Quantifying the impacts of invasive non-native species using key functional traits

William Norman Whitlock Fincham

The candidate confirms that the work submitted is their own and that appropriate credit has been given where reference has been made to the work of others.

Chapter 2 - data were collected by WNW Fincham and guidance was provided by H Hesketh. WNWF carried out all statistical analyses and wrote the manuscript with guidance and comments provided by AM Dunn, LE Brown, HH and HE Roy. Additional feedback was received from two anonymous reviewers.

Chapter 3 - data used as part of this chapter were collected by professional and citizen scientists as part of the UK Ladybird Survey and Rothamsted Insect Survey. C Shortall and HER aided in the selection of suitable aphid species. WNWF performed all statistical analyses with guidance from NJB Isaac. WNWF wrote the manuscript with feedback and comments provided by AMD and HER.

Chapter 4 - data collection, statistical analyses and the writing of the manuscript were undertaken by WNWF with guidance from LEB and AD throughout.

Chapter 5 - WNWF led the data collection for data used as part of this chapter and was aided by B Pile, C Taylor, S Bradbeer, and D Warren. Guidance was provided by AMD and LEB. Leaf stoichiometry analyses were undertaken by the School of Geography Research Technician Team, University of Leeds. WNWF conducted all statistical analyses and wrote the manuscript with comments and guidance from AMD and LEB.

William N. W. Fincham

Acknowledgements

My sincere thanks go to my supervisory team, Alison Dunn, Lee Brown and Helen Roy, you've each taught me so much and nurtured my enthusiasm. You've dealt with so many of my scientific and not so scientific questions while continuing to encourage me and allowing me to develop further as a PhD student.

My family also deserve my sincere thanks, for providing constant encouragement and support throughout my education in addition to teaching me to grab opportunities and 'go for it'.

I'm grateful to the members of the Dunn and Hassall labs and members of the Geography department at the University of Leeds. Specifically, thanks to Dan Warren, Ben Pile, Steph Bradbeer, Caitriona Shannon, Myrna Barjau Pérez Milicua, Giovanna Villalobos-Jiménez, Tom Doherty-Bone, Jamie Bojko, Lucy Anderson, Katie Arundell, Nigel Taylor and Sarah Fell who collectively put up with my endless questions at the start of the project, provided a welcome distraction when needed and sounded suitably interested when I'd found something I thought was cool. Thanks also to the community of researchers and PhD students at CEH Wallingford and the University of Edinburgh for providing such friendly and welcoming environments in which I was lucky enough to spend time working during the project.

Lastly, without the unwavering support of Charlotte Regan this thesis would likely never have come to fruition. I've lost count of the number of scientific and panicked discussion we have had about this thesis as well as the many evenings spent in the lab, trips to collect samples, and reading of drafts. You did it all without a second thought - Thank you.

Contents

List of Tables

List of Figures

Abstract

Invasive non-native species place high pressures on native communities and can result in ecological impacts often associated with differences in key functional behaviours that mediate top-down and bottom-up forces. In this thesis, I use two model systems, the UK Coccinellidae system and the UK freshwater amphipod system, to quantify *per-capita* differences between native and invasive non-native species. I scale these studies up to more complex ecological communities and attempt to account for additional environmental pressures (e.g. pathogenic infection).

First, I present a laboratory experiment to quantify the *per-capita* differences in predatory behaviour between native and invasive non-native Coccinellidae with a pathogen (*Beauveria bassiana*) exposure treatment. *H. axyridis* was the most efficient predator and pathogenic infection reduced the forage ability in all species.

Second, I used existing *H. axyridis* distribution and aphid abundance data to quantify *H. axyridis'* impact through top-down forces. The arrival of *H. axyridis* is correlated with significant changes in aphid abundance and, of the 14 species studied, five declined in abundance, four increased, while the remaining five showed no significant change.

Third, I measured the *per-capita* differences in detrital processing rates between native and invasive freshwater amphipods when provided with three diets of differing resource quality and maintained at three temperatures. The rates of detrital processing varied between the native and invasive non-native species and between the temperature and resource quality treatments.

Fourth, I applied native and invasive amphipods at two density treatments (high and low) to a field mesocosm experiment to measure how the *per-capita* differences impacted more complex ecological systems. The presence of invasive amphipods changed the macroinvertebrate community composition and ecosystem functioning.

I finish by highlighting that our understanding as to how the pressures of invasive non-native species interact with additional environmental stressors remains limited and an area that warrants further investigation.

Chapter 1

General introduction

Native communities face increasing pressure from a variety of sources which is driving population declines, species extinctions, and ultimately biodiversity losses (Millennium Ecosystem Assessment 2005; Butchart *et al.* 2010). Primary causes of biodiversity declines include habitat loss (Brook *et al.* 2008; Ducatez & Shine 2017), overexploitation (Millennium Ecosystem Assessment 2005), climate change (Bellard *et al.* 2012; Butchart *et al.* 2010), and the spread of invasive non-native species (Bellard *et al.* 2016; Butchart *et al.* 2010; Millennium Ecosystem Assessment 2005). In addition to their ecological impacts, invasive non-native species can also impose further costs in terms of economic welfare (Pimentel *et al.* 2000; Williams *et al.* 2010) and human health (Juliano & Philip Lounibos 2005). While estimating the true cost of invasive non-native species is difficult, Pimentel *et al.* (2005) estimated the cost invasive non-native species in the USA to be almost $120 billion per year while Bradshaw *et al.* (2016) suggest that the global cost of invasive non-native insects alone is in excess of $70.0 billion per year with, in excess of, an additional $6.9 billion per year in associated health costs. It is because of these significant economic, human health and environmental costs that invasive species are prioritised as part of various national and international legal frameworks (for example, Convention on Biological Diversity 2006; European Comission 2017; United Nations 2015). Despite these efforts however, Seebens *et al.* (2017) have shown that the rates of global species invasions shows no sign of slowing.

1.1 What are invasive non-native species?

Considerable debate still remains around the terminology used in invasion ecology. For the purpose of this thesis, I refer to 'non-native species' synonymously with 'alien species' as defined by the Convention on Biological Diversity (2006) as a species introduced outside its natural distribution. I further refer to 'invasive non-native species' which I define as a non-native species (as defined previously) that has the ability to spread further and result in damage to either the economy, human health or the environment, as defined by the GB Non-Native Species Secretariat (2018) and similar to the European Comission (2017) definition of 'invasive alien species'.

Europe is home to in excess of 10,000 non-native species with just over 100 of these occurring in the UK (European Comission 2018). Non-native species can be introduced intentionally, for example for as a biological control agent, or inadvertently by 'hitch-hiking' on goods (Anderson *et al.* 2014; Hulme 2009). For example, *Rhododendron ponticum* was introduced as an ornamental plant and has since become invasive throughout the UK (Rotherham 2001) whereas *Coccinella septempunctata* was introduced into North America as a biological control agent and has since become a problematic invasive non-native predator (Majerus & Kearns 1994; Harmon *et al.* 2007). These new arrivals can pose major risks for native species. For example, American signal crayfish (*Pacifastacus leniusculus*), initially introduced in 1975 as a food source, are a host and reservoir of crayfish plague (*Aphanomyces astaci*), an Oomycete. The native white-clawed crayfish (*Austropotamobius pallipes*) have shown significant declines of between 50-80% (Füreder *et al.* 2010), which have been attributed to *P. leniusculus* and *A. astaci* (Dunn *et al.* 2009). The rates of species introductions varies around the world with invasion hotspots correlating with increased GDP and human population density (Dawson *et al.* 2017). Most species introductions are human mediated and so increases in both GDP and human population densities are likely to contribute to more frequent species invasions in the future (Seebens *et al.* 2017).

1.2 Are there costs to invasive non-native species?

The debate around the potential impacts of invasive non-native species is ongoing (Thomas & Palmer 2015; Briggs 2017; Crowley *et al.* 2017; Davis & Chew 2017; Tassin *et al.* 2017; Russell & Blackburn 2017b,a; Ricciardi & Ryan 2018a,b; Sagoff 2018) and, while scientific debate and critique of scientific findings and theories is that drives science forwards, the potential rise of scientific denialism is arguably to the detriment to scientific progression. Denialism has been defined as the use of arguments lacking evidence in the face of valid evidence to the contrary, often with the aim of discrediting specific idea, scientific finding or belief (Russell & Blackburn 2017a; Ricciardi & Ryan 2018a), and is therefore substantially different from rigorous scientific debate. Studies of varying scales

have provided evidence as to the impacts posed by invasive non-native species. For example, Clavero & García-Berthou (2005) provide evidence that invasive non-native species are associated with over 50% of extinctions of species on the IUCN Red List, for which causes were known. Similarly, Doherty *et al.* (2016) show that invasive non-native mammals are implicated in the extinctions of 58% of recent global bird, mammal, and reptile extinctions. Despite these findings Russell & Blackburn (2017a) and Ricciardi & Ryan (2018a) both report a rise in denialism regarding the impacts of invasive non-native species. The authors report invasive species denialism is prevalent within literature such as opinion pieces, popular science articles and books - potentially due to the lack of rigorous peer review - and link this movement to similar movements in other scientific fields, such as climate change and evolution. The miscommunication of scientific findings, specifically within invasion ecology, has been highlighted as a potential future challenge and one that could impede our ability to undertake mitigation or control activities (Ricciardi *et al.* 2017). Therefore, providing further evidence as to the impacts, or lack thereof, of invasive non-native species is of increasing importance so as to further this debate and ensure the use of accurate scientific data within such debates.

1.3 What are the costs of invasive non-native species?

Invasive non-native species can be hugely costly to human populations and native communities. The costs imposed by invasive non-native species can be broken down into three primary categories; 1) economic costs, 3) human-health costs, and 3) environmental costs.

1.3.1 Economic costs

Economic costs imposed by invasive non-native species are often associated with their control and removal but additional costs can also be imposed, such as through damage to property or agricultural crops. For example, invasive non-native Asian longhorn beetles (*Anoplophora glabripennis*) can result in mass tree mortality (Nowak *et al.* 2001) and *Harmonia axyridis* will inhabit grape clusters which results in tainted wines (Kögel

et al. 2011; Pickering *et al.* 2005), both species can and do result in significant costs in invaded regions. It is estimated that invasive non-native species result in economic costs in excess of \$120 billion per year in the USA, as a result of control costs and the costs associated with losses and damages (Pimentel *et al.* 2005). In Europe, damage caused by invasive non-native species is thought to cost at least €12.5 billion per year while extrapolating to fill gaps in available data increases this figure to an estimated cost of €20 billion per year (Kettunen *et al.* 2008). Williams *et al.* (2010) suggests that invasive non-native species cost to the UK economy in excess of £1.68 billion per year.

1.3.2 Human-health costs

Invasive non-native species can impact human health directly and indirectly. For example, the Asian tiger mosquito (*Aedes albopictus*) can directly impact human health via its role as a disease vector. *A. albopictus* is one of the most invasive non-native vector species in the world and is known to transmit multiple disease causing infections including the Dengue and Chikungunya viruses (see Bonizzoni *et al.* 2013). Recent outbreaks of Dengue and Chikungunya viruses in at least four regions, in addition to the first externally sourced outbreak of Dengue virus in Europe have all been attributed to *A. albopictus* (Bonizzoni *et al.* 2013). Conversely, Common Gorse (*Ulex europaeus*) is a widespread non-native plant species that can impact human health indirectly, through increasing the risk of fire to human populations with its extensive and flammable vegetation (Brooks *et al.* 2004; Coombs *et al.* 2004).

1.3.3 Environmental costs

Invasive non-native species often negatively impact native communities and lead to declines in biodiversity and species extinctions (Clavero & García-Berthou 2005; Blackburn *et al.* 2012, but also see Gurevitch & Padilla 2004; Didham *et al.* 2005). Mcneely (2001) provide evidence that 20% of vertebrate species at risk of extinction are negatively impacted by invasive non-native species. Invasive non-native species, such as the American mink (*Neovison vison*), are known to affect a diverse range of native

species including seabirds, small mammals, amphibians and crustaceans (Bonesi &
Palazon 2007). In the UK, *N. vison* not only out competes the endangered European
mink (*Mustela lutreola*) but also predates other native species of conservation concern,
including water voles (*Arvicola terrestris*) (Bonesi & Palazon 2007; Keller *et al.* 2011).
Chinese mitten crabs (*Eriocheir sinensis*), another UK invasive non-native species, is
also likely to impact native macroinvertebrate communities directly, through predation,
while its burrowing behaviour could further disrupt the community indirectly, with
changes in flow dynamics and siltation likely impeding breeding fish (Yang *et al.* 2018;
GB Non-Native Species Secretariat 2018). These ecological impacts can be realised
through factors such as naïve native prey species, for example invasive rat (*Rattus spp.*)
populations pose significant risks to island species and seabird colonies (Harper &
Bunbury 2015, National Trust for Scotland, pers. comms.). Invasive species often reach
much higher densities than their native counterparts (Laverty *et al.* 2017b; Parker *et al.*
2013; Snyder & Evans 2006) with Hansen *et al.* (2013) showing invasive populations can
be on average three times more abundant than their native counterparts. For example,
invasive non-native populations of the freshwater amphipod *Dikerogammarus villosus* in
the Netherlands are, at least, two fold more abundant that native amphipods (Josens
et al. 2005). As part of this thesis I will be using two study systems containing high
profile invasive non-native species thought likely to impact native communities. The UK
Coccinellidae system contains the invasive non-native predator *Harmonia axyridis*
(Section 1.7.1) while the UK freshwater amphipod systems includes the invasive
non-native omnivores *Dikerogammarus villosus* and *Dikerogammarus haemobaphes*
(Section 1.7.2).

1.4   How are the environmental impacts of invasive non-native species realised?

As has been alluded to, invasive non-native species can impose impacts on native
communities through a variety of means and both directly and indirectly. Invasive

non-native species can interact with the native community via the interruption of interactions between species for example, parasitism, direct and indirect competition, herbivory, mutualisms, and commensalisms. The interruption of such interactions can be via the modification of or engaging in key functional behaviours. As part of this thesis I will address how invasive non-native species can impact native communities though top-down forces, through predation, and bottom-up forces, through detritivory.

1.4.1 Predation

Invasive predators can be a major cause of worldwide biodiversity declines (Doherty *et al.* 2016; Snyder & Evans 2006). The invading predators can often benefit from the naïvety of native prey species, which often have no evolutionary history with the invader. The inability of native species to recognise the European brown trout (*Salmo trutta*) as a predator has resulted in the species having a detrimental impact on native freshwater communities in New Zealand and South America (Cox & Lima 2006). The Asian hornet (*Vespa velutina*) is expected to colonise the UK and continue to expand its invaded range in Europe in the coming years (Keeling *et al.* 2017; GB Non-Native Species Secretariat 2018). In addition to the human health impacts of stings, *V. velutina* is also highly predatory and poses a significant risk to native insect pollinators including bumblebees and honeybees (Monceau *et al.* 2014). In addition to predating individuals of lower trophic levels individuals can also engage in specialised predatory behaviours such as intra-guild predation, whereby predators prey on other potential competitors. Invasive *Harmonia axyridis* has led to declines in native Coccinellidae throughout its invaded range through competition for food and via intra-guild predation (Koch & Galvan 2008; Roy *et al.* 2012; Grez *et al.* 2016), but little is known about the wider impacts including native aphid prey species (Roy & Brown 2015; Roy *et al.* 2016a), the pest species it was initially introduced to control.

1.4.2 Detritivory

Nutrient cycling is an important process for communities with resource availability being an important determinant of individual fitness and community metrics, such as community composition (Wardle *et al.* 2004). Dead organic matter, or detritus, will enter the detrital processing pathway and be broken down before being disseminated throughout the community, commonly as primary production. While not limited to plant matter, approximately 90% of plant biomass will evade herbivory and enter the detrital processing pathway (Gessner *et al.* 2010). While all communities have a detrital pathway, freshwater bodies, which are commonly net heterotrophic, rely heavily on the processing of detrital matter to provide available resources to the wider community (Marcarelli *et al.* 2011). Invasive species can alter these cycles by modifying the biomass or nutritional components of matter entering the system or at multiple stages thought the detrital breakdown process. For example, Himalayan Balsam (*Impatiens glandulifera*), an invasive annual plant introduced throughout North America, Europe and New Zealand, is associated with reduced invertebrate abundance (Tanner *et al.* 2013) which is likely to result in reduced rates of detritivory. Similarly, the New Zealand flatworm (*Arthurdendyus triangulatus*), another European non-native species, is also capable of impacting the detrital pathway in invaded sites (Boag & Yeates 2001). *A. triangulatus* has a patchy but widespread distribution in the UK and will predate native earthworms which not only impacts native earthworm density but other native species that also feed on native earthworms, such as moles (*Talpa europaea*), badgers (*Meles meles*), and blackbirds (*Turdus merula*) (Boag & Yeates 2001; Boag & Neilson 2006).

1.4.3 Omnivory

Invasive non-native omnivorous species have the potential to disrupt energy flows throughout native communities via their top-down and bottom-up regulatory processes (Klose & Cooper 2013; Tumolo & Flinn 2017). Omnivores are able to undertake detrital processing of leaf matter and predation behaviours which can result in invasive non-native omnivores having wide reaching impacts that are often difficult to predict.

For example, in North America the rusty crayfish (*Orconectes rusticus*), has resulted in a 99.99% decrease in snail density and, through direct consumption and as a by product of predation behaviour, reduced macrophyte biomass by up to 75% (Lodge *et al.* 1994; Wilson *et al.* 2004). The invasive amphipods *Dikerogammarus villosus* and *Dikerogammarus haemobaphes*, invasive across large areas of Europe, can modify the rates of community detrital processing through slower rates of detrital breakdown (MacNeil *et al.* 2011; Jourdan *et al.* 2016; Piscart *et al.* 2011; Constable & Birkby 2016; Kenna *et al.* 2016). As yet however, our understanding as to how this change impacts the wider community remains incomplete.

1.4.4   Interaction with other environmental stressors

1.4.4.1   Climate change

While in many cases the impacts of invasive non-native species are well understood, our understanding as to how the pressures of invasive non-native species interact with other environmental stressors remains insufficient. One of the biggest pressures facing the natural world is climate change which has been linked with projected biodiversity losses and species extinctions (Bellard *et al.* 2012; Thomas 2010). In addition to facilitating future range shifts, and potentially species invasions, climate change could also result in changes that may favour invasive species and/or increase their impact on native communities. Gallardo & Aldridge (2013) showed that by 2050, under current climate change projections, the native, and endangered, depressed mussel (*Pseudanodonta complanata*) is likely to show range decreases of between 14-36% while the invasive non-native zebra mussel (*Dreissena polymorpha*) is expected to increase its range by 15-20% leading to an overall increase in the overlap between the two species of up to 24%. In addition to shifting species ranges, the associated increase in the frequency and intensity of extreme climatic events (e.g. wildfires and flooding) are likely to increase disturbance levels in many areas. Disturbed habitats are considered at an increased risk of species invasion as the invading species is often able to better capitalise on disturbance, potentially associated with wider environmental tolerance thresholds (Strayer 2010).

1.4.4.2 Parasites and pathogens

In addition to climate change species also face pressures associated to parasitic and pathogenic infections (Dunn *et al.* 2012; Prenter *et al.* 2004). Despite being widespread and often linked with biological invasions, they are often absent from such studies (Dunn *et al.* 2012; Dunn & Hatcher 2015; Lafferty *et al.* 2006, 2008; Prenter *et al.* 2004). Parasites can play multiple roles in species invasions including modifying interactions between invasive and native species (Dunn *et al.* 2012; Dunn & Hatcher 2015). For example, as previously mentioned, *A astaci*, the cause of crayfish plague, was introduced to Europe with its host *P. leniusculus* (Reynolds 1988) and has imposed density effects by reducing native *A. pallipes* density (Hatcher & Dunn 2011). This system also demonstrates spill-over with *A. astaci* being transmitted from the *P. leniusculus* host reservoir to the native *A. pallipes* (Reynolds 1988). In the absence of *P. leniusculus*, in Northern Ireland, *A. astaci* successfully invaded however, in the absence of *P. leniusculus* the pathogen subsequently became extinct (Reynolds 1988). Parasites can further mediate species invasions though modifying host behaviour, therefore imposing a trait-mediated effect (Hatcher & Dunn 2011). In Northern Ireland, invasive populations of the freshwater amphipod *Gammarus pulex* competitively exclude the native *Gammarus duebeni celticus*. Infection by *Eechinorhynchus truttae* increases the predation rates of invasive *G. pulex* leading to an increased impact on the native species within the invaded community (Dick *et al.* 2010; Hatcher & Dunn 2011). We also know that invasive species can be less susceptible to parasitic and pathogenic infection, for example, *H axyridis* is known to be less susceptible than certain native species to *Beauveria bassiana* (Cottrell & Shapiro-Ilan 2003; Roy *et al.* 2008b), a widespread entomopathogen; however, little is known about how this impacts the species predatory ability.

1.5 How can we quantify the environmental impacts of invasive non-native species?

Due to the range and intensity of costs imposed by invasive non-native species, research efforts to quantify their environmental impacts have been extensive. While these studies range in their research aims, they can commonly be categorised into three scales; 1) microcosms, 2) mesocosms, and 3) field or landscape studies.

1.5.1 Laboratory microcosm experiments

Microcosms are simplified ecological systems that contain key features of larger ecological systems or communities. Due to the commonly small size, these manipulation experiments benefit from being highly replicable while providing the ability to quantify mechanistic links in a highly controlled environment without the confounding effects present in field studies (e.g. temperature fluctuations) (Benton *et al.* 2007; Drake & Kramer 2012; Schindler 1998). Ecological systems are highly complex and the ability of such simplistic interactions, as present in laboratory microcosms, to represent more complex field communities remains the subject of debate (Drake & Kramer 2012; Srivastava *et al.* 2004). Microcosms have been a valuable resource in understanding community interactions, specifically accurate *per-capita* measures which are difficult to obtain from field communities. Within invasion ecology, microcosms have been invaluable in furthering our understanding as to the differences between invasive non-native species and their native analogues. For example, functional response experiments have been used to quantify and compare the predatory ability of species (for example, Dick *et al.* 2013). To better understand how these *per-capita* estimates scale-up to field populations, investigators are reliant on scaling predictions (Dick *et al.* 2017b; Laverty *et al.* 2017b) or further experiments with increasingly complex ecological systems, for example mesocosms.

1.5.2 Laboratory or field mesocosm experiments

Similar to microcosm designs, mesocosms are also simplified ecological systems created to represent key features of more complex ecological systems. While mesocosms are commonly larger and more ecologically complex than microcosms, their validity to accurately represent the complexities of larger field communities has also been debated (Brown *et al.* 2011a; Lamberti & Steinman 1993; Schindler 1998). Mesocosms however, represent a compromise between the experimental manipulations possible in microcosms and the greater ecological complexity present in field communities. In freshwater systems, the ability of mesocosms to better represent more ecologically complex systems can be improved through the use of a flow-through design, whereby water in the mesocosms is constantly cycled through input and outflow via a nearby waterbody. For example, Piggott *et al.* (2015) describes the use of such an experimental design to quantify the impacts of multiple environmental stressors with the mesocosm communities being highly representative of those of the adjoining waterbody. While being more representative, these experimental designs can be inappropriate for example, when working with invasive species which are liable to spread and result in ecological damage. Mesocosms can be utilised to scale-up, often simplistic but accurate, *per-capita* microcosm studies to better account for the increased ecological complexity characteristic of field communities and environmental stochasticity.

1.5.3 Field or landscape field studies

Lastly and at the largest spatial scale, field studies commonly allow for the least experimental manipulation but do represent the most complex ecological systems and environmental stochasticity. Comparison between field sites can often be associated with additional confounding variables such as variation in abiotic factors (e.g. temperature), species diversity, and invasion history (for example, Kueffer *et al.* 2013). Similar to flow-through mesocosm designs, field studies may have limitations when working with invasive species. Due to the highly complex nature of field communities, identifying changes of interest can be difficult (for example, Melbourne & Hastings 2008). For

instance, native aphid populations are impacted by a wide variety of pressures including climatic variables, host plant availability, changing agricultural practices, and changes in predator abundance (van Emden & Harrington 2017). When available, long-term datasets can be especially beneficial as they can allow for statistical signals to be disseminated from environmental stochascity.

1.6 The importance of interacting pressures on native communities

While we understand that, and in many cases how, invasive non-native species impact native communities, our understanding as to how the pressures of invasive non-native species interact with other environmental stressors, such as climate change and pathogenic infections, is far from complete (Brook *et al.* 2008; Strayer 2010). As the environmental pressures arising from continued climate change and the increased spread of invasive non-native species, filling this knowledge gap is of great importance (Foden *et al.* 2013; Gallardo & Aldridge 2014; Seebens *et al.* 2017). The widespread omission of parasites and pathogens from trophic food webs and experimental studies is also likely to leave our understanding of species interactions incomplete. To better understand such interactions, I argue, a multi-scale approach is essential. Such an approach would make use of multiple experimental designs across spatial scales for example, laboratory microcosms, field mesocosms, and records from the field. The use of both top-down and bottom-up approaches could also allow for potential generalisations to be drawn. I suggest that such an approach is essential to better inform our current understanding as to the impacts imposed by invasive non-native species in real world complex ecological communities but also allow for inference as to how these may change under projected climate change and scale-up from *per-capita* effects to community or landscape scales.

1.7   Study systems

Invasive non-native species impact native communities through a variety of processes, including top-down and bottom-up forces. The risk of species invasions is also known to vary between habitats, for example, freshwater habitats are often more susceptible to species invasions than those in terrestrial environments (Moorhouse & Macdonald 2015). Due to this variation, I suggest that to understand how these forces affect native communities, in isolation and when interacting with existing environmental stressors, the use of multiple study systems is important. As part of this thesis, I will use two study systems containing three of the UK's most recent and damaging invasive non-native species to the UK; the freshwater invaders *Dikerogammarus villosus* and *Dikerogammarus haemobaphes* and *Harmonia axyridis*, an invasive Coccinellidae.

1.7.1   UK Coccinellidae systems

The UK is home to 44 native Coccinellidae, 24 of these being conspicuous and relatively easily identifiable as ladybird species (Roy *et al.* 2011). Commonly regarded as charismatic or even iconic species, ladybirds are also seen as beneficial to humans through their predation of aphids which are regarded as pests. Throughout their life-cycle, ladybirds undergo complete metamorphosis as they transition from egg to larvae to pupae and, finally, to fully grown adults (Hodek *et al.* 2012). While ladybirds are active predators during their larval and adult life-stages, it is only the adults that are capable of flight and therefore able to disperse further and more readily.

The UK is also home to two non-native ladybird species, the herbivorous bryony ladybird (*Henosepilachna argus*) whose range remains localised and patchy and the harlequin ladybird (*Harmonia axyridis*) (Figure 1.1) which, following its arrival, has spread rapidly. In the UK, records of Coccinellidae in the UK have been collected as part of the UK Ladybird Survey (2018) which began in 2005 and replaced the Coccinellidae Recording Scheme, which began in 1964. This long-term recording of UK Coccinellidae has resulted in a valuable dataset that has tracked the arrival and spread

Figure 1.1: The UK Coccinellidae study system consisting of invasive non-native *Harmonia axyridis* (the harlequin ladybird, top), and native *Adalia bipunctata* (the 2-spot ladybird, bottom-left) and *Coccinella septempunctata* (the 7-spot ladybird, bottom-right).

of the invasive non-native *H. axyridis*, in addition to the subsequent ecological impacts on native Coccinellidae (Roy *et al.* 2012).

The harlequin ladybird (*H. axyridis*), native Asia, has been described as the most invasive ladybird on Earth (Roy *et al.* 2006) and is now present across the world, including Europe (Honek *et al.* 2016; Roy *et al.* 2012), North (Koch & Galvan 2008) and South America (Grez *et al.* 2016), South Africa (Stals & Prinsloo 2007), and New Zealand (Ministry for Primary Industries 2016). *H. axyridis* is a large ladybird (7-8 mm in diameter) and can take several morphs (Hodek *et al.* 2012). The morphs of *H. axyridis* can vary in terms of the number of spots (between zero and 21) and have a base colour though yellow to red to black (Hodek *et al.* 2012). *H. axyridis* was released throughout much of Europe and North America as a biological control agent against aphid pest species and has since spread further via a proposed 'bridge-head' effect, by which subsequent invasions stem from particularly successful non-native populations rather than the native range (Lombaert *et al.* 2010). The arrival of *H. axyridis* into the

UK, in 2004, is considered likely to have been through individuals arriving from invaded regions of Europe (Brown *et al.* 2008), however, the spread of *H. axyridis* to the Shetland Islands was facilitated by the transportation of goods (Ribbands *et al.* 2009). Following its arrival into the UK, *H. axyridis* has spread rapidly and now occupies much of England and Wales, with its northern expansion being impeded by upland areas such as the Pennines. *H. axyridis* now dominates many UK ladybird communities (Brown & Roy 2017).

The arrival of *H. axyridis* has been associated with declines in native Coccinellidae, for example, Roy *et al.* (2012) showed that native *Adalia bipunctata* have declined by 44 and 30% in the UK and Belgium. This impact of native Coccinellidae is also mirrored throughout much of the species' non-native range, with declines in native ladybirds in North (Koch & Galvan 2008) and South America (Grez *et al.* 2016) as well as throughout Europe (for example, Roy *et al.* 2012). In addition to other Coccinellidae and aphid species, *H. axyridis* is also know to predate other insect species including the eggs of the monarch butterfly (*Danaus plexippus*) (Koch *et al.* 2003). Ultimately, this results in *H. axyridis* having substantial ecological impacts throughout its invaded range. While we understand how *H. axyridis* impacts native Coccinellidae and other non-target species, our understanding as to how native prey species have been affected remains poorly understood (Roy & Brown 2015; Roy *et al.* 2016a). We also know little as to how infection with a pathogen may increase or decrease the predatory pressure imposed by *H. axyridis*. Together the species impact on native communities, high dispersal rate, and the wealth of data throughout the UK invasion process makes the UK Coccinellidae system a valuable tool for answering questions as to the impacts of invasive non-native species.

1.7.2 UK freshwater amphipod systems

The UK is home to three dominant native freshwater amphipod species; *Gammarus lacustris* is widespread and common in northern England and Scotland, *Gammarus duebeni* is common in Ireland and localised to coastal regions of Britain, and *Gammarus pulex* which is widespread and abundant throughout much of Britain and non-native in Northern Ireland. These species are often highly abundant, omnivorous and fulfil an

important ecological niche within freshwater systems, the shredding of coarse detrital matter. The UK is also home to five non-native amphipod species, potentially the two most notable being *Dikerogammarus villosus* and *Dikerogammarus haemobaphes* whose spread has been tracked throughout Europe (for example, Bij de Vaate *et al.* 2002).

Native to the Ponto-Caspian region, both *D. villosus* and *D. haemobaphes* (Figure 1.2) are large freshwater amphipods (10-20 mm), in comparison to native amphipod species including *G. pulex*, and are generalists in terms of both their prey and habitat preferences. Both species are also recent arrivals to the UK, with *D. villosus* first recorded in 2010 and *D. haemobaphes* in 2012 (GB Non-Native Species Secretariat 2018). *D. villosus* is highly localised with populations in Grafham Water, Cambridgeshire, Cardiff Bay and Eglwys Nunydd, South Wales, and the Norfolk Broads, Norfolk. Conversely, *D. haemobaphes* is more widely distributed within the UK, with the species known to occupy many lotic water bodies including the River Thames, River Great Ouse, River Nene, River Trent and the Leeds-Liverpool Canal (GB Non-Native Species Secretariat 2018). The range expansion of Ponto-Caspian invaders, including both *Dikerogammarus* species, was facilitated by the connectivity of the water bodies across Europe, specifically the opening of the Rhine-Main-Danube canal in 1992 which connected the Rhine and Danube river basins (Bij de Vaate *et al.* 2002). *D. villosus* and *D. haemobaphes* expanded their ranges across Europe at similar times with *D. haemobaphes* first recorded in the Rhine-Main-Danube canal in 1993. *D. haemobaphes* and *D. villosus* were first recorded in the Netherlands (River Rhine) in 2000 and 1994, respectively. The colonisation of the UK from European populations is considered to have been facilitated by human activity, including accidental transportation in shipping ballast water and recreational activities such as angling (Anderson *et al.* 2015). Arundell *et al.* (2015) provide evidence that the arrival of *D. villosus* into the UK was via multiple invasion events, and with both species spreading at the same time and via the same routes, it is also likely that the same is true for *D. haemobaphes*.

Throughout their spread across Europe, the impact the *Dikerogammarus* species has been the subject of much research attention, primarily as to their predatory impacts (for example, Bacela-Spychalska & Van Der Velde 2013; Berezina 2007; Josens *et al.* 2005;

Jourdan *et al.* 2016; Kenna *et al.* 2016). The arrival of *Dikerogammarus* species into previously non-invaded communities has been correlated with declines in native macroinvertebrates and replacement of amphipod species (Dick & Platvoet 2000; MacNeil *et al.* 2013). While we understand the predatory impact of both *Dikerogammarus* species, our understanding as to the pressures the species impose through bottom-up forces, via altering the rates of detrital processing, remains incomplete (Constable & Birkby 2016; Kenna *et al.* 2016). Specifically, *D. villosus* and *D. haemobaphes* are considered more predatory than native amphipods and can show lower rates of detrital processing (Constable & Birkby 2016; Kenna *et al.* 2016). Less detrital processing behaviour could result in fewer available nutrients for the wider community, however, this effect could be exacerbated by the predation and displacement of other macroinvertebrate shredders, for example *G. pulex* and *Asellus aquaticus*. Projections as to future global mean climatic warming are between 0.4 and 4.8°C (IPCC 2014), under the various projection scenarios, with extreme climatic events likely to increase in frequency and severity. Freshwater systems are expected to track these changes in temperature however extreme climatic events such as drought and flooding are also likely to significantly modify the flow regimes of impacted water bodies. In the coming years both *D. villosus* and *D. haemobaphes* are expected to expand the non-native range within the UK (Gallardo & Aldridge 2014); however, we understand little about how the bottom-up impacts of either *Dikerogammarus* species will vary under projected climate change scenarios and this is likely to become even more important in the coming years (Brook *et al.* 2008; Gallardo & Aldridge 2014). It was, at least partially, because of the substantial impacts these *Dikerogammarus* species posed to native communities that the UK Department for Food, Environment and Rural Affairs (Defra) launched the 'Check, Clean, Dry' campaign (Madgwick & Aldridge 2011). While these efforts may have succeeded in raising the profile of the species and biosecurity practices, *D. villosus* subsequently colonised the Norfolk Broads (2012), two years after its first record in the UK, suggesting that the species is likely to continue its spread.
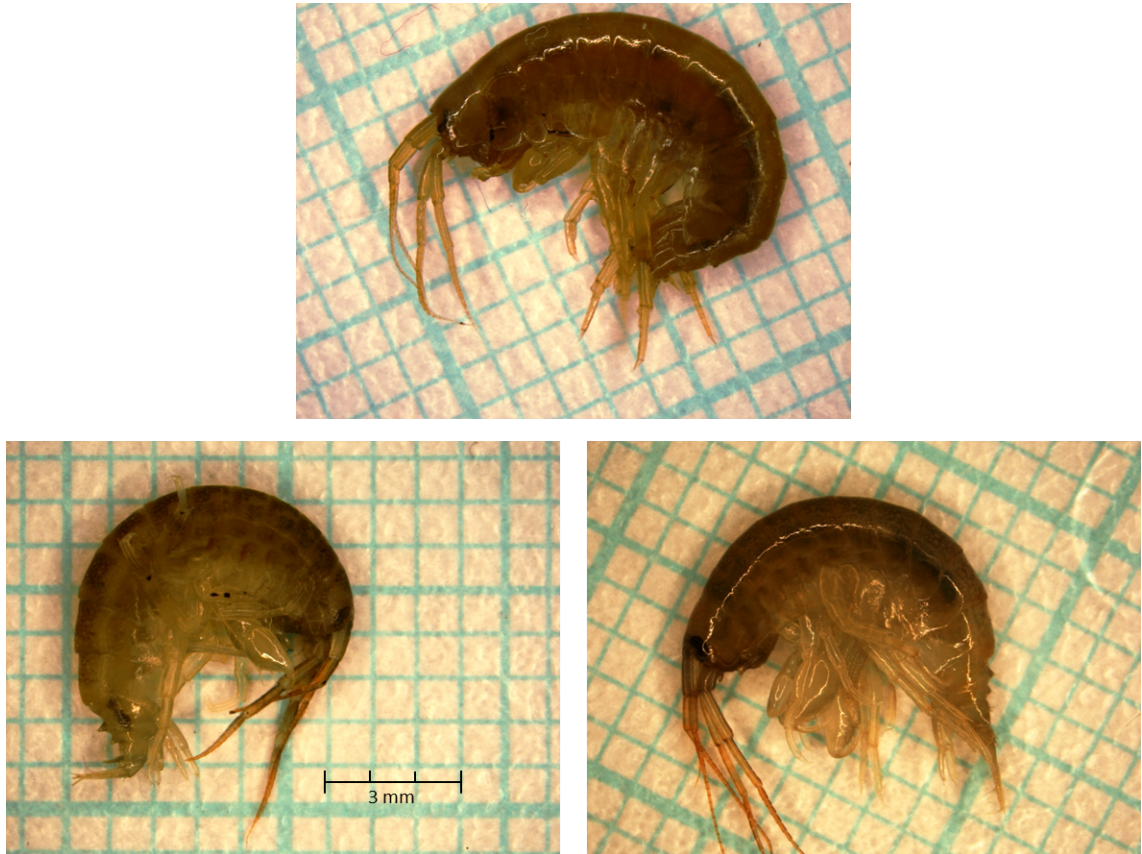
Figure 1.2: The UK freshwater amphipod study system containing native *Gammarus pulex* (top), and invasive non-native *Dikerogammarus villosus* (bottom-left) and *Dikerogammarus haemobaphes* (bottom-right).

1.8 Research aims

Throughout this thesis, I will investigate the ecological impacts invasive non-native species have on native communities, through top-down and bottom-up forces, and how these are modified by the additional environmental stressors of climate change and pathogenic infection. I will address these questions by using two complimentary study systems; the UK Coccinellidae system (Figure 1.1), to quantify the impacts of predation, and the UK freshwater amphipod system (Figure 1.2), to measure the impacts of detrital processing.

In Chapters 2 and 3 I aim to quantify the impact of a widespread invasive non-native predatory insect, the harlequin ladybird (*H. axyridis*), via top-down pressures. I use two methods to quantify the species impact. I begin (Chapter 2) by questioning how the predatory abilities of native and invasive-non native predators (Coccinellidae) differ when subjected to the pressure of a pathogenic infection. I aimed for this analysis to inform our current understanding as to the success and ongoing ecological impact of the invasive non-native *H. axyridis* in the UK. To date, our understanding as to how the predatory abilities of native and invasive non-native Coccinellidae remains limited while we know little about how these predatory abilities vary with respect to pathogenic infection. I quantify and compare the predatory behaviour and efficiency of the invasive non-native *H. axyridis* with two historically common and widespread UK Coccinellidae, the 2-spot (*A. bipunctata*) and 7-spot (*C. septempunctata*) ladybirds. In addition to quantifying and comparing the behaviours of apparently healthy individuals, I further investigate how the additional environmental stressor of pathogenic infection impacts my findings using the widespread entomopathogen *Beauveria bassiana*. In Chapter 3 I scale-up my investigation, as to the impacts of *H. axyridis* on native prey, to the landscape scale. While the impacts of *H. axyridis* on native insects (e.g. Coccinellidae) has been well reported (Koch *et al.* 2003; Koch & Galvan 2008; Roy *et al.* 2012; Grez *et al.* 2016), we know little about how the arrival and subsequent spread of *H. axyridis* has impacted native aphid species - the very species they were introduced to control. I use long-term datasets collected by expert and citizen scientists as part of the UK

Ladybird Survey and Rothamsted Insect Survey, to measure any changes in native aphid population abundance across England before and after the arrival of *H. axyridis*. As far as we are aware, this is the first study to combine these two datasets.

In Chapters 4 and 5 I make efforts to quantify the bottom-up impacts of two freshwater invasive non-native amphipods (*D. villosus* and *D. haemobaphes*). In Chapter 4 I discuss a laboratory manipulation experiment where I quantify the differences in detrital processing rates of *D. villosus*, *D. haemobaphes*, and the native *Gammarus pulex*. While previous work has suggested that both *Dikerogammarus* species could undertake detrital processing at different rates to that of native *G. pulex* (Constable & Birkby 2016; Kenna *et al.* 2016), our understanding as to how these rates vary across different leaf diets and at temperature extremes remains poor. As part of this experiment I also investigate how both resource quality, through three diets of differing resource quality, and at temperature extremes, with three different temperature treatments, impacts the detrital processing and survival rates of the amphipod species.

Finally, in Chapter 5 I develop Chapter 4, in an attempt to account for the wider community impacts of the top-down and bottom-up forces imposed by the *Dikerogammarus* omnivores, by conducting a field mesocosm experiment. Our current understanding as to the impacts of invasive non-native *Dikerogammarus* species is predominantly through either small scale, often *per capita* laboratory microcosm studies or field studies, that are commonly observational in nature. The use of field mesocosms in this chapter aimed to fill this research gap and extend our detailed, highly controlled, yet ultimately simplistic laboratory microcosm experiment to measure the community impacts of the invasive *Dikerogammarus* species. As part of this experiment I measure how the three amphipod species, at two density treatments, alter community measures such as detrital processing, community diversity measures and primary production.

Chapter 2

Predators under pressure: predicting the impacts of an invasive non-native predator under pathogen pressure

2.1   Abstract

Invasive non-native species (INNS) can drive community change through key functional behaviours, such as predation. Parasites and pathogens can play an important role in community function including mitigating or enhancing INNS impacts. Despite this, few studies have quantified the impacts of INNS key functional behaviours when subject to pathogen pressure. Here we questioned whether the predatory ability of native and invasive non-native predators differed between species and between individuals subject to pathogenic infection and those not. We quantified the predatory behaviour of the highly invasive non-native harlequin ladybird (*Harmonia axyridis*) and two UK native species, the 7-spot (*Coccinella septempunctata*) and 2-spot (*Adalia bipunctata*) ladybirds using comparative functional response experiments. We investigated the impacts of pathogen infection on the predatory ability of native and invasive non-native ladybirds by exposing individuals to *Beauveria bassiana*, a widespread entomopathogen. Invasive *H. axyridis* was a more efficient predator than both the native *A. bipunctata* and *C. septempunctata*, often having higher attack and/or lower prey handling time coefficients. Native *A. bipunctata* were the least efficient predators, often having lower attack coefficients and/or higher prey handling coefficients. These differences were found in both adult and larval life-stages. *B. bassiana* infection significantly altered the predatory efficiency of adult and larval ladybird predators. The effects of pathogenic infection differed between species and life-stage but in many cases infection resulted in a reduced predatory ability. Our work suggests that the synergistic effects and interactions between INNS, parasites and pathogens are integral to determining invasion success and impact. Incorporating such species interactions in laboratory manipulation experiments can provide insight into how *per-capita* differences may vary between native and invasive non-native species.

2.2 Introduction

Global biodiversity is under an increasing threat from multiple anthropogenic pressures including climate change, habitat loss and the spread of invasive non-native species (INNS) (Bellard *et al.* 2012; Butchart *et al.* 2010; Simberloff *et al.* 2013). The global spread of non-native species can impose pressures on native biodiversity, with the Convention on Biological Diversity (2006) suggesting 40% of species extinctions in the last 400 years are directly attributable to the impacts of non-native species. Furthermore, INNS can result in significant economic costs through their impacts on infrastructure and both human and animal health (Williams *et al.* 2010; Juliano & Philip Lounibos 2005).

The rate of species invasions has increased in recent decades with the expansion of global trade and movement, and further rate increases appear likely (Hulme 2009; Levine & D'Antonio 2003; Seebens *et al.* 2017). As a result, understanding the impacts of species invasion events has rarely been more important. The impacts that INNS can impose on native systems are thought to vary with respect to their trophic position and key functional behaviours, such as predation, which can also facilitate invader success (Bellard *et al.* 2016; Salo *et al.* 2007). Although characteristics of the invader can influence its effects, they can also differ according to characteristics of the community in which they find themselves. Parasites and pathogens play key roles within communities and can provide resistance to species invasions and modify the impacts of invading species, in addition to colonising novel areas as INNS themselves (Hatcher *et al.* 2014; Roy *et al.* 2016b; Vilcinskas 2015). Key functional roles are undertaken by parasites and pathogens through lethal and sub-lethal trait effects (Dunn & Hatcher 2015). Lethal effects of parasites can affect host population densities and result in population declines whereas the sub-lethal effects of infection can result in more complex impacts (Hatcher *et al.* 2014; Dunn & Hatcher 2015). For example, Roy *et al.* (2008b) provided evidence that harlequin ladybirds (*Harmonia axyridis*) infected with *Beauveria bassiana* showed reduced egg production. Sub-lethal effects of parasites can also affect species with which hosts interact; for example, Dick *et al.* (2010) showed that *Gammarus pulex* infected with *Echinorhynchus truttae* consume prey at an increased rate compared to uninfected

conspecifics. Despite their widespread presence within communities, parasites and pathogens are often absent from studies investigating the impacts of INNS (Hatcher *et al.* 2012), potentially resulting in oversimplified study systems that are unlikely to be representative of those in the field. Accounting for these effects not only provides insight as to species behaviours at suboptimal health, but also the role of parasites and pathogens during species invasions.

Predation can be a key way in which INNS can influence native communities. The quantification of predatory behaviour is an established method and can use predatory functional responses to describe the relationship between a species' resource use and the availability of that resource (Holling 1959). Specifically, functional responses enable us to measure how a predator's rate of prey consumption varies with respect to changes in prey density. This provides a *per-capita* measure of predatory ability and subsequently predatory pressure imposed on the prey species which can be compared between species and/or treatments and used to estimate population level impacts (Dick *et al.* 2017b; Laverty *et al.* 2017b). Predatory functional responses have historically been used in population and community ecology in addition to pest management via biological control (for example Sabelis 1992; O'Neill 1990), however, they have more recently been applied within invasion ecology to understand and predict the impacts of invasive species (Alexander *et al.* 2014; Dick *et al.* 2017a). Functional response experiments allow for predation behaviour to be quantified and described as one of three well defined response types (I, II and III) in addition to the calculation of predatory coefficients; handling time (h) and attack rate (a) (Holling 1959) across a range of prey densities. The functional response type can inform the ecological impact of the predator on the prey population. For example a predator displaying a type II relationship could be expected to predate a prey population to low densities or localised extinction. Conversely, a type III relationship suggests that the predator could show prey switching behaviour when the primary prey species reaches low densities. The UK Coccinellidae system provides an ideal opportunity to study the impacts of an INNS that is amenable to laboratory, field and citizen science data collection methods (Roy *et al.* 2016a).

Functional response studies aim to replicate one part of a complex interaction

between predator and prey individuals in a more simplistic form, specifically, how a predators rate of prey consumption changes with respect to prey density. This simplistic representation allows for this relationship to be accurately quantified, something that is difficult and liable to additional external of variation, for example temperature or competition. The use of functional response experiments has been shown to accurately predict the impact of multiple invasive non-native species, for example the 'bloody red' shrimp (*Hemimysis anomala*) (Dick *et al.* 2013). As a simplistic representation of one part of a complex ecological network, functional response experiments are often limited in their ability to account for the wider complexities of ecological systems, for example additional species interactions and resources.

The harlequin ladybird (*H. axyridis*) is a highly invasive coccinellid predator that has invaded throughout the world aided by multiple releases as a biological control agent (Brown *et al.* 2008, 2011c; Grez *et al.* 2016). *H. axyridis* has been described as a voracious aphid predator (Majerus *et al.* 2006), however, the impacts on prey populations within the invaded range are less studied (Roy & Brown 2015; Roy *et al.* 2016a). However, previous research has shown that *H. axyridis* will predate the immature stages of the monarch butterfly (*Danaus plexippus*) (Koch *et al.* 2003). In addition to impacting prey species, *H. axyridis* has also led to declines of native ladybird populations through, at least in part, intra-guild predation (Katsanis *et al.* 2013; Ware & Majerus 2008). *H. axyridis* now dominates many Coccinellidae assemblages throughout its invaded range (e.g. Brown & Roy 2017) which has resulted in reduced species diversity (Harmon *et al.* 2007; Koch & Galvan 2008; Bahlai *et al.* 2014; Grez *et al.* 2016). Following the arrival of *H. axyridis* in the UK in 2004, the 2-spot ladybird (*Adalia bipunctata*) showed a decline of 44% while 7-spot ladybird (*Coccinella septempunctata*) populations showed no significant change (Roy *et al.* 2012). Both of these species are historically common in the UK. The predatory ability of *H. axyridis* is believed to have been instrumental in the population declines of native Coccinellidae whilst giving the invasive species a competitive advantage, therefore facilitating its continued spread (for example Majerus *et al.* 2006).

Beauveria bassiana is a widespread entomopathogenic fungus and a common

pathogen in the UK ladybird system that is known to be a major cause of overwinter mortality in native *C. septempunctata* (Ormond *et al.* 2011). Infection at lower doses can also be long lasting and result in sub-lethal trait mediated effects; for example, Roy *et al.* (2008b) showed that *B. bassiana* infection reduces egg production of *H. axyridis*. Despite having reason to expect *H. axyridis* would be affected by *B. bassiana* in some way, there has been no attempt to understand how *B. bassiana* infection could affect the predatory behaviour of *H. axyridis* in relation to those natives which have coevolved with this pathogen.

In this study we aimed to compare the predatory behaviour of the invasive non-native *H. axyridis*, and two UK native ladybird species; *A. bipunctata* and *C. septempunctata*, during their larval and adult life stages, so as to better understand the ecological impact of the *H. axyridis* invasion and any potential insights as to *H. axyridis*' invasion success. We also investigated how pathogenic infection impacts the predatory ability of the three species across their larval and adult life stages by infecting individuals with a sub-lethal dose of *B. bassiana*. We hypothesised that: the invasive non-native *H. axyridis* would demonstrate more efficient predatory behaviour than the native species. Efficient predatory behaviour was defined as having a higher overall functional response relationship, increased attack rate or reduced handling time. We further investigated how sub-lethal *B. bassinana* pathogenic infection would impact the predatory efficiency of the three ladybird species as this could inform our understanding as to the success of *H. axyridis*. The loss of native natural enemies could facilitate *H. axyridis*' success, as predicted by the enemy release hypothesis. However, it remains the subject of debate as to weather *H. axyridis* benefits from the loss of natural enemies, lost through the invasion process, or a generally low susceptibility to natural enemies, potentially due to its advanced chemical defences (Ceryngier *et al.* 2018; Koyama & Majerus 2007; Roy *et al.* 2008a; Shapiro-Ilan & Cottrell 2011).

2.3   Materials and methods

2.3.1   Insect cultures

We collected first and second larval instars of *H. axyridis* and adult *C. septempunctata* in Oxfordshire (51°60'N; -1°11'W) through visual and sweep net sampling of vegetation. Due to their scarcity, we purchased *A. bipunctata* first and second instar larvae from an industrial supplier (Green Gardener, UK) and we collected *C. septempunctata* as adults and they were therefore not used in larval experiments as they were also found to be scarce in this particular field season. All individuals were maintained at constant conditions (20°C, 16:8 L:D cycle) for at least seven days prior to experimentation. We reared *H. axyridis* and *A. bipunctata* larvae in control conditions until their use in either larval or adult experiments. We fed individuals a mixed diet of sycamore aphids (frozen, mixed age classes), *Ephestia kuehniella* eggs (Entofood, Koppert, the Netherlands) and an artificial diet (detailed by Roy *et al.* 2013). We purchased English grain aphids (*Sitobion avenae*) from a commercial supplier (Ervibank, Koppert, the Netherlands) and reared them in the same conditions on the wheat plants on which they where received. We sexed adult ladybirds using established physical characteristics (McCornack *et al.* 1980; Roy *et al.* 2011). We used females in experimental trials as they are known to predate at higher rates than males (Xue *et al.* 2009; Gupta *et al.* 2012). Due to the inability to sex ladybird larvae, the larval treatments were of mixed sex. All larval treatments used fourth instar ladybird larvae.

2.3.2   *Beauveria bassiana* infection

We cultured *Beauveria bassiana* from a commercially available product (Botanigard WP, strain GHA) on Sabouraud dextrose agar (SDA) in Petri dishes in darkness at 25°C. We prepared single spore isolations from these cultures, and subsequently sub-cultured under the same conditions before being stored at -20°C in 10% glycerol (v/v sterile milli-Q water) as a cryoprotectant. Thawed sub-cultures were macerated, spread onto fresh SDA plates and cultured for approximately 14 days until sporulation. We prepared spore

suspensions in 0.03% Tween 20 (v/v in sterile water) surfactant to reduce spores clumping together and the concentration of the resulting suspension was estimated using a Neubauer improved haemocytometer. We produced a 10^6 spores ml^{-1} dilution from the stock suspension approximately 16 hours prior to the experiment, stored on-ice and homogenised before use in experiments. This dose was aimed to provide an ecologically relevant dose that could feasibly impact predatory behaviour (Roy *et al.* 2008b).

We inoculated ladybird predators with one of two treatment solutions; a control treatment of 0.03% Tween 20 or a 10^6 spores ml^{-1} *B. bassiana* spore suspension for infection treatments. Roy *et al.* (2008b) report the LD$_{50}$ (median lethal dose) of native *C. septempunctata* and *A. bipunctata* were similar at 10^6 and $10^{6.2}$ spores ml^{-1} respectively whereas invasive non-native *H. axyridis* had an LD$_{50}$ of $10^{9.6}$ spores ml^{-1}. Individuals were inoculated by inversion (five times) in one ml of inoculum and were placed on filter paper (Whatman No.1) in a Büchner funnel to remove excess inoculum. All equipment was cleaned with 95% ethanol between treatments. Following exposure to *B. bassiana*, treatment groups were housed separately to prevent contamination and starved for eight hours to standardise gut contents before the start of the experiment.

2.3.3   Experimental methods

Experimental arenas consisted of a Petri dish (90 mm) and contained blades of winter wheat (*Triticum aestivum*; ten strips, 40 mm in length) embedded in 2% water agar, approximately four mm in depth, so as in increase habitat heterogeneity and therefore better represent natural environments. Filter papers (Whatman No.1) were positioned in the lids to moderate moisture levels. Wheat was grown from seed (Syngenta) for 14 days before use. Grain aphids (*Sitobion avenae*) were provided as a prey resource at known densities of live second and third instar individuals. Fourth instar larval treatments were provided with prey densities of; 1, 2, 4, 8, 16, 32, 64 and 128 individuals. Adult treatments received prey densities of 1, 2, 4, 8, 16, 32, 64, 128 and 256 individuals. The doubling sequence of prey densities is required to correctly define and quantify the treatments functional response (Dick *et al.* 2014, e.g.). Specifically, fine scale accuracy at lower prey densities is required to correctly distinguish between type II and type III

Table 2.1: Sample sizes for each of the ladybird species, *B. bassiana* infection, and aphid prey density treatment combinations for both adult and larval treatments.

(a) Ladybird larvae

Species	B. bassiana	Prey density							
		1	2	4	8	16	32	64	128
A. bipunctata	-	5	5	5	5	5	5	6	5
	+	5	5	5	5	5	5	5	4
H. axyridis	-	4	5	5	5	5	6	5	5
	+	5	4	5	5	5	5	5	5

(b) Ladybird adults

Species	B. bassiana	Prey density								
		1	2	4	8	16	32	64	128	256
A. bipunctata	-	5	5	5	5	5	5	5	5	4
	+	5	5	5	5	5	5	5	5	5
C. septempunctata	-	4	5	6	5	5	5	4	5	4
	+	5	4	4	5	5	5	5	5	5
H. axyridis	-	5	5	5	5	5	5	5	5	4
	+	5	5	5	5	5	5	5	5	4

functional responses. Adults received an additional prey density treatment as they are known to consume more prey then larvae. We aimed to replicate each treatment combination five times, due to ladybird mortality the total number of treatment replicates varied between four and six (Table 2.1).

Predators were weighed after their starvation period before being added to the experimental arenas. The experiment ran for 24 hours at constant conditions during which time predation of aphid prey could occur. After 24 hours the ladybirds were removed from the arenas and remaining live and dead prey were counted. No cases of partial consumption were observed. Individuals were starved for a further 12 hours before resuming a mixed diet and were monitored for mortality over the next 14 days. Adult cadavers, collected within the 14 day post-experiment observation period, were surface sterilised using a 1% bleach solution to reduce contamination, before being plated out on 2% water agar and incubated in darkness at 25°C. Incubated cadavers were visually checked for signs of fungal sporulation for a period of 14 days.

2.3.4   Statistical analysis

All statistical analyses were undertaken in R version 3.3.2 and RStudio version 1.0.136 (R Core Team 2016; RStudio 2016). We compared ladybird masses with respect to species and treatment using ANOVA and TukeyHSD post-hoc statistical tests for both life stages. The masses of adult ladybird species did not differ between the infection treatment groups (ANOVA; $F_{2,257} = 0.836$, $P = 0.434$) and for each of the three ladybird species, ladybird masses were not significantly different between treatment groups (ANOVA; $F_{1,259} = 1.117$, $P = 0.291$), as a result we did not account for mass in subsequent models. Adults of each species differed significantly in mass (ANOVA; $F_{2,260} = 500.9$, $P < 0.001$) and TukeyHSD results indicated this is driven by *A. bipunctata* (mean $\pm$ SD, 9.97 mg $\pm$ 2.4, n = 89) being significantly smaller than *H. axyridis* (36.05 mg $\pm$ 7.38, n = 88) and *C. septempunctata* (37.22 mg $\pm$ 8.34, n = 86). Similarly, we found no evidence that larvae masses varied significantly with *B. bassiana* infection treatments whether we accounted for species differences or not (ANOVA; $F_{1,152} = 2.912$, $P = 0.09$ and $F_{1,153} = 2.461$, $P = 0.119$). As with adult predators, larval *A. bipunctata* (5.87 mg $\pm$ 2.41, n = 80) were significantly smaller than *H. axyridis* (mean $\pm$ SD, 19.51 mg $\pm$ 8.91, n = 76) (ANOVA; $F_{1,154} = 174.2$, $P < 0.001$). The number of prey surviving in predator treatments was compared to the control treatments using linear regression with, in response to signs of overdispersion, a quasipoisson error structure. We compared the number of prey consumed in the predator treatments between species and treatments, for both larvae and adult predators, using generalised linear models with quasipoisson error structures.

