The impacts of multiple stressors on a vital ecosystem process in temperate freshwater ecosystems

Benjamin Pile

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Abstract

Anthropogenic change is exerting increasing pressures, impacting the structure and function of ecosystems. Anthropogenic stressors of ecosystems, along with natural stressors, such as parasitism, may interact as stressors rarely occur in isolation. Therefore, it is important that the impacts of combinations of multiple stressors are studied, however these interactions are currently under-represented in ecological research.

Climate warming and introductions of invasive species are main drivers of ecological disturbance, altering the function of ecosystems globally. Parasites are ubiquitous biotic stressors in ecosystems, that can have impacts on host survival and behaviour. I investigated combined impacts of these stressors on freshwater ecosystems. The nutrient cycles in temperate freshwater ecosystems are skewed towards allochthonous leaf litter as the basal resource, requiring a guild of macroinvertebrate shredders to disperse nutrients through trophic webs. I investigated the productivity of keystone amphipod shredders, both native and invasive, through rates of shredding and predation, in interaction with temperature and parasites.

In chapter 2, I use a laboratory study to investigate the impacts of temperature and parasitism on survival and shredding rates, comparing the native amphipod and keystone shredder *Gammarus duebeni celticus* and an invasive amphipod *Gammarus pulex* from Northern Irish freshwater ecosystems. The most important factor affecting rates of shredding was

temperature, with shredding doubling from the lowest rate, at 5°C for each amphipod and parasite combination, to their highest rate at 20, 25 (for parasitised and unparasitised *G. pulex* respectively) or 30°C (*G. D. celticus*). However, survival was negatively affected by temperature; the lower survival of *G. pulex* in increasing temperatures indicates the warming climate may be less favourable for the invasive species. However, the consequences of extreme warming are likely to be severe as both species suffered 100% mortality at 30°C. Therefore, whilst increases in temperature will increase *per capita* shredding (up to an optimum, before decreasing with extreme temperatures), amphipod abundances are likely to decline, leading to reduced productivity at an ecosystem level.

The impact of the invasive species and parasitism on resource processing in freshwater ecosystems depends on both leaf shredding capability and on relative abundance, with invasive species often achieving much higher abundance than native species. In chapter 3, I focused on another UK study system, where *G. pulex* is the native in Great Britain, and the invasive amphipod is the Ponto-Caspian species *Dikerogammarus villosus*. I used the Relative Impact Potential (RIP) metric to incorporate relative consumer abundance response as a means of scaling relative *per capita* effects to compare the relative impact potential of the biological invasion. Although *per capita* shredding was higher for *G. pulex* than *D. villosus* at all temperatures, the higher abundances of the invader led to higher impact scores for *D. villosus* at all temperatures; hence replacement of the native by the invasive amphipod is predicted to drive an increase in shredding. This could be interpreted as a positive effect on ecosystem function, leading to faster accumulation of amphipod biomass and a greater rate of fine particulate organic matter (FPOM) provisioning for the ecosystem. However, the high density of invaders compared to natives may lead to exhaustion of the resource in sites with relatively low leaf detritus levels.

In addition to invasive macroinvertebrates, riparian zones are commonly subjected to human-mediated introductions of invasive non-native plants. In chapter 4, I investigated the interactions of the stressors of invasive allochthonous leaf detritus (*Rhododendron ponticum* and *Fallopia japonica* compared to native *Alnus glutinosa*), invasive shredders (*D. villosus* compared to native *G. pulex*) and temperature. Despite lower nutritional value, and possible toxicity, it was found that amphipods will feed on the invasive leaf species selected, which indicates ecosystem function can be maintained despite riparian invasion by these non-native plants. However, both invasive plant species were shredded at a lower rate than native *A. glutinosa.* Temperature was positively correlated with shredding, with rates of shredding for native *A. glutinosa* and invasive *F. japonica* leaf litter doubling between the lowest (5°C) and highest (25°C) temperatures. Hence a change in the dominant riparian leaf litter source as a result of invasion could alter the productivity of the ecosystem.

Many amphipods are omnivorous. In addition to shredding leaf material, both the native *G. pulex* and invasive *D. villosus* predate other macroinvertebrates. Optimal foraging theory states that animals will seek to maximise the benefit gained from foraging, which may include only eating the most nutritious parts of prey if there is plentiful availability. In chapter 4, I compared the predatory behaviour of the native *G. pulex* and invasive species *D. villosus,* the effect of temperature on predation and on the discarding of uneaten prey. The native and invasive amphipods had similar rates of predation on *Asellus aquaticus* prey but *D. villosus* had a higher rate of predation on chironomid larvae than *G. pulex,* resulting in more successful attacks and more discarded dead chironomid tissues for the invasive amphipod. This preference, along with the much greater densities of the invasive amphipod, is likely to impact the chironomid population. The interaction of temperature and amphipod predator species in discard rates for both *A. aquaticus* and chironomid prey indicate that *G. pulex* may contribute well to animal detritus at low temperatures but *D. villosus* will be better able to maintain the supply of animal detritus at higher temperatures.

Temperature had a significant impact on amphipod survival and trophic behaviour, increasing rates of shredding and predation up to an optimum temperature, beyond which rates fell. The most elevated temperatures used caused mortality in all amphipods. Although consumed at a lower rate, invasive riparian plants may be able to maintain freshwater ecosystems, with the leaf detritus lasting longer than rapidly consumed native species. The gammarid amphipods investigated in this thesis appear to be closely related in behaviour. However, small differences in behaviour are greatly amplified as a result of the higher densities of the invasive species.

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AIC	Akaike's information criterion
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
BMR	Basal metabolic rate
CPOM	Coarse particulate organic matter > 1mm
DOC	Dissolved organic carbon < 0.45 µm
FPOM	Fine particulate organic matter < 1mm
FR	Functional response
GAM	General additive model
GAMLSS	Generalised additive model for location, scale and shape
REML	Restricted maximum likelihood model
RIP	Relative impact potential
SD	Standard deviation
SE	Standard error

Chapter 1: General introduction and background

1.1 Introduction

Ecosystems worldwide are subjected to increasing pressures from multiple stressors which can impact their structure and function, altering productivity and affecting connected ecosystems (Sala *et al.*, 2000; Pereira *et al.*, 2010; Orr *et al.*, 2020). In this thesis I investigate interactions of stressors and quantify impacts to productivity to enable better predictions for future ecosystem function. This introduction describes some of the most common stressors that act upon ecosystems which may affect their function and productivity. That these stressors co-occur in natural systems and may interact is underrepresented in research, which this thesis seeks to address. I outline the stressors of temperature due to climate warming, invasive species, parasites and the possible interactions of those stressors to affect ecosystem function. Background is provided for the focal temperate freshwater ecosystems examined, and the crustacean amphipods which hold a keystone role in nutrient cycling in those ecosystems.

1.2 Stressors

Ecosystems are exposed to stressors that can alter their structure and function, with anthropogenic drivers increasing pressures globally. Human activity is altering natural environments and has already caused significant changes to global biodiversity and climate (Vitousek *et al.*, 1997). Anthropogenic change is causing extinctions at a rate that exceeds normal background rates of species loss, which can limit the productivity of ecosystems due to the loss of biodiversity limiting the exploitation of resources in natural systems (Vitousek *et al.*, 1997; Hooper *et al.*, 2012). Biodiversity supports the function, productivity and stability of ecosystems, which can be compromised by human activity (Cardinale, Palmer and Collins, 2002; Cardinale *et al.*, 2012). Multiple stressors act on ecosystems concurrently, altering processes within those systems due to drivers both natural and anthropogenic (Orr *et al.*, 2020). The impacts of such stressors are expected to increase with predicted increases in the pressure from anthropogenic drivers of global change, such as climate change, land-use change and introductions of invasive species (Chapin *et al.*, 2000; Pereira *et al.*, 2010; Pachauri *et al.*, 2014).

Multiple stressors can interact in unpredictable ways, with insufficient research into how these interactions may affect ecosystems (O'Brien *et al.*, 2019). Assuming a pair of stressors both have a negative impact on a system or process (figure 1.1), if the impact of the pair of stressors in combination is the sum of the negative effect of each individual stressor the relationship of the stressors can be described as additive. If the impact of the pair of stressors is less than the sum of the individual stressors it can be termed as an antagonistic relationship.

When the impact of the pair of stressors is greater than the sum of the individual stressors their relationship can be described as synergistic (Crain, Kroeker and Halpern, 2008). Additionally, changing a factor such as temperature, may have a positive or a negative impact depending on how this relates to species' thermal optima. This thesis investigates how some common stressors, natural and anthropogenic, biotic and abiotic, interact using freshwater systems as models. I aim to provide predictions on whether projected increases in stressors are likely to mitigate or amplify impacts when combined.

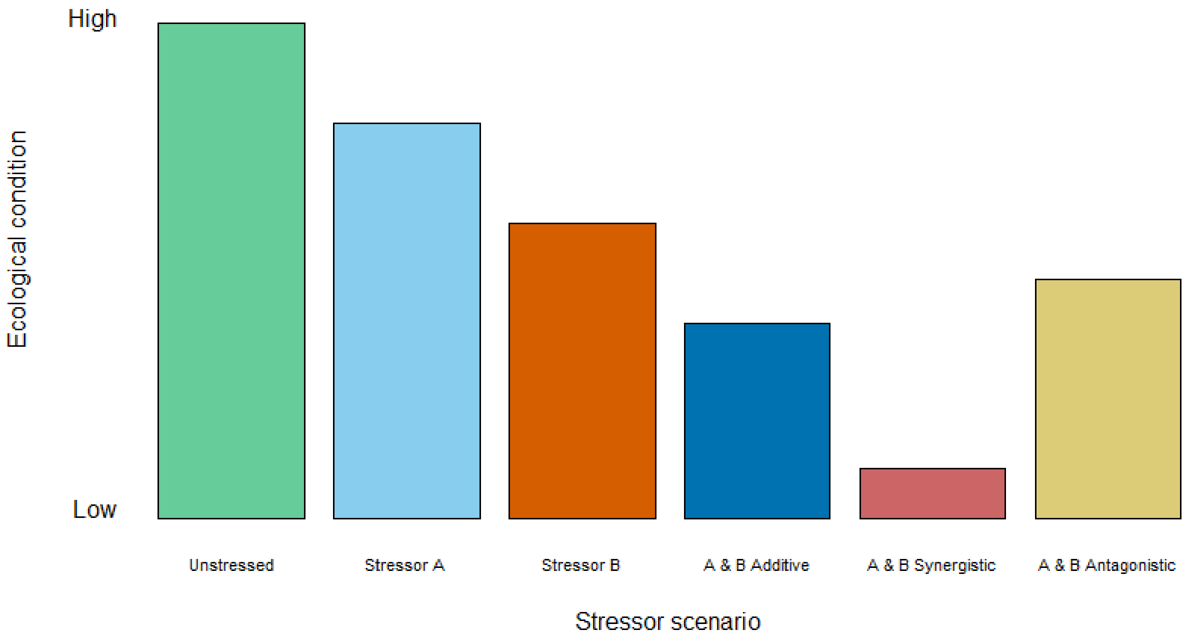

Figure 1.1: Illustration of the potential impacts on ecological condition of different interaction types in an ecosystem with two stressors, adapted from: (Spears *et al.*, 2021)

1.2.1 Climate change

Climate change is an anthropogenic, abiotic factor that is having a significant impact on the natural environment globally. Climate change, caused by increases in emissions of greenhouse gasses since the Industrial Revolution, has increased mean temperatures and increased the frequency and intensity of extreme weather events, such as flooding and heatwaves (Jenkins *et al.*, 2009; Pachauri *et al.*, 2014). Alterations to climate influence the physiology of species. When a species' physiological limits are exceeded this can result in local extirpations, with potentially cascading impacts to ecosystem function (Chapin *et al.*, 2000; Cardinale *et al.*, 2012). Sub-lethal effects of climate warming can also affect animals' behaviour and reproduction, which can cause impacts which ripple through trophic webs, creating problems for species at higher and lower trophic levels (Moore, Folt and Stemberger, 1996). Phenological differences between species may become amplified by the changing climate, or new relationships may be forged due to climate disturbances' effects on the timings of life history events, as the magnitude of phenological shifts can vary widely between species (Primack *et al.*, 2009). Alterations to any cyclical weather patterns due to climate change can have consequences for the function of ecosystems. For example, changes to the timing and intensity of seasonal storms due to climate change may cause phenological mismatches in ecosystems which require the regular cyclical influx of water and dissolved organic content for normal function (Keller, 2007). This thesis investigates how temperature affects the behaviour of animals that maintain a key role in processes within their ecosystems, with each chapter using a range of temperatures to examine the influence of warming and its potential to interact with other common stressors.

1.2.2 Invasive species

Invasive species are among the main drivers of biodiversity loss and alterations to ecosystem function globally (Sala *et al.*, 2000). Anthropogenic introductions of non-native species have been taking place for thousands of years, but globalisation and increases in trade routes have caused an unprecedented increase in the rate of introductions (Vitousek *et al.*, 1996; Seebens *et al.*, 2017). The term “invasive” is used within this thesis to describe a species that is non-native to a location, has spread rapidly and is producing a significant negative impact to ecological, economic or health factors, which the EU defines as adversely impacting biodiversity and ecosystem services (Davis, 2009; Blackburn *et al.*, 2011; European Parliament, 2015). A minority of species that are introduced go on to establish a population in their novel range, with a minority of established non-native species going on to become invasive. The tens rule estimates that 10% of introduced alien species are able to establish a population, and of those, only approximately 10% become damagingly invasive (Williamson and Fitter, 1996). Each stage of progression to potential invasiveness, from transport to a novel range, introduction of a propagule, establishment of a population and spread of the species in the invaded range, acts as a filter or barrier to progression of the majority of non-native introductions (Davis, 2009; Jaric´ and Cvijanovic´, 2012). In reality, the stages may be concurrent and are imposed by human observers to more easily define progression of the introduction of a non-native species, but it remains a useful framework (Ricciardi *et al.*, 2013).

Invasive species are introduced non-native species which cause a measurable deleterious impact to the recipient ecosystem, as per the EICAT framework. Impacts can be defined as reductions in the biodiversity of native species and alterations to ecosystem processes within the affected ecosystem (Blackburn *et al.*, 2014). Impacts of invasive species may be considered as the per capita effect of individuals of the species, multiplied by the abundance or density of individuals in an invasive population, over the area of the range in which it is found (Parker *et al.*, 1999). Each of the chapters in this thesis measures per capita effects of invasive species, and chapter 3 also considers the density of the species at an invaded location to better appreciate its potential for negative impacts. Impacts from invasive species incur substantial costs for economies due to reductions in productivity and ecosystem services. Costs of management of invasive species are also significant, in order to control or eradicate introduced pest species. Invasive species are reported to have incurred global mean costs of US$ 26.8 billion per year between 1970 and 2017, with a trend of increasing costs meaning the estimate of incurred costs for 2017 may total US$ 162.7 billion (Diagne *et al.*, 2021). Total global expenditure on management of invasive species since 1960 has been estimated at US$ 95.3 billion, with the damage caused by invasive species during that period being conservatively estimated as US$ 1130.60 billion (Cuthbert *et al.*, 2022). The cost in losses and expenses in the UK alone due to the impacts of invasive species is conservatively estimated to be £ 122.1 million, on average, per year (Cuthbert *et al.*, 2021). Research is required into how some specific invasive species interact with stressors and whether their presence will exert positive or negative impacts on a changing environment, which may aid in assessing likely costs from invasions.

1.2.3 Parasites

Parasites are ubiquitous biotic stressors of ecosystems which can have effects on host survival and behaviours (Hatcher and Dunn, 2011; Hatcher, Dick and Dunn, 2012). Trait mediated effects exerted by parasites can alter host feeding rates, distribution and anti-predator behaviours, which can affect rates of ecosystem processes, especially when keystone species are affected (Mouritsen and Poulin, 2005; Hatcher, Dick and Dunn, 2014). Parasites can thus significantly influence interspecies interactions and nutrient flows within trophic webs through effects on host behaviours, though their presence and influence may not be immediately apparent (Lafferty, Dobson and Kuris, 2006; Hatcher, Dick and Dunn, 2012). Behavioural manipulation of hosts by parasites can be subtle alterations of behaviours or dramatic phenotypic shifts of multiple traits (Thomas, Poulin and Brodeur, 2010). For example, manipulations may be complex, such as parasitic freshwater hairworms which infect terrestrial cricket hosts but need to enter water to complete their reproductive cycle. The parasite manipulates its host into jumping into a water body, where the parasite can exit the host and mate (Ponton *et al.*, 2011). Simpler alterations to host behaviours are involved when the presence of parasites can increase (Dick *et al.*, 2010) or decrease feeding behaviours (Wood *et al.*, 2007), either of which could lead to trophic cascades or alter ecosystem function if the hosts hold keystone roles. Chapters 2 and 3 incorporate parasites as additional stressors to investigate what impact they have on their hosts and key host behaviours.

1.3 Interactions

Ecosystems are subject to challenges from stressors, which may exert pressures upon their structure and function (Folke *et al.*, 2004). The complexity of natural systems means they are rarely challenged by single stressors in isolation, but rather a suite of biotic and abiotic factors which can combine in unpredictable ways to create synergies that may have amplified impacts (Brook, Sodhi and Bradshaw, 2008; Jackson, 2015). Climate change, parasitism, pollution, land use change and invasive species are common stressors in ecosystems in much of the world. Stressors have combined to have synergistic impacts on amphibian populations, which have experienced major declines and extinctions worldwide. Pathogens, such as the parasitic *Batrachochytrium dendrobatidis*, have interacted with climate change and chemical pollutants, such as pesticides and nitrogenous fertilisers, leading to significant mortality rates in amphibian species (Blaustein and Kiesecker, 2002; Boone *et al.*, 2007). As species which experience both aquatic and terrestrial stressors, which have permeable skin and lay eggs without protective shells, they represent sentinel species which are sensitive early indicators of the threats of multiple stressors and possibly greater imminent ecological disturbances (Roy, 2002; Davidson and Knapp, 2007).

Different species have different plasticity in adapting to challenges, and multiple stressors exert multiple pressures on phenotypes, which may compromise a species' survival and behaviour (Sih, Ferrari and Harris, 2011). At an ecosystem level, stressors such as temperature may serve as a disturbance which facilitates the establishment of a damaging invasive species through extirpation or suppression of native competitors, to make vacant niche space and reduce an ecosystem’s biotic resistance to invasion (Brook, Sodhi and Bradshaw, 2008). Climate change is impacting virtually all ecosystems globally, and is predicted to alter the distribution of parasites while also increasing the transmission and virulence of many, which may have cascading effects on interspecies interactions and trophic webs (Marcogliese, 2008). Research into stressors must take into account the complexity of natural systems by investigating the impacts of multiple stressors acting on focal interactions or ecosystems in order to obtain better predictive power for more realistic scenarios (Côté, Darling and Brown, 2016; Orr *et al.*, 2020). This thesis investigates combinations of natural and anthropogenic stressors, both biotic and abiotic, to address knowledge gaps arising from previous research that has generally adhered to studying single stressors and has potentially missed the complexity of more realistic interactions.

1.4 Focal ecosystems

Freshwater habitats occupy approximately 1% of the world’s surface, while marine environments occupy approximately 70%, however they each support almost the same number of known species (Strayer and Dudgeon, 2010; Wiens, 2015). The high biodiversity of freshwater ecosystems is threatened and decreasing, with high risks of population reductions and extinctions (Dudgeon *et al.*, 2006; Vörösmarty *et al.*, 2010; Reid *et al.*, 2019). Human activity exploits a large proportion of freshwater systems for many uses, including transport, food production, waste disposal, recreational activities and energy generation (Albert *et al.*, 2021). Human activity also degrades these ecosystems, causing impacts to biodiversity and function through mechanisms such as climate change, land use change, introductions of invasive species and pollution (Birk *et al.*, 2020; Faghihinia *et al.*, 2021). Human requirements for fresh water and the vulnerability of fragmented, relatively low volume water bodies mean freshwater habitats are increasingly degraded and threatened with little prospect of damaging pressures easing in the foreseeable future (Reid *et al.*, 2019).

Freshwater ecosystems have experienced greater declines in biodiversity than other ecosystem types, possibly due to their relatively high levels of biodiversity and the limited dispersal opportunities for species afforded by the structure of many freshwater habitats as species' temperature thresholds are exceeded (Heino, Virkkala and Toivonen, 2009; Woodward, Perkins and Brown, 2010). Freshwater ecosystems are also dominated by ectothermic species, which may be less able to mitigate increasing temperatures (Woodward, Perkins and Brown, 2010). Temperature plays a key role in rates of energy use and generation in ecosystems at all levels. Warming influences all levels of organisation, from determining the structure of trophic webs, for example, increasing beta-diversity and algal growth in Arctic lakes, down to affecting metabolic rates in individuals (Brown *et al.*, 2004; Smol *et al.*, 2005). I investigated changes to metabolic demands in macroinvertebrates due to temperature in chapter 3, to examine whether this small-scale alteration can explain resulting behavioural differences.

In England and Wales mean water temperatures increased by 0.03°C per annum between 1990 and 2006, whilst U. S. data indicated mean water temperature increased by up to 0.08°C per annum between 1965 and 2007(Kaushal *et al.*, 2010; Orr *et al.*, 2015). A study on Welsh waters found mean temperatures to have risen by a total of 1.5°C in forest waters and 1.7°C in moorland waters between 1981 and 2005. This increase correlates with reductions in macroinvertebrate abundance, which is predicted to decrease by 21% for each 1°C increase in mean temperature (Durance and Ormerod, 2007). Each experiment in this thesis investigates the impacts of temperature on aquatic systems to better understand how projected climate warming may affect freshwater ecosystems.

High levels of human activity around freshwater systems means they are vulnerable to species introductions through propagules attached to transport, in ballast water, attached to recreational vehicles and equipment and also through water transfers (Sala *et al.*, 2000; Gallardo and Aldridge, 2018). The aquarium trade is also a growing threat to freshwater, as potentially invasive animal and plant species are freely traded through unregulated internet channels and released into novel natural ecosystems intentionally or unintentionally (Strecker, Campbell and Olden, 2011). Introduction and spread of highly successful, tolerant and adaptable invasive species is leading to a homogenisation of freshwater biota across ecosystems connected by human activity (Strayer, 2010).

The relative endemism and isolation of many freshwater ecosystems means species may be more naïve to novel species interactions, increasing vulnerability to major impacts from invasive predators (Cox and Lima, 2006). Adjoining habitats may also contain invasive species which impact aquatic ecosystems, such as riparian plants providing a leaf litter food source to freshwater invertebrates. European bird cherry has been introduced to riparian zones in

Alaska and has been found to support lower size and density of freshwater leaf consumers than native plant species (Roon, Wipfli and Wurtz, 2014). In this thesis I used the common Amphipoda order of freshwater invertebrates, which contains species both native (*Gammarus pulex, Gammarus duebeni celticus*) and invasive (*Dikerogammarus villosus*) in the U. K., to compare survival and behaviours of native and invasive species. Additionally, chapter 4 investigates the impacts of invasive plant species rhododendron and Japanese knotweed as a food source for aquatic macroinvertebrates.

As most biota requires water for survival, freshwater sites become locations for high concentrations of interspecies and intraspecies interactions, which can facilitate parasite transmission (Reid *et al.*, 2019). There have been numerous, relatively recent outbreaks of disease in freshwater ecosystems, such as chytridiomycosis causing high mortality of frogs and toads, and porcelain disease causing local extirpations of native crayfish in Europe (Edgerton *et al.*, 2004; Rödder *et al.*, 2009). Additionally, parasites often exert sub-lethal effects on hosts, such as altering feeding rates, changing social aggregation behaviour or affecting activity rates (Hernandez and Sukhdeo, 2008; Lewis *et al.*, 2012; Friesen *et al.*, 2017). In this thesis chapters 2 and 3 feature parasites (*Echinorynchus truttae, Pleistophera mulleri*) alongside other stressors, to investigate the impact that these natural biotic stressors can have in combination with anthropogenic disturbances to freshwater ecosystems.

Trophic webs in freshwater ecosystems in temperate zones are most often detritus-based, with allochthonous inputs of leaf litter vital for energy and nutrients (Gessner *et al.*, 2010). However tough cellulose-rich leaf litter requires some decomposition before its nutrients are released (Cornwell *et al.*, 2008). Fungal microbes colonise the leaf litter, penetrating the tissues with hyphae and initialising decomposition, breaking down cellulose with digestive enzymes (Gulis, Su and Kuehn, 2019). These aquatic hyphomycetes are vital intermediaries, increasing palatability of allochthonous leaf litter to macroinvertebrate species and concentrating nitrogen-rich material on the detritus surface to further increase its attractiveness to consumers (Graça *et al.*, 1993; Bärlocher, 2016). A guild of macroinvertebrate shredder species have mouthparts able to consume the softened leaf detritus, fragmenting the resource and releasing small particulates to provision other consumers in the ecosystem (Cummins and Klug, 1979; Cummins *et al.*, 1989; Covich *et al.*, 2004). These shredders hold a keystone role in temperate freshwater ecosystems, converting the dominant basal resource into useable form by fragmentation, provision of faeces and accumulation of biomass which supplies predatory species (Wallace and Webster, 1996; Ruetz, Newman and Vondracek, 2002). This thesis investigates the survival and behaviour of shredder species, both native and invasive, to study the likely impacts of common freshwater stressors on the key ecosystem process of shredding (chapters 2, 3 and 4) and the predatory provisioning of animal detritus by omnivorous shredders (chapter 5). Rates of leaf detritus shredding are used in chapters 2, 3 and 4 as a proxy for productivity to demonstrate good ecosystem function (Gessner and Chauvet, 2002). This method was adapted for chapter 5 to investigate how multiple stressors may affect the provisioning of animal detritus rather than shredded leaf matter.

1.5 Focal species

The species of detritivorous shredder investigated in this thesis are members of the order Amphipoda, which are important species in temperate freshwater ecosystems, often dominant by abundance (Glazier, 2009). Amphipods often perform the keystone role of shredding leaf litter in detritus-based freshwater ecosystems, distributing nutrients as particulates through the trophic web to collector species and other detritivores and providing a source of prey to predatory species, such as fish (Väinölä *et al.*, 2008; Covich and Thorp, 2010). These amphipods are generalist omnivores, but their vital role in the processing of the basal resource in freshwater ecosystems means research is required to investigate how their function as ecosystem provisioners is maintained when subjected to stressors. In mainland Great Britain the amphipod *G. pulex* (Linnaeus, 1758) is the most common native freshwater amphipod shredder. Found across much of Europe, *G. pulex* was introduced to Northern Ireland in the late 1950s, where it has become invasive (Pinkster, 1970; Strange and Glass, 1979; Macneil *et al.*, 1999). The native analogue species of amphipod in Northern Ireland is *Gammarus duebeni celticus* (Lilljeborg, 1852), which lives in the flowing waters of rivers and streams but is threatened by *G. pulex* (MacNeil *et al.*, 2001).

Numerous factors, both biotic and abiotic, influence the distribution of the native *G. d. celticus* and the invasive *G. pulex* in Northern Ireland since the introduction of the invader. Intraguild predation plays a highly significant role in species distributions of the native and the invasive amphipod, with the invader imparting a greater predatory pressure upon the native (Dick, Montgomery and Elwood, 1993). *Gammarus pulex* is a more aggressive predator, especially of recently moulted congeneric females, and is a more aggressive defender in interactions with the less aggressive *G. d. celticus* (Dick *et al.*, 1995). However, this relationship does not explain fully the patterns of distribution of the two species, as *G. d. celticus* persists at some sites alongside the invasive species. It appears that the intraguild predation pressures exerted by both are mediated by interactions with other biotic and abiotic factors (Dick, 2008).

An abiotic factor which can influence the invasion success of *G. pulex* is water quality. Replacement of the native by the invader has not been complete, with some sites containing populations of only *G. pulex* or *G. d. celticus* exclusively, and some locations supporting both species in sympatry (MacNeil *et al.*, 2001). Temperature is negatively correlated with concentrations of dissolved oxygen in water. The invader is more likely to be present in poorer quality water conditions, particularly with lower concentrations of dissolved oxygen, than the native species, while the native is more likely to dominate in water with a higher dissolved oxygen content (MacNeil, Prenter, *et al.*, 2004). Water quality can mediate interactions between the amphipod species, determining predatory efficiency of *G. pulex* on recently moulted *G. d. celticus* females. The alteration of asymmetry in intraguild predation by an environmental factor then determines whether the species co-exist at a particular site, or whether the native or invasive species dominates, with the invasive species able to replace the native or co-exist in water with lower organic quality (Piscart *et al.*, 2009; MacNeil and Campbell, 2018). The relative success of the invasive amphipod in lower quality conditions means that the lower percentage of dissolved oxygen in warmer water may indicate a shift towards conditions which favour expansion of the invader’s range in a warming climate.

The amphipod *D. villosus* (Sowinsky, 1894) (Figure 1.2) originates from the Ponto-Caspian region but has become highly invasive across a wide range. First detected in the Danube in 1926, the species spread through connected waterways throughout much of eastern Europe. In 1992 a canal opened to connect the Danube to the Rhine and *D. villosus* spread further to now occupy many of the main river and canal systems in mainland Europe (Rewicz *et al.*, 2014). First detected in the UK in 2010, the "killer shrimp" *D. villosus* has established several significant populations in Great Britain and threatens to spread throughout the UK freshwater network (MacNeil, Boets and Platvoet, 2012). *D. villosus* grows and reproduces at a high rate, with an extended breeding season, relatively high tolerance for a range of abiotic conditions and is a flexible feeder. These characteristics enable the species to be highly invasive and mean it can potentially disrupt trophic webs in ecosystems to which it is introduced (Platvoet *et al.*, 2009; Pöckl, 2009; Rewicz *et al.*, 2014). This disruption can be in the form of reductions in biodiversity of native species, which may compromise ecosystem function (van Riel *et al.*, 2007; Bollache *et al.*, 2008). Additionally, *D. villosus* in the U. K. is benefitting from enemy release, as natural enemies from its home range, such as parasites, do not appear to have made the journey to the novel range along with the host amphipod (Arundell *et al.*, 2015). Therefore, the invasive amphipod suffers fewer negative pressures in invaded locations, which has enabled greater population growth (Warren, Bradbeer and Dunn, 2021). Stopping the spread is the best defence against invasion by *D. villosus*, with post-invasion eradication potentially impossible (Bradbeer *et al.*, 2020).

Figure 1.2: *Dikerogammarus villosus*, total length 20mm. Image c/o Environment Agency

Previous studies have found that *D. villosus* tends to shred leaf litter at a lower rate than native species, which may have a significant impact on ecosystem productivity due to the keystone role in trophic webs held by amphipod shredders (Piscart, Mermillod-Blondin, *et al.*, 2011; Boeker and Geist, 2015). While intraguild predation by *G. pulex* can extirpate *G. d. celticus*, the highly invasive and predatory *D. villosus* will predate the Great Britain native *G. pulex*, with alternative food sources of prey or detrital matter only slowing the rate of species replacement (MacNeil and Platvoet, 2005). The high densities at which the Ponto-Caspian amphipod can be found may also mean that indirect effects of consumption of shared resources will impact numerous native species (Rewicz *et al.*, 2014). Such intraguild interactions, and interactions with the wider community, can be altered by stressors. Parasitic infection can increase the predation rate of *G. pulex* on macroinvertebrates while decreasing its predation rate on the native *G. d. celticus* (Hatcher, Dick and Dunn, 2014). Temperature has also been found to increase the predatory rate and rate of leaf litter shredding of *G. pulex* and *D. villosus*, (Truhlar, Dodd and Aldridge, 2014; Kenna *et al.*, 2017; Laverty *et al.*, 2017). In this thesis the effects of stressors on rates of ecosystem provisioning, through leaf shredding or partial predation and discarding of prey carcasses are investigated to better inform predictions of impacts of projected climate change and species distributions.

1.6 Research aims

In this thesis I investigate multiple stressors that are exerting pressures on freshwater ecosystems. Climate change and introductions of invasive species are anthropogenic disturbances which are having significant impacts on natural systems globally, with projected increases in the intensity of challenges faced by ecosystems (Chapin *et al.*, 2000; Sala *et al.*, 2000). Additionally, ecosystems contain natural stressors, such as parasites which, though often invisible, can have significant effects on interactions of organisms and thus impact the function of complex trophic webs (Lafferty, Dobson and Kuris, 2006; Kuris *et al.*, 2008). These stressors of temperature, invasive species and parasitism have most often been studied in isolation, taking a single factor and attempting to measure its impacts (Côté, Darling and Brown, 2016; Orr *et al.*, 2020). However, I attempt to investigate whether these factors interact with one another to mediate outcomes. Temperate freshwater ecosystems are used as the model systems due to their vulnerability to stressors and the ongoing relative lack of research into these systems, which are vital to the natural environment (Darwall *et al.*, 2011; Lowry *et al.*, 2013; Maasri *et al.*, 2022). Impacts are measured by effects on key ecosystem processes which are indicators of ecosystem function and productivity. Leaf detritus shredding is a keystone role performed by the amphipod shredder species investigated in this thesis. Shredding allows the efficient cycling of nutrients through the ecosystem and is vital to supporting most current temperate freshwater ecosystems (Gessner and Chauvet, 2002; Gessner *et al.*, 2010). This role of provisioning the ecosystem is also explored by investigating how stressors of temperature and invasive species may alter rates of the provisioning of animal detritus.

Chapter 2: Impacts of temperature and parasitism on survival and shredding rates, comparing a native and an invasive amphipod from Northern Irish freshwater ecosystems

2.1 Introduction

Multiple stressors affect ecosystems worldwide, impacting natural processes, driven by the increasing influence of anthropogenic change to the natural world (Orr *et al.*, 2020). Stressors of ecosystems are predicted to have increasing impacts globally on ecosystem services, processes and function (Chapin *et al.*, 2000; Pereira *et al.*, 2010). The impacts of stressors may combine to challenge an ecosystem's resistance, which is its capacity to withstand challenges and retain its structure and processes to maintain ecosystem function (Folke *et al.*, 2004). Biotic stressors, such as introductions of invasive species, parasites and predation, may interact with abiotic stressors, such as climate change, land use change and pollution to alter the structure or processes within an ecosystem, impacting its outputs and productivity. These impacts are predicted to increase in the foreseeable future, due in large part to increasing intensities of anthropogenic stressors, such as climate warming and introductions of invasive species (Sala *et al.*, 2000; Pachauri *et al.*, 2014).

Freshwater ecosystems are extremely biodiverse, with high productivity and importance for connected ecosystems (Dudgeon et al., 2006; Reid et al., 2005). However, these systems are highly vulnerable to anthropogenic stressors due to their exploitation for human uses and the connectedness of many freshwater habitats. These human-borne stressors rarely occur in isolation, and may interact with each other and natural stressors, such as parasites, predation and disease to affect ecosystem structure and productivity (Birk et al., 2020; Ormerod et al., 2010; Vörösmarty et al., 2010). Interactions between stressors may be additive, antagonistic or synergistic, and outcomes can be difficult to predict, meaning research is required into many species and stressor combinations (Côté et al., 2016; Orr et al., 2020; Paine et al., 1998).

