date

Date	*n*	Prevalence (%)	Mean abundance of parasites (± S.E.)	Range	Mean weight of parasite burden (percentage of hosts weight ± S.E.)	Range (%)
Oct 12	23	61	10·7 ± 2·3	0–35	3·9 ± 0·8	0–9·5
Apr 13	24	58	3·4 ± 0·9	0–14	2·2 ± 0·9	0–19·4
Oct 13	25	60	5·4 ± 1·5	0–26	1·7 ± 0·5	0–8·8

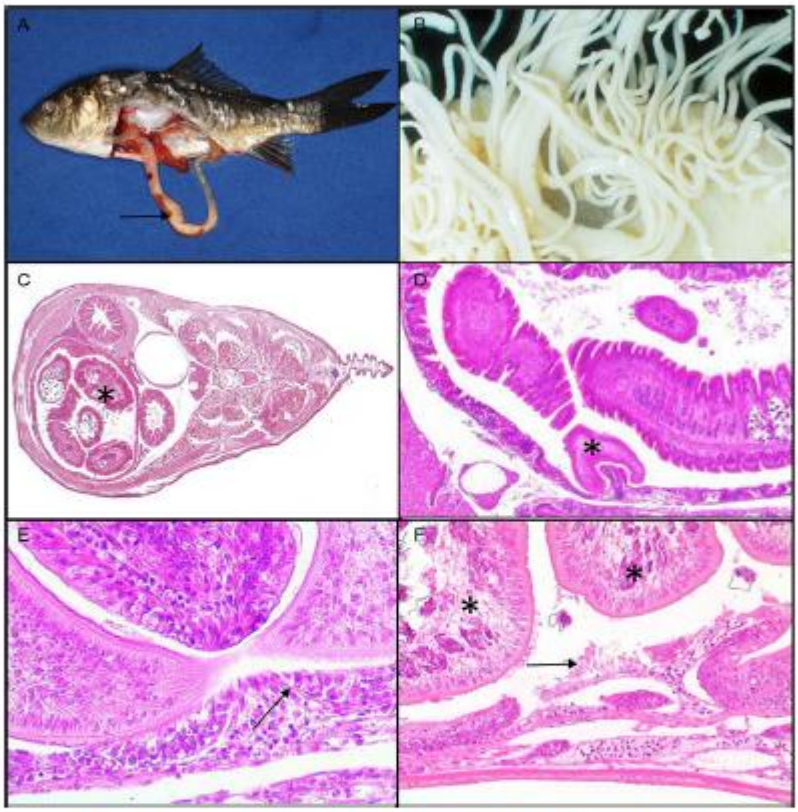

Fig. 1. (A) *Bothriocephalus acheilognathi* infection in juvenile common carp, with resulting pale distended intestine. (B) Attachment of multiple *B. acheilognathi* within the intestine, many with mature proglottids. (C) Transverse section through juvenile carp showing *B. acheilognathi* occupying the anterior intestine (*), with compression of the gut wall and displacement of internal organs, including the swim bladder. (D) *B. acheilognathi* attachment site showing the scolex (*) pinching the gut wall and flattening of normal intestinal folds throughout infected regions of the gut. (E) Pronounced compression of epithelium at the apex of scolex attachment, with loss of epithelium, thinning of musculature and near exposure of the basement membrane (arrow). Lymphocytes may be seen within the lamina propria (F) Flattening of intestinal folds with epithelial erosion (arrow) as a consequence of pressure exerted by the body of tapeworms (*) within the intestine.

2013 and October 2013 (ANOVA: $F_{1,45} = 0{\cdot}22$, $P > 0{\cdot}05$) (Table 1). Of other parasites recorded these were all native species that would be considered as the expected parasite fauna of these fishes in a UK community and were recorded at levels that were considered as not high enough to cause clinical pathology (Hoole *et al.* 2001).

Histopathology

Histopathological examinations revealed consistent pathological changes associated with *B. acheilognathi* infection. The presence of *B. acheilognathi* within the gut of infected carp was usually evident prior to dissection of the intestine, with the mass of pale

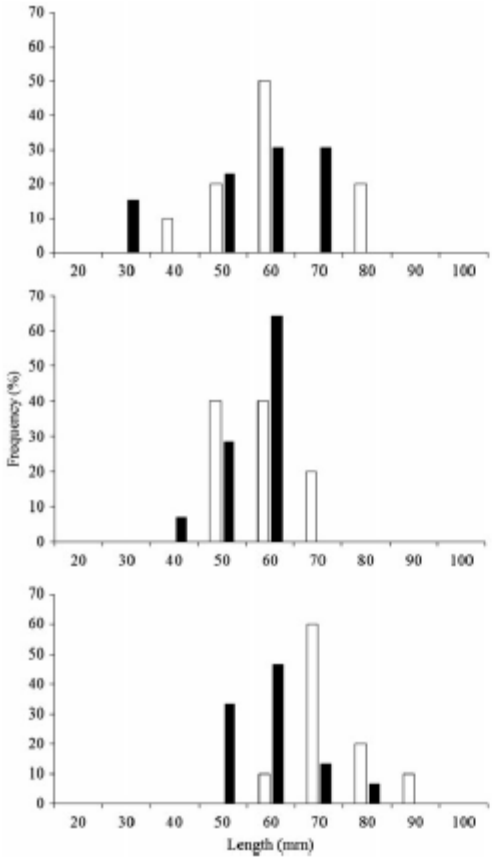

Fig. 2. Length frequency histograms of infected (black) and uninfected (white) *C. carpio*. (A) October 2012, $n = 23$; (B) April 2013, $n = 24$; and (C) October 2013, $n = 25$.

tapeworms visible through the distended gut wall (Fig. 1A). Dissection of the intestinal tract revealed attachment sites of *B. acheilognathi* within the anterior region of the tract with mass of proglottids filling a large proportion of the gut lumen (Fig. 1B and C). Heavy infections caused near complete occlusion of the intestinal tract. Histopathological observations confirmed thinning and compression of the gut wall with displacement of internal organs, including the swim bladder (Fig. 1C). During attachment, the scoleces of *B. acheilognathi* engulfed the intestinal folds, leading to marked compression of the epithelium (Fig. 1D). At the point of attachment, the intestine was severely compressed, with loss of normal gut architecture, loss of epithelium and near exposure of the basement membrane (Fig. 1E and F). Infection was frequently accompanied by an increase in lymphocytes throughout the epithelium and lamina propria (Fig. 1E) compared with uninfected fish. In very heavy infections, pressure exerted by

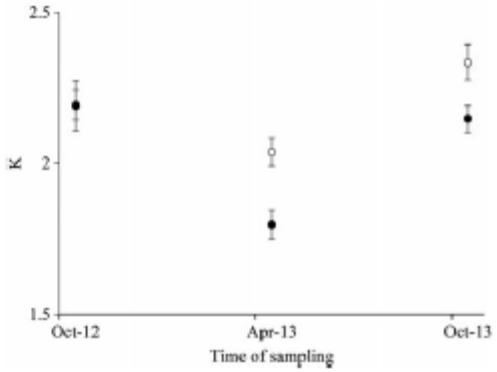

Fig. 3. Fulton's condition factor (K) of infected (black circles) and uninfected (white circles) *C. carpio* over the study period. Error bars represent standard error.

the mass of parasites within the intestine caused thinning of the musculature and forced the gut wall against the inside of the body cavity (Fig. 1F).