2.3.4.1   Functional responses

Functional response curve fitting was undertaken using the bbmle and emdbook statistical packages (Bolker & R Development Core Team 2014; Bolker 2016). Defining predatory functional response relationships can be difficult. In an attempt to overcome the difficulties of correctly defining a functional response type we used three statistical techniques; linear regression, LOESS curve fitting, and AICc scores. Our use of linear

regression allows us to determine the relationship, specifically the gradient of the slope, between the proportion of prey consumed and the number of prey available (Juliano 2001). Conversely, LOESS curve fitting has less restrictive assumptions than linear regression techniques however, this results in this method being less statistically powerful (Juliano 2001). Lastly, we used AICc scores to determine the 'best fitting' model from a set of candidate models, in this case being type I, II, and II functional response relationships. Using AICc scores over the similar AIC scores better accounts for smaller samples sizes often present within ecological studies.

Functional response relationships were fitted using Holling's original type I equation (Equation 2.1), Rogers' type II equation (Equation 2.2), and Hassell's type III equation (Equation 2.3). Hassall's type III and Rogers' type II equations are similar however, while Rogers' type II includes an attack rate parameter (a), Hassall's type III assumes the attack rate varies with prey density via a hyperbolic relationship. Rogers' type II and Hassell's type III equations both account for prey depletion (Rogers 1972; Hassell 1978) and rely on the Lambert W function (Bolker 2016).

$$N_e = aTN_0 \tag{2.1}$$

$$N_e = N_0(1 - e^{(a(N_e h - T))}) \tag{2.2}$$

$$N_e = N_0(1 - e^{\frac{(d + bN_0(hN_e - T))}{1 + cN_0}}) \tag{2.3}$$

In all equations N_e denotes prey consumed, N_0 is the number of prey provided, T is the time during which behaviours occurred, a and h are attack rate and handling time coefficients of the predators. In Hassell's type III equation (Equation 2.3) b, c and d are used to calculate the hyperbolic a. The attack rate constant (a) is defined as the rate of prey consumption and informs the gradient of the functional response curve whereas the handling time coefficient (h) is the rate of saturation and provides insight as to the time predators spend handling prey between attacks. Together these parameters define the

Table 2.2: Results of a logistic regression of the total prey consumed in each prey density treatment for each species. Results indicate each species consumed significantly more prey than control treatments in which predators were absent. Prey density was also found to be significant, with more prey consumed at higher prey densities. The analysis was carried out using a quasi-poisson error structure with prey density values scaled and centred. Asterisks denote significance of P values; * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

	Coefficient ($\pm$ SE)	t	P
Intercept	-3.266 ($\pm$ 2.021)	-1.616	0.107
Density	0.689 ($\pm$ 0.030)	23.029	< 0.001***
Adalia bipunctata	5.029 ($\pm$ 2.023)	2.487	0.014*
Coccinella septempunctata	5.708 ($\pm$ 2.022)	2.823	0.005**
Harmonia axyridis	5.907 ($\pm$ 2.021)	2.922	0.004**

predator's overall functional response.

2.3.4.2 Comparing predatory behaviours

Predatory statistics of attack rates (a) and handling times (h) were calculated and compared using nonlinear least squares regression (as described by Juliano 2001). The number of prey consumed was regressed against the initial density, density^2 and density^3. Type I and II responses would be indicated by a significant and negative first order term (density) and a type III response would be indicated by a significant and positive first order (density) and quadratic term (density^2) or a significant third order term (density^3) (Juliano 2001). Confidence intervals were calculated for each functional response relationship through bootstrapping (n = 999). Separate models were fitted for fourth instar larvae and adult predator treatments.

2.4 Results

Prey survival in control treatments was 86.9%, which was significantly higher than predator treatments (*H. axyridis* = 48.8%, *C. septempunctata* = 50% and *A. bipunctata* = 70.8%) (Table 2.2). Prey mortality was therefore attributed to predatory behaviour of the focal predators. *B. bassiana* infection was confirmed in 63% of adult and 48.5% of larvae infection treatment individuals that died following experiments. 5.9% of uninfected treatment adults and no larvae showed infection.

In adult treatments, the three ladybird species consumed prey at significantly different rates (GLM; $F_{(2,259)}$ = 11.952, P <0.001), with invasive *H. axyridis* consuming the most and *A. bipunctata* consuming the least, and more prey were consumed with the increasing prey density treatments (GLM; $F_{(2,259)}$ = 268.848, P <0.001). Pathogen exposure did not significantly impact the number of prey consumed by adult ladybirds with each of the interaction terms containing the pathogen treatment (density*species*pathogen, species*pathogen, density*pathogen and density*species) and the main effect all being removed at P >0.05. Although ultimately removed from the final model, a marginally significant species*pathogen interaction term (GLM; $F_{(2,256)}$ = 2.965, P = 0.054) suggested that the pathogen exposure might have changed the prey consumption of the ladybird species differently. In larval treatments, the number of prey consumed with increasing density treatment changed significantly when ladybird predators were subject to pathogen exposure (GLM; $F_{(1,151)}$ = 1075.8, P = 0.010). Similar to the adult treatments, larvae of the three ladybird species consumed significantly different numbers of prey (GLM; $F_{(1,153)}$, = 65.962, P <0.001). As with the adult ladybird analyses, all other terms were removed from the final model at significance values (P) of more than 0.05.

2.4.1 Functional responses

All species treatments showed type II functional responses (Figure 2.1). Logistic regression of the proportion of prey consumed against prey density indicated that 7 of the 10 treatments showed a type II functional response through a significant and negative first order term (density) (Tables 2.3a and 2.3b). Two of these analyses showed a significant second order term (density^2), however, these were positive and did not indicate a type III response. No density terms were significant in three treatments; uninfected *A. bipunctata* and infected *C. septempunctata* adults and infected *H. axyridis* larvae. This could suggest either a type I relationship or that the functional response relationship was undetectable. Further investigation of these treatments using AICc values of the fitted functional response equations (Equations 2.1, 2.2 and 2.3) suggested a type II response for uninfected *A. bipunctata* adults (Table 2.4). AICc values for

infected *C. septempunctata* adults suggested a type III relationship and an indistinguishable type II/III relationship for infected *H. axyridis* larvae. Visual inspection of fitted LOESS curves provided qualitatively similar results (Figure 2.2). As the majority of methods and treatments showed type II responses, this was accepted for all species-treatment combinations.

Table 2.3: Results from multiple logistic regressions of the proportion of prey consumed by ladybirds against polynomials of initial prey density to determine suitable functional response types (I, II or III) for each species-treatment. As suggested by Juliano (2001), a significant negative first order term indicates a type I or II response while a significant positive first order term and a significant negative second order term indicates a type III response. A type III response could also have been suggested by a significant third order term. For each species-treatment combination, the proportion of prey consumed was modelled against first, second and third order polynomials of initial prey density using logistic regression with binomial error structures. Non-significant higher order terms were removed from the analysis through step-wise model simplification. Prey densities values scaled and centred and a quasi-binomial error structure was used. Coefficients are reported with associated standard errors in parenthesis and asterisks denoting significance of P values; * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

(a) Adult ladybirds

	H. axyridis		*A. bipunctata*		*C. septempunctata*	
	Infected	Uninfected	Infected	Uninfected	Infected	Uninfected
Intercept	0.464	0.045	-0.843***	-1.134***	-0.050	0.412
	(0.293)	(0.316)	(0.270)	(0.317)	(0.391)	(0.439)
Density	-0.006***	-0.003*	-0.005***	-0.001	-0.001	-0.019**
	(0.002)	(0.002)	(0.002)	(0.002)	(0.002)	(0.008)
Density^2						<0.001*
						(<0.001)
n	44	44	45	44	43	43

(b) Larval ladybirds

	H. axyridis		*A. bipunctata*	
	Infected	Uninfected	Infected	Uninfected
Intercept	0.326	0.868*	-1.167**	-1.049***
	(0.332)	(0.428)	(0.527)	(0.252)
Density	-0.003	-0.013***	-0.034*	-0.010***
	(0.003)	(0.005)	(0.019)	(0.003)
Density^2			<0.001**	
			(<0.001)	
n	39	37	39	41

Table 2.4: AICc measures of model fit for fitted type I, II and III functional response (FR) models. The lowest AICc values suggest the best model fit with a difference of 2 suggesting a significantly different model fit. The lowest AICc values are highlighted in bold.

			FR types		
Life-stage	Species	Treatment	I	II	III
Adult	*H. axyridis*	infected	798.667	653.083	**648.585**
		uninfected	805.539	**764.532**	**764.385**
	C. septempunctata	infected	1132.086	1123.759	**1096.16**
		uninfected	671.747	567.453	**558.788**
	A. bipunctata	infected	521.994	**458.479**	460.510
		uninfected	570.760	**568.597**	570.968
Larvae	*H. axyridis*	infected	431.930	**427.978**	**429.693**
		uninfected	694.134	**594.867**	597.088
	A. bipunctata	infected	**250.698**	256.090	255.229
		uninfected	248.034	**221.335**	**222.075**
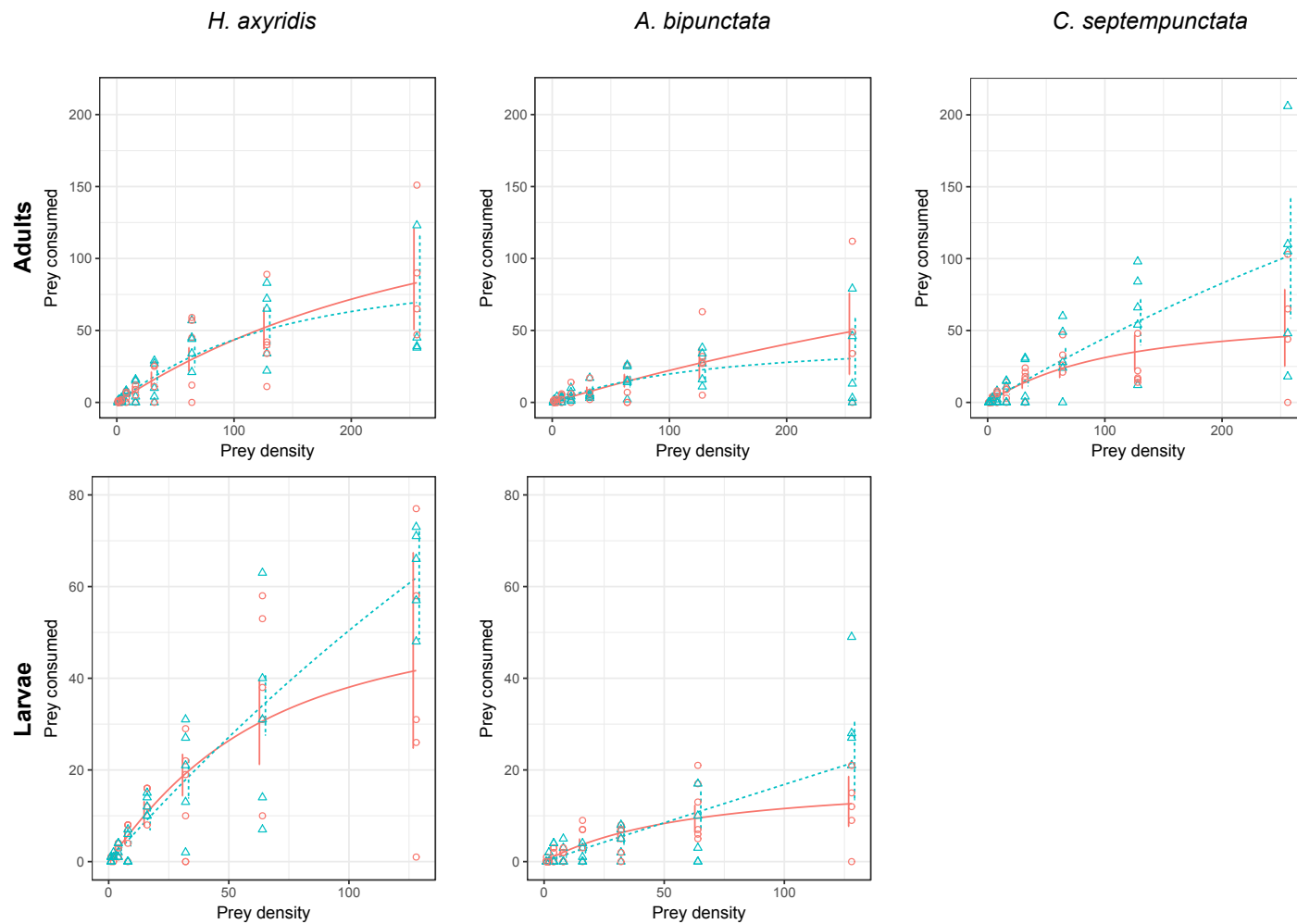
Figure 2.1: Predatory functional response curves for three ladybird predators; invasive non-native *H. axyridis* (left) and native *A. bipunctata* (middle) and *C. septempunctata* (right) across their adult (top) and larval (bottom) life-stages. Functional response curves (lines) are displayed with replicate data (points; Table 2.1) and bootstrapped 95% confidence intervals (n = 999) (vertical lines). Uninfected predators (red solid lines and circles) were inoculated with a control dose of Tween 20 and *B. bassiana* infected predators (blue dashed lines and triangles) were inoculated with a 10^6 suspended spore solution.
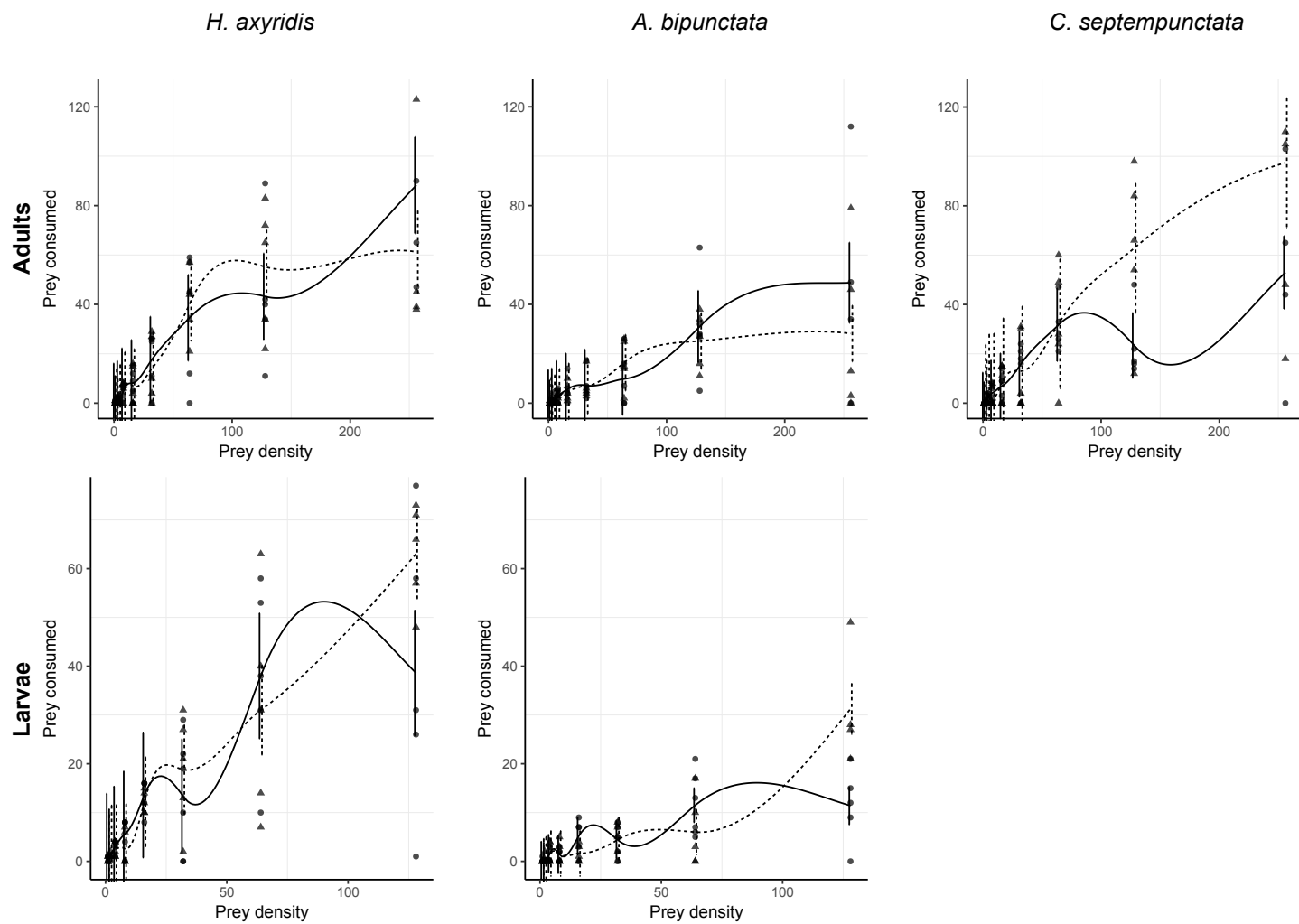
Figure 2.2: Locally weighted non-parametric scatterpot smoothing (LOESS) plots of prey consumed against the initial prey density for the three ladybird predators; invasive non-native *H. axyridis* (left) and native *A. bipunctata* (middle) and *C. septempunctata* (right) across their adult (top) and larval (bottom) life-stages. Fitted LOESS models are displayed (lines) with the number of prey consumed at each density and replicate datapoints for both infected (dashed lines and triangles) and uninfected (solid lines and circles) treatments. 95% confidence intervals are presented as vertical lines (dashed = infected, solid = uninfected).

2.4.2   Comparing predatory behaviours

Visual inspection of functional response curves suggested between-species differences in predatory behaviour, as well as different responses to infection. The functional response curves suggested that *H. axyridis* predated at a higher rate than native *A. bipunctata* and *C. septempunctata* (Figure 2.1) and this was associated with increased attack rate and handling time coefficients which suggest a greater forage ability (Table 2.5). A similar result was also seen in larval treatments with invasive *H. axyridis* consuming more prey than native *A. bipunctata* (Figure 2.1).

Predators responded to *B. bassiana* infection differently, varying with species and life-stage (Table 2.6). Infected *H. axyridis* and *A. bipunctata* adults showed lower functional response curves than uninfected conspecifics. In contrast, larval treatments showed the opposite response with infected individuals consuming more prey than uninfected individuals. Adult *C. septempunctata* showed an opposing response to infection than other adult treatments, instead infected individuals ate more than uninfected individuals. Pathogenic infection also increased the variation in predation, with infected individuals eating at both higher and lower rates than uninfected treatments. In all pairwise comparisons between infected and uninfected treatments, functional response curves differed the most in the higher prey density treatments (Figure 2.1).

Predatory behaviour appeared to differ between treatments but as the confidence intervals for the fitted functional response relationships overlapped we explored these relationships further through comparison of predatory statistics (attack rates (a) and handling times (h)). Within species treatments, *B. bassiana* infection resulted in increased attack rates (a) in adult *H. axyridis* (uninfected = 0.762, infected = 1.005, z = -2.696, P = 0.007) and *A. bipunctata* (uninfected = 0.281, infected = 0.392, z = 2.189, P = 0.029) (Table 2.6). Adult *C. septempunctata* showed no significant change in attack rate when subjected to pathogen pressure (P = 0.323). Conversely, infected ladybird larvae showed lower attack rates in comparison to their uninfected conspecifics (Table 2.6). However, when adult ladybirds were subjected to pathogen infection *C.*

Table 2.5: Maximum likelihood comparisons of functional response parameters (attack rate (a) and handling time (h)) between species and treatments. Functional response parameters were calculated through the fitting of the Rogers' 'random predator' type II functional response equation (Eq. 2.2). Maximum likelihood comparisons are made using methods described by Juliano (2001). Asterisks denote significance of P values; * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

Life stage	Base species-treatment		Contrast species-treatment		Metric	Estimate ±SE	z	P
Adult	Infected	*A. bipunctata*	Infected	*C. septumpunctata*	*a*	-0.273 (±0.06)	-4.520	<0.001***
					h	0.020 (± 0.003)	6.925	< 0.001***
				H. axyridis	*a*	-0.614 (± 0.083)	-7.404	< 0.001***
					h	0.012 (± 0.003)	4.259	< 0.001***
			Uninfected	*A. bipunctata*	*a*	0.110 (± 0.050)	2.189	0.029*
					h	0.017 (± 0.004)	4.834	< 0.001***
				C. septumpunctata	*a*	-0.354 (± 0.083)	-4.284	< 0.001***
					h	0.006 (± 0.003)	1.943	0.052.
				H. axyridis	*a*	-0.371 (± 0.070)	-5.280	< 0.001***
					h	0.016 (± 0.003)	5.628	< 0.001***
		C. septumpunctata	Infected	*H. axyridis*	*a*	-0.342 (± 0.083)	-4.128	< 0.001***
					h	-0.008 (± 0.001)	-7.138	< 0.001***
			Uninfected	*A. bipunctata*	*a*	0.383 (± 0.050)	7.642	< 0.001***
					h	-0.003 (± 0.002)	-1.036	0.300
				C. septumpunctata	*a*	-0.082 (± 0.083)	-0.989	0.323
					h	-0.014 (± 0.002)	-8.105	< 0.001***
				H. axyridis	*a*	-0.097 (± 0.070)	-1.392	0.164
					h	-0.004 (± 0.001)	-3.168	0.002**
	Uninfected	*A. bipunctata*	Infected	*H. axyridis*	*a*	-0.724 (± 0.076)	-9.553	< 0.001***
					h	-0.005 (± 0.002)	-2.099	0.036*
			Uninfected	*C. septumpunctata*	*a*	-0.464 (±0.076)	-6.147	<0.001***
					h	-0.011 (± 0.003)	-4.075	< 0.001***
				H. axyridis	*a*	-0.481 (± 0.061)	-7.802	< 0.001***
					h	-0.001 (± 0.002)	-0.409	0.683
		C. septumpunctata	Infected	*H. axyridis*	*a*	-0.259 (± 0.100)	-2.586	0.010**
					h	0.006 (± 0.002)	3.544	< 0.001***
			Uninfected	*H. axyridis*	*a*	-0.017 (± 0.090)	-0.183	0.855
					h	0.010 (± 0.002)	5.797	< 0.001***
		H. axyridis	Infected	*H. axyridis*	*a*	-0.243 (± 0.090)	-2.696	0.007**
					h	-0.004 (± 0.001)	-3.475	< 0.001***
Larvae	Infected	*H. axyridis*	Infected	*A. bipunctata*	*a*	0.781 (± 0.079)	9.929	< 0.001***
					h	0.052 (± 0.013)	3.900	< 0.001***
			Uninfected	*A. bipunctata*	*a*	0.546 (± 0.095)	5.747	< 0.001***
					h	-0.050 (± 0.010)	-4.829	< 0.001***
				H. axyridis	*a*	-0.486 (± 0.151)	-3.219	0.001**
					h	-0.013 (± 0.002)	-5.833	< 0.001***
	Uninfected	*H. axyridis*	Infected	*A. bipunctata*	*a*	0.816 (± 0.078)	10.545	< 0.001***
					h	0.049 (± 0.012)	4.276	< 0.001***
			Uninfected	*A. bipunctata*	*a*	1.033 (± 0.143)	7.237	< 0.001***
					h	-0.037 (± 0.011)	-3.574	< 0.001***
	Uninfected	*A. bipunctata*	Infected	*A. bipunctata*	*a*	0.224 (± 0.060)	3.770	< 0.001***
					h	0.103 (± 0.017)	6.079	< 0.001***

Table 2.6: Comparison of predicted attack rate (a) and handling time (h) coefficients between infected and uninfected predator treatments. Coefficients were calculated by fitting Rogers' 'random predator' type II functional response equation (Equation 2.2) and compared using maximum likelihood. Asterisks denote significance of P values; $*$ = $P < 0.05$, $**$ = $P < 0.01$ and $***$ = $P < 0.001$.

Life-stage	Species	Metric	Uninfected ($\pm$ SE)	Infected ($\pm$ SE)	P
Adult	*A. bipunctata*	a	0.281 ($\pm$ 0.027)	0.392 ($\pm$ 0.043)	0.029[*]
		h	0.005 ($\pm$ 0.002)	0.022 ($\pm$ 0.003)	< 0.001[***]
	C. septempunctata	a	0.746 ($\pm$ 0.071)	0.664 ($\pm$ 0.042)	0.323
		h	0.016 ($\pm$0.002)	0.002 ($\pm$0.001)	<0.001[***]
	H. axyridis	a	0.762 ($\pm$ 0.056)	1.005 ($\pm$ 0.071)	0.007[**]
		h	0.006 ($\pm$ 0.001)	0.010 ($\pm$ 0.001)	< 0.001[***]
Larvae	*A. bipunctata*	a	0.333 ($\pm$ 0.057)	0.192 ($\pm$ 0.023)	0.032[*]
		h	0.054 ($\pm$ 0.010)	0.003 ($\pm$ 0.009)	< 0.001[***]
	H. axyridis	a	1.366 ($\pm$ 0.131)	0.879 ($\pm$ 0.076)	0.001[**]
		h	0.017 ($\pm$ 0.002)	0.004 ($\pm$ 0.002)	< 0.001[***]

septempunctata showed a shortening of handling times whereas *A. bipunctata* and *H. axyridis* both showed increases (Table 2.6). Larval treatments of both species showed shorter handling times when exposed to the pathogen (Table 2.6). It is important to note that the predatory coefficients (a, h, and maximum feeding rates) are intrinsically linked and combined result in the overall predatory behaviour exhibited by the species. For example, an increase in a predators handling time will result in a decreased maximum feeding rate.

Pairwise comparisons of attack rate (a) and handling time (h) showed significant differences between species treatment combinations (Table 2.5). Species differed significantly with respect to their predatory behaviour with 36 of 42 pairwise comparisons between handling time and attack rate coefficients being significantly different ($P < 0.001$) and in each comparison at least one of the predatory statistics (a and h) was significantly different (Table 2.5).

2.5   Discussion

Consistent with our hypothesis, we have shown that a widespread invasive non-native predator (*Harmonia axyridis*) consumes more prey than two native species (*Adalia bipunctata* and *Coccinella septempunctata*), as adults and larvae. *H. axyridis*' higher consumption rate was linked with better forage ability including higher attack rate and shorter handling time coefficients. Typical efficient predatory behaviour would consist of high rates of attack on prey and short periods of time spent handling and consuming prey. We suggest this *per-capita* difference in predatory consumption and forage ability between native and INNS could shed light on the ecological impacts of *H. axyridis* that have been documented throughout its invaded range. Specifically, these attributes could give *H. axyridis* an ecological advantage over native competitors (e.g. other Coccinellidae) and prey (e.g. aphid) species. Previous literature has suggested that the invasive *H. axyridis* is an efficient predator of aphid pests (Xue *et al.* 2009; Abbott *et al.* 2014; Seko *et al.* 2014) and this is likely to have facilitated the species' spread through multiple releases as a biological control agent. We show that *H. axyridis* is indeed an

effective predator, in keeping with our initial hypothesis and previous literature.

Whilst finding that *H. axyridis* consumed more prey than both native species was in keeping with our initial hypothesis, the degree to which the species differed, while linking well with the wider literature, was somewhat unexpected. We suggest that the predatory behaviour of *H. axyridis* could, at least in part, be predicted by their size. Invasive *H. axyridis* and native *C. septempunctata* are both large ladybirds and were found to be generally more similar in their predatory behaviours than the smaller native *A. bipunctata*. The similarity between *H. axyridis* and *C. septempunctata* is in accordance with previous literature findings (Abbott *et al.* 2014; Xue *et al.* 2009). Features that facilitate more efficient predatory behaviour are known to scale with predator size. For example, size commonly correlates with greater predator speed, which can increase predator attack rates, and less time spent consuming and digesting prey, which will reduce a predators handling time (Woodward & Warren 2007; Gergs & Ratte 2009). A similar relationship was also noted in larval treatments, with *H. axyridis* and *A. bipunctata* predatory behaviours being significantly different. Metabolic theory, as discussed by Brown *et al.* (2004), suggests that the energetic demands of an organism is correlated with the organism's mass. While this is in keeping with our findings, no further investigation of the relationship between consumption rate and predator mass was undertaken as; 1) while *H. axyridis* and *C. septempunctata* do overlap with respect to their masses, neither overlap with the masses of *A. bipunctata* and this would result in complete separation in statistical models and 2) the functional response fitting procedures are currently unable to account for non-integer consumption rates, that would result from predator mass standardised predation rates.

For the first time, to our knowledge, we also show the impact of a widespread pathogen on the predatory ability of ladybirds and, specifically, how this impacts the relative predatory abilities of native and invasive ladybirds. *B. bassiana* infection resulted in significant changes in predator forage ability. Invasive non-native *H. axyridis* and native *A. bipunctata* adults showed an increase in attack rate and handling time coefficients when exposed to the pathogen. While an increase in the attack rate coefficient would suggest an increase in prey consumption, the increase in the handling

time coefficient would suggest the opposite with the predator spending more time handling and consuming prey individuals. Visual inspection of the functional response relationship shows these coefficients result in a lower overall functional response curve. Contrary to our expectations, native *C. septempunctata* adults showed reduced prey handling times when exposed to the pathogen and no significant change in attack rates. Both larval treatments (*H. axyridis* and *A. bipunctata*) demonstrated significantly reduced attack rate and handling time coefficients when subject to the pathogen treatment. We suggest that considering the evolutionary histories of the species could further inform the differences between species in their response to the pathogen. Native ladybird species are likely to have an evolutionary history with *B. bassiana* and are more likely to have behavioural or chemical defences while INNS are more likely to be naïve to the novel pathogen (Hatcher & Dunn 2011). For example *B. bassiana* is a significant cause of *C. septempunctata* overwintering mortality and, while unable to demonstrate such avoidance behaviours within this experiment, *C. septempunctata* are known to avoid *B. bassiana* infected cadavers in the field (Ormond *et al.* 2011). It is possible that other ladybird species also encounter *B. bassiana*, as the pathogen has been isolated from other habitats such as hedgerows, and could also show adaptations to avoid infection (Meyling & Eilenberg 2007). However, it should also be noted that *H. axyridis* is known to have advanced chemical defences that could also protect individuals against *B. bassiana* infection (Röhrich *et al.* 2012; Schmidtberg *et al.* 2013) and may have contributed to the species' success.

We have shown that *B. bassiana* infection changes ladybird predatory behaviour and results in different predatory functional response curves. While low prey density treatments are important to effectively differentiate between type II and III functional responses, many aphid species are known to aggregate and reach high densities on host plants (van Emden & Harrington 2017). Our finding that the predatory behaviour between infected and uninfected individuals of the same species, is greatest at our higher prey densities could be as a result of the predatory behaviour at the lower prey densities or could be indicative of the likely predatory behaviour present at higher prey densities in the field. Optimal foraging theory suggests species are likely to aggregate to areas of

high resource availability (MacArthur & Pianka 1966; Charnov 1976). This would likely result in *H. axyridis* and the native ladybird predators aggregating around high densities of aphid prey, as many aphid species are known to reach high densities. This could result in our measures of predation at our highest prey densities being more representative than those at the lower density treatments.

B. bassiana pathogenic infection resulted in different results between adult and larval life-stages. It could be possible that *B. bassiana* infection, through its hyphal growth throughout the host, would impose physiological damage that would impede the predatory ability of individuals, resulting in reduced prey consumption, attack rates and an increase in handling times. However, predatory behaviour may either increase, as the host attempts to mitigate the costs of infection (for example Dick *et al.* 2010 and Bunke *et al.* 2015), or decrease as the costs of infection rise from either the damage or increased metabolic demand associated with the infection process (for example, Haddaway *et al.* 2012; Toscano *et al.* 2014; MacNeil *et al.* 2003). We suggest that the physiological damage and increased metabolic demand resulted in a decreased ability to consume prey in adult treatments whereas the mechanism driving the observed changes in infected larvae is less clear. We suggest that desiccation or other fungal, viral or bacterial infections could be a contributing factor. Upon infection, *B. bassiana* conidia germinate and penetrate the hosts outer integument before commencing extensive hyphal growth throughout the host's internal cavity. Vey & Jacques (1977) and Poprawski *et al.* (1999) suggest the repeated penetration of the outer cuticle or soft body of the host can result in an increased risk of desiccation and subsequent infections which would result in additional costs to the host and subsequently affect the host's predatory behaviours. We suggest the larvae are responding to these increased costs, specifically desiccation, by increasing their consumption rates however, further investigation would be required to explicitly establish this relationship.

In light of our findings, we propose that the invasion of *H. axyridis* is likely to have imposed an increased level of novel predatory pressure on prey species (e.g. aphids) and indirect effects on competitors (for example other Coccinellidae). *H. axyridis* is known to be highly abundant and commonly dominates invaded Coccinellidae assemblages (Brown

& Roy 2017), it is likely therefore that the *per-capita* differences identified here will scale up and result in larger community impacts in field populations as *H. axyridis'* numerical response to prey density is taken into account (see Dick *et al.* 2017b). While not quantified here, the numerical response, a measure of how predator abundance changes with respect to changes in prey abundance, is likely to impact native prey in a similar way the the predators *per capita* functional response. Accounting for the demographics of wild populations is essential to further our understand of any potential impacts posed by a predator and overcome limitations within functional response studies. For example, functional response studies, do not allow for prey switching which could otherwise be expected in natural communities. However, it has been suggested that generalist predators, such as Coccinellidae are likely to show less pronounced prey switching than predators foraging on a fewer prey (Van Leeuwen *et al.* 2013). Additionally, measures of predatory behaviour attained through functional response studies rely on those predator individuals being representative of the wider predator community. The Coccinellid predators used as part of this study were all of similar ages (fourth instar larvae or recently emerged adults) however, it could be expected that unhealthy or otherwise suboptimal individuals, including those becoming increasingly moribund, could show lower rates of predatory behaviour. It is likely that *H. axyridis* will impact some species more than others, for example Roy *et al.* (2012) attribute the decline of native *A. bipunctata* (44% in Britain and 30% in Belgium) to the arrival and subsequent spread of *H. axyridis*. In contrast, *C. septempunctata* populations showed no significant change. Kenis *et al.* (2017) use a collection of risk measures (for example, the likelihood of encountering *H. axyridis*) to predict the native species at most risk from *H. axyridis*. Native *A. bipunctata* were identified as being at 'very high' risk while native *C. septempunctata* were identified as being at 'medium risk'. We propose that our findings suggest that higher predatory ability of *H. axyridis* may be one of the mechanisms underpinning the findings of Kenis *et al.* (2017) and Roy *et al.* (2012). We also suggest the increased predatory behaviour exhibited by *H. axyridis* could have facilitated the species' initial spread and success throughout its invaded range. Our second key finding was that pathogen infection impacted the predatory behaviour of ladybirds in a species

and life-stage specific way. Despite being known to mediate invasion success and impact through their lethal and sub-lethal effects (Dunn *et al.* 2012; Strauss *et al.* 2012), the effects of parasites and pathogens are rarely accounted for and current literature shows that their impacts can vary. For example, invasive *Gammarus pulex* harbouring acanthocephalan infection show increased intake of prey (Dick *et al.* 2010). Conversely, other infected species have also been shown to have significantly reduced consumption rates (Toscano *et al.* 2014; Wright *et al.* 2006). In keeping with the enemy release hypothesis, *H. axyridis* is known to be resistant to some native natural enemies (Vilcinskas *et al.* 2013) and is a poor host to others, such as the parasitic wasp *Dinocampus coccinellae* (Berkvens *et al.* 2010). While instances of infection by native parasites and pathogens may be low in *H. axyridis*, understanding how infection can modify the key functional behaviours of native and INNS is key to furthering our understanding of the effects of infection on the success and impacts of invasion events (Brook *et al.* 2008; Strayer 2010). Previous literature has shown a lower lethal effect of parasitic infection in the invasive *H. axyridis* (Cottrell & Shapiro-Ilan 2003; Roy *et al.* 2008b). Here we provided evidence that pathogenic infection affects a key functional trait, predation, and impacts *H. axyridis* to a lesser degree than two native species.

We have provided evidence that the invasive non-native *H. axyridis* displays significantly more efficient predatory behaviour than two native predators in both adult and larval life-stages. Pathogenic infection significantly changed the foraging ability of ladybird predators in a species and life-stage specific way but resulted in no measurable change in overall prey consumption. This could be due to the conflicting pressures of increased metabolic demand and physiological damage sustained through the infection process. We suggest the impacts of *H. axyridis* are at least partially explained by the more efficient predatory behaviour detailed here.

Chapter 3

Invasive non-native predator is correlated with changes in native aphid populations

3.1 Abstract

Despite species invasions being a major environmental pressure, commonly our understanding as to the rate of spread and the impacts throughout the invaded range are limited by the poor availability of spatio-temporal data. The arrival and subsequent spread of the highly invasive generalist predator *Harmonia axyridis* (the harlequin ladybird) has been documented as part of a long running biological recording scheme, the UK Ladybird Survey, therefore providing a valuable opportunity to investigate such questions. Despite being introduced throughout the world as a biological control agent against aphid pest species, little research has investigated the impact of *H. axyridis* on native aphid populations after its initial release or in invaded regions. We aimed to understand how the arrival of *H. axyridis* has impacted the abundance of 14 common UK aphid species. To do this we quantified the impact of *H. axyridis* on 14 common native aphid prey species throughout England by using long-term datasets collected as part of the UK Ladybird Survey and the Rothamsted Insect Survey. We compared annual changes in aphid population abundance for a total of nine sites before and after the initial arrival of *H. axyridis* into the UK. We show that the arrival of *H. axyridis* is associated with declines of five aphid species, increases in four, and no change in the remaining five. We suggest that these changes are, at least partially, explained by expected habitat overlap with *H. axyridis*, which is likely to result in increased predatory pressure experienced by the overlapping aphid prey species. As far as we are aware, this is the first study to quantify the impacts of *H. axyridis* on native prey species using field collected data.

3.2   Introduction

Invasive non-native species are key drivers of environmental change and have been linked with biodiversity losses and species extinctions (Bellard *et al.* 2016; Kenis *et al.* 2009). Native species face increasing pressures, which are linked with ever increasing species extinctions and population declines (Bellard *et al.* 2016; Simberloff *et al.* 2013). Invasive non-native species often impose impacts on native communities through key functional behaviours, such as predation, which can adversely affect native species (Beggs *et al.* 2011; Doherty *et al.* 2016; Ocasio-Torres *et al.* 2015; Snyder & Evans 2006).

Historically, invasive non-native predators have been shown to drive species extinctions. For example, feral cats are considered responsible for the extinction of 22 Australian mammal species (Woinarski *et al.* 2015). Currently, 20 of the 26 invasive alien animal species of European Union concern are predators (European Comission 2017). Insect predators are of particular concern due to their high dispersal rate, small size and rapid reproductive ability with respect to their initial colonisation but also their subsequent spread and therefore impact on native species and the wider community (Snyder & Evans 2006). As the rate of species invasions continues to rise, with no sign of slowing (Seebens *et al.* 2017), our understanding of the potential impacts of invasive non-native species is becoming even more important.

Harmonia axyridis (harlequin ladybird) is a highly invasive insect and a generalist predator, with its consumption of a wide variety of aphid species resulting it its application as a biological control agent (Brown *et al.* 2011c; Roy & Brown 2015). Native to central and eastern Asia, the species was released throughout much of Europe and the USA as a biological control agent (Majerus *et al.* 2006). *H. axyridis* then continued to expand its non-native range, inadvertently facilitated by human activity in at least some cases (Ribbands *et al.* 2009). In the UK, *H. axyridis* first established in 2004 and has since spread to occupy much of the UK and dominate coccinellid communities (Brown & Roy 2017). The arrival and subsequent spread of *H. axyridis* has been linked with declines in native Coccinellidae through competition and intra-guild predation (Bahlai *et al.* 2014; Roy *et al.* 2012). For example, Roy *et al.* (2012) found that the arrival of *H.*

axyridis was linked with 44% and 30% declines of UK and Belgian populations of native *Adalia bipunctata* respectively. In addition to other Coccinellidae, *H. axyridis* has been shown to consume the immature stages of *Danaus plexippus* (the Monarch butterfly) (Koch *et al.* 2003) and other aphidophagous predators, such as *Episyrphus balteatus* (Ingels *et al.* 2015). While efforts have been made to quantify the predatory ability of *H. axyridis*, these are commonly with respect to biological control applications (Lee & Kang 2004; Seko *et al.* 2014) and nearly all of these studies consists of laboratory, often *per-capita*, microcosm studies. Our understanding as to the in-field predatory impacts of *H. axyridis* on aphid species, the very group of species they were initially released to control, remains poorly understood (Roy & Brown 2015; Roy *et al.* 2016a).

Britain is home to at least 600 aphid species, many of which are considered pests species in agricultural systems, causing damage directly through feeding activities or indirectly through the transmission of disease to the host plant (van Emden & Harrington 2017). Aphids are able to show rapid population growth when experiencing optimal conditions and while associating with a primary host plant species, many species have the ability to switch host plants either with season or under suboptimal conditions (van Emden & Harrington 2017). These features of aphid populations, in addition to their phenology, results in the location and density of aphids being difficult to predict and liable to change throughout and between years (van Emden & Harrington 2017; Rothamstead Research 2015). It is likely that predators will track these changes in aphid abundance, especially considering UK ladybirds and aphids are most active during the summer months. Unfortunately, data relating to the spatial and temporal abundance peaks for aphid species is often absent or unreliable and we therefore make no effort to include this data as art of this analysis.

We aimed to quantify the realised field impact of *H. axyridis* on native aphid prey populations in England, UK. We compared annual aphid populations at suction-trap sites across England, for 14 common and widespread native aphid species, before and after the establishment of *H. axyridis*. We hypothesised that the colonisation of an area by *H. axyridis* would result in lower aphid abundance, but that this would vary between habitat types, specifically how these habitat types overlap with those used by *H. axyridis*.

Broadleaf trees and the perennial herb, *Urtica dioica*, are primary habitats for *H. axyridis* (Roy *et al.* 2011). Conversely, while *H. axyridis* inhabits agricultural crops, another primary habitat, it rarely dominates the community (Roy *et al.* 2016a). *H. axyridis* is known to inhabit coniferous woodland in its native range, however evidence suggests that coniferous woodlands in the UK are secondary habitats (Brown *et al.* 2011b; McClure 1986; Purse *et al.* 2014). Firstly, we hypothesise that aphid species inhabiting primary *H. axyridis* habitats (broadleaf trees; *Drepanosiphum platanoidis*, *Periphyllus testudinaceus*, *Eucallipterus tiliae*, and *U. dioica*; *Microlophium carnosum*) will experience the greatest predatory pressure from *H. axyridis* and therefore show the largest declines in abundance. We further hypothesise that aphids occupying agricultural crops (agricultural crops; *Sitobion avenae*, *Metopolophium dirhodum*, *Rhopalosiphum padi*, *Rhopalosiphum oxyacanthae*, *Aphis fabae*, *Acyrthosiphon pisum*, *Brevicoryne brassicae*, *Macrosiphum euphorbiae*, *Myzus persicae*) will also show declines in aphid abundance due to the predatory pressure imposed by *H. axyridis*, albeit to a lesser degree than aphids in other primary habitats. Lastly, we hypothesise that aphids inhabiting secondary *H. axyridis* habitats (coniferous woodlands; *Elatobium abietinum*) will experience less predatory pressure than those in other habitats. While we also suggest that aphids in agricultural and secondary habitats could further be impacted by *H. axyridis* indirectly by the displacement of native predators, we expect these decreases to be less than those of aphid species inhabiting primary *H. axyridis* habitats.

Through this work we aimed to question how the arrival and subsequent spread of the globally invasive non-native *H axyridis* has impacted 14 widespread and previously common aphid species in England and Wales. As these species cover a broad range of habitat types (e.g. trees and agricultural crops) we hoped to further our understanding as to the impacts *H axyridis* has had on native aphids across habitats, specifically between those habitats used heavily by *H axyridis* and those considered less favourable. To date, we know very little about how *H axyridis* has impacted UK aphid species despite *H axyridis* being introduced around the world to control aphids considered as pest.

3.3   Materials and methods

Distribution records of *H. axyridis* was obtained from the UK Ladybird Survey (2018) which uses volunteers to report sightings of UK Coccinellidae. Individual records are geo-referenced to a 1 km resolution and verified by experts from photos. The first record of *H. axyridis* in the UK was in 2004 and since then the UK Ladybird Survey has collected more than 161,000 records, with over 31,000 of these being *H. axyridis*. We calculated the annual distribution of *H. axyridis* by extracting the date and locations for records of *H. axyridis* between the species' arrival in 2004 and 2014.

Aphid abundance data was collected as part of the Rothamsted Insect Survey using a network of 12.2 m suction-traps (Bell *et al.* 2015) located across England (Figure 3.1). Aphids were collected and identified daily during the aphid flying season (April-November) and weekly at other times of the year (Bell *et al.* 2015). Due to our records of *H. axyridis* being collected in an non-stratified manner by citizen scientists, our records of *H. axyridis* presence/pseudo-absence are unreliable over shorter temporal scales. We therefore used annual aphid population counts for a total of 14 common and widespread aphid species spanning a variety of habitat types. Our 14 focal aphid species comprised of; four tree species (*Drepanosiphum platanoidis*, *Periphyllus testudinaceus*, *Eucallipterus tiliae*, and *Elatobium abietinum*), one perennial herb species (*Microlophium carnosum*), and nine agricultural crop species, spanning grain crops (*Sitobion avenae*, *Metopolophium dirhodum*, *Rhopalosiphum padi*, and *Rhopalosiphum oxyacanthae*), legumes (*Aphis fabae* and *Acyrthosiphon pisum*), and other crops including brassicas, potatoes, and beets (*Brevicoryne brassicae*, *Macrosiphum euphorbiae*, and *Myzus persicae*).

The first record of *H. axyridis* within a 10 km^2 grid square around each of the suction-trap sites was calculated. I response to potential 'recorder fatigue', whereby recorders may slow or cease entirely in their reporting of species that are seen as being increasingly common, it was assumed that this was the date of local establishment and that *H. axyridis* persisted in this location in subsequent years. To account for variation in aphid abundance due to climatic variables (Harrington *et al.* 2007, for example), the
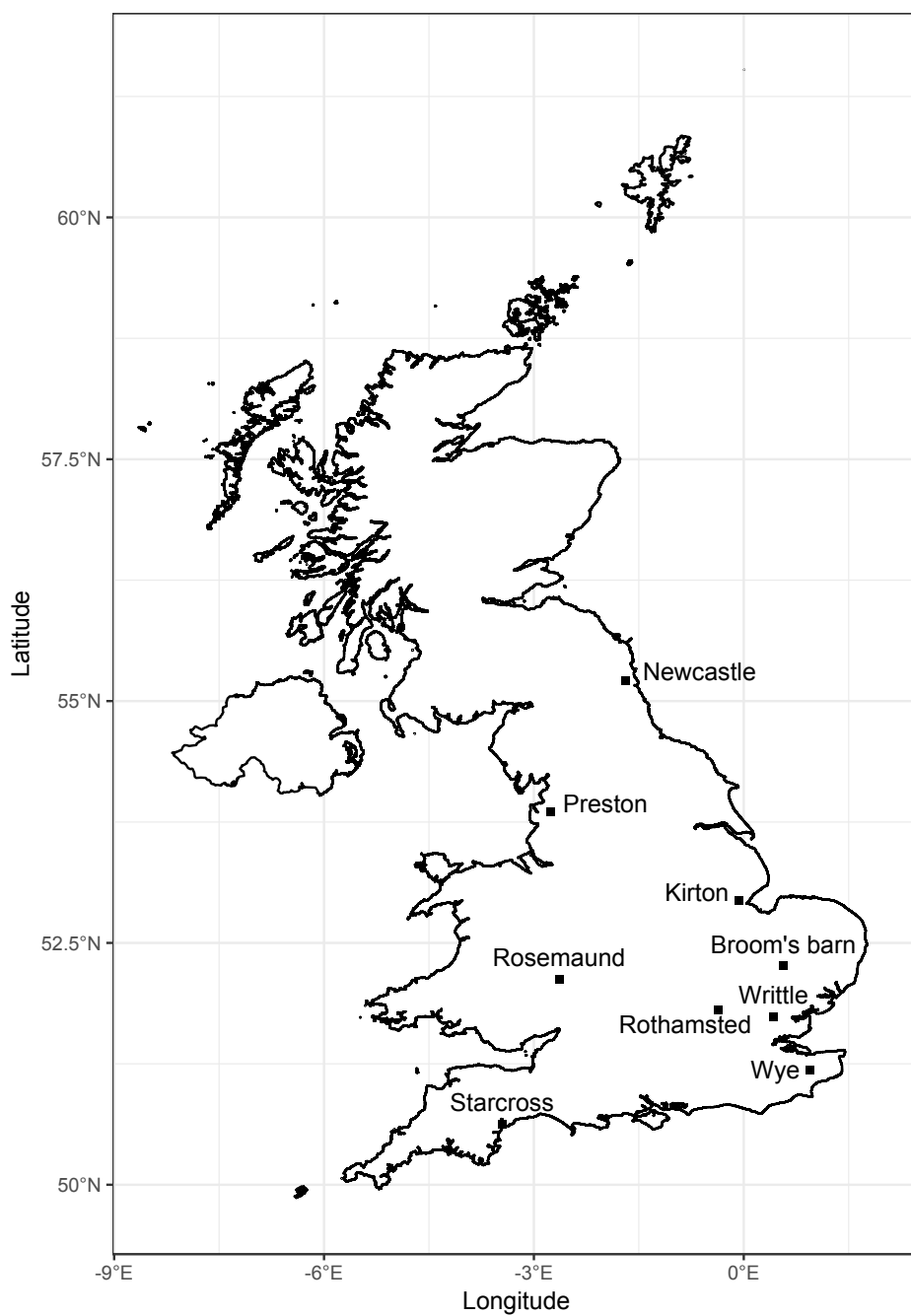
Figure 3.1: Locations of the nine 12.2 m aphid suction-traps, operated by the Rothamsted Insect Survey (Bell *et al.* 2015), used as part of this study which are spread across England.

mean annual temperature (°C) and precipitation (mm) climatic variables were extracted from UKCP09 (Met Office *et al.* 2017) for each of the aphid suction-traps.

3.3.1   Statistics

All statistical analyses were undertaken in R version 3.3.2 and RStudio version 1.0.136 (R Core Team 2016; RStudio 2016). We used the the MASS package for the negative binomial generalised linear models (Venables & Ripley 2002).

We created five candidate models containing combinations of a binary *H. axyridis* term, denoting the presence/pseudo-absence of *H. axyridis* in the 10 km^2 grid square of the suction-trap, the suction-trap ID, the year of sampling, and mean annual temperature (°C) and precipitation (mm) terms. All models contained a year term and the null model contained only this year term and the mean annual aphid population abundance. Candidate models were compared for each of the aphid species via second-order Akaike information criterion (AICc), with the 'best fit' model having the lowest AICc score. Notable candidate models were defined as being within two AICc scores of the 'best fit' model. All models were parametrised in terms of annual growth rates, rather than actual abundance measures, as discussed by Freeman & Newson (2008).

3.4   Results

The year of local colonisation by *H. axyridis* at the suction-trap locations varied from 2004 to 2012 and the mean (and median) year of local colonisation was 2006. Visual inspection of local colonisation at the suction-trap sites suggested that, overall, the year of colonisation in the suction-trap grid square was consistent with the first records from the adjacent grid squares (Figure 3.2). Annual aphid species counts varied between 0 and 39,310 at the suction-trap locations.
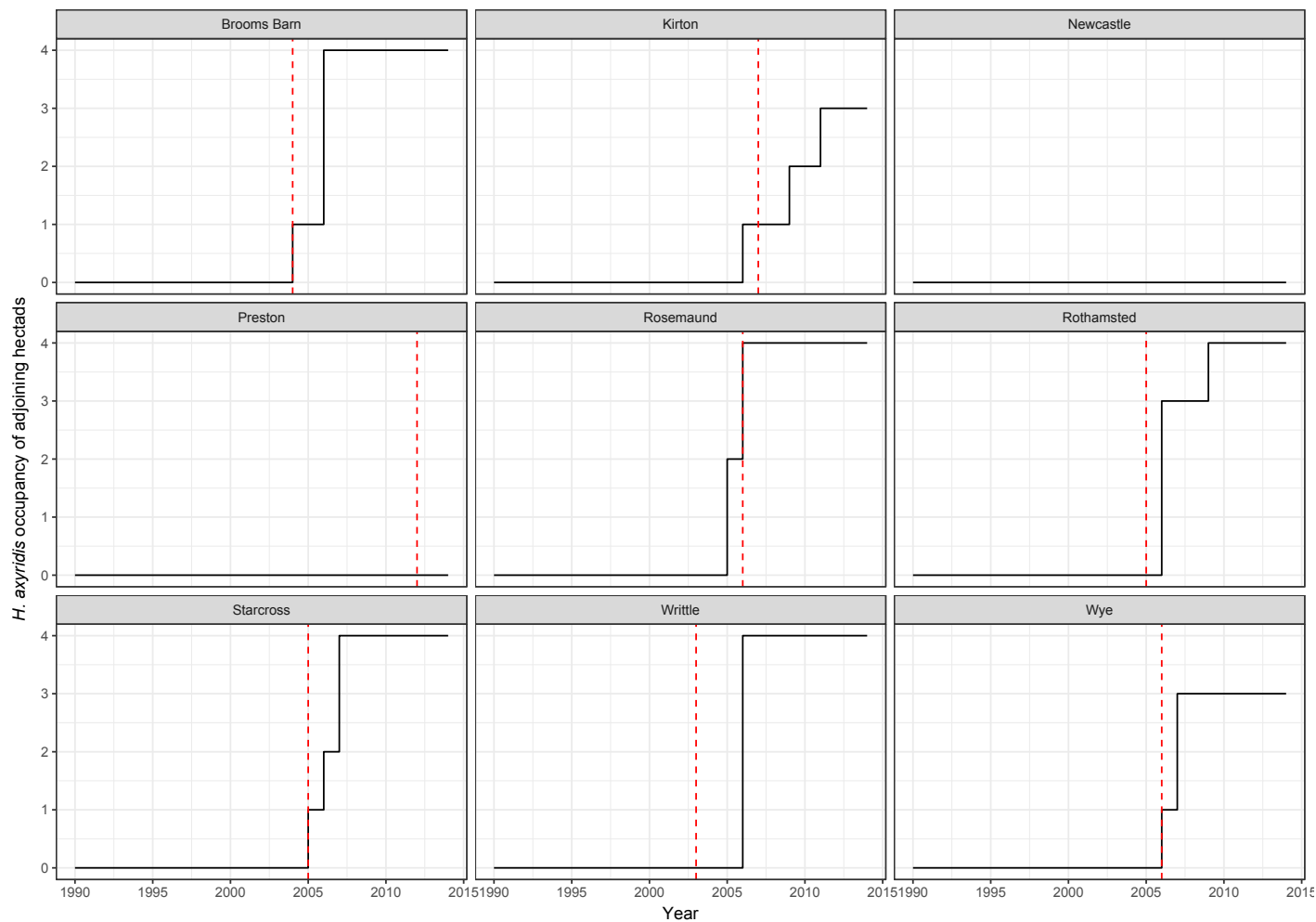
Figure 3.2: Local colonisation of *H. axyridis* at each of the suction-trap locations and the adjacent 10 km^2 grid squares. The first records for the suction-trap grid square is denoted by the vertical dashed red line and records for the four adjacent grid squares is shown as a black step line. Overall, the plot shows that the first records of *H. axyridis* at the suction-trap sites is in accordance with the adjacent grid squares. There were no records of *H. axyridis* in the Newcastle suction-trap hectad or in the adjoining hectads. Similarly, while *H. axyridis* was first recorded in the Preston suction-trap hectad in 2012 it was not recorded in any of the adjoining hectads.

Including the *H. axyridis* term significantly improved the null model for all species other than *E. tiliae*, *M. carnosum* and *M. euphorbiae* (Table 3.2). Including the suction-trap location significantly improved the null model for all aphid species (Table 3.2). The 'best fit' models for *A. fabae*, *A. pisum*, *M. dirhodum*, *M. persicae*, and *S. avenae*, contained only the suction-trap location term which suggests that after accounting for suction-trap location the arrival of *H. axyridis* didn't affect their annual population abundance (Table 3.2). 'Best fit' models for *D. platanoidis*, *M. carnosum*, *M. euphorbiae*, and *P. testudinaceus* all contain *H. axyridis*, suction-trap location and sampling year terms (Table 3.2). 'Best fit' models for the remaining aphid species (*B. brassicae*, *E. abietinum*, *E. tiliae*, *R. oxyacanthae*, and *R. padi*) contained the same models terms (*H. axyridis*, suction-trap location and sampling year), with the addition of two climatic variable terms describing mean annual temperature and precipitation (Table 3.2). These 'best fit' models suggested that increased mean annual precipitation was correlated with increases in *B. brassicae*, *E. tiliae*, *R. oxyacanthae*, and *R. padi* aphid abundances (Table 3.3). Conversely, increased annual precipitation was correlated with a decrease in aphid abundance for *E. abietinum* (Table 3.3). It should be noted that while deemed significant, changes in aphid abundance correlated with precipitation were generally small (Table 3.3). Higher mean annual temperatures were correlated with increased aphid abundance for both *B. brassicae* and *E. abietinum* while, conversely, increased mean annual temperatures were correlated with decreased abundance values for *E. tiliae*, *R. oxyacanthae*, and *R. padi*. The 'best fit' models suggest that *H. axyridis* was associated with declines in aphid population abundance for *B. brassicae*, *E. tiliae*, *M. euphorbiae*, *M. carnosum*, and *R. oxyacanthae*. Conversely, *D. platanoidis*, *E. abietinum*, *P. testudinaceus*, and *R. padi* all show increases in annual population abundance following the arrival of *H. axyridis* (Figure 3.3).

Table 3.1: Coefficients and standard errors for the 'best fit' models for each of the 14 aphid species. Only terms included in the 'best fit' model are represented in the table. All models used a negative binomial error structure. Asterisks denote significance of P values; *** $= P < 0.001$, ** $= P < 0.01$ and * $= P < 0.05$.