Allochthonous inputs of riparian leaf litter are a vital source of energy and nutrients in many freshwater ecosystems in temperate regions (Gessner et al., 2010). Decomposition of this resource is vital for nutrient cycling and is connected to secondary production in these ecosystems, therefore the rate of decomposition of allochthonous leaf litter is an indicator of ecosystem function (Gessner & Chauvet, 2002). Mechanical and microbial decomposition can slowly release energy from the detrital resource, but a guild of shredder species are important to maintain the speed and efficiency of energy cycling through these trophic webs (Covich et al., 2004; Cummins et al., 2005; Cummins et al., 1989). The phenology of detrital resource availability is maintained by shredder species which mediate the release of carbon and other nutrients through fragmenting leaf litter, excreting consumed detritus and accumulating biomass. This shredding activity is vital to the freshwater community, such as collector species which feed on released fine particulates of shredded detritus and predators of the shredder species that accumulate biomass from detrital nutrients (Alp et al., 2016). Shredders are therefore keystone species in temperate freshwater systems, at the centre of trophic webs, and vital to the persistence of processes and outputs from these important ecosystems (Cummins & Klug, 1979; Reid et al., 2019).

In Northern Ireland, *Gammarus d. celticus* (Lilljeborg, 1852) is the sole native amphipod present in flowing waters, where it is the dominant species of keystone shredder (MacNeil et al., 2004). However, in many rivers it has been replaced by the invasive species *Gammarus pulex* (Linnaeus, 1758) since its introduction in the 1950s from mainland Great Britain, intended to provide a food source to facilitate fish growth for anglers (Strange and Glass, 1979). *Gammarus pulex* is common across much of Europe, but has been found to be invasive in some areas to which it has been introduced, potentially affecting ecosystem structure and function (Kelly *et al.*, 2006a; Piscart, *et al.*, 2011). Both species are host to a range of macro and micro parasites which mediate the interactions between the native and the invasive amphipod species, as well as interactions with other species in the ecosystem (Fielding *et al*., 2005; Macneil *et al*., 2003; MacNeil *et al*., 2004).

Density-mediated survival effects and trait-mediated behavioural effects exerted by parasites upon their hosts can interact to influence host species' abundances and impact on the ecosystem (Dunn *et al.*, 2012). *Gammarus duebeni celticus* is host to the muscle-wasting microsporidian parasite *Pleistophera mulleri* (Pfeiffer, 1895), which can reach high prevalence (Fielding *et al*., 2005; MacNeil *et al*., 2004). *Pleistophera mulleri* is host-specific, not infecting the invasive *G. pulex.* However, in the native species, *G. d. celticus*, it reduces host survival and decreases activity and aggression, though the parasite's effect on shredding has not been explored (MacNeil *et al.*, 2003). The acanthocephalan *Echinorhynchus truttae* (Schrank, 1788) uses amphipods as its intermediate hosts and reaches higher prevalence in *G. pulex* than in *G. d. celticus* (Macneil et al., 2003). Parasitism of the invader has been found to increase the amphipod's predatory behaviour, but has little effect on its detritus shredding behaviour (Dick et al., 2010; Fielding et al., 2003).

Climate change is predicted to have negative impacts on biodiversity and ecosystem function globally due to higher mean temperatures and more frequent and intense extreme weather events (Jenkins *et al.*, 2009; Murphy *et al.*, 2010; Pachauri *et al.*, 2014). In particular, high temperatures confer high stresses upon ecosystems, exerting pressures on the structure and function of those systems, as well as potentially interacting with other stressors (Jankowski *et al.*, 2006; Daufresne, Bady and Fruget, 2007; Bertani, Primicerio and Rossetti, 2016; Hupało *et al.*, 2018). For example, a characteristic of invasive species, which increases their likelihood of establishment and spread, is their tolerance of a wide range of conditions (Dukes and Mooney, 1999; Zerebecki and Sorte, 2011). Tolerance of higher temperatures may offer an advantage to an introduced species in comparison to a native analogue. Temperature can affect the survival and behaviour of species and may alter the course and impacts of an invasion by affecting interspecies relationships, and altering processes within the invaded ecosystem (Diez et al., 2012; Iacarella et al., 2015; Rahel & Olden, 2008). It is predicted that *G. pulex* may be less impacted by climate warming than the native *G. d. celticus* in Northern Ireland due to the invasive species' greater tolerance of lower dissolved oxygen concentrations, which are a consequence of increasing temperatures (Laverty et al., 2014).

Relationships between parasites and their hosts are also subject to stressors such as temperature. Interactions between temperature and host-parasite relationships can alter patterns of survival (Gehman et al., 2018; Greenspan et al., 2017; Musgrave et al., 2019), and also may affect behaviour, such as feeding rates (Larsen and Mouritsen, 2009). For example, an interaction between temperature and infection with *E. truttae* led to increased predation by parasitised *G. pulex* at elevated temperatures, compared to unparasitised conspecifics

(Laverty *et al.*, 2017). However, there is a lack of research into how parasitic infection may interact with extreme temperatures to impact rates of shredding in freshwater ecosystems.

Any alteration to a food web can have far-reaching consequences, especially when involving keystone species. The vital ecosystem process of shredding may be affected if the survival or behaviour of keystone shredder species are altered by stressors. The aim of this study is to explore the effect of temperature and parasitism on shredding by the native amphipod *G. d. celticus* compared to the invasive *G. pulex*. This study will examine how interacting stressors are likely to differentially affect *G. d. celticus* and *G. pulex* in Northern Ireland, and how this will affect the shredding activity of amphipods, which plays a keystone role in energy and nutrient cycling in temperate freshwaters.

2.2 Methods

2.2.1 Experimental material

G. pulex were collected by kick-sampling from the Minnowburn River, Northern Ireland (54.5484° N, -5.9531° E). *G. d. celticus* were collected by kick-sampling from a stream in Portaferry, Northern Ireland (54.3556° N, -5.5297° E). Amphipods were transported to the University of Leeds (53.8045° N, -1.5548° E) within 48 hours in an insulated cool box lined with water-saturated paper to ensure sufficient hydration and aeration. Parasitised animals were identified by visual examination. Individuals of *G. pulex* parasitised by *E. truttae* were identifiable by an orange acanthocephalan cystacanth visible through the cuticle (Awachie, 1966; Macneil, Fielding, *et al.*, 2003) . Only hosts with a mature cystacanth were used to enable pre-experimental identification. Infection status was confirmed by dissection and visual identification under light microscopy at 10x magnification after completion of the experimental procedures (Perrot-Minnot *et al.*, 2011). Individuals of *G. d. celticus* parasitised by *P. mulleri* were identified by the presence of white spore masses in tissue on the abdomen under light microscopy at 10x magnification (MacNeil *et al.*, 2003). All amphipods were kept for a minimum of 5 days acclimatisation period prior to experiments, in host species-specific, parasite-specific, communal tanks in a controlled temperature room at 15 ± 0.1°C S.D., with a 12:12 hour light/dark cycle (08:00-20:00). Tanks were filled with 2 litres of aerated aged tap water and study organisms were fed *ad libitum* with leaf litter.

Common alder, *Alnus glutinosa*, was collected as autumn leaf fall and dried for use in the experiment. *A. glutinosa* is favoured by shredder species in temperate freshwater ecosystems skewed towards allochthonous leaf litter as a basal resource (Waring and Running, 2007; Bloor, 2011). The leaves were conditioned in stream water from Meanwood Beck, West Yorkshire (53.8301°N, -1.5746°E) for two weeks to increase palatability through microbial colonisation and decomposition (Bloor, 2011; Foucreau *et al.*, 2013). A cork borer was used to cut 6 mm diameter discs from the conditioned leaves, avoiding the tougher midrib tissue, and discs were then air-dried, collected into sets of 15 and weighed (mean mass = 27.35 ± 1.77 mg S.D.). Leaf discs were again conditioned in stream water for 48 hours immediately prior to use in the experiment. The reconditioning was done in order to restore some live microbial film that was killed by drying, which is vital to normal trophic interactions between shredders and detrital leaf resources (Hall and Meyer, 1995).

2.2.2 Experimental design

Leaf shredding and survival rates of *G. pulex* that were either unparasitised or parasitised by *E. truttae,* and *G. d. celticus* that were either unparasitised or parasitised by *P. mulleri*, were measured at a range of temperatures. Temperature treatments were between 5 and 30°C in 5°C increments to investigate impacts of temperature from mean winter temperatures to extreme heatwaves (Garner *et al.*, 2014). The range of temperatures was intended to identify thermal optima of species by exceeding standard temperature regimes. Amphipods had excess water removed on a paper towel and were weighed before being placed individually in transparent, circular plastic containers (diameter 7 cm, depth 5 cm) with 250 ml aged tap water. Two transparent glass beads were placed in the containers to provide a refuge and prevent excess swimming associated with thigmotactic behaviours (Perrot-Minnot, Banchetry and Cézilly, 2017; Kohler, Parker and Ford, 2018). The containers were placed in incubators at 15°C with a 12:12 hour light/dark cycle and the animals underwent a 24-hour starvation period to standardise hunger, during which the temperature was gradually increased or decreased at a rate of 1°C every 2 hours, until the desired temperature for the treatment was reached. A total of 87 *G. pulex* and 96 *G. d. celticus* were allocated to the range of temperatures, as tabulated below (Tables 2.1 and 2.2).

Table 2.1: Numbers of each amphipod species and parasitised state treatment by temperature for the shredding data

		Temperature (°C)					
		5	10	15	20	25	30
Amphipod and parasite	*G. pulex*, unparasitised	6	10	6	9	6	2
	G. pulex with *E. truttae*	4	8	4	7	3	0
	G. duebeni, unparasitised	6	10	6	10	6	6
	G. duebeni with *P. mulleri*	4	10	6	9	6	6

Table 2.2: Numbers of each amphipod species and parasitised state treatment by temperature for the survival data

		Temperature (°C)					
		5	10	15	20	25	30
Amphipod and parasite	*G. pulex*, unparasitised	6	10	11	10	6	8
	G. pulex with *E. truttae*	5	8	7	8	5	3
	G. duebeni, unparasitised	6	10	10	10	6	6
	G. duebeni with *P. mulleri*	6	10	10	10	6	6

After the starvation period, 15 weighed leaf discs were added to each container. Animals were checked and mortality was recorded every 24 hours. Mortality was identified as a lack of pleopod beating and absence of reaction to a physical stimulus.

Some mortality occurred during the initial 24-hour hunger standardisation period, before leaf litter was supplied to the subjects, so the shredding experiment had a lower number of replicates than the initial total for the survival data.

After 6 days the experiment was ceased and any remaining coarse particulate organic matter (CPOM) was removed and stored in ethanol in order to halt any continued microbial decomposition prior to weighing. Samples were heated in a drying oven at 60°C for 24 hours in previously weighed aluminium weighing boats and the CPOM mass was then calculated.

2.3 Data analysis

All analyses were carried out in R, with plots for shredding rate generated with the package “ggplot2” (Wickham, 2016; R, 2019).

2.3.1 Shredding

Shredding rate was calculated by dividing leaf mass reduction by the time in days, as amphipod survival was checked every 24 hours. Shredding was adjusted for size of individual by dividing shredding rate by amphipod mass. The “mgcv” package in R was used to construct a general additive model (GAM) to assess the impact of the factors of temperature, amphipod species and parasitised status on shredding rate (Wood, 2011). The restricted maximum likelihood method (REML) of smoothing the continuous temperature variable using a cyclic cubic regression spline produced the best model fit. Stepwise removal of non-significant factors was carried out, and Akaike's information criterion (AIC) was used to compare model fits.

2.3.2 Survival

The "survival" package in R was used to produce Kaplan Maier product limit estimator curves for plots, and Cox proportional hazards models were used to calculate differences in survival between treatments and interactions (Therneau, 2021).

2.4 Results

2.4.1 Shredding

Temperature had a significant effect on shredding rate, with an overall positive correlation between temperature and shredding rate ($F_{(2.89,149)} = 230.3$, $p < 0.001$) modelled using a cyclic cubic regression spline to account for non-linear interactions. Parabolic curves of shredding rate related to temperature are observed, with a peak mean shredding rate observed at 25°C for unparasitised *G. pulex* 52 milligrams of leaf per gram of amphipod per day, hereafter referred to as mg per day, more than double the lowest rate at 5°C (22 mg per day). For parasitised *G. pulex* the highest shredding rate at 20°C (64 mg per day) was double the lowest rate, at 5°C (31 mg per day). A lower rate of shredding was observed beyond the optimal shredding temperatures of 25°C for unparasitised *G. pulex* and 20°C for parasitised *G. pulex*. The highest shredding rate for unparasitised *G. d. celticus* at 30°C (47 mg per day) was double the lowest rate, at 5°C (23 mg per day). Parasitised *G. d. celticus* also had a highest mean shredding rate at 30°C (42 mg per day), which was approximately double the lowest rate at 5°C (20 mg per day) (Figure 2.1). There was no difference in shredding rate between *G. d. celticus* and *G. pulex* ($t_{(1,149)}$ = 0.07, p = 0.95), and no interaction between temperature and species ($t_{(11,149)}$ = 1.12, p = 0.27).

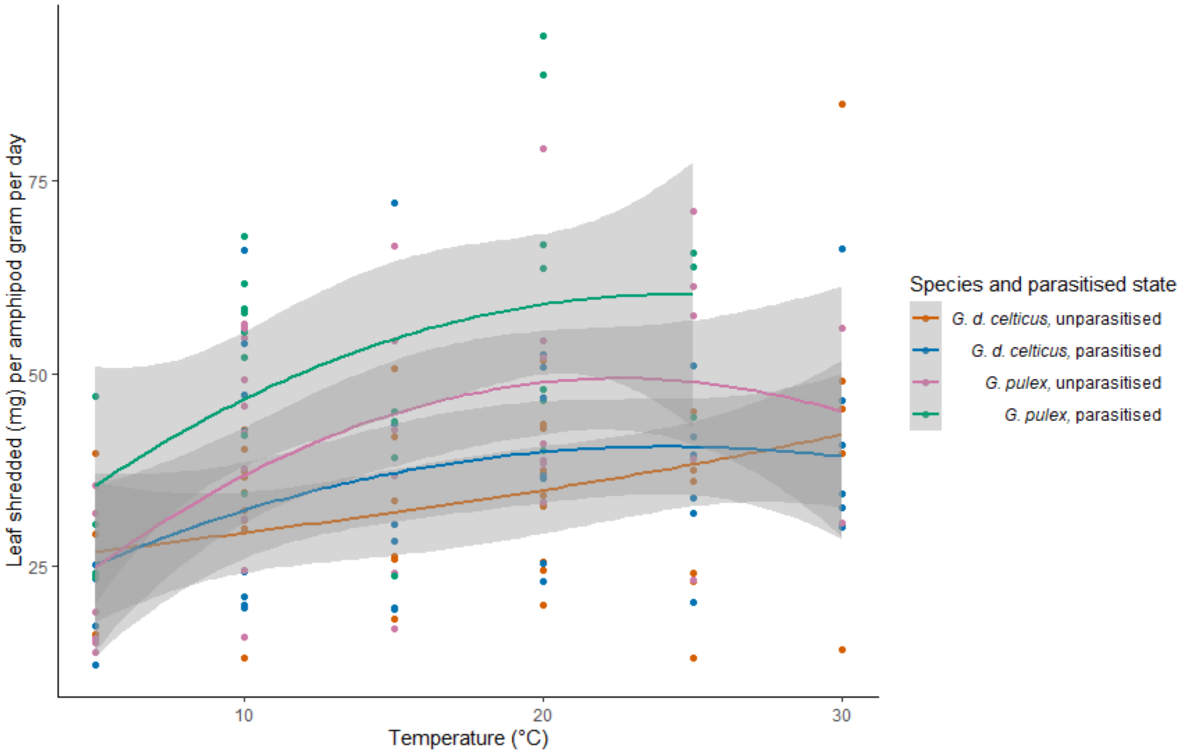

Figure 2.1: Relationship between temperature and rates of shredding for each amphipod treatment (species and parasitised state), displaying one standard error with Loess-smoothed curves.

No significant effect of parasitism on shredding was found, with no difference in the shredding rates of unparasitised *G. pulex*, and *G. pulex* parasitised by *E. truttae* ($t_{(1,64)}$ = 1.62, p = 0.11) and similarly, *G. d. celticus* that were unparasitised and those parasitised by *P. mulleri* did not differ in rates of shredding ($t_{(1,84)}$ = -0.14, p = 0.89). No interaction was found between temperature and parasitised state in either *G. pulex* ($t_{(11,64)}$ = -0.52, p = 0.61) or *G. d. celticus* ($t_{(11,84)}$ = 0.47, p = 0.64).

2.4.2 Survival

Temperature had a significant effect on survival (z = 7.09, p < 0.001), with a negative correlation observed, (figures 2.2 a-d). Increasing temperature was associated with a 1.14 greater hazard of mortality (95% CI: 1.10, 1.18). *G. d. celticus* experienced greater survival than *G. pulex* (z = 2.46, p = 0.01) but there was no interaction between temperature and species (z = 0.07, p = 0.95). Unparasitised *G. d. celticus* experienced greater survival than unparasitised *G. pulex* (z = 2.55, p = 0.01) however, there was no significant interaction between temperature and parasitised state. Parasitised and unparasitised *G. pulex* did not experience significantly different survival (z = 0.92, p = 0.36) and similarly, survival did not differ between parasitised and unparasitised *G. d. celticus* (z = -1.68, p = 0.09).

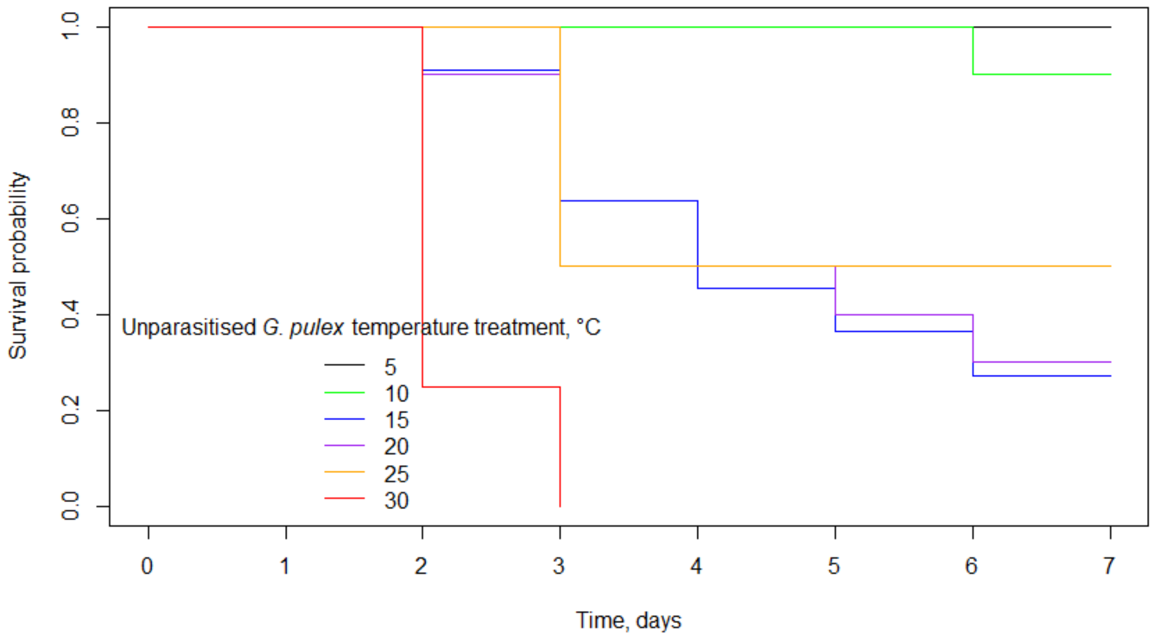

Figure 2.2 a): Kaplan Meier plot of survival of unparasitised *G. pulex*

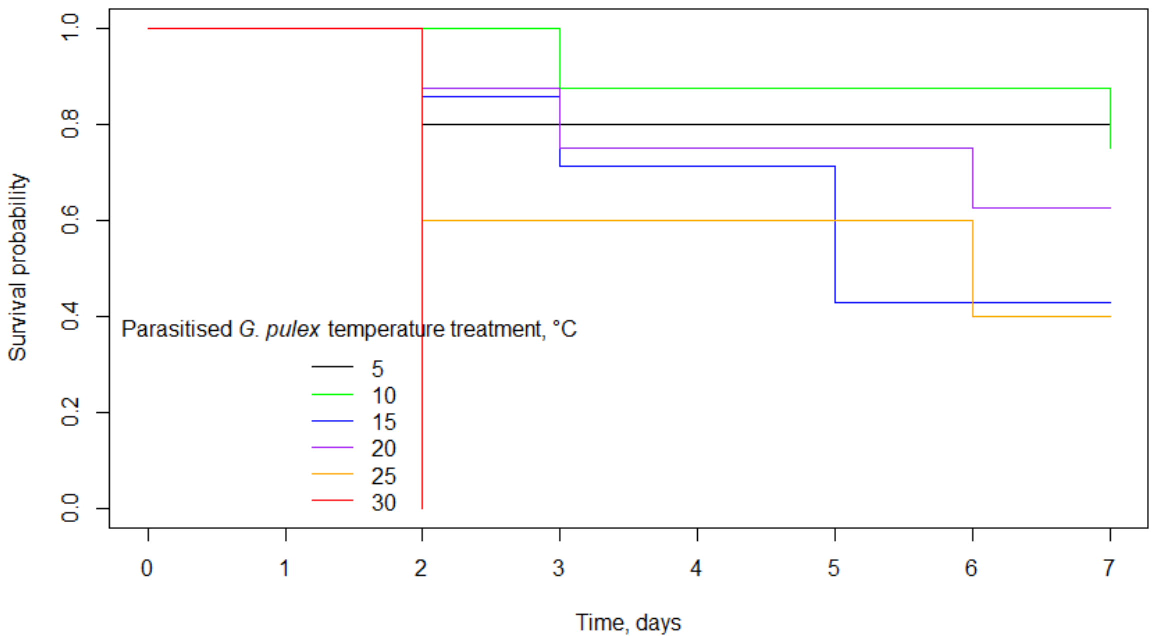

Figure 2.2 b): Kaplan Meier plot of survival of *G. pulex* parasitised by *E. truttae*

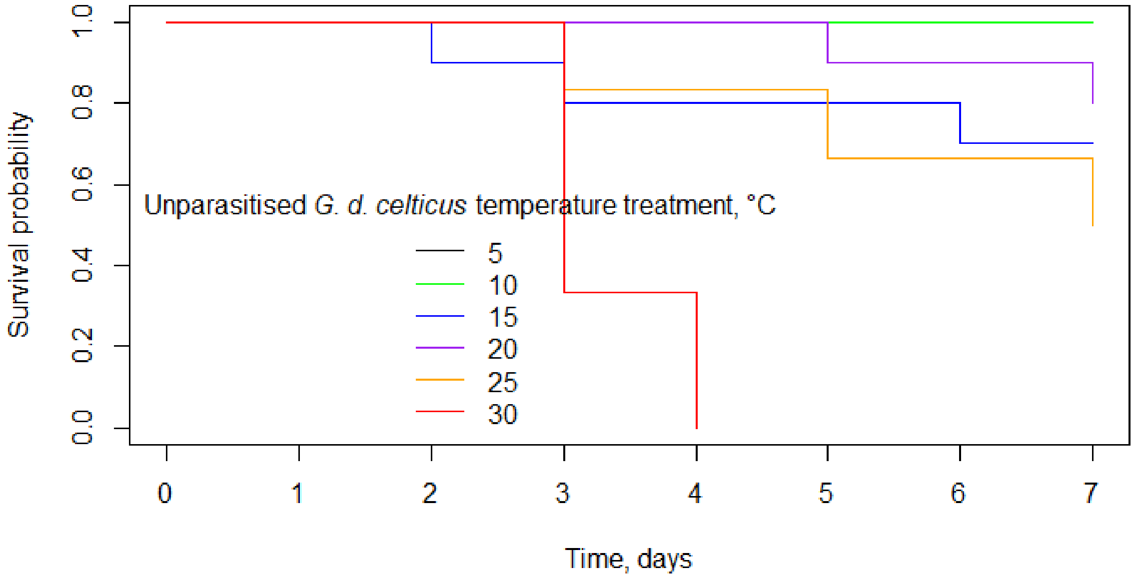

Figure 2.2 c): Kaplan Meier plot of survival of unparasitised *G. d. celticus*

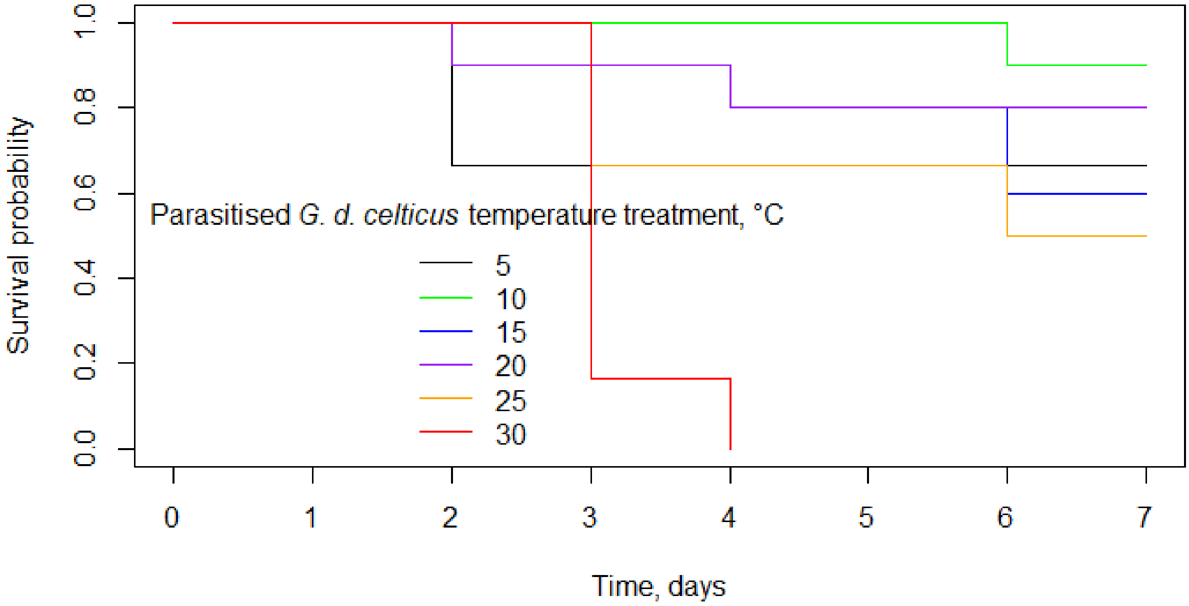

Figure 2.2 d): Kaplan Meier plot of survival of *G. d. celticus* parasitised by *P. mulleri*

2.5 Discussion

The most important factor affecting rates of shredding was temperature, with shredding doubling from the lowest rate, at 5°C for each amphipod and parasite combination, to their highest rate at 20, 25 (parasitised and unparasitised *G. pulex*) or 30°C (*G. D. celticus*). Similarly, increasing temperature was found to increase predatory pressure from amphipods (Laverty et al., 2017; Pellan et al., 2016). Temperature had a significant effect on shredding. However, survival was negatively affected by temperature. Therefore, whilst increases in temp will increase per capita shredding (up to an optimum before decreasing), amphipod abundances are likely to decline. Hence productivity may not be altered at an ecosystem level.

The native and invasive amphipod species used in this experiment are closely related and share a similar diet, which consists of leaf detritus, some wood and algae and some invertebrate tissue (Piscart, Roussel, *et al.*, 2011). Previous studies have found that the invasive *G. pulex* is more predatory and more aggressive in intra-guild interactions than the native *G. d. celticus* (Dick *et al.*, 1995; MacNeil, Dick, *et al.*, 2004). In contrast, we found no difference in shredding rates between the two species. Temperature was also the factor which had the greatest impact on survival in this experiment. Although the highest rate of shredding for *G. d. celticus* was at 30°C, amphipods did not survive longer than 4 days at that temperature. Therefore, the increase in shredding rate driven by increasing temperatures may be mediated by decreasing survival.

The slightly higher rates of shredding in *E. truttae*-infected *G. pulex,* though not statistically significant, is in accord with observations of higher rates of predation by *G. pulex* infected with *E. truttae* and is likely to reflect the increased activity of infected individuals and metabolic demands of this parasite (Dick *et al.*, 2010). Temperature and parasitism had an additive effect on shredding rate, in accord with a previous study, but only temperature had an impact strong enough to be statistically identified here (Laverty *et al.*, 2017). However, as survival decreased at higher temperatures, overall ecosystem productivity is unlikely to increase under warming. Warming may therefore favour other components of trophic webs over amphipod shredders, such as algal growth, altering the structure and the flow of energy and nutrients through this freshwater trophic web (Daufresne, Lengfellner and Sommer, 2009; Coyne *et al.*, 2021).

Though the native *G. d. celticus* experienced greater survival rates than the invasive *G. pulex* with increasing temperature, both suffered 100% mortality after 4 days at 30°C. *G. pulex* has previously been found to tolerate conditions of reduced oxygen at a fixed temperature of 12°C better than *G. d. celticus* (MacNeil et al., 2004), suggesting that the lower survival of the invasive here is related to a separate physiological effect of temperature and not simply the lower dissolved oxygen content of warmer water. The lower survival of *G. pulex* in increasing temperatures indicates the warming climate may be less favourable for the invasive species in this case, potentially facilitating coexistence of both amphipod species in Northern Ireland.

Northern Irish freshwater ecosystems are affected by stressors including temperature and invasive species. The warming climate will have a major impact on the structure and function of these communities, affecting the productivity of freshwaters and connected ecosystems. This chapter indicates that temperature is likely to have the greatest impact on keystone amphipod shredders, which maintain the flow of nutrients from allochthonous leaf litter through the ecosystem. Invasion by *G. pulex* has led to local extinction of the native *G. d. celticus*, as well as declines in macroinvertebrate abundance, diversity and richness of taxa other than gammarid species (Kelly et al., 2006b). However, the native *G. d. celticus* and the invasive *G. pulex* were found to shred leaf litter at similar rates at all temperatures tested. Although we found no difference in per capita resource processing by the invasive species, in the field *G. pulex* often attains much higher densities than does the native species, with densities found in the River Lissan in Northern Ireland for *G. pulex* over one hundred times higher than *G. d. celticus* in some locations (Kelly et al., 2006b). The higher relative abundance of the invasive species may have a significant impact on rates of shredding in an ecosystem, and this is addressed in the next chapter.

Chapter 3: The invasive amphipod *Dikerogammarus villosus* impacts detritus processing through high abundance rather than differential response to temperature

3.1 Introduction

Ecosystems worldwide are subject to impacts from multiple abiotic and biotic stressors which stem from a combination of natural and anthropogenic drivers (Orr *et al.*, 2020). Abiotic factors, such as climate warming, ocean acidification, pollution, and land use change act by influencing species' physiology and extirpate those organisms for which conditions shift beyond physiological limits. Biotic stressors, such as species introductions, predation, parasitism, disease, and spatiotemporal decoupling from food resources, act at a population level to influence demographic processes (Chapin *et al.*, 2000; Cardinale *et al.*, 2012; Fincham *et al.*, 2019). To reflect the complexity of natural systems, more research is needed on the effects of multiple factors in synergy, rather than considering the impacts of single stressors in isolation (Ormerod *et al.*, 2010; Strayer, 2010; O'Brien *et al.*, 2019).

Freshwater habitats are among the most threatened by these interacting stressors. These ecosystems are biodiverse, occupying only 0.8% of the world's surface and 0.01% of the world's water, but harbouring 6% of described species (Dudgeon *et al.*, 2006). This biodiversity supports high productivity and provides important resources to adjacent ecosystems and human-kind (Millennium Ecosystem Assessment, 2005; Larsen, Muehlbauer and Marti, 2016). In temperate freshwater ecosystems the basal energy resource is often heavily skewed towards allochthonous riparian leaf litter (Gessner *et al.*, 2010), with macroinvertebrate shredders contributing to the release of nutrients, dispersing shredded leaf particulates and transferring biomass up through trophic webs (Covich *et al.*, 2004; Cummins, Merritt and Andrade, 2005; Alp *et al.*, 2016). However, freshwater ecosystems are vulnerable to multiple anthropogenic stressors (Ormerod *et al.*, 2010; Vörösmarty *et al.*, 2010; Gutowsky *et al.*, 2019).

Among the most significant biotic stressors facing natural systems are invasive species (Gallardo *et al.*, 2016; Fletcher *et al.*, 2019; Pereira and Vitule, 2019). Successful invaders often outcompete native species in the new habitat, monopolising resources, and may also prey upon natives, rapidly altering community structure (Vitousek *et al.*, 1996; Sakai *et al.*, 2001; Rewicz *et al.*, 2014). Alterations of this kind may affect ecosystem function and resource processing, causing cascading effects across trophic levels (de Omena, Srivastava and Romero, 2017; DeBoer, Anderson and Casper, 2018; Doherty-Bone *et al.*, 2019). Amphipods are important shredder species in temperate freshwaters (MacNeil, Dick and Elwood, 1997; Dangles and Malmqvist, 2004) but those from the Ponto-Caspian region, such as the ‘killer shrimp’ *Dikerogammarus villosus* (Sowinsky, 1894), have proved highly invasive. *D. villosus* has invaded major waterways throughout western Europe, has been present in the UK since at least 2010, and is predicted to invade North America and Ireland from horizon scanning (Grabowski, Bacela and Konopacka, 2007; *Species alerts - GB non-native species secretariat*, 2012; Rewicz *et al.*, 2014). This invasion has resulted in replacement of native amphipods and impacted wider macroinvertebrate communities through competition and predation (Grabowski, Bacela and Konopacka, 2007; Dodd *et al.*, 2014; Rewicz *et al.*, 2015). *Gammarus* *pulex* (Linnaeus, 1758) is the dominant native freshwater amphipod shredder in Great Britain, however it is outcompeted and preyed on by the invasive *D. villosus*, leading to species replacement (MacNeil and Platvoet, 2005; van Riel *et al.*, 2007; Bollache *et al.*, 2008; Kinzler *et al.*, 2009; Dodd *et al.*, 2014).

In addition to anthropogenic stressors, species face a suite of stressors as a result of their place within ecological networks of interactions. Parasites are a ubiquitous part of natural communities that may alter patterns of host survival and can also affect host traits, including behaviour and feeding rates (Fielding *et al.*, 2003; Hatcher and Dunn, 2011; Dunn *et al.*, 2012; Grabner, 2017). The native amphipod *G. pulex* is commonly parasitised by acanthocephalans, including *Echinorhynchus truttae* (Schrank, 1788), which uses the amphipod as an intermediate host, and can have a prevalence of up to 70 % in *G. pulex* host populations (Macneil, Fielding, *et al.*, 2003). Parasite manipulation by *E. truttae* alters anti-predator behaviour in amphipods (Macneil, Fielding, *et al.*, 2003; Baldauf *et al.*, 2007; Perrot-Minnot, Kaldonski and Cézilly, 2007; Lagrue, Gueventam and Bollache, 2013), and has also been shown to influence the predatory behaviour of the amphipod host (Fielding *et al.*, 2003; Dick *et al.*, 2010).