Effect of infection on fish length and condition

There was no significant difference in lengths of the uninfected and infected fish sampled in October 2012 and April 2013 (ANOVA: October 12: $F_{1,21} = 1{\cdot}04$, $P > 0{\cdot}05$; April 13: $F_{1,22} = 2{\cdot}31$, $P > 0{\cdot}05$; Fig. 2). In October 2013, however, the uninfected fish were significantly larger than infected fish (ANOVA: October 13: $F_{1,23} = 14{\cdot}38$, $P < 0{\cdot}01$; Fig. 2). Whilst there were no significant differences in the condition (K) of infected and uninfected *C. carpio* in October 2012 (ANOVA: $F_{1,21} = 0{\cdot}00$, $P > 0{\cdot}05$), there was in April 2013 (ANOVA: $F_{1,22} = 11{\cdot}68$, $P < 0{\cdot}01$) and this significant difference remained in October 2013 (ANOVA: $F_{1,23} = 6{\cdot}57$, $P < 0{\cdot}05$) (Fig. 3).

Stable isotope metrics

The mean values of $\delta^{13}C$ and $\delta^{15}N$ of the infected and uninfected fish were significantly different in April 2013 (ANOVA $\delta^{13}C$: $F_{1,22} = 10{\cdot}62$, $P < 0{\cdot}01$, $\delta^{15}N$: $F_{1,22} = 10{\cdot}94$, $P < 0{\cdot}01$) and October 2013 (ANOVA $\delta^{13}C$: $F_{1,23} = 20{\cdot}88$, $P < 0{\cdot}01$, $\delta^{15}N$: $F_{1,23} = 21{\cdot}77$, $P < 0{\cdot}01$) (Table 2). By contrast, in October 2012, only $\delta^{13}C$ was significantly different between the groups (ANOVA $\delta^{13}C$: $F_{1,21} = 13{\cdot}83$, $P < 0{\cdot}01$, $\delta^{15}N$: $F_{1,21} = 3{\cdot}39$, $P > 0{\cdot}05$) (Fig. 4). In all cases where differences between the isotopes of the groups were significant, the infected fish had enriched $\delta^{15}N$ and depleted $\delta^{13}C$.

The outputs of the mixing models predicting the diet composition of the uninfected and infected fish revealed some significant differences between the two groups (Table 3). In both April and October 2013, infected fish were predicted to have

Table 2. Sample size, mean lengths of sub-sampled fish and mean stable isotope data

Date	Species	*n*	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
Oct 12	Uninfected *C. carpio*	9	66·1 ± 3·32	−32·31 ± 0·59	17·79 ± 1·19
	Infected *C. carpio*	14	58·7 ± 4·57	−33·14 ± 0·48	18·60 ± 0·90
Apr 13	Uninfected *C. carpio*	6	64·6 ± 1·92	−32·44 ± 0·67	18·00 ± 1·11
	Infected *C. carpio*	10	60·4 ± 1·91	−33·69 ± 0·78	19·61 ± 0·84
	Arthropoda	11		−33·65 ± 1·39	13·42 ± 0·37
	Plankton <250 μm	3		−36·54 ± 0·76	18·68 ± 1·24
	Plankton more than 250 μm	3		−30·63 ± 1·25	17·42 ± 0·47
Oct 13	Uninfected *C. carpio*	9	78·7 ± 2·84	−32·07 ± 0·94	17·93 ± 1·31
	Infected *C. carpio*	14	64·67 ± 2·35	−34·03 ± 1·12	20·02 ± 0·93
	Arthropoda	8		−34·33 ± 0·99	10·13 ± 0·41
	Plankton <250 μm	2		−36·37 ± 0·15	19·38 ± 0·74
	Plankton more than 250 μm	2		−30·09 ± 0·97	17·16 ± 1·16

a significantly higher proportion of plankton <250 μm in their diet compared with uninfected fish (mean 41 ± 6% in April and 57 ± 2% in October; ANOVA April: $F_{1,22} = 863·33$, $P < 0·01$, October: $F_{1,23} = 372·70$, $P < 0·01$). Arthropoda were predicted to comprise a significantly higher proportion of the diets of uninfected fish on both sampling dates (mean 50 ± 4% in April and 32 ± 3% in October; ANOVA April: $F_{1,22} = 874·04$, $P < 0·01$, October: $F_{1,23} = 173·33$, $P < 0·01$). Plankton > 250 μm made up a smaller proportion of the diet of uninfected fish than infected fish in April (29 ± 4% cf. 33 ± 6%; ANOVA $F_{1,22} = 143·43$, $P < 0·01$) and a larger proportion in October (45 ± 2% cf. 24 ± 2%; ANOVA $F_{1,23} = 448·76$, $P < 0·01$) (Table 3).

DISCUSSION

Sampling of the juvenile fish over the 12 month period revealed that infection by *B. acheilognathi* resulted in the development of long-term pathological and ecological consequences. Although the hosts sampled at the end of their first summer revealed little difference in lengths and condition compared with their uninfected conspecifics, the outputs of stable isotope analysis revealed they already had a significantly different diet composition. The condition of infected fish was significantly reduced after their first winter and by the end of their second summer, they were significantly smaller than uninfected fish and remained in significantly reduced condition. The diet of these two subsets of fish also remained significantly different over this time.

Other studies on *B. acheilognathi* have also suggested that infection causes a range of foraging consequences for hosts, including impairment of their ability to capture prey (Scott and Grizzle, 1979; Britton *et al.* 2011, 2012; Scholz *et al.* 2012). The shift towards foraging on less motile, more easily available food sources by hosts has also been observed in other parasitized populations. For example, the freshwater amphipod *Gammarus roeseli* infected with the acanthocephalan *Polymorphus minutus* (as an intermediate host) consumed equivalent numbers of dead isopods as uninfected conspecifics, but fewer live isopods (Medoc *et al.* 2011). In stickleback *Gasterosteus aculeatus*, parasitism by the cestode *Schistocephalus solidus* tends to lead to selection of smaller prey items (Barber *et al.* 1995). Shifts in host feeding behaviours arise through a variety of mechanisms; for example, parasites utilize energy reserves of their hosts, infection may increase metabolic costs or be associated with increases in energetically demanding immune functions (Barber *et al.* 2000). Hosts infected with strongly debilitating parasites may also exhibit reduced activity levels that impact foraging behaviours (Britton *et al.* 2011; Britton, 2013). Thus, infection consequences frequently manifest as changes in energy budgets expenditure and, subsequently, appetite, foraging and diet composition (Barber *et al.* 2000). Moreover, in fish populations, the frequency distribution of phenotypic trait values often follows a normal distribution, reflecting genotypic differences and environmental noise, but parasitic infection can shift the mean value of traits, increasing their variance at the population level (Poulin and Thomas, 1999). This was apparent in the *C. carpio* of our study where the increase in the trophic niche size of the host population was related to it comprising two, almost discrete niches that corresponded with uninfected and infected fish.