Model term		A. pisum	A. fabae	B. brassicae	D. platanoidis	E. abietinum	E. tiliae	M. euphorbiae	M. dirhodum	M. carnosum	M. persicae	P. testudinaceus	R. oxyacanthae	R. padi	S. avenae
	(Intercept)	5.258 (0.235)***	4.915 (0.318)***	2.045 (3.983)	7.447 (0.270)***	-6.215 (2.899)*	9.941 (3.449)**	4.552 (0.220)***	7.076 (0.258)***	5.129 (0.343)***	5.953 (0.277)***	4.448 (0.279)***	5.518 (2.620)*	13.564 (2.352)***	5.599 (0.234)***
Site:	Rosemaund	-1.394 (0.176)***	-0.277 (0.237)	-0.477 (0.262)	0.617 (0.203)**	0.477 (0.187)*	-0.189 (0.216)	-0.397 (0.171)*	-1.000 (0.194)***	-0.657 (0.265)*	-1.522 (0.208)***	0.491 (0.217)*	-0.136 (0.172)	0.126 (0.154)	-0.452 (0.174)*
	Kirton	0.493 (0.174)**	-0.310 (0.237)	0.121 (0.291)	-1.331 (0.204)***	-0.470 (0.210)*	-0.089 (0.229)	0.213 (0.166)	-0.196 (0.193)	-0.508 (0.262)	-0.493 (0.207)*	-0.025 (0.201)	0.959 (0.198)***	0.132 (0.168)	0.411 (0.174)*
	Newcastle	-2.559 (0.180)***	-1.093 (0.238)***	-0.581 (0.276)*	-0.686 (0.204)***	2.918 (0.365)***	-2.452 (0.437)***	0.249 (0.166)	-1.314 (0.194)***	-1.922 (0.284)***	-2.561 (0.210)***	-1.561 (0.205)***	0.407 (0.188)*	-0.152 (0.296)	-1.170 (0.175)***
	Preston	-1.935 (0.177)***	-1.303 (0.239)***	-3.071 (0.511)***	-0.292 (0.219)	2.184 (0.344)***	-0.747 (0.308)	-0.786 (0.177)***	-1.305 (0.194)***	-0.219 (0.272)	-1.959 (0.209)***	-1.885 (0.218)***	2.028 (0.330)***	1.025 (0.282)***	-1.164 (0.175)***
	Rothamsted	-0.580 (0.174)***	0.013 (0.237)	-2.788 (0.483)***	1.408 (0.213)***	0.640 (0.208)*	1.475 (0.223)***	-0.836 (0.169)***	-1.162 (0.194)***	-1.121 (0.263)***	-0.675 (0.207)***	-0.158 (0.200)	0.024 (0.190)	-0.714 (0.169)***	-0.286 (0.174)
	Starcross	-0.658 (0.175)***	0.151 (0.237)	-0.293 (0.278)	-0.505 (0.203)*	-0.069 (0.253)	-1.464 (0.310)***	-0.442 (0.167)**	-0.911 (0.194)***	-0.749 (0.261)**	-1.571 (0.208)***	-2.994 (0.214)***	0.703 (0.231)**	0.030 (0.207)	-0.873 (0.175)***
	Writtle	0.014 (0.174)	-0.232 (0.237)	-0.311 (0.337)	-2.334 (0.203)***	-0.321 (0.189)	1.290 (0.200)***	-0.148 (0.166)	-0.641 (0.194)***	-0.054 (0.259)	-0.131 (0.206)	0.127 (0.199)	-0.103 (0.172)	0.047 (0.153)	0.026 (0.174)
	Wye	-0.749 (0.175)***	-0.885 (0.238)***	0.735 (0.248)**	-1.026 (0.202)***	0.248 (0.439)	0.249 (0.512)	-0.872 (0.169)***	-1.316 (0.194)***	-1.156 (0.420)**	-0.721 (0.207)***	-0.988 (0.201)***	-0.158 (0.194)	0.121 (0.356)	-0.414 (0.174)*
Year:	1991	0.981 (0.287)***	1.309 (0.388)***	-2.804 (0.612)***	-0.530 (0.330)	1.204 (0.325)***	-1.047 (0.368)**	0.759 (0.265)**	0.769 (0.315)*	0.453 (0.421)	-0.580 (0.341)	0.305 (0.344)	1.202 (0.397)**	1.244 (0.263)***	1.899 (0.286)***
	1992	0.792 (0.284)**	-0.867 (0.387)*	2.482 (0.448)***	-0.291 (0.330)	0.599 (0.317)	-0.576 (0.385)	-0.093 (0.264)	1.518 (0.315)***	2.290 (0.416)***	1.452 (0.340)***	-0.084 (0.342)	-0.031 (0.293)	-1.601 (0.263)***	1.571 (0.284)***
	1993	-1.727 (0.286)***	0.569 (0.388)	-1.151 (0.433)	0.406 (0.330)	-1.741 (0.360)***	0.260 (0.449)	-1.489 (0.270)***	-2.430 (0.315)***	-0.968 (0.414)*	-1.615 (0.340)***	0.215 (0.341)	0.724 (0.292)*	1.326 (0.295)***	-1.353 (0.284)***
	1994	1.631 (0.286)***	0.755 (0.387)	1.687 (0.489)***	-0.427 (0.330)	1.238 (0.316)***	1.207 (0.388)**	1.520 (0.270)***	1.466 (0.315)***	1.450 (0.414)***	1.596 (0.340)***	-0.120 (0.340)	-0.088 (0.328)	-1.250 (0.261)***	1.094 (0.284)***
	1995	0.488 (0.283)	-0.959 (0.387)*	1.675 (0.422)***	1.069 (0.330)**	-2.493 (0.470)***	-0.771 (0.537)	-0.539 (0.265)*	-1.921 (0.316)***	-3.971 (0.423)***	0.814 (0.337)*	0.761 (0.337)*	-0.980 (0.291)***	0.177 (0.371)	-0.161 (0.284)
	1996	-0.149 (0.283)	0.440 (0.387)	-3.843 (0.629)***	-0.927 (0.330)**	2.653 (0.479)***	-0.553 (0.579)	0.963 (0.264)***	1.780 (0.316)***	0.017 (0.433)	-0.107 (0.336)	0.912 (0.340)**	-0.374 (0.414)	1.351 (0.378)***	0.818 (0.284)**
	1997	-0.939 (0.284)***	-0.911 (0.388)*	-0.943 (0.649)	-0.347 (0.331)	-0.972 (0.317)**	1.056 (0.409)*	-1.367 (0.266)***	0.845 (0.315)*	2.393 (0.424)***	-2.283 (0.340)***	0.452 (0.332)	0.656 (0.422)	-0.677 (0.263)**	-1.714 (0.284)***
	1998	-1.095 (0.287)***	0.305 (0.388)	2.184 (0.442)***	0.327 (0.331)	-0.285 (0.317)	0.158 (0.367)	-0.892 (0.276)**	-0.271 (0.318)	-0.953 (0.416)*	-0.427 (0.345)	-2.211 (0.349)***	0.726 (0.293)*	-0.590 (0.261)*	-1.669 (0.285)***
	1999	0.232 (0.289)	0.217 (0.388)	-0.088 (0.427)	-0.969 (0.331)**	1.429 (0.332)***	-0.328 (0.388)	0.342 (0.279)	1.727 (0.319)***	-0.215 (0.418)	0.369 (0.345)	3.222 (0.347)***	-0.950 (0.291)***	-0.064 (0.275)	1.320 (0.285)***
	2000	-0.464 (0.289)	0.454 (0.387)	-1.311 (0.456)**	1.789 (0.331)***	-1.583 (0.314)***	-1.000 (0.379)**	-0.413 (0.280)	-0.874 (0.317)**	1.380 (0.417)***	-0.044 (0.343)	-4.951 (0.398)***	0.224 (0.307)	-0.063 (0.258)	-0.633 (0.285)*
	2001	1.192 (0.288)***	-1.535 (0.389)***	1.157 (0.426)**	-2.321 (0.332)***	-1.017 (0.329)**	1.503 (0.424)**	1.091 (0.276)***	0.189 (0.318)	-0.915 (0.416)*	1.081 (0.341)**	3.650 (0.401)***	-0.281 (0.287)	0.030 (0.286)	0.947 (0.285)***
	2002	-2.112 (0.294)***	1.207 (0.389)**	-1.704 (0.481)***	2.492 (0.332)***	-0.127 (0.317)	1.419 (0.355)***	-1.478 (0.281)***	-0.422 (0.318)	0.475 (0.417)	-1.449 (0.343)***	-1.504 (0.345)***	0.835 (0.318)*	1.007 (0.261)***	-1.229 (0.285)***
	2003	1.266 (0.295)***	0.676 (0.387)	1.761 (0.453)***	-1.163 (0.331)***	-0.960 (0.320)**	-0.490 (0.327)	0.808 (0.286)**	0.486 (0.318)	-0.410 (0.417)	1.026 (0.343)**	1.256 (0.346)***	-1.556 (0.305)***	-0.942 (0.254)***	0.810 (0.285)**
	2004	0.596 (0.287)*	0.159 (0.386)	-0.558 (0.428)	2.330 (0.331)***	0.712 (0.313)*	0.026 (0.351)	-0.136 (0.279)	1.895 (0.316)***	1.680 (0.419)***	-0.145 (0.340)	-1.861 (0.355)***	0.079 (0.293)	1.650 (0.264)***	0.916 (0.285)***
	2005	0.294 (0.285)	-1.549 (0.387)***	2.223 (0.415)***	-3.266 (0.334)***	0.095 (0.335)	-0.206 (0.345)	0.578 (0.279)*	-1.741 (0.316)***	-2.918 (0.423)***	0.752 (0.339)*	3.222 (0.348)***	0.736 (0.285)**	-1.119 (0.259)***	-0.430 (0.284)
	2006	0.655 (0.284)*	-0.009 (0.389)	-2.352 (0.433)***	2.714 (0.331)***	-0.098 (0.307)	-1.812 (0.305)***	0.264 (0.272)	0.781 (0.316)*	1.990 (0.427)***	-1.041 (0.340)***	1.020 (0.341)**	0.111 (0.295)	-0.508 (0.277)	-0.595 (0.285)*
	2007	-2.313 (0.289)***	0.083 (0.389)	1.054 (0.425)*	-1.506 (0.333)***	0.914 (0.434)*	1.779 (0.358)***	-0.711 (0.278)*	-2.362 (0.320)***	-4.484 (0.508)***	0.861 (0.340)*	0.643 (0.331)	1.309 (0.288)***	1.167 (0.253)***	-2.089 (0.287)***
	2008	-0.508 (0.299)	-0.365 (0.389)	-0.831 (0.456)	-0.900 (0.330)**	-1.461 (0.591)*	-1.779 (0.498)***	-0.402 (0.286)	0.776 (0.322)*	7.205 (0.507)***	-1.506 (0.343)***	0.484 (0.327)	-0.704 (0.308)*	-1.542 (0.355)***	0.608 (0.287)*
	2009	1.901 (0.294)***	-0.467 (0.391)	0.729 (0.415)	1.235 (0.330)***	1.685 (0.505)***	1.560 (0.700)*	1.219 (0.280)***	1.090 (0.318)***	-6.597 (0.469)***	2.005 (0.342)***	-2.074 (0.337)***	0.130 (0.282)	1.570 (0.483)**	0.884 (0.286)**
	2010	1.670 (0.284)***	0.808 (0.390)*	-0.870 (0.591)	1.674 (0.329)***	4.339 (1.690)*	-3.563 (2.070)	-0.523 (0.273)	1.710 (0.316)***	4.456 (0.469)***	-1.036 (0.339)**	0.599 (0.343)	-1.858 (0.396)***	-1.330 (0.416)***	0.814 (0.285)**
	2011	-1.705 (0.284)***	0.550 (0.388)	0.612 (0.814)	-1.718 (0.329)***			0.393 (0.273)	0.154 (0.315)	1.880 (0.428)***	1.250 (0.339)***	0.312 (0.335)	0.762 (0.539)	-4.210 (1.381)**	0.384 (0.285)
	2012	-2.127 (0.297)***	-2.006 (0.392)***	-1.759 (0.704)*	-2.218 (0.332)***			-1.550 (0.291)***	-3.740 (0.320)***		-1.007 (0.338)***		0.034 (0.464)		0.680 (0.286)*
	2013	1.455 (0.298)***	-0.026 (0.397)	3.591 (2.354)	1.446 (0.332)***			1.072 (0.294)***	0.943 (0.322)**		0.137 (0.339)		-1.036 (1.538)		
Precipitation				0.010 (0.009)		-0.009 (0.006)	0.025 (0.007)**						0.021 (0.006)***	0.001 (0.005)	
Temperature				0.353 (0.378)		0.934 (0.274)***	-0.832 (0.327)*						-0.222 (0.248)	-0.612 (0.223)	

Table 3.2: Second-order Akaike Information Criterion scores (AICc) and associated weights (Weight) for the five candidate models and the 14 aphid species. All models used a negative binomial error structure and the null model compared the change in annual aphid population abundance in relation only to the mean annual change in growth rate, denoted by 1. 'Best fit' models, with the lowest AICc score are highlighted in bold to aid interpretation.

Aphid species

Model	A. pisum		A. fabae		B. brassicae		D. platanoidis		E. abietinum		E. tiliae		M. euphorbiae	
	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight
~1	2797.428	0.000	2700.798	0.000	2523.702	0.000	3424.216	0.000	2497.941	0.000	1694.463	0.000	2061.152	0.000
~H. axyridis	2781.648	0.000	2692.796	0.000	2513.478	0.000	3419.683	0.000	2488.300	0.000	1695.958	0.000	2063.619	0.000
~Site	**2582.212**	**0.706**	**2667.181**	**0.611**	2378.219	0.290	3201.100	0.043	2328.693	0.024	1470.115	0.260	1982.884	0.196
~H. axyridis + Site	2584.335	0.244	2668.331	0.344	2377.855	0.347	**3195.948**	**0.563**	2326.101	0.088	1472.332	0.086	**1980.307**	**0.712**
~H. axyridis + Site + Precipitation + Temperature	2587.505	0.050	2672.421	0.045	**2377.768**	**0.363**	3196.665	0.394	**2321.472**	**0.888**	**1468.274**	**0.654**	1984.397	0.092

Aphid species

Model	M. dirhodum		M. carnosum		M. persicae		P. testudinaceus		R. oxyacanthae		R. padi		S. avenae	
	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight	AICc	Weight
~1	3036.359	0.000	2387.320	0.000	2700.554	0.000	2374.283	0.000	3583.825	0.000	3828.512	0.000	3451.098	0.000
~H. axyridis	3032.540	0.000	2389.784	0.000	2682.351	0.000	2348.578	0.000	3517.944	0.000	3811.873	0.000	3446.671	0.000
~Site	**2970.639**	**0.787**	2355.162	0.189	**2561.922**	**0.745**	2122.292	0.255	3162.374	0.040	3694.786	0.342	**3358.374**	**0.755**
~H. axyridis + Site	2973.402	0.198	**2352.627**	**0.672**	2564.325	0.224	**2120.361**	**0.669**	3164.693	0.012	3696.417	0.151	3361.106	0.193
~H. axyridis + Site + Precipitation + Temperature	2978.545	0.015	2355.789	0.138	2568.288	0.031	2124.693	0.077	**3156.019**	**0.948**	**3694.003**	**0.506**	3363.692	0.053

Table 3.3: Coefficients and standard errors for the 'best fit' models for each of the 14 aphid species. Only terms included in the 'best fit' model are represented in the table. All models used a negative binomial error structure. Asterisks denote significance of P values; $*** = P < 0.001$, $** = P < 0.01$ and $* = P < 0.05$. The year term is not reported here to facilitate interpretation.

Model term		A. pisum	A. fabae	B. brassicae	D. platanoidis	E. abietinum	E. tiliae	M. euphorbiae	M. dirhodum	M. carnosum	M. persicae	P. testudinaceus	R. oxyacanthae	R. padi	S. avenae
	(Intercept)	5.258 (0.235)***	4.915 (0.318)***	2.045 (3.983)	7.447 (0.270)***	−6.215 (2.899)*	9.941 (3.449)**	4.552 (0.220)***	7.076 (0.258)***	5.129 (0.343)***	5.953 (0.277)***	4.448 (0.279)***	5.518 (2.620)***	13.564 (2.352)***	5.599 (0.234)***
H. azyrdis															
Site:	Rosemaund	−1.394 (0.176)***	−0.277 (0.237)	−0.477 (0.262)	0.617 (0.203)**	0.477 (0.187)*	−0.189 (0.216)	−0.397 (0.171)*	−1.000 (0.194)***	−0.657 (0.265)*	−1.522 (0.208)***	0.491 (0.217)*	−0.136 (0.172)	0.126 (0.154)	−0.452 (0.174)**
	Kirton	0.493 (0.174)***	−0.310 (0.237)	0.121 (0.291)	−1.331 (0.204)***	1.454 (0.217)***	−1.571 (0.262)***	0.213 (0.166)	−0.196 (0.193)	−0.313 (0.261)	−0.493 (0.207)*	−0.025 (0.201)	0.959 (0.198)***	−0.220 (0.177)	0.411 (0.174)*
	Newcastle	−2.559 (0.180)***	−1.093 (0.238)***	−3.071 (0.511)***	−0.686 (0.204)***	−0.470 (0.210)*	−0.089 (0.229)	0.249 (0.166)	−1.314 (0.194)***	−0.508 (0.262)	−2.561 (0.210)***	−1.561 (0.205)***	0.407 (0.188)*	0.132 (0.168)	−1.170 (0.175)***
	Preston	−1.935 (0.177)***	−1.303 (0.239)***	−2.788 (0.483)***	1.408 (0.213)***	2.184 (0.344)***	−0.747 (0.308)	−0.786 (0.177)***	−1.305 (0.194)***	−0.219 (0.272)	−1.959 (0.209)***	−1.885 (0.218)***	3.172 (0.314)***	1.025 (0.282)***	−1.164 (0.175)***
	Rothamsted	−0.580 (0.174)***	0.013 (0.237)	−0.293 (0.278)	−0.505 (0.203)*	0.640 (0.208)**	1.475 (0.223)***	−0.836 (0.169)***	−1.162 (0.194)***	−1.121 (0.263)***	−0.675 (0.207)**	−0.158 (0.200)	0.024 (0.190)	−0.714 (0.169)***	−0.286 (0.174)
	Starcross	−0.658 (0.175)***	0.151 (0.237)	−0.311 (0.337)	−2.334 (0.203)***	−0.069 (0.253)	−1.464 (0.310)***	−0.442 (0.167)**	−0.911 (0.194)***	−0.749 (0.261)**	−1.571 (0.208)***	−2.994 (0.214)***	0.703 (0.231)**	0.030 (0.207)	−0.873 (0.175)***
	Writtle	0.014 (0.174)	−0.232 (0.237)	0.735 (0.248)**	−1.026 (0.202)***	−0.321 (0.189)	1.290 (0.200)***	−0.148 (0.166)	−0.641 (0.194)***	−0.054 (0.259)	−0.131 (0.206)	0.127 (0.199)	−0.103 (0.172)	0.047 (0.153)	0.026 (0.174)
	Wye	−0.749 (0.175)***	−0.885 (0.238)***	−0.462 (0.283)	−1.745 (0.203)***	0.129 (0.212)	−1.195 (0.249)***	−0.872 (0.169)***	−1.316 (0.194)***	0.300 (0.258)	−0.721 (0.207)***	−0.988 (0.201)***	−0.158 (0.194)	−0.274 (0.173)	−0.414 (0.174)*
Precipitation				0.010 (0.009)		−0.009 (0.006)	0.025 (0.007)***						0.021 (0.006)***	0.001 (0.005)	
Temperature				0.353 (0.378)		0.934 (0.274)***	−0.832 (0.327)*						−0.222 (0.248)	−0.612 (0.223)**	

Aphid species

61
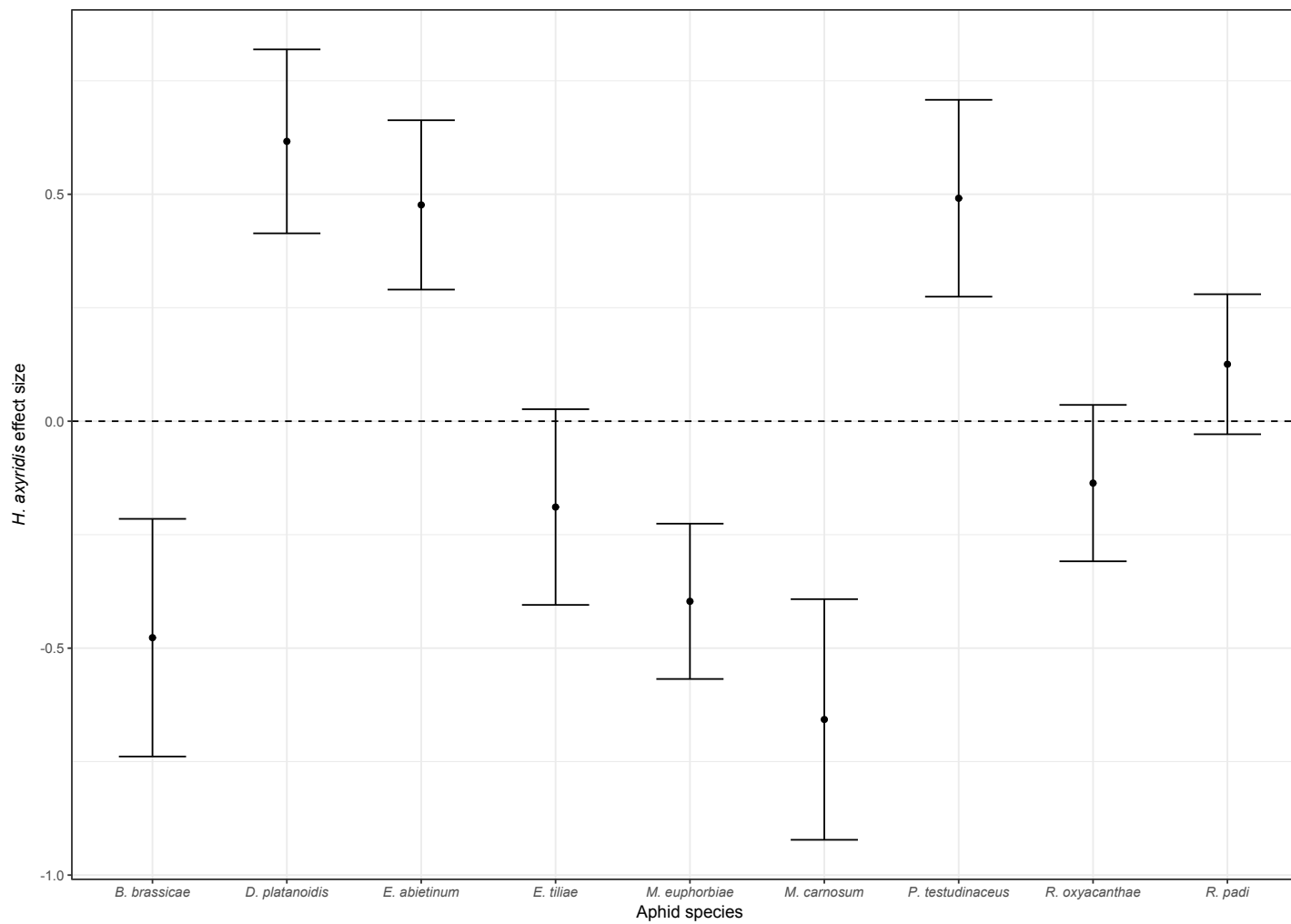
Figure 3.3: Effect sizes and standard errors for the *H. axyridis* model term, which denotes the presence/absence of *Harmonia axyridis*, for each of the nine aphid species whose 'best fit' model contained the *H. axyridis* term. Positive effect sizes denote increases in annual aphid abundance and negative effect sizes show decreases following the arrival of *H. axyridis*.

3.5   Discussion

Invasive non-native species are often linked with species declines and biodiversity impacts through key functional behaviours, such as predation (Beggs *et al.* 2011; Snyder & Evans 2006). *Harmonia axyridis* is a widespread invasive non-native predator that has spread rapidly and now dominates the majority of invaded communities (for example Brown & Roy 2017). Prior to its release, many studies aimed to quantify the predatory efficiency of *H. axyridis* in laboratory studies with a focus on pest species (for example Lee & Kang 2004; Seko *et al.* 2014). As far as we are aware, this is the first study to quantify the impacts of *H. axyridis* on native prey species using field collected data.

We have provided evidence that the arrival of *H. axyridis* in the UK was correlated with changes in aphid annual abundance. Using long-term datasets to quantify changes in annual population abundance rates of native aphid species and the presence/pseudo-absence of *H. axyridis*, we show that, of the 14 aphid species studied, that the arrival of *H. axyridis* correlated with declines of five aphid species, increases in four, and no significant change in the remaining five (Table 3.3 and Figure 3.3). After accounting for the site location and climatic variables (where appropriate), our reported average declines following the arrival of *H. axyridis* varied between -13.6 and -65.7% while our reported increases were between 12.6 and 61.7%. We suggest our reported changes in aphid abundance are associated with changing predatory pressure, likely as a result of the arrival of *H. axyridis*.

H. axyridis is highly generalist, consuming multiple prey species and inhabiting a variety of habitat types (Roy & Brown 2015; Brown *et al.* 2008). We had hypothesised that native aphid abundance would be impacted through the predatory pressure of *H. axyridis*. In accordance with Kenis *et al.* (2017), who found that habitat overlap was a key determinant of native Coccinellidae being negatively impacted by *H. axyridis*, we suggested that aphids with a greater habitat overlap with *H. axyridis* would be more impacted than those with less overlap. Specifically, we hypothesised that aphids inhabiting primary *H. axyridis* habitats (broadleaf trees, *U. dioica*, and agricultural crops) (Roy *et al.* 2011) would show the greatest declines in abundance.

Our finding that *M. carnosum*, *E. tiliae*, *B. brassicae*, *M. euphorbiae*, *R. oxyacanthae* show decreased population growth rates of -65.7, -18.9, 47.7, 39.7, and 13.6% (after accounting for site location and climatic variation, where appropriate) in correlation with the arrival of *H. axyridis* is in agreement with this hypothesis. However, we have also shown that the other species inhabiting these primary habitats, *D. platanoidis*, *P. testudinaceus*, and *R. padi* have increased in abundance (61.7, 49.1, and 12.6%) while the remaining species have shown no significant change. We had further hypothesised that aphids inhabiting secondary habitats of *H. axyridis* would be less impacted than those inhabiting primary habitats and therefore show smaller decreases in abundance. In accordance with this hypothesis, *E. abietinum* was found to have increased in abundance following the arrival of *H. axyridis*. It should also be noted that several of these aphid species will adopt multiple hosts when their primary host is unavailable for example, *R. oxyacanthae* and *S. avenae* will commonly inhabit agricultural crops (a primary *H. axyridis* habitat) but will also switch hosts to uncultivated grasses (a secondary *H. axyridis* habitat) (Harrington *et al.* 2007). This could therefore further inform our findings and suggest that, due to their habitat switching behaviours, there is a more complex relationship between *H. axyridis* and aphid species with respect to habitat overlap.

We propose that the impacts of *H. axyridis* on aphid prey species, presented here, are likely to be indicative of complex interactions between *H. axyridis*, native aphid predators, aphid prey, in addition to aphid host plants, wider agricultural practices and climate change. We suggest that our reported decreases in aphid abundance, following the arrival of *H. axyridis*, are likely due to increased predatory pressure imposed by the invader, a species known to be highly predatory (Chapter 2, Ingels *et al.* 2015; Koch *et al.* 2003; Lee & Kang 2004; Seko *et al.* 2014). The arrival of *H. axyridis* could also have resulted in decreased aphid abundance via imposing additional predatory pressure on the prey species, along side the pressure previously imposed by the native predators (Snyder 2009, e.g.). It is also possible that *H. axyridis* could displace the native predators to other habitats (Harmon *et al.* 2007; Roy *et al.* 2012, e.g.), where they could then impose more predatory pressure than was previously experienced. With respect to

those aphid species that have shown no significant change in abundance, we suggest this could be due to two possible scenarios. Firstly, this could be indicative of no direct (e.g. predation) or indirect (e.g. displacement of native predators) impact of *H. axyridis* on the aphid species. The displacement of native species by those invading has been previously documented with the displacement native Coccinellidae by *H. axyridis* being reported in Europe (Roy *et al.* 2012; Kenis *et al.* 2017) and North America (Harmon *et al.* 2007). Beggs (2001) also report the displacement of invasive German wasps (*Vespula germanica*) in New Zealand by additional invasive species, common wasps (*Vespula vulgaris*). Secondly, the complete or near-complete replacement of native predators by *H. axyridis* could result in the predatory pressure experienced by aphid prey remaining constant over time. It should also be noted that aphid abundance is known to be increasing with climate change (Bell *et al.* 2015; Martay *et al.* 2017) which could result in no significant change, were the rates of predation by *H. axyridis* similar to the climate change induced increases in abundance. We do, however, believe this is unlikely due to the increase in abundance being less than the expected levels of predation by *H. axyridis* in addition to these reported changes in aphid abundance occurring over longer time scales than any predation by *H. axyridis* would be expect to take effect. Lastly, with respect to the four aphid species that have shown increases in abundance, we suggest this could be due to the aphids having little or no interaction with *H. axyridis* with the increased abundance due to abiotic factors including changes in agricultural practices or climate change (Bell *et al.* 2015; Harrington *et al.* 2007; Martay *et al.* 2017; van Emden & Harrington 2017). Aphid abundance and distribution is known to vary with respect to multiple abiotic processes such as land use and fertilisation practices within agricultural systems. Harrington *et al.* (2007) highlight the association between land use and aphids, specifically their first flight time. However, the authors do concede their land use categories (by necessity) generalise habitats (e.g. Arable) which show significant variation in the host plants and therefore likely aphid species. Similarly, Newman (2005) provide evidence that fertiliser application practices may interact with projected climate change and negatively impact cereal aphids and drive their population declines. It could also be possible that the predatory pressure imposed by *H. axyridis*,

following the displacement of native predators, is less than was previously experienced before the arrival of *H. axyridis* and therefore enables aphid abundance to increase.

Aphid abundance is also impacted by bottom-up processes, in addition to top-down predation forces. Agricultural practices, such as the prevalence of host plants sown in a given year or amounts of pesticide applied, will impact aphid abundance (van Emden & Harrington 2017). Climate change has also been shown to increase aphid abundance in addition to the duration and phenology of the aphid flight period (Harrington *et al.* 2007). Climate variables are also linked with agricultural choices, such as crop and pesticide treatments and the timing of agricultural events, such as sowing (van Emden & Harrington 2017). These factors, in addition to others, are likely to further complicate our ability to quantify the impact of *H. axyridis* on native prey species.

Developments of this work could further our understanding of the processes underlying the effects we have found. For example, we used annual aphid population counts which will not account for the phenology of aphid abundance peaks or the duration of the aphid flight period which will not only vary between species but also the availability and quality of resources and climate change (van Emden & Harrington 2017). It is likely that phenological shifts in prey abundance will result in changes in the predatory pressure experienced by other aphid prey, in addition to the pressures resulting from herbivory experienced by host plants. As part of this study we only quantified the impact of *H. axyridis* on 14 common aphid species. As we have reported a significant change in aphid abundance for nine of the 14 aphid species included in this study, we suggest it is also likely that other UK and European aphid species are also impacted by *H. axyridis*.

Better understanding the impacts of widespread invasive non-native species is of increasing importance, with respect to informing policy and conservation management decisions. We have shown that the arrival of a widespread invasive non-native predator (*H. axyridis*) into the the UK is correlated with significant changes in the abundance of a number of native aphid species. Future research efforts would benefit from identifying and quantifying the underlying mechanisms of the predator-prey relationships identified as part of this study. Large spatial and long-term studies, such as the UK Ladybird

Survey (UK Ladybird Survey 2018) and the Rothamsted Insect Survey (Rothamstead Research 2015), are invaluable in addressing questions of this type, in addition to engaging non-specialists in ecological issues. The arrival of *H. axyridis* into the UK provided a valuable opportunity to study the impacts of a highly invasive non-native predator. It is hoped that we continue to learn from such events to better reduce the chances of and/or mitigate future invasion events.

Chapter 4

Interactive effects of resource quality and temperature drive differences in detritivory among native and invasive freshwater amphipods

4.1   Abstract

Invasive non-native species and climatic variation are two of the biggest pressures facing native communities; however, our understanding as to how they interact is poorly understood. These pressures are likely to impact detritivory, a key functional behaviour which is particularly important in temperate freshwater lotic systems which are typically net heterotrophic. We sought to understand how the detrital processing rates varied between native and invasive non-native amphipods across leaf diets and temperature extremes. We further assessed how any difference in rates could be realised by measuring their survival in each treatment combination. We therefore quantified the rates of detrital processing and survival of one UK native (*Gammarus pulex*) and two invasive non-native (*Dikerogammarus villosus* and *Dikerogammarus haemobaphes*) freshwater amphipod species, across three temperatures (8, 14, and 20°C), and with three leaf diets of varying resource quality (oak, sycamore, and alder) in a laboratory microcosm experiment. We provide evidence that the rates of detrital processing vary between the native and invasive non-native amphipod species, with native *G. pulex* undertaking more processing behaviour than both invasive non-native species at the lower temperatures. However, as the temperature treatments increased we found that between-species differences in detrital processing decreased, while the differences between diets of differing resource qualities become larger. We also show that, although the amphipod species do not differ in their survival probability, the chances of survival did differ between the temperature and resource quality treatments, in addition to the size of the amphipod. We suggest that the current impact of the invasive non-native *Dikerogammarus* species, specifically through lower rates of detrital processing, could be reduced under predicted climate change warming. We also predict that, under predicted climatic warming, the impact of resource quality is likely to become increasingly important in determining the rates of detrital processing and the survival of the three amphipod species investigated here.

4.2 Introduction

Biodiversity and species communities are under increasing pressure from a variety of anthropogenic sources with invasive non-native species being one of the most prominent (Simberloff *et al.* 2013). Specifically, we still have much to learn about how invasive non-native species impact native communities across climatic conditions (Bellard *et al.* 2012). Invasive non-native species can impact native communities directly, through interactions such as predation, and indirectly, for example through altering the ecosystems energy flow (Salo *et al.* 2007; Kenis *et al.* 2009). It is also likely that changing climatic conditions, under projected climate change scenarios, will interact with current environmental stressors, such as invasive species which, together, may place an increased environmental stress on communities, with freshwater systems often highlighted as being particularly at risk (see Woodward *et al.* 2010). While the negative effects of invasive non-native species are the subject of ongoing research effort, we still have much to learn about how their affects interact with climatic variables, particularly climatic extremes (Bellard *et al.* 2016; Sorte *et al.* 2013).

Freshwater systems are particularly at risk as their connectivity, flow regimes and biodiversity lead to their risk of initial invasion, subsequent spread and detrimental impact being higher than many other communities (Moorhouse & Macdonald 2015). While the impacts of climatic extremes and invasive non-native species are well documented, the degree to which these two stressors interact and impact ecosystem functioning remains poorly understood.

Freshwater communities gain the majority of their nutrient input from allochthonous sources, often in the form of organic detrital matter. The largest of these nutrient sources is the windfall of leaves in autumn which results in an annual nutrient input 'pulse'. Windfall leaves are quickly colonised by microbial biofilms (e.g. hyphomycete fungi) and macroinvertebrates (e.g. *Gammarus* spp.) (McArthur & Barnes 1988) which break down the leaves from coarse particulate organic matter (CPOM, > 1 mm) to fine particulate organic matter (FPOM, < 1 mm). This initial stage of the detritus pathway is essential as, for the vast majority of the freshwater community, the CPOM remains

and inaccessible resource until broken down into FPOM. The importance of leaf shredding macroinvertebrates have been demonstrated, for example Cuffney *et al.* (1990) measured a 50-74% decline in leaf litter processing rates and an associated 33% decline in FPOM when streams were treated with insecticide which removed macroinvertebrates. Macroinvertebrate shredders are therefore important keystone species and play an important role in facilitating energy flow within freshwater communities (Wallace & Webster 1996).

Gammarus pulex is a widespread freshwater amphipod in Europe that undertakes detrital leaf shredding behaviour. In recent years two novel freshwater invaders have arrived in Europe; *Dikerogammarus villosus* and *Dikerogammarus haemobaphes*, arriving in the UK in 2010 and 2012. Both species originate from the Ponto-Caspian region and are significantly larger than the native *G. pulex* (Devin *et al.* 2003). Following their arrival, both *Dikerogammarus* species have spread and now dominate the invaded water bodies. Studies have suggested that the invasive amphipods could impact the native community through direct predation (for example Dodd *et al.* 2014), indirect predation effects (MacNeil *et al.* 2011), and through differences in detrital processing rates (for example Jourdan *et al.* 2016). Specifically, the *Dikerogammarus* species show higher rates of predation and lower rates of detrital processing than native amphipods. For example, MacNeil *et al.* (2011) shows that *G. pulex* undertake significantly more detrital processing than *D. villosus*, when provided with sycamore leaves (See also Jourdan *et al.* 2016; Piscart *et al.* 2011). Truhlar *et al.* (2014) compared the leaf breakdown rates of native *G. pulex* and non-native *D. villosus* at low and high temperature extremes (5 and 25°C) and reported that both amphipod species behaved similarly at low temperatures but at the upper temperature extreme *D. villosus* broke down more leaf matter than *G. pulex*. Kenna *et al.* (2016) demonstrated that the mean rate of detrital processing increased with temperature in both *G. pulex* and *D. villosus* with lower amphipod survival as temperature increased. Despite having many of the same characteristics, *D. haemobaphes* remains little studied (Constable & Birkby 2016). Constable & Birkby (2016) suggest that *D. haemobaphes* could be functionally similar to *D. villosus* and have a slower rate of leaf breakdown than *G. pulex*. Despite the current body of

literature, questions remain as to how different vegetation types, of different qualities, impact detrital processing rates between native and invasive amphipods.

Previous studies have suggested that the arrival of novel amphipod species could impact nutrient cycling through modifying the rates of detrital processing (Jourdan *et al.* 2016; Truhlar *et al.* 2014; Kenna *et al.* 2016). Such studies have often consisted of only pairwise comparisons of amphipod species, and with amphipods only supplied with one leaf species as a detrital resource at only a single temperature regime. We aimed to quantify the *per-capita* detrital processing rates of three amphipod species, when provided with three diets of differing resource quality and kept at three temperatures. We also compared amphipod mortality between treatment combinations as changes in population density are likely to also impact detrital shredding rates. We hypothesised that; (H_1) the rate of detrital breakdown will differ between the amphipod species, resource quality, through three leaf species diets, and temperature treatments. In line with previous literature findings, we expected native *G. pulex* to process detritus of all three species at a higher rate (more CPOM loss and more FPOM creation) than both *Dikerogammarus* species (for example MacNeil *et al.* 2011). Generally, we expected the rate of leaf consumption to increase as temperature increased due to increased metabolic activity. However, previous work has suggested the upper thermal tolerance of both *Dikerogammarus* species to be much higher than that of the native *G. pulex* (Van der Velde *et al.* 2009; Maazouzi *et al.* 2011; Truhlar *et al.* 2014), leading us to suggest that due to being outside their optimal range, *G. pulex* will show significantly reduced detrital processing rates in comparison to the two non-native amphipods at the higher temperature extreme.

While previous studies have investigated how amphipods perform at temperature extremes (e.g. Truhlar *et al.* (2014); Kenna *et al.* (2016)) little attention has been paid to the impacts amphipod survival could have on overall rates of detrital processing (Maazouzi *et al.* 2011, but see). For example, any between-species *per-capita* difference in detrital shredding rate could either be exacerbated or negated through variation in between-species survival rates. Under warming conditions species are likely to experience increasing and differing pressures which could impact the ecophysiology of individuals

(Gardner *et al.* 2017). For example, body size is considered likely to play an important role in an individuals survival when faced with extreme environmental conditions with smaller individuals predicted to cope better with warming conditions due to their surface area to volume ration facilitating more efficient heat dissipation (Gardner *et al.* 2011, 2017). We therefore further hypothesised that; (H_2) the survival of amphipods would vary between amphipod species, leaf species and temperature treatment combinations, as well with amphipod size. We had expected that, due to their broad thermal tolerance range (for example Bruijs *et al.* 2001), both *Dikerogammarus* species would be more tolerant to any further stressor encountered as part of the experiment (poor quality resources or higher temperatures). We also expected that the survival of smaller amphipods will be higher than those of larger individuals. Temperature extremes would likely be associated with increased metabolic stress which, we suggest, would result in a decrease in amphipod survival as the treatment temperature increases. Specially, we hypothesise that amphipod survival would be the highest in the lower temperature treatment and lowest at the highest temperature. We also suggest that poor resource quality would impact amphipod survival with individuals provided with a better quality resource having a higher probability of survival than individuals provided with a resource of poorer quality. Further, while previous studies have often quantified the rate of detrital processing through the loss of coarse particulate organic matter (CPOM), we have used both the loss of CPOM and the creation of fine particulate organic matter (FPOM), the essential by-product of detrital processing which provides a key nutritional resource throughout the wider community.

Here, we aimed to quantify how the detrital processing rates (generation of FPOM and consumption of CPOM) varied between native and invasive non-native amphipods when provided with three different leaf species diets and maintained at three different temperature regimes. Additionally, in an effort to inform how our *per capita* measures of detrital processing could scale-up to small populations, we quantified the differences in survival between then three amphipod species, three leaf diets, and three temperature regimes as any *per capita* differences could either be exacerbated or negated by differences in survival.

4.3   Materials and methods

4.3.1   Animal husbandry

We collected invasive non-native *D. villosus* from Grafham Water, Cambridgeshire (52°29'N; -0°32'W), *D. haemobaphes* from the Leeds-Liverpool canal, Saltaire (53°84'N; -1°80'W), and native *G. pulex* were collected from Meanwood Beck, Leeds (53°83'N; -1°58'W), UK. We used standard kick sampling methods for collecting both *D. haemobaphes* and *G. pulex* while, due to their high abundance, we collected *D. villosus* by hand from artificial substrate. Following collection, we kept amphipods in control conditions of 14°C 12:12 light:dark cycle in oxygenated five litre tanks of dechlorinated tap water. Before use in experiments, amphipods were brought to the treatment temperatures at a rate of 2°C change every six hours. We acclimatised amphipods to the treatment temperatures for 24 hours before they were starved for a further 24 hours to standardise gut contents.

4.3.2   Leaf material

We collected windfall leaves and air-dried them at room temperature before use. We collected English oak (*Quercus robur*) leaves from Meanwood Park, Leeds (53°83'N; -1°58'W), alder (*Alnus glutinosa*) at the University of Leeds, Leeds (53°80'N; -1°55'W) and sycamore (*Acer pseudoplatanus*) from Woodhouse Moor, Leeds (53°80'N; -1°56'W). We conditioned leaves in stream water, collected from Meanwood Beck, Leeds, for 14 days at 14°C and 12:12 L:D. We cut leaves into discs (8 mm in diameter), avoiding midribs, dabbed them dry, weighed them, before adding them to the experimental arenas.

4.3.3   Experimental microcosms

Experimental arenas consisted of two stacked 12 oz plastic containers (Solo, diameter = 117 mm, depth = 61 mm). The base of the upper container was replaced with a 1 mm

plastic mesh to retain particles larger than 1 mm (CPOM) in the upper container, while particles smaller than 1 mm (FPOM) were collected in the lower container. So as to encourage more natural behaviours, we provided the amphipods with a refuge in the form of a glass bead (approx 20 mm in diameter) in the upper container in addition to a diet of 12 conditioned leaf discs of known weight. Experimental arenas were filled with 300 ml dechlorinated tap water.

4.3.4   Experimental methods

Individuals of each of the three amphipod species (native *G. pulex* and invasive non-native *D. villosus* and *D. haemobaphes*) were subject to one of three leaf species treatments (oak, alder and sycamore) and one of three temperature treatments (8, 14 or 20°C). These temperature treatments were chosen to provide a representative range of current (8 and 14°C) (Hammond & Pryce 2007) and an upper and lower extreme within the boundaries of current predicted climate change scenarios (20°C) (Orr *et al.* 2010). Control treatments were constructed in the same way except no amphipods were added to these arenas to enable the rate of leaf breakdown not attributable to amphipod shredding to be measured (for example, microbial and fungal breakdown). After replicates were removed where the amphipod died within 24 hours of the experimental start time, each treatment combination was replicated between 14 and 30 times. After the 24 hour starving period amphipods were weighed before being added to the experimental arenas. The experiment ran for 14 days and amphipods were checked daily for mortality and moulting. When an amphipod died, the replicate was stopped and the date of death recorded. When we observed that individuals had moulted, their moults were removed to avoid an additional food resource impacting our measures of FPOM creation and CPOM loss. After 14 days, amphipods were removed from arenas and weighed (dabbed dry). The remaining leaf discs were removed from the arenas and oven-dried to a constant mass (105°C for 24 hours) and weighed. Water samples, containing suspended FPOM, were filtered through a filter paper (Whatmann GF/F, pore size = 0.7 μm), oven-dried (105°C for 24 hours), and weighed.

4.3.5 Leaf nutrient content

We oven-dried (105°C for 24 hours) conditioned leaves of each of the three species (oak, alder and sycamore) to a constant dry mass and analysed their carbon, nitrogen and total phosphorous content using standardised laboratory protocols (Allen 1989). We analysed carbon and nitrogen contents with an Elementar vario micro cube combustion analyser (n = 3, species = mean (mg) ($\pm$ SE); oak = 3.926 ($\pm$ 0.025), alder = 3.992 ($\pm$ 0.062), sycamore = 3.949 ($\pm$ 0.012)) and we used a Skalar continuous-flow auto-analyser for the total phosphorous analysis (n = 2, species = mean (mg) $\pm$ SE; oak = 199.9 ($\pm$ 0.000), alder = 0.200 ($\pm$ 0.000), sycamore = 200.1 ($\pm$ 0.300)).

4.3.6 Statistical analysis

All statistical analyses were undertaken in R version 3.3.2 and RStudio version 1.0.136 (R Core Team 2016; RStudio 2016). Weights of the three amphipod species were significantly different (ANOVA; $F_{(2,554)}$ = 195.1, P <0.001), with a Tukey HSD test indicating that each pairwise comparison was significantly different ($P < 0.001$). Invasive non-native *D. villosus* individuals were the largest, while native *G. pulex* were the smallest (mean mg ($\pm$ SE); *D. villosus* = 85.38 ($\pm$ 2.19), *D. haemobaphes* = 52.74 ($\pm$ 1.26), *G. pulex* = 43.88 ($\pm$ 0.92)). This difference in size makes it difficult to separate the effects of amphipod mass and amphipod species, we therefore standardised the individual amphipods rate of detrital shredding (FPOM production and CPOM loss) by the mass of the individual amphipod (mg).

4.3.6.1 Shredding rates

Rates of amphipod leaf breakdown, measured as FPOM generated and CPOM lost, were standardised by both the individual amphipods weight (mg) and the number of shredding days the individual experienced. The number of shredding days was defined as the number of days the amphipod was alive minus one. An accurate weight of the initial mass of CPOM provided to the experimental replicates is impossible to measure as the conditioning process adds weight to the samples as microbial biofilms develop and any

drying of these conditioned samples would destroy the biofilm and therefore render the conditioning process fruitless. We therefore converted the CPOM leaf discs (dabbed dry) weights into predicted oven dry weights using regression coefficients from a set of 30 replicates and these were used to calculate the rate of CPOM loss. Amphipods that died within 24 hours of the start of the experiment, therefore having zero shredding days, were excluded from detrital breakdown analyses. The rates of detrital breakdown were analysed by generating 15 candidate linear regression models with all combinations of; amphipod species, leaf species and temperature as fixed and interaction terms. We then compared these models, using AICc scores, to the null model which contained the mean of the response variable (rate of FPOM generation or CPOM loss) as a fixed effect.

4.3.6.2 Amphipod survival

We analysed amphipod survival using Cox proportional hazard models using the survival r package (Therneau 2015). Similar to our other analyses, we selected 14 candidate models, containing interaction and main effect term combinations of; amphipod species, leaf species and temperature treatment terms. We then compared these models, using AICc scores, to the null model which contained the mean of the response variable as a fixed effect. We included amphipods that died within 24 hours of the experiment start time in this analysis to prevent bias in mortality estimates. We compared the 'best fit' model, using AICc values, with the same model with a scaled and mean centred amphipod weight term included, to test the importance of amphipod weight on survival. As the three amphipod species were significantly different sizes, we scaled and mean centred the amphipod weight term to allow for their comparison without this being confounded with the amphipod species term. This process also facilitates interpretation as the mean weight of an amphipod species is 0, with negative values being individuals smaller than average for their species and positive values being larger than average.

4.4 Results

4.4.1 Leaf nutrient content

Alder leaf diet contained a higher concentration of nitrogen, and therefore had a lower carbon:nitrogen ratio (percentage composition) than both the oak and sycamore diets (mean C:N ($\pm$ SE); alder = 22.16 ($\pm$ 0.14), sycamore = 40.12 ($\pm$ 1.42) and oak = 40.93 ($\pm$ 1.20)). Our results show that the amount total phosphorus (mg/g) was the lowest in the alder leaves and highest in the sycamore leaves (mean total phosphorus (mg/g) ($\pm$ SE); alder = 0.22 ($\pm$ 0.00), oak = 0.33($\pm$ 0.02) and sycamore = 0.50 ($\pm$ 0.03)).

4.4.2 Detrital shredding rates

Control treatments showed negligible FPOM production (species = mean (mg) $\pm$ SE; oak = 0.704 ($\pm$ 0.111), alder = 1.125 ($\pm$ 0.195), sycamore = 0.878 ($\pm$ 0.118)), suggesting that breakdown in amphipod treatments was attributable to amphipod shredding rather than microbial or fungal breakdown.

Our linear regression of the rate of FPOM production (mg per mg amphipod per shredding day) suggested that after accounting for amphipod species, temperature and leaf species significantly improved the null model (Δ AICc = 56.63, 125.36 and 133.01). We found that the best model (AICc weight = 1 and Δ AICc = 464.48 contained the full three-way interaction term (amphipod species * leaf species * temperature interaction), suggesting that effect of each of the treatments, on the rate of FPOM production, was dependent on the combination of the other two treatment variables (Table 4.1 and Figure 4.1). For example, when provided with an alder leaf diet and kept at 8°C, *D. haemobaphes* produced FPOM 63% less, and *D. villosus* 56% less, than *G. pulex* under the same conditions. However, when maintained at 20 °C, *D. haemobaphes* produced 0.4% more FPOM and *D. villosus* produced 27% less than *G. pulex* also at the same conditions. We also see that as temperature increases there is less of a difference in FPOM production between amphipod species while the differences between leaf species increases with temperature (Figure 4.1).

Table 4.1: Three-way factorial ANOVA of fine particulate organic matter (FPOM) generated per mg of amphipod shredder per shredding day. A three-way interaction, between amphipod species, temperature and leaf species was included in the model.

	Df	Sum Sq	Mean Sq	F value	P
Species	2	0.202	0.101	70.046	<0.000
Leaf species	2	0.435	0.218	151.005	<0.000
Temperature	2	0.376	0.188	130.428	<0.000
Species * Leaf species	4	0.019	0.005	3.365	0.010
Species * Temperature	4	0.039	0.010	6.687	<0.000
Leaf species * Temperature	4	0.041	0.010	7.200	<0.000
Species * Leaf species * Temperature	8	0.070	0.009	6.087	<0.000
Residuals	525	0.756	0.001		
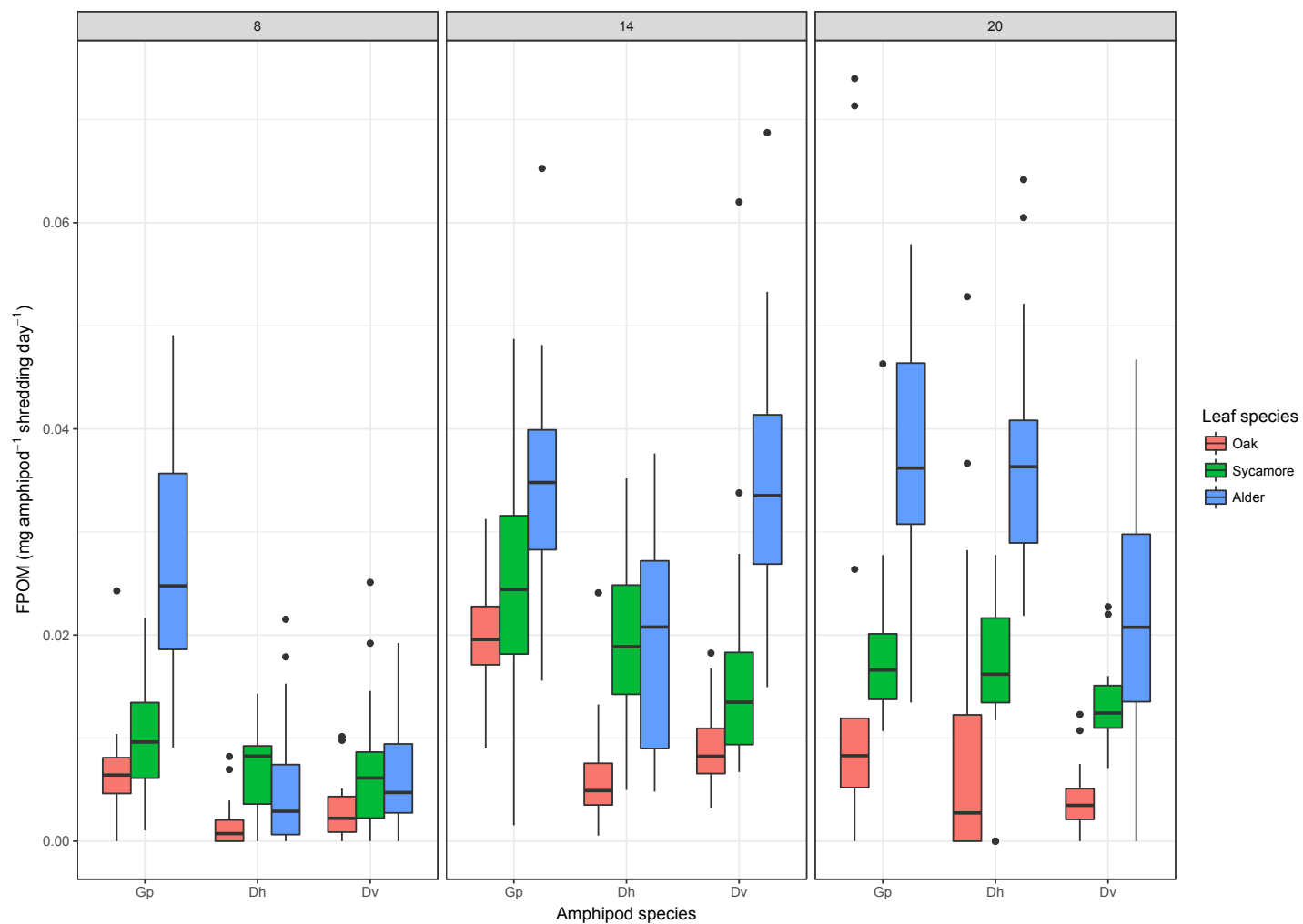
Figure 4.1: Mass of fine particulate organic matter (FPOM) generated per mg of amphipod per shredding day. Shredding day is defined as the day of death -1 as it is assumed that amphipods were likely to have displayed atypical behaviour prior to death. Gp = *Gammarus pulex*, Dh = *Dikerogammarus haemobaphes* and Dv = *Dikerogammarus villosus*.
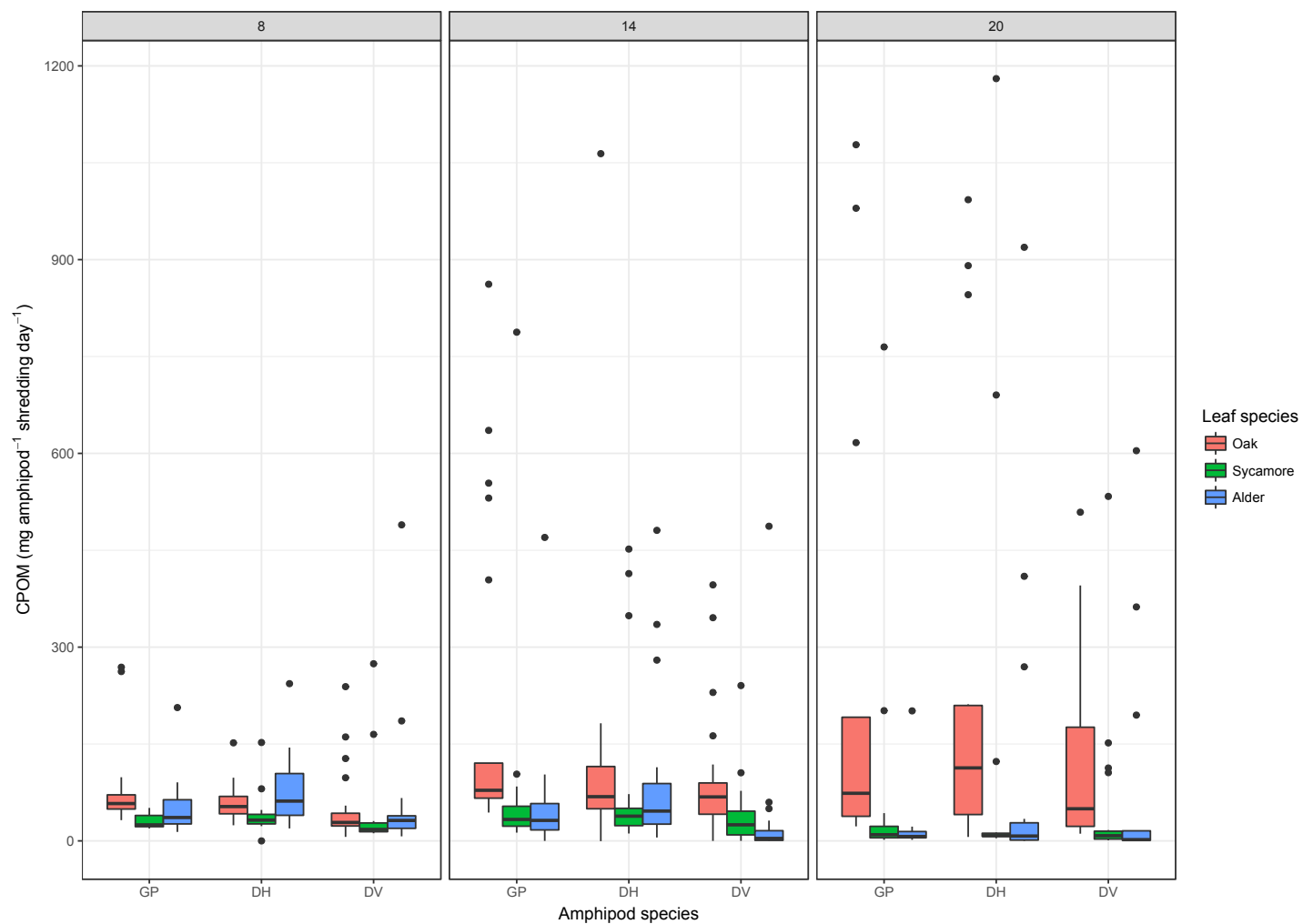
Figure 4.2: Mass of coarse particulate organic matter (CPOM) consumed per mg of amphipod per shredding day. Shredding day is defined as the day of death -1 as it is assumed that amphipods were likely to have displayed atypical behaviour prior to death. Gp = *Gammarus pulex*, Dh = *Dikerogammarus haemobaphes* and Dv = *Dikerogammarus villosus*.
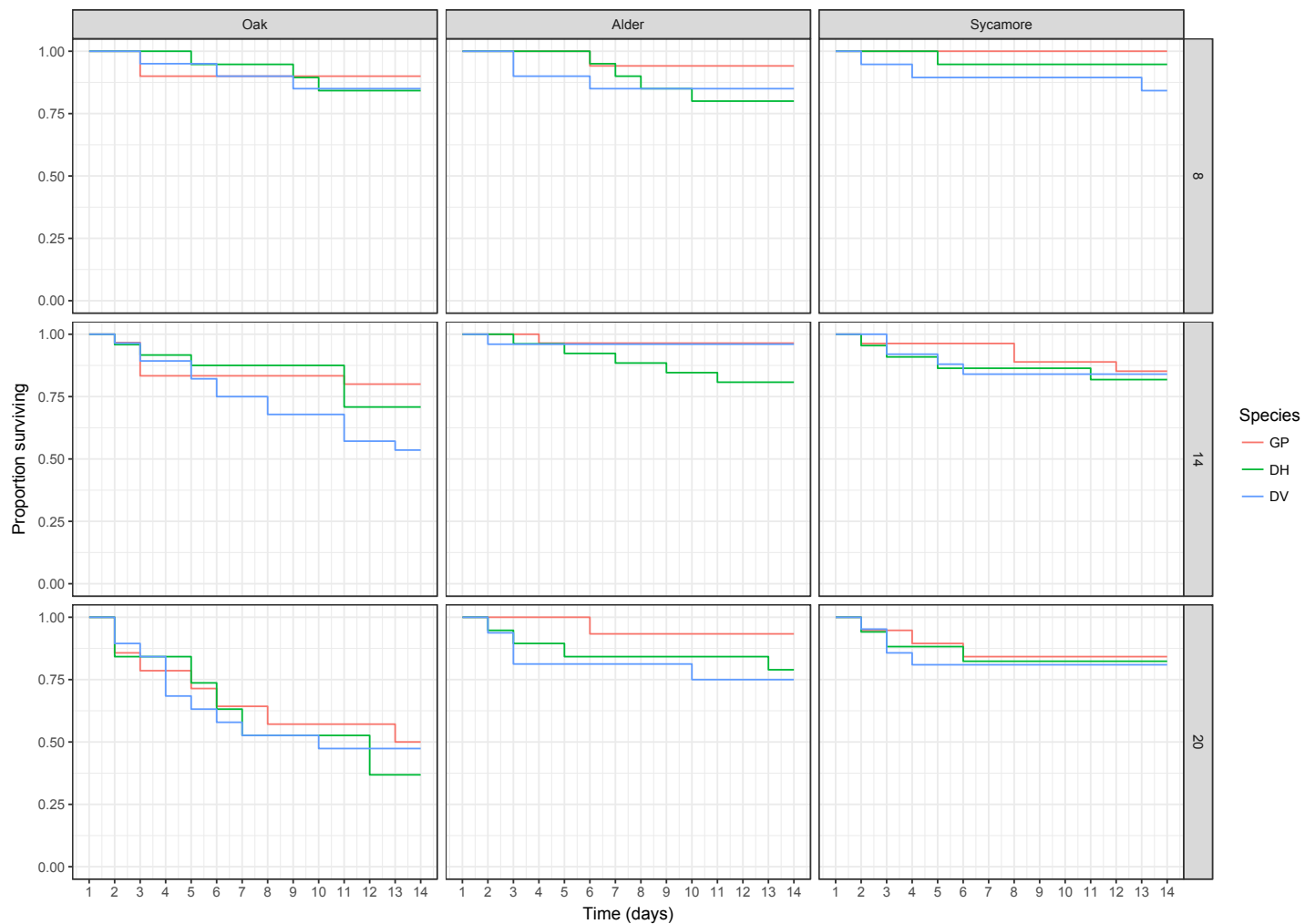
Figure 4.3: Kaplan–Meier survival curves showing the proportion of amphipods surviving for each amphipod species (*G. pulex* = Gp (red), *D. haemobaphes* = Dh (green), and *D. villosus* = Dv (blue)), in each of the temperature (8 (top row), 14 (middle row), and 20°C (bottom row)), and leaf species (Oak (*Quercus robur*; left row), Alder (*Alnus glutinosa*; middle row), and Sycamore (*Acer pseudoplatanus*; right row)) treatment combination.