Alterations to host feeding behaviours may be due to energetic costs exerted by parasites, reflected in changes to metabolic rate. The effect of acanthocephalan infection of amphipods by *Polymorphus minutus* and *Pomphorhynchus laevis* on basal metabolic rate (BMR) has previously been studied, but *E. truttae* has not previously been studied in relation to its effect on host BMR (Labaude *et al.*, 2015; Perrot-Minnot, Maddaleno and Cézilly, 2016). In contrast to the native species, *D. villosus* has benefitted from enemy release in much of its new range, with some parasites from its native range absent from invader populations of *D. villosus*, and no evidence of infection by *E. truttae* in invaded areas (Gendron, Marcogliese and Thomas, 2012; Arundell *et al.*, 2015).

Climate change has long been established as a threat to biodiversity due to higher mean temperatures, as well as an increase in the frequency and intensity of extreme events (Jenkins *et al.*, 2009; Pachauri *et al.*, 2014). High temperatures experienced during heatwaves can have impacts on the structure and function of communities (Bertani, Primicerio and Rossetti, 2016; Leung, Connell and Russell, 2017). Climate change may also make ecosystems more vulnerable to the impact of additional stressors, such as invasive species (Jankowski *et al.*, 2006; Daufresne, Bady and Fruget, 2007; Hupało *et al.*, 2018), which may have different thermal optima than their native analogues. Tolerance to a wide range of conditions is a characteristic of successful invasive species, facilitating establishment and spread; however survival and behaviour become less predictable at high temperatures (Diez *et al.*, 2012; Tepolt and Somero, 2014; Iacarella *et al.*, 2015). Experimental work is therefore required to investigate how invasive species survive and function in high temperatures in comparison to native analogues. Previous studies comparing shredding rates in *G. pulex* and *D. villosus* report a positive correlation between shredding rates and temperature, but comparisons between the native and invader conflict (Truhlar, Dodd and Aldridge, 2014; Kenna *et al.*, 2017). . Interactions between parasites and hosts can also be influenced by temperature, with outcomes difficult to predict when thermal optima of hosts or parasites are exceeded due to species-specific interactions (Greenspan *et al.*, 2017; Fernández-Loras *et al.*, 2019; Gehman and Harley, 2019; Musgrave, Bartlow and Fair, 2019). Previous studies have not considered the impact of extreme temperatures and of parasitic infection on shredding efficiency.

This study aims to investigate how survival and shredding differs between native and invasive amphipods as a function of two significant stressors: temperature and a common parasite of native amphipods, *E. truttae*. We hypothesise that the invasive *D. villosus* outcompetes the native *G. pulex* partly through higher rates of shredding, which may be underpinned by differences in metabolic rate. The native species suffers more parasitism than the invasive in Great Britain, so the impact of the common parasite *E. truttae* is tested, in order to determine whether the metabolic demands of the parasite alter the behaviour and survival of the native amphipod. The lesser competitive power of the native is hypothesised to be exacerbated by parasitism. We aimed to identify whether species and parasitised status interact with temperature to alter survival and behaviour to investigate whether multiple stressors can have amplified impacts on the ecosystem process of shredding. The impact of the invasive species and parasitism on resource processing depends on both leaf shredding capability and relative abundance. The Relative Impact Potential (RIP) metric (Dick *et al.*, 2017) incorporates relative consumer abundance response as a means of scaling relative *per capita* effects to compare the relative impact potential of amphipod treatments. We hypothesise that the high densities of the invasive species will indicate a higher impact potential for *D. villosus*.

3.2 Methods

3.2.1 Experimental material

Gammarus pulex were collected by kick-sampling from Meanwood Beck at Golden Acre Park (53.8687°N, -1.5884°E) (figure 3.1) and Meanwood Park, West Yorkshire (53.8301°N, -1.5746°E). *Dikerogammarus villosus* were collected from Grafham Water, Cambridgeshire (52.2909°N, -0.0323°E) (figure 3.2). All amphipods were kept for a minimum of 5 days acclimatisation period prior to experiments, in species-specific communal tanks in a controlled temperature room at 15 ± 0.1°C S.D., with a 12:12 hour light/dark cycle (08:00-20:00).

Figure 3.1: Meanwood Beck, Golden Acre Park c/o Wikimedia commons

Tanks were filled with aerated aged tap water and study organisms were fed *ad libitum* with leaf litter. Parasitised animals were identified by visual examination. Individuals parasitised by *E. truttae* were identifiable by an orange acanthocephalan cystacanth visible through the cuticle (Awachie, 1966). Only hosts with a mature cystacanth were used, which facilitated pre-experimental identification. Infection status was confirmed after experiments by dissection and visual identification (Perrot-Minnot *et al.*, 2011).

Figure 3.2: Jetty from which *D. villosus* samples were collected at Grafham Water, Cambridgeshire. Image c/o Anglian Water Services Ltd

Leaf material selected for experimental use was common alder (*Alnus glutinosa*) as amphipod species have demonstrated a preference for the leaf detritus of this species (Bloor, 2011).

Alder is a nitrogen-fixing species, producing nutrient-rich leaf litter, relatively low in carbon, which is favoured by detritivorous species in fresh waters (Waring and Running, 2007; Bloor, 2011). Alder is also a common riparian species throughout temperate areas of the northern hemisphere (Bjelke *et al.*, 2016), and is found in locations where the experimental animals were collected. Leaves were collected as natural autumn leaf fall and dried. Leaves were then conditioned for two weeks in water from Meanwood Beck, West Yorkshire, to promote microbial colonisation and increase the palatability of the detritus for amphipods (Bloor, 2011). Once conditioned, a cork-borer was used to cut 6 mm diameter discs of leaf, avoiding the lignified and less palatable midrib and veins. Leaf discs were air-dried and then weighed out in sets of 15 (mean mass = 27.0 ± 1.9 mg S.D.) and subsequently conditioned in Meanwood Beck stream water for 48 hours immediately prior to the experimental period. The drying of the discs prior to weighing killed the microbes that had accumulated as biofilm during the initial leaf conditioning, while still leaving a nitrogen-rich mass. The reconditioning was carried out so that some live microbial film was present when feeding to experimental animals, in addition to the previously accumulated nitrogen-rich biomass.

3.2.2 Experimental design

Leaf shredding and survival rates of *D. villosus* and *G. pulex,* that were either unparasitised or parasitised by *E. truttae,* were measured at a range of temperatures. Individual amphipods were placed on a paper towel to remove excess water and weighed. Animals were placed individually in transparent, circular plastic containers (diameter 7 cm, depth 5 cm) with 250 ml aged tap water. Two transparent glass beads were placed in the containers to provide a refuge, and prevent excess swimming due to thigmotactic behaviours (Perrot-Minnot, Banchetry and Cézilly, 2017; Kohler, Parker and Ford, 2018), while still allowing observation (figure 3.3). The containers were then placed in incubators at 15°C with a 12:12 hour light/dark cycle and the animals underwent a 24-hour starvation period to standardise hunger, during which the temperature was gradually increased or decreased at a rate of 1°C every 2 hours, until the desired temperature for the treatment was reached. Temperature treatments were between 5 and 30°C in 5°C increments with the following number of replicates for the initial survival experimental data: 5°C (unparasitised *G. pulex* n = 16, parasitised *G. pulex* n = 13, *D. villosus* n = 16), 10°C (unparasitised *G. pulex* n = 16, parasitised *G. pulex* n = 14, *D. villosus* n = 16) , 15°C (unparasitised *G. pulex* n = 15, parasitised *G. pulex* n = 16, *D. villosus* n = 16) , 20°C (unparasitised *G. pulex* n = 16, parasitised *G. pulex* n = 15, *D. villosus* n = 16) , 25°C (unparasitised *G. pulex* n = 16, parasitised *G. pulex* n =14, *D. villosus* n = 16) and 30°C (unparasitised *G. pulex* n = 16, parasitised *G. pulex* n = 16, *D. villosus* n = 16). After the starvation period, 15 weighed leaf discs were added to each container (figure 3.3). Animals were checked every 24 hours, and mortality was recorded. Mortality was identified as a lack of pleopod beating and absence of reaction to a physical stimulus. These treatments were selected to test outcomes at a range of temperatures up to and beyond known thermal limits. Water levels in each container were maintained with oxygenated water at the relevant temperature being added if required.

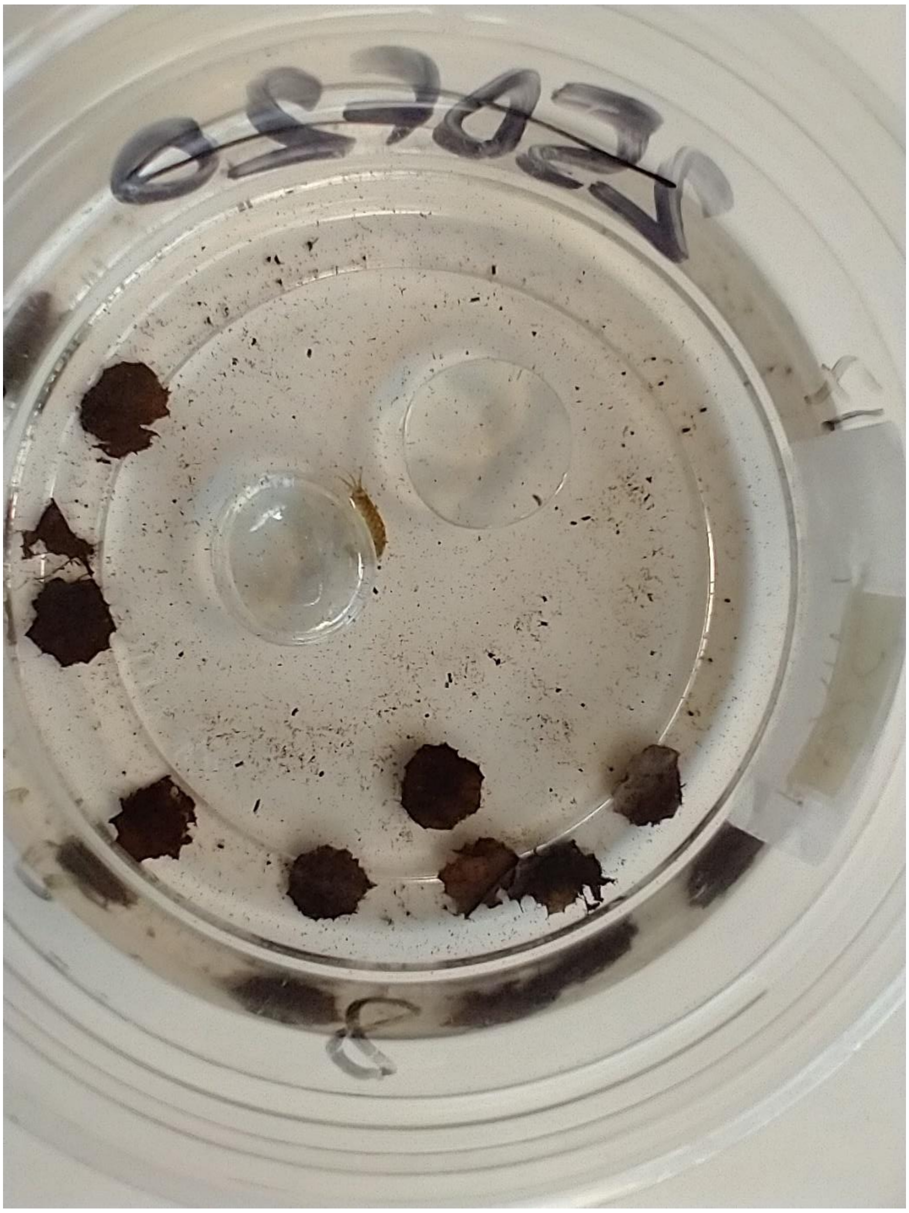

Figure 3.3: An individual *D. villosus* sheltering by a glass bead in a container with partially shredded *A. glutinosa* leaf discs. Shredded leaf matter FPOM and amphipod faeces are visible.

After 6 days the experiment was halted and any remaining leaf discs, from here on identified as coarse particulate organic matter (CPOM), were stored in ethanol to stop further microbial decomposition before reweighing. For measurement of CPOM samples, metal weighing boats were heated in a drying oven for 24 hours at 60°C and weighed. Individual CPOM samples were allocated to a weighing boat, to then be heated in the drying oven for 24 hours at 60°C. Boats and leaves were then weighed together and the mass of CPOM was calculated.

3.2.3 Metabolic rate measurements

The metabolic rates of *D. villosus* (unparasitised) and *G. pulex* (unparasitised/parasitised) were measured at 10°C (unparasitised *G. pulex* n = 17, parasitised *G. pulex* n = 17, *D. villosus* n = 12), 20°C (unparasitised *G. pulex* n = 21, parasitised *G. pulex* n = 11, *D. villosus* n = 18) and 25°C (unparasitised *G. pulex* n = 17, parasitised *G. pulex* n = 16, *D. villosus* n = 20). Animals were placed individually in plastic containers, as used in the shredding experiment above, with glass beads and aged tap water, with temperature gradually changed as required. The animals underwent a 24 hour starvation period ensuring that measurements were post-absorptive and unaffected by metabolism of food (Rowe *et al.*, 2001). Amphipods were dabbed dry on paper towels before weighing and placing in a closed circuit respirometry vial (diameter 15 mm, height 48 mm, volume 4 ml; © OXVIAL4) containing fully aerated water at the relevant temperature (figure 3.4). A small section of plastic mesh was also inserted into the vial to restrict amphipod movement and encourage natural clinging and resting behaviour to allow measurement of basal metabolic rate (Becker *et al.*, 2016). The amphipods were acclimatised for 30 minutes before measurements were taken. An optical oxygen sensor (Pyroscience© Piccolo2) was used to measure dissolved oxygen content of the water in mg/l (ppm) at the beginning of the test, and again after a 30-minute period, giving decrease in milligrams of oxygen per litre of water. This figure was converted to a rate by calculating reduction in oxygen per hour and adjusted for amphipod size to give a rate of oxygen consumption per gram of amphipod.

Figure 3.4: closed circuit respirometry vial as used to measure change in dissolved oxygen

c/o Pyroscience.com

<u>3.2.4 Invader relative impact potential metric</u>

The Relative Impact Potential Metric (RIP) incorporates relative consumer abundance as a means of scaling relative *per capita* effects (Dick *et al.*, 2017). The metric was used to compare the relative impact potential of these freshwater amphipod species. To calculate the relative impact potential the functional response asymptotes, or maximum feeding rates, of native and invasive species, as well as their relative abundances, are compared:

$$\text{RIP} \quad = \quad \left(\frac{\text{Invader FR}}{\text{Native FR}}\right) \times \left(\frac{\text{Invader abundance}}{\text{Native abundance}}\right)$$

Where the result of this equation is > 1 the invader can be regarded as having a greater potential impact on the invaded ecosystem than that exerted by the native resident. The higher the RIP score, the higher the impact of the invasive species, relative to the native. The RIP metric has previously been used to compare the relative impact of invasive predators (Warren, Bradbeer and Dunn, 2021) and algal uptake by filter feeders (Kemp, Tang and Aldridge, 2018). Here, we apply this metric for the first time to explore the impact of biological invasion on the key process of leaf shredding. As leaf detritus was supplied in excess, the feeding rate on leaf matter was used as a measure of consumption representative of the functional response curve asymptote maximum feeding rate. Abundance data were taken from Warren *et al* (2021), with additional data on parasite prevalence calculated as a percentage of the animals collected for this study.

3.2.5 Data analysis

All analyses were produced using R, with plots for shredding, metabolic rate and relative impact potential created using the package “ggplot2” (Wickham, 2016; R, 2019).

3.2.5.1 Shredding

Because of the significantly higher mass of *D. villosus* compared to *G. pulex* (Student’s t-test; $t = -14.36$, $p < 0.001$) and unparasitised *G. pulex* being larger than parasitised conspecifics ($t = 4.19$, $p < 0.001$) data were standardised by body mass (g). A general additive model (GAM) was constructed, using the “mgcv” package, to assess the effect of amphipod species, temperature, and parasitised status on rates of shredding (Wood, 2011). Temperature was modelled using a tensor smooth which improved model fit, and mass of leaf consumed per gram of amphipod was transformed using the natural log, which reduced heteroscedacity and improved residual distribution.

3.2.5.2 Survival

Survival statistics were modelled using a Cox proportional hazards model, with plots produced using Kaplan Maier product limit estimator curves using the “survival” package in R (Therneau, 2021).

3.2.5.3 Metabolic Rate

Metabolic rate was calculated by measuring milligrams of oxygen consumed per hour per gram of subject amphipod. To test for differences in metabolic rate between amphipod species and parasitised status a Quade's ANCOVA was carried out, with Wilcoxon pairwise post hoc tests. A one-way ANOVA was carried out to test for differences in metabolic rate for amphipods between temperature regimes, and post hoc Tukey's tests were used for pairwise comparisons of metabolic rate between temperatures.

3.2.5.4 Invader Relative Impact Potential

In the field, the abundance of *G. pulex* parasitised by *E. truttae*, was extremely low, at a mean of 4.38 individuals per m^2, compared to 164 per m^2 for unparasitised individuals, and 1176 individuals per m^2 for *D. villosus*. Therefore, due to low abundance, RIP analysis was not carried out using parasitised *G. pulex.* The abundance data showed that there are differences in the abundances of the native and the invasive amphipods in the field. Estimates were made based on multiple counts at multiple locations, with mean abundances of 83.280 (± 15.710) individuals per m^2 for *D. villosus* and 17.378 (± 4.486) per m^2 for *G. pulex* (Warren, Bradbeer and Dunn, 2021). Mortality did not differ between species by temperature treatment, therefore abundance data did not need to be adjusted, as the ratio of species' abundances remained the same, as both suffer mortality at the same rate under each temperature regime.

Variation and uncertainty were accounted for by using standard deviations of all data in probability density functions (Dick *et al.*, 2017).

3.3 Results

3.3.1 Shredding

Shredding rate differed significantly between amphipod species ($F_{(1,244)}$ = 142.30, $p < 0.001$), with *G. pulex* having a higher rate of shredding than *D. villosus* (Figure 3.5). Temperature had a significant effect on rates of shredding ($F_{(5,240)}$ = 76.07, $p < 0.001$). There was no significant interaction between temperature and species ($F_{(5,244)}$ = 2.37, $p = 0.13$), but temperature and parasitism status were found to significantly interact to affect rates of shredding ($F_{(10,228)}$ = 4.25, $p = 0.02$). The shredding rate of unparasitized *G. pulex* and *D. villosus* peaked at 15 and 20°C respectively, while the shredding of *G. pulex* infected with *E. truttae* had an accelerating rate of increase as temperature increased, with greater variation than the other treatments (figure 3.1). Comparing *G. pulex* treatments, there was no difference between parasitised and unparasitised amphipods ($F_{(1,155)}$ = 0.65, $p = 0.42$), but parasitised status interacted significantly with temperature ($F_{(11,155)}$ = 7.50, $p = 0.01$).

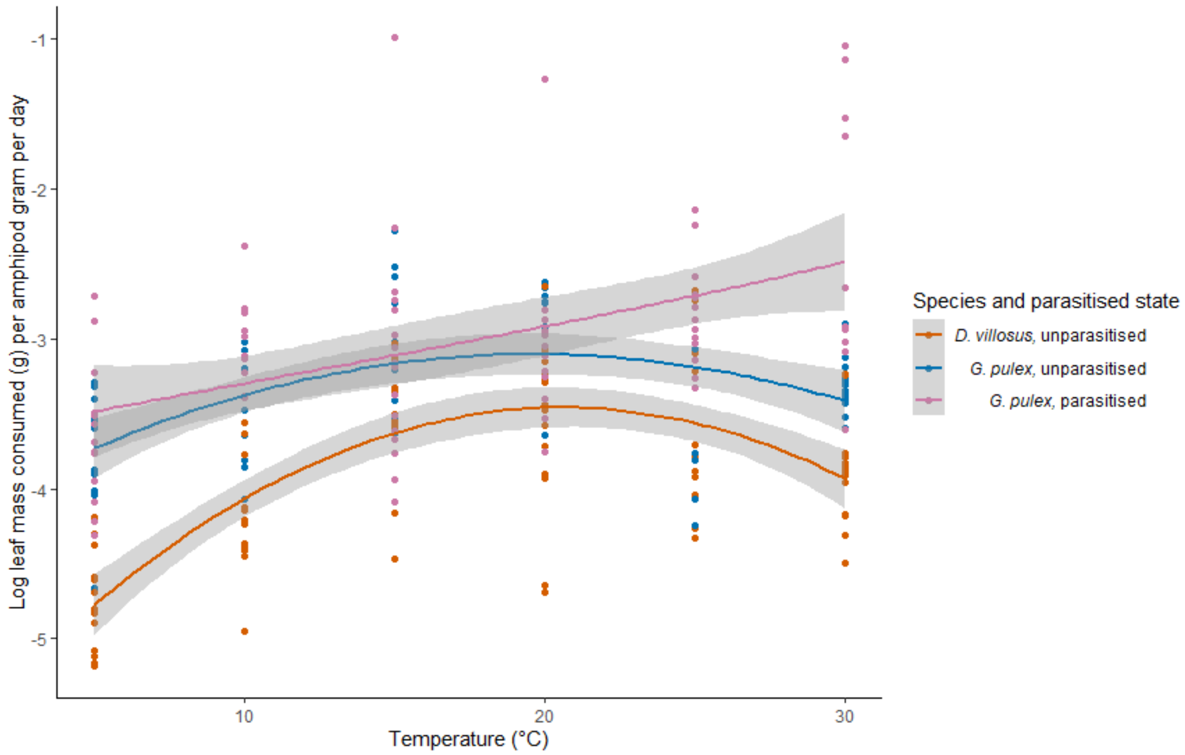

Figure 3.5: Relationship between rates of ln-transformed shredding for each amphipod treatment (species and parasitised state) and temperature, displaying standard error for Loess smoothed curves.

3.3.2 Survival

Temperature had a significant effect on survival (*z* = 7.79, *p* < 0.001), with a strong negative correlation identified (figures 3.6 a-c). Increasing temperature was associated with a 1.25 greater hazard of mortality (95% CI: 1.18, 1.32). Survival did not differ between species of amphipods (*z* = 0.73, *p* = 0.47), and no interaction was found between temperature and species (*z* = -1.32, *p* = 0.19). Survival over the experimental period did not differ between parasitised and unparasitised *G. pulex* (*z* = -1.28, *p* = 0.20), but an interaction was detected between temperature and time to mortality for unparasitised *G. pulex* (*z* = 2.66, *p* = 0.01). There was an increased likelihood of mortality occurring earlier in *G. pulex* which were not parasitised by *E. truttae*, than parasitised conspecifics, with a 1.06 greater hazard of earlier mortality (95% CI: 1.02, 1.11) (figure 3.6 b).

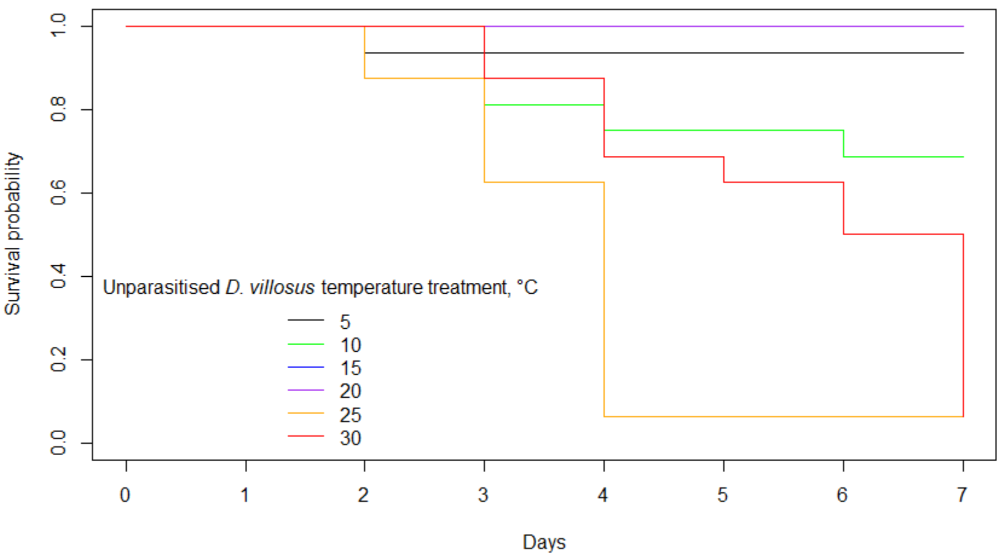

Figure 3.6 a): Kaplan Meier plot of survival by temperature treatment of unparasitised *Dikerogammarus villosus*

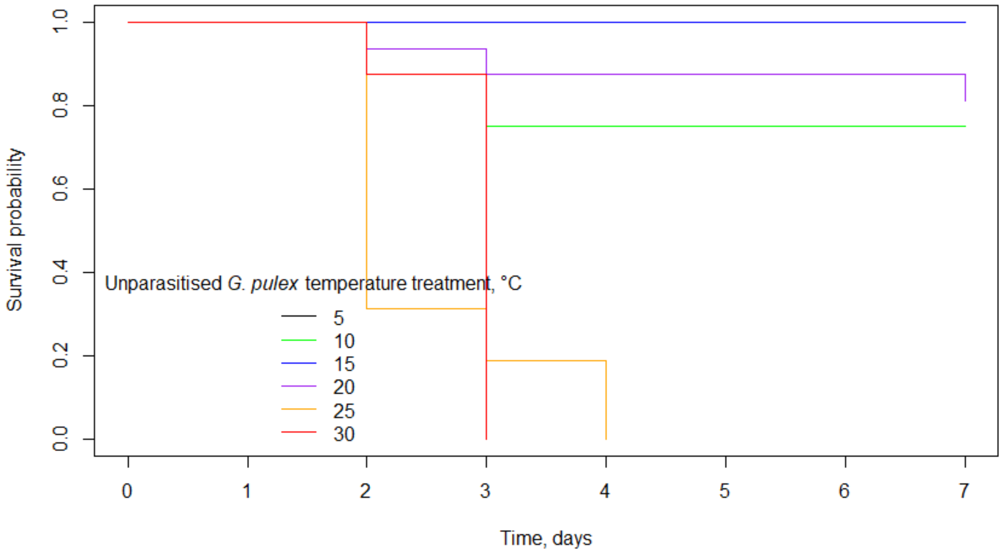

Figure 3.6 b): Kaplan Meier plot of survival by temperature treatment

of unparasitised *G. pulex*

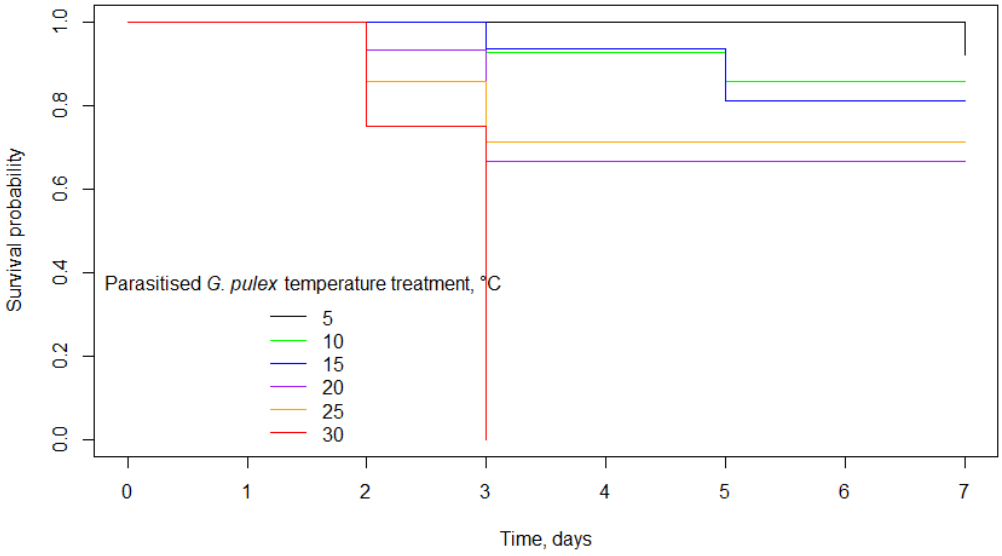

Figure 3.6 c): Kaplan Meier plot of survival by temperature treatment

of *G. pulex* parasitised by *E. truttae*

3.3.3 Metabolic rate

There was a significant difference in rate of oxygen consumption between temperature treatments (ANOVA $F_{(2,\ 147)}$ = 18.36, p < 0.001). Post hoc Tukey's tests found significant differences between all temperature treatments (10°C, 20°C, 25°C) in pairwise tests: 10 and 20°C (p < 0.001), 10 and 25°C (p < 0.001), 20 and 25°C (p < 0.01). No significant interaction between species and temperature on metabolic rate was found using Quade's ANCOVA: $F_{(2,4)}$ = 5.64, p = 0.07. Post hoc Wilcoxon pairwise tests revealed no significant differences in metabolic rate between species (p = 0.75) or parasitised status (p = 1) (figure 3.7).

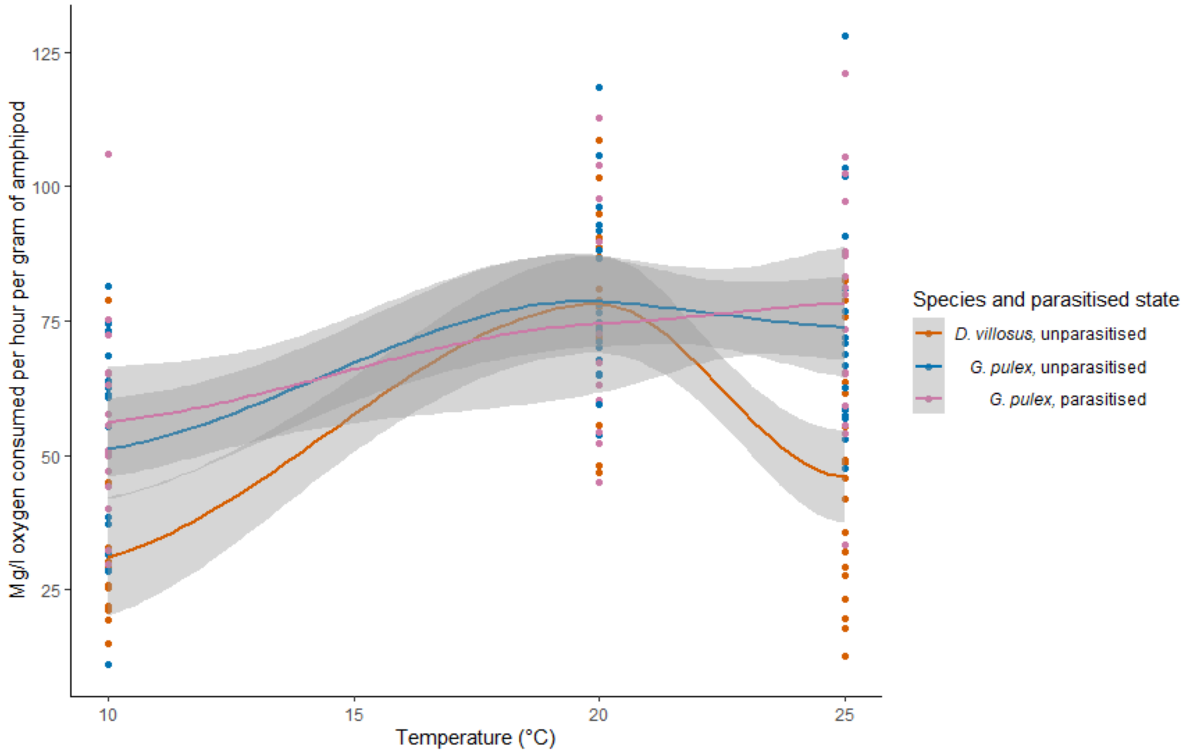

Figure 3.7: Oxygen consumption for parasitised and unparasitised *Gammarus pulex* and unparasitised *Dikerogammarus villosus* at 10, 20 and 25°C displaying standard error for Loess smoothed curves.

3.3.4 Invader relative impact potential

In all temperature regimes, the invasive *D. villosus* had mean RIP scores > 1 (Table 3.1). This indicates greater density-scaled shredding rates in the invader relative to the native species, with an increasing RIP score indicating a greater relative shredding rate. There was a general trend of increasing mean RIP scores with increasing temperature, from 4.82 at 5°C, with the highest RIP of 20.85 at 25°C, with RIP then falling to 7.56 at 30°C (Table 3.1).

Table 3.1: Relative impact potential (RIP) results by temperature treatment comparing the invasive *Dikerogammarus villosus* to the native *Gammarus pulex*. RIP scores > 1 indicate a predicted impact of the invasive compared to the native species in leaf shredding rate.

Temperature (°C)	Mean RIP	95% Confidence Interval		% Probability RIP > 1	% Probability RIP > 10
		Lower Limit	Upper Limit		
5	4.82	0.08	29.83	61.0	10.8
10	6.80	0.10	42.31	68.6	15.4
15	11.07	0.16	69.42	78.2	23.9
20	8.01	0.12	49.92	72.3	18.1
25	20.85	0.24	133.94	86.0	36.3
30	7.56	0.13	46.49	72.3	17.4

The higher potential impact of *D. villosus* is due to the extremely high densities at which this species is found in invaded sites, compared to the much lower densities at which *G. pulex* is found. Despite the lower maximum shredding rate of the invasive species, the higher abundance of the invader leads to higher RIP values which predict higher rates of shredding in ecosystems where the invader is present (Figure 3.8).