Over the study period, temporal changes were also detected in parasite burden. These tended to reduce over time, despite being sufficient to incur pathological and ecological consequences. Although this reduction might relate to the mortality of hosts with high parasite abundances, seasonal shifts in aspects of fish parasite infections are often apparent in temperate regions due to its influence on the behaviours, habitat utilization and immune responses of potential hosts (Bromage *et al.* 2001; Bowden *et al.* 2007). Given these can vary between host species then parasite prevalence and abundance can show considerable variability across species within communities. For example, in reservoirs in North

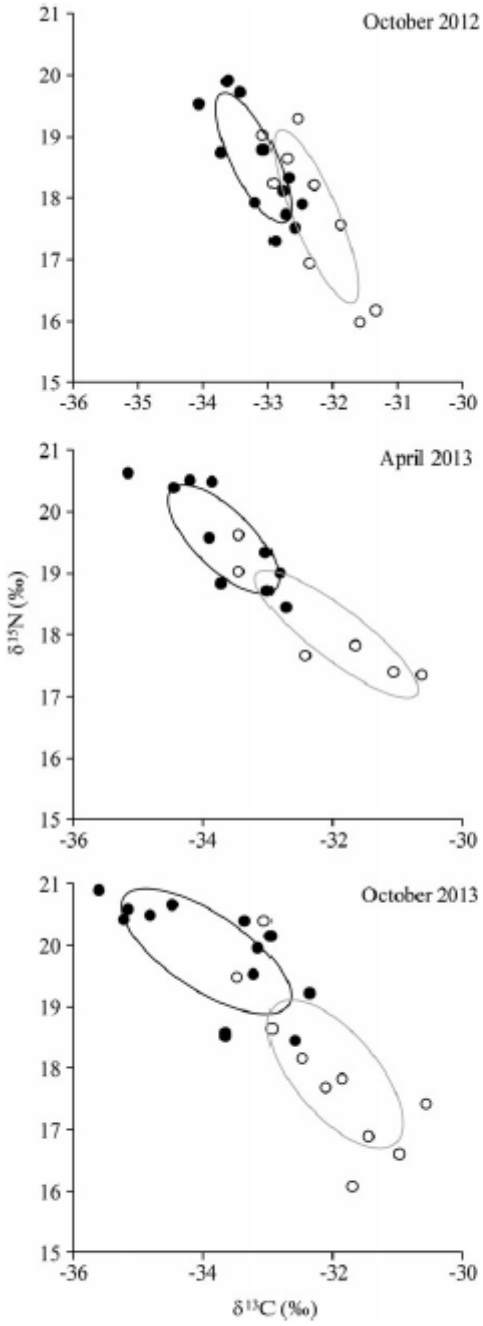

Fig. 4. Trophic niche width (as SEAc) of infected and uninfected *C. carpio* sampled in (A) October 2012, (B) April 2013 and (C) October 2013. The black circles mark the infected individuals and the black line the SEAc of infected individuals. The white circles mark the uninfected individuals and the grey line represents the SEAc of uninfected individuals.

Carolina, USA, *B. acheilognathi* abundance was highest in fathead minnow *P. promelas* and red shiner *Notropis lutrensis* in autumn, whereas it was highest in winter in mosquito fish *G. affinis* (Riggs *et al.* 1987). For parasites whose transmission to final hosts is through trophic links, the phenology of intermediate hosts is also important, with seasonal changes in copepod communities identified as a driver of the different infection levels of *B. acheilognathi* observed in fish host communities (Riggs *et al.* 1987). Temporal and spatial changes in the definitive host infection level that result from varying transmission success due to shifts in the dynamics of intermediate host populations have also been recorded across a range of fishes and their parasites (Amundsen *et al.* 2003; Jiménez-Garcia and Vidal-Martínez, 2005).

The divergence in the lengths of the infected and uninfected fish that developed over time has the potential to restrict host fitness, as in most fish species, maturation is associated with size and thus faster growing individuals will mature earlier in life (Scott, 1962; Bagenal, 1969; Ali and Wootton, 1999). Furthermore, larger fish are more fecund, and thus contribute more to the population (Hislop, 1988; Beldade *et al.* 2012). Whilst a reduction in growth associated with parasitism has been recorded in a variety of species, such as the rainbow smelt *Osmerus mordax* infected by protocephalid parasites (Sirois and Dodson, 2000), and farmed and wild salmonids infected with sea lice (e.g. *Lepeophtheirus salmonis*) (Costello, 2006), it is not the universal response to parasitism (Loot *et al.* 2001). Indeed, rapid growth aligned with parasitic castration in hosts is the response recorded in other cestode parasites, such as *Ligula intestinalis* (Thompson and Kavaliers, 1994; Loot *et al.* 2001) and *S. solidus* (Arnott *et al.* 2000; Barber *et al.* 2000).

In summary, significant differences in the condition and body lengths of infected and uninfected populations developed over the course of the study, with histopathology revealing substantial local damage in the intestine of hosts. Analyses then revealed the diet composition of the infected fish was predicted to comprise of a significantly higher proportion of smaller items (<53 μm) than uninfected fish. Thus, it was demonstrated that in this cohort of juvenile *C. carpio*, sub-lethal impacts of parasitism included substantial histopathological consequences that resulted in significant growth and trophic impacts whose development could have been overlooked had the temporal context of the study been lacking. It is thus especially important to investigate the temporal influence of parasitism in any evaluation of potential parasite impacts on trophic niche and condition of the host.

Table 3. Summary of the Bayesian mixing models outputs predicting the proportions of each major food item to the diet of infected and uninfected fish on each sample occasion, and the F value from ANOVA, where $^{**}P < 0{\cdot}01$

Date	Food item	Modelled diet proportion (±S.E.) Uninfected	Infected	F
Apr-13	Arthropoda	0·50 ± 0·04	0·26 ± 0·04	874·0**
	Plankton <250 μm	0·21 ± 0·03	0·41 ± 0·06	863·3**
	Plankton more than 250 μm	0·29 ± 0·04	0·33 ± 0·06	143·4**
Oct-13	Arthropoda	0·32 ± 0·03	0·18 ± 0·02	173·3**
	Plankton <250 μm	0·23 ± 0·03	0·57 ± 0·02	372·7**
	Plankton more than 250 μm	0·45 ± 0·02	0·24 ± 0·02	448·7**

Values of the predicted proportions represent their mean and standard error. Sample sizes as Table 2.

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Views expressed in the paper are those of the authors and not their parent organizations.