Figure 4.4: Cox proportional hazard ratios for amphipod survival with respect to the leaf species diet and temperature treatment combinations. The three amphipod species did not differ significantly in terms of their survival and therefore they are not represented here.

Table 4.2: Second-order Akaike Information Criterion scores (AICc), delta AICc (Δ AICc), and associated weights (AICc Wt.) for the 15 candidate models for the rate of FPOM production. The null model contained only one fixed term, the mean rate of FPOM production, which is denoted by '1'. The 'best fit' model is highlighted in bold.

Model	AICc	Δ AICc	AICc Wt.
˜1	-1549.10	464.48	0
˜Amphipod species	-1605.73	407.85	0
˜Temperature	-1674.46	339.12	0
˜Leaf diet	-1682.10	331.48	0
˜Amphipod species + temperature	-1737.75	275.83	0
˜Amphipod species + leaf diet	-1760.83	252.75	0
˜Temperature + leaf diet	-1848.70	164.88	0
˜Amphipod species + temperature + leaf diet	-1944.76	68.82	0
˜Temperature * leaf diet	-1865.05	148.53	0
˜Temperature * amphipod species	-1743.95	269.63	0
˜Amphipod species * leaf diet	-1758.85	254.73	0
˜Amphipod species + Temperature * leaf diet	-1963.78	49.80	0
˜Leaf diet + temperature * amphipod species	-1959.90	53.68	0
˜Temperature + amphipod species * leaf diet	-1948.13	65.45	0
˜Amphipod species * temperature * leaf diet	**-2013.58**	**0.00**	**1**

Our analysis of the rate of CPOM loss (CPOM (mg) mg amphipod^{-1} day $^{-1}$) also showed that accounting for amphipod species, temperature and leaf species all improved the model fit (Δ AICc = 4.03, 11.58 and 2.90). We found that the 'best fit' model contained species as a fixed effect and an interaction between temperature and leaf species (Δ AICc = 20.9).
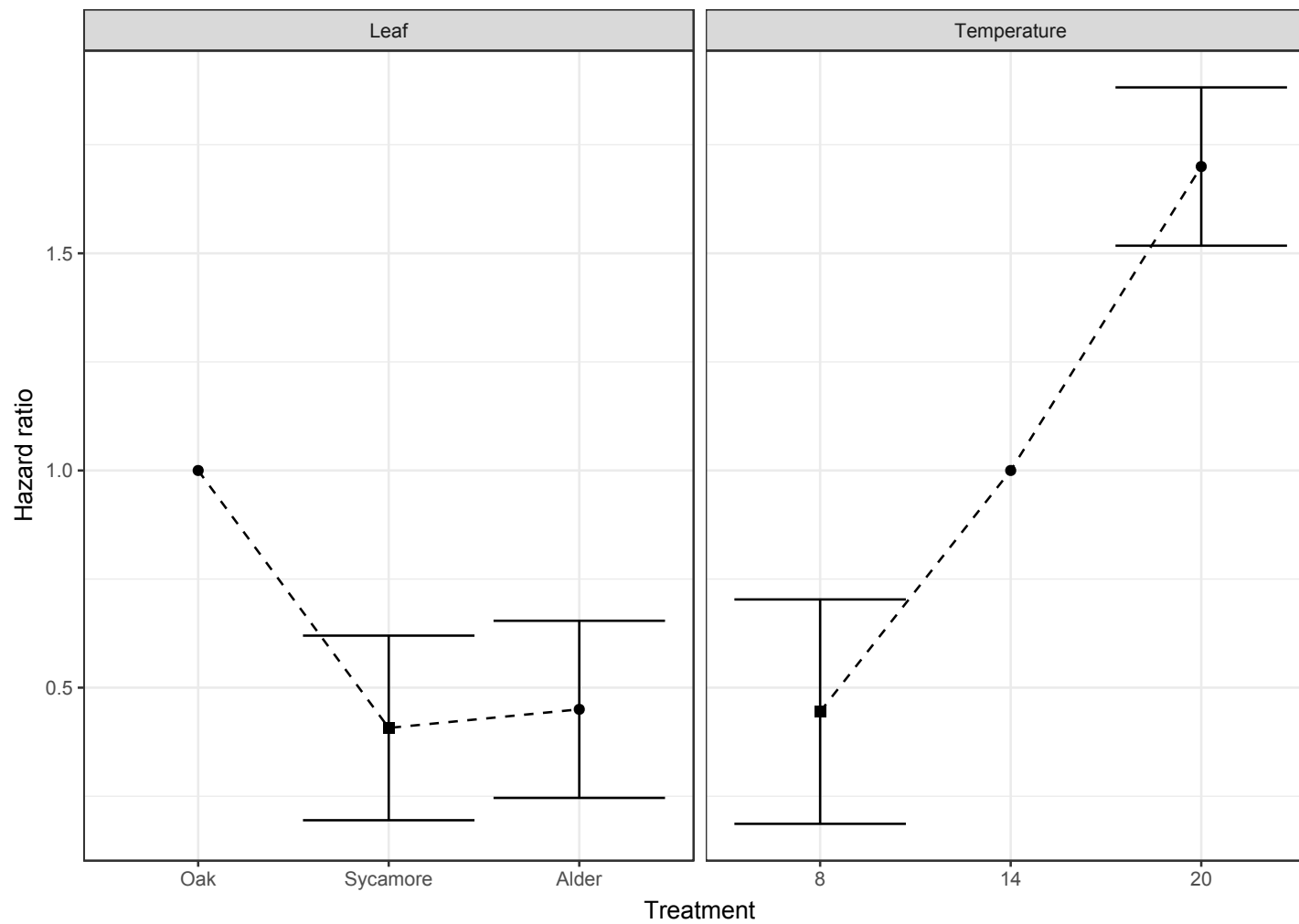
4.4.3 Amphipod survival

Amphipod survival was affected by resource quality and temperature but did not differ between amphipod species. We found that the comparisons of candidate models suggested that the temperature and leaf species terms improved the null model (Δ AICc = 25.2 and 18.14). Contrary to our expectations, accounting for amphipod species did not improve the null model (Δ AICc = 2.49), which suggests the three amphipod species did not differ in their risk of mortality. The 'best fit' model suggested that the leaf species diet resulted in different survival probabilities in amphipods, with those individuals provided with a diet of oak leaves having a lower survival rate than those provided with alder or sycamore leaves (HR = 0.407, 95% CI = 0.269-0.618 and HR =

Table 4.3: Second-order Akaike Information Criterion scores (AICc), delta AICc (Δ AICc), and associated weights (AICc Wt.) for the 15 candidate models for the rate of CPOM consumption (mg). The null model contained only the mean rate of FPOM production, which is denoted by '1'. The 'best fit' model is indicated in bold.

Model	AICc	Δ AICc	AICc Wt.
~1	-1428.16	20.90	0.00
~Amphipod species	-1432.19	16.88	0.00
~Temperature	-1439.74	9.32	0.01
~Leaf diet	-1431.06	18.01	0.00
~Amphipod species + temperature	-1442.59	6.48	0.02
~Amphipod species + leaf diet	-1434.99	14.08	0.00
~Temperature + leaf diet	-1442.92	6.15	0.03
~Amphipod species + temperature + leaf diet	-1445.64	3.43	0.11
~Temperature * leaf diet	-1446.51	2.55	0.17
~Temperature * amphipod species	-1438.85	10.21	0.00
~Amphipod species * leaf diet	-1428.77	20.30	0.00
~Amphipod species + Temperature * leaf diet	**-1449.07**	**0.00**	**0.63**
~Leaf diet + temperature * amphipod species	-1441.89	7.18	0.02
~Temperature + amphipod species * leaf diet	-1439.48	9.58	0.01
~Amphipod species * temperature * leaf diet	-1426.93	22.13	0.00

Table 4.4: ANOVA table of the 'best fit' model for CPOM consumption (mg) containing single order terms of Amphipod species, leaf diet, and temperature, in addition to an interaction term between temperature and leaf diet.

	Df	Sum Sq	Mean Sq	F value	P)
Amphipod species	2	0.03	0.02	4.24	0.0149
Temperature	2	0.06	0.03	7.44	0.0006
Lead diet	2	0.03	0.01	3.61	0.0278
Temperature * leaf diet	4	0.05	0.01	2.91	0.0212
Residuals	540	2.22	0.00		

Table 4.5: Second-order Akaike Information Criterion scores (AICc), delta AICc (Δ AICc), and associated weights (AICc Wt.) for the 15 candidate Cox proportional hazards models. These models compare the relative survival of amphipods with the null model containing only the mean survival rate which is denoted by '1'. The 'best fit' model is in bold.

Model	AICc	Δ AICc	AICc Wt.
˜1	1761.07	45.97	0.00
˜Amphipod species	1763.56	48.46	0.00
˜Temperature	1735.85	20.75	0.00
˜Leaf diet	1742.93	27.83	0.00
˜Amphipod species + temperature	1738.24	23.14	0.00
˜Amphipod species + leaf diet	1745.33	30.23	0.00
˜Temperature + leaf diet	1715.10	0.00	0.46
˜Amphipod species + temperature + leaf diet	1717.56	2.46	0.14
˜Temperature * leaf diet	**1716.01**	**0.90**	**0.30**
˜Temperature * amphipod species	1742.92	27.82	0.00
˜Amphipod species * leaf diet	1752.53	37.43	0.00
˜Amphipod species + Temperature * leaf diet	1718.56	3.46	0.08
˜Leaf diet + temperature * amphipod species	1721.68	6.58	0.02
˜Temperature + amphipod species * leaf diet	1725.16	10.06	0.00
˜Amphipod species * temperature * leaf diet	1742.06	26.96	0.00

Table 4.6: ANOVA table for the 'best fit' Cox proportional hazards model. This model contains the single order terms of temperature and leaf diet treatments. Amphipods were not found to differ in their chances of survival.

		coef	exp(coef)	se(coef)	Z	P
Temperature:	8°	-0.81	0.44	0.26	-3.14	0.00
	20°	0.53	1.70	0.18	2.91	0.00
Leaf diet:	Sycamore	-0.90	0.41	0.21	-4.23	0.00
	Alder	-0.80	0.45	0.20	-3.91	0.00

0.450, 95% CI = 0.302-0.671). Amphipod survival was greatest in the 8°C treatment and lowest at 20°C (8°C; HR = 0.445, 95% CI = 0.268-0.738 and 20°C; HR = 1.700, 95% CI = 1.190-2.429). There was some indication that the assumption of a constant relative hazard was violated within the 'best fit' model ($P = 0.032$). This was driven by the alder leaf treatment ($P = 0.026$) within which amphipods risk of mortality increased with time. Our comparison of candidate models also suggested that a second model was similar at explaining the observed variation (Δ AICc = 0.9). This second model was the same as the 'best fit' model but also contained a leaf species * temperature interaction term. The most 'best fit' model was used to generate further candidate models to test the importance of amphipod weight in determining amphipod survival. The best model (AICc weight = 0.74) contained a temperature * amphipod species interaction term suggesting that the effect of amphipod size on amphipod survival varied between the temperature treatments (Figure 4.5 and table 4.5). Visual inspection of this interaction term showed that in the 8°C treatment the size of amphipods appeared to have minimal impact on amphipod survival whereas opposing relationships were seen at both 14 and 20°C treatments (Figure 4.5). At 14°C smaller amphipods were more likely to survive whereas at 20°C the opposite was true with larger amphipods having much a lower risk of mortality (Figure 4.5).
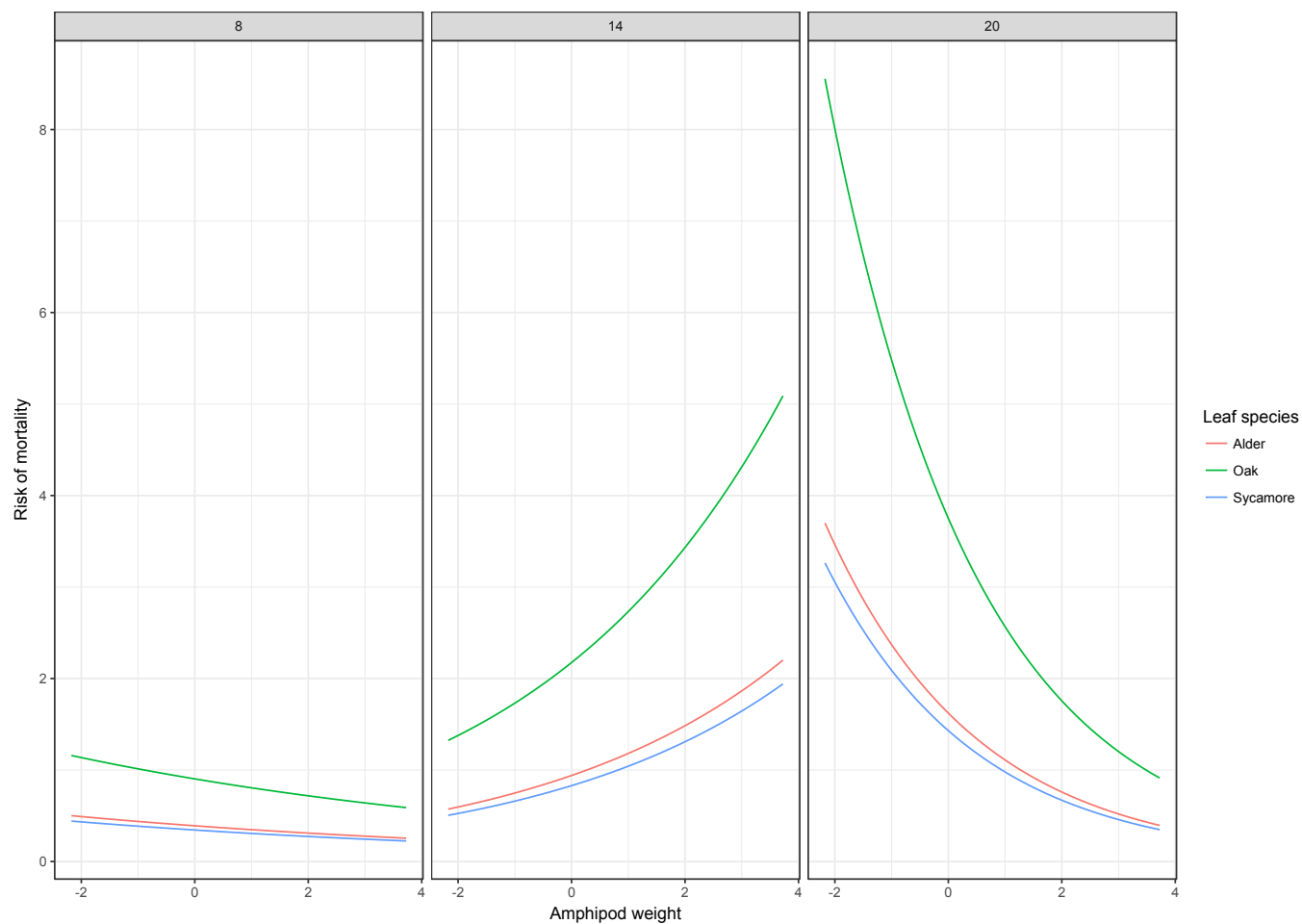
Figure 4.5: Cox proportional hazards model of the risk of amphipod mortality with respect to the resource quality (line colours; red = alder, green = oak and blue = sycamore) and temperature (8°C = left, 14°C = middle, 20°C = right) treatments and amphipod weight. This 'best fit' model contained a temperature*size interaction term in addition to a resource quality term. The amphipod species term was not indicated as being informative through the model selection procedure and is therefore not included. Amphipod weight was scaled and mean centred within amphipod species meaning that 0 represents the mean mass of the amphipod species with lower numbers showing smaller than average amphipods and higher numbers showing those larger than average.

4.5   Discussion

We have shown that the combined effects of invasive non-native species, climate extremes and resource quality could all change the detrital processing regime and therefore the energy flow throughout a freshwater system. Overall, we found that the rates of detrital leaf shredding and, more specifically, the amount of FPOM generated is significantly different between native and invasive amphipod species, across a range of temperature regimes and a variety of leaf species diets of varying resource quality. At lower temperatures (8°C) we see a notable difference in the rate of FPOM production between the three amphipod species, with native *G. pulex* producing FPOM at a higher rate than both the invasive non-native *Dikerogammarus* species and a difference between the resource quality types for example, alder diets resulted in a higher rate of FPOM production than oak in all species. However, as the temperature treatments increase, the difference in the rate of FPOM production between the amphipod species reduces while the degree to which the resource quality treatments differ becomes greater. We further show that the survival of amphipods varied with respect to the composition of their leaf diet, water temperature and their size, with the effect of the latter differing with respect to temperature. For example, the risk of amphipod mortality when provided with a poor nutritional resource (oak) was higher than amphipods provided with a better quality resource (alder or sycamore) (Figure 4.1) and while larger amphipods were at an increased risk of mortality at 14°C, at 20°C this effect was reversed with smaller amphipods having an increased risk (Figure 4.5).

Our finding that the three amphipod species undertake detrital processing at significantly different rates provides further insight into the current debate surrounding the likely effects of invasive non-native species on ecosystem functioning. In accord with Kenna *et al.* (2016) we find that detrital processing is undertaken at a higher rate by native *G. pulex* than by *D. villosus*. We develop on the work of Kenna *et al.* (2016) by including an additional UK non-native *Dikerogammarus* species, *Dikerogammarus haemobaphes* and accounting for different leaf species diets, where the original authors only provided one diet (alder leaves). We further develop the work of Kenna *et al.* (2016)

and other previously published literature (e.g. MacNeil2011,Truhlar2014,Constable2016) through the quantification of both the loss of CPOM and the generation of FPOM.

We had hypothesised that differences in resource quality, through the three leaf diets, would result in changes in the rate of leaf shredding behaviour (H_1). Specifically, we hypothesised that amphipods provided with higher quality resource diet would consume more of that resource, so as to capitalise on its availability, and therefore produce FPOM at an increased rate. Our analyses suggest that this is indeed the case, with the rates of FPOM creation being highest for amphipods on alder leaf diets and lowest for those on oak diets. The detrital matter stoichiometry is likely to be a key determinant of food quality, in addition to the physical characteristics of the leaves such as toughness. Carbon, nitrogen and phosphorus are three of the most prominent chemical elements essential for life. For example, phosphorus is often a limiting factor in the rate of primary production (Schindler 1977) and Frost (2002) show that mayflies show decreased growth rates when provided with a phosphorus deficient diet. Plant stoichiometry is known to vary between species and, to a lesser degree, within species subject to factors such as environmental conditions and plant age (Ågren & Weih 2012).

Alder leaves commonly have high nitrogen content due to their nitrogen fixing fungal symbionts, which can make them a popular resource in an otherwise nutrient poor allochthonous diet (Webster & Benfield 1986). Our analyses show that the alder leaves did indeed have the lowest C:N ratio, meaning a greater proportion of nitrogen and therefore a better quality resource (Wurzbacher *et al.* 2016), however, they also had the lowest phosphorus content of the three leaf species. Oak leaves are a physically tough leaf resource and commonly contain high levels of tannins which can make them a suboptimal food resource (Gulis *et al.* 2006; Foucreau *et al.* 2013). Our analyses show that these leaves also had a higher C:N ratio and an average total phosphorus content, showing they are also a suboptimal nutrient source. However, Foucreau *et al.* (2013) suggest that oak leaves fill an important role in detrital litter as their slower breakdown provides a long lasting nutritional resource in the winter, when the majority of the other detrital matter has been processed. Therefore, the inclusion of resources of varying quality, in studies of this type, is essential in furthering our understanding as to the interactions of

changing climatic conditions and invasive non-native species. In addition to

Amphipod survival varied with resource quality however, survival between the three amphipod species did not differ. Specifically, amphipods provided with a high quality resource (alder) diet had the highest chances of survival whereas amphipods supplied with a poor quality resource (oak) had the lowest chances of survival. While leaf stoichiometry is known to vary between species, ratios will also vary within species subject various factors, such as environmental conditions and/or climate change (Ågren & Weih 2012; Yuan & Chen 2015). This could result in changes in resource quality for freshwater systems that are commonly reliant on allochthonous material due to many being net heterotrophic (Marcarelli *et al.* 2011). Our results suggest that resource quality impacts amphipod survival and any changes in resource quality would also be likely to impact freshwater communities. However, the fact that native and invasive non-native amphipods did not differ in their survival suggests that changes in resource quality may not offer either species a competitive advantage.

Contrary to our expectations, we found no difference in survival between the three amphipod species. This is in contrast to findings presented by Kenna *et al.* (2016) that suggest a difference in survival between native *G. pulex* and *D. villosus* as temperature treatments increase. It is possible this is due to the difference in experiment length between the two studies with amphipod survival observed over 72 hours by Kenna *et al.* (2016) and 14 days this study. Our finding suggests that at high temperature extremes, native *G. pulex* may be impacted less than was predicted and that the current dynamic between native *G. pulex* and invasive *Dikerogammarus* species would be expected to continue. Ultimately, this is likely to result in the continued spread and domination of both *Dikerogammarus* species which will, subsequently, result in the decline of native *G. pulex*.

Here we have provided evidence that the rates of FPOM generation varied between the three amphipod species, with respect to their leaf species diet and the water temperature. At the lower temperature treatments *G. pulex* produces FPOM at a higher rate than both *Dikerogammarus* species, which perform similarly (Figure 4.1). As the temperature increases we show that the difference in FPOM production between-species

is reduced, with the three amphipod species producing FPOM at similar rates at 20°C, and the differences in FPOM production between the three leaf species diets becoming larger, with amphipods provided with a diet of alder leaves producing FPOM at higher rates than those of sycamore, with both producing more than those on diets of oak leaves (Figure 4.1). While outside the temperature range addressed in this study, these results are in contrast to those provided by Truhlar *et al.* (2014) who showed that at higher temperature extremes (25°C) the rate of leaf shredding (CPOM loss) varied between native (*G. pulex*) and invasive (*D. villosus*) amphipods, while no such difference was seen at lower temperatures. Similarly, Kenna *et al.* (2016) also provide evidence that, while *G. pulex* and *D. villosus* show different detrital processing rates, the difference between the two species as temperature increases remains consistent. Based on our findings, we predict that whilst replacement of *G. pulex* by *D. villosus* and *D. haemobaphes* may lead to reduced FPOM availability at lower temperatures, this effect may be ameliorated by increased temperatures which could be predicted under climate change. Specifically, in warmer water bodies, the two *Dikerogammarus* species are predicted to break down detrital leaf matter at similar rates to native *G. pulex*. Furthering this study to include a wider thermal range or short-term extreme climatic events could shed further light on these suggested differences.

We have provided a *per-capita* quantification of detrital processing rates that show a species difference. While we did not find a difference in the rates of amphipod survival, the densities of amphipods are known to vary substantially with invasive non-native species often reported to reach higher densities than their native counterparts. For example, a species numerical response is only one constituent part of the species overall impact potential, only when combined with a *per capita* measure of resource use (e.g. functional response) does this measure truly reflect the species potential realised impact (Dick *et al.* 2017b, e.g.). Due to the variable nature of many invasive non-native species populations and the current lack of data regarding the densities of both *Dikerogammarus* species within the UK, we make no effort to account for varying densities. It should be noted that a species numerical response would likely impact the *per capita* differences reported here by orders of magnitude. Here, we instead provide a *per capita* baseline for

the three amphipod species. In this study we selected only male amphipods, which have been shown to consume more resources than females (Dick & Platvoet 2000). This results in our estimates being a 'worst case scenario'. Further efforts to scale up the *per-capita* effect, presented here, to represent known field densities and account for the wider community impacts could allow for the impacts of the species to be calculated spatially and temporally. However, any such estimation would be reliant on the recorded population densities which currently are rarely available.

Due to the nature of our study, we have made no effort to account for the dynamic interactions that occur between the amphipod species under natural conditions, as well as between the detrital resources and other individuals in the community. For example, many studies have focussed on the predatory nature of *D. villosus* which could impact macroinvertebrate communities directly through predation (Dodd *et al.* 2014, e.g.) or indirectly through increased anti-predator behaviours (MacNeil *et al.* 2011, e.g.).

We have provided evidence that the impacts of invasive species and climate extremes, in addition to resource quality, can alter the rates of detrital processing in freshwater systems. We suggest that under current climate conditions, native *G. pulex* undertake significantly more detrital processing than the invasive non-native *D. villosus* and *D. haemobaphes*, but under future projected climate conditions between-species differences will reduce. We have further shown that the current differences between leaf species diets will increase, with overall rates of detrital processing also likely to increase, resulting in higher resource availability to the wider community. Scaling up from *per-capita* laboratory manipulations to larger scale field studies, is a key next step in understanding the complex impacts we can have on our natural world.

Chapter 5

Impacts of non-native aquatic amphipods on invertebrate community structure and ecosystem processes

5.1 Abstract

Invasive non-native *Dikerogammarus* species are recent arrivals in western Europe and are known to show different rates of detrital shredding and predation behaviours. The spread of the species has resulted in native species declines; however, to date, there remains a research gap as to how findings from *per-capita* laboratory microcosm experiments scale-up to large scale field studies. We hoped to assess how invasive non-native amphipods impacted the wider freshwater community through their omnivorous behaviours by measuring multiple community measures. To this aim, we quantified how native communities containing *Dikerogammarus villosus* and *Dikerogammarus haemobaphes*, changed in their community diversity and functioning measures at two different amphipod densities (high and low). We measured changes in macroinvertebrate communities (abundance, diversity and richness), primary production (biofilm mass and chlorophyll *a*), microbial activity (cotton strip breakdown), and detrital processing (leaf breakdown) in a field mesocosm experiment. We show that mesocosms containing *Dikerogammarus* species had different macroinvertebrate community compositions than non-invaded communities containing native amphipods and that, while not differing with respect to amphipod species, microbial breakdown rates were higher in mesocosms with a higher density of amphipods. Conversely, we found no evidence of differing leaf breakdown rates or changes in primary production with respect to either amphipod species or density treatments. We suggest that our study highlights the difficulties of scaling laboratory microcosm experiments to larger mesocosm studies, which may be more suitable for identifying wider community impacts.

5.2   Introduction

Invasive non-native species have been shown to alter native communities through direct and indirect effects including predation (e.g. Doherty *et al.* 2016), herbivory (Gandhi & Herms 2010; Tanentzap *et al.* 2009), competition (Dangremond *et al.* 2010) and parasitism (Dunn & Hatcher 2015). These effects are likely to be at the root of declines in biodiversity throughout the invaded range (Bellard *et al.* 2016; Sorte *et al.* 2013). Invasive non-native species can also initiate community change though interrupting or modifying key functional behaviours, such as the breakdown of detrital matter and the subsequent release of nutrients, which can have far reaching impacts by altering trophic linkages and energy flow (Gallardo *et al.* 2016). Invasive non-native omnivores have the potential to impact native communities via acting as predators, detrivores and the relative dominance of these two behaviours, for example by undertaking more predatory behaviours than their native counterparts.

The majority of temperate freshwater systems are net heterotrophic (Marcarelli *et al.* 2011) and rely on the nutritional input from allochthonous sources, with the largest of these inputs being windfall leaves in autumn (Cummins *et al.* 1989), which makes detritivory an important functional behaviour. After entering a freshwater system, the leaves are colonised by bacterial and fungal biofilms and the initial stages of their breakdown begins. These microbial biofilms also provide a valuable nutritional resource for macroinvertebrate detrital shredders in the community, such as amphipods (Graca *et al.* 2001). Macroinvertebrate shredders are the responsible for the next stage of the breakdown process and convert the coarse leaf matter (CPOM), which is a largely inaccessible resource to the majority of the freshwater community, into fine particulate organic matter (FPOM) that is a usable nutrient resource. It is for this detrital processing behaviour that macroinvertebrate shredders are important members of freshwater communities and are responsible for the vast majority of detrital processing. For example, Cuffney *et al.* (1990) showed that macroinvertebrate leaf shredders were responsible for 50-74% of leaf processing, with their exclusion resulting in a 33% decline in FPOM. Species invasion events that interrupt or modify key functional behaviours,

such as detritivory, are likely to impose higher impacts on the wider native community than those invasions that have no effect on such processes. Invasive non-native *Dikerogammarus* sp. are one group of freshwater amphipods that are likely to impose significant impacts on native communities through displacing native amphipods, altering trophic linkages, and disrupting the freshwater detrital processing pathway. Importantly, *Dikerogammarus* sp. are omnivorous and are therefore able to consume detrital leaf matter and native detritivores (for example MacNeil *et al.* 2011; Kenna *et al.* 2016, Chapter 4). The omnivorous behaviour of the *Dikerogammarus* species could impacts communities via multiple routes. For example, as previously mentioned, many members of freshwater communities rely on other species to breakdown the course allochthonous detrital matter entering the system to release essential nutrients. A reduced rate of this breakdown could impact the community through a trophic cascade via reduced primary production (due to limiting resource availability stemming from reduced detrital breakdown).

Previous work has shown that native and invasive non-native amphipods can show different *per capita* rates of detrital processing (for example MacNeil *et al.* 2011; Constable & Birkby 2016, Chapter 4). However, changes in a species numerical response, whereby a species changes in density with respect to the availability of a specific resource, have the potential to increase any *per capita* impacts by many orders of magnitude (Dick *et al.* 2017b). Understanding how these known *per capita* differences are realised, in terms of their impact on the wider ecological community, in complex systems is essential to furthering our understanding the risks posed by omnivorous *Dikerogammarus* species. The amphipod densities used within this study are lower than would be expected in natural systems and could make identifying density-dependant effects difficult, they were chosen due to high rates of predation by the *Dikerogammarus* species which have been previously documented (Dick *et al.* 2002; Bovy *et al.* 2015). While using higher densities of amphipods would likely be more representative of field densities, the high rate of predation imposed on the macroinvertebrate community could have likely resulted in the complete consumption of many species.

Gammarus pulex is a common freshwater amphipod, and keystone species for its

detrital processing behaviour, throughout Europe. In recent years, the arrival and subsequent spread of the invasive amphipods *Dikerogammarus villosus* and *Dikerogammarus haemobaphes* have led to the declines of native macroinvertebrates (Dick *et al.* 2002; Boets *et al.* 2010) and many other taxa in the freshwater communities, including predation of fish eggs (Taylor & Dunn 2016; Casellato *et al.* 2007), and the replacement of native amphipods including *G. pulex* (Dick & Platvoet 2000; Rewicz *et al.* 2014). *Dikerogammarus* sp. may also affect detrital processing as research suggests both species undertake detrital leaf shredding at slower rates than native *G. pulex* (MacNeil *et al.* 2011; Jourdan *et al.* 2016; Piscart *et al.* 2011; Constable & Birkby 2016; Kenna *et al.* 2016, Chapter 4). *Dikerogammarus* sp. will also undertake more predation than *G. pulex*, including predation of other shredding macroinvertebrates (Dodd *et al.* 2014; MacNeil *et al.* 2011). Both *D. villosus* and *D. haemobaphes* are now present at multiple sites within the UK with *D. villosus* being first recorded in 2010 and *D. haemobaphes* in 2012. Despite the impacts of these invasive non-native amphipods being relatively well documented through laboratory experiments, their impact on the wider community, including primary production and community composition remains poorly understood.

We aimed to quantify the effects of the invasive non-native *D. villosus* and *D. haemobaphes* amphipods on detrital processing, invertebrate community structure and community function through use of a mesocosm experiment to explore how the small scale interactions observed in laboratory scale studies scale-up to the community level. We subjected mesocosms containing native macroinvertebrate communities to one of three amphipod species treatments; native *G. pulex*, non-native *D. villosus* or non-native *D. haemobaphes* to quantify the impact of species invasions on native community function. In an effort for further our understanding as to how differences in an individuals *per capita* rate of resource processing may scale-up to small communities (Laverty *et al.* 2017b, for example), we also used two amphipod density treatments; high (12 amphipods per experimental mesocosm), or low (6 amphipods per experimental mesocosm).

We hypothesised that (H$_1$) the invasive non-native amphipod species (*D. villosus* and *D. haemobaphes*) would consume less CPOM than native amphipods (*G. pulex*),

consistent with previous laboratory studies (Piscart *et al.* 2011; Constable & Birkby 2016; Kenna *et al.* 2016), we also expected that this effect would vary with amphipod density, with the effect being approximately additive, with more amphipods breaking down more CPOM.

We further hypothesised that (H$_2$) the abundance and diversity of macroinvertebrates would be higher in native amphipod treatments as evidence suggests the non-native *Dikerogammarus* sp. are more predatory than the native *G. pulex* (for example MacNeil *et al.* 2011). We also expected the density of amphipods to impact native macroinvertebrates, but that this would likely be species specific with less mobile individuals being particularly at risk from the fast moving *Dikerogammarus* species (Boets *et al.* 2010). In the non-native amphipod treatments, we expected a higher density of amphipods would result in reduced biodiversity and abundance whereas native amphipod treatments were expected to show an opposing relationship, with higher density treatments showing higher biodiversity and abundance values. We suggest that native *G. pulex* would undertake more detrital processing behaviour than the two invasive *Dikerogammarus* sp. which could result in increased resource availability for the wider community. Conversely, in *Dikerogammarus* sp. treatments we expected there would be less resource availability, due to less detrital processing behaviour, and an increased predatory pressure imposed by the invasive amphipods.

Lastly, we hypothesised that (H$_3$) due to expected differences in detrital processing rates, invasive non-native amphipod treatments, which were expected to produce less FPOM, which would result in lower nutrient availability, will be associated with less microbial activity (biofilm biomass and cotton strip tensile strength) and primary production (chlorophyll *a* concentration) than native amphipod treatments.

Through testing of these hypotheses we aimed to understand how invasive non-native *Dikerogammarus* species impact community macroinvertebrate species diversity and richness, primary production (through measuring microbial biofilms), leaf breakdown rates (through use of leaf packs), and microbial detrital processing rates (through the use of cotton strips) in comparison to communities containing only native amphipods. This would inform, not only our understanding of the current impacts of *D. villosus* and *D.*

haemobaphes throughout Europe but also the potential impacts of future invasive non-native omnivores.

5.3 Materials and methods

5.3.1 Animal husbandry

We collected native *G. pulex* from Meanwood Beck, Leeds (53°83'N; -1°58'W) and invasive non-native *D. haemobaphes* from the Leeds-Liverpool canal, Saltaire (53°84'N; -1°80'W) using standard kick sampling methods. Invasive non-native *D. villosus* were collected from artificial substrate at Grafham Water, Cambridgeshire (52°29'N; -0°32'W), UK. We collected macroinvertebrates from non-invaded freshwater communities at Meanwood Beck, Leeds (53°83'N; -1°58'W), Golden Acre Park, Leeds (53°87'N; -1°59'W), and Wothersome Lake, Leeds (53°87'N; -1°59'W) using standard kick sampling methods. Following collection, we kept animals in control conditions of 14°C 12:12 light:dark (L:D) cycle in oxygenated five litre tanks of dechlorinated tap water.

5.3.2 Leaf material

We collected windfall *Alnus glutinosa* (European alder) leaves at the University of Leeds, Leeds (53°80'N; -1°55'W) and air-dried them at room temperature. Dry leaves were weighed and used to create 10 g coarse mesh leaf packs (10 mm aperture). Before use, we conditioned leaf packs in stream water, collected from Meanwood Beck, for 14 days at 14°C and 12:12 L:D.

5.3.3 Experimental mesocosms

Experimental mesocosms were positioned at Spen Farm, University of Leeds (53°86'N; -1°34'W) and consisted of a 75 litre bucket (XL Gorilla tub, depth = 37 cm, diameter = 57 cm and filled volume of 0.076 m^3) with a layer (approximately 5 cm) of 20 mm gravel

(Diall brand) to provide refugia and habitat heterogeneity. Mesocosms were seeded with 3 litres of source water from each of the three macroinvertebrate sample sites (Golden Acre Park, Meanwood Beck and Wothersome Lake), totalling 9 litres of source water per mesocosm to introduce microbial and fungal species that would otherwise occur in the water column, and filled with local bore hole water. Each mesocosm contained four stone tiles (23 mm x 23 mm), for biofilm growth, one cotton strip, prepared as described by Tiegs *et al.* (2013), to measure microbial breakdown rates, and a coarse mesh leaf pack, to quantify detrital processing of the community (CPOM loss). The use of cotton strips has been shown to be an effective measure of organic matter decomposition primarily through microbial and fungal detritivory (Clapcott & Barmuta 2010a; Tiegs *et al.* 2013), although, Clapcott & Barmuta (2010b) do report instances of macroinvertebrates consuming cotton strips in carbon limited environments. We introduced a standardised macroinvertebrate community into each mesocosm which was representative of the local freshwater communities sampled and contained; 18 *Asellus aquaticus*, eight cased caddis larvae (*Trichoptera*), 10 nematodes (*Nematoda*), five mayfly (*Ephemeridae sp*), 70 planarian worms (*Planariidae sp*) and five spire shell snails (*Potamopyrgus sp*). These species represent a range of BMWP scores which range from pollution-tolerant (lower BMWP scores) and pollution-sensitive (higher BMWP scores) species (Paisley *et al.* 2014). We arranged 48 mesocosms in a randomised block design, with each block containing one replicate of each experimental treatment, which resulted in each amphipod species-density treatment being replicated eight times.

5.3.4   Experimental methods

Once filled with water, we allowed the mesocosms to acclimatise for five days before the macroinvertebrate communities were introduced. We then left the mesocosms, now containing the macroinvertebrate communities, to reach equilibrium for a further 20 days before the amphipod treatments were added. Prior to the amphipod treatments being added, we randomly sampled two of the tiles from each mesocosm to quantify biofilm mass and chlorophyll *a* content before the treatments were applied. We subjected each mesocosm to one of three amphipod species at one of two densities; high (12 individuals

per experimental mesocosm) and low (6 individuals per experimental mesocosm). The experiment ran from October to December. Due to the predation risk posed by *D. villosus* and *D. haemobaphes* to the macroinvertebrate community (Dick *et al.* 2002; Bovy *et al.* 2015), the amphipod treatments were in the mesocosms for 14 days.

We selected one mesocosm, at random, from each block that had water temperature recorded by three TinyTag data loggers positioned through the water column (top, middle and bottom) so as to identify/quantify any thermal stratification. We shielded the uppermost logger from direct sunlight by a section of opaque plastic pipe (white, approximately 6 cm in diameter and 16 cm in length). We measured the water temperature and dissolved oxygen (DO_2) concentration, at three depths (top, middle and bottom of the water column) in every mesocosm, each week using a YSI Environmental ProODO probe. The measuring of temperature and DO_2 enabled us to quantify the degree to which DO_2 was stratified by depth in the mesocosms and compare the temperature logger measures of temperature with those of the other mesocosms.

At the end of the experiment, we emptied the mesocosms. The leaf packs were removed and stored in 70% ethanol so as to stop microbial and fungal breakdown. The macroinvertebrates were sampled by filtering the water and rinsing the gravel substrate through a muslin cloth and stored in 70% ethanol. The remaining two tiles were removed and stored at -20°C to stop biofilm growth and degradation.

5.3.5   Organic matter decomposition

We washed the cotton strips in 80% ethanol to halt microbial and fungal activity, after they were removed from the mesocosms, before drying and storing them in a desiccator (Tiegs *et al.* 2013). We determined the maximum tensile strength of each cotton strip using an Instron Universal Strength Tester at a rate of 20 mm min^{-1}. The tensile strength of cotton strips can be used as a measure of the cellulose decomposition potential via microbial and fungal activity (Clapcott & Barmuta 2010b,a; Tiegs *et al.* 2013).

5.3.6 Leaf breakdown

We searched the leaf packs for additional macroinvertebrates, which were added to the existing macroinvertebrate samples from the mesocosm, before drying the remaining leaf matter. Initially, we calculated dry mass (DM) of the remaining leaf matter (unused CPOM) by oven drying the leaves at 105°C for 24 hours. We then calculated the ash free dry mass (AFDM), to quantify the the mass of the organic matter in the sample, by ashing the leaf samples at 500°C for four hours in a muffle furnace (Hauer & Lamberti 2007). The use of AFDM overcomes the risk of the leaf samples containing non-organic contaminants form the mesocosms (e.g. sand).

5.3.7 Microbial biofilm mass and chlorophyll *a* content

We removed the biofilm from the upper surface (529 mm^2) of the tiles using a scalpel and a plastic brush in deionised water to create a 50 ml solution of suspended biofilm (Hauer & Lamberti 2007). We filtered a 5 ml aliquot of this suspension through a 0.45 μm nylon filter (Sigma Aldrich) and determined the chlorophyll *a* concentration by absorbance spectroscopy using standard methods (Steinman *et al.* 1996; APHA 2005). The remaining biofilm suspension (45 ml) was filtered through a pre-weighed 0.7 μm (Watman GF/F) filter and oven dried before being weighed to give the mass of the biofilm (Hauer & Lamberti 2007).

5.3.8 Statistics

All statistical analyses were undertaken in R version 3.3.2 and RStudio version 1.0.136 (R Core Team 2016; RStudio 2016). The lme4 package was used for linear mixed effects models, the vegan package was used for NMDS and PERMANOVA analyses and betapart package was used for β diversity calculations. In the β diversity analyses, the null model contained only the mesocosm block term. In all other analyses, the null model contained only the mean value of the response variable. Unless otherwise stated, sets of 5 candidate models were generated using single and interaction terms of

amphipod species and amphipod density terms. All models, including the null model, contained a mesocosm block random effect term. The 'best fit' model was chosen as having the lowest second-order Akaike information criterion (AICc) value and other notable good candidate models were defined as having an AICc score within 2 AICc points of the 'best fit' model.

5.3.8.1 Temperature and DO_2

We tested for thermal and DO_2 stratification, in the subset of mesocosms with TinyTag loggers, by creating a set of five candidate models containing combinations of single and interaction terms of date and position in the water column terms. Each candidate model had a nested random effect of mesocosm in experimental block. We compared the temperatures from the TinyTag loggers to the weekly temperature measures by comparing temperatures from both methods to a null model by AICc scores. We also tested for any differences in temperature between experimental treatment combinations by using the same statistical methods. All models had a nested random effect of mesocosm in experimental block.

5.3.8.2 Macroinvertebrate detrital processing

To identify differences in detrital processing, we compared the loss of CPOM and the remaining mass of CPOM (AFDM) in the mesocosms against the amphipod species and density terms using linear mixed effects models.

5.3.8.3 Macroinvertebrate community composition

We compared the Shannon and Simpson diversity indices in addition to species richness and the total number of macroinvertebrate individuals using linear mixed effects models. We also used non-metric multidimensional scaling (NMDS) with Bray-Curtis distance, to identify clustering of mesocosm macroinvertebrate communities. We compared pairwise mesocosm macroinvertebrate β diversity, using Bray-Curtis distances, with PERMANOVA analyses.

5.3.8.4 Microbial biofilm mass, cotton tensile strength and chlorophyll *a* content

We compared the change in AFDM and chlorophyll *a* content over the treatment period using linear mixed effects models with a nested mesocosm-block random effect. The maximum tensile strength of the cotton strips was tested using linear mixed effects models.

5.4 Results

5.4.1 Temperature and DO_2

Mesocosm water temperature, across each of the mesocosm depths, varied over the full duration of the experiment from 0.012 to 12.880°C (mean ($\pm$ SE) = 4.814°C ($\pm$ 0.004)). Our weekly measures of water temperature were representative of the continuously recorded (temperature logger) water temperature measures recorded in the subset of mesocosms (R^2 = 0.996). The inclusion of a depth term did not improve the null model of DO_2 concentration or water temperature, suggesting there is no evidence of thermal or DO_2 stratification in the mesocosms. Both DO_2 and water temperature were shown to vary over the course of the experiment as the week term significantly improved both models (Δ AICc = -644.97 and -371.38) (Figures 5.1 and 5.2). As the experiment continued, the amount of DO_2 increased by an average of 1.044 mg/L per week and while mesocosm temperatures fluctuated throughout the experiment overall they decreased by an average of -1.3°C each week (Figures 5.1 and 5.2). However our analyses suggest there was no difference in water temperature between the amphipod species-amphipod density treatments over the duration of the experiment as the 'best fit' model contained only a week term (*Delta* AICc = 0.00), denoting the week of the experiment (amphipod density term; *Delta* AICc = 2.01 and amphipod species term; *Delta* AICc = 4.03).
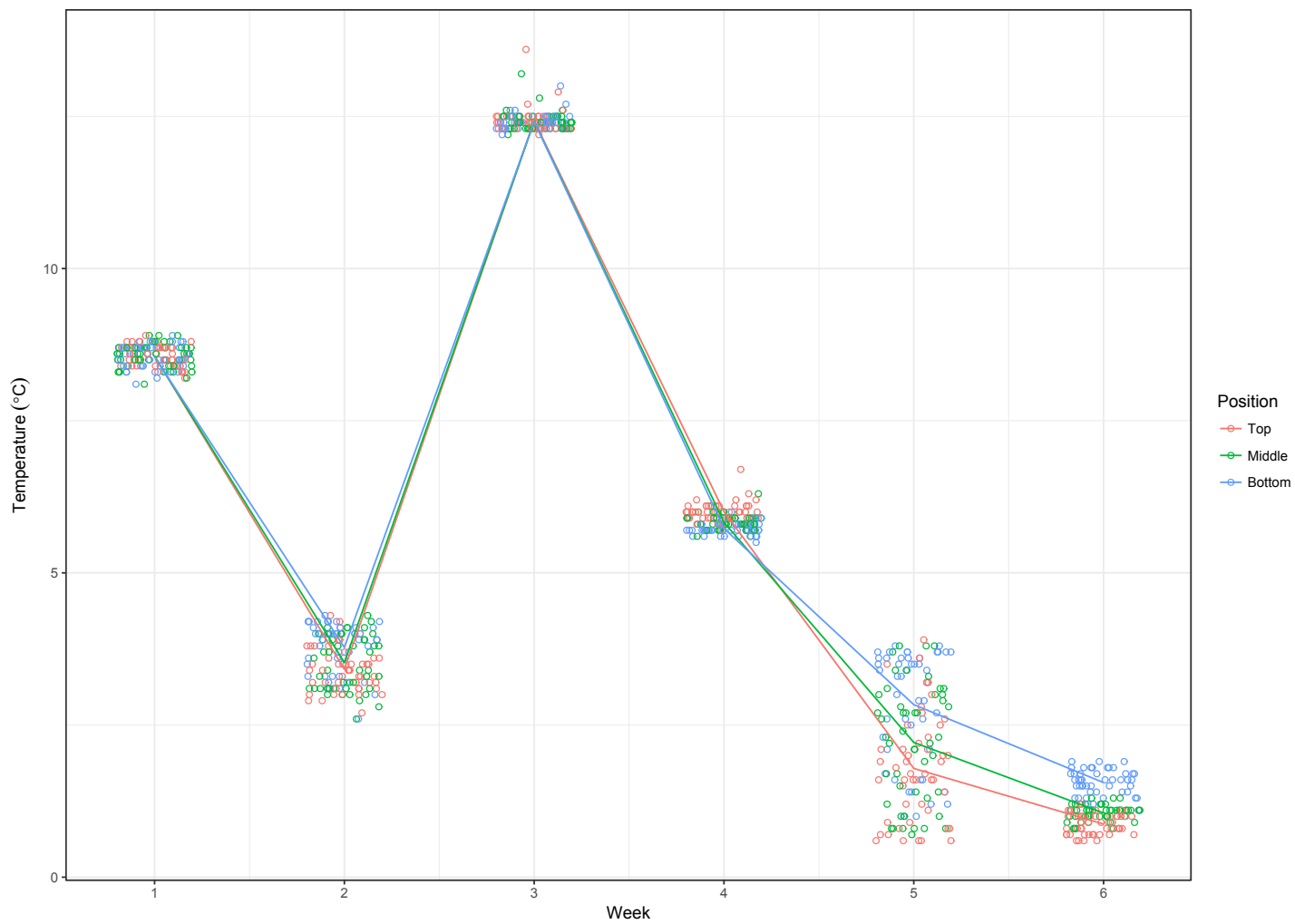
Figure 5.1: Change in mesocosm water temperature (°C) over the experiment, as measured weekly using a YSI Environmental ProODO probe. Points represent weekly mesocosm measurements with colours signifying the depth of the measurement (red = top, green = middle and blue = bottom). Trend lines, passing through the mean temperature value of each week, for each water column position are also shown. Mesocosm data points are jittered on the x axis to facilitate interpretation.

Figure 5.2: Change in mesocosm dissolved oxygen (DO$_2$ mg/L) over the experiment, as measured weekly using a YSI Environmental ProODO probe. Points represent weekly mesocosm measurements with colours signifying the depth of the measurement (red = top, green = middle and blue = bottom). Trend lines, passing through the mean temperature value of each week, for each water column position are also shown. Mesocosm data points are jittered on the x axis to facilitate interpretation.
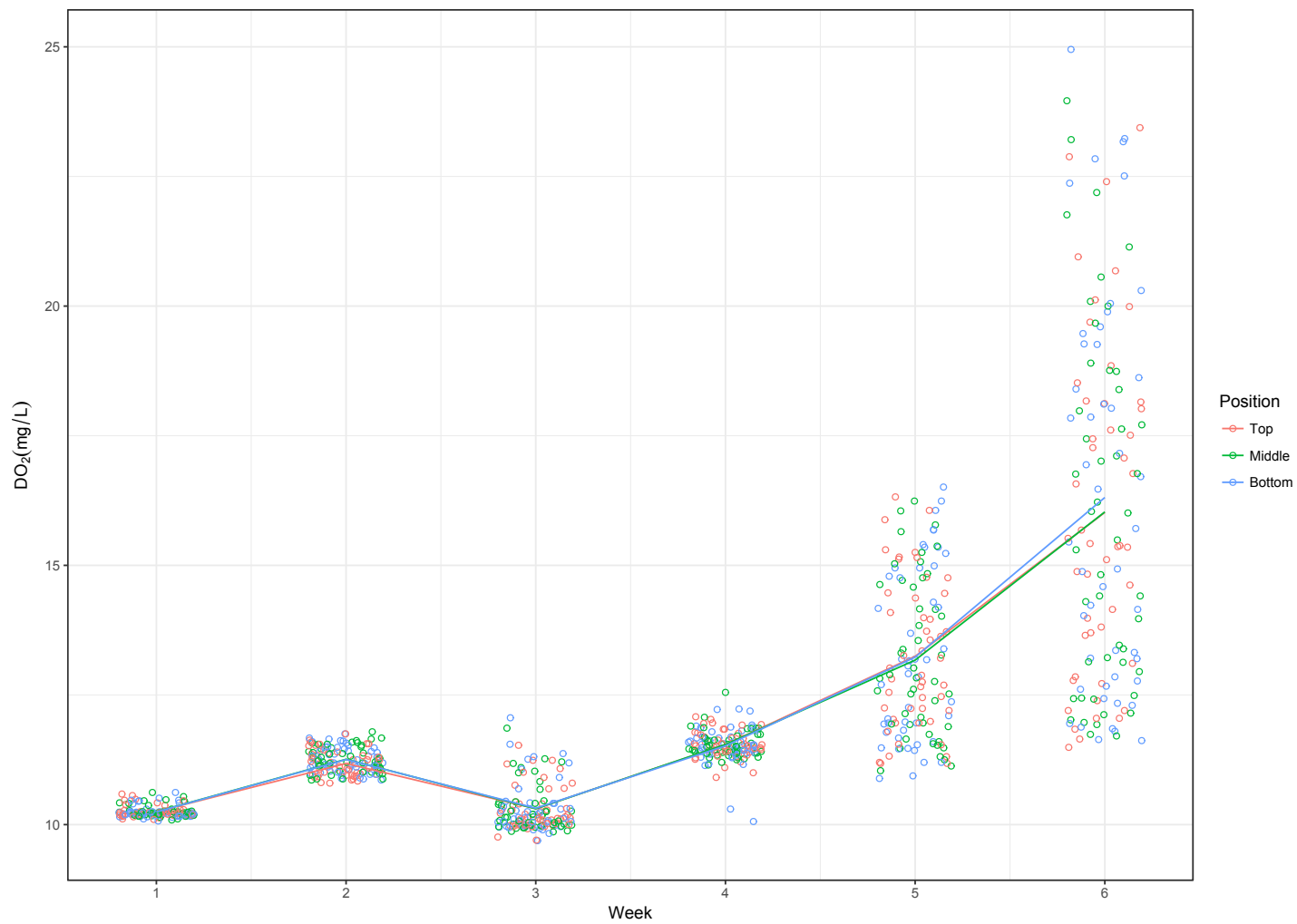
5.4.2   Macroinvertebrate detrital processing

Comparison of candidate models of the AFDM of consumed CPOM suggested that neither the amphipod species nor amphipod density terms improved the null model (Δ AICc = +3.06 and +2.33). The null model was ultimately the 'best fit' model, which suggests there was difference in detrital processing between the mesocosm treatment combinations. Overall, the loss of CPOM was low (mean loss (DM) ($\pm$ SE) = 3.176 g ($\pm$ 0.067)) (Figure 5.3).

