The Ecological Impact of the Invasive Freshwater Killer Shrimp *Dikerogammarus villosus* on Native UK Amphibians

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

Chapter 2 is based on a jointly authored research article which was recently accepted for publication in Scientific Reports, co-authored by Daniel Warren (lead author), Stephanie Bradbeer and Dr Alison Dunn (co-authors). Daniel Warren developed the idea, conducted the experiments, analysed the data, and wrote the manuscript. Stephanie Bradbeer and Dr Alison Dunn advised on experimental design and contributed to the interpretation of results and writing of the manuscript, led by Daniel Warren. Additional feedback was provided by Dr William Fincham. *Xenopus laevis* embryos were provided by the European Xenopus Resource Centre (EXRC; University of Portsmouth). This work was partially funded by the Water@Leeds SPRING grant.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

AICc	Finite sample corrected Akaike's information criterion
ANOVA	Analysis of variance
Bd	*Batrachochytrium dendrobatidis*
CI	Confidence interval
df	Degrees of freedom
FR	Functional response
GLM	Generalised linear model
IAS	Invasive Alien Species
NR	Numerical Response
RIP	Relative Impact Potential
SD	Standard deviation
SE	Standard error

Abstract

Invasive alien species are one of several drivers implicated in the ever-increasing rate of global amphibian declines, contributing through intense predation of early life stages, but also through the spread of pathogenic agents. In this thesis I investigate the potential for the invasive freshwater amphipod crustacean *Dikerogammarus villosus* to adversely affect UK amphibians.

First, I examine the possibility for *D. villosus* to impact upon amphibians directly as a predator of early life stages. Using functional response (FR) analyses, I quantify differences in *per capita* predatory behaviours between invasive *D. villosus* and the native amphipod *Gammarus pulex* towards the embryos and larvae of several UK anurans. Using the relative impact potential metric (RIP), I supplement FR measurements with native and invasive amphipod abundance data, recorded during the surveillance of uninvaded and invaded UK field sites, and from previously published literature. Compared to native *G. pulex*, invasive *D. villosus* demonstrated an exceptionally stronger predicted predatory impact, as a result of differential body size and population abundance.

Second, I examine how interactions between conspecific invaders may alter the predicted ecological impact of *D. villosus* towards larval anurans. *Per capita* predation decreased as a function of increasing predator abundance, caused by strong conspecific aggregation and the emergence of mutual interference between amphipod predators. Non-aggressive interference between interacting conspecifics is predicted to cause a reduction in predatory pressures by *D. villosus* towards larval amphibians.

Third, I explore how predatory pressures imposed by *D. villosus* towards larval UK anurans may change when alternative prey species are present, either in equal quantities or at varying provisioning ratios. *Dikerogammarus villosus* exhibited significantly greater selectivity towards alternative prey rather than anuran larvae, regardless of relative abundance. In the absence of frequency-dependent prey-switching behaviours, larval amphibians are expected to experience a reduced risk of predation by *D. villosus* when more preferred prey types are available.

Fourth, I address the possibility for *D. villosus* to indirectly affect native amphibian populations, as a potential carrier of the amphibian chytrid fungus, *Batrachochytrium dendrobatidis* (*Bd*). I verify that *D. villosus* can acquire infections, localised to the exterior surface of the cuticle only. Infections did not cause any discernible pathologies in amphipod hosts, but instead appeared to be transient, with some amphipod hosts clearing infections by shedding the infected cuticle.

I finish by highlighting that, although these findings suggest the potential for invasive *D. villosus* to directly and/or indirectly affect amphibian populations, evidence of previous field-based interactions between larval amphibians and invasive amphipods is limited, and as such is an area that warrants further investigation.

Chapter 1

General Introduction

Over the past 450 million years, the Earth has experienced five mass extinction events which led to substantial declines in global biodiversity, within a relatively short period of time (Jablonski, 1994; Erwin, 2001; Alroy, 2008). Scientists now believe that we may be experiencing a sixth mass extinction (Wake and Vredenburg, 2008; Butchart et al., 2010; Barnosky et al., 2011; Dirzo et al., 2014), for which human activity has been identified as a predominant driving force (Barnosky et al., 2011). Aptly referred to as the "Anthropocene epoch", human-caused declines in global biodiversity are happening across all major taxa. For invertebrates, at least 42% of described terrestrial species, and a further 25% of marine species, have been classified as threatened with extinction (Collen et al., 2012). For vertebrates, ~543 species have gone extinct since 1900, with a further 515 vertebrate species on the brink of collapse (Ceballos et al., 2020). Between 1970 and 2016, global vertebrate populations experienced average declines of approximately 68% (WWF, 2020).

Amphibians are one of the most heavily affected groups of vertebrate organisms to have experienced declines and/or extinctions to-date – a fact which was first noted at the First World Congress of Herpetology in 1989 (Barinaga, 1990). Since then, substantial evidence has been collected, illustrating the true extent of global amphibian declines – a phenomenon which appears to be accelerating (McCallum, 2007). Indeed, current extinction rates are estimated to be 211 times greater than background extinction rates (McCallum, 2007). However, this number rises drastically if the number of amphibian species on the brink of extinction are considered, becoming four orders of magnitude greater – somewhere in the realms of between 25,000 – 45,500 times – than background extinction rates (McCallum, 2007; Alroy, 2015). Of the ~8,200 described species of amphibians, recorded at the time-of-writing (AmphibiaWeb, 2020), 35 species are formally classified as extinct, with an additional 134 species considered as "possibly extinct" after having disappeared from the wild (IUCN, 2020). Approximately 3,100 amphibian species (~38%) are threatened with extinction of which ~500 species may be endangered, critically or otherwise (González-del-Pliego et al., 2019).

Global declines can be attributed to five major, anthropogenically-driven factors; 1) climate change, 2) habitat loss, 3) environmental pollution, 4) invasive alien species, and 5) parasites, pathogens and infectious diseases (Hayes et al., 2010). These factors typically do not contribute independently, but rather synergistically (Hayes et al., 2010). In recent years, studies have focussed on the impact of invasive alien species, as well as their role in dispersing pathogens which, when considered synergistically, has caused one of the greatest documented losses of biodiversity attributable to a pathogen (O'Hanlon et al., 2018; Scheele et al., 2019; Fisher and Garner, 2020). Throughout this thesis, I investigate the potential ecological impact of invasive alien species towards amphibians and examine the potential for invaders and pathogens to operate synergistically.

1.1 Biological Invasions and Invasive Alien Species – Definitions, Distributions and Damages

1.1.1 What are biological invasions?

Biological invasions are a ubiquitous consequence of globalisation (Meyerson and Mooney, 2007; Hulme, 2009; Simberloff et al., 2013), and describe the process by which species are moved, introduced, become established, and subsequently spread beyond their native ranges (Blackburn et al., 2011; Dunn and Hatcher, 2015).

As humanity has strived to create a globalised society, establishing increasingly complex international networks through which both people and products move around the world at an ever-increasing rate, so too has there been a dramatic increase in the movement of non-human organisms across the globe (Vitousek et al., 1997; Ricciardi, 2007; Hulme, 2009; Richardson, 2010; Seebens et al., 2017). Over the past 200 years, there has been an exponential increase in the number and diversity of plant and animal species being translocated beyond their natural biogeographic range through human action (Richardson et al., 2000; Kettunen et al., 2008; Brunel et al., 2013; Seebens et al., 2017). Classified as alien (i.e. non-native) species (Secretariat of the Convention on Biological Diversity, 2006; Kettunen et al., 2008), these organisms are propagated via a number of transmission pathways, all of which can be categorised into three general mechanisms of introduction; i) accidental or deliberate importation as or on a commodity (e.g. biological control agent or parasite), ii) carried by a transport vector (e.g. ballast water), and iii) dissemination from an area of previous introduction, either via human-made infrastructures (e.g. roads, railways and canals) or naturally (Hulme et al., 2008; Wilson et al., 2009). Many of these organisms fail to subsist within novel regions, and as such are termed to be *casual* species. However, some alien species form reproductively active populations in the absence of human intervention, with between 10 and 50% of introduced species surviving and becoming successfully *established* (Williamson and Fitter, 1996; Jeschke and Strayer, 2005).

Europe, a historically important centre for global commerce, is now home to more than 10,000 established alien species (Scalera et al., 2012), of which more than 3,000 species can be found in the UK alone (GB non-native species secretariat, 2020). In most cases, established alien species appear to have no discernible impact within introduced regions; although this may be due to limitations in the available data (Jeschke et al., 2014). Some alien species can even be beneficial, modifying community dynamics, functioning as surrogate ecosystem engineers, facilitating the restoration of extirpated native species, and providing ecosystem services (Goodenough, 2010; Schlaepfer et al., 2011). For example, the African honeybee (*Apis mellifera*) can be a vital pollinator in some regions, promoting agricultural production (Delaplane and Mayer, 2000; Morse and Calderone, 2000) and the recovery of native flora (Sanguinetti and Singer, 2014). However, a small, yet apparently increasing proportion of alien species (DAISIE, 2009; Pascal et al., 2010), likely ranging from 10-50% of known species, progress from *established* to *invasive* (Williamson and Fitter, 1996; Jeschke and Strayer, 2005), disseminating beyond the boundaries of their initial introduction, becoming established within a novel geographic region (Figure 1.1; Blackburn et al., 2011; Dunn and Hatcher, 2015). For example, of the >10,000 alien species currently established in Europe, approximately 11% of these are regarded as invasive (Vilà et al., 2010). Although biological invasions are a global phenomenon, occurring in most regions inhabited by humanity, both the number, and rate, at which invasive species are introduced into novel regions is often asymmetric, with greater incidence of invasions typically recorded in densely populated countries (Dawson et al., 2017), countries of high economic growth and development (e.g. USA), as well as small, geographically isolated islands (e.g. Réunion; Turbelin et al., 2017). Beyond their natural range, some invasive species can have a significant detrimental effect upon the local ecology, economy and/or human health, although there is no correlation between invasiveness and invader impact (see Ricciardi and Cohen, 2007).

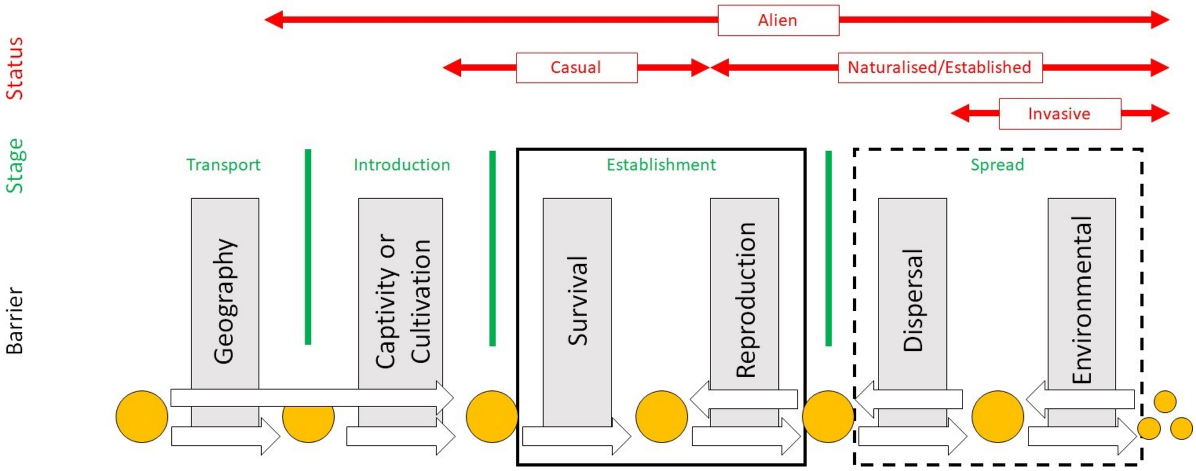

Figure 1.1. Summary of the invasion process, as adapted from Blackburn et al. (2011), which describes the progression of alien populations (yellow circles) across different stages of invasion (green text) by crossing invasion barriers (black text). Terminology describing populations at each stage of invasion are given in red. White arrows define the transition of populations across invasion barriers which can occur in either direction (temporal shifts in status). Black squares indicate the areas of focus for this thesis, particularly the impact of invasive alien species post-establishment (solid black square), but also the potential implications for future dispersal (dashed black square).

1.1.2 What are invasive alien species?

There is considerable debate regarding the definition of invasiveness, with some ecologists defining it within a biological context (i.e. geographic distribution and population abundance; Colautti and MacIsaac, 2004), others within a policy context (i.e. negative environmental and/or anthropocentric impacts; IUCN, 2020), and even some considering the potential for native species to demonstrate invasive capabilities (Valéry et al., 2009). However, in this thesis, I adopt the definition used by the

European Commission (2014), defining an invasive alien species (IAS) – a term synonymous with invasive non-native species (INNS; GB non-native species secretariat, 2020) – as any species that is introduced by humans, either accidentally or deliberately, outside its natural range, which becomes established, spreads, and adversely impacts upon biodiversity and related ecosystem services (i.e. ecological impact), human health and/or the economy. In this thesis I primarily focus on assessing the ecological impact of invasive alien species, here considered as any biological process which negatively affects native biodiversity, thereby resulting in a measurable change in the population(s) of affected species (Dick et al., 2017). I also extend the definition of an invasive alien species to include microorganisms, including bacteria, viruses and fungi, as IAS (Roy et al., 2017); which I will address further in later sections (Section 1.3.2.1) and chapters (Chapter 5).

Throughout this thesis I will be focussing on the ecological impact of IAS on native species, post-establishment (Figure 1.1; black solid box), considering invader impact as a function of its *per capita* effect on native species (E), its abundance within invaded regions (A) and its range (R; Parker et al., 1999). These aspects of invader impact will be addressed further in later sections (Section 1.3.1.2 – 3). In this thesis, I will explore the ecological impact of the invasive 'killer shrimp' *Dikerogammarus villosus* on UK amphibians; with respect to *per capita* effect (Chapters 2, 3 and 4), and abundance (Chapters 2 and 3), in particular. Based on these findings I will discuss the potential implications for future invader dispersal (Figure 1.1; black dashed box).

1.2 What are the costs of invasive alien species?

Invasive alien species are renowned for substantially affecting environments into which they are introduced, and subsequently become established in. Within novel regions, established IAS detrimentally impact those communities that naturally inhabit, and/or are reliant upon, these new ecosystems (European Commission, 2014). Globally, the costs associated with IAS can be assigned to three principal categories; 1) economic costs, 2) human-health costs, and 3) environmental costs (Diagne et al., 2020).

1.2.1 Economic costs of invasive alien species

Invasive alien species typically generate substantial economic costs, often associated with their control and/or removal. Additional monetary costs incurred as a result of biological invasions frequently include costs to human health (e.g. zoonoses and allergens) and wellbeing (e.g. restrictions to recreational activities), damages to human infrastructure (e.g. flood defences and buildings) and financial losses for the various production industries, such as agriculture, horticulture, aquaculture, livestock and forestry (van der Veer and Nentwig, 2015). For example, global costs associated with the control of invasive insects, damaging to production industries and cultural services (e.g. tourism), has been estimated at least $70 billion (USD) per year, with further annual costs of more than $6.9 billion (USD) related to health implications of insect invaders (Bradshaw et al., 2016).

Annual economic costs of IAS control, and the damages sustained to marketable goods, have been estimated at approximately $120 billion in the United States (Pimentel et al., 2005), €12 billion in Europe (Altmayer, 2015), and £1.7 billion in the UK (Williams et al., 2010). Combined, the global economic cost of IAS has been estimated to exceed $1.4 trillion (USD), equating to approximately 5% of the global gross world product (GWP) at the time of estimation (Pimentel et al., 2001). This global figure was derived from costings estimated for more than 120,000 invasive plant and animal species, and included costings associated with pathogens and diseases spread by IAS (Pimentel et al., 2001; Hoffmann and Broadhurst, 2016). Estimated based on data collated in 1998, the projected global economic cost of biological invasions is likely to be much higher; although accurate, more recent estimations are yet to be published (but see InvaCost project; Diagne et al., 2020).

1.2.2 Human-health costs of invasive alien species

Invasive alien species can have a dramatic impact upon human health, directly and/or indirectly affecting not only physical health, but also mental health (Peyton et al., 2019). Allergic reactions induced by bites, stings and/or direct contact with compounds produced by IAS can have a significant direct effect on human health, sometimes resulting in death. For example, anaphylaxis caused by stings from the Asian hornet (*Vespa mandarinia*) is associated with up to 50 deaths per year in Japan (Yanagawa et al., 2007). Zoonotic infectious diseases – that is diseases caused by the transmission of pathogenic agents between animal reservoirs and human hosts – are also a primary factor in IAS-mediated declines in physical human health (Mazza et al., 2014). Invasive alien species are thought to be directly implicated in the increasing global incidence of zoonotic diseases, for which more than 850 infectious organisms are known to infect humans, and include 132 species that are regarded as emerging (i.e. recently discovered yet rapidly spreading; Taylor et al., 2001; Jones et al., 2008; Hulme, 2014; Mazza et al., 2014; Young et al., 2017). For example, emerging zoonotic diseases, such as West Nile virus, yellow fever and Lyme disease, have all been attributed to the spread of invasive haemophagous arthropods, including fleas, ticks, and of course mosquitoes (Juliano and Lounibos, 2005; Hulme, 2014; Mazza et al., 2014; Young et al., 2017). In some cases, zoonoses may themselves exhibit characteristics of an invasive alien species, although they are rarely recognised as such. For example, the spread of SARS-CoV-2, the emerging zoonotic coronavirus responsible for causing a significant number of human deaths and the current global pandemic crisis, displays strong parallels with the typical biological invasion process (Nuñez et al., 2020).

1.2.3 Environmental costs of invasive alien species

Biological invasions and the spread of IAS are acknowledged as a primary driver of global biodiversity loss, ranked second only to habitat loss (Wilcove et al., 1998; Baillie et al., 2004), or food production (e.g. agriculture and aquaculture; Bellard et al., 2016a), in terms of impact. However, determining the extent to which IAS impact upon biodiversity remains a key point of discussion. For example, Gurevitch and Padilla (2004) determined that IAS were directly associated with the extinction of only 6% of species, listed on the IUCN red list. However, reanalysis of the data suggested this to be a gross underestimation of invader impact, instead revealing that IAS likely contributed to the extinction of more than 50% of species listed (Clavero and García-Berthou, 2005). Wilcove et al. (1998) reported that invasive species threatened 49% of species, listed as endangered in the United States, whereas more recent assessments indicated only ~6% at risk (Dueñas et al., 2018). Nevertheless, with evidence to suggest that the rate of biological invasions is increasing exponentially, and is projected to continue for many years, with little evidence of stabilisation, the impact of spreading IAS is now regarded as a global conservation concern (Simberloff et al., 2013; Seebens et al., 2017; Sardain et al., 2019).

The impacts of biological invasions are experienced throughout both terrestrial and aquatic (freshwater and marine) ecosystems (Simberloff et al., 2013). Despite being grossly under-represented in the literature (Gherardi, 2007; Ricciardi and MacIsaac, 2010; Lowry et al., 2013), aquatic ecosystems often experience a considerably greater incidence of invasions (Dudgeon et al., 2006; Moorhouse and Macdonald, 2015; Thomaz et al., 2015; Tricarico et al., 2016). For example, between 1970 and 2007, a period corresponding to one of the most dramatic increases in alien species introductions worldwide (Seebens et al., 2017; Seebens et al., 2020), the rate at which alien species were introduced into European aquatic ecosystems increased by approximately 76% (Butchart et al., 2010), and is predicted to increase further in the future (Sardain et al., 2019; Seebens et al., 2020).

Freshwater ecosystems are especially susceptible to biological invasions (Gherardi, 2007; Ricciardi and MacIsaac, 2010). Although spatially restricted, making up only 0.01% of the world's water and occupying 0.8% of the Earth's surface, freshwater ecosystems contain a disproportionately high diversity of flora and fauna, supporting at least 6% of all described species (Dudgeon et al., 2006), 9.5% of all described animal species, and ~35% of the world's vertebrate species (Balian et al., 2008). Humanity is also reliant upon freshwater ecosystems, utilising more than 50% of global freshwater runoff for economic, cultural and/or aesthetic purposes (Jackson et al., 2001; Dudgeon et al., 2006). However, extensive modification to freshwater habitats, such as the construction of dams and canals, and the overexploitation of this natural resource, has facilitated the introduction, establishment, and subsequent spread of many invasive freshwater species (Dudgeon et al., 2006; Johnson et al., 2008), either intentionally or unintentionally as or with commodity species, or from vectors such as ballast water and recreational equipment (Hulme et al., 2008; Anderson et al., 2014; Smith et al., 2020). Due to a comparative lack of dispersal boundaries in some freshwater environments (e.g. rivers), relative to terrestrial ecosystems, established IAS are able to disseminate rapidly, enhanced by the high intrinsic dispersal ability often demonstrated by freshwater IAS, and facilitated by the hydrology of lotic systems, and the natural catchment linkages of rivers, streams and lakes (Lodge et al., 1998; Shurin and Havel, 2002; Dudgeon et al., 2006; Chapman et al., 2020). As a result, freshwater ecosystems experience an inordinately greater incidence of biological invasions, particularly by ecologically and/or economically damaging high-impact IAS, when compared to marine (Ricciardi and Kipp, 2008; Ricciardi and MacIsaac, 2010; Vilà et al., 2010; Tricarico et al., 2016), and terrestrial (Sala et al., 2000; Dudgeon et al., 2006; Strayer, 2010; Moorhouse and Macdonald, 2015; Thomaz et al., 2015; Anton et al., 2020), ecosystems. For example, on average, at least 11% of IAS situated in freshwater ecosystems around the world are found to be high-impact, as opposed to the 4% found in global marine ecosystems (Ricciardi and MacIsaac, 2010). Similarly, no less than 30% of European freshwater invaders are regarded as ecologically damaging, when compared to the ~16% found in marine ecosystems (Vilà et al., 2010).

Due to the highly variable and insular nature of some freshwater habitats, particularly lakes and small rivers which tend to be hydrologically, and therefore biotically, distinct from one another, freshwater biotas can be endemic, much in the same way that remote oceanic islands frequently contain distinctive, highly diverse communities (Cox and Lima, 2006; Strayer and Dudgeon, 2010). However, these isolated freshwater communities are often evolutionarily naïve towards invaders which are functionally distinct from native congeneric species, making them vulnerable to introduced IAS (Cox and Lima, 2006; Anton et al., 2020). As a result, freshwater ecosystems often experience significantly greater IAS-mediated declines in both the abundance and diversity of resident species (Gallardo et al., 2016). Between 1970 and 2012 IAS contributed, in-part, to population reductions of approximately 81%, recorded in vertebrate populations present in global freshwaters. By comparison, estimated declines of 36% and 38% were recorded for marine and terrestrial ecosystems, respectively (WWF, 2016). Most recently, population declines of freshwater vertebrates have been estimated at 84% (WWF, 2020). Within the freshwater environment, amphibians are particularly vulnerable to invasive alien species, which have contributed, in part, to the extinction of at least 10 amphibian species, and threaten at least 16% of extant amphibian species; of which 11% of species categorised as vulnerable, endangered, or critically endangered (Bellard et al., 2016b; Nunes et al., 2019; IUCN, 2020).

Crustaceans (Arthropoda: Crustacea) are a particularly problematic group of high-impact freshwater invaders, in terms of both their distribution and impact (Karatayev et al., 2009; Strayer, 2010), and are most frequently identified as having the greatest overall impact on amphibians (reviewed in Nunes et al., 2019). Invasive crustaceans are typically over-represented in many invaded regions, dominating native crustaceans in terms of diversity (Devin et al., 2005; Karatayev et al., 2009; Keller et al., 2009;

Strayer, 2010) and abundance (Josens et al., 2005; van Riel et al., 2006; van Riel, 2007; Arndt et al., 2009; Hansen et al., 2013). This is particularly apparent with respect to invasive decapods (Order: Decapoda), mysids (Order: Mysida) and also amphipods (Order: Amphipoda; Hänfling et al., 2011), of which a disproportionately high number of amphipod species – primarily gammarids (Family: Gammaridae) – originate from a single region, the Ponto-Caspian, and have largely invaded Eurasian waters (Cuthbert et al., 2020a). Of those freshwater invaders present in North America and Europe, crustaceans constitute approximately 38% and 53%, respectively. Conversely, native crustaceans only comprise approximately 7% of the native biodiversity present in North America, and 12% in Europe (Karatayev et al., 2009). In some regions, invasive crustaceans compose up to 90% of the total abundance of all macroinvertebrates (Arndt et al., 2009). In Europe alone, ten species of invasive freshwater crustaceans are regarded amongst the 100 worst invaders, including the red swamp crayfish (*Procambarus clarkii*), Chinese mitten crab (*Eriocher sinensis*), American signal crayfish (*Pacifastacus leniusculus*) and the killer shrimp (*Dikerogammarus villosus*; Nentwig et al., 2018). In this thesis, I will be focussing on invasive *D. villosus*.

1.3 How do invasive alien species impact native ecology?

Invasive alien species can detrimentally affect native communities through a variety of direct and indirect means, resulting in the extirpation of vulnerable native species and the subsequent taxonomic homogenisation of native biota (McKinney and Lockwood, 1999; Olden et al., 2004). Invasive alien species can directly impact native species through predation of incumbent species, competition over available resources (e.g. habitat or food) or through hybridisation with functionally similar natives. Invasive alien species can also affect native communities indirectly, through modification of habitat form or function (e.g. the water table), or through the transmission of parasites, pathogens and diseases (Manchester and Bullock, 2000; Olden et al., 2004; Lymbery et al., 2014). As part of this thesis, I will address how the invasive freshwater crustacean, *Dikerogammarus villosus* (see Section 1.4.1), can impact native species, directly through predation (Chapter 2, 3, and 4), and indirectly as a potential carrier of generalist pathogens (Chapter 5).

1.3.1 Impact of invasive alien species as predators

Predation plays an important role as a primary determinant of complex ecological communities (Messinger and Ostling, 2013); fundamental in regulating population dynamics (Belgrad and Griffen, 2016), shaping the structure of resident assemblages (Start and Gilbert, 2017), and dictating the functioning of natural ecosystems (Hawlena and Schmitz, 2010; Hammerschlag et al., 2019). Whilst predation is applicable when describing the consumption of faunal (i.e. carnivory) and floral (i.e. herbivory) prey, aspects of this thesis pertaining to the ecological impact of invasive predators (i.e. Chapter 2, 3, and 4), will focus exclusively on the consumption of animal prey.

Predators are categorised into two distinct classifications of feeding strategy; specialist and generalist (Smith et al., 2011). Specialist predators have evolved a narrow dietary niche, comprising of a low diversity of prey types, often larger than the predator themselves, whereas generalist predators exhibit a broader dietary niche, capable of consuming a diverse range of generally smaller prey types (Smith et al., 2011; Michálek et al., 2017). By extension, predatory generalism can also include omnivorous predators, which consume a diversity of prey types, across several trophic levels (Pimm and Lawton, 1978). Although dietarily restricted, specialist predators are typically more efficient in their ability to hunt prey, when compared to generalists, having developed highly specialised morphological and/or behavioural adaptations (Michálek et al., 2017). However, given that generalist predators readily capture most encountered prey types, spending a relatively short period of time handling and ingesting said prey, generalists characteristically expend less energy than specialists – which tend to invest more time and energy hunting for specific prey, and spend considerably longer time when handling and ingesting captured prey (Michálek et al., 2017). For example, comparisons between two species of predatory spiders, a specialist (*Nops* cf *variabilis*) and a generalist (*Harpactea rubicunda*), have shown *N. variabilis* to demonstrate active selectivity towards certain prey (alternative spider species), utilising a simple hunting strategy to efficiently capture and immobilise desired prey within a relatively short period of time, before spending a comparable greater period feeding. Conversely, the generalist *H. rubicunda* displays indiscriminate feeding across a range of invertebrate prey types, employing a more complicated sequence of hunting behaviours, before feeding for a far shorter period (García et al., 2018).

Whilst a diverse diet can be beneficial for generalists, allowing individuals to exploit a wide range of food types, energy acquisition (per individual prey consumed) can vary significantly when compared to specialists, which typically gain a relatively large amount of energy from each prey item consumed (Michálek et al., 2017). As such, generalist predators often engage in hunting activities more frequently (Pompozzi et al., 2019). This is particularly problematic with respect to invasive alien predators, which are typically characterised as generalist or, in the case of freshwater invaders, omnivorous predators (Park, 2004; Gallardo et al., 2016); although some successful invasive specialists have been reported (e.g. the vendace (*Coregonus albula*); Bøhn and Amundsen, 2001).

Within the context of biological invasions, predation is regarded as a primary mechanism through which established invaders can directly impact upon native communities (Moyle and Light, 1996). Indeed, invasive alien predators have been identified as a major driver in the decline of global biodiversity (Snyder and Evans, 2006; Doherty et al., 2015; Doherty et al., 2016a; Mollot et al., 2017). For example, invasive mammalian predators have been implicated in the extinction of approximately 58% of known vertebrate species, with invaders such as the feral cat (*Felis catus*) and the black rat (*Rattus rattus*) dramatically affecting birds, reptiles and other mammals; although this is likely to be an underestimation (Doherty et al., 2016a).

For amphibians, predation by aquatic invaders has been most frequently reported as contributing to global amphibian declines (reviewed in Nunes et al., 2019), with population declines predominantly attributed to the consumption of vulnerable embryos and larvae by invasive fish (e.g. rainbow trout – *Oncorynchus mykiss*), amphibians (e.g. American bullfrog – *Rana catesbeiana*), and crustaceans (e.g. red swamp crayfish – *Procambarus clarkii;* (Kats and Ferrer, 2003; Pilliod et al., 2012; Nunes et al., 2019). Invasive crustaceans, particularly crayfish, have been reported to have the greatest overall impact (Nunes et al., 2019); likely ascribed to high levels of aggression often displayed by invasive crustaceans (Gamradt et al., 1997; Weis, 2010), which has also contributed to the success and impact of these invasive aquatic predators (Holway and Suarez, 1999; Ricciardi and MacIsaac, 2010; Lodge et al., 2012).

For example, studies conducted in Sweden have reported a significant negative predatory effect by established populations of *P. leniusculus* towards the embryos and larvae of multiple native amphibian species (Axelsson et al., 1997; Nyström and Åbjörnsson, 2000; Nyström et al., 2001). These invasive crayfish were also found to affect the breeding behaviours of some adult species (Nyström et al., 2002). Similar studies concerning the predatory impact of invasive *P. clarkii*, established in regions of Spain and Portugal, report evidence of intense predation towards the embryos and larvae of numerous amphibian species (Cruz and Rebelo, 2005; Cruz et al., 2006a; Cruz et al., 2006b), resulting in the extirpation of several, previously abundant species (Rodríguez et al., 2005; Cruz et al., 2008). Indigenous to the Southern States of North America, the introduction of invasive *P. clarkii* into mountain streams of California has also led to considerable declines in the abundance and distribution of local California newts (*Taricha torosa*), resulting from excessive predation of early life stages (Gamradt and Kats, 1996), and deterred breeding within invaded systems (Gamradt et al., 1997).

The success and impact of invasive alien predators is often attributed to the naivety of native prey species which have no shared eco-evolutionary history with invaders, or with functionally similar congeneric native predators. Naïve native prey species often lack the ability to recognise invasive predators as a threat, or to express any effective anti-predatory responses; thereby making them more susceptible to predation by invaders (i.e. consumptive effect; Sih et al., 2010; Carthey and Banks, 2014; Hettyey et al., 2016). In some cases, native prey species may recognise the potential threat of invasive predators – if similar to native congeners – or may develop predator recognition and/or adaptive anti-predatory responses over multiple generations (Anton et al., 2020), limiting the direct consumptive effects imposed by novel predators (Sih et al., 2010). However, by adapting to the presence of invasive predators, whether through modifications to prey behaviour (e.g. activity levels), prey morphology (e.g. defensive spines), and/or prey life history (e.g. accelerated development), invaders can also impose a potentially substantial indirect effect on prey fitness (i.e. non-consumptive effect; Sih et al., 2010).

Native inhabitants of aquatic ecosystems frequently exhibit far greater naivety, and therefore vulnerability, to invasive alien predators, when compared to terrestrial systems (Cox and Lima, 2006; Anton et al., 2020). In freshwater ecosystems, increased naivety is primarily due to biogeographic isolation between habitats, as well as highly varied predation regimes; with freshwaters often containing only a few or no predators (Cox and Lima, 2006). Increased naivety is particularly apparent in larval amphibians (Kats and Ferrer, 2003; Nunes et al., 2019).

Within invaded communities, ecological impacts imposed by invasive alien species are often defined by differential resource use; a characteristic which has been reported in various invasive fauna (reviewed in Dick et al., 2014) and flora (see Funk and Vitousek, 2007; Heberling and Fridley, 2013). When compared to native species, invaders are typically capable of efficiently utilising local resources at higher rates, causing affected resource populations (e.g. prey) to experience potentially acute declines or extinctions (Dick et al., 2014). Indeed, differential resource use between native and invasive species has been identified as a fundamental principle of 28 out of 29 established invasion hypotheses (Catford et al., 2009). For example, in Bahamian reefs invaded by the Indo-Pacific red lionfish (*Pterois volitans*), populations of the native fairy basslet (*Gramma loreto*) have suffered significant declines when compared to populations present in reefs inhabited by native predators; with predation by *P. volitans* leading to declines of more than 50%, and also the extirpation of *G. loreto* populations from some invaded reefs (Ingeman, 2016).

As typical generalist predators, impacts imposed by invaders as a result of superior consumptive ability may be dictated by patterns of resource use (Shea and Chesson, 2002). Having evolved a wide dietary breadth, invaders can exert predatory pressures across multiple prey species. Whilst this may ultimately reduce invader impacts towards any given prey species, with predatory burdens distributed across multiple species, it might also intensify invader impacts, with populations of invasive predators able to sustain themselves when any given prey species becomes depleted or is lost (Shik and Dussutour, 2020). The ability for invaders to exploit a variety of resource types, often at a higher rate when compared to natives (e.g. Dick et al., 2014), can also provide invaders with a competitive advantage over functionally similar native consumers when accessing shared resources. As such, competition can often result in reductions in native prey and predator populations and ultimately, the potential extirpation of native predators. For example, in East African riverine systems colonised by populations of both invasive *P. clarkii* and functionally similar native crab species, increased predation of available prey by *P. clarkii* has led to significant reductions in the abundance and biodiversity of native macroinvertebrate communities and caused substantial declines in native crab abundance, subsequently resulting in the extirpation of native crabs from some regions (Jackson et al., 2016). In some instances, the displacement of native predators may be further exacerbated via the expression of intraguild predation by invaders; a specialised predatory response through which invaders actively target native competitors as a consumable prey resource (e.g. Dick, 2008).

Greater access to a wide range of available resources, coupled with the ability to exploit resources at a markedly higher rate, can lead to successful invasive alien species often establishing superabundant populations within invaded regions, further contributing to their ecological impact towards native communities (Ricciardi and MacIsaac, 2010; Dick et al., 2017; Blackburn et al., 2019; Dickey et al., 2020). For example, following the introduction of the invasive killer shrimp (*D. villosus*) into the River Rhine in 1995, this omnivorous invasive amphipod rapidly established highly abundant populations (~10,000 individuals/m^2), and significantly altered the composition of native macroinvertebrate assemblages through the opportunistic consumption of various available resource populations (van Riel et al., 2006; Koester et al., 2016). I will discuss the ecological impacts associated with spread of invasive *D. villosus* in later sections (see Section 1.4.1), outlining its importance as the focal study organism in this thesis.

Whilst there is strong empirical evidence concerning the detrimental effect that invasive alien predators can have upon the abundance and diversity of amphibians worldwide, it is important to note that there **is** an apparent bias in the published literature, with respect to the range of invasive predators assessed. In a recent systematic review conducted by Nunes et al. (2019), 110 published studies were collated, consisting of 1062 cases whereby native amphibians were negatively affected by invasive plants (n = 263 cases), mammals (n = 4 cases), reptiles (n = 54 cases), fish (n = 279 cases), amphibians (n = 144 cases), and invertebrates (n = 318 cases). Of the 318 cases pertaining to invasive invertebrates, 299 cases focused on only four species of invasive crayfish; *Astacus leptodactylus* (narrow-clawed crayfish), *Orconectes rusticus* (rusty crayfish), *P. clarkii* and *P. leniusculus*. *Procambarus clarkii* had the greatest representation, reported in 267 cases (89%), followed by *P. leniusculus* (n = 23 cases; 7.7%). Although this provides consistent evidence, identifying invasive *P. clarkii* as a major driver of global amphibian declines, it also highlights a considerable knowledge gap concerning alternative high-impact invasive freshwater crustaceans, for which it may still be undetermined as to whether they demonstrate the potential to impose equivalent predatory impacts upon native amphibians, or if they are simply under-represented in the literature (Nunes et al., 2019). This thesis aims to address the potential ecological impact imposed by *D. villosus*, a voracious, high-impact invasive predatory amphipod (see Section 1.4.1). In Chapters 2, 3, and 4, I will quantify the predatory impact of invasive *D. villosus* upon the early life-stages of UK amphibians, utilising predictive methodologies in order to ascertain potential ecological impact.

1.3.1.1 Quantifying the impact of invasive alien predators – functional response

Over the years there have been several attempts at developing a generalised framework for assessing the ecological impact of invasive alien species, particularly with respect to the mechanisms by which invaders might affect native communities and ecosystems through differential resource use (see Parker et al., 1999) Until relatively recently, studies assessing the ecological impact of invasive alien species have traditionally examined how the presence of an already established invader alters the composition and functioning of an invaded community, when compared to an uninvaded one; thereby deriving predictions concerning future impacts based solely on invasion history (e.g. Ricciardi, 2003; Kulhanek et al., 2011). Although effective when predicting future ecological impacts of particularly problematic, widespread, and historically well-documented invaders (e.g. *P. clarkii*; Nunes et al., 2019), this method fails somewhat when considering emerging invaders (e.g. round goby (*Neogobius melanostomus*); Gebauer et al., 2019), as well as those with limited impact histories (e.g. walking catfish (*Clarias batrachus*); Kulhanek et al., 2011). This method also does not allow for the testing of potential variations in the local ecology (e.g. extirpation of apex predator; reviewed in Ritchie and Johnson, 2009) or environment (e.g. pollution; Crooks et al., 2011). Alternatively, some studies have attempted to derive invader impacts using laboratory-based experimentation, providing prey – often at a fixed density or in excess – to native and invasive consumers, and comparing consumption rates (e.g. Renai and Gherardi, 2004; Rehage et al., 2005; Stoffels et al., 2011). However, by utilising such 'snapshot' experimental designs, these studies often fail to consider how predatory behaviours can change in response to varying resource availability, and as such, often cannot differentiate between native and invader consumption rates. As a result, these studies may fail to ascertain the true ecological impact of an invasive consumer (Figure 1.2; Dick et al., 2014).