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Head morphology and piscivory of European eels, *Anguilla anguilla*, predict their probability of infection by the invasive parasitic nematode *Anguillicoloides crassus*

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SUMMARY

1. The morphology of animal body structures influences their function; intrapopulation plasticity in diet composition can occur where head morphology limits gape size. The European eel, *Anguilla anguilla*, a critically endangered catadromous fish, shows significant intrapopulation variations in head width, with broader headed individuals being more piscivorous.
2. Infection of eels during their freshwater phase by *Anguillicoloides crassus*, an invasive nematode parasite, involves paratenic fish hosts. We tested the relationships between their infection status, head functional morphology (as head width/total length ratio; HW:TL) and the proportion of fish in diet (estimated by stable isotope mixing models) across three populations.
3. The extent of piscivory in the diets of individual eels increased significantly as their HW:TL ratios increased. There were no significant differences between infected and uninfected eels in their total lengths and hepatic–somatic indices. However, the HW:TL ratios of infected eels were significantly higher than those of uninfected eels and, correspondingly, their diet comprised a higher proportion of fish.
4. Logistic regression revealed that head morphology and diet were significant predictors of infection status, with models correctly assigning up to 78% of eels to their infection status. Thus, eel head functional morphology significantly influenced their probability of being infected by invasive *A. crassus*, most likely through increased exposure to fish paratenic hosts. Accordingly, the detrimental consequences of infections are likely to be focussed on those individuals in freshwater populations whose functional morphology enables greater specialisation in piscivory.

Keywords: complex life cycle, individual specialisation, non-native parasite, paratenic host, stable isotope analysis

Introduction

Phenotypic differences in morphology, physiology and behaviour are frequently observed between parasitised and non-parasitised individuals (Lafferty, 1999; Krist, 2000; Miura *et al.*, 2006). Although often considered in the context of parasite-induced changes to the host post-infection (Blanchet *et al.*, 2009), some traits increase the susceptibility of individuals to infection, resulting in a small number of hosts harbouring the majority of parasites (Viljoen *et al.*, 2011). These traits include the following: host body size, where increased size favours the development of larger parasite loads (Lindenfors *et al.*, 2007); social behaviours, where increased social interactions increase parasite transmission (Viljoen *et al.*, 2011); and sex, as oestrogens can stimulate immunity whereas testosterone can act as an immunosuppressant (Folstad & Karter, 1992) so that males often have higher parasite loads (Schalk & Forbes, 1997; Moore & Wilson, 2002). Functional traits that enable the development of specialised feeding behaviours in individuals can also increase the risk of infection by trophically transmitted parasites through increased exposure to intermediate hosts (Bolnick *et al.*, 2003). For example, different feeding speciali-

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sations of individuals within Arctic charr (*Salvelinus alpinus*) populations result in aggregations of helminth parasites in those individuals that persistently forage on the pelagic copepods that act as intermediate hosts (Knudsen, Curtis & Kristoffersen, 2004).

Paratenic hosts can play important roles in the transmission of trophically transmitted parasites (Ewald, 1995; Galaktionov, 1996), as they increase parasite fitness and ensure that larvae that would otherwise be 'lost' in unsuitable hosts are recovered (Morand, Robert & Connors, 1995). They can assist transmission when obligate intermediate hosts are not represented strongly in the diet of final hosts (Moehl *et al.*, 2009; Medoc *et al.*, 2011; Benesh, Chubb & Parker, 2014), and thus facilitate parasite transfer along food chains and across trophic levels (Marcogliese, 2007). For example, *Alaria* trematode parasites, whose obligate amphibian intermediate hosts are rarely consumed by their canine final host, also have mammalian and bird paratenic hosts that substantially increase their transmission rates (Moehl *et al.*, 2009). Paratenic hosts also increase the time over which potential hosts are vulnerable to infection. For example, because the obligate intermediate hosts of *Bothriocephalus barbatus* and *Bothriocephalus gregarious* are copepods, their flatfish final hosts are vulnerable to infection during their planktonic juvenile stages (Robert *et al.*, 1988). However, as *B. gregarious* also has a gobiid fish paratenic host, the predaceous adult stages of potential hosts continue to be exposed to the parasite, resulting in higher prevalence rates than for *B. barbatus* (Robert *et al.*, 1988; Morand *et al.*, 1995).

The nematode parasite *Anguillicoloides crassus* was introduced from Asia into Europe in the 1980s, where it infects the freshwater life stages of the European eel, *Anguilla anguilla*, (Kirk, 2003), a critically endangered species (Jacoby & Gollock, 2014). A number of factors have been suggested as contributing to the decline of European eel populations, including *A. crassus* infections as these affect swim bladder function (Lefebvre *et al.*, 2013). This parasite has a complex life cycle; in the native range, infection of Japanese eel (*Anguilla japonica*) is via ingestion of crustacean intermediate hosts (Nagasawa, Kim & Hirose, 1994), but in Europe a wide range of species, primarily fishes, also act as paratenic hosts (Szekely, 1994; Kennedy, 2007). Although not evident in the native range (Thomas & Ollevier, 1992), studies suggest that the consumption of paratenic fish hosts has contributed to increased transmission rates and prevalence of *A. anguilla* (Szekely, 1994; Sures & Streit, 2001; Kirk, 2003; Knopf & Mahnke, 2004).

Within populations, *A. anguilla* exhibits considerable variation in head width, with 'broad-headed individuals' and 'narrow-headed individuals' (Tesch, 1977, 2003; Lammens & Visser, 1989; Proman & Reynolds, 2000), although a recent study suggests that there is continuous morphological variation rather than a dichotomy (Cucherousset *et al.*, 2011). As with other species where head morphology limits energy acquisition (Smith & Skulason, 1996; Bulte, Irschick & Blouin-Demers, 2008), these differences in head morphology have been related to individual specialisation, with broader headed *A. anguilla* individuals being more piscivorous (Cucherousset *et al.*, 2011). We investigated how *A. anguilla* head morphology, diet and trophic ecology influence the infection status and parasite load with *A. crassus* over three river populations. We predicted that variation in the functional head morphology of *A. anguilla* leads to significant differences in individual diet composition and trophic niche, significantly influencing the probability of infection by *A. crassus* in broader headed individuals through their increased parasite exposure via fish paratenic hosts.

Methods

Sample collection and initial data collection

The three study sites were all lowland rivers in England where *A. anguilla* was known to be infected with *A. crassus*, and the eel population was abundant and, thus, destructive sampling would not be detrimental to their status. The sites were the River Huntspill (Site 1; 8 to 12 m width, maximum depth 3 m; Lat: 51.198440N, Long: 2.993181W), the St. Ives Chub stream (Site 2; 4–8 m width, maximum depth 1.5 m; Lat: 52.331143N, Long: 0.061219E), and a side channel of the River Frome (Site 3; 4–8 m width, maximum depth 1.5 m; Lat: 50.679668N, Long: 2.181917W).

Sampling was completed in August 2013 (sites 1 and 2) and August 2014 (Site 3), and methods were dependent on site characteristics. At Site 1, a series of fyke nets (6.5-mm mesh, 50-cm-diameter front hoop, 3-m leader) was placed across the width of the river and all captured eels removed after 24 h. At sites 2 and 3, sampling was by electric fishing, using a back-mounted Smith-Root LR-24 Backpack (50-MHz pulsed DC at approximately 2 Amps). At all sites, silver eels (sexually mature, pre-spawning eels) were returned without processing. Yellow eels were retained in water-filled containers, and a maximum of 24 individuals were selected randomly and taken back to the laboratory for processing. This sample size avoided removal from small river populations of excessive numbers of a critically endangered apex predator. Samples of

putative food items were also collected from each site, including samples of small prey fishes (*Phoxinus phoxinus*, *Cottus gobio* and *Gymnocephalus cernua*, presence dependent on site, maximum 10 individuals per species) and macroinvertebrates, collected using a combination of electric fishing, kick sampling and a 40-m micromesh seine net. Triplicate samples were taken of each macroinvertebrate species where possible. Thus, these samples either comprised a single individual (fish) or were pooled samples of single species (macroinvertebrates; n = 5–20 individuals per sample).

