A new look at factors influencing intraguild predation and cannibalism between native and invasive species.

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The candidate confirms that the work submitted is his own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

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Bunke M: (candidate) designed experiment, practical work, analysis, wrote paper
Dunn AM: (supervisor) contributed to experimental design and paper writing
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Abstract

Biological invasions are a major ecological problem with freshwater environments particularly susceptible to their impacts. Invasive species interact with their native analogues and have direct and indirect impacts on their population dynamics. These interactions between natives and invasives are also influenced by parasitism.

Cannibalism and intraguild predation are important interaction between individuals, because they can affect the dynamics of the prey population. They may offer the predator the advantage associated with the removal of potential competitors. In this thesis I use the predatory functional response approach to investigate the cannibalism and intraguild predation interactions between native and invasive amphipods and the influence of parasitism on these interactions.

I explored cannibalism in the native *Gammarus duebeni celticus* and the invasive *Gammarus pulex* in Northern Ireland. The rate of cannibalism of juveniles by adults did not differ between the species. However, I found that in *G. d. celticus* this maximum consumption rate is doubled by an infection with microsporidian parasite *Pleistophora mulleri.* Investigation of cannibalism in *G. pulex* revealed that the effect of the acanthocephalan parasite *Echinorhynchus truttae* was influenced by other environmental factors: in the presence of habitat structure and of higher order predator cures, the cannibalistic maximum consumption rate of infected *G. pulex* to be double that of their uninfected counter parts. This means that parasite infection can have a negative impact on the population dynamics of its host and might even cause population collapse if too many juveniles are consumed.

I also used the comparative functional response approach to compare intraguild predation between the native and the invasive species. Overall I found the IGP maximum consumption to be higher than the cannibalistic maximum consumption rate. This means that the invader can affect the population dynamics of the native and the native can affect on the population dynamics of the invader. No difference was found in the maximum consumption rate by adults of heterospecific juveniles. I found that parasitism causes the maximum consumption rate of *G. d. celticus* to increase while it does not impact on the maximum consumption rate *of G. pulex*. This may indicate that parasitism facilitates the coexistence between the native and invasive.

I also investigated the IGP between the native *Gammarus pulex* and the recently arrived invader *Dikerogammarus villosus* in England. I used the comparative functional response approach to investigate how an increase in temperature, as might be caused by climate change, affects the interaction between the two species. Overall the maximum consumption rate of heterospecific juveniles was five times higher in the invader than in the native. An increase in water temperature caused the maximum consumption rate in the invader to increase while it showed a deceasing trend in the native's maximum consumption rate. This may mean that climate change might speed up the invasion process in this case. I also investigated how an parasitic infection in the native impacts IGP interactions in adults and found that increases the occurrence of IGP. In this case parasitism may facilitate the invasion process and the replacement of the native by the invader.

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Chapter One

General Introduction

Freshwater ecosystems are important because they provide us with numerous ecosystem services (Aylward et al. 2005). They are disproportionally important for biodiversity because even through freshwater systems only cover 1% of earth's surface; they contain approx. 10% of all known species (Strayer and Dudgeon 2010).

Freshwater ecosystems are among the most threatened by human activity (WWF 2014). They are especially at risk because their fragmented nature means that their populations are relatively isolated compared to terrestrial or marine equivalents. This isolation is similar to that of islands. This higher degree of isolation means that individuals are also more isolated and may be evolutionary naïve to new archetypes of invasive species. This could be one of the reasons why impact of invasive species are stronger when compared to marine and terrestrial realms (Cox and Lima 2006). They are also particularly vulnerable to invasive species introduction as a result of transport, trade, tourism and recreational activities such as fishing (Gallardo and Aldridge 2013a).

Invasive species are species which have been introduced either intentionally or unintentionally by human action and which have established, reproduced and spread at multiple sites within their introduced range (Blackburn et al. 2011). Aquatic invasive species are one of the leading causes of biodiversity loss (Collen et al. 2013) and it is estimated that their control comes at an annual coast of 26.5 million which likely to increase with future spread of invasive species (Gallardo and Aldridge 2013b).

The knowledge we have about how invasive species impact on fresh water ecosystems is limited because research carried out is biased towards terrestrial biomes (Lowry et al. 2013). This is especially detrimental because we know that invasive species have caused a higher rate of biodiversity loss in freshwater systems 76% than in in marine and terrestrial systems (39%) each over the past 40 years (WWF 2014, Collen et al 2013).

Invasive species affect native communities as a result of a range of trophic interactions including competition, predation, intraguild predation and parasitism. In this chapter I briefly review ways in which invasive species interact with species from their receptive ecosystems and how parasitism may influence these interactions. I will then introduce the work that will be presented in my thesis.

1.1. Invasive species

Freshwater ecosystems are particularly impacted by invasive species in many parts of the world (Strayer and Dudgeon 2010; Moorhouse and Macdonald 2014). Aquatic ecosystems are particularly vulnerable to invasions because they are physicly interconnected and have a high level of human disturbance (Dudgeon et al. 2006). Climate change and invasive species are two of the most prominent environmental stressors to biodiversity and species communities (Bellard et al. 2012; Simberloff et al 2013). Invasive species include species which have arrived by accident such as in ship ballast waters and accidental release or are introduced intentionally (Keller et al.2009). Freshwater systems can become dominated by invasive species. For example 90% of river Rhine's invertebrate species are invasive (Riel et al. 2006). Invasive species can impact native communities directly (predation) and indirectly (altering energy flow of ecosystems) (Salo et al. 2007).

Invasive species include species from all functional guilds (figure 1.1.) causing impact through their trophic interactions (Strayer 2010; Gallardo et al. Vilà 2016). They can impact on biotic properties that include species diversity and population size of native species. Impacts are caused by trophic interactions, competition and by habitat-invader interaction (Gallardo et al. 2016). Abiotic factors affected include flow and turbidity of the water (Strayer 2010). Invasive plants can for example shade out water bodies and thus outcompete other plant species for light resulting in a loss of primary production in the system (Strayer 2010; Gallardo et al.2016). Invasive filter feeders can reduce the biomass of phyto and zooplankton resulting in more light in the water column, and thus in more bentic primary production and invertebrates, but can also cause loss of free organic matter from system to move up the food web (Strayer 2010). Invasive grazers such as snails have been found to reduce benthic biomass and increase phytoplankton production by enriching water column with nutrients (Carlsson et al. 2004). Grazing fish are likely to increase water turbidity by disturbing the sediment (Matsuzaki et al.2007). Invasive omnivores have been observed to be more predatory which results in reduced decomposition rates of litter (Piscart et al. 2011; MacNeil et al.2011).

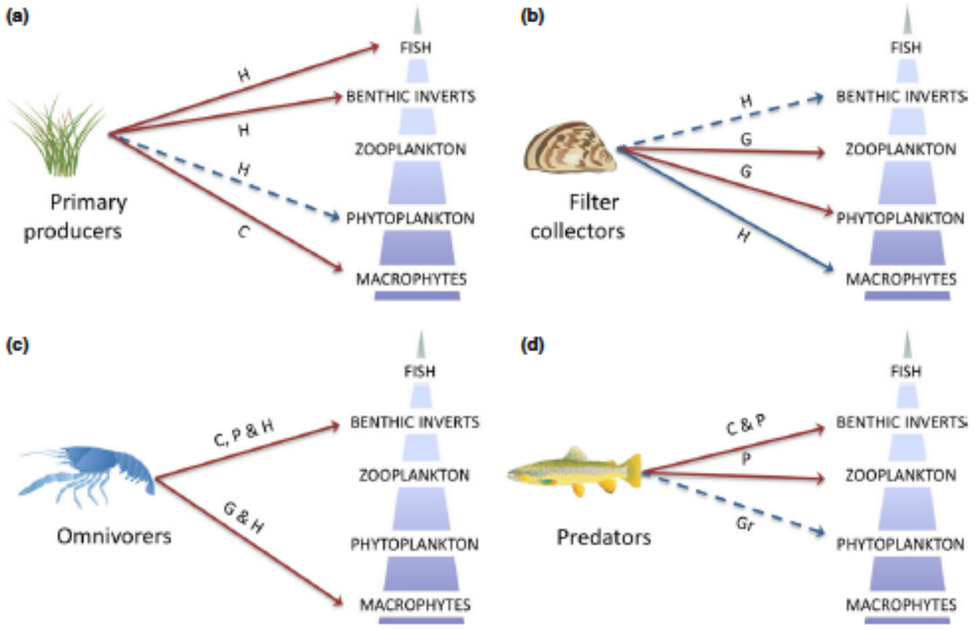

Figure 1.7. Impacts of the different functional guilds of freshwater invasive species on the different levels of the food web. From Gallardo et al. (2016) . The arrows reflect the negative (unbroken lines) or positive (dashed lines) impacts of the invasive species on the abundance of the functional elements of the native community. Impacts are the result of the direct ecological (C- competition, P-predation, G-grazing) and indirect impacts of invasive species (Gr – grazer release, H – habitat alteration).

Invasive predators are also known to have impacts on populations of native prey. For example introduced salmonids led to a decrease in the number of stream invertebrates present (Simon and Townsend 2003). Another example is that the mosquitofish (*Gambusia spp*.) has reduced densities of native invertebrates and outcompete native fishes (Pyke 2008). Like predatory fish, benthic amphipods such as *Dikerogammarus villosus, Gammarus tigrinus, corophium curvispinum* and *Gammarus pulex* have been widely established in Europe outside their native ranges. They have been found to have large predator impacts causing a decline in macroinvetebrates diversity and abundance (Kinzler and Maier 2003; Kelly and Dick 2005; Berezina 2007) as well as being a leading cause behind replacement of native amphipods (Dick et al. 1999; Dick and Platvoet 2000).

Overall, there is strong evidence that successful invaders have a higher consumption than native analogues (Ella et al. 2016). The invasion process tends to favour ecological traits that promote adaptability and tolerance to environmental stress (Lee 2002). Species with a high rate of consumption are able to sustain rapid growth rates and early reproductive maturity. This helps establishment and spread. Changes caused by invasive species can cause changes in ecosystem functioning such as decomposition rates, primary production and nutrient cycling (Strayer 2010; Gallardo et al. 2016). The success and impact of an invasive species can be understood through comparisons with native analogues, with the precise questions answered depending upon the choice of analogue (Dick et al. 2014).

There is considerable empirical evidence for the effect of invasive predators on populations of native prey species, as illustrated by the examples above. However, other trophic interactions are less well studied. Intra guild predation (IGP) is the predation between members of the same ecological guild; i.e. species that are potential competitors (Polis 1981). Cannibalism is another widespread process (Polis 1981; Crump 1990; Elgar and Crespi 1992; MaNeil et al. 1997) that is common in populations (such as amphipods) which are stage structured and in which generations overlap in space and time (Crump 1990; Rudolf 2007a). In this thesis I explore cannibalism and intraguild predation within and between native and invasive amphipods.

Species invasion can also cause novel host parasite interactions, which also affect the invasion impact. This can be the result of density effects, for example when the invasive signal crayfish *Pacifastacus leniusculus,* which was introduced in the UK is a vector to the highly pathogenic crayfish plague *Aphamomyces astaci* (Alderman 1993)*,* it caused the white clawed crayfish *Austropotamobius pallipes* to become endangered across Europe (Svoboda et al. 2017), while itself is immune to the disease and has managed to replace the native in many locations.

Parasites can also influence species invasions by mediating native invader interactions, through trait mediated effects, i.e. changes in their hosts behaviour, morphology, life history or physiology (Hatcher and Dunn 2011; Dunn et al. 2012). For example the outcome of competitive interaction between the native mosquito *Ochlerotatus triseriatus* and the invasive mosquito *Aedes albopictus* in North America is modified by the gregarine parasite *Ascogregarina taiwanensis*, with infected invasive individuals having a lower impact on the native (Aliabadi and Juliano 2002). However, because prevalence in the first years of invasion was low it allowed the invader to be more competitive during the initial phase of its invasion increasing the likelihood of successful invasion (Juliano and Lounibos 2005).

Parasites can also alter the predatory impact of invasive species. For example infection with the acanthocephalan parasite *Echinorhynchus truttae* in *G. pulex* has been shown to increase the maximum consumption of invertebrate in its host (Dick et al. 2010). Whilst there are a number of examples of parasites affecting competition and predatory interactions, less is known about the effect of parasitism on other trophic interactions. In this thesis I explore the effect of parasitic infection on cannibalism and on intraguid predation.

1.2. Parasitism

Parasites represent close to half of all biodiversity (Poulin and Morand 2000; Dobson et al. 2008). They can play pivotal roles in ecosystems (Thomas et al. 1996; Hatcher et al. 2012). Parasites are known to be a powerful force in the structuring of communities (Hudson et al. 2006). Their potential to influence population and community structure is increasingly recognised (Tompkins et al. 2003). It is now known that Parasites might increase or decrease ecosystem processes by reducing host abundance (Frainer et al. 2018). It is also thought that they could cause an increase in trait diversity by supressing dominant species or by increasing host diversity (Frainer et al. 2018). This range of effects makes it difficult to predict the net effects parasite have on the ecosystem.

In the last 20 years there has been a steady advancement in our understanding of how important parasites are as integral parts of ecosystems (Poulin 1999; Hudson et al. 2006; Tompkins et al. 2011) but we are still far away from having a complete picture which connects the ecological phenomena to parasites (Sures et al. 2017). There are still only a limited number of examples of parasites that affect population dynamics or of how the behavioural manipulations by parasites affect energy and biomass flows in ecosystems and food webs (Sures et al. 2017). So far only a limited number of studies into how parasites affect population dynamics of their hosts have been carried out and we are only just at the beginning how they affect the biomass flow in ecosystems and food webs (Sures et al. 2017).

1.2.1. Alteration of host behaviour by parasites

Parasites can affect the behaviour of their hosts. For example, the microsporidian *P. mulleri* infects the muscle of its *G. d. celticus* host, causing reduced motility (MacNeil et al. 2003a) . Infected *G. d. celticus* are less strong predators than are those uninfected (MacNeil et al. 2003b). Furthermore, some parasites manipulate their host behaviour in order to enhance their own fitness, in particular by increasing the probability of their transmission from one host to another, at the expense of their host (Thomas et al. 2005). For example, *E. truttae* causes increased motility and altered phototaxis in the *G. pulex* host, making the intermediate host susceptible to predation by fish, the final host for the parasites (MacNeil et al. 2003d). It has been highlighted that this manipulation can have an impact on a large scale ranging from the host population to ecosystems (Lefèvre et al. 2009). In *G. pulex, E. truttae* infection causes increased rate of predation of *Asellus aquaticus* (Fielding et al. 2003).

However, the manipulation itself may also be influenced by environmental factors. With factors interacting to shape interactions between individuals in the ecosystem (figure 1.2.) (Labaude et al. 2015).

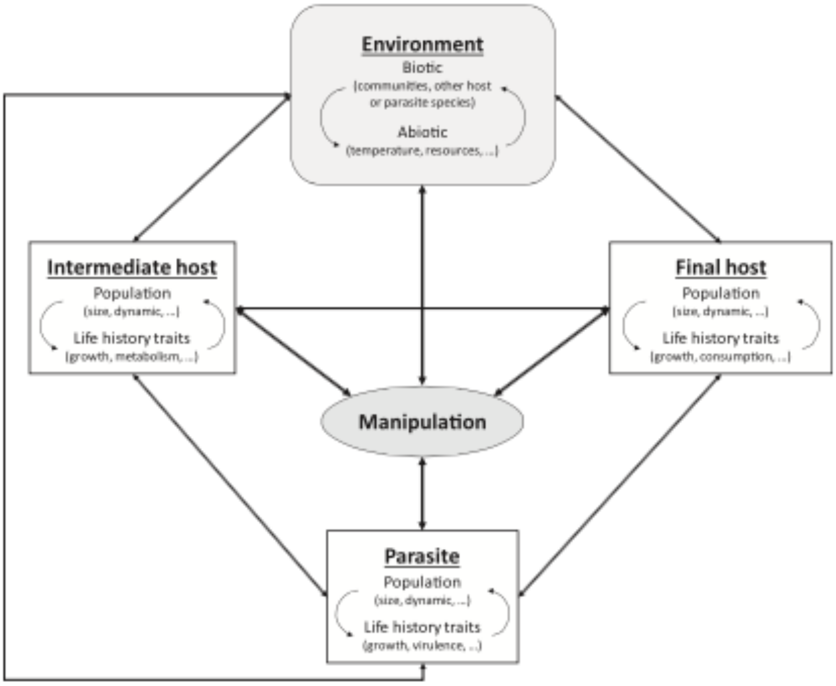

Figure 1.8. Schematic representation of all the interacting factors in a system involving parasite manipulation from Labaude et al. (2015).

Manipulative parasites can induce modifications in their host which can impact on their hosts population dynamics and structure (Labaude et al. 2015) as well as modifying predatory-prey dynamics, Mathematical modelling suggests that this is likely to have consequences for other populations in the ecosystem (Fenton and Rands 2006). For example parasites may alter the behaviour and morphology of their host which in turn might cause them to occupy different ecological niches, *Gammarus insensibilis* infected by the trematode *Microphallus papillorobustus* were found in surface waters of salt marsh, whilst those uninfected were found lower in the water column (Ponton et al. 2005). This may cause an ecological segregation between the infected and uninfected proportion of the population.

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1.2.2. Influence on species invasion

Parasites are now recognised to be able to affect the outcome of species interactions. This can have consequences on species invasions. They can facilitate or limit invasions, and have positive or negative impacts on native species (Dunn et al.2012).

There are three ways in which parasites determine success of initial stages of invasion. The first way is that they can cause what is called a "spill over". This happens when new pathogens are introduced along with the invasive host and spill-over to infect the native population (Tompkins,et al. 2011; Hatcher et al. 2012).

Parasites can also cause a phenomena known as "spill back", this happens when the invader functions as a reservoir for the parasites, thus causing an increase in the prevalence of the infection (Kelly et al. 2009; Poulin et al. 2011). The third way parasites influence the invasion success of a species is through enemy release (Keane and Crawley 2002). It happens when the invader is no longer burdened by the effects of parasites that affected it in its native range. Invasive animals may escape up to 75% of parasites and pathogens from their native range (Torchin and Mitchell 2004; Sarabeev et al. 2017).

Once the invasive species is established parasites may influence competition, predation and other interactions between them and native species (Dunn et al.2012). Related host species are commonly susceptible to the same parasite but often with differences in prevalence and virulence (Dunn and Dick 1998; Young et al. 2017). This means that competitors may be affected to different levels (Thomas et al.1996). The extent to which parasites impact on their host is also likely to be influenced by environmental factors. The combination of these factors may exacerbate or negate the effects that the invader has in the recipient ecosystem.

Most empirical studies have focused on pairwise interactions between parasites and hosts.. This oversimplification could result in misleading conclusions because the impact of parasites affects populations throughout communities (Roy and Lawson Handley 2012). A large amount of theoretical work on the regulation of host population by parasites has been carried out (Roy et al. 2012) but the empirical evidence of the effects at population level is limited (Tompkins et al. 2010). In this thesis I will use comparative functional responses to examine how parasitism affects IGP and cannibalism in two study systems and how it interacts with other environmental stressors.

1.3. Climate change

It is predicted that over the next decades climate change will lead to a warming of between 0.3 and 4.8°C in the UK (IPCC 2013). Warming is likely to cause changes in water chemistry, hydrological changes and community composition (van de Waal et al. 2009; Woodward et al. 2010). It is likely that climate change will interact with other current environmental stressors and may place increased environmental stress on communities.

Temperature influences all biological processes (Sentis, Hemptinne, and Brodeur 2012). It affects animal metabolism and behaviour (Bale 2002; Woodward et al. 2010). Temperature affects life history of animals (eg Bale 2002). Theoretical models have shown that temperature can influence the strength of interactions (Vasseur and McCann 2005). That the effects of warming can cascade to have effects at the population and community levels (Petchey et al. 2010).

It is likely that climate change will interact with other environmental stressors such as invasive species. Native and invasive species might differ in their thermal limits. This means that they might also differ in their response to a rise in watertemperature Invasive species often differ in their size and tolerance to temps compared to native counterparts (Bates,et al. 2013). This means that climate change could facilitate invasion events (Gallardo,et al. 2012; Bates et al. 2013). Impacts of invasive species could either be negated or excavated under the influence of climate change There is still much to learn about how these stressors interact (Sorte et al. 2013; Bellard et al. 2016). In this thesis I explore how temperature affects the functional response of intraguild predation between the native *Gammarus pulex* and the invasive *Dikerogammarus villosus*.

1.4. Species Interactions in biological invasions

The trophic links been levels in an ecosystem are heavily size structured (Woodward et al. 2010). Invasive species interact with natives directly and indirectly (Salo et al. 2007). This happens through a number of forces of inter and interspecific nature, such as predation, and competition, as well as through the less well studied interactions of cannibalism, intraguild predation and parasitism. This means that removing one consumer can have indirect effects on other trophic levels via trophic cascades (Polis et al. 2000). An interesting study by Britton et al. (2018) found that after the introduction of a new species niche partioning between native and invasive compeitors led to minimised trophic interactions between competing species. This is why it is important to consider structural and functional ecosystem consequences of invaders (Townsend 2003; Jackson et al. 2014). In this thesis, I focus on two key interactions: cannibalism and intraguild predation and how they are affected by parasitism.

1.4.1. Intraguild predation

Invaders are known to have a big predatory impact on the native species in their receptive ecosystem (Strayer 2010). Less well studied is intraguild predation between natives and their invasive analogues. (Dick et al. 2014). Intra guild predation (IGP) is the predation between members of an ecological guild; i.e. species that are potential competitors (Polis 1981). IGP is found across taxa and ecosystems- from IGP between dogs and wolves (Polis et al. 1989) to IGP between ciliates (Diehl and Feißel 2000). It can be uni- or bidrirectional and often occurs among closely related species (Dick et al. 1993). Recent analyses of real food webs indicate that IGP is widespread and important in the structuring of communities (Arim and Marquet 2004; Bascompte and Melián 2005). IGP has been shown to be key driver of exclusion of native amphipods by invaders (Dick et al. 1993).

IGp is also context dependent. Worked carried out investigating the IGP between 2 species of larval salamanders using mesocosm experiments found that the IGP between the species was affected by the presence of a higher order predator but not the presence of habitat (Anderson and Semlitsch 2016). In addition to this work carried out by Ball et al. (2015) found that the IGP occurring between five species of ground beetles depended on the body size of the IGpredator, with bigger predators being more predatory on smaller animals. These are just two examples of studies that show that IGP is affected by a number of factors. In this thesis, I will explore how environmental factor affect IGP between species of amphipods.

Theoretical work predicts that IGP can only persist in an ecosystem if the species which is the weaker IG predator is otherwise strongly competitive dominant, or it will be eliminated by the stronger predator (Holt and Polis 1997). It is still unclear if this prediction is upheld in many real ecosystems (Rosenheim 2007). However, theoretical work shows that the inclusion of parasitism in models of IGP increases the likelihood of coexistence between species (Hatcher et al. 2006).

IGP is often associated with cannibalism (Dick et al. 1993) because the ability to kill and consume conspecifics may easily transcribe into predation on congenerics (Dick et al. 1993). IGP appears to be particularly common when species that compete have age or stage structure (Holt and Polis 1997). The vulnerability of a species to IGP varies with its life history stage (Polis et al. 1989).

Generally speaking larger amphipods prey on smaller heterospecifics, particularly males (the larger sex) on females. This asymmetric loss of females potentially has a big impact on the population because it reduces population size as well as causes a loss of recruitment (e.g. Dick et al. 1993). Juvenile amphipods are also subject to IGP by adults. This interaction has clear implications for the recruitment of the population and the dynamics of coexistence.

It has been demonstrated that environmental factors are able to modulate IGP (Piscart et al. , 2009) this is why studying IGP in various environmental contexts will help to understand and predict the structuring of communities and possible outcomes of species invasions (Kestrup et al. 2011).

Intraguild predation is known to occur between invasive species and their native analogues. (e.g. Dick et al. 1993; Dick and Platvoet 2000; Piscart et al. 2009b). For example, IGP It is thought to be a major driving force behind the exclusion of the native *Gammarus duebeni celticus* by the invasive *Gammarus pulex* in northern Ireland (Dick et al.1993).

Whilst IGP is gaining in appreciation as a factor in community structure, the role of parasitism in mediating IGP has received less attention. It is important because all the direct and indirect effects of IGP may be changed by parasite modification of IGP interaction strength (Hatcher et al. 2006). The effects of parasitism on the host condition, behaviour or habitat use could alter the prey or predatory aspects of IGP relationships, whether the parasites are shared or not (Hatcher et al. 2006). The studies by MacNeil et al. (2003b, 2003d) have shown that the coexistence between the native *G. d. celticus* and invasive *G. pulex* is enhanced when the invader is infected with the parasite *E. truttae*. The studies deployed one on one lab experiments and mesocosm experiments in the field. In this thesis I use the comparative functional response approach to quantify the IGP between the two species and how it is affected by parasitism.

1.4.2. Cannibalism

Cannibalism has been observed to occur in more than 3000 species, including a range of aquatic species (Polis 1981; Crump 1990; Elgar and Crespi 1992; MacNeil 1997). It is especially common in populations which are stage structured and in which generations overlap in space and time (Crump 1990; Rudolf 2007a). This is because the smaller individuals are vulnerable to cannibalism by larger individuals, while posing less risk of injury for the attacker (Pfennig et al. 1991). For example cannibalism is widespread in amphipods, where adults consume juveniles (Dick et al. 1993).