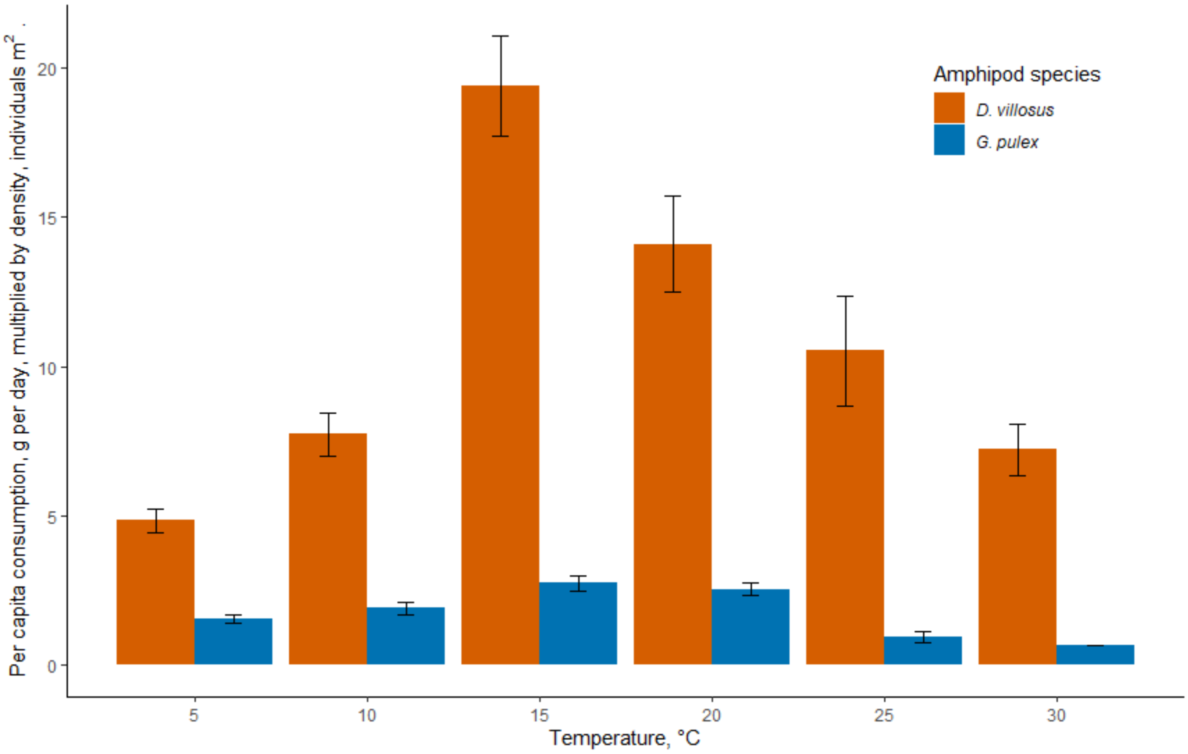

Figure 3.8: Relative impact potential of *Dikerogammarus villosus* and *Gammarus pulex* at different temperatures, calculated by multiplying *per capita* consumption of leaf detritus by amphipod density in field locations. Error bars represent ± 1 standard error.

3.4 Discussion

Impact on shredding rates of the invasive amphipod *D. villosus* is due to its abundance, rather than the shredding behaviour of individuals, as *G. pulex* has a higher *per capita* shredding rate, but exists in Great Britain at lower densities than the invasive species. The enemy release experienced by *D. villosus* in Great Britain facilitates high density populations which is likely to alter shredding and nutrient flow in invaded freshwater systems. Parasitism affected amphipod shredding, with the highest rate of shredding in *G. pulex* parasitised with *E. truttae*. At lower temperatures, parasitised *G. pulex* maintained a similar shredding rate to the other amphipod treatments, however at 20°C and above the shredding rate for parasitised *G. pulex* individuals increased rapidly. This correlates with a previous study that found predatory behaviour to increase with temperature in *G. pulex* parasitised with *E. truttae* (Laverty *et al.*, 2017). Although this increased food intake could suggest a higher energy demand by parasitised individuals, this was not reflected in an increase in metabolic rate. The relatively low prevalence of *E. truttae* infection in many locations means the increased shredding at higher temperatures is not likely to have a significant ecosystem effect, especially as shredding rates increase most at temperatures at which survival is reduced through species' thermal limits being exceeded.

Temperature was the significant factor affecting amphipod survival, with no interaction with species, as was found in a previous study (Wijnhoven *et al.*, 2003). The lack of difference in survival of high temperatures between the native and invasive species should be considered in light of the much higher population density of *D. villosus*. The mortality caused by sustained temperatures will cause a lesser relative impact to the invasive species population, which may facilitate replacement on invasion fronts. However, these predictions are based on current population densities, which does not take into account the impact of temperature on fecundity and development. Data is lacking for the focal amphipod species of this study, but research on other amphipod species indicates that higher temperatures generally decrease brood size, but also decrease development time (Maranhão and Marques, 2003; Jacobson, Prevodnik and Sundelin, 2008; Cardoso *et al.*, 2018). Additional research is required to investigate how this would alter the population size and structure of both native and invasive amphipods, as impacts of temperature may be amplified or lessened by changes in density over time. No effect on survival was exerted by parasitism in *G. pulex*. Acanthocephalan parasites are transmitted from the intermediate invertebrate host when it is predated by the definitive host; survival of the intermediate host is vital to allow for trophic transmission of the parasite.

Parasitism did not affect the metabolic rate of the host, in contrast with findings of Labaude *et al*. (2015), who reported increased metabolic rate in *G. pulex* infected with the acanthocephalan *Pomphorhynchus laevis*. The relatively large size of *E. truttae* means it is surprising that metabolic rate was not affected, but it may reflect an equivalence in metabolic activity for *G. pulex* and *E. truttae* tissues by mass, as well as selection on the parasites to trade-off energetically and metabolically costly processes, such as growth, with the survival of their host (Dianne *et al.*, 2012). The oxygen requirements and temperature-related mortality did not differ between the native and invasive species, therefore suggesting the invasive species is not better adapted to climate change warming or increasing frequency of high temperature extremes.

The impact of the invasive *D. villosus* on resource processing will depend on both its leaf shredding capability, relative to the native species that it replaces, its predation of native shredders to alter shredder community structure, and on its relative abundance in an invaded location. *D. villosus* typically reaches much higher densities in their invaded range compared to densities reported for *G. pulex* (Rewicz *et al.*, 2014; Warren, Bradbeer and Dunn, 2021). The RIP metric has previously been used to predict the potential ecological impact of predation by invasive species (Dick *et al.*, 2017; Dickey *et al.*, 2018). Here we use the RIP metric to explore the potential impact of species and temperature on amphipod shredding. Although *per capita* shredding was higher for *G. pulex* than *D. villosus* at all temperatures, the higher abundances of this invader led to higher RIP scores for *D. villosus* at all temperatures; hence replacement of the native by the invasive amphipod is predicted to drive an increase in shredding. This could be interpreted as a positive effect on ecosystem function, with a higher rate of shredding leading to faster accumulation of amphipod biomass and a greater rate of FPOM provisioning for the ecosystem. However, the high density of invaders compared to natives may lead to exhaustion of the resource in sites with relatively low leaf detritus levels. In addition, the flexible feeding habits of *D. villosus* could affect community structure, as competition for resources may lead to increased predation of macroinvertebrates and fish eggs as the detrital resource reduces, the *D. villosus* population matures and its trophic level increases (Platvoet *et al.*, 2009; Mancini *et al.*, 2021). The high density of invasive shredders may also increase the nutrient load of affected water courses, with the increase in FPOM leading to possible eutrophication in freshwater systems with high seasonal allochthonous inputs (Doherty-Bone, Dunn, Liddell, *et al.*, 2018). Such increases in the nutrient load can alter planktonic communities, leading to algal blooms and cascading impacts to food webs (Adamczuk *et al.*, 2019).

The results demonstrate that an interaction of the stressors of temperature, invasive species and parasites can affect the ecosystem process of shredding. The invasive amphipod *D. villosus* has a lower *per capita* rate of shredding than the native *G. pulex* but exists in higher densities in the environment which means that its replacement of the native species is predicted to lead to increased processing of detritus resources. Although rates of shredding were found to increase with temperature, it is likely that processing of detritus will decline severely if temperatures exceed the thermal tolerances of the amphipod shredders. Once thermal optima are exceeded it is likely that shredders will seek thermal refugia, which may limit shredding activity compared to normal foraging behaviour. Thus, shredding activity would be limited by mortality or amphipods moving away from detrital resources to seek a more favourable thermal situation (Kenna *et al.*, 2017). In previous studies the native *G. pulex* has been found to be slightly more tolerant of high temperatures than *D. villosus*, with mortality of all individuals at 34.9 ± 0.2°C compared to the invasive's 32.3 ± 0.7°C in a study of relatively rapid temperature increases (Verberk *et al.*, 2018). In sustained high temperatures *G. pulex* experienced mortality of all individuals at 30°C compared to 27°C for *D. villosus* (Maazouzi *et al.*, 2011). These results are similar to what was found in this study, with the effects of sustained high temperatures leading to high mortality below a theoretical thermal limit for the species. This may be due to cumulative deleterious impacts on mitochondria, as a previous experiment has found temperatures of 30°C and above limited ATP production in *G. pulex* (Cottin *et al.*, 2012).

The fine particulate organic matter (FPOM) produced by shredding activity supports a community of collector species, from gathering collectors including mayfly nymphs and midge larvae, to filtering collectors such as blackfly larvae and mussels (Cummins and Klug, 1979). The combined effects of invasive species and increasing temperatures could have significant impacts on freshwater communities and potentially cascading effects to connected ecosystems (de Omena, Srivastava and Romero, 2017).

Chapter 4: Invasive riparian plants may support freshwater ecosystem function alongside stressors of temperature and invasive shredder species

4.1 Introduction

Multiple stressors exert pressures upon ecosystems, affecting the efficiency and productivity of processes within those systems (Brook, Sodhi and Bradshaw, 2008; Orr *et al.*, 2020). Biotic and abiotic stressors can interact to impact the structure and function of ecosystems, with anthropogenic factors accelerating the rate of environmental change (Chapin *et al.*, 2000; Sala *et al.*, 2000). The biotic stressor of introductions of invasive species is increasing, with globalisation facilitating increasing rates of introduction and greater propagule pressures (Seebens *et al.*, 2017; Essl *et al.*, 2020; Pyšek *et al.*, 2020). Temperature, due to climate change, is an abiotic factor that is an increasing stressor of ecosystems due to increasing mean temperatures, as well as the increasing frequency and severity of extreme events such as heatwaves (Jenkins *et al.*, 2009; Pachauri *et al.*, 2014). More research is required into how combinations of these stressors interact, as drivers of diversity and function loss rarely occur in isolation, with studies of interacting factors better reflecting the conditions to which natural ecosystems are subjected (Brook, Sodhi and Bradshaw, 2008; Côté, Darling and Brown, 2016; Birk *et al.*, 2020; Orr *et al.*, 2020). In this chapter I explore interactions of native and invasive species, and how temperature can affect those interactions to impact a vital ecosystem process.

Invasive species are one of the main drivers of biodiversity loss and environmental degradation globally, altering the structure and function of ecosystems (Sala *et al.*, 2000; Clavero and García-Berthou, 2005; Bellard, Cassey and Blackburn, 2016). Introductions of species to novel ranges have been occurring for thousands of years. However, the rapid opening of trade and transport routes due to globalisation has accelerated the rate of introductions, which are driving extirpations of native species and impacting the functioning of ecosystem processes and services (Bright, 1999; Cardinale, Palmer and Collins, 2002; Pejchar and Mooney, 2009; Early *et al.*, 2016). The introduction of invasive species can lead to novel ecological interactions between invasives and the native species already present in the ecosystem, as well as interactions between multiple invasive species introduced to the same novel range (Heger *et al.*, 2019). Novel species interactions can be unpredictable, owing to a lack of a shared evolutionary history for the organisms. Possible advantages or disadvantages are conferred by species traits and adaptability to novel interaction partners and environments, including environmental conditions such as temperature (Sih, Ferrari and Harris, 2011; Saul, Jeschke and Heger, 2013; Saul and Jeschke, 2015). Novel interactions between species can be neutral or may also be antagonistic, with species inhibiting each other's success through direct trophic mechanisms such as predation, or indirect interactions (Jackson, 2015; Kuebbing and Nuñez, 2015). Interactions between species may also be positive or synergistic, with mutualisms between species amplifying the impact they have on an ecosystem. Such interactions can increase the abundance and activity of interacting species, including introduced invasives, and may alter ecosystem processes and suppress the biotic resistance of the ecosystem to further invasions (Simberloff and Von Holle, 1999; Green *et al.*, 2011).

Riparian zones are important borders between terrestrial and aquatic habitats, acting as an interface through which energy and biomass move between adjacent ecosystems. Temperate freshwater ecosystems are often reliant upon the supply of allochthonous leaf litter from riparian habitats (Webster and Benfield; Gessner *et al.*, 2010). The breakdown of this detrital resource by physical and microbial decomposition, and importantly the activity of a guild of shredder species, is the foundation of trophic webs in such ecosystems (Cummins and Klug, 1979; Covich *et al.*, 2004). However, riparian zones are commonly subjected to human-mediated disturbance, including introductions of invasive non-native plant species (Gerber *et al.*, 2008; Hladyz *et al.*, 2011). The alteration of allochthonous inputs by invasive plants to temperate freshwater systems can have an impact on the structure and function of the aquatic ecosystems, affecting energy flows within and between the riparian and aquatic habitats, reflecting the close links between the neighbouring ecosystems (Dekanová et al., 2021; Richardson et al., 2007; Seeney et al., 2019; Vilà et al., 2011). For example, a study found invasive riparian plants are likely to negatively impact growth of aquatic insects due to poor nutritional content and short duration of leaf litter (Going and Dudley, 2007). The high carbon : nitrogen ratio of many invasive plant species, such as knotweeds, may have cascading effects, reducing the growth of species which rely on the allochthonous leaf litter, and the predators which prey on them in turn (Graça, 2001; Urgenson et al., 2009).

As well as invasive leaf litter, aquatic ecosystems are also subject to additional stressors, such as species of invasive macroinvertebrates. A guild of macroinvertebrate shredder species play a key role in breaking down leaf litter in freshwater ecosystems (Cummins et al., 1989; W. Cummins, 2019). The invasive amphipod *Dikerogammarus villosus* (Sowinsky, 1894), from the Ponto-Caspian region (Rewicz et al., 2014) is replacing native amphipod shredders in Western Europe. Alteration of the primary leaf resource or the shredder processors of that resource could disrupt trophic webs in aquatic ecosystems with consequences for productivity and energy supply to neighbouring ecosystems as different shredder species have different leaf preferences and different physiological digestive capabilities, which may change rates of shredding in novel leaf-shredder combinations (Bärlocher and Porter, 1986). The unpredictability of novel interactions means that outcomes of different combinations of native and invasive species, whether allochthonous leaf litter or the shredder species which process that resource, require further research.

In the UK, riparian zones have been subject to numerous plant invasions, particularly rhododendrons and knotweeds. *Rhododendron ponticum* is an invasive shrub now found throughout the UK since its introduction in 1763 as an ornamental plant for gardens and parks and cover for game birds (Elton, 1958). The species is commonly found in riparian habitats in the UK, dominating the leaf litter in invaded sites (Dehnen-Schmutz et al., 2004). The species grows rapidly and limits the growth of competing native plants by light reduction, acidification of soil and allelopathic chemicals to deter herbivory and retard growth of competing plants, resulting in monocultures dominating invaded riparian habitats (Cross, 1975; En et al., 2006). Phenolic compounds and grayanotoxins in the leaves of *R. ponticum*, as well as its relatively poor quality as a food source, could reduce the productivity of shredding activity in waterways adjacent to invaded riparian zones and impact the survival of species engaging in herbivory (Cross, 1975; En et al., 2006; Hladyz et al., 2011; Hladyz et al., 2009). *R. ponticum* toxins have been found to be lethal for the European honey bee, *Apis mellifera,* to have sublethal toxic effects on a mining bee, *Andrena carantonica*, but to have no toxic effect on *Bombus terrestris* bumblebees (Tiedeken *et al.*, 2016). The invasive shrub *R. ponticum* has affected freshwater communities in Ireland, having a lower decomposition rate than native leaf litter, and supporting a lower abundance of shredder species, such as the native amphipod *Gammarus duebeni* (Hladyz *et al.*, 2011). Similarly, Doherty-Bone (2018) found that the decomposition rate of *R. ponticum* in invaded waterways was lower than that of other native leaf and non-native sycamore leaf litter. However, there is a lack of research into the effect of *R. ponticum* leaf litter on aquatic invertebrates.

Another allelopathic invasive plant present in North American, continental European and UK riparian zones is *Fallopia japonica*, or Japanese knotweed (Lecerf *et al.*, 2011; Kato-Noguchi, 2021). The exudation of allelochemicals to inhibit competing species can cause dense monocultural stands of *F. japonica*, which leads to domination of allochthonous leaf litter inputs to waterways adjacent to invaded habitats (Kato-Noguchi, 2021). *F. japonica* has been shown to reduce diversity of terrestrial macroinvertebrates in affected ecosystems (Gerber *et al*., 2008; Seeney *et al*., 2019a), but effects on aquatic macroinvertebrates vary, with a study finding decreases in diversity (Seeney *et al*., 2019) and others finding similar macroinvertebrate diversity in waterways invaded by F. japonica (Christopher *et al*., 2014; Fogelman *et al*., 2018). Vitally however, the rate of decomposition of leaf litter was found to be higher with increasing dominance of riparian *F. japonica* (Lecerf *et al.*, 2011), or to occur at a similar rate to that of native leaf species in several other studies, which may help maintain ecosystem processes and productivity (Braatne *et al*., 2007; Dangles *et al*., 2002; Fogelman *et al.*, 2018).

Amphipods are key shredder species in leaf litter dominated temperate freshwaters, with a central role in trophic webs (Cummins *et al.*, 1989). These amphipod shredders, responsible for much of the energy and carbon cycling in these ecosystems, contains numerous highly invasive species of amphipods which have altered freshwater communities across Europe in recent years (Bacela-Spychalska & Van Der Velde, 2013; Bij De Vaate et al., 2002; Rewicz et al., 2015). For example, the killer shrimp *D. villosus*, has become invasive throughout most major mainland European waterways since the 1990s, and is now found in the UK (Rewicz et al., 2014). *D. villosus* is a highly successful invader, replacing many native species through high individual and population growth rates, tolerance of a wide range of conditions and strong predatory behaviours (Pöckl, 2009). Replacement of native shredders by an invasive species, such as *D. villosus*, can lead to changes in the structure of the trophic web in the ecosystem, and impact productivity by affecting rates of shredding of the vital leaf detritus resource (Krisp and Maier, 2005; Little and Altermatt, 2018). However, more research is required into interactions of *D. villosus* and invasive leaf litter species, which I seek to investigate with this study.

Temperature plays a significant role in the functioning of freshwater detritus-based ecosystems (Gessner *et al.*, 2010). Increasing temperatures due to climate change can disrupt aquatic assemblages, and may favour tolerant invasive species, affecting macroinvertebrate communities and associated traits (Daufresne, Bady and Fruget, 2007). Temperature and shredding rate have been shown to be positively correlated in our previous chapters, as well as other studies investigating the invasive *D. villosus* and its native analogue *Gammarus pulex* (Linnaeus, 1758) (Kenna et al., 2017; Truhlar et al., 2014). A change of shredder species may make the rate of shredding more temperature-sensitive and alter the phenology of the availability of leaf detritus (Alp et al., 2016). Some studies have found invasive amphipods to shred less than native species, but to increase their shredding rate faster than natives at higher temperatures (Truhlar, Dodd and Aldridge, 2014; Constable and Birkby, 2016; Kenna *et al.*, 2017; Little and Altermatt, 2018). However, I found no difference in shredding rates between the native and the invasive at a range of temperatures in chapters 2 and 3, and although increasing temperature may increase the rate of shredding in amphipods it can also lead to decreased survival if thermal limits are exceeded (Galic and Forbes, 2017).

The impacts of interactions of these stressors of invasive species and climate warming on freshwater ecosystems are currently understudied (Gallardo et al., 2016) Furthermore , few studies have investigated the impacts of invasive leaf detritus and invasive shredder species in combination (Doherty-Bone, Dunn, Brittain, *et al.*, 2018), and no current studies combine these factors with the additional stressor of elevated temperature. This study investigates the interactions of the stressors of invasive allochthonous leaf detritus (*R. ponticum* and *F. japonica* compared to native alder, *Alnus glutinosa*), invasive shredders (*D. villosus* compared to native *G. pulex*) and temperature. Comparisons are made between the survival of the native and invasive shredders with each leaf type at a range of temperatures, and temperature-linked rates of leaf shredding, which relates to ecosystem function. The invasive leaf species *R. ponticum* and *F. japonica* contain allelopathic chemicals (Cross, 1975; En, Nilsen and Oktay Yildiz, 2006; Kato-Noguchi, 2021)), and have a lower nutritional content than native *A. glutinosa* (Aguilera et al., 2010; Hladyz et al., 2011). Therefore, I predict that *A. glutinosa* leaf detritus will be more palatable than the invasive leaf species, leading to greater shredding of *A. glutinosa* leaf litter and a higher survival rate for amphipods supplied with the native leaf litter. In macroinvertebrates, metabolic rate is positively correlated with temperature, up to an optimum level and I have previously found that increasing temperatures increases rates of shredding. Here I therefore predict that there will be higher rates of shredding at higher temperatures. In chapter 3 I found that there was no difference in patterns of survival or shredding related to temperature between *G. pulex* and *D. villosus*. Similarly, I expect to find the same, predicting there will be no difference between the invasive and the native amphipod species in survival or shredding rates.

4.2 Methods

4.2.1 Experimental material

G. pulex were collected by kick-sampling from Meanwood Beck, West Yorkshire (53.8301°N, -1.5746°E) (figure 4.1). *D. villosus* were collected from Grafham Water, Cambridgeshire (52.2909°N, -0.0323°E). All amphipods were kept for a minimum of 5 days acclimatisation period prior to experiments, in species-specific communal tanks in a controlled temperature room at 15 ± 0.1°C S.D., with a 12:12 hour light/dark cycle (08:00-20:00). Tanks were filled with 5l aerated aged tap water and study organisms were fed *ad libitum* with native leaf litter containing *A. pseudoplatanus*, *Q. robur* and *A. glutinosa*.

Figure 4.1: Meanwood Beck, Meanwood Park c/o Wikipedia

Native leaf material selected for experimental use was common alder (*A. glutinosa*) as amphipod species have demonstrated a preference for the leaf detritus of this species, which is commonly found in riparian habitats throughout both amphipod species' ranges (Waring and Running, 2007; Bloor, 2011). *R. ponticum* was selected as an invasive leaf species as it is probably the "major alien environmental weed in the British Isles" (Preston *et al.*, 2011), which can form dominant stands, excluding other species (Cross, 1975). *R. ponticum* grows well in wet conditions and can out-compete native plants to dominate allochthonous inputs to aquatic ecosystems in riparian habitats (Hladyz *et al.*, 2011). The second invasive leaf species selected was *F. japonica* (Japanese knotweed), which can also form monocultures in invaded riparian zones, replacing native plants and altering allochthonous inputs to detritus-dependent freshwater ecosystems (Aguilera *et al.*, 2010; Murrell *et al.*, 2011).

Leaves were collected as autumn leaf fall and air-dried, followed by conditioning in stream water from Meanwood Beck, West Yorkshire for two weeks in order to increase palatability through microbial colonisation and decomposition (Bloor, 2011). A cork-borer was used to cut discs of diameter 12 mm, avoiding midribs and veins from each leaf species, which were then air-dried and weighed. Due to differing leaf thicknesses and densities an equal mass (44.43 ± 7.44 mg S.D.) of each leaf type was approximated by grouping into different numbers of leaf discs: *A. glutinosa* n = 10, *F. japonica* n = 7, *R. ponticum* n = 3. Prior to use in the experiment the leaf discs were reconditioned for 48 hours in Meanwood Beck stream water to allow microbial recolonisation, increasing palatability and nutrition of the detrital resource (Nelson, 2011).

4.2.2 Experimental design

Leaf shredding and survival rates of *G. pulex* and *D. villosus* with leaf discs of either *A. glutinosa*, *R. ponticum* or *F. japonica* were measured at a range of temperatures. Amphipods were weighed after excess water was removed by placing the individual on a paper towel. Animals were placed individually in transparent, circular plastic containers (diameter 7 cm, depth 5 cm) with 250 ml aged tap water. Two transparent glass beads were placed in the containers, which allows observation whilst preventing excess thigmotactic swimming behaviours (Perrot-Minnot, Banchetry and Cézilly, 2017; Kohler, Parker and Ford, 2018). The containers were placed in incubators at 15°C with a 12:12 hour light/dark cycle and the animals underwent a 24-hour starvation period to standardise hunger. The incubator temperature was either increased or decreased at a rate of 1°C every 2 hours, until the relevant treatment temperature was reached. Four temperature treatments were selected as close to equally spaced between 5 and 25°C as possible, given the incubator's 0.5°C gradations: 5°C, 11.5°C, 18°C and 25°C.

Table 4.1: Numbers of replicates of each combination of amphipod and leaf species at each experimental temperature for the survival element of the experiment

	G. pulex			*D. villosus*		
Temperature (°C)	*A. glutinosa*	*F. japonica*	*R. ponticum*	*A. glutinosa*	*F. japonica*	*R. ponticum*
5	20	20	20	20	19	20
11.5	20	20	20	20	20	20
18	20	20	20	20	20	20
25	20	20	20	20	20	20

After the starvation period, leaf discs were added to each container. Animals were checked every 24 hours, and mortality was recorded. Mortality was identified by a lack of pleopod beating and lack of reaction to a physical stimulus. Some mortality occurred during the initial 24-hour hunger standardisation period, before leaf litter was supplied to the subjects, so the shredding experiment had a lower number of replicates than the initial total used for the survival data.

Table 4.2: Numbers of replicates of each combination of amphipod and leaf species at each experimental temperature for the shredding element of the experiment

	G. pulex			*D. villosus*		
Temperature (°C)	*A. glutinosa*	*F. japonica*	*R. ponticum*	*A. glutinosa*	*F. japonica*	*R. ponticum*
5	19	20	20	20	19	20
11.5	20	20	19	20	20	20
18	19	19	20	20	20	20
25	15	13	18	20	20	20

After 6 days the experiment was halted and the remaining coarse particulate organic matter (CPOM) was stored in ethanol to stop further microbial decomposition. CPOM samples were placed in aluminium weighing boats and heated in a drying oven for 24 hours at 60°C before reweighing.

4.2.3 *Rhododendron ponticum* toxicity

As our initial observations suggested lower survival of amphipods when provided with *R. ponticum* leaf litter, I conducted an additional experiment to explore whether mortality was related to ingestion of *R. ponticum* leaf material or toxic leachates passively absorbed by amphipods. Effects of grayanotoxins from *R. ponticum* has been researched in humans, farmed mammals and some bee species, but there is a lack of research into the effects of *R. ponticum* on aquatic invertebrates (Bhardwaj *et al.*, 2013; Jansen *et al.*, 2012; Tiedeken *et al.*, 2016). I compared survival of *G. pulex* and *D. villosus* when exposed to R. ponticum leaf litter, but unable to consume it, with survival in control conditions. In both treatments, with or without *R. ponticum*, *A. glutinosa* leaf litter was provided as food. Containers were constructed using two transparent, circular plastic containers (diameter 7 cm, depth 5 cm), placed one inside the other. The bottom of the inner container was removed and replaced with 1mm plastic mesh which allowed water movement between containers but not movement of amphipods or coarse particulates of leaf detritus. A cork borer was used to cut 12 mm discs of *R. ponticum* leaf, and 15 discs were placed in the outer container, whilst control containers were left empty of *R. ponticum*. A single amphipod of either native *G. pulex* or invasive *D. villosus* was placed in the inner container with a section of *A. glutinosa* leaf litter, 250 ml of aged tap water and two glass beads (Figure 4.2). Subjects were placed in incubators at 15°C with a 12:12 hour light/dark cycle and survival was recorded every 24 hours for seven days. Each of the 4 treatment groups, *G. pulex* with *R. ponticum*, *G. pulex* control group, *D. villosus* with *R. ponticum* and *D. villosus* control group, had 25 replicates.

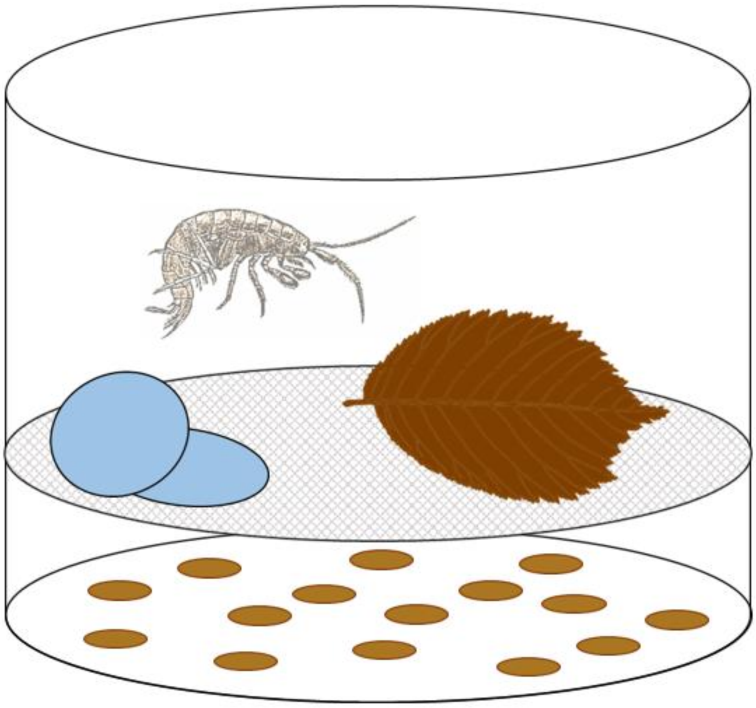

Figure 4.2: Experimental arena for *R. ponticum* toxicity experiment, showing amphipod, *A. glutinosa* leaf, glass beads above the mesh layer to exclude amphipod from physical access to discs of *R. ponticum* below the mesh (*R. ponticum* not present in control groups). Amphipod image courtesy of Florida Centre for Instructional Technology, University of South Florida.

4.2.4 Data analysis

All analyses were produced using R, with plots for shredding created using the package “ggplot2” (Wickham, 2016; R, 2019).

4.2.4.1 Shredding

Data were standardised for amphipod size by dividing shredding rates by amphipod body mass (g). The R package “mgcv” was used to construct a general additive model (GAM) to assess the impact on rates of shredding of amphipod species, leaf litter species and temperature (Wood, 2011). The mass reduction of leaf litter per gram of amphipod, divided by the number of days of leaf consumption, was transformed using the natural log, which improved model fit, reducing heteroscedacity and improving residual distribution. Temperature was modelled using restricted maximum likelihood (REML) smoothing with a cyclic cubic regression spline, which provided the best model fit. Removal of non-significant factors was carried out and models were compared using Akaike’s Information Criterion (AIC).

4.2.4.2 Survival

Survival plots were created with Kaplan Maier product limit estimator curves using the “survival” package in R. Survival statistics were modelled using a Cox proportional hazards model using the “survival” package in R (Therneau, 2021).

4.2.4.3 *Rhododendron ponticum* toxicity

R. ponticum toxicity was assessed using the R package "survival" by constructing a Cox proportional hazards model (Therneau, 2021). Kaplan Maier product limit estimator curves were produced using the "survival" package to plot survival curves for *G. pulex* and *D. villosus* amphipods exposed to dissolved exudates of *R. ponticum*, or control groups with no *R. ponticum* exposure.

4.4 Results

4.4.1 Shredding

There was no interaction of temperature and species of amphipod ($t_{(7,460)}$ = -0.89, p = 0.38), therefore this interaction was removed from the model (AIC Δ = 3.6). Species of amphipod and species of leaf did not interact: *G. pulex* and *F. japonica* ($t_{(5,460)}$ = 1.31, p = 0.19), *G. pulex* and *R. ponticum* ($t_{(5,460)}$ = 0.26, p = 0.79), in comparison to the baseline reference species *A. glutinosa* and *D. villosus*. Therefore, this interaction was removed from the model (AIC Δ = 2.0). Temperature was significantly positively correlated with rates of shredding ($F_{(2.58,460)}$ = 1377, p < 0.01), modelled using restricted maximum likelihood (REML) for log-transformed shredding rates (Figure 4.3). Mean shredding rates, including all amphipod and leaf species, were lowest at 5°C (30.1 ± 15.9 S.D. milligrams of leaf consumed per gram of amphipod per day, hereafter referred to as mg per day) and were more than doubled at their highest at 25°C (68.7 ± 35.0 SD. mg per day). Amphipod species was also a significant factor, with native *G. pulex* shredding at a higher rate than invasive *D. villosus* ($t_{(1,460)} = 13.44$, $p < 0.01$) (Table 4.3).

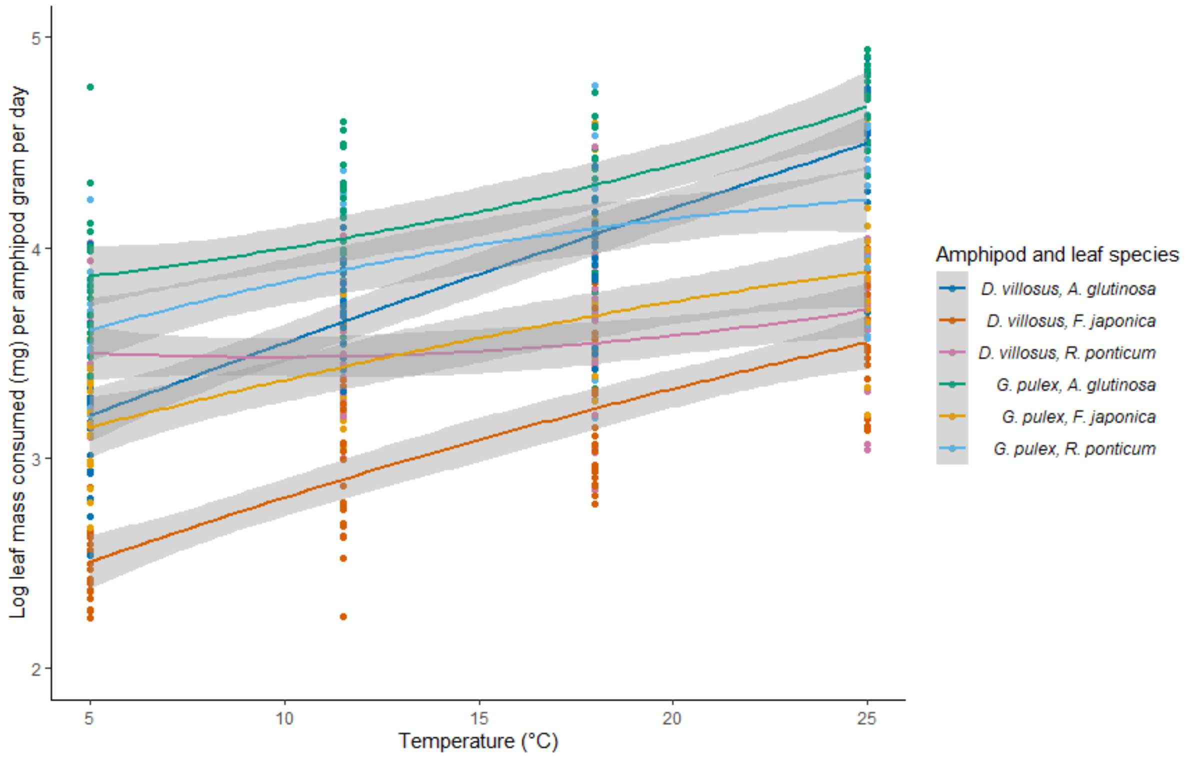

Figure 4.3: Relationship between temperature and log-transformed rates of shredding for each amphipod and leaf detritus species combination, displaying one standard error with Loess-smoothed curves.