5.4.3   Macroinvertebrate community composition

Macroinvertebrate species diversity and the total number of macroinvertebrate individuals was higher in the native *G. pulex* treatments than the invasive treatments. The amphipod species term significantly improved the null models for Shannon and Simpson diversity indices and the total number of species analyses (Δ AICc = -4.36, -5.03 and -0.47) (Table 5.2 and Figure 5.4). For both Shannon and Simpson diversity indices the 'best fit' model contained only an amphipod species term, with mesocosms subject to the native *G. pulex* having higher diversity values (1.507 and 0.741). The 'best fit' model for the total number of macroinvertebrate species also contained only an amphipod species term, with invasive *D. haemobaphes* having an average total number of individuals of 29, which was greater than both *G. pulex* (28) and *D. villosus* (24). There was an indication that density could also have an effect on the Simpson diversity index as a model with both amphipod species and amphipod density terms was also a good candidate model (Δ AICc = +0.87). Analysis of the total number of macroinvertebrate individuals suggested that the null model was also a good candidate model (Δ AICc = +0.47). The 'best fit' model for species richness was the null model however, another model with an amphipod density term was also a good candidate model (Δ AICc = +1.67). β diversity did not differ between treatments, as neither the amphipod density or amphipod species terms improved the null model, the 'best fit' model was the null model. NMDS clustering showed no separation between the amphipod density and species treatments (Figure 5.5). This suggests that the macroinvertebrate communities
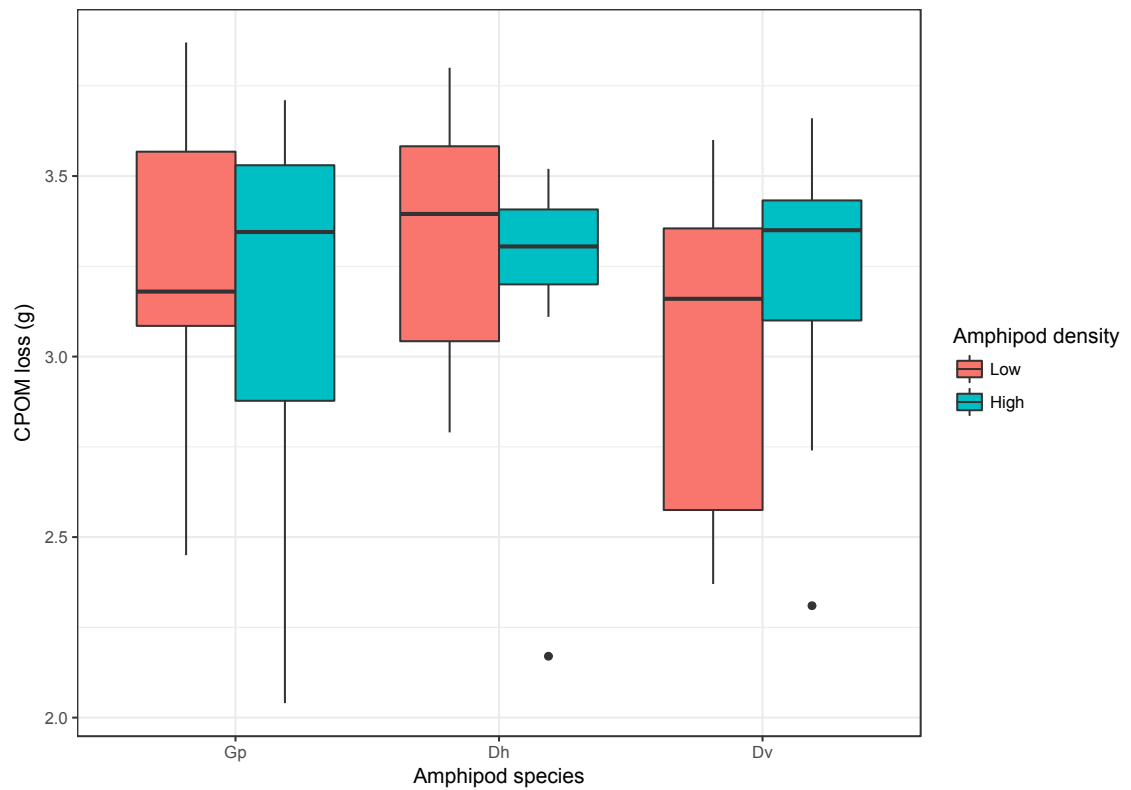
Figure 5.3: Mass of CPOM loss from the mesocosms leaf packs, as a measure of detrital leaf matter processed, in each of the mesocosms subject to one of three amphipod species treatments (native *G. pulex* (Gp) or invasive non-native *D. haemobaphes* (Dh), and *D. villosus* (Dv)) and at one of two amphipod density treatments (Low (red, 6 individuals) and High (blue, 12 individuals)). Overall, there was no evidence of a significant difference between the detrital processing rates of either the amphipod species of density treatments.

Table 5.1: Second-order Akaike Information Criterion scores (AICc) and associated weights (AICc Wt.) for each of the community functioning and diversity variables measured. The null model contained the mean response variable, denoted by '1'. 'Best fit' models, with the lowest AICc score are highlighted in bold to aid interpretation.

Model terms	Shannon			Simpson			Total individuals			Species Richness		
	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.
~1	-20.25	4.36	0.07	-136.48	4.54	0.05	343.88	0.47	0.34	**153.60**	**0.00**	**0.52**
~Species	**-24.61**	**0.00**	**0.65**	**-141.01**	**0.00**	**0.49**	**343.40**	**0.00**	**0.43**	155.75	2.15	0.18
~Density	-18.16	6.45	0.03	-135.52	5.49	0.03	346.24	2.84	0.10	155.27	1.67	0.23
~Species + Density	-22.36	2.25	0.21	-140.14	0.87	0.32	346.00	2.60	0.12	157.60	4.01	0.07
~Species * Density	-18.82	5.79	0.04	-138.08	2.93	0.11	350.66	7.25	0.01	162.33	8.74	0.01

Model terms	CPOM loss (mg AFDM)			Tensile strength (N)			Change in biofilm mass (mg)			Change in chlorophyll *a* (mg)		
	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.	AICc	Δ AICc	AICc Wt.
~1	**63.78**	**0.00**	**0.63**	441.59	1.01	0.33	**125.17**	**0.00**	**0.50**	**-260.47**	**0.00**	**0.51**
~Species	66.83	3.06	0.14	445.60	5.02	0.04	129.53	4.35	0.06	-258.33	2.13	0.18
~Density	66.10	2.33	0.20	**440.58**	**0.00**	**0.55**	125.59	0.42	0.40	-258.79	1.67	0.22
~Species + Density	69.39	5.62	0.04	444.74	4.16	0.07	130.16	4.99	0.04	-256.56	3.91	0.07
~Species * Density	73.36	9.58	0.01	450.01	9.43	0.00	135.58	10.40	0.00	-253.56	6.91	0.02

Table 5.2: Model outputs, containing the model coefficients and standard errors, for the measures of macroinvertebrate diversity (Simpson and Shannon diversity indices and the total number of macroinvertebrate individuals), microbial breakdown (maximum tensile strength of cotton strips (N)) and primary production (biofilm AFDM (mg)). Only models that were significantly better than the null model (with a lower AICc score) are shown. The biofilm mass model contained a mesocosm-block nested random effect while all other models contain a mesocosm block random term.

| Term		Macroinvertebrate diversity		Microbial detritivory	Primary production
	Simpson	Shannon	Total macroinvertebrates	Tensile strength	Biofilm mass
(Intercept)	0.765***	1.507***	28.187***	340.508***	2.414***
	(0.012)	(0.043)	(2.559)	(6.451)	(0.260)
Species: *D. haemobaphes*	−0.052**	−0.174**	1.063		
	(0.017)	(0.057)	(2.378)		
D. villosus	−0.045*	−0.135*	−4.312		
	(0.017)	(0.057)	(2.378)		
Density: Low				10.422	−0.187
				(5.533)	(0.120)
Log Likelihood	76.221	18.019	-165.986	-215.824	-234.433
Var: block (Intercept)	0.000	0.002	29.753	210.440	
Var: Residual	0.002	0.026	45.230	367.404	0.682
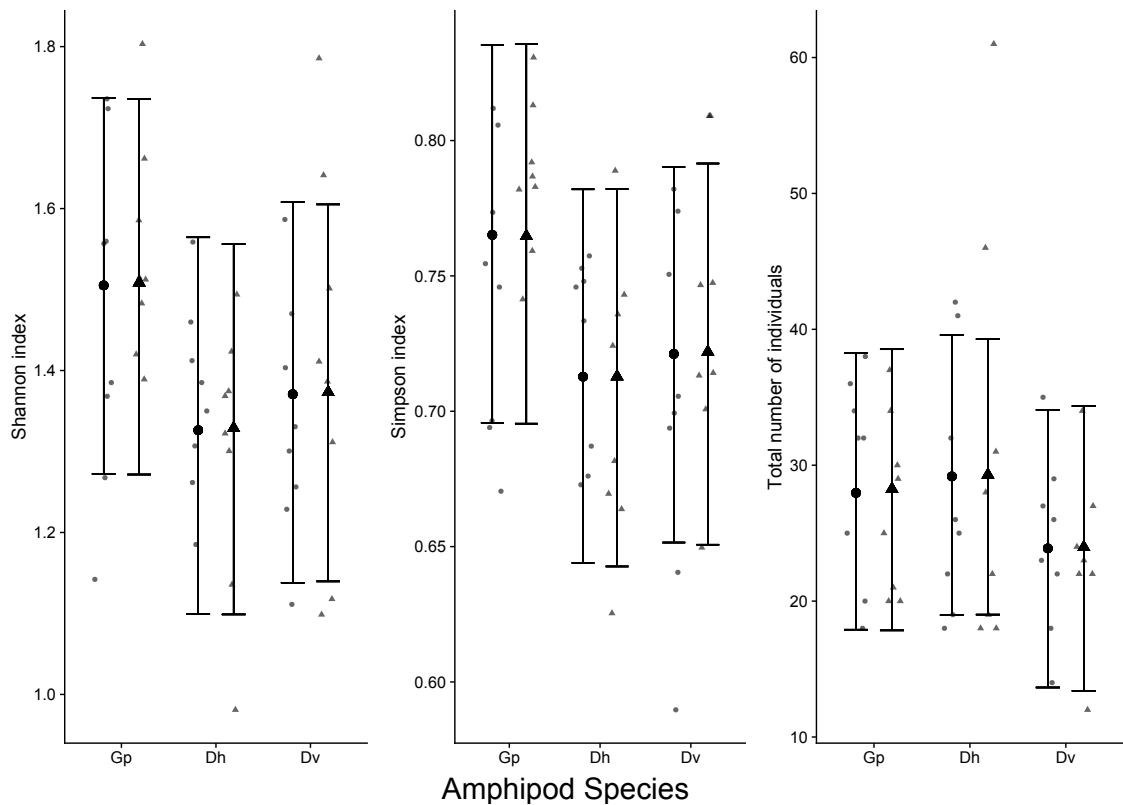
Figure 5.4: Macroinvertebrate community measures (Shannon and Simpson diversity indices and the total number of macroinvertebrates) with respect to each of the amphipod density and species treatments. Community measures for each mesocosm are shown as grey points for each of the amphipod species (x axis) and density (circles = high and triangles = low) treatments with the model predicted mean value for each treatment as a black point. Confidence intervals, calculation through permutation analyses (n = 999) are shown as black lines.

between the amphipod species and density treatments were not significantly different.

5.4.4 Microbial biofilm mass and chlorophyll *a* content

There was little evidence that biofilm mass changed between treatments (mean biomass (mg) = 2.79 ($\pm$ 0.06)). Neither amphipod species nor amphipod density terms improved the null model for the mean change in biofilm mass over the treatment period, however, there was some indication that an amphipod density term could explain some variation in this analysis (Δ AICc = +0.42). Consistent with our previous analyses, the higher amphipod density treatment resulted in a higher biofilm mass (AFDM). Another candidate model, containing an amphipod density term (Δ AICc = +1.61) was also indicated as being a good fit.

Similar to the analysis of biofilm mass, there was no evidence that biofilm chlorophyll
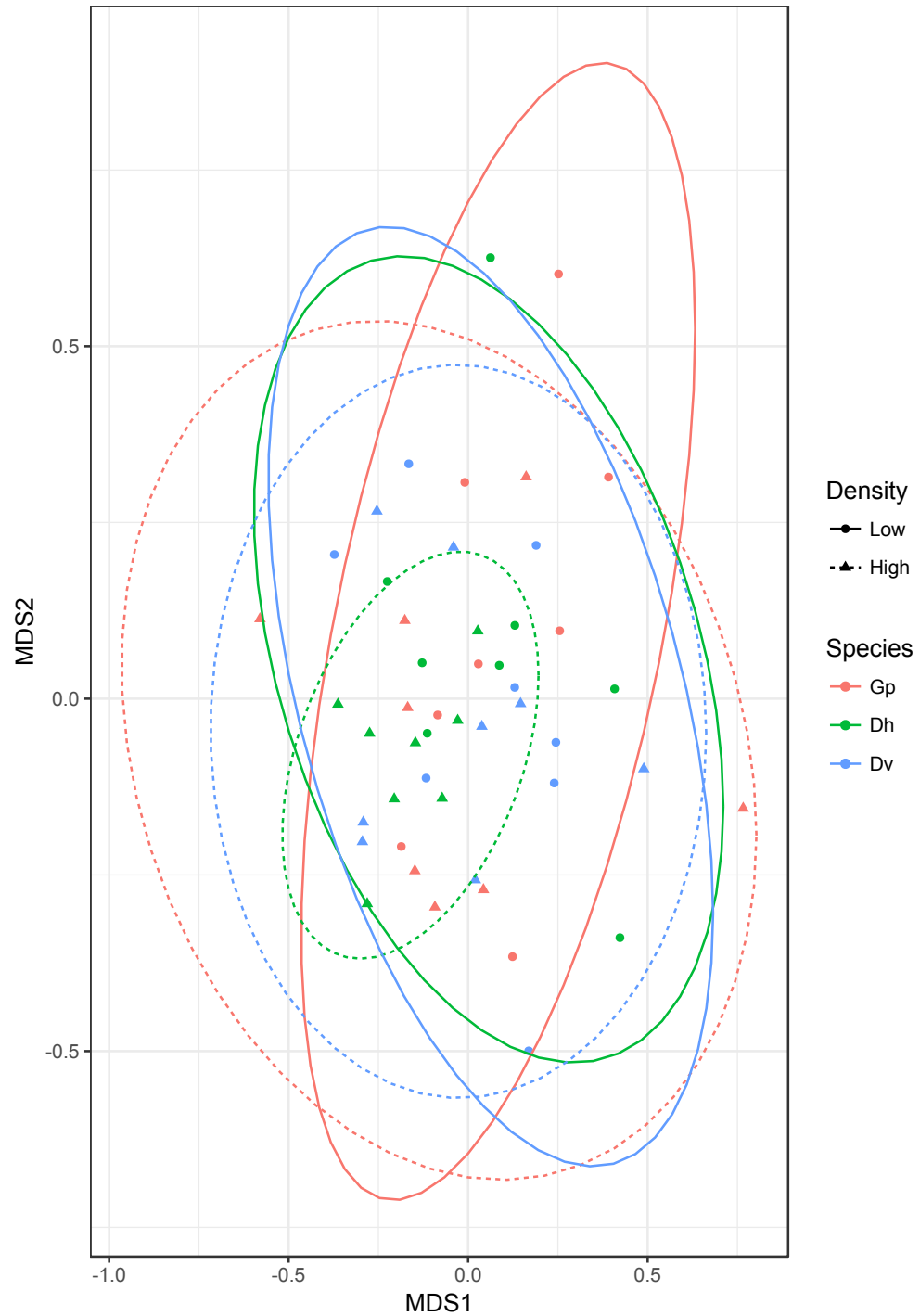
Figure 5.5: Non-metric multidimensional scaling (NMDS) ordination plot of the dissimilarity of macroinvertebrate communities, represented as NMDS scores 1 (NMDS1) and 2 (NMDS2). The lack of separation between the macroinvertebrate communities suggests the macroinvertebrate communities did not differ between the amphipod species and density treatments. Point shapes and line types represent amphipod densities (solid lines and circles = low, dashed lines and triangles = high) and point and line colours represent amphipod species (red = *G. pulex*, green = *D. haemobaphes*, and blue = *D. villosus*).
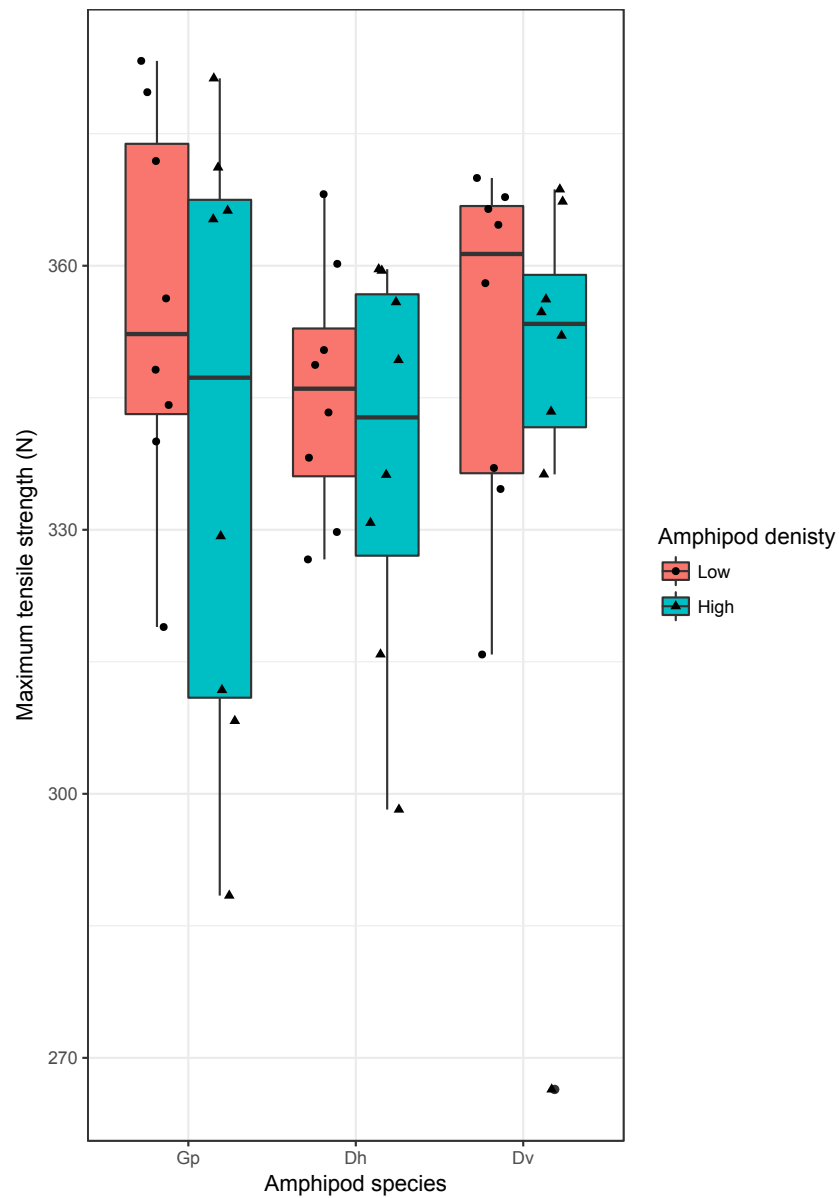
Figure 5.6: Maximum tensile strength (Newtons) of cotton strips, as a measure of microbial and fungal organic matter breakdown, in each of the mesocosms subject to one of three amphipod species treatments (native *G. pulex* (Gp) or invasive non-native *D. haemobaphes* (Dh) and *D. villosus* (Dv)) and at one of two amphipod density treatments; Low (6 individuals, red and circles) and High (12 individuals, blue and triangles)). Overall, the maximum tensile strengths of the cotton strips did not differ between the treatments.

a varied between treatments (mean chlorophyll *a* (mg) = 0.10 (± 0.01)) as neither amphipod species or density terms significantly improved the null model which resulted in the null model being the 'best fit' model.

The amphipod density term improved the null model for the maximum tensile strength of the cotton strips (Δ AICc = -1.01), although, the null model remained a good candidate model (Table 5.2 and Figure 5.6). Cotton strips from the low amphipod density treatment had a higher tensile strength (350.930 N) than the high amphipod density treatment (340.508 N) (Figure 5.6).

5.5   Discussion

We have presented evidence that communities containing invasive non-native amphipods differed in their macroinvertebrate community diversity when compared to those containing native amphipods . We have also shown that the density of amphipods resulted in different microbial breakdown rates. Mesocosms subject to the native amphipod treatment (*G. pulex*) had, on average, more diverse macroinvertebrate communities than those subject to experimental species invasions by *D. villosus* and *D. haemobaphes.* The breakdown rates of cotton strips by microbial detritivores, as shown by their maximum tensile strength, was higher in mesocosms with more amphipods, suggesting that more amphipod shredders resulted in more microbial breakdown of the cotton strips (Figure 5.6).

Contrary to our expectations, we found no effect of amphipod species or amphipod density on the detrital processing of leaf matter (CPOM). Previous studies have shown that both *D. villosus* and *D. haemobaphes* undertake detrital breakdown at slower rates than native *G. pulex*, instead favouring predatory behaviour (Piscart *et al.* 2011; Constable & Birkby 2016; Kenna *et al.* 2016, Chapter 4). Our experiment differs from many of these previous studies in that ours is a field mesocosm experiment and is using a small community of amphipods (6 or 12 individuals) rather than measuring a *per-capita* rate in a laboratory microcosm. We suggest that our findings could reflect the additional variation experienced outside of the controlled conditions of such environments.

Laboratory studies are often undertaken in controlled conditions and therefore lack natural perturbations in climatic variables (e.g. precipitation, temperature, and wind) that would otherwise be experienced in more complex ecological systems (Benton *et al.* 2007; Drake & Kramer 2012; Schindler 1998). Laboratory systems are commonly space limited and can therefore lack the ecological complexity present in field systems, something that can ultimately call the use of laboratory systems into question (Drake & Kramer 2012; Srivastava *et al.* 2004). Specifically, a greater diversity and complexity of species interactions are present within the mesocosms as amphipods and other macroinvertebrates are able to engage in within- and between-species interactions including, for example, competition for food and predation. Our measures of detrital processing in the mesocosms are an overall community measure and include the shredding behaviour of both the amphipods and the wider macroinvertebrate community (e.g. *A. aquaticus*) and therefore also account for any direct (predation) or indirect (anti-predator) impacts of the amphipod species. We therefore suggest that caution should be used in assuming that *per-capita* microcosm experimental results scale completely to community or ecosystem level but would rather be indicative of the overall relative difference in detrital breakdown.

We have shown that communities containing invasive amphipods had significantly lower macroinvertebrate diversity than those containing native amphipods. Field studies have yielded similar results with Dick & Platvoet (2000) showing that, in the Netherlands, *D. villosus* has replaced the dominant native amphipod (*Gammarus duebeni*) and another non-native amphipod (*Gammarus tigrinus*), which until the arrival of *D. villosus* had been dominant. Declines in native macroinvertebrate communities have been documented throughout the literature (Dick & Platvoet 2000; MacNeil & Platvoet 2005) however, as previously stated, much of the literature has focussed on *D. villosus* rather than *D. haemobaphes*. As we found no significant difference between the detrital processing rates of native and invasive amphipods, we are unable to attribute this change in biodiversity to changes in FPOM availability. Instead, we suggest that macroinvertebrates in the *Dikerogammarus* treatments are subject to higher predation pressure than those in the *G. pulex* treatments as the invasive amphipods have been

documented as being more predatory than their native counterparts (Van Riel *et al.* 2006). Our results also suggest that *D. haemobaphes* could be more similar to the native amphipods, exhibiting less predatory behaviour, than *D. villosus* as communities containing *D. haemobaphes* had a higher overall number of macroinvertebrate species than both *G. pulex* and *D. villosus*. We suggest that native *G. pulex* is less predatory and this, along with the production of FPOM, results in increased macroinvertebrate diversity. Overall, our measured impacts on community composition and diversity were lower than expected considering previous evidence of substantial macroinvertebrate declines in *Dikerogammarus* sp. invaded communities (see MacNeil *et al.* 2013; Dick & Platvoet 2000). Again, we suggest our result could be due to the additional complexity of the mesocosms or due to the relatively short duration of the experiment.

Invasive non-native species can reach high densities and are often recorded at higher densities than their native counterparts, for example, Josens *et al.* (2005) provides evidence that *D. villosus* populations reached higher densities (200-500 per artificial substrate) than the previous native amphipod communities (50-120 per artificial substrate) in the river Rhine. It is therefore likely that our amphipod densities (6 and 12 individuals per experimental mesocosm) are much lower than those commonly experienced in invaded systems. We applied our amphipods at these densities in light of the previously reported rates of predation. We had hypothesised that applying amphipods at such densities would enable us to measure both predation and detrital processing rate changes and reduce the chances of local extinctions due to excessive predation. Due to the changeable nature, in terms of population density and sex ratios, representing field relevant population dynamics in laboratory and field manipulation experiments is difficult. The need for longer and more field representative experiments into the impacts of widespread invasive non-native species, such as those *Dikerogammarus* species, is further highlighted by our findings.

Contrary to our expectations we found no effect of either amphipod species or density on the biomass or chlorophyll content of the microbial biofilms. We had expected a significant difference in shredding behaviours between the three amphipod species, as has been found in laboratory studies (Chapter 4). We had anticipated that differing

rates of detrital processing, as measured as CPOM loss, would result in a trophic cascade with more leaf breakdown resulting in increased mass and chlorophyll *a* concentration of the biofilms, indicating increased levels of primary production, and increased rates of microbial breakdown, through decreased cotton strip tensile strength (Tiegs *et al.* 2013). The use of cotton strips has been shown to be a reliable and standardised measure of microbial and fungal breakdown which can avoid variation in leaf stoichiometry and physical characteristics (e.g. toughness). We suggest our finding, of no significant change in microbial biomass or chlorophyll content, is likely linked with our previous finding that the loss of CPOM did not differ between the amphipod species or density treatments. Specifically, we suggest that as the rates of CPOM consumption did not differ the amounts of FPOM generated also did not differ between the treatments and this resulted in the availability of nutrients being consistent across mesocosms. While our results limit our ability to provide evidence as to the effects of differing detrital processing rates have on primary production, we suggest that such impacts are still likely. Questions remain however as to the longer term impacts of the invasive non-native amphipods which can often reach higher densities and may result in changes to trophic interactions which, may in turn, impact primary production.

We have shown the difficulty in scaling *per-capita* microcosm experiments up to community mesocosms and therefore we suggest that further work is required to identify if the findings here represent issues with experimental design or rather the fact that pre capita differences, described in microcosm experiments, do not translate to wider community effects. Ultimately it is the community level effect that is important in quantifying the impacts of invasive non-native species and, while it has been suggested that microcosms could be advantageous to identify *per-capita* differences if these differences do not materialise in more complex field systems then their use is called into question. The use of mesocosms has been shown to be representative of field systems (Cooper & Barmuta 1993; Englund & Cooper 2003); however, our results could call into question how we would expect *per-capita* differences to manifest in more complex communities. The use of microcosms and mesocosms are of particular importance with respect to freshwater communities as water bodies are highly heterogeneous. For

example, they often differ in their hydrodynamics, water chemistry, levels of anthropogenic pressure, riparian vegetation and community structure. Manipulation experiments also allow for the impacts of species invasions to be quantified in otherwise complicated systems; however, studies of this type rely on the assumption that *per-capita* effects scale up to field systems. Here we show this may not always be the case and despite studies reporting *per-capita* differences in detrital processing, we still have little understanding as to how these differences will manifest in more complex field relevant systems.

Chapter 6

General discussion

Throughout this thesis I aimed to quantify the impacts of invasive non-native species, through both top-down and bottom-up processes, while also questioning how these impacts vary with respect to two other environmental stressors, climate change and pathogenic infection. I initially quantified *per-capita* differences in key functional behaviours (predation and detritivory), before making attempts to scale these *per-capita* differences up to community and landscape scales. I have provided evidence that the invasive non-native predator, *Harmonia axyridis*, shows significantly greater forage ability to two native Coccinellidae (Chapter 2). Similarly, I also have also shown that two invasive non-native *Dikerogammarus* species show significantly slower detrital processing rates than the native amphipod species (Chapter 4). Finally, as I scaled-up these studies, I have shown that these *per-capita* differences in key functional behaviours do result in community changes (Chapters 3 and 5). These realised community impacts suggest a complex of interactions within the community, as could be expected, and also suggests that *per-capita* differences can be indicative of wider ecosystem impacts. However, I suggest that the use of *per-capita* measures in combination with community measures will better improve our understanding as, focussing on one scale alone could be unreliable and ultimately unrepresentative of more complex ecological systems.

6.1 Impacts of invasive non-native predators and omnivores

Invasive non-native species are known to impact native communities through top-down and bottom-up forces with predators capable of imposing top-down regulation and omnivores capable of imposing both top-down and bottom-up regulation (for example Keeler *et al.* 2006). While the outside the focus of this study, there remains much debate as to whether top-down or bottom-up forces are more dominant in regulating communities (Heath *et al.* 2014; Wollrab *et al.* 2012) in addition as to whether invasive non-native species impact native communities at all (Russell & Blackburn 2017b; Ricciardi & Ryan 2018b, e.g.). I hope to inform our current understanding of both the

impacts my study systems pose within their invaded range in addition to more general questions within invasion ecology, for example informing the invasion denialism debate. The UK Coccinellidae system has been impacted by top-down regulation through the predation behaviour of *H. axyridis* (Roy & Brown 2015) whereas the impacts of *Dikerogammarus* species in the UK freshwater amphipod system is considered likely to regulate by both bottom-up and top-down forces (Van Riel *et al.* 2006), with both top-down and bottom-up forces able to initiate large scale trophic cascades (Pace *et al.* 1999).

6.1.1   Top-down effects of predation

Top-down impacts imposed by many invasive non-native predators are known to significantly impact native communities (Ocasio-Torres *et al.* 2015; Van Riel *et al.* 2006). Invasive arthropod predators, such as *H. axyridis*, have been suggested as one of the clearest cases of top-down regulation (Snyder & Evans 2006). The arrival and subsequent spread of *H. axyridis* has received much research attention with this resulting in valuable long-term datasets and research findings being available (UK Ladybird Survey 2018) which together make the UK ladybird system a valuable resource for quantifying the impacts of an invasive non-native predator via top-down forces. While the arrival of *H. axyridis* in the UK has been be correlated with declines in native Coccinellidae (Roy *et al.* 2012), little is known about how *H. axyridis* impacts native prey populations in its invaded range (Roy & Brown 2015; Roy *et al.* 2016a). The predatory ability *H. axyridis* has previously been studied prior to use as a biological control agent however, these studies are dominated by *per-capita* microcosm studies (for example Lee & Kang 2004; Seko *et al.* 2014). Firstly, while previous studies have shown *H. axyridis* to be an efficient predator (Abbott *et al.* 2014; Kögel *et al.* 2013; Lee & Kang 2004; Seko & Miura 2008; Xue *et al.* 2009), I've shown that the predatory ability of *H. axyridis* is greater than two native two native Coccinellidae and secondly, that in addition to impacting native Coccinellidae (Bahlai *et al.* 2014; Roy *et al.* 2012) and non-target species (Ingels *et al.* 2015; Koch *et al.* 2003). Using long-term field data, I have shown that *H. axyridis* is also correlated with significant changes in native aphid

prey populations in the UK. My *per-capita* functional response study (Chapter 2) showed that *H. axyridis* is a more efficient predator than two native Coccinellidae, I therefore suggest that the decrease in aphid abundance demonstrated using field collected data (Chapter 3) is realised through top-down regulation.

Top-down regulation has been shown to be important in biological invasions for example, invasive non-native wasps (*Vespula* spp.) (Beggs 2001; Beggs *et al.* 2011; Lester *et al.* 2013). My findings not only further our understanding as to the impacts of this globally invasive non-native species, but also make use of long-term datasets which are often unavailable for such studies and can therefore provide greater insight. The UK Coccinellidae system also provides a valuable opportunity to, not only measure the impact of a highly invasive non-native species (*H. axyridis*), but also other environmental stressors, such as agricultural practices or climate change on the abundance of native aphids. As far as I am aware, this is the first study to quantify the impacts of *H. axyridis* on native prey species using field collected data.

6.1.2   Top-down and bottom-up effects of omnivory

Similar to top-down effects, the bottom-up impacts of invasive non-native species can be equally damaging for native communities (Boag & Yeates 2001; Boag & Neilson 2006; Gallardo *et al.* 2016). Omnivores have the potential to impact native communities by imposing a complex of both top-down and bottom-up forces on communities. Recent arrivals to the UK freshwater amphipod system, *Dikerogammarus villosus* and *Dikerogammarus haemobaphes* have both been suggested to impact native communities, through bottom-up forces, through reduced detrital breakdown which results in fewer available nutrients (MacNeil *et al.* 2011; Jourdan *et al.* 2016; Piscart *et al.* 2011; Constable & Birkby 2016; Kenna *et al.* 2016), and top-down forces via predation (Bacela-Spychalska & Van Der Velde 2013; Dick *et al.* 2002; MacNeil & Platvoet 2005).

Through a laboratory microcosm experiment, I have provided additional evidence that both omnivorous *Dikerogammarus* species do indeed undertake less detrital processing behaviour than the dominant native amphipod species. I also provide new evidence as to the impact of resource quality and different temperature regimes on the

rates of detrital processing. I've shown that with increasing temperature, the differences in detrital processing rates between native and invasive non-native amphipods decreases while differences due to resource quality increase. By scaling-up my microcosm experiment to a community mesocosm scale, I've also been able to provide further evidence that *D. villosus* and *D. haemobaphes* significantly impact native communities, through reducing the diversity and abundance of macroinvertebrate species. I suggest both top-down and bottom-up forces are likely to have resulted in these findings and shape native communities. As omnivores, *Dikerogammarus* species could impact native communities via two routes. Firstly, differing rates of detrital processing could reduce nutrient availability and drive bottom-up forces. Secondly, predation of other detritivorous species (e.g. *Asellus aquaticus*), via top-down pressures, could result in reduced food availability and a further reduction in community detrital processing.

Field studies have shown a significant decrease in native macroinvertebrates in response to the arrival of *D. villosus* and *D. haemobaphes* (e.g. Josens *et al.* 2005; Jourdan *et al.* 2016). My laboratory microcosm study indicated that different rates of detrital processing, resulting in less resource availability, might be an important underlying mechanism for these documented changes. This result could also suggest the invasive non-native omnivores are more likely to undertake predatory over detrital behaviour. Scaling up my microcosm study to a community mesocosm, to better allow for the full quantification of the omnivorous behaviours, I found that while neither *Dikerogammarus* species resulted significantly lower rates of community detrital processing, both *Dikerogammarus* species were associated with declines in macroinvertebrate abundance and diversity, likely through predation. It is therefore likely that *D. villosus* and *D. haemobaphes*, through their role as omnivores, are able to impact native communities through a complex of both top-down and bottom-up forces.

I further suggest that the UK amphipod system provides a valuable opportunity to investigate the impacts of invasive non-native omnivores on native communities. Such an understanding is essential as many potential future invaders are also omnivorous, for example the rusty crayfish (*Orconectes rusticus*), the common yabby (*Cherax destructor*), and the Asian paddle crab (*Charybdis japonica*) have all been identified as

having a very high risk of arriving, becoming established, expanding their invaded range, and then impacting European biodiversity before 2025 (Roy *et al.* 2015a). I suggest that applying the combined methods of laboratory microcosms, mesocosms and field studies, as have been demonstrated here, to these potentially invasive non-native species.

6.1.3   Effects of interacting environmental pressures

While we understand how major environmental pressures such as climate change, invasive species, and pathogens impact native communities in isolation (Clavero & García-Berthou 2005; Blackburn *et al.* 2012; Lafferty *et al.* 2008; Bellard *et al.* 2012), our understanding as to how these pressures interact remains poorly understood (Brook *et al.* 2008; Strayer 2010). The ecological impacts of climate change are expected to worsen (Foden *et al.* 2013) and this is likely to result in species range shifts, including those of invasive species, and the level of disturbance experienced by habitats (Diez *et al.* 2012; Early *et al.* 2016). Ultimately, this increased habitat disturbance, for example as a by-product of more frequent and extreme climatic events, could combine with the other factors, such as increased international trade, and increase the rate of species invasions still further (Brook *et al.* 2008). I've provided evidence that climate change induced warming is likely to impact the detrital processing rates, and therefore likely resource availability in freshwater communities. At current water temperatures (8°C) native *G. pulex* had the highest rate of detrital processing, significantly higher than both *Dikerogammarus* species. However, as temperature treatments increased the differences between the three amphipod species decreased and at the highest temperature treatment (20°C) the three species undertook detrital processing at similar rates. Rates of detrital processing were highest in amphipods receiving the diet of greatest resource quality however, as temperatures increased, these resource quality diets resulted in a greater difference in detrital shredding rates. I suggest this could result in climate change mediating the current impacts imposed by the invasive *Dikerogammarus* species. Further insight could be gained by addressing how the stoichiometry of allochthonous detrital matter may change under project climate change scenarios. In addition to warming, climate change is also expected to impact freshwater communities via increased

atmospheric CO_2 and variation in flow rates, as a by product of more frequent and extreme climatic events (e.g. drought) (Woodward *et al.* 2010). I suggest, that while my findings begin to address how climate change could interact with invasive species pressures, that greater integration of such pressures in experimental studies is necessary to better prepare for and understand future invasion events and their impacts.

Parasites and pathogens are known to be highly prevalent throughout ecological communities and while their pressures on individuals hosts are generally well understood, the functional group is often omitted from many studies (Lafferty *et al.* 2006, 2008) including those investigating the impact of invasive non-native species, despite their known importance (Dunn *et al.* 2012; Dunn & Hatcher 2015). The pressures of pathogens and parasites are also likely to change in the future, under projected climate change and as new non-native parasites or pathogens are introduced (Roy *et al.* 2016b). I've been able to show that pathogenic infection significantly impacted the forage ability of native and invasive non-native Coccinellidae alike. This, I suggest, means that such infection is unlikely to favour either the native or non-native species but may benefit native prey species. While *B. bassiana* is unlikely to be an appropriate biological control for *H. axyridis*, due to it's broad host range, it is likely that the two species do interact in the wild, due to habitat overlap with both the pathogen and other hosts (Ormond *et al.* 2011; Roy *et al.* 2011), as yet however our understanding of how they interact and what impacts such interactions may have remain limited (but see Cottrell & Shapiro-Ilan 2003; Roy *et al.* 2008b). I suggest that future studies should make similar efforts to increase the complexity of similar *per-capita* studies, for example though increasing habitat heterogeneity and the number of species interactions, so as to better reflect the more complex ecological systems to which they are modelled.

6.2 Using functional traits to quantify impact

Key functional traits or behaviours have been linked with invasive species impacts (Dick *et al.* 2017a; Ricciardi *et al.* 2013) and are often used to asses the impact non-native species can have on native communities (for example Crowder & Snyder 2010; Dick *et al.*

2017b; Van Kleunen *et al.* 2010). As part of this thesis, I focussed primarily on two key functional behaviours, predation and detritivory, which are able to impact native communities via top-down and bottom-up forces. While species invasions can impact native communities through complex interactions, the use of functional traits or behaviours to predict and describe species impacts have been shown to be effective. For example, functional response studies have been used to quantify and compare *per-capita* differences in predation rates (Barrios-O'Neill *et al.* 2014; Dick *et al.* 2013, 2014, 2017a; Dodd *et al.* 2014; Laverty *et al.* 2015; Lee & Kang 2004; Shipp & Whitfield 1991 but also see Vonesh *et al.* 2017), in addition to being used to scale up effects to population levels (Dick *et al.* 2017b; Laverty *et al.* 2017b). Functional response studies have provided an opportunity to quantify and compare the potential impacts an invasive non-native species could have have within an invaded environment, via predatory behaviour, in the absence of an invasion history. The extension of such methods to include other traits (e.g. detritivory), account for more of the complexities present in ecological systems (e.g. pathogens; Laverty *et al.* 2017a, Chapter 2), and the interaction of multiple environmental stressors (e.g. climate change; Laverty *et al.* 2015), has the potential to greatly improve our understanding as to the risks associates with species invasions.

6.3   How does invasion ecology inform ecology?

Species invasions have provided a valuable opportunity for ecologists to investigate and further support long standing ecological theories which would have otherwise been impossible or impractical. The very nature of species invasions results in multiple unplanned experimental situations across both spatial and temporal scales (Sax *et al.* 2007). During these invasions scientists also have the ability to investigate ecological and evolutionary processes in real-time, with well defined dates of arrival, rather than having to make inferences about possible historical events (Sax *et al.* 2007). For example, the large spatial scales at which species invasions often occur have provided evidence that simple climatic envelope matching is likely to be a unreliable estimate of species distributions with the importance of geographic barriers being highlighted. The spread

of *Harmonia axyridis* and *Coccinella septempunctata* throughout their non-native ranges demonstrates the ability of species to spread rapidly when unimpeded by barriers (e.g. geographical) (Evans 2000; Brown & Roy 2017).

The spread if invasive non-native species, while posing substantial ecological and economic risks, also provides an opportunity for scientific investigation that would otherwise be unethical (Sax *et al.* 2007). For example the introduction of a novel species into an ecological system at large spatial scales would be likely to negative impact the native community, resulting in such experiments being hard to justify. The spread of invasive non-native species has provided such an experiment which has provided evidence for long standing ecological theories as postulated by Charles Darwin and others (Darwin 1859). Darwin (1859) suggested that species are unlikely to be optimally adapted to their ecological niche and the arrival and dominance of invasive non-native species supports such a theory. A more recent finding from invasion ecology for the benefit of wider ecological disciplines is that of ecological saturation in species communities and what features drive community assemblages. For example, freshwater fish richness in Hawaii has increased by 800% due to the successful invasion of 40 non-native fish species and the loss of no native species (Sax & Gaines 2003). However, these results are not consistent across all ecological systems. Terrestrial birds could provide support for a saturation hypothesis as there has been no net change in species richness with species lost, through extinctions, and gained, through species invasions, at similar rates (Sax *et al.* 2002). It is clear that species invasions have significant potential to further our understanding of general ecological principles, for example our understanding of predator-prey systems and the implications of the creation of novel species interactions, across ecological disciples, from population to evolutionary ecology.

6.4 Where next for invasion ecology?

Invasion ecology has and will continue to develop with the innovation and adoption of novel experimental protocols (e.g. Matthews *et al.* 2017), technologies (e.g. Blackman *et al.* 2017; Goldberg *et al.* 2016), and statistical methods (e.g. Isaac *et al.* 2014) to

prevent and mitigate the impacts of future species invasions. Such advancements are essential if efforts to slow the spread and reduce the impacts of future invasive non-native species are to be successful. I suggest that future efforts within invasion ecology should prioritise; 1) efforts to control the spread of invasive non-native species through horizon scanning and biosecurity, 2) improve our understanding as to how the pressures imposed by invasive non-native species are realised in ecological systems, and 3) making use of existing long-term ecological datasets, technology, and citizen scientists to better understand the long-term impacts and complexities of species invasions.

Global efforts to combat species invasions have successfully resulted in multiple national and international legal frameworks (Millennium Ecosystem Assessment 2005; Convention on Biological Diversity 2006; European Comission 2017; United Nations 2015) however, despite these efforts, the rate of successful species invasions continues to rise (Seebens *et al.* 2017). Predicting future species through horizon scanning has been shown to be effective, for example in predicting the arrival of the quagga mussel (*Dreissena rostriformis bugensis*) and the Asian hornet (*Vespa velutina*) into the UK shortly before their recorded arrival (Roy *et al.* 2014). While horizon scanning efforts have proven their importance, I suggest a proactive and pre-emptive approach is required to avoid further species invasions. However, these are likely to result in economic costs or losses which would make them unpopular as the costs of successfully avoided invasions will never be realised. Considering global connectivity and major trade routes are an important step in reducing the spread of invasive species (Brenton-Rule *et al.* 2016; Hulme 2009; Perrings *et al.* 2010), yet governments will need to pro-actively restrict high risk products or trade routes. Such global cooperation and coordination efforts would likely benefit from being politically neutral, so as to facilitate international cooperation and avoid decisions based on short-term economic gains. At a national level, adoption of biosecurity procedures by professionals and members of the public is likely to reduce the spread of non-native species within geographic regions (Anderson *et al.* 2015). Complications arise, however, as to how to encourage uptake and adherence to any such strategies in addition to any such enforcement. Nevertheless, it is likely that an invested interest in those ecological systems, perceived to be at risk from invasive species, will aid

in good biosecurity practice.

In order to further inform potential national and international initiatives, a comprehensive understanding of the impacts a non-native species is likely to impose is necessary. I believe that future efforts to quantify the impacts of recent or potential future invasive species would benefit from using a multi-scale approach. I suggest such an approach allows for *per-capita* differences to be calculated in a controlled environment. These *per-capita* effects can then be scaled with known population densities (for example Dick *et al.* 2017b), or developed further to asses how these *per-capita* differences impact native communities. Such an approach, combined with horizon scanning methods, could dramatically improve our ability to mitigate and predict future invasions and their potential impacts.

Lastly, long-term datasets have been invaluable to this thesis and continue to improve our understanding of species invasions (e.g. Roy & Brown 2015). The UK, and Europe, is in an enviable position to tackle environmental crises with our long history of environmental data collection (Pocock *et al.* 2015) providing a rich ecological history. The creation and use of long-term datasets has huge potential in increasing our ability to answer questions as to the impacts of future species invasions. The collection of such data can also be facilitated by using citizen science methods, with the use of mobile and internet applications making data collection, validation, and organisation increasingly simple (Roy *et al.* 2015b). For example, the UK Ladybird Survey (www.ladybird-survey.org) has received sightings of Coccinellidae from over 14,000 citizen scientists via on-line submissions and the mobile application (UK Ladybird Survey 2018). Similarly, the Zooniverse project (www.zooniverse.org) uses an on-line application to allow citizen scientists to contribute to ongoing research, for example by identifying key features in images. To date, the zooniverse project has over 1.6 million registered citizen scientists who have contributed to 166 peer reviewed publications. It should be noted, however, that cryptic species or those that are not easily identifiable are likely to be less suitable for such applications. The continued application of citizen science initiatives to assist research efforts should be encouraged and is likely to be of great benefit to our future understanding of invasive non-native species.

6.5   Concluding remarks

Invasive species are, and are likely to continue to be, a major ecological issue throughout the world (Bellard *et al.* 2016; Butchart *et al.* 2010; Ducatez & Shine 2017; Hulme 2007; Seebens *et al.* 2017) with the impacts of future invasions likely to become increasingly interlinked with other existing environmental pressures (Brook *et al.* 2008; Seebens *et al.* 2015; Strayer 2010). Here, I have shown that invasive non-native species can impose top-down and bottom-up pressures on communities. I also show that, broadly, our *per-capita* results scale to show an impact at the community scale. I hope that the evidence presented here will also further bolster efforts to finalise the debate surrounding invasive species denialism. I believe it is essential to make every effort to resolve such a fundamental argument within invasion ecology in an effort to reduce the chances of the discipline being marred by denialism moving forwards. For example, immunology, climate change, and to a lesser extent, evolution disciples are all marred with denialism which could greatly impede the future progress of invasion ecology due to the great advancements already facilitated by successful public engagement. Future efforts to further our understanding of the impacts invasive species can have on native communities should continue with the integration of research efforts, for example horizon scanning, biosecurity, and quantifications of impact potential. While it is hoped that a greater understanding as to the ecological impacts of invasive species will aid in reducing and mitigating the arrival and impacts of future invaders, pre-emptive action including better controls on imported products and biosecurity applications at a national and international level is likely to be required to have any notable effect on the ever increasing rate of species invasions in the the UK and Europe.

References

Abbott, K.C., Harmon, J.P. & Fabina, N.S. (2014) The challenge of predicting temperature effects on short-term predator-prey dynamics. *Population Ecology* **56**, 375–392.

Ågren, G.I. & Weih, M. (2012) Plant stoichiometry at different scales: Element concentration patterns reflect environment more than genotype. *New Phytologist* **194**, 944–952.

Alexander, M.E., Dick, J.T.A., Weyl, O.L.F., Robinson, T.B. & Richardson, D.M. (2014) Existing and emerging high impact invasive species are characterized by higher functional responses than natives. *Biology Letters* **10**, 20130946.

Allen, S.E. (ed.) (1989) *Chemical Analysis of Ecological Materials.* Blackwell Scientific Publications, Oxford, second edn.

Anderson, L.G., Rocliffe, S., Haddaway, N.R. & Dunn, A.M. (2015) The role of tourism and recreation in the spread of non-native species: A systematic review and meta-analysis. *Plos One* **10**, e0140833.

Anderson, L.G., White, P.C.L., Stebbing, P.D., Stentiford, G.D. & Dunn, A.M. (2014) Biosecurity and vector behaviour: evaluating the potential threat posed by anglers and canoeists as pathways for the spread of invasive non-native species and pathogens. *PloS one* **9**, e92788.

APHA (2005) *Standard methods for the examination of water and wastewater.* American Public Health Agency, Washington, DC, 21st edn.

Arundell, K., Dunn, A., Alexander, J., Shearman, R., Archer, N. & Edward, J. (2015) Enemy release and genetic founder effects in invasive killer shrimp populations of Great Britain. *Biological Invasions* **17**, 1439–1451.

Bacela-Spychalska, K. & 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**, 730–741.

Bahlai, C.A., Colunga-Garcia, M., Gage, S.H. & Landis, D.A. (2014) The role of exotic ladybeetles in the decline of native ladybeetle populations: evidence from long-term monitoring. *Biological Invasions* **17**, 1005–1024.

Barrios-O'Neill, D., Dick, J.T.A., Emmerson, M.C., Ricciardi, A. & Macisaac, H.J. (2014) Predator-free space, functional responses and biological invasions. *Functional Ecology* **29**, 377–384.

Beggs, J. (2001) The ecological consequences of social wasps (*Vespula* spp.) invading an ecosystem that has an abundant carbohydrate resource. *Biological Conservation* **99**, 17–28.

Beggs, J.R., Brockerhoff, E.G., Corley, J.C., Kenis, M., Masciocchi, M., Muller, F., Rome, Q. & Villemant, C. (2011) Ecological effects and management of invasive alien Vespidae. *BioControl* **56**, 505–526.

Bell, J.R., Alderson, L., Izera, D., Kruger, T., Parker, S., Pickup, J., Shortall, C.R., Taylor, M.S., Verrier, P. & Harrington, R. (2015) Long-term phenological trends, species accumulation rates, aphid traits and climate: Five decades of change in migrating aphids. *Journal of Animal Ecology* **84**, 21–34.

Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W. & Courchamp, F. (2012) Impacts of Climate Change on the Future of Biodiversity. *Ecology Letters* **15**, 365–377.

Bellard, C., Cassey, P. & Blackburn, T.M. (2016) Alien species as a driver of recent extinctions. *Biology Letters* **12**, 20150623.

Benton, T.G., Solan, M., Travis, J.M.J. & Sait, S.M. (2007) Microcosm experiments can inform global ecological problems. *Trends in Ecology and Evolution* **22**, 516–521.

Berezina, N.A. (2007) Invasions of alien amphipods (Amphipoda: Gammaridea) in aquatic ecosystems of North-Western Russia: Pathways and consequences. *Hydrobiologia* **590**, 15–29.

Berkvens, N., Moens, J., Berkvens, D., Samih, M.A., Tirry, L. & De Clercq, P. (2010) *Dinocampus coccinellae* as a parasitoid of the invasive ladybird *Harmonia axyridis* in Europe. *Biological Control* **53**, 92–99.

Bij de Vaate, A., Jazdzewski, K., Ketelaars, H.A., Gollasch, S. & Van der Velde, G. (2002) Geographical patterns in range extension of Ponto-Caspian macroinvertebrate species in Europe. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1159–1174.

Blackburn, T.M., Cassey, P., Duncan, R.P., Evans, K.L. & Kevin, J. (2012) Avian Extinction and Mammalian Introductions on Oceanic Islands. *Science* **305**, 1955–1958.

Blackman, R.C., Constable, D., Hahn, C., Sheard, A.M., Durkota, J., Hänfling, B. & Handley, L.L. (2017) Detection of a new non-native freshwater species by DNA metabarcoding of environmental samples – first record of *Gammarus fossarum* in the UK. *Aquatic Invasions* **12**, 177–189.

Boag, B. & Neilson, R. (2006) Impact of New Zealand Flatworm on agriculture and wildlife in Scotland. Tech. rep.

Boag, B. & Yeates, G.W. (2001) The Potential Impact of the New Zealand Flatworm, a Predator of Earthworms, in Western Europe. *Ecological applications* **11**, 1276–1286.

Boets, P., Lock, K., Messiaen, M. & Goethals, P.L. (2010) Combining data-driven methods and lab studies to analyse the ecology of *Dikerogammarus villosus*. *Ecological Informatics* **5**, 133–139.

Bolker (2016) emdbook: Ecological models and data analysis in R; R package.

Bolker, B. & R Development Core Team (2014) bbmle: Tools for general maximum likelihood estimation.

Bonesi, L. & Palazon, S. (2007) The American mink in Europe: Status, impacts, and control. *Biological Conservation* **134**, 470–483.

Bonizzoni, M., Gasperi, G., Chen, X. & James, A.A. (2013) The invasive mosquito species *Aedes albopictus*: current knowledge and future perspectives. *Trends in Parasitology* **29**, 460–468.

Bovy, H.C., Barrios-O'Neill, D., Emmerson, M.C., Aldridge, D.C. & Dick, J.T.a. (2015) Predicting the predatory impacts of the "demon shrimp" *Dikerogammarus haemobaphes*, on native and previously introduced species. *Biological Invasions* **17**, 597–607.

Bradshaw, C., Leroy, B., Bellard, C., Albert, C., Roiz, D., Barbet-Massin, M., Fournier, A., Salles, J.M., Simard, F. & Courchamp, F. (2016) Massive yet grossly underestimated global costs of invasive insects. *Nature Communications* **7**.

Brenton-Rule, E.C., Barbieri, R.F., Lester, P.J., Vitousek, P., D'Antonio, C., Loope, L., Rejmanek, M., Westbrooks, R., Ehrenfeld, J., Towns, D., Atkinson, I., Daugherty, C., Hulme, P., Essl, F., Pimentel, D., Zuniga, R., Morrison, D., Goldson, S., Tittensor, D., Lodge, D., Organization, W.T., Halkos, G., Tzeremes, N., Gallardo, B., Aldridge, D., Vilà, M., Pujadas, J., Liebhold, A., Brockerhoff, E., Garrett, L., Parke, J., Britton, K., Sakalidis, M., Slippers, B., Wingfield, B., Hardy, G., Burgess, T., Team, R.C., Margolis, M., Shogren, J., Fischer, C., Ding, J., Mack, R., Lu, P., Ren, M., Huang, H., Kolar, C., Lodge, D., Marchetti, M., Moyle, P., Levine, R., Pheloung, P., Williams, P., Halloy, S., Myers, N., Mittermeier, R., Mittermeier, C., da Fonseca, G., Kent, J., Kleijn, D., Sutherland, W., Hulme, P., Mauro, P., Stohlgren, T., Schnase, J., Lester, P., Perrings, C., Dehnen-Schmutz, K., Touza, J. & Williamson, M. (2016) Corruption, development and governance indicators predict invasive species risk from trade. *Proceedings of the Royal Society* **283**.

Briggs, J.C. (2017) Rise of invasive species denialism? A response to Russell and Blackburn. *Trends in Ecology and Evolution* **32**, 231–232.

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

Brooks, M.L., D'Antonio, C.M., Richardson, D.M., Grace, J.M., Keeley, J.E., DiTomaso, J.M., Hobbs, R.J., Pellant, M. & Pyke, D. (2004) Effects of invasive alien plants on fire regimes. *BioScience* **54**, 677–688.

Brown, J.H., Gillooly, J.F., Allen, A.P., Savage, V.M. & West, G.B. (2004) Toward a metabolic theory of ecology. *Ecology* **85**, 1771–1789.

Brown, L.E., Edwards, F.K., Milner, A.M., Woodward, G. & Ledger, M.E. (2011a) Food web complexity and allometric scaling relationships in stream mesocosms: Implications for experimentation. *Journal of Animal Ecology* **80**, 884–895.

Brown, P.M.J., Adriaens, T., Bathon, H., Cuppen, J., Goldarazena, A., Hägg, T., Kenis, M., Klausnitzer, B.E.M., Kovář, I., Loomans, A.J.M., Majerus, M.E., Neved, O., Pedersen, J., Rabitsch, W., Roy, H.E., Ternois, V., Kakharov, I.A. & Roy, D.B. (2008) *Harmonia axyridis in Europe: spread and distribution of a non-native coccinellid.* *BioControl* **53**, 5–21.

Brown, P.M.J., Frost, R., Doberski, J., Sparks, T., Harrington, R. & Roy, H.E. (2011b) Decline in native ladybirds in response to the arrival of Harmonia axyridis: early evidence from England. *Ecological Entomology* **36**, 231–240.

Brown, P.M.J. & Roy, H.E. (2017) Native ladybird decline caused by the invasive harlequin ladybird *Harmonia axyridis*: evidence from a long-term field study. *Insect Conservation and Diversity* .

Brown, P.M.J., Thomas, C.E., Lombaert, E., Jeffries, D.L., Estoup, A. & Lawson Handley, L.J. (2011c) The global spread of *Harmonia axyridis* (Coleoptera: Coccinellidae): distribution, dispersal and routes of invasion. *BioControl* **56**, 623–641.

Bruijs, M.C.M., Kelleher, B., Van der Velde, G. & bij de Vaate, A. (2001) Oxygen consumption, temperature and salinity tolerance of the invasive amphipod *Dikerogammarus villosus*: Indicators of further dispersal via ballast water transport. *Archiv für Hydrobiologie* **152**, 633–646.

Bunke, M., Alexander, M.E., Dick, J.T.A., Hatcher, M.J., Paterson, R.A. & Dunn, A.M. (2015) Eaten alive: cannibalism is enhanced by parasites. *Royal Society of London* **2**, 140369.

Butchart, S.H.M., Walpole, M., Collen, B., van Strien, A., Scharlemann, J.P.W., Almond, R.E.A., Baillie, J.E.M., Bomhard, B., Brown, C., Bruno, J., Carpenter, K.E., Carr, G.M., Chanson, J., Chenery, A.M., Csirke, J., Davidson, N.C., Dentener, F., Foster, M., Galli, A., Galloway, J.N., Genovesi, P., Gregory, R.D., Hockings, M., Kapos, V., Lamarque, J.F., Leverington, F., Loh, J., McGeoch, M.A., McRae, L., Minasyan, A., Morcillo, M.H., Oldfield, T.E.E., Pauly, D., Quader, S., Revenga, C., Sauer, J.R., Skolnik, B., Spear, D., Stanwell-Smith, D., Stuart, S.N., Symes, A., Tierney, M., Tyrrell, T.D., Vié, J.C. & Watson, R. (2010) Global Biodiversity: Indicators of Recent Declines. *Science* **328**, 1164 – 1168.

Casellato, S., Visentin, A. & La Piana, G. (2007) The predatory impact of *Dikerogammarus villosus* on fish. *Biological invaders in inland waters: profiles, distribution and threats* (ed. F. Gherardi), pp. 495–507.

Ceryngier, P., Nedvěd, O., Grez, A.A., Riddick, E.W., Roy, H.E., San Martin, G., Steenberg, T., Veselý, P., Zaviezo, T., Zúñiga-Reinoso, Á. & Haelewaters, D. (2018) Predators and parasitoids of the harlequin ladybird, Harmonia axyridis, in its native range and invaded areas. *Biological Invasions* **20**, 1009–1031.

Charnov, E. (1976) Optimal foraging theory: the marginal value theorem. *Theoretical Population Biology* **9**, 129–136.

Clapcott, J.E. & Barmuta, L.A. (2010a) Forest clearance increases metabolism and organic matter processes in small headwater streams. *Journal of the North American Benthological Society* **29**, 546–561.

Clapcott, J.E. & Barmuta, L.A. (2010b) Metabolic patch dynamics in small headwater streams: Exploring spatial and temporal variability in benthic processes. *Freshwater Biology* **55**, 806–824.

Clavero, M. & García-Berthou, E. (2005) Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* **20**, 110.

Constable, D. & Birkby, N.J. (2016) The impact of the invasive amphipod *Dikerogammarus haemobaphes* on leaf litter processing in UK rivers. *Aquatic Ecology* **50**, 273–281.

Convention on Biological Diversity (2006) *Global Biodiversity Outlook 2*. Secretariat of the Convention on Biological Diversity, Montreal.

Coombs, E.M., Markin, G.P., Pratt, P.D. & Rice, B. (2004) Gorse. *Biological control of invasive plants in the United States* (eds. E.M. Coombs, J.K. Clark, G.L. Piper & J. Cofrancesco, Alfred F.), pp. 178–179, Oregon State University Press, Corvallis, Oregon.