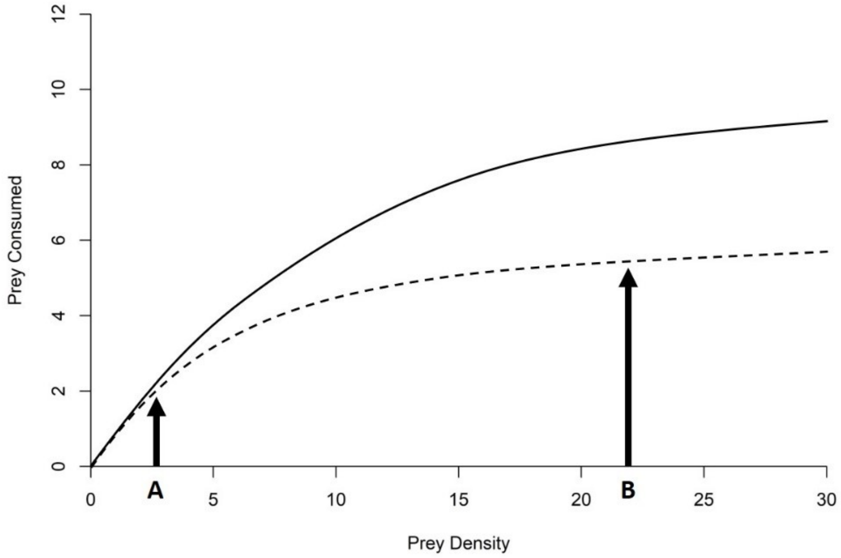

Figure 1.2. Hypothetical example in which a 'snapshot' study design can fail to distinguish between native (dashed line) and invasive (solid line) consumption rates, relative to prey availability. Arrows indicate points at which a fixed density might fail to discern differential feeding rates (arrow A), when compared to higher densities (arrow B). Adapted from examples provided in Dick et al. (2014).

In more recent years, alternative predictive methodologies have been suggested, one of which is the comparative functional response approach; first promoted by Dick et al. (2014) as a rapid, reliable, and cost-effective tool for predicting the ecological impact of biological invaders. Historically used as a means of quantifying the efficacy of non-native biological control agents (e.g. parasitoids; reviewed in Fernández-Arhex and Corley, 2003), Dick et al. (2014) determined that the application of functional response analyses, within the context of biological invasions, could fulfil the same purpose; with comparisons between the FRs of current and/or emerging invaders, and those recorded for trophically analogous natives, predicting the ecological impact of invasive alien consumers.

The functional response (FR) of a consumer (e.g. predator) describes the relationship between the rate at which a resource (e.g. prey) is consumed, *per capita*, and the availability (i.e. density) of said resource (Solomon, 1949; Holling, 1959a). With *per capita* consumption rates assumed to be a function of prey density alone, consumer FRs are considered "prey-dependent" (Arditi and Ginzburg, 2012). Consumer FRs are typically characterised as one of three classic FR types (Type I, II, and III), with each response type describing a different consumer-resource relationship, as reflected by the shape of resultant FR curves (Figure 1.3), and each having different implications for the stability of prey populations (Holling, 1959a; Hassell, 1978). A Type I FR describes a positive linear, density-independent relationship between *per capita* consumption rate and prey density, and is a phenomenon typically expressed by filter-feeding organisms (e.g. bivalves; Jeschke et al., 2004). A Type II FR is represented by a saturating hyperbolic curve and describes an inversely density-dependent relationship between consumption rate and prey density, with *per capita* consumption rates increasing at a continuously decelerating rate, establishing an asymptote at higher densities as consumption becomes limited by handling times (Holling, 1966). Type II FRs are often regarded as a potentially destabilising predator-prey dynamic, resulting from the near-complete, or total, consumption of prey at low densities (Hassell, 1978). A Type III FR, as represented by a sigmoidal curve, describes a density-dependent relationship between consumption rate and prey density, in which consumption is limited at lower prey densities, gradually accelerating as density increases before decelerating to an asymptote as consumption becomes limited by handling times (Holling, 1966). Unlike a Type II FR, Type III FRs are considered more stabilising, with limited mortality risk at lower densities providing prey with a low-density refuge (Murdoch and Oaten, 1975).

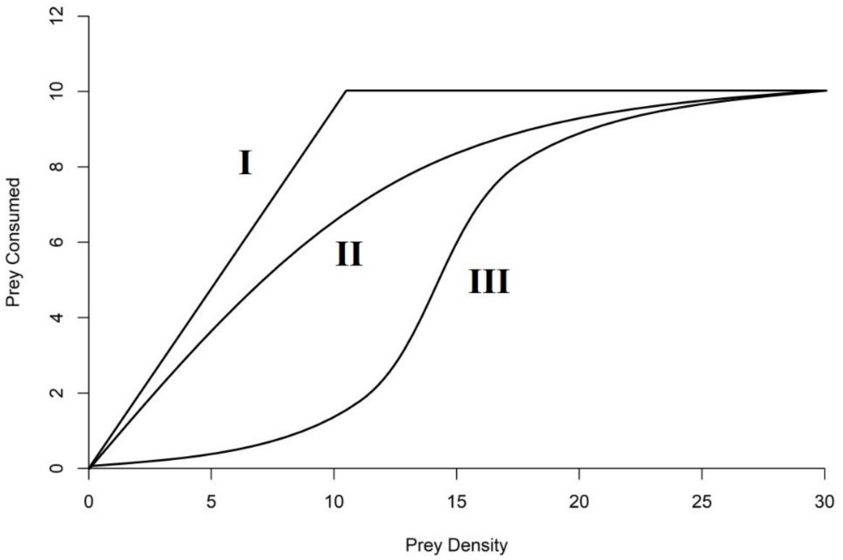

Figure 1.3. Three classic functional response forms; Type I (linear), Type II (saturating hyperbolic) and Type III (sigmoidal).

Whilst the shape of consumer FR curves provides phenomenological insights into the type and stability of consumer-resource interactions, the projected magnitude of these curves can highlight their strength, as determined by mechanistically quantified, biological parameters such as the attack rate (a) and handling time (h; Jeschke et al., 2002; Pritchard et al., 2017). The attack rate, also known as attack coefficient, describes the efficiency by which a predator searches, locates and attacks prey, and corresponds to the initial slope of resultant FR curves. Predators which demonstrate particularly high attack rates may potentially destabilise prey populations at lower densities. Conversely, the handling time describes the time taken to capture, restrain, ingest and digest individual prey (Jeschke et al., 2002), and can be reciprocated to derive inferential maximum feeding rates ($1/h$) – corresponding to the asymptote of resultant FR curves (Dick et al., 2017). By comparing the *per capita* effects of native and invasive consumers towards focal resource populations, it is possible to determine the potential ecological impact of invasive alien species (Parker et al., 1999).

Within the field of invasion ecology, there is a growing body of literature in which FR analyses have been conducted when assessing the ecological impact of invasive alien species. At the time of writing, a total of 93 published studies can be found (see Appendix 1, Table A1.1), in which FR analyses were applied in order to evaluate the *per capita* impact of an invasive consumer (n = 73 studies), or as a means of quantifying the efficacy of current and/or potential biological control agents in managing invasive prey species (n = 20 studies). For a number of studies, FRs have been examined under different abiotic and/or biotic context-dependencies, including climate change (e.g. Pellan et al., 2016), habitat complexity (e.g. Barrios-O'Neill et al., 2015), the presence of other predators (e.g. Barrios-O'Neill et al., 2014a), and the presence of parasites (e.g. Iltis et al., 2018). In ~77% of studies, FR analyses have been used whilst assessing the impact of invasive freshwater species, with crustaceans – particularly amphipods – being the most represented taxon. Across all available literature, FR analyses have frequently revealed the expression of Type II FRs by invasive alien predators, indicating the potential for invaders to destabilise affected prey populations. For those studies which utilised the CFR approach (n = 40 studies), invaders characteristically displayed either higher magnitude FRs – demonstrating markedly higher attack rates (i.e. steep initial slope) and/or asymptotic maximum feeding rates – or a more destabilising FR type (Type II instead of Type III) when compared to trophically similar native analogues, signifying a greater predicted ecological impact towards local prey populations (Dick et al., 2014). If an invasive alien species is functionally novel within an invaded range, then the expression of a superior FR, when compared to native consumers with which an affected prey population shares an eco-evolutionary experience, may explain and predict a substantial invader impact (Dick et al., 2014).

Under the influence of different context-dependencies, studies showed that the magnitude and/or form of native and invader FRs can become modified, either intensifying (e.g. Dick et al., 2010; Oyugi et al., 2012; Pellan et al., 2016) or reducing (e.g. Haddaway et al., 2012; Barrios-O'Neill et al., 2015; Laverty et al., 2015a) predicted ecological impact. In some cases, predictions of potentially greater invader impacts, as suggested by superior Type II FRs when compared to native consumers, were consistent with observed field impacts, providing support for the predictive power of the FR approach. For example, the invasive bloody red shrimp (*Hemimysis anomala*) has been shown to express a markedly higher Type II FR towards native cladocerans, when compared to native mysids. Following the introduction of *H. anomala* into Europe, native cladocerans became practically extinct (Dick et al., 2013). In Chapter 2 I use phenomenological and mechanistic FR methodologies to quantify the predatory impact of invasive *D. villosus* towards the vulnerable early life-stages of several UK amphibians. Using the CFR approach, I compare predatory FRs of invasive *D. villosus* and the native amphipod comparator *Gammarus pulex*, thereby deriving predictions concerning the potential ecological impact of this invasive freshwater amphipod.

In natural ecological field systems, predators rarely forage alone. Instead, predators typically forage upon resource populations that are shared by multiple predators. These resources may be shared across multiple predator species (e.g. aphids and aphidophagous hoverflies, ladybirds and midges; Hindayana et al., 2001), or amongst individuals of the same species (e.g. elk and wolves; Hebblewhite, 2013); although this is often dictated by the spatial scale of the natural system and the distribution of these resource populations throughout the foraging landscape (Arditi and Ginzburg, 2012; Northfield et al., 2017). When accessing these shared resources, predators will often interact with neighbouring predators, interfering with their foraging behaviours. Interference caused by these interactions invariably alters the foraging efficiencies of those individuals involved, with predators allocating a greater proportion of time to interacting with other predators; time which would otherwise be allocated to foraging for prey (Arditi and Ginzburg, 2012; Médoc et al., 2013). Depending on the nature of these interactions (competition, mutualism, commensalism, amensalism, etc.) and their intensity, as determined by the relative abundance of available resources, interactions amongst predators can either enhance *per capita* feeding through mutual facilitation/cooperation – as observed between owls and snakes whilst hunting for small mammalian prey (Embar et al., 2014) – or depress *per capita* feeding through competition – as observed between free-ranging domestic dogs and native foxes (Vanak et al., 2009).

Advancements to predation theory has seen the development of alternative FR model classes, incorporating conspecific interference, commonly referred to as "mutual interference", as an additional parameter when quantifying the *per capita* effect of a consumer. Unlike classic FR models, which consider *per capita* consumption rates to be "prey-dependent" (i.e. a function of prey density alone), these alternative models consider *per capita* consumption to be a function of both prey and predator density; a phenomenon known as "predator-dependence" (Arditi and Ginzburg, 1989, 2012). By incorporating mutual interference (c or m) as a quantifiable parameter, predator-dependent models enable consumer FRs to become adaptable, transitioning between prey-dependence and predator-dependence depending on the intensity of interference amongst interacting conspecifics (Figure 1.4; Arditi and Ginzburg, 2012). In the absence of interference ($c = 0$), predators are assumed to exhibit prey-dependent FRs. If interference does occur between conspecific predators ($c > 0$), predators are likely to display predator-dependent FRs. However, predator-dependence can develop further to include a modified type of predator-dependence, known as "ratio-dependence"; whereby *per capita* consumption is considered as a function of *per capita* prey availability, or rather the prey-to-predator ratio. Ratio-dependence is regarded as a special case of predator-dependence through which mutual interference is implicit ($c = 1$), developing as a result of perfect prey sharing (Arditi and Ginzburg, 1989, 2012). Indeed, ratio-dependence has been suggested as the "null model of predation" (Arditi and Ginzburg, 2012).

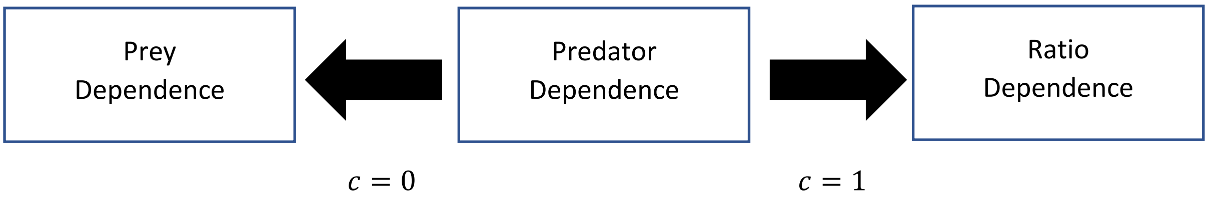

Figure 1.4. Example illustrating the potential for predatory functional responses to transition between prey-, predator, and ratio-dependence, depending on the intensity of mutual interference amongst conspecific predators.

The expression of predator-dependent and/or ratio-dependent *per capita* consumer effects, resulting from the emergence of mutual interference amongst conspecific predators, has been studied across all ecosystem types, examined in aquatic invertebrates (e.g. Kratina et al., 2009; Médoc et al., 2013, 2015; Prokopenko et al., 2017), terrestrial invertebrates (e.g. Arditi and Ginzburg, 1989; Spataro et al., 2012), and large terrestrial mammals (e.g. Vucetich et al., 2002; Jost et al., 2005; Hebblewhite, 2013; Zimmermann et al., 2015). By accounting for the density-dependent effects of predator abundance, relative to the availability of prey resources, both laboratory- and field-based studies have typically identified predator-dependence, or ratio-dependence, as the most common FR class (reviewed in Arditi and Ginzburg, 2012), with predator density, and the associated emergence of mutual interference, often found to have an overall negative effect on *per capita* consumption, causing predator-prey dynamics to become more stable (Arditi et al., 2004).

Given that invasive alien species typically exist at high densities within invaded communities (e.g. van Riel et al., 2006; Hansen et al., 2013), interactions amongst conspecific invaders are likely to occur, modifying *per capita* consumption rates, and thus predictions concerning potential ecological impact. However, there are very few studies to-date which consider the potential effects of predator-predator interactions, whilst examining invader impacts; although alternative metrics have been developed which consider predator density when predicting the relative impact of invaders (see Section 1.3.1.3). In Chapter 3 I explore how interactions amongst invasive *D. villosus* can influence *per capita* impacts towards larval amphibians and examine one of the underlying mechanisms by which mutual interference may emerge – namely predatory spatial dynamics.

As typical generalist predators, the ability to select from, and consume a wide diversity of resident prey species can contribute to the success and impact of many invasive alien species; with invaders possessing a broad dietary niche able to impose predatory pressures across multiple prey species (Snyder and Evans, 2006; Courant et al., 2017; Shik and Dussutour, 2020). However, by exploiting multiple prey types, the *per capita* consumptive effects imposed by generalist invaders towards any given prey species, may be expected to become reduced, with some invaders feeding indiscriminately across available prey species – if prey are of a similar nutritional and/or energetic quality, or available at similar relative abundances (e.g. Haddaway et al., 2012; Dodd et al., 2014) – or switching from less abundant prey species to more abundant, yet often nutritionally poorer, prey (Murdoch, 1969; Shik and Dussutour, 2020).

Prey-switching, as a form of frequency-dependent predation, is regarded as a fundamental factor of functional response theory, predicted to cause consumers to express a Type III FR towards numerically rare prey types, thereby conveying a more stabilising effect on affected prey populations, particularly within diverse communities (Murdoch, 1969; Oaten and Murdoch, 1975; Hanski et al., 2001; van Leeuwen et al., 2013). Indeed, the potential for consumers to actively and/or passively switch between different prey species has been incorporated into classic FR methodologies, resulting in the development of alternative multiple prey species functional response models (MSFRs); with switching considered implicitly as a function of relative abundance (Oaten and Murdoch, 1975; Chesson, 1983; van Leeuwen et al., 2007), or as a quantifiable parameter as derived based on consumer preference (van Leeuwen et al., 2013; Morozov and Petrovskii, 2013; Baudrot et al., 2016).

Multiple prey species functional response methodologies have been used in several studies whilst assessing the *per capita* effects of terrestrial (e.g. canids; Baudrot et al., 2016), and marine (e.g. cetaceans; Smout, 2007; Smout et al., 2010) generalist predators. However, to-date, the use of MSFR methodologies, within the context of biological invasions, is scarce; although alternative approaches examining how the relative abundance of alternative prey can affect *per capita* impacts have been used (Cuthbert et al., 2018a; Cuthbert et al., 2018b; Taylor and Dunn, 2018; Joyce et al., 2019). Instead, studies have often examined the implications of multi-prey systems, by presenting invaders with alternative prey species at a fixed density (e.g. Médoc et al., 2018), or at equal abundance (e.g. Dodd et al., 2014) or biomass (e.g. Haddaway et al., 2012; Taylor and Dunn, 2017). Whilst some invasive alien predators have been shown to adopt an indiscriminate feeding pattern, when presented with multiple prey species (e.g. Haddaway et al., 2012; Dodd et al., 2014; Rosewarne et al., 2016), in some cases invaders have also demonstrated an active selectivity for preferred prey types – a foraging strategy often determined by the relative ease by which certain prey are captured, as well as their relative nutritional and/or energetic quality (Eubanks and Denno, 2000; Taylor and Dunn, 2017). Interestingly, when presented with alternative prey at differential relative abundances, invaders can demonstrate an alternative form of prey-switching, negative prey-switching, which is indicative of strong, persistent predatory pressures being applied towards a given prey type, regardless of relative abundance, thereby imposing far higher *per capita* effects (van Leeuwen et al., 2007; Taylor and Dunn, 2018). In Chapter 4, I investigate how the presence of alternative prey species might affect the *per capita* effect of invasive *D. villosus* towards the early life-stages of UK amphibians. I use MSFR methodologies in order to detect, quantify and examine possible prey-switching behaviours, and determine how potential prey-switching might influence ecological predictions.

1.3.1.2 Quantifying the impact of invasive alien predators – relative impact potential (RIP)

To adequately predict the absolute potential for an invasive alien species to negatively impact native species, classic ecological theory deems it necessary to not only consider the consumer functional response (FR), but also the numerical response (NR), which describes the consumer population response to changes in prey density, via changes in spatial arrangement (i.e. aggregation) or changes to reproduction rate (Solomon, 1949; Holling, 1959a, 1959b). When considered concurrently, the total response (TR) of a consumer is derived, whereby:

$$TR = FR\,x\,NR$$

However, quantifying NRs is typically more challenging, given the ambiguous nature of population dynamics (e.g. time lags; Dick et al., 2017; Dickey et al., 2020). Instead, it has been suggested that alternative proxies for NR, such as consumer abundance (AB), can be utilised, allowing a modified version of TR to be derived – known as the Impact Potential (IP; Dick et al., 2017; Dickey et al., 2020), for which:

$$IP = FR\ x\ AB$$

IP, as an absolute metric, fails to assess an invader's potential impact towards a prey population, given the lack of comparison with a native analogue, with which the affected prey population shares an eco-evolutionary history. However, IP can be further modified, allowing effective assessment of the potential impact of an invasive consumer's population, relative to the existing baseline impacts of a trophically analogous, co-evolved native consumer population. Therefore, invader IP becomes relative to native IP, generating the Relative Impact Potential (RIP):

$$RIP = \left(\frac{Invader\ FR}{Native\ FR}\right)\ x\ \left(\frac{Invader\ AB}{Native\ AB}\right)$$

When RIP = 1, invader and native impact potentials are expected to be equal, whilst RIP < 1 predicts the potential impact of native consumers to exceed invader impact. Conversely, when RIP > 1, this is indicative that an invasive consumer's impact potential is likely to be greater than that of a native congener. As such, the RIP metric can effectively highlight present or emerging invasive species which demonstrate the potential to impose a high ecological impact when compared to natives, such as invaders which possess a higher relative abundance, a higher relative *per capita* consumption rate, or both (Figure 1.5; Dick et al., 2017; Dickey et al., 2020).

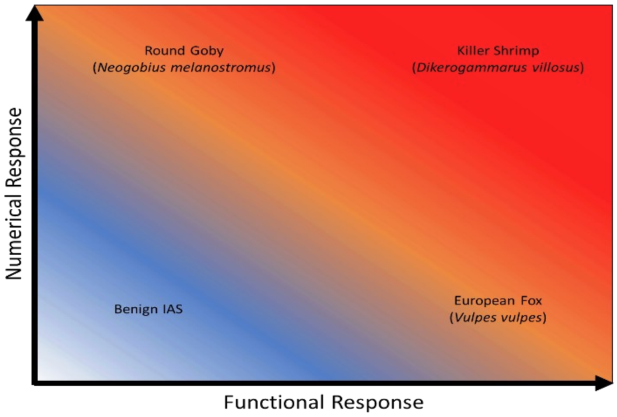

Figure 1.5. Heat map illustrating different examples of high-impact invasive alien species, depending on differential estimations of functional and numerical responses, when compared to native species. Ecological impact increases towards the top right. Adapted from Dickey et al. (2020).

One assumption of the RIP approach is the complete replacement of the native comparator species by an invader (Dick et al., 2017), as observed following the introduction of *Gammarus pulex* into Irish waters inhabited by native *Gammarus duebeni celticus* (Kelly et al., 2006). However, following the arrival of an invasive species, there is often a lag period before invaders impose any ecological impacts upon recipient populations and/or communities (Coutts et al., 2018), with a further delay prior to the partial (or complete) replacement of native species. As such, the relative total potential impact of an invasive alien species is expected to fluctuate over the course of the invasion process (i.e. pre-arrival → arrival → replacement → spread; reviewed in Dickey et al., 2020). Prior to the arrival of an invasive species, predatory impact is baseline, dictated solely by native predators. Following the arrival of an invasive species into a region, total potential impact is predicted to temporarily increase, caused by the combined effect of both invasive and native predator populations, before decreasing as the invader proceeds to replace native consumers. Once the invader is established, and the replacement of native consumers is complete, total potential impact is then driven solely by the invasive population, with the magnitude of invader impact determined by their foraging efficiency. The invasive species may subsequently spread, elevating total potential impact as invaders propagate throughout a region (reviewed in Dickey et al., 2020).

Following the inception of the RIP metric by Dick et al. (2017), it has been used to assess a number of invasive alien species, highlighting markedly greater overall potential impact by invasive reptiles (Dickey et al., 2018), molluscs (Kemp et al., 2018; Joyce et al., 2019) and fish (Faria et al., 2019; Mofu et al., 2019a). In Chapter 2 I utilise the RIP metric to predict the potential overall impact of invasive *D. villosus* towards the early life-stages of UK amphibians, when compared to native *G. pulex*. I incorporate estimates of native and invasive FRs alongside estimates of native and invasive amphipod abundance, recorded in several field sites in the UK.

1.3.2 Impact of invasive alien species as carriers of parasites, pathogens and emerging infectious diseases

Parasites – a term used herein to describe macro- and micro-parasites, as well as pathogenic agents – can often play a significant role in the ecological impact of IAS; contributing to the successful introduction, establishment, and subsequent expansion of invaders, as well as contributing directly and/or indirectly to the substantial ecological pressures typically imposed by invaders post-establishment (Dunn et al., 2012; Lymbery et al., 2014; Dunn and Hatcher, 2015; Blackburn and Ewen, 2017). As a result, biological invasions can lead to changes in the distribution of parasites, as well as the emergence of novel host-parasite interactions (Tompkins et al., 2011); however, parasites are rarely considered when examining the distribution and/or impact of IAS, and as such are grossly under-represented in the literature (Poulin, 2017). In Chapter 5, I investigate a potential host-parasite dynamic between invasive *D. villosus* and an invasive aquatic fungal parasite and consider the potential implications of this dynamic for the future conservation of amphibians.

Following the introduction of IAS into a novel region, the prevalence, diversity and abundance of parasites in an invasive population is often lower when compared to conspecific populations, existing within their native range (Torchin and Mitchell, 2004), but also when compared to native host species. present within the invaded range (Dunn and Dick, 1998; Torchin et al., 2005). Parasites may be lost as a result of sub-sampling of host organisms from within their native range, and through selective pressures experienced by hosts and/or parasites during the invasion process (Tompkins et al., 2011; Dunn and Hatcher, 2015). Known as enemy release, a reduced parasitic presence in invasive populations may provide invaders with a competitive fitness advantage over native host species, thereby facilitating their establishment within invaded communities (Mitchell and Power, 2003; Colautti et al., 2004; Torchin and Mitchell, 2004; Tuttle et al., 2017). For example, having lost a significant proportion of its native metazoan parasites during its introduction into regions of Australasia, the invasive green crab (*Carcinus maenas*) has demonstrated increased performance and fitness, when compared to populations of *C. maenas* present within its native European range (Torchin et al., 2001).

However, over time invaders may acquire parasites native to the invaded region, which in turn may cause the rate of infections amongst native hosts to subsequently increase (spillback) or decrease (dilution), depending on the competency of invasive hosts (Dunn and Hatcher, 2015; Young et al., 2017). For example, in Australia the invasive Cane toad (*Rhinella marina*) has played an important spillback role in facilitating the dispersal and transmission of myxosporean parasites in two native bell frog species (*Litoria aurea* and *L. raniformis*), contributing to their decline in the wild (Hartigan et al., 2011). In Ireland, acquisition of the acanthocephalan parasite *Pomphorhynchus tereticollis* by invasive dace (*Leuciscus leuciscus*) may reduce the prevalence and intensity of helminth infections in native brown trout (*Salmo trutta*), with the invader acting as a potential sink for infective life stages (Tierney et al., 2020).

Conversely, IAS may introduce novel parasites into invaded regions, having previously acquired them in their native range, or whilst inhabiting a previously invaded region (Chalkowski et al., 2018). Once introduced, these parasites may be transmitted to native host species, resulting in the emergence of new diseases amongst natives (spillover or pathogen pollution; Daszak et al., 2000; Taraschewski, 2006; Roy et al., 2017). Within the invaded range, native host species may be more susceptible to these novel parasites as they often have no shared co-evolutionary history and may therefore experience dramatic population declines (Prenter et al., 2004; Taraschewski, 2006; Dunn, 2009). For example, the squirrel parapoxvirus (Chordopoxviridae), introduced into the UK alongside the invasive North American grey squirrel (*Sciurus carolinensis*), has caused significant mortality in native red squirrel populations (*Sciurus vulgaris*), facilitating the replacement of native *S. vulgaris* by invasive *S. carolinensis* throughout much of the UK (Tompkins et al., 2003; Rushton et al., 2006). Another example, and perhaps one of the most well-known examples of parasite-invader spread, is the role that the North American bullfrog (*Rana catesbeiana*) has had in facilitating the spread of the aquatic chytrid fungus (*Batrachochytrium dendrobatidis*) – a highly virulent fungal parasite responsible for causing significant declines in amphibian populations worldwide (Garner et al., 2006; Fisher and Garner, 2007).

1.3.2.1 The Amphibian Chytrid Fungus – *Batrachochytrium dendrobatidis*

Batrachochytrium dendrobatidis (*Bd*) is a non-hyphal, non-mycelial aquatic Chytridiomycete fungus, belonging to the Chytridiomycota phylum; a primitive phylum of 'lower fungi', comprising of approximately 1000 described chytrid species (James et al., 2006a; James et al., 2006b). Most chytrid fungi typically reside in moist soil or freshwaters, as saprobes (i.e. feed on decaying organic matter), or parasites of plants, algae or invertebrates (Longcore and Simmons, 2020). However, *Bd* is distinct from most chytrids, as one of only two members of the Rhizophydiales order to have recently evolved, capable of parasitising vertebrate host organisms – the other being *Batrachochytrium salamandrivorans* (Longcore et al., 1999; Martel et al., 2013).

Batrachochytrium dendrobatidis was originally discovered in 1998, and later named in 1999 (Longcore et al., 1999), following sudden mass die-off events in regions of Central America and Australia, resulting in the extirpation of many affected amphibian species (Berger et al., 1998). Since then, extensive research into the impact and distribution of *Bd* has shown it to be highly transmissible, capable of infecting more than 1000 amphibian species, including at least 600 anurans (i.e. frogs and toads), 90 urodeles (i.e. newts and salamanders), and 10 caecilians (i.e. slow-worms); thus earning it the moniker of the "amphibian chytrid fungus" (Olson and Ronnenberg, 2014; Fisher and Garner, 2020). For those amphibians susceptible to *Bd* infections, for example bufonids (true toads), ranids (true frogs) and hylids (tree frogs), infections are often highly virulent and pathogenic, rapidly developing into chytridiomycosis, a cutaneous disease – often fatal to amphibian hosts – which causes significant population declines, and in some cases, extinctions (Olson et al., 2013; reviewed in Van Rooij et al., 2015).

Batrachochytrium dendrobatidis typically has an entirely asexual life cycle, although evidence of a possible sexual phase has previously been reported (see Morgan et al., 2007). The *Bd* life cycle comprises of two primary life stages; i) a motile, free-living zoospore – spherical or ovate in shape with a posterior directed flagellum – which characterises the infective stage of the *Bd* life cycle; and ii) a maturing, encysted, reproductively active thallus – termed zoosporangium – which characteristically develops on or in the outer epidermal layers of amphibian hosts, and generates new, asexual zoospores (Longcore et al., 1999; Berger et al., 2005a).

Motile *Bd* zoospores, free-living in freshwater ponds and streams, are chemotactically attracted to the keratinised epidermal regions of aquatic amphibian hosts, in response to sugars, proteins and/or lipids present on the surface of the skin, or in the skin mucus (Moss et al., 2008; Van Rooij et al., 2015). Zoospores subsequently adhere to the outer keratinised surface and encysts, absorbing the flagellum and developing a thick cell-wall (Berger et al., 2005a). It is unknown as to the exact mechanism by which *Bd* zoospores adhere to host epidermis, although it may be attributable to the expression of specific adhesion genes (Rosenblum et al., 2012a), or the synthesis of substrate-binding modular surface proteins (Abramyan and Stajich, 2012). Depending on the susceptibility of amphibian hosts to *Bd* infections – as dictated by their ability to generate an immune responses (reviewed in Van Rooij et al., 2015; Eskew et al., 2018), but also determined by factors such as host age (e.g. Bradley, P. et al., 2019a), life-history stage (e.g. Ortiz-Santaliestra et al., 2013), composition of commensal cutaneous microbiome (e.g. Harris et al., 2009; Bates et al., 2018), and several environmental factors (e.g. temperature, altitude, etc.; Sapsford et al., 2013; Bradley, P. et al., 2019b) – infections by *Bd* can progress along two different potential routes; endobiotically in *Bd*-susceptible hosts (Figure 1.6), or epibiotically in *Bd*-resistant hosts (Figure 1.7; Van Rooij et al., 2015).

In those amphibian species susceptible to infections, the *Bd* infection cycle often progresses endobiotically, proceeding with the germination of encysted zoospores and the development of a germ tube, which penetrates the superficial epidermal layer (i.e. *stratum corneum*) and extends into host epidermal cells (Figure 1.6; Berger et al., 2005a; Van Rooij et al., 2015). From the tip of this germ tube, a novel intracellular thallus (i.e. zoosporangium) develops, into which fungal genetic materials are transplanted. The donor thallus recedes, and this intracellular process continues, propagating immature zoosporangia deeper into epidermal tissues (e.g. *stratum granulosum*). These maturing zoosporangia subsequently migrate back to the superficial *stratum corneum* via differentiating epidermal cells, whereupon they develop a discharge tubule, subsequently releasing new clonal zoospores into the aquatic environment. These zoospores may then either re-infect host tissues or infect a new amphibian host (Berger et al., 2005a; Van Rooij et al., 2015). Transmission amongst amphibian hosts may also occur through direct contact, for example during amplexus (i.e. mating), or territorial confrontations (Rowley and Alford, 2007). Colonisation of amphibian host skin by *Bd* is typically limited to those regions in which the outer epidermal layers are keratinised (Daszak et al., 1999; Olsen et al., 2004; Van Rooij et al., 2015). Whilst this constitutes a relatively large area of adult host skin, it also means that *Bd* can effectively colonise keratinised regions of some larval amphibians (e.g. anurans). Colonisation of larval structures is typically localised to the mouthparts only – the only structures to undergo keratinisation in early-stage larvae – until metamorphosis is achieved, at which point other regions become keratinised (e.g. hindlimbs), and thus infectable (Marantelli et al., 2004; McMahon and Rohr, 2015).

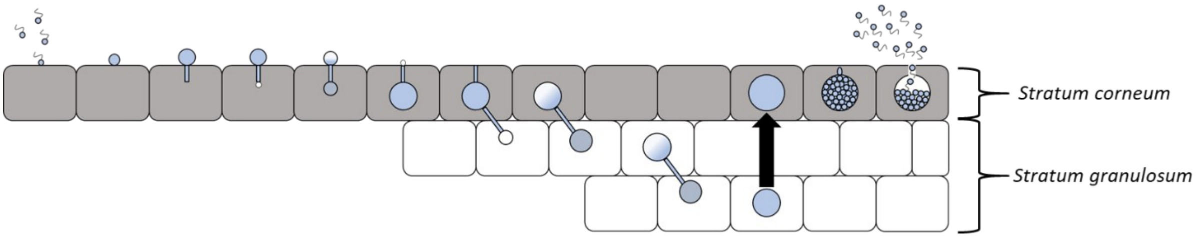

Figure 1.6. The putative endobiotic infection cycle of *Batrachochytrium dendrobatidis* within the superficial keratinised epidermal layers of amphibian host epidermis. Adapted from Van Rooij et al. (2015).

By comparison, for those amphibians which are comparably more resilient/tolerant of *Bd*, for example the African long-clawed frog (*Xenopus laevis*), infections typically develop epibiotically (Figure 1.7; Van Rooij et al., 2012, 2015). Like the endobiotic infection cycle, zoospores become encysted on the surface of amphibian host tissues and develop a germ tube, which penetrates into the epidermal cells of the *stratum corneum*. However, rather than transmitting fungal genetic materials, as observed in endobiotic *Bd* infection cycle, this germ tube develops into an increasingly more complex intracellular network of filamentous rhizoids. These rhizoids digest the cytoplasm of host epidermal cells, utilising the acquired nutrients to fuel the development of the externalised zoosporangium, and the asexual development of novel zoospores. Subsequently, the zoosporangium ruptures, discharging the clonal zoospores back into the aquatic environment (Van Rooij et al., 2012, 2015). For those amphibians in which *Bd*-infections are epibiotic (e.g. *X. laevis*), infections are often mild, with hosts exhibiting no obvious clinical pathologies nor experiencing die-offs attributed to infections (Weldon et al., 2004; Ramsey et al., 2010).

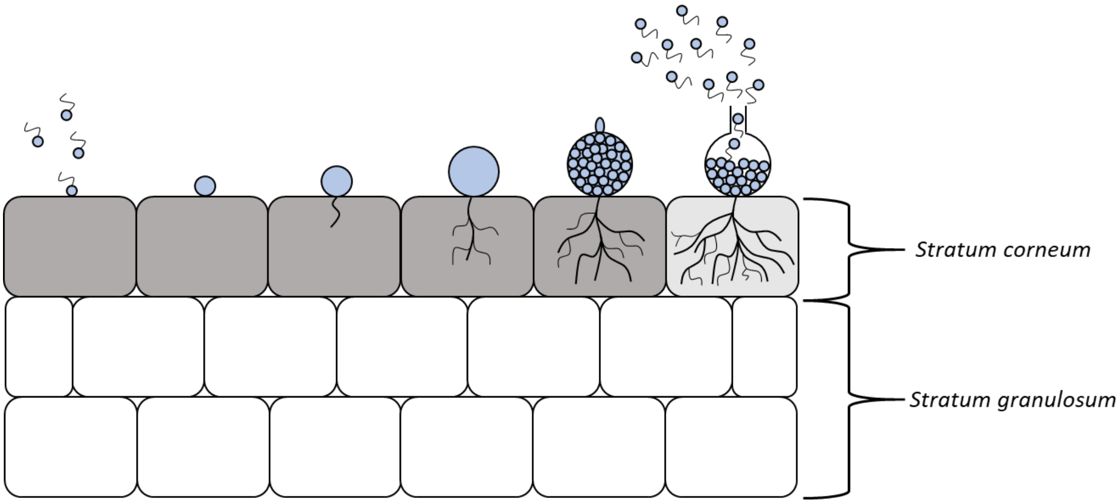

Figure 1.7. The epibiotic infection cycle of *Batrachochytrium dendrobatidis*, as observed in some *Bd*-tolerant amphibian species. Adapted from Van Rooij et al. (2015).

For those amphibians in which chytridiomycosis develops as a result of endobiotic infections, subsequent pathological developments are typically localised to the skin. Characteristically pathologies may develop as mild-to-severe hyperkeratosis (i.e. irregular skin thickening) occurring in the *stratum corneum* and *stratum granulosum*, hyperplasia (i.e. increased skin growth) of the *stratum spinosum* (situated beneath the *stratum granulosum*), occasional ulcerations of the skin, spongiosis (i.e. intercellular oedema), necrosis of epidermal tissues and vacuolation of deeper cell layers (Nichols et al., 2001; Berger et al., 2005a; Berger et al., 2005b). As the disease progresses, infections by *Bd* may lead to increased rates of skin sloughing – an immune response typically associated with the replacement of damaged or old skin, by newly synthesised skin. In some cases, skin sloughing has been shown to reduce, and sometimes clear, infections of *Bd* within some amphibian host species (Ohmer et al., 2015; Ohmer et al., 2017). However, increased skin sloughing can inadvertently exacerbate infections, causing greater cutaneous water loss (Russo et al., 2018), but also a drastic decline in the quality and functionality of renewed skin, as a result of *Bd*-related suppression of genes associated with the production of integral epidermal components, such as keratin, collagen, and fibrinogen (Rosenblum et al., 2012b). In larval amphibians, infections of developing mouthparts often fail to induce pathologies, except perhaps for minor hyperkeratosis (Berger et al., 1998; Marantelli et al., 2004), and/or the depigmentation of mouthparts (Knapp and Morgan, 2006). Typically, *Bd*-induced pathologies, and associated mortalities occur post-metamorphosis (Marantelli et al., 2004); although mortality can occur in some larval species (e.g. Blaustein et al., 2005).