Cannibalism has direct benefits for consumers such as faster growth (Elgar and Crespi 1992), but there are also some indirect positive effects associated with it. It may reduce intraspecific competition and hence benefit growth and reproduction (Claessen et al. 2004). It also eliminates potential rival mates, competitors for food and aggressors (Getto et al. 2005).

It is thought that in situations in which food is scarce cannibalism might function as a lifeboat for the population by ensuring survival of individuals (Bosch,et al. 1988). However, this can only function if the consumption of individual provides enough energy for recruitment without reducing populations numbers too low.

However, cannibalism is also associated with a number of costs, such as risk of injury from struggling conspecifics. Cannibals might acquire parasite and toxins if they consume individuals which were carrying them (Polis 1980; Pfennig et al. 1991).

Parasites are known to influence predatory interactions (MacNeil et al. 2003d; Dick et al. 2010) as well as affecting their hosts vulnerability to predation (Hudson,et al. 1992; Hatcher and Dunn 2011). Hence, it is likely that cannibalism is affected too. This is likely to have implications for the structure of populations and the community dynamics (Rudolf 2008a). However, there are few/no studies on the impact of parasitism on cannibalism. In this thesis I explore the effect that parasitism has in two study systems. The impact of the microsporidian parasite *P. mulleri* on cannibalism in the native *G. d. celticus* and the impact of the acanthocephalan parasite *E. truttae* on cannibalism in the invasive *G. pulex*.

1.4.3. Functional responses

In order to determine the potential impact factors such as parasitism and invasive species have on freshwater ecosystems, we first need to quantify how it affects resource consumption. This can be achieved using a functional response (FR) approach which measures/explores the relationship between the availability of a resource and the consumption of it (Holling 1959).

Functional responses can be used to inform us how factors impact on populations (Holling 1959). This can be done by determining the type of functional response which occurs. There are three main types of functional response (figure 1.3.). Type-I is traditionally associated with filter feeders, who have a constant rate of consumption in relation to food density. In a Type-II FR the amount of prey at first rapidly increases with an increase in the resource density before levelling off. The levelling off happens because the predator reaches the maximum amount that it can uptake. In a Type-III FR the rate of consumption increases slowly until the resource density reaches a threshold, the consumption rapidly increases before reaching the maximum consumption plateau. Theoretical ecology predicts Type-II and III to have different effects on the dynamics of a population. A Type-II FR is predicted to have a destabilizing effect on populations because the predation pressure on the population is highest when the population density is at its lowest. This puts the population at a high risk of collapsing and becoming extinct. A Type-III FR on the other hand is predicted to have a stabilising effect on populations (Murdoch et al. 2003; Williams and Martinez 2004). Because the predation pressure on the prey population is low when population density is low and only steadily increases as prey population density increases. This effectively gives the population a refuge from predation. This means that the population is less likely to become extinct through predation.

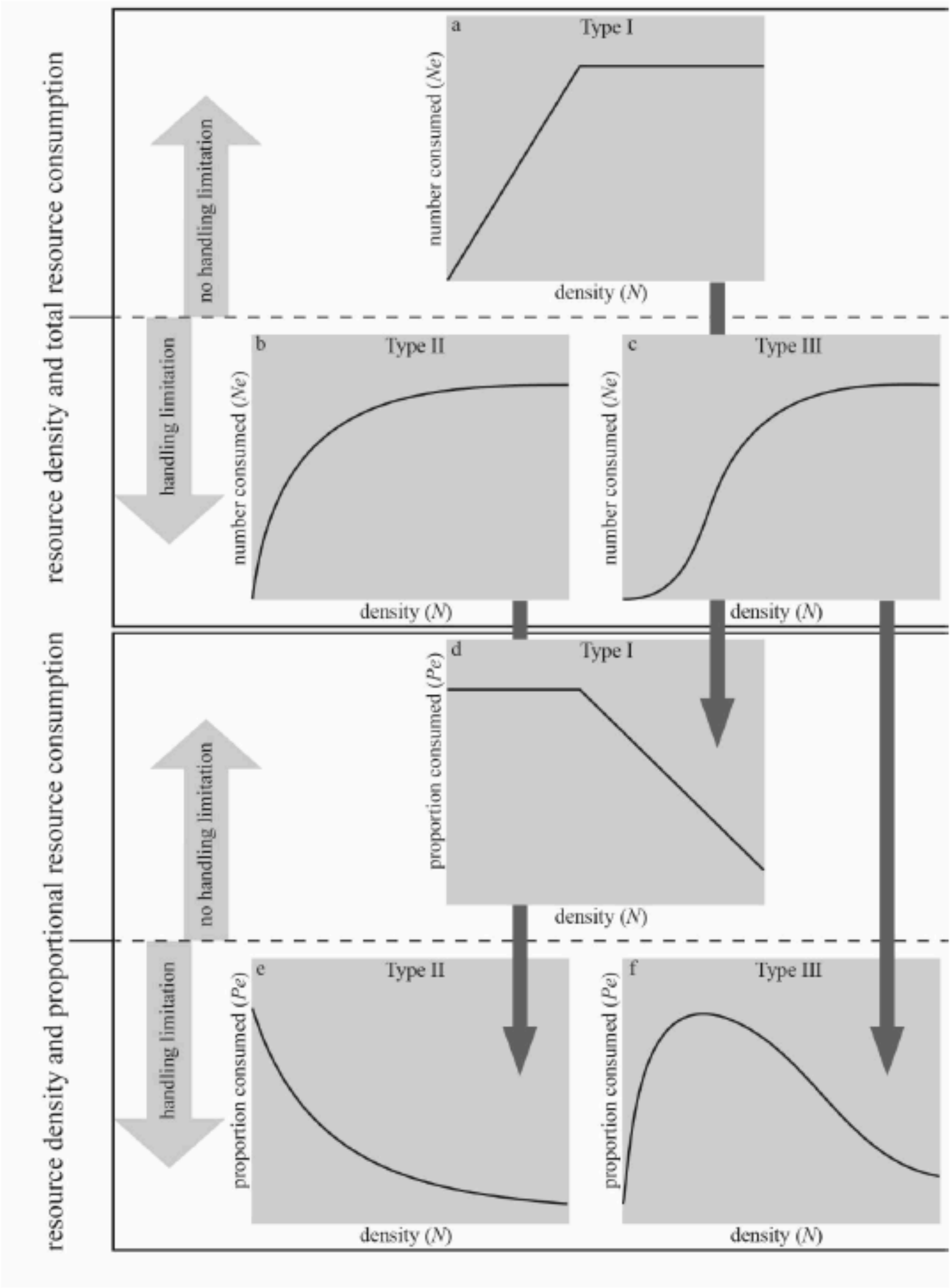

Figure.1.3. Categorical forms of the functional response according to Holling. Relationships between prey density and consumption for: (a) Type I; (b) Type II; and (c) Type III functional responses. These correspond to relationships between prey density and *proportional* consumption for: (d) Type I; (e) Type II; and (f) Type III. From Barrios-O'Neill (2014)

Once the types of FR observed and their maximum consumption rate have been determined, they can be used for comparisons (Comparative functional response). The support for the use of FRs to understand and predict the impacts of invasive species is growing (Haddaway et al. 2012; Alexander et al. 2014; Dodd et al. 2014; Paterson et al., 2015a). This experimental approach has the advantage that using FRs avoids errors, which are associated with choosing a single starting resource density (Dick et al. 2014).

Functional responses have been widely used to compare the predatory impact between natives and invasive species (Dick et al. 2014). For example the invasive signal crayfish *P. leniusculus* was found to vice versa have a higher FR feeding on the amphipod *G. pulex* than the native white-clawed crayfish *A. pallipes* (Haddaway et al. 2012). They can also be used to look at the influence of environmental factors on predator/prey interactions. For example, Laverty et al. (2017) found that increase in temperature caused an increase in the FR of *G. pulex* feeding on chironomid larvae. Also Dick et al (2010) found that parasitism can affect the FR of *G. pulex* feeding on *Asellus aquaticus* if the amphipod is infected with the acanthocephalan parasite *E. truttae*. Iltis et al. (2018) found that the FR of *Dikerogammerous villosus* was also affected when it was infected with the microsporidian parasite *Cucumispora dikerogammeri*.

Overal FRs have been used across a diverse range of taxonomic groups including 22 invader/native systems with 47 individual comparisons. Out of these high impact invaders were associated with higher FRs when compared to their native trophic analouges (Dick, et al. 2017).

Previous studies have examined IGP between individual adult amphipods, (Dick et al. 1993, Bovy et al. 2015) and in field manipulation experiments (MacNeil et al. 2003b). However, so far the FR approach has not been deployed to assess the impact native and invasive species will have on each other through IGP.. Similarly, the effect of parasitism on IGP has yet to be quantified using the FR approach., and is explored in this thesis The comparative FR approach can also be used to investigate the impact cannibalism has on a population, as cannibalism is a special case of predation. In this thesis I use two different parasite study systems to explore cannibalism using the FR approach and also investigate the effect of paraisitsm on this trophic interaction. this thesis..

1.5. Study system

Crustacean amphipods such as Gammarids are widespread throughout a large range of freshwater habitats (MacNeil et al. 1997; Piscart et al. 2009). They are considered to be a key species in food webs because they are often one of the dominant macroinvertebrate taxa in terms of biomass (MacNeil et al. 1997), are an important prey for many other species

(Degani et al. 1987; Friberg et al. 1994), are an important predator for many species (MacNeil 1997; Kelly et al. 2002) and they are known to be involved in maintain the water quality as well as recycling of organic matter (Piscart et al. 2009; Constable and Birkby 2016).

In Great Britain crustaceans comprise approximately 18% of established alien freshwater species and 24% of all species (Keller et al. 2009). Range of invasive freshwater species is likely to increase further with climate change over the next 100 years (Bellard et al. 2013). This potential expansion extension makes a mechanistic understanding of success and impact of invasives particularly important, yet detailed understanding is lacking. In this thesis I will use the comparative FR approach to gain such a mechanistic insight into the interactions in two invasive – native study systems and how they are affected by parasitism and other environmental stressors.

Amphipod crustaceans provide an ideal model to test if and how parasites mediate predation during invasions. Amphipods feature in many invasions, because of coincidental transport with humans and deliberate introductions for fish farming, angling and even ecological experiments (Dick and Nelson 1997; MacNeil et al. 1999).

1.5.1. Gammaridian Amphipods

The body of Gammaridian amphipods is curved, laterally compressed and divided into 4 main parts: head, peron, pleon and urosome (figure 1.4.). The head has two pairs of antenna and a pair of compound-eyes; the peron has 7 pairs of jointed legs which are used for swimming, crawling and grasping; the gills attaches the thoracic legs in the peron; the pleon consists of three pairs of pleopods, used for circulating water and swimming; the urosome has three pairs of uropods for swimming as well.

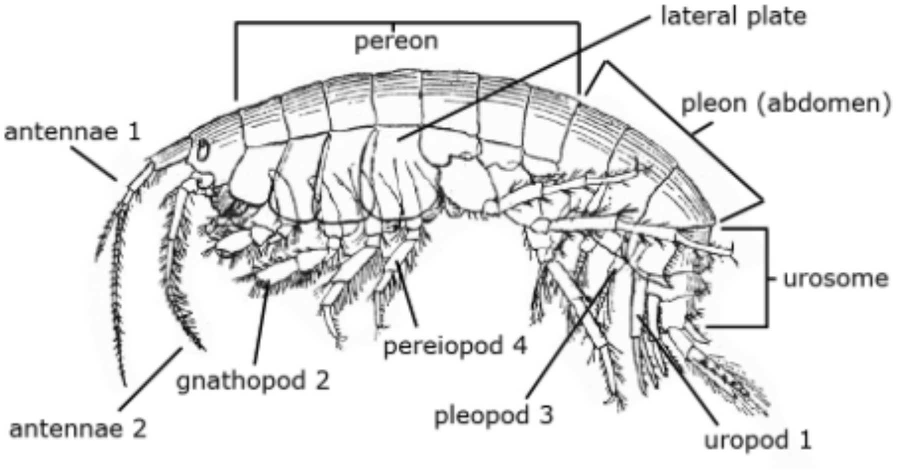

Figure 1.4. Drawing of freshwater amphipod with anatomy. From Stephensen 1929

Like other amphipods, *Gammarus* partake in a behaviour called precopulatory mateguarding. During this the larger males carries the smaller female of the species (figures 1.5.). They partake in this behaviour because copulation is only possible within a few hours after the female moult, when the new cuticle is still soft and insemination passages are open (Dick and Elwood 1989) and the male wants to ensure access to the female in order to reproduce.

After insemination the female carries the embryos in the brood pouch until they are released at the next moult. At the time of release juveniles are between 1 and 2mm in size and fully formed. They reach sexually maturity at a size of between 6 to 8 mm in size. Females are smaller in size than male individuals (Hynes 1954, 1955). The average length of adult males is about 21 mm, while females are approximately 14 mm (Pinkster 1970; Sutcliffe 1992; Maltby and Crane 1994). They have an average life span of 1-2 years (Sutcliffe et al. 1981).

Figure 1.5. photo *Gammarus* male carrying a female in mateguard credit: Jean-Francois Cart published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

For a long time amphipods were thought to be shredders of plant material and only for the past 20 years has it been recognised that they have a far broader dietary spectrum. They are now know to be omnivores preying on other macroinvetebrates including amphipods (MacNeil et al. 1997). They are also known to partake in intraguild predation and cannibalism (MacNeil et al. 1997).

1.5.1.1. Gammarus duebeni celticus (Lilljeborg, 1852)

Figure 1.6. Adult *Gammarus duebeni celticus*.

The amphipod *Gammarus duebeni celticus* is native to freshwater streams and lakes in Northern Ireland. Since the early 1990s a number of invasive amphipods have invaded northern Ireland and excluded the native from some sites while it has maintained long-term population in others. Sometimes the native has been observed to coexist with the invaders, in other locations it has been excluded see figure 1.7. for example of species distribution (MacNeil et al. 2003b)

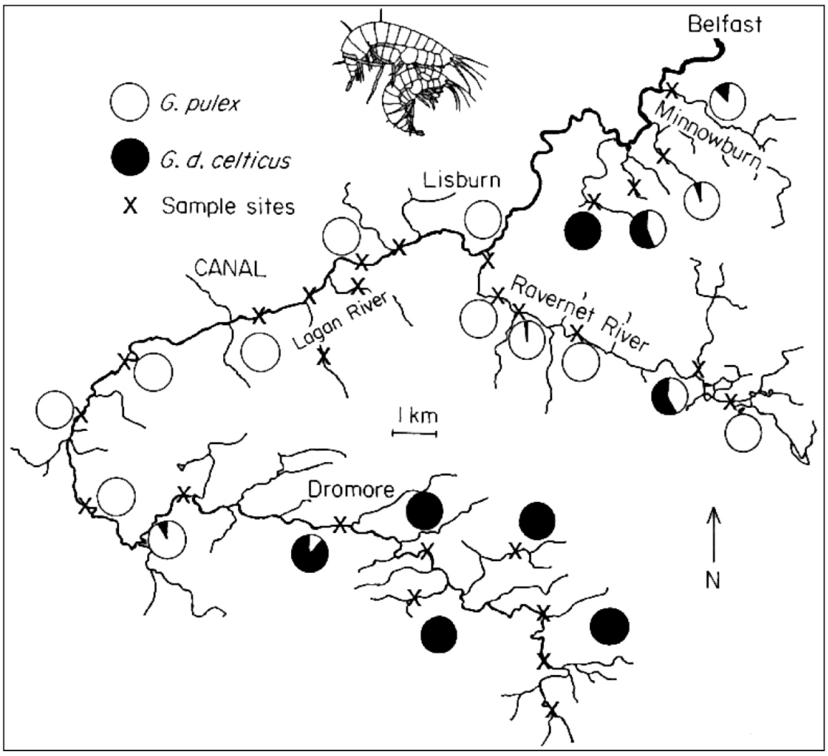

Figure 1.7. Distribution of *Gammarus duebeni celticus* and *Gammarus pulex* in the Lagan river system, including the composition of amphipod species; taken from Dick (2008)

1.5.1.2. Gammarus pulex (Linnaeus, 1758)

Gammarus pulex has a widespread distribution in Europe, British Isles, North Africa and Northern Asia in many rivers, streams and stagnant ponds under leaves, stones, and wood. While it is a native species in England, it is an invader in Northern Ireland. The species was transplanted from North Yorkshire in England in order to supplement fish diets in Northern Ireland (Strange and Glass 1979). It has been shown to displace native amphipod populations in Ireland (Kelly et al. 2006)...

Figure 1.8. *Gammarus pulex* adult male. Credit: Jim Rae published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

Hynes (1954,1955) has suggested that *G. d. celticus* will eventually be invariably replaced by *G. pulex* when the two species meet. Evidence of replacements have been observed in a number of river runs and lakes in Northern Ireland (Dick et al 1993, Dick 2008). This makes the two species an ideal opportunity to assess the role that biotic factors have on the coexistence and replacement between the native and the invader (MacNeil, Prenter, Briffa, Fielding, Dick, Riddell, Hatcher, and Dunn 2004).

1.5.1.3. Dikerogammarus villosus (Sowinsky, 1894)

Figure 1.9. *Dikerogammarus villosus* male. Credit: Michael Grabowski published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

Dikerogammarus villosus is native to the Ponto-Caspian region of south-eastern Europe. It has rapidly spread across Europe through the network of rivers and channels (figure 1.9.). It is thought that its spread through Europe was facilitated by accidental transport on commercial and recreational equipment and may be assisted by natural vectors such as birds (Madgwick and Aldridge 2011; Gallardo and Aldridge 2013b.). *D. villosus* was first recorded in the UK in Grafham Water in 2010 (MacNeil et al 2010), and subsequently recorded in two sites in South Wales during 2011 (Madgwick and Aldridge 2011) and Barton Broad, Norfolk in March 2012 (Gallardo and Aldridge 2013b). Because the UK has a high connectivity in its hydrological network and its climate is highly suitable to *D. villosus*, the invader is expected to further spread (Gallardo,et al. 2012).

. Along its path of invasion. D. villosus t has caused replacement/reduction in native amphipod species and led to changes in community structure (Rewicz et al. 2014).

. *D. villosus* is omnivorous, feeding on detritus and macroinvertevrates including other amphipod species (Madgwick and Aldridge 2011) and is a physiologically tolerant species (Rewicz et al. 2014). *D. villosus* has been nicknamed the 'killer shrimp' because of its tendency to rapidly consume a wide range of macroinvertebrate taxa in the laboratory (Dick et al. 2002; Platvoet et al. 2009; Dodd et al. 2014) and evidence from stable isotope analyses in the field (van Riel et al. 2006). This predatory behaviour is likely to contribute to the strong impacts of *D. villosus* on macroinvertebrate abundance and biomass, which have been observed in the field, especially isopods, tubificids and resident amphipods (Dick and Platvoet 2000; Dick et al. 2002; Kley and Maier 2003; Josens et al. 2005; Gergs and Rothhaupt 2015). Its effect on ecosystem diversity and functioning has led to it being listed as one of the worst invasive species in Europe (DAISIE 2009)

While the interactions of *D. villosus* with a number of prey species have been studied, there have been few studies of IGP and of its interactions with *G. pulex* (which is native to the UK) (Kinzler et al. 2008).

In the UK, *D. villosus* appears to have undergone enemy release, with very few of the parasites recorded in the native or European invasive range being found in invasive UK populations (Bojko et al. 2013; Arundell et al. 2015).

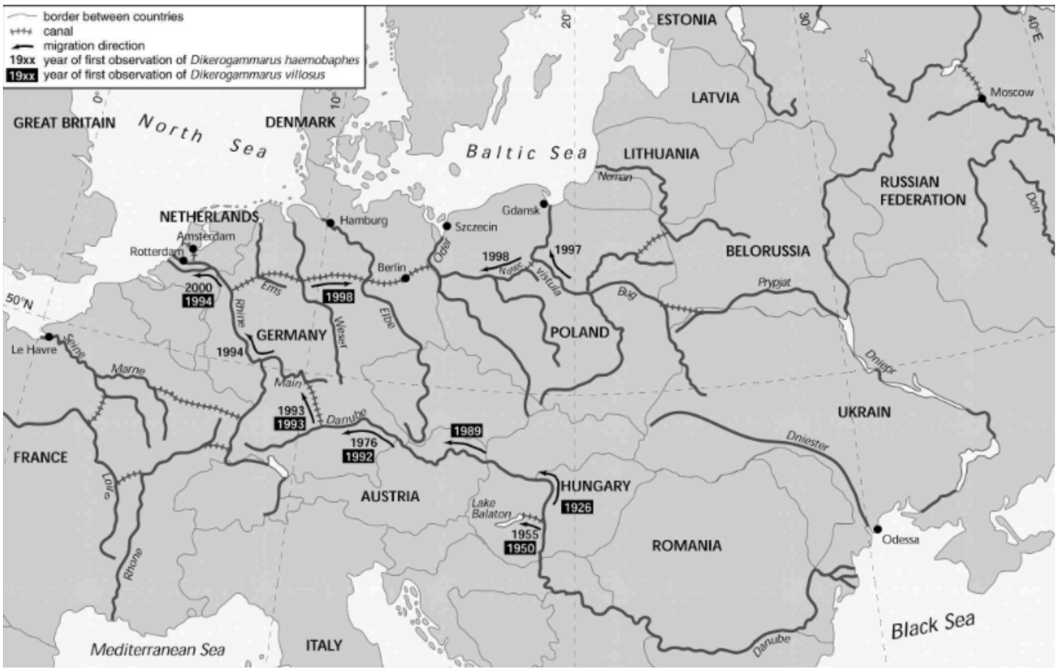

Figure 1.10. Map showing the migration pattern of *Dikerogammarus villosus* in Europe. Reproduced from Bij de Vaate et al. 2002

1.5.2. Parasites

Amphipods are hosts to an astonishing diversity of parasites (Sures et al. 2017). They can have very high prevalence in some parasites with some populations having been observed to have a 100% prevalence for some parasite taxa (Grabner 2017).

Parasites are known to have multiple effects of their *Gammarid* hosts, such as altering their behaviour (Bauer et al. 2000; Kaldonski et al. 2007), their immune system (Cornet et al. 2009), energetic reserves (Plaistow et al. 2001) and metabolic rate (Labaude et al. 2015). All these changes can ultimately modify the role that *Gammarids* have in the ecosystem.
In this thesis I will focus on parasites that are present in my study amphipods. The microsporidian parasite Pleistophora mulleri which is specific to the host amphipod Gammarus duebeni, and the acanthocephlan parasite Echinorhyncus truttae which uses fish as the definitive host and can use individualss from several species of amphipod as an intermediate host

1.5.2.1. Microsporidian parasites

Microsporidian parasites occur in many taxa in the animal kingdom (Keeling and Fast 2002), and are widespread in amphipods. Microsporidian parasites were found in all *Gammarus* species with prevalence between 20 and 100% and helmith were also found to appear with high prevalence (Sures et al. 2017). In an study of microsporidian diversity in an urban region in Germany, eight amphipod species were found to host a total of 10 microsporidian species (Grabner et al., 2015; Terry et al 2003).

The microsporidian parasite *Pleistophora mulleri* is specific to the amphipod *Gammarus duebeni celticus*. It infects the abdominal muscle tissue of individuals degrading it (Terry et al. 2003). An infection with the parasite is easily identified because the infected abdominal tissue appears opaque as can be seen in figure 1.11. (MacNeil et al. 2003b). It is horizontally transmitted (MacNeil et al. 2003e) with a transmission efficiency of 23% (MacNeil et al. 2003a). so far no other pathways of transmission for the parasite have been discovered (MacNeil et al. 2003e).

Figure 1.11. Adult *Gammarus duebni celticus* infected with the microsporidian parasite *Pleistophora mulleri*. Spores visible through carapace.

The parasite is common in *G. d. celticus* with its prevalence reaching as high as 90% (MacNeil et al. 2003b). The prevalence in populations can change with respect to season and was found to correlate with the abundance of the host (Fielding et al. 2005). Females have been observed to have a higher prevalence than males (Fielding et al. 2005).

Infection with the parasite has been observed to have a number of effects on its host. Infection has been observed to reduce the activity of infected animals, which could be caused by damages of the abdominal tissue and thus reduced ability to swim (Fielding et al. 2005). Infected individuals have also been observed to be less able to feed on juvenile *Asellus aquaticus* (Fielding et al. 2005). Infected animals also seem more likely to die as marked declines of its host populations have been observed to occur when a large proportion if the population had a heavy parasite burden (Fielding et al. 2005).

It has also been observed that the parasite affects how its host is interacting with other species. Infection has been observed to render the host more vulnerable to predation by the invader *G. pulex* (MacNeil et al. 2003b) as well as making them less capable to prey on individuals of the invader (Fielding, MacNeil, Robinson, Dick, Elwood, Terry, Ruiz, and Dunn 2005). It has also been shown that infection weakened *G. d. celticus'* competitive abilities (MacNeil et al.2003b). Intraspecific interactions have also been observed to be affected. Infected animals were found to be both more cannibalistic and more likely to be cannibalised (MacNeil, Dick, Hatcher, and Dunn 2003a, c) while these findings are interesting, experiments were of a qualitative rather than an quantitative nature. A quantitative approach is needed in order to as how these alterations in interactions could impact on the dynamics of host population and ecosystem dynamics.

1.5.2.2. Acanthocephalan parasite

Acanthocephalan parasites are characterized by a complex 2-host life cycle. They mature and sexually reproduce in the definitive vertebrate host. Eggs are then released in the water with the host faeces, these must then be consumed by an amphipod crustacean intermediate host. The life cycle of the parasite is completed when the infected amphipod is then consumed by the appropriate definitive host (Crompton and Nickol 1985).