The species of leaf detritus significantly affected shredding rates, with both invasive species *F. japonica* ($t_{(2,460)} = -7.83$, $p < 0.01$) and *R. ponticum* ($t_{(2,460)}$, $p = 0.01$) being shredded at a lower rate than native *A. glutinosa*. The rate of shredding of *A. glutinosa* was more than double that of *F. japonica* at all temperatures. The mean shredding rate for *A. glutinosa* and

F. japonica more than doubled between the lowest and highest temperatures 5 and 25°C, correlated with lowest and highest shredding rates (*A. glutinosa*: 36.9 ± 19.5 S.D. to 104 ± 26.1 S.D. mg per day; *F. japonica*: 17.9 ± 7.9 S.D. to 42.2 ± 15.4 S.D. mg per day), while *R. ponticum* shredding rate increased by approximately 60% (35.5 ± 9.9 S.D. to 58.2 ± 26.3 S.D. mg per day). The native leaf *A. glutinosa* and the invasive *F. japonica* had a similar relationship to temperature ($t_{(5,460)}$ = -1.23, p = 0.22), however, invasive *R. Ponticum* differed from the other leaf species in interaction with temperature ($t_{(5,460)}$ = -6.55, p < 0.01).

Table 4.3: Mean and standard deviation of shredding rate in milligrams per amphipod gram per day for each treatment temperature, amphipod and leaf detritus species

		Temperature (°C)							
		5		11.5		18		25	
Amphipod spp.	Leaf spp.	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
G. pulex	*A. glutinosa*	49.1	20.0	64.6	19.7	72.2	23.9	115.0	20.7
	F. japonica	23.3	7.5	30.8	10.2	45.1	20.0	50.7	19.3
	R. ponticum	36.8	10.3	56.7	13.7	58.2	23.4	76.1	27.4
D. villosus	*A. glutinosa*	25.3	9.4	42.5	8.1	55.1	13.0	96.5	27.5
	F. japonica	12.1	2.0	20.8	8.4	25.00	8.8	36.5	8.6
	R. ponticum	34.3	9.6	33.6	9.2	37.2	15.6	42.00	10.1

4.4.2 Survival

Temperature significantly impacted the survival of amphipods (*z* = 3.55, *p* < 0.01), with a negative correlation between temperature and survival (HR = 1.12, 95% CI: 1.05, 1.19) (Figures 4.4 a-f). Survival was not found to be impacted by the species of leaf litter supplied to experimental amphipods, either *F. japonica* (*z* = 0.35, *p* = 0.73) or *R. ponticum* (*z* = 1.31, *p* = 0.19), compared to the native leaf *A. glutinosa*. There was no interaction between temperature and species of leaf litter for the invasives *F. japonica* (*z* = 1.23, *p* = 0.22) or *R. ponticum* (*z* = 0.08, *p* = 0.94) compared to native *A. glutinosa*. Similarly, no interaction was found between species of amphipod and species of leaf litter (*G. pulex* and *F. japonica*: *z* = -0.07, *p* = 0.94; *G. pulex* and *R. ponticum*: *z* = -0.59, *p* = 0.56) compared to the baseline reference species *D. villosus* and *A. glutinosa*. Survival did not differ between the native *G. pulex* and the invasive amphipod *D. villosus* (*z* = -0.43, *p* = 0.66), however, there was an interaction between temperature and amphipod species (*z* = 2.84, *p* < 0.01), with lower survival for native G. pulex with increasing temperature (HR = 1.12, 95% CI: 1.04, 1.22).

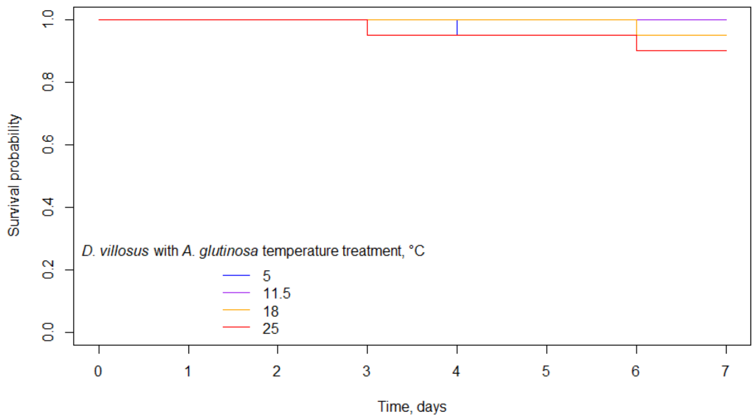

Figure 4.4 a): Kaplan Meier plot of survival of invasive *D. villosus* amphipods supplied with native *A. glutinosa* leaf litter at each treatment temperature

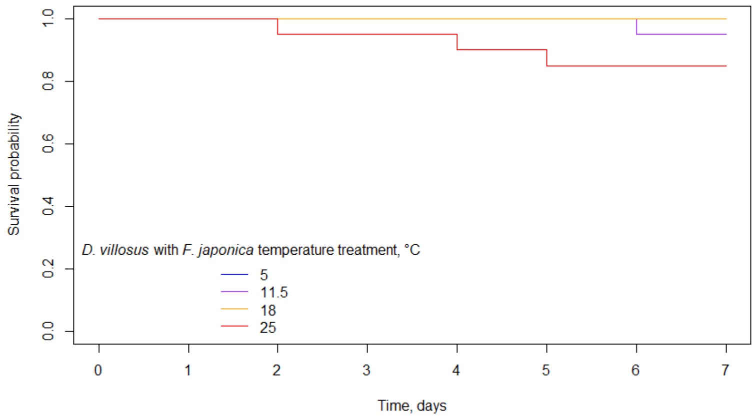

Figure 4.4 b): Kaplan Meier plot of survival of invasive *D. villosus* amphipods supplied with invasive *F. japonica* leaf litter at each treatment temperature

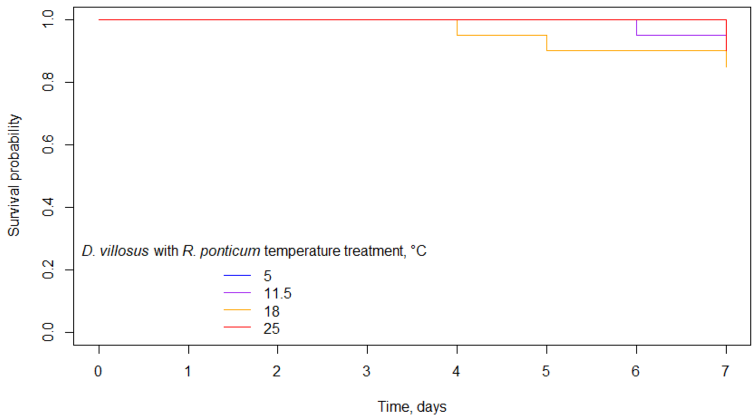

Figure 4.4 c): Kaplan Meier plot of survival of invasive *D. villosus* amphipods supplied with invasive *R. ponticum* leaf litter at each treatment temperature

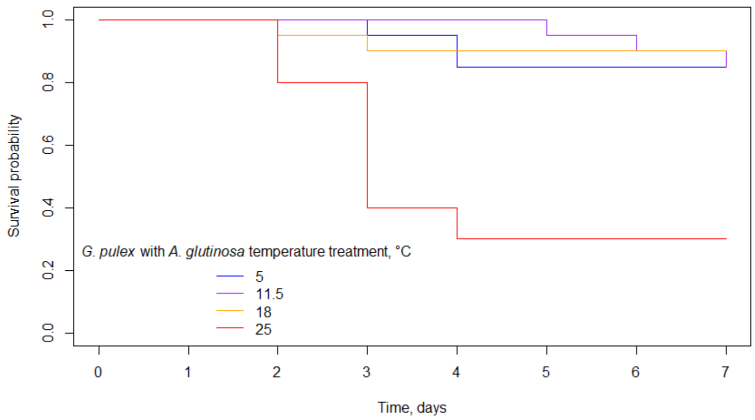

Figure 4.4 d): Kaplan Meier plot of survival of native *G. pulex* amphipods supplied with native *A. glutinosa* leaf litter at each treatment temperature

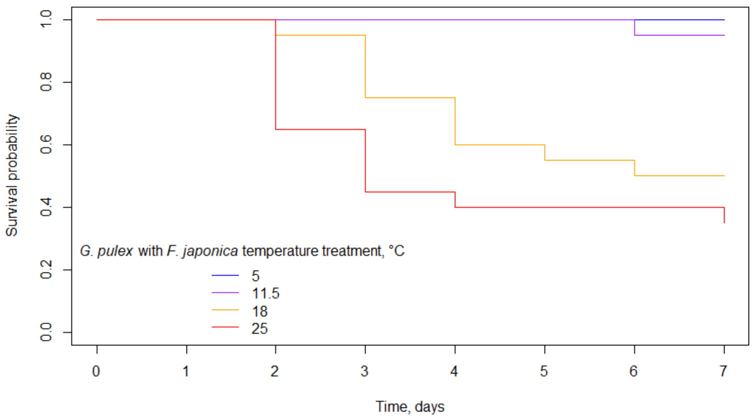

Figure 4.4 e): Kaplan Meier plot of survival of native *G. pulex* amphipods supplied with invasive *F. japonica* leaf litter at each treatment temperature

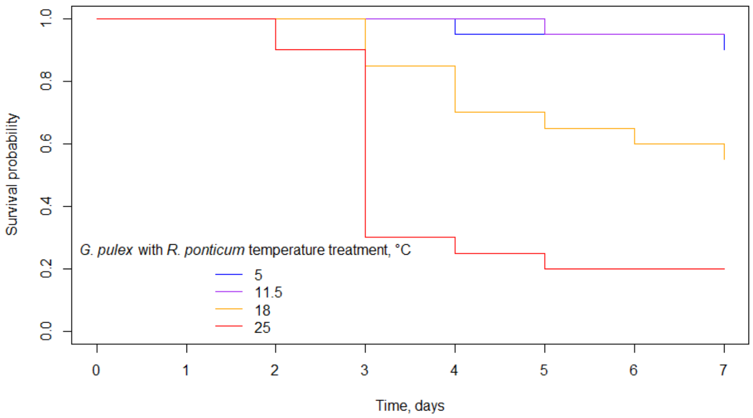

Figure 4.4 f): Kaplan Meier plot of survival of native *G. pulex* amphipods supplied with invasive *R. ponticum* leaf litter at each treatment temperature

4.4.3 *Rhododendron ponticum* toxicity

Survival did not differ significantly between amphipods that were exposed to *R. ponticum* leaf litter and those that were not exposed to the invasive leaf (z = -1.48, p = 0.1) (Figure 4.5). Species of amphipod was a significant factor in survival in this experiment (z = 2.81, $p < 0.01$), with greater hazard of mortality for native *G. pulex* compared to invasive *D. villosus* (HR = 8.40, 95% CI: 1.91, 37.03), however, there was no significant interaction detected between species of amphipod and exposure to *R. ponticum* leaf litter (z = 1.40, p = 0.16).

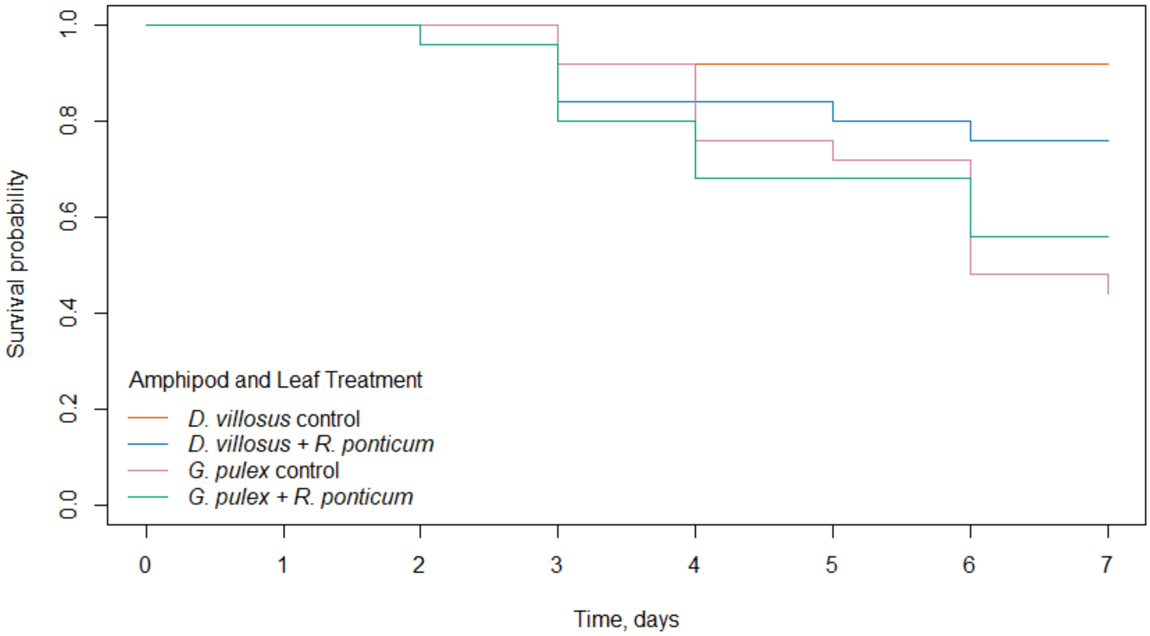

Figure 4.5: Kaplan Meier plot of survival of native *G. pulex* and invasive *D. villosus* amphipods either exposed to dissolved *R. ponticum* leachates or control treatments with no *R. ponticum* present.

4.5 Discussion

The results of this experiment demonstrate that a change in the identity of dominant shredder or leaf litter species can have a significant impact on the productivity of a freshwater ecosystem reliant on allochthonous leaf material and may make processes within the system more temperature sensitive. The phenology of the availability of the detrital leaf resource may be altered, which could have cascading impacts on the trophic web in the affected ecosystem (Alp *et al.*, 2016). Shredding of *F. japonica* was more than two-fold lower than that of *A. glutinosa* at all temperatures. The lower shredding rate of the invasive leaf species, as found in another study on *F. japonica*, is likely to reduce the resources made available to other macroinvertebrates that rely on FPOM , but would also likely lead to an extended period of leaf availability, which would favour multivoltine species, such as *D. villosus*, facilitating an extended breeding period (Lecerf *et al.*, 2011).

Dikerogammarus villosus demonstrated similar feeding patterns to the native *G. pulex*, although the native shredded more leaf litter of all species than the invasive (Platvoet *et al.*, 2009). However, the native amphipod was found to be more sensitive to temperature in relation to survival. This contrasts with the findings of chapter 3, wherein *D. villosus* and *G. pulex* had no difference in survival across the temperatures investigated. In this experiment the differences in survival between the amphipod species were most obvious at 25°C, but also to an extent at 18°C, for *G. pulex* with invasive leaf litter rather than amphipods feeding on *A. glutinosa*. This may reflect the lower nutritional value of the invasive leaves being less able to support the metabolic demands of *G. pulex* as energy requirements increased with increasing temperature.

Dikerogammarus villosus was found to shred less than *G. pulex*, even at 25°C, in contrast to what Truhlar et al. (2014) found. However, results for shredding rates and survival here matched the findings of Kenna et al. (2017), with higher rates of shredding, but lower survival at high temperature for *G. pulex*. The lower shredding rate of the invasive amphipod is likely to be mediated by its much higher abundance in invaded sites (Rewicz *et al.*, 2014; Warren, Bradbeer and Dunn, 2021). The high densities that *D. villosus* reaches, compared to native *G. pulex* densities, may be reflected in higher total ecosystem-level shredding rates, including shredding of the less favoured invasive leaves *F. japonica* and *R. ponticum*. Though high temperature was found to reduce survival, as found in previous chapters and other studies, the slightly higher survival, though not significant, as well as higher densities, indicate the invasive *D. villosus* may better preserve shredding rates in a warming climate (Wijnhoven *et al.*, 2003). However, this is likely to further degrade the macroinvertebrate community. The biodiversity reduction due to invasive allochthonous leaf supply may be amplified due to the invasive amphipod's predatory behaviour (Krisp and Maier, 2005; Dodd *et al.*, 2014; Seeney, Pattison, *et al.*, 2019).

Rhododendrons are known to produce numerous toxic compounds that protect the plant from competitors and deter predators, inhibiting insect herbivores (En, Nilsen and Oktay Yildiz, 2006). Some rhododendron species have also been used to poison fish in aquatic environments, though that has not been documented for *R. ponticum* (Bhardwaj *et al.*, 2013).

Grayanotoxins found in *R. ponticum* have been found to cause pathology in humans who have ingested “mad honey” produced by bees feeding on *R. ponticum* nectar (Jansen *et al.*, 2012). However, I found no evidence for decreased survival in amphipods either feeding on *R. ponticum* leaf litter or exposed to dissolved leachates from sectioned leaves. This may be due to a physiological resistance of *G. pulex* and *D. villosus* to the toxins within *R. ponticum* leaf tissues, or dilution and low consumption causing sub-lethal effects. Although amphipods consumed more *R. ponticum* than *F. japonica*, the increase in shredding rate related to temperature was less for *R. ponticum*. It is not clear whether this lower increase in temperature-related consumption is due to toxins, leaf toughness or another factor.

Dangles *et al*. (2002) stated that ecosystem function can persist if shredder diversity is maintained following riparian invasion by *F. japonica*. The invasive leaf litter may be slower to break down, but the generalist feeding behaviours of *G. pulex* and *D. villosus* are likely to maintain nutrient cycling. Despite the lower leaf litter quality of *F. japonica*, the amphipod shredding demonstrated here should support trophic webs in aquatic ecosystems (Braatne *et al.*, 2007). The lower shredding rate of *F. japonica* may be connected to slower microbial colonisation of the non-native leaf due to the novel weapons hypothesis, as the lack of a shared evolutionary history between the non-native leaf and native microbes lead to inhibited decomposition due to secondary compounds produced in the leaf litter to which decomposers are naïve (Callaway and Ridenour, 2004; Fogelman *et al.*, 2018). Microbial conditioning is important for increasing the palatability of leaf litter for amphipod shredders, but this experiment has demonstrated that *F. japonica* can support *G. pulex* and *D. villosus* shredding, albeit at a lower rate than native *A. glutinosa* (McInerney and Rees, 2017).

The abilities of the invasive *F. japonica* and *R. ponticum* to dominate invaded locations and to form dense monocultures are likely to have long-term impacts in invaded locations. Such a shift may have cascading impacts on the ecosystem community, as amphipod shredders are an important prey species for fish and other aquatic predators, as well as providing FPOM for collector species (Cummins and Klug, 1979; Glazier, 2009). This vital keystone role of amphipod shredders means that a change in the identity of amphipods, or the dominant riparian leaf litter source for the aquatic system could greatly alter the productivity of that ecosystem. The lower shredding rate of *D. villosus* amphipods and the lower shredding of *R. ponticum* and *F. japonica* leaf litter would mean a decrease in energy flows and reduced productivity, may change the period of leaf litter availability, and decrease macroinvertebrate and fish abundance, affecting adjacent ecosystems (Alp *et al.*, 2016; Gallardo *et al.*, 2016; Little and Altermatt, 2018).

As predicted, temperature had a significant positive correlation with shredding rates, with the native leaf *A. glutinosa* being shredded at a higher rate than the invasive *F. japonica* or *R. ponticum*. However, the positive correlation between temperature and shredding rate was lesser for *R. ponticum* than the other species of leaf litter. The reason for this is not clear but may relate to differences in palatability or bacterial colonisation between the species of leaf litter used. Contrary to initial predictions, native *G. pulex* had higher shredding rates than the invasive *D. villosus*, and exhibited an interaction with temperature, with lesser survival at higher temperatures than the invasive amphipod. Despite lower nutritional value, and possible toxicity, it was found that amphipods will feed on the invasive leaf species selected, which indicates ecosystem function can be maintained despite riparian invasion by these non-native plants. However, dominance of allochthonous leaf detritus inputs to a freshwater ecosystem by a less favoured invasive species may lead to increased resource switching. The omnivorous natures of these amphipod species indicate switching to predation of the macroinvertebrate community may occur in the presence of invasive leaf litter. The high densities that *D. villosus* is commonly found in will likely offset any per capita shredding reduction in an invasion scenario, but the invasive amphipod’s impact on macroinvertebrates is to be explored in the next chapter.

Chapter 5: Changes to the structure of freshwater ecosystems due to species-specific predation by an invasive amphipod may be accelerated by climate warming

5.1 Introduction

Multiple ecosystem stressors are an increasing problem, imparting pressures on the natural environment, altering function and productivity (Orr *et al.*, 2020). Anthropogenic disturbance is altering ecosystem processes through multiple factors, such as climate change and introductions of invasive non-native species, which are increasing in intensity and causing shifts in biodiversity, species' distributions and ecosystem function (Chapin *et al.*, 2000; Pereira *et al.*, 2010). Abiotic stressors, such as climate change, land use change and pollution are predicted to increase in intensity, impacting trophic webs and processes within ecosystems through environmental modifications which may impact species' behaviour or survival (Pachauri *et al.*, 2014). Biotic stressors, such as introductions of non-native species and parasites, tend to exert pressures at the population level, altering the biotic structure of an ecosystem (Chapin *et al.*, 2000; Cardinale *et al.*, 2012). If multiple stressors interact it may cause a greater synergistic impact, but understanding of how such pressures interact is not sufficiently understood, requiring further research to enable more accurate forecasts for predicted stressor increases (Jenkins *et al.*, 2009; Côté, Darling and Brown, 2016; Birk *et al.*, 2020).

Anthropogenic climate change is exerting considerable stresses on ecosystems globally, with forecasts of increases in the intensity of warming in the future (Murphy *et al.*, 2010; Pachauri *et al.*, 2014). High temperature events are also predicted to increase in frequency, with associated impacts to the function of ecosystems (Bertani, Primicerio and Rossetti, 2016). Disturbances, such as mean temperature increases, are known to facilitate additional stressors, such as invasive non-native species (Jankowski *et al.*, 2006; Daufresne, Bady and Fruget, 2007; Iacarella *et al.*, 2015). Invasive species are a significant stressor of ecosystems, with freshwater habitats particularly vulnerable due to high levels of human activity around fresh water (Ormerod *et al.*, 2010; Vörösmarty *et al.*, 2010). The adaptability of many invasive species often allows them to more successfully exploit resources and outcompete native species in their novel range, which can alter community structure and induce trophic cascades, affecting ecosystem productivity (Vitousek *et al.*, 1996; Sakai *et al.*, 2001; de Omena, Srivastava and Romero, 2017). Freshwater ecosystems are particularly vulnerable to stressors due to their geographic fragmentation, high levels of anthropogenic disturbance and being relatively sensitive to changes in air temperature, with temperate freshwater systems likely to be affected by increasing temperatures before more tropical and biodiverse ecosystems at lower latitudes (Woodward, Perkins and Brown, 2010; Capon, Stewart-Koster and Bunn, 2021).

Temperate freshwater ecosystems often rely on a basal resource of allochthonous leaf litter (Abelho, 2001; Gessner *et al.*, 2010). Autumnal leaf abscission means a resource pulse enters the ecosystem from riparian vegetation, but the nutrients and energy within the leaf resource remain inaccessible due to the toughness of the leaves (Benfield, 1997; Cornwell *et al.*, 2008).

Microbial decomposers colonise the leaf litter, increasing its palatability to macroinvertebrates and producing a nitrogen-rich biomass attractive to macroinvertebrate consumers (McInerney and Rees, 2017). A guild of shredder species equipped with suitable mouthparts consumes the softened leaf detritus, producing fragments of fine particulate organic matter (FPOM) and faeces that can then be processed by other macroinvertebrates (Covich *et al.*, 2004; Cummins, 2019). The shredders can be dominant by biomass in many freshwater ecosystems, accumulating large populations which provide a valuable food source for predatory species (Wallace and Webster, 1996; Ruetz, Newman and Vondracek, 2002; Nery and Schmera, 2016).

Amphipods are common shredder species in temperate freshwaters, which often hold the keystone role in the centre of their trophic webs (Väinölä *et al.*, 2008; Glazier, 2009; Covich and Thorp, 2010). This role involves converting the nutrients locked in allochthonous leaf litter into a more useable form for the ecosystem, provisioning species throughout the trophic web with FPOM from shredding activity or providing their own biomass as prey for predators. However, many amphipods are omnivorous and predatory themselves (MacNeil, Dick and Elwood, 1997; Kelly, Dick and Montgomery, 2002; Bacela-Spychalska and Van Der Velde, 2013; Warren, Bradbeer and Dunn, 2021). The invasive Ponto-Caspian amphipod *Dikerogammarus villosus* (Sowinsky, 1894), also known as the killer shrimp, has spread through western European waters, impacting the structure of freshwater ecosystems (Grabowski, Bacela and Konopacka, 2007; Rewicz *et al.*, 2014). The invasive *D. villosus* has been found by Dick et al. (2002) to often inflict bites on invertebrate prey without consuming the whole of the animal. It is therefore possible that omnivorous amphipods also generate prey animal tissue detritus, provisioning the ecosystem with partially predated remains, as well as serving as providers through shredding activity and biomass accumulation.

Optimal foraging theory states that animals will seek to maximise the benefit gained from foraging, which may include only eating the most nutritious parts of prey if there is plentiful availability and handling time is not a limiting factor (Pulliam, 1974; Sih, 1980; Breed and Moore, 2016). For example, brown bears (*Ursus arctos*) often select the most nutritionally valuable parts of salmon prey to consume preferentially, such as the brain and gonad-containing belly, while discarding the rest, when prey is plentiful and of high quality (Lincoln and Quinn, 2019). If the cost of giving up a portion of a meal and catching another is outweighed by the high fat and protein availability of abundant and easily captured salmon prey, partial remains of fish will be discarded and will provision scavengers and plants in the ecosystem with a vital resource (Levi *et al.*, 2020). The provision of leaf detritus dominates energy cycles in many temperate freshwater ecosystems, however the detritus quality rather than quantity is often the limiting factor for many detritivorous species (Anderson and Cargill, 1987; Gessner *et al.*, 2010). Decomposing leaf litter does not provide a large supply of proteinaceous material compared to dead animal tissue's higher nutritional value, though animal detritus is usually provisioned at a lower level (Anderson and Cummins, 1979). Animal carcasses decompose at a faster rate than leaf litter in aquatic environments, releasing more nutrients and supporting a greater microbial community, which supports greater productivity in ecosystems and larger populations of higher trophic levels in food webs (Mastrantonio *et al.*, 2021). Animal detritus contains more nitrogen than leaf litter and nutrients may be more easily accessible to a wider range of detritivores than can be obtained from relatively tough leaf litter. For these reasons detritus derived from animal sources has been found to support greater abundances and diversity of aquatic invertebrates (Yee and Juliano, 2012). Therefore, partial predation of animal prey by amphipods may significantly contribute to ecosystem productivity.

When an aquatic system contains animal carcass detritus it leads to higher productivity, for example faster growth of insect larvae and greater survivorship and population growth for other species in the community (Yee and Juliano, 2006; Yee, Kesavaraju and Juliano, 2007). Mosquitoes preferentially select sites containing evidence of predation through proliferation of bacteria from egested faeces and decomposing animal carcasses, which enhance the survival and growth of their offspring compared to conspecifics that are raised with a solely plant detritus based diet (Albeny-Simões *et al.*, 2014; Beasley and Walton, 2016). Nutrients in animal tissues must go through microbial decomposition to be made available to primary plant producers through mineralisation, but are readily available for detritivorous species to benefit from, and are easier to access and assimilate than nutrients in leaf detritus (Vanni, 2002). Small particles of animal detritus can also provide a nutrient-rich food source for filter feeding species of the collector functional feeding guild (Wallace and Webster, 1996; Vanni, 2002). Through predatory behaviour both *D. villosus* and *G. pulex* can have a beneficial effect on their ecosystems through provisioning of diverse nutrient sources, including animal detritus, faeces, shredded leaf litter and their own biomass.

The stressors of temperature and invasive species may interact in unpredictable ways and freshwater systems are dominated by ectothermic species, the behaviours of which are sensitive to temperature change (Woodward, Perkins and Brown, 2010). Invasive species are often tolerant of a wider range of environmental conditions than their native analogues, properties which increases their capability to become invasive (Bates *et al.*, 2013; Kelley, 2014). However, it is difficult to predict responses of native and invasive species to a stressor, such as temperature, without experimental studies (Hellmann *et al.*, 2008; Diez *et al.*, 2012; Tepolt and Somero, 2014). This study aims to investigate how temperature affects predatory behaviours of a common UK native amphipod *Gammarus pulex* (Linnaeus, 1758) and the invasive *D. villosus*. Amphipod predators were provided with *Asellus aquaticus* (Linnaeus, 1758) or chironomid larval prey at a range of temperatures and predatory behaviour was recorded, including partial predation leading to provisioning of the environment with animal detritus. Interactions of temperature and species were investigated to compare predatory foraging behaviours between *G. pulex* and *D. villosus* to predict whether temperature is likely to affect impacts on the ecosystem's productivity and nutrient flows.

5.2 Methods

5.2.1 Experimental material

Invasive *D. villosus* were collected from a population in Grafham Water, Cambridgeshire (52.2909°N, -0.0323°E) by removal from a submerged blanket attached to a pontoon. Native *G. pulex* and *A. aquaticus* were collected at Meanwood Beck, West Yorkshire (53.8301°N, -

1.5746°E) by kick-sampling. Chironomid larvae were purchased from suppliers as live fish food supplies. Amphipods, isopods and chironomid larvae were placed in species-specific tanks of 5l aerated aged tap water in a controlled-temperature room. All animals were maintained for a minimum of 5 days acclimatisation at 15 ± 0.1°C S.D., with a 12:12 hour light/dark cycle (08:00-20:00). Animals were fed *ad libitum* with *Alnus glutinosa* leaf litter, a species of leaf present in the locations animals were collected from and favoured by the shredder species investigated (Bloor, 2011).

5.2.2 Experimental design

Amphipods were placed individually in containers with aged tap water. Containers were transparent, circular plastic pots (diameter 7 cm, depth 5 cm) holding 250 ml water and contained 2 transparent glass beads to provide refugia for amphipods, reducing excessive thigmotactic swimming whilst permitting observation (Perrot-Minnot, Banchetry and Cézilly, 2017; Kohler, Parker and Ford, 2018). The containers housing the amphipods were then placed in incubators with a 12:12 hour light/dark cycle (08:00-20:00) for a 24-hour period of starvation to standardise hunger. Incubators were initially at 15°C and temperature was either increased or decreased at a rate of 1°C every 2 hours until the treatment temperature was reached. Treatment temperatures of 5, 10, 15, 20 and 25°C were then maintained until experiments were complete.

Amphipods were placed on a paper towel, to remove excess water, and weighed. The glass beads were removed from their container, to deny refugia and promote active foraging behaviours. The water was replaced with aged tap water at the correct treatment temperature, to ensure experimental water was not deoxygenated from the respiration of the subject amphipod during the 24-hour acclimatisation period. The amphipod was placed in the container and left for a 10-minute acclimatisation period in the incubator. Three prospective prey individuals of *A. aquaticus* were then added to each pot with an amphipod predator, with isopods selected by eye to be approximately 50%, or less, of the size of the amphipod.

Observations of predatory activity were observed and recorded for an hour, collecting data on time elapsed before attack (unsuccessful), time elapsed before attack (successful), duration of feeding by predator on prey, percentage of prey discarded after predation. If prey was killed or incapacitated, any remainder was removed from the trial arena in order that the predator could not return to feed on the carrion. Prey were not considered incapacitated if they remained mobile from a leg or legs being removed by the predator and the isopod could continue walking. It was assumed flowing water in the natural environment would transport the carcass away from the predator to provision the ecosystem. If an easy carrion source were available, it may inhibit further predatory attack behaviours on live prey, therefore, injured prey isopods were removed from the test arena. If predation was taking place at the end of the hour observation period, the feeding behaviour would be allowed to continue to completion and the time and percentage of the prey discarded were recorded.

Table 5.1: Number of replicates of *A. aquaticus* predation experiment for each amphipod predator and temperature treatment combination

	Treatment temperature				
	5°C	10°C	15°C	20°C	25°C
G. pulex	48	50	49	54	50
D. villosus	47	56	56	56	54

Table 5.2: Number of replicates of chironomid larva predation experiment for each amphipod predator and temperature treatment combination

	Treatment temperature	
	15°C	20°C
G. pulex	22	14
D. villosus	16	16

5.2.3 Data analysis

All analyses were produced using R, with plots for shredding created using the package "ggplot2" (Wickham, 2016; R, 2019).

5.2.3.1 Successful attacks on *Asellus aquaticus*

Due to the large number of zeroes in the attack count data a zero-inflated Poisson regression (R package “pscl”) was performed, with a Vuong test confirming the better fit of the zero-inflated model compared to a standard Poisson ($z = -3.55$, $p < 0.01$) and acceptable coefficient of dispersion of 1.07.

5.2.3.2 Successful attacks on chironomid larvae

A Poisson regression was performed using the R package “AER” for the number of successful attacks during the experimental period. Dispersion was satisfactory (< 1.10) and a non-significant factor was removed after comparing models by AIC.

5.2.3.3 Remains of *Asellus aquaticus* prey discarded

Data for the remains of prey discarded after a lethal attack were collected as a percentage of the intact animal that was released after the prey was killed and partially consumed. Percentages were summed in the case of multiple lethal predation events by any individual predator amphipod during the hour observation period. A generalised additive model (GAM) with a zero-inflated Poisson distribution with a restricted maximum likelihood (REML) approach was constructed using the "mgcv" package in R. Temperature was modelled with cyclic cubic regression splines for best fit.

5.2.3.4 Remains of chironomid larvae prey discarded

A GAMLSS model was built using the "gamlss" package in R, with a zero-altered negative binomial type I distribution (ZANBI) producing the best fit for the data. Amphipod mass was modelled with a 2-power polynomial basis matrix and the iterative model run with starting parameters: nu = 0.7, sigma = 3.0, mu = 10, tau = 1. Models were compared by residual worm plots.

5.3 Results

5.3.1 Successful attacks on *Asellus aquaticus*

Though a one-way ANOVA indicated a significant difference in mass between *G. pulex* and *D. villosus* ($F_{(1,520)} = 328.60$, $p < 0.01$), with the invasive *D. villosus* being larger than the native *G. pulex* (*D. villosus*: 96.3 ± 2.5 mg, *G. pulex*: 47.8 ± 0.5 mg), amphipod mass was found to not affect the number of successful attacks on *A. aquaticus* ($z = 1.50$, $p = 0.13$), so was removed from the model. Temperature was found to have a significant effect on the number of successful predatory attacks by amphipods ($z = -2.88$, $p < 0.001$) with a 1°C increase in temperature correlating to 0.07 fewer successful attacks. Amphipod species did not have an effect ($z = 1.52$, $p = 0.13$) and a significant interaction of species and temperature was not detected ($z = -1.78$, $p = 0.08$) (Figure 5.1).

Amphipod species did not affect the chances of zero successful attacks taking place in the zero-inflated model ($z = 0.17$, $p = 0.87$). However, temperature had a significant effect, with increasing temperature increasing the chances of zero successful attacks taking place in any individual trial ($z = -3.07$, $p < 0.01$). Amphipod species and temperature did not interact to increase likelihood of zeroes in the data ($z = -0.001$, $p = 0.99$).