Depending on the intensity of infections, malformations of the amphibian host integument can cause significant disruptions to vital cutaneous functioning. Despite having lungs, the skin of metamorphosed amphibians also fulfils important respiratory processes, with cutaneous respiration contributing to the exchange of up to 95% of respiratory gases (i.e. oxygen and carbon dioxide), especially within the aquatic environment (Tattersall, 2007; Vitt and Caldwell, 2009). The skin also plays a crucial role in osmoregulation, which in turn regulates electrolyte transport (Rick et al., 1980; Larsen and Ramløv, 2013). However, disruption of the cutaneous surface, resulting from the development of *Bd*-mediated pathologies, can impair these vital homeostatic processes, causing significant cutaneous water loss, severe electrolyte imbalances, hypovolemia (i.e. low blood volume) – ultimately leading to cardiac arrest and the death of heavily infected amphibian hosts (Voyles et al., 2009; Campbell et al., 2012).

In a recent meta-analytical study *Bd* was reported to have directly contributed to the declines of 501 amphibian species (6.5% of all amphibian species), an estimate based on published literature, published data and expert opinion spanning back approximately 40 years – to before the identification of *Bd* as a pathogenic agent (Scheele et al., 2019). Of those 501 amphibian species, 124 species were found to have experienced extreme declines (>90% decline in global abundance), and a further 90 presumed or confirmed to be extinct in the wild, including the Chilean Darwin's frog (*Rhinoderma* *rufum* – last seen 1980's), the Australian sharp snouted day frog (*Taudactylus acutirostris* – last seen 1997) and the Kihansi spray toad (*Nectophrynoides asperginis* – last seen 2000's; Scheele et al., 2019). However, this study was subsequently questioned over claims of insufficient evidence (Lambert et al., 2020; but see Scheele et al., 2020). Nevertheless, *Bd* is regarded as one, if not the greatest driver of global amphibian declines in current history and is a source of major conservation concern (Skerratt et al., 2007; Bellard et al., 2016b; Fisher and Garner, 2020).

Pinpointing the exact origin of the *Bd* fungus has been a point of debate over the past two decades, with discussions surrounding two conflicting theories of emergence; 1) the 'novel pathogen hypothesis' – which predicts that *Bd* emerged locally, following its introduction through global trade, and 2) the 'endemic pathogen hypothesis' – which predicts that *Bd* is a endemic widespread commensal of native amphibians, which developed virulence through global change (Skerratt et al., 2007; Fisher and Garner, 2020). With five major genetic *Bd* lineages, each of varying virulence, discovered to-date – including a globally present lineage associated with mass mortality of global amphibian populations (global pandemic lineage; GPL), and four regionally endemic lineages (*Bd*CAPE – South Africa, *Bd*ASIA-1 – South Korea, *Bd*Brazil/ASIA-2 – South America and *Bd*ASIA-3 – East Asia) – in addition to several hybridised and/or recombinant sub-lineages, there is evidence for both arguments (reviewed in Fisher and Garner, 2020).

Historically, *Bd* was thought to have originated from Africa, following the detection of chytrid DNA in tissue samples collected in 1938 (Weldon et al., 2004). Since then, several alternative geographic sites of origin have been subsequently proposed, including Japan (Goka et al., 2009), East Asia (Bataille et al., 2013), South America (Rodriguez et al., 2014) and North America (Talley et al., 2015). Most recently, O'Hanlon et al. (2018) reported the discovery of a previously unidentified lineage (*Bd*ASIA1), which demonstrated an ancestral origin in the Korean peninsula from which other major lineages diverged, including the *Bd*GPL lineage – which is estimated to have diverged most recently, between 50-120 years ago. Further support for the Asiatic origin of the *Bd* fungus was presented with the discovery of the *Bd*ASIA-3 lineage, thought to be the first lineage to have diverged from the ancestral *Bd*ASIA-1 lineage (Byrne et al., 2019).

Batrachochytrium dendrobatidis is now regarded as a panzootic parasitic fungus, established across the four major continents, and present in most regions inhabited by amphibians ($\geq$ 71 countries; Olson and Ronnenberg, 2014), including remote, yet megadiverse islands such as Madagascar (Kolby, 2014; Bletz et al., 2015; but see Kolby and Skerratt, 2015). Despite having only recently diverged within the last 120 years, the highly virulent *Bd*GPL lineage has the greatest global presence, existing in all regions which have experienced epizootics, and the greatest *Bd*-related amphibian declines (Figure 1.8; O'Hanlon et al., 2018; Fisher and Garner, 2020).

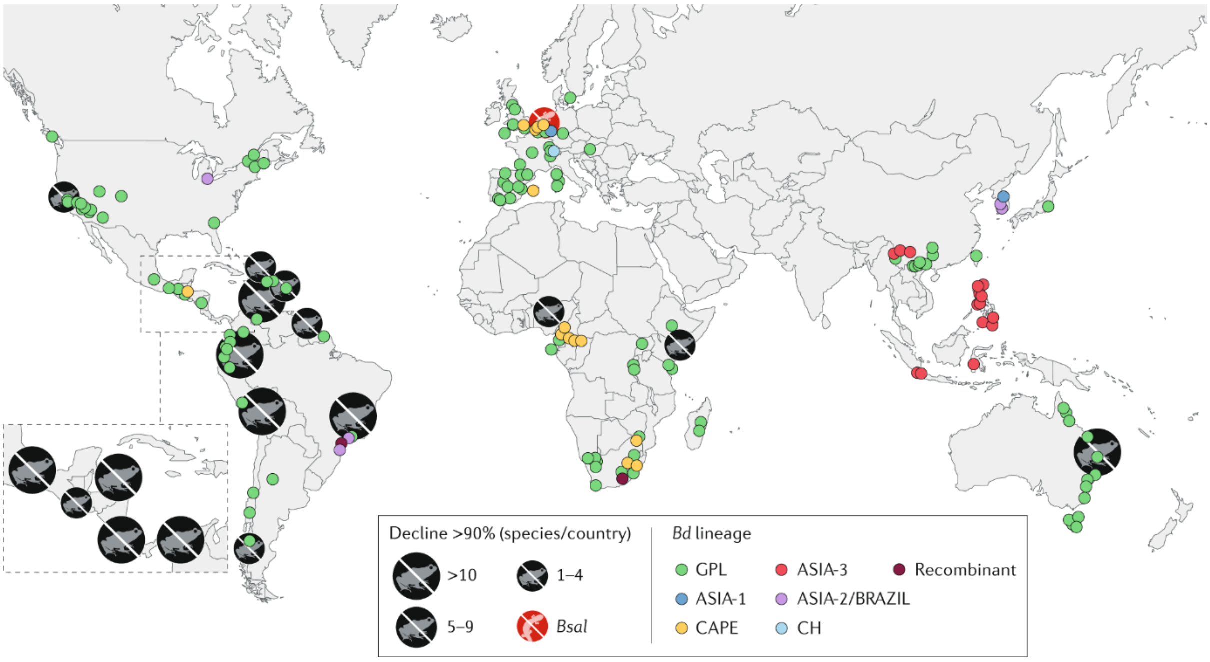

Figure 1.8. Global distribution of the five major lineages of *Batrachochytrium dendrobatidis* (*Bd*), as well as several *Bd*-recombinants and *Batrachochytrium salamandrivorans* (*Bsal*). CH lineage was recently combined with ASIA-1 lineage (see O'Hanlon et al., 2018). ⊘ denotes the number of species (per country) which have experienced *Bd*-attributed declines of >90%. Taken from Fisher and Garner (2020).

The global expansion of *Bd* can be largely attributed to the international trade of exotic amphibians, in which infections of *Bd* are often found (Fisher and Garner, 2007; O'Hanlon et al., 2018). Contributing to an industry worth approximately $300 billion (USD), and up to an additional $20 billion (USD) when considering the illegal animal trade (Smith et al., 2017), the sale of exotic amphibians - whether it be for the pet trade (e.g. Kolby, 2014; Wombwell et al., 2016), food industry (e.g. Schloegel et al., 2009) or research (e.g. Weldon et al., 2004) – has facilitated the unimpeded, international translocation of *Bd* (Figure 1.9; Fisher and Garner, 2007). This is particularly true of some alien amphibian species, such as the American bullfrog (*R. catesbeiana*) and the African long-clawed frog (*X. laevis*) which, having historically been traded in their millions (Fisher and Garner, 2007), are directly implicated in the introduction of *Bd* into regions of Canada (Garner et al., 2006), North America (Garner et al., 2006; Huss et al., 2013; Vredenburg et al., 2013), South America (Hanselmann et al., 2004; Garner et al., 2006; Schloegel et al., 2010; Solís et al., 2010; Soto-Azat et al., 2016), Asia (Goka et al., 2009; Bai et al., 2010), the UK (Cunningham et al., 2005; Garner et al., 2006; Tinsley et al., 2015a), and Continental Europe (Garner et al., 2006; Ouellet et al., 2012). Traded as a food commodity (*R. catesbeiana*), or for research purposes (*X. laevis*), both species have been shown to develop and maintain infections in captivity – with infective zoospores likely released into the environment through the disposal of contaminated materials (e.g. water) – and as feral introduced populations (Hanselmann et al., 2004; Fisher, 2009; Tinsley et al., 2015a; Ribeiro et al., 2019). With both species typically asymptomatic carriers of *Bd*, suffering minimal or no *Bd*-associated pathologies (but see Gervasi et al., 2013), *R. catesbeiana* and *X. laevis* can act as effective reservoir hosts, facilitating the global establishment of *Bd*, whilst also enabling *Bd* to persist within novel regions (Weldon et al., 2004; Garner et al., 2006; Valenzuela-Sánchez et al., 2018).

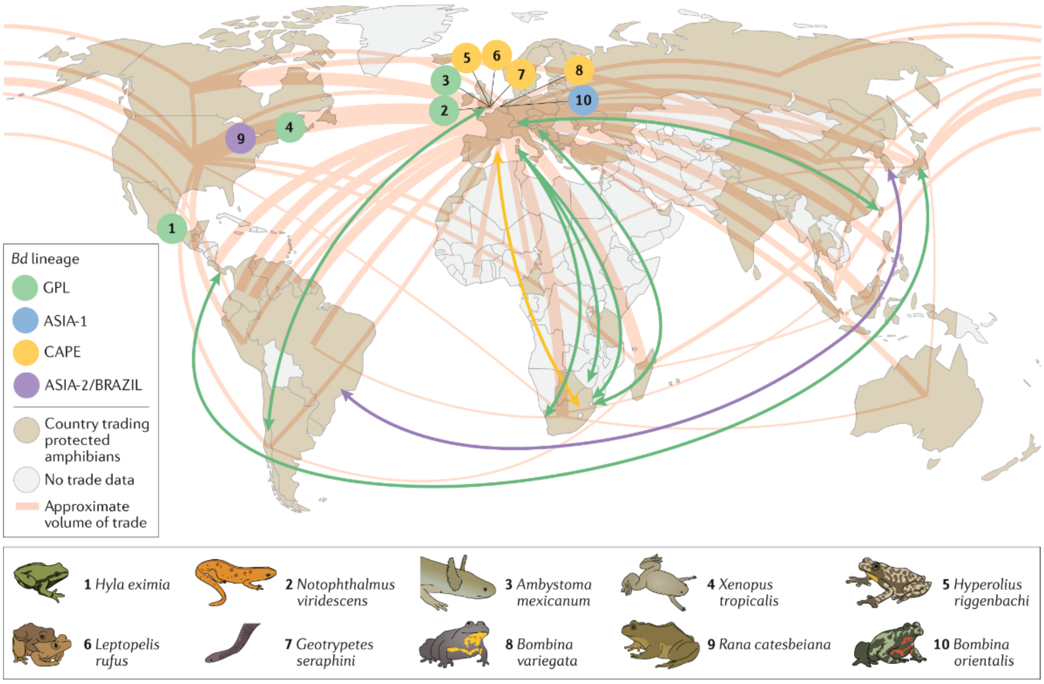

Figure 1.9. The projected intercontinental movement of *Bd*-carrying alien amphibian species – translocated between continents via global trade routes (orange lines). Map displays the inferred movement of different *Bd* lineages, as determined through genomic sequencing of geographically separated samples, by different exotic amphibian species. Numbers indicate localities where isolates of *Bd*-lineages (colour-coded) were recovered from traded amphibian species (see coloured illustrations). Taken from Fisher and Garner (2020).

Whilst the global trade of *Bd*-carrying alien amphibian species may primarily explain the extent to which *Bd* has become established, it alone may not necessarily explain the success and impact of this invasive chytrid fungus within global freshwater environs. Indeed, the ability for *Bd* to thrive within freshwaters may be more complicated than the simple parasite-host dynamic, previously assumed to be exclusive to amphibians. In the absence of suitable amphibian hosts *Bd* might persist in the environment, with several *in vitro* studies demonstrating that *Bd* can survive in sterile water and river sediment (e.g. moist sand and soil), and can remain infective for up to 12 weeks (Johnson and Speare, 2003, 2005; Walker et al., 2007). As with many chytrid species, *Bd* can adopt a saprobic life strategy in the environment, capable of growing on the remains of dead aquatic microorganisms, including algae and invertebrates (Johnson and Speare, 2003).

Although regarded as a generalist parasite of amphibians, several studies have shown that this generalist nature may also extend to non-amphibian hosts. For example, *Bd* can grow saprophytically on sterile birds' feathers (Johnson and Speare, 2005), as well as the keratinised toe scales of waterfowl (Garmyn et al., 2012; Hanlon et al., 2017). When considered in conjunction with a relatively high prevalence of *Bd*, detected in populations of wild waterfowl (Garmyn et al., 2012; Hanlon et al., 2017) and most recently in museum specimens (Burrowes and De la Riva, 2017), migratory aquatic birds may serve as a potential non-amphibian reservoir and/or vector for this fungal pathogen. Reptiles may also represent a potential reservoir, with *Bd* having been shown to grow saprophytically on sterile snakeskin (Longcore et al., 1999), and has even been detected in wild reptile populations (Kilburn et al., 2011); although it is uncertain whether *Bd* is able to grow on reptile skin under natural conditions. Most recently, fish have been identified as a potential host of *Bd*, with empirical studies having demonstrated the ability for live zebrafish (*Danio rerio*) to experimentally acquire *Bd* infections, causing dose-dependent pathologies within infected hosts, such as erosion along the caudal fin and degeneration of adjacent tissues, and subsequent mortality in some individuals (Liew et al., 2017).

Alternatively, invertebrates might also be suitable reservoir hosts for *Bd*, with several studies having reported infections – and associated mortalities – in nematodes (Shapard et al., 2012), and decapod crustaceans (McMahon et al., 2013; Brannelly et al., 2015; Oficialdegui et al., 2019). Even though *Bd* has previously been found to colonise the keratinised regions of vertebrate non-/amphibian host skin – a trait historically attributed to the use of keratin as a source of nutrition in *Bd* development (Daszak et al., 1999) – McMahon et al. (2013) reported the ability for *Bd* to colonise and proliferate on non-keratinised organic materials, namely the chitinous carapace and gastrointestinal (GI) tract of several crayfish species; the blue crayfish (*Procambarus alleni*), the red swamp crayfish (*P. clarkii*) and the virile crayfish (*Orconectes virilis*). In the lab, McMahon et al. (2013) found that *Bd* was capable of growing saprophytically on sterile carapace and GI tract and confirmed that *Bd* was able to infect live crayfish following exposures to infective zoospores. Infections were maintained for at least 3 months, before crayfish either cleared infections, or died as a result of *Bd*-related pathologies (e.g. gill recession). McMahon et al. (2013) also confirmed the potential for infected crayfish to transmit infections to naïve amphibian larvae, with *Bd* detected in the mouthparts of infected larvae, suggesting the possibility for non-amphibian hosts to contribute in the transmission dynamics of chytrid. In the field, populations of *Procambarus spp.* and *O. virilis*, present in Louisiana and Colorado (USA), were found to naturally acquire infections by *Bd*, detected at relatively high prevalence (≤29%) in populations by McMahon et al. (2013), and later by Brannelly et al. (2015). Interestingly, Brannelly et al. (2015) found *Bd* prevalence to fluctuate seasonally, with the highest prevalence recorded in Spring (≤6%). This happened to coincide with the breeding season of native amphibian populations and as such, was likely to facilitate infections in amphibian populations. Outside of the amphibian breeding season, *Bd* was found to persist in crayfish populations, even in the absence of amphibian hosts, indicating the possibility for decapods to act as non-amphibian reservoirs (McMahon et al., 2013; Brannelly et al., 2015). Similar findings have subsequently been reported in an invasive population of *P. clarkii*, present in Spain (Oficialdegui et al., 2019).

Given the range of potential non-amphibian hosts identified to-date, current rates of global amphibian declines may likely worsen. This is especially true when considering potential invasive non-amphibian hosts, such as *P. clarkii*, which are often conveyed globally through international trade (Westphal et al., 2008; Loureiro et al., 2015), or can enter novel regions undetected via transport vectors (Morais and Reichard, 2018). Crustaceans are typically over-represented amongst IAS (Karatayev et al., 2009; Strayer, 2010), and as such could pose an additional threat in the spread of *Bd* to new amphibian populations. *Dikerogammarus villosus* has spread rapidly throughout much of Europe and is also regarded as a horizon species in North America (Rewicz et al., 2014). In Chapter 5, I conduct an experimental infection study to determine the potential for the invasive non-amphibian *D. villosus* to become infected by *Bd*, therefore determining its potential to act as a carrier.

1.4 Study System

Throughout this thesis, I focus on assessing the potential ecological impact of the invasive freshwater amphipod crustacean *Dikerogammarus villosus* towards UK anurans, particularly the larvae of the native common frog (*Rana temporaria*). Through the application of laboratory-based experimentation I investigate the potential for invasive *D. villosus* to impact anurans directly, as a possible predator of early life-stages (Chapter 2, 3, and 4), as well as indirectly as a potential carrier of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (Chapter 5). Where possible, I compare the *per capita* effects of invasive *D. villosus* with the native amphipod *Gammarus pulex*.

1.4.1 Focal Predator – Killer Shrimp (*Dikerogammarus villosus*) (Sowinsky, 1894)

Figure 1.10. The invasive 'killer shrimp' *Dikerogammarus villosus*. Image credit: Alchetron, licensed under CC BY-SA

Dikerogammarus villosus (Crustacea: Gammaridae) is a freshwater gammaridean amphipod crustacean native to the Ponto-Caspian region of south-eastern Europe, where it naturally inhabits the lower courses of large rivers in the Black and Caspian Sea basins (Rewicz et al., 2015). However, following the opening of the Main-Danube-Rhine canal in 1992, *D. villosus* began to disperse north-westerly, facilitated initially by transport vectors (e.g. ballast water; Mayer et al., 2009), before naturally dispersing through the highly connected European waterways (Bij de Vaate et al., 2002; Rewicz et al., 2014). Rapidly disseminating throughout much of Western Europe, *D. villosus* was then transported across the English Channel, likely conveyed in contaminated ballast water or upon recreational equipment (e.g. angling; Anderson et al., 2015a), whereupon it was introduced into Grafham Water Reservoir (Cambridgeshire), became an established population, and was first detected in 2009 (MacNeil et al., 2010). Since then highly localised populations have been found in four other locations; including Cardiff Bay and Eglwys Nunydd (South Wales), and Norfolk Broads (Norfolk; Gallardo et al., 2012a) and as of 2015, Pitsford Reservoir (Northampton; Clinton et al., 2018). Future expansion within the UK is predicted, with over a quarter of Great Britain identified as suitable for potential introductions (Gallardo et al., 2012a), likely propagated by recreational equipment (e.g. angling; Anderson et al., 2014; Smith et al., 2020). Intercontinental translocation is also predicted, with the introduction of *D. villosus* into the Laurentian Great Lakes (USA) expected in the near future (U.S. Fish and Wildlife Service, 2016; Kramer et al., 2017).

Within invaded regions, *D. villosus* typically dominates native macroinvertebrate assemblages, in terms of abundance and biomass (van Riel et al., 2006; van Riel, 2007; MacNeil et al., 2013a). Enhanced life-history traits, including rapid growth, early maturation with interbrood intervals, high fecundity, and a high generational turnover, enable *D. villosus* to rapidly colonise novel habitats, generating highly abundant populations (Devin et al., 2004; van Riel et al., 2006; Pöckl, 2007).

Following the establishment of *D. villosus* in a novel region, native communities can often experience restructuring, resulting from an overall reduction in the abundance and/or diversity of resident macroinvertebrate species (van Riel et al., 2006; MacNeil et al., 2013a). Declines in native macroinvertebrate assemblages are primarily attributed to strong predatory behaviours, characteristically displayed by *D. villosus*. Having developed a large body size (~30mm in length), typically larger than other European gammaridean species (Figure 1.11), coupled with large generalist mouthparts, *D. villosus* – aptly nicknamed as the killer shrimp – can consume a diverse range of macroinvertebrate taxa in the laboratory, including other amphipods, larval dipterans, larval odonates, isopods, cladocerans, aquatic bugs, juvenile crayfish and leeches (reviewed in Rewicz et al., 2014). As flexible omnivores, *D. villosus* can act as an effective detritivore (Truhlar and Aldridge, 2015; Kenna et al., 2017). Such diversity in the diet of invasive *D. villosus* can often be reflected in the field (van Riel et al., 2006; Hellmann et al., 2015). Predation can also extend to some aquatic vertebrates, with studies demonstrating the ability for *D. villosus* to consume fish eggs and fry (Casellato et al., 2007; Platvoet et al., 2009a; Taylor and Dunn, 2017).

Figure 1.11. Natural size differences between invasive *Dikerogammarus villosus* (top) and native *Gammarus pulex* (bottom). Scale = 5mm.

Now present in most main inland freshwater bodies throughout Western Europe, *D. villosus* is regarded as one of more than 100 worst invaders in Europe (Nentwig et al., 2018).

1.4.2 Native Analogue – *Gammarus pulex* (Linnaeus, 1758)

Gammarus pulex (Crustacea: Gammaridae) was selected as an appropriate native analogue, chosen as a comparator when assessing the predatory impact of *D. villosus* towards early-stage UK amphibians. *Gammarus pulex* is common and widespread throughout much of Britain, inhabiting a range of freshwater systems, including those colonised by amphibians during the breeding season (pers. obs.). Although regarded as a typical detritivorous shredder, important in the processing of organic matter and the release of nutrients into freshwater ecosystems, *G. pulex* is also an effective predator, capable of feeding upon various macroinvertebrates, including other amphipods (Kelly et al., 2002a, 2002b, 2006; Dodd et al., 2014), as well as some vertebrates (fish eggs and fry; Taylor and Dunn, 2017). Whilst native to Great Britain and Western Europe (Piscart et al., 2007), *G. pulex* is an invader of Northern Ireland, displacing native amphipod populations (i.e. *Gammarus duebeni celticus*) through competition and intraguild predation, and impacting upon native communities (MacNeil et al., 2004; Kelly et al., 2006). However, within its natural range, *G. pulex* is also at risk of disturbance by invasive alien species, such as *D. villosus*; often driven by intense intraguild predation (MacNeil et al., 2011, 2012). Comparatively, *G. pulex* and *D. villosus* exhibit considerably different behavioural characteristics, whether it be hunting strategies (sit-and-wait versus active pursuit; Dodd et al., 2014), sociality, boldness or activity (Truhlar and Aldridge, 2015). As such, the displacement of *G. pulex* by *D. villosus* from native freshwater systems, is likely to lead to novel predator-prey interactions, thereby impacting upon native prey populations, including larval amphibians (Saul and Jeschke, 2015).

1.4.3 Focal Prey – European Common Frog (*Rana temporaria*)

In Chapters 2, 3, and 4 I utilise freshly hatched, early-stage *R. temporaria* larvae as the focal prey organism (Figure 1.12a). Widespread throughout much of the UK and mainland Europe, *R. temporaria* are a relatively abundant anuran species categorised as 'least concern' by IUCN (IUCN, 2020). Although adults are predominantly terrestrial, they will colonise a variety of urban and rural freshwater habitats – including ponds, lakes, streams, rivers, canals and marsh (Beebee and Griffiths, 2001; Reid et al., 2014) – in order to breed, rapidly producing and fertilising large quantities of eggs (i.e. explosive breeding; Hartel et al., 2007; Loman, 2016). Once hatched, the resultant larvae will remain in the aquatic environment until metamorphosis (Walsh, 2010), during which larvae remain vulnerable to predation by both native (e.g. odonates; Hossie and Murray, 2016), and invasive (e.g. decapods; Cruz and Rebelo, 2005) predators. These sites may be frequently visited by anglers (pers. obs.) which, given the potential for IAS to be conveyed by recreational equipment (Anderson et al., 2014; Smith et al., 2020), may provide the opportunity for invader introductions, and subsequent interactions between native and invasive species.

In Chapter 2, the focus of my research extends to address the potential for *D. villosus* to predate upon the encapsulated embryos of *R. temporaria* (Figure 1.12b). I also consider the possibility for *D. villosus* to threaten the embryos of alternative amphibian species. I utilise the embryos of the invasive African long-clawed frog (*X. laevis*; Figure 1.12c) – commercially available in the UK (European *Xenopus* Resource Centre; EXRC), but for which several feral populations have previously been established in the UK (Tinsley et al., 2015a, 2015b).

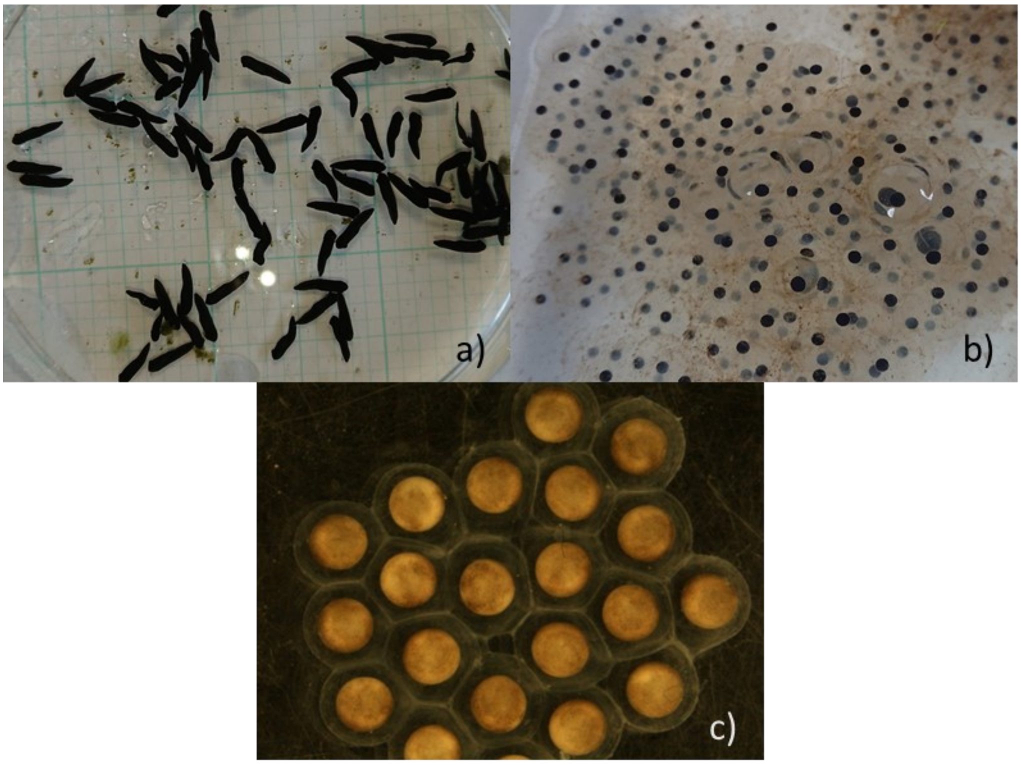

Figure 1.12. Focal amphibian prey organisms used in research. (a) Native *Rana temporaria* larvae, (b) *R. temporaria* embryos, and (c) invasive *Xenopus laevis* embryos.

1.5 Research Aims and Thesis Plan

In this thesis I present four novel studies investigating, in detail, the potential ecological impacts imposed by invasive *D. villosus* towards UK amphibians. I examine the possibility for *D. villosus* to affect amphibians, directly via the consumption of vulnerable embryonic and/or larval life-stages, or indirectly as a potential carrier of amphibian pathogens. By addressing these questions, I aim to improve our understanding regarding the breadth of freshwater fauna adversely affected by this high-impact invader, and the mechanisms by which these species are affected. It is my hope that the findings presented herein will help to inform upon the future management of IAS (Kumschick and Richardson, 2013), as well as policies concerning the conservation of native amphibian biodiversity (Nunes et al., 2019).

There is a wide body of evidence demonstrating the predatory impact of *D. villosus* towards native macroinvertebrates (reviewed in Rewicz et al., 2014), yet there is comparably little research concerning vertebrates (Casellato et al., 2007; Taylor and Dunn, 2017). In chapters 2, 3 and 4, my research focusses on assessing the direct predatory impact of invasive *D. villosus* towards early-stage UK amphibians using predictive FR methodologies.

In chapter 2, I investigate the potential predatory impact of invasive *D. villosus* towards amphibians, when compared to native amphipods. I apply traditional FR modelling techniques, measuring *per capita* predation as a function of prey density alone (i.e. prey-dependence; see Dick et al., 2014), to compare the predatory impact of invasive *D. villosus* and native *G. pulex* towards the embryos and larvae of several UK anuran species; *R. temporaria*, native to the UK and Europe, and *X. laevis*, a globally established high-impact invader, for which several feral populations have existed in the UK (Tinsley et al., 2015a; but see Tinsley et al., 2015b). In addition, I supplement FR models with estimates of native and invasive amphipod abundance – a proxy for the numerical response (NR) – measured in several UK field populations, and recorded in previously published literature. I incorporate both FR and abundance data into the RIP metric (see Dick et al., 2017), in order to obtain population-level predictions concerning the relative impact of invasive *D. villosus*.

Invasive alien species often exist at higher densities within invaded communities (van Riel et al., 2006; Hansen et al., 2013). As such, invaders are likely to interact with conspecifics, which can modify their *per capita* foraging success (Arditi and Ginzburg, 2012). In chapter 3, I examine how interactions amongst conspecific amphipods may influence the predatory success of invasive *D. villosus*. Using a bivariate experimental design, in which both prey (i.e. amphibian larvae) and predator (i.e. amphipod) abundance are manipulated, I apply a variety of FR model variants, modelling *per capita* predation as a function of i) prey abundance only (i.e. prey-dependence), ii) the absolute prey and predator abundance (i.e. predator-dependence), and iii) the prey-to-predator ratio (i.e. ratio-dependence), By doing so, I determine how *per capita* consumption by invasive *D. villosus* may change in response to variations in the relative availability of native *R. temporaria* larvae, and explore how the emergence of mutual interference amongst interacting conspecific predators may alter the predicted ecological impact of *D. villosus* towards native *R. temporaria* larvae. I also investigate how mutual interference between conspecific predators might alter the spatial dynamics of *D. villosus* over time.

Invasive alien predators frequently exhibit the potential to destabilise native prey populations, as indicated by the expression of a Type II FR (Dick et al., 2014; see Appendix 1, Table A1.1). However, in the field, alternative prey types are likely to be available, which can cause predator to switch between prey; thereby providing a refuge for prey at lower densities (Oaten and Murdoch, 1975; van Leeuwen et al., 2007). In chapter 4 I explore how predatory pressures towards larval UK amphibians might change when invasive *D. villosus* have access to multiple prey types. I examine how diet composition and prey choice may differ between invasive *D. villosus* and native *G. pulex*, when presented with native *R. temporaria* larvae as the focal prey, alongside several alternative macroinvertebrate prey types (isopods and dipteran larvae), in equal quantities. I go on to present invasive amphipods with several experimental prey combinations – consisting of *R. temporaria* larvae and either isopodan or dipteran prey – when offered at varying relative densities. Using prey selectivity indices, I investigate how predatory behaviours towards larval amphibians may change in response to proportional availability and determine whether *D. villosus* demonstrates a propensity for frequency-dependent prey-switching – choosing to feed upon the more abundant prey species. I develop this concept further by applying complex multiple-prey FR methodologies in order to ascertain how predatory pressures towards larval amphibians are effected, when *D. villosus* can choose between prey.

Invasive alien species can have multiple effects on native organisms (Manchester and Bullock, 2000; Nunes et al., 2019). In addition to predation, invaders can act as vectors of parasites, facilitating disease transmission (Dunn and Hatcher, 2015). *Batrachochytrium dendrobatidis* is a high-impact fungal pathogen which has caused many amphibian species to suffer from drastic declines and/or extinctions (Scheele et al., 2019). Primarily spread by invasive amphibians (e.g. *R. catesbeiana*; Fisher and Garner, 2020), more recent studies have identified the potential for various non-amphibian carriers (Kilburn et al., 2011; Garmyn et al., 2012; McMahon et al., 2013; Liew et al., 2017). In chapter 5, the focus of my research shifts to exploring the possibility for invasive *D. villosus* to indirectly affect native amphibian populations. Through the application of quantitative molecular diagnostics, supplemented with histological analysis of amphipod tissues, I investigate the potential for *D. villosus* to act as a carrier of *Bd*. Using infection experiments, I verify whether *D. villosus* is capable of acquiring and maintaining infections by *Bd*, following exposures to infectious life stages, and examine how pathologies might manifest within infected amphipods over time. I also conduct a mortality experiment, to assess how infections by *Bd* might affect amphipod host survival.

Chapter 2

Superior Predatory Ability and Abundance Predicts Potential Ecological Impact towards Early-Stage Anurans by Invasive Killer Shrimp (*Dikerogammarus villosus*)

2.1 Abstract

Invasive alien species negatively impact upon biodiversity and generate significant economic costs worldwide. Globally, amphibians have suffered considerable losses, with a key driver being predation by large invasive invertebrate and vertebrate predators. However, there is no research regarding the potential ecological impact of small invertebrate invaders. The invasive freshwater amphipod *Dikerogammarus villosus* can act as a top predator capable of displacing native amphipods and preying heavily upon a range of native species. Listed as one of Europe's top 100 worst invaders, *D. villosus* has significantly restructured freshwater communities across western Europe and is expected to invade North America in the near future. Here I explore the ecological impact of invasive *D. villosus* upon UK native and invasive amphibians (*Rana temporaria* and *Xenopus laevis* respectively) using the "Relative Impact Potential" (RIP) metric. By combining estimations of *per capita* effects (i.e. functional response; FR) and relative field abundances, I apply the RIP metric to quantity the potential ecological impact of invasive *D. villosus* upon embryonic and larval amphibian prey, compared to the native amphipod *Gammarus pulex*. Both native and invasive amphipods consumed early-stage amphibians and exhibited potentially destabilising Type II FRs. However, larger body size in invasive *D. villosus* translated into a superior FR through significantly lower handling times and subsequently higher maximum feeding rates – up to seven times greater than native *G. pulex*. Higher invader abundance also drove elevated RIP scores for invasive *D. villosus*, with potential impact scores predicted up to 15.4 times greater than native *G. pulex*. Overall, *D. villosus* is predicted to have a greater predatory impact upon amphibian populations than *G. pulex*, due primarily to its larger body size and superior field abundance, potentially reducing amphibian recruitment within invaded regions.

2.2 Introduction

Biological invasions are of increasing global concern, with invasive alien species (IAS) generating a substantial cost to the global economy, estimated to be more than $974 billion USD (Hoffmann and Broadhurst, 2016). IAS can have an immense, often irreversible effect upon native communities and ecosystems, ranked second only to habitat destruction in terms of impact (Dueñas et al., 2018). Freshwater ecosystems are spatially restricted (occupying ~0.8% of the Earth's surface) yet highly biodiverse, supporting approximately 6% of all described species (Dudgeon et al., 2006). However, freshwaters experience a disproportionate incidence of IAS invasions (Ricciardi and MacIsaac, 2010), with invader impacts typically more severe when compared to terrestrial ecosystems (Moorhouse and Macdonald, 2015). IAS influence native communities through a variety of trophic interactions, of which predation is key (Rosewarne et al., 2016). Compared to trophically analogous native species, invasive predators often consume prey at a higher rate (reviewed by Dick et al., 2014, 2017; Cuthbert et al., 2019a). Furthermore, IAS typically reach higher abundances in comparison to native analogues (Dick et al., 2017), applying even greater predatory pressures upon local prey populations and assemblages.

Amphipod crustaceans (Order: Amphipoda) are frequently identified as high-impact freshwater invaders (Devin et al., 2004). The killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894) is listed as one of the 100 worst invaders in Europe (Nentwig et al., 2018), and is a species of high concern in Great Britain (Gallardo and Aldridge, 2015), and North America (Kramer et al., 2017). *Dikerogammarus villosus* threatens freshwater biodiversity and ecosystem functioning throughout Western Europe, permanently altering the structure of invaded native assemblages across multiple trophic levels (van Riel et al., 2006; MacNeil et al., 2013a; Dodd et al., 2014). The invasive success of *D. villosus* is attributable to several life history characteristics, including a wide ecophysiological tolerance (Bruijs et al., 2001), rapid growth and high fecundity (Devin et al., 2004; Pöckl, 2007), an effective anti-predator strategy (Rolla et al., 2020), and a strong competitive ability (Kobak et al., 2016). Acknowledged for its large body size, large mouthparts, flexible omnivory and superior predatory capabilities (Rewicz et al., 2014), *D. villosus* is a voracious, high trophic predator (van Riel et al., 2006). In the laboratory, *D. villosus* readily consumes a wide range of freshwater macroinvertebrates (reviewed in Rewicz et al., 2014). This wide dietary range is also seen in the field, as confirmed by stable isotope analyses (van Riel et al., 2006; Hellmann et al., 2015). Aquatic vertebrates may also be at risk, with reports of predation on fish eggs and larvae (Platvoet et al., 2009a; Taylor and Dunn, 2017). However, to our knowledge there are no studies concerning the predatory impact of invasive *D. villosus* towards amphibians.

Regarded globally as a critical conservation concern, amphibians have experienced substantial declines over the past 40 years (Alford, 2011). Current amphibian extinction rates are estimated to be four orders of magnitude greater than background extinction rates (Alroy, 2015), with approximately 32% of known amphibian species threatened with imminent extinction (Hayes et al., 2010). Amphibian declines are driven by various factors, including climate change, environmental pollutants, habitat loss, pathogens (e.g. *Batrachochytrium dendrobatidis*; Fisher and Garner, 2020) and invasive species (Hayes et al., 2010). Of the 6,771 amphibian species listed on the International Union for Conservation of Nature (IUCN) Red List, 17% of species are directly threatened by invasive alien species, of which 11% of species are categorised as vulnerable, endangered or critically endangered (Bellard et al., 2016b; IUCN, 2019; Nunes et al., 2019). Predation of embryonic and larval amphibians by large invasive freshwater predators, particularly fish, crayfish (Ilhéu et al., 2007), and other amphibians (e.g. bullfrogs) is one of the major contributors in the decline and extirpation of amphibian populations (Kats and Ferrer, 2003). Whilst amphibians typically breed in ponds, lakes, streams, rivers and canals (Beebee and Griffiths, 2005), they can also occupy the same habitat as *D. villosus*, having previously been recorded in large invaded freshwater bodies in the UK (National Biodiversity Network, 2017; Anglian Water, pers. comm.; Cardiff Harbour Authority, pers. comm.), and also in mainland Europe (Uehlinger et al., 2009; Gergs and Rothhaupt, 2014; Koester et al., 2016; Haubrock et al., 2019). Given that stable isotope analysis suggests that *D. villosus* can occupy the same trophic level as many predatory fish species (Marguillier, 1998), this invasive amphipod may pose a potential risk to larval amphibians.