Gammarids are intermediate hosts of several acanthocephalan parasites that are known to have multiple effects of their host. They are known to change the use of shelter (Médoc et al. 2006; Médoc et al. 2009) cause an increase in activity (MacNeil et al. 2003d) and reduce predator avoidance (Lewis 2012). These behavioural manipulations all increase the vulnerability of the gammarid host to predation, thus aiding trophic transmission of the parasite to its definitive host.

1.5.2.2.1. *Echinorhyncus truttae*

The acanthocephalan parasites *Echinorhyncus truttae* has a complex life cycle in which an amphipod serves as the intermediate and the brown trout *Salmo trutta* as the final host (figure 1.12.). In Northern Ireland the parasite uses both the native *G..d. celticus* and *G. pulex* as hosts but the prevalence is much higher in the invader. It has been observed to have a high prevalence with up to 70% of individuals infected in populations in Northern Irelandd (MacNeil et al. 2003d). The parasite form a large orange cycst which is clearly visible through the cuticle of infected individuals (figure 1.12.) and can comprise up to 24% of a hosts body mass (Dick et al. 2010). It is thus likely that the parasite poses a metabolic burden on its host.

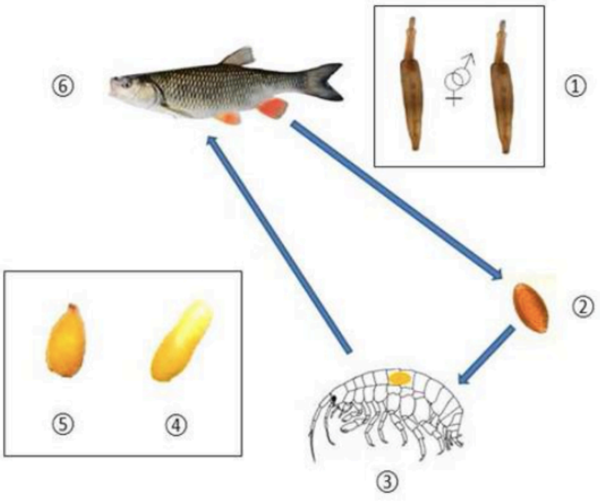

Figure 1.12. Schematic representation of the life cycle of *Echinoryncus truttae* including the final host brown trout ... and the intermediate host amphipod *Gammarus pulex*. Replicated from....1. Mating/laying of egg; 2. Egg with larvae of acanthocephalan; 3. Consumption by intermediate host; 4. Acanthella; 5. Cystacanth; 6. Consumption by final host and development into mature individual

Figure 1.13 . *Gammarus pulex* adult infected with the Acanthecephelan parasite *Echinoryncus truttae*. Credit: Hippocampus Bildarchiv published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

It has been found that an infection with a parasite causes changes in the intermediate hosts behaviour, which is likely to aid the trophic transmission to its final fish host (MacNeil et al. 2003d). Infected individuals have been observed to be more active (MacNeil et al. 2003d) less photophobic (MacNeil et al. 2003d) and a less effective predator (MacNeil et al. 2003d).,

Less well studied is the effect that this infection has on the hosts own predatory behaviour. Comparative functional response studies have found that an infection with the parasite affects the predatory impact *G. pulex* has on *A. aquaticus*. Infected individuals consumeg more prey than their uninfected counterparts (Dick et al. 2010). In contrast, field manipulations have shown reduced Igpredation by G pulex infected with E truttae, Leading to enhanced coexisteance.. However, there have been no studies that explore how parasitism affects IGP FRs , or of how this in turn is. In this thesis I will use the FR approach to explore how infection with *E. truttae* affects cannibalism and IGP and I will explore how its impact on cannibalism in affected by environmental stressors.

1.6. Thesis plan

In this thesis I used quantitative experiments to investigate how cannibalism and IGP of native and invasive species are affected by parasitism and other environmental stressors. This provides information on how population dynamics of species may be impacted on by species invasions and provides a mechanistic insight into the invasion process..

In chapters one and two , I investigate cannibalism within native and invasive amphipods and the effect of parasitism on cannabilistic FRs.

In *Chapter Two*, I investigate how cannibalism in the amphipod *Gammarus duebeni celticus* is affected by the microsporidian parasite *Pleistophora mulleri*. Because parasitism is known to affect predatory FRs, leading to a reduced prey intake rate, I propose it will similarly affect the cannibalism FR. I also conducted a prey choice experiment to test the hypothesis that, as this parasite is transmitted through consumption, *G. d. celticus* should avoid cannibalising parasitized individuals.

In *Chapter Three*, I explore the effect of an infection with *E. truttae* on the cannibalistic behaviour of the invasive *G. pulex*. Previous research has shown that E truttae lead to enhanced predation rates on smaller invertebrate prey (Macneil et al) likely reflecting the metabolica demands of the parasite. terefore I I used the comparative functional response approach to test the hypothesis that cannibalism in amphipod *Gammarus pulex* would be increased as a result of infection by the acanthocephalan parasite *Echinoryncus truttae*. Furthermore, as recent research has shown that predation rates are affected by environmental factors REF, I propose that cannibalism FR will be affected by these environmental factors. I include factors such as habitat structure and a predator cue from a higher order predator. This addition to the experiment should make extrapolation of experimental results to the real world more probable (Schindler, 1998).

In *Chapter Four*, I used the comparative functional response approach to quantify intraguild predation between the native amphipod *Gammarus duebeni celticus* and the invasive amphipod *Gammarus pulex* in Northern Ireland. I also looked at how infection with the parasites *Pleistophora mulleri* (which is specific to the native amphipod host) and

Echinoryncus truttae (which is more prevalent in the invasive host) affects the interspecific interaction and how it compares to the level of cannibalism occurring in the species.

In *Chapters Five* and *Six*, I looked at similar questions in a second invasive-native amphipod study system. I carried out a set of experiments to investigate intraguild predation between the native *Gammarus pulex* and the recently arrived invader *Dikerogammarus villosus*. In *Chapter Five* I used the comparative functional response approach to quantify the IGP occurring between the two species. As temperatures are predicted to increase with climate change, I also investigated how IGP it is affected by a warming in temperature. This could provide an insight into how the invader might impact on the ecosystem biodiversity. In *Chapter Six* I investigate how intraguild predation between the two species is affected if the native is infected with the acanthocephalan parasite *Echinoryncus truttae*. No experiments with *Dikerogammarus villosus* infected with the parasite were carried out as it does not appear to be a host to the local parasite and seems to have undergone "enemy release" resulting in no visually detectable parasites being present in the population (Bojko et al. 2013; Arundell et al. 2015).

In *Chapter Seven*, I outline and summarise the findings of the above-mentioned research and discuss their potential implications on the coexistence between native and invasive populations.

Chapter Two

Eaten alive: cannibalism is enhanced by parasites

Abstract

Cannibalism is ubiquitous in nature and especially pervasive in consumers with stage-specific resource utilization in resource limited environments. Cannibalism is thus influential in the structure and functioning of biological communities. Parasites are also pervasive in nature and, I hypothesize, might increase cannibalism since infection can alter host foraging behaviour. I investigated the effects of a common parasite, the microsporidian *Pleistophora mulleri*, on the cannibalism rate of its host, the freshwater amphipod *Gammarus duebeni celticus*. Parasitic infection increased the rate of cannibalism by adults towards uninfected juvenile conspecifics, as measured by adult functional responses, that is, the rate of resource uptake as a function of resource density. This may reflect the increased metabolic requirements of the host as driven by the parasite. Furthermore, when presented with a choice, uninfected adults preferred to cannibalize uninfected rather than infected juveniles conspecifics, probably reflecting selection pressure to avoid the risk of parasite acquisition. By contrast, infected adults were indiscriminate with respect to infection status of their victims, probably owing to metabolic costs of infection and the lack of risk as the cannibals were already infected. Thus parasitism, by enhancing cannibalism rates, may have previously unrecognized effects on stage structure and population dynamics for cannibalistic species and may also act as a selective pressure leading to changes in resource use.

2.1. Introduction

Cannibalism has been recorded in more than 3000 species (Polis 1981; Crump 1990; Elgar and Crespi 1992; MacNeil, Dick, and Elwood 1997) and may be influential at the levels of individuals, populations and communities. It is especially common in stage-structured populations where generations overlap in time and space (Crump 1990; Rudolf 2007b). Direct individual benefits of cannibalism include increased growth and survival (Elgar and Crespi 1992), while indirect positive effects include the elimination of competitors (Claessen, de Roos, and Persson 2004). Cannibalism may also enhance population persistence when resources are limited; for example, cannibalism may function as a ‘lifeboat mechanism' whereby cannibalistic adults have access to resources and energy accrued by the cannibalized juveniles (van den Bosch, de Roos, and Gabriel 1988). There are, however, a number of costs associated with cannibalism, including the acquisition of parasites via consumption of infected conspecifics (Pfennig, Loeb, and Collins 1991).

Parasitism is also pervasive in nature (Hatcher and Dunn 2011) and influences a number of intra- and interspecific interactions, including competition and predation, through both density- and trait-mediated effects (Hatcher, Dick, and Dunn 2006, 2014; Hatcher and Dunn 2011). In particular, parasites can modify the rate of predatory interactions (MacNeil, Dick, Hatcher, Terry, Smith, and Dunn 2003; Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010) as well as alter the vulnerability of infected hosts to predation (Hudson, Dobson, and Newborn 1992; Hatcher and Dunn 2011). Parasitism, I propose, may therefore also be an important determinant in cannibalistic interactions with implications for population structure and community dynamics. This may be evidenced through changes in host behaviour as a result of metabolic costs (Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010), parasite manipulation to increase transmission likelihood (Poulin 1995; Lefèvre, Lebarbenchon, Gauthier-Clerc, Missé, Poulin, and Thomas 2009; Cézilly, Thomas, Médoc, and Perrot-Minnot 2010), or can reflect selection on hosts to avoid costs of infection (Cézilly, Thomas, Médoc, and Perrot-Minnot 2010).

The microsporidian parasite *Pleistophora mulleri* is specific to the amphipod *Gammarus duebeni celticus*. It has a prevalence of up to 90% and can alter predation hierarchies among species (MacNeil, Dick, Hatcher, Terry, Smith, and Dunn 2003) with both parasitized and unparasitized individuals occurring in close proximity to one another (MacNeil, Dick, Johnson, Hatcher, and Dunn 2004). There is a large body of evidence that indicates *G. d. celticus* commonly engages in cannibalism in the field (Dick 1995). In addition, the only known route for the transmission of the microsporidian is cannibalism, providing further evidence of field cannibalism (MacNeil, Dick, Hatcher, Fielding, Hume, and Dunn 2003). Therefore, as the parasite is transmitted orally, with an efficiency rate of 23% (MacNeil, Dick, Hatcher, Fielding, Hume, and Dunn 2003) and, as cannibalism in this species is common, it imparts a risk of infection of *P. mulleri* (MacNeil, Dick, Hatcher, Fielding, Hume, and Dunn 2003). As such, parasite mediation of cannibalism may occur with important implications for host populations. I therefore investigated whether the cannibalistic rate and preferences of *G. d. celticus* are affected by infection with *P. mulleri*.

I used a 'functional response' approach (FR; resource uptake as a function of resource density), which can inform on consumer impacts on resource populations (Holling 1959).

First, I investigated the impact of parasitism on cannibalistic propensity by deriving FRs for individuals with and without the parasite. Second, I used an intraspecific prey choice experiment to test whether infected and uninfected *G. d. celticus* showed any preferences with respect to the infection status of juvenile conspecific victims.

2.2. Material and methods

Adult male and juvenile *G. d. celticus* were collected from Downhill River, County Antrim, Northern Ireland (55.166674 N, 6.8201185 W) in November 2010 and April 2011 (figure 2.1.). No permissions are required for this sampling activity. Males were selected for experiments owing to the wide variation in female cannibalism that can occur due to factors relating to egg and embryo brooding (Lewis, Dick, Lagerstrom, and Clarke 2010). Parasite status was determined by the presence/absence of *P. mulleri* spore mass visible through the exoskeleton (status confirmed by later dissection) and parasitized individuals all had visible infection of one to two segments (Fielding, MacNeil, Robinson, Dick, Elwood, Terry, Ruiz, and Dunn 2005). Animals were separated according to infection status and maintained in aquaria with water and leaf material from their source at 12°C and a 12 L : 12D cycle.

Figure 2.1. Map showing the location of the fieldsite used for the collection of *Gammarus duebeni celticus* in Northern Ireland. Location marked by black circle on the northern coastline.

For FR experiments, I selected similar-sized infected and uninfected adult male *G. d. celticus* (body mass (mg) ± s.e., infected 52.57 ± 1.49, uninfected 50.90 ± 1.23; two-sample t-test, t=0.86, p>0.05). I presented single infected and visually uninfected males (starved for 48 h) with visually uninfected juveniles (4–6mm body length) at seven juvenile densities (2, 4, 6, 8, 10, 15, 20; n=3 per density) in plastic dishes (8 cm diameter) containing 200 ml of aerated water from the amphipod source river (figure 2.2.). The densities of juvenile prey used were informed by previous FR studies on gammarids in combination with known densities from the wild that are hypervariable and can reach several thousand per square metre (Kelly and Dick 2005). Controls were three replicates of each juvenile density without adults. Replicates were initiated at 17.00 h and prey consumption was examined after 40 h.

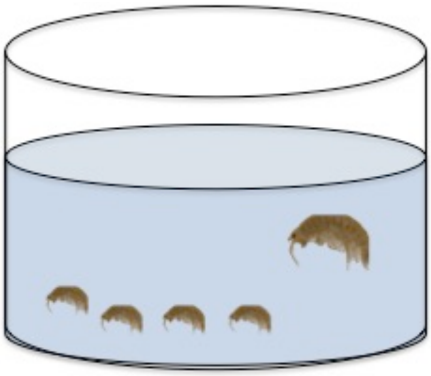

Figure 2.2. Schematic of the experimental setup used in the study. Example of an adult male supplied with 4 juveniles as prey.

Mean number of juveniles killed was examined with respect to adult infection status and juvenile density using generalized linear models (GLMs) with quasi-poison error distribution in R v. 3.0.1 that were simplified via a step-deletion process. I determined FR types using logistic regression of the proportion of prey consumed against initial prey density (Juliano 2001) and modelled FRs using the Rogers' random predator equation for a Type-II FR, which accounts for non-replacement of prey as they are consumed (Rogers 1972). FR data were bootstrapped (n=15) and the parameters attack rate *a*, handling time *h* and maximum feeding rate 1/hT (T =experimental time) compared using GLMs.

Preferences of infected and visually uninfected adults for cannibalism of infected versus uninfected juveniles were investigated by presenting adult males (n=30 visually uninfected and 30 infected individuals; sizes as above; starved for 72 h) with a choice between an infected and uninfected juvenile individual (6mm body length; matched by weight) in plastic dishes (10 cm diameter, 150 ml volume). Trials began from the addition of the prey and were terminated when a prey item had been selected. Prey choice by the adults with respect to juvenile infection status was determined using χ^2-tests.

2.3. Results

Control juvenile *G. d. celticus* survival was high (99.5%), thus experimental deaths were attributed to cannibalism by adults. This was further evidenced through observation and amphipod body parts littering the aquarium floor. Significantly more juveniles were eaten by infected than uninfected adults ($F_{1,40}$=5.03, p<0.05; figure 2.3.) and both FRs were found to follow a Type-II curve (figure 2.3.). Infected adults had significantly greater attack rates a (t=5.87, p<0.001) and significantly lower handling times *h* (t=3.67, p<0.01). This translated into significantly higher maximum feeding rates (1/hT) (t =2.71, p< 0.05) in comparison to uninfected individuals (figure 2.3.).

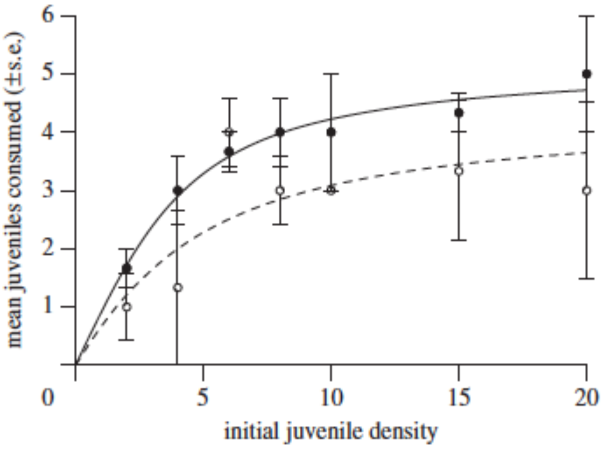

Figure 2.3. Functional response curves of infected (filled circles, solid line) and uninfected (open circles, dashed line) *Gammarus duebeni celticus* adults towards juvenile conspecific prey. Lines are modelled by Rogers' random predator equation for a Type-II response. Data points are mean number of juveniles consumed at each density ± s.e.

Uninfected adults more frequently consumed uninfected than infected juveniles (χ^2_1=4.8, p<0.0285; figure 2.4.), whereas infected adults showed no preference (χ^2_1=1.333, p>0.05; figure 2.4.).

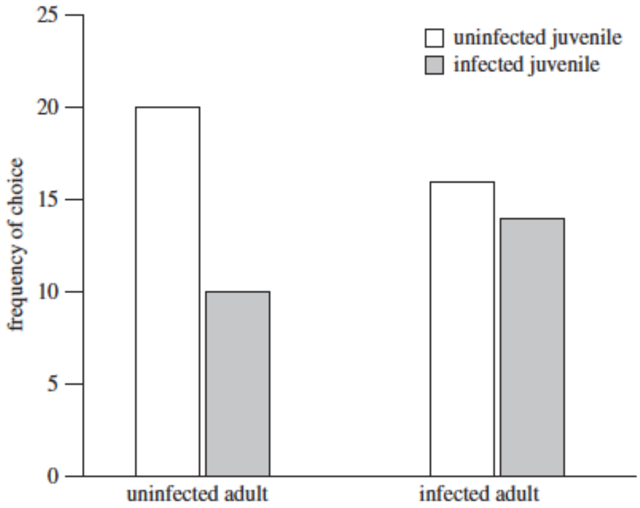

Figure 2.4. The frequency of consumption of uninfected versus infected juveniles by uninfected and infected adult *Gammarus duebeni celticus*.

2.4. Discussion

Although the role of parasitism in interspecific predator–prey interactions has been studied in a number of systems (MacNeil et al- 2003; Dick et al. 2010), the influence of parasites in mediating cannibalism has received far less attention, despite cannibalism and parasitism both being widespread and pervasive in natural communities (Elgar and Crespi 1992; Hatcher and Dunn 2011). Parasites may affect cannibalism since they have been shown to affect foraging behaviour, both increasing and decreasing host consumption of resources, with potential implications for population dynamics and community structure in such taxa (Dick et al. 2010).

Here, the FR of the amphipod *G. d. celticus* infected with the microsporidian parasite *P. mulleri* towards juvenile (uninfected) conspecific prey was significantly higher in comparison to uninfected adults. Furthermore, infected amphipods had significantly greater attack rates, decreased handling times and hence heightened maximum feeding rates, demonstrating that infected amphipods are more efficient than their uninfected counterparts at cannibalizing juveniles. This probably reflects the metabolic burden imposed by the parasite, leading to higher feeding rates (Dick et al. 2010). The degraded abdominal tissues in infected individuals, mean it is more strenuous for infected individuals to swim, thus increasing their metabolic need for food. That infected individuals are such efficient foragers is despite the fact that this parasite degrades host tissue and substantially debilitates its host (Terry et al. 2003). The preferential consumption of uninfected juveniles by uninfected adults probably reflects selection for avoiding cannibalizing infected juveniles and therefore reducing the risk of parasite acquisition (Pfennig et al. 1991; Pfennig 1997). On the other hand, infected adults showed no such discrimination. One explanation for this lack of discrimination may be that immune priming or immune upregulation protects infected individuals from further infection (Masri and Cremer 2014). However, Terry et al. (2003) found no evidence of encapsulation or other immune responses in *P. mulleri* infected hosts. Rather, one might speculate the lack of discrimination in cannibalism of infected versus uninfected juveniles by infected adults again reflects the metabolic burden of infection whereby parasitized individuals cannot afford to be as selective in what prey they consume. Furthermore, as they are already infected with the parasite, there is no advantage to avoiding infection risk by preferentially consuming uninfected prey.

Overall, I show that infection of *G. d. celticus* with the parasite *P. mulleri* altered cannibalism rates and feeding preferences on juvenile conspecific victims. This in turn may increase the rate of juvenile mortality (over and above conventional virulence effects), which could lead to changes in population stage structure and density (Rudolf 2007b; Rudolf and Armstrong 2008; Hatcher, Dick, and Dunn 2014). Furthermore, this interplay between cannibalism and parasitism could have powerful impacts on population and community resilience in changing environments, whereby cannibalism becomes an important mechanism in preserving populations (van den Bosch, de Roos, and Gabriel 1988), although in the wild, population outcomes will also depend on the relative importance of interspecific predation and cannibalism. Cannibalism and intraguild predation co-occur frequently in a broad range of systems (Polis 1981; Rudolf 2007b) and the balance of these intra- versus interspecific interactions is key to species coexistence and replacement patterns (Dick 1996). Parasites are also recognized as having important indirect and pervasive effects on communities and ecosystems, often owing to their density and trait-mediated indirect effects on species that interact with their hosts (Hatcher, Dick, and Dunn 2012). Further exploration of parasite-modified cannibalism thus has potential to further understand and predict population dynamics and community processes.

Chapter Three

The effect of parasitism and environmental factors on the cannibalistic predatory functional response in *Gammarus pulex*

Abstract

Predator prey interactions are known to be important forces which play a part in shaping ecological communities. Cannibalism is an important part of these interactions as it can have profound impacts on populations and thus communities. Cannibalism may be affected by biotic and abiotic factors. Here I investigate the effect of combined biotic and abiotic factors on the cannibalistic functional response of *Gammarus pulex* adults feeding on conspecific juveniles. In the absence of additional factors, individuals infected with the acanthocephalan parasite *Echinorynchus truttae* displayed a 50% reduction in cannibalism in comparison with their uninfected counterparts, reflecting longer handling time. However, in an environment which contained habitat structure and cues of a higher predator simultaneously, this difference was reversed and infected individuals had a significantly higher functional response than their uninfected counterparts. This means that individuals infected with *E. truttae* have the potential to consume significantly more conspecific juveniles, which could have a strong impact on the population especially in situations where parasite prevalence is high. This study makes a strong case for conducting functional response experiments in as natural situations as possible in order to avoid overestimation of impacts.

3.1. Introduction

Predator prey interactions are important forces that structure ecological communities (Dayton 1971; Paine 1980). Most studies describe predator prey interactions between species from different tropic levels and how these affect the species population dynamics and or structure. Intraguild predation (IGP) and cannibalism are both special cases of predator prey interactions, in which prey consumption removes a potential competitor (Polis et al. 1989; Holt and Polis 1997) and as such may carry additional benefits for the predator (Pfennig, Loeb, and Collins 1991; Wise 2006). Because IGP and cannibalism result in the removal of a potential competitor the consequences it has on population dynamics are more complex than that of ordinary predator prey interactions (Polis et al. 1989).

Foodweb analyses reveal that IGP and cannibalism are common and important in structuring of communities (Arim and Marquet 2004; Bascompte and Melián 2005; Thompson et al. 2007) and are often closely associated with each other. Cannibalism is a special form of IGP, in which an individual consumes another conspecific individual from its population. It is especially common in populations, which are stage structured and in which stages overlap in time and space (Crump 1990; Rudolf 2008b). Cannibalism is known to hold a large potential to shape the structure of a population. It can sustain a population in times of food scarcities; a theory known as the lifeboat hypothesis (van den Bosch, de Roos, and Gabriel 1988). However it may also have a negative impact on the population if too high a number of juveniles are consumed and may even threaten population extinction (Cushing 1992). Another risk of cannibalism is that pathogens and parasites can be acquired if they are present in the prey (Pfennig, Loeb, and Collins 1991). Like other predator prey dynamics, cannibalism may also be also affected by biotic and abiotic factors.

Biotic factors, which can reduce the predatory impact on a species are its possible defences such as spines or poison, which prevent the predator from successfully consuming the prey. An example for such a factor is that the exoskeleton of the amphipod *G. pulex,* which makes it difficult for intraguild or cannibalistic predators to prey on individuals of larger or equal size. This may be why most predation in these cases occurs between adults and juveniles which are smaller (Polis 1981) or between adults just after an individual (prey) has moulted leaving them vulnerable to predation (Dick and Platvoet 1996). The presence of higher order predators may affect the behaviour of both predators and prey, has been shown to affect predation by *Gammarus* of smaller macroinvertebrates (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015a). I propose that the presence of higher order predators might also affect cannibalistic interactions

Abiotic factors have also been shown to impact the predator prey interactions. For example predation by the lionfish *Pterois volitans* has been found to be higher at elevated temperatures (South and Dick 2017) and to be reduced at low levels of dissolved oxygen (Laverty, Dick, Alexander, and Lucy 2015). It has also been found that the availability of shelter for the prey results in a lower predatory success (Alexander et al 2013, Wassermann et al 2016). I propose that presence of shelter will reduce the cannibalistic predator-prey interactions.