In the laboratory, all fish were euthanised through an anaesthetic overdose (MS-222), with weight, total length and head width of the eels measured (Cucherousset *et al.*, 2011). A detailed post-mortem was then conducted on the eels and other fishes using a standard protocol (Hoole *et al.*, 2001) to detect infections by native and non-native parasites. Skin scrapes and internal organs were examined with the aid of low- and high-power microscopy to enable parasite identification. Eel swim bladders were removed and the numbers of male, female and juvenile *A. crassus* counted. As *A. crassus* exhibits marked sexual dimorphism, with females at least 10 times larger than males, and it is the female parasites that primarily cause the gross pathological damage of the swim bladder (Lefebvre *et al.*, 2013), only counts of the large, female nematodes were used in subsequent analyses as the measure of parasite abundance. These female parasites were also the dominant form of *A. crassus* encountered in the swim bladders. In addition, as the life cycle of the parasite is relatively short (a few months) compared with the duration of the freshwater life phase of eels (minimum 3 years), then the absence of *A. crassus* at post-mortem does not preclude that an eel has been repeatedly infected and severely affected in the past. Consequently, uninfected eels were identified by both an absence of *A. crassus* and a swim bladder wall of transparent-yellowish coloration (i.e. undamaged, indicating no previous infection), as per Lefebvre, Contournet & Crivelli (2002). The liver was also removed and weighed, and a sample of dorsal muscle was taken for stable isotope analysis. The muscle samples, along with samples from other fishes and the putative food resources, were then oven-dried at 60 °C until they achieved constant weight, before processing and analysis at the Cornell Isotope Laboratory, New York, U.S.A. Note that due to financial constraints, only 60 of the 86 eels were analysed. The initial stable isotope data were in the format of delta (δ) isotope ratios expressed per mille (‰).

Data analysis

Infection levels of *A. crassus* in *A. anguilla* were described as their prevalence (number of infected individuals/total number of female *A. crassus* × 100) and abundance (number of mature female *A. crassus* per eel). Hereafter, where an *A. anguilla* individual is referred to as either infected or non-infected, it refers to the presence/absence of *A. crassus* in that individual during the post-mortem. Ratios of head width to total length (HW:TL) in the *A. anguilla* populations were determined (Proman & Reynolds, 2000) and were used as a morphological index (Cucherousset *et al.*, 2011). To standardise HW:TL ratios across the sites, their values within each site were expressed as their standardised residual values from their population mean. Hepatic–somatic index (HSI), a measure of energy storage, was then calculated for each individual *A. anguilla* using the formula: HSI = liver weight (g)/total bodyweight (g). Note this could not be completed for *A. anguilla* from Site 3.

Anguilla anguilla diet composition and trophic niche size was investigated at each site using the stable isotope data. Diet composition was assessed using Bayesian mixing models that estimated the relative contribution of each putative food resource to the diet of each individual *A. anguilla* per site (Moore & Semmens, 2008). The models were run using the MixSIAR GUI package in the R computing program (R Development Core Team, 2013; Stock & Semmens, 2013). Given that excessive putative food resources can cause mixing models to underperform, the data for resources with similar isotope values were combined *a priori*, whilst respecting the taxon and functional affiliation of the individual species, as per Phillips, Newsome & Gregg (2005). Accordingly, models at each site always included 'prey fishes'. At Site 1, they also included one macroinvertebrate group, 'Arthropoda' (*Gammarus pulex*, Hydropsychidae and Simuliidae spp.). At Site 2, differences in stable isotope data within the Arthropoda enabled inclusion of two groups in the mixing model (1: *Gammarus pulex* and *Asellus aquaticus*; 2: other Arthropoda), and at Site 3, two groups of Arthropoda (as Site 2), plus Lymnaea sp. Isotopic fractionation factors between resources and consumers in the models were 3.4 ‰ (±0.98 ‰) for $\delta^{15}N$ and 0.39 ‰ (±1.3 ‰) for $\delta^{13}C$ (Post, 2002). Outputs were the predicted proportion of each resource to eel diet (0–1), with the predicted proportion of fish used as a measure of the extent of piscivory in each individual *A. anguilla*. The stable isotope data were then used to calculate the standard ellipse area (SEA_c) for the infected and uninfected eels at each site using

the SIAR package (Parnell *et al.*, 2010) 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, 2012). The subscript 'c' in SEA_c indicated that a small sample size correction was used due to the limited number of *A. anguilla* sampled. Where SEA_c overlapped between the infected and uninfected eels, the percentage of overlap was calculated to indicate the extent to which they shared food resources.

Statistical analysis

Differences in $\delta^{13}C$ and $\delta^{15}N$ between infected and uninfected *A. anguilla* at each site were tested using generalised linear models (GLMs); the stable isotope data were dependent variables and infection status was the independent variable. The effect of total *A. anguilla* length was included in initial models but removed if it was not significant. In subsequent analyses, as the data used were standard for all sites, they were combined and used in linear mixed models. 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 *A. anguilla* were used as true replicates, models were fitted with site as a random effect on the intercept. Thus, the model testing for difference in *A. anguilla* weight according to *A. crassus* infection used weight as the dependent variable, infection status as the independent variable, site as the random effect and total length as the covariate (Garcia-Berthou, 2001). The significance of the difference in weight between the groups was determined by pairwise comparisons of estimated marginal means, adjusted for multiple comparisons (Bonferroni). Differences in hepatic–somatic index, mean HW:TL ratios, total lengths and the extent of piscivory in diet between infected and uninfected *A. anguilla* were then tested using the same model structure, but without length as a covariate. Finally, the effect of HW:TL ratios on the extent of piscivory in eel diet was tested across the sites using linear regression.

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 data of each individual eel on their (i) HW:TL ratio, and (ii) estimated proportion of fish in their diet, using eqn 1

$$e^{(a+bx)}/1 + e^{(a+bx)}, \quad (1)$$

where a and b were the regression coefficients, and x is either HW:TL ratio or proportion of fish in diet. A final PoI model used both HW:TL ratios and estimated proportion of fish in their diet (D) in eqn 2

$$e^{(a+bHW:TL+cD)}/1 + e^{(a+bHW:TL+cD)}, \quad (2)$$

where a, b and c were the regression coefficients. Predicted group membership and its probability (infected or uninfected) were stored as model outputs, with differences in probabilities tested between groups using Mann–Whitney *U*-tests. Predicted group membership was compared with the actual data set and expressed as the proportion that were correctly assigned.

The relationships of parasite abundance (as number of mature female *A. crassus*) with total length, body mass, hepatic–somatic index, HW:TL ratios and extent of piscivory were then tested in two ways. Firstly, the abundances were grouped by the number of mature female parasites present in the swim bladder, where low = 1–3 parasites, medium = 4–6 and high >7. These groups were then used in linear mixed models using the same model structures as already described for infected and uninfected eels. The abundance data were then used as the continuous variable in multiple regression, where total length, body mass, hepatic–somatic index, HW:TL ratios and extent of piscivory were used as explanatory variables. Outputs were assessed according to the values of the standardised β coefficients (higher values indicate a greater contribution to the variance of the data) and the significance of the explanatory variables.