I present the first empirical study comparing the ecological impacts of invasive and native freshwater amphipod predators upon the early, aquatic life-stages of two amphibian species. The ecological impact of invasive predators is dependent on predatory capability, relative to native analogues, as well as relative abundance (Dick et al., 2017). Here I compare the predatory functional responses of invasive *D. villosus* and native *Gammarus pulex* (Linnaeus, 1758) towards the embryos and larvae of native *Rana temporaria* (Linnaeus, 1758) (European Common Frog) and invasive *Xenopus laevis* (Daudin, 1802) (African Long-Clawed Frog). I also estimate relative abundances of native *G. pulex* and invasive *D. villosus* in field populations in Great Britain, and supplement these values using published estimates. I apply the Relative Impact Potential metric (see Dick et al., 2017), which incorporates relative consumer abundance as a means of scaling relative *per capita* effects to compare the relative impact potential of these freshwater amphipod species towards amphibians present in Great Britain.

2.3 Materials and Methods

I compared the predatory impacts of invasive *D. villosus*, and the British-native amphipod, *G. pulex*, upon the early life-stages of amphibians. Initial experiments used invasive *X. laevis* embryos as a focal prey organism and established the potential for native and invasive amphipods to predate upon early-stage anurans. Therefore, experiments proceeded utilising the embryonic and larval forms of native *R. temporaria* as focal prey types. *Rana temporaria* have been recorded occupying the same habitat as *D. villosus*, both in UK invaded sites (National Biodiversity Network, 2017; Anglian Water, pers. comm; The Wildlife Trust for Bedfordshire, Cambridgeshire & Northamptonshire, pers. comm), and in mainland Europe (Uehlinger et al., 2009; Koester et al., 2016).

I compared size-matched amphipods to examine intrinsic differences between species, as well as significantly larger *D. villosus* to reflect natural differences in amphipod size (Dodd et al., 2014; Taylor and Dunn, 2017). A comparative functional response (FR) approach was utilised to quantify amphipod predation upon invasive and native amphibian embryos and larvae. FRs are a fundamental measure of resource use frequently applied in invasion ecological research as a metric to assess trophic interactions; quantifying the relationship between *per capita* predation rate and prey abundance (i.e. FR). By comparing FRs of IAS and native analogues predictions can be made as to how differential predator behaviours might impact upon prey populations in the field (Dick et al., 2014, 2017).

When considering the absolute ecological impact of invasive predators, total invader impact should consider predatory capability, relative to native analogues, as well as relative abundance (Dick et al., 2017). Based on the classic total response equation (Total Response = Functional Response x Numerical Response), the Relative Impact Potential (RIP) metric has recently been developed and incorporates relative consumer abundance or biomass – a proxy for numerical response – as a means of scaling relative *per capita* effects (i.e. FR) to predict the relative impact of an invasive predator in comparison with a native analogue (RIP = FR x Abundance; Dick et al., 2017).

2.3.1 Experimental Organisms

Ethical consent was sought from Natural England, the Home Office and the University of Leeds Ethics Committee. However, ethical approval was not required, given that the use of freshly hatched, pre-feeding *R. temporaria* larvae for experimentation fell outside the remit of the Wildlife and Countryside Act 1981 (section 9.5; protected against sale only), and the UK Animal Scientific Procedures Act 1986 (ASPA; section 1.4.2). Animals were maintained in compliance with guidelines stated in the Code of Practice for the Housing and Care of Animals Bred, Supplied or Used for Scientific Purposes (section 3, chapter 11). All experimental work was conducted in accordance with relevant guidelines and regulations, including the maintenance, use and termination of study organisms.

2.3.1.1 Amphibians

Xenopus laevis embryos were sourced from adult females, commercially reared by the European Xenopus Resource Centre (EXRC, University of Portsmouth). In December 2016, embryos were transported to the laboratory, stored in isotonic 1x Modified Barth's Saline (MBS) solution (Appendix 2, Table A2.1). Upon receipt, embryos were gradually transferred into aerated dechlorinated tap water over the course of several hours and kept at 14.0 ± 0.1°C, under a 12:12 h light:dark regime, as recommended by the EXRC.

Freshly deposited native *R. temporaria* embryos (approximately 36 h post-fertilisation) were collected between February and March 2017 from several freshwater sites around West Yorkshire (Appendix 2, Table A2.2). Embryos were removed as whole clutches and approximately halved, with half of the clutch transferred to 2L sterile storage containers with site-sourced water, and half of the clutch returned to the site. Harvested embryos accounted for less than 10% of the total population of embryos present at each site.

Embryos were transported to the laboratory in insulated boxes and stored as individual half-clutches in aerated aquaria with dechlorinated tap water at 4.0 ± 0.2°C under a 12:12 h light:dark regime. By maintaining the embryos at 4°C, the rate of embryonic development was reduced considerably (~30 days to hatching), maximising the potential experimental period whilst enabling greater control over developmental progress.

To obtain *R. temporaria* larvae for experimentation, embryos were reared to early-stage larvae. When larvae, still encapsulated in vitelline jelly, began to develop external gill filaments and exhibited neuromuscular reflex responses (i.e. Gosner stage or G 18-19; see Gosner, 1960), they were transferred to 14 ± 0.1°C (consistent with ambient temperatures recorded during field sampling) in preparation for hatching. Transference to the higher temperature regime, conducted gradually over the course of 24 h, accelerated development, with hatching typically occurring within approximately 24 h of changing temperature regimes. Shifting temperature regimes also allowed larvae to acclimatise to warmer conditions prior to experimentation. Conditions were sufficient to produce high rates of larval hatching (>75%) and survival (~70%). Embryonic and larval stock tanks were cleaned twice weekly. Only recently hatched, pre-feeding larvae (i.e. G. 19-20; Gosner, 1960), lacking any obvious functioning mouthparts and relying solely on the yolk sac for nutrition (Currie et al., 2016), were used for experimentation.

2.3.1.2 Amphipods

Invasive *D. villosus* were sampled from artificial substrates in Grafham Water, Cambridgeshire (52°17′31.2″N 0°19′23.6″W), and native *G. pulex* were kick-sampled from Meanwood Beck, West Yorkshire (53°49′49.2″N 1°34′31.3″W). Amphipod species were identified based on urosome morphology (Müller et al., 2002; Blackman et al., 2017). Each species was independently maintained in the laboratory in 4L aquaria with aerated, dechlorinated tap water, and provided an *ad libitum* diet of sycamore leaves (*Acer pseudoplatanus* L.), stream-conditioned for approximately one month. Amphipod specimens were maintained at 14.0 ± 0.1°C under a 12:12 h light:dark regime for at least 96 h before experimental use.

Male amphipods were used in experimental treatments as females may exhibit variations in predatory behaviour- (van der Velde et al., 2009). Male *G. pulex* were identified via precopulatory pairings, whilst male *D. villosus* were identified by the presence of genital papillae, and the absence of oostegites (i.e. brood plates). Amphipods exhibiting visible parasitic infections were excluded from experimentation, controlling for potential variations in behaviour caused by infections (Dick et al., 2010; Iltis et al., 2018). Amphipods were kept in sex-specific communal tanks for at least 24 h prior to their use in experimental trials and were only used once in each experimental treatment.

Given the significantly larger natural body size of *D. villosus* when compared to other European gammaridean amphipods (Rewicz et al., 2014), amphipods were categorised into three size groups; large *G. pulex*, intermediate *D. villosus* and large *D. villosus*. Controlling for amphipod body size enabled fundamental comparisons of inherent differences in predatory impact between size-matched native (large *G. pulex*) and invasive (intermediate *D. villosus*) amphipod groups, whilst also considering the predicted amplificatory effect that larger natural body size in *D. villosus* may have on maximal predatory impact (Dodd et al., 2014).

Amphipods were blotted dry, weighed and photographed in a resting curved state, with measurements taken approximately 2 h prior to the starvation of amphipods in preparation of experimental trials. Body length was measured as a curved line from the rostrum tip to urosome base in Image J (http://rsbweb.nih.gov/ij/). Rarefaction of datasets using size parameters recorded for amphipod groups used across all experimental systems indicated appropriate size-matching between large *G. pulex* (mean ± standard error (SE), length = 16.356 ± 0.121mm; weight = 57.461 ± 0.779mg) and intermediate *D. villosus* (length = 16.656 ± 0.132mm; weight = 57.314 ± 0.856mg; $p > 0.05$ for both body parameters). Large *D. villosus* were significantly larger (23.481 ± 0.130mm) and heavier (146.218 ± 1.963mg) than size-matched *D. villosus* and *G. pulex* in both experiments ($p < 0.001$ for both; see Appendix 2, Table A2.3).

2.3.2 Experimental Design – Functional Response (FR)

To compare predatory FRs of native and invasive amphipods against amphibian prey, three independent experiments were conducted in which amphipods were presented with amphibian prey in varying densities. The first experiment compared FRs between native and invasive amphipods towards invasive *X. laevis* embryos (mean ± SE diameter = 2.19 ± 0.02mm). The second experiment compared amphipod FRs towards native *R. temporaria* embryos (mean ± SE diameter = 7.83 ± 0.16mm). The third experiment assessed amphipod FRs towards *R. temporaria* larvae (mean ± SE length = 14.82 ± 0.31mm).

Prior to experimentation, individual amphipods were placed in clear plastic arenas (90mm diameter, 50mm height) with 250ml of dechlorinated tap water, and starved for 24 h. A single glass bead (20mm diameter, 9mm height) was placed in arenas as substrate, providing amphipods with shelter and to prevent continuous swimming. Amphipods were then transferred to experimental arenas, identical to those described above, containing a known number of invasive *X. laevis* embryos (2, 4, 6, 8, 10, 15, 20, 40 or 70 embryos), native *R. temporaria* embryos (1, 2, 3, 5, 8, 10 or 15 embryos) or freshly hatched native *R. temporaria* larvae (1, 2, 3, 5, 8, 10 or 15 larvae; see Appendix 2, Table A2.4 for details concerning developmental stages). Prey were situated in arenas two hours prior to the introduction of amphipod predators and the commencement of trials.

Experimental trials began with the introduction of a single amphipod predator and were conducted at 14.0 ± 0.1°C under a 12:12 h light:dark regime. Trials continued for 24 h (*X. laevis*/*R. temporaria* embryos) or 48 h (*R. temporaria* larvae), without replacing consumed prey. Trials concluded with the removal of amphipod predators and the enumeration of alive, dead or consumed prey. Dead prey which did not exhibit signs of predation were assumed to reflect background mortality (< 1.24% in all experiments). After terminating experimental trials, amphipods were monitored for a further 24 h. Amphipods that moulted or died were excluded from analysis. Following rarefaction to ensure appropriate size-matching, data pertaining to embryonic prey treatments was retained for five replicates, whilst the larval prey treatment comprised up to eleven replicates at all prey densities. Controls consisted of five (embryos) or eleven (larvae) replicates of each prey density, without amphipod predators present.

2.3.3 Field Sampling – Estimating Amphipod Abundance

In November 2017, field sampling was undertaken at several un/invaded freshwater sites within Great Britain to estimate field abundances of native *G. pulex* and invasive *D. villosus* within these regions. *Dikerogammarus villosus* were sampled from six sites situated around the perimeter of Grafham Water, Cambridgeshire (Appendix 2, Table A2.5). Sampling was conducted approximately 2m from the shoreline within a 50 x 50cm area ($0.25m^2$), using a modified, bottomless receptacle (65cm height, 50cm width, 80L volume) which allowed access to the underlying substrate. Over a five-minute period, the substrate was agitated and netted, followed by two minutes of netting through the water column.

Gammarus pulex were sampled from five sites along Adel Beck and Meanwood Beck, West Yorkshire (Appendix 2, Table A2.5). Sampling was conducted in the centre of these lotic systems, within a 50 x 50cm area (0.25m^2). The substratum was agitated for 5 minutes and any amphipods dislodged were collected in a surber sampler. A further two minutes were spent hand sampling larger rocks present within the sampling area.

Amphipod specimens were stored in 70% ethanol. In the laboratory, amphipod specimens were sorted into size categories, matching those selected for FR trials, and enumerated.

Abundance data was supplemented using estimates reported in previously published literature; recorded for *G. pulex* within native ranges (Welton, 1979; Oertli, 1993; Lods-Crozet and Reymond, 2006; Harkness, 2008; Leberfinger and Herrmann, 2010; Lods-Crozet, 2014; Johns et al., 2018), and *D. villosus* within invaded ranges (Haas et al., 2002; Krisp and Maier, 2005; Lods-Crozet and Reymond, 2006; Mulattieri, 2006; Muskó et al., 2007; Platvoet et al., 2009b; Tricarico et al., 2010; Gergs and Rothhaupt, 2014; Lods-Crozet, 2014; Hellmann et al., 2017; Clinton et al., 2018). Data was taken from studies of amphipod abundance at sites where the presence of native *R. temporaria*, and other European amphibian species, had also been recorded (amphibian occurrence taken from recording databases; GBIF.org, 2004; National Biodiversity Network, 2017; INaturalist.org, 2020). Using published abundance estimations, the number of large *G. pulex*, intermediate *D. villosus* and large *D. villosus* was calculated, based on the proportional abundance of each amphipod size group recorded during field sampling.

2.3.4 Statistical Analysis

Statistical analyses were performed in R studio, version 3.3.2 (R Core Team, 2018). FR analyses were conducted using an integrated package for functional response analysis in R (*frair*, version 0.5.100; Pritchard et al., 2017).

2.3.4.1 Phenomenological Functional Response Analysis

A phenomenological approach was applied to each experimental combination (amphipod x prey type) to determine FR type (I, II, III) based on the general shape of the response curve. For each amphipod x prey type combination, logistic regressions of proportional prey consumption against prey density were performed, fitted with a quasibinomial error distribution to account for overdispersion. A significant negative first-order term was indicative of a Type II FR, whilst a significant positive first order term, superseded by a significant negative second order term denoted a Type III FR (Juliano, 2001).

2.3.4.2 Mechanistic Functional Response Analysis

Where analyses suggested that Type II FRs were appropriate, FRs were modelled using the Rogers' random predator equation (Eqn. 1; Rogers, 1972). This model accounts for the depletion and non-replacement of prey (Juliano, 2001).

$$N_e = N_O\left(1 - exp\left(a(N_e h - T)\right)\right) \qquad \text{(Eqn. 1)}$$

where N_e is the number of prey consumed, N_O is the initial density of prey, a and h represent the mechanistically explicable coefficients for attack coefficient (a) and handling time (h), and T is the total experimental period in days.

Using these parameters, maximum feeding rate was calculated as $1/Th$. FR models were fitted using the *frair_fit* function, which utilises maximum likelihood estimations (*bbmle::mle2*, version 1.0.20; Bolker and R Core Team, 2017), and a modified version of Eqn. 1, incorporating the *Lambert W* function to resolve the presence of N_e on either side of the equation (Eqn. 2).

$$N_e = N_O - \frac{lambertW\left(a \cdot h \cdot N_O \cdot exp\left(-a \cdot (T - h \cdot N_O)\right)\right)}{(a \cdot h)} \qquad \text{(Eqn. 2)}$$

Comparisons of attack coefficient (a) and handling time (h) were conducted between amphipod groups (within each prey type) using an 'indicator variable' approach to explicitly model differences in the parameter estimates for each amphipod group (*frair_compare* function; see Juliano, 2001; Pritchard et al., 2017; Taylor and Dunn, 2017).

Each fitted FR model was non-parametrically bootstrapped (n = 2000) to generate 95% confidence intervals, thereby visualising model variability. Additional non-parametric bootstrapping (n = 30) was applied to models, allowing multiple estimates of handling time (h), and thus maximum feeding rate ($1/Th$) to be calculated (Dodd et al., 2014; Laverty et al., 2017a; Cuthbert et al., 2018c). This generated mean (±SE) estimated maximum feeding rates for RIP calculations.

In FR experiments which utilised invasive *X. laevis* embryos as focal prey, negligible predation recorded in native *G. pulex* (5 embryos consumed across all replicates), relative to intermediate and large *D. villosus* (133 and 213 embryos consumed, respectively), prevented effective comparisons of FR curves and parameters between native and invasive amphipods. Instead, Chi-square (χ^2) tests were applied to compare the frequency of predation (i.e. proportion of individuals that consumed embryos) recorded between amphipods. Chi-squared tests were also conducted for FR experiments with native

R. temporaria larvae, given the relatively low incidence of predation recorded in size-matched amphipod groups, compared to large *D. villosus*. In FR experiments with native *R. temporaria* embryos, a complete absence of predation prevented statistical analyses.

2.3.4.3 Amphipod Field Abundance Estimates

Field abundance estimates for native and invasive amphipods were compared using a generalised linear model (GLM), fitted with a quasipoisson error distribution to account for overdispersion. A *post-hoc* Tukey HSD test (α = 0.05; *multcomp::glht*, version 1.4-8; Hothorn, Bretz and Westfall, 2008), was subsequently conducted to compare field abundance estimations between amphipods.

2.3.4.4 Relative Impact Potential (RIP) Analysis

Mean (±SE) estimates for maximum feeding rates (i.e. FR), generated from bootstrapped models, and field abundances (AB) were incorporated into the RIP metric (Eqn. 3), enabling pairwise comparisons of relative impact potential between invasive and native amphipods. Using a probability density function (pdf), standard errors were incorporated into the RIP metric, allowing confidence intervals (60% and 80% CIs) and RIP probabilities (RIP > 1) to be generated for invasive amphipods, when compared to native *G. pulex* (Dick et al., 2017).

$$RIP = \left(\frac{Invader\ FR}{Native\ FR}\right) x \left(\frac{Invader\ AB}{Native\ AB}\right) \qquad \text{(Eqn. 3)}$$

Due to the potential ecological significance of invader predation upon native amphibian species, RIP analyses focussed on FR models pertaining to the predation of native *R. temporaria* larvae. ‘RIP biplots’ were generated, presenting the RIP values of the three amphipod groups using field abundance estimates as a proxy for numerical response (see Laverty et al., 2017a; Dickey et al., 2020).

2.4 Results

2.4.1 Predation of Invasive *X. laevis* Embryos

Prey survival was 100% in all control treatments, which was significantly higher than within invasive amphipod treatments (intermediate *D. villosus* = 84.8% and large *D. villosus* = 75.7% survival; Fisher’s exact test $p < 0.001$ for both), but not large *G. pulex* (99.4% survival; $p = 0.06$). Therefore, mortality was attributed to amphipod predation. When presented with invasive *X. laevis* embryos, predation by large native *G. pulex* was minimal. Only 5 of 45 individuals consumed embryos. By comparison, a significantly higher incidence of predation was observed in intermediate (i.e. size-matched with *G. pulex*) and large invasive *D. villosus* (44 of 45 individuals for both; $\chi^2 = 105.138$, df = 2, $p < 0.001$).

2.4.1.1 Functional Responses

Logistic regressions revealed significantly negative first order terms by invasive *D. villosus* against *X. laevis* embryos, confirming the expression of Type II FRs (Figure 2.1a; Appendix 2, Table A2.6). When compared to intermediate *D, villosus*, large *D. villosus* displayed significantly lower handling times on *X. laevis* embryos, whilst attack coefficients (i.e. initial slope of FR curves; see Figure 2.1a) did not differ statistically (Table 2.1-2.2). Estimated maximum feeding rates (i.e. asymptote of FR curve; Figure 2.1a) were substantially higher in large *D. villosus*, consuming considerably more prey items during the experimental period, compared to intermediate invaders (Table 2.3). Negligible predation by large *G. pulex* prevented an FR curve from being plotted or compared with invasive amphipods. Significantly lower handling times and higher maximum feeding rates estimated for large *D. villosus* translated into a noticeably higher FR curve, compared to intermediate *D. villosus* (Figure 2.1a). Although 95% confidence intervals overlapped at lower prey densities, the expression of a steep initial FR gradient by larger individuals resulted in the separation of confidence intervals at higher densities.

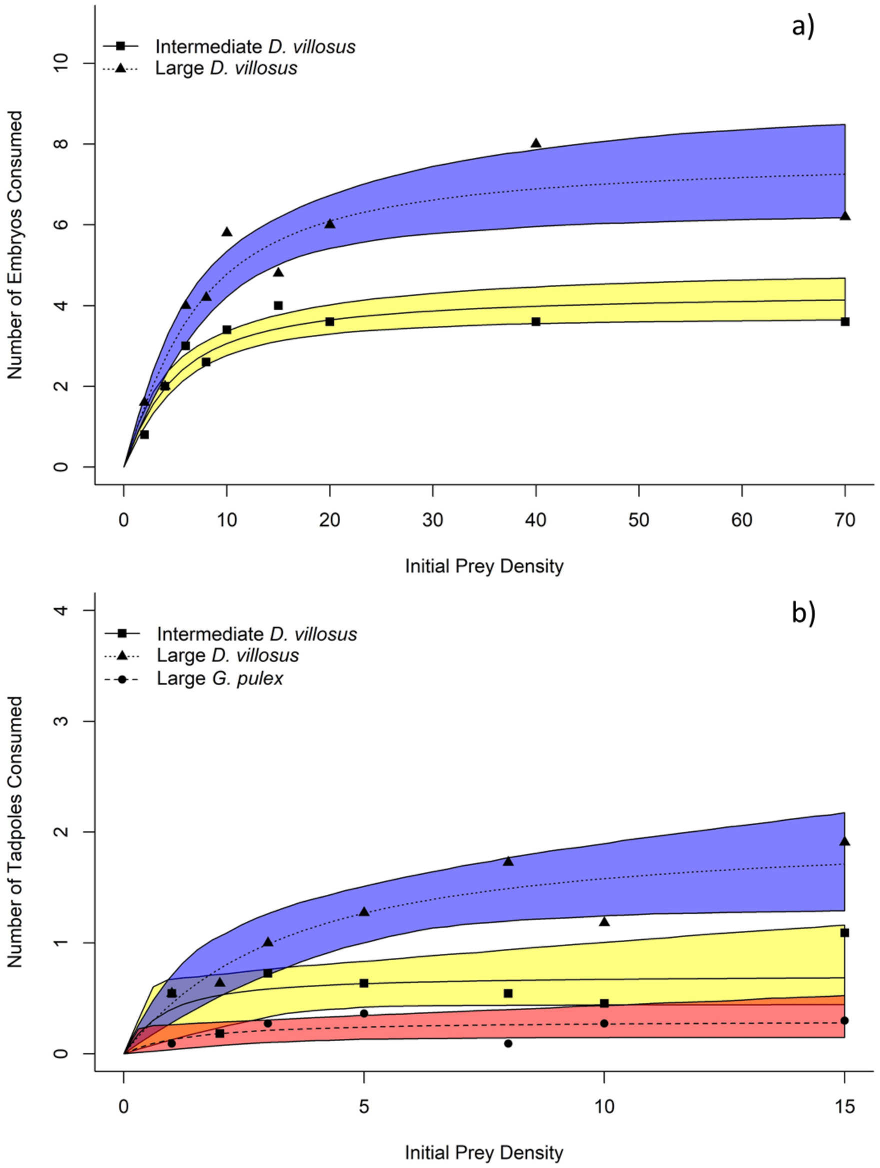

Figure 2.1. Type II functional response curves for large *G. pulex* (filled circles with dot-dash black line), intermediate *D. villosus* (filled squares and solid black line) and large *D. villosus* (filled triangle and dotted black line) towards (a) non-native *X. laevis* embryos (n = five replicates per prey density), and (b) native *R. temporaria* larvae (n = up to 11 replicates per prey density). *Shaded Regions* display bootstrapped 95% confidence intervals (red: large *G. pulex*, yellow: intermediate *D. villosus*, blue: large *D. villosus*).

Table 2.1: Functional response parameter estimates for each amphipod size group (intermediate *D. villosus*, large *D. villosus* and large *G. pulex*) feeding upon invasive *X. laevis* embryos and native *R. temporaria* larvae as prey. Estimates extracted from the Rogers' Random Predator Equation, fitted in the *frair* package (Pritchard et al., 2017).

Prey treatment	Amphipod group	Parameter	Estimate (±SE)	z	P
X. laevis embryos	Intermediate *D. villosus*	a	0.616 (±0.186)	3.311	< **0.001*****
		h	0.459 (±0.061)	7.590	< **0.001*****
	Large *D. villosus*	a	0.850 (±0.183)	4.650	< **0.001*****
		h	0.258 (±0.028)	9.114	< **0.001*****
R. temporaria larvae	Large *G. pulex*	a	0.120 (±0.141)	0.853	0.394
		h	6.600 (±2.721)	2.425	**0.015***
	Intermediate *D. villosus*	a	0.597 (±0.719)	0.529	0.407
		h	2.803 (±0.709)	3.955	< **0.001*****
	Large *D. villosus*	a	0.392 (±0.150)	2.612	< **0.01****
		h	0.988 (±0.213)	4.631	< **0.001*****

a – attack coefficient, h - handling time (days prey item^{-1}), SE – Standard error.

Significant differences from null (i.e. zero) are indicated in bold. Asterisks indicate significance level of P values; * = $P < 0.05$, ** = $P < 0.01$, and *** = $P < 0.001$.

Table 2.2: Comparison of functional response parameter estimates for three amphipod size groups (intermediate *D. villosus*, large *D. villosus* and large *G. pulex*) feeding upon invasive *X. laevis* embryos and native *R. temporaria* larvae as prey. Comparisons based on analyse conducted using 'indicator' variables in the *frair* package (Pritchard et al., 2017).

Prey treatment	Base group	Comparator group	Parameter	Estimate (±SE) of difference (Da or Dh)	z	P
X. laevis embryos	Large *D. villosus*	Intermediate *D. villosus*	a	0.233 (0.262)	0.890	0.373
			h	-0.202 (0.067)	-3.016	**0.003**
R. temporaria larvae	Large *D. villosus*	Large *G. pulex*	a	0.272 (0.205)	1.328	0.184
			h	-5.606 (2.726)	-2.056	**0.040**
	Intermediate *D. villosus*	Large *G. pulex*	a	0.477 (0.731)	0.652	0.515
			h	-3.795 (2.811)	-1.350	0.177
	Large *D. villosus*	Intermediate *D. villosus*	a	-0.204 (0.735)	-0.278	0.781
			h	-1.814 (0.740)	-2.451	**0.014**

a – attack coefficient, h - handling time (days prey item^{-1}), SE – Standard Error

Significant differences between FR parameters, measured between base and comparator groups, are indicated in bold.

2.4.2 Predation of Native *R. temporaria* Embryos

Survivorship of native *R. temporaria* embryos within both control and experimental amphipod treatments was absolute. In 105 replicated trials (across the three amphipod treatments), no embryos were consumed, although evidence of attempted predation by invasive *D. villosus* was observed (Figure 2.2). To determine whether unsuccessful predation affected embryonic viability, embryos which exhibited signs of predation by *D. villosus* were maintained at 14.0 ± 0.1°C, and reared until hatching (i.e. G. 19-20; Gosner, 1960). Predation attempts by *D. villosus* did not appear to adversely affect embryonic development and/or survival, with all larvae successfully hatching, without any apparent deformities. As such, analysis could not be conducted further.

In an additional trial to confirm that *R. temporaria* embryos were palatable, amphipods were offered ten *R. temporaria* embryos which had been subjected to considerable mechanical damage (n = 3 replicates per amphipod group). Predation by invasive amphipods was observed, with large *D. villosus* consuming an average of 3.6 embryos and intermediate *D. villosus* consuming 2.3 embryos. *Gammarus pulex* did not consume any embryos.

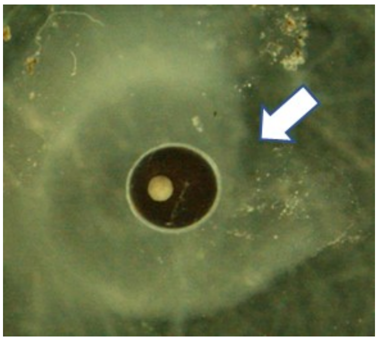

Figure 2.2. *Rana temporaria* embryo exhibiting signs of attempted predation upon vitelline jelly capsule. Note: White arrow indicates area of attempted predation

2.4.3 Predation of Native *R. temporaria* Larvae

Native *R. temporaria* larvae experienced negligible mortality in control treatments (1.2%), whilst mortality was significantly higher when exposed to large *D. villosus*, intermediate *D. villosus* and large *G. pulex* (18.8%, 9.5% and 3.6%, respectively; Fisher's exact test $p < 0.05$ for all). Predation of larvae was observed, as well as fragments of partially consumed larvae, following the removal of amphipod predators (Figure 2.3a-c). Therefore, mortality was attributed to predation by amphipods. Predatory frequency was highest for large *D. villosus*, with 53 of 77 individuals (68.83%) consuming larvae. This was significantly higher than frequencies recorded in intermediate *D. villosus* ($\chi^2 = 11.55$, df = 1, $p < 0.001$), for which 31 of 77 individuals (40.26%) were observed consuming native *R. temporaria* larvae. Predation was significantly less frequent in large *G. pulex*, with only 16 of 76 individuals (21.05%) consuming larvae ($p < 0.001$ for both).

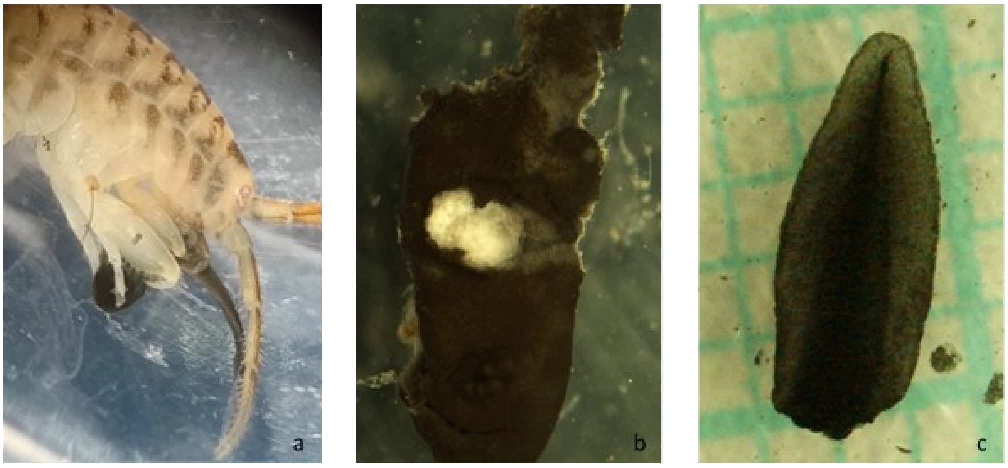

Figure 2.3. a) Observed predation by invasive *D. villosus* upon an early stage *R. temporaria* larva; b) a dead *R. temporaria* larva with tail missing following predation event with amphipod predator and signs of predation around head-body; c) a dead *R. temporaria* with head-body missing following predation event with amphipod predator.

2.4.3.1 Functional Responses

Logistic regressions identified significantly negative first order terms in all amphipod groups ($p < 0.05$ for all), indicating that native and invasive amphipods expressed Type II FRs towards native *R. temporaria* larvae (Figure 2.1b; Appendix 2, Table A2.6). Estimates for attack coefficients were statistically similar between amphipod groups ($p > 0.05$; Table 2.1-2.2). Comparisons between size-matched native and invasive amphipods revealed non-significant differences in estimates of handling time. In contrast, handling times were significantly lower in large *D. villosus* when compared to intermediate *D. villosus* and large *G. pulex* ($p < 0.05$ for both). Maximum feeding rates estimated for large *D. villosus* were considerably higher than size-matched native and invasive amphipods (Table 2.3), up to seven times greater when compared to large *G. pulex*. Superior maximum feeding rates translated into a higher FR curve with a distinct separation from smaller amphipods (Figure 2.1b).

2.4.3.2 Relative Impact Potential

Comparisons of total field abundance estimations revealed statistically significant differences in the abundances of native (mean ± SE, 170 ± 43.83 ind/m^2) and invasive (870 ± 259.79 ind/m^2) populations (GLM; $F_{(1,68)} = 17.589$, $p < 0.001$). When categorised based on body size, statistical comparisons also indicated significant differences between the field abundance estimates for the three amphipod groups ($F_{(2,92)} = 21.395$, $p < 0.001$; Table 2.3). *Post hoc* analyses revealed that intermediate *D. villosus* were significantly more abundant when compared to large *D. villosus* and *G. pulex* ($p < 0.001$), whilst abundance estimates did not differ between *G. pulex* and large *D. villosus* ($p = 0.925$).

The RIP metric returned a substantially greater impact potential in invasive *D. villosus*, relative to native *G. pulex* (Table 2.3). The RIP metric indicated that considerably higher RIP scores estimated for large and intermediate *D. villosus*, compared to *G. pulex*, were driven by different biological characteristics. Despite the non-significant differences in FR parameters between size-matched amphipods (Table 2.1), significantly superior field abundance estimates recorded for intermediate *D. villosus* generated a greater RIP score, than large *G. pulex* (Table 2.3). The RIP biplot illustrates this, with differential field abundance estimates generating a substantial vertical shift for intermediate *D. villosus* in comparison with the RIP for large *G. pulex* (Figure 2.4). Large *D. villosus* displayed similar field abundances, when compared to large *G. pulex* (Table 2.3). However, significantly lower handling times, and subsequently higher maximum feeding rates, resulted in a superior RIP scores, with the RIP biplot highlighting a substantial shift to the right when compared to *G. pulex* (Figure 2.4).

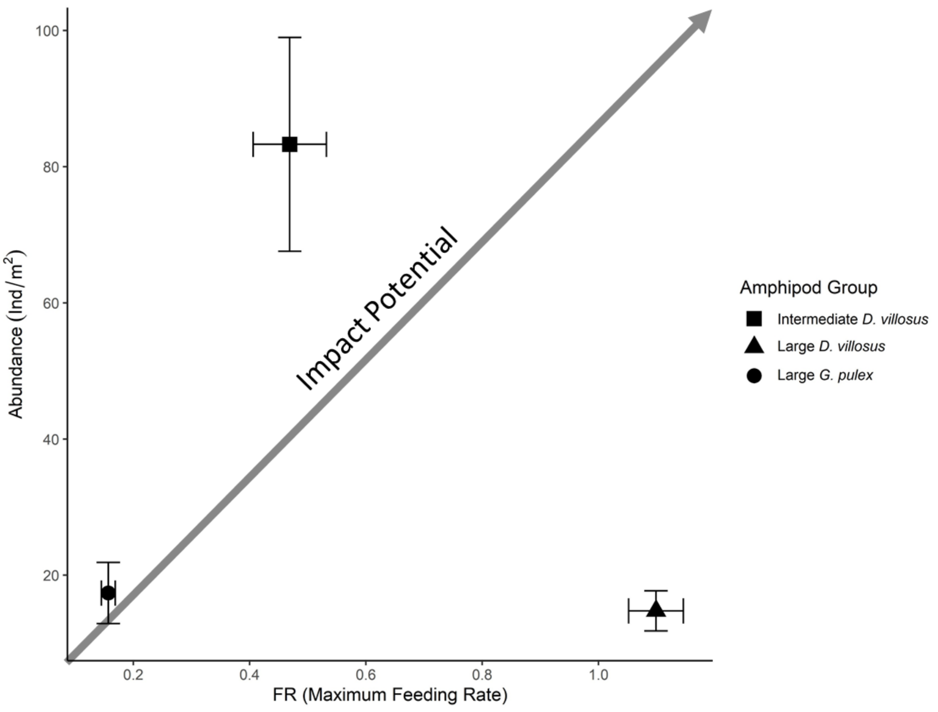

Figure 2.4. RIP biplots comparing intermediate *D. villosus* (filled square), large *D. villosus* (filled triangle) and large *G. pulex* (open circle) when feeding upon native *R. temporaria* larvae as prey. Biplots generated using mean ± standard errors (SE) estimates for FRs (i.e. maximum feeding rates) and field abundances (ind/m^2) recorded in each amphipod size treatment. Mean (±SE) FR parameters are generated from bootstrapped estimates (n = 30 bootstraps).

Table 2.3: Mean Relative Impact Potential (RIP) scores, generated using mean ± standard error (SE) estimates of maximum feeding rate (FR) and field abundance (ind/m^2), recorded for each amphipod group (intermediate *D. villosus*, large *D. villosus* and large *G. pulex*) whilst feeding upon native *R. temporaria* as prey. RIP scores are presented alongside estimates of uncertainty (60% and 80% confidence intervals; CIs) and the probability (%) that the RIP output will exceed 1. Mean (±SE) estimates of maximum feeding rates obtained through bootstrapping FR model n = 30.

Comparison								
Predator A (Base Group)	Predator B (Comparator Group)	Predator A Mean FR parameter (±SE)	Predator B Mean FR parameter (±SE)	Predator A Mean field abundance (ind/m^2 ±SE)	Predator B Mean field abundance (ind/m^2 ±SE)	RIP	Uncertainty 80% CI 60% CI	$P_{RIP} > 1$ (%)
Large *D. villosus*	Large *G. pulex*	1.099 (± 0.047)	0.157 (± 0.012)	14.760 (± 2.955)	17.378 (± 4.486)	6.379	4.555 – 7.990 5.544 – 6.565	100
Intermediate *D. villosus*	Large *G. pulex*	0.469 (± 0.063)	0.157 (± 0.012)	83.280 (± 15.710)	17.378 (± 4.486)	15.359	10.748 – 19.404 13.213 – 15.784	100
Intermediate *D. villosus*	Large *D. villosus*	0.469 (± 0.063)	1.099 (± 0.047)	83.280 (± 15.710)	14.760 (± 2.955)	2.509	1.849 – 3.098 2.215 – 2.587	99.780

2.5 Discussion

Over the past 20 years, *D. villosus* has spread rapidly throughout Europe (MacNeil et al., 2013a). Within invaded communities, *D. villosus* has a significant ecological impact, with considerable declines in native macroinvertebrate populations and altered ecosystem functioning ascribed to its competitive and predatory capabilities (Dick and Platvoet, 2000; van Riel et al., 2006; Bollache et al., 2008; MacNeil et al., 2010; MacNeil et al., 2013a; Jourdan et al., 2016; Worischka et al., 2018).