Predatory impact can be measured using predatory 'functional response' (FR) that descriptive the relationship between resource use and resource availability to provide a measure of per capita predatory ability (Holling 1959). Comparative (FR) provides a way to make comparisons of feeding rates not only between species but also between circumstances (Dick et al. 2010). The FR analysis also provides the chance to extract the *a* and *h* parameters which provide information on the predator prey interaction which may be used to further infer impact of the biotic and abiotic factors on the consumption by the predator. The *a* parameter is a scaling parameter which is associated with the attack rate of the predator on its prey and is known to define the initial slope of the functional response curve (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015a). The *h* parameter stands for the handling time of the prey by the predator and limits the maximum number of prey consumed (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015a).

Comparative FRs have been used to investigate the impact of possible invaders on native prey species. For example *Dikerogammarus villosus* have been found to have a significantly higher FR feeding on *Asellus aquaticus* than the native amphipods *Gammarus pulex*, *Gammarus roeseli* and *Gammarus duebeni* (Bollache, Dick, Farnsworth, and Montgomery 2008; Dodd, Dick, Alexander, MacNeil, Dunn, and Aldridge 2014). Comparative FRs have also been used to investigate the impact of parasitism on predator prey interactions. Dick et al (2010) found that an infection with the parasite *Echinorynchus truttae* increases the FR of *G. pulex* feeding on *A. aquaticus* and in chapter two I found that infection with *Pleistophora mulleri* increased the cannibalism of *Gammarus duebeni celticus* on its juveniles. Predatory FRs can also be context dependent, with the relationship affected by abiotic and biotic factors. For example, the presence of higher order predators can affect the predator FR (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015b). For example it has been found that both the presence of a higher predator and availability of shelter reduced the functional response (FR) of *Echinogammarus marinus* on its prey *Joera nordnanni* (Alexander et al 2013). Only a few studies have explored the impact of multiple environmental factors on predator prey interactions and to my knowledge, none have considered their effect on cannibalistic interactions. I present a novel study to assess how the cannibalistic FR of *G. pulex* feeding on conspecific juveniles is affected by three factors: infection with *Echinorhycus truttae,* habitat and the presence of a higher predator.

The occurrence of parasitism is very widespread in nature (Hatcher and Dunn 2011). It is known to impact on intra and interspecific interactions including cannibalism (Hatcher et al. 2006; Bunke et al. 2015). Parasitic infection has been shown to modify the rate of predatory interactions (Dick et al 2010) and can also increase the vulnerability of their hosts to predation (MacNeil et al 2003). Here I investigate the effect of parasitism on cannibalism in *G. pulex* in a multifactorial experiment using the comparative FR method.

The acanthocephalan parasite *E. truttae* has a complex life cycle in which the amphipod *G. pulex* and the brown trout *Salmo trutta* function as intermediate and final host respectively. In the past it has been found to have a high prevalence in northern Irish populations of *G. pulex* with up to 70% of the population being infected (MacNeil, Fielding, Dick, Briffa, Prenter, Hatcher, and Dunn 2003). The parasite forms a large orange cyst that is visible through the cuticle and that can take up to 24% of a hosts body mass (Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010) and thus is likely to pose a metabolic burden on the host organism. It has also been found that infection with the parasite causes changes in the behaviour of the intermediate host, likely to aid trophic transmission to the final host (MacNeil, Fielding, Hume, and Dick 2003). A previous study investigating the predatory FR of *G. pulex* found that infection with the parasite caused a higher FR feeding on *A. aquaticus* (Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010). I propose that since cannibalism is a special case of predation behaviour it will be affected by an infection with the parasite *E. truttae.*.I also propose that environmental factors will interact with the parasitic infection of the host and affect the cannibalistic FR of *G. pulex* feeding on conspecific juveniles. .

3.2. Methods

3.2.1. Animals

G. pulex were kick sampled from the river Lagan, UK at Shaw's Bridge (54'548509N, 5'9526063W, figure 3.1.). On arrival at the lab, juvenile and adult individuals were separated and in maintained in separate tanks. Animals were kept in tanks with substrate, leaf material and aerated river water from their source at 13°C at a 12h:12h light:dark regime. Only adult males were used as predators in the experiments because the predatory ability of females is affected by other factors such as the presence of offspring in their brood pouch. Animals were sexed based on the presence of copulatory papillae in males, under a light microscope. Before the start of the experiment the adult male individuals were starved for 48h in order to standardize hunger.

Figure 9.1. Map showing the location of the fieldsite used for the collection of *Gammarus pulex* in Northern Ireland. Location marked by black circle to the south of Belfast.

The fish used in the experiments, as a source for predator cue, were fingerling brown trout *S. truttae*, which were obtained from the Cookstown trout hatchery and no bigger than 10cm in size. The fish were kept in tanks with aerated, filtered stream water on a diet of commercial fish pellets. They represent the final host for the parasite *E. truttae* (Fielding et al. 2003).

3.2.2. Experimental design

During the experiment *G. pulex* male adults either uninfected or infected with the acanthocephalan parasite *E. truttae* were randomly allocated to one of two predator treatments (visual and olfactory predator cue vs no predator cue) and one of two habitat treatments (with or without habitat). Hence there were a total of 8 treatments as shown in table 1.

Table 3.1. Experimental set up. Combinations of experimental factors used in the functional response experiment treatments.

Parasite status	Uninfected				Infected			
Higher order predator treatment	No Predator		Predator		No Predator		Predator	
Habitat treatment	No Habitat	Habitat	No Habitat	Habitat	No Habitat	Habitat	No Habitat	Habitat

The experiment was carried out in clear glass arenas (circular glass dishes with a circumference of 8 cm) containing 150ml of river water. Habitat was provided by adding 100g of small sized gravel into the glass dishes, which allowed for a cover of the bottom about 1cm deep. Control treatment arenas had no gravel. To produce fish cues, a single fish was placed into the experimental tanks with 4l river water at least 4h prior to the start of the experiment to ensure that olfactory cues had spread through the water, before the glass arenas containing the amphipods were placed into these larger tanks. Thus the amphipods were exposed to both olfactory and visual cues but the fish could not predate the amphipods. In the control treatments, the arenas were also placed into larger tanks with no fish present (figure 3.2.).

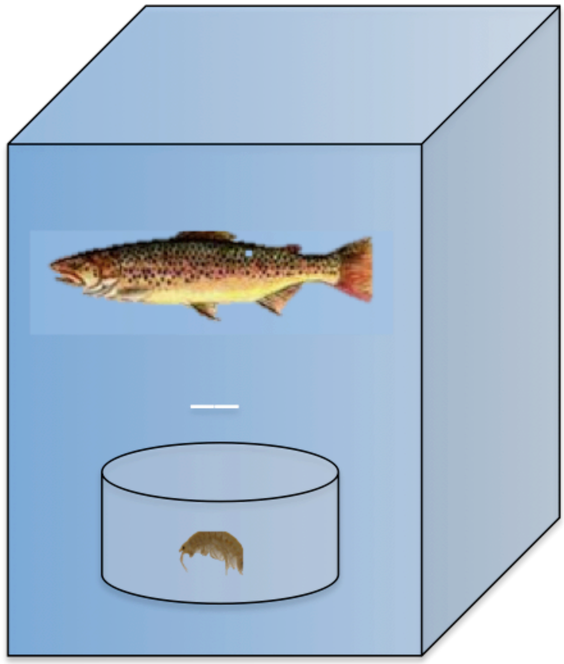

Figure 3.2. Schematic of an example experimental setup used in the study. Example chosen is using an uninfected adult male, in an experimental arena without habitat structure, with the presence of a higher predator.

Arenas were provided with a known number of prey items (2, 4, 6, 8, 10, 15, 20, 30 , 40 , 50 or 60), with 3 replicates at each density. Prey were juveniles of the same species in the size range of between 4-6 mm and were allowed to acclimatize for 30 mins before the predator was added. The experiment was left to run for 40 hours, before it was ended by removing first the arena from the tank and then second the adult *G. pulex* from the experimental arena. The number of prey items left in the glass dish was noted and the adult individuals were dissected in order to confirm the infection status of the individual. Only individuals infected with a single *E. truttae* parasite cyst were considered in the analysis. As only one individual was excluded due to a double infection, this did not affect the outcome of the analysis.

In addition adult-free controls were also set up containing juveniles at the experimental densities to investigate their survival in the absence of adult predators

3.2.3. Analysis

All statistical analyses were undertaken in R version 3.3.3 (R Foundation for Statistical Computing 2017). The type of predatory functional response (the relationship between proportional mortality of prey and prey density) occurring was analysed using the frair_test function of the frair package in R (Pritchard 2016) for each of the treatments.

Subsequently the predatory functional responses were modelled using a maximum likelihood estimation with the Rogers'-random predator equation (e.g. 3.1.)

$$(N = N_o(1-e^{a(Nh-PT)})) \qquad \text{eq 3.1.}$$

where N is the number of prey eaten, No is the initial prey density, a is the attack constant, h is the handling time and T is the total time available for predation.

The equation assumes non-replacement of prey, in order to obtain estimates of the saturation parameter *h* and the scaling parameter *a* for each of the treatments. FRs were modelled using the R package frair (Pritchard 2016) which utilizes maximum likelihood estimation within the bbmle package (Bolker 2014).

A comparison of the *a* and *h* parameter values between the different treatments was carried out, using the frair_compare function which is part of the frair package. It implements the delta or difference method of Juliano (2001). Raw consumption data were compared between treatments using a GLM under an assumed poisson error distribution.

3.3. Results

The logistic regression analysis revealed that all eight treatments were observed to be Type-II (logistic regression first order coefficient significant negative (table 3.2.). The subsequent fitting of the non-linear model with use of the frair package provided some interesting insights into how an infection with the acanthocephalan parasite *E. truttae* influences the cannibalistic behaviour of *G. pulex* adults on juveniles in the presence of available shelter and cues from a predator which is also the final host to the parasite (figure 3.3.).

In the absence of shelter and predators, individuals infected with the acanthocephalan parasite were observed to consume significantly lower amounts of juveniles than their uninfected counterparts (GLM, $X^2_{1,262}$=10.57, p=0.001; figure 3.3.). This relationship does not seem to be affected by the presence of a predator in the experimental arena (GLM, $X^2_{1,260}$=0.2, p=0.656). The inclusion of a habitat structure was observed to cause a significant drop in predation in both the infected individuals and their uninfected counterparts (GLM, $X^2_{1,261}$=335.27, p<0.001). This dropped even further for the uninfected individuals but not infected ones when a predator cue was included in the treatment. This lead to the observation that, when habitat and higher order predator cues were present, infected individuals were observed to have a significantly higher FR than their uninfected counterparts (figure 3.3a), a reversal in order of FRs between uninfected and infected individuals in the absence of these cues (figure 3.3d) (GLM, $X^2_{1,248}=4.12$, $p=0.042$). It is interesting to point out that this was not caused by an increase in predation by the infected individuals; rather the difference was caused by a reduction in predation by uninfected individuals (figure 3.3).

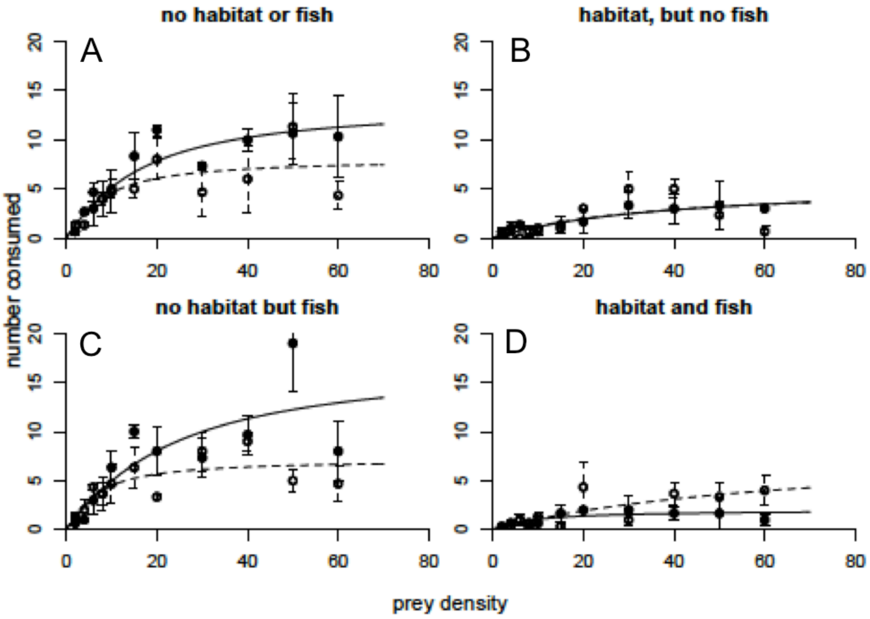

Figure 3.3. Predatory functional response curves as influenced by the factors of habitat structure and predator cue. Full circles and solid lines represent uninfected individuals of *G. pulex*; empty circles and dashed lines represent individuals of the same species infected with the acanthocephalan parasite *E. truttae*

The differences observed in these curves are also reflected in the differences in the values of the saturation and scaling parameters *a* and *h* (table 3.2.).

Table 3.2. Parameter estimates and significance levels from first-order logistic regression analysis of the proportion of prey killed against initial prey density, with functional response parameters. Estimates for *G. pulex* adult males feeding on juvenile *G. pulex*; extracted from Rogers' random predator equation fitted to data in the frair package. P – value referring to the first order term, a - attack coefficient, h – handling time, SE – standard error

Parasite status	Habitat treatment	Higher order predator treatment	First-order term	p	a	SE	H	SE
uninfected	Control	Control	-0.0343	<0.001	0.689	0.126	0.122	0.015
	Habitat	Control	-0.0155	0.03567	0.091	0.033	0.295	0.141
	Control	Predator	-0.0284	<0.001	0.571	0.097	0.096	0.014
	Habitat	Predator	-0.0351	<0.001	0.140	0.078	0.834	0.250
Infected	Control	Control	-0.0417	<0.001	0.853	0.213	0.205	0.025
	Habitat	Control	-0.0241	0.0011	0.097	0.030	0.310	0.115
	Control	Predator	-0.0460	<0.001	0.956	0.265	0.233	0.028
	Habitat	predator	-0.0112	<0.001	0.079	0.026	0.203	0.128

In the absence of both habitat and the cue of the higher predator, uninfected and infected individuals did not differ from one another in their *a* parameter. In contrast, The *h* parameter was observed to be almost doubled for infected individuals (frair_compare, p=0.004).

Adding only a predator cue did not cause a change in the *a* and *h* parameters when compared to the control treatment. However, when the predator cue was added, the *h* parameter was observed to be more than twice as high in uninfected individuals than in infected individuals (frair_compare, p<0.001). For uninfected individuals the inclusion of both habitat and predator cue caused a significant drop in *a* (frair_compare, p<0.001) and a significant rise in the *h* parameter (frair_compare, p=0.005). In the presence of habitat and predator cue the *a* parameter did not differ between infected and uninfected individuals in the study. However, a significant difference was observed for the *h* parameter, which was found to be four times higher in uninfected individuals than in infected ones (frair_compare, p=0.025).

3.4. Discussion

This study shows that environmental factors and parasitism influence the cannibalistic tendencies of *G. pulex*. In the absence of habitat structure and predator cue we observed that the infection with the acanthocephalan parasite caused a 50% drop in cannibalism of juveniles by adult male individuals. This finding is in contrast to the finding of Dick et al

(2010) who found that an infection by *E. truttae* caused an increase in the FR of *G. pulex* feeding upon *A. aquaticus*. However, it is in accord with the finding of MacNeil et al (2003) who found that *G. pulex* infected with the parasite were less predatory on juveniles of the co-occurring native amphipod *G. duebeni celticus*. One explanation for this is that the impact an infection with *E. truttae* has on *G. pulex* FR is dependent on the type of prey.

The drop in the cannibalism observed in this study could be caused by the burden that the parasite posts on its host (Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010). It has been previously shown that the acanthocephalan worms comprise up to 24% of the hosts biomass (Dick et al 2010). Another possible explanation for the lower predatory functional response of the infected individuals could be that the infection with the parasite causes a shift in the distribution of the animals in the water column, *E. truttae* causes a reversal in phototaxis, with infected individuals spending more time in the water column and closer to the water surface (MacNeil, Fielding, Hume, and Dick 2003; Fielding, MacNeil, Dick, Elwood, Riddell, and Dunn 2003). This behavioural manipulation is likely to increase vulnerability to predation by the definitive fish host and thus to enhance parasite transmission (Poulin, Wise, and Moore 2003; MacNeil, Fielding, Dick, Briffa, Prenter, Hatcher, and Dunn 2003). This change in habitat use could mean that the infected individuals simply are less likely to encounter their prey, which results in a lower FR. A similar finding was observed by (Fielding, MacNeil, Dick, Elwood, Riddell, and Dunn 2003) who observed that individuals with *E. truttae* consumed less of the bottom dwelling *A. aquaticus.* Looking at the scaling *a* and saturation *h* parameters it becomes clear that the reduced predatory strength is not caused by a reduction in activity (infected individuals have a higher attack rate of prey) but probably results from an increase in the handling time which is almost twice that when compared to that of their uninfected counter parts. Biologically this longer handling time in the infected individuals is likely caused by the parasite placing a burden on its host.

The most interesting observation of the experiment was made when the presence of a habitat structure and predator cue was combined in the experimental set up. The predatory functional response curves of for the uninfected group declined significantly. This drop in FR on inclusion of shelter was also observed in other FR studies e.g. (Alexander, Dick, and O'Connor 2013) In part this likely caused by the inclusion of habitat in the experimental arena which provides shelter for the prey hence their prey encounter rate drops which in return causes a lower predatory functional response (Ferris and Rudolf 2007).

The lower drop of FR for the infected group in the set up could be caused by parasite manipulation as it has been shown *E. truttae* causes a reduction in the predator avoidance behaviour of the intermediate host, to increase the chance that it is consumed by the predator that constitutes its final host (MacNeil, Dick, Hatcher, Fielding, Hume, and Dunn 2003).

It is important to note that if the experimental set up had not included the habitat structure and predator cue simultaneously in order to simulate a more natural setting, the higher FR of *G. pulex* individuals infected with the parasite *E. truttae* feeding on juveniles in comparison to uninfected individuals would have been missed. The impact of infection with the parasite on the predatory functional of cannibalism on their juveniles would thus have been underestimated. This makes a strong argument for designing experimental set up in such as manner as to match them as closely to the natural setting of a species' habitat as possible in order to be able to gain an insight into inter and intra-specific interactions. It would be interesting to see FR experiments carried out in nature to investigate how FRs obtained there compare to the ones obtained from lab experiments.

The fact that all FRs observed were found to by Type-II FR gives an indication that cannibalism can have a strong impact on the dynamics of the population. This is the case because in Type-II FRs cannibalism occurs at constant levels even at low population densities and in the presence of shelter. In contrast, Alexander et al. (2003) study also found a change in the type of FR from Type-II to a Type-III when shelter was provided in *E. marinus /J. nordanni* predator-prey experiments. However, other studies have not observed this change in type of FR in the presence of shelter or complex habitat (MacNeil et al, 2003; Wasserman *et al.*, 2016; South *et al.*, 2017).

A Type-II FR suggests that the presence of shelter does not provide a refuge at low prey densities, and hence that cannibalism has the potential to exert strong negative pressure on population numbers. This is because in a Type-II FR predation pressure on the prey remains high even at low population densities. This poses a high risk of to the population of becoming extinct if too many individuals are removed from the population. This means that a Type-II FR has the potential to drive populations to extinction (Rindone and Eggleston 2011).

Chapter Four

Another look inside the box: parasites influence interactions between *Gammarus pulex* and *Gammarus duebeni celticus*

Abstract

In Northern Ireland the native and invasive amphipods *Gammarus duebeni celticus* and *Gammarus pulex* have been observed to coexist in some places whilst in others the native has been replaced by the invader. Previous studies have shown that intraguild predation (IGP) and cannibalism can be important factors in structuring populations and communities. Parasitism has been found to facilitate coexistence between species by altering host behaviours. In this study I used the comparative functional response approach to investigate the impact parasitism has on the IGP between *G. d. celticus* and *G. pulex* and on cannibalism within each species. I found that while infection with the microsporidian parasite *Pleistophora mulleri* caused an increase in the FRs of *G. d. celticus,* an infection with the acanthocephalan parasite *Echinorynchus truttae* did not alter the FRs of *G. pulex*. Overall IGP FRs were observed to be higher than cannibalism FRs. This finding, in combination from the results of prey choice experiment enables us to conclude that these species of amphipods prefer heterospecifics rather than conspecifics prey. This is likely to be selectively advantageous as feeding on heterospecific prey removes possible future competitors without the risk of consuming juvenile kin or acquiring parasites that comes with consuming conspecific prey. From the results I can also infer that infection of the native *G. d. celticus* with *P. mulleri* might, by enhancing its IGP, facilitate the coexistence between the two species.

4.1. Introduction

In Northern Ireland *Gammarus pulex* transplanted from England have been observed to have invaded a number of populations of the native *Gammarus duebeni celticus* (Strange and Glass 1979). Intraguild predation (IGP), the predation between individuals of the same trophic guild which removes a potential competitor (Polis et al. 1989; Holt and Polis 1997) has been observed to occur between the two species (Dick 1992). The species are also known to be highly cannibalistic (Dick, Montgomery, and Elwood 1993) . The removal of potential competitors is thought to have consequences for the population dynamics of the species, which are more complex than those of ordinary predator prey interactions (Polis et al 1989). Previous studies have found *G. pulex* are stronger IGP predators than *G. d. celticus* and this differential IGP is related to the widespread replacement of the native (Dick 1996; Dick, Montgomery, and Elwood 1999; MacNeil, Montgomery, Dick, and Elwood 2001).

Native and invasive amphipods are both known to be hosts to a range of acanthocephalan, and microsporidian parasites (Dunn and Dick 1998). Infection with a parasite is known to affect a range of behaviours in their hosts. For example infection with the parasite *E. truttae* have been shown to make *G. pulex* more active and less photophobic (MacNeil, Dick, Hatcher, Terry, Smith, and Dunn 2003). Parasites have also been shown to modify the rate of predatory interactions (Dick et al 2010) and can also increase the vulnerability of their host's predation (MacNeil et al 2003).

The native *G. d. celticus* is the specific host to the microsporidian parasite *P. mulleri* which locally infects and degrades the abdominal muscle resulting in reduced activity (MacNeil, Dick, Hatcher, and Dunn 2003). It is known to be transmitted orally with a transmission rate of 23% (MacNeil, Dick, Hatcher, Fielding, Hume, and Dunn 2003). The prevalence of the parasite in the field has been observed to be as high as 45% (Fielding, MacNeil, Robinson, Dick, Elwood, Terry, Ruiz, and Dunn 2005). Previous work has shown that an infection with the parasite causes its host to be significantly more cannibalistic feeding on conspecific juveniles (Bunke et al. 2015), chapter 2..

The invasive *G. pulex* is host to the acanthocephalan parasite *E. truttae*, which has a complex lifecycle in which *G. pulex* is the intermediate host whilst the brown trout *Salmo trutta* functions as the final host. It is known to have a high prevalence with up to 70% of the amphipod population in the wild having been observed to be infected (MacNeil, Fielding, Dick, Briffa, Prenter, Hatcher, and Dunn 2003). In previous studies it has been found that an infection with the parasite causes change in behaviour of *G. pulex* which is likely to facilitate transmission to the final host (MacNeil, Fielding, Hume, and Dick 2003). Another study investigating the predatory FR of *G. pulex* feeding on *Asellus aquaticus* found than an infection with the parasite caused a higher FR (Dick, Armstrong, Clarke, Farnsworth, Hatcher, Ennis, Kelly, and Dunn 2010), whilst in chapter three I found a context dependent impact of *E. truttae* infection on the canniblism FR of its *G. pulex* host.

The predatory impact of a species can be measured using predatory functional responses. They describe the relationship between the use of a resource and its availability (Holling 1959). Comparative FR provides a way to make comparisons of feeding rates not only between species but also between circumstances (Dick et al. 2010). The FR analysis also enables the extraction of the *a* and *h* parameters which provide information on the predator prey interaction which may be used to further infer impact of the biotic and abiotic factors on the consumption by the predator. The *a* parameter is a scaling parameter which is associated with the attack rate of the predator on its prey and is known to define the initial slope of the functional response curve (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015a). The *h* parameter stands for the handling time of the prey by the predator and limits the maximum number of prey consumed (Paterson, Dick, Pritchard, Ennis, Hatcher, and Dunn 2015a).

Comparative FRs have been used to investigate the impact of possible invaders on native prey species. For example *Dikerogammarus villosus* have been found to have a significantly higher FR feeding on *A. aquaticus* than the native amphipods *G. pulex*, *Gammarus roeseli* and *Gammarus duebeni* (Bollache, Dick, Farnsworth, and Montgomery 2008).

To my knowledge this is the first study using the comparative FR approach to investigate the impact of parasitism on cannibalism and IGP within and between the invasive *G. pulex* and the native *G. d. celticus*. I propose that parasite infection will alter IGP interactions between these two species.

4.2. Methods

G. d. celticus were collected from Downhill River, County Antrim, Northern Ireland (55.166674 N, 6.8201185 W; figure 4.1.). *G. pulex* were kick sampled from the river Lagan system at Shaw's Bridge (54_548509N, 5_9526063W; figure 4.1.). On arrival at the lab, juvenile and adult individuals were separated and in separate tanks from the adults. Parasite status was determined by the presence/absence of *P. mulleri* spore mass visible through the exoskeleton of *G. d. celticus* and parasitized individuals all had visible infection of one to two segments. Infection with the parasite *E. truttae* was determined with the acanthocephalan being visible as an orange line through the exoskeleton of *G. pulex* individuals. The infection with the parasite was confirmed by dissection after the experiment. Individuals with a visually confirmed parasite infection status using a light microscope, were maintained in separate tanks by species. Animals were kept in tanks with substrate, leaf material and aerated river water from their source at 13°C at a 12h:12h light:dark regime. Adults were sexed based on the presence of copulatory papillae under a light microscope., Only adult males were used as predators in the experiments because the predatory ability of females is affected by other factors such as the presence of offspring in their brood pouch. Before the start of the experiment the adult male individuals were starved for 48h in order to standardize hunger.