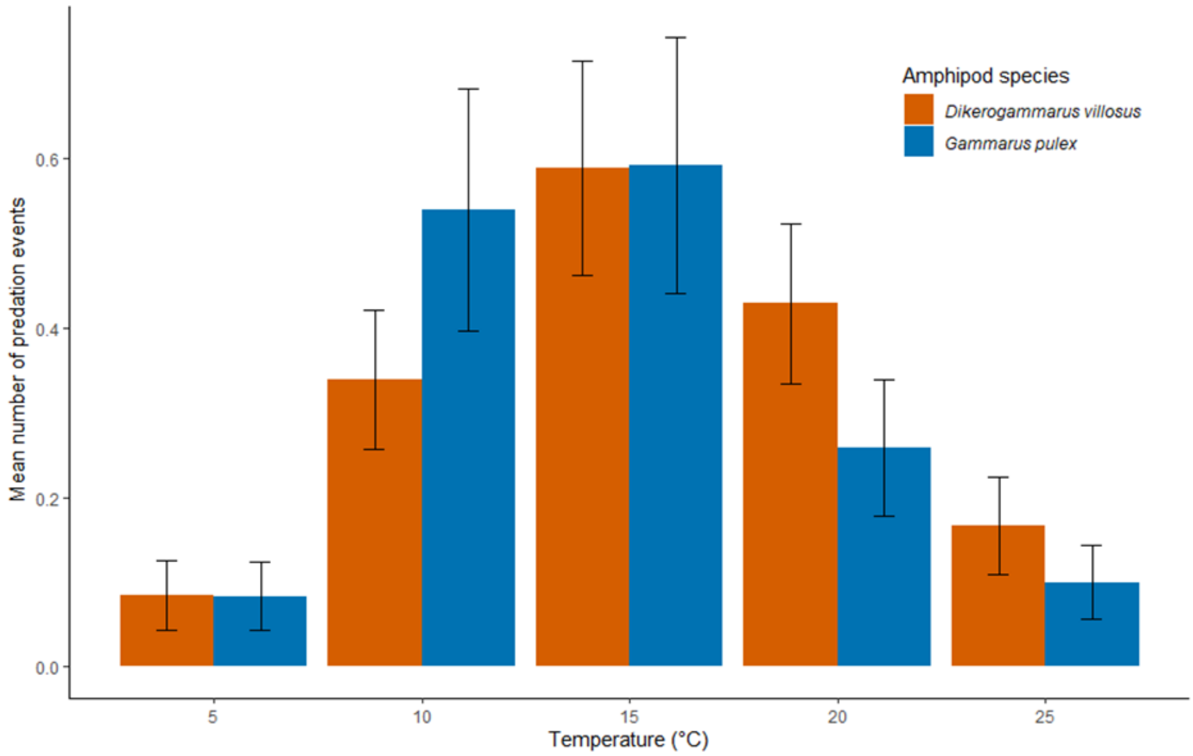

Figure 5.1: Mean number of successful predatory attacks on *A. aquaticus* isopods during the 1-hour observation period at each treatment temperature by invasive *D villosus* and native *G. pulex* amphipods with bars displaying ± 1 standard error.

5.3.2 Successful attacks on chironomid larvae

Amphipod mass had no effect on the rate of successful attacks (z = 0.63, p = 0.53) and was removed from the model (AIC Δ = 1.8). Amphipod species had a significant effect on the number of successful attacks during the experimental period (z = -2.19, p = 0.03), with *D. villosus* having a higher successful attack rate (15°C: 1.94 mean attacks per hour ± 0.19 S. E., 20°C: 2.62 mean attacks p. h. ± 0.13 S. E.) than *G. pulex* (15°C: 1.45 mean attacks p. h. ± 0.24

S. E., 20°C: 1.57 mean attacks p. h. ± 0.25 S. E.). Temperature did not affect attack rate (*z* = 1.16, *p* = 0.24) and no interaction of amphipod species and temperature was detected (*z* = -0.62, *p* = 0.53).

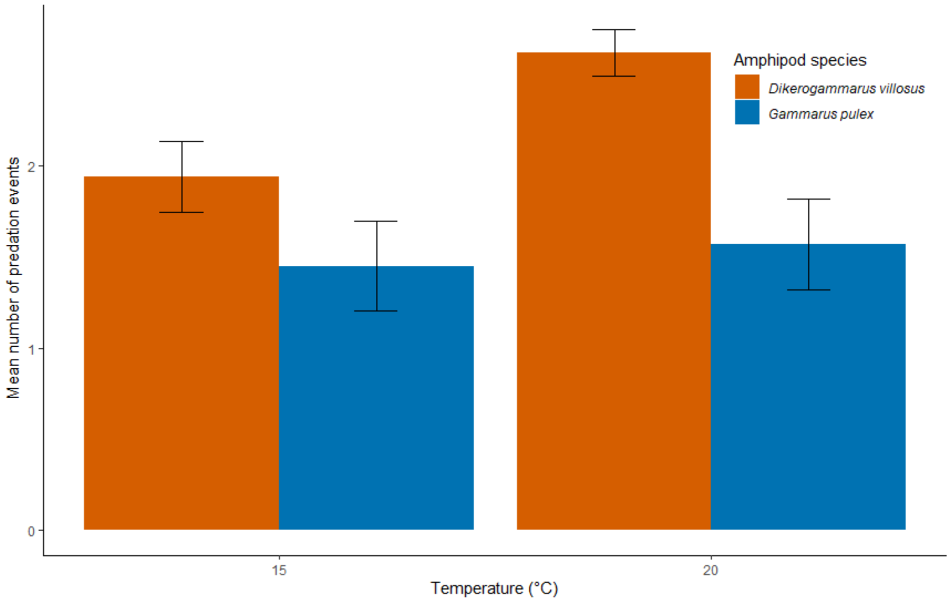

Figure 5.2: Mean number of successful predatory attacks on chironomid larvae during the 1-hour observation period at each treatment temperature by invasive *D villosus* and native *G. pulex* amphipods with bars displaying ± 1 standard error.

5.3.3 Remains of *Asellus aquaticus* prey discarded

Species of amphipod did not significantly affect how much dead tissue was dropped after *D. villosus* and *G. pulex* predators had finished consuming *A. aquaticus* prey (z = -1.53, *p* = 0.13)

(Figure 5.3). Temperature significantly affected discard rate ($z = -5.67$, $p < 0.01$), with highest rates at 15°C for both species of amphipod (*G. pulex*: 7.0% per hour ± 3.0% S. E.) (*D. villosus*: 10.9% p. h. ± 3.4% S. E). An interaction of temperature and species was detected ($z = -2.16$, $p = 0.03$) where native *G. pulex's* lowest mean discard rate was at 25°C (1.3% per hour ± 1.0% S. E.) whereas invasive *D. villosus'* lowest mean discard rate was at 5°C (1.90% p. h. ± 1.90% S. E.). Amphipod mass had a significant impact on prey discard rate ($z = -5.02$, $p < 0.01$) with a negative correlation.

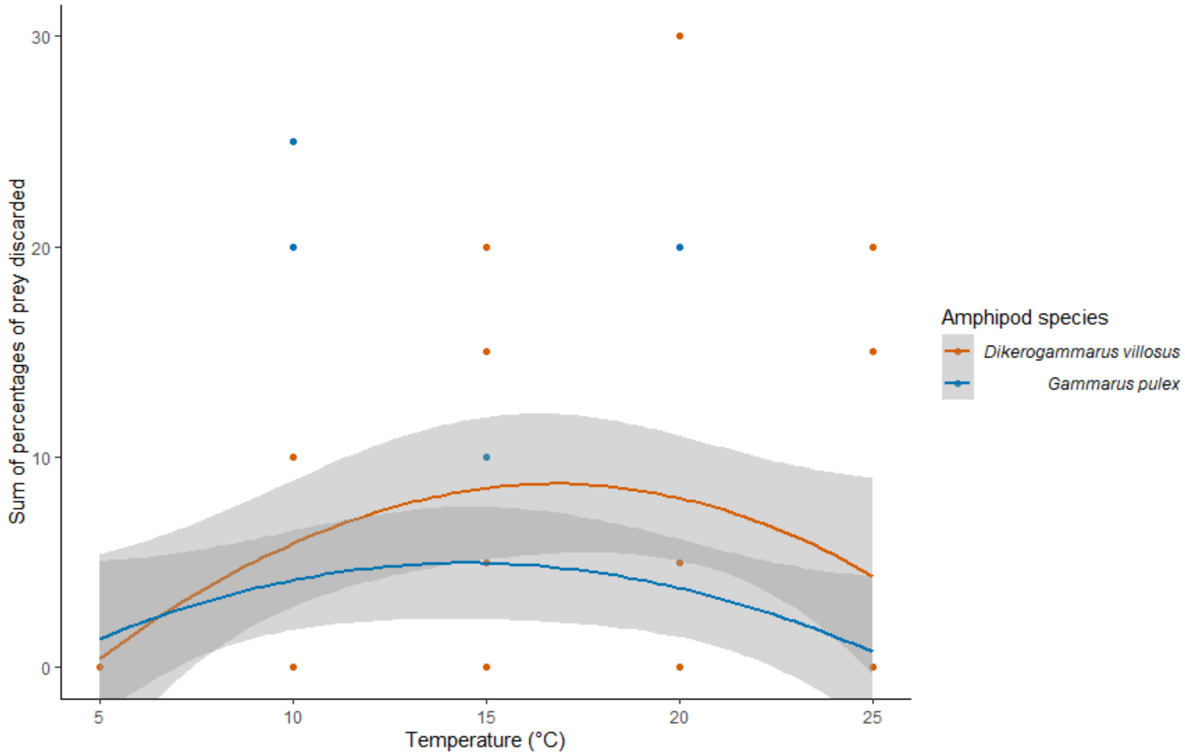

Figure 5.3: Percentages of prey carcasses discarded after each successful lethal attack were summed. Curves represent mean values of the sum of percentages of *A. aquaticus* isopod prey released after lethal attack and partial consumption by invasive *D. villosus* and native *G. pulex* amphipods at each treatment temperature, displaying standard error for Loess smoothed curves.

5.3.4 Remains of chironomid larvae prey discarded

Species of amphipod significantly affected the amount of dead tissue discarded by predators during the hour test period (*t* = 2.15, *p* = 0.04) with invasive *D. villosus* having a higher mean discard rate (13.4% per hour ± 5.6%S. E.) than native *G. pulex* (9.2% p. h. ± 3.9% S. E.). Temperature did not affect the amount of discarded tissue (*t* = 0.55, *p* = 0.59), however species of amphipod predator and temperature had an interaction (*t* = -2.36, *p* = 0.02) (table 5.3) with a negative correlation between temperature and discard rate for *G. pulex* and a positive correlation between temperature and discard rate for *D. villosus*. Amphipod mass did not influence the discard rate of dead chironomid prey tissue by amphipod predators (*t* = 0.89, *p* = 0.38) and no interaction was found between amphipod mass and temperature in affecting prey discard rates (*t* = -0.24, *p* = 0.81).

Table 5.3: Means and standard errors of the sums of percentages of chironomid prey discarded by amphipod predators after a successful lethal attack at each treatment temperature.

		Gammarus pulex		*Dikerogammarus villosus*	
		Mean	S. E.	Mean	S. E.
Treatment temperature	15°C	10.7	4.9	12.5	8.0
	20°C	6.8	6.4	14.4	8.1

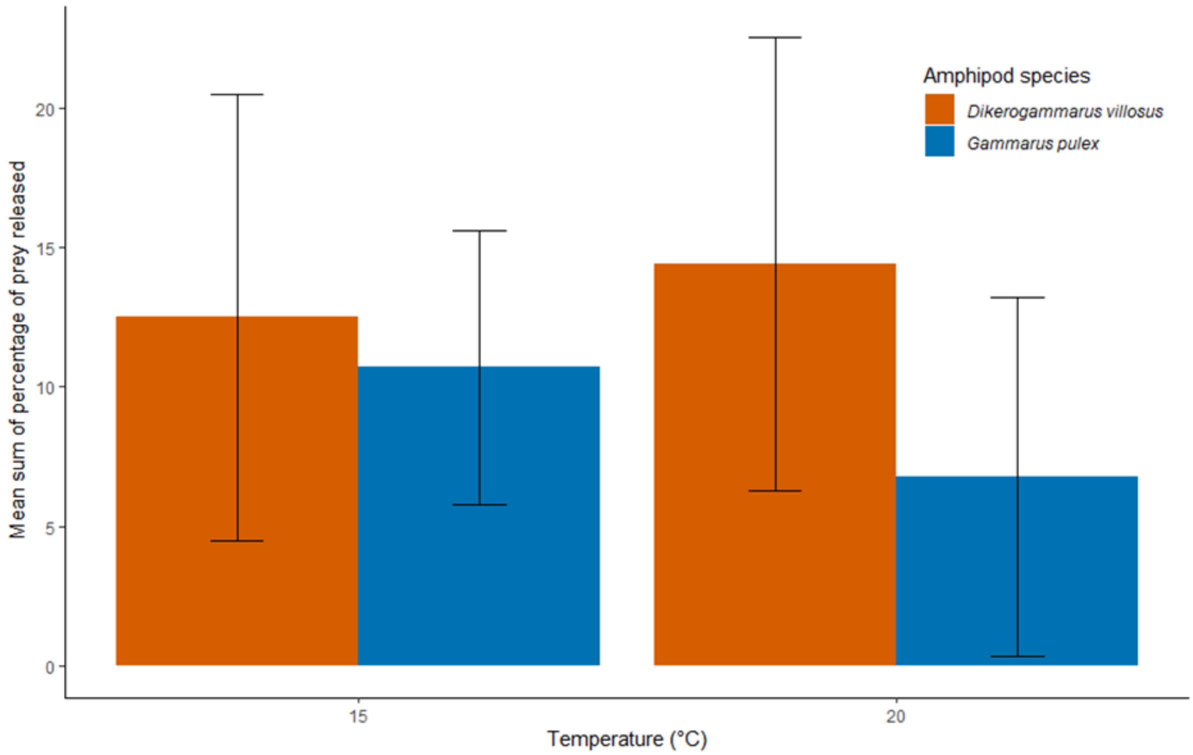

Figure 5.4: Percentages of chironomid larval prey discarded after each successful lethal attack were summed. Bars represent mean values of sums of prey discarded by invasive *D. villosus* and native *G. pulex* amphipods at each treatment temperature with error bars displaying ± 1 standard error.

5.4 Discussion

The species of amphipod predator had no effect on the rate at which isopod carcasses were discarded to contribute to animal detritus and to potentially provision the ecosystem. Similarly, no difference was found in the rate of successful lethal attacks on isopod prey between the native *G. pulex* and the invasive *D. villosus*. However, *D. villosus* had more successful attacks on chironomid prey than *G. pulex* and discarded more dead chironomid tissues. Numerous previous studies have found *D. villosus* to have a higher predatory functional response (FR) than *G. pulex,* in experiments with a single predator and multiple prey, which may have become evident for both prey species in a longer-running experiment than the hour observation investigated here (Bollache *et al.*, 2008; Taylor and Dunn, 2017; Warren, Bradbeer and Dunn, 2021). However, in a relatively small laboratory experiment without flowing water, discarded carcasses would likely become a food source to the predator when foraging recommences after a successful attack and feeding event, which may not occur in a more complex natural environment. The lack of difference in isopod lethal attack and carcass discard rates found between the native and invasive amphipods may indicate *G. pulex* and *D. villosus* are likely to occupy similar trophic levels in natural systems.

The higher rate of *D. villosus* predation on chironomid prey accords with a previous study, which found that the invasive amphipod had a higher FR than the native for several prey types, including chironomid larvae (Dodd *et al.*, 2014). However, trophic level estimation by analyses of δ^{15}N levels has found that *D. villosus* has a similar trophic level to coexisting amphipod species, including a native, in two tributaries of the River Rhine (Sahm *et al.*, 2021). Other studies have found that, in established populations, *D. villosus* tends to omnivory, with a low trophic position (Koester, Bayer and Gergs, 2016). Predatory behaviour by *D. villosus* in the Rhine is dependent on community structure, not an intrinsic predatory property of the amphipod species, which may relate to this study's results finding similar rates of isopod attack, but higher rates of attacks on chironomid larvae (Hellmann *et al.*, 2015, 2017; Koester *et al.*, 2018). If *D. villosus* has a higher rate of provisioning the ecosystem with animal detritus than native amphipod species in some cases, this may be balanced by the lower rate of leaf detritus shredding I have found in previous chapters. However, the higher abundance of the invasive amphipod, especially in the earlier stages of invasion establishment, may have significant impacts on freshwater communities, with favoured prey being severely reduced in density (Rewicz *et al.*, 2014; Warren, Bradbeer and Dunn, 2021). More replicates of this experiment, and more temperature treatments, may provide more information on interactions of temperature and amphipod species in chironomid larva predation.

Temperature affected rates of successful attacks on *A. aquaticus* prey in both amphipod species, with no difference between native *G. pulex* and invasive *D. villosus*. The optimal temperature for the rate of successful attacks during the one-hour experimental period was 15°C (Figure 5.1), with similar declines as temperature increased or decreased away from the optimum. Though only two temperature treatments were tested for chironomid prey, the rate of successful attacks was slightly higher, though not significantly so, at 20°C compared to 15°C. The 5°C increase in temperature is likely to increase metabolic demands for the amphipods but *A. aquaticus* is relatively difficult to predate compared to the chironomid larvae. The relatively easy chironomid prey can be captured at the higher temperature but the greater energetic costs of capturing *A. aquaticus* may outweigh the energy recouped on increased consumption of the isopod prey. Discard rates of *A. aquaticus* carcass detritus were highest at 15°C, with lower rates below and above that temperature. The interaction of temperature and amphipod predator species in discard rates for both *A. aquaticus* and chironomid prey indicate that *G. pulex* may contribute well to animal detritus at low temperatures but *D. villosus* will be better able to maintain the supply of animal detritus at higher temperatures. In a warming climate this greater supply of nutrient-rich carcasses may better maintain ecosystem productivity.

In natural ecosystems interspecies and intraspecies interactions affect rates of predation and related behaviours. The invasive *D. villosus* has been found to aggregate socially more than the native *G. pulex*, with interference between individuals potentially decreasing their predatory impact, which was not investigated here (Truhlar and Aldridge, 2015). Aggregation behaviours and defence against kleptoparasitism have been shown to reduce *D. villosus* predation rates when multiple amphipods are present, with *G. pulex* having a higher predatory functional response on dipteran larvae than the invasive species when there are multiple predators (Médoc, Albert and Spataro, 2015). Therefore, the single predators used in this investigation may not provide a definitive result for these interactions as they would occur in a natural ecosystem.

The presence of *D. villosus* has been shown to increase anti-predator behaviours in prey species, limiting activity and affecting survival and fecundity of prey populations (Sitvarin, Rypstra and Harwood, 2016; MacNeil and Briffa, 2019). For example, the presence of predators has been shown to increase refuge-seeking behaviour in chironomid larvae, altering the sediment layer in aquatic environments (Stief and Hölker, 2006). Therefore, the presence of *D. villosus* may cause unpredictable cascades due to context-related alterations to community composition through selective predation and animal detritus provisioning, or anti-predator behaviours in prey species resulting in altered ecosystem engineering and ecosystem processes. Habitat complexity and availability of refugia may affect outcomes and may be valuable additions to further work. Further studies may also benefit from the presence of multiple predators to ascertain whether behaviours are affected by social interactions.

The potential impact of invasive *D. villosus* amphipods in the UK must be considered with the density in which this invader is found. A relatively recent introduction, benefitting from enemy release in its new range, the species is found at extremely high densities at invasion sites (Arundell *et al.*, 2015; Warren, Bradbeer and Dunn, 2021). When the biomass of a population is growing rapidly, the species is more likely to be a nutrient sink than a source, despite the potential for increased predatory behaviour and provisioning of the ecosystem with animal detritus, compared to native analogues (Vanni, 2002; Devin *et al.*, 2004; Pöckl, 2007). However, the high density of the invasive amphipod has the potential to greatly impact community structure at invaded sites. Selective predation, here demonstrated as a higher predation pressure on chironomid larvae than exerted by native amphipods, may alter ecosystem function in ways that are difficult to predict. It appears likely that negative effects of trophic cascades will be more impactful than a degree of extra provisioning of nutrient-rich animal detritus in freshwater ecosystems. The higher rate of *D. villosus* predating chironomid larvae may be amplified in a warming climate, meaning structural changes to ecosystems may be accelerated by predicted increases in mean temperature.

6 Concluding remarks

Ecosystems are subject to multiple stressors which can affect the species within them and the vital ecosystem processes which maintain their function and productivity. Climate warming is a stressor in the majority of ecosystems globally, while invasive species are one of the leading drivers of biodiversity loss, which can have a detrimental impact on the functioning of natural systems (Sala *et al.*, 2000). Parasites are an omnipresent stressor for most free-living species, with potential impacts to host population dynamics, growth and behaviour (Wood and Johnson, 2015). Interactions of these stressors can be difficult to predict, and in this thesis I have investigated how common stressors in freshwater ecosystems may impact the behaviour of keystone species and how those stressors may interact.

Temperature was a significant factor in each experiment, increasing rates of shredding up to an optimum temperature, beyond which rates fell. Previous research has generally concentrated on a lesser range of temperatures, but in this thesis I have used a wide range of temperatures with relatively small temperature intervals to produce a high resolution of results. Temperatures were increased to a point where performance was negatively impacted which identified the thermal optima for each amphipod, leaf or parasite treatment, producing novel data for thermal optima and tolerances. The most elevated temperatures used caused mortality in all amphipods. However, the only interaction between temperature and parasitism was an increase in shredding for *G. pulex* infected with *E. truttae*. The prevalence of this parasite in the host population from which samples were taken was extremely low and would not have a significant impact on the ecosystem's productivity during a period of elevated temperatures. Temperature also interacted with amphipod species in discarding of chironomid prey. Discard rate was positively correlated with temperature in the invasive *D. villosus*, but negatively correlated with temperature in *G. pulex*. This appears unlikely to greatly impact provisioning of the ecosystem by the invasive amphipod as predator evasion is likely to increase in a more complex natural habitat.

The gammarid amphipods investigated in this thesis appear to be closely related in behaviour. However, small differences in behaviour will be amplified over time and by differences in numerical response. The invasive species of amphipod are generally found at much higher densities than the natives in the novel range, as shown by *D. villosus* densities in England and *G. pulex* invasive populations in Northern Ireland (Kelly *et al.*, 2006; Warren et al., 2021). The use of the relative impact potential (RIP) in chapter 3 shows that despite the native amphipod's higher per capita rates of shredding, the far greater density of invasive amphipods will result in a far greater rates of shredding. The higher reproductive rate of *D. villosus* may further amplify these differences over time, with research required into how stressors impact population dynamics for native and invasive amphipods.

Chapter 5 investigated partial predation by amphipods, developing a novel method for measuring this feeding behaviour and how it may provision an ecosystem. The invasive *D. villosus* was found to predate selectively. The native and invasive amphipods had similar rates of predation on *A. aquaticus* prey but *D. villosus* had a higher rate of predation on chironomid larvae than *G. pulex*. This preference, along with the much greater densities of the invasive amphipod, is likely to impact the chironomid population. Chironomid larvae abundance may be reduced and the larvae may increase predator evasion behaviours by burrowing into benthic sediments and spending more time in refugia burrows (Stief and Hölker, 2006). This activity increases the quantity of dissolved organic carbon (DOC) in the water column, which can amplify eutrophication and reduce water quality for drinking water (Ledesma, Köhler and Futter, 2012; Solomon *et al.*, 2015; Kritzberg *et al.*, 2020). Such ecosystem engineering due to predators may induce trophic cascades, with unpredictable alterations to the recipient ecosystem.

In chapter 4 the invasive riparian leaves, *R. ponticum* and *F. japonica*, were found to be shredded at a lower rate than the native *A. glutinosa*. However, both invasive leaves were shredded, with no impact to amphipod survival. This indicates that, despite a slower rate of shredding, the invasive plants will sustain trophic webs in neighbouring aquatic ecosystems. As amphipods become habituated to the invasive leaves, which may be a novel foodstuff, shredding rates may increase if no ill-effects are experienced. Invasive riparian plants may be able to maintain freshwater ecosystems, with the leaf detritus lasting longer than rapidly consumed native species.

The experiments carried out for this thesis were all relatively small-scale laboratory-based work, using individuals of the focal amphipod species in isolation from one another. This was to ensure better control of the multiple factors that were being investigated but avoids interactions likely to occur in more complex real-world situations. For example, intraspecies interference competition is common in *D. villosus*, with conspecifics engaging in social aggregation and theft of prey (Médoc et al., 2015; Truhlar and Aldridge, 2015). These behaviours can reduce the predatory pressure exerted by the amphipod, which could alter predictions of impacts. Future work should add layers of complexity to investigate impacts of multiple stressors with multiple amphipods and a representative community of macroinvertebrates. The laboratory experiments also reduced the complexity of habitat structure. Different species, and even morphologically indistinguishable cryptic species of amphipod, can favour different habitat structures, which will further influence behaviour (Eisenring *et al.*, 2016). More complex habitat and community structures are likely to better simulate natural systems.

The major differences in impacts between native and invasive amphipods are likely to be due to the greater densities at which invasive populations can often be found. The higher predation of chironomid larvae by *D. villosus* compared to *G. pulex* shows that prey preferences may also cause differing outcomes. Interactions of amphipods with a range of other freshwater species and riparian plants may uncover more interactions that can potentially result in impacts to ecosystem structure or function. The high reproductive rate of invasive *D. villosus* should also be considered (Rewicz *et al.*, 2014). The experiments carried out as part of this thesis involved the measurement of behaviours of individuals or multiplied those individual results by an abundance count generated as a snapshot of amphipod density at a single point in time. It would be ideal to be able to carry out a longer term experiment which could take into account population dynamics because temperature, amphipod species, parasites and leaf detritus resources may all affect rates of reproduction differentially, as well as impacts to mortality and feeding rate.

References

Abelho, M. (2001) ‘From Litterfall to Breakdown in Streams: A Review’, *The Scientific World Journal*. Hindawi Limited, 1, p. 656. doi: 10.1100/TSW.2001.103.

Adamczuk, M. *et al.* (2019) ‘Allochthonous subsidies as driving forces for development of plankton in an autotrophic, temperate, and small lake’, *Hydrobiologia*. Springer, 846(1), pp. 59–73. doi: 10.1007/S10750-019-04052-9.

Aguilera, A. G. *et al.* (2010) ‘Impacts of the invasive plant Fallopia japonica (Houtt.) on plant communities and ecosystem processes’, *Biological Invasions*. Springer, 12(5), pp. 1243–1252. doi: 10.1007/S10530-009-9543-Z/TABLES/5.

Albeny-Simões, D. *et al.* (2014) ‘Attracted to the enemy: Aedes aegypti prefers oviposition sites with predator-killed conspecifics’, *Oecologia*. Springer Verlag, 175(2), pp. 481–492. doi: 10.1007/S00442-014-2910-1/FIGURES/3.

Albert, J. S. *et al.* (2021) ‘Scientists’ warning to humanity on the freshwater biodiversity crisis’, *Ambio*. Springer Science and Business Media B.V., 50(1), pp. 85–94. doi: 10.1007/S13280-020-01318-8/FIGURES/2.

Alp, M. *et al.* (2016) ‘Phenological response of a key ecosystem function to biological invasion’, *Ecology Letters*. Edited by R. Sterner. John Wiley & Sons, Ltd, 19(5), pp. 519–527. doi: 10.1111/ele.12585.

Anderson, N. H. and Cargill, A. S. (1987) ‘Nutritional Ecology of Aquatic Detritivorous Insects’, in Slansky, F. J. and Rodriguez, J. G. (eds) *Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates*. New York, pp. 903–925.

Anderson, N. H. and Cummins, K. W. (1979) 'Influences of Diet on the Life Histories of Aquatic Insects', *Journal of the Fisheries Research Board of Canada*. Canadian Science Publishing, 36(3), pp. 335–342. doi: 10.1139/F79-052.

Arundell, K. *et al.* (2015) 'Enemy release and genetic founder effects in invasive killer shrimp populations of Great Britain', *Biological Invasions*, 17(5), pp. 1439–1451. doi: 10.1007/s10530-014-0806-y.

Awachie, J. (1966) 'The development and life history of Echinorhynchus truttae Schrank, 1788 (Acanthocephala).', *Journal of helminthology*. University of Leeds, 40(1), pp. 11–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6006726.

Bacela-Spychalska, K. and Van Der Velde, G. (2013) 'There is more than one "killer shrimp": Trophic positions and predatory abilities of invasive amphipods of Ponto-Caspian origin', *Freshwater Biology*, 58(4), pp. 730–741. doi: 10.1111/fwb.12078.

Baldauf, S. A. *et al.* (2007) 'Infection with an acanthocephalan manipulates an amphipod's reaction to a fish predator's odours', *International Journal for Parasitology*. Pergamon, 37(1), pp. 61–65. doi: 10.1016/J.IJPARA.2006.09.003.

Bärlocher, F. (2016) 'Aquatic hyphomycetes in a changing environment', *Fungal Ecology*, 19, pp. 14–27. doi: 10.1016/j.funeco.2015.05.005.

Bärlocher, F. and Porter, C. W. (1986) 'Digestive Enzymes and Feeding Strategies of Three Stream Invertebrates', *Journal of the North American Benthological Society*. North American Benthological Society, 5(1), pp. 58–66. doi: 10.2307/1467747.

Bates, A. E. *et al.* (2013) 'Geographical range, heat tolerance and invasion success in aquatic species'. The Royal Society, 280(1772), p. 20131958. doi: 10.1098/RSPB.2013.1958.

Beasley, D. A. and Walton, W. E. (2016) ‘Suitability of monotypic and mixed diets for Anopheles hermsi larval development’, *Journal of Vector Ecology*. John Wiley & Sons, Ltd, 41(1), pp. 80–89. doi: 10.1111/JVEC.12197.

Becker, J. *et al.* (2016) ‘Metabolic activity and behavior of the invasive amphipod Dikerogammarus villosus and two common Central European gammarid species (Gammarus fossarum, Gammarus roeselii): Low metabolic rates may favor the invader’, *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. Pergamon, 191, pp. 119–126. doi: 10.1016/J.CBPA.2015.10.015.

Bellard, C., Cassey, P. and Blackburn, T. M. (2016) ‘Alien species as a driver of recent extinctions’, *Biology Letters*. Royal Society of London, 12(4), p. 20150623. doi: 10.1098/rsbl.2015.0623.

Benfield, E. F. (1997) ‘Comparison of litterfall input to streams’, *Journal of the North American Benthological Society*. North American Benthological Society, 16(1), pp. 104–108. doi: 10.2307/1468242.

Bertani, I., Primicerio, R. and Rossetti, G. (2016) ‘Extreme Climatic Event Triggers a Lake Regime Shift that Propagates Across Multiple Trophic Levels’, *Ecosystems*. Springer US, 19(1), pp. 16–31. doi: 10.1007/s10021-015-9914-5.

Bhardwaj, A. *et al.* (2013) ‘The genus Rhododendron: An ethnopharmacological and toxicological review Cite this paper The genus Rhododendron: An ethnopharmacological and toxicological review’, *Journal of Ethnopharmacology*, 147, pp. 42–62. doi: 10.1016/j.jep.2013.02.022.

Bij De Vaate, A. *et al.* (2002) ‘Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe 1’, *Can J Fish Aquat Sci*, 59, pp. 1159–1174. doi: 10.1139/F02-098.

Birk, S. *et al.* (2020) ‘Impacts of multiple stressors on freshwater biota across spatial scales and ecosystems’, *Nature Ecology & Evolution 2020 4:8*. Nature Publishing Group, 4(8), pp. 1060–1068. doi: 10.1038/s41559-020-1216-4.

Bjelke, U. *et al.* (2016) ‘Dieback of riparian alder caused by the Phytophthora alni complex: Projected consequences for stream ecosystems’, *Freshwater Biology*, 61(5), pp. 565–579. doi: 10.1111/fwb.12729.

Blackburn, T. M. *et al.* (2011) ‘A proposed unified framework for biological invasions’, *Trends in Ecology and Evolution*, 26(7), pp. 333–339. doi: 10.1016/J.TREE.2011.03.023.

Blackburn, T. M. *et al.* (2014) ‘A Unified Classification of Alien Species Based on the Magnitude of their Environmental Impacts’, *PLoS Biology*. Public Library of Science, 12(5), p. e1001850. doi: 10.1371/journal.pbio.1001850.

Blaustein, A. R. and Kiesecker, J. M. (2002) ‘Complexity in conservation: Lessons from the global decline of amphibian populations’, *Ecology Letters*, 5(4), pp. 597–608. doi: 10.1046/J.1461-0248.2002.00352.X.

Bloor, M. C. (2011) ‘Dietary Preference of Gammarus pulex and Asellus aquaticus during a Laboratory Breeding Programme for Ecotoxicological Studies’, *International Journal of Zoology*. Hindawi, 2011, pp. 1–5. doi: 10.1155/2011/294394.

Boeker, C. and Geist, J. (2015) ‘Effects of invasive and indigenous amphipods on physico-chemical and microbial properties in freshwater substrates’, *Aquatic Ecology*. Kluwer Academic Publishers, 49(4), pp. 467–480. doi: 10.1007/S10452-015-9539-Y/TABLES/2.

Bollache, L. *et al.* (2008) ‘Comparison of the functional responses of invasive and native amphipods’, *Biology Letters*, 4(2), pp. 166–169. doi: 10.1098/rsbl.2007.0554.

Boone, M. D. *et al.* (2007) ‘Multiple stressors in amphibian communities: effects of chemical contamination, bullfrogs and fish’, *Ecological Applications*, 17(1), pp. 291–301.

Braatne, J. H. *et al.* (2007) ‘Leaf Decomposition and Stream Macroinvertebrate Colonisation of Japanese Knotweed, an Invasive Plant Species’, *International Review of Hydrobiology*, 92(6), pp. 656–665. doi: 10.1002/iroh.200611009.

Bradbeer, S. J. *et al.* (2020) ‘The effectiveness of disinfectant and steam exposure treatments to prevent the spread of the highly invasive killer shrimp, Dikerogammarus villosus’, *Scientific Reports*, 10(1), p. 1919. doi: 10.1038/s41598-020-58058-8.

Breed, M. D. and Moore, J. (2016) ‘Foraging’, in Breed, M. D. and Moore, J. (eds) *Animal Behavior*. Second edi. London: Academic Press, pp. 293–323. doi: 10.1016/B978-0-12-801532-2.00009-X.

Bright, C. (1999) ‘Invasive Species: Pathogens of Globalization’, *Foreign Policy*. JSTOR, (116), p. 50. doi: 10.2307/1149643.

Brook, B. W., Sodhi, N. S. and Bradshaw, C. J. A. (2008) ‘Synergies among extinction drivers under global change’, *Trends in Ecology & Evolution*. Elsevier Current Trends, 23(8), pp. 453–460. doi: 10.1016/J.TREE.2008.03.011.