Whilst previous evidence concerning the predation of early stage amphibians by amphipods is scarce (see Fries and Tesch, 1965; Hudgens and Harbert, 2019), I provide the first empirical evidence that amphipods can kill and consume both embryonic and larval amphibians. Greater *per capita* feedings rates combined with higher densities in the field lead us to predict that invasive *D. villosus* might also exert a population-level effect upon amphibians within invaded communities.

Predation of invasive *X. laevis* embryos was observed, with *D. villosus* consuming a significantly larger number of embryos – increasing with invader body size – whilst predation by native *G. pulex* was negligible. When presented with native *R. temporaria* embryos, both native and invasive amphipods appeared incapable of consuming these larger embryos, although there was evidence of attempted predation. Predation of native *R. temporaria* embryos by invasive amphipods was only observed when embryos were damaged prior to exposure. *Rana temporaria* embryos are surrounded by a comparatively thick vitelline jelly capsule (Räsänen et al., 2003), which protects the eggs from some predators (Ward and Sexton, 1981). *Rana temporaria* embryos may be susceptible to predators with piercing, sucking mouthparts, yet reasonable invulnerable to predators which possess chewing, biting mouthparts (Henrikson, 1990), such as amphipods, thereby escaping potential predatory pressures exerted by invasive amphipods upon embryonic amphibians.

Invasive *X. laevis* embryos are comparably smaller than those generated by native *R. temporaria* (diameter = ~2mm and 8mm, respectively; pers. obs.), and other anurans (Duellman and Trueb, 1994). Our findings suggest that *D. villosus* may present a predatory threat to other native amphibian species with relatively small embryos, such as the great crested newt (*Triturus cristatus*; embryo diameter = ~4.5mm), the smooth newt and the palmate newt (*Lissotriton vulgaris* and *L. helveticus*; embryo diameter = ~3mm; Latham et al., 2005); of which several species have been recorded in regions invaded by *D. villosus* (e.g. Grafham Water and Pitsford Reservoir; Anglian Water, pers. comm; The Wildlife Trust for Bedfordshire, Cambridgeshire & Northamptonshire, pers. comm). Our findings also retain ecological relevance with regards to invasive *X. laevis* populations, given the previous existence of several feral populations in Great Britain (Tinsley et al., 2015a).

Native and invasive amphipods readily preyed upon *R. temporaria* larvae. Large *D. villosus* expressed significantly lower handling times, consuming early-stage amphibians at a greater rate than smaller amphipods, which translated into substantially higher maximum feeding rates, seven times greater than large *G. pulex*. As such, the higher *per capita* prey intake observed in significantly larger invaders is likely explained by the naturally larger body size of *D. villosus*, rather than any interspecific differences in innate predatory ability (Dodd et al., 2014; Taylor and Dunn, 2017). Our observation of higher consumption by larger amphipods is consistent with previous studies (Dodd et al., 2014; Taylor and Dunn, 2017), and by extension, general biological theory (Rall et al., 2012). Metabolic theory predicts that metabolic rate typically exhibits positive allometric scaling relative to size (Glazier, 2010), with greater metabolic demand requiring high resource intake to maintain fundamental biological processes (Brown et al., 2004).

Superior consumptions rates by larger amphipods may be facilitated by larger mouthparts and gnathopods, allowing individuals to capture and consume a wide range of prey (Mayer et al., 2008), or a larger gut capacity required to efficiently digest food items (Vucic-Pestic et al., 2010). The presence of large glycogen reserves in *D. villosus*, compared to other amphipod species, allow this invader to overcome various anti-predatory evasion behaviours demonstrated by larval amphibians, such as burst-swimming (Maazouzi et al., 2011); a trait which appears superior in ranids, compared to other anurans (e.g. bufonids; Álvarez and Nicieza, 2009). Given that *R. temporaria* larvae remain vulnerable to predation until complete metamorphosis (Ward and Webster, 2016), I posit that the potential impact of invasive *D. villosus* could persist throughout development, with prolonged predation on growing larvae in the field continuing until they achieve a size or developmental stage which is invulnerable to *D. villosus*.

The comparative FR approach revealed that, when compared to native *G. pulex*, invasive *D. villosus* generally exhibited a higher Type II FR. This differential predatory response became more apparent in larger invaders, with significantly higher FRs exhibited by large *D. villosus* feeding upon embryonic and larval amphibians. Separation between FR curves generated for large invaders and size-matched amphipods across both prey systems would imply the potential for *D. villosus* to impose a greater predatory impact upon native amphibian populations, compared to its native analogue.

Type II FRs are indicative of potentially unstable predator-prey interactions (Price et al., 2011). At higher prey densities, *per capita* predation rates decelerates to an asymptote as consumption become limited by consumer handling times (Price et al., 2011). As a result, an unstable equilibrium is attained, centred on the asymptotic point (Price et al., 2011). If predation levels exceed the growth capacity of prey populations existing at densities below the established equilibrium point, predator-prey dynamics may destabilise resulting in the extirpation of affected prey populations (Juliano, 2001; Price et al., 2011). Differential Type II FRs identified in comparative laboratory-based studies of predation on macroinvertebrate prey are consistent with observed field patterns of reduced macroinvertebrate diversity and abundance (Bollache et al., 2008; MacNeil et al., 2013a). Our findings suggest a similar threat may extend to amphibians in the field.

When compared to native amphipods, *D. villosus* exhibits notably higher fecundity and a short interbrood interval (Pöckl, 2007), allowing this invader to rapidly form “super-abundant” populations in invaded regions (van Riel et al., 2006). The RIP metric highlighted a significantly greater impact potential by invasive *D. villosus* than by native *G. pulex*, driven by both larger body size and greater abundance of this invasive species. Large *D. villosus* exhibited a higher *per capita* impact than native *G. pulex*, translating into an RIP score 6.4 times stronger than native *G. pulex*. A superior RIP score was also predicted for intermediate *D. villosus*, when compared to *G. pulex*. Whilst the comparative FR approach identified no significant difference between *per capita* effects recorded in size-matched native and invasive amphipods, inclusion of field abundance estimates into the RIP metric detected a substantially higher impact potential for intermediate *D. villosus*, with significantly higher field densities (4.8 times higher than *G. pulex*) generating a predicted impact score approximately 15 times greater than its native counterpart.

The RIP metric considers the effect of differential field abundances of natives and invaders, but assumes that consumer interactions are explicitly advantageous (Dick et al., 2017). In reality, interactions between consumers may be additive (Barrios-O’Neill et al., 2014a), synergistic (Sentis and Boukal, 2018), or antagonistic (Médoc et al., 2015). By incorporating such context dependencies into FR models, we might further refine predictions. Nevertheless, the RIP metric has proven to be an effective predictive tool when applied to previous literature. Estimations of invader RIP support alternative impact measurements (e.g. Laverty et al., 2015b) and correspond to observed field impacts (Dick et al., 2017). As such, the RIP metric has formed the foundation for a range of alternative quantitative metrics (see Dickey et al., 2020).

In the current study, the RIP metric highlighted a significantly greater impact potential by invasive *D. villosus* towards native early-stage amphibians, when compared to native *G. pulex*. These findings are consistent with similar magnitudinal patterns of differential impacts identified in *D. villosus* towards other freshwater organisms (see Dick et al., 2017). However, our estimates for the abundance of *D. villosus* in Grafham Water Reservoir were considerably lower than those recorded in other European and UK localities in which *R. temporaria* have been reported. The potential for *D. villosus* to reach higher densities indicate that the potential impact of this invader upon early-stage amphibians may be even stronger in other invaded regions.

2.6 Conclusions

This is the first empirical evidence of predation of early-stage amphibians by freshwater amphipods. The invasive *D. villosus* exhibited consistently higher *per capita* predation rates upon invasive amphibian embryos and native amphibian larvae, with predation increasing as a function of invader body size. The detection of Type II FRs, significantly higher in large-bodied invaders, are indicative of the potential ecological impact of *D. villosus*, with higher predation rates predictive of a depletive, potentially destabilising effect upon amphibian populations, through the consumption of vulnerable embryos and larvae. This higher ecological impact, predicted for invasive *D. villosus*, is further intensified when the higher field abundances of this invasive amphipod are considered.

Large-bodied invasive predators are acknowledged as primary drivers of global amphibian declines (Kats and Ferrer, 2003; Ilhéu et al., 2007). With evidence of potential co-occurrence between *D. villosus* and native amphibians, recorded in both UK freshwaters (Anglian Water, pers. comm; Cardiff Harbour Authority, pers. comms) and in mainland Europe (Uehlinger et al., 2009; Gergs and Rothhaupt, 2014; Koester et al., 2016; Haubrock et al., 2019), the findings of the current study predict that the highly predacious killer shrimp may further contribute to declining amphibian populations through the predation of early life-stages. Given the projected expansion of *D. villosus* into British freshwaters (Gallardo et al., 2012b; Gallardo and Aldridge, 2020), likely facilitated by recreational water users (e.g. anglers; Smith et al., 2020), we might predict the introduction of *D. villosus* into amphibian-rich areas in the near future, with consequences for amphibian populations expected to follow. However, further research is required to determine the suitability of different freshwater habitats which are typically used by breeding amphibians.

Chapter 3

Prey-to-Predator Ratio Dependence in the Functional Response of Invasive *Dikerogammarus villosus* Predicts a *Per Capita* Reduction in Biotic Pressures towards Native UK Amphibians

3.1 Abstract

Invasive predators typically impose strong detrimental impacts upon invaded communities, often through the consumption of native species. Comparisons of functional response (FR) – the relationship between individual feeding rate and resource availability – have previously been applied as a means of predicting the ecological impact of invasive predators upon native prey species. However, invasive predators also often dominate invaded communities in terms of biomass and abundance. As such, individuals may interact with one another whilst accessing shared prey, thus altering *per capita* consumption through interference. Here I examine how mutual interference amongst interacting invaders may alter the predicted ecological impact of the invasive freshwater amphipod *Dikerogammarus villosus* towards larvae of the native amphibian *Rana temporaria*, by measuring *per capita* predation under different combinations of *D. villosus* and *R. temporaria* densities. I compared a range of different FR models, consisting of the three main forms of FR (Type I, II, and III), and the three main theoretical FR classes which consider *per capita* predation to be, a) prey dependent (a function of prey density), b) predator-dependent (a function of prey and predator density), or c) ratio-dependent (a function of prey-to-predator ratio). I found that invasive *D. villosus* may exhibit a Type II ratio-dependent FR towards native *R. temporaria*, with a reduction in *per capita* predation predicted at higher predator densities, and the emergence of mutual interference expected to lead to perfect prey-sharing. Spatial arrangement of invasive *D. villosus* in each prey-predator combination was also examined using the index of aggregation (i.e. variance-to-mean ratio). *Dikerogammarus villosus* exhibited high levels of gregariousness, with aggregation towards conspecifics increasing over time, resulting from increasing rates of prey depletion occurring at higher predator densities. These findings predict a reduction in predatory pressures by invasive *D. villosus* towards larval amphibians, resulting from non-aggressive interferential behaviours (e.g. kleptoparasitism).

3.2 Introduction

Predation is a fundamental ecological process responsible for determining the composition, stability and functioning of communities (Start and Gilbert, 2017). As such, studying the complexities of predator-prey systems is central to understanding the role that predation can play in driving community dynamics (Hassell, 1978; Turchin, 2013). This is especially important when assessing the ecological impact of invasive alien species (IAS; Dick et al., 2014). IAS are renowned for altering the composition of invaded native communities (Simberloff et al., 2013), particularly within freshwater ecosystems, which are disproportionately affected (Moorhouse and Macdonald, 2015). Following the introduction of invasive predators, communities often experience a reduction in abundance and diversity across multiple trophic levels, driven primarily by intense predation (Doherty et al., 2016b).

When compared to trophically analogous native predators, invaders typically consume prey at a much higher efficiency (reviewed in Dick et al., 2014, 2017). The comparative functional response (FR) approach has been applied extensively when assessing differential predation by native and invasive predators. By examining the relationship between *per capita* prey consumption and prey availability (i.e. FR), comparisons of invader FRs relative to those of trophically analogous native species, can provide useful, quantifiable insights when assessing and/or predicting invader impacts upon local prey populations (Dick et al., 2014). Typically, consumers exhibit one of three classic FR types (Type I, II, III); each describing a different consumer-resource relationship. A Type I response denotes a positive linear relationship between *per capita* consumption and resource availability, and is characteristically observed in filter-feeding organisms (Jeschke et al., 2004). A Type II response describes consumption increasing at a decelerating rate (i.e. saturating hyperbolic curve) and is frequently displayed by predators with the potential to destabilise prey populations; often resulting from “boom-burst” population dynamics (Médoc et al., 2015). A Type III response is characterised by limited consumption at low prey densities, increasing at a decelerating rate at higher densities (i.e. sigmoidal curve) and is indicative of more stabilising predator-prey dynamics, providing prey with refugia from predation at low densities due to prey-switching (Sarnelle and Wilson, 2008). In the laboratory, the expression of Type II FRs by invaders have been recorded across a variety of aquatic taxa (reviewed in Dick et al., 2014). When compared to trophically analogous native predators, invaders often demonstrate a higher rate of consumption across prey densities, as reflected in greater magnitudes of FR. Superior Type II FRs demonstrated by invaders frequently reflect observed declines of certain prey species, and the subsequent extirpation of affected species from invaded field communities (reviewed in Dick et al., 2014, 2017).

Within invaded regions, invasive predators often exist at greater densities when compared to native analogues (Hansen et al., 2013), which may exacerbate invader impacts (reviewed in Dick et al., 2017; Dickey et al., 2020). Typically, the presence of other predators is associated with the sharing of available resources, which can directly or indirectly interfere with individual feeding behaviours, subsequently effecting *per capita* consumption rates. Interference amongst conspecific predators – commonly referred to as "mutual interference" – can amplify or impair individual foraging success, depending upon the context of predator-predator interactions (Médoc et al., 2013). When considered in conjunction with superior invader FRs, interactions amongst invasive conspecifics that are driven by greater invader abundance, may accelerate the decline of native species (Dick et al., 2017; Dickey et al., 2020).

The invasive killer shrimp *Dikerogammarus villosus* (Sowinsky, 1894) is considered to be one of the 100 worst invasive species in Europe (Nentwig et al., 2018). *Dikerogammarus villosus* is a voracious predatory amphipod, renowned for exerting a strong negative impact upon local aquatic biota and driving the extensive and irreversible reformation of native freshwater communities (MacNeil et al., 2013a). In the laboratory, *D. villosus* consumes a broad range of native macroinvertebrate species (reviewed in Rewicz et al., 2014). Predation can also extend to some aquatic vertebrates, including embryonic and juvenile fish and amphibians (e.g. Taylor and Dunn, 2017; Chapter 2). In the field, this flexible carnivorous diet is conserved, with stable isotope analysis identifying a wide diversity in the type of prey consumed (van Riel et al., 2006). As such, *D. villosus* is acknowledged as a top predator similar to many fish species (van Riel et al., 2006), with intense predation thought to lead directly to the loss of native prey species, and the subsequent destabilisation of invaded freshwater communities and ecosystems (Bollache et al., 2008; MacNeil et al., 2013a).

Previous studies have reported evidence of *D. villosus* expressing potentially destabilising Type II FRs towards a range of freshwater prey species, with significantly greater predation rates recorded when compared to native amphipods (reviewed in Dick et al., 2014, 2017; also see Taylor and Dunn, 2017; Chapter 2). Superior predatory responses exhibited by this invader corroborate observed declines, and the extirpation of native prey in the field (Bollache et al., 2008; Dodd et al., 2014). However, many of these FR studies focus only on a single predator provided with a range of prey densities, in order to predict population-level impacts. Known as "prey dependence" – this form of FR model assumes that predatory FR is a function of prey abundance (N) only, excluding considerations of other biologically important traits, such as the effects of predator population abundance (Arditi and Ginzburg, 2012).

Gammarids are highly gregarious organisms, known to form dense aggregations (Beermann et al., 2015). Within invaded regions, *D. villosus* can form 'super-abundant' populations, with densities of up to 10,000 individuals per m^2 having been recorded (e.g. van Riel et al., 2006). With reports suggesting that *D. villosus* may contribute up to 90% of total community abundance in some areas (see Arndt et al., 2009), this invader frequently dominates native assemblages in terms of relative abundance and biomass (van Riel et al., 2006; van Riel, 2007). Interactions amongst densely aggregated conspecifics may modify predatory behaviours, leading to substantial alterations to previous predictions concerning the ecological consequences of *D. villosus* within invaded field systems (see Dick et al., 2017; but also Médoc et al., 2015).

Recent studies have attempted to incorporate the effects of predator abundance on *per capita* predation by utilising adapted versions of the original "prey-dependent" FR model class; known as "predator-dependent" and "ratio-dependent" (reviewed in Arditi and Ginzburg, 2012). Predator dependence – and, by extension ratio dependence – considers *per capita* predation to be a function of the absolute abundance of prey and predators, or the ratio of prey-to-predators, respectively. Both modified FR classes take into account variations in predator and prey abundance and consider the effects of mutual interference between interacting predators (Médoc et al., 2015).

Over the years several FR mathematical formulations have been developed to account for predator interference when predicting consumer responses. However, for each model variant developed, the process by which interference is considered and/or incorporated differs. For example, the Holling Type II FR – a standard model belonging to the classic prey-dependent FR class – can be modified through the addition of predator abundance (P) and a mutual interference coefficient (c), thereby forming the additive, predator-dependent Beddington-DeAngelis Type II FR model variant (Beddington, 1975; DeAngelis et al., 1975; see Table 3.1). Conversely, by incorporating the ratio of prey-to-predator abundance (N/P) into the classic Holling II model, we arrive at the ratio-dependent Arditi-Ginzburg II model, which is regarded as a special case of predator dependence in which mutual interference is considered implicitly (Arditi and Ginzburg, 1989). From these different model variants consumer FRs can be viewed along a spectrum, depending on the effect that predator interference has upon *per capita* consumption rates. At one end of the spectrum the purely prey-dependent Holling models can be found, at which point mutual interference is essentially non-existent. At the other end lies the ratio-dependent Arditi-Ginzburg model, whereupon mutual interference leads to perfect prey-sharing. In between these two extremes, various predator-dependent FR models can be found (e.g. Beddington-DeAngelis model; Arditi and Ginzburg, 2012). There are also several predator-dependent FR variants which are dynamic in their expression of mutual interference. For example, the Arditi-Akçakaya model and the Hassell-Varley model both consider predator interference as a generic multiplicative process (Novak and Stouffer, 2020). By incorporating a mutual interference coefficient (c) as an exponent, this parameter becomes dimensionless, enabling models to position themselves at any point along the FR spectrum, depending on the strength of interference. As a result, both the Arditi-Akçakaya and Hassell-Varley models have the ability to reflect prey dependence when interference is absent, predator dependence when the level of interference is intermediate, or ratio dependence when interference is high in magnitude (Hassell and Varley, 1969; Arditi and Akçakaya, 1990). A more recent predator-dependent formulation, the Tyutyunov II model, also incorporates mutual interference as a continuous process, and as such can resemble prey-, predator- or ratio dependence. However, unlike the Arditi-Akçakaya and Hassell-Varley models, the Tyutyunov II model incorporates an interference coefficient (c) which quantifies the level of predator density at which ratio dependence gradually replaces prey dependence, rather than the intensity of interference between conspecific predators. At low predator densities, the Tyutyunov model approximates to the prey-dependent Holling model, whereas at high predator densities it resembles the ratio-dependent Arditi-Ginzburg model (Tyutyunov et al., 2008).

By measuring predation rates, relative to controlled variations in prey and predator abundance, predator dependence – and by extension ratio dependence – has been reported to be the primary predatory response displayed by a range of predatory taxa (reviewed in Arditi and Ginzburg, 2012). Here I examine the density-dependent effects of predator-prey population dynamics on the predatory impact of invasive *D. villosus* towards the early life-stages of the native amphibian *Rana temporaria* (European common frog). For the past 50 years, amphibians have suffered significant declines (Alford, 2011), with invasive predators recognised as primary contributors (see Nunes et al., 2019). Recently, invasive *D. villosus* has been shown to consume *R. temporaria* larvae (Chapter 2). Here I investigate how the presence of interacting conspecific predators may influence the *per capita* effect of invasive *D. villosus* towards native *R. temporaria* larvae. Through controlled variations of both prey and predator densities, I fit a range of different FR model variants to determine whether invasive *D. villosus* exhibits a prey-, predator-, or ratio-dependent FR towards early-stage *R. temporaria* in response to changing predator-prey dynamics. I evaluate how shifting dynamics may drive the emergence of mutual interference amongst predators, thereby altering predictions regarding the potential ecological impact of this invader. Furthermore, I examine the mechanisms underpinning observed conspecific interference by investigating how individual amphipods arrange themselves spatially over time under different combinations of prey and predator densities. This study was originally designed to be comparative, exploring differential feeding rates between invasive *D. villosus* and the native amphipod *Gammarus pulex* towards native *R. temporaria* larvae. However, following observations of negligible predation by native *G. pulex*, compared to the frequent consumption of larvae recorded in invasive *D. villosus*, the focus of this study shifts to evaluate the predatory impact of invasive *D. villosus* only.

3.3 Material and Methods

3.3.1 Collection and Maintenance of Study Organisms

3.3.1.1 Native *Rana temporaria* embryos/larvae

Between February and April 2018, *R. temporaria* embryos were collected, within ~36 hrs of fertilisation, from freshwater sites throughout Yorkshire (see Appendix 3, Table A3.1 for sampling sites). Whole clutches of embryos were removed and approximately halved, with half of the clutch stored for transport to the laboratory in an insulated container containing site-sourced water. The other half of each clutch was returned to the site. To minimise the effect of sampling on local *R. temporaria* populations, no more than 5% of embryos at each site were harvested.

In the laboratory, embryos were stored as half-clutches in individual aerated aquaria with dechlorinated tap water. Tanks were cleaned and waters changed twice weekly. Embryos were reared under controlled laboratory conditions (4.0 ± 0.2°C under a 12:12 h light:dark regime), until they reached late embryonic stages consisting of a pre-hatched larval form still encapsulated within the vitelline jelly envelope. Pre-hatched larvae possessed functionally developed gills and circulatory system and displayed reflexive neuromuscular responses (i.e. Gosner stage or G. 18-19; Gosner, 1960). At G. 18-19, embryos were transferred and gradually acclimatised over 24 h to 14°C in preparation of hatching and use in experimentation. Only freshly hatched *R. temporaria* larvae (i.e. G. 19-20; mean ± SEM length = 13.005 ± 0.082mm) were used in experimental trials.

3.3.1.2 Amphipods

Between April and May 2018, invasive *D. villosus* were collected from submerged artificial substrates at Grafham Water Reservoir, Cambridgeshire (52°17′31.2″N 0°19′23.6″W). In the laboratory, amphipods were housed in aquaria with aerated dechlorinated tap water, maintained at 14 ± 0.2°C under a 12:12 hr light:dark regime. Amphipods received conditioned sycamore leaves (*Acer pseudoplatanus*), *ad libitum*, for food. Amphipods were maintained in communal tanks for approximately 96 hrs prior to use in experimental trials. Prior to experimentation, amphipods were individually starved for 24 hrs in order to standardise hunger levels.

Only male amphipods were used in experimental trials, thereby avoiding sex-associated variability in predatory behaviours. Males were identified by the presence of genital papillae and the absence of brood plates (i.e. oostegites). Only large male amphipods were selected for use in experimental trials (mean ± SEM weight = 114.64 ± 2.40mg), thereby eliminating potential size-effects. Individuals were clear of visibly apparent parasitic infections, thereby avoiding potential parasite-mediated changes in predatory behaviours (e.g. Iltis et al., 2018). Individual amphipods were blotted dry and weighed at the end of experimental trials.

3.3.2 Experimental Setup

3.3.2.1 Amphipod Functional Response

Experimental trials were conducted at 14 ± 0.2°C and 12:12 hr light:dark regime. Experimental tanks were established, within which 5, 10 ,15, 20 or 30 *R. temporaria* larvae, and 1, 2, 5 or 10 invasive *D. villosus* were placed to test 20 combinations of prey (*N*) and predator (*P*) densities, corresponding to 14 prey-to-predator ratios (N/P = 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7.5, 10, 15, 20 and 30). Plastic tanks, measuring at 150 x 90 x 50mm (L x W x H), were filled with approximately 500ml of dechlorinated water. On the underside of each experimental tank, a grid comprising of 15 equal-sized squares was present, with each square measuring at 30.0 x 30.0mm. Amphipods have been shown to be positively thigmotactic and so will exhaust itself swimming around an empty experimental arena, where tactile stimuli are absent (Kohler et al., 2018). To avoid this problem, eight glass beads were placed in each experimental tank to provide shelter for amphipods whilst also allowing organisms to be observed. Glass beads were placed at equal distances from one another at positions where grid lines intersected (Figure 3.1), to minimise any effect that habitat may have on the spatial arrangement of amphipods (e.g. individuals selecting to aggregate around densely clustered substrates; see Kobak et al., 2015).

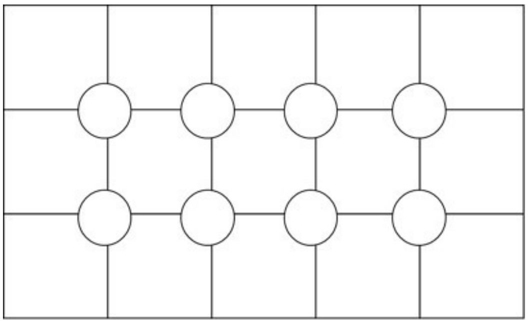

Figure 3.1. Diagram illustrating the design of experimental arenas.

This study was initially designed to be comparative, assessing the predation rates of invasive *D. villosus* relative to the native amphipod analogue *Gammarus pulex* under different combinations of prey and predator densities. However, following an initial set of trials using the aforementioned combinations of prey-predator densities (excluding combinations with 30 prey), with four replicates per combination tested (n = 64 trials in total), only a single predation event was recorded for native *G. pulex*, in which a single larva was killed and partially consumed. A relatively high incidence of amphipod mortality was also recorded whilst using native *G. pulex*, with deaths occurring in 23 of 64 replicated trials (35.94%). Of the 23 instances of native amphipod mortality, 17 were recorded in multi-predator treatments and were directly attributable to cannibalism of individuals by conspecifics (73.91%). Conversely, mortality in invasive *D. villosus* was comparably lower, occurring in only 15 of 120 replicated trials. Of these 15 instances, cannibalism was recorded in only two (13.33%). As such, the focus of this study shifted to examining the predatory impact of invasive *D. villosus* towards native *R. temporaria* larvae exclusively.

Prior to the commencement of experimental trials, larval *R. temporaria* prey were placed in arenas for 30 minutes to acclimate. Trials commenced with the introduction of amphipods, released from a single introductory position from which they then could dispersed into the remaining arena-space. Trials continued for 24 hours, after which amphipods were removed and the larvae enumerated for the following categories: “alive”, “dead”, “completely consumed” and “partially consumed”. Prey consumed during experimental trials were not replaced. Amphipods were used in experimental trials only once. If amphipods died during the experimental study period, that replicate was excluded from the dataset and a replacement trial was carried out.

Trials were replicated five times for each combination of prey and predator density. Five additional replicates were conducted for the one predator: five prey treatment (n = 10 replicates in total) due to scarce encounters between the predator and prey resulting in highly variable data. Controls, consisting of larvae present at the same densities as experimental treatments without amphipods, were conducted for each prey density (n = 5 replicates per density).

3.3.2.2 Amphipod Spatial Arrangement

To evaluate the effects of predator and prey abundance upon the spatial arrangement of amphipods over time, tanks established for each prey-predator combination of the FR experiment were photographed every 30 minutes over the initial eight hours of each experimental trial (n = 16 images per tank; 1680 images in total). From these images, the distribution of amphipods within the grid of 15 squares (i.e. the number of squares occupied and the number of individuals occupying each square) was recorded. From this, I calculated the index of aggregation (i.e. variance-to-mean ratio) as a representative metric for amphipod aggregation:

$$d = \frac{\sigma^2}{\bar{x}}$$

where σ^2 denotes variance, and $\bar{x}$ represents the mean. Aggregation indices <1 indicate a uniform distribution, whereas values >1 indicate aggregated patterns of distribution. If an index of 1 is calculated, individuals are randomly positioned in space. Due to the developmental age of native *R. temporaria* used in experimental trials prey spatial arrangement was not considered.

3.3.3 Statistical Analyses

Data analysis was conducted using the R software (version 3.5.1; R Core Team, 2018), with a significance level (α) of 0.05.

3.3.3.1 Amphipod Functional Response

Fourteen FR models, including a null model, were fitted and compared to explain the observed predation rates of *R. temporaria* larvae by invasive *D. villosus*. These competing FR models correspond to the three of the main classes of FR theory (prey-, predator-, and ratio-dependent), and describe the three main FR forms – Type I (linear), Type II (hyperbolic), Type III (sigmoidal; Table 3.1).

Table 3.1. Equations for the 14 candidate functional response models used to assess the functional response of invasive *D. villosus* whilst feeding upon native *R. temporaria* larvae. Models include the null, prey-dependent, predator-dependent and ratio-dependent FR classes, and correspond to Type I (linear), Type II (hyperbolic) and Type III (sigmoidal) FR forms. N is initial number of prey, P is predator density, a is the attack coefficient, h is the handling time, and c is the mutual interference coefficient.

Model Name	Dependence	Equation	Reference
Null Model		$f(N) = a$	
Holling Type I	Prey	$f(N) = aN$	(Holling, 1959b)
Holling Type II	Prey	$f(N) = \frac{aN}{1 + ahN}$	(Holling, 1959b)
Holling Type III	Prey	$f(N) = \frac{aN^2}{1 + ahN^2}$	(Holling, 1959b)
Beddington-DeAngelis Type I	Predator	$f(N,P) = \frac{aN}{1 + cP}$	
Beddington-DeAngelis Type II	Predator	$f(N,P) = \frac{aN}{1 + ahN + cP}$	(Beddington, 1975; DeAngelis et al., 1975)
Beddington-DeAngelis Type III	Predator	$f(N,P) = \frac{aN^2}{1 + ahN^2 + cP}$	(Kratina et al., 2009)
Hassell-Varley (Arditi-Akçakaya Type I)	Predator	$f(N,P) = \frac{aN}{P^c}$	(Hassell & Varley, 1969)
Arditi- Akçakaya Type II	Predator	$f(N,P) = \frac{aNP^c}{1 + ahNP^c}$	(Arditi & Akçakaya, 1990)
Arditi- Akçakaya Type III	Predator	$f(N,P) = \frac{aN^2P^c}{1 + ahN^2P^c}$	(Kratina et al., 2009)
Tyutyunov Type II	Predator	$f(N,P) = \frac{aN}{\frac{P}{c} + \exp\left(\frac{-P}{c}\right) + ahN}$	(Tyutyunov et al., 2008)
Arditi-Ginzburg Type I	Ratio	$f\left(\frac{N}{P}\right) = aN$	(Arditi & Ginzburg, 1989)
Arditi-Ginzburg Type II	Ratio	$f\left(\frac{N}{P}\right) = \frac{a\frac{N}{P}}{1 + ah\frac{N}{P}}$	(Arditi & Ginzburg, 1989)
Arditi-Ginzburg Type III	Ratio	$f\left(\frac{N}{P}\right) = \frac{a\left(\frac{N}{P}\right)^2}{1 + ah\left(\frac{N}{P}\right)^2}$	(Kratina et al., 2009)

The shape of each FR model formulation is determined by two main biological parameters; i) *attack coefficient* or searching efficiency (a) which describes the rate at which predators search for and attack prey; and ii) *handling time* (h) which describes the time needed to capture, ingest and digest each prey item. Predator-dependent FR variants incorporate a third parameter – the *mutual interference coefficient* (c) – which typically quantifies the strength of predator interference emerging from conspecific interactions (DeLong and Vasseur, 2011), or in the case of the Tyutyunov II model, quantifies the threshold predator density at which prey dependence transitions to ratio dependence (Tyutyunov et al., 2008; Arditi and Ginzburg, 2012). Characteristically, the mutual interference coefficient can vary between 0 and 1, but can also vary between -1 and 1 in the case of the Arditi-Akçakaya and Hassell-Varley models (Arditi et al., 2004; DeLong and Vasseur, 2011; Prokopenko et al., 2017).

For the Beddington-DeAngelis models, in which c is incorporated as an additive effect, models become identical to the prey-dependent Holling model variants when $c = 0$ (i.e. *per capita* consumption rates are dependent upon prey density only). However, models become approximately ratio-dependent at higher predator densities, when in conjunction with high interference ($c \approx 1$); at which point part of the model's denominator ($ahN + cP$ in the case of the Type II formulation) becomes much greater than 1, indicating a *per capita* reduction in consumption rates (Cosner et al., 1999; Arditi and Ginzburg, 2012). For the Arditi-Akçakaya and Hassell-Varley models, in which the mutual interference coefficient is incorporated as a dimensionless exponent, models reflect perfect prey dependence when $c = 0$, becoming identical to Holling variants (Hassell and Varley, 1969; Arditi and Akçakaya, 1990; Arditi and Ginzburg, 2012). As competition between interacting predators increases, thereby limiting *per capita* consumption rates, models become purely ratio-dependent as interference levels reach extremis – as evidenced by a interference coefficient of $c = -1$, or $c = 1$ depending upon its expression within the model formulation (i.e. P^{c} or P^{-c}; see DeLong and Vasseur, 2011) – whereupon the Arditi-Akçakaya models develop into the Arditi-Ginzburg variants (Arditi and Akçakaya, 1990; Kratina et al., 2009; Arditi and Ginzburg, 2012; Prokopenko et al., 2017). For the Tyutyunov II model formulation, in which c is incorporated as a gradual process, a consumer's FR can transition from prey dependence to ratio dependence, depending on predator abundance. At low predator densities, when interference is essentially non-existent, the term $P/c + \exp(-P/c)$ in the model denominator becomes close to 1, causing the Tyutyunov II model to resemble the prey-dependent Holling II model. At high predator densities the term $\exp(-P/c)$ approaches 0, causing the Tyutyunov II model to transition to ratio dependence, thereby resembling the Arditi-Ginzburg II model (Tyutyunov et al., 2008; Arditi and Ginzburg, 2012).

To account for prey depletion, ordinary differential equations (ODE's) were used, integrating changes in prey density over time into the original FR formulae. I designed and fitted ODE-based FR model variants, using fine-scale numerical integration (*deSolve::lsoda*; version 1.21; Soetaert et al., 2010), to simulate prey depletion over a 24 h period, divided into 100 time intervals (i.e. every 0.01 days or 14.4 minutes). Maximum likelihood estimations were used (*bbmle::mle2*; version 1.0.20; Bolker and

R Core Team, 2017) to generate best-fit parameter estimations for FR parameters – attack coefficient (a), handling time (h) and interference coefficient (c).

For the three-parameter predator-dependent FR models (i.e. Beddington-DeAngelis Type II and Type III models, Arditi-Akçakaya Type II and Type III models, and Tyutyunov Type II model), arbitrary covariation between attack coefficient and interference coefficient prevented realistic estimations of the three FR parameters simultaneously. Instead, it was necessary to fix the attack coefficient at the estimated value for the single predator treatment (P = 1), in order to estimate handling time and interference coefficient for the whole dataset – including all prey-to-predator treatment combinations (Barrios-O'Neill, 2019, pers. comm.). Each FR model variant was non-parametrically bootstrapped (n = 5000) to generate approximate 95% confidence intervals for each free FR parameter.

With the inclusion of the mutual interference coefficient as a free parameter in predator-dependent FR model variants, these models were also refitted with the mutual interference parameter fixed at c = -1/1, thereby simulating ratio-dependence (Arditi and Ginzburg, 2012). Using Likelihood-Ratio Tests (LRTs), these refitted models were compared to the original models – containing free estimates of mutual interference – to determine whether estimations of the mutual interference coefficient significantly differed from -1/1. If models were not significantly different, they were considered to exhibit ratio-dependent properties. All 14 model formulations were compared using the finite sample corrected Akaike's information criterion (AICc) and Akaike weights. The model with the lowest AICc, and the greatest Akaike weight, was identified as the best-fit model, whilst models within 2 AICcs of the best fitting model were considered statistically indistinguishable.

3.3.3.2 Amphipod Spatial Arrangement

Changes in the spatial arrangement of amphipods over time, as represented by the index of aggregation, were analysed using a generalised linear mixed effect model – fitted via Penalised Quasi-likelihood due to non-normal data (*MASS::glmmPQL*; version 7.3-51.4; Venables and Ripley, 2003). A gamma distribution with a log-link function was fitted to the data, accounting for a continuous response variable. I examined the effects of prey depletion, predator density and time on the spatial arrangement of amphipods. Repeated observations were nested within replicated trial and assigned as a random effect. A full factorial model was initially generated, including a three-way interaction (prey depletion x predator density x time), and all corresponding two-way interactions. Given the nature of quasi-likelihood models, standard methods of comparison (i.e. AIC, QAIC, BIC, ANOVA, Likelihood Ratio Tests, etc.) are not applicable when assessing nested models. Therefore, I conduct model simplification through the removal of non-significant interaction terms, until a minimum adequate model was established, comprising of main effect variables and significant interaction terms.

3.4 Results

In control treatments, survivorship of native *R. temporaria* larvae exceeded 99%, with only a single death recorded out of 400 larvae. By comparison, survivorship in experimental trials, whilst in the presence of invasive *D. villosus*, was significantly lower (88.92%; Fisher's exact test $p < 0.001$). Therefore, any mortality of *R. temporaria* larvae, recorded in experimental trials, was attributed to amphipod predation. Predation of native *R. temporaria* larvae was also directly observed in experimental trials.

3.4.1 Amphipod Functional Response

Type II FR models were consistently identified as the better fitting models, when compared to Type I and Type III variants; with the exception of the prey-dependent Holling III model which ranked marginally higher compared to the Holling II model (AICc = 411.87 vs 412.51, respectively; Table 3.1). The ratio dependent Arditi Ginzburg III model was identified as the least representative FR model variant (ΔAICc = 132.15). According to AICc comparisons and Akaike weightings, the model which yielded the most appropriate fit for the empirical data was the ratio-dependent Arditi-Ginzburg II model (AICc = 402.97), followed by the predator-dependent Tyutyunov II model (ΔAICc = 0.48), the Arditi-Akçakaya II model (ΔAICc = 0.55) and finally the Beddington-DeAngelis II model (ΔAICc = 1.17). However, all three predator-dependent Type II FR model variants appeared to be statistically indistinguishable from the ratio-dependent Arditi-Ginzburg II model, with ΔAICc < 2 (Table 3.2; Figure 3.2a – d). All predator-dependent Type II model variants exhibited relatively similar estimations for a – except for the Beddington-DeAngelis II model – and h. Estimates of c recorded for all Type II predator-dependent variants approximated to 1, or -1 in the case of the Arditi-Akcakaya II model. Comparisons of the original predator-dependent FR models, comprising of multiple free parameters (h and c), with simplified models refitted with an interference coefficient fixed at $c = 1$ (Tyutyunov II and Beddington-DeAngelis II), or $c = -1$ (Arditi-Akçakaya II) revealed that estimates of c in the original models did not differ significantly from 1 (Tyutyunov II $p = 0.998$; Beddington-DeAngelis II $p = 0.576$), or -1 (Arditi-Akçakaya II $p = 0.831$), indicating that all predator-dependent Type II FR models predicted high levels of interference between conspecific amphipod predators, despite being mathematically different.