Figure 10.1. Map showing the location of the field sites used for the collection of amphipods in Northern Ireland. *Gammarus pulex* were collected at the location marked by the black circle to the south of Belfast. *Gammarus duebeni celticus* were collected at the location marked by the black circle on the north coast.

4.2.1. Functional response experiments

The FR experiments were carried out in clear circular plastic arenas with a circumference of 8 cm. These pots were filled with 200ml water,.For the IGP FR the water was made up in equal parts from the *G. pulex* and the *G. d. celticus* field sites. For the cannibalism the arena contained water from the field site according to species source. For the IGP FR experiments single adult male individuals were offered juveniles of the other species at a number of densities (2, 4, 6, 8, 10, 15, 20, 30 and 40) with three replicates at each density. For the cannibalism FR experiments single adult male individuals were offered juveniles of their own species at a number of densities (2, 4, 6, 8, 10, 15 and 20) with three replicates for each density. I only needed seven densities for cannibalism instead of the nine that were used for the IGP FR experiments, because the number of prey items consumed reached a plateau at a lower density for the cannibalism than for the IGP set up. During both types of FR experiments the prey individuals were placed into the experimental arena 30 minutes prior to the start of the experiment to allow them to acclimatise. Then adult males were placed into the arena and left for 40h. Removing the adult individuals and counting how many items of prey remained, ended the experiment.

All statistical analyses for the FR experiments were undertaken in R version 3.3.3 (R Foundation for Statistical Computing 2017). The type of predatory functional response (the relationship between proportional mortality of prey and prey density) occurring was analysed using the frair_test function of the frair package in R (Pritchard 2016) for each of the treatments.

Subsequently the predatory functional responses were modelled using the Rogers'-random predator equation (eq 4.1.) this was appropriate in this situation because the FRs were type two and prey was not replaced during the cause of the experiment. (Juliano, 2001).

$$(N = N_o(1-e^{a(Nh-PT)})) \qquad \text{eq 4.1}$$

where N is the number of prey eaten, No is the initial prey density, a is the attack constant, h is the handling time and T is the total time available for predation.

The equation assumes non-replacement of prey, in order to obtain estimates of the saturation parameter h and the scaling parameter a for each of the treatments. FRs were modelled using the R package frair (Pritchard 2016) which utilizes maximum likelihood estimation within the bbmle package (Bolker 2014) .

The FR curves were bootstrapped and plotted with CIs in order to visualise the variability. The *a* and *h* parameters were compared between the predator groups and infection status using the indicator variables (function frair_compare; Paterson et al. 2015b).

A comparison of the *a* and *h* parameter values between the different treatments was carried out, using the frair_compare function which is part of the frair package. It implements the delta or difference method of Juliano (2001). The mean number of juveniles killed was examined with respect tot he adult infection status, prey type and juvenile density using generalized linear models (GLMs) with quasi-poison error distribution in R v. 3.3.3 that were simplified via a step-deletion process.

4.2.2. Prey choice

To explore whether adult males preferred to predate juveniles of the competing species, or to cannibalise conspecific juveniles, a prey choice experiment was conducted. Two size matched juveniles, one juvenile of each species, were placed into a small plastic pot containing water from both amphipod sources. After 30 minutes an adult individual (either

G. pulex or *G d. celticus*) was placed into the experimental arena and left there until one juvenile was consumed. Only visually uninfected adult males were used in this experiment. The remaining juvenile was then checked under a dissection microscope to establish which species the consumed individual belonged to. This was done based on the shapes of the animal's eyes. While *G. pulex's* eyes are round in shape, the eyes of *G.d. celticus* are more kidney shaped (Gledhill et al. 1993). The results of 30 replicates for each species were analysed using a chi-squared test.

4.3. Results

All FR for experiments carried out were observed to be Type-II FRs with logistic regression first order coefficients significantly negative as can be seen in figure 4.2. in which lines are fitted to average data points at each density (table 4.1.).

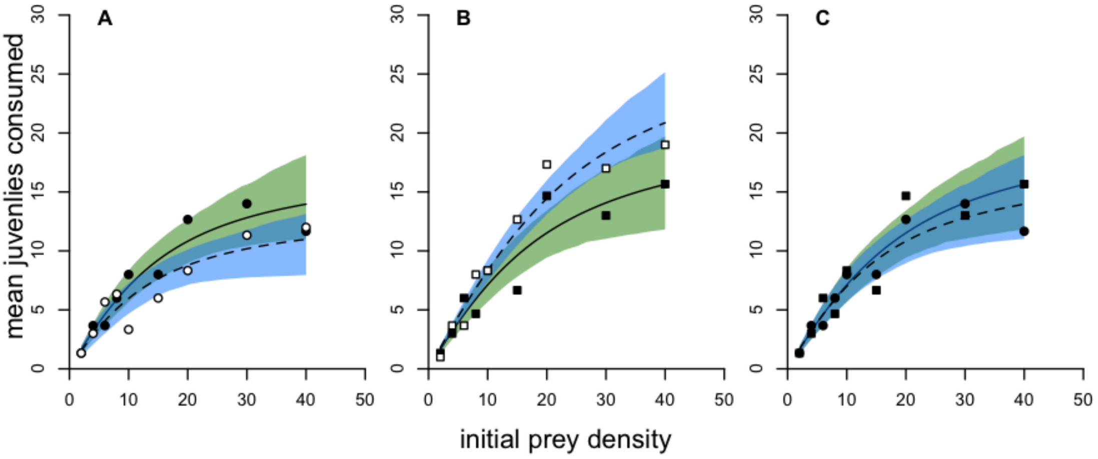

Figure 4.2. Comparative FR curves with bootstrapping polygons for IGP between *G. pulex* and *G. d. celticus*. Circles represent *G. pulex*, squares represent *G. d. celticus*. In A and B Full shapes and lines represent uninfected individuals and empty shapes and dotted lines represent individuals infected with *E. truttae* and *P. mullerei* respectively. The direct species comparison uses data from uninfected individuals only (full lines *G. pulex*, dotted lined *G. d. celticus*) A) Comparison of FRs between uninfected and infected *G. pulex*. B) Comparison of FRs between uninfected and infected *G. d. celticus* C) Comparison of FRs between uninfected *G. pulex* and *G. d. celticus.* The coloured areas are the bootstrapping polygons. In A and B green represents the unfinfected individuals and blue the infected individuals. The third colour in the figure is caused by an overlap of the polygons. In C green is the polygon for *G.d.celticus* and blue for *G. pulex*. Again the third colour in the figure is caused by an overlap of the polygons

The analysis of the FRs investigating the impact of parasitism on IGP showed that overall there is no sig. difference between the IGP predation by *G. pulex* and *G. d. celticus* (figure.

4.2c*)*. In *G. pulex* an infection with the parasite *E. truttae* did not cause an increase in the IGP FR (figure 4.2.a). In contrast, *G. d. celticus* infected individuals were observed to have a higher IGP FR than uninfected individuals (GLM, $X^2_{1,100}$ =33.189, p=0.0178; figure 4.2b.).

Using the comparative approach to compare the IGP FR to cannibalism FR showed the IGP FR for both *G. pulex* and *G. d. celticus* is higher than the cannibalism FR observed for each species (*G. pulex,* GLM, $X^2_{1,45}$=11.968, p=0.006; *G d. celticus*, GLM, $X^2_{1,98}$=34.52, p<0.001; figure 4.3.).

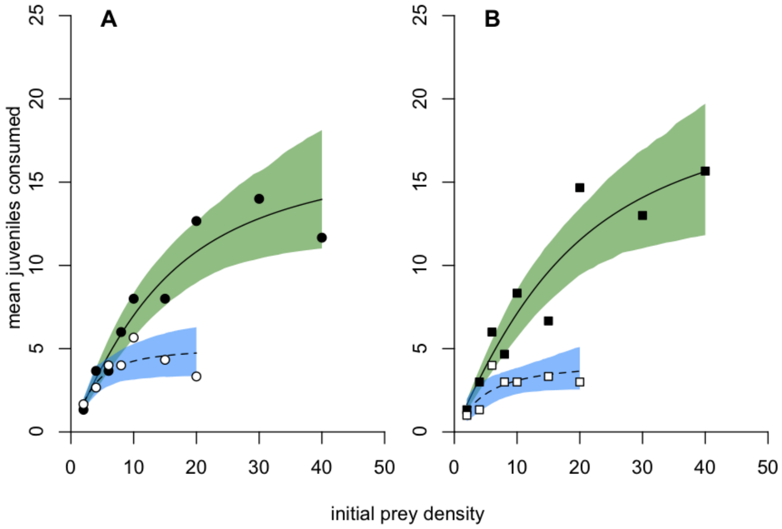

Figure 4.3. Comparison of FR between IGP and cannibalism. Full shapes represent IGP and empty circles represent cannibalism datapoints. A) comparison for *G. pulex* B) comparison of FRs for *G. d. celticus*. Shaded areas are the bootstrapping polygons. Only data points from uninfected individuals are used for the figures and analysis. The cannibalism data used was taken from chapters 2 and 3.

The comparison of the *h* and *a* parameters showed that *h* parameters were significantly higher in cannibalism than in IGP for both *G. pulex* (frair_compare, p<0.001) and *G. d. celticus* (frair_compare, p=0.002). A difference in the *a* parameter was not observed (frair_compare, p=0.316).

Table 4.1. Parameter estimates and significance levels from first-order logistic regression analysis of the proportion of prey killed against initial prey density, with functional response parameters. Estimates extracted from Rogers' random predator equation fitted to data in the frair package. P – value referring to the significance value of to the first order term, a - attack coefficient, h – handling time, SE – standard error

Predator	Prey	First order term	p	a	SE	h	SE
G. pulex	IGP	-0.057	<0.001	1.189	0.211	0.093	0.013
uninfected	Canni	-0.159	<0.001	1.838	0.777	0.321	0.053
G. pulex	IGP	-0.042	<0.001	0.964	0.231	0.121	0.022
infected	canni	-0.057	0.037	0.620	0.210	0.149	0.064
G. duebeni	IGP	-0.047	<0.001	1.123	0.195	0.078	0.012
uninfected	Canni	-0.110	<0.001	0.773	0.357	0.385	0.097
G. duebeni	IGP	-0.059	<0.001	1.499	0.209	0.056	0.007
infected	Canni	-0.120	<0.001	1.752	0.826	0.320	0.057

Using the comparative FR approach to investigate the impact of parasitism with the help of the bootstrapping graphs showed that cannibalism of *G. pulex* is not affected by an infection with the parasite *E. truttae* (figure 4.4a). As discussed in chapter two an infection with *P. mullerei* caused a significant increase of the cannibalism FR in *G. d. celticus* (figure 4.4b). Even with the bootstrapping polygon overlapping a difference between the groups is still possible as the bootstrapping is based on 3 replicates and is less clear cut than the GLM analysis. A direct comparison of the cannibalism FR between *G. d. celticus* and *G. pulex* did not show a difference between the two species (figure 4.4c). The direct comparison showed that *G. pulex* and *G. d. celticus* have similar cannibalistic abilities when feeding on conspecific juveniles.

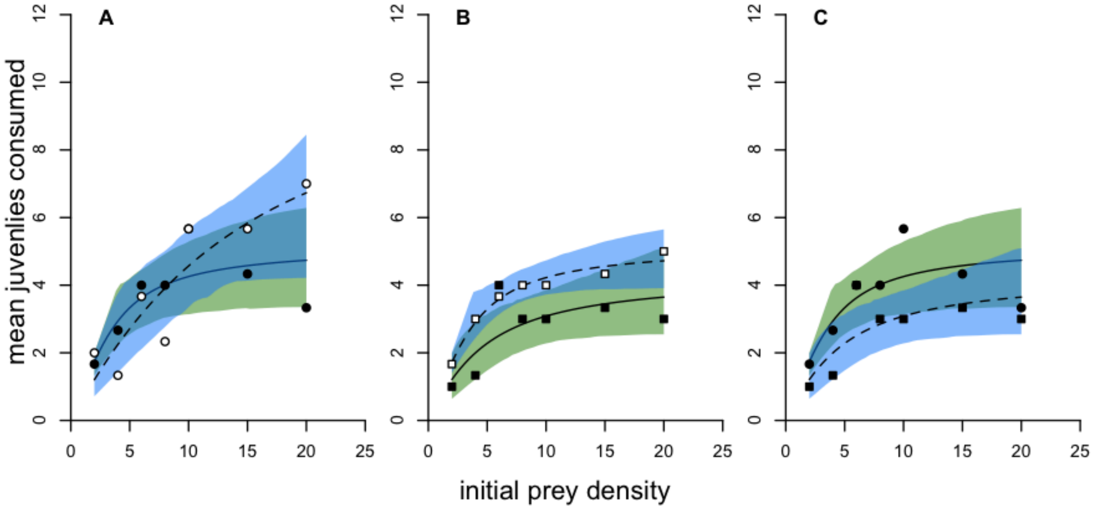

Figure 4.4. Comparison of cannibalism FR curves. A) comparison of *G. pulex* uninfected green vs individuals infected with *E. truttae* blue B) comparison of *G. duebeni celticus* uninfected green vs infected with *P. mulleri* green C) comparison of cannibalistic FR between *G. pulex* green and *G. duebeni celticus* blue with data from uninfected individuals only. The third colour present in the figure occurs when the two polygons present overlap.

The prey choice experiment showed that both *G. pulex* and *G. d. celticus* preferred to feed on prey of the other species ($X^{2}_{1}=32.26$, $p<0.001$; figure 4.5), with 87% of Adults feeding on heterospecific juveniles. The number was the same for adults of both species.

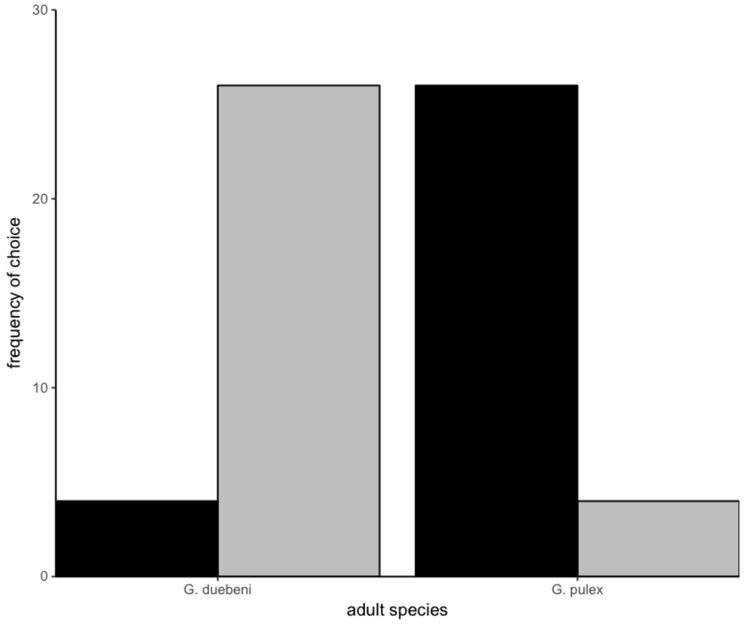

Figure 4.5. The frequency of consumption of conspecific versus herterospecific juveniles by uninfected adults of *G. pulex* and *G. d. celticus*. Black bars represent *G. d. celticus* juveniles and grey bars represent *G. pulex* juveniles

4.4. Discussion

The comparative FR experiment showed that all FR curves were Type-II, which means that IGP can have a possible destabilizing impact on the prey population. This is the case because the predation pressure on the population remains constant even when the density becomes low. This can potentially cause the extinction of a prey population (Rindone and Eggleston 2011). This could be a possible cause behind the exclusion of one species from a system by another as is often observed when one species invades a new ecosystem and pushes out a native species. In the past this has been observed in Northern Ireland where the invasive amphipod *G. pulex* has been observed to exclude the native *G. d. celticus* from a large number of sites (Dick and Platvoet 1996; Dick et al. 1999; MacNeil et al. 2001).

I found that both *G. pulex* and *G. d. celticus* are equally good at IGP of juveniles of the competing species. This result differs from the findings of Dick et al (1996) who found that *G. pulex* was the better IGP predator. However, Dick et al. (1996) looked at IGP of adult females (singletons and in pairs). It could be that the success of IGP is dependent on the size of prey. In our FR experiment the IGP prey were juveniles. It could be at this size difference both species are equally capable IGP predators.

An infection with the acanthocephalan parasite *E. truttae* did not impact the FR of *G. pulex* feeding on *G. d. celticus* juveniles. This result is in contrast to the finding of MacNeil et al (MacNeil et al. 2003). In a field experiment, they found that the infection with the parasite lowered the predatory ability of *G. pulex* on *G. d. celticus* adults. They also observed that an infection caused *G. pulex* to have a lower ability to prey on *G. d. celticus* females, which are carried in precopulatory pairs. It could be that the ability to predate adults of the competing species is reduced because the infection places a burden on its host, which lowers its ability or effectiveness at being an IGP predator on large adult individuals. This might not be such an important role if the prey is smaller such as in juveniles used in the current experiment. Indeed, it has also been observed that an infection with *E. truttae* causes *G. pulex* to have a higher FR feeding on the smaller species *A. aquaticus* (Dick et al 2010). This suggest that the impact infection has is dependent on the type and size of prey. In chapter three I found that infection with *E. truttae* causes cannibalistic FR of *G. pulex* to double in the presence of a higher predator and habitat structure.

The finding that *G. d. celticus* infected with *P. mulleri* had a higher FR feeding on *G. pulex* could be caused by the fact that an infection with the parasite puts a burden on its host causing it to have a greater need for consumption (Fielding, MacNeil, Dick, Elwood, Riddell, and Dunn 2003). It also matches the finding of chapter two, which found that an infection with the parasite makes the species more cannibalistic when feeding on conspecific juveniles.

Using the comparative FR approach to compare IGP to cannibalism showed that for both *G. pulex* and *G. d. celticus* the FRs feeding on heterospecific juveniles (IGP) were higher than the FRs feeding on conspecific juveniles (Cannibalism). This makes sense for a number of reasons. The first is that cannibalism carries a higher risk than intraguild predation of acquiring an infection with a parasite through consumption (Pfennig et al. 1998; MacNeil et al. 2003). Secondly, IGP removes a possible competitor without the risk that it might be ones own offspring, as could be the case during cannibalism. The finding is further supported by the finding of the prey choice experiment. It showed clearly that when given the choice individuals for both species preferred to prey on juveniles of the other species rather than conspecific juveniles. This finding is in concurrence with (MacNeil and Dick 2014) who observed that when given a choice both *G. pulex* and *G. duebeni* prefer to feed on hetero specific rather than conspecific prey.

Taken together the findings from our study show that parasitism can potentially have a strong influence not only on the dynamics of its host population but also impact on the population of a similar species which is a possible competitor. In such situation it can cause a reversal of dominance relationships between species making its host the superior predator. It thus can mediate the outcome of species interactions such as predator prey interactions and might be able to reverse them. In these instances this change in interactions is very likely to have an impact on the ecosystem at a broader range. It could for example allow for species to co-exist in situations in which one species might have otherwise excluded the other from the ecosystem (Hatcher, et al. 2008).

Chapter Five

Temperature skews predatory strength between native and invasive predators

Abstract

Climate change and invasive species are key threats to biodiversity and community function. Climate change is predicted to lead to warming in UK of between 0.3 and 4.8°C. Temperature affects biological processes and so may influence interactions between species. Changes in temperature might impact native and invasive species differently if they have different temperature tolerance. I investigated how an increase in water temperature affects the intraguild predation between the native amphipod *Gammarus pulex* and the invasive *Dikerogammarus villosus*. I observed mutual, albeit skewed intraguild predation between the two species. The invader was observed to consume significantly more juveniles of the native then in the reverse set up. Predation between adults was unidirectional, with *D. villosus* males feeding on *G. pulex* females, but very little predation of *D. villosus* by *G. pulex* The two species reacted differently to the increase in water temperature, with the invader showing a stronger increase in predation, which could reflect its greater thermal tolerance. Our results suggest that the effect of the invader on native species may be exacerbated under changed climatic conditions in which water temperatures are increased. .

5.1. Introduction

Biological communities are under increasing pressure from a variety of anthropogenic sources. Two of the most important stressors affecting biodiversity and community structure are climate change and invasive species. (Bellard et al. 2012; Simberloff et al. 2013; Sorte et al. 2013) with freshwater systems highlighted as being particularly at risk (Woodward et al. 2010). Biological invasions are a major driver of biodiversity loss with the rate of invasions increasing as a result of anthropogenic activity. Invasive non-native species can affect native species through competitive and trophic interactions including predation (Orrock et al. 2015; Sugiura 2016; David et al. 2017; Falcão et al. 2017).

Climate change is likely to increase the level of environmental stress placed on organisms (Mooney et al. 2009) because temperature influences all biological processes (Sentis et al.2012), affecting animal metabolism and behaviour (Bale 2002; Woodward et al. 2010; Gilbert et al. 2015). These effects might result in changes which can cascade to have effects at the population and community levels (Petchey et al. 2010) and allows new species to survive. It is also likely that climate change will interact with current environmental stressors, such as invasive species. Invasive non-native species often have a wider thermal tolerance than their native counter parts, (Bates et al. 2013), with climate warming potentially facilitating invasion events (Sorte et al., 2013; Gallardo et al. 2012). Climate change is predicted to alter the distribution of invasive species (Gallardo et al. 2012,,Gallardo and Aldridge 2013c) Gallardo and Aldridge 2013. However, few studies have looked into climate change will affect the impact of invasive species and the potential biodiversity loss of the invaded ecosystem (McCary et al. 2016; Doherty et al. Dickman 2016). We still have much to learn about how these stressors interact (Bellard et al., 2016; Sorte et al., 2013).

Temperature has been shown to affect the life history of animals (e.g. Bale 2002; Dale et al. 2015; Tordesillas et al. 2016; Tremont et al. 2016). However, few studies have investigated the links between temperature and trophic interactions such as predation. These studies are especially important because theoretical mathematical models have shown that temperature can strongly influence the strength of interactions, which can have important effects on the dynamics between predator and prey (Vasseur and McCann 2005). Invasive species and the native species with which they interact may differ in their thermal tolerance, and hence in their response to temperature. This chapter considers the predatory interactions between an invasive and native species and the influence of temperature on this interaction.

The Ponto–Caspian amphipod *Dikerogammarus villosus* (Sowinsky, 1894), is spreading rapidly across Europe leading to replacement/reduction in native amphipod species and led to changes in community structure (Rewicz et al. 2014). Its effect on ecosystem diversity and functioning has led to it being listed as one of the worst invasive species in Europe (DAISIE 2009). *D. villosus* is omnivorous, feeding on detritus and macroinvertevrates including other amphipod species (Madgwick and Aldridge 2011) and is a physiologically tolerant species, although with a temperature tolerance similar to that of other European amphipods (Rewicz et al. 2014). It was first recorded in the UK in 2010 (MacNeil et al. 2010), and has since established in other parts of England and Wales (MacNeil et al. 2012). Its introduction has already led to community-level changes at invaded sites, including the displacement of the native amphipod *Gammarus pulex* (Linnaeus, 1758) (Madgwick and Aldridge 2011).

Previous research comparing the predatory functional response of *D. villosus* with the native *Gammarus pulex*, revealed that *D. villosus* has higher predatory impact than has the native *species* on a range of invertebrate prey (Bovy et al. 2014) including *Chelicoporphium curvispinum*, *Asellus aquaticus, Daphnia magna* and *Chaoborus larvae* (Bollache et al. 2008; Dodd et al. 2014; Iltis et al. 2017).

Amphipods also show intraguild predation (IGP) (predation between individuals, which also compete with each other Polis et al., 1989). Predation is size dependent and predation of juveniles by heterospecific adults is common, as is predation of females (the smaller sex) by males (Wissinger 1992; Dick et al. 1993). Studies on individual predator-prey interactions have shown that *D. villosus* is a stronger IG predator than the native *G. pulex* and *Echinogammarus berilloni, in* Germany (Kinzler et al. 2008). However, to my knowledge the comparative functional response approach has not yet been used to investigate IGP between *D. villosus* and sympatric native amphipod species. Functional response curves can be seen as simple models of the predator prey interaction strength (Sentis et al. 2012). The functional response (FR) is the proportion of provided prey consumed by a predator. Their derivation illustrates predator behaviour and the impacts on their prey (Holling 1959). Comparative FRs can be used to explain and predict the impacts that invasive species can have on native species and their communities (Bollache et al. 2008).

The predatory functional response approach can also be used to explore the effects of temperature on predatory interactions (e.g. Wasserman et al. 2016; South et al. 2017) Past studies have found that FRs usually increase with a warming in temperature (Zamani et al. 2006). Theoretical work predicts that this is likely to be caused by a direct link between both the search/attack and handling time and biochemical processes which are influenced by temperature (Gillooly et al. 2001). Further theoretical work predicted that warming in temperature will cause the attack rate to exponentially increase and the handling time to drop exponentially (Brown et al. 2004). Here I use a predatory FR approach alongside 1:1 predation experiments to investigate IGP between *D. villosus* and *G. pulex* and the effect of elevated temperature on this mutual predatory-prey interaction

5.2. Methods

We carried out a series of experiments to investigate the influence temperature has on intraguild predation between the native *Gammarus pulex* and the invasive *Dikerogammarus villosus*. *Dikerogammarus villosus* were collected from Grafham Water, Cambridgeshire, UK (52.1703100N, 0.1902400W, figure 5.1.), where it was picked from pontoon mooring ropes. *Gammarus pulex* were collected from DuloeBrook, Cambridgeshire, UK (52.1304900N, 0.1802600W, figure 5.1.) by kicksampling. In the laboratory all animals were maintained in separate tanks in a temperature controlled room at 14°C and a 12:12h light:dark cycle at Cambridge University. Each pot was supplied with aerated source water, substrate and food in the form of gravel, local autochthonous and allochthonous vegetation, which included willow leaves (Salix sp.). The animals were kept in these individual pots for a minimum of 24h before the start of the experiments.