Brown, J. H. *et al.* (2004) ‘Toward a metabolic theory of ecology’, *Ecology*, 85(7), pp. 1771–1789. Available at: https://www.esa.org/history/Awards/papers/Brown_JH_MA.pdf

Callaway, R. M. and Ridenour, W. M. (2004) ‘Novel weapons: invasive success and the evolution of increased competitive ability’, *Frontiers in Ecology and the Environment*. Ecological Society of America, 2(8), pp. 436–443. doi: 10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2.

Capon, S. J., Stewart-Koster, B. and Bunn, S. E. (2021) ‘Future of Freshwater Ecosystems in a 1.5°C Warmer World’, *Frontiers in Environmental Science*. Frontiers Media S.A., 9, p. 596. doi: 10.3389/FENVS.2021.784642/BIBTEX.

Cardinale, B. J. *et al.* (2012) ‘Biodiversity loss and its impact on humanity’, *Nature*, 486(7401), pp. 59-67. doi: 10.1038/nature11148.

Cardinale, B. J., Palmer, M. A. and Collins, S. L. (2002) ‘Species diversity enhances ecosystem functioning through interspecific facilitation’, *Nature*, 415(6870), pp. 426–429. doi: 10.1038/415426a.

Cardoso, P. G. *et al.* (2018) ‘Interactive effects of increased temperature, pCO2 and the synthetic progestin levonorgestrel on the fitness and breeding of the amphipod Gammarus locusta’, *Environmental Pollution*. Elsevier, 236, pp. 937–947. doi: 10.1016/J.ENVPOL.2017.10.065.

Chapin, F. S. *et al.* (2000) ‘Consequences of changing biodiversity’, *Nature*, 405(6783), pp. 234–242. doi: 10.1038/35012241.

Christopher, F. J., Argent, D. G. and Kimmel, W. G. (2014) ‘Temporal Macroinvertebrate Community Structure in Leaf Packs from a Stream Ohio River Basin Assessment View project’, *Keystone Journal of Undergraduate Research*, 2(1), pp. 29–36. Available at: https://www.researchgate.net/publication/271211144 (Accessed: 8 April 2022).

Clavero, M. and García-Berthou, E. (2005) ‘Invasive species are a leading cause of animal extinctions’, *Trends in Ecology & Evolution*, 20(3), p. 110. doi: 10.1016/j.tree.2005.01.003.

Constable, D. and Birkby, N. J. (2016) ‘The impact of the invasive amphipod Dikerogammarus haemobaphes on leaf litter processing in UK rivers’, *Aquatic Ecology*. Springer Netherlands, 50(2), pp. 273–281. doi: 10.1007/s10452-016-9574-3.

Cornwell, W. K. *et al.* (2008) ‘Plant species traits are the predominant control on litter decomposition rates within biomes worldwide’, *Ecology Letters*. John Wiley & Sons, Ltd, 11(10), pp. 1065–1071. doi: 10.1111/J.1461-0248.2008.01219.X.

Côté, I. M., Darling, E. S. and Brown, C. J. (2016) ‘Interactions among ecosystem stressors and their importance in conservation’, *Proceedings of the Royal Society B: Biological Sciences*. The Royal Society, 283(1824). doi: 10.1098/RSPB.2015.2592.

Cottin, D. *et al.* (2012) ‘Disentangling the effects of local and regional factors on the thermal tolerance of freshwater crustaceans’, *Naturwissenschaften*. Springer-Verlag, 99(4), pp. 259–264. doi: 10.1007/s00114-012-0894-4.

Covich, A. P. *et al.* (2004) ‘The role of biodiversity in the functioning of freshwater and marine benthic ecosystems’, *BioScience*, 54(8), pp. 767–775. doi: 10.1641/0006-3568(2004)054[0767:TROBIT]2.0.CO;2.

Covich, A. P. and Thorp, J. H. (2010) ‘Introduction to the Subphylum Crustacea’, in Covich, A. P. and Thorp, J. H. (eds) *Ecology and Classification of North American Freshwater Invertebrates*. Third edit. London: Elsevier Inc, pp. 695–722. Available at: https://books.google.co.uk/books?hl=en&lr=&id=gKHmGKqMrgQC&oi=fnd&pg=PP1&ots=lUXXYQ_GZU&sig=4oNqlD3L9r8PkY26215aa5s-LnY&redir_esc=y#v=onepage&q&f=false

Cox, J. G. and Lima, S. L. (2006) ‘Naiveté and an aquatic–terrestrial dichotomy in the effects of introduced predators’, *Trends in Ecology & Evolution*. Elsevier Current Trends, 21(12), pp. 674–680. doi: 10.1016/J.TREE.2006.07.011.

Coyne, K. J. *et al.* (2021) ‘Interactive effects of light, CO2 and temperature on growth and resource partitioning by the mixotrophic dinoflagellate, Karlodinium veneficum’, *PLOS ONE*. Public Library of Science, 16(10), p. e0259161. doi: 10.1371/JOURNAL.PONE.0259161.

Crain, C. M., Kroeker, K. and Halpern, B. S. (2008) ‘Interactive and cumulative effects of multiple human stressors in marine systems’, *Ecology Letters*, 11(12), pp. 1304–1315. doi: 10.1111/J.1461-0248.2008.01253.X.

Cross, J. R. (1975) ‘Rhododendron Ponticum L.’, *Source: Journal of Ecology*, 63(1), pp. 345–364.

Cummins, K. W. *et al.* (1989) ‘Shredders and Riparian Vegetation’, *BioScience*. Oxford University Press (OUP), 39(1), pp. 24–30. doi: 10.2307/1310804.

Cummins, K. W. (2019) ‘Functional Analysis of Stream Macroinvertebrates’, in *Limnology*. IntechOpen. doi: 10.5772/intechopen.79913.

Cummins, K. W. and Klug, M. J. (1979) ‘Feeding Ecology of Stream Invertebrates’, *Annual Review of Ecology and Systematics*, 10, pp. 147–172. Available at: https://about.jstor.org/terms (Accessed: 24 May 2021).

Cummins, K. W., Merritt, R. W. and Andrade, P. C. N. (2005) ‘The use of invertebrate functional groups to characterize ecosystem attributes in selected streams and rivers in south Brazil’, *Studies on Neotropical Fauna and Environment*, 40(1), pp. 69–89.

Cuthbert, R. N. *et al.* (2021) ‘Economic costs of biological invasions in the United Kingdom’, *NeoBiota 67: 299-328*. Pensoft Publishers, 67, pp. 299–328. doi: 10.3897/NEOBIOTA.67.59743.

Cuthbert, R. N. *et al.* (2022) ‘Biological invasion costs reveal insufficient proactive management worldwide’, *Science of the Total Environment*. Elsevier B.V., 819. doi: 10.1016/J.SCITOTENV.2022.153404.

Dangles, O., Jonsson, M. and Malmqvist, B. (2002) ‘The Importance of Detritivore Species Diversity for Maintaining Stream Ecosystem Functioning Following the Invasion of a Riparian Plant’, *Biological Invasions*, 4, pp. 441–446. Available at: https://www.academia.edu/13238817/The_Importance_of_Detritivore_Species_Diversity_for_Maintaining_Stream_Ecosystem_Functioning_Following_the_Invasion_of_a_Riparian_Plant

Dangles, O. and Malmqvist, B. (2004) ‘Species richness-decomposition relationships depend on species dominance’, *Ecology Letters*. John Wiley & Sons, Ltd (10.1111), 7(5), pp. 395–402. doi: 10.1111/j.1461-0248.2004.00591.x.

Darwall, W. R. *et al.* (2011) ‘Implications of Bias in Conservation Research and Investment for Freshwater Species Recommended Citation’, *Conservation Letters*, 4, pp. 474–482. doi: 10.1111/j.1755-263X.2011.00202.x.

Daufresne, M., Bady, P. and Fruget, J.-F. (2007) ‘Impacts of global changes and extreme hydroclimatic events on macroinvertebrate community structures in the French Rhône River’, *Oecologia*. Springer-Verlag, 151(3), pp. 544–559. doi: 10.1007/s00442-006-0655-1.

Daufresne, M., Lengfellner, K. and Sommer, U. (2009) ‘Global warming benefits the small in aquatic ecosystems’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 106(31), p. 12788. doi: 10.1073/PNAS.0902080106.

Davidson, C. and Knapp, R. A. (2007) ‘Multiple stressors and amphibian declines: dual impacts of pesticides and fish on yellow-legged frogs’, *Ecological Applications*, 17(2), pp. 587–597.

Davis, M. A. (2009) *Invasion Biology*. First diti. Oxford. Available at: https://books.google.co.uk/books?hl=en&lr=&id=41jS4xjy3P8C&oi=fnd&pg=PR7&ots=5apcDFC9C_&sig=pTxDiVU9Z2cdGuRly-9VSLYYUQc&redir_esc=y#v=onepage&q&f=false (Accessed: 26 May 2022).

DeBoer, J. A., Anderson, A. M. and Casper, A. F. (2018) ‘Multi-trophic response to invasive silver carp (Hypophthalmichthys molitrix) in a large floodplain river’, *Freshwater Biology*. John Wiley & Sons, Ltd (10.1111), 63(6), pp. 597–611. doi: 10.1111/fwb.13097.

Dehnen-Schmutz, K., Perrings, C. and Williamson, M. (2004) ‘Controlling Rhododendron ponticum in the British Isles: An economic analysis’, *Journal of Environmental Management*. Academic Press, 70(4), pp. 323–332. doi: 10.1016/j.jenvman.2003.12.009.

Dekanová, V. *et al.* (2021) ‘Litter breakdown of invasive alien plant species in a pond environment: Rapid decomposition of Solidago canadensis may alter resource dynamics’, *Limnologica*. Elsevier GmbH, 90. doi: 10.1016/J.LIMNO.2021.125911.

Devin, S. *et al.* (2004) ‘Life History Traits of the Invader Dikerogammarus villosus (Crustacea: Amphipoda) in the Moselle River, France’, *International Review of Hydrobiology*. John Wiley & Sons, Ltd, 89(1), pp. 21–34. doi: 10.1002/IROH.200310667.

Diagne, C. *et al.* (2021) ‘High and rising economic costs of biological invasions worldwide’, *Nature*, 592, pp. 571-576. doi: 10.1038/s41586-021-03405-6.

Dianne, L. *et al.* (2012) ‘Larval size in acanthocephalan parasites: Influence of intraspecific competition and effects on intermediate host behavioural changes’, *Parasites & Vectors*. BioMed Central, 5(1), p. 166. doi: 10.1186/1756-3305-5-166.

Dick, J. T. A. (2008) ‘Role of behaviour in biological invasions and species distributions; lessons from interactions between the invasive *Gammarus pulex* and the native *G. duebeni* (Crustacea : Amphipoda)’, *Contribution to Zoology*, 77(2), pp. 91–98.

Dick, J. T. A. *et al.* (2010) ‘Parasitism may enhance rather than reduce the predatory impact of an invader.’, *Biology letters*, 6(5), pp. 636–638. doi: 10.1098/rsbl.2010.0171.

Dick, J. T. A. *et al.* (2017) ‘Invader Relative Impact Potential: a new metric to understand and predict the ecological impacts of existing, emerging and future invasive alien species’, *Journal of Applied Ecology*. Blackwell Publishing Ltd, 54(4), pp. 1259–1267. doi: 10.1111/1365-2664.12849.

Dick, J. T. A., Montgomery, I. and Elwood, R. W. (1993) ‘Replacement of the indigenous amphipod Gammarus duebeni celticus by the introduced G. pulex: differential cannibalism and mutual predation’, *Journal of Animal Ecology*, 62, pp. 79–88.

Dick, Jaimie T A *et al.* (1995) ‘The behavioural basis of a species replacement: differential aggresssion and predation between the introduced Gammarus pulex and the native G. duebeni celticus (Amphipoda)’, *Behavioral Ecology and Sociobiology*. Springer, 37(6), pp. 393–398. doi: 10.1007/BF00170586.

Dickey, J. W. E. *et al.* (2018) ‘Assessing the relative potential ecological impacts and invasion risks of emerging and future invasive alien species’, *NeoBiota*. Pensoft Publishers, (40), pp. 1–24. doi: 10.3897/neobiota.40.28519.

Diez, J. M. *et al.* (2012) ‘Will extreme climatic events facilitate biological invasions?’, *Frontiers in Ecology and the Environment*, 10(5), pp. 249–257. doi: 10.1890/110137.

Dodd, J. A. *et al.* (2014) ‘Predicting the ecological impacts of a new freshwater invader: Functional responses and prey selectivity of the “killer shrimp”, Dikerogammarus villosus, compared to the native Gammarus pulex’, *Freshwater Biology*, 59(2), pp. 337–352. doi: 10.1111/fwb.12268.

Doherty-Bone, T. M., Dunn, A. M., Brittain, J., *et al.* (2018) ‘Invasive alien shredders clear up invasive alien leaf litter’, *Ecology and Evolution*. John Wiley and Sons Ltd, 8(20), pp. 10049–10056. doi: 10.1002/ece3.4430.

Doherty-Bone, T. M., Dunn, A. M., Liddell, C., *et al.* (2018) ‘Transformation of detritus by a European native and two invasive alien freshwater decapods’, *Biological Invasions*. Springer, 20(7), p. 1799. doi: 10.1007/S10530-018-1661-Z.

Doherty-Bone, T. M. *et al.* (2019) ‘Multi-faceted impacts of native and invasive alien decapod species on freshwater biodiversity and ecosystem functioning’, *Freshwater Biology*. John Wiley & Sons, Ltd (10.1111), 64(3), pp. 461–473. doi: 10.1111/fwb.13234.

Dudgeon, D. *et al.* (2006) ‘Freshwater biodiversity: importance, threats, status and conservation challenges’, *Biological Reviews*, 81(02), p. 163. doi: 10.1017/S1464793105006950.

Dukes, J. S. and Mooney, H. A. (1999) ‘Does global change increase the success of biological invaders?’, *Trends in Ecology & Evolution*. Elsevier Current Trends, 14(4), pp. 135–139. doi: 10.1016/S0169-5347(98)01554-7.

Dunn, A. M. *et al.* (2012) ‘Indirect effects of parasites in invasions’, *Functional Ecology*. John Wiley & Sons, Ltd, 26(6), pp. 1262–1274. doi: 10.1111/j.1365-2435.2012.02041.x.

Durance, I. and Ormerod, S. J. (2007) ‘Climate change effects on upland stream macroinvertebrates over a 25-year period’, *Global Change Biology*, 13(5), pp. 942–957. doi: 10.1111/j.1365-2486.2007.01340.x.

Early, R. *et al.* (2016) ‘Global threats from invasive alien species in the twenty-first century and national response capacities’, *Nature Communications*. Nature Publishing Group, 7(1), pp. 1–9. doi: 10.1038/ncomms12485.

Edgerton, B. F. *et al.* (2004) ‘Understanding the Causes of Disease in European Freshwater Crayfish’, *Conservation Biology*. John Wiley & Sons, Ltd, 18(6), pp. 1466–1474. doi: 10.1111/J.1523-1739.2004.00436.X.

Eisenring, M. *et al.* (2016) ‘Habitat requirements and ecological niche of two cryptic amphipod species at landscape and local scales’, *Ecosphere*. John Wiley & Sons, Ltd, 7(5), p. e01319. doi: 10.1002/ECS2.1319.

Elton, C. S. (1958) *The Ecology of Invasions by Animals and Plants*. Boston, MA: Springer US. doi: 10.1007/978-1-4899-7214-9.

En, D. E., Nilsen, E. T. and Oktay Yildiz, • (2006) ‘Ecology, Competitive Advantages, and Integrated Control of Rhododendron: An Old Ornamental yet Emerging Invasive Weed Around the Globe’, in SilvaJ.A., T. da (ed.) *Floriculture, Ornamental and Plant Biotechnology Volume III*. Middlesex: Global Science Books Ltd, pp. 407–421.

Essl, F. *et al.* (2020) ‘Drivers of future alien species impacts: An expert-based assessment’, *Global Change Biology*. Blackwell Publishing Ltd, 26(9), pp. 4880–4893. doi: 10.1111/GCB.15199.

European Parliament, C. of the E. U. (2015) *Regulation (EU) No 1143/2014 of the European Parliament and of the Council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species*. Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?qid=1417443504720&uri=CELEX:32014R1143.

Faghihinia, M. *et al.* (2021) ‘Freshwater biodiversity at different habitats: Research hotspots with persistent and emerging themes’, *Ecological Indicators*. Elsevier, 129, p. 107926. doi: 10.1016/J.ECOLIND.2021.107926.

Fernández-Loras, A. *et al.* (2019) ‘Infection with Batrachochytrium dendrobatidis lowers heat tolerance of tadpole hosts and cannot be cleared by brief exposure to CTmax’, *PLoS ONE*. Public Library of Science, 14(4). doi: 10.1371/journal.pone.0216090.

Fielding, N. J. *et al.* (2003) ‘Effects of the acanthocephalan parasite Echinorhynchus truttae on the feeding ecology of Gammarus pulex (Crustacea: Amphipoda)’, *Journal of Zoology*. Blackwell Publishing Ltd, 261(3), pp. 321–325. doi: 10.1017/S0952836903004230.

Fielding, N. J. *et al.* (2005) ‘Ecological impacts of the microsporidian parasite Pleistophora mulleri on its freshwater amphipod host Gammarus duebeni celticus’, *Parasitology*, 131, pp. 331–336. doi: 10.1017/S0031182005007754.

Fincham, W. *et al.* (2019) ‘Invasion success of a widespread invasive predator may be explained by a high predatory efficacy but may be influenced by pathogen infection’, *Biological Invasions*, 21, pp. 3545–3560. doi: 10.1007/s10530-019-02067-w.

Fletcher, R. A. *et al.* (2019) ‘Invasive plants negatively impact native, but not exotic, animals’, *Global Change Biology*. John Wiley & Sons, Ltd, 25(11), pp. 3694–3705. doi: 10.1111/gcb.14752.

Fogelman, K. J. *et al.* (2018) ‘Decomposition and benthic macroinvertebrate communities of exotic Japanese knotweed (Fallopia japonica) and American sycamore (Platanus occidentalus) detritus within the Susquehanna River’, *https://doi.org/10.1080/02705060.2018.1458660*. Taylor & Francis, 33(1), pp. 299–310. doi: 10.1080/02705060.2018.1458660.

Folke, C. *et al.* (2004) ‘Regime Shifts, Resilience, and Biodiversity in Ecosystem Management’, *http://dx.doi.org/10.1146/annurev.ecolsys.35.021103.105711*. Annual Reviews, 35, pp. 557–581. doi: 10.1146/ANNUREV.ECOLSYS.35.021103.105711.

Foucreau, N. *et al.* (2013) ‘Effect of leaf litter characteristics on leaf conditioning and on consumption by Gammarus pulex’, *Freshwater Biology*, 58(8), pp. 1672–1681. doi: 10.1111/FWB.12158.

Friesen, O. C. *et al.* (2017) ‘Differential impacts of shared parasites on fitness components among competing hosts’, *4682 | Ecology and Evolution*, 7, pp. 4682–4693. doi: 10.1002/ece3.3062.

Galic, N. and Forbes, V. E. (2017) ‘Effects of temperature on the performance of a freshwater amphipod’, *Hydrobiologia*. Springer International Publishing, 785(1), pp. 35–46. doi: 10.1007/s10750-016-2901-9.

Gallardo, B. *et al.* (2016) ‘Global ecological impacts of invasive species in aquatic ecosystems’, *Global Change Biology*. John Wiley & Sons, Ltd (10.1111), 22(1), pp. 151–163. doi: 10.1111/gcb.13004.

Gallardo, B. and Aldridge, D. C. (2018) ‘Inter-basin water transfers and the expansion of aquatic invasive species’, *Water Research*. Pergamon, 143, pp. 282–291. doi: 10.1016/J.WATRES.2018.06.056.

Garner, G. *et al.* (2014) ‘River temperature regimes of England and Wales: Spatial patterns, inter-annual variability and climatic sensitivity’, *Hydrological Processes*. John Wiley & Sons, Ltd, 28(22), pp. 5583–5598. doi: 10.1002/hyp.9992.

Gehman, A.-L. M. L. M., Hall, R. J. and Byers, J. E. (2018) ‘Host and parasite thermal ecology jointly determine the effect of climate warming on epidemic dynamics’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 115(4), pp. 744–749. doi: 10.1073/pnas.1705067115.

Gehman, A. L. M. and Harley, C. D. G. (2019) ‘Symbiotic endolithic microbes alter host morphology and reduce host vulnerability to high environmental temperatures’, *Ecosphere*. Wiley-Blackwell, 10(4). doi: 10.1002/ecs2.2683.

Gendron, A. D., Marcogliese, D. J. and Thomas, M. (2012) ‘Invasive species are less parasitized than native competitors, but for how long? The case of the round goby in the Great Lakes-St. Lawrence Basin’, *Biological Invasions*, 14(2), pp. 367–384. doi: 10.1007/s10530-011-0083-y.

Gerber, E. *et al.* (2008) ‘Exotic invasive knotweeds (Fallopia spp.) negatively affect native plant and invertebrate assemblages in European riparian habitats’, *Biological Conservation*. Elsevier, 141(3), pp. 646–654. doi: 10.1016/J.BIOCON.2007.12.009.

Gessner, M. and Chauvet, E. (2002) ‘A Case for Using Litter Breakdown to Assess Functional Stream Integrity’, *Ecological Applications*, 12(2), pp. 498–510. doi: 10.1890/10510761(2002)012.

Gessner, M. O. *et al.* (2010) ‘Diversity meets decomposition’, *Trends in Ecology and Evolution*. Elsevier Ltd, 25(6), pp. 372–380. doi: 10.1016/j.tree.2010.01.010.

Glazier, D. S. (2009) ‘Amphipoda’, in Mehner, T. (ed.) *Encyclopedia of Inland Waters*. First edit. Academic Press, pp. 89–115. doi: 10.1016/B978-012370626-3.00154-X.

Going, B. M. and Dudley, T. L. (2007) ‘Invasive riparian plant litter alters aquatic insect growth’, *Biological Invasions 2007 10:7*. Springer, 10(7), pp. 1041–1051. doi: 10.1007/S10530-007-9182-1.

Grabner, D. S. (2017) ‘Hidden diversity: parasites of stream arthropods’, *Freshwater Biology*, 62(1), pp. 52–64. doi: 10.1111/fwb.12848.

Grabowski, M., Bacela, K. and Konopacka, A. (2007) ‘How to be an invasive gammarid (Amphipoda: Gammaroidea)–comparison of life history traits’, *Hydrobiologia*, 590, pp. 75–84. doi: 10.1007/s10750-007-0759-6.

Graça, M. A. S. *et al.* (1993) ‘Importance of fungi in the diet of Gammarus pulex and Asellus aquaticus’, *Oecologia*. Springer-Verlag, 96(3), pp. 304–309. doi: 10.1007/BF00317498.

Graça, M. A. S. (2001) ‘The role of invertebrates on leaf litter decomposition in streams–a review’, *International Review of Hydrobiology*, 86(4–5), pp. 383–393. doi: 10.1002/1522-2632(200107)86:4/5<383::AID-IROH383>3.0.CO;2-D.

Green, P. T. *et al.* (2011) ‘Invasional meltdown: Invader-invader mutualism facilitates a secondary invasion’, *Ecology*, 92(9), pp. 1758–1768. doi: 10.1890/11-0050.1.

Greenspan, S. E. *et al.* (2017) ‘Infection increases vulnerability to climate change via effects on host thermal tolerance’, *Scientific Reports*. Nature Publishing Group, 7(1). doi: 10.1038/s41598-017-09950-3.

Gulis, V., Su, R. and Kuehn, K. A. (2019) ‘Fungal Decomposers in Freshwater Environments’, in Hurst, C. (ed.) *The structure and function of aquatic microbial communities*. Volume 7. Cham: Springer, pp. 121–155. doi: 10.1007/978-3-030-16775-2_5.

Gutowsky, L. F. G. *et al.* (2019) ‘Quantifying multiple pressure interactions affecting populations of a recreationally and commercially important freshwater fish’, *Global Change Biology*. John Wiley & Sons, Ltd, 25(3), pp. 1049–1062. doi: 10.1111/gcb.14556.

Hall, R. O. and Meyer, J. L. (1995) ‘The trophic significance of bacteria in a detritus-based stream food web’, *Ecology*, 79(6), pp. 1995–2012.

Hatcher, M. J., Dick, J. T. A. and Dunn, A. M. (2012) ‘Diverse effects of parasites in ecosystems: Linking interdependent processes’, *Frontiers in Ecology and the Environment*, 10(4), pp. 186–194. doi: 10.1890/110016.

Hatcher, M. J., Dick, J. T. A. and Dunn, A. M. (2014) ‘Parasites that change predator or prey behaviour can have keystone effects on community composition’, *Biology Letters*, 10(1), pp. 20130879–20130879. doi: 10.1098/rsbl.2013.0879.

Hatcher, M. J. and Dunn, A. M. (2011) *Parasites in ecological communities : from interactions to ecosystems*. Cambridge University Press. Available at: http://www.cambridge.org/gb/academic/subjects/life-sciences/ecology-and-conservation/parasites-ecological-communities-interactions-ecosystems?format=PB&isbn=9780521718226#JH1rGOCg3MTEkhXe.97.

Heger, T. *et al.* (2019) 'Towards an Integrative, Eco-Evolutionary Understanding of Ecological Novelty: Studying and Communicating Interlinked Effects of Global Change', *BioScience*, 888(11). doi: 10.1093/biosci/biz095.

Heino, J., Virkkala, R. and Toivonen, H. (2009) 'Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions', *Biological Reviews*. John Wiley & Sons, Ltd, 84(1), pp. 39–54. doi: 10.1111/J.1469-185X.2008.00060.X.

Hellmann, C. *et al.* (2015) 'The trophic function of Dikerogammarus villosus (Sowinsky, 1894) in invaded rivers: A case study in the Elbe and Rhine', *Aquatic Invasions*, 10(4), pp. 385–397. doi: 10.3391/ai.2015.10.4.03.

Hellmann, C. *et al.* (2017) 'River-specific effects of the invasive amphipod Dikerogammarus villosus (Crustacea: Amphipoda) on benthic communities', *Biological Invasions*. Springer International Publishing, 19(1), pp. 381–398. doi: 10.1007/s10530-016-1286-z.

Hellmann, J. J. *et al.* (2008) 'Five Potential Consequences of Climate Change for Invasive Species', *Conservation Biology*, 22(3), pp. 534–543. doi: 10.1111/j.1523-1739.2008.00951.x.

Hernandez, A. D. and Sukhdeo, M. V. K. (2008) 'Parasite effects on isopod feeding rates can alter the host's functional role in a natural stream ecosystem', *International Journal for Parasitology*, 38(6), pp. 683–690. doi: 10.1016/j.ijpara.2007.09.008.

Hladyz, S. *et al.* (2009) 'Resource quality and stoichiometric constraints on stream ecosystem functioning', *Freshwater Biology*, 54(5), pp. 957–970. doi: 10.1111/J.1365-2427.2008.02138.X.

Hladyz, S. *et al.* (2011) ‘Impacts of an aggressive riparian invader on community structure and ecosystem functioning in stream food webs’, *Journal of Applied Ecology*, 48(2), pp. 443–452. doi: 10.1111/j.1365-2664.2010.01924.x.

Hooper, D. U. *et al.* (2012) ‘A global synthesis reveals biodiversity loss as a major driver of ecosystem change’, *Nature*, 486, pp. 105–108. doi: 10.1038/nature11118.

Hupało, K. *et al.* (2018) ‘Climate change as a possible driver of invasion and differential in HSP70 expression in two genetically distinct populations of the invasive killer shrimp, Dikerogammarus villosus’, *Biological Invasions*. Springer International Publishing, 20(8), pp. 2047–2059. doi: 10.1007/s10530-018-1679-2.

Iacarella, J. C. *et al.* (2015) ‘Ecological impacts of invasive alien species along temperature gradients: Testing the role of environmental matching’, *Ecological Applications*. Ecological Society of America, 25(3), pp. 706–716. doi: 10.1890/14-0545.1.

Jackson, M. C. (2015) ‘Interactions among multiple invasive animals’, *Ecology*. John Wiley & Sons, Ltd, 96(8), pp. 2035–2041. doi: 10.1890/15-0171.1.

Jacobson, T., Prevodnik, A. and Sundelin, B. (2008) ‘Combined effects of temperature and a pesticide on the Baltic amphipod Monoporeia affinis’, *Aquatic Biology*, 1(3), pp. 269–276. doi: 10.3354/AB00028.

Jankowski, T. *et al.* (2006) ‘Consequences of the 2003 European heat wave for lake temperature profiles, thermal stability, and hypolimnetic oxygen depletion: Implications for a warmer world’, *Limnology and Oceanography*. Wiley-Blackwell, 51(2), pp. 815–819. doi: 10.4319/lo.2006.51.2.0815.

Jansen, S. A. *et al.* (2012) ‘Grayanotoxin Poisoning: “Mad Honey Disease” and Beyond’, *Cardiovascular Toxicology*. Springer, 12(3), p. 208. doi: 10.1007/S12012-012-9162-2.

Jaric´, I. and Cvijanovic´, G. (2012) ‘The Tens Rule in Invasion Biology: Measure of a True Impact or Our Lack of Knowledge and Understanding?’, *Environmental Management*. Springer, 50(6), pp. 979–981. doi: 10.1007/S00267-012-9951-1.

Jenkins, G. J. *et al.* (2009) *UK Climate Projections: Briefing Report*. Exeter, UK. Available at: http://ukclimateprojections.defra.gov.uk (Accessed: 20 January 2018).

Kato-Noguchi, H. (2021) ‘Allelopathy of Knotweeds as Invasive Plants.’, *Plants (Basel, Switzerland)*. MDPI, 11(1). doi: 10.3390/plants11010003.

Kaushal, S. S. *et al.* (2010) ‘Rising stream and river temperatures in the United States’, *Frontiers in Ecology and the Environment*, 8(9), pp. 461–466. doi: 10.1890/090037.

Keller, W. (2007) ‘Implications of climate warming for Boreal Shield lakes: a review and synthesis’, *Environmental Reviews*, 15, pp. 99–112. doi: 10.1139/A07-002.

Kelley, A. L. (2014) ‘The role thermal physiology plays in species invasion’, *Conserv Physiol*. Kelley AL, 2(1). doi: 10.1093/conphys/cou045.

Kelly, D. W. *et al.* (2006) ‘Invasion by the amphipod Gammarus pulex alters community composition of native freshwater macroinvertebrates’, *Diversity and Distributions*. John Wiley & Sons, Ltd, 12(5), pp. 525–534. doi: 10.1111/J.1366-9516.2006.00275.X.

Kelly, D. W., Dick, J. T. A. and Montgomery, W. I. (2002) ‘Predation on mayfly nymph, Baetis rhodani, by native and introduced Gammarus: direct effects and the facilitation of predation by salmonids’, *Freshwater Biology*, 47, pp. 1257–1268.

Kemp, J. S., Tang, F. and Aldridge, D. C. (2018) ‘Quantifying invader impact: Applying functional response metrics to a rapidly spreading non-native species’, *Freshwater Biology*. Blackwell Publishing Ltd, 63(12), pp. 1514–1522. doi: 10.1111/fwb.13180.

Kenna, D. *et al.* (2017) ‘Antagonistic effects of biological invasion and environmental warming on detritus processing in freshwater ecosystems’, *Oecologia*. Springer Berlin Heidelberg, 183(3), pp. 875–886. doi: 10.1007/s00442-016-3796-x.

Kinzler, W. *et al.* (2009) ‘Mutual predation between and cannibalism within several freshwater gammarids: Dikerogammarus villosus versus one native and three invasives’, *Aquatic Ecology*. Springer Netherlands, 43(2), pp. 457–464. doi: 10.1007/s10452-008-9206-7.

Koester, M. *et al.* (2018) ‘Is The invasive amphipod Dikerogammarus villosus the main factor structuring the benthic community across different types of water bodies in the River Rhine system?’, *Limnologica*. Urban & Fischer, 71, pp. 44–50. doi: 10.1016/J.LIMNO.2018.06.001.

Koester, M., Bayer, B. and Gergs, R. (2016) ‘Is Dikerogammarus villosus (Crustacea, Gammaridae) a “killer shrimp” in the River Rhine system?’, *Hydrobiologia*, 768(1), pp. 299–313. doi: 10.1007/s10750-015-2558-9.

Kohler, S. A., Parker, M. O. and Ford, A. T. (2018) ‘Shape and size of the arenas affect amphipod behaviours: Implications for ecotoxicology’, *PeerJ*. PeerJ Inc., 2018(7). doi: 10.7717/peerj.5271.

Krisp, H. and Maier, G. (2005) ‘Consumption of macroinvertebrates by invasive and native gammarids: a comparison’, *Journal of Limnology*, 64(1), p. 55. doi: 10.4081/jlimnol.2005.55.

Kritzberg, E. S. *et al.* (2020) ‘Browning of freshwaters: Consequences to ecosystem services, underlying drivers, and potential mitigation measures’, *Ambio*. Springer, 49(2), pp. 375–390. doi: 10.1007/S13280-019-01227-5/FIGURES/6.

Kuebbing, S. E. and Nuñez, M. A. (2015) ‘Negative, neutral, and positive interactions among nonnative plants: Patterns, processes, and management implications’, *Global Change Biology*, 21(2), pp. 926–934. doi: 10.1111/GCB.12711.

Kuris, A. M. *et al.* (2008) ‘Ecosystem energetic implications of parasite and free-living biomass in three estuaries’, *Nature*, 454(7203), pp. 515–518. doi: 10.1038/nature06970.

Labaude, S. *et al.* (2015) ‘Influence of host nutritional condition on post-infection traits in the association between the manipulative acanthocephalan Pomphorhynchus laevis and the amphipod Gammarus pulex’, *Parasites & Vectors*. BioMed Central, 8(1), p. 403. doi: 10.1186/s13071-015-1017-9.

Lafferty, K. D., Dobson, A. P. and Kuris, A. M. (2006) ‘Parasites dominate food web links.’, *Proceedings of the National Academy of Sciences of the United States of America*. National Academy of Sciences, 103(30), pp. 11211–6. doi: 10.1073/pnas.0604755103.

Lagrue, C., Gueventam, A. and Bollache, L. (2013) ‘Manipulative parasites may not alter intermediate host distribution but still enhance their transmission: field evidence for increased vulnerability to definitive hosts and non-host predator avoidance’, *Parasitology*, 140(2), pp. 258–265. doi: 10.1017/S0031182012001552.

Larsen, M. H. and Mouritsen, K. N. (2009) ‘Increasing temperature counteracts the impact of parasitism on periwinkle consumption’, *Marine Ecology Progress Series*, 383, pp. 141–149. doi: 10.3354/MEPS08021.

Larsen, S., Muehlbauer, J. D. and Marti, E. (2016) ‘Resource subsidies between stream and terrestrial ecosystems under global change’, *Global Change Biology*. Blackwell Publishing Ltd, 22(7), pp. 2489–2504. doi: 10.1111/gcb.13182.

Laverty, C. *et al.* (2015) ‘Differential ecological impacts of invader and native predatory freshwater amphipods under environmental change are revealed by comparative functional responses’, *Biological Invasions*. Kluwer Academic Publishers, 17(6), pp. 1761–1770. doi: 10.1007/S10530-014-0832-9.