High estimations of mutual interference suggested that each of the three best-fit predator-dependent FR models exhibited characteristics comparable to ratio dependence. A value of $c \approx -1$ meant that the Arditi-Akçakaya II model was structurally very similar to the ratio-dependent Arditi-Ginzburg II model; however, because the Arditi-Akçakaya II model was fitted with an attack coefficient (a) estimated from the single predator treatment (i.e. $P = 1$), estimations for parameters a and h were noticeably different between the two models. A value of $c \approx 1$ implied that the Beddington-DeAngelis II model tended towards the Arditi-Ginzburg II model as predator density increased ($ahN + cP >> 1$;

range = 12.9 – 70.6). The Tyutyunov II model, with an estimated interference coefficient of $c = 1$, resembled the prey-dependent Holling II model at low predator densities ($P/c + \exp(-P/c) \approx 1$), but became approximately ratio-dependent at higher densities ($P \geq 5$ amphipods), tending towards the Arditi-Ginzburg II model ($\exp(-P/c)$ = 0.0150 and 0.0002 for 5 and 10 amphipods, respectively).

All best-fit models predicted a similar asymptotic increase in *per capita* predation rates by invasive *D. villosus*, relative to increasing larval *R. temporaria* abundance. At lower predator densities, functional response curves rapidly decelerated to an asymptote (Figure 3.2a, b) At higher predator densities deceleration was dampened somewhat, with models attaining a higher asymptote at higher larval densities (Figure 3.2c, d). Overall, the average total number of *R. temporaria* larvae consumed by *D. villosus* increased, relative to predator abundance, increasing from 0.7 larvae day^{-1} (P = 1 amphipod) to 3.0 larvae day^{-1} (P = 10 amphipods). However, as the number of amphipod predators increased, *per capita* consumption rates declined, decreasing from 0.7 larvae amphipod^{-1} day^{-1} (1 amphipod) to 0.3 larvae amphipod^{-1} day^{-1} (10 amphipods; Figure 3.2a-d).

Table 3.2. Best-fit parameter estimations, the number of estimated parameters (K), finite sample corrected Akaike's Information criterion (AICc), ΔAICc, and AIC weight for fitted functional response models using the per capita consumption rates (i.e. prey consumed per unit time) recorded for invasive *D. villosus* whilst feeding upon native *R. temporaria* larvae.

	Parameter Estimation [95% Confidence Intervals; CIs]					
Model Name	*a*	*h*	*c*	K	AICc (ΔAICc)	AIC Weight
Null Model	0.365 [0.300 – 0.435]			1	413.69 (10.72)	<0.01
Holling Type I	0.027 [0.020 – 0.036]			1	478.58 (75.61)	<0.01
Holling Type II	0.367 [0.101 – 0.996]	2.328 [1.539 – 2.902]		2	412.51 (9.54)	<0.01
Holling Type III	0.120 [0.024 – 0.333]	2.453 [1.832 – 3.038]		2	411.87 (8.90)	<0.01
Beddington-DeAngelis Type I	0.060 [0.032 – 0.097]		0.207 [0.017 – 0.474]	2	461.28 (58.31)	<0.01
Beddington-DeAngelis Type II	***1.263***	**1.827** **[1.231 – 2.551]**	**1.326** **[0.270 – 2.647]**	**2**	**404.14** **(1.17)**	**0.18**
Beddington-DeAngelis Type III	*0.278*	2.343 [1.732 – 2.922]	0.474 [0.134 – 2.453]	2	409.72 (6.74)	0.01
Hassell-Varley (Arditi- Akçakaya Type I)	0.055 [0.035 – 0.081]		0.429 [0.138 – 0.709]	2	460.50 (57.53)	<0.01
Arditi- Akçakaya Type II	***0.660***	**1.724** **[1.096 – 2.500]**	**-0.964** **[-1.478 – -0.102]**	**2**	**403.53** **(0.55)**	**0.24**
Arditi-Akcakaya Type III	*0.142*	2.318 [1.534 – 2.911]	-0.512 [-1.719 – 0.575]	2	410.40 (7.42)	0.01
Tyutyunov Type II	***0.839***	**1.813** **[1.018 – 2.404]**	**1.001** **[0.310 – 2.382]**	**2**	**403.46** **(0.48)**	**0.25**
Arditi-Ginzburg Type I	0.117 [0.089 – 0.151]			1	500.64 (97.66)	<0.01
Arditi-Ginzburg Type II	**0.896** **[0.259 – 2.143]**	**1.851** **[1.021 – 2.435]**		**2**	**402.97** **(0.00)**	**0.31**
Arditi-Ginzburg Type III	0.023 [0.009 – 0.219]	4.743 [1.268 – 15.751]		2	535.13 (132.15)	<0.01

Note: **Bold** identifies four most parsimonious FR models. *Italicised* estimations for attack coefficients (*a*) correspond to models for which attack coefficients were fixed, using estimates obtained from single predator treatments (P = 1). This was due to arbitrary co-variation occurring between attack coefficients and mutual interference coefficients (*c*), whilst fitting models using the whole dataset. Values within square brackets are 95% confidence intervals obtained by boostrapping models (n = 5000).

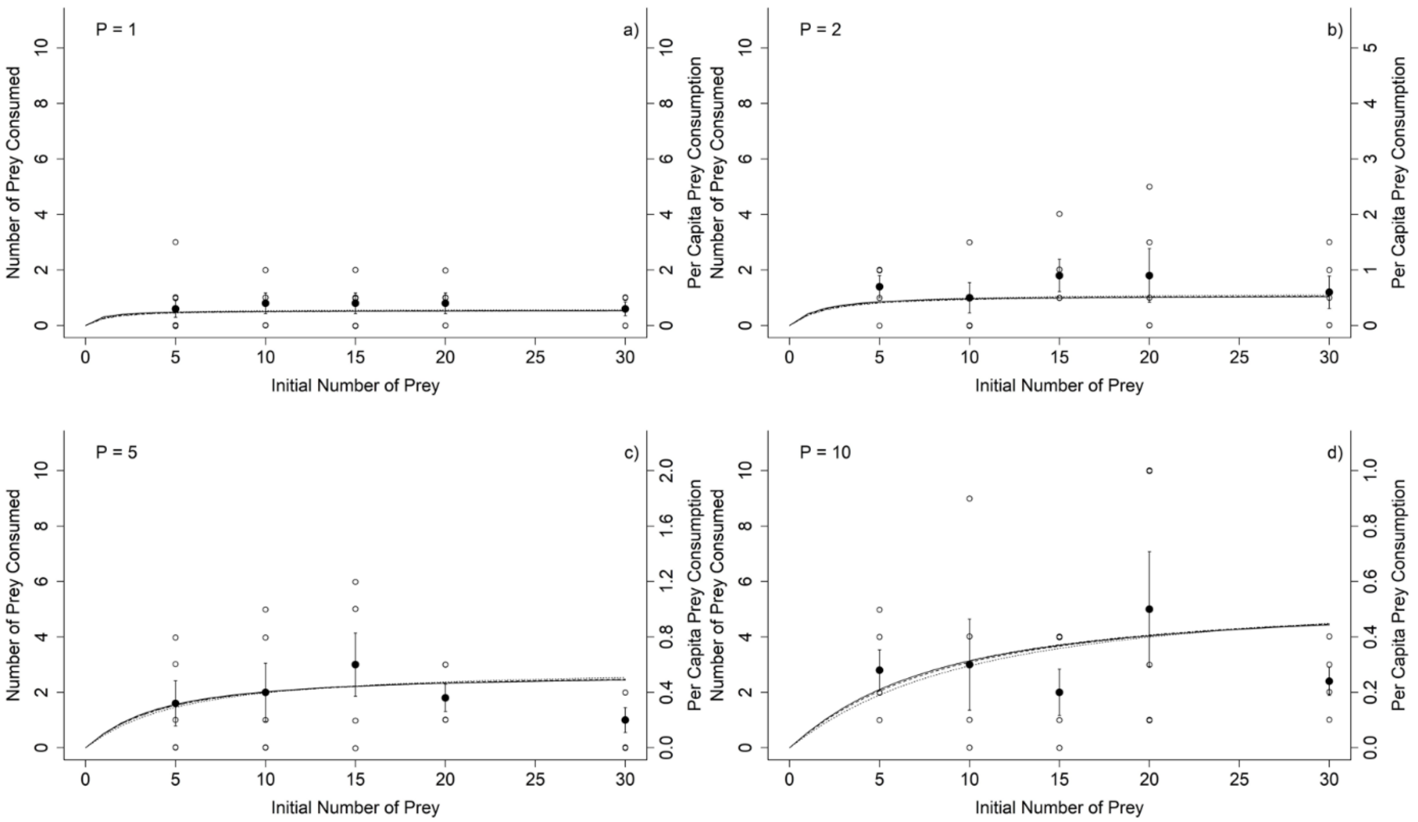

Figure 3.2. Functional response curves generated for the four best-fit FR models – Arditi-Ginzburg II (solid line; Akaike weight = 0.314), Tyutyunov II (dashed line; Akaike weight = 0.246), Arditi-Akçakaya II (dotted line; Akaike weight = 0.238), Beddington-DeAngelis II (dot-dash line; Akaike weight = 0.175) – illustrating the relationship between the number of *R temporaria* larvae consumed, and prey density supplied, measured at four predator densities (P = 1, 2, 5 and 10; panels a-d). Open circles are observations and black circles represent binned means (± SEM). Secondary y-axis indicates *per capita* prey consumption (i.e. number of prey consumed / number of predators).

3.4.2 Amphipod Spatial Arrangement

Predator spatial arrangement was analysed with respect to prey depletion, predator abundance and experimental time. Following the removal of a non-significant three-way interaction ($p = 0.317$) and a non-significant two-way interaction between prey depletion and time ($p = 0.930$), the minimum adequate model (MAM) was established. Predator spatial arrangement was significantly affected by predator abundance ($t = 12.17$, $p < 0.001$), with *D. villosus* exhibiting greater aggregation as the number of predators increased within each experimental system ($D > 1$). Analysis of the MAM revealed a significant interaction term between prey depletion and predator density ($t = -2.692$, $p < 0.01$), suggesting that invasive *D. villosus* became considerably more aggregated with conspecifics as predation of native *R. temporaria* larvae increased relative to predator density. Additionally, a significant interaction term was found between predator density and time (t = 3.886, $p < 0.001$), indicating that *D. villosus* aggregated more with conspecifics as experimental trials progressed, with aggregation increasing considerably at higher predator densities (Figure 3.3).

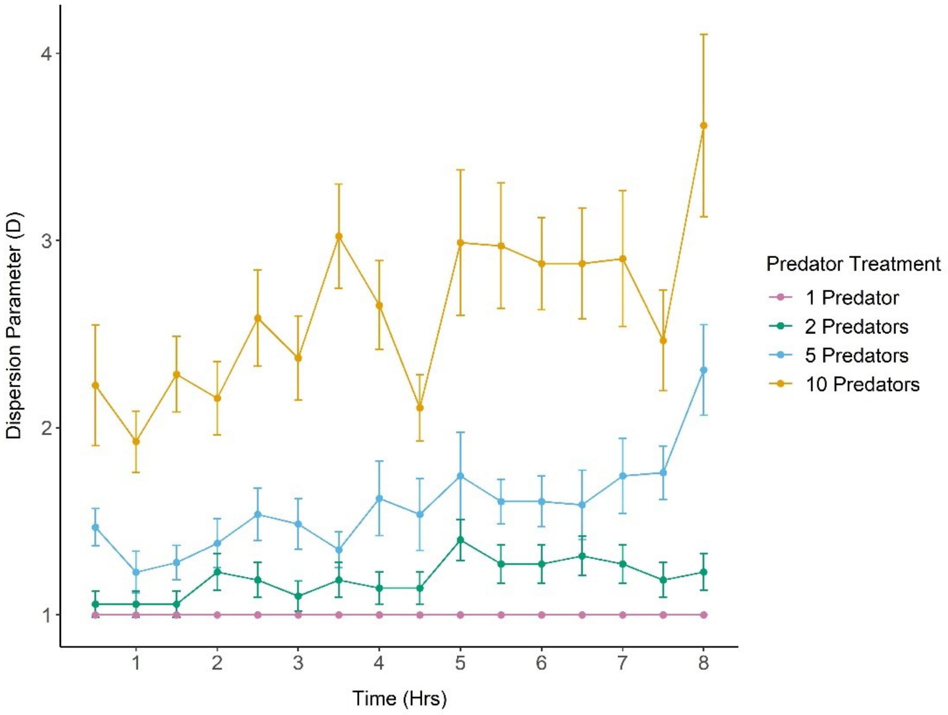

Figure 3.3. Mean ± SEM dispersion of invasive *D. villosus*, as represented by the index of aggregation (D), in experimental arenas, when present as 1 predator (pink), 2 predators (green), 5 predators (blue) and 10 predators (orange). Dispersion measured every 30 minutes over 8 hours.

3.5 Discussion

In this study I evaluated the potential predatory impact of invasive *D. villosus* towards larval *R. temporaria* and explored how predator density might modify predictions concerning the ecological impact of this invader. I also examined how conspecific predator interactions may affect the feeding success and spatial organisation of individual amphipods. When provided with native *R. temporaria* larvae at varying densities, invasive *D. villosus* exhibited a Type II FR across all predator densities, with *per capita* predation rates increasing relative to prey availability, before decelerating to an asymptote as prey ingestion became limited by handling time. The expression of a Type II FR by invasive *D. villosus* is consistent with previous studies (Bollache et al., 2008; Dick et al., 2010; Dodd et al., 2014; Taylor and Dunn, 2017; Chapter 2), and is indicative of a potentially destabilising predator-prey dynamic (Neutel and Thorne, 2016), as excessive consumption of prey at lower densities may result in the collapse of affected prey populations (Juliano, 2001; Price et al., 2011). As such, the expression of a Type II response would suggest that *D. villosus* may have the capacity to detrimentally affect populations of *R. temporaria* in the field (see Dick et al., 2014; Chapter 2). However, I demonstrate that *per capita* effects may be moderated by predator interference at higher predator densities.

When I considered the potential density-dependent effects of both prey and predator abundance, analyses revealed *per capita* consumption by invasive *D. villosus* to be ratio-dependent, with the Arditi-Ginzburg II FR model appearing to be the most parsimonious fit to recorded data. Ratio-dependence is regarded as a 'special' form of predator-dependence, and its emergence is centred around the 'gradual interference hypothesis' (Ginzburg and Jensen, 2008; Tyutyunov et al., 2008; Trần, 2009; Arditi and Ginzburg, 2012). At low predator densities (e.g. $P = 1$), *per capita* predation is predicted to be exclusively prey-dependent, with negligible interference ($c \approx 0$) occurring between conspecific predators (i.e. Holling II). However, as predator density increases, conspecific interference is also expected to increase gradually, with *per capita* predation becoming exclusively ratio-dependent at high predator densities ($c = -1/1$) – as represented by the Arditi-Ginzburg II model (DeLong and Vasseur, 2011; Arditi and Ginzburg, 2012).

The findings of the current study appeared to corroborate the gradual interference hypothesis, with prey-dependent formulations identified as poorer representations of *per capita* predation in relation to changing prey and predator densities, whereas ratio-dependent and predator-dependent variants performed considerably better; including the Tyutyunov II model which is regarded as a gradual interference model (Tyutyunov et al., 2008). Despite being mathematically different, each of the three best-fit predator-dependent Type II FR variants estimated high levels of interference amongst conspecific *D. villosus*, predicting a density-mediated reduction in amphipod feeding rates in response to changes in the proportional availability of larval prey. For the Beddington-DeAngelis II model, predator interference was actually estimated to exceed the typical limits of the interference coefficient (i.e. $0 \leq c \leq 1$), suggesting that high conspecific interference might lead to a greater reduction in *per capita* predation rates than expected by proportional prey availability (i.e. ratio dependence). Known as overcompensation, this phenomenon predicts an unstable and temporary predator-prey equilibrium, with the complete loss of equilibrium expected at low prey densities, resulting in extinctions (Arditi et al., 2004). However, given that the estimated interference coefficient did not significantly differ from $c = 1$, and 95% confidence intervals encompassed $c = 1$, overcompensating interference amongst conspecific *D. villosus* whilst feeding upon *R. temporaria* larvae seemed unlikely. Instead, the Beddington-DeAngelis II model appeared to demonstrate characteristics tending towards ratio dependence (Arditi and Ginzburg, 2012). Interference estimates recorded for both the Arditi-Akçakaya II and Tyutyunov II formulations also indicated similar ratio-dependent properties, despite both model variants being conceptually different in their consideration of predator interference (Arditi and Ginzburg, 2012).

It is important to note that estimates obtained from each predator-dependent FR model, were generated based on restricted model parameterisation (i.e. attack coefficient a estimated for single predator treatment). By controlling for arbitrary covariation between the attack and interference coefficients, systematic biases were invariably imposed, somewhat limiting the extent to which statistical comparison could be performed. In the current study, parameter covariation may be attributable to several factors, such as; i) functional non-independence between attack and interference coefficients in some model formulations (e.g. Arditi-Akçakaya II); ii) large, heteroskedastic variance in amphipod feeding rates (particularly at lower prey densities); iii) insufficient replication at lower prey densities; or iv) spacing between prey densities too broad to effectively constrain parameters (Novak and Stouffer, 2020; Uszko et al., 2020). With greater replication, shorter intervals between density treatments, and appropriate data transformation (e.g. logarithmic transformation) it may be possible to attain more accurate estimations of consumer FRs, to better differentiate between FR formulations (i.e. ratio- vs predator-dependent), and to ultimately obtain a better description of predator behaviours (Uszko et al., 2020). Nevertheless, based on the frequency with which ratio dependence and/or predator dependence is reported in previously published studies, whilst assessing the *per capita* response of various predatory species (reviewed in Arditi and Ginzburg, 2012), including several amphipod species (Médoc et al., 2013, 2015), I believe that the current best-fit FR formulations provide a strong heuristic indication that interference from conspecific predators plays an important role in shaping the functional response of *D. villosus* towards native larval amphibians. I also believe that there is sufficient qualitative evidence to suggest that best-fit predator-dependent FR models exhibited properties consistent with, or approximate to, ratio dependence.

In the field, ratio dependence is predicted to develop as a consequence of prey sharing (Arditi and Ginzburg, 1989). With *per capita* prey availability inversely proportional to predator abundance, access to shared prey by individuals is expected to become limited when conspecific predators are present at high densities. As a result, interference amongst conspecific predators is likely to emerge as individuals attempt to access available prey (Trần, 2009). Interference amongst conspecific *D. villosus* is therefore likely to cause a reduction in *per capita* consumption rates, as evidenced here, moderating the ecological impact toward larval *R. temporaria*.

A major determinant in the emergence and intensity of mutual interference amongst predators is the spatial dynamics of conspecifics (Cosner et al., 1999). In the field, individuals often aggregate towards conspecifics in response to i) the availability and distribution of resources (Arditi and Ginzburg, 2012), ii) risk of predation (Jermacz and Kobak, 2018), and iii) microhabitat preference (Platvoet et al., 2009b; Jermacz et al., 2015). Here, I analysed the spatial dynamics of invasive *D. villosus* with respect to resource availability only. I found that invasive *D. villosus* exhibited significantly higher aggregation towards conspecifics over time and as the number of predators increased within experimental systems. Furthermore, I found that aggregation amongst invasive *D.*

villosus also appeared to be in response to increased consumption of native *R. temporaria* larvae, observed at higher predator densities.

Mutual interference amongst predators can emerge as a result of direct interactions that affect the foraging success of conspecifics through social behaviours, agonistic behaviours (e.g. cannibalism; Rudolf, 2008) and interference competition (e.g. kleptoparasitism, prey-hoarding/guarding; Iyengar, 2008; Holdridge et al., 2016). With respect to ratio-dependence, interference can emerge as an indirect consequence of non-random prey searching behaviours (i.e. *pseudo-interference*; see Free et al., 1977), and/or predator-mediated prey depletion, thereby restricting the availability of prey to all predators within a system (i.e. exploitative competition; Holdridge et al., 2016). Moreover, predator interference can also develop as an indirect effect of anti-predator prey responses (Anholt and Werner, 1995). Predator-induced prey responses by larval anurans can include changes in morphology, life-history and behaviour (Nunes et al., 2013; Nunes et al., 2014). However, given the developmental age of larvae used in this study (G. 19-20), such changes were unlikely to occur.

In the current study, the emergence of interference amongst conspecific *D. villosus*, whilst feeding upon native *R. temporaria* larvae, appeared to be attributable to both indirect and direct mechanisms. Predator interference was likely an indirect product of predation – as indicated by observations of notably increased aggregation amongst *D. villosus*, in response to prey depletion at higher predator densities. Proximity to conspecific predators may have led to more frequent encounters, interactions and therefore interference with neighbouring predators whilst attempting to access *R. temporaria* larvae, resulting in a reduction in *per capita* feeding success and the emergence of predator- or ratio-dependence (Ginzburg and Jensen, 2008). Mutual interference appeared to promote an increase in *per capita* handling times. By refitting the top four FR models using data concerning the single predator treatment only (i.e. $P = 1$), comparisons to the original models – generated using the entire dataset (see Médoc et al., 2015) – revealed an overall increase in *per capita* handling times as the number of predators increased within experimental systems (single predator model(s) $h = 1.26 \pm 0.52$ vs Arditi-Ginzburg II original model $h = 1.85 \pm 0.28$; Tyutyunov II original model $h = 1.81 \pm 0.27$; Arditi-Akçakaya II original model $h = 1.72 \pm 0.25$; Beddington-DeAngelis II original model $h = 1.83 \pm 0.28$). Considering the minimal incidence of cannibalism recorded amongst invasive *D. villosus*, increased *per capita* handling times may be attributed to competitive behaviours such as kleptoparasitism and prey-guarding, with individuals spending more time defending prey resources from conspecific predators (Iyengar, 2008; Médoc et al., 2015; Holdridge et al., 2016).

In recent years, the application of FR analyses has proven to be an effective tool when evaluating the potential ecological impact of invasive predators, with predictions often consistent with observed impacts to invaded field communities (reviewed in Dick et al., 2014). Superabundance is a common characteristic of high-impact invasive species (Dick et al., 2017; Blackburn et al., 2019), but few studies account for possible interference amongst interacting conspecific predators when assessing invader impact; instead regarding consumer FRs to be entirely prey-dependent. Whilst this may be appropriate for solitary predators, such as the Eurasian lynx (*Lynx lynx*; Nilsen et al., 2009) or the Pikeperch (*Stizostedion lucioperca*; Nilsson, 2001), the use of the prey-dependent FR may misrepresent the potential ecological impact of gregarious, group-living organisms; which are predicted to display some form of interferential behaviours when closely associated with feeding conspecifics, thereby altering potential *per capita* responses (Cosner et al., 1999; Arditi and Ginzburg, 2012). Given the highly social nature of invasive *D. villosus* (see Jermacz and Kobak, 2018), it is vital that such behavioural contexts be incorporated, when assessing the ecological impact of this top invasive predator. In the current study interference, emerging from interactions driven by increased aggregation amongst conspecific *D. villosus*, was predicted to cause *per capita* feeding rates to decline, reducing potential biotic pressures imposed by individual amphipods towards native UK early-stage amphibians. Whilst this may be indicative of a lower-than-expected potential ecological impact, when compared to previous studies (see Chapter 2), it might also denote a possible time-delay in the extirpation of larval amphibians from regions invaded by *D. villosus*, with amphipods imposing predatory pressures over a protracted period of time (Parshad et al., 2016); however, based on the current available data, this remains uncertain.

Ratio dependence has been identified in a variety of taxonomic groups (reviewed in Arditi and Ginzburg, 2012; also see Spataro et al., 2012; Médoc et al., 2013, 2015; Prokopenko et al., 2017), suggesting that interference may be a critical factor in predicting the foraging success of consumers in general. To my knowledge, this is the first study to apply such a fully comprehensive assessment whilst examining the FR of an invasive freshwater predator. Utilising 14 widely regarded FR model variants, across the three main theoretical FR classes, this study provides evidence for ratio dependence and/or predator dependence when describing the functional response of invasive *D. villosus* towards native *R. temporaria* larvae; thereby highlighting the importance of both prey and predator abundance, as well as the emergence of mutual interference, as fundamental determinants of predation. Predatory responses may be further modified under additional ecological contexts, such as the presence of parasites or heterospecifics predators (e.g. Paterson et al., 2015), changes in habitat complexity (e.g. Barrios-O'Neill et al., 2015), and climate change (e.g. Pellan et al., 2016).

However, whilst interference amongst interacting conspecific predators is predicted to lead to a reduction in *per capita* feeding success, particularly at higher predator densities, it is important to consider how predator abundance might also affect an invader's overall impact (i.e. population-level effect). When assessing the total impact potential (IP) of an invasive alien species, towards a given prey species, functional response is but one determinant of ecological impact: another being the numerical response (NR), or a suitable proxy, such as abundance (AB, IP = FR x AB; Dick et al., 2017; Dickey et al., 2020). By incorporating both measurements into metrics, like the relative impact potential (RIP), invasion ecologists have identified a number of high-impact invasive alien species, such as *D. villosus*, which are capable of imposing strong individual- and/or population level effects (reviewed in Dick et al., 2017; but also see Chapter 2); including those invaders which might exhibit a reduced functional response, yet exist at high densities in the field (Dickey et al., 2020). In the current study, similar patterns were observed across the different amphipod densities. Despite a reduction in *per capita* predation rates by *D. villosus*, likely due to frequent spatially mediated interferential interactions occurring at higher predator densities, there was an overall increase in the total number of larvae consumed, relative to amphipod abundance. This would suggest that the limiting effect of interference on the foraging behaviours of individual *D. villosus* may be offset by amphipod abundance, translating into a potentially greater total predatory impact towards amphipod larvae overall (Médoc and Spataro, 2015; Dick et al., 2017; Dickey et al., 2020). Given that *D. villosus* typically exist at high densities within invaded regions (see Chapter 2), the effect of predator abundance on total invader impact may be compounded further, with invasive amphipod populations imposing a strong ecological impact upon early-stage amphibian populations.

Regardless, the findings of the current study provide a unique insight concerning the potential ecological impact of invasive *D. villosus* when feeding upon native *R. temporaria* larvae; a possible predator-prey dynamic which has only recently been determined, yet is predicted to significantly impact upon populations of native *R. temporaria* in the field (Chapter 2). With evidence of ratio dependence as the dominant FR class, this study provides a key refinement to previous ecological predictions and emphasises the need to consider predator-predator interactions and the potential emergence of mutual interference when assessing invader impacts. As such, I advocate the use of alternative FR model variants, in addition to classic prey-dependence, in order to garner accurate predictions concerning the potential ecological impact of invasive predators in more natural contexts. However, one must also consider the underlying mechanisms through which mutual interference may develop, such as spatial dynamics, and how these may likely translate within natural field systems (Arditi and Ginzburg, 2012). This is particularly important given that predator and prey populations typically exist at far higher field densities than that which is feasible to replicate in laboratory studies.

Chapter 4

Strong Preferential Feeding of Alternative Macroinvertebrate Prey Limits Potential Ecological Impact by Invasive *Dikerogammarus villosus* towards Larval UK Anurans

4.1 Abstract

As invasive alien predators continue to spread throughout the world, native biodiversity continues to experience the severe detrimental effects linked to these invasions. As generalists, invasive predators can impose strong predatory pressures across a range of resident prey species, often leading to local declines and/or extinctions. However, the strength of trophic interactions can be dictated by intricate mechanisms, often associated with the composition and complexity of native prey communities. When multiple prey species co-occur, predation may be frequency-dependent, with predators switching between prey species in response to relative availability; thereby providing prey with a low-density refuge. Conversely, predators might demonstrate strong preferential feeding towards certain prey species, regardless of relative availability; thereby driving the decline of affected prey populations. In this chapter, I use prey preference and prey-switching experiments to determine how predatory pressures imposed by invasive *Dikerogammarus villosus* towards native *Rana temporaria* larvae may be affected by the presence of alternative macroinvertebrate prey species (*Asellus aquaticus* and *Chironomus* sp. larvae). Using multiple prey species functional response modelling (MSFR) I also examine how *per capita* predation rates towards each prey species are influenced by relative prey abundance (i.e. functional response; FR). When presented with all three prey species in equal quantities, *D. villosus* demonstrated clear preferential feeding patterns, consuming prey in the following hierarchical order (*Chironomus* sp. larvae > *A. aquaticus* > *R. temporaria* larvae). Comparisons between invasive *D. villosus* and the native amphipod *Gammarus pulex* revealed there was no significant difference in the consumption of *Chironomus* sp. larvae between amphipod species; although *D. villosus* consumed significantly more isopod and anuran prey, and substantially more prey overall. When presented with experimental combinations of prey species, consisting of *R. temporaria* larvae and either *A. aquaticus* or *Chironomus* sp. larvae, at varying ratios, *D. villosus* continued to exhibit a strong preference towards macroinvertebrate prey, regardless of relative availability. Using MSFR models, *D. villosus* exhibited a potentially destabilising Type II FR across all prey species, with preferential feeding of *A. aquaticus* and *Chironomus* sp. larvae reflected by higher attack rates, shorter handling times, and greater maximum feeding rates, when compared to *R. temporaria* larvae. Preferential feeding in the absence of prey-switching indicate greater predatory risks by *D. villosus* towards macroinvertebrate prey communities – potentially causing local extinctions. Larval anurans may likely experience limited predatory pressures by *D. villosus* directly; however, with the removal of key macroinvertebrate species (e.g. *A. aquaticus*) expected, the survival of larval anuran populations may be indirectly affected.

4.2 Introduction

Predation is a major determinant in the structure of ecological communities (Messinger and Ostling, 2013), fundamental in regulating population dynamics (Belgrad and Griffen, 2016), shaping the structure of resident communities (Start and Gilbert, 2017), and dictating the functioning of natural ecosystems (Hawlena and Schmitz, 2010; Hammerschlag et al., 2019). Predators are often categorised into two distinct classifications of feeding strategy; specialist and generalist (Smith et al., 2011). Specialist predators have evolved a narrow dietary niche, comprising a low diversity of prey types/species (Smith et al., 2011). However, specialists are typically more efficient in their ability to hunt prey items when compared to generalist predators, having developed highly specialised morphological and/or behavioural adaptations (Michálek et al., 2017). By comparison, generalists have evolved a broad dietary niche, and as such are capable of readily exploiting a wide range of prey types (Smith et al., 2011); although the amount of energy acquired from each prey type consumed can vary significantly when compared to specialist predators (Schmidt et al., 2012; Michálek et al., 2017; Potter et al., 2018). As a result, generalist predators often engage in hunting activity more frequently (Pompozzi et al., 2019).

Most invasive alien species (IAS) are ecological generalists; a trait which has facilitated their establishment across a wide range of novel regions (Dammhahn et al., 2017), and has contributed to the severe detrimental impacts frequently incurred by native communities (Snyder and Evans, 2006). Within invaded regions, the success and impact of invasive predators is often associated with predatory interactions (Salo et al., 2007; Crowder and Snyder, 2010; Hentley et al., 2016; Fincham et al., 2019) and the over-exploitation of prey resources (Catford et al., 2009; Ricciardi et al., 2013; Dick et al., 2014; Dick et al., 2017; Dickey et al., 2020). Invasive predators frequently impose high predatory pressures upon native communities through the consumption of a diverse range of prey species, often leading to the displacement/removal of affected natives, with subsequent changes to the abundance and diversity of native prey assemblages (Park, 2004; Salo et al., 2007; Gallardo et al., 2016). This is particularly apparent in freshwater ecosystems, which experience a disproportionately high incidence of biological invasions, by a relatively large number of ecologically damaging high-impact invaders, when compared to terrestrial (Moorhouse and Macdonald, 2015) and marine ecosystems (Ricciardi and Kipp, 2008).

Variability in the predatory capabilities of native and invasive predators is often associated with invader impact and has previously been assessed via the application of the comparative functional response (FR) approach, evaluating the relationship between *per capita* predation and prey availability (Dick et al., 2014). The implementation of comparative FR analyses has predominantly been centred around assessing the ecological impact of invasive freshwater predators, with high-impact invaders typically characterised by a Type II FR (reviewed in Dick et al., 2014, 2017). A Type

II FR is described by a hyperbolic saturating response curve, whereby *per capita* predation increases at a decelerating rate, establishing an asymptote at higher prey densities as predators become satiated (Holling, 1966). Type II FRs are indicative of a potentially destabilising predator-prey dynamic, where high or complete consumption of prey at low prey densities may lead to the extirpation of affected prey species (Hassell, 1978; Dick et al., 2014). When compared to trophically analogous native predators, invaders frequently express superior Type II FRs, with invaders displaying significantly greater *per capita* predation rates (i.e. curve asymptote) upon focal prey species. Differences in the magnitude of native and invader FRs, often reflect the known ecological impacts of many invasive predators, observed within invaded sites (Dick et al., 2014; Dick et al., 2017). Moreover, there is a growing body of evidence demonstrating the importance of "context-dependence" when determining the impact of invasive predators. For example, studies have shown that the magnitude and type of FR generated by invasive predators can be influenced by environmental stressors (e.g. temperature; South et al., 2018), substrate type (Cuthbert et al., 2019b; South et al., 2019), habitat structure (Dodd et al., 2014; Alexander et al., 2015a; Barrios-O'Neill et al., 2015; DeRoy et al., 2020a), parasitism (Dick et al., 2010; Haddaway et al., 2012; Paterson et al., 2015; Fincham et al., 2019), and the presence of other predators (Arditi and Ginzburg, 2012; Médoc et al., 2013, 2015; Barrios-O'Neill et al., 2014a; Paterson et al., 2015; Chapter 3). As generalist predators, the presence of alternative prey types may also influence *per capita* predation (e.g. Médoc et al., 2018; Taylor and Dunn, 2018). However, to-date, most FR studies concerning invasive predator impacts have often focussed upon single predator-single prey species interactions. Here we explore how predatory response may change when multiple prey types are available.

When multiple prey types are present in equal proportions, generalist predators may demonstrate indiscriminate feeding across all available prey types. However, as the relative abundance of each prey type begins to vary, generalist predators may develop a preference for the more common prey type, leading to modifications in predator FR type and the emergence of a Type III FR via prey switching (Murdoch and Oaten, 1975; Tschanz et al., 2007). A Type III FR describes a sigmoidal response curve, with limited consumption occurring at low prey densities, increasing at an accelerating rate at intermediate densities, before decelerating to an asymptote at higher densities as the predator becomes satiated (Holling, 1966). Compared to the Type II FR, Type III FRs signify more stabilising predator-prey dynamics, with reduced consumption at lower prey densities providing prey with a low-density refugium (Holling, 1966; van Leeuwen et al., 2007). Whilst indiscriminate feeding has previously been reported in some invasive predators, contributing to the success and subsequent detrimental impact of some invaders (see Haddaway et al., 2012; Dodd et al., 2014), evidence surrounding prey-switching behaviours by invaders is relatively scarce. Instead, invasive predators may demonstrate continuous selective predation of certain prey types, regardless of relative availability (Ruscoe et al., 2005; Taylor and Dunn, 2017, 2018; Cuthbert et al., 2018a; Médoc et al., 2018). Persistent preference for specific prey types by invasive predators maintains potentially destabilising Type II FRs even when prey are scarce, driving the decline and subsequent collapse of affected prey populations (Dick et al., 2014). Continual selection for certain prey may be attributed to dissimilarities with other co-existing prey types, whether it be differences in morphology, behaviour, location, habitat choice, etc. (see Morozov and Petrovskii, 2013; van Leeuwen et al., 2013). With that in mind, I explore prey selectivity and investigate how potential prey-switching behaviours may influence the FR of the high-impact invasive freshwater killer shrimp, *Dikerogammarus villosus* (Sowinsky, 1894) (Crustacea: Amphipoda).

Dikerogammarus villosus is a high-impact invader of significant ecological concern, regarded as one of the top 100 worst invaders in Europe (Nentwig et al., 2018). Established throughout most of Western Europe, present in the UK, and predicted to invade North America (reviewed in Rewicz et al., 2014), *D. villosus* is a voracious predator, responsible for reductions in the abundance and diversity of resident assemblages, resulting from the extirpation of native prey species through intense predation (van Riel et al., 2006; MacNeil et al., 2013a). In the laboratory, *D. villosus* has been observed consuming a wide range of freshwater macroinvertebrates (reviewed in Rewicz et al., 2014). Moreover, diversity in the range of macroinvertebrate prey consumed by *D. villosus* appears to be conserved in the field, with stable isotope analyses revealing invasive *D. villosus* to be on the same trophic level as predatory fish (Marguillier, 1998; van Riel et al., 2006; Maazouzi et al., 2007; but see Hellmann et al., 2015, 2017; Koester et al., 2016; Haubrock et al., 2019). Predation by invasive *D. villosus* may also extend to some aquatic vertebrates, with predation of embryonic and larval fish (Casellato et al., 2007; Platvoet et al., 2009a; Taylor and Dunn, 2017), and amphibians (Chapter 2 and 3), having been observed. When presented with a single focal prey type/species, comparative FR analyses have consistently reported *D. villosus* expressing a significantly greater Type II FR, when compared to trophically analogous native comparators. However, as a generalist predator, invasive *D. villosus* are capable of predating upon a diverse range of prey types/species (e.g. Dick et al., 2002). As such, the ecological impact of this invader is likely to be affected by prey selectivity and/or potential prey switching behaviours.

Here I present a novel laboratory-based study to determine how predatory pressures exerted by invasive *D. villosus* towards vulnerable, freshly hatched native *Rana temporaria* (Anura: Ranidae) larvae are affected when alternative macroinvertebrate prey types are present. Firstly, I explore how predation risk towards native *R. temporaria* larvae is effected by the presence of *Chironomus* sp. larvae (Diptera: Chironomidae) and *Asellus aquaticus* (Isopoda: Asellidae) as alternative prey types, by comparing prey selectivity by invasive *D. villosus* and the native amphipod *Gammarus pulex*, when all three prey types are present in equal quantities. I test whether invasive *D. villosus* demonstrates indiscriminate feeding across the three prey types, or preferential selectivity towards specific prey. Due to negligible predation of native *R. temporaria* larvae by *G. pulex*, further examination of predatory behaviours focussed solely upon invasive *D. villosus*, via the implementation of FR analyses. Here I assess the potential ecological impact of invasive *D. villosus* towards native *R. temporaria* larvae by utilising multi-prey species FR models (i.e. MSFRs). By comparing model types, I determine how predation of native *R. temporaria* by invasive *D. villosus* is affected by the presence of an alternative prey type (*Chironomus* sp. larvae or *A. aquaticus*) when provided under varying relative abundances, and ascertain whether *D. villosus* exhibits "prey-switching" behaviours in response to changes in proportional availability, or continual selectivity of certain prey types regardless of relative prey abundance.