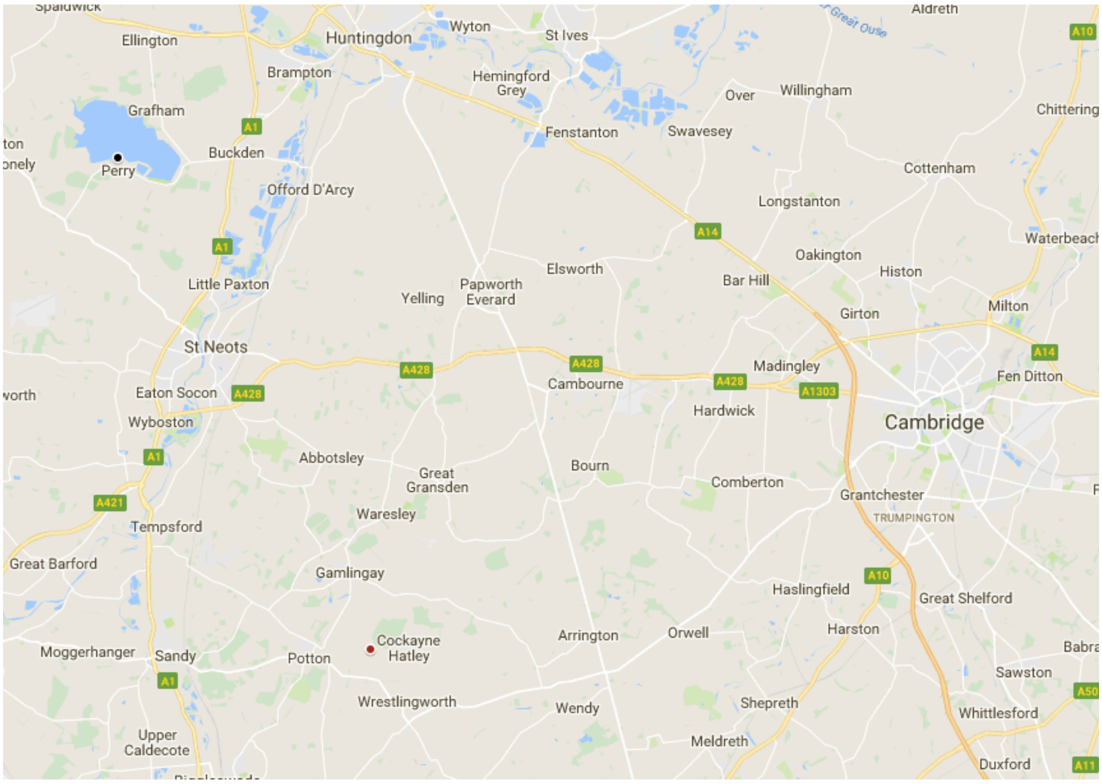

Figure 11.1. Map showing the location of the sampling sites used for the study. Black circle shows the site used to collect *Dikerogammarus villosus* at Grafham waters. Red circle shows the site *Gammarus pulex* were collected from at DuloeBrook.

5.2.1. IGP between adult singletons

An experiment was carried out to investigate whether temperature has an impact on IGP occurring between single adult males preying on single heterospecific adult females. The experimental set up consisted of a small opaque plastic pot (8 cm diameter) as an arena, lined with gravel to provide habitat structure and 150 ml water in equal parts from each amphipod source. During the experiment food was provided to excess in the form of dried leaves. The experiment was carried out at two temperatures; 8°C and 20°C. The temperatures were selected to represent water temperatures occurring in spring and summer respectively (Garner, et al.. 2013). At the start of the experiment single female of one species were placed into the experimental arena and left to acclimatize for 30 minutes, before a single heterospecific male was added to the arena (figure 5.2., N= 30 replicates per treatment) (male: female weight ratio of 2.2 to 1.9 which has been observed in natural population of both species (Devin et al. 2004; Dunn et al. 2008). The experiment was checked twice daily for five days for signs of predation or moult. Gravid females were used as prey because a) theoretically speaking this makes them most valuable from population dynamics point of view and b) this means that they are not likely to moult during the duration of the experiment. Any replicates where moulting occurred were excluded from the analysis as moulting increases vulnerability to predation.

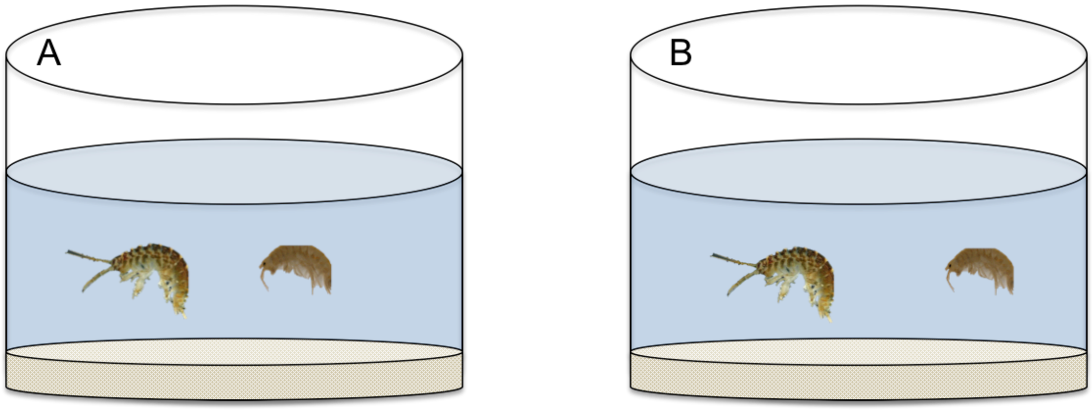

Figure 5.2. Schematic of experimental set up used in the experiment. Experimental set up of glass dish with gravel, water and amphipod individuals.

As a control to the experiment 30 single females of each species were kept in experimental arenas at each temperature without an IG predator being present.

GLMs with a binomial error distribution were performed on the results of the experiment and the control to investigate whether temperature affects the occurrence of IGP between the two species and the survival of the individual females.

5.2.2. IGP by adults on juveniles

The comparative functional response approach was used to investigate the effect of temperature on IGP of *G. pulex* juveniles by *D. villosus* adults and GP of *D. villosus* juveniles by *G. pulex* adults. FR experiments were carried out at two temperatures (8 and 20°C). Prior to the experiment, animals were allowed to acclimatize for at least 24 hours. Male individuals (IG predators) were starved for 48 hours before the experiment to standardize hunger. The experiment was carried out in small plastic pots with a diameter of 8cm, filled with 100ml river water taken in equal parts from both amphipod sources. Prey was provided in the form of heterospecific juveniles at the seven densities of 2, 4, 6, 8, 10, 15 and 20. Three replicates were carried out at each density and at each temperature. The number of prey items consumed was noted after 40 hours. FR experiments using adult prey were not undertaken as data from the first experiment revealed low numbers consumed.

The statistical analysis for the FR experiments was carried out in R version 3.3.3 (R Foundation for Statistical Computing 2017). The type of predatory functional response occurring was analysed using the frair_test function of the frair package in R (Pritchard 2016) for each of the treatments. Subsequently the predatory functional responses were modelled using a maximum likelihood estimation with the Rogers'-random predator equation

$$(N = N_o(1-e^{a(Nh-PT)})) \qquad \text{eq 5.1}$$

where N is the number of prey eaten, No is the initial prey density, a is the attack constant, h is the handling time and T is the total time available for predation.

The equation assumes non-replacement of prey, in order to obtain estimates of the saturation parameter *h* and the scaling parameter *a* for each of the treatments. FRs were modelled using the R package frair (Pritchard, 2016) which utilizes maximum likelihood estimation within the bbmle package (Bolker 2014). The *a* and *h* parameters were compared between the treatments using generalized linear models (GLMs) with a gamma error distribution on 1000 bootstrapped values obtained from the frair_ package. The *a* parameter is a scaling parameter which is associated with the attack rate of the predator on its prey and is known to define the initial slope of the functional response curve (Paterson et al. 2015). The *h* parameter stands for the handling time of the prey by the predator and limits the maximum number of prey consumed (Paterson et al. 2015).

In the *G. pulex* FR experiments, insufficient prey were consumed to successfully fit a FR curve using the frair package. For the data available I obtained the AIC values for each type of FR for each treatment in order to manually see which type of FR fits the data best. The mean number of juveniles killed was examined with respect to temperature; predator species and juvenile density using generalized linear models (GLMs) with a quasipoisson error distribution.

5.3. Results

The overall survival of the control individuals in the absence of intraguild predators was high (94%) with no difference in survival between the species ($X^2_{1,117}$=0.069, p=0.792) and at the two different temperatures ($X^2_{1,118}$=1.735, p=0.1877). At 8°C survival of *G. pulex* and *D. villosus* was 87% and 97% respectively, while at 20°c it was 90% and 87% respecitvely. From this it follows that death can be primarily attributed to IGP in the experiments carried out.

There was asymmetry in the IGP between adults of the native and invasive species, and temperature affected the rate of IGP. *D. villosus* was a stonger IGpredator than was *G. pulex* ($X^2_{1,100}$=21.94, p<0.001; figure 5.3) At the lower temperature, there was very little IGpredation by either species, whereas IGPredation was higher at the higher temperature ($X^2_{1,99}$=13.23, p=<0.001; figure 5.3). Inspection of figure 5.3 suggests that *D. villosus* showed a greater increase in IGPredation at the higher temperature than did the native species, although the species*temperature interaction was not significant p= 0.107.

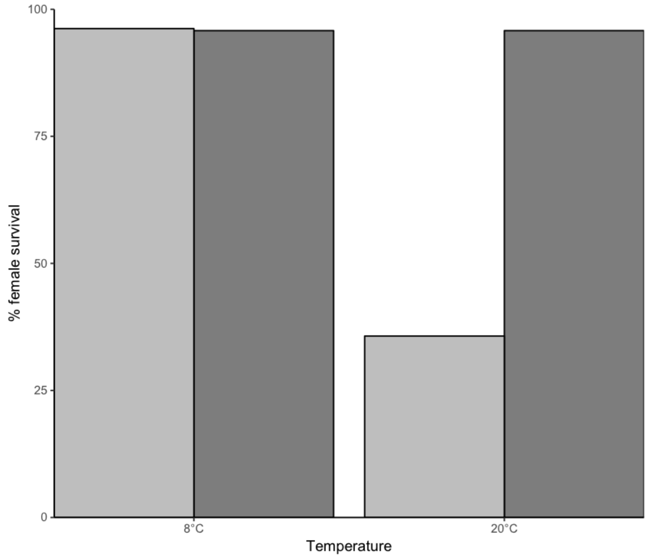

Figure 5.3. Female survival in singleton IGP experiments. Light grey bars show the survival of individual *G. pulex* females (IGprey) in the presence of *D. villosus* males (IG predator). Dark grey bars show the survival of individual *D. villosus* females (IG prey) in the presence of *G. pulex* males (IG predator).

The two FRs of *D. villosus* feeding on *G. pulex* juveniles at 8 and 20°C were observed to be Type-II. The predation level of IGP of *G. pulex* feeding on *D. villosus* juveniles were too low to be expressly identified as Type-II FR by the frair package. However, I decided to proceed with the data analysis under the assumption of a Type-II because the logistic regression analysis had a negative first order term (table 5.1.), the AIC value was best for Type-II (table 5.2), after excluding Type-I because *G. pulex* are not filter feeders for who this type of FR is generally used. In addition to this *G. pulex* have been shown to display a Type-II FR over a range of preys and in a number of different conditions (Dick et al. 2010; Paterson et al. 2015, chapter 3 and 4 of this thesis).

Table 5.1. Results from FR analysis of the IGP FR as performed in frair package in R. First order term and p value are the results from the logistic regression analysis to determine the type of FR. other values are estimates for a and h parameters and their standard error extracted from Rodgers' random predator equation. a – attack coefficient, h – handling time, SE – standard error

Predator Species	Temperature treatment	First-order term	P of first order term	a	SE	H	SE
D. villosus	8°C	-0.179	<0.001	5.65	3.17	0.18	0.02
	20°C	-0.242	<0.001	11.20	8.15	0.15	0.02
G. pulex	8°C	-0.036	0.279	0.17	0.08	0.28	0.26
	20°C	-0.042	0.262	0.14	0.09	0.49	0.44

However, because the failure to automatically fit a type of FR to the data prevented us from comparing the treatments to each other within the package, instead I performed the GLM on the output of the bootstrapped coefficients provided by the frair_package to enable this comparison to be carried out outside of the package.

The GLM of the FR data between *D. villosus* and *G. pulex* found that IGP between the native and invasive species was asymmetric. *D. villosus* consumed significantly more *G. pulex* juveniles than *G. pulex* consumed *D. villosus* juveniles (GLM, $X^2_{1,82} = 167.974$, p<0.001). For both species, the FR was higher at 20°C than at 8°C (figure 5.4). An interaction between the factors of species and temperature was also observed in the model (GLM, $X^2_{1,79}=2.395$, p=0.033), reflecting a stronger effect of temperature on IGPredation by *D. villosus* than on *G. pulex*. Overall the FR of *D. villosus* was higher that that of *G. pulex*. At 8°C it was about 4 times higher while at 20°C it was around 5 times higher than that of *G. pulex*.

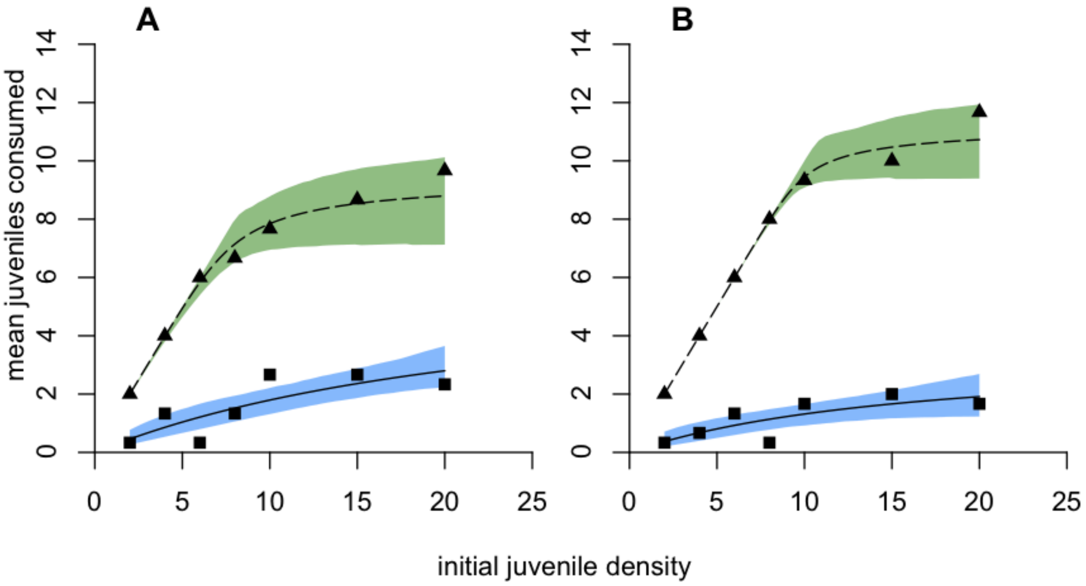

Figure 5.4. Predatory functional responses of adult males feeding on heterospecific juveniles at A). 8°C B) FR IGP at 20°C. Datapoints in the graph refer to the mean number of juveniles consumed with polygons giving the 95 CI from the bootstrapping function. Triangles and green polygons refer to IG predation by *D. villosus* on *G. pulex* juveniles, and squares and blue polygons refer to IG predation by *G. pulex* on *D. villosus*

Overall the *a* parameters were observed to be higher for the FRs in which *D. villosus* was the predator, while the *h* parameter were higher for the FRs in which *G. pulex* was the IG predator. The attack rate parameter differed between species (GLM $X^2_{1,5631}$=14491, p<0.001) and was also significantly affected by temperature ($X^2_{1,5632}$= 434, p<0.001), but no interaction between the two factors was observed. For the *h* parameter there was a significant interaction between temperature and species (temp*spp) ($X^2_{1,5630}$=93.08, p<0.001).

Overall the *a* parameter in *D. villosus* is more than a magnitude higher than in *G. pulex*. The *h* parameter is *D. villosus* is only about half as high is *G. pulex (*table 5.1*)*. In *D. villosus* the increase in temperature doubled the *a* parameter and caused a slight drop in the *h* parameter. In *G. pulex* the increase in temperature caused a non significant reduction in the *a* parameter, while the *h* parameter nearly doubled.

Table 5.2. Comparison of data fitted to different types of FR. AIC values for each treatment and type of functional response. Obtained using the frair package in R.

Predator Species	Temperature treatnent	Type I	Type II	Type III
D. villosus	8°C	90.21	50.98	52.98
	20°C	79.15	32.49	34.49
G. pulex	8°C	57.39	58.33	60.22
	20°C	50.02	50.81	52.79

5.4. Discussion

Mutual IGP between the two species was observed during the experiments I carried out. It was of asymmetric nature with *D. villosus* being the stronger IG predator. The functional response of *D. villosus* feeding on *G. pulex* juveniles was much higher than the FR of the reverse situation. This fits in with the results from previous studies, which found that *D. villosus* displays higher predatory FR than native amphipods on a number of species (Bollache and Cezilly 2004; Bovy et al. 2014; Dodd et al. 2014). At the lower temperature I observed very little IGP between adult individuals of the two species. This could be due to the fact that both species are less active at the lower temperature (Maazouzi et al. 2011).

The increase in water temperature affected the native and invader differently. The increase caused the FR of *D. villosus* adults feeding on *G.pulex* juveniles to increase but did not have the same effect on the FR of *G. pulex* feeding on *D. villosus*. The observation that FR for *D. villosus* is higher at higher temperature fits in with previous FR studies, which have found that a warming in temperature causes an increase in a maximum consumption rate (Laverty et al. 2017; South and Dick 2017; South et al. 2017).

However, the FR of *G. pulex* feeding on *D. villosus* juveniles seems to drop at the higher temperature, through this finding was not statistically significant. It could be that the increase in temperature pushes *G. pulex* close to the limits of its temperature tolerance. Previous studies of *G. pulex* from Rhone River near Saint-Alban-du-Rhône, have indicated that this limit would be around 25°C (Maazouzi et al. 2011). However, it could be that populations in more northern locations have lower upper temperature limit. This has previously been observed for other species (Cottin et al. 2012). Being pushed to the edge of the temperature tolerance can cause a reduction in predatory activity (Maazouzi et al. 2007), which would cause a drop in the FR.

It is interesting to observe that, although there was negligible evidence of IGP of adults, *G. pulex* does have the ability to prey on *D. villosus* juveniles. This suggests that its ability to do so is influenced by the size of its prey. As long as the prey is small enough it is able to capture and consume them. The lower FR at the higher temperature could either be caused by a behavioural of the prey making them better at evading predation or by a behavioural change in the predator decreasing their predatory ability. Unfortunately our data does not allow us to say which one of the two is more likely. However, the outcome means that a warming in temperature increase the skewedness of IGP occurrence further in favour of *D. villosus*.

The exponential increase of the *a* parameter and decrease of the *h* parameter I observed for *D. villosus,* with an increase in temperature fits in with the predictions made by other studies (Gillooly et al. 2001). Their general models based on biochemical kinetics and allometry showed that metabolic rates are a function from temperature and body size. This means with increasing temperatures, metabolic rates go up. This leads to an increase in energetic demands, which means animals need to consume more food in order to survive. The observed increase in attack and decline in handling time could indicate that this has occurred here . Brown *et al.* (2004) predicted that a warming in temperature would lead to an exponential increase in the *a* parameter and an exponential drop in the *h* parameter. The change I observed in the *a* and *h* parameters is likely to have been caused by a direct link between these parameters and biochemical processes which are influenced by temperature (Gillooly et al. 2001).

The fact that our observations of *a* decreasing with an increasing temperature for *G. pulex* do not fit this prediction could be explained as follows. It has been suggested in the past that the a parameter might be described by a number of different shapes including linear and humped shaped functions (Cave and Gaylor 1989; Xia et al. 2003). The observation of the decrease in *a* with a warming in temperature could be an indicator for the situation that the parameter follows a humped shaped function. In this case the higher temperature could position the parameter value on the downward slope of the function. This would be an indicator that *G. pulex* might be close to the edge of its temperature tolerance.

Individuals affected by the increase in temperature will spend more time searching for prey and attacking said prey as well as spending less time handling it. This change in behaviour in response to an increase in water temperature has also been observed by Pellan et al (2016). They observed *Gammarus tigrinus* spending more time looking for prey and *D. villosus* spending less time handling their prey. This would mean a higher impact on their prey species. This change in behaviour could be caused by a strain that an increase in temperature places on the individual, which causes an increase in metabolic need and means an individual needs to consume more prey in order to satisfy energetic needs (Pellan et al. 2016).

At the higher temperature unidirectional IGP between adult individuals occurred in the direction that only *D. villosus* males preyed on *G. pulex* females. Such a unidirectional IGP between *D. villosus* and other native gammaridian species has been observed in other studies previously (MacNeil and Platvoet 2005). This ability could be caused by the fact that *D. villosus* has larger mouth parts (Platvoet 2007) which might enable them to bite through the hardened exoskeleton of its victims, thus killing them.

The invader is a stronger IG predator, which may contribute to species replacement as has been observed in the field (Riel et al. 2009; Boets et al. 2010). The invader has also been observed to be a stronger predator on *G. pulex* (Madgwick and Aldridge 2011). Hence it can affect amphipods and other invertebrates adversely. Scenarios of increases in temperature caused by climate change by the Intergovernmental Panel on Climate change (IPCC) predict an increase in temperature between 0.3 and 4.8°C by 2100 (IPCC 2013). This will cause an increase in water temperature and even a minor increase will influence the physiology and behaviour of individuals (Maazouzi et al.t 2011). Success of the invasive species correlates strongly with the species ability to tolerate environmental stressors (Velde et al. 2000). This means that the impact of invasive could be greater in the future, especially if it is better at tolerating the change than the native species.

Such an influence of climate change on species invasions has been widely discussed (Occhipinti-Ambrogi 2007; Sorte et al. 2013). It is especially likely in such cases in which the invader stems from a region in which higher water temperatures are more common, meaning that the invader is better able to cope with increasing temperatures than the native (Maazouzi et al.. This holds even more true for freshwater systems. They are particularly vulnerable to climate change and non native species invasions because they are relatively isolated and physically fragmented (Woodward et al. 2010). Therefore that the populations present cannot move in order to select new habitats in order to cope with new climatic conditions. In addition, space is limited, therefore the introduction of a new species results in competition for limited space and other resources such as shelter and food.

Chapter Six

Parasitism influences IGP between the native *Gammarus pulex* and the invasive *Dikerogammarus villosus*

Abstract

Intraguild predation, predation between species that are in competition for resources with each other, is a widespread trophic interaction and is often observed to occur between invasive species and their native analogues. Parasitism can alter the behaviour of the hosts, and its interaction with other species. I used laboratory experiments to investigate how infection with the acanthocephalan parasite *Echinorhynchus truttae* affects intraguild predation of the amphipod *Gammarus pulex* by an invasive amphipod, *Dikerogammarus villosus*. Intraguild predation of singleton *G. pulex* females was high and *E. truttae* infection did not affect predation likelihood. *G. pulex* females that were being guarded in precopula were less likely to be preyed upon than were single females. However, females that were guarded in precopula by parasitized males suffered a 2-fold increase in the risk of predation in comparison with those guarded by uninfected males. These findings suggest that *E. truttae* infection may increase the negative impact *D. villosus* has on the native *G. pulex*, increasing the likelihood of its replacement.

6.1. Introduction

Biological invasions are a global problem (Mack et al. 2000). They can disrupt native communities both directly and indirectly (White et al. 2006). Invasive species can affect native species directly through competition and predation (Gallardo et al. 2016) as well as intraguild predation (IGP) (Bampfylde and Lewis 2007). These interactions can generate ecological impacts that can propagate along the food web and can trigger trophic cascades (Strayer 2010). For example, an invasion by the zebra mussel *Dreissena polymorpha* in the Hudson river in 1991 reduced the primary production in the river by 80% and as a consequence to the pelagic food web of the river withered, while the littoral part of the food web thrived as a consequence to the water clarity (Strayer 2008).

IGP, the predation between individuals of the same trophic guild is a common trophic interaction which also removes a potential competitor (Polis et al. 1989; Holt and Polis

1997) The removal of potential competitors is thought to have consequences for the population dynamics of the species which are more complex than those of ordinary predator prey interactions (Polis et al. 1989). IGP may occur between invasive species and their native analogues. For example, IGP by the invasive *Harmonia axyridis* is a driver behind the decline of native ladybirds in Europe (Majerus et al. 2006), and IGP by the invasive *Dikerogammarus villosus* is a driver behind the replacement of native amphipods in Europe (Dick and Platvoet 2000).