Laverty, C. *et al.* (2017) ‘Temperature rise and parasitic infection interact to increase the impact of an invasive species’, *International Journal for Parasitology*. Australian Society for Parasitology, 47(5), pp. 291–296. doi: 10.1016/j.ijpara.2016.12.004.

Lecerf, A. *et al.* (2011) ‘Stream ecosystems respond to riparian invasion by Japanese knotweed (Fallopia japonica)’, *Can J Fish Aquat Sci*. NRC Research Press Ottawa, Canada, 64(9), pp. 1273–1283. doi: 10.1139/F07-092.

Ledesma, J. L. J., Köhler, S. J. and Futter, M. N. (2012) ‘Long-term dynamics of dissolved organic carbon: Implications for drinking water supply’, *Science of The Total Environment*. Elsevier, 432, pp. 1–11. doi: 10.1016/J.SCITOTENV.2012.05.071.

Leung, J. Y. S., Connell, S. D. and Russell, B. D. (2017) ‘Heatwaves diminish the survival of a subtidal gastropod through reduction in energy budget and depletion of energy reserves’, *Scientific Reports*, 7(1). doi: 10.1038/s41598-017-16341-1.

Levi, T. *et al.* (2020) ‘Community Ecology and Conservation of Bear-Salmon Ecosystems’, *Frontiers in Ecology and Evolution*. Frontiers Media S.A., 8, p. 433. doi: 10.3389/FEVO.2020.513304/BIBTEX.

Lewis, S. E. *et al.* (2012) ‘Impact of acanthocephalan parasites on aggregation behavior of amphipods (Gammarus pseudolimnaeus)’, *Behavioural Processes*. Elsevier, 91(2), pp. 159–163. doi: 10.1016/J.BEPROC.2012.07.009.

Lincoln, A. E. and Quinn, T. P. (2019) ‘Optimal foraging or surplus killing: selective consumption and discarding of salmon by brown bears’, *Behavioral Ecology*. Oxford Academic, 30(1), pp. 202–212. doi: 10.1093/BEHECO/ARY139.

Little, C. J. and Altermatt, F. (2018) ‘Species turnover and invasion of dominant freshwater invertebrates alter biodiversity-ecosystem-function relationship’, *Ecological Monographs*. Wiley-Blackwell, 88(3), pp. 461–480. doi: 10.1002/ecm.1299.

Lowry, E. *et al.* (2013) ‘Biological invasions: a field synopsis, systematic review, and database of the literature’, *Ecology and Evolution*. John Wiley & Sons, Ltd, 3(1), pp. 182–196. doi: 10.1002/ECE3.431.

Maasri, A. *et al.* (2022) ‘A global agenda for advancing freshwater biodiversity research’, *Ecology Letters*. John Wiley & Sons, Ltd, 25(2), pp. 255–263. doi: 10.1111/ELE.13931.

Maazouzi, C. *et al.* (2011) ‘Ecophysiological responses to temperature of the “killer shrimp” Dikerogammarus villosus: Is the invader really stronger than the native Gammarus pulex?’, *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*. Elsevier Inc., 159(3), pp. 268–274. doi: 10.1016/j.cbpa.2011.03.019.

Macneil, C. *et al.* (1999) ‘Differential microdistributions and interspecific interactions in coexisting Gammarus and Crangonyx amphipods’, *Ecography*. John Wiley & Sons, Ltd, 22(4), pp. 415–423. doi: 10.1111/J.1600-0587.1999.TB00578.X.

Macneil, C., Dick, J. T. A., *et al.* (2003) ‘Parasite-mediated predation between native and invasive amphipods’, *Proceedings of the Royal Society B: Biological Sciences*, 270, pp. 1309–1314. doi: 10.1098/rspb.2003.2358.

Macneil, C., Fielding, N., *et al.* (2003) ‘Parasite altered micro-distribution of Gammarus pulex (Crustacea: Amphipoda)’, *International Journal for Parasitology*, 33, pp. 57–64. doi: 10.1016/S0020-7519(02)00229-1.

MacNeil, C. *et al.* (2001) ‘Factors influencing the distribution of native andintroduced Gammarus spp. in Irish river systems’, *Archiv für Hydrobiologie*. Schweizerbart’sche Verlagsbuchhandlung, 151(3), pp. 353–368. doi: 10.1127/ARCHIV-HYDROBIOL/151/2001/353.

MacNeil, C. *et al.* (2003) ‘Parasite transmission and cannibalism in an amphipod (Crustacea)’, *International Journal for Parasitology*. Pergamon, 33(8), pp. 795–798. doi: 10.1016/S0020-7519(03)00110-3.

MacNeil, C., Dick, J. T. a., *et al.* (2004) ‘A species invasion mediated through habitat structure, intraguild predation, and parasitism’, *Limnology and Oceanography*, 49(5), pp. 1848–1856. doi: 10.4319/lo.2004.49.5.1848.

MacNeil, C., Prenter, J., *et al.* (2004) ‘The replacement of a native freshwater amphipod by an invader: Roles for environmental degradation and intraguild predation’, *Canadian Journal of Fisheries and Aquatic Sciences*, 61(9), pp. 1627–1635. doi: 10.1139/F04-091.

MacNeil, C., Boets, P. and Platvoet, D. (2012) '"Killer Shrimps", Dangerous Experiments and Misguided Introductions: How Freshwater Shrimp (Crustacea: Amphipoda) Invasions Threaten Biological Water Quality Monitoring in the British Isles', *Freshwater Reviews*. Freshwater Biological Association, 5(1), pp. 21–35. doi: 10.1608/FRJ-5.1.457.

MacNeil, C. and Briffa, M. (2019) 'Fear alone reduces energy processing by resident "keystone" prey threatened by an invader; a non-consumptive effect of "killer shrimp" invasion of freshwater ecosystems is revealed', *Acta Oecologica*. Elsevier B.V., 98, pp. 1–5. doi: 10.1016/j.actao.2019.05.001.

MacNeil, C. and Campbell, M. (2018) 'A long-term study (1949–2015) of the aftermaths of the deliberate experimental introduction of an invasive freshwater amphipod (Crustacea) to a small Island', *Aquatic Invasions*, 13(4), pp. 491–500. doi: 10.3391/ai.2018.13.4.07.

MacNeil, C., Dick, J. T. A. and Elwood, R. W. (1997) 'The trophic ecology of freshwater Gammarus spp. (Crustacea: Amphipoda): Problems and perspectives concerning the functional feeding group concept', *Biological Reviews of the Cambridge Philosophical Society*. Cambridge University Press, 72(3), pp. 349–364. doi: 10.1017/S0006323196005038.

MacNeil, C. and Platvoet, D. (2005) 'The predatory impact of the freshwater invader Dikerogammarus villosus on native Gammarus pulex (Crustacea: Amphipoda); influences of differential microdistribution and food resources', *Journal of Zoology*. John Wiley & Sons, Ltd, 267(01), p. 31. doi: 10.1017/S0952836905007351.

Mancini, F. *et al.* (2021) 'Ontogenetic shift in the trophic role of the invasive killer shrimp Dikerogammarus villosus : a stable isotope study', *Biological Invasions 2021 23:6*. Springer, 23(6), pp. 1803–1817. doi: 10.1007/S10530-021-02472-0.

Maranhão, P. and Marques, J. C. (2003) ‘The influence of temperature and salinity on the duration of embryonic development, fecundity and growth of the amphipod Echinogammarus marinus Leach (Gammaridae)’, *Acta Oecologica*. Elsevier Masson, 24(1), pp. 5–13. doi: 10.1016/S1146-609X(02)00003-6.

Marcogliese, D. J. (2008) ‘The impact of climate change on the parasites and infectious diseases of aquatic animals’, *Rev. sci. tech. Off. int. Epiz*, 27(2), pp. 467–484. Available at: https://pdfs.semanticscholar.org/219d/8e86f333f2780174277b5e8c65d1c2aca36c.pdf (Accessed: 29 January 2018).

Mastrantonio, V. *et al.* (2021) ‘Cannibalism and Necrophagy Promote a Resource Loop and Benefit Larval Development in Insects of Temporary Waters’, *Insects*, 12(7), p. 657. doi: 10.3390/insects12070657.

McInerney, P. J. and Rees, G. N. (2017) ‘Co-invasion hypothesis explains microbial community structure changes in upland streams affected by riparian invader’, *Freshwater Science*. University of Chicago Press, 36(2), pp. 297–306. doi: 10.1086/692068.

Médoc, V., Albert, H. and Spataro, T. (2015) ‘Functional response comparisons among freshwater amphipods: ratio-dependence and higher predation for Gammarus pulex compared to the non-natives Dikerogammarus villosus and Echinogammarus berilloni’, *Biological Invasions*. Springer International Publishing, 17(12), pp. 3625–3637. doi: 10.1007/s10530-015-0984-2.

Millennium Ecosystem Assessment (2005) *Ecosystems and Human Well-being: Synthesis.* Washington D. C.

Moore, M. V., Folt, C. L. and Stemberger, R. S. (1996) ‘Consequences of elevated temperatures for zooplankton assemblages in temperate lakes’, *Archiv für Hydrobiologie*. Schweizerbart’sche Verlagsbuchhandlung, 135(3), pp. 289–319. doi: 10.1127/ARCHIV-HYDROBIOL/135/1996/289.

Mouritsen, K. N. and Poulin, R. (2005) ‘Parasites boost biodiversity and change animal community structure by trait-mediated indirect effects’, *OIKOS*, 108(2), pp. 344–350.

Murphy, J. *et al.* (2010) *Climate change projections*, *Climate Change and Agriculture*. doi: 10.1787/9789264086876-5-en.

Murrell, C. *et al.* (2011) ‘Invasive knotweed affects native plants through allelopathy’, *American Journal of Botany*. John Wiley & Sons, Ltd, 98(1), pp. 38–43. doi: 10.3732/AJB.1000135.

Musgrave, K., Bartlow, A. W. and Fair, J. M. (2019) ‘Long-term variation in environmental conditions influences host-parasite fitness’, *Ecology and Evolution*. Wiley, 9(13), pp. 7688–7703. doi: 10.1002/ece3.5321.

Nelson, D. (2011) ‘Gammarus-microbial interactions: A review’, *International Journal of Zoology*, 2011. doi: 10.1155/2011/295026.

Nery, T. and Schmera, D. (2016) ‘The effects of top-down and bottom-up controls on macroinvertebrate assemblages in headwater streams’, *Hydrobiologia*. Springer International Publishing, 763(1), pp. 173–181. doi: 10.1007/S10750-015-2371-5/FIGURES/2.

O’Brien, A. L. *et al.* (2019) ‘After decades of stressor research in urban estuarine ecosystems the focus is still on single stressors: A systematic literature review and meta-analysis’, *Science of The Total Environment*. Elsevier, 684, pp. 753–764. doi: 10.1016/J.SCITOTENV.2019.02.131.

de Omena, P. M., Srivastava, D. S. and Romero, G. Q. (2017) ‘Does the strength of cross-ecosystem trophic cascades vary with ecosystem size? A test using a natural microcosm’, *Freshwater Biology*. Blackwell Publishing Ltd, 62(4), pp. 724–736. doi: 10.1111/fwb.12897.

Ormerod, S. J. *et al.* (2010) ‘Multiple stressors in freshwater ecosystems’, *Freshwater Biology*. John Wiley & Sons, Ltd (10.1111), 55, pp. 1–4. doi: 10.1111/j.1365-2427.2009.02395.x.

Orr, H. G. *et al.* (2015) ‘Detecting changing river temperatures in England and Wales’, *Hydrological Processes*. John Wiley & Sons, Ltd, 29(5), pp. 752–766. doi: 10.1002/HYP.10181.

Orr, J. A. *et al.* (2020) ‘Towards a unified study of multiple stressors: divisions and common goals across research disciplines’, *Proceedings of the Royal Society B*. The Royal Society, 287(1926). doi: 10.1098/RSPB.2020.0421.

Pachauri, R. K. *et al.* (2014) *IPCC, 2014: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, *IPCC, Geneva, Switzerland*. IPCC. Available at: https://www.ipcc.ch/pdf/assessment-report/ar5/syr/SYR_AR5_FINAL_full_wcover.pdf (Accessed: 20 January 2018).

Paine, R. T., Tegner, M. J. and Johnson, E. A. (1998) ‘Compounded Perturbations Yield Ecological Surprises’, *Ecosystems*, 1(6), pp. 535–545. Available at: https://www.jstor.org/stable/3658754 (Accessed: 15 February 2022).

Parker, I. M. *et al.* (1999) ‘Impact: Toward a framework for understanding the ecological effects of invaders’, *Biological Invasions*, 1, pp. 3–19. doi: 10.1023/A:1010034312781.

Pejchar, L. and Mooney, H. A. (2009) ‘Invasive species, ecosystem services and human well-being’, *Trends in Ecology and Evolution*, 24(9), pp. 497–504. doi: 10.1016/J.TREE.2009.03.016.

Pellan, L. *et al.* (2016) ‘Feeding choice and predation pressure of two invasive gammarids, Gammarus tigrinus and Dikerogammarus villosus, under increasing temperature’, *Hydrobiologia*, 781(1), pp. 43–54. doi: 10.1007/s10750-015-2312-3.

Pereira, F. W. and Vitule, J. R. S. (2019) ‘The largemouth bass Micropterus salmoides (Lacepède, 1802): impacts of a powerful freshwater fish predator outside of its native range’, *Reviews in Fish Biology and Fisheries*. Springer International Publishing, 29(3), pp. 639–652. doi: 10.1007/s11160-019-09570-2.

Pereira, H. M. *et al.* (2010) ‘Scenarios for Global Biodiversity in the 21st Century’, *Science*, 330, pp. 1496–1501. doi: 10.1126/science.1196624.

Perrot-Minnot, M.-J. *et al.* (2011) ‘Interspecific differences in carotenoid content and sensitivity to UVB radiation in three acanthocephalan parasites exploiting a common intermediate host’, *International Journal for Parasitology*. Pergamon, 41(2), pp. 173–181.

Perrot-Minnot, M.-J., Kaldonski, N. and Cézilly, F. (2007) ‘Increased susceptibility to predation and altered anti-predator behaviour in an acanthocephalan-infected amphipod’, *International Journal for Parasitology*. Pergamon, 37(6), pp. 645–651. doi: 10.1016/J.IJPARA.2006.12.005.

Perrot-Minnot, M.-J., Maddaleno, M. and Cézilly, F. (2016) ‘Parasite-induced inversion of geotaxis in a freshwater amphipod: a role for anaerobic metabolism?’, *Functional Ecology*. John Wiley & Sons, Ltd, 30(5), pp. 780–788. doi: 10.1111/1365-2435.12516.

Perrot-Minnot, M. J., Banchetry, L. and Cézilly, F. (2017) ‘Anxiety-like behaviour increases safety from fish predation in an amphipod crustacea’, *Royal Society Open Science*. Royal Society Publishing, 4(12). doi: 10.1098/rsos.171558.

Pinkster, S. (1970) ‘Redescription of Gammarus Pulex (Linnaeus, 1758) Based On Neotype Material (Amphipoda)’, *Crustaceana*. Brill, 18(2), pp. 177–186. doi: 10.1163/156854070X00798.

Piscart, C. *et al.* (2009) ‘Environmental mediation of intraguild predation between the freshwater invader gammarus pulex and the native G. duebeni celticus’, *Biological Invasions*. Springer, 11(9), pp. 2141–2145. doi: 10.1007/S10530-009-9497-1/FIGURES/2.

Piscart, C., Roussel, J. M., *et al.* (2011) ‘Effects of coexistence on habitat use and trophic ecology of interacting native and invasive amphipods’, *Freshwater Biology*. Blackwell Publishing Ltd, 56(2), pp. 325–334. doi: 10.1111/j.1365-2427.2010.02500.x.

Piscart, C., Mermillod-Blondin, F., *et al.* (2011) ‘Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems’, *Biological Invasions*. Springer, 13(12), pp. 2861–2868. doi: 10.1007/S10530-011-9969-Y/TABLES/4.

Platvoet, D. *et al.* (2009) ‘Flexible omnivory in Dikerogammarus villosus (Sowinsky, 1894) (Amphipoda)’, *Crustaceana*, 82(6), pp. 703–720. doi: 10.1163/156854009X423201.

Pöckl, M. (2007) ‘Strategies of a successful new invader in European fresh waters: Fecundity and reproductive potential of the Ponto-Caspian amphipod Dikerogammarus villosus in the Austrian Danube, compared with the indigenous Gammarus fossarum and G. roeseli’, *Freshwater Biology*, 52(1), pp. 50–63. doi: 10.1111/J.1365-2427.2006.01671.X.

Pöckl, M. (2009) ‘Success of the invasive Ponto-caspian amphipod Dikerogammarus villosus by life history traits and reproductive capacity’, *Biological Invasions*, 11(9), pp. 2021–2041. doi: 10.1007/s10530-009-9485-5.

Ponton, F. *et al.* (2011) ‘Water-seeking behavior in worm-infected crickets and reversibility of parasitic manipulation’, *Behavioral Ecology*. Oxford Academic, 22(2), pp. 392–400. doi: 10.1093/BEHECO/ARQ215.

Preston, C. *et al.* (2011) *Biological Invasions: Economic and Environmental Costs of Alien Plant, Animal, and Microbe Species*. Second edi. Edited by D. Pimentel. Boca Raton: CRC Press. Available at: https://www.nhbs.com/new-atlas-of-the-british-and-irish-flora-book (Accessed: 2 February 2022).

Primack, R. B. *et al.* (2009) ‘Spatial and interspecific variability in phenological responses to warming temperatures’, *Biological Conservation*, 142(11), pp. 2569–2577. doi: 10.1016/J.BIOCON.2009.06.003.

Pulliam, H. R. (1974) ‘On the Theory of Optimal Diets’, *The American Naturalist*. University of Chicago Press, 108(959), pp. 59–74. doi: 10.1086/282885.

Pyšek, P. *et al.* (2020) 'Scientists' warning on invasive alien species', *Biological Reviews*. John Wiley & Sons, Ltd, 95(6), pp. 1511–1534. doi: 10.1111/BRV.12627.

R, C. T. (2019) 'R: A language and environment for statistical computing.' Vienna: R Foundation for Statistical Computing, Vienna, Austria. Available at: https://www.r-project.org/.

Rahel, F. J. and Olden, J. D. (2008) 'Assessing the effects of climate change on aquatic invasive species', *Conservation Biology*. John Wiley & Sons, Ltd, 22(3), pp. 521–533. doi: 10.1111/j.1523-1739.2008.00950.x.

Reid, A. J. *et al.* (2019) 'Emerging threats and persistent conservation challenges for freshwater biodiversity', *Biological Reviews*. John Wiley & Sons, Ltd, 94(3), pp. 849–873. doi: 10.1111/BRV.12480.

Reid, W. V *et al.* (2005) *Millennium Ecosystem Assessment: Ecosystems and Human Well-Being: Synthesis*. Washington DC. Available at: https://www.millenniumassessment.org/documents/document.356.aspx.pdf (Accessed: 20 January 2018).

Rewicz, T. *et al.* (2014) 'The profile of a "perfect" invader – the case of killer shrimp, Dikerogammarus villosus', *Aquatic Invasions*, 9(3), pp. 267–288. doi: 10.3391/ai.2014.9.3.04.

Rewicz, T. *et al.* (2015) 'Out of the Black Sea: Phylogeography of the Invasive Killer Shrimp Dikerogammarus villosus across Europe', *PLOS ONE*. Edited by D. Heath. Public Library of Science, 10(2), p. e0118121. doi: 10.1371/journal.pone.0118121.

Ricciardi, A. *et al.* (2013) ‘Progress toward understanding the ecological impacts of nonnative species’, *Ecological Monographs*. John Wiley & Sons, Ltd, 83(3), pp. 263–282. doi: 10.1890/13-0183.1.

Richardson, D. M. *et al.* (2007) ‘Riparian vegetation: degradation, alien plant invasions, and restoration prospects’, *Diversity and Distributions*. John Wiley & Sons, Ltd, 13(1), pp. 126–139. doi: 10.1111/J.1366-9516.2006.00314.X.

van Riel, M. C. *et al.* (2007) ‘Interference competition among native and invader amphipods’, *Acta Oecologica*, 31(3), pp. 282–289. doi: 10.1016/j.actao.2006.12.006.

Rödder, D. *et al.* (2009) ‘Global Amphibian Extinction Risk Assessment for the Panzootic Chytrid Fungus’, *Diversity 2009, Vol. 1, Pages 52-66*. Molecular Diversity Preservation International, 1(1), pp. 52–66. doi: 10.3390/D1010052.

Roon, D. A., Wipfli, M. S. and Wurtz, T. L. (2014) ‘Effects of invasive European bird cherry (Prunus padus) on leaf litter processing by aquatic invertebrate shredder communities in urban Alaskan streams’, *Hydrobiologia*. Kluwer Academic Publishers, 736(1), pp. 17–30. Available at: https://link.springer.com/article/10.1007/s10750-014-1881-x (Accessed: 2 June 2021).

Rowe, C. L. *et al.* (2001) ‘Metabolic costs incurred by crayfish (Procambarus acutus) in a trace element-polluted habitat: further evidence of similar responses among diverse taxonomic groups’, *Comparative Biochemistry and Physiology Part C*, 129, pp. 275–283. Available at: https://www.ecophys.fishwild.vt.edu/wp-content/uploads/rowe-et-al.-2001-CBP.pdf (Accessed: 23 April 2019).

Roy, D. (2002) ‘Amphibians as environmental sentinels’, *Journal of Biosciences 2002 27:3*. Springer, 27(3), pp. 187–188. doi: 10.1007/BF02704906.

Ruetz, C. R. I., Newman, R. M. and Vondracek, B. (2002) ‘Top-down control in a detritus-based food web: fish, shredders, and leaf breakdown Received’:, *Oecologia*, 132, pp. 307–315. doi: 10.1007/s00442-002-0953-1.

Sahm, R. *et al.* (2021) ‘Compound-specific δ15N analyses of amino acids for trophic level estimation from indigenous and invasive freshwater amphipods’, *International Review of Hydrobiology*. John Wiley & Sons, Ltd, 106(1), pp. 41–47. doi: 10.1002/IROH.202002058.

Sakai, A. K. *et al.* (2001) ‘The Population Biology of Invasive Species’, *Annual Review of Ecology and Systematics*, 32, pp. 305–332. doi: 10.1146/annurev.ecolsys.32.081501.114037.

Sala, O. E. *et al.* (2000) ‘Global Biodiversity Scenarios for the Year 2100 Global Biodiversity Scenarios for the Year 2100’, *Science*, 287(March), pp. 1770–1774. doi: 10.1126/science.287.5459.1770.

Saul, W.-C., Jeschke, J. and Heger, T. (2013) ‘The role of eco-evolutionary experience in invasion success’, *NeoBiota 17: 57-74*. Pensoft Publishers, 17, pp. 57–74. doi: 10.3897/NEOBIOTA.17.5208.

Saul, W.-C. and Jeschke, J. M. (2015) ‘Eco-evolutionary experience in novel species interactions’, *Ecology Letters*, 18(3), pp. 236–245. doi: 10.1111/ELE.12408.

Seebens, H. *et al.* (2017) ‘No saturation in the accumulation of alien species worldwide’. Nature Publishing Group, 8(1). doi: 10.1038/ncomms14435.

Seeney, A., Eastwood, S., *et al.* (2019) ‘All change at the water’s edge: invasion by non-native riparian plants negatively impacts terrestrial invertebrates’, *Biological Invasions*. Springer International Publishing, 21(6), pp. 1933–1946. doi: 10.1007/S10530-019-01947-5/TABLES/2.

Seeney, A., Pattison, Z., *et al.* (2019) ‘Stream invertebrate diversity reduces with invasion of river banks by non-native plants’, *Freshwater Biology*. Blackwell Publishing Ltd, 64(3), pp. 485–496. doi: 10.1111/FWB.13236.

Sih, A. (1980) ‘Optimal Foraging: Partial Consumption of Prey’, *The American Naturalist*. University of Chicago Press, 116(2), pp. 281–290. doi: 10.1086/283626.

Sih, A., Ferrari, M. C. O. and Harris, D. J. (2011) ‘Evolution and behavioural responses to human-induced rapid environmental change’, *Evolutionary Applications*. Wiley-Blackwell, 4(2), p. 367. doi: 10.1111/J.1752-4571.2010.00166.X.

Simberloff, D. and Von Holle, B. (1999) ‘Positive Interactions of Nonindigenous Species: Invasional Meltdown?’, *Biological Invasions*. Kluwer Academic Publishers, 1(1), pp. 21–32. doi: 10.1023/A:1010086329619.

Sitvarin, M. I., Rypstra, A. L. and Harwood, J. D. (2016) ‘Linking the green and brown worlds through nonconsumptive predator effects’, *Oikos*. Blackwell Publishing Ltd, 125(8), pp. 1057–1068. doi: 10.1111/OIK.03190.

Smol, J. P. *et al.* (2005) ‘Climate-driven regime shifts in the biological communities of arctic lakes’, *Proceedings of the National Academy of Sciences of the United States of America*, 102(12), pp. 4397–4402. doi: 10.1073/PNAS.0500245102/ASSET/826ACB4C-C552-4678-B7B5-44FD4C5EB256/ASSETS/GRAPHIC/ZPQ0100575720003.JPEG.

Solomon, C. T. *et al.* (2015) ‘Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges’, *Ecosystems*. Springer New York LLC, 18(3), pp. 376–389. doi: 10.1007/S10021-015-9848-Y/FIGURES/6.

Spears, B. M. *et al.* (2021) ‘Making waves. Bridging theory and practice towards multiple stressor management in freshwater ecosystems’, *Water Research*. Pergamon, 196, p. 116981. doi: 10.1016/J.WATRES.2021.116981.

Species alerts - GB non-native species secretariat (2012). Available at: http://www.nonnativespecies.org/alerts/index.cfm?id=3 (Accessed: 17 June 2018).

Stief, P. and Hölker, F. (2006) ‘Trait-Mediated Indirect Effects of Predatory Fish on Microbial Mineralization in Aquatic’, *Source: Ecology*, 87(12), pp. 3152–3159.

Strange, C. D. and Glass, G. B. (1979) ‘The Distribution of Freshwater Gammarids in Northern Ireland’, *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science*, 79, pp. 145–153. Available at: https://about.jstor.org/terms (Accessed: 21 January 2022).

Strayer, D. L. (2010) ‘Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future’, *Freshwater Biology*. John Wiley & Sons, Ltd, 55(SUPPL. 1), pp. 152–174. doi: 10.1111/j.1365-2427.2009.02380.x.

Strayer, D. L. and Dudgeon, D. (2010) ‘Freshwater biodiversity conservation: recent progress and future challenges’, *Journal of the North American Benthological Society*, 29(1), pp. 344–358. doi: 10.1899/08-171.1.

Strecker, A. L., Campbell, P. M. and Olden, J. D. (2011) ‘The Aquarium Trade as an Invasion Pathway in the Pacific Northwest’, *Fisheries*. John Wiley & Sons, Ltd, 36(2), pp. 74–85. doi: 10.1577/03632415.2011.10389070.

Taylor, N. G. and Dunn, A. M. (2017) ‘Size matters: predation of fish eggs and larvae by native and invasive amphipods’, *Biological Invasions*. Springer International Publishing, 19(1), pp. 89–107. doi: 10.1007/s10530-016-1265-4.

Tepolt, C. K. and Somero, G. N. (2014) ‘Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, Carcinus maenas’, *Journal of Experimental Biology*, (217), pp. 1129–1138. doi: 10.1242/jeb.093849.

Therneau, T. M. (2021) ‘A Package for Survival Analysis in R. R package version 3.2-11, https://CRAN.R-project.org/package=survival.’ Available at: https://cran.r-project.org/package=survival.

Thomas, F., Poulin, R. and Brodeur, J. (2010) ‘Host manipulation by parasites: a multidimensional phenomenon’, *Oikos*. Blackwell Publishing Ltd, 119(8), pp. 1217–1223. doi: 10.1111/j.1600-0706.2009.18077.x.

Tiedeken, E. J. *et al.* (2016) ‘Nectar chemistry modulates the impact of an invasive plant on native pollinators’, *Functional Ecology*. John Wiley & Sons, Ltd, 30(6), pp. 885–893. doi: 10.1111/1365-2435.12588.

Truhlar, A. M. and Aldridge, D. C. (2015) ‘Differences in behavioural traits between two potentially invasive amphipods, Dikerogammarus villosus and Gammarus pulex’, *Biological Invasions*. Springer International Publishing, 17(5), pp. 1569–1579. doi: 10.1007/s10530-014-0816-9.

Truhlar, A. M., Dodd, J. A. and Aldridge, D. C. (2014) ‘Differential leaf-litter processing by native (Gammarus pulex) and invasive (Dikerogammarus villosus) freshwater crustaceans under environmental extremes’, *Aquatic Conservation: Marine and Freshwater Ecosystems*, 24(1), pp. 56–65. doi: 10.1002/aqc.2375.

Urgenson, L. S., Reichard, S. H. and Halpern, C. B. (2009) ‘Community and ecosystem consequences of giant knotweed (Polygonum sachalinense) invasion into riparian forests of western Washington, USA’, *Biological Conservation*, 142(7), pp. 1536–1541. doi: 10.1016/J.BIOCON.2009.02.023.

Väinölä, R. *et al.* (2008) ‘Global diversity of amphipods (Amphipoda; Crustacea) in freshwater’, *Hydrobiologia*. Springer, 595(1), pp. 241–255. Available at: https://decapoda.nhm.org/pdfs/27701/27701.pdf (Accessed: 12 August 2019).

Vanni, M. J. (2002) ‘Nutrient cycling by animals in freshwater ecosystems’, *Annu. Rev. Ecol. Syst*, 33, pp. 341–70. doi: 10.1146/annurev.ecolsys.33.010802.150519.

Verberk, W. C. E. P. *et al.* (2018) ‘Thermal limits in native and alien freshwater peracarid Crustacea: the role of habitat use and oxygen limitation’, *Functional Ecology*, 32(4), pp. 926–936. doi: 10.1111/1365-2435.13050.

Vilà, M. *et al.* (2011) ‘Ecological impacts of invasive alien plants: a meta-analysis of their effects on species, communities and ecosystems’, *Ecology Letters*. John Wiley & Sons, Ltd, 14(7), pp. 702–708. doi: 10.1111/J.1461-0248.2011.01628.X.

Vitousek, P. M. *et al.* (1996) ‘Biological Invasions as Global Environmental Change’, *American Scientist*. Sigma Xi, The Scientific Research Society, 84(5), pp. 468–478. doi: 10.2307/29775751.

Vitousek, P. M. *et al.* (1997) ‘Human Domination of Earth’s Ecosystems’, *Science*. American Association for the Advancement of Science, 277(5325), pp. 494–499. doi: 10.1126/SCIENCE.277.5325.494.

Vörösmarty, C. J. *et al.* (2010) ‘Global threats to human water security and river biodiversity’, *Nature*. Nature Publishing Group, 467(7315), pp. 555–561. doi: 10.1038/nature09440.

Wallace, J. B. and Webster, J. R. (1996) ‘The role of macroinvertebrates in stream ecosystem function’, *Annual Review of Entomology*, 41, pp. 115–139. Available at: www.annualreviews.org (Accessed: 26 April 2022).

Waring, R. H. and Running, S. W. (2007) *Forest ecosystems: analysis at multiple scales*. 3rd Edition. San Diego: Elsevier/Academic Press.

Warren, D. A., Bradbeer, S. J. and Dunn, A. M. (2021) ‘Superior predatory ability and abundance predicts potential ecological impact towards early-stage anurans by invasive “Killer Shrimp” (Dikerogammarus villosus)’, *Scientific Reports*, 11, p. 4570. doi: 10.1038/s41598-021-82630-5.

Webster, J. R. and Benfield, E. F. ‘Vascular Plant Breakdown in Freshwater Ecosystems’, 17(1), pp. 567–594. Available at: https://www.annualreviews.org/doi/abs/10.1146/annurev.es.17.110186.003031 (Accessed: 25 September 2018).

Wickham, H. (2016) ‘ggplot2: Elegant Graphics for Data Analysis’. New York: Springer-Verlag. Available at: https://ggplot2.tidyverse.org.

Wiens, J. J. (2015) ‘Faster diversification on land than sea helps explain global biodiversity patterns among habitats and animal phyla’, *Ecology Letters*, 18, pp. 1234–1241. doi: 10.1111/ele.12503.

Wijnhoven, S. *et al.* (2003) ‘Exotic and indigenous freshwater gammarid species: Physiological tolerance to water temperature in relation to ionic content of the water’, *Aquatic Ecology*. Kluwer Academic Publishers, 37(2), pp. 151–158. doi: 10.1023/A:1023982200529.

Williamson, M. and Fitter, A. (1996) ‘The varying success of invaders’, *Ecology*, 77(6), pp. 1661–1666.

Wood, C. L. *et al.* (2007) ‘Parasites alter community structure’, *PNAS*. National Academy of Sciences, 104(22), pp. 9335–9339. doi: 10.1073/PNAS.0700062104.

Wood, C. L. and Johnson, P. T. T. J. (2015) ‘A world without parasites: exploring the hidden ecology of infection’, *Frontiers in ecology and the environment*. NIH Public Access, 13(8). doi: 10.1890/140368.

Wood, S. N. (2011) ‘Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models’, *Journal of the Royal Statistical Society: Series B (Statistical Methodology)*, 73(1), pp. 3–36. doi: 10.1111/J.1467-9868.2010.00749.X.

Woodward, G., Perkins, D. M. and Brown, L. E. (2010) ‘Climate change and freshwater ecosystems: impacts across multiple levels of organization’, *Philosophical Transactions of the Royal Society B: Biological Sciences*. The Royal Society, 365(1549), p. 2093. doi: 10.1098/RSTB.2010.0055.

Yee, D. A. and Juliano, S. A. (2006) ‘Consequences of detritus type in an aquatic microsystem: effects on water quality, micro-organisms and performance of the dominant consumer’, *Freshwater biology*. NIH Public Access, 51(3), p. 448. doi: 10.1111/J.1365-2427.2005.01504.X.

Yee, D. A. and Juliano, S. A. (2012) ‘Concurrent effects of resource pulse amount, type, and frequency on community and population properties of consumers in detritus-based systems’, *Oecologia*. NIH Public Access, 169(2), p. 511. doi: 10.1007/S00442-011-2209-4.

Yee, D. A., Kesavaraju, B. and Juliano, S. A. (2007) ‘Direct and Indirect Effects of Animal Detritus on Growth, Survival, and Mass of Invasive Container Mosquito Aedes albopictus (Diptera: Culicidae)’, *Journal of medical entomology*. NIH Public Access, 44(4), p. 580. doi: 10.1603/0022-2585(2007)44[580:daieoa]2.0.co;2.

Zerebecki, R. A. and Sorte, C. J. B. (2011) ‘Temperature Tolerance and Stress Proteins as Mechanisms of Invasive Species Success’, *PLOS ONE*. Public Library of Science, 6(4), p. e14806. doi: 10.1371/JOURNAL.PONE.0014806.