4.3 Materials and Methodology

4.3.1 Collection and Maintenance of Study Organisms

4.3.1.1 Amphipods

Between February and April 2019, native *G. pulex* were sampled from Meanwood Beck, West Yorkshire (53°49'50.0"N, 1°34'33.3"W), netted from submerged leaf-litter and rocky substrates, and invasive *D. villosus* were sampled from Grafham Water Reservoir, Cambridgeshire (52°17'31.1"N, 0°19'23.6"W), removed by hand from submerged artificial substrates. Each species was transported to Leeds in insulated containers with site-sourced water. In the laboratory, native and invasive amphipods were independently housed in communal aquaria containing dechlorinated tap water. Aquaria received constant aeration and were maintained at 14°C in a 12h: 12h light: dark photoperiod. Amphipods were fed a diet of stream-conditioned sycamore leaves (*Acer pseudoplatanus L.*), provided *ad libitum*, for a period of at least 96h prior to use in experimentations.

Only adult male amphipods, clear of visually apparent macro-parasites, were selected for use in experimental trials, thereby avoiding potential sex-mediated (e.g. Dick and Platvoet, 2000; van der Velde et al., 2009), and/or parasite-associated variations (e.g. Dick et al., 2010; Iltis et al., 2018) in predatory behaviours. Males were identified via pre-copulatory mate-guarding (*G. pulex*), or the presence of genital papillae and the absence of oostegites (*D. villosus*). Only large-bodied native *G. pulex* and invasive *D. villosus* were selected for use in experiments, thereby providing an accurate picture of the situation in invaded environments (e.g. Rewicz et al., 2014). The decision to compare large-bodied native and invasive amphipods was also based on previous observations concerning an apparent lack of statistically significant differences in the predation rates of size-matched *G. pulex* and *D. villosus* whilst predating upon native *R. temporaria* larvae (see Chapter 2). Prior to use in experimental trials, amphipods were blotted dry and individually weighed. Statistical comparisons of native and invasive amphipod weights revealed highly significant differences between amphipod species (Mann-Whitney U = 4860, $p < 0.001$). *Dikerogammarus villosus* were substantially heavier (mean ± SEM; 124.895 ± 0.920mg) than *G. pulex* (54.247 ± 1.322mg).

4.3.1.2 Prey Organisms

Three native freshwater species were selected for use as prey organisms; *Rana temporaria* larvae, *Chironomus* sp. larvae and *Asellus aquaticus*. *Rana temporaria* larvae were used as the focal prey organism, and *Chironomus* sp. larvae and *A. aquaticus* as appropriate alternative prey organisms; representative of macroinvertebrate species typically found in habitats used by adult *R. temporaria* during the spawning season (e.g. ponds).

With prior consent from Natural England, the Home Office and the University of Leeds Ethics Committee, freshly deposited *R. temporaria* embryos were sampled from several field sites throughout Yorkshire (see Appendix 4, Table A4.1), collected within approximately 36 h of fertilisation. At each field site, embryos were removed as whole clutches and approximately halved, with half of each clutch transferred to insulated boxes containing site-sourced water, and the other "half-clutch" returned to the site. Samples were transported to the laboratory and stored as independent half-clutches in aquaria, still situated in site-sourced water. Aquaria received constant aeration and were maintained at 4°C under a 12 h: 12 h light: dark photoperiod. After 24 h site-sourced water was substituted with dechlorinated tap water, and water changes were conducted twice weekly.

Embryos were reared under controlled laboratory conditions until development had progressed to pre-hatched, early-stage larvae (i.e. Gosner stage or G. 18 – 19; see Gosner, 1960). Still encased in the vitelline jelly capsule, G. 18 – 19 larvae had acquired functioning circulatory systems, gradually-developing external gill filaments and exhibited reflexive neuromuscular responses. Pre-hatched G. 18 – 19 larvae were transferred to 14°C in preparation for hatching, which typically occurred within 36h of transfer. Transfer to the higher temperature also provided pre-hatched *R. temporaria* larvae with time to acclimate to the new thermal regime, prior to use in experimentation.

Only recently hatched, pre-feeding *R. temporaria* larvae (i.e. G. 20 –21; Gosner, 1960) were used in experimental trials; lacking any visibly apparent functioning mouthparts and instead obtaining sustenance from an internalised yolk sac (Currie et al., 2016). Freshly hatched larvae from successive clutches were used on successive days of the experiment, such that clutch one was used on day one, clutch two on day two, etc. This ensured approximate standardisation in the developmental stage of *R. temporaria* larvae used, with larvae belonging to the same clutch often hatching synchronously and developing at a more uniform rate when associated with kin (Orizaola and Laurila, 2008). Only healthy larvae were used in experimental trials, free of any obvious morphological malformations (e.g. stunted tail fin, distended head-body, etc.). Larvae were used only once in each experimental trial.

Asellus aquaticus were sampled from the same location as native *G. pulex* – Meanwood Beck, West Yorkshire (53°49’50.0”N, 1°34’33.3”W). *Asellus aquaticus* were sampled via kick-sampling and hand-searching of submerged materials (e.g. rocky substrates and leaf-litter). Isopods were transported in insulated boxes with site-sourced water. In the laboratory, *A. aquaticus* were housed in aerated communal aquaria containing dechlorinated tap water and were maintained under the same environmental conditions as native and invasive amphipods (14°C under a 12 h: 12 h light: dark photoperiod). Isopods were fed a diet of sycamore leaves and were kept in communal aquaria for at least 72h prior to experimentation. Both male and female isopods were used in experimental trials, although attempts were made to standardise for body size. Females selected for use were non-gravid. Due to difficulties in obtaining sufficient numbers of *A. aquaticus* for experimental trials, healthy individuals were used up to two times across each experimental study. However, isopods had a minimum of 24h to recover in communal tanks prior to re-use.

Chironomus sp. larvae were purchased from a commercial live-food supplier (Northampton Reptile Centre; Northampton). Larvae were sourced from freshwater sites throughout the UK and were delivered in fresh water, maintained at approximately 10°C. Upon receipt, *Chironomus* sp. larvae were transferred to aerated aquaria, still situated in the same water they were delivered in, for a period of 24h before replacing source water with dechlorinated tap water. Larvae were used within 72h of receipt, with individuals used only once in each experimental trial. Individuals selected for use in experimental trials were of a comparable length and demonstrated similar levels of motility.

4.3.2 Experimental Design

All experiments were carried out at 14°C under a 12 h: 12 h light: dark photoperiod. Experimental trials were conducted in clear plastic arenas (diameter = 115mm, depth = 70mm), containing approximately 300ml dechlorinated tap water, and ran for a set period of 24h (prey selectivity experiment), or 48h (multiple prey species FR experiment). A single clear glass bead was placed in each experimental arena, providing substrate for animals to use as shelter, thereby deterring continual swimming behaviours, whilst remaining visible to the researcher. The mean ± SEM length of prey organisms used in experiments were as follows; *R. temporaria* larvae = 10.304 ± 0.084mm; *Chironomus* sp. larvae = 9.722 ± 0.144mm; *A. aquaticus* = 9.142 ± 0.162mm. Prior to the commencement of experimental trials, amphipod predators were individually starved for 24h to standardise hunger levels.

4.3.2.1 Prey Selectivity Experiment

In the first experiment individual, pre-starved native and invasive amphipods were simultaneously provided with five individuals of each of the three different prey types (*R. temporaria* larvae, *Chironomus* sp. larvae and *A. aquaticus*) to test for preferences (e.g. Haddaway et al., 2012).

Experimental trials were carried out over three days, spanning a single week, with a block of ten replicates established for each amphipod species per experimental day. Prey organisms were situated in experimental arenas one hour prior to the introduction of amphipod predators, providing time for prey to acclimate and settle. Experimental trials commenced with the introduction of amphipod predators and concluded with the removal of amphipods after an experimental period of 24h. Following the removal of predators, prey items were classified into four categories – 'partially consumed', 'completely consumed', 'alive' and 'dead' – and counted. Prey items were considered partially consumed if individuals were found dead, exhibiting extensive signs of predation (i.e. missing appendages), or if fragments of prey items were located which accounted for more than 5% of the entire organism. Conversely, prey items which had died without exhibiting signs of predations were simply considered as dead and reflected background mortality.

Amphipod predators were independently maintained for a further 24 h and observed in case of moulting or death. Amphipods which moulted and/or died during this post-experimental observation period were excluded from analyses, and an additional replicate was conducted as a replacement. The final dataset comprised 30 replicates recorded for native *G. pulex* and invasive *D. villosus*. Fifteen control replicates were also performed, in which amphipod predators were absent from experimental arenas. Controls were set up as blocks of five replicates carried out per experimental day, with controls conducted alongside experimental amphipod treatments over the three days of experimentation.

4.3.2.2 Multiple Prey Species Functional Response Experiment

This experiment initially began as a comparative study between native *G. pulex* and invasive *D. villosus*. However, preliminary trials revealed negligible predation by native *G. pulex* towards *R. temporaria* larvae, when offered in conjunction with *Chironomus* sp. larvae. Of the 35 replicated pilot trials conducted, comprising of five replicates for each combination of *R. temporaria* and *Chironomus* sp. larvae supplied, only 11 amphibian larvae, out of a possible 525 individuals provided across all preliminary trials, were consumed over the course of 48 h (~2%). By comparison, *Chironomus* sp. larvae were readily consumed (196/525 individuals supplied; ~37%). Native *G. pulex* also experienced a relatively high incidence of moulting and/or death whilst provided with experimental combinations of *R. temporaria* larvae and *Chironomus* sp. larvae, occurring in 34.29% of replicated trials. This prevented comparative FR analyses from being conducted between native *G. pulex* and invasive *D. villosus*. As such, the focus of the second experiment shifted to concentrate exclusively on assessing the predatory response of invasive *D. villosus* towards pairwise combinations of different prey types, when provided at different relative abundances.

In this experiment, individual pre-starved invasive *D. villosus* were provisioned with two experimental treatments in which native *R. temporaria*, the focal prey species, was provided alongside two alternative freshwater native macroinvertebrate prey species – *Chironomus* sp. larvae or *A. aquaticus*. For each experimental prey combination, individual *D. villosus* were supplied with a total of 30 individual prey (*R. temporaria* larvae – *Chironomus* sp. larvae) or 12 individual prey (*R. temporaria* larvae – *A. aquaticus*) at seven different ratios (*R. temporaria* larvae: *Chironomus* sp. larvae = 0:30, 5:25, 10:20, 15:15, 20:10, 25:5, 30:0; and *R. temporaria* larvae: *A. aquaticus* = 0:12, 2:10, 4:8, 6:6, 8:4, 10:2, 12:0). Prey organisms were placed in experimental arenas one hour prior to amphipod predators. Experimental trials began with the introduction of amphipod predators and ended with the removal of amphipods after an experimental period of 48 h. Prey consumed during the experimental period were not replaced. Following the removal of predators, each prey type was classified as 'partially consumed', 'completely consumed', 'alive' and 'dead', and enumerated. As with the prey preference experiment, amphipod predators were monitored for a further 24 h and amphipods which moulted and/or died during this observation period were removed, leaving a final dataset comprising of 6 – 13 replicates for each experimental prey combination. Controls for each prey combination comprised of five replicated arenas without amphipod predators.

4.3.3 Statistical Analysis

All statistical analyses were conducted in R version 3.5.1 (R Core Team, 2018), with a baseline significance level (α) = 0.05.

4.3.3.1 Prey Selectivity Experiment

Total consumption of all three prey types (*R. temporaria* larvae, *Chironomus* sp. larvae, and *A. aquaticus*) by native *G. pulex* and invasive *D. villosus*, was analysed using a generalised linear model fitted with a Poisson error distribution. Due to considerable under-dispersion of residuals (residual deviance / degrees of freedom = 0.172; (Crawley, 2007), the total number of prey items consumed was fitted, with regards to amphipod species, using a Conway-Maxwell Poisson regression model (*glm.cmp:: mpcmp*, version 0.1.3; Fung et al., 2019); thereby correcting for under-dispersed count data.

Differential consumption of each prey type, recorded for both native and invasive amphipods, was analysed using a GLM fitted with a binomial error distribution. Proportional consumption was analysed with respect to amphipod species (two levels) and prey type (three levels) and included a two-way interaction term (amphipod species x prey type). If the interaction term was non-significant, it was removed from the model, with model simplification assessed via analysis of deviance using χ^2. However, if the interaction term was statistically significant, *post-hoc* analysis was conducted comparing differences in marginal means estimated for prey types between native and invasive amphipods, as well as marginal means estimated for prey types within native and invasive amphipods (*emmeans::emmeans*, version 1.5.0; Lenth, 2020). *Post-hoc* analysis was conducted with a False Discovery Rate adjustment of p-value.

Within each amphipod species, compositional diet analysis was used to determine non-random predation of available prey types by native and invasive amphipods, and to derive prey choice hierarchies; ranking prey species based on their proportional contribution to amphipod diets (e.g. Strain et al., 2014; Orłowski et al., 2015; Taylor and Dunn, 2017). Compositional diet analyses were carried out using the R package *adehabitatHS* (version 0.3.15; Calenge, 2006). This analysis was conducted under the assumption of independence between amphipod predators and equal availability of all prey species to individual amphipods. Availability for each prey species was provided as a percentage of the total number of prey types initially supplied to amphipods at the start of experimental trials (n = 5 items per prey species; total number of prey items = 15). The proportional consumption of each prey type was summarised as a percentage of the total number of prey items consumed by individual amphipods. To facilitate the use of standard statistical analyses based upon multivariate normality, the percentage data was converted to log-ratios, thus making data pertaining to each prey species linearly independent (Aitchison, 1982). In the absence of predation of certain prey types by individual amphipods, zero percentages were replaced with a small value (0.01%), thereby enabling log-ratio transformations (Aebischer et al., 1993). Multivariate analysis of variance (MANOVA) was conducted, testing for significant difference in food selection behaviours by comparing compositional dietary data with the null hypothesis (i.e. random food consumption) using Wilk's lambda (Λ). Statistical significance was determined via randomisation (n = 2500 permutations). For statistically significant MANOVAs, prey types were ranked based on differences between consumption and availability (as log-ratios) for each pair of prey types. Prey species were ranked in order of importance in amphipod diets, based on mean differences across individual amphipods. Significant rankings were identified via randomisation tests (n = 2500 permutations).

4.3.3.2 Multiple Prey Species Functional Response Experiment

Overall consumption of each prey species by invasive *D. villosus* was analysed using a GLM fitted with a Poisson error distribution. Where necessary, GLMs were also fitted with a 'quasi-poisson' error distribution to account for overdispersion of residuals. Independent GLMs were conducted for experimental combinations of (i) *R. temporaria* larvae and *Chironomus* sp. larvae, and (ii) *R. temporaria* larvae and *A. aquaticus*. Consumption was analysed with regards to "prey species" and the proportion of each prey species supplied. A two-way interaction term (prey species x proportion supplied) was also included, and subsequently removed if statistically non-significant. Following the removal of a non-significant interaction term, model simplification was assessed via analysis of deviance using χ^2.

For each pairwise combination of prey species, prey preference by invasive *D. villosus* with respect to the relative abundance of each prey species provided, was determined using Manly's alpha (α) selectivity index, assuming no replacement of consumed prey items (Eqn. 1):

$$a_i = (\ln((n_{i0} - r_i)/n_{i0}))/\sum_{j=1}^{m}(\ln((n_{j0} - r_j)/n_{j0})) \quad \text{Eqn. 1}$$

where a_i is Manly's alpha selectivity index for prey species *i*, n_{i0} is the initial number of prey species *i* provided at the start of the experimental period, r_i is the number of prey species *i* consumed after 48 hours, *m* is the total number of prey species available (i.e. two), n_{j0} is the initial number of prey species *j* provided at the start of the experimental period, and r_j is the number of prey species *j* consumed. The value of a_i and a_j were bounded between 0 and 1, with 0 indicating absolute avoidance of corresponding prey species by invasive *D. villosus*, and 1 indicating absolute selectivity towards a specific prey species. In either two-prey system, a value of 0.5 was indicative of equal selectivity of both prey species (i.e. no preference).

Manly's α indices could not be calculated when a single prey species was supplied at extreme ratios (i.e. 0:12, 12:0, 0:30 and 30:0), or in cases where both prey species were present, yet one species experienced absolute predation (i.e. all prey items supplied were consumed). To overcome this issue datasets were modified to simulate the inclusion of an additional 0.001 of a single prey item for each prey species (n = 2 species) and for each provisioning treatment (n = 7 ratios). With the inclusion of such a small decimal value, modified initial prey densities still approximated to actual prey densities offered, whilst enabling the Manly's α index to be calculated across all experimental ratios. Non-parametric comparisons using the Mann-Whitney U test – due to non-normal residuals – revealed no statistically significant difference between estimations of the Manly's α index, calculated for each prey combination using the original and modified datasets (*R. temporaria* larvae vs *A. aquaticus*: U = 6418, $p = 0.382$; *R. temporaria* larvae vs *Chironomus* sp. larvae: U = 3760.5, $p = 0.061$). This would suggest that the simulated additions did not cause significant deviations in estimations of Manly's α. As such, further analyses concerning Manly's α indices were conducted using the adjusted estimates, calculated using modified datasets. To reduce the effects of extreme values (i.e. 0s and 1s), Manly's α indices calculated using modified datasets were transformed prior to analysis (Eqn. 2):

$$a_t = (a_i(n - 1) + 0.5/n \qquad \text{Eqn. 2}$$

where a_i is the untransformed index, a_t is the transformed index and *n* is the sample size. Transformed Manly's α indices calculated for each prey species, across both pairwise prey combinations, were analysed via beta regression (*betareg:: betareg*, version 3.1-3; Cribari-Neto and Zeileis, 2010). Selectivity indices were analysed with respect to "prey species" and the "proportion" of prey provided and included a two-way interaction term (prey species x proportion of prey) – which was subsequently removed if statistically non-significant. Model simplification was assessed via likelihood ratio testing (*lmtest::lrtest* version 0.9-38; Zeileis and Hothorn, 2002).

For each experimental combination of prey types, FR analyses were conducted to determine the relationship between the number of each prey type consumed and the initial number of each prey type provided, and to ascertain how the presence of alternative macroinvertebrate prey types may influence the *per capita* predation of native *R. temporaria* by invasive *D. villosus*, when present at varying relative abundances. Functional response analyses were performed upon datasets, modified to exclude extreme provisioning ratios (i.e. 0:12, 12:0; 0:30 30:0), for which only a single prey type was supplied. Multiple prey species FR models (i.e. MSFRs) were fitted to rarefied datasets; modelling *per capita* predation rates as a saturating hyperbolic Type II response (Eqn. 3), or a sigmoidal Type III response (Eqn. 4), relative to initial provisioning ratio:

$$f_i(N_i, N_j) = \left. a_i N_i \middle/ 1 + \sum_{j=1}^{n} a_j h_j N_j \right. \qquad \text{Eqn. 3}$$

$$f_i(N_i, N_j) = \left. a_i N_i^2 \middle/ 1 + \sum_{j=1}^{n} a_j h_j N_j^2 \right. \qquad \text{Eqn. 4}$$

where n is the number of available prey types, N is initial prey density, a is the predator's attack rate, h is the predator's handling time and all notations are prey-specific, corresponding to either prey species i or j. Originally developed by Murdoch (1973) and Chesson (1983), these models incorporate the consumption of multiple prey types, thus providing the potential for prey-switching behaviours by predators. In the absence of prey-switching, the ratio of prey consumed is proportional to the ratio of prey available; coinciding with the expression of a Type II response (van Leeuwen et al., 2013). However, if preference for a specific prey type increases as the relative density of that prey type also increases, predators are predicted to demonstrate prey-switching behaviours, selecting for the more abundant prey type, and resulting in the expression of a Type III response (Chesson, 1983).

To account for prey depletion over the course of experimental trials, each MSFR model was fitted using *ordinary differential equations* (ODE's; *deSolve::lsoda*, version 1.28; Soetaert et al., 2010), allowing prey depletion to be numerically simulated, and thus integrated into FR models, as a continuous process over the duration of experimental trials (i.e. 48h; see Bolker, 2012; Prokopenko et al., 2017; Rosenbaum and Rall, 2018). To obtain best-fit parameter estimations for the attack rate (a) and handling time (h) of invasive *D. villosus* towards each prey type, ODE-derived FR models were fitted with a binomial distribution via maximum likelihood estimations (*bbmle::mle2*, version 1.0.23.1; Bolker and R Core Team, 2017). Maximum feeding rate was also inferred, calculated as the reciprocal of predatory handling time ($1/h$). MSFR models were non-parametrically bootstrapped (n = 5000) to generate 95% confidence intervals for FR parameter estimations. To determine which

MSFR type best represented predatory responses by invasive *D. villosus* towards each prey type, across each experimental pairwise prey combination, Type II and Type III MSFR models were compared using the small sample corrected Akaike's information criterion (i.e. AICc), whereby ΔAICc < 2 is indicative of indistinguishable model performance.

Where analyses indicated a Type II response to be the most appropriate MSFR form, further supplementary modelling was conducted by fitting an alternative, mechanistically derived closed-form MSFR model, in which prey-switching behaviours were incorporated as a quantifiable parameter – formally known as the "similarity index" (s_{ij}; van Leeuwen et al., 2013; see Appendix 4, Section A4.2 for methodology). This model was fitted for each experimental prey combination, with s_{ij} provided as a fixed parameter, and as a free parameter to be estimated. However, when compared to Type II MSFR models described above, these additional models performed similarly to previous models when s_{ij} was provided as a fixed parameter (ΔAICc < 1), or worse when s_{ij} was provided as a free parameter – generating inconsistent estimates of FR parameters (see Appendix 4, Section A4.3 for results and outputs from model fitting).

4.4. Results

4.4.1 Prey Preference Experiment

Overall prey mortality was negligible within control treatments (4.89%). In the absence of amphipod predators, each prey type experienced similar, low levels of mortality (*R. temporaria* larvae = 2.67%; *A. aquaticus* = 5.33%; *Chironomus* sp. larvae = 6.67%). By comparison, overall prey mortality was significantly higher whilst in the presence of native *G. pulex* and invasive *D. villosus* (34.44% and 43.78% respectively; Fisher's exact test p < 0.001 for both). Therefore, deaths recorded in experimental treatments were assumed to be the result of predation by amphipod species, as supported by observations of direct predation upon each prey type, and the presence of partially consumed prey items following the removal of amphipod predators.

Total predation of all three prey types significantly differed between native and invasive amphipods. When compared to native *G. pulex*, invasive *D. villosus* consumed significantly greater numbers of prey overall (Conway-Maxwell Poisson GLM; $\chi^2 = 58.6$, df = 1, p < 0.001). When predation was assessed with respect to amphipod predator and prey type, analysis indicated a highly significant difference in overall consumption between native and invasive amphipods (Binomial GLM; $\chi^2 = 15.626$, df = 1, p < 0.001), with invasive *D. villosus* consuming considerably more prey items on average. The mean number of prey items consumed for each prey type also differed significantly (Binomial GLM; $\chi^2 = 206.621$, df = 2, p < 0.001), indicating a possible selection bias (i.e. prey preference hierarchy) by amphipods. A statistically significant 'amphipod x prey species' interaction term (Binomial GLM; $\chi^2 = 17.093$, df = 2, p < 0.001) indicated that, while native *G. pulex* and invasive *D. villosus* demonstrate similar prey preference hierarchies (*Chironomus* sp. larvae > *A. aquaticus* > *R. temporaria* larvae), the strength of preference was greater in *G. pulex* (Figure 4.1).

Native *G. pulex* and invasive *D. villosus* showed a substantially greater preference for *Chironomus* sp. larvae above *A. aquaticus* ($p < 0.001$ for both) and *R. temporaria* ($p < 0.001$ for both), with natives consuming marginally more prey overall when compared to invasive *D. villosus* (98.67% and 94.67% respectively; $p = 0.074$). The second most consumed prey species was *A. aquaticus*, with both native and invasive amphipods demonstrating intermediate levels of selectivity. Invasive *D. villosus* consumed significantly greater numbers of *A. aquaticus* than *R. temporaria* larvae ($p = 0.001$), and consumed a substantially greater number of isopods when compared to native *G. pulex* (26% and 4% respectively; $p < 0.001$). There was no significant difference in the consumption of *A. aquaticus* and *R. temporaria* larvae by native *G. pulex* ($p = 0.093$). *Rana temporaria* were the least selected prey species by both native and invasive amphipods (0.667% and 10.667% respectively), although again invasive *D. villosus* consumed significantly more individuals when compared to native *G. pulex* ($p = 0.006$).

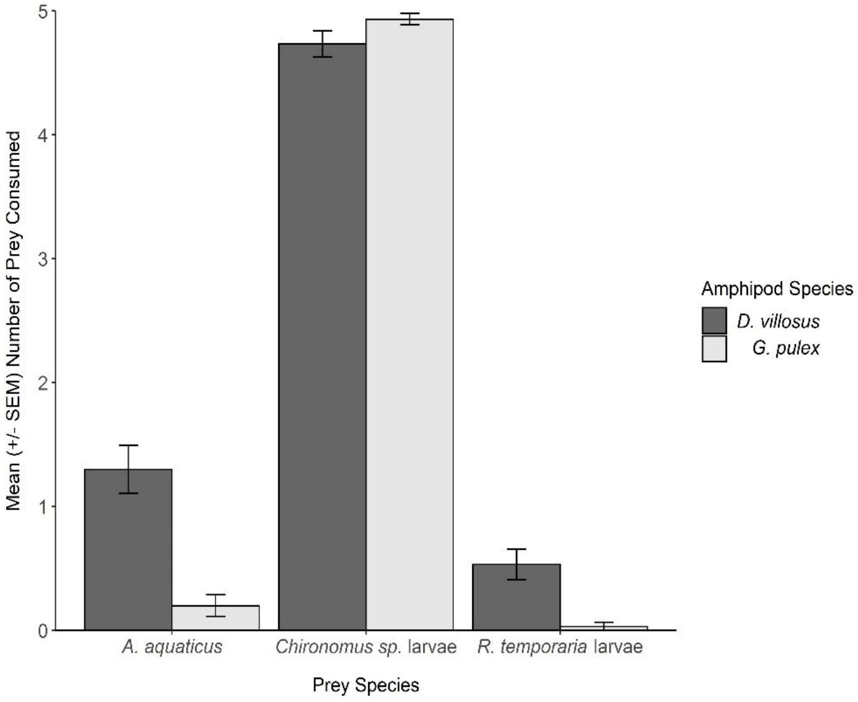

Figure 4.1. Mean (±SEM) number of prey items consumed by invasive *D. villosus* and native *G. pulex*, when provided with native *R. temporaria* larvae, *A. aquaticus* and *Chironomus* sp. larvae in equal quantities (n = 5 individuals of each species).

Comparisons concerning the proportional contribution of each prey species to native and invasive amphipod diets also indicated prey preference hierarchies. Compositional diet analysis confirmed that native *G. pulex* and invasive *D. villosus* both demonstrated significantly non-random feeding behaviours, when presented with each prey species in equal proportions (*G. pulex*: Wilks $\Lambda = 0.020$, p

< 0.001; *D. villosus*: Wilks Λ = 0.283, p < 0.001). Analyses confirmed that *Chironomus* sp. larvae made the greatest contribution to native and invasive amphipod diets, with 100% of native *G. pulex* and invasive *D. villosus* having consumed these dipteran larvae, followed by *A. aquaticus* (16.67% of *G. pulex* and 76.67% of *D. villosus*), and finally *R. temporaria* larvae (3.33% of *G. pulex* and 43.33% of *D. villosus*; Table 4.1a,b).

Table 4.1. Ranking matrices generated by compositional diet analysis (Aebischer et al., 1993), ranking prey species based upon their proportional contribution to (a) native and (b) invasive amphipod diets. Symbols (+ or -) denote whether a prey species is ranked above or below another prey species respectively, and triple symbols (+++ or ---) indicate whether rankings between prey species are significant. Matrices generated via randomisation/permutation testing (n = 2500 resamples).

a)

G. pulex	*R. temporaria* larvae	*A. aquaticus*	*Chironomus* sp. larvae
R. temporaria larvae	**0**	**-**	**---**
A. aquaticus	+	**0**	**---**
Chironomus sp. larvae	+++	+++	**0**

b)

D. villosus	*R. temporaria* larvae	*A. aquaticus*	*Chironomus* sp. larvae
R. temporaria larvae	**0**	**---**	**---**
A. aquaticus	+++	**0**	**---**
Chironomus sp. larvae	+++	+++	**0**

4.4.2 Multiple Prey Species Functional Response Experiment

Prey mortality was negligible in control treatments, with total prey mortality <5% for each experimental pairwise prey combination (*R.temporaria – A. aquaticus* = 4.52% mortality; *R. temporaria – Chironomus* sp. larvae = 1.33% mortality). Overall prey mortality within experimental treatments was significantly higher whilst in the presence of *D. villosus*, with 31.62% total mortality recorded for *R. temporaria* larvae and *A. aquaticus*, and 28.47% total mortality recorded for *R. temporaria* larvae and *Chironomus* sp. larvae (Fisher's exact test p < 0.0001 for both pairwise prey combinations). As such, prey mortality recorded in experimental treatments was assumed to be the product of predation by *D. villosus*, as evidenced by observed predation of each prey species and the presence of fragmented, partially consumed prey items.

Across both experimental pairwise prey combinations, *D. villosus* exhibited a strong preference for alternative macroinvertebrate prey types over focal amphibian larvae. When presented with *R. temporaria* and *Chironomus sp.* larvae, preferential selection of dipteran larvae was consistently observed across all provisioning ratios; except for extreme ratios (0:30, 30:0) when only a single prey species was offered (Table 4.2; Figure 4.2). When supplied with *R. temporaria* larvae and *A. aquaticus*, *D. villosus* displayed no obvious preferential feeding patterns towards either prey species when the proportional availability of amphibian larvae was low (2:10, 4:8). However, as the relative abundance of *R. temporaria* larvae increased, feeding patterns became more selective, with amphipods exhibiting a stronger preference for isopod prey overall (Table 4.3; Figure 4.3).

Overall consumption of *R. temporaria* and *Chironomus sp.* larvae increased significantly in response to increasing proportional availability (Poisson GLM; $\chi^2 = 282.07$, df = 6, $p < 0.0001$). However, significantly fewer *R. temporaria* larvae were consumed, when compared to *Chironomus sp.* larvae (7.95% and 56.73%, respectively; $\chi^2 = 347.39$, df = 1, $p < 0.0001$), indicating a greater selection bias towards dipteran prey (Figure 4.2). A non-significant 'prey species x proportion' interaction was identified ($\chi^2 = 0.960$, df = 6, $p = 0.987$) and removed from the model. Analyses pertaining to the consumption of *R. temporaria* larvae and *A. aquaticus* indicated similar patterns as those described above (Figure 4.3). Overall consumption increased significantly with respect to proportional availability (Poisson GLM; $\chi^2 = 70.783$, df = 1, $p < 0.001$), and *D. villosus* consumed substantially greater numbers of *A. aquaticus* (47.41%) when compared to *R. temporaria* larvae (14.54%; $\chi^2 = 77.383$, df = 1, $p < 0.001$). Again the 'prey species x proportion' interaction term was statistically non-significant ($\chi^2 = 0.977$, df = 6, $p = 0.987$), and was therefore removed from the model.

Analysis of Manly's α prey preference indices revealed a significantly greater selection for *Chironomus sp.* larvae by *D. villosus*, above *R. temporaria* larvae (Beta Regression; $\chi^2 = 144.58$, df = 1, $p < 0.001$; Table 4.2). Values estimated for the Manly's α were also significantly affected by the proportion of prey available ($\chi^2 = 291.62$, df = 6, $p < 0.001$). Inclusion of a 'prey species x proportion' interaction term proved to be statistically significant ($\chi^2 = 203.07$, df = 6, $p < 0.001$), with predation of dipteran prey greatest at intermediate provisioning ratios. In the absence of prey-switching patterns, this would indicate an overall preference for *Chironomus sp.* larvae as an alternative non-amphibian prey species (Figure 4.2). When supplied with both *R. temporaria* and *A. aquaticus*, *D. villosus* also exhibited a significantly greater preference for isopods over amphibian larvae (Beta Regression; $\chi^2 = 26.655$, df = 1, $p < 0.001$), with Manly's α index values notably affected by proportional availability ($\chi^2 = 49.879$, df = 6, $p < 0.001$; Table 4.3). Again, a statistically significant 'prey species x proportion' interaction term was identified ($\chi^2 = 13.826$, df = 6, $p = 0.032$), reflected by a reduced preference for *A. aquaticus* when amphibian larvae were available at low relative abundances (2:10, 4:8). However, given the disproportionate preference for isopod prey overall ($\alpha > 0.5$; Table 4.3), there was no evidence of prey-switching (Figure 4.3).

Table 4.2. Mean values for the untransformed Manly's α selectivity index displayed by invasive *D. villosus* towards native *R. temporaria* larvae (focal prey) and *Chironomus* sp. larvae (alternative prey) across varying provisioning rations.

Prey Species			
R. temporaria larvae		*Chironomus sp.* larvae	
Proportional Availability	Manly's α + SEM	Proportional Availability	Manly's α + SEM
0.17	0.27 + 0.09	0.83	0.73 + 0.09
0.33	0.19 + 0.07	0.67	0.81 + 0.07
0.50	0.10 + 0.03	0.50	0.90 + 0.03
0.67	0.05 + 0.02	0.33	0.95 + 0.02
0.83	0.03 + 0.02	0.17	0.97 + 0.02

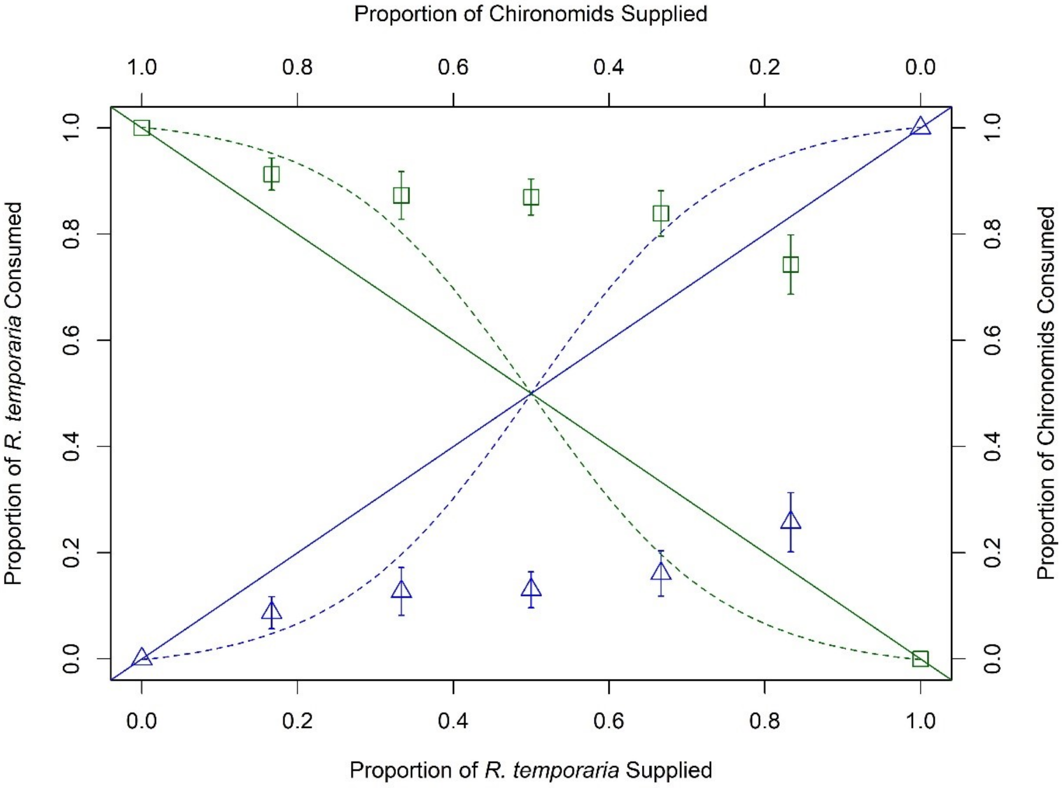

Figure 4.2. Proportional consumption (mean + SEM) of *R. temporaria* larvae (blue open triangles) and *Chironomus* sp. larvae (green open squares) by invasive *D. villosus*, as a function of the proportional availability of each prey type supplied. Solid lines represent a relationship for which there is no preferential selectivity between the two prey types and the dashed lines represents a hypothetical switching pattern between prey types. The colour of each line type corresponds to each prey type.

Table 4.3. Mean values for the untransformed Manly's α selectivity index displayed by invasive *D. villosus* towards native *R. temporaria* larvae (focal prey) and *A. aquaticus* (alternative prey) across varying provisioning rations.