Other than the stable isotope mixing models, all analyses were completed in IBM SPSS v. 21.0 (Armonk, New York, USA). In all analyses, the assumptions of normality of residuals and homoscedasticity were checked, and response variables were log-transformed to meet the assumption if necessary.

Results

Across the three *A. anguilla* populations, the prevalence of *A. crassus* ranged between 58 and 70% per population, with abundance between 1 and 13 mature female parasites per infected individual (Table 1). Of the 86 eels sampled across all the sites, 54 were infected with *A. crassus* (63%). Nine native parasites were also recorded on the eels across the sites, all at minor levels of infection, and thus were considered inconsequential. *Gymnocephalus cernua* was recorded as a paratenic host of *A. crassus* at sites 1 and 2. The application of stable

isotope mixing models to the stable isotope data (Table 2) revealed a significant increase in the proportion of fish in diet as HW:TL ratio increased ($R^2 = 0.28$, $F_{1,58} = 4.82$, $P = 0.03$; Fig. 1).

Differences in the stable isotope values for infected and uninfected *A. anguilla* were significant for $\delta^{13}C$ from sites 1 and 2 (GLM: Site 1: Wald $\chi^2 = 6.84$, mean difference 1.14 ± 0.30 ‰, $P < 0.01$; Site 2: Wald $\chi^2 = 6.13$, mean difference 1.05 ± 0.42 ‰, $P < 0.01$) and for $\delta^{15}N$ from Site 3 (GLM: Wald $\chi^2 = 8.49$, mean difference 0.59 ± 0.21 ‰, $P < 0.01$) (Table 2; Fig. 2). Across all sites, infected eels had significantly larger HW:TL ratios and higher estimated proportions of fish in their diet compared with uninfected eels ($P < 0.01$; Tables 3 and 4; Fig. 1). There were, however, no significant differences between infected and uninfected eels in their total lengths, body mass and hepatic–somatic index ($P > 0.05$; Table 4). Trophic niche size, as SEAc, was higher in infected *A. anguilla* than in uninfected *A. anguilla* from sites 1 (3.11 versus 2.61 ‰^{2}) and 3 (3.10 versus 1.10 ‰^{2}), with the converse for Site 2 (2.65 versus 1.63 ‰^{2}). The amount of overlap in the trophic niches of the uninfected and infected *A. anguilla* was relatively low, with infected *A. anguilla* sharing 34.8, 15.4 and 9.2% of trophic niche space with uninfected *A. anguilla* in sites 1, 2 and 3, respectively (Fig. 2).

The binary logistic regression models were all significant, revealing both HW:TL ratios and the extent of piscivory had significant effects on *A. crassus* infection (Table 5). Comparison of predicted group membership revealed that HW:TL ratio correctly assigned 72% of *A. anguilla* to their observed infection status, HW:TL ratio and extent of piscivory correctly assigned 76%, and extent of piscivory 78%. In the latter model, the difference in the mean probability of infection between uninfected and infected *A. anguilla* was significant (uninfected: 0.34 ± 0.05; infected: 0.71 ± 0.04; Mann–Whitney *U*-test $Z = -4.72$, $P < 0.01$) (Table 5).

The linear mixed models testing the significance of differences in biometrics according to light, medium and heavy *A. crassus* infections across the 32 infected *A. anguilla* revealed some significant differences in lengths between these groups (Table 6). However, there were no significant differences in HW:TL ratios, extent of piscivory in diet, hepatic–somatic index and weight (Table 6), where the effect of length as a covariate was significant in the latter model ($P < 0.01$). When these variables were used in a multiple regression with parasite abundance used as a continuous variable, the overall model was not significant ($R^2 = 0.17$; $F_{4,27} = 1.19$, $P > 0.05$), and none of the variables had significant effects on parasite abundance ($P > 0.05$ in all cases). Total length had the highest standardised β coefficient ($\beta = 0.39$, $P > 0.05$).

Table 1 Prevalence and abundance of *Anguillicoloides crassus* in the *Anguilla anguilla* populations

Site	*n*	Prevalence (%)	Mean abundance of female parasites (± SE)	Range
1	30	70	2.61 ± 0.52	0–8
2	30	63	2.05 ± 0.54	0–5
3	26	58	2.66 ± 0.70	0–13

Table 2 Sample sizes and mean total lengths, and $\delta^{13}C$ and $\delta^{15}N$, of infected and uninfected *Anguilla anguilla* at each site, plus the mean $\delta^{13}C$ and $\delta^{15}N$ values of their putative food resources used in mixing models. Error around the mean is standard error

Site	Species	*n*	Mean length (mm)	Mean $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
1	Infected *A. anguilla*	9	467 ± 73	−31.14 ± 0.29	21.48 ± 0.23
	Uninfected *A. anguilla*	9	460 ± 81	−32.28 ± 0.36	20.54 ± 0.74
	Prey fishes			−32.33 ± 0.10	22.72 ± 0.66
	Arthropoda			−30.66 ± 0.18	19.88 ± 0.22
2	Infected *A. anguilla*	10	422 ± 143	−29.22 ± 0.16	21.00 ± 0.28
	Uninfected *A. anguilla*	9	433 ± 152	−30.27 ± 0.41	20.68 ± 0.21
	Prey fishes			−29.93 ± 0.30	20.00 ± 0.45
	Arthropoda 1			−31.61 ± 0.43	14.94 ± 0.14
	Arthropoda 2			−31.62 ± 0.13	16.33 ± 0.17
3	Infected *A. anguilla*	9	363 ± 86	−30.18 ± 0.53	13.65 ± 0.20
	Uninfected *A. anguilla*	14	321 ± 102	−29.48 ± 0.28	13.06 ± 0.08
	Prey fish			−30.53 ± 0.31	12.30 ± 0.24
	Arthropoda 1			−32.44 ± 0.09	8.34 ± 0.18
	Arthropoda 2			−29.92 ± 0.33	8.72 ± 0.23
	Lymnaea			−21.96 ± 0.11	7.73 ± 0.01

Discussion

Anguilla anguilla head morphology is related to intrapopulation diet specialisation whereby broader headed fish are more piscivorous (Cucherousset *et al.*, 2011). Consequently, that head width/total length ratios were significantly higher in eel infected by *A. crassus* in the three populations suggests this was associated with their increased piscivory. This then infers that the consumption of paratenic fish hosts by *A. anguilla* was important for *A. crassus* transmission in these populations. This inference was also supported by the outputs of the sta-