Cooper, S.B. & Barmuta, L.A. (1993) Field experiments in biomonitoring. *Freshwater biomonitoring and benthic macroinvertebrates* (eds. D.M. Rosenberg & V.H. Resh), pp. 339–441, Chapman & Hall, London.

Cottrell, T.E. & Shapiro-Ilan, D.I. (2003) Susceptibility of a native and an exotic lady beetle (Coleoptera: Coccinellidae) to *Beauveria bassiana*. *Journal of Invertebrate Pathology* **84**, 137–144.

Cox, J.G. & Lima, S.L. (2006) Naiveté and an aquatic-terrestrial dichotomy in the effects of introduced predators. *Trends in Ecology and Evolution* **21**, 674–680.

Crowder, D.W. & Snyder, W.E. (2010) Eating their way to the top? Mechanisms underlying the success of invasive insect generalist predators. *Biological Invasions* **12**, 2857–2876.

Crowley, S.L., Hinchliffe, S., Redpath, S.M. & McDonald, R.A. (2017) Disagreement about invasive species does not equate to denialism: A response to Russell and Blackburn. *Trends in Ecology and Evolution* **32**, 228–229.

Cuffney, T.F., Wallace, J.B. & Lugthart, G.J. (1990) Experimental evidence quantifying the role of benthic invertebrates in organic matter dynamics of headwater streams. *Freshwater Biology* **23**, 281–299.

Cummins, K.W., Wilzbach, M.A., Gates, D.M., Perry, J.B., Bruce, W., Cummins, K.W., Wilzbach, M.A., Gates, D.M., Perry, J.B. & Taliaferro, W.B. (1989) Shredders and riparian vegetation stream invertebrates. *BioScience* **39**, 24–30.

Dangremond, E.M., Pardini, E.a. & Knight, T.M. (2010) Apparent competition with an invasive plant hastens the extinction of an endangered lupine. *Ecology* **91**, 2261–2271.

Darwin, C. (1859) *On the origin of species by means of natural selection, or preservation of favoured races in the struggle for life*. John Murray, London.

Davis, M.A. & Chew, M.K. (2017) 'The denialists are coming!' Well, not exactly: A response to Russell and Blackburn. *Trends in Ecology and Evolution* **32**, 229–230.

Dawson, W., Moser, D., Van Kleunen, M., Kreft, H., Pergl, J., Pyšek, P., Weigelt, P., Winter, M., Lenzner, B., Blackburn, T.M., Dyer, E.E., Cassey, P., Scrivens, S.L., Economo, E.P., Guénard, B., Capinha, C., Seebens, H., García-Díaz, P., Nentwig, W., García-Berthou, E., Casal, C., Mandrak, N.E., Fuller, P., Meyer, C. & Essl, F. (2017) Global hotspots and correlates of alien species richness across taxonomic groups. *Nature Ecology and Evolution* **1**.

Devin, S., Piscart, C., Beisel, J.N. & Moreteau, J.C. (2003) Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. *Archiv für Hydrobiologie* **158**, 43–56.

Dick, J.T., Alexander, M.E., Jeschke, J.M., Ricciardi, A., MacIsaac, H.J., Robinson, T.B., Kumschick, S., Weyl, O.L.F., Dunn, A.M., Hatcher, M.J., Paterson, R.A., Farnsworth, K.D. & Richardson, D.M. (2014) Advancing impact prediction and hypothesis testing in invasion ecology using a comparative functional response approach. *Biological Invasions* **16**, 735–753.

Dick, J.T., Gallagher, K., Avlijas, S., Clarke, H.C., Lewis, S.E., Leung, S., Minchin, D., Caffrey, J., Alexander, M.E., Maguire, C., Harrod, C., Reid, N., Haddaway, N.R., Farnsworth, K.D., Penk, M. & Ricciardi, A. (2013) Ecological impacts of an invasive predator explained and predicted by comparative functional responses. *Biological Invasions* **15**, 837–846.

Dick, J.T. & Platvoet, D. (2000) Invading predatory crustacean *Dikerogammarus villosus* eliminates both native and exotic species. *Proceedings of the Royal Society of London* **267**, 977–983.

Dick, J.T., Platvoet, D. & Kelly, D.W. (2002) Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 1078–1084.

Dick, J.T.A., Alexander, M.E., Ricciardi, A., Laverty, C., Paul, O., Xu, M., Jeschke, J.M., Saul, W.C., Hill, M.P., Neill, D.B.O., Weyl, O.L.F. & Shaw, R.H. (2017a) Functional responses can unify invasion ecology. *Biological Invasions* **5**, 1667–1672.

Dick, J.T.A., Armstrong, M., Clarke, H.C., Farnsworth, K.D., Hatcher, M.J., Ennis, M., Kelly, A. & Dunn, A.M. (2010) Parasitism may enhance rather than reduce the predatory impact of an invader. *Biology Letters* **6**, 636–638.

Dick, J.T.A., Laverty, C., Lennon, J.J., Barrios-O'Neill, D., Mensink, P.J., Britton, J.R., Médoc, V., Boets, P., Alexander, M.E., Taylor, N.G., Dunn, A.M., Hatcher, M.J., Rosewarne, P.J., Crookes, S., MacIsaac, H.J., Xu, M., Ricciardi, A., Wasserman, R.J., Ellender, B.R., Weyl, O.L.F., Lucy, F.E., Banks, P.B., Dodd, J.A., MacNeil, C., Penk, M.R., Aldridge, D.C. & Caffrey, J.M. (2017b) 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* **54**, 1259–1267.

Didham, R.K., Ewers, R.M. & Gemmel, N.J. (2005) Comment on "Avian Extinction and Mammalian Introductions on Oceanic Islands". *Science* **307**, 1412a.

Diez, J.M., D'Antonio, C.M., Dukes, J.S., Grosholz, E.D., Olden, J.D., Sorte, C.J., Blumenthal, D.M., Bradley, B.A., Early, R., Ibáñez, I., Jones, S.J., Lawler, J.J. & Miller, L.P. (2012) Will extreme climatic events facilitate biological invasions? *Frontiers in Ecology and the Environment* **10**, 249–257.

Dodd, J.A., Dick, J.T.A., Alexander, M.E., MacNeil, C., Dunn, A.M. & Aldridge, D.C. (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**, 337–352.

Doherty, T.S., Glen, A.S., Nimmo, D.G., Ritchie, E.G. & Dickman, C.R. (2016) Invasive predators and global biodiversity loss. *Proceedings of the National Academy of Sciences* **113**, 11261–11265.

Drake, J.M. & Kramer, A.M. (2012) Mechanistic analogy: How microcosms explain nature. *Theoretical Ecology* **5**, 433–444.

Ducatez, S. & Shine, R. (2017) Drivers of Extinction Risk in Terrestrial Vertebrates. *Conservation Letters* **10**, 186–194.

Dunn, A.M. & Hatcher, M.J. (2015) Parasites and biological invasions: parallels, interactions, and control. *Trends in Parasitology* **31**, 189–199.

Dunn, A.M., Torchin, M.E., Hatcher, M.J., Kotanen, P.M., Blumenthal, D.M., Byers, J.E., Coon, C.A.C., Frankel, V.M., Holt, R.D., Hufbauer, R.A., Kanarek, A.R., Schierenbeck, K.A., Wolfe, L.M. & Perkins, S.E. (2012) Indirect effects of parasites in invasions. *Functional Ecology* **26**, 1262–1274.

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

Early, R., Bradley, B.A., Dukes, J.S., Lawler, J.J., Olden, J.D., Blumenthal, D.M., Gonzalez, P., Grosholz, E.D., Ibañez, I., Miller, L.P., Sorte, C.J.B. & Tatem, A.J. (2016) Global threats from invasive alien species in the twenty-first century and national response capacities. *Nature Communications* **7**, 12485.

Englund, G. & Cooper, S.B. (2003) Scale Effects and Extrapolation in Ecological Experiments. *Advances in ecological research* **33**, 161–213.

European Comission (2017) Invasive Alien Species of Union Concern. Tech. rep.

European Comission (2018) Delivering Alien Invasive Species In Europe (DAISIE).

Evans, E.W. (2000) Morphology of invasion: Body size patterns associated with establishment of *Coccinella septempunctata* (Coleoptera: Coccinellidae) in western North America. *European Journal of Entomology* **97**, 469–474.

Foden, W.B., Butchart, S.H.M., Stuart, S.N., Vié, J.C., Akçakaya, H.R., Angulo, A., DeVantier, L.M., Gutsche, A., Turak, E., Cao, L., Donner, S.D., Katariya, V., Bernard, R., Holland, R.A., Hughes, A.F., O'Hanlon, S.E., Garnett, S.T., Şekercioğlu, Ç.H. & Mace, G.M. (2013) Identifying the world's most climate change vulnerable species: A systematic trait-based assessment of all birds, amphibians and corals. *PLoS ONE* **8**.

Foucreau, N., Piscart, C., Puijalon, S. & Hervant, F. (2013) Effect of climate-related change in vegetation on leaf litter consumption and energy storage by *Gammarus pulex* from Continental or Mediterranean populations. *PloS one* **8**, e77242.

Freeman, S.N. & Newson, S.E. (2008) On a log-linear approach to detecting ecological interactions in monitored populations. *Ibis* **150**, 250–258.

Frost, P.C. (2002) Growth responses of littoral mayflies to the phosphorus content of their food. *Ecology Letters* **5**, 232–240.

Füreder, L., Gherardi, F., Holdich, D., Reynolds, J., Sibley, P. & Souty-Grosset, C. (2010) *Austropotamobius pallipes*, White-clawed Crayfish. *IUCN Red List of Threatened Species* .

Gallardo, B. & Aldridge, D.C. (2013) Evaluating the combined threat of climate change and biological invasions on endangered species. *Biological Conservation* **160**, 225–233.

Gallardo, B. & Aldridge, D.C. (2014) Is Great Britain heading for a Ponto-Caspian invasional meltdown? *Journal of Applied Ecology* **52**, 41–49.

Gallardo, B., Clavero, M., Sánchez, M.I. & Vilà, M. (2016) Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology* **22**, 151–163.

Gandhi, K.J. & Herms, D.A. (2010) Direct and indirect effects of alien insect herbivores on ecological processes and interactions in forests of eastern North America. *Biological Invasions* **12**, 389–405.

Gardner, J.L., Peters, A., Kearney, M.R., Joseph, L. & Heinsohn, R. (2011) Declining body size: A third universal response to warming? *Trends in Ecology and Evolution* **26**, 285–291.

Gardner, J.L., Rowley, E., de Rebeira, P., de Rebeira, A.D. & Brouwer, L. (2017) Effects of extreme weather on two sympatric Australian passerine bird species. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**.

GB Non-Native Species Secretariat (2018) GB Non-Native Species Information Portal.

Gergs, A. & Ratte, H.T. (2009) Predicting functional response and size selectivity of juvenile *Notonecta maculata* foraging on *Daphnia magna*. *Ecological Modelling* **220**, 3331–3341.

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

Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cornman, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E. & Taberlet, P. (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* **7**, 1299–1307.

Graca, M.A.S., Cressa, C., Gessner, M.O., Feio, M.J., Callies, K.A. & Barrios, C. (2001) Food quality , feeding preferences , survival and growth of shredders from temperate and tropical streams. *Freshwater Biology* **46**, 947–957.

Grez, A.A., Zaviezo, T., Roy, H.E., Brown, P.M.J. & Bizama, G. (2016) Rapid spread of *Harmonia axyridis* in Chile and its effects on local coccinellid biodiversity. *Diversity and Distributions* **22**, 982–994.

Gulis, V., Ferreira, V. & Graca, M.A.S. (2006) Stimulation of leaf litter decomposition and associated fungi and invertebrates by moderate eutrophication: implications for stream assessment. *Freshwater Biology* **51**, 1655–1669.

Gupta, R., Pervez, A., Guroo, M. & Srivastava, K. (2012) Stage-specific functional response of an aphidophagous ladybird, *Coccinella septempunctata* (Coleoptera: Coccinellidae), to two aphid species. *International Journal of Tropical Insect Science* **32**, 136–141.

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

Haddaway, N.R., Wilcox, R.H., Heptonstall, R.E.A., Griffiths, H.M., Mortimer, R.J.G., Christmas, M. & Dunn, A.M. (2012) Predatory functional response and prey choice identify predation differences between native/invasive and parasitised/unparasitised crayfish. *PloS ONE* **7**, e32229.

Hammond, D. & Pryce, A. (2007) *Climate change impacts and water temperature.* Environment Agency, Bristol.

Hansen, G.J., Vander Zanden, M.J., Blum, M.J., Clayton, M.K., Hain, E.F., Hauxwell, J., Izzo, M., Kornis, M.S., McIntyre, P.B., Mikulyuk, A., Nilsson, E., Olden, J.D., Papeş, M. & Sharma, S. (2013) Commonly rare and rarely common: Comparing population abundance of invasive and native aquatic species. *PLoS ONE* **8**, e77415.

Harmon, J.P., Stephens, E. & Losey, J. (2007) The decline of native coccinellids (Coleoptera: Coccinellidae) in the United States and Canada. *Journal of Insect Conservation* **11**, 85–94.

Harper, G.A. & Bunbury, N. (2015) Invasive rats on tropical islands: Their population biology and impacts on native species. *Global Ecology and Conservation* **3**, 607–627.

Harrington, R., Clark, S.J., Welham, S.J., Verrier, P.J., Denholm, C.H., Hullé, M., Maurice, D., Rounsevell, M.D. & Cocu, N. (2007) Environmental change and the phenology of European aphids. *Global Change Biology* **13**, 1550–1564.

Hassell, M.P. (1978) The dynamics of arthropod predator-prey systems. *Functional responses* (ed. M.P. Hassell), pp. 28–49, Princeton University Press, Princeton.

Hatcher, M. & Dunn, A. (2011) *Parasites in ecological communities: from interactions to ecosystems.* Cambridge University Press, Cambridge.

Hatcher, M.J., Dick, J.T. & Dunn, A.M. (2012) Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment* **10**, 186–194.

Hatcher, M.J., Dick, J.T.A. & Dunn, A.M. (2014) Parasites that change predator or prey behaviour can have keystone effects on community composition. *Biology Letters* **10**, 20130879.

Hauer, F.R. & Lamberti, G.A. (2007) *Methods in Stream Ecology.*

Heath, M.R., Speirs, D.C. & Steele, J.H. (2014) Understanding patterns and processes in models of trophic cascades. *Ecology Letters* **17**, 101–114.

Hodek, I., van Emden, H.F. & Honek, A. (eds.) (2012) *Ecology and Behaviour of the Ladybird Beetles (Coccinellidae)*. Wiley-Blackwell Publishing.

Holling, C.S. (1959) Some characteristics of simple types of predation and parasitism. *The Canadian Entomologist* **91**, 385–398.

Honek, A., Martinkova, Z., Dixon, A.F., Roy, H.E. & Pekár, S. (2016) Long-term changes in communities of native coccinellids: Population fluctuations and the effect of competition from an invasive non-native species. *Insect Conservation and Diversity* **9**, 202–209.

Hulme, P.E. (2007) Biological invasions in Europe: Drivers, pressures, states, impacts and responses. *Biodiversity Under Threat* (eds. R. Hester & R.M. Harrisson), 25, pp. 56–80, Cambridge University Press, Cambridge.

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

Ingels, B., Van Hassel, P., Van Leeuwen, T. & De Clercq, P. (2015) Feeding history affects intraguild interactions between *Harmonia axyridis* (Coleoptera: Coccinellidae) and *Episyrphus balteatus* (Diptera: Syrphidae). *Plos One* **10**, e0128518.

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*. Geneva, Switzerland.

Isaac, N.J., Van Strien, A.J., August, T.A., de Zeeuw, M.P. & Roy, D.B. (2014) Statistics for citizen science: Extracting signals of change from noisy ecological data. *Methods in Ecology and Evolution* **5**, 1052–1060.

Josens, G., De Vaate, A.B., Usseglio-Polatera, P., Cammaerts, R., Chérot, F., Grisez, F., Verboonen, P. & Vanden Bossche, J.P. (2005) Native and exotic Amphipoda and other Peracarida in the River Meuse: New assemblages emerge from a fast changing fauna. *Hydrobiologia* **542**, 203–220.

Jourdan, J., Westerwald, B., Kiechle, A., Chen, W., Streit, B., Klaus, S., Oetken, M. & Plath, M. (2016) Pronounced species turnover, but no functional equivalence in leaf consumption of invasive amphipods in the river Rhine. *Biological Invasions* **18**, 763–774.

Juliano, S.A. (2001) Nonlinear curve fitting: Predation and functional response curves. *Design and analysis of ecological experiments* (eds. S.M. Scheiner & J. Gurevitch), pp. 178–196, Oxford University Press, New York, second edn.

Juliano, S.A. & Philip Lounibos, L. (2005) Ecology of invasive mosquitoes: Effects on resident species and on human health. *Ecology Letters* **8**, 558–574.

Katsanis, A., Babendreier, D., Nentwig, W. & Kenis, M. (2013) Intraguild predation between the invasive ladybird *Harmonia axyridis* and non-target European coccinellid species. *BioControl* **58**, 73–83.

Keeler, M.S., Chew, F.S., Goodale, B.C. & Reed, J.M. (2006) Modelling the impacts of two exotic invasive species on a native butterfly: Top-down vs. bottom-up effects. *Journal of Animal Ecology* **75**, 777–788.

Keeling, M.J., Franklin, D.N., Datta, S., Brown, M.A. & Budge, G.E. (2017) Predicting the spread of the Asian hornet (*Vespa velutina*) following its incursion into Great Britain. *Scientific Reports* **7**.

Keller, R., Geist, J., Jeschke, J. & Kuhn, I. (2011) Invasive species in Europe: ecology, status, and policy. *Environmental Sciences Europe* **23**.

Kenis, M., Adriaens, T., Brown, P.M.J., Katsanis, A., Martin, G.S., Branquart, E., Maes, D., Eschen, R., Zindel, R., van Vlaenderen, J., Babendreier, D., Roy, H.E., Hautier, L. & Poland, R.L. (2017) Assessing the ecological risk posed by a recently established invasive alien predator: *Harmonia axyridis* as a case study. *BioControl* **62**, 341–354.

Kenis, M., Auger-Rozenberg, M.A., Roques, A., Timms, L., Péré, C., Cock, M.J.W., Settele, J., Augustin, S. & Lopez-Vaamonde, C. (2009) Ecological effects of invasive alien insects. *Biological Invasions* **11**, 21–45.

Kenna, D., Fincham, W.N.W., Dunn, A.M., Brown, L.E. & Hassall, C. (2016) Antagonistic effects of biological invasion and environmental warming on detritus processing in freshwater ecosystems. *Oecologia* .

Kettunen, M., Genovesi, P., Gollasch, S., Pagad, S., Starfinger, U., ten Brink, P. & Shine, C. (2008) Technical support to EU strategy on invasive alien species (IAS) - Assessment of the impacts of IAS in Europe and the EU (final module report for the European Commission). Tech. rep.

Klose, K. & Cooper, S.D. (2013) Complex impacts of an invasive omnivore and native consumers on stream communities in California and Hawaii. *Oecologia* **171**, 945–960.

Koch, R., Hutchison, W., Venette, R. & Heimpel, G. (2003) Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biological Control* **28**, 265–270.

Koch, R.L. & Galvan, T.L. (2008) Bad side of a good beetle: The North American experience with *Harmonia axyridis*. *Biocontrol* **53**, 23–35.

Kögel, S., Gross, J. & Hoffmann, C. (2011) The harlequin ladybird *Harmonia axyridis* – Sensory thresholds of the "ladybird taint" in wine under different processing conditions. *Nature*.

Kögel, S., Schieler, M. & Hoffmann, C. (2013) The ladybird beetle *harmonia axyridis* (Coleoptera: Coccinellidae) as a possible predator of grape phylloxera *Daktulosphaira vitifoliae* (Hemiptera: Phylloxeridae). *European Journal of Entomology* **110**, 123–128.

Koyama, S. & Majerus, M.E.N. (2007) Interactions between the parasitoid wasp Dinocampus coccinellae and two species of coccinellid from Japan and Britain. *BioControl* **53**, 253–264.

Kueffer, C., Pyšek, P. & Richardson, D.M. (2013) Integrative invasion science: Model systems, multi-site studies, focused meta-analysis and invasion syndromes. *New Phytologist* **200**, 615–633.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P., Dunne, J.a., Johnson, P.T.J., Kuris, A.M., Marcogliese, D.J., Martinez, N.D., Memmott, J., Marquet, P.a., McLaughlin, J.P., Mordecai, E.a., Pascual, M., Poulin, R. & Thieltges, D.W. (2008) Parasites in food webs: The ultimate missing links. *Ecology Letters* **11**, 533–546.

Lafferty, K.D., Dobson, A.P. & Kuris, A.M. (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 11211–11216.

Lamberti, G.A. & Steinman, A.D. (1993) Research in artificial streams: Applications, uses, and abuses. *Journal of the North American Benthological Society* **12**, 313–384.

Laverty, C., Brenner, D., McIlwaine, C., Lennon, J.J., Dick, J.T., Lucy, F.E. & Christian, K.A. (2017a) Temperature rise and parasitic infection interact to increase the impact of an invasive species. *International Journal for Parasitology* **47**, 291–296.

Laverty, C., Dick, J.T.A., Alexander, M.E. & Lucy, F.E. (2015) Differential ecological impacts of invader and native predatory freshwater amphipods under environmental change are revealed by comparative functional responses. *Biological Invasions* **17**, 1761–1770.

Laverty, C., Green, K.D., Dick, J.T.A., Barrios-O'Neill, D., Mensink, P.J., Médoc, V., Spataro, T., Caffrey, J.M., Lucy, F.E., Boets, P., Britton, J.R., Pegg, J. & Gallagher, C. (2017b) Assessing the ecological impacts of invasive species based on their functional responses and abundances. *Biological Invasions* pp. 1653–1665.

Lee, J.H. & Kang, T.J. (2004) Functional response of Harmonia axyridis (Pallas) (Coleoptera: Coccinellidae) to Aphis gossypii Glover (Homoptera: Aphididae) in the laboratory. *Biological Control* **31**, 306–310.

Lester, P.J., Beggs, J. & Brown, R.L. (2013) The outlook for control of New Zealand's most abundant, widespread and damaging invertebrate pests: social wasps. *New Zealand Science Review* **70**, 56–62.

Levine, J.M. & D'Antonio, C.M. (2003) Forecasting biological invasions with increasing international trade. *Conservation Biology* **17**, 322–326.

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

Lombaert, E., Guillemaud, T., Cornuet, J.M., Malausa, T., Facon, B. & Estoup, A. (2010) Bridgehead effect in the worldwide invasion of the biocontrol *Harlequin Ladybird*. *PLoS ONE* **5**, e9743.

Maazouzi, C., Piscart, C., Legier, F. & Hervant, F. (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* **159**, 268–274.

MacArthur, R.H. & Pianka, E.R. (1966) On optimalbuse of a patchy environment. *The American Naturalist* **100**, 603–609.

MacNeil, C., Boets, P., Lock, K. & Goethals, P.L.M. (2013) Potential effects of the invasive 'killer shrimp' (*Dikerogammarus villosus*) on macroinvertebrate assemblages and biomonitoring indices. *Freshwater Biology* **58**, 171–182.

MacNeil, C., Dick, J.T.a., Platvoet, D. & Briffa, M. (2011) Direct and indirect effects of species displacements: an invading freshwater amphipod can disrupt leaf-litter processing and shredder efficiency. *Journal of the North American Benthological Society* **30**, 38–48.

MacNeil, C., Fielding, N.J., Hume, Kevin, D., Dick, J.T., Elwood, R.W., Hatcher, M.J. & Dunn, A.M. (2003) Parasite altered micro-distribution of *Gammarus pulex*(Crustacea: Amphipoda). *International Journal for Parasitology* **33**, 57–64.

MacNeil, C. & 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* **267**, 31–38.

Madgwick, G. & Aldridge, D.C. (2011) Killer shrimps in Britain: Hype or horror? The facts about our latest invasive animal. *British Wildlife* pp. 408–412.

Majerus, M., Strawson, V. & Roy, H. (2006) The potential impacts of the arrival of the harlequin ladybird, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), in Britain. *Ecological Entomology* **31**, 207–215.

Majerus, M.E. & Kearns, P. (1994) *Ladybirds. No 81. New Naturalist Series.* Harper Collins, London.

Marcarelli, A.M., Baxter, C.V., Mineau, M.M. & Hall, R.O. (2011) Quantity and quality: unifying food web and ecosystem perspectives on the role of resource subsidies in freshwaters. *Ecology* **92**, 1215–1225.

Martay, B., Brewer, M.J., Elston, D.A., Bell, J.R., Harrington, R., Brereton, T.M., Barlow, K.E., Botham, M.S. & Pearce-Higgins, J.W. (2017) Impacts of climate change on national biodiversity population trends. *Ecography* **40**, 1139–1151.

Matthews, J., Beringen, R., Creemers, R., Hollander, H., van Kessel, N., van Kleef, H., van de Koppel, S., Lemaire, A., Odé, B., Verbrugge, L., Hendriks, J., Schipper, A., van der Velde, G. & Leuven, R. (2017) A new approach to horizon-scanning: identifying potentially invasive alien species and their introduction pathways. *Management of Biological Invasions* **8**, 37–52.

McArthur, J. & Barnes, J. (1988) Community Dynamics of Leaf Litter Breakdown in a Utah Alpine Stream. *Journal of the North American Benthological Society* **7**, 37–43.

McClure, M.S. (1986) Role of predators in regulation of endemic populations of *Matsucoccus matsumurae* (Homoptera: Margarodidae) in Japan. *Environmental Entomology* **15**, 976–983.

McCornack, B.P., Koch, R.L. & Ragsdale, D.W. (1980) A simple method for in-field sex determination of the multicolored Asian lady beetle *Harmonia axyridis*. *Journal of Insect Science* **7**, 1–12.

Mcneely, J. (2001) Invasive species: a costly catastrophe for native biodiversity. *Land Use and Water Resources Research* **1**, 1–10.

Melbourne, B.A. & Hastings, A. (2008) Extinction risk depends strongly on factors contributing to stochasticity. *Nature* **454**, 100–103.

Met Office, Hollis, D. & McCarthy, M. (2017) UKCP09: Met Office gridded and regional land surface climate observation datasets.

Meyling, N.V. & Eilenberg, J. (2007) Ecology of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* in temperate agroecosystems: Potential for conservation biological control. *Biological Control* **43**, 145–155.

Millennium Ecosystem Assessment (2005) Ecosystems and human well-being:biodiversity synthesis. Tech. rep., World Rescources Institute, Washtington, DC.

Ministry for Primary Industries (2016) Pest watch: 11 May – 30 June 2016. *Surveillance* **43**, 75.

Monceau, K., Bonnard, O. & Thiéry, D. (2014) *Vespa velutina*: A new invasive predator of honeybees in Europe. *Journal of Pest Science* **87**, 1–16.

Moorhouse, T.P. & Macdonald, D.W. (2015) Are invasives worse in freshwater than terrestrial ecosystems? *WIREs Water* **2**, 1–8.

Newman, J.A. (2005) Climate change and the fate of cereal aphids in Southern Britain. *Global Change Biology* **11**, 940–944.

Nowak, D.J., Pasek, J.E., Sequeira, R.A., Crane, D.E. & Mastro, V.C. (2001) Potential Effect of *Anoplophora glabripennis* (Coleoptera: Cerambycidae) on Urban Trees in the United States. *Journal of Economic Entomology* **94**, 116–122.

Ocasio-Torres, M.E., Crowl, T.A. & Sabat, A.M. (2015) Effects of the presence of a predatory fish and the phenotype of its prey (a shredding shrimp) on leaf litter decomposition. *Freshwater Biology* **60**, 2286–2296.

O'Neill, R.J. (1990) Functional response of arthropod predators and its role in the biological control of insect pests in agricultural systems. *New directions in biological control: alternatives for suppressing agricultural pests and diseases.* (eds. P.E. Dunn & R.R. Baker), pp. 83–96, Alan R. Liss Inc., New York.

Ormond, E.L., Thomas, A.P.M., Pell, J.K., Freeman, S.N. & Roy, H.E. (2011) Avoidance of a generalist entomopathogenic fungus by the ladybird, *Coccinella septempunctata*. *FEMS Microbiology Ecology* **77**, 229–237.

Orr, H., des Clers, S., Simpson, G., Hughes, M., Battarbee, R., Cooper, L., Dunbar, M., Evans, R., Hannaford, J., Hannah, D., Laize, C., Richards, K., Watts, G. & Wilby, R. (2010) Changing water temperatures: a surface water archive for England and Wales. *Role of Hydrology in Managing Consequences of a Changing Global Environment* (ed. C. Kirby), British Hydrological Society.

Pace, M.L., Cole, J.J., Carpenter, S.R. & Kitchell, J.F. (1999) Trophic cascades revealed in diverse ecosystems. *Trends in Ecology and Evolution* **14**, 483–488.

Paisley, M.F., Trigg, D.J. & Walley, W.J. (2014) Revision of the biological monitoring working party (BMWP) score system: Derivation of present-only and abundance-related scores form field data. *River Research and Applications* pp. 887–904.

Parker, J., Torchin, M., Hufbauer, R., Lemonine, N., Alba, C., Blumenthal, D., Jarosik, V., Kanarek, A., Martin, L., Perkins, S., Pysek, P., Schierenbeck, K., Schloder, C., van Klinkin, R., Vaughn, K., Williams, W. & Wolfe, L. (2013) Do invasive species perform better in their new ranges? *Ecology* **94**, 985–994.

Perrings, C., Burgiel, S., Lonsdale, M., Mooney, H. & Williamson, M. (2010) International cooperation in the solution to trade-related invasive species risks. *Annals of the New York Academy of Sciences* **1195**, 198–212.

Pickering, G.J., Lin, Y., Reynolds, A., Soleas, G., Riesen, R. & Brindle, I. (2005) The influence of *Harmonia axyridis* on wine composition and aging. *Journal of Food Science* **70**, 128–135.

Piggott, J.J., Townsend, C.R. & Matthaei, C.D. (2015) Climate warming and agricultural stressors interact to determine stream macroinvertebrate community dynamics. *Global Change Biology* **21**, 1887–1906.

Pimentel, D., Larch, L., Zuniga, R. & Morrison, D. (2000) Environmental and economic costs of nonindigenous species in the United States. *BioScience* **50**, 53–65.

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

Piscart, C., Mermillod-Blondin, F., Maazouzi, C., Merigoux, S. & Marmonier, P. (2011) Potential impact of invasive amphipods on leaf litter recycling in aquatic ecosystems. *Biological Invasions* **13**, 2861–2868.

Pocock, M.J., Roy, H.E., Preston, C.D. & Roy, D.B. (2015) The Biological Records Centre: A pioneer of citizen science. *Biological Journal of the Linnean Society* **115**, 475–493.

Poprawski, T.J., Parker, P.E. & Tsai, J.H. (1999) Laboratory and field evaluation of hyphomycete insect pathogenic fungi for control of brown citrus aphid (Homoptera: Aphididae). *Environmental Entomology* **28**, 315–321.

Prenter, J., MacNeil, C., Dick, J.T.A. & Dunn, A.M. (2004) Roles of parasites in animal invasions. *Trends in Ecology and Evolution* **19**, 385–390.

Purse, B.V., Comont, R., Butler, A., Brown, P.M.J., Kessel, C. & Roy, H.E. (2014) Landscape and climate determine patterns of spread for all colour morphs of the alien ladybird *Harmonia axyridis*. *Journal of Biogeography* **42**, 1–14.

R Core Team (2016) Computing, R: A Language and Environment for Statistical Computing.

Rewicz, T., Grabowski, M., MacNeil, C. & Karolina, B. (2014) The profile of a 'perfect' invader – the case of killer shrimp, *Dikerogammarus villosis*. *Aquatic Invasions* **9**, 267–288.

Reynolds, J.D. (1988) Crayfish extinctions and crayfish plague in central Ireland. *Biological Conservation* **45**, 279–285.

Ribbands, B., Brown, P.M.J. & Majerus, M.E. (2009) The most northerly record of the harlequin ladybird in the British Isles. *Entomologist's Monthly Magazine* pp. 43–44.

Ricciardi, A., Blackburn, T.M., Carlton, J.T., Dick, J.T., Hulme, P.E., Iacarella, J.C., Jeschke, J.M., Liebhold, A.M., Lockwood, J.L., MacIsaac, H.J., Pyšek, P., Richardson, D.M., Ruiz, G.M., Simberloff, D., Sutherland, W.J., Wardle, D.A. & Aldridge, D.C. (2017) Invasion science: A horizon scan of emerging challenges and opportunities. *Trends in Ecology and Evolution* **32**, 464–474.

Ricciardi, A., Hoopes, M.F., Marchetti, M.P. & Lockwood, J.L. (2013) Progress toward understanding the ecological impacts of nonnative species. *Ecological Applications* **83**, 263–282.

Ricciardi, A. & Ryan, R. (2018a) Invasive species denialism revisited: response to Sagoff. *Biological Invasions* pp. 1–8.

Ricciardi, A. & Ryan, R. (2018b) The exponential growth of invasive species denialism. *Biological Invasions* **20**, 549–553.

Rogers, D. (1972) Random search and insect population models. *Journal of Animal Ecology* **41**, 369–383.

Röhrich, C.R., Ngwa, C.J., Wiesner, J., Schmidtberg, H., Degenkolb, T., Kollewe, C., Fischer, R., Pradel, G., Vilcinskas, A., Rohrich, C.R., Ngwa, C.J., Wiesner, J., Schmidtberg, H., Degenkolb, T., Kollewe, C., Fischer, R., Pradel, G. & Vilcinskas, A. (2012) Harmonine, a defence compound from the harlequin ladybird, inhibits mycobacterial growth and demonstrates multi-stage antimalarial activity. *Biology letters* **8**, 308–311.

Rothamstead Research (2015) Rothamsted Insect Survey.

Rotherham, I. (2001) Rhododendron gone wild: Conservation implications of *Rhododendron ponticum* in Britain. *Biologist (London, England)* **48**, 7–11.

Roy, E., Adriaens, T., Aldridge, D.C., Blackburn, T.M., Branquart, E., Brodie, J., Carboneras, C., Cook, E., Copp, G.H., Dean, H., Eilenberg, J., Essl, F., Gallardo, B., Garcia, M., Garcia-Berthou, E., Genovesi, P., Hulme, P.E., Kenis, M., Kerckhof, F., Kettunen, M., Minchin, D., Nentwig, W., Nieto, A., Scalera, R., Schindler, S., Schonrogge, K., Sewell, J., Solarz, W., Stewart, A., Tricarico, E., Vanderhoeven, S., van der Velde, G., Vilà, M., Wood, C. & Zenetos, A. (2015a) Invasive alien species - prioritising prevention efforts through horizon scanning ENV.B.2/ETU/2014/0016. Tech. rep., European Commission.

Roy, H., Brown, P.M.J. & Majerus, M.E. (2006) Harmonia axyridis: a successful biocontrol agent or an invasive threat? *An Ecological and Societal Approach to Biological Control*, pp. 295–309, Springer, Dordrecht.

Roy, H.E., Adriaens, T., Isaac, N.J.B., Kenis, M., Onkelinx, T., Martin, G.S., Brown, P.M.J., Hautier, L., Poland, R., Roy, D.B., Comont, R., Eschen, R., Frost, R., Zindel, R., Van Vlaenderen, J., Nedvěd, O., Ravn, H.P., Grégoire, J.C., de Biseau, J.C. &

Maes, D. (2012) Invasive alien predator causes rapid declines of native European ladybirds. *Diversity and Distributions* **18**, 717–725.

Roy, H.E., Baverstock, J., Ware, R.L., Clark, S.J., Majerus, M.E.N., Baverstock, K.E. & Pell, J.K. (2008a) Intraguild predation of the aphid pathogenic fungus *Pandora neoaphidis* by the invasive coccinellid *Harmonia axyridis*. *Ecological Entomology* **33**, 175–182.

Roy, H.E. & Brown, P.M.J. (2015) Ten years of invasion: *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Britain. *Ecological Entomology* .

Roy, H.E., Brown, P.M.J., Adriaens, T., Berkvens, N., Borges, I., Clusella-Trullas, S., Comont, R.F., De Clercq, P., Eschen, R., Estoup, A., Evans, E.W., Facon, B., Gardiner, M.M., Gil, A., Grez, A.A., Guillemaud, T., Haelewaters, D., Herz, A., Honek, A., Howe, A.G., Hui, C., Hutchison, W.D., Kenis, M., Koch, R.L., Kulfan, J., Lawson Handley, L., Lombaert, E., Loomans, A., Losey, J., Lukashuk, A.O., Maes, D., Magro, A., Murray, K.M., Martin, G.S., Martinkova, Z., Minnaar, I.A., Nedved, O., Orlova-Bienkowskaja, M.J., Osawa, N., Rabitsch, W., Ravn, H.P., Rondoni, G., Rorke, S.L., Ryndevich, S.K., Saethre, M.G., Sloggett, J.J., Soares, A.O., Stals, R., Tinsley, M.C., Vandereycken, A., van Wielink, P., Viglášová, S., Zach, P., Zakharov, I.A., Zaviezo, T. & Zhao, Z. (2016a) The harlequin ladybird, *Harmonia axyridis*: global perspectives on invasion history and ecology. *Biological Invasions* **18**, 997–1044.

Roy, H.E., Brown, P.M.J., Comont, R.F., Poland, R.L., Sloggett, J.J., Allington, S. & Shields, C. (2013) *Ladybirds (Naturalists Handbook)*. Pelagic Publishing, second edn.

Roy, H.E., Brown, P.M.J., Frost, R. & Poland, R. (2011) *Ladybirds (Coccinellidea) of Britain and Ireland*. FSC Publications, Shrewsbury.

Roy, H.E., Brown, P.M.J. & Rothery, P. (2008b) Interactions between the fungal pathogen *Beauveria bassiana* and three species of ladybird: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. *BioControl* **53**, 265–276.

Roy, H.E., Hesketh, H., Purse, B.V., Eilenberg, J., Santini, A., Scalera, R., Stentiford, G.D., Adriaens, T., Bacela-Spychalska, K., Bass, D., Beckmann, K.M., Bessell, P., Bojko, J., Booy, O., Cardoso, A.C., Essl, F., Groom, Q., Harrower, C., Kleespies, R., Martinou, A.F., van Oers, M.M., Peeler, E.J., Pergl, J., Rabitsch, W., Roques, A., Schaffner, F., Schindler, S., Schmidt, B.R., Schönrogge, K., Smith, J., Solarz, W., Stewart, A., Stroo, A., Tricarico, E., Turvey, K.M.A., Vannini, A., Vilá, M., Woodward, S., Wynns, A.A. & Dunn, A.M. (2016b) Alien pathogens on the horizon: Opportunities for predicting their threat to wildlife. *Conservation Letters* **10**, 477–484.

Roy, H.E., Peyton, J., Aldridge, D.C., Bantock, T., Blackburn, T.M., Britton, R., Clark, P. & Cook, E. (2014) Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. *Global Change Biology* **20**, 3859–3871.

Roy, H.E., Rorke, S.L., Beckmann, B., Booy, O., Botham, M.S., Brown, P.M.J., Harrower, C., Noble, D., Sewell, J. & Walker, K. (2015b) The contribution of volunteer recorders to our understanding of biological invasions. *Biological journal of the Linnean Society* **115**, 678–689.

RStudio (2016) RStudio: Integrates development environment for R.

Russell, J.C. & Blackburn, T.M. (2017a) Invasive alien species: denialism, disagreement, definitions, and dialogue. *Trends in Ecology and Evolution* **32**, 312–314.

Russell, J.C. & Blackburn, T.M. (2017b) The rise of invasive species denialism. *Trends in Ecology and Evolution* **32**, 3–6.

Sabelis, M.W. (1992) Arthropod predators. *Natural enemies, the population biology of predators, parasites and diseases* (ed. M.J. Crawley), pp. 225–264, Blackwell Publishing Ltd, Oxford.

Sagoff, M. (2018) Invasive species denialism: a reply to Ricciardi and Ryan. *Biological Invasions* pp. 1–7.

Salo, P., Korpimäki, E., Banks, P.B., Nordström, M. & Dickman, C.R. (2007) Alien predators are more dangerous than native predators to prey populations. *Proceedings of the Society* **274**, 1237–1243.

Sax, D.F. & Gaines, S.D. (2003) Species diversity: From global decreases to local increases. *Trends in Ecology and Evolution* **18**, 561–566.

Sax, D.F., Gaines, S.D. & Brown, J.H. (2002) Species invasions exceed extinctions on islands worldwide: A comparative study of plants and birds. *The American Naturalist* **160**, 766–783.

Sax, D.F., Stachowicz, J.J., Brown, J.H., Bruno, J.F., Dawson, M.N., Gaines, S.D., Grosberg, R.K., Hastings, A., Holt, R.D., Mayfield, M.M., O'Connor, M.I. & Rice, W.R. (2007) Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution* **22**, 465–471.

Schindler, D.W. (1977) Evolution of phosphorus limitation on lakes. *Science* **195**, 260–262.

Schindler, D.W. (1998) Whole-ecosystem experiments: Replication versus realism: The need for ecosystem-scale experiments. *Ecosystems* **1**, 323–334.

Schmidtberg, H., Röhrich, C., Vogel, H. & Vilcinskas, A. (2013) A switch from constitutive chemical defence to inducible innate immune responses in the invasive ladybird *Harmonia axyridis*. *Biology Letters* **9**, 1–4.

Seebens, H., Blackburn, T.M., Dyer, E., Genovesi, P., Hulme, P.E., Jeschke, J.M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., Kartesz, J., Kenis, M., Kreft, H., Kühn, I., Lenzner, B., Liebhold, A., Mosena, A., Moser, D., Nishino, M., Pearman, D., Pergl, J., Rabitsch, W., Rojas-Sandoval, J., Roques, A., Rorke, S., Rossinelli, S., Roy, H.E., Scalera, R., Schindler, S., Štajerová, K., Tokarska-Guzik, B., van Kleunen, M., Walker, K., Weigelt, P., Yamanaka, T. & Essl, F. (2017) No saturation in the accumulation of alien species worldwide. *Nature Communications* **15**, 14435.

Seebens, H., Essl, F., Dawson, W., Fuentes, N., Moser, D., Pergl, J., Pyšek, P., van Kleunen, M., Weber, E., Winter, M. & Blasius, B. (2015) Global trade will accelerate plant invasions in emerging economies under climate change. *Global Change Biology* **21**, 4128–4140.

Seko, T. & Miura, K. (2008) Functional response of the lady beetle *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) on the aphid *Myzus persicae* (Sulzer) (Homoptera: Aphididae). *Applied Entomology and Zoology* **43**, 341–345.

Seko, T., Sumi, A., Nakano, A., Kameshiro, M., Kaneda, T. & Miura, K. (2014) Suppression of aphids by augmentative release of larvae of flightless *Harmonia axyridis*. *Journal of Applied Entomology* **138**, 326–337.

Shapiro-Ilan, D.I. & Cottrell, T.E. (2011) Susceptibility of lady beetles (Coleoptera: Coccinellidae) to entomopathogenic nematodes. *Journal of invertebrate pathology* **89**, 150–156.

Shipp, J.L. & Whitfield, G.H. (1991) Functional response of the predatory mite, *Amblyseius cucumeris* (Acari: Phytoseiidae), on western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae). *Environmental Entomology* **20**, 694–699.

Simberloff, D., Martin, J.l., Genovesi, P., Maris, V., Wardle, D.A., Aronson, J., Courchamp, F., Galil, B., Pascal, M. & Pys, P. (2013) Impacts of biological invasions: what's what and the way forward. *Trends in Ecology & Evolution* **28**, 58 – 66.

Snyder, W.E. (2009) Coccinellids in diverse communities: Which niche fits? *Biological Control* **51**, 323–335.

Snyder, W.E. & Evans, E.W. (2006) Ecological effects of invasive arthropod generalist predators. *Annual Review of Ecology, Evolution, and Systematics* **37**, 95–122.

Sorte, C.J.B., Ibáñez, I., Blumenthal, D.M., Molinari, N.a., Miller, L.P., Grosholz, E.D., Diez, J.M., D'Antonio, C.M., Olden, J.D., Jones, S.J. & Dukes, J.S. (2013) Poised to prosper? A cross-system comparison of climate change effects on native and non-native species performance. *Ecology Letters* **16**, 261–270.

Srivastava, D.S., Kolasa, J., Bengtsson, J., Gonzalez, A., Lawler, S.P., Miller, T.E., Munguia, P., Romanuk, T., Schneider, D.C. & Trzcinski, M.K. (2004) Are natural microcosms useful model systems for ecology? *Trends in Ecology and Evolution* **19**, 379–384.

Stals, R. & Prinsloo, G. (2007) Discovery of an alien invasive, predatory insect in South Africa: the multicoloured Asian ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae). *South African Journal Of Science* **103**, 123–126.

Steinman, A.D., Lamberti, G.A. & Leavitt, P. (1996) Biomass and pigments of benthic algae. *Methods in Stream Ecology* (eds. F. Hauer & G. Lamberti), pp. 357–359, Elsevier Inc., Oxford.

Strauss, A., White, A. & Boots, M. (2012) Invading with biological weapons: The importance of disease-mediated invasions. *Functional Ecology* **26**, 1249–1261.

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

Tanentzap, A.J., Burrows, L.E., Lee, W.G., Nugent, G., Maxwell, J.M. & Coomes, D.A. (2009) Landscape-level vegetation recovery from herbivory: Progress after four decades of invasive red deer control. *Journal of Applied Ecology* **46**, 1064–1072.

Tanner, R.A., Varia, S., Eschen, R., Wood, S., Murphy, S.T. & Gange, A.C. (2013) Impacts of an invasive non-native annual weed, *Impatiens glandulifera*, on above- and below-ground invertebrate communities in the United Kingdom. *PLoS ONE* **8**, e67271.

Tassin, J., Thompson, K., Carroll, S.P. & Thomas, C.D. (2017) Determining whether the impacts of introduced species are negative cannot be based solely on science: A response to Russell and Blackburn. *Trends in Ecology and Evolution* **32**, 230–231.

Taylor, N.G. & Dunn, A.M. (2016) Size matters: predation of fish eggs and larvae by native and invasive amphipods. *Biological Invasions* pp. 1–19.

Therneau, T. (2015) A package for survival analysis in S.

Thomas, C.D. (2010) Climate, climate change and range boundaries. *Diversity and Distributions* **16**, 488–495.

Thomas, C.D. & Palmer, G. (2015) Non-native plants add to the British flora without negative consequences for native diversity. *Proceedings of the National Academy of Sciences* **2015**, 1–6.

Tiegs, S.D., Clapcott, J.E., Griffiths, N.A. & Boulton, A.J. (2013) A standardized cotton-strip assay for measuring organic-matter decomposition in streams. *Ecological Indicators* **32**, 131–139.

Toscano, B.J., Newsome, B. & Griffen, B.D. (2014) Parasite modification of predator functional response. *Oecologia* **175**, 345–352.

Truhlar, A.M., Dodd, J.A. & 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**, 56–65.

Tumolo, B.B. & Flinn, M.B. (2017) Top-down effects of an invasive omnivore: detection in long-term monitoring of large-river reservoir chlorophyll-*a*. *Oecologia* **185**, 293–303.

UK Ladybird Survey (2018) UK Ladybird Survey.

United Nations (2015) Transforming our world: The 2030 agenda for sustainable development. Tech. rep.

Van der Velde, G., Leuven, R.S.E.W., Platvoet, D., Bacela, K., Huijbregts, M.A.J., Hendriks, H.W.M. & Kruijt, D. (2009) Environmental and morphological factors influencing predatory behaviour by invasive non-indigenous gammaridean species. *Biological Invasions* **11**, 2043–2054.

van Emden, H.F. & Harrington, R. (eds.) (2017) *Aphids as Crop Pests*. CABI, Cambridge, MA, USA, 2nd edn.

Van Kleunen, M., Weber, E. & Fischer, M. (2010) A meta-analysis of trait differences between invasive and non-invasive plant species. *Ecology Letters* **13**, 235–245.

Van Leeuwen, E., Brannstrom, A., Jansen, V.A.A., Dieckmann, U. & Rossberg, A.G. (2013) A generalized functional response for predators that switch between multiple prey species. *Journal of Theoretical Biology* **328**, 89–98.

Van Riel, M.C., Velde, G.V.D., Rajagopal, S., Marguillier, S., Dehairs, F. & De Vaate, A.B. (2006) Trophic relationships in the Rhine food web during invasion and after establishment of the Ponto-Caspian invader *Dikerogammarus villosus*. *Hydrobiologia* **565**, 39–58.

Venables, W.N. & Ripley, B.D. (2002) *Modern Applied Statistics with S*. Springer, New York, fourth edn.

Vey, A. & Jacques, F. (1977) Histological and ultrastructural studies of *Beauveria bassiana* infection in *Leptinotarsa decemlineta* larvae during ecdysis. *Journal of Invertebrate Pathology* **30**, 207–215.

Vilcinskas, A. (2015) Pathogens as biological weapons of invasive species. *PLoS Pathogens* **11**, e1004714.

Vilcinskas, A., Stoecker, K., Schmidtberg, H., Röhrich, C.R., Vogel, H., Rohrich, C.R. & Vogel, H. (2013) Invasive harlequin ladybird carries biological weapons against native competitors. *Science* **340**, 862–863.

Vonesh, J., McCoy, M., Altwegg, R., Landi, P. & Measey, J. (2017) Functional responses can't unify invasion ecology. *Biological Invasions* .

Wallace, J.B. & Webster, J.R. (1996) The role of macroinvertebrates in stream ecosystem function. *Annual review of entomology* **41**, 115–139.

Wardle, D.A., Bardgett, R.D., Klironomos, J.N., Setälä, H., Wim, H., Putten, V.D. & Wall, D.H. (2004) Ecological linkages between aboveground and belowground biota. *Science* **304**, 1629–1633.

Ware, R.L. & Majerus, M.E.N. (2008) Intraguild predation of immature stages of British and Japanese coccinellids by the invasive ladybird *Harmonia axyridis*. *BioControl* **53**, 169–188.

Webster, J.R. & Benfield, E.F. (1986) Vascular plant breakdown in freshwater ecosystems. *Annual Review of Ecology and Systematics* **17**, 567–594.

Williams, F., Eschen, R., Harris, A., Djeddour, D., Pratt, C., Shaw, R., Varia, S., Lamontagne-Godwin, J., Thomas, S. & Murphy, S. (2010) The economic cost of invasive non-native species on Great Britain. Tech. rep., CABI.

Wilson, K.A., Magnuson, J.J., Lodge, D.M., Hill, A.M., Kratz, T.K., Perry, W.L. & Willis, T.V. (2004) A long-term rusty crayfish (*Orconectes rusticus*) invasion: dispersal patterns and community change in a north temperate lake. *Canadian Journal of Fisheries and Aquatic Sciences* **61**, 2255–2266.

Woinarski, J.C.Z., Burbidge, A.A. & Harrison, P.L. (2015) Ongoing unraveling of a continental fauna: Decline and extinction of Australian mammals since European settlement. *Proceedings of the National Academy of Sciences* **112**, 4531–4540.

Wollrab, S., Diehl, S. & De Roos, A.M. (2012) Simple rules describe bottom-up and top-down control in food webs with alternative energy pathways. *Ecology Letters* **15**, 935–946.

Woodward, G., Perkins, D.M. & Brown, L.E. (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 2093–2106.

Woodward, G. & Warren, P. (2007) Body size and predatory interactions in freshwaters : scaling from individuals to communities. *Body Size: The structure and function of aquatic ecosystems*, pp. 98–117.

Wright, H.A., Wootton, R.J. & Barber, I. (2006) The effect of *Schistocephalus solidus* infection on meal size of three-spined stickleback. *Journal of Fish Biology* **68**, 801–809.

Wurzbacher, C., Wannicke, N., Grimmett, I.J. & Bärlocher, F. (2016) Effects of FPOM size and quality on aquatic heterotrophic bacteria. *Limnologica* **59**, 109–115.

Xue, Y., Bahlai, C.A., Frewin, A., Sears, M.K. & Schaafsma, A.W. (2009) Predation by *Coccinella septempunctata* and *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Aphis glycines* (Homoptera: Aphididae). *Environmental Entomology* **38**, 708–714.

Yang, N., Haiying, O., Yan, C., Clark, P. & Gollasch, S. (2018) *Eriocheir sinensis* (Chinese mitten crab) Data Sheet.

Yuan, Z.Y. & Chen, H.Y. (2015) Decoupling of nitrogen and phosphorus in terrestrial plants associated with global changes. *Nature Climate Change* **5**, 465–469.

Appendix A

Publications produced during this project

RESEARCH ARTICLE

Water Quality Is a Poor Predictor of Recreational Hotspots in England

Guy Ziv[1]*, Karen Mullin[1], Blandine Boeuf[2], William Fincham[3], Nigel Taylor[3], Giovanna Villalobos-Jiménez[3], Laura von Vittorelli[4], Christine Wolf[5], Oliver Fritsch[1], Michael Strauch[6], Ralf Seppelt[6,7], Martin Volk[6], Michael Beckmann[6]

1 University of Leeds, School of Geography, Leeds, LS2 9JT, United Kingdom, 2 University of Leeds, School of Earth and Environment, Leeds, LS2 9JT, United Kingdom, 3 University of Leeds, School of Biology, Leeds, LS2 9JT, United Kingdom, 4 UFZ, Helmholtz Centre for Environmental Research, Department of Environmental and Planning Law, Permoserstraße 15, 04318 Leipzig, Germany, 5 UFZ, Helmholtz Centre for Environmental Research, Department of Economics, Permoserstraße 15, 04318 Leipzig, Germany, 6 UFZ, Helmholtz Centre for Environmental Research, Department of Computational Landscape Ecology, Permoserstraße 15, 04318 Leipzig, Germany, 7 Institute of Geoscience & Geography, Martin-Luther-University Halle-Wittenberg, 06099 Halle (Saale), Germany

* g.ziv@leeds.ac.uk

Abstract

Maintaining and improving water quality is key to the protection and restoration of aquatic ecosystems, which provide important benefits to society. In Europe, the Water Framework Directive (WFD) defines water quality based on a set of biological, hydro-morphological and chemical targets, and aims to reach good quality conditions in all river bodies by the year 2027. While recently it has been argued that achieving these goals will deliver and enhance ecosystem services, in particular recreational services, there is little empirical evidence demonstrating so. Here we test the hypothesis that good water quality is associated with increased utilization of recreational services, combining four surveys covering walking, boating, fishing and swimming visits, together with water quality data for all water bodies in eight River Basin Districts (RBDs) in England. We compared the percentage of visits in areas of good water quality to a set of null models accounting for population density, income, age distribution, travel distance, public access, and substitutability. We expect such association to be positive, at least for fishing (which relies on fish stocks) and swimming (with direct contact to water). We also test if these services have stronger association with water quality relative to boating and walking alongside rivers, canals or lakeshores. In only two of eight RBDs (Northumbria and Anglian) were both criteria met (positive association, strongest for fishing and swimming) when comparing to at least one of the null models. This conclusion is robust to variations in dataset size. Our study suggests that achieving the WFD water quality goals may not enhance recreational ecosystem services, and calls for further empirical research on the connection between water quality and ecosystem services.

Introduction

Water is one of the most regulated areas in European Union (EU) environmental policy, covering topics as diverse as drinking water [1], bathing water [2], and groundwater [3]. However, early attempts to regulate Europe's aquatic environment were characterized by serious deficits in policy implementation and effectiveness [4]. Through a combination of substantive and procedural measures the EU Water Framework Directive (WFD) [5] represents a major effort to tackle the challenges that have long frustrated endeavours of EU and national policy-makers to improve water quality in Europe. With respect to procedures, the WFD advocates, amongst others, river basin management, i.e. management activities at hydrological rather than administrative scales—the so-called River Basin Districts (RBDs)–as well as the establishment of participatory forums in water planning. The WFD thus responds to the insight that coordination problems lay at the heart of previous failures to effectively reduce water pollution in EU member states.

Over the past decades, water quality standards have evolved from unidimensional characteristics (e.g. water clarity) to multidimensional metrics that account for biological, hydro-morphological and chemical criteria [6]. For surface waters, the WFD quality assessment is based on a measurement scale that rates ecological characteristics as 'high', 'good', 'moderate', 'poor' or 'bad', and chemical characteristics as either 'good' or 'fail'. These metrics are assessed against reference conditions before "major industrialisation, urbanisation and intensification of agriculture" [7]. For instance, 'high' status is characterized by the presence of "no, or only very minor, anthropogenic alterations [. . .] from those normally associated with that type under undisturbed conditions" [8]. The overall substantive policy goal of the WFD is to achieve 'good' or 'high' overall status of both surface- and groundwaters across Europe by 2027, and to protect water bodies from further deterioration. For surface waters, good overall status is defined by high/good state in both ecological and chemical conditions.

The implementation of the WFD has been studied from various disciplinary angles and perspectives [9,10]; for example, its legal [11], ecological [12] and economical [13] implications have been addressed. However, we know little about the social benefits (ecosystem services) generated by the WFD and its outcomes. Furthermore, over the past five years, the European Commission has expressed in a number of policy documents the view that achieving 'good' water status will not only "allow aquatic ecosystems to recover", but will also "deliver the ecosystem services that are necessary to support life and economic activity that depend on water" [14–17] (also see S1 Table). Yet so far, empirical evidence is scarce as to whether improved water status does actually enhance the provision and utilisation of ecosystem services [18–20].

In this paper, we test whether reaching WFD targets enhances cultural ecosystem services, specifically recreation, which is of significant economic and cultural importance in England and across Europe. Various attempts have been made to link water quality to the recreational value of inland waters (e.g. [21–26]), however, these come with a number of shortcomings. First, they typically assess the perceived value of a water body, usually with reference to economic proxies such as willingness-to-pay or the travel-cost method [21,22], rather than actual utilization. Second, the recreational value commonly comes in an aggregated form and does not distinguish between different recreational services (e.g. walking vs. swimming) that may have different water quality requirements [23,24]. Thus, few studies explicitly explore the relationship between actual indicators of water quality and a specific recreational use. As one of the few examples, Vesterinen et al. [25] found an effect of water clarity (Secchi depth) on participation in fishing, and on the frequency of fishing and/or swimming visits across a number of lakes and coasts in Finland. In a U.S. study by Ribaudo & Piper [26] total suspended

sediment, total nitrogen and total phosphate had an effect on the probability that an individual went fishing but not the frequency of trips they make.

While most of the indicators in the abovementioned research are part of the composite WFD 'overall water status' indicator, not all WFD criteria are included. A fuller range of metrics as included in WFD water status are considered in the literature review by Vidal-Aberca et al. [27], who argue that the majority of the hydromorphological and biological indices used in WFD are likely factors in provision and use of recreational services. Nevertheless, to our knowledge only one study [19] has explicitly correlated the WFD ecological status metric to an ecosystem service: fish catch measured as catch per unit effort, in different locations along a one large boreal Finnish lake. Thus, whether the composite WFD overall water status is, as argued by European Commission official documents, an indicator that correlates with societal benefits is still unknown.