Parasites are known to be a powerful force in shaping ecological communities (Hatcher et al. 2006). They achieve this by altering species interactions such as competition and predation (Hatcher and Dunn 2011). If a parasite affects its host's mortality and/or fecundity this can affect population size. For example, Rabbit Haemorrhagic disease caused declines in rabbit populations in Spain, and consequent declines of the Iberian lynx (*Lyn pardinus*) and the eagle (*Auila adalberti)* which prey upon rabbits (Ferrer and Negro 2004). In addition to these density-mediated effects, a parasite can also affect predator-prey interactions by changing the host's behaviour. An infection with a parasite may increase the vulnerability of its host to predation (Thomas et al. 2005). For example, many vertically transmitted parasites manipulate their host's behaviour in order to increase the likelihood of it being consumed by its final host. The acanthocephalan parasite *Polymorphus minutus* makes the intermediate host *Gammarus pulex* more active and less photophobic, leading to an increase likelihood of being consumed by the final host to the parasite mallard ducks *Anas platyrhynchos* (MacNeil et al 2003c).

6.1.1. Study system

D. villosus is an amphipod crustacean native to the Ponto-Caspian region which has spread rapidly across mainland Europe (Rewicz et al, 2014). *D. villosus* is an omnivore but also displays traits such as powerful mouthparts and a fast ambush technique which make it an effective predator (Madgwick and Aldridge 2011). Its role in altering communities which it invaded (Dick and Platvoet, 2000, Dick *et al.*, 2002) has lead to its inclusion of the 100 worst invasive species in Europe (www.europe-aliens.org). It is thought that the appearance of *D. villosus* is connected to the disappearance of the native Bullhead *Cottus gobio* populations in Lake Gouwzee, in The Netherlands, by consuming the eggs of the species (Platvoet et al. 2009). Communities invaded by *D. villosus* have been observed to undergo a large reduction in invertebrate diversity (Riel et al. 2006; Boets et al. 2010) and this invader displaces native amphipods (Dick and Platvoet 2000).

D. villosus was first recorded in the UK in Grafham Water in 2010 (MacNeil et al. 2010), and subsequently recorded in two sites in South Wales during 2011 (Madgwick and Aldridge 2011) and Barton Broad, Norfolk in March 2012 abundant (Gallardo and Aldridge 2013b). Because the UK has a high connectivity in its hydrological network and its climate is highly suitable to *D. villosus*, the invader is expected to further spread, particularly in the South and East of the country (Gallardo et al. 2012). It could be that the lower temperatures present in the UK region during autumn and winter provides a form of thermal barrier, preventing *D. villosus* from becoming established in other UK regions. Something similar was observed by Hesselschwerdt and Wantzen (2018), who found that winters provided a thermal barrier preventing *G. roselli* from being excluded by *D. villosus* in Lake Constance.

Intraguild predation is common between amphipods. Amphipods show sexual dimorphism with the female being the smaller sex, and females are more vulnerable to IGP. In addition, IGP is particularly common when the prey is vulnerable during its moult period (Dick et al.1990). *D. villosus* is a strong IGPredator and has been observed to consume females of *G. duebeni* and *G. tigrinus*. Unusually, *D. villosus* have been observed to consume heterospecific amphipod females even during intermoult stages of their lifecycle (Dick and Platvoet 2000) and in chapter five I observed them consuming juveniles of the native *G. pulex*. While the interactions of *D. villosus* with a number of species have been studied, IGP interactions with *G. pulex* (which is native to the UK) have only been investigated using freshly moulted individuals (Kinzler et al. 2008).

Gammarids show precopula mate guarding in which the male guards the females for several days before she moults when fertilisation takes place (Conlan 1991). Precopulatory guarding is advantageous to the male as it ensures that he is able to fertilise the eggs during the short period of receptivity immediately following the females moults (Greenwood and Adams 1987) However, precopula pairs are more vulnerable to higher order predators (Cothran 2004). Here I investigate the effect of precopula pairing on vulnerability of *G. pulex* to IGP by *D. villosus*.

In UK, *D. villosus* appears to have undergone enemy release, with very few of the parasites recorded in the native or European invasive range being found invasive UK populations (Bojko et al. 2013; Arundell et al 2015). In contrast the native *G. pulex* is host to a suite of parasites including the acanthocephalan parasite *E. truttae,* which can reach high prevalence in populations of the native *G. pulex* (MacNeil et al 2003e) but has not been recorded in UK *D. villosus* populations.

E. truttae is tropically transmitted from the intermediate host *G. pulex* to the definitive brown trout *Salmo trutta* host. *E. truttae* manipulates the antipredatory behaviour of its host, making it more active and less photophobic (MacNeil et al. 2003b), thus enhancing he likelihood of predation by the fish definitive host. Hover, it is possible that infection also alters the vulnerability of the host to predation by other predators including to IGP by *D. villosus*. Our study aims to assess IGP between the native *G. pulex* and the invasive *D. villosus* in England and how it might be affected by an infection of the native species with the acanthocephalan parasite *E. truttae.*

6.2. Methods

I carried out a series of experiments to investigate the influence that precopula pairing and parasite infection has on intraguild predation of the native *G. pulex* by the invasive *D. villosus*. *D. villosus* were collected from Grafham Water, Cambridgeshire, UK (52.1703100N, 0.1902400W; figure 6.1.), where it was picked from pontoon mooring ropes. *G. pulex* were collected from DuloeBrook, Cambridgeshire, UK (52.1304900N, 0.1802600W; figure 6.1.) by kick-sampling. In the laboratory all animals were maintained in separate tank in a temperature controlled room at 14 °C and a 12:12 h light:dark cycle at Cambridge University. Each tank was supplied with aerated source water, substrate and food in the form of gravel, local autochthonous and allochthonous vegetation, which included willow leaves (*Salix sp.).*

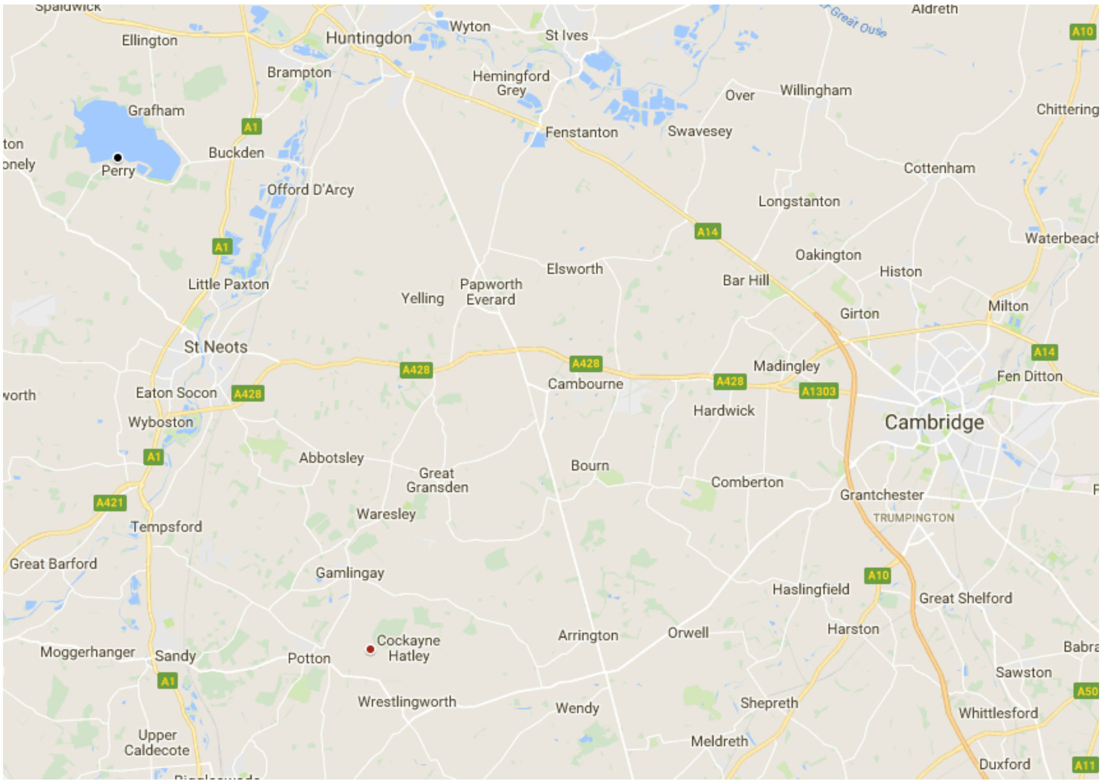

Figure 12.1. Map showing the location of the sampling sites used for the study. Black circle shows the site used to collect *Dikerogammarus villosus* at Grafham waters. Red circle shows the site *Gammarus pulex* were collected from at DuloeBrook.

The experimental arena consisted of a small opaque plastic pot (8cm diameter) lined with gravel to provide habitat structure and 150ml water comprised in equal parts from each amphipod source. Food was provided to excess in the form of dried leaves.

In the first experiment, the influence of parasitism on IGP of *G. pulex* singleton females by *D. villosus* males was investigated. For this a *G. pulex* female either uninfected or infected with the acanthocephalan parasite *E. truttae*, was placed into the arena and left to acclimatize for 30 minutes before a single *D. villosus* male was added (N=30, figure 6.2c). The female:male weight ratios used in this experiment were between 1.9 and 2.2 which is within the range of weight ratios observed in the natural populations of both species (Devin et al. 2004; Dunn et al. 2008). Arenas were checked twice daily for five day for signs of predation or moult.

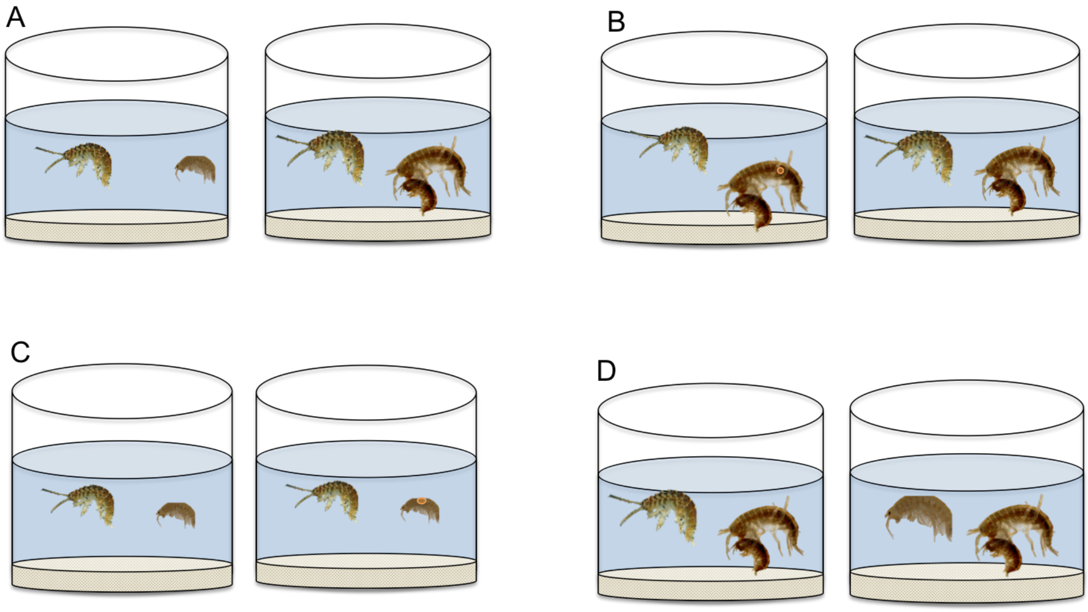

Figure 6.2. Schematics of the experimental set up used in this study to investigated IGP between *Gammarus pulex* and *Dikerogammarus villosus*. A) Investigation into if mateguarding decreases IGP between the two species. B) Does parasite infection reduce protection offered through mateguarding. C) Does parasite infection affect survival of female *G. pulex*. D) Comparison between IGP and Cannibalism in *G. pulex* mateguard pairs.

In a second set of experiments I investigated whether precopula mate-guarding provides the female *G. pulex* protection from IGP by a single male *D. villosus,* and if this was affected when the guarding male was parasitized by *E. truttae*. To test whether mate guarding reduced the vulnerability of female *G. pulex* to IGP, I placed either a single female or a precopula pair (which had formed naturally in the field) into the arena for 30 minutes before adding a single male *D.* villosus (30 replicates of each treatment, figure 6.2a).

To explore the effect of parasitism on the vulnerability to IGP, I also measured IGP by *D. villosus* of *G. pulex* females guarded by parasitized males (30 replicates) and compared the likelihood of predation of a female who was being guarded in pre-copula by either an uninfected or *E. truttae* infected male (figure 6.2b). The males of both species used in these experiments were matched for size before the experiment and weighed after the experiment finished. Arenas were checked twice daily for five day for signs of predation or moult.

The final experiment carried out compared the occurrence of IGPredation by *D. villosus* males of *G. pulex* in precopula, to that of cannibalism by *G. pulex* males of *G. pulex* in precoupla. For this a precopula pair of uninfected *G. pulex* was placed into the experimental arena for 30 minutes before adding either a conspecific or heterospecific single male (figure 6.2d). The males of both species used in these experiments were matched for size before the experiment and weighed after the experiment finished. Arenas were checked twice daily for five day for signs of predation or moult (N=30).

The data in the experiments were analysed by performing GLM analysis with a binominal error distribution with regards to female survival.

6.3. Results

D. villosus males predated singleton *G. pulex* females as well as females which were guarded in precopula and parasitism affected vulnerability of *G. pulex* to predation. Single and paired female *G. pulex* were predated by *D. villosus*. However, females that were guarded in precopula were less vulnerable to IGP than were singleton females (GLM, $X^2_{1,56}=13.645$, $p<0.001$; figure 6.3a). Precopula guarding reduced predation of the female *G. pulex* . However, this protection from IGP was reduced when the male carrying the female was infected with the parasite (GLM, $X^2_{1,51}=4.627$, $p=0.0315$; figure 6.3b).

IGpredation by *D. villosus* on *G. pulex* female singletons was high (60% of females predated) and there was no difference between the predation of uninfected or *E. truttae* infected females (GLM, $X^2_{1,56}=0.0031$, $p=0.955$; figure 6.3c). However, the greater the size difference between the female and male the more likely predation was to occur (GLM, $X^2_{1,55}=9.561$, $p=0.002$).

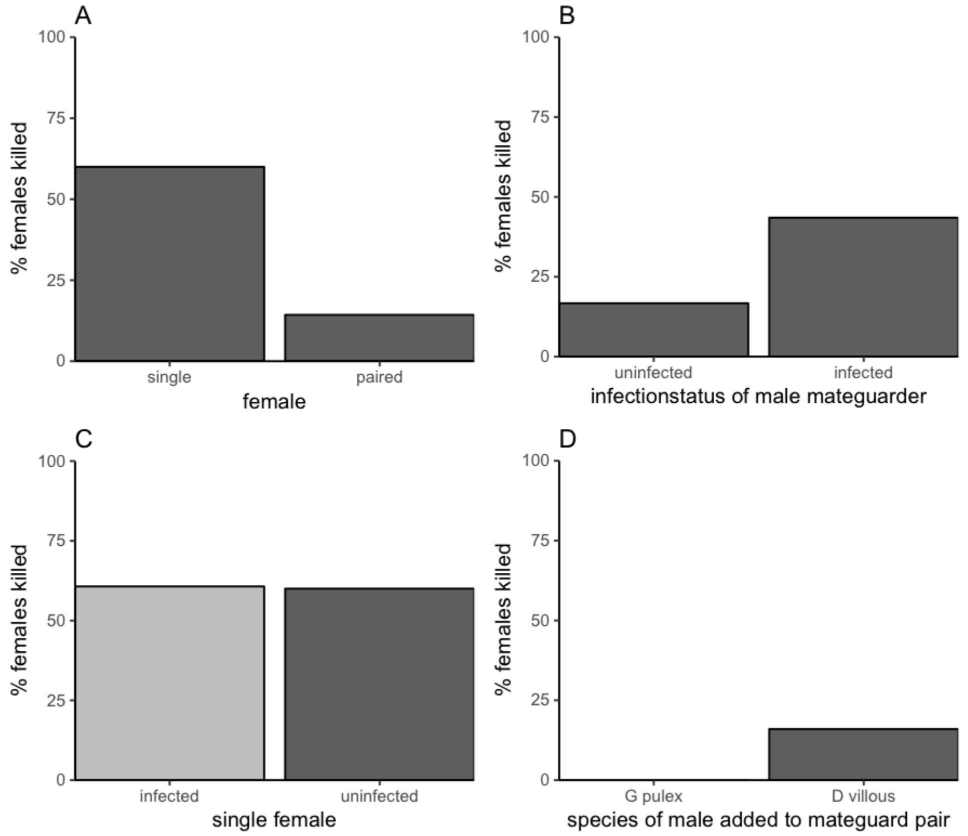

Figure 6.3. The percentage of females killed as a result of intraguild predation by *Dikerogammarus villosus.* A) IGP of singleton females and of females in precopula mate guarding B) IGP of *G. pulex* females that were guarded in precopula by uninfected or *Echinorhynchus truttae* infected males C) IGP of singleton females either uninfected or infected with *E. truttae* D) Predation of *G. pulex* females from precopula pairs by *G. pulex* versus *D. villosus* males. Dark grey bars represent uninfected females, light grey bars represent females infected with *E. truttae*.

IGP by male *D. villosus* on *G. pulex* pairs occurred in 16% of cases (figure 6.3a). In contrast, there were no incidences of cannibalism between *G. pulex* males and conspecific precoupla pairs. However, the difference was not observed to be statistically significant (GLM, $X^2_{1,58}$=3.2076, p=0.073; figure 6.3d).

6.4. Discussion

D. villosus showed strong IG predation of singleton *G. pulex* females and also predated females from precopula pairs. Predation of singleton females was high and was unaffected by *E. truttae* infection. This is in contrast with MacNeil *et al.*, (2003) who found that an infection of *Gammarus duebeni celticus* with the parasite *Pleistophora mulleri* doubles its vulnerability to predation by *G. pulex*. It is also in contrast with a study of snails infected with a trematode which found that infected individuals occurred a 40% reduction in predation pressure (Wood et al. 2007).

The similar rates of predation on infected and uninfected individuals could reflect the predatory strength of *D. villosus. P*reying on a single female *G. pulex* may be so easy for male *D. villosus* that it does not matter for its success if the female is infected or not. Furthermore, consumption of infected prey does not pose a risk of infection to the *D. villosus* predator (consumption by the definitive fish host is required to complete the *E. truttae* life cycle), hence there will be no selective pressure to avoid infected IG prey.

D. villosus also predated females from precopula pairs, although at a lower frequency than singletons. Similarly, mateguarding was found to offer *G. duebeni* protection from IGP by *D. villosus* in Holland (Dick and Platvoet 2000). The lower rate of predation of precopula pairs could occur because the male is able to fend off attacks on the female by the invader (Dick and Platvoet 2000) or it may be that the pair forms a large target which is less likely to be attacked. Nonetheless, mateguarading offered only limited protection and females from precopula pairs were predated in 16% of cases, suggesting that *D. villosus* is such a strong and able predator that it is still able to “steal” the female from the male and kill it.

Interestingly, if the male *G. pulex* carrying the female in mateguard is infected with the parasite its ability to protect the female from IGP by *D. villosus* is reduced. This suggests that the burden that the parasite places on the host may reduce its swimming ability and makes it less able to defend the female from the invader, which decreases her chance of survival. This in turn could place a selective pressure on females to avoid parasitized mates.

Parasite induced trait mediated effects on IGP have the potential to influence native-invader interactions, which could lead to facilitation of species coexistence or exclusion (Hatcher et al. 2006). For example, when native *G. duebeni celticus* are infected with the micosporidian parasite *Pleistophora mulleri* they have an increased vulnerability to IGP by the *G. pulex* (which is invasive in Ireland) leading to coexistence of the two species in field trails (MacNeil et al 2003b). In Ireland, *E. truttae* infection of *G. pulex* was also found to facilitate the coexistence between the invader and the native *G. duebeni celticus* because it led to a reduction in IGP of the former on the later (MacNeil et al 2003d). In these studies in which parasite infection facilitates coexistence, the stronger IG predator is the host to the parasite, which causes a reduction in its predatory ability and thus releases the prey from some predation pressure allowing for coexistence between the species.

However, in my study the strong asymmetrical IGP by *D. villosus* on *G. pulex* may be enhanced rather than mitigated by an infection with the parasite *E. truttae*. This could be due to the fact that the stronger IG predator *D. villosus* is not host to the parasite while the IG prey *G. pulex* is. It appears infection with the parasite makes G. pulex more vulnerable to IG predation by *D. villosus*. This additional increase in predation pressure on the native, could lead to replacement of the native by the invader being accelerated by the infection with *E. truttae*. This replacement of the native amphipod can have serious consequences for the structure of the local species community (Dick and Platvoet 2000) and could lead to a loss in biodiversity in the UK as has been observed in other Europenan aquatic ecosystems which previously have been invaded by *D. villosus* (Dick et al. 2002).

Chapter Seven

General Discussion

The aim of this thesis was to quantify intraspecific and interspecific interactions of native and invasive amphipod species to gain an insight into their implication for population and community dynamics of the species. I also looked at how these interactions are influenced by the environmental factors of parasitism, habitat structure, cues from higher predators and temperature. In this section I aim to summarize my findings and discuss implications for invasion process of species and how invasive species impact their receptive ecosystems.

7.1. Intraspecific interaction – Cannibalism

Cannibalism frequently occurs in animals (Fox 1975) especially in species in which generations overlap in time and space (Crump 1990; Rudolf 2008c). While it provides benefits to the predator (Polis 1980) it also carries risks (Pfennig et al. 1991). The level of cannibalism occurring within population will impact on the population dynamics especially if cannibalism focuses on the part of the population associated with recruitment such as juveniles and females.

Amphipod crustaceans are known to be cannibalistic. The functional response approach I used to quantify the cannibalism of the native *Gammarus duebeni celticus* and invasive *Gammarus pulex* in Northern Ireland revealed that both species are cannibalistic on their own juveniles. The level of cannibalism did not differ between the native and invasive species, with both functional response curves reaching an asymptote at similar levels (figure 4.3.). Although cannibalism was important for both species, the cannibalism FR was lower than the IGP FR for both and, when offered both prey, both species preferred to prey on heterospecifics. This makes sense for a number of reasons. For example, IGP carries the benefits of removing a potential competitor and does not carry he risks that cannibalism carries such as consuming your own kin and acquisition of parasites from the prey (Pfennig et al. 1998).

7.2. Intraguild predation

Both the native *G. d. celticus* and the invasive *G. pulex* are known to partake in intraguild predation (IGP) with each other. Previous work looking at interactions between individual adults, showed that *G. pulex* was the stronger IG predator of the two (Dick 1996). From this it was suggested that this could be the reason behind the exclusion of the native by the invader (Dick and Platvoet 1996). However, the IGP has not been previously quantified using the FR approach, and nor has the (more common) predation of adults on juveniles. When I used the comparative functional response approach to quantify the IGP between the two species, I found that they do not differ in the amount they consume of juveniles from the other species (figure 4.2.). This would suggest that the native and invader have an equal predator impact on each other's population. However, since this rate is per capita, the invader might still have a higher impact on the native if the invader is present in higher numbers than the native. It has indeed been observed that *G. pulex* occurs in densities which are up to 8 times than those of *G. duebeni celticus* in the field (Dick, et al., 2017a). Such a big difference in the abundances of the two species would mean that the invader has a much higher impact on the population of the native than in the reverse situation.

7.3. Information gained from functional response analysis

The quantitative approach is a useful tool to gain an insight into the interactions between native and invasive species. My work is novel in that I used the predatory FR approach to explore IGP and cannibalistic interactions in a quantitative manner. Functional response analysis does not only quantify the maximum consumption of a predator it also provides us with information as to which type of functional response the interaction falls into. The type of functional response occurring provides information as to how it may affect the dynamics of the prey population. A Type-II response is associated with unstable predator-prey dynamics in which the prey is at risk of becoming locally extinct, while a Type-III functional response is seen as stabilising which allows both predator and prey populations to persist (Williams and Martinez 2004). All functional response curves observed in this thesis, in both cannibalism and IGP experiments were Type-II functional responses. This means that when densities of the prey populations are low they are at danger of becoming extinct. This fits in with the situation that IGP between native and invasive species is often thought to be one of the driving factors of species replacement in species invasions (Dick et al. 1993; MacNeil et al. 2003b). It also fits in with other studies investigating the FR of Amphipods feeding of other prey. Most of these studies have observed Type II FRs to occur (e.g. Bovy et al. 2015; Paterson et al. 2015a; Taylor and Dunn 2017).

When fitting the functional response curves to the data we can also obtain information on the *a* and *h* parameters. They stand in for the attack/ search rate and the handling time of the predator. This might provide information how environmental factors affect the predator and how this influences the predator interaction. However, it is important to not overstate the impacts here as they are scaling parameters used to fit the FR curves to the data points and not experimentally measured rates.

While FR curves are very useful tools when comparing the predator prey interactions between species, they appear to be certain limitations to the technique. . Most FR studies, including those in my thesis, consider the impact of an individual predator on prey at different densities. However, in the field multiple predators will be present. Recent studies reveal that predation impact may not scale linearly with multiple predators, but that predators may interfere or may act synergistically (Médoc et al. 2013). Multiple predators could for example cause a higher FR . especially in species which aggregate in nature. It could also happen that an aggregation in predator causes a drop in the FR when individuals of a species inhibit each other predatory behaviour.My work has shown that they are influenced not only by the type of prey but also by its size and environmental conditions they are measured in. .The presence of habitat and of higher order predators likely to affect not only predatory behaviour but also prey behaviour. Hence it is important to keep set ups alike when wanting to draw up comparisons between species. .