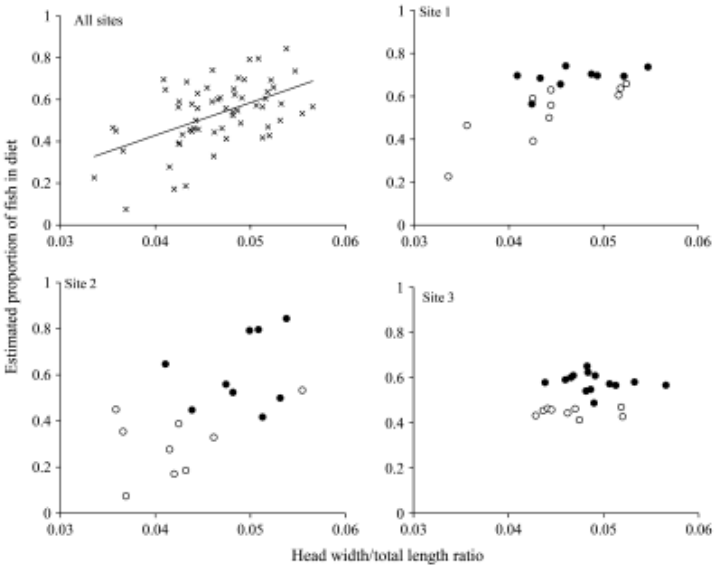

Fig. 1 Relationship between head width and total length (HW:TL) ratio and estimated extent of piscivory in the diet of *Anguilla anguilla* in all sites (×), where the solid line represents the significant relationship between the variables according to linear regression, and for sites 1 to 3 according to their infection status by *Anguillicoloides crassus* (infected: ●; uninfected: ○).

ble isotope mixing models. Whilst these indicated that all of the eels were facultative piscivores, individuals with higher estimated proportions of fish in their diet had greater probabilities of being infected with *A. crassus*. Thus, both head width/total length ratios and the estimated proportion of fish in diet were significant predictors of infection status, with up to 78% of eels correctly assigned by the models.

The trophic fractionation between the eels and their prey fishes was often low and highly variable, but generally below the 3.4 ‰ δ^{15}N that would be expected had their diet been based entirely on fish, that is one trophic level (Grey, 2006). This variability in fractionation was then reflected in the predictions from the mixing models of the proportions of fish in the diet of individual eels, where the mean for all eels was 0.53 (± 0.02 SE) and range 0.08–0.84. It should be noted that the mixing models provided estimates of diet composition based on standard isotopic fractionation factors, and given that mixing models are sensitive to the fractionation factors used (Phillips *et al.*, 2014), then these might have influenced their outputs. Had species-specific fractionation factors been available, then some absolute differences in the dietary proportions might have resulted (Bond & Diamond, 2011; Phillips *et al.*, 2014). Whilst this suggests some uncertainty in the extent of the actual differences in piscivory between the infected and infected eels, it remains that broader headed eels tend to be more piscivorous (e.g. Cucherousset *et al.*, 2011) and the study outputs revealed that the probability of infection increased significantly as head width increased, irrespective of diet predictions. An alternative approach to providing robust estimates of the extent of piscivory in *A. anguilla* diet would have been stomach contents analysis, although this was not feasible with the low *A. anguilla* sample numbers available. Indeed, the sample sizes used per population in the study were relatively low compared with other recent studies on *A. crassus* (e.g. Lefebvre *et al.*, 2013), but this was unavoidable given the endangered status of eel populations generally allied with the sampled populations being from small rivers. Consequently, although the study outputs were relatively unambiguous across the sites with consistent infection patterns apparent, the use of small sample sizes and the diet estimates being derived from mixing models does introduce some inherent uncertainties in the overall output.

The recent study in southern France of Lefebvre *et al.* (2013) revealed that *A. anguilla* with severe swim bladder damage due to *A. crassus* infections had greater body lengths and mass compared to non-infected individuals of the same age. The authors postulated that their findings were most likely due to the most active foragers growing faster and having a greater probability of becoming repeatedly infected via trophic transmission and with infection having a low energetic burden. Our study did not reveal a similar significant difference in body length and mass between infected and non-

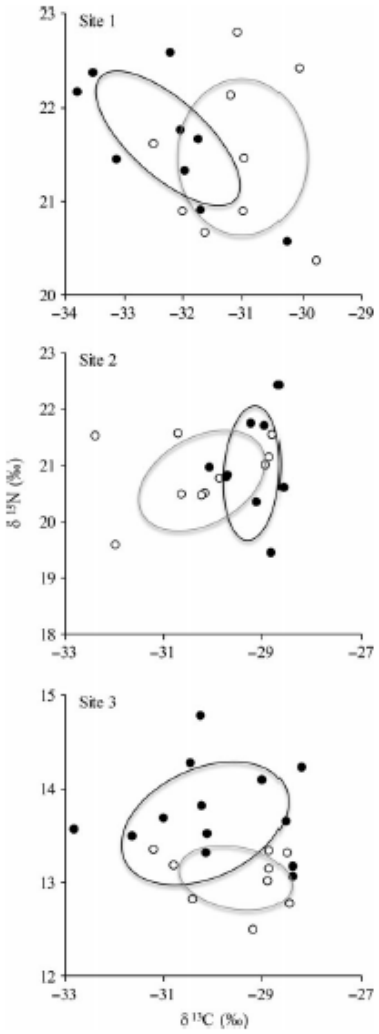

Fig. 2 Stable isotope bi-plots of infected (●) and uninfected *Anguilla anguilla* (○) at each site. Black ellipses represent the trophic niche size (as standard ellipse area) of infected eels, and grey ellipses represent those of uninfected eel. Note different *X* and *Y* axes values for the sites.

infected individuals, or any effect of parasite abundance on biometrics, although the mean infection levels we recorded (<3.0) were lower than those (4.1 ± 4.4) reported by Lefebvre *et al.* (2013). Whilst we cannot discount this being a potential effect of a smaller sample size in our study, our findings are consistent with other studies (Koops & Hartmann, 1989; Wuertz, Knopf & Taraschewski, 1998). Irrespective, we argue that our outputs provide empirical support for the interpretations of Lefebvre *et al.* (2013). However, rather than the most active foragers being most vulnerable to the parasite, we suggest it is the more piscivorous individuals that are repeatedly exposed to the parasite, most likely via increased consumption of paratenic fish hosts, facilitated by their head functional morphology. We speculate that the consequent greater energetic intake associated with piscivory would then facilitate the faster growth rates observed by Lefebvre *et al.* (2013).

Notwithstanding these significant relationships between functional morphology, diet and *A. crassus* infections, we acknowledge that the extent of piscivory of individual *A. anguilla* at the time of infection could not be determined. Consequently, we cannot definitively conclude that infection was a causal consequence of

Table 3 Mean head width/total length ratios (HW:TL) and mean proportion of fish in the diet of *Anguilla anguilla* uninfected and infected with *Anguillicoloides crassus* in the three study sites. Error around the mean is standard error

Site	*A. anguilla* infection status	HW:TL	Proportion of fish in diet
1	Uninfected	0.042 ± 0.002	0.31 ± 0.05
	Infected	0.049 ± 0.001	0.61 ± 0.06
2	Uninfected	0.044 ± 0.002	0.53 ± 0.04
	Infected	0.048 ± 0.001	0.69 ± 0.02
3	Uninfected	0.046 ± 0.001	0.45 ± 0.01
	Infected	0.049 ± 0.001	0.58 ± 0.01