To shed light on the putative association between cultural services and WFD overall water status, this paper uses data from several nationwide surveys in eight RBDs in England, which give us a unique opportunity to perform an empirical statistical analysis for different dimensions of cultural ecosystem services across a large land area. Recreation is an important ecosystem service in the United Kingdom (UK), as demonstrated by the UK National Ecosystem Assessment and its follow-on project [28,29]. Within each RBD, we use a statistical analysis comparing the frequency of four recreational activities (walking alongside water bodies, boating, fishing and swimming) in locations of good/high overall water status to different null models (see Methods and overview Fig 1). These null models account for factors such as site access, demography (population density and age distribution) and socio-economic factors (income, ethnicity or people with disability). One would expect that if good water quality is important for recreational ecosystem services there will be a positive association between WFD overall water status and locations of all or some recreational services—hereafter referred to as the 'water quality—recreational ecosystem services hypothesis'. The association should be strongest for those services more dependent on ecological conditions that are measured/reflected by the WFD status assessment. Therefore, we also test whether the strength of association between overall good/high water status and ecosystem services is greater for fishing (which relies on fish stock) and swimming (which involves significant contact with water), and weaker for boating, and walking along rivers, canals or lakeshores (where the relationship with water is less direct).

Methods

Study River Basin Districts and their characteristics

Within the UK, regulation of the environment is devolved, with responsibility allocated to separate authorities for England, Northern Ireland, Scotland, and Wales, each implementing distinct policies and approaches. This results in slightly different monitoring schemes across the UK. Because of this, and the geographic limit of one of the largest datasets (MENE; Natural England's Monitoring of Engagement with the Natural Environment), this article focuses on England and its eight RBDs only. We analysed only RBDs which are wholly within or cover large areas of England, and are under the remit of the English Environment Agency. Two further RBDs—Dee and Solway Tweed—which cover small areas of England but are principally managed by Natural Resources Wales and the Scottish Environmental Protection Agency, were excluded from the analysis.

At approximately 139,000 km in length, England's rivers and canals, in addition to 5,700 lakes and extensive coastal, estuarine and ground waters, are a critical source of multiple and diversified ecosystem services. There are, however, dominant human activities characteristic
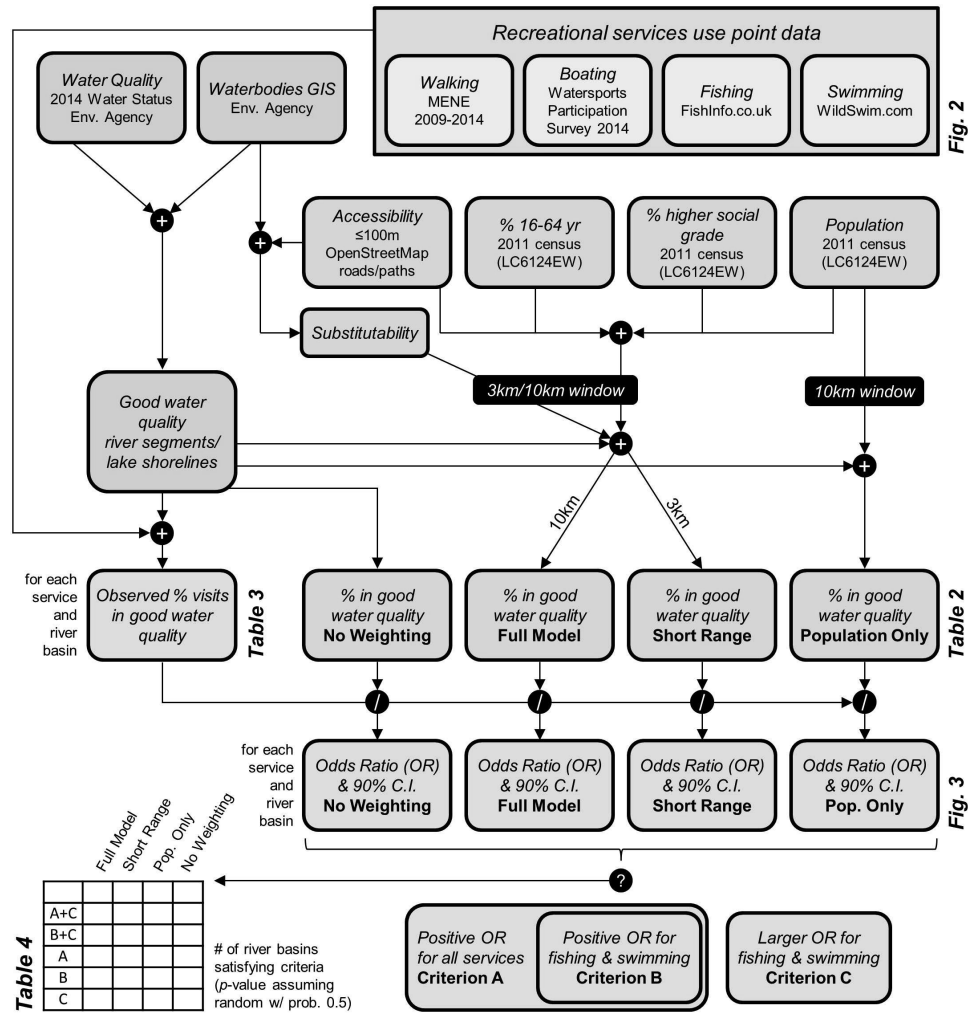
Fig 1. Schematic diagram of the different steps undertaken within the analysis. Multiple data sources were combined (+), compared relative to each other (/) and tested against defined criteria (?). Colors match respective Methods sections: (i) Recreation use data curation (green); (ii) Water status and geospatial data (blue); (iii) Null models (orange); (iv) Statistical analysis (purple).

doi:10.1371/journal.pone.0166950.g001

of each basin, highlighted within the individual River Basin Management Plans [30], which may be synergistic or competitive with recreational use. The main drivers affecting water bodies across all RBDs include urbanization, agriculture, flow modification, invasive species and mining. Table 1 gives an overview of some characteristics of the RBDs and the threats impacting their water bodies, providing some context for the use of waterbodies for recreation purposes.

The extent of different pressures upon the waterbodies in each RBD provides some insight into the past and present uses of land and water. For example, differences in the relative importance of rural and urban/industrial activities to the local economy and the dominance of particular types of industries are key determinants in the usage of water and land. Approximately 70% of land use in England is agricultural [32], and across all eight RBDs, the majority of land is rural. The Thames RBD which includes Greater London is the most urbanised catchment, supporting the largest population and number of visitors, but the predominant economic activity—financial—does not directly utilise the river as a resource. The North West RBD similarly contains some of the most highly populated, previously industrial, urban centres in England, and its aquifers provide a crucial public water supply. However, in the North West, use of water resources is mixed as there is also a large rural economy, for which tourism to its lakes is critical. For the principally rural based economies of the Southwest and Anglian basins, water based tourism constitutes one of the main industries. This is due to the location of the Norfolk Broads (Anglian) and over half of England's bathing waters (Southwest) within these districts.

Recreation use data curation

We used geospatial locations of actual use of inland water (rivers, canals and freshwater lakes) for recreational services (walking, boating, fishing and swimming). Locations were obtained from nationwide surveys conducted between 2002–2014 by different agencies and an online website reporting outdoor swimming sites (Fig 2 and S1 Text).

For walking, we used data from the MENE survey [33], specifically the raw visitation data, in order to obtain locations of outdoor visits. We selected visits whose 'visit location' related to rivers, lakes or canals and the 'outdoor activity' included walking with or without a dog. For boating, we used data from the 2014 Watersports Participation Survey conducted by British Marine Federation (BMF), Royal Yachting Association (RYA), Maritime and Coastguard Agency (MCA), Royal National Lifeboat Institution (RNLI), British Canoe Union (BCU), and the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) [34], from which we selected those locations where the major activity related to boating. For fishing we used a geospatial database of fisheries/venues produced by the Angling Trust [35]. We selected locations where water type was defined as river, canal or still water, excluding transitional and saltwater fisheries. Finally, for swimming we used the locations of reported swimming sites provided by the Outdoor Swimming Society [36]. Further technical details on each data source and preprocessing steps are found in the S1 Text.

To avoid issues related to uneven sampling effort across RBDs which could arise in both in-house surveys and online databases, we statistically analysed each RBD separately, and examined how many of the RBDs agree with the water quality-recreational services hypothesis. To account for uneven sampling effort within RBDs, we repeated the analysis with equal-sized subsamples for each service (see Statistical analysis).

Water status and geospatial data

The Environment Agency (EA) reports annually on the status of individual water bodies in England based on a national standard implementation of the WFD water status classification

Table 1. Data obtained from individual River Basin Management Plans (Environment Agency, 2015) and Land Cover Map of Great Britain 2007 [31]. See inset in Fig 2 for location of River Basic Districts.

River Basin District	Size (km²)	Population (million)	% urban area	% agricultural land	% woodland	Physical modifications	Waste pollution	% water bodies under pressure from:				
								Pollution from towns, cities & transport	Changes to natural flow and level of water	Negative effects of invasive species	Pollution from rural areas	Pollution from abandoned mines
Northumbria	9,000	2.5	7	46	11	38	13	4	2	<1	10	9
North West	13,200	7	12	42	7	50	24	13	2	<1	18	3
Humber	26,100	10.8	10	65	7	42	38	16	6	<1	32	4
Anglian	27,900	7.1	5	74	6	51	50	10	10	6	47	N/A
Severn	21,000	5	6	66	10	27	29	12	7	<1	40	2
Thames	16,200	15	17	63	13	44	45	17	12	3	27	N/A
South East	10,200	3.5	7	55	13	43	40	9	7	2	30	N/A
South West	21,000	5.3	4	62	9	22	33	4	3	1	44	4

doi:10.1371/journal.pone.0166950.t001

Fig 2. Available datasets for cultural ecosystem services use in rivers, canals and lakes across England. Geo-referenced visitation data from the Managing Engagement with the Natural Environment (MENE, Natural England 2009–2014; n = 4459); boating visits in the Watersports Participation Survey (British Marine, MCA, RNLI, RYA, British Canoeing and CEFAS 2014; n = 1298); fishing sites on fishinginfo.co.uk (Angling Trust 2015; n = 816); and outdoor swimming sites on wildswim.com (Outdoor Swimming Society 2015; n = 565). Inset shows the locations of the eight River Basin Districts in England (north to south): Northumbria (NB), North West (NW), Humber (HU), Anglian (AN), Severn (SV), Thames (TM), South East (SE) and South West (SW). Only points near (≤1km) of a river body with a reported 'overall water status' (i.e. WFD water quality standard) in 2014 were included.

doi:10.1371/journal.pone.0166950.g002

[37]. WFD water status combines metrics on ecological integrity (e.g. fish, blooms, littoral invertebrates), physio-chemical elements (e.g. temperature, pH), geomorphology, and over 70 specific pollutants or chemicals compounds (see S1 Text for details). Some of these, for example temperature or phytoplankton blooms, can be directly sensed by people, whereas e.g. the thresholds for most chemicals are below visual and/or olfactory detection limits. As the most recently completed dataset, we used the 2014 water status classification of waterbodies, available on the EA's website [38]. Geospatial data on the location of monitored rivers and lakes are publicly available from the EA, whereas geospatial data on the location of canals, not publicly available, were provided courtesy of the EA. All canals, rivers, and lakeshores were divided into linear segments (average length 5.3 m), resulting in about 9.5 million 'potential sites' for freshwater-based recreational activities. To assign water status to each record in the recreational use data, we matched the location of the visit with the nearest waterbody, keeping only those visits in our dataset which occurred within 1km of a waterbody with a valid water status. In this way we excluded water bodies whose status has not been assessed.

Null models

According to Natural England's MENE survey data of 2013–2014, the following factors affected the participation of people in outdoor recreation activities: age, social grade, ethnic origin, level of deprivation, and whether or not a person had a limiting illness or disability [39]. Amongst others, it was found that people over 65, in social grade DE ("Semi-skilled & unskilled manual occupations, Unemployed and lowest grade occupations"; UK Office for National Statistics (ONS)), of non-white ethnicity or with any disability are underrepresented in outdoor recreational activities. The analysis also shows that 68% of all visits were within 2 miles (3.2 km) and 83% of all visits within 5 miles (8 km) of a respondent's home. Using the MENE data, Bateman et al. [40] similarly found that income, percentage of retired people, percentage of non-white ethnicity, total population and travel time to be highly statistically significant, in addition to variables reflecting land cover and substitutions within a 10 km radius. In contrast to the MENE analysis, however, the effect of proportion of retired people was positive rather than negative. Neither analysis focused specifically on water nor considered the importance of water status in people's choice of recreation sites. Narrowing MENE (and the other datasets) to include only locations nearby water bodies limits the applicability of the approach used by Bateman et al. which requires very large sample sizes. Instead, we developed a null model of the predicted percentage of visits to good/high status sites within a RBD, and compared that with actual use data. We developed four variants of this null model (Table 2), variously including the effect of demand (population, age, income/social grade), substitutability (alternative options within short travel distance from home), and accessibility (distance to OSM road layer features). The four variants test the sensitivity and robustness of our results to null model assumptions.

The general form of the null model for the percentage of visits to locations with good/high water status in RBD j is

$$e_{j,good} = \frac{\sum_{i \in S(j)} w_i g_i}{\sum_{i \in S(j)} w_i}$$

where $g_i = 1$ for potential sites i within the RBD ($S(j)$) where overall water status was classified as 'good' or 'high'. Variants of the model were created using different weighting functions $w_i - w_i = 1$ ('No Weighting'), $w_i = \sum_{k \in A_{10}(i)} p_k$ where $A_x(i)$ is a radius of 10 km around site i and p_k the population density in pixel k on a 100m resolution map of the UK ('Population Only'), and $w_i = \sum_{k \in A_x(i)} r_k p_k a_k i_k / \sum_{k \in A_x(i)} r_k l_k$ where a_k is the percent of population between 16–65

Table 2. Null models for the expected % of visits in good/high overall water status sites.

Null model	Description	Expected % in Good/High Overall Water Status ($e_{j,good}$)							
		Northumbria	North West	Humber	Anglian	Severn	Thames	South East	South West
Full	Weight each river body segment by the ratio of demand and substitutability. Demand is calculated as a 10km radius aggregated population density of adults (age 16–64) with higher income (excluding social grade DE). Substitutability is a 10km (proximity to home) aggregated linear kilometres of rivers, canals and lakeshores. To account for public accessibility, only river/canal/lakeshore segments closer than 100m of a road/path/trail in Open Street Map are included	11.9%	21.7%	17.5%	12.1%	17.9%	3.9%	14.7%	17.3%
Short Range	Same as 'Full' model but assuming shorter trips, with a 3.3km radius (proximity to home) buffer around each river body segment	15.6%	17.4%	15%	9.7%	15.2%	2.5%	13.4%	16.7%
Population Only	Weighting based on 10km radius aggregated population density, includes all river body segments regardless of accessibility	11%	22.1%	24.3%	11.1%	20.6%	4.4%	15.6%	17.3%
No Weighting	All river body segments included with equal probability	27.9%	28.3%	15.1%	10.2%	17.2%	9.5%	12%	18.9%

years of age, i_k is the percentage of working age people not in social grade DE, r_k = 1 if a site is within 100m from a road/pathway and zero otherwise and l_k is the sum of linear length of accessible rivers, canals and lakeshores within pixel k ('Full' model x = 10; 'Short Range' model x = 3.3).

Spatial data for the null models were processed as follows (see also Fig 1). To proxy demand, UK census population data of 2011 [41] were converted and gridded at 100m resolution to create a map of population density. In the 'Full' and 'Short Range' models, this was further filtered by age (including only population aged 16–64 years) and social grade (namely excluding social grade DE) based on UK census data [42]. Social grade is a system of demographic classification in the UK, ranging from upper middle class (A), middle class (B), lower middle class (C1), skilled lower middle class (C2), working class (D), and non-working (E). To analyse the accessibility of sites we used the Open Street Map (OSM) road layer [43], which displays roads, footpaths, and bridleways. We define accessibility as the distance to the nearest feature in the OSM road layer. Nearly 90% of visit data describes locations within a distance of 100m from OSM road layer features (S1 Fig). Unfortunately, information related to public access rights were not consistently available for all roads. We therefore assumed that all OSM features are publically accessible, and so is any river, canal or lakeshore stretch within 100m of these. Substitutability was defined as the total linear length of accessible water bodies (i.e. potential recreational sites) in the vicinity of a site. As a simple proxy for travel time and travel distance, we performed a spatial integration of substitutability and demand over a radius of 10 km (or 3.3 km in the 'Short Range' null model).

Statistical analysis

Our analysis relies on the Odds Ratio (*OR*), contrasting the odds that a member of a specified population will fall into a certain category with the odds that a member of another population will fall into the same category. To this end, we distinguished visits to sites with good/high water status and visits to sites characterized by moderate/poor/bad status. We then compared the actual visitation data to data derived from random sampling, based on the null models described

above, for all potential recreation sites. Formally, $OR_j = n_{j,good}/(n_j − n_{j,good})·(1 − e_{j,good})/e_{j,good}$ where $n_{j,good}$ is the total number of visits to water bodies with a good/high status in RBD j. An OR_j value larger than 1 means positive association, namely that recreational activities are more likely to take place in locations with good/high overall water status. Following Grissom & Kim [44], we calculated a 90% confidence interval, assuming the natural logarithm of OR is normally distributed, or $ln(OR) ± z_{\alpha/2}S_{ln(OR)}$ where $z_{\alpha/2} = 1.645$ is the value of a two-sided standard normal distribution at 90 per cent, and $S_{ln(OR)}^2 \simeq (n_{j,good} + 0.5)^{-1}(n_j − n_{j,good} + 0.5)^{-1}$ where we neglect the terms arising from the much larger sample of population 2 and use the standard bias correction constant [46]. We independently calculate $OR_{j,i}$ for each of the four recreational services ($i = walk$, $boat$, $fish$, $swim$) in each RBD j.

To test the 'water quality—recreational ecosystem services' hypothesis, we considered three quantitative criteria. First (a), we expect all recreational services to have a positive association with WFD overall water status ($OR_{j,i} > 1$). Secondly (b), if the former does not hold, we at least expect that both swimming and fishing—services with direct and prolonged contact with water—would show positive association ($min(OR_{j,swim}, OR_{j,fish}) > 1$). Finally (c), we expect walking and boating to have weaker association than swimming and fishing, where $max(OR_{j,walk}, OR_{j,boat}) < min(OR_{j,swim}, OR_{j,fish})$. Expecting that at least 50% of the RBDs would agree to criteria if the hypothesis is true, we calculated p-values based on binomial probabilities to observe an equal or smaller number of RBDs meeting the criteria by random. To test if the different n for the four datasets affected our results, we repeated this analysis with randomly sub-sampled datasets for walking, boating and fishing with same n as swimming (see S1 Text).

Results

The location and number of site visits related to four ecosystem services—walking, boating, fishing and swimming—as determined by the four surveys used, comprised of a total of 7,177 data points (Table 3). According to these data sets, 22.8% of all walking (alongside a waterbody), 17.9% of all boating, 13.7% of all fishing, and 15.7% of all swimming visits in England took place at sites classified as good/high water status. However, we observe a great degree of variation between the eight RBDs in England. For example, the percentage of walking visits made to good/high water status sites ranges from 5.7% in Anglia to 47.9% in the North-West (Table 3). Likewise, few visits (for all activities) in the Thames RBD take place in sites characterized by a good water status (2.6 to 7%), whilst users in Northumbria and the North-West recreate more often at sites with good/high water status (17.9 to 47.9%).

Expected frequencies of visits to sites with good/high water quality, as predicted by the null models, similarly differ between the river basins but, to an extent, are also dependent upon which null model is applied (Table 2). According to the basic 'No Weighting' model, expected visits to sites with good/high status range from 9.5 to 27.9% across all eight RBDs. However, incorporating population density, household income, substitutability, accessibility and proximity to home (within a 10km radius) substantially changes these rates. Most notably, expected visits to good/high status sites in Northumbria decreased from 27.9 to 11.9%, in the North West from 28.3 to 21.7%, and in the Thames RBD from 9.5 to 3.9% ('No Weighting' model compared to the 'Full' model, Table 2). Assuming a shorter travel distance (3.3 km radius), by applying the 'Short Range' model, only slightly reduces expected rate of good/high status site visits when compared to the 'Full' model. Furthermore, there were no notable differences between the 'Population Only' model and the 'Full' model (Table 2). All null models predict that the rate of visits to good/high water status sites is lowest in the Thames RBD and generally high (>15%) in the North West, Humber, Severn, and South West RBDs.

Table 3. Number of visits and percent in good/high overall water status sites in all eight River Basin Districts.

Dataset	Survey (Year/s)	Source of Data	Criteria for inclusion	n_j (% in Good/High Overall Water Status = $n_{j,good}$ / n_j)							
				Northumbria	North West	Humber	Anglian	Severn	Thames	South East	South West
Walking	MENE (2009–14)	Natural England	*Question 5* option 4 ("Specific visit location included—River, Lake or Canal") positive & *Question 4* option 15 (Walking Without a Dog) and/or option 16 (Walking With a Dog) positive	186 (20.4%)	624 (47.9%)	1467 (24.5%)	597 (5.7%)	657 (23.7%)	527 (7.0%)	138 (25.4%)	282 (22.3%)
Boating	Watersports Participation Survey (2014)	British Marine	*Activity* marked "total boating visits"	71 (25.4%)	175 (28.0%)	273 (16.8%)	160 (9.4%)	105 (23.8%)	182 (6.0%)	121 (14.9%)	229 (21.4%)
Fishing	FishingInfo.co.uk (accessed 8/15)	Angling Trust	*Water type* is river, canal or stillwater	17 (35.3%)	78 (17.9%)	244 (13.1%)	115 (10.4%)	115 (14.8%)	116 (3.4%)	58 (8.6%)	74 (21.6%)
Swimming	WildSwim.com (accessed 8/15)	Outdoor Swimming Society	*Site type* is river (include canals) or lake	14 (42.9%)	74 (18.9%)	118 (9.3%)	61 (19.7%)	62 (11.3%)	117 (2.6%)	25 (32.0%)	95 (23.2%)

doi:10.1371/journal.pone.0166950.t003

The Odds Ratio (*OR*) analysis showed that the actual number of visits by walkers to good/high status sites is higher than expected (under weighted null models) in seven RBDs, and significantly so in most (Fig 3, red boxes). For boating, the 'Full' model suggests higher probabilities of visits to good/high status sites in only five RBDs, with the difference significant in only three (Northumbria, North West, South West; blue boxes in Fig 3). Using the 'Short Range' model, the other two positive associations (Severn and Thames RBDs) become significant, while in the Humber RBD the 'Population Only' model shows a significant negative association. Across all null models, fishing is positively linked to water status in Northumbria and the South West, but negatively associated in the Humber, Severn and South East (Fig 3, green boxes). However, significant associations are only generated by some models in Northumbria and the Humber. Finally, under the 'Full' model, swimming visits are positively associated with water status in four RBDs (significantly so in three) but negatively associated in the other four RBDs (significantly so in only the Humber; yellow boxes in Fig 3). In the 'Short Ranged' model, the association between water status and swimming visits is significantly positive in one additional RBD (South West). In the 'Population Only' and 'No Weighting' models, the correlation between water status and swimming visits is significantly negative in up to three additional RBDs.

We test the 'water quality—recreational ecosystem services' hypothesis by examining the number of RBDs agreeing to different quantitative criteria (see Methods). Postulating that the hypothesis implies positive association of water quality with all services, and stronger association for swimming and fishing, we find that at most one RBD (Northumbria; NB) agrees to both criteria ('(a)+(c)'; Table 4). Even if one expects as few as 50% of RBDs tested to agree to all criteria (assuming the hypothesis is true), this result is highly unlikely by chance alone ($p < 0.05$ based on a binomial distribution). Relaxing the criteria, demanding only swimming and fishing are positively associated with status ('(b)+(c)') we get either 1 or 2 RBDs agreeing to both criteria (p between 0.035 and 0.145) which is still unlikely. Further relaxing those criteria, and using a null model which favours shorter trips ('Short-Ranged' model) would gradually increase the number of RBDs that match. Still, in 17 of 20 combinations of Table 4, the
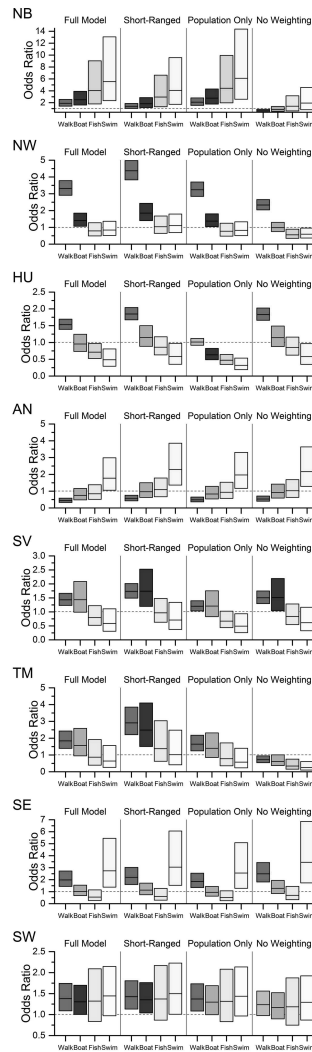
Fig 3. Association of good/high overall water status and use of cultural ecosystem services for the eight River Basin Districts. The Odds Ratio (*OR*) of each River Basin District measures the likelihood that actual visits take place in sites characterized by good/high overall water status compared to random locations selected under a null model accounting for demand and substitutability ([Table 2](#)). *OR* exhibits a statistically significant positive (negative) association (i.e. visits in good/high overall water status sites are more (less) common than random; solid colours) if the 90% confidence interval is completely above (below) the line

probability to observe the same or fewer RBDs in agreement with results is less than 15%. If one increases the expected probability from 50% to 60% (or more) we find that 17 (or more) of these 20 combinations have a $p < 0.05$. Demanding the positive associations are statistically significant (i.e. 90% C.I. is above $OR = 1$), we get only one RBD (Northumbria) ($p = 0.035$) that meets the criteria (a, b and c and their combinations as in Table 4) for the 'Full', 'Short-Ranged' and 'Population-Only' models, and none for the 'No Weighting' model.

To ensure these results are not affected by differences in sampling between services, we performed similar analysis with randomly sub-sampled visitation data for walking, boating and fishing (S2 Table) with similar *n* to those of swimming. We find that between 0.7±0.5 (mean and standard deviation for 'Full' model for '(a)+(c)' criteria) RBDs to 1.3±0.8 RBDs ('Short-Ranged' model, '(b)+(c)' criteria) conform with the more stringent sets of criteria of the water quality-recreational ecosystem services hypothesis. Furthermore, in 16 of 20 combinations of S2 Table, we get at most 2 RBDs ($p < 0.15$) agreeing with criteria for 9 or more of 10 random realizations. These results are similar to results based on the full datasets.

Discussion

Our results do not support our original 'water quality—recreational ecosystem services hypothesis' that there would be a consistent positive association between WFD water status and service utilization. Moreover, of all four recreational ecosystem services, walking is most consistently and strongly associated with good/high water status. In other words, the association is strongest for the activity with the least direct relationship with water. In testing our hypothesis, we controlled for a variety of socio-economic and geographical factors that could also affect site choice, such as population density, age, ethnic characteristics, income, substitutability of sites, and site access. The results held, even when controlling for different null models, quantitative criteria, and dataset sizes. We offer four possible explanations for these somewhat counter-intuitive findings.

Table 4. Number (and codes) of River Basin Districts agreeing with the water quality—recreational ecosystem services hypothesis (or variants thereof): if good water quality is important for recreational ecosystem services we expect either (a) a positive association with water quality for all services or (b) positive association at least for services with significant direct contact with water—swimming and fishing, (c) stronger association with water quality for swimming and fishing relative to walking and boating. The *p*-values denote the probability of getting equal or fewer RBDs meeting the criteria by chance alone, assuming a binomial distribution with 0.5 probability of success per trial.

Criteria held	Full Model	Short-Ranged	Population Only	No Weighting
(a)+(c)	1* ($p = 0.035$) (NB)	1* ($p = 0.035$) (NB)	1* ($p = 0.035$) (NB)	0* ($p = 0.004$)
(b)+(c)	1* ($p = 0.035$) (NB)	2 ($p = 0.145$) (NB, AN)	1* ($p = 0.035$) (NB)	2 ($p = 0.145$) (NB, AN)
(a) only	2 ($p = 0.145$) (NB, SW)	4 ($p = 0.637$) (NB, NW, TM, SW)	2 ($p = 0.145$) (NB, SW)	1* ($p = 0.035$) (SW)
(b) only	2 ($p = 0.145$) (NB, SW)	5 ($p = 0.855$) (NB, NW, AN, TM, SW)	2 ($p = 0.145$) (NB, SW)	3 ($p = 0.363$) (NB, AN, SW)
(c) only	2 ($p = 0.145$) (NB, AN)	2 ($p = 0.145$) (NB, AN)	2 ($p = 0.145$) (NB, AN)	2 ($p = 0.145$) (NB, AN)

doi:10.1371/journal.pone.0166950.t004

First, water status as defined by the WFD may not adequately reflect water quality priorities of the wider public. For example, the biodiversity of aquatic invertebrates, one of the WFD metrics, may be irrelevant for swimmers and boaters. The presence of litter or debris, in contrast, might discourage water use although it has little impact on the status of a water body [45–49]. Water temperature contributes to status assessments, but reference conditions may be cooler than those preferred by swimmers [50]. Water users seem to prefer clearer waters [25,47,48], but good ecological status may be associated with relatively poor clarity in certain water body types, for instance in humic inland lakes [25].

Second, the relationship between water quality and cultural ecosystem services may be non-linear [51]. For example, the difference between poor and medium water status may be more noticeable than the difference between medium and good/high status. In other words, although achievement of good overall water status is the objective of the WFD, this transition may not be the most critical for recreational site use.

Third, WFD water status may simply be unimportant when making site choices—or at least much less important than other factors. In our null models we controlled for a number of socio-economic and geographical factors, but possibly missed some important local effects. To illustrate, a water body must meet certain fundamental criteria to facilitate recreational use: sufficiently large to launch a boat, reasonably safe for swimmers, or with relevant permissions for angling. Furthermore, some ecosystem services require specific types of infrastructure, for instance boating ramps or convenient swimming access points. Natural resource management decisions (e.g. fish stocking) and strategies related to the touristic marketing of sites may further affect site choice. Finally, site choice could be driven by non-water environmental characteristics such as surrounding land use [40], naturalness/wildness, presence or absence of shade, and wind [50]. Together, these infrastructure and management factors may limit site choice, meaning water status must be compromised in favour of practicality.

Fourth, it could be hypothesized that the status of waters in England has improved quickly in the more recent past. Society, however, has a long 'memory' for preferred recreational sites. People thus keep visiting places with potentially poorer water quality because locations with good/high water status have not yet been 'discovered' or become well known. The plausibility of this argument, however, is undermined by the fact that, according to the EA, water has not improved significantly between 2008 and 2012 (S3 Table). However, given the actual implementation of the WFD in the UK is very recent, with the first round of River Basin Management Plans published in 2009, it is possible some new measures may still impact recreational use in the future.

Nevertheless, we found, across all null models and in all but one RBD, a remarkably consistent association between water status and walking visits (Fig 3). Walkers may be more responsive to water status (since they are less restricted to specific water bodies by factors such as hydromorphology and infrastructure), and not as influenced by inter-service competition as are boaters, swimmers or fishers. Furthermore, they have the option of walking in other 'green spaces', not along water bodies, so may be more selective as to the water quality when choosing 'blue space' recreation sites.

Our data also highlight regional differences in the association between water status and recreational use (Fig 3). Most notably, Northumbria and the South West are the only RBDs in which all activities are positively related to water status (when compared to the weighted null models). In most RBDs we find a pattern of decreasing association from walking-boating-fishing-swimming, but in the Northumbrian and Anglian RBDs this pattern is reversed. Detailed exploration of these regional differences is beyond the scope of this paper, but as potential explanations we suggest regional idiosyncrasies in (i) demography, with younger people being more critical of water quality [49], (ii) frequency of recreational water use, with more frequent

water users more sensitive to differences in water status [52], (iii) relative importance of site choice factors, in that residents of more urbanized RBDs may be less sensitive to water status, (iv) differences in typical travel distances (willingness to travel farther) for different populations in different regions of England, (v) attachment to specific sites irrespective of water quality, through force of habit [53,54] or marketing of specific sites [55].

Conclusion

Using the case of England our analysis shows no, or even negative, correlation between WFD water status and spatial patterns of recreational services, in particular fishing and swimming. This undermines recent arguments about the benefits of the WFD, and warns that achieving 'good' or 'high' overall water status may not, in fact, improve the provision and utilization of ecosystem services. Extending the analysis to other parts of the UK and Europe, perhaps using citizen science approaches to collect recreational use data [56], is necessary to validate the generality of our findings and explore the spatial variation across RBDs. Further research should also explore if the relationship between water quality and recreational services is different in developing countries, where water quality is generally poorer than in present-day Europe. Necessary datasets (see schematic Fig 1) may possibly include a combination of crowdsourced water quality data (e.g. E. coli crowdsourced testing in India [57]), social-media (e.g. Flickr) for recreational use data, and emerging global datasets (e.g. world population [58], remote-sensed poverty map [59]).

The ecological integrity of Europe's aquatic ecosystems is threatened by a range of anthropogenic and natural pressures. This article suggests that if the aim of water legislation in the EU is to maintain the services these waters provide to society, it is necessary to improve the WFD monitoring system to capture other dimensions affecting supply and demand, especially of cultural services. This will necessitate involving also social scientists and the public in defining metrics and targets, not only freshwater ecologists and ecotoxicologists, to form a truly trans-disciplinary water framework for Europe.

Supporting Information

S1 Fig. Cumulative distribution for the distance of individual visits from Open Street Map road/trail/path. The vast majority of visits are near a road/trail/path which demonstrates a strong dependence of these cultural ecosystem services on accessibility. The percent of visits within 100m of a road/trail/path is 92.5% for walking (red), 92.9% for boating (blue), 60.9% for fishing (green), 80.0% for swimming and 87.9% in the combined dataset comprising all data (black dotted).
(DOCX)

S1 Table. Ecosystem services as an integral part of the WFD: evidence in official documents.
(DOCX)

S2 Table. Number of River Basin Districts agreeing with the water quality—recreational ecosystem services hypothesis (or variants thereof) with sub-sampling of Walking (12.67% of available data), Boating (43.52%) and Fishing (69.24%) to match Swimming n. Values are averages and standard deviations of the number of RBDs agreeing with criteria in 10 random boot-strapping realizations, and percentage of realizations with 2 or less RBDs ($p < 0.15$) matching criteria.
(DOCX)

S3 Table. Status classifications of UK surface water bodies in percent (including Wales and Scotland) under the WFD.
(DOCX)

S1 Text. Supporting Information.
(DOCX)

Acknowledgments

Author Contributions

Conceptualization: GZ KM BB WF NT GVJ LV CW OF MS RS MV MB.

Data curation: GZ KM.

Formal analysis: GZ KM BB WF NT GVJ LV CW MS MB.

Funding acquisition: GZ MB.

Methodology: GZ KM NT GVJ MS MB.

Visualization: GZ.

Writing – original draft: GZ KM BB WF NT GVJ LV CW OF MS RS MV MB.

Writing – review & editing: GZ KM BB WF NT GVJ LV CW OF MS RS MV MB.

References

1. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.

2. Council Directive 76/160/EEC of 8 December 1975 concerning the quality of bathing water.

3. Council Directive 80/68/EEC of 17 December 1979 on the protection of goundwater against pollution caused by certain dangerous substances.

4. Grant W, Matthews D, Newell P (2000) The Effectiveness of European Union Environmental Policy: Palgrave Macmillan UK.

5. Council Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy (Water Framework Directive)

6. Perry J, Vanderklein EL (1996) Water Quality: Management of a Natural Resource: Wiley-Blackwell.

7. European Commission (2003) Common Implementation Strategy for the Water Framework Directive (2000/60/EC). Guidance Document No. 10. River and Lakes—Typology, Reference Conditions and Classification Systems. Luxembourg: Office for Official Publications of the European Communities. pp. 29.

8. Council Directive 2000/60/EC of 23 October 2000 establishing a framework for Community action in the field of water policy (Water Framework Directive)

9. Boeuf B, Fritsch O (2016) Studying the Implementation of the Water Framework Directive in Europe: a Meta-Analysis of 89 Journal Articles. Ecology and Society 21: 19.

10. Hering D, Borja A, Carstensen J, Carvalho L, Elliott M, Feld CK, et al. (2010) The European Water Framework Directive at the age of 10: A critical review of the achievements with recommendations for the future. Science of The Total Environment 408: 4007–4019. doi: 10.1016/j.scitotenv.2010.05.031 PMID: 20557924

11. Albrecht J (2016) Legal framework and criteria for effectively coordinating public participation under the Floods Directive and Water Framework Directive: European requirements and German transposition. Environmental Science & Policy 55, Part 2: 368–375.

12. Ball T (2012) Wetlands and the water environment in Europe in the first decade of the water framework directive. are expectations being matched by delivery? pp. 255–274.

13. Martin-Ortega J (2012) Economic prescriptions and policy applications in the implementation of the European Water Framework Directive. Environmental Science & Policy 24: 83–91.

14. European Commission (2012) Report from the Commission to the European Parliament and the Council on the Implementation of the Water Framework Directive (2000/60/EC) River Basin Management Plans. COM(2012) 670 final. Brussels. pp. 5.

15. European Commission (2012) Commission Staff Working Document. The Fitness Check of EU Freshwater Policy. SWD(2012) 393 final. Brussels.

16. European Commission (2012) A Blueprint to Safeguard Europe's Water Resources. COM(2012) 673 final. Brussels.

17. European Commission (2012) Commission Staff Working Document. Impact Assessment. A Blueprint to Safeguard Europe's Water Resources. SWD(2012) 382. Brussels.

18. Blackstock KL, Martin-Ortega J, Spray CJ (2015) Implementation of the European Water Framework Directive: What Does Taking an Ecosystem Services-Based Approach Add? In: Martin-Ortega J, Ferrier R C, Gordon IJ, Khan S, editors. Water Ecosystem Services: a Global Perspective. Cambridge: Cambridge University Press. pp. 57–64.

19. Tolonen KT, Hämäläinen H, Lensu A, Meriläinen JJ, Palomäki A, Karjalainen J (2014) The Relevance of Ecological Status to Ecosystem Functions and Services in a Large Boreal Lake. Journal of Applied Ecology 51: 560–571.

20. Volkery A, Geeraerts K, Farmer A, Merlino C, Chalsege L, Vandresse B, et al. (2011) European Commission—General Directorate Environment. Support to Fitness Check Water Policy. Project: Request for Services in the Context of the Framework Contract on Evaluation and Evaluation-Related Services. ABAC N˚101934. Deloitte Consulting and Institute for European Environmental Policy.

21. Crase L, Gillespie R (2008) The Impact of Water Quality and Water Level on the Recreation Values of Lake Hume. Australasian Journal of Environmental Management 15: 21–29.

22. Egan KJ, Herriges JA, Kling CL, Downing JA (2009) Valuing Water Quality as a Function of Water Quality Measures. American Journal of Agricultural Economics 91: 106–123.

23. Heiskary SA, Walker WW (1988) Developing Phosphorus Criteria for Minnesota Lakes. Lake and Reservoir Management 4: 1–9.

24. Koteen J, Alexander SJ, Loomis JB (2002) Evaluating Benefits and Costs of Changes in Water Quality. General Technical Report PNW-GTR-548. United States Department of Agriculture.

25. Vesterinen J, Pouta E, Huhtala A, Neuvonen M (2010) Impacts of Changes in Water Quality on Recreation Behavior and Benefits in Finland. Journal of Environmental Management 91: 984–994. doi: 10.1016/j.jenvman.2009.12.005 PMID: 20036782

26. Ribaudo MO, Piper SL (1991) Estimating Changes in Recreational Fishing Participation from National Water Quality Policies. Water Resources Research 27: 1757–1763.

27. Vidal-Abarca MR, Santos-Martín F, Martín-López B, Sánchez-Montoya MM, Suárez Alonso ML (2016) Exploring the Capacity of Water Framework Directive Indices to Assess Ecosystem Services in Fluvial and Riparian Systems: Towards a Second Implementation Phase. Environmental Management 57: 1139–1152. doi: 10.1007/s00267-016-0674-6 PMID: 26884142

28. UK National Ecosystem Assessment (2011) The UK National Ecosystem Assessment: Synthesis of the Key Findings. UNEP-WCMC. Cambridge.

29. UK National Ecosystem Assessment (2014) The UK National Ecosystem Assessment Follow-on: Synthesis of the Key Findings. UNEP-WCMC, LWEC. Cambridge.

30. Environment Agency (2015) River Basin Management Plans. www.gov.uk/government/collections/river-basin-management-plans-2015

31. Centre for Ecology and Hydrology (2011) Land cover map of Great Britain 2007. https://www.ceh.ac.uk/services/land-cover-map-2007

32. UK National Ecosystem Assessment (2011) The UK National Ecosystem Assessment: Synthesis of the Key Findings. UNEP-WCMC, Cambridge.

33. Natural England (2015) Monitor of Engagement with the Natural Environment: the National Survey on People and the Natural Environment. Natural England Joint Report JP015.

34. Arkenford (2014) Watersports Participation Survey 2014 Full Report.

35. Angling Trust (2015) http://fishinginfo.co.uk/index.html. Downloaded August 2015.

36. Outdoor Swimming Society (2015) http://wildswim.com. Downloaded July 2015.

37. DEFRA (2014) Water Framework Directive implementation in England and Wales: new and updated standards to protect the water environment.

38. Environment Agency (2015) data.gov.uk.

39. Natural England (2015) Monitor Engagement with the Natural Environment: Annual Report from the 2013–2014 Survey. JP009.

40. Bateman IJ, Harwood AR, Mace GM, Watson RT, Abson DJ, Andrews B, et al. (2013) Bringing Ecosystem Services into Economic Decision-Making: Land Use in the United Kingdom. Science 341: 45–50. doi: 10.1126/science.1234379 PMID: 23828934

41. Office for National Statistics (2011) Usual resident population. https://www.nomisweb.co.uk/census/2011/ks101ew.

42. Office for National Statistics (2011) Approximated Social Grade by Sex by Age. https://www.nomisweb.co.uk/census/2011/lc6124ew.

43. OpenStreetMap http://download.geofabrik.de/europe/great-britain.html. Downloaded July 2015.

44. Grissom RJ, Kim JJ (2011) Effect Sizes for Research: Univariate and Multivariate Applications: Taylor & Francis.

45. David EL (1971) Public Perceptions of Water Quality. Water Resources Research 7: 453–457.

46. Happs J (1986) Constructing an Understanding of Water-Quality: Public Perceptions and Attitudes Concerning Three Different Water-Bodies. Research in Science Education 16: 208–215.

47. Smith DG, Cragg AM, Croker GF (1991) Water Clarity Criteria for Bathing Waters Based on User Perception. Journal of Environmental Management 33: 285–299.

48. Smith DG, Davies-Colley RJ (1992) Perception of Water Clarity and Colour in Terms of Suitability for Recreational Use. Journal of Environmental Management 36: 225–235.

49. House MA (1996) Public Perception and Water Quality Management. Water Science and Technology 34: 25–32.

50. Ditton RB, Goodale TL (1973) Water Quality Perception and the Recreational Uses of Green Bay, Lake Michigan. Water Resources Research 9: 569–579.

51. Dallimer M, Irvine KN, Skinner AMJ, Davies ZG, Rouquette JR, Maltby LL, et al. (2012) Biodiversity and the Feel-Good Factor: Understanding Associations between Self-Reported Human Well-being and Species Richness. BioScience 62: 47–55.

52. Faulkner H, Green A, Pellaumail K, Weaver T (2001) Residents' Perceptions of Water Quality Improvements Following Remediation Work in the Pymme's Brook Catchment, North London, UK. Journal of Environmental Management 62: 239–254. doi: 10.1006/jema.2001.0435 PMID: 11475083

53. Doherty E, Campbell D, Hynes S, van Rensburg TM (2013) Examining Labelling Effects within Discrete Choice Experiments: an Application to Recreational Site Choice. Journal of Environmental Management 125: 94–104. doi: 10.1016/j.jenvman.2013.03.056 PMID: 23648268

54. Hailu G, Boxall PC, McFarlane BL (2005) The Influence of Place Attachment on Recreation Demand. Journal of Economic Psychology 26: 581–598.

55. Connell J (2005) Toddlers, Tourism and Tobermory: Destination Marketing Issues and Television-Induced Tourism. Tourism Management 26: 763–776.

56. Wood SA, Guerry AD, Silver JM, Lacayo M (2013) Using Social Media to Quantify Nature-Based Tourism and Recreation. Scientific Reports 3: 2976. doi: 10.1038/srep02976 PMID: 24131963

57. Brown J, Loo A (2015) Crowdsourcing Water Quality Data Using Low-Cost Quantal E. coli Tests with Ambient-Temperature Incubation. Water and Health Conference 2015: Where Science Meets Policy.

58. Center for International Earth Science Information Network—CIESIN—Columbia University. 2016. Gridded Population of the World, Version 4 (GPWv4): Population Density. Palisades, NY: NASA Socioeconomic Data and Applications Center (SEDAC). http://dx.doi.org/10.7927/H4NP22DQ

59. Jean N, Burke M, Xie M, Davis WM, Lobell DB, Ermon S (2016) Combining satellite imagery and machine learning to predict poverty. Science 353: 790–794. doi: 10.1126/science.aaf7894 PMID: 27540167

CrossMark

GLOBAL CHANGE ECOLOGY – ORIGINAL RESEARCH

Antagonistic effects of biological invasion and environmental warming on detritus processing in freshwater ecosystems

Daniel Kenna[1] · William N. W. Fincham[1] · Alison M. Dunn[1] · Lee E. Brown[2] ·
Christopher Hassall[1]

Abstract Global biodiversity is threatened by multiple anthropogenic stressors but little is known about the combined effects of environmental warming and invasive species on ecosystem functioning. We quantified thermal preferences and then compared leaf-litter processing rates at eight different temperatures (5.0–22.5 °C) by the invasive freshwater crustacean *Dikerogammarus villosus* and the Great Britain native *Gammarus pulex* at a range of body sizes. *D. villosus* preferred warmer temperatures but there was considerable overlap in the range of temperatures that the two species occupied during preference trials. When matched for size, *G. pulex* had a greater leaf shredding efficiency than *D. villosus*, suggesting that invasion and subsequent displacement of the native amphipod will result in reduced ecosystem functioning. However, *D. villosus* is an inherently larger species and interspecific variation in shredding was reduced when animals of a representative size range were compared. *D. villosus* shredding rates increased at a faster rate than *G. pulex* with increasing temperature suggesting that climate change may offset some of the reduction in function. *D. villosus*, but not *G. pulex*, showed evidence of an ability to select those temperatures at which its shredding rate was maximised, and the activation energy for shredding in *D. villosus* was more similar to predictions from metabolic theory. While per capita and mass-corrected shredding rates were lower in the invasive *D. villosus* than the native *G. pulex*, our study provides novel insights in to how the interactive effects of metabolic function, body size, behavioural thermoregulation, and density produce antagonistic effects between anthropogenic stressors.

Introduction

Biological invasions are a widespread and significant component of human-induced global environmental change, and are having a major impact on the Earth's ecosystems (Simberloff et al. 2013; Dunn and Hatcher 2015). Invasions also impact world economies, with financial costs reaching over $120 billion per year in the United States (Pimentel et al. 2005) and €12bn per year in Europe (Altmayer 2015). The current rate of alien species introductions is unprecedented, due mainly to globalisation and growth in the volume of trade and tourism (Anderson et al. 2015). These effects make urgent the need to generate a better understanding of the mechanisms that underpin the impacts of invasive species on native species and recipient ecosystems, and how those invasions might interact with other anthropogenic stressors. Invasions by alien species are increasingly being recognised as one of the major threats to biodiversity and ecosystem functioning in freshwater ecosystems (Strayer and Dudgeon 2010). Invasive species can have a variety of effects on the structure of recipient

✉ Christopher Hassall
c.hassall@leeds.ac.uk

1 School of Biology and water@leeds, University of Leeds, Leeds, UK

2 School of Geography and water@leeds, University of Leeds, Leeds, UK

⌂ Springer

freshwater communities, such as displacing native species (Dick et al. 2002) and altering the diversity and abundance of macroinvertebrate assemblages (Ricciardi 2001). These direct effects, and their underlying mechanisms such as predation and competition, are relatively easy to identify (MacNeil and Platvoet 2005). While the consequences of invasions for ecosystem functioning are less readily understood, research in this area is increasing and there are well-described case studies, such as *Dreissena polymorpha* in the Hudson river (Strayer et al. 1999) and *Corbicula fluminea* in the Plata river (Sousa et al. 2008), that have shed light on how freshwater invaders can dramatically affect ecosystem processes (Strayer 2010).

In both terrestrial and freshwater habitats, macroinvertebrates influence whole ecosystem functioning by accelerating detritus decomposition, and by releasing bound nutrients through their feeding activities and burrowing behaviours (Wallace and Webster 1996; Covich et al. 1999). In freshwater food webs, energy flows from leaf-litter processing are enhanced significantly by shredder consumption, particle fragmentation, and faeces production which convert coarse particulate organic matter (CPOM; organic material >1 mm diameter) into fine particulate organic matter (FPOM; 50 μm–1 mm) (Vannote et al. 1980; Graça 2001; Truhlar et al. 2014). This process makes allochthonous energy inputs accessible to invertebrates that feed directly on FPOM, facilitating trophic energy transfer (Vannote et al. 1980; Graça et al. 2001; MacNeil et al. 2011). Functionally, freshwater amphipods (Crustacea) play significant roles as shredders exerting strong control over the rate of leaf processing (Newman 1990; Navel et al. 2010; Truhlar et al. 2014). Alterations to amphipod assemblage composition can therefore have major consequences for aquatic ecosystem functioning (Piscart et al. 2011).

When introduced to a new area, invasive amphipods often displace their native counterparts due to competition for resources or direct predation pressure (Piscart et al. 2009; Truhlar et al. 2014). This process of displacement has been observed with the Ponto–Caspian amphipod *Dikerogammarus villosus* (Sowinsky, 1894), which has replaced or disrupted the distribution of many resident amphipod species, including previously successful invaders, at numerous sites across Europe (Rewicz et al. 2014). Known as the 'killer shrimp' due to its predatory nature, *D. villosus* is a highly voracious, omnivorous, and physiologically tolerant species (Rewicz et al. 2014). It is capable of surviving in ship ballast water promoting its dispersal (Bruijs et al. 2001), and is regarded as one of the worst invasive species in Europe in terms of its negative impact on the functioning and biodiversity of invaded ecosystems (DAISIE 2009). It is expected to expand its range and eventually reach North America (Ricciardi and Rasmussen 1998). In September 2010, *D. villosus* was recorded outside mainland Europe

for the first time, in a reservoir called Grafham Water in the UK (MacNeil et al. 2010), and has since established in other parts of England and Wales (MacNeil et al. 2012). Its introduction has already led to community-level changes at invaded sites, including the displacement of the native amphipod *Gammarus pulex* (Linnaeus, 1758) (Madgwick and Aldridge 2011; Truhlar et al. 2014). Previous research into how this invasion may affect ecosystem functioning in freshwaters has indicated that *D. villosus* has a lower leaf shredding efficiency than other amphipod species, including the native *G. pulex* (MacNeil et al. 2011; Jourdan et al. 2016). Consequently, the introduction of *D. villosus* may threaten the fundamental role played by native macroinvertebrate shredders in determining energy flow in these invaded ecosystems (MacNeil et al. 2011).

Life history traits of *D. villosus*, such as early sexual maturity, large reproductive capacity, and high growth rates (Pöckl 2009), as well as its predatory capabilities combined with an omnivorous nature (Dick et al. 2002; Rewicz et al. 2014) confer a large competitive advantage over many other amphipods (Rewicz et al. 2014). For poikilothermic animals such as *D. villosus* and *G. pulex*, the temperature of the surroundings strongly modulates their performance, by driving variation in metabolic rate (Brown et al. 2004; Maazouzi et al. 2011). Increasing metabolic rate with temperature necessarily drives enhanced consumption, and metabolic theory of ecology (MTE) predicts that the activation energy of these consumer-resource interactions should vary around 0.60–0.70 eV similar to those of the underlying biochemical reactions of individual metabolism (e.g., Brown et al. 2004). Deviations from these predictions may provide unique insights into the linkages between biodiversity and ecosystem functioning (e.g., Yvon-Durocher and Allen 2012; Perkins et al. 2015), but studies marrying the functional effects of invaders and native species with metabolic theory have yet to be undertaken.

Behavioural studies on the thermal avoidance and preference of crustaceans have indicated that they exhibit distinct temperature preferences and their thermosensitivity may be in the range of 0.2–2 °C (Lagerspetz and Vainio 2006; González et al. 2010). Devin et al. (2003) demonstrated that *D. villosus* and *G. pulex* prefer similar substratum types, and that the spatial niches of these two species overlap significantly. If these amphipods also demonstrate preferences for similar thermal ranges, then this could further promote direct competition between the two, and increase the threat of the displacement of the native *G. pulex*.

This study investigated the thermal preferences and leaf shredding efficiencies of both the invasive *D. villosus* and the native *G. pulex*, to better understand the combined impacts that species invasion and warming could have on ecosystem functioning in freshwater habitats. This study

specifically aimed to test the following predictions concerning our study system: (1) *D. villosus*, characteristic of the eurythermic Ponto–Caspian species, exhibits a broader thermal preference and greater preference for higher temperatures than *G. pulex*; (2) leaf shredding efficiencies for both species increase with temperature in line with predictions of MTE, but are overall higher for *G. pulex* due to its greater preference for plant-based food sources; and (3) both species select temperatures at which they perform optimally. This study provides a comprehensive investigation into the thermal biology of an invasive species relative to a displaced native species, which provides the basis for understanding better the complexity of impacts that both climate change and biological invasions will have on freshwater ecosystem functioning.

Methods

Collection and maintenance of animals

Test animals were collected during June and July 2015 through standard sweep sampling, with *D. villosus* collected from Grafham Water in Cambridgeshire (52°18′N; 0°19′W) and *G. pulex* collected from a small stream adjacent to Meanwood Beck in Yorkshire (53°50′N; 1°35′W). Air and water temperature data suggest minimal differences between the sites (Fig. S1). Each species was maintained separately in the laboratory in aerated tanks (30 × 18 × 15 cm) filled with dechlorinated aged tap water at 15 °C under a 16:8 lighting regime. Shelter was provided in the form of gravel and pebbles (Bruijs et al. 2001). Leaves of naturally conditioned alder (*Alnus glutinosa*) and sycamore (*Acer pseudoplatanus*) were provided as a food source, and air stones provided smooth water movement and sufficient dissolved oxygen concentrations (Kley et al. 2009).

Thermal preference experiments

We used the 'acute' method to derive thermal preferenda for each species (Reynolds and Casterlin 1979), whereby three different acclimation temperatures were used (5, 15, or 20 °C) for a 4-day period prior to a 135 min testing phase in a thermal gradient. Temperature selection behaviour was examined using four toroidal (annular) thermal gradient tracks (Fig. S2) modified from Kivivuori and Lagerspetz (1990). Each track was 120 cm (L) × 11 cm (W). An ice bath was used to cool one end of the track, with a water bath heating the opposite end. The resultant water temperature gradient ranged from 4 to 24 °C (Fig. S3, raw data in Table S1), measured using 16 evenly spaced digital thermometers (Avax DT-1) The bottom of the apparatus was lined with a thin layer of gravel (ca. 2 mm particle size), and water depth was 2 cm to prevent thermal stratification of the water column. All tracks were illuminated evenly to prevent dark-seeking behaviour.

For each acclimation temperature, 30 *G. pulex* and 30 *D. villosus* were introduced to the gradient apparatus in pairs to determine thermal preferenda. A 1:1 male:female ratio was used, and individuals were selected that represented the full range of body sizes for adults. After the experiment, amphipods were preserved in 70% ethanol, weighed, and then photographed to determine body length using ImageJ software. As both body length (Lewis et al. 2010) and mass (Navel et al. 2010) have been used as predictors of energetic demands for amphipods, these two correlated metrics were combined into a single index of amphipod 'body size' using principal component analysis (Truhlar et al. 2014). Individuals were introduced to the section of the gradient corresponding to their acclimation temperature to reduce stress caused to the animal, and were left for a 30 min period initially to reduce the impact of handling on behaviour. The water temperature of the position of each individual within the track was then recorded every 3 min for a period of 45 min. To ensure that both species showed no preference for any particular position in the track, control experiments were carried out using six animals from each species and recording amphipod locations every 2 min when the water was a uniform temperature of 20 °C. A concern arising from test animals being introduced in pairs is that they may interact socially so cannot be treated as independent individuals (Karlsson et al. 1984); however pilot data comparing individual and paired animals suggested that grouping did not affect thermal behaviour in the gradients.

Leaf shredding experiments

Leaves of the sycamore tree (*A. pseudoplatanus*) were provided as the food source, as this tree is common at the collection sites of both species and its leaves have been shown to be highly palatable to amphipods (MacNeil and Platvoet 2005). Leaves were conditioned in stream water for two weeks at 15 °C, which allowed the leaching of soluble components, softening, and encouraged fungal growth (Bloor 2011). Leaves were cut into 6 mm-diameter leaf discs using a cork-borer, with midribs and any obvious infected areas avoided, and these were then air-dried, sorted into batches of five, and weighed (leaf batch air-dry mass = 16.00 ± 3.27 mg, *n* = 320).

Dikerogammarus villosus are an inherently larger species than *G. pulex* (animals used in this study were: *G. pulex* length = 12.10 ± 0.10 mm, range 7.35–17.86 mm; *D. villosus* length = 15.89 ± 0.18 mm, range 9.13–25.77 mm). Therefore, all shredding experiments were

conducted with the full range of body sizes, but at least half of all replicates used size-matched individuals to avoid the confounding effects of variation in body size and reproductive cycle (Truhlar et al. 2014). Full data on the sizes of all specimens can be found in Table S4 along with the results of the experiment.