Prey Species			
R. temporaria larvae		*A. aquaticus*	
Proportional Availability	Manly's α ± SEM	Proportional Availability	Manly's α ± SEM
0.17	0.43 ± 0.12	0.83	0.57 ± 0.12
0.33	0.34 ± 0.11	0.67	0.66 ± 0.11
0.50	0.19 ± 0.06	0.50	0.82 ± 0.06
0.67	0.19 ± 0.05	0.33	0.81 ± 0.05
0.83	0.26 ± 0.16	0.17	0.74 ± 0.16

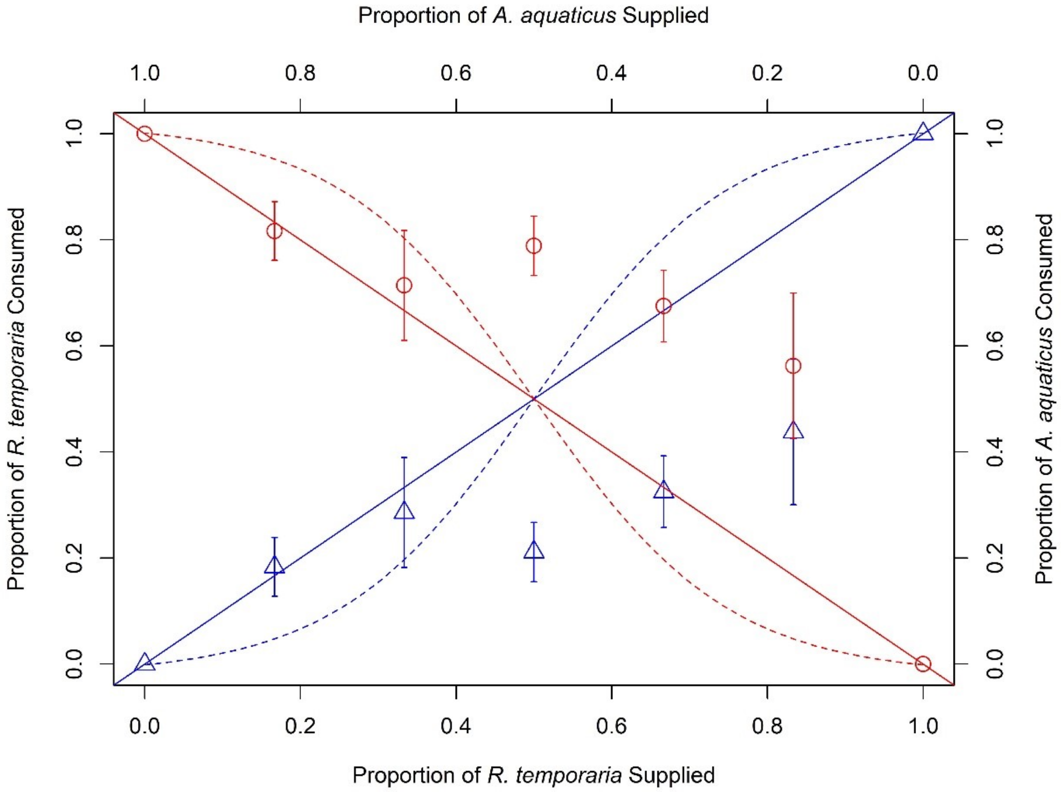

Figure 4.3. Proportional consumption (mean ± SEM) of *R. temporaria* larvae (blue open triangles) and *A. aquaticus* (red open circles) by invasive *D. villosus*, as a function of the proportional availability of each prey type supplied. Solid lines represent a relationship for which there is no preferential selectivity between the two prey types and the dashed lines represents a hypothetical switching pattern between prey types. The colour of each line type corresponds to each prey type.

For each experimental prey combination, *D. villosus* appeared to display a saturating Type II response to all prey types provided. When compared to the Type III MSFR model variant, Type II models performed substantially better when fitting recorded data (ΔAICc > 2; Table 4.4). Greater selectivity towards alternative macroinvertebrate prey types translated into differential estimations of FR parameters (Table 4.4). Whilst feeding upon native *R. temporaria* larvae, *A. aquaticus* and/or *Chironomus* sp. larvae, provided at varying relative densities, *D. villosus* attacked *Chironomus* sp.

larvae and *A. aquaticus* at much higher rates when compared to larval *R. temporaria* (estimated attack rates 17.1 and 4.9 times greater respectively), with clear separation between 95% confidence intervals indicative of a significant difference. Conversely, *D. villosus* spent less time when handling individual dipteran and isopodan prey, when compared to larval anurans (2 and 1.5 times lower, respectively); although comparisons of 95% confidence intervals suggested a significant difference between *R. temporaria* and *Chironomus* sp. larvae only. By inference, *D. villosus* demonstrated greater maximum feeding rates towards *Chironomus* sp. larvae (6.4 prey/day) and *A. aquaticus* (2.9 prey/day), when compared to *R. temporaria* larvae (3.1 and 2.0 prey/day, respectively).

Differential estimations of FR parameters were reflected by variations in the predicted trajectories of predatory response curves, with *per capita* consumption of *Chironomus sp.* larvae (Figure 4.4a) and *A. aquaticus* (Figure 4.4b) increasing at a much steeper rate, relative to proportional prey availability, before establishing a higher asymptote. By comparison, FR curves generated for *D. villosus* whilst feeding upon native *R. temporaria* larvae were much shallower, reaching an asymptote sooner. Interestingly, the projected response curve generated for *R. temporaria* larvae, when supplied alongside *Chironomus* sp. larvae, exhibited an apparent ‘hump’, as predation of anuran larvae began to plateau (Figure 4.4a). Preferential consumption of larval dipterans, even at lower relative densities, appeared to lower the effective attack rate towards *R. temporaria* larvae, thereby underestimating predicted predation of *R. temporaria* larvae, when compared to recorded data. By comparison, FR curves generated for *R. temporaria* larvae and *A. aquaticus* did not exhibit similar projections, instead describing a typical Type II curve relative to relative prey availability (Figure 4.4b).

Table 4.4. Best-fit estimates for FR parameters, obtained using ODE-derived Type II (hyperbolic) and Type III (sigmoid) multi-prey species functional response (MSFR) models, regarding per capita consumption by invasive *D. villosus* towards several experimental prey combinations (*R. temporaria* larvae – *Chironomus sp.* larvae; *R. temporaria* larvae – *A. aquaticus*), when supplied at varying provisioning ratios. Parameters were obtained using maximum likelihood estimations, and 95% confidence intervals generated via non-parametric bootstrapping (n = 5000). Models comparisons conducted using small sample corrected Akaike's information criterion (AICc).

Prey Combination	Prey Species	Type II MSFR Model (Murdoch, 1973)				Type III MSFR Model (Chesson, 1983)			
		Parameter Estimate [95% CI]		*1/h*	AICc	Parameter Estimate [95% CI]		*1/h*	AICc
		a	*h*			*a*	*h*		
1	*R. temporaria* larvae	0.301 [0.185 – 0.458]	0.319 [0.214 – 0.402]	3.1	355.055	0.069 [0.030 – 0.136]	0.384 [0.306 – 0.480]	2.6	441.637
	Chironomus sp. larvae	5.140 [3.456 – 7.555]	0.157 [0.143 – 0.174]	6.4		4.482 [2.068 – 7.626]	0.172 [0.157 – 0.188]	5.8	
2	*R. temporaria* larvae	0.319 [0.193 – 0.504]	0.512 [0.317 – 0.689]	2.0	345.173	0.104 [0.047 – 0.186]	0.620 [0.457 – 0.762]	1.6	392.454
	A. aquaticus	1.565 [0.979 – 2.236]	0.347 [0.282 – 0.406]	2.9		0.873 [0.419 – 1.428]	0.407 [0.358 – 0.460]	2.5	

a = attack rate
h = handling time
1/h = maximum feeding rate

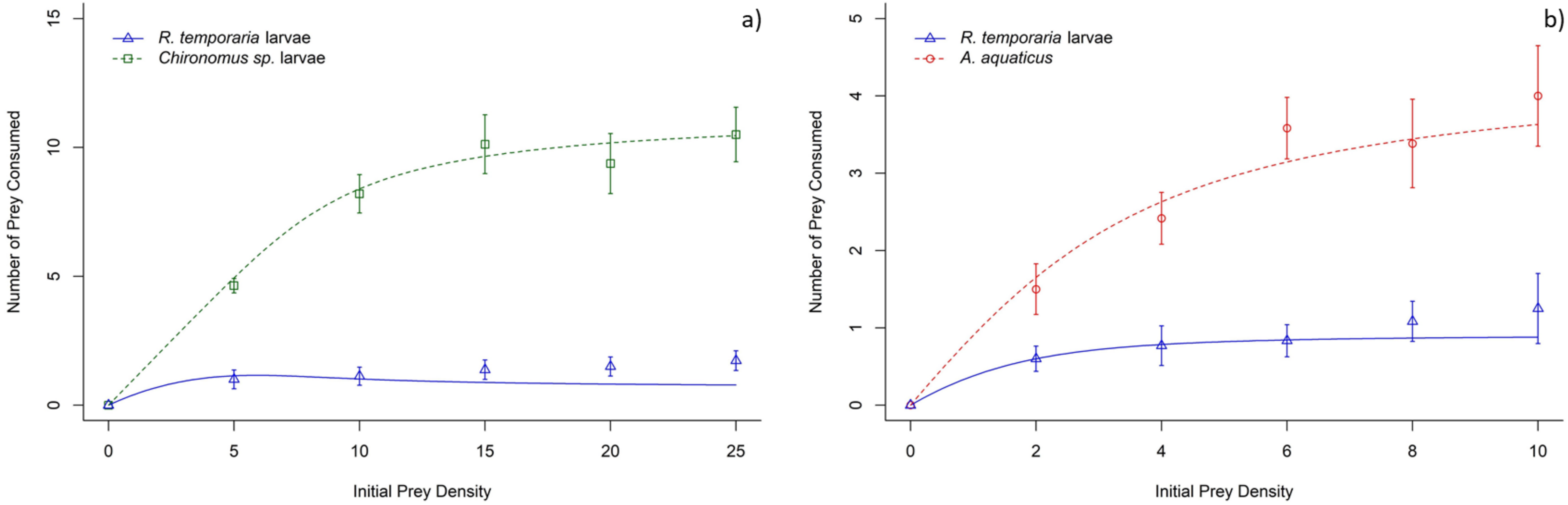

Figure 4.4. Type II multi-prey species functional response (MSFR) curves for per capita consumption by invasive *D. villosus* towards a) *R. temporaria* larvae (blue open triangles and solid line) and *Chironomus* sp. larvae (green open squares and dashed line), and b) *R. temporaria* larvae (blue open triangles and solid line) and *A. aquaticus* (red open circles and dashed line), when supplied at varying provisioning ratios. Curves obtained using an ODE-derived MSFR model (Murdoch, 1973), fitted via maximum likelihood estimations. Points denote mean (± SEM) number of preys consumed.

4.5 Discussion

Dikerogammarus villosus is regarded as a top predatory invader, renowned for its voracious appetite and exceptional predatory capabilities (Rewicz et al., 2014). *Dikerogammarus villosus* demonstrates the potential to impose greater ecological impacts, when compared to native amphipods that it replaces, across a range of invertebrate and vertebrate prey (Dick et al., 2002; MacNeil et al., 2013a; Dodd et al., 2014; Taylor and Dunn, 2017), including amphibians (Chapter 2). Here, I utilise prey choice experiments, to show that native *G. pulex* and invasive *D. villosus* express similar hierarchies in prey preference, but that *D. villosus* has a more generalist diet. Specifically, *D. villosus* makes greater use of amphibian larvae and isopod prey when compared to native counterparts, which demonstrated a strong preference for dipteran larvae only. Using prey-switching experiments, providing individual *D. villosus* with anuran larvae and either macroinvertebrate prey species at varying ratios, amphipod predators were shown to display a disproportionately greater preference for macroinvertebrates overall; although selectivity towards *A. aquaticus* became less apparent when *R. temporaria* larvae were supplied at a low relative abundance. Functional response analyses revealed attack rates by *D. villosus* to be higher, and handling times to be lower towards isopod and dipteran prey when compared to anuran larvae. Differential predation towards paired prey species translated into markedly steeper FR curves with respect to macroinvertebrate prey, reaching considerably higher asymptotes (i.e. maximum feeding rates) as the proportional availability of prey species increased. Despite an overall reduction in the *per capita* predation of *R. temporaria* larvae, preference for anurans was relatively high when present at low relative abundance alongside isopod prey, although evidence of possible prey-switching was negligible. Taken together, these findings indicate that predatory impacts exerted by *D. villosus* towards native amphibian populations are likely to be relatively low, depending on alternative prey communities. However, there is some evidence to suggest that, under certain circumstances, predation of larval anurans could be higher at low relative abundance, which could potentially destabilise populations.

As a generalist predator, invasive *D. villosus* is predicted to display indiscriminate feeding across available prey types (see Dodd et al., 2014); a trait thought to contribute to the success of this high-impact invader (Hänfling et al., 2011). However, the findings of the current study appear to conflict with these predictions, with invasive *D. villosus* demonstrating strong non-random feeding behaviours when provided with *Chironomus* sp. larvae, *A. aquaticus* and *R. temporaria* larvae in equal proportions. Evidence of hierarchical prey selectivity would suggest that predatory pressures exerted by this invader are unlikely to be evenly distributed amongst resident species (see Taylor and Dunn, 2017). Although *D. villosus* displayed a significantly higher preference towards *Chironomus* sp. larvae, when compared to *A. aquaticus* and *R. temporaria* larvae, similarities in the level of consumption exhibited by native and invasive amphipods would indicate that any disturbance experienced by dipteran larvae, while in the presence of *D. villosus* is likely to be negligible. Whilst consistent with previous empirical works, as well as observations recorded within invaded field systems (see Dodd et al., 2014), comparable predation by native *G. pulex* and invasive *D. villosus* may also reflect possible limitations in experimental design (e.g. 'snapshot' comparisons; see Dick et al., 2014); as indicated by near absolute predation by both native and invasive amphipods (~99% and ~95%, respectively). Conversely, a significantly greater preference by invasive *D. villosus* towards both *A. aquaticus* and *R. temporaria* larvae, when compared to native *G. pulex*, would suggest that native isopods and larval anurans may experience notably greater predatory impact, even though selectivity towards both prey types was considerably lower than dipteran larvae.

Examination of invader *per capita* predation, in response to the proportional availability of larval *R. temporaria*, *A. aquaticus* and/or *Chironomus* sp. larvae, provided further evidence of non-random feeding by *D. villosus*. Regardless of relative abundance, *D. villosus* demonstrated preferential feeding across each experimental prey combination, exhibiting a significantly greater preference for alternative macroinvertebrate prey types, and decreased selectivity towards *R. temporaria* larvae. *Dikerogammarus villosus* appeared to demonstrate no definitive prey-switching behaviours across experimental prey combinations, indicating that predatory pressures exerted towards isopod and dipteran prey may be maintained even as proportional availability varies, possibly resulting in the extirpation of macroinvertebrate prey from invaded field communities (Murdoch and Oaten, 1975; van Leeuwen et al., 2007). Conversely, decreased selectivity towards native *R. temporaria* larvae, across all available provisioning ratios, indicated limited predatory impact being imposed by *D. villosus*, whilst in the presence of alternative macroinvertebrate prey species. As such, populations of larval anurans, present in communities invaded by *D. villosus*, may be expected to experience negligible/minimal disturbance.

Through the application of MSFR modelling, preferential selectivity in conjunction with an absence of positive prey-switching (i.e. switching to a more abundant prey type; Oaten and Murdoch, 1975) – behaviours consistent with previous empirical works concerning invader impacts (see Cuthbert et al., 2018a; Taylor and Dunn, 2018) – translated into substantially different predatory responses by *D. villosus* towards *R. temporaria*, *A. aquaticus* and *Chironomus* sp. larvae; thus altering ecological predictions. *Dikerogammarus villosus* were found to express a Type II FR towards all prey types; indicative of a potentially destabilising predator-prey dynamic (Dick et al., 2014). Whilst consistent with previous studies, which utilised single-prey FR modelling to predict the ecological impact of invasive *D. villosus* (e.g. Bollache et al., 2008; Dodd et al., 2014; Chapter 2), these findings appear to conflict with prior theoretical works, which expect predators to demonstrate positive prey-switching relative to proportional abundance, thereby resulting in the emergence of a Type III FR; predicted to stabilise predator-prey dynamics (see Oaten and Murdoch, 1975; van Leeuwen et al., 2007). As such, predatory impacts demonstrated by *D. villosus* are likely to persist within invaded field communities, regardless of the availability of alternative prey (e.g. Bollache et al., 2008; Dodd et al., 2014).

Significantly higher attack rates, estimated towards *A. aquaticus* and *Chironomus* sp. larvae, may denote preferential selectivity by *D. villosus* (see Allan and Flecker, 1988; Smout et al., 2010; Baudrot et al., 2016), indicating a strong tendency for this invader to target macroinvertebrate prey types, when compared to *R. temporaria* larvae. When considered in conjunction with considerably lower handling times, higher attack rates demonstrated towards *A. aquaticus* and *Chironomus* sp. larvae, would suggest a greater predicted impact by *D. villosus* towards macroinvertebrate prey, when compared to *R. temporaria* larvae (Cuthbert et al., 2019a). Increased selectivity by *D. villosus* towards macroinvertebrate prey may likely reflect a greater proficiency in capturing *A. aquaticus* and *Chironomus* sp. larvae, resulting from this invader's predatory foraging mode (Sih, 1993), but may also be attributed to marked dissimilarities between prey species; particularly morphology and/or behaviour (see van Leeuwen et al., 2013).

Dikerogammarus villosus is an efficient "high-speed" sit-and-wait predator (Maazouzi et al., 2011), and as such will often remain static, utilising lateral line sensory organs (see Platvoet et al., 2007), as well as elongated secondary antennae, to detect proximate prey items, before aggressively striking and subduing (Platvoet et al., 2009a). Therefore, the success of this invader in consuming prey items is largely determined by prey mobility, with more active prey types likely to experience more frequent encounters with *D. villosus*, thereby stimulating a stronger predatory response (Sih, 1993). *Asellus aquaticus* is a slow-moving, hard-bodied benthic macroinvertebrate (Dick et al., 2002), known for implementing random, sporadic movement patterns (i.e. Lévy walk; Augusiak and Van den Brink, 2015). *Chironomus* sp. larvae often demonstrate highly energetic, undulating movements; a behaviour associated with gaseous exchange (Panis et al., 1996; Roskosch et al., 2012) and locomotion (Brackenbury, 2000). Although chironomid larvae may lessen mobility in order to avoid predation, some species also strongly associate with substrates, burrowing into sediment (Hölker and Stief, 2005). Whilst this may succeed in deterring large predators (e.g. fish; Stief and Hölker, 2006), *D. villosus* is capable of excavating *Chironomus* sp. larvae, enabling it to consume these soft-bodied larval dipterans with relative ease (Platvoet et al., 2009a). In the presence of sit-and-wait predators, *R. temporaria* larvae often exhibit a significant, prolonged reduction in activity (pers. obs.; Laurila et al., 2008; Álvarez and Nicieza, 2009; Maag et al., 2012); a behaviour which may likely limit encounters with invasive *D. villosus*. However, if captured, *D. villosus* may overpower *R. temporaria* larvae, utilising enhanced morphological structures (i.e. large mouthparts and gnathopods) to restrain and consume individual prey (Chapter 2). Although *R. temporaria* larvae possess somewhat higher nutritional contents (i.e. C: N: P), when compared to *A. aquaticus* and *Chironomus* sp. larvae (see Fink et al., 2006; Liess et al., 2013; Norlin et al., 2016), the potential energetic costs associated with overcoming vigorous antipredator behavioural responses by larval *R. temporaria*, may deter *D. villosus* from attempting to frequently hunt these native anuran prey. Instead, invasive *D. villosus* may be expected to favour alternative, easy-to-capture prey when available, such as *A. aquaticus* and

Chironomus sp. larvae; although, based upon the findings of the first experiment, and previous empirical works, increased predation by *D. villosus* towards larval dipterans, may not necessarily result in a greater ecological impact, when compared to native *G. pulex* (see Dodd et al., 2014).

The findings of the current study predict a low direct ecological impact by invasive *D. villosus* towards native *R. temporaria*, resulting from substantially greater selectivity towards more motile, easily handled *A. aquaticus* and *Chironomus* sp. larvae. However, increased predation of alternative macroinvertebrate prey types may, in turn, indirectly impact upon the survival of native UK anurans, with potential far-reaching implications for the stability of invaded field systems. As stated previously, predation of *Chironomus* sp. larvae by *D. villosus* is unlikely to disturb populations of larval dipterans in the field, with evidence of consumption rates comparable to *G. pulex* indicative of similar predatory pressures (Dodd et al., 2014). However, markedly higher consumption rates towards *A. aquaticus*, demonstrated by invasive *D. villosus* when compared to native *G. pulex* in the first experiment, corroborates observations concerning the extirpation of freshwater isopods from invaded field sites within the UK (see Dodd et al., 2014). *Asellus aquaticus* is often regarded as a highly effective omnivorous shredder, occupying the same ecological niche as native *G. pulex* (Bloor, 2011; MacNeil et al., 2011; Hunting et al., 2012). As a keystone shredder, *A. aquaticus* plays a vital role in nutrient cycling, processing coarse organic matter and releasing crucial nutrients into the environment, thereby making them available to lower trophic organisms (e.g. filter-feeders; MacNeil et al., 2011). Shredding is also an important ecological process in regulating periphyton communities, with the presence of *A. aquaticus* shown to enhance microorganism community richness (see Hunting et al., 2012). Periphytons are a vitally important primary resource utilised by a wide range of freshwater organisms (Azim, 2009), including early stage *R. temporaria* larvae which are predominantly herbivorous grazers (Brönmark et al., 1991; Griffiths, 1991). As such, the predator-induced extirpation of *A. aquaticus*, and other shredders, from invaded freshwaters by invasive *D. villosus* – as observed in previous field studies (Madgwick and Aldridge, 2011; Dodd et al., 2014; Truhlar et al., 2014) – may cause considerable disruptions to nutrient flow, subsequently impacting upon periphyton communities, as well as organisms which are reliant upon these algal resources, and/or the nutritional by-products of detritivory (MacNeil et al., 2011). As a result, the removal of a functionally important species, such as *A. aquaticus*, could trigger a cascade of secondary extinctions across multiple trophic levels (i.e. *sequential extinctions*), potentially causing the eventual collapse of an entire community (see Ebenman and Jonsson, 2005; Dunne and Williams, 2009)

Disruption to nutrient cycling, and its subsequent impact upon periphyton abundance, may lead to higher mortality in early stage *R. temporaria* larvae (see Nyström et al., 2001). In response, *R. temporaria* larvae may instead switch to a more carnivorous diet, accessing alternative resources by altering intestinal morphology in order to accommodate a switch in diet (e.g. shortening of gut; see Ruthsatz et al., 2019); although switching to an animal-based diet at an early developmental age may impact considerably upon larval performance, affecting individual fitness post-metamorphosis (Craioveanu et al., 2019). The removal of macroinvertebrate prey species from invaded field communities by *D. villosus*, whether it be through direct predation or as a result of sequential extinctions, may also impact upon the survival of *R. temporaria* larvae at later developmental stages, whereupon larvae undergo a dietary shift, transitioning from herbivory to exclusive carnivory, following the development of hindlimbs (Linzey, 2012). As carnivores, late-stage *R. temporaria* larvae feed upon small macroinvertebrate prey, such as cladocerans (Savage, 1952; Savage, 1962). Although predation of cladocerans by invasive *D. villosus* was not examined in the current study, previous literature has demonstrated significantly higher predation when compared to native *G. pulex*, with increased predation by *D. villosus* expected to lead to dramatic declines of cladocerans, under certain circumstances (see Dodd et al., 2014). As such, the removal of key macroinvertebrate prey types by *D. villosus* may have a detrimental impact upon the larvae of other amphibian species, particularly those which rely on carnivory throughout larval development (e.g. *Rana sylvaticus* and *Rana clamitans*; Petranka and Kennedy, 1999). Alternatively, intense predation of macroinvertebrate prey by *D. villosus* may likely limit the availability of prey to alternative freshwater predators. As a result, *R. temporaria* larvae may become the target of more common, more effective predators, such as dragonfly larvae (see Hossie and Murray, 2016), fish, and possibly other invaders (e.g. signal crayfish; see Nyström and Åbjörnsson, 2000; Nyström et al., 2001).

To-date, this is the first empirical study to utilise MSFR modelling as a means of predicting the potential ecological impact of invasive *D. villosus*. Compared to previous works, which formerly used the single-prey FR approach in order to assess *per capita* predation, applied to a range of freshwater prey species (reviewed in Dick et al., 2014), including early-stage fish (e.g. Taylor and Dunn, 2017), and anurans (e.g. Chapter 2), the MSFR approach takes into consideration differential predation across multiple prey types, and incorporates potential frequency-dependent prey-switching behaviours (Oaten and Murdoch, 1975; van Leeuwen et al., 2007; van Leeuwen et al., 2013; Baudrot et al., 2016). Whereas previous studies have predicted that, when compared to native *G. pulex*, invasive *D. villosus* may impose significantly greater predatory pressures towards *R. temporaria* larvae when present as a single prey type (Chapter 2), the findings of the current study would suggest that, in actuality, predatory pressures exerted by this invader may likely be dampened within invaded field systems, resulting in *D. villosus* displaying a significantly greater preference for alternative, easily caught macroinvertebrate prey. With evidence of strong preferential feeding, *D. villosus* is expected to consistently exert substantially greater predatory pressures upon macroinvertebrate prey, regardless of the relative abundance of *R. temporaria* larvae; thereby providing these larval anurans with a potential escape from invader predation. However, it must be noted that ecological predictions obtained via laboratory-based empirical studies may not necessarily reflect actual observed field impacts (Morozov and Petrovskii, 2013). In the field, freshwater ecosystems typically contain complex communities, consisting of large numbers of interacting species; many of which belong to the same trophic level (Hall and Raffaelli, 1993; Morozov and Petrovskii, 2013). Moreover, with *D. villosus* often existing at high densities within invaded regions (e.g. van Riel et al., 2006), the presence of alternative predators may also influence invader impact (see Dick et al., 2017; Chapter 2), with interactions amongst conspecifics expected to alter *per capita* feeding behaviours (Médoc et al., 2015; Chapter 3). Nevertheless, the findings of the current study provide a unique insight into the predatory behaviours of invasive *D. villosus*, enhancing previous predictions concerning the ecological impact of this invader upon amphibians; a group which has suffered considerable global declines as a result of invasive predators (see Kats and Ferrer, 2003; Ilhéu et al., 2007; Nunes et al., 2019). This study demonstrates the efficacy of the MSFR approach, when utilised in conjunction with prey choice experiments, in obtaining accurate predictions regarding *per capita* predation, and determining the likely impacts of invader predation in recipient communities.

Chapter 5

Molecular Diagnostics and Tissue Histology Reveal the Invasive Killer Shrimp (*Dikerogammarus villosus*) as a Potential Carrier of *Batrachochytrium dendrobatidis*

5.1 Abstract

Invasive alien species are increasingly recognised as drivers in the spread of emerging infectious diseases, representing a significant threat to native wildlife. *Batrachochytrium dendrobatidis* (*Bd*), the causative agent behind chytridiomycosis, is considered to be one of the most important causes of global amphibian declines. Typically regarded as an amphibian specialist, *Bd* can infect a range of alternative non-amphibian hosts, including invasive crustaceans; a trait which may explain the virulence, distribution, and maintenance of *Bd* in the environment, even in the absence of suitable amphibian hosts. As such, it is crucial to identify potential biological reservoirs and to establish their role in pathogen persistence; particularly when widespread invasive species are involved. In this chapter I investigate the potential for the invasive freshwater amphipod *Dikerogammarus villosus* to act as a carrier of *Bd*. Using experimental infection and mortality trials, I aimed to (1) determine whether *D. villosus* can acquire and maintain infections, (2) examine how infections might manifest within amphipod hosts, and (3) explore how infections may affect host survival. Following repeated exposures to infectious *Bd* zoospores, amphipods became infected, with low-intensity infections detected in ~14% of exposed individuals. Histological analysis revealed the presence of zoosporangia, interacting with the exterior surface of the amphipod cuticle and the gill lamellae; there was no evidence of intracellular infections. Infected individuals appeared asymptomatic, with no obvious evidence of *Bd*-mediated pathologies, nor induced immune responses. Exposure to *Bd* zoospores did not affect amphipod survival, when compared to controls, although there was some evidence to suggest greater mortality associated with infections. A number of amphipods moulted following exposures to *Bd* zoospores, and whilst post-moult host animals tested negative for *Bd*-infections, surprisingly, *Bd* was prevalent on exuviae (>90%), suggesting that amphipods may clear infections through ecdysis. These results indicate that invasive *D. villosus* may be suitable, asymptomatic carriers of *Bd*, with the potential to facilitate its persistence in the environment, either as an epibiotic parasite of amphipod hosts, or as a saprobe on contaminated exuviae. As a widely established invader, these findings suggest that *D. villosus* may have the potential to indirectly affect amphibian populations via the spread and/or maintenance of *Bd*.

5.2 Introduction

Global declines of amphibian populations were first acknowledged in the late 1980's (Barinaga, 1990; Wake, 1991). Since then, amphibians have become an international conservation concern, with substantial evidence highlighting patterns of global decline spanning the past half century (Stuart et al., 2004; Stuart, 2012). At the time of writing, 35 of the ~8,200 known amphibian species are formally confirmed to be extinct (AmphibiaWeb, 2020; IUCN, 2020), although taking into consideration additional species which have disappeared from the wild, listed as "possibly extinct", this number may increase to 171 species (IUCN, 2020). A further 38% (~3100 species) of known amphibian species are also considered to be threatened with extinction, of which ~500 species may be endangered, critically or otherwise (González-del-Pliego et al., 2019). As such, most recent estimations indicate current extinction rates to be four orders-of-magnitude greater than background extinction rates (Alroy, 2015), with some estimates suggesting amphibian extinction rates to be 25,000 – 45,500 times higher than background rates (McCallum, 2007). Global amphibian declines are predominantly attributed to five major interacting factors; climate change, environmental pollution, habitat degradation, invasive species and pathogenic diseases (Hayes et al., 2010).

Invasive alien species (IAS) are a primary driver of global amphibian declines (Kats and Ferrer, 2003; Bellard et al., 2016a; Bellard et al., 2016b; Nunes et al., 2019). Despite having received less attention when compared to alternative factors (e.g. habitat loss) – likely due to the localised scale of impact often associated with invaders (Adams, 2000; Vredenburg, 2004; Hayes et al., 2010) – IAS threaten a considerably higher number of amphibian species, when compared to other vertebrate taxa (Bellard et al., 2016b). Invasive amphibians, fish and invertebrates contribute significantly to amphibian declines, with invertebrates having the greatest overall impact (Nunes et al., 2019). In many instances, IAS-mediated declines are caused by intense predation of vulnerable embryos and larvae, resulting in impaired recruitment, drastic reductions in local abundance and in some cases, the extirpation of affected amphibian populations (reviewed in Kats and Ferrer, 2003; Ilhéu et al., 2007). However, IAS are also renowned for facilitating the spread of highly pathogenic, invasive amphibian diseases (Daszak et al., 1999; Garner et al., 2006; Fisher and Garner, 2020).

Batrachochytrium dendrobatidis, an invasive non-hyphal aquatic chytrid fungus commonly referred to as *Bd* (Berger et al., 1999; Berger et al., 2005a), is the causative agent behind the emergent amphibian disease chytridiomycosis, and the single greatest driver of amphibian declines worldwide (Bellard et al., 2016b). Believed to have originated in East Asia (see O'Hanlon et al., 2018), *Bd* is now present across six continents, found in most countries inhabited by amphibians (Olson et al., 2013), including those which were previously thought isolated (Bletz et al., 2015). Considered to be one of the 100 worst invaders in the world (Lowe et al., 2000), *Bd* is directly implicated in the decline and/or extinction of a large number of amphibian species (Scheele et al., 2019), although obtaining accurate estimations regarding the number of affected species has become the focus of some debate (see Lambert et al., 2020; Scheele et al., 2020). Nevertheless, *Bd* is known to infect at least 520 species of amphibians, infiltrating the skin of adults via a motile, flagellated infectious zoosporic life stage. Colonising the superficial keratinised layers of the host epidermis, *Bd* zoospores become encysted and develop into zoosporangia, within which fresh zoospores are generated via asexual reproduction, before being discharged into the environment (Van Rooij et al., 2015). Adults infected by *Bd* often experience irregular thickening in the keratinised layers of the epidermis (i.e. hyperkeratosis), abnormal growth in tissues situated immediately beneath infected areas (i.e. hyperplasia), and excessive skin sloughing of the outermost epidermal layers, as the disease progresses**.** Disruption in the functionality of amphibian skin can impair crucial regulatory processes, impacting upon cutaneous respiration and osmoregulation, and ultimately resulting in the death of infected adults (Berger et al., 1998; Voyles et al., 2009; Van Rooij et al., 2015). *Bd* can also infect the larvae of some anuran species. However, unlike adults, *Bd* infiltration is localised to mouthparts; the only keratinised structure present in early-stage larvae. *Bd* will proliferate within the keratinised mouthparts before colonising the hindlimbs, where the epidermis becomes keratinised during later developmental stages (Marantelli et al., 2004; McMahon and Rohr, 2015). Infiltration of larval mouthparts does not appear to induce mortality, when compared to other keratinised body regions (Berger et al., 1998; Rachowicz and Vredenburg, 2004), but it may impact upon foraging efficiencies, subsequently affecting larval growth and development (Hanlon et al., 2015).

Originally regarded as a pathogen specific to amphibians (Berger et al., 1998; Piotrowski et al., 2004; Wake, 2007), the introduction of *Bd* into novel regions was previously attributed solely to the movement of invasive amphibians, facilitated via the pet trade and/or the use of animals for scientific research (Weldon et al., 2004; Fisher and Garner, 2007). Released from captivity, either accidentally or intentionally, invasive amphibians such as the American bullfrog (*Rana catesbeiana*), African long-clawed frog (*Xenopus laevis*) and Cane toad (*Bufo marinus*), have all been implicated as primary vectors in the spread of *Bd* (Weldon et al., 2004; Garner et al., 2006; Tinsley et al., 2015a), as asymptomatic carriers infected with *Bd* yet resistant to associated pathologies (Fisher and Garner, 2007). However, *Bd* has been shown to exist in some regions in the absence of suitable amphibian hosts, persisting in the environment on various substrates (Johnson and Speare, 2005), detritus (Johnson and Speare, 2003), and vegetation (Kolby et al., 2015; but see Stoler et al., 2016). A number of studies have also demonstrated the possibility for *Bd* to experimentally, and naturally, infect a range of non-amphibian host species, identifying a variety of potential alternative reservoir hosts, including waterfowl (Garmyn et al., 2012; Burrowes and De la Riva, 2017; Hanlon et al., 2017), reptiles (Kilburn et al., 2011), fish (Liew et al., 2017) and nematodes (Shapard et al., 2012). Moreover, several invasive decapods have been shown to be capable of harbouring *Bd*, with the pathogen having been detected in the red swamp crayfish (*Procambarus clarkii*), and the virile crayfish (*Orconectes virilis*; McMahon et al., 2013; Brannelly et al., 2015; Oficialdegui et al., 2019). Crayfish were also found to successfully transmit *Bd* to vulnerable anuran larvae (McMahon et al., 2013), highlighting the capacity for non-amphibian reservoir hosts to transmit *Bd* to natural amphibian hosts, possibly via predator-prey interactions (Oficialdegui et al., 2019).

Invasive alien species are frequently implicated in the introduction of novel diseases to invaded communities (e.g. Dunn et al., 2012). As such, identifying the possibility for non-amphibian invaders to acquire and transmit *Bd* to amphibians emphasises the importance of determining the potential for alternative invasive predators to act as prospective reservoir hosts. Recently, *Dikerogammarus villosus*, a high-impact predatory amphipod renowned for consuming a wide range of native prey species (reviewed in Rewicz et al., 2014), including some vertebrates (Taylor and Dunn, 2017), was found to consume UK larval anurans (Chapter 2). Under certain circumstances, *D. villosus* demonstrates the capacity to inflict a substantial predatory impact towards amphibian larvae, when compared to native amphipod species (Chapter 2; but also see Chapter 3 and 4). Such predator-prey interactions may provide the ideal opportunity for amphipod predators to become infected by *Bd*, acting as potential carriers through which this invasive fungal pathogen may proliferate and spread to native amphibians. Present throughout most of continental Europe, and most recently discovered in the UK (Rewicz et al., 2015), identification of *D. villosus* as a potential carrier would greatly expand our knowledge regarding the host-pathogen dynamics of *Bd*, highlighting an additional threat to native amphibian populations.

In this chapter I assess the potential for *D. villosus* to act as a potential non-amphibian carrier of *Bd*. Through the application of laboratory-based infection experiments, I determine whether *D. villosus* can acquire *Bd* infections, following direct exposure to infectious *Bd* zoospores. Using molecular diagnostics and histological analyses I aim to (i) test for the presence of *Bd* in amphipod hosts, (ii) measure parasitic burden, and (iii) explore how *Bd*-infections might develop in infected hosts. Furthermore, I assess the effects of *Bd*-infections on the survivorship of *D. villosus* over time.

5.3 Materials and Methods

5.3.1 General Biosecurity

All aspects of experimental works concerning the handling, culturing and disposal of *Bd* was carried out under aseptic conditions in a Class II Microbiological Sterile Culture Hood. *Batrachochytrium dendrobatidis* cultures, and all consumables which came into contact with *Bd* contaminants (e.g. culture flasks, media, experimental tanks, etc.) were disinfected using a 1% solution of Virkon S, prior to disposal. All equipment used in the culturing and/or distribution of *Bd* inocula (e.g. pipette filler) were sterilised and autoclaved between uses.

5.3.2 Culturing of *Bd* inoculum

Inocula containing viable *Bd* zoospores, henceforth referred to as *Bd* positive (i.e. *Bd*$^+$), were generated from three 10ml master liquid stock cultures provided by the Institute of Zoology (IOZ; Zoological Society of London). These stock cultures contained the UK CORN'12 IA strain of the Global Pandemic Lineage (GPL) complex, obtained whilst screening invasive alpine newts (*Ichthyosaura alpestris*) in Cornwall (UK). To ensure a sufficient volume of viable *Bd* zoospores were generated for amphipod inoculations, the original master stock cultures were passaged a total of three times over the course of six days, producing a minimum of five sub-cultures from each established master stock. From each established master stock, 1ml of *Bd*-containing media was removed and transferred into 10ml of freshly autoclaved TGhL culture broth (16g tryptone, 2g gelatin hydrolysate, 4g lactose and 1000ml distilled water). Passaged sub-cultures were then incubated for a total of seven days at 20°C.

5.3.3 Generating the *Bd* inoculum

To generate the *Bd*$^+$ inocula, incubated sub-cultures were passed through filter paper (7µm diameter), removing mature zoosporangia whilst retaining viable motile zoospores. To determine the concentration of zoospores present in each *Bd*$^+$ inoculum, a 10µl aliquot of the inoculate was stained using 10µl of Trypan Blue dye – a cheap and effective alternative to more commonly used DNA intercalating fluorescent dyes (e.g. SYBR-14 and propidium iodide; see McMahon and Rohr, 2014). Stained samples of the *Bd*$^+$ inocula were viewed under a compound microscope, at 400x magnification (40x objective), allowing viable (unstained) and non-viable (stained) zoospores to be differentiated (Figure 5.1). Viable zoospores were counted using a haemocytometer, and the concentration of zoospores determined (zoospores/µl; zsp μl^{-1}). Counting was conducted twice. A separate inoculum lacking any *Bd* materials was simultaneously prepared, henceforth referred to as *Bd* negative (i.e. *Bd*$^-$). Comprising entirely of TGhl culture broth without the introduction/passaging of viable zoospores, the *Bd*$^-$ inoculum was produced using the same procedure as *Bd*$^+$ inocula and was maintained under the same conditions.