7.4. Impact of parasitism

Parasites may alter the behaviour of their hosts (Hatcher, Dick, and Dunn 2006), influencing a number of intra and interspecific interactions such as predation through density and trait mediated effects (Hatcher et al. 2006, 2014; Hatcher and Dunn 2011). It has been shown that infection with a parasite can modify the rate of predatory interactions (MacNeil et al. 2003b; Dick et al. 2010). As both IGP and cannibalism are special cases of predatory interactions I proposed that parasitism would have an influence on these interactions too. I found that parasitism affected both cannibalistic and IGP Frs and that the effect of parasitism varied between species and was affected by environmental context

In chapter two I quantified the impact that infection with the microsporidian parasite *Pleistophora mulleri* has on the juvenile cannibalism functional response of *G. d. celticus*. I found that infected individuals had a functional response, which was double that of uninfected counterparts (figure 2.3.). . It may be difficult to predict the impact of parasites on consumption rate generatlly.. this is the case because the impact parasites have does not only vary between parasites but also between hosts and the type of prey which is consumed. For example, while being infected with *E. truttae* decreases the FR of *G .pulex* feeding on A. aquaticus (Dick et al. 2010) it increases the IGP FR of *G. pulex* feeding on *G. dueben*i celticus juveniles (Chapter 4 this thesis).

This means that infection has a big potential to impact on the population dynamics of the native. In chapter four I looked into how an infection with the acanthocephalan parasite *Echinorhyncus truttae* affects the cannibalism functional response of the invasive *G. pulex*. Although there appeared to be a tendency for infected individual to have a higher functional response this was not found to be significant (figure 4.4.), Most interesting result was the change in comparative FR in presence of both habitat and higher order predators. This is probably caused by a change in the behaviour of infected individuals. Uninfected individuals will reduce their activity in the presence of a predator as a form of predator avoidance. This predator avoidance behaviour has been observed to be reduced in infected individuals (MacNeil et al. 2003a). It is likely that this difference in behaviour in the presences of a higher predator is behind the difference in the FR.

7.4.1. Facilitation of coexistence between native and invasive species

In chapter four, I investigated the impact that parasite infection has on IGP. . IP between coexisting poulations is widespread in nature, yet theoretical models predict that 2 species that show IGP unlikely to coexist (Hatcher et al. 2006). Hatcher et al (2006) predicted theoretically that parasites, through their effects on host survival and host behaviour, should facilitate coexistence under IGP (Hatcher et al. 2006). My studies support this predication„The infection with the parasite *P. mulleri* caused an increase in the IGP functional response of *G. d. celticus* feeding on *G. pulex* juveniles (figure 4.2.) The maximum consumption of infected individuals is around one quarter higher than that of uninfected individuals (figure 4.2.). In *G. pulex* on the other hand there appears to be a tendency that individuals infected with the acanthocephalan parasite causes a drop in the IGP functional response feeding on G. *d. celticus* juveniles (figure 4.2.). The differential impact that parasitism has on the functional response of the native and invasive is likely to have an impact on the population and thus community dynamics between the two species. In this case it appears to facilitate coexistence between the two species. It does appear to achieve this by making the native a stronger IG predator while it makes the invader a worse IG predator. This decreases predation pressure on the native in two ways. Firstly by reducing the population of the invader and second by reducing the predatory impact the remaining invader population has on the native.

However, the two parasites I studies in my experiments are not the only two parasites, which are know to use *G. pulex* and *G. duebeni celticus* as their hosts. It is possible that individuals used in the experiments were also infected with other parasites which could not be visually detected by visually inspection under a light microscope. It could be that undetected parasite infections influence the FR curves of the species investigated. Some parasite taxa such as microsporodiai are particularly common in amphipods. One way to exclude this uncertainty in future experiments would be to utlise pcr with general microsporidian primers, in order to screen for non target parasitic infections. the end of any expts.

7.4.2. Facilitation of species replacement of native by invader

Whilst parasitism might enhance coexistence as a result of its impact on IGP as descried above, I also found evidence that parasitism might enhance exclusion of the native by the invader.. In chapter six I investigated the impact that parasite infection with the parasite *E. truttae* in *G. pulex* (the native species, in England) has on the IGP interaction with the invasive *D. villosus*. While the infection status did not affect vulnerability to IGP in single *G. pulex* females it did affect the vulnerability of those carried in mateguard. Females carried by an infected male were three times as likely to preyed on by *D. villosus* (figure 6.3.).

In chapter five I found that the IGP occurring between the native adult *G. pulex* and adults of the invasive *D. villosus* in England was unidirectional, with only *D. villosus* feeding on *G. pulex* (figure 5.3.). In chapter six I observed that being carried in mateguard provides some protection for female *G. pulex*. However, if parasitism negates this protection and increases vulnerability it will have a negative impact on population dynamics of *G. pulex* in two ways. The first way being that it directly cause the population to decline by removing female individuals. But this removal of females could also cause a further decline in population numbers because it removes potential future recruitment from the population. This means that in this case of native invader interaction, the infection with the parasite could facilitate the replacement of the native by the invader and thus may aid the invasion process.

7.5. Impact of environmental factors

Predator prey interactions can be affected by other environmental factors including abiotic and biotic factors. In chapter three of this thesis I looked into how habitat structure and cues from a higher predator affect the cannibalistic predation rates of *G. pulex* infected with the parasite *E. truttae*. As observed in chapter four, in the absence of the additional environmental factors, the parasite was not found to affect the cannibalism functional response. However, in the presence of addition factors in the form of a combination of habitat structure and cues from a higher predator, it was found that parasitism causes the cannibalism functional response to double (figure 3.3.). Hence parasitism, in this system, is likely to increase the rate of cannibalism. These changes likely reflect changes parasite-manipulation of the host behaviour in order to facilitate the transmission to its final host (Hatcher et al. 2006). The fact that the impact of parasitism on this intraspecific interaction only became apparent upon inclusion of the additional factors in the experimental set up makes a case for making experiments as realistic as possible in order to be able to translate their outcomes to the real world.

Were possible it might be a good idea to run experiments in the field, as this would make their results the most translatable. However, this is not always possible or advisable. For example experiments investigating the impact of temperature increases such as caused by climate change, can not be carried out in the field as would be nearly impossible to simulate this artificially. Also with experiments which investigate the impacts of possible invasive species it might not be advisable to carry out experiments in the field, as this would pose the risk of an accidental introduction of the species should individuals manage to escape from the experimental set up.

7.6. Impact of temperature

The other environmental factor that I explored was temperature. Temperature affects how animals behave (Bale 2002; Woodward et al. 2010) and thus it also affects the way species interact with each other such as in predatory interactions (Laverty et al. 2017). Due to the difference in their evolutionary histories caused by the differences in geographical origin it is likely that native and invasive species will be differently affected by changes in temperature (Bates et al. 2013).

In chapter five of this thesis I investigated how an increase in temperature affects the IGP functional response of *G. pulex* feeding on *D. villosus* juveniles and the reverse set up. I found that overall *D. villosus* has a much higher functional response feeding on juveniles of the native four to five times higher than that of the native feeding on juveniles of the invader (figure 5.4.). This means that the invader has a great negative impact on the population dynamics of the native by exerting such a high predation pressure on them. I further found that while an increase in temperature caused the functional response of the invader to increase further it did not affect the functional response of the native species in the same way. Indeed I found a tendency that an increase in temperature would cause the functional response of the native feeding on the invasive to drop.

A similar finding was observed by Kenna et al. (2017) who lloked into how shredding in *G. pulex* and *D. villosus* was affected by an increase in water temperatures. This difference in the reaction to the increase in temperature observed in my study, and in Kenna et al (2017) could be related to the evolutionary histories of the species. The invasives native range has a greater range of temperatures occurring (Gallardo et al 2012). This could mean that it is better at coping with these higher temperatures. The native on the other hand might experience stress caused by this increase in temperature, causing it to feed less. This broadens the gap in predatory ability between the species. The increase in temperature also caused the increase in the IGP of *D. villosus* on *G. pulex* females while it did not affect the IGP of *G. pulex* on *D. villosus* females.

These experiments suggest that an increase in temperature will increase the impact that the invasive has on the native's population through predatory interactions and by reducing future recruitment. This means that increases in temperatures can facilitate species invasions by increasing the impact invasive species have on native populations, possibly causing a speeding up of the exclusion of the native species.

7.8. Concluding remarks

My data support the use of comparative functional response as a tool to quantify intra and interspecific interactions between native and invasive species and how these interactions are impacted on by parasitism and other environmental factors. A solid understanding of the invasion process is key when trying to make predictions on future invasions and can be used to parameterized models to investigate how future environmental changes might affect interactions between natives and already present invasive species.

Biological invasions are one of the largest threats to biological diversity especially to that of freshwater systems (Dudgeon et al. 2006; WWF 2014). Through their influence on population dynamics, parasites may play a key role in influencing the outcome of species invasions (Hatcher et al. 2008; Dunn et al. 2012). I show that key trophic interactions of cannibalism and IGP between native and invasive species are affected by parasitism, as well as by abiotic environmental factors of temperature and habitat. However, native and invasive species are affected to different extends. Due to this difference the outcome of invasions may be affected. This means that factors might aid or hinder the invasion process. Hence, it is important to consider parasitism as well as environmental factors that may interact when trying to predict the impact of future invasions.

My findings on the IGP between *G. pulex* and *G. duebeni celticus* could help to further understand this exclusion of the native by the invasive in some areas while they coexist in other areas. It would be interesting to investigate if the IGP FRs between those areas differ and what might be the cause behind these differences.

I would also be interesting to compare the FRs of *G. pulex* feeding on a range of prey items between their native and invasive ranges to see if maybe a change in predator aptitude has occurred in the years since it has been introduced in Northern Ireland.

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```
rm(list=ls())
library(bbmle)
library(frair)


# read the data in and look at the structure
data<-read.csv("4a lr.csv")
attach(data)
str(data)


#this checks for the type of functional response present in the data
frair_test(formula=eatenu~density, data=data)
frair_test(formula=eateni~density, data=data)


#this outputs the a and h parameters for the predators
g_fit <- frair_fit(formula = eatenu~density, data = data,
response = "rogersII",
start = list(a=1, h=0.1),
fixed = list(T=40/24))
with(data, plot(density, eatenu, xlab= "Prey D ensity",
ylab="No. Prey Eaten"))
lines(g_fit, lty = 1 , col = "grey25")
print(g_fit)
summary(g_fit$fit)


g_fit <- frair_fit(formula = eateni~density, data = data,
response = "rogersII",
start = list(a=1, h=0.1),
fixed = list(T=40/24))
with(data, plot(density, eateni, xlab= "Prey D ensity",
ylab="No. Prey Eaten"))
lines(g_fit, lty = 1 , col = "grey25")
print(g_fit)
```

```
summary(g_fit$fit)



# Compares a and h between the two groups of the dataset
st <- list(a = 1, h = 0.1)
fx <- list(T = 40/24)
a_fit <- frair_fit(eatenu~density, data =data,
response = 'rogersII',
start = st, fixed = fx)
b_fit <- frair_fit(eateni~density, data=data,
response='rogersII',
start = st, fixed = fx)

#compares parameters between uninfected and infected predators
frair_compare(a_fit, b_fit) r script
```

Bootstrapping

```
library(bbmle)
library(frair)
library(gplots)

#read the data into R and look at its structure
data<-read.csv("4combi.csv")
attach(data)
str(data)


#this gets me a and h parameters, need this step to be able to bootstrap the data
a_fit <- frair_fit(formula = eaten1~density, data = data,
response = "rogersII",
start = list(a=1, h=0.1),
fixed = list(T=40/24))
print(a_fit)
```

```
summary(a_fit$fit)


#enable ploting of the bootstrap polygons onto the graph
afitb<-frair_boot(a_fit)
confint(afitb)

# this calculates the mean and se and plots them on a plot
mean.u<-aggregate(eaten1,list(density),mean)
mean.i<-aggregate(eaten2,list(density),mean)
colnames(mean.u)<-c("density","mean.eatenu")
colnames(mean.i)<-c("density","mean.eateni")
se=function(x)sd(x)/sqrt(length(x))
se.u<-aggregate(eaten1,list(density),se)
se.i<-aggregate(eaten2,list(density),se)
plot.unpa<-cbind(mean.u[,],se.u[,2])
colnames(plot.unpa)[3]<-"se"
plot.unpa
plot.para<-cbind(mean.i[,],se.i[,2])
colnames(plot.para)[3]<-"se"
plot.para

#draw the graph of the bootstrap polygons,
plot(afitb, type='n', xlab=", ylab=", main='A',line=-0.5,adj=0.1, axes=F, xaxs="i",yaxs="i",
 xlim=c(0,50), ylim=c(0,30))
drawpoly(afitb, col=rgb(0.75,0.75,0.75,0.5),border="NA")
lines(a_fit, lty = 2 , col = "black")
drawpoly(bfitb, col=rgb(0.24,0.24,0.24,0.5),border="NA")
lines(b_fit, lty=4, col="black")
axis(1)
axis(2)

points(mean.eatenu~density,plot.unpa,
 pch=15,bg="black")
points(mean.eateni~density,plot.para,
 pch=12,bg="white")
```

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Eaten alive: cannibalism is enhanced by parasites

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1. Summary

Cannibalism is ubiquitous in nature and especially pervasive in consumers with stage-specific resource utilization in resource-limited environments. Cannibalism is thus influential in the structure and functioning of biological communities. Parasites are also pervasive in nature and, we hypothesize, might affect cannibalism since infection can alter host foraging behaviour. We investigated the effects of a common parasite, the microsporidian *Pleistophora mulleri*, on the cannibalism rate of its host, the freshwater amphipod *Gammarus duebeni celticus*. Parasitic infection increased the rate of cannibalism by adults towards uninfected juvenile conspecifics, as measured by adult functional responses, that is, the rate of resource uptake as a function of resource density. This may reflect the increased metabolic requirements of the host as driven by the parasite. Furthermore, when presented with a choice, uninfected adults preferred to cannibalize uninfected rather than infected juvenile conspecifics, probably reflecting selection pressure to avoid the risk of parasite acquisition. By contrast, infected adults were indiscriminate with respect to infection status of their victims, probably owing to metabolic costs of infection and the lack of risk as the cannibals were already infected. Thus parasitism, by enhancing cannibalism rates, may have previously unrecognized effects on stage structure and population dynamics for cannibalistic species and may also act as a selective pressure leading to changes in resource use.

2. Introduction

Cannibalism has been recorded in more than 3000 species [1–4] and may be influential at the levels of individuals, populations and communities. It is especially common in stage-structured populations where generations overlap in time and space [2,5]. Direct individual benefits of cannibalism include increased growth and survival [3], while indirect positive effects include

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the elimination of competitors [6]. Cannibalism may also enhance population persistence when resources are limited; for example, cannibalism may function as a 'lifeboat mechanism' whereby cannibalistic adults have access to resources and energy accrued by the cannibalized juveniles [7]. There are, however, a number of costs associated with cannibalism, including the acquisition of parasites via consumption of infected conspecifics [8].

Parasitism is also pervasive in nature [9] and influences a number of intra- and interspecific interactions, including competition and predation, through both density- and trait-mediated effects [9–11]. In particular, parasites can modify the rate of predatory interactions [12,13] as well as alter the vulnerability of infected hosts to predation [9,14]. Parasitism, we propose, may therefore also be an important determinant in cannibalistic interactions with implications for population structure and community dynamics. This may be evidenced through changes in host behaviour as a result of metabolic costs [12], parasite manipulation to increase transmission likelihood [15–17], or can reflect selection on hosts to avoid costs of infection [17].

The microsporidian parasite *Pleistophora mulleri* is specific to the amphipod *Gammarus duebeni celticus*. It has a prevalence of up to 90% and can alter predation hierarchies among species [13] with both parasitized and unparasitized individuals occurring in close proximity to one another [18]. There is a large body of evidence that indicates *G. d. celticus* commonly engages in cannibalism in the field [19]. In addition, the only known route for the transmission of the microsporidian is cannibalism, providing further evidence of field cannibalism [20]. Therefore, as the parasite is transmitted orally, with an efficiency rate of 23% [20] and, as cannibalism in this species is common, it imparts a risk of infection of *P. mulleri* [20]. As such, parasite mediation of cannibalism may occur with important implications for host populations. We therefore investigated whether the cannibalistic rate and preferences of *G. duebeni celticus* are affected by infection with *P. mulleri*.

We used a 'functional response' approach (FR; resource uptake as a function of resource density), which can inform on consumer impacts on resource populations [21]. First, we investigated the impact of parasitism on cannibalistic propensity by deriving FRs for individuals with and without the parasite. Second, we used an intraspecific prey choice experiment to test whether infected and uninfected *G. d. celticus* showed any preferences with respect to the infection status of juvenile conspecific victims.

3. Material and methods

Adult male and juvenile *G. d. celticus* were collected from Downhill River, County Antrim, Northern Ireland (55.166674 N, 6.8201185 W) in November 2010 and April 2011. No permissions are required for this sampling activity. Males were selected for experiments owing to the wide variation in female cannibalism that can occur due to factors relating to egg and embryo brooding [22]. Parasite status was determined by the presence/absence of *P. mulleri* spore mass visible through the exoskeleton (status confirmed by later dissection) and parasitized individuals all had visible infection of one to two segments [23]. Animals were separated according to infection status and maintained in aquaria with water and leaf material from their source at 12° C and a 12 L : 12 D cycle.

For FR experiments, we selected similar-sized infected and uninfected adult male *G. d. celticus* (body mass (mg) ± s.e., infected 52.57 ± 1.49, uninfected 50.90 ± 1.23; two-sample t-test, $t = 0.86$, $p > 0.05$). We presented single infected and uninfected males (starved for 48 h) with uninfected juveniles (4–6 mm body length) at seven juvenile densities (2, 4, 6, 8, 10, 15, 20; $n = 3$ per density) in plastic dishes (8 cm diameter) containing 200 ml of aerated water from the amphipod source river. The densities of juvenile prey used were informed by previous FR studies on gammarids in combination with known densities from the wild that are hypervariable and can reach several thousand per square metre [24]. Controls were three replicates of each juvenile density without adults. Replicates were initiated at 17.00 h and prey consumption was examined after 40 h.

Mean number of juveniles killed was examined with respect to adult infection status and juvenile density using generalized linear models (GLMs) with quasi-poison error distribution in R v. 3.0.1 that were simplified via a step-deletion process. We determined FR types using logistic regression of the proportion of prey consumed against initial prey density [25] and modelled FRs using the Rogers' random predator equation for a Type II FR, which accounts for non-replacement of prey as they are consumed [26]. FR data were bootstrapped ($n = 15$) and the parameters attack rate a, handling time h and maximum feeding rate $1/hT$ ($T =$ experimental time) compared using GLMs.

Preferences of infected and uninfected adults for cannibalism of infected versus uninfected juveniles were investigated by presenting adult males ($n = 30$ uninfected and 30 infected individuals; sizes as

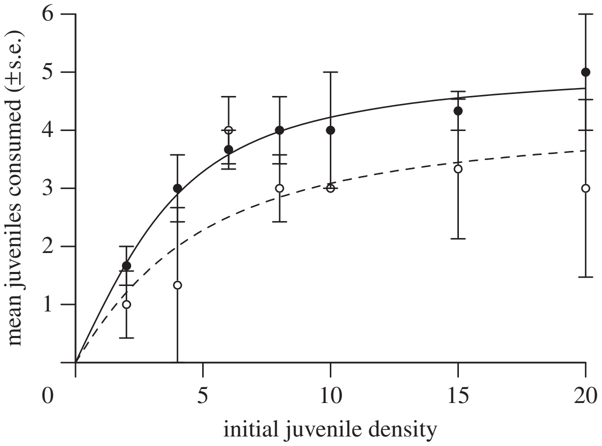

Figure 1. FRs of infected (filled circles, solid line) and uninfected (open circles, dashed line) *Gammarus duebeni celticus* adults towards juvenile conspecific prey. Lines are modelled by the Rogers' random predator equation for a Type II response. Data points are mean numbers of juveniles consumed at each density ± s.e.

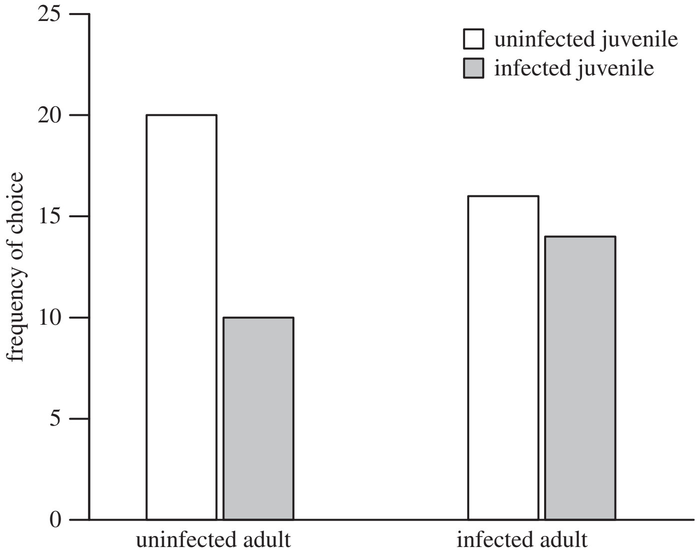

Figure 2. The frequency of consumption of uninfected versus infected juveniles by uninfected and infected adult *Gammarus duebeni celticus*.

above; starved for 72 h) with a choice between an infected and uninfected juvenile individual (6 mm body length; matched by weight) in plastic dishes (10 cm diameter, 150 ml volume). Trials began from the addition of the prey and were terminated when a prey item had been selected. Prey choice by the adults with respect to juvenile infection status was determined using χ^2-tests.

4. Results

Control juvenile *G. d. celticus* survival was high (99.5%), thus experimental deaths were attributed to cannibalism by adults. This was further evidenced through observation and amphipod body parts littering the aquarium floor. Significantly more juveniles were eaten by infected than uninfected adults ($F_{1,40} = 5.03$, $p < 0.05$; figure 1) and both FRs were found to follow a Type II curve (figure 1). Infected adults had significantly greater attack rates a ($t = 5.87$, $p < 0.001$) and significantly lower handling times h ($t = 3.67$, $p < 0.01$). This translated into significantly higher maximum feeding rates ($1/hT$) ($t = 2.71$, $p < 0.05$) in comparison to uninfected individuals (figure 1). Uninfected adults more frequently consumed uninfected than infected juveniles ($\chi^2_1 = 4.8$, $p < 0.0285$; figure 2), whereas infected adults showed no preference ($\chi^2_1 = 1.333$, $p > 0.05$; figure 2).

5. Discussion

Although the role of parasitism in interspecific predator–prey interactions has been studied in a number of systems [12,13], the influence of parasites in mediating cannibalism has received far less attention, despite cannibalism and parasitism both being widespread and pervasive in natural communities [3,9]. Parasites may affect cannibalism since they have been shown to affect foraging behaviour, both increasing and decreasing host consumption of resources, with potential implications for population dynamics and community structure in such taxa [12].

Here, the FR of the amphipod *G. d. celticus* infected with the microsporidian parasite *P. mulleri* towards juvenile (uninfected) conspecific prey was significantly higher in comparison to uninfected adults. Furthermore, infected amphipods had significantly greater attack rates, decreased handling times and hence heightened maximum feeding rates, demonstrating that infected amphipods are more efficient than their uninfected counterparts at cannibalizing juveniles. This probably reflects the metabolic burden imposed by the parasite, leading to higher feeding rates [12]. That infected individuals are such efficient foragers is despite the fact that this parasite degrades host tissue and substantially debilitates its host [27].

The preferential consumption of uninfected juveniles by uninfected adults probably reflects selection for avoiding cannibalizing infected juveniles and therefore reducing the risk of parasite acquisition [8,28]. On the other hand, infected adults showed no such discrimination. One explanation for this lack of discrimination may be that immune priming or immune upregulation protects infected individuals from further infection [29]. However, Terry *et al.* [27] found no evidence of encapsulation or other immune responses in *P. mulleri* infected hosts. Rather, we suggest that the lack of discrimination in cannibalism of infected versus uninfected juveniles by infected adults again reflects the metabolic burden of infection whereby parasitized individuals cannot afford to be as selective in what prey they consume. Furthermore, as they are already infected with the parasite, there is no advantage to avoiding infection risk by preferentially consuming uninfected prey.

Overall, we show that infection of *G. d. celticus* with the parasite *P. mulleri* altered cannibalism rates and feeding preferences on juvenile conspecific victims. This in turn may increase the rate of juvenile mortality (over and above conventional virulence effects), which could lead to changes in population stage structure and density [5,11,30]. Furthermore, this interplay between cannibalism and parasitism could have powerful impacts on population and community resilience in changing environments, whereby cannibalism becomes an important mechanism in preserving populations [7], although in the wild, population outcomes will also depend on the relative importance of interspecific predation and cannibalism. Cannibalism and intraguild predation co-occur frequently in a broad range of systems [1,5] and the balance of these intra- versus interspecific interactions is key to species coexistence and replacement patterns [31]. Parasites are also recognized as having important indirect and pervasive effects on communities and ecosystems, often owing to their density and trait-mediated indirect effects on species that interact with their hosts [32]. Further exploration of parasite-modified cannibalism thus has potential to further understand and predict population dynamics and community processes.

Data accessibility. The datasets supporting this article have been uploaded as part of the electronic supplementary material.

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Author contributions. All authors gave final approval for publication.

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