Table 4 Outputs of linear mixed models testing the significance of (a) *Anguilla anguilla* total length, (b) *A. anguilla* body mass, (c) hepatic–somatic index (HSI), (d) standardised ratio of head width to total length and (e) extent of piscivory in diet on the infection status of *A. anguilla* from three populations. Site was the random effect on the *y* intercept

Pairwise comparison	Mean difference (estimated marginal means)
(a) Infection status ~ total length: AIC = 721.0; log likelihood = 717.0	
Infected versus uninfected	11.2 ± 28.1 mm, $P > 0.05$
(b) Infection status ~ body mass: AIC = 634.7; log likelihood = 630.7	
Infected versus uninfected	1.5 ± 13.1 g, $P > 0.05$
(c) Infection status ~ HSI: AIC = −138.6; log likelihood = −142.6	
Infected versus uninfected	0.01 ± 0.01, $P > 0.05$
(d) Model: Infection status ~ HW:TL: AIC = −447.1; log likelihood = −451.1	
Infected versus uninfected	0.002 ± 0.001, $P = 0.003$
(e) Model: Infection status ~ Extent of piscivory: AIC = −57.8; log likelihood = −61.8.	
Infected versus uninfected	0.18 ± 0.04, $P < 0.001$

Table 5 Binary logistic regression coefficients (eqn 1) and their statistical significance for the probability of infection of *Anguilla anguilla* by *Anguillicoloides crassus* according to (a) ratio of head width to total length (HW:TL), (b) predicted proportion of fish in *A. anguilla* diet and (c) both variables

Parameter	Symbol in equation 1	Coefficient	Standard error	*P*
(a)				
Constant	*a*	0.15	0.28	0.58
HW:TL	*x*	176.10	7.37	<0.01
(b)				
Constant	*a*	−8.49	2.47	<0.01
Diet	*x*	18.61	0.33	<0.01
(c)				
Constant	*a*	−8.50	2.60	<0.01
HW:TL	*b*	169.85	81.57	0.03
Diet	*c*	18.57	5.54	<0.01

Table 6 Outputs of linear mixed models testing the significance of *Anguillicoloides crassus* abundance (low, medium, heavy infections) on (a) total length, (b) body mass, (c) hepatic-somatic index (HSI), (d) standardised ratios of head width to total length and (e) extent of piscivory. Site was the random effect on the y intercept

Pairwise comparison	Mean difference (estimated marginal means)
(a) Parasite abundance ~ total length: AIC = 355.5; log likelihood = 351.5, $P = 0.01$	
Low/medium	121.9 ± 37.7 mm, $P = 0.01$
Low/high	87.8 ± 45.6 mm, $P > 0.05$
Medium/high	34.0 ± 46.0 mm, $P > 0.05$
(b) Parasite abundance ~ body mass: AIC = 315.2; log likelihood = 311.2, $P > 0.05$	
Low/medium	15.3 ± 21.1 g, $P > 0.05$
Low/high	7.9 ± 23.5 g, $P > 0.05$
Medium/high	7.4 ± 22.0 g, $P > 0.05$
(c) Parasite abundance ~ HSI: AIC = −102.9; log likelihood = −106.9, $P > 0.05$	
Low/medium	0.01 ± 0.01, $P > 0.05$
Low/high	0.01 ± 0.01, $P > 0.05$
Medium/high	0.01 ± 0.01, $P > 0.05$
(d) Model: Parasite abundance ~ HW:TL: AIC = −229.0; log likelihood = −233.0, $P > 0.05$	
Low/medium	0.01 ± 0.01, $P > 0.05$
Low/high	0.01 ± 0.01, $P > 0.05$
Medium/high	0.01 ± 0.01, $P > 0.05$
(e) Model: Parasite abundance ~ piscivory: AIC = −59.89; log likelihood = −63.86; $P > 0.05$	
Low/medium	0.03 ± 0.03, $P > 0.05$
Low/high	0.03 ± 0.03, $P > 0.05$
Medium/high	0.06 ± 0.04, $P > 0.05$

head functional morphology. Moreover, in some fishes, parasitism causes shifts in feeding behaviour and trophic position through mechanical processes and/or changes in energy demand (Barber, Hoare & Krause, 2000; Britton, Pegg & Williams, 2011), and can induce changes in habitat utilisation that can influence foraging behaviours (Blanchet *et al.*, 2009; Britton, Jackson & Harper, 2009). Thus, we cannot discount that the shift to piscivory in *A. anguilla* occurred post-infection. However, we consider this scenario unlikely, as *A. anguilla* head morphology is a well-recognised functional trait known to enable greater individual specialisation in piscivory (Proman & Reynolds, 2000; Cucherousset *et al.*, 2011), and was documented in their populations prior to the introduction of *A. crassus* into Europe (Moriarty, 1974; Tesch, 1977). In addition, the development of the trait of 'broad-headedness' is apparent throughout the life of individual eels (from glass eel to maturity; Proman & Reynolds, 2000) and thus is unlikely to be a parasite-induced trait (Decharleroy *et al.*, 1990; Moravec *et al.*, 1994). As such, we propose that the higher extent of piscivory that was apparent through this functional morphology in infected *A. anguilla* at the time of sampling was most likely a causal factor in their infection, with their increased consumption of fish paratenic hosts at least partially responsible. However, we also recognise that other factors, such as individual differences in MHC genes and differences in cytokine regulation, might have also influenced the host qualities of these eels, so that vulnerability to *A. crassus* infection is likely to depend on more complex factors than diet and functional morphology alone (Knopf & Lucius, 2008).

Several studies of *A. crassus* in *A. anguilla* have suggested that body size is a strong predictor of infection, with larger *A. anguilla* having higher levels of prevalence and abundance than smaller *A. anguilla* (Barus & Prokes, 1996; Schabuss *et al.*, 2005; Lefebvre *et al.*, 2013). In German populations, however, there was no correlation between infection status and *A. anguilla* length and weight (Wuertz *et al.*, 1998), as with our findings. Overall, we suggest body length and mass are relatively crude metrics to test against *A. crassus* infection, as *A. anguilla* growth rates in their freshwater life stage can be extremely variable [e.g. 14 to 152 mm per year (Aprahamian, 2000)], and the duration of the freshwater life stage can be as low as 3–5 years (Camargue Lagoon, France; Melia *et al.*, 2006) and as high as 33–57 years (Burrishole, Ireland; Poole & Reynolds, 1996). Therefore, assessing infection levels using a metric that is subject to such variability over time and space might be limited in its utility for understanding the infection dynamics. We suggest that measurements that incorporate head functional morphology are a more appropriate metric due to its influence on diet composition and the apparent importance of paratenic hosts in *A. crassus* transmission.

Whilst the actual role of *A. crassus* in the decline of *A. anguilla* populations remains unclear, the pathology associated with infections has been related to increased freshwater mortality in populations exposed to additional environmental stressors (Kirk, 2003). Additionally, the damage to the swim bladder severely impacts on swimming performance (Palstra *et al.*, 2007) and can thus potentially disrupt spawning migrations (Barry *et al.*, 2014; Pelster, 2015). Thus, in conclusion, we suggest these consequences of parasitism in *A. anguilla* are focussed on those individuals in populations whose functional morphology enables greater specialisation in piscivory, through a mechanism of greater parasite exposure via higher consumption of paratenic fish hosts.

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