Eight experimental temperatures (5, 8, 10, 12.5, 15.5, 17.5, 20, and 22.5 °C, chosen to cover the range of UK river temperatures, Garner et al. 2014) were used to assess the effect of temperature on shredding efficiency for both species of amphipod. All trials were subject to a uniform photoperiod of 16:8, and the water temperature at each experimental temperature was recorded for the duration of the trials using Tinytag Plus 2 TGP-4017 dataloggers (Gemini Data Loggers). Each species was tested separately with 10 replicates of size-matched individuals and 10 replicates containing amphipods covering the remaining range of each species' body sizes, giving 8 temperature treatments, 2 species treatments, and 2 size treatments, each replicated 10 times for 320 replicates in total. Each replicate was established in a plastic container [8 cm (ϕ) × 7 cm (D)] filled with dechlorinated tap water along with three clear glass pebbles (2-cm diameter) to provide animals a retreat whilst still permitting observation (MacNeil and Platvoet 2005). Two animals were placed in each pot and were subjected to a 24 h starvation period at their experimental temperature prior to testing. Each replicate involved two animals for two reasons: (1) mortality was relatively high at higher temperatures and so multiple animals gave a higher chance of the 72 h incubation yielding at least one animal alive at the end, and (2) shredding rates were measured over a relatively short period and so the combined shredding of two animals gave a stronger signal. At the start of each trial, a pre-weighed batch of five leaf discs was added to each pot. Each trial lasted for 72 h, with amphipod deaths recorded every 24 h and dead animals being removed (Truhlar et al. 2014). At the end of each experiment, the animals were weighed and photographed for their body length to be measured using ImageJ software. Animals were retained for 3 days post-experiment, and any that moulted was removed from subsequent data analyses (Paterson et al. 2015). Remaining leaf discs were dried for 24 h at 90 °C and weighed. Control pots established at each temperature consisted of amphipod-free pots with only leaf discs added.

Data analysis

Thermal preferences

In the control experiment with the gradient apparatus held at 20 °C, animal locations were classified into regions of the track of length 10 cm and Chi-squared tests were used to assess preference. For each species, 30 recordings were taken of 6 specimens, giving 180 recordings for each species. In the main experiment with thermal gradient, the median selected temperature during the period of observation was calculated to avoid pseudoreplication and provide a measure of preference for each individual (Karlsson et al. 1984). Median preferenda were then examined with respect to amphipod species, acclimation temperature, body size, and sex in linear mixed effects model using the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2015) packages in R v3.2.0 (R Core Team 2015), with time of experiment and track as random effects, and slopes and intercepts allowed to vary at random. Following data transformation to account for leptokurtosis in the residuals, model selection carried out on this global model using the dredge function in the MuMIn package (Barton 2015) in R, and model averaging was used to take the weighted averages of the parameters of those models with ΔAIC < 4, providing a final mixed effects model of 'Species + Acclimation temperature + Species × Acclimation temperature' with 'experimental track' and 'time of run' accounted for as random effects.

At each acclimation temperature, the average acute thermal preferendum for each species was calculated as the mean of the selected temperatures (Reiser et al. 2014). The final thermal preferendum derived by the acute method is defined as that temperature where preference equals acclimation temperature (Fry 1947). Therefore, to determine this value for each species, acute thermal preferenda were plotted with a 1:1 regression line (where response temperatures and acclimation temperatures are equal), and the final thermal preferendum of each species was calculated as the point of intersection between this line of equality and the trend line describing the acute thermal preferenda (Reynolds and Casterlin 1979).

Leaf shredding

Leaf shredding efficiency was measured as the dry mass of leaf consumed per amphipod/day (Truhlar et al. 2014). To account for the effects of amphipod deaths, the leaf mass consumed in each replicate was standardised by the number of amphipod days in that replicate, where amphipod days was equal to the number of surviving amphipods on a given day summed over all 3 days of the experiment. To compare shredding efficiency between species, size-matched male amphipods were used. The two correlated metrics of wet mass and body length were combined using PCA into a single index of 'body size'. The species scores from PC1 were then analysed using one-way ANOVA to confirm successful size-matching. Leaf shredding efficiency was then

Fig. 1 Position records of
G. pulex (90 animals) and *D.
villosus* (90 animals) in the
temperature gradients. Prior
to the experiments, *G. pulex*
(*solid lines*) and *D. villosus*
(*dashed lines*) individuals were
acclimated to either 5 °C (*light
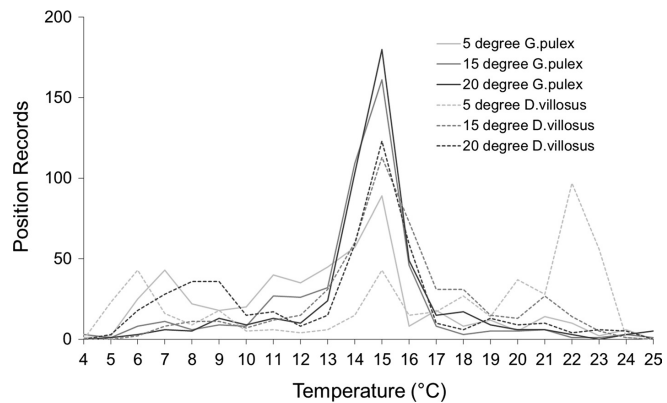grey*), 15 °C (*medium grey*), or
20 °C (*dark grey*)

Table 1 Subset of linear mixed effects models with ΔAIC < 4 that describe thermal preferences in two species of amphipods, *G. pulex* and *D. villosus*

Model	df	AICc	ΔAIC	W_i
Species + Acc.Temp + Species × Acc.Temp	7	555.9	0.00	0.660
Species	5	557.9	1.98	0.246
Species + Acc.Temp + Sex + Species × Acc.Temp	8	559.8	3.89	0.094

All models include 'experimental track' and 'time of run' as random effects. "Acc.Temp" = acclimation temperature

examined with respect to amphipod species and temperature in a two-way ANOVA. Non-significant terms were removed via stepwise deletion. Data were then pooled to incorporate the results from the full size ranges for both species, and leaf shredding efficiency was again examined with respect to species and temperature in a two-way ANOVA. Post-hoc testing for both the above models was conducted using Tukey's HSD tests.

For each experimental temperature treatment, water temperature was converted to $1/kT_c - 1/kT$ where k is the Boltzmann constant (8.62×10^{-5} eV K^{-1}), T is temperature in °K (Yvon-Durocher et al. 2012), and c denotes the intercept temperature for 15 °C (288.15 °K); higher values of this standardised variable therefore relate to higher water temperature. Temperatures were plotted against ln transformed leaf shredding efficiency and relationships determined using linear regression in R2.14.0. Regression multipliers provide estimates of the activation energy of leaf shredding efficiency. ANCOVA was used to assess whether the relationship between temperature and leaf shredding differed between the two species.

Thermal preference versus shredding performance

Temperature zones (ranging 1 °C either side) were assigned to each experimental temperature tested in the shredding trials (i.e., the zone for 15.5 °C temperature would be 14.5–16.5 °C). Then, for each species, the relationship between habitat use (mean number of position records per individual in a temperature zone for 15 °C acclimated animals) and functional performance (mean leaf mass consumed per individual in the corresponding temperature zone) was examined using orthogonal non-linear least squares regression (ONLS, as both variables were measured with error) to test for an asymptote that would indicate that the amphipod species selected temperatures at which they performed optimally. Specifically, the model fitted using the ONLS approach was 'shredding ~ $\alpha + \beta$/habitat use'. The measure of functional performance was taken as mean leaf mass consumed per individual over 72-h, as opposed to mean shredding efficiency, as this measure partially accounted for the increased mortality rates that were observed with increasing temperatures for both species.

Results

Thermal preference experiments

At all acclimation temperatures, both *G. pulex* and *D. villosus* displayed a distinct preference for a narrow temperature range between 13 and 16 °C (Fig. 1, raw data can be found in Table S3). From the linear mixed effects model, the acute thermal preferences differed significantly between the two species, with species featuring in all top models (Table 1) and being statistically significant in the top

Table 2 Results of the final minimum linear mixed effects model (Sp + Acc.Temp + Sp × Acc.Temp) for temperature preference, with 'experimental track' and 'time of run' as random effects

Parameter	Estimate	SE	T	i
Intercept	0.974	0.261	3.736	<0.001
Species	−2.028	0.369	−5.500	<0.001
Acc.Temp	−0.057	0.018	−3.231	0.001
Species × Acc.Temp	0.111	0.025	4.419	<0.001

"Acc.Temp" = acclimation temperature
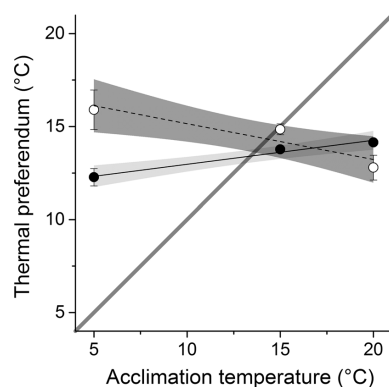
Fig. 2 Acute thermal preference of *G. pulex* (*filled symbols*, *solid line*, *light grey* area denotes 95% CI) and *D. villosus* (*open symbols*, *dashed line*, *dark shaded area* denotes 95% CI). *Error bars* denote ±1 SE. *Grey line* indicates the line of equality (i.e. where acclimation temperature and preferred temperature are equal). The point of intersection between these *lines* indicates the final thermal preferendum for each species

Table 3 Results of two-way analysis of variance testing the effects of species and temperature on overall leaf shredding efficiency in size-matched individuals and for all individuals pooled

Size classes	Source	df	F	p
Size-matched	Species	1148	169.580	<0.001
	Temperature	1148	92.386	<0.001
	Species × temperature	1148	0.095	0.759
All individuals	Species	1302	93.551	<0.001
	Temperature	1302	57.833	<0.001
	Species × temperature	1302	3.917	0.049

model (Table 2). *D. villosus* preferred higher temperatures to *G. pulex*. Acclimation temperature also had a significant effect on thermal preferences, and interestingly, there was a significant difference between the species × acclimation

temperature interaction, indicating that the effect of acclimation temperature on preference temperature was different for each amphipod species. Specifically, as acclimation temperature increased the preference temperature of *G. pulex* also increased, but this pattern was reversed for *D. villosus*, with its preference temperature decreasing with increasing acclimation temperature (Fig. 2). Based on the thermal preferenda derived for the three acclimation temperatures (Fig. 2), the final thermal preferendum using the acute method was calculated at 13.4 °C for *G. pulex* and 14.3 °C for *D. villosus*. Neither *G. pulex* ($\chi^2 = 6.93$, $df = 11$, $p = 0.805$) nor *D. villosus* ($\chi^2 = 15.13$, $df = 11$, $p = 0.176$) showed a preference for any particular section of the track when the water temperature was held at a uniform temperature of 20 °C (i.e., control conditions, Fig. S4, full data can be found in Table S2).

Leaf shredding experiments

Leaf shredding by size-matched individuals

Two-way ANOVA showed that leaf shredding efficiency was significantly affected by both amphipod species and temperature (Table 3). The interaction between these two variables was not significant, indicating that both species responded the same way to increasing temperature with respect to their shredding efficiencies, and this interaction was thus removed from the model. *G. pulex* displayed a significantly greater leaf shredding efficiency than *D. villosus*, and leaf shredding efficiency increased with temperature for both species (Fig. 3, raw data can be found in Table S4). The observed activation energy of shredding efficiency was 0.83 eV for *D. villosus* although the theoretically predicted range (0.6–0.7 eV) fell within the 95% confidence intervals of the regression (0.56–1.10 eV). In contrast, *G. pulex* estimates were outside of MTE predictions (0.40 eV; 95% CI 0.46–0.34). ANCOVA showed a significant effect of species identity on the relationship between temperature and shredding ($F_{3,12} = 14.19$, $p = 0.003$). PC1 explained 93.8% of the variance of body length and wet mass in the amphipods, making it a highly reliable index of overall body size. The one-way ANOVA of PC1 scores with respect to species for the size-matched individuals showed no significant difference ($F_{1,319} = 0.708$, $p = 0.401$) between the body sizes of *G. pulex* (length = 13.29 ± 1.46 cm, weight = 0.037 ± 0.011 g) and *D. villosus* (length = 13.37 ± 1.60 cm, weight = 0.039 ± 0.013 g), therefore the size-matching was considered successful. Leaf discs in the control aquaria (no animals present) had a negligible mass loss (<2% of the mass of initial discs added) over the duration of the experiment, and so loss of leaf mass due to microbial breakdown or leaching was discounted (MacNeil et al. 2011).

Fig. 3 Relationship between temperature and **a** survival in size-matched amphipods, **b** survival in the whole sample of amphipods, **c** shredding rates for size-matched amphipods, and **d** shredding rates for the whole sample of amphipods. Points are mean values (±1 SE for shredding; 95% CI for survival), for *G. pulex* (*filled symbols*) and *D. villosus* (*open circles*)
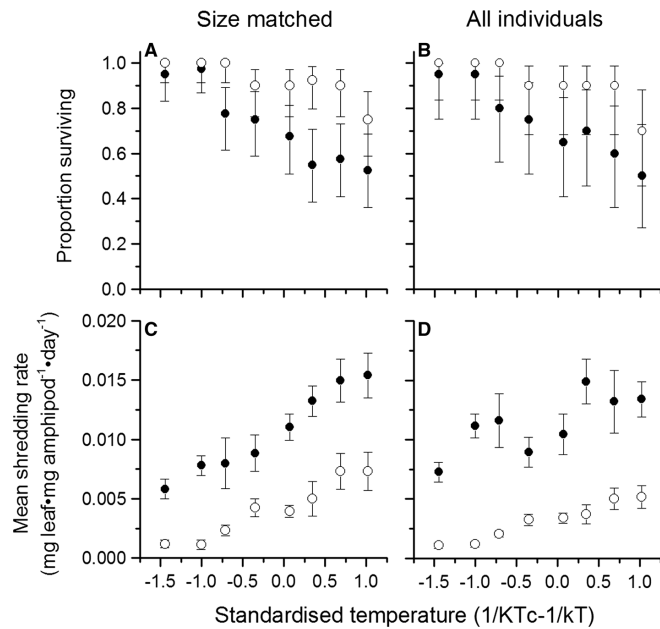

Table 4 Regression parameters for ln mean shredding rates as a function of water temperature [$1/kT$ (15 °C) − $1/kT$]

Species	Data type	Intercept (±95% CI)	Multiplier (±95% CI)	p	R^2
G. pulex	Size-matched	−4.53 (±0.05)	0.40 (±0.06)	<0.001	0.98
	All data	−4.46 (±0.15)	0.21 (±0.18)	0.03	0.57
D. villosus	Size-matched	−5.57 (±0.22)	0.83 (±0.27)	<0.001	0.91
	All data	−5.80 (±0.16)	0.68 (±0.20)	<0.001	0.92

Leaf shredding across all size classes

The large *D. villosus* consumed more leaf mass per amphipod day over all temperatures compared to smaller conspecifics, and the large-sized *G. pulex* only consumed more at temperatures of 12.5 °C and above (Fig. 3). Similar to the results for size-matched individuals, the two-way ANOVA found that leaf shredding efficiency was significantly affected by both amphipod species and temperature. However, in contrast to results from size-matched animals, the interaction between these two variables was significant indicating that the two species differed in the nature of the relationship between shredding and temperature (Table 3).

When all sizes of individuals were taken into consideration, *G. pulex* still displayed a significantly greater leaf shredding efficiency than *D. villosus* (Fig. 3). Leaf shredding efficiency increased with temperature for *D. villosus*. However, this tendency was much less pronounced for *G. pulex* owing mainly to the reduced leaf shredding efficiency of smaller individuals at higher temperatures. Increasing temperatures had a greater effect on the mortality rate of *G. pulex* than on *D. villosus* (Fig. 3). The observed activation energy of shredding efficiency for *D. villosus* was within the range predicted by MTE (0.68 ± 0.20 eV, Table 4). In contrast, *G. pulex* estimates were outside of MTE predictions (0.21 eV; 95% CI 0.03–0.39). ANCOVA showed a significant effect of species identity on the relationship between temperature and shredding ($F_{3,12} = 18.82$, $p < 0.001$), as was seen for the raw shredding data (Table 3).

Fig. 4 Relationship between habitat use and functional performance for **a** *G. pulex* and **b** *D. villosus*. Fitted line in **b** is the result of an orthogonal non-linear least squares regression that takes into account error in both *x* and *y* variables (see text for details). *Error bars* denote ±1 SE for both variables
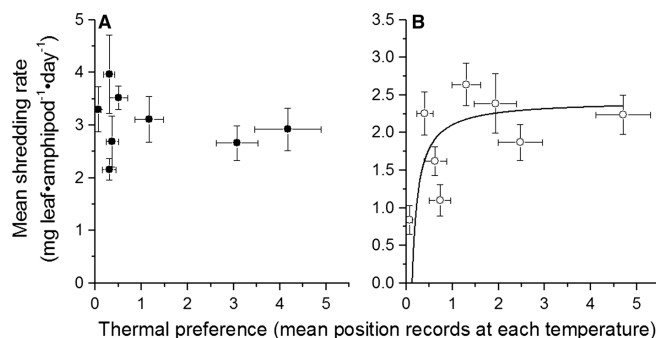
Thermal preference versus shredding performance

ONLS regressions showed that there was no relationship between our measure of thermal preference (the time over which a particular thermal microclimate was used) and the measure of performance (per capita leaf shredding) in *G. pulex* ($t = 0.830$, $p = 0.438$), but that the preference did explain the increase to asymptote in *D. villosus* ($t = -2.915$, $p = 0.027$; Fig. 4).

Combining the shredding rates, mortality rates, and body size measurements allows prediction of the potential consequences of replacement of *G. pulex* by *D. villosus* (Fig. 5). Population-level shredding capacity shows no relationship with temperature in *G. pulex* ($r = 0.091$, $p = 0.830$), demonstrating that increased mortality at higher temperatures cancels-out any increase in shredding efficiency. However, population-level shredding in *D. villosus* continues to increase approximately linearly with temperature ($r = 0.913$, $p = 0.002$; Fig. 5). The regression lines suggest *G. pulex* shreds 200% more leaf matter at 5 °C but only 20% more at 22.5 °C, hence replacement by *D. villosus* is predicted to result in smaller declines in resource processing at warmer temperatures.

Discussion

Animal invasions are being recognised increasingly as a major threat to biodiversity and ecosystem function in freshwater ecosystems (Simberloff et al. 2013). Here, we have demonstrated that the invasive amphipod *D. villosus* shreds less leaf mass than the native species *G. pulex*. However, we show that any decline in ecosystem function following replacement of the native by the invasive is likely to be offset by the greater size of the invasive species, climate-induced warming of the aquatic environment, and the ability of the invasive species to select those microclimates that
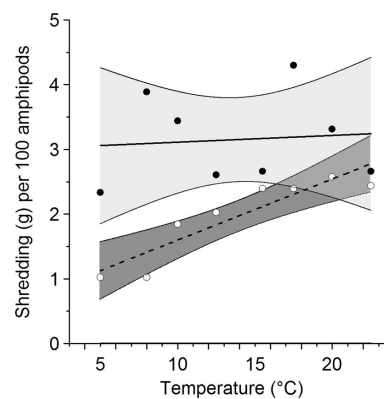
Fig. 5 Predictions of shredding (g leaf mass per 72 h) in theoretical populations of 100 *G. pulex* (*filled symbols*, *solid line*, *light grey area* denotes 95% CI) and 100 *D. villosus* (*open symbols*, *dashed line*, *dark shaded area* denotes 95% CI) over a 72 h period. Shredding capacity is the product of mass-specific shredding rate over 72 h and the mean mass of each species (30.5 mg in *G. pulex*, 68.2 mg in *D. villosus*), multiplied by the survival rate at that temperature. Per capita post-mortality rates are multiplied by 100 to give an estimated mortality for the hypothetical population

optimise its performance which is absent from the native species.

Thermal preference experiments

The results from this study clearly demonstrate thermal preference behaviour in both *D. villosus* and *G. pulex*, consistent with previous work on crustaceans (Lagerspetz and Vainio 2006; González et al. 2010; Reiser et al. 2014). Neither body size nor sex had a significant effect

on temperature preference, indicating that the final thermal preferenda derived from this study appear to be representative of all individuals of these species, at least individuals from the populations where we collected specimens. The thermal preference of a species depends on its evolutionary thermal history (Lagerspetz and Vainio 2006), which may account for the slightly higher thermal preferendum found for *D. villosus*, as it is native to the Ponto–Caspian basin where summer water temperatures may reach 29 °C at its peak (Rewicz et al. 2014). The native and the invasive amphipods spent the majority of their time in similar water temperatures ranging between 13 and 16 °C, suggesting similar thermal niches and therefore a high potential for direct competition (McMahon et al. 2006). Previous research has shown that when both *G. pulex* and *D. villosus* are present in microcosm and mesocosm experiments, *G. pulex* suffer severe intraguild predation from *D. villosus* with no reciprocal predation observed (Dick et al. 2002; MacNeil et al. 2011). Field studies have also shown that populations of native *G. pulex* decline after *D. villosus* invasion (Madgwick and Aldridge 2011). Therefore, in invaded ecosystems, direct competition resulting from overlapping thermal niches would likely result in the displacement of *G. pulex* by *D. villosus*.

Leaf shredding experiments

The invasive *D. villosus* exhibited lower leaf shredding efficiency than the native *G. pulex*, consistent with previous studies (MacNeil et al. 2011; Piscart et al. 2011). In isolation, this observation may lead to the prediction of serious implications for ecosystem functioning in invaded waterways, as a decrease in leaf-litter processing would result in a reduction of FPOM production, consequently reducing energy inputs accessible to other macroinvertebrates and disrupting energy transfer between trophic levels (Vannote et al. 1980; Graça et al. 2001). In contrast to these results, Truhlar et al. (2014) observed that *D. villosus* was significantly more efficient at shredding leaves than *G. pulex* when experiments were carried out at 25 °C. Potential explanations for this difference are that the experimental temperatures in the present study only reached 22.5 °C, while 25 °C may have greater associated costs for *G. pulex* than *D. villosus*, and that Truhlar et al. (2014) used unconditioned *Salix* leaves as the food source in their shredding experiments. The present study used conditioned *Acer* leaves and *D. villosus* may be able to utilise unconditioned leaf more effectively than *G. pulex* (Truhlar et al. 2014).

Leaf shredding efficiency of both *G. pulex* and *D. villosus* increased significantly with temperature but MTE-predicted activation energies applied only to *D. villosus* and not to *G. pulex*. This poses the question of why this is the case and what are the wider implications? *G. pulex*

is a cool-adapted species and is seemingly unable to maintain its rate of shredding at higher temperatures, contributing to the lower activation energy and enhanced mortality. One potential reason for its elevated consumption across all temperatures (and confirmed by the higher intercept from the regressions) is that the nutrient stoichiometry of sycamore is inadequate for *G. pulex*; hence it has to consume more leaf to meet its metabolic demands (c.f. Tuchman et al. 2002). This would suggest *G. pulex* to be more selective in terms of detrital matter than *D. villosus*. Further experiments with other types of leaf litter are needed to test this hypothesis, but sycamore has previously been shown to underpin slower growth rates amongst *G. pulex* compared with elm leaf (Sutcliffe et al. 1981). An increase in detrital leaf shredding by *D. villosus* is likely to have wider implications within aquatic communities, for example by increasing available nutrients after leaf decomposition and thus potentially increasing primary productivity. A net result of this interspecific difference in leaf consumption would be more successful invasion by *D. villosus* as it spends less time foraging and feeding, and can allocate more resource to growth and reproduction.

For *G. pulex*, no relationship was found between habitat use and functional performance, however for *D. villosus* there was evidence of a positive relationship, indicating that individuals may spend a greater proportion of their time within thermal limits where they had a greater functional performance: *G. pulex* only spent 8.7% of their time in the temperatures where they performed best, compared to *D. villosus* that spent 29.7% of their time there. This result provides evidence that *D. villosus*, but not the native *G. pulex*, may optimise its performance through selective use of microclimates. Coupled with this was the finding that *D. villosus* had a lower mortality rate than *G. pulex* at every temperature. These eurythermic traits demonstrated by *D. villosus* are common in Ponto–Caspian invaders, which are also commonly euryoecious and euryhaline species tolerant of rapid environmental change (Rewicz et al. 2014). These traits are likely to have contributed to its invasion success in the thermally heterogeneous freshwater environments of Europe. These findings are important in relation to global warming, as not only will temperatures increase over the coming years (UK Met Office 2011), but there will also be an increased variation in daily temperatures (Schar et al. 2004), and this appears to favour the invasive *D. villosus* over the native *G. pulex*.

Summary

The main findings of this study suggest that invasion by *D. villosus* and the consequent displacement of *G. pulex* will result in reduced leaf decomposition rates due to the lower shredding efficiency of the invader. However, for

this system, at least, it appears that the larger size of the invasive species and the effect of environmental warming will partly offset this negative effect through increased resource processing in the invasive species at higher temperatures. Uniquely, this study has shown that the replacement may not impact ecosystem functioning as much as previously thought if other factors enhance the shredding activity of the invasive species, although the higher predatory efficiency of *D. villosus* may reduce overall shredding through predation on other macroinvertebrate shredders (Dodd et al. 2014). Our findings therefore constitute a case of antagonistic stressors (Jackson et al. 2016) and provide new insights into the interactions that link environmental thermal regimes with ecological responses across multiple levels of organisation (i.e., metabolic processes of individuals, populations dynamics of invasive and native species, and ecosystem functioning; cf. Woodward et al. 2010). The wider application of MTE analysis, with respect to invasive species, could prove beneficial in terms of identifying 'risk' species during horizon scanning. The results of this study will help predict the possible effect that *D. villosus* will have on freshwater ecosystems as it displaces native species under a warming climate. While estuaries, lakes, and stream outlets represent the current strongholds of *D. villosus*, suitable habitats exist in lower order streams (especially where channelised) and colonisation may be restricted only by stochastic processes (Altermatt et al. 2016), hence further colonisation of headwaters is likely to be a matter of time for this and many other Ponto–Caspian species (Gallardo and Aldridge 2015). Studying and understanding these complex linkages and feedbacks in more detail is vital if ecologists are to deliver more effective modelling of invasion dynamics to inform prevention and mitigation measures.

Authors contribution statement DK, CH, WF, AD, and LB conceived the experiment, DK carried out the experiment, DK and CH analysed the data, WF and LB performed the metabolic scaling analysis, and DK, CH, WF, AD, and LB wrote the paper.

References

Altermatt F, Alther R, Mächler E (2016) Spatial patterns of genetic diversity, community composition and occurrence of native and non-native amphipods in naturally replicated tributary streams. BMC Ecol 16:1–11. doi:10.1186/s12898-016-0079-7

Altmayer A (2015) Invasive alien species: List of species of Union concern. PE 573.889, European Parliamentary Research Service

Anderson LG, Rocliffe S, Haddaway NR, Dunn AM (2015) The role of tourism and recreation in the spread of non-native species: a systematic review and meta-analysis. PLoS ONE 10:e0140833. doi:10.1371/journal.pone.0140833

Barton K (2015) MuMIn: multi-model inference, R package version 1.15.1 edn. R package version 1.15.1. http://CRAN.R-project.org/package=MuMIn

Bates D, Maechler M, Bolker B, Walker S (2015) lme4: linear mixed-effects models using Eigen and S4, R package version 1.1-8 edn. R package version 1.1-8. https://CRAN.R-project.org/package=lme4

Bloor MC (2011) Dietary preference of *Gammarus pulex* and *Asellus aquaticus* during a laboratory breeding programme for ecotoxicological studies. Int J Zool 2011:294394

Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology 85:1771–1789. doi:10.1890/03-9000

Bruijs MCM, Kelleher B, Van der Velde G, De Vaate AB (2001) Oxygen consumption, temperature and salinity tolerance of the invasive amphipod *Dikerogammarus villosus*: indicators of further dispersal via ballast water transport. Arch Hydrobiol 152:633–646

Covich AP, Palmer MA, Crowl TA (1999) The role of benthic invertebrate species in freshwater ecosystems: zoobenthic species influence energy flows and nutrient cycling. Bioscience 49:119–127. doi:10.2307/1313537

DAISIE (2009) Handbook of alien species in Europe. Springer, Berlin

Devin S, Piscart C, Beisel JN, Moreteau JC (2003) Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. Arch Hydrobiol 158:43–56. doi:10.1127/0003-9136/2003/0158-0043

Dick JTA, Platvoet D, Kelly DW (2002) Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea: Amphipoda). Can J Fish Aquat Sci 59:1078–1084. doi:10.1139/f02-074

Dodd JA, Dick JTA, Alexander ME, MacNeil C, Dunn AM, Aldridge DC (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*. Freshw Biol 59:337–352. doi:10.1111/fwb.12268

Dunn AM, Hatcher MJ (2015) Parasites and biological invasions: parallels, interactions, and control. Trends Parasitol 31:189–199. doi:10.1016/j.pt.2014.12.003

Fry FEJ (1947) Effects of the environment on animal activity. University of Toronto Press, Toronto

Gallardo B, Aldridge DC (2015) Is Great Britain heading for a Ponto–Caspian invasional meltdown? J Appl Ecol 52:41–49. doi:10.1111/1365-2664.12348

Garner G, Hannah DM, Sadler JP, Orr HG (2014) River temperature regimes of England and Wales: spatial patterns, inter-annual variability and climatic sensitivity. Hydrol Process 28:5583–5598. doi:10.1002/hyp.9992

González RA, Díaz F, Licea A, Denisse Re A, Noemí Sánchez L, García-Esquivel Z (2010) Thermal preference, tolerance and oxygen consumption of adult white shrimp *Litopenaeus vannamei* (Boone) exposed to different acclimation temperatures. J Therm Biol 35:218–224. doi:10.1016/j.jtherbio.2010.05.004

Graça MAS (2001) The role of invertebrates on leaf litter decomposition in streams—a review. Int Rev Hydrobiol 86:383–393

Graça MAS, Cressa CMOG, Gessner TMO, Feio MJ, Callies KA, Barrios C (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Freshw Biol 46:947–957

Jackson MC, Loewen CJG, Vinebrooke RD, Chimimba CT (2016) Net effects of multiple stressors in freshwater ecosystems: a meta-analysis. Glob Change Biol 22:180–189. doi:10.1111/gcb.13028

Jourdan J et al (2016) Pronounced species turnover, but no functional equivalence in leaf consumption of invasive amphipods in the river Rhine. Biol Invasions 18:763–774. doi:10.1007/s10530-015-1046-5

Karlsson L, Ekbohm G, Steinholtz G (1984) Comments on a study of the thermal behaviour of the American eel (*Anguilla rostrata*) and some statistical suggestions for temperature preference studies. Hydrobiologia 109:75–78. doi:10.1007/BF00006299

Kivivuori L, Lagerspetz KYH (1990) Temperature selection behaviour of the isopod *Saduria entomon* (L.). J Therm Biol 15:83–86. doi:10.1016/0306-4565(90)90052-J

Kley A, Kinzler W, Schank Y, Mayer G, Waloszek D, Maier G (2009) Influence of substrate preference and complexity on co-existence of two non-native gammarideans (Crustacea: Amphipoda). Aquat Ecol 43:1047–1059. doi:10.1007/s10452-009-9242-y

Kuznetsova A, Brockhoff PB, Christensen RHB (2015) lmerTest: tests in linear mixed effects models, R package version 2.0-29 edn. R package version 2.0-29

Lagerspetz KYH, Vainio LA (2006) Thermal behaviour of crustaceans. Biol Rev 81:237–258. doi:10.1017/S1464793105006998

Lewis SE, Dick JTA, Lagerstrom EK, Clarke HC (2010) Avoidance of filial cannibalism in the amphipod *Gammarus pulex*. Ethology 116:138–146. doi:10.1111/j.1439-0310.2009.01726.x

Maazouzi C, Piscart C, Legier F, Hervant F (2011) Ecophysiological responses to temperature of the "killer shrimp" *Dikerogammarus villosus*: Is the invader really stronger than the native *Gammarus pulex*? Comp Biochem Physiol A Mol Integr Physiol 159:268–274. doi:10.1016/j.cbpa.2011.03.019

MacNeil C, 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. J Zool 267:31–38. doi:10.1017/S0952836905007351

MacNeil C et al (2010) The Ponto–Caspian 'killer shrimp', *Dikerogammarus villosus* (Sowinsky, 1894), invades the British Isles. Aquat Invasions 5:441–445

MacNeil C, Dick JTA, Platvoet D, Briffa M (2011) Direct and indirect effects of species displacements: an invading freshwater amphipod can disrupt leaf-litter processing and shredder efficiency. J N Am Benthol Soc 30:38–48. doi:10.1899/10-056.1

MacNeil C, Boets P, 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. Freshw Rev 5:21–35. doi:10.1608/FRJ-5.1.457

Madgwick G, Aldridge DC (2011) Killer shrimps in Britain: hype or horror? The facts about our latest invasive animal. Br Wildl 22:408

McMahon TE, Bear BA, Zale AV (2006) Comparative thermal preferences of westslope cutthroat trout and rainbow trout. Final report to the Wild Fish Habitat Initiative, Montana Water Center at Montana State University-Bozeman and Partners to Fish and Wildlife Program, US Fish and Wildlife Service. Montana State University, Bozeman

Navel S et al (2010) Interactions between fauna and sediment control the breakdown of plant matter in river sediments. Freshw Biol 55:753–766. doi:10.1111/j.1365-2427.2009.02315.x

Newman RM (1990) Effects of shredding amphipod density on watercress *Nasturtium officinale* breakdown. Ecography 13:293–299. doi:10.1111/j.1600-0587.1990.tb00621.x

Paterson RA, Dick JTA, Pritchard DW, Ennis M, Hatcher MJ, Dunn AM (2015) Predicting invasive species impacts: a community module functional response approach reveals context dependencies. J Anim Ecol 84:453–463. doi:10.1111/1365-2656.12292

Perkins DM et al (2015) Higher biodiversity is required to sustain multiple ecosystem processes across temperature regimes. Glob Change Biol 21:396–406. doi:10.1111/gcb.12688

Pimentel D, Zuniga R, Morrison D (2005) Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273–288. doi:10.1016/j.ecolecon.2004.10.002

Piscart C, Dick JA, McCrisken D, MacNeil C (2009) Environmental mediation of intraguild predation between the freshwater invader *Gammarus pulex* and the native *G. duebeni celticus*. Biol Invasions 11:2141–2145. doi:10.1007/s10530-009-9497-1

Piscart C, Roussel J-M, Dick JTA, Grosbois G, Marmonier P (2011) Effects of coexistence on habitat use and trophic ecology of interacting native and invasive amphipods. Freshw Biol 56:325–334. doi:10.1111/j.1365-2427.2010.02500.x

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

R Core Team (2015) R: A language and environment for statistical computing, R foundation for statistical computing edn. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Reiser S, Herrmann J-P, Temming A (2014) Thermal preference of the common brown shrimp (*Crangon crangon* L.) determined by the acute and gravitational method. J Exp Mar Biol Ecol 461:250–256. doi:10.1016/j.jembe.2014.08.018

Rewicz T, Grabowski M, MacNeil C, Bacela-Spychalska K (2014) The profile of a 'perfect' invader–the case of killer shrimp, *Dikerogammarus villosus*. Aquat Invasions 9:267–288

Reynolds WW, Casterlin ME (1979) Behavioral thermoregulation and the "final preferendum" paradigm. Am Zool 19:211–224

Ricciardi A (2001) Facilitative interactions among aquatic invaders: is an "invasional meltdown" occurring in the Great Lakes? Can J Fish Aquat Sci 58:2513–2525. doi:10.1139/f01-178

Ricciardi A, Rasmussen JB (1998) Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can J Fish Aquat Sci 55:1759–1765. doi:10.1139/f98-066

Schar C et al (2004) The role of increasing temperature variability in European summer heatwaves. Nature 427:332–336

Simberloff D et al (2013) Impacts of biological invasions: what's what and the way forward. Trends Ecol Evol 28:58–66. doi:10.1016/j.tree.2012.07.013

Sousa R, Antunes C, Guilhermino L (2008) Ecology of the invasive Asian clam *Corbicula fluminea* (Müller, 1774) in aquatic ecosystems: an overview. Annals of Limnology 44:85–94

Strayer DL (2010) Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshw Biol 55:152–174. doi:10.1111/j.1365-2427.2009.02380.x

Strayer DL, Dudgeon D (2010) Freshwater biodiversity conservation: recent progress and future challenges. J N Am Benthol Soc 29:344–358. doi:10.1899/08-171.1

Strayer DL, Caraco NF, Cole JJ, Findlay S, Pace ML (1999) Transformation of freshwater ecosystems by bivalves: a case study of zebra mussels in the Hudson River. Bioscience 49:19–27

Sutcliffe DW, Carrick TR, Willoughby LG (1981) Effects of diet, body size, age and temperature on growth rates in

the amphipod *Gammarus pulex*. Freshw Biol 11:183–214. doi:10.1111/j.1365-2427.1981.tb01252.x

Truhlar AM, Dodd JA, Aldridge DC (2014) Differential leaf-litter processing by native (*Gammarus pulex*) and invasive (*Dikerogammarus villosus*) freshwater crustaceans under environmental extremes. Aquat Conserv Mar Freshw Ecosyst 24:56–65. doi:10.1002/aqc.2375

Tuchman NC, Wetzel RG, Rier ST, Wahtera KA, Teeri JA (2002) Elevated atmospheric CO_2 lowers leaf litter nutritional quality for stream ecosystem food webs. Glob Change Biol 8:163–170. doi:10.1046/j.1365-2486.2002.00460.x

UK Met Office (2011) Climate: observations, projections and impacts: United Kingdom. Met Office, Exeter

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE (1980) The river continuum concept. Can J Fish Aquat Sci 37:130–137. doi:10.1139/f80-017

Wallace JB, Webster JR (1996) The role of macroinvertebrates in stream ecosystem function. Annu Rev Entomol 41:115–139. doi:10.1146/annurev.en.41.010196.000555

Woodward G, Perkins DM, Brown LE (2010) Climate change and freshwater ecosystems: impacts across multiple levels of organization. Philos Trans R Soc Lond B Biol Sci 365:2093–2106. doi:10.1098/rstb.2010.0055

Yvon-Durocher G, Allen AP (2012) Linking community size structure and ecosystem functioning using metabolic theory. Philos Trans R Soc Lond B Biol Sci 367:2998–3007. doi:10.1098/rstb.2012.0246

Yvon-Durocher G et al (2012) Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487:472–476. http://www.nature.com/nature/journal/v487/n7408/abs/nature11205.html#supplementary-information

Woodland ladybirds

Rachel Farrow & William Fincham

Harlequin ladybird *(Harmonia axyridis)*

Woodlands host a rich variety of plant species which in turn attracts a wide diversity of animal species. This is particularly true for insect species and it has been shown that woodland (especially deciduous) provides vital habitats for hundreds of notable and rare insect species, such as the black hairstreak butterfly, golden hoverfly and bearded false darkling beetle. Together with the vast number of insect species that are not at risk, this makes woodlands a treasure trove for insect enthusiasts. A multitude of beetle species make their homes in woodland, including one very well-known beetle family, the ladybirds (Coccinellidae).

Endearing insects

Ladybirds are captivating – their colourful wing casings endear them to wildlife enthusiasts and their consumption of aphid pests ensures their popularity with gardeners and farmers. Some of the UK ladybird species are commonly found and easily recognisable to most people, such as the 7-spot *(Coccinella septempunctata)* and 2-spot *(Adalia bipunctata)*. There are 47 ladybird species in the UK and Ireland, with 26 species that are conspicuous and readily identifiable. The other 21 species are generally quite small (less than 3.5mm long) and inconspicuous[1]. Some of the common species are less well known, for example, the kidney-spot ladybird *(Chilocorus renipustulatus)* and the eyed ladybird *(Anatis ocellata)*, as these tend to be found in more specialised habitats than the 7-spot and 2-spot ladybirds. There are also rare ladybird species in the UK: the 5-spot ladybird *(Coccinella quinquepunctata)* is only found on unstable river shingle in Wales and Scotland, and the scarce 7-spot *(Coccinella magnifica)* is only found living near wood ant nests.

Approximately 90% of UK ladybird species are predators and consume a range of aphid species and scale insects. The remaining species have a diet of mildew, plants or pollen. Some species will also prey on the eggs and larvae of other ladybird species, in what is known as intraguild predation. Ladybirds defend against natural enemies by releasing a fluid from their leg joints called reflex blood. The reflex blood is yellow in colour and contains bitter and toxic alkaloids to deter predators.

Woodland ladybirds

Ten of the most common woodland ladybirds in adult and larval form are detailed in Figure 1. Five of the native ladybird species listed are considered to be generalists, indicating that they can be found on a wide variety of vegetation and will also consume a wide variety of prey[1]. These five generalists can all be found in woodlands, for example on lime, sycamore or field maple.

The 10-spot (*Adalia decempunctata*) can also be found on oak and hawthorn, while the 2-spot ladybird prefers mature trees, both deciduous and coniferous. In addition to inhabiting these tree species, the 7-spot and 14-spot (*Propylea quattuordecimpunctata*) also frequent herbaceous understorey. The pine ladybird (*Exochomus quadripustulatus*), as suggested by its name, is found in coniferous woodland, but also in deciduous woodland, on the tree species listed above as well as ash, beech, birch and hazel. Both the 7-spot and 10-spot can also be found in coniferous woodland, particularly on Scots pine. The eyed ladybird is the largest UK ladybird (7-8.5mm) and is a specialist in coniferous woodland, specifically Scots pine, Douglas fir and larch. In late autumn, however, it is possible to see this ladybird on oak and lime trees. The orange ladybird (*Halyzia quadripunctata*) and cream spot ladybird (*Calvia quattuordecimguttata*) are both deciduous woodland specialists and prefer ash, sycamore, lime, sallow and hawthorn. The kidney spot ladybird is also a deciduous woodland specialist and is more likely to be found on field maple, oak, ash and willow, especially on the bark rather than the foliage.

Figure 1. 10 most common woodland ladybirds

Ladybird	Adult	Larva	Habitat	Food
Harlequin ladybird *Harmonia axyridis*			Generalist near human buildings such as churches as well as deciduous & coniferous woodland	Aphids, scale insects, ladybirds, fruit
7-spot ladybird *Coccinella septempunctata*			Generalist in deciduous, mixed & coniferous woodland	Aphids
2-spot ladybird *Adalia bipunctata*			Generalist in deciduous & coniferous woodland	Aphids
14-spot ladybird *Propylea quattuordecimpunctata*			Generalist in deciduous woodland	Aphids
10-spot ladybird *Adalia decempunctata*			Generalist in deciduous, mixed & coniferous woodland	Aphids
Pine ladybird *Exochomus quadripustulatus*			Generalist in deciduous, mixed & coniferous woodland	Scale insects
Eyed ladybird *Anatis ocellata*			Specialist in coniferous woodland	Aphids
Orange ladybird *Halyzia sedecimguttata*			Specialist in deciduous woodland	Mildew
Cream spot ladybird *Calvia quattuordecimguttata*			Specialist in deciduous woodland	Aphids
Kidney spot ladybird *Chilocorus renipustulatus*			Specialist in deciduous woodland	Scale insects

David Lovejoy

Harlequin and single 2-spot *(Adalia bipunctata)* ladybirds

An invasive ladybird

Of the 26 conspicuous ladybird species, the majority are native to the UK. Two of the non-native species currently in the UK are the bryony ladybird *(Henosepilachna argus)* and the cream-streaked ladybird *(Harmonia quadripunctata)*. These species have not been found to have a negative impact on native ladybirds and are not considered invasive. The same cannot be said for the harlequin ladybird *(Harmonia axyridis)*, which has spread rapidly through the UK since its arrival in 2003.

The harlequin ladybird was introduced to the USA and some European countries as a biological control agent in an attempt to control aphids on crops, but its introduction into the UK is thought to have been accidental. The harlequin ladybird is native to central and eastern Asia. It is a large ladybird (5-8mm) and is highly diverse in its colouration and so individuals can look very different from one another. The number of spots can range from zero to 21 and the background colour can vary from yellow to red to black. Similar to the native 7-spot, the harlequin is strong during flight and can fly at speeds of up to 30km per hour. This invasive species is also less susceptible to parasites and fungal infections that are common in native UK ladybird species. The harlequin ladybird is a generalist predator consuming a large number of aphid species – up to 60 different aphid species have been recorded as prey of the harlequin[2]. If its preferred prey is unavailable, the harlequin ladybird will also engage in intraguild predation by consuming the eggs, larvae and pupae of other ladybirds. Even though other ladybird species do the same, research has determined that the harlequin ladybird is more successful in these interactions, partly as it has better physical and chemical defences[3].

The harlequin ladybird is also a generalist in terms of its habitat preference. The species is found in many habitats covering both urban and rural areas. In woodland, the harlequin can be found predominantly on sycamore and lime, but also on several other tree species including oak, field maple, Scots pine, ash and yew[1]. Since it arrived in the UK, the spread of the harlequin ladybird has been closely documented by scientists with the engagement of citizen scientists as part of the UK Ladybird Survey (ladybird-survey.org). Data collected through the UK Ladybird Survey has shown declines in several native ladybird

species following the arrival of the harlequin ladybird. The 44% decline in 2-spot ladybird numbers is attributed to the increase in harlequin numbers and increased competition for prey.

Overwintering ladybirds

Ladybirds are most likely to be spotted on warm, sunny days, although it is possible to find them during colder months. In temperate climates, such as that of the UK, ladybird species must either migrate during the winter or become dormant. In the UK, ladybirds become dormant and choose a suitable site in which to overwinter. Prior to entering this dormant phase, ladybirds will consume as much food as possible to ensure they have the reserves needed to get them through to spring, when they can emerge ready to reproduce[4].

In woodland, the harlequin can overwinter in a variety of sheltered locations, including tree crevices and spaces under bark. However, the favoured location for an overwintering harlequin ladybird is inside houses. Many people will be familiar with large groups, or aggregations, of ladybirds congregating in their houses on windows, ceilings, sheds or fence posts. These are often large groups of harlequin ladybirds, but can include a mix of harlequins and other species such as the 2-spot ladybird, which also likes elevated positions such as attics. Where possible, harlequins tend to move from woodlands to overwinter near human settlements in sheltered locations such as churches, sheds and houses. This is when the species is often noticed, especially as harlequin aggregations can be up to thousands strong.

Native woodland ladybird species tend to remain near their usual habitats. Here, the majority of ladybird species tend to overwinter in leaf litter, low herbage or shrubs, such as gorse. Some, like the cream spot, pine and 2-spot ladybird, prefer to spend winter in the crevices within tree bark. Depending on the winter conditions, many species can be found where the branches meet the tree trunk.

There is some evidence that ladybirds are accurate long-term weather forecasters. Research has shown that the proportion of ladybirds that remained on trees each year was positively correlated to the summed minimum daily temperature for November to February inclusive. The interesting aspect of this is that once they have chosen their overwintering sites in early October, the vast majority of ladybirds rarely move from these sites[2].

Overwintering sites can be revisited year after year. It is thought that pheromones released by previously overwintering ladybirds persist as markers for individuals the following year[4]. Overwintering ladybirds indoors are very unlikely to cause anything more than a nuisance. While allergic reactions are possible, they are rare, and staining from the yellow reflex blood is a more likely side effect.

Pine ladybirds (*Exochomus quadripustulatus*)

How can you contribute?

It is well known that human actions have impacted the UK's wildlife, and ladybirds are no exception. Habitat loss attributed to urbanisation and intensification of agricultural practices, as well as the arrival of invasive non-native species, such as the harlequin ladybird, impose an increasing pressure on native ladybirds. Records of ladybirds from members of the public are invaluable, not only in the warmer months, but also during the winter period. Recording ladybirds (adults, pupae and larvae) is relatively quick and simple and can be done by several means, especially via the free iRecord Ladybirds recording app (iPhone or Android) or website (ladybird-survey.org/recording.aspx).

Rachel Farrow is a PhD student at Anglia Ruskin University. Her work researches the effects of invasive non-native species and how best to implement conservation measures for native species.

William Fincham is a PhD student at the University of Leeds and the Centre for Ecology & Hydrology. His work focuses on the impacts of invasive non-native species.

1. Roy, H.E., Brown, P.M.J., Frost, R. and Poland, R. 2011. Ladybirds (Coccinellidae) of Britain and Ireland: an atlas of the ladybird of Britain, Ireland, the Isle of Man and the Channel Islands. FSC Publications, Shrewsbury.

2. Majerus, M.E.N., Roy, H.E. and Brown, P.M.J. 2016. A natural history of ladybird beetles. Cambridge University Press, Cambridge.

3. Brown, P.M.J., Frost, R., Doberski, J., Sparks, T., Harrington, R. and Roy, H.E. 2011. Decline in native ladybirds in response to the arrival of Harmonia axyridis: early evidence from England. Ecological Entomology, 36: 231-240.

4. Roy, H.E., Brown, P.M.J., Comont, R.F., Poland, R.L. and Sloggett, J.J. 2013. Ladybirds. Second edition. Pelagic Publishing, Exeter.

Essex, 2004. The harlequin ladybird (*Harmonia axyridis*) first arrives in Britain and begins to spread, rapidly. Over the next four years the species will be found in all regions of England and Wales, and will occupy 1,022 10km square sections of Britain. At its peak, the harlequin will spread at a rate of 105km a year.

Described at the time as 'the most invasive ladybird on Earth', the harlequin ladybird and its rapid spread posed multiple questions for scientists. What enabled the species to capitalise on Britain? What factors, if any, have held the species back? And what impact has the harlequin ladybird had on native species?

A decade after the first report of the harlequin ladybird in Essex, we have been able to reflect on the success of this species,

They can survive on many food sources ranging from aphids to pollen. Scientists analysed the gut contents of the ladybird to investigate 'in-field' feeding and found among pollen and aphids evidence of hoverflies and other ladybird species. As the harlequin can eat almost everything, there is the potential for competition between it and other species that share food resources, which include hoverflies, lacewings and other ladybirds. This could, in part, explain the recent decline in two-spot ladybirds in Britain: populations are estimated to have fallen by 44% following the arrival of the harlequin ladybird.

The harlequin ladybird has spread especially rapidly throughout the south of England. Generally speaking, Britain's climate becomes increasingly wet and cold with increasing latitude. There are fewer

"The most INVASIVE ladybird on Earth"

The harlequin has spread across the entire British Isles in just a decade. William Fincham and colleagues look at why this beetle has thrived here

thanks to citizen science initiatives such as the UK Ladybird Survey. These projects use data gathered with the help of the public and have contributed hugely to our understanding of this invader.

WHY SO SUCCESSFUL?

Factors that dictate where a species lives can be diverse, but commonly come down to habitat, climatic requirements and interactions with existing species, including competitors and natural enemies (predators, parasites and pathogens). Important traits include the ability of a species to adapt to novel environments and – potentially most importantly for invasive non-native species – the ability to disperse.

The harlequin ladybird is often described as eurytopic – it is found in a wide variety of habitats. UK Ladybird Survey records show individuals are present in nearly all habitat types within Britain, but they appear to prefer urban areas, while commonly avoiding areas of coniferous woodland.

records of this invader in northern England and Scotland, suggesting climate could become limiting with increasing latitude.

Perhaps the clearest indication of the harlequin's phenotypic adaptability is the existence of a wide range of colour morphs. There is some evidence to suggest that climate can drive the relative dominance of these morphs. The common colour morphs in Britain are the non-melanic (form *succinea*) and melanic morphs (f. *spectabilis* and f. *conspicua*). Records from the UK Ladybird Survey suggest 80% of UK harlequins are the non-melanic morph.

The melanic morph's colouration is considered beneficial in colder climates, as it helps the ladybird retain the heat of the sun, yet is costly in warmer climes.

In addition to climatic conditions, it is also likely that physical barriers have restricted the spread of the harlequin ladybird. Key physical barriers include the Pennines in northern England and the Cambrian mountains in Wales. These obstacles likely prove challenging for

Harlequins and humans

Despite its size there are many ways this small beetle can affect humans. In autumn, these colourful beetles aggregate and find shelter in your shed, garage and house.

When disturbed, ladybirds often release reflex blood (a yellow fluid), which has a strong smell and stains walls and furniture. In a few rare cases, these aggregations have also been reported to cause allergies. These little beetles can also damage soft fruit (yet another potential food source), which includes grape vines used in wine production.

While never released intentionally in the UK, the species had been used across Europe as a biological control agent against crop pests.

MARIS MIDGLEY: LORI HANDLEY

even the most able-bodied coccinellid, although hitchhiking with humans is always a possibility.

The harlequin's great adaptability does not fully address how individuals were capable of such a significant spread. The answer is simple: harlequins are great fliers. They can maintain flight for up to two hours and at altitudes of 1,100m, so individuals have the potential to disperse up to 120km per flight. Such flights, while possible, are likely to be rare, however, with flight distances commonly shorter and at lower altitudes for short-distance travel between vegetation.

Despite their dispersal ability, this isn't always required: harlequins can easily become 'stowaways' on transported goods. The first recorded individual in mainland Scotland is reported to have arrived in a suitcase, and others reached the Orkney Islands and Northern Ireland on vegetables sent from mainland Britain. This ability to stow away, linked with their aggregation behaviour, likely aids in colonisation of new areas.

ON ENEMY SOIL

Ladybirds have many natural enemies. In the UK, these range from bacterial and fungal pathogens to larger parasites, including flies and wasps, one of which turns the ladybird host into an unsuspecting and zombie-like bodyguard. The wasp (*Dinocampus coccinellae*) lays its egg in the

Harlequins can easily become stowaways on transported goods

ladybird, giving rise to a larva that feeds inside the ladybird until it is ready to pupate. At this stage, the larva burrows out of the ladybird and begins to spin a cocoon to the underside of the ladybird, attaching it to the leaf, wall or wherever the unsuspecting ladybird is at the time. The ladybird is rendered a bodyguard for the parasite, protecting it from predators.

The invasive harlequin ladybird is less susceptible to natural enemies in the UK than our native species. It is thought that the harlequin may have undergone 'enemy release', with many of the species' enemies failing to spread during the invasion process with the host. Natural enemies of UK ladybird species are maladapted to attack the harlequin ladybird, giving the invader a potential advantage.

The parasitic wasp *D. coccinellae* is one such natural enemy. It is less abundant in the harlequin ladybird than in other native species, possibly due to the wasp's inability to counter the harlequin's internal defences. The harlequin also has an increased resistance to other enemies, including the fungal pathogen *Beauvaria bassiana*. Details

LADYBIRD GUIDE: MICHAEL KILNER; CAT HUMPHRIES; MIKE BIDWELL; MARIS MIDGLEY; REMY POLAND

concern to an 'Alert' email or online. So far, there have been no confirmed sightings.

From an ecological perspective, the harlequin ladybird has revealed the complexity of the invasion process. It is intuitive to consider the ecology of non-native species in their native range when assessing potential invasive traits. However, there is increasing evidence that this is perhaps overly simplistic and that so-called 'bridgehead effects' are relevant. Bridgehead effects are situations where an initial invasion event has occurred and given rise to a particularly successful invasive population. This population can then bring about future invasion events in isolation from the original native population. Evidence suggests the harlequin ladybird has demonstrated such effects during its invasion history with invasive North American populations contributing to invasions in South America, South Africa and Europe.

THE NEXT 10 YEARS

The arrival of the harlequin ladybird was met with concern, both in the scientific community and the media. The species has been the focus of substantial research effort, which will undoubtedly continue to further our understanding of the species and the invasion process.

It is exciting to consider how much has been revealed about the harlequin ladybird over the last 10 years. And it is inspiring to reflect on the contributions of volunteers to our understanding of the ecology of this species, with tens of thousands of observations submitted by people across the UK. It is not only the observations that people submit, but also the thought-provoking questions and discussions along the way that stimulate research ideas.

During the next decade, there will be opportunities to explore the interactions between harlequin ladybirds and other species, with the potential to address some of the big questions in ecology. For example, how does the arrival of an invasive non-native species alter the ecological function and resilience of species assemblages? The advance of molecular techniques coupled with field observations will enable us to delve deeper into the interactions between harlequin ladybirds and other species.

www.ladybird-survey.org

---

of how the harlequin ladybird could escape natural enemies are largely unknown, but it is hoped laboratory and field research will improve our understanding.

The control of the harlequin ladybird has been the focus of significant research effort, yet a solution is still lacking. Many control measures have been suggested, including various traps and the release of natural enemies. However, no control measure has been found to be both successful enough and species specific so as to pose no risk to native species.

The advances in our understanding of invasion by the harlequin ladybird have relevance more widely for invasion biology. The success of the UK Ladybird Survey in tracking the spread of the harlequin ladybird inspired the development of an online system for surveillance of other invasive non-native species. For example, the Asian hornet (*Vespa velutina*) is a non-native species not yet present in the UK but anticipated to arrive in the coming years. The public are being encouraged to look out for this species and report sightings of

Alison M Dunn is interested in how invasive species and parasitic disease affect trophic links and ecosystem processes. She has worked on aquatic invasive species such as killer shrimp and signal crayfish.

William Fincham is a PhD student at the University of Leeds and the Centre for Ecology & Hydrology. He is interested in the impacts of invasive non-native species on native species and communities.

Katie Murray is interested in the effect parasites have on their hosts, with particular focus on the harlequin ladybird. She is in the final year of her PhD at the University of Stirling.

Professor Helen Roy is a principal scientist at the Centre for Ecology & Hydrology and a visiting professor with Reading University. She co-leads the UK Ladybird Survey.

Harlequins can eat almost anything, giving them a competitive advantage over native species

LADYBIRD GUIDE: KEN DOLBEAR; EDWARD BARTOSZEWICZ; RICHARD COMONT; MICHAEL MAJERUS

Bug buster
Some of the most common of the UK's 43 resident ladybird species

TWO-SPOT LADYBIRD
Adalia bipunctata

SEVEN-SPOT
Coccinella septempunctata

10-SPOT
Adalia decempunctata

14-SPOT
Propylea 14-punctata

22-SPOT
Psyllobora 22-punctata

CREAM STREAKED
Harmonia 4-punctata

CREAM SPOT
Calvia 14-guttata

EYED LADYBIRD
Anatis ocellata

KIDNEY SPOT
Chilocorus renipustulatus

ORANGE LADYBIRD
Halyzia 16-guttata

PINE LADYBIRD
Exochromus 4-pustulatus

STRIPED
Myzia oblongoguttata

Appendix B

Activities and outreach

2017   **Co-authored magazine article** Wood Wise, The Woodland Trust
Discovery zone Leeds University
A regional outreach event for local school pupils
Cafe Scientifique Leeds
Public outreach event discussing the impacts of invasive non-native species
NERC DTP SPHERES student conference Runner-up best talk
BES annual meeting, Ghent, Belgium Poster
ESA annual meeting, Portland OR, USA Conference talk

2016   **Co-authored magazine article** The Biologist, Royal Society of Biology
Discovery Zone Leeds University
Freshwater Bio-assessment University of Stirling
A residential course on freshwater bio-assessment and it's application in
management with respect to the EU Water Framework Directive
NERC DTP SPHERES student conference Best 'talking poster'
BES annual meeting, Liverpool, UK Conference talk

2015   **FAWKES I** Leeds University and Helmholtz Centre for Environmental
Research. The project investigated the interaction between ecosystem
services and the Water Framework Directive
Freshwater taxonomy Natural History Museum, London
Discovery Zone Leeds University
Science communication training The British Ecological Society
Ideas put forward were incorporated into the societies Festival of Science
BES annual meeting, Edinburgh, UK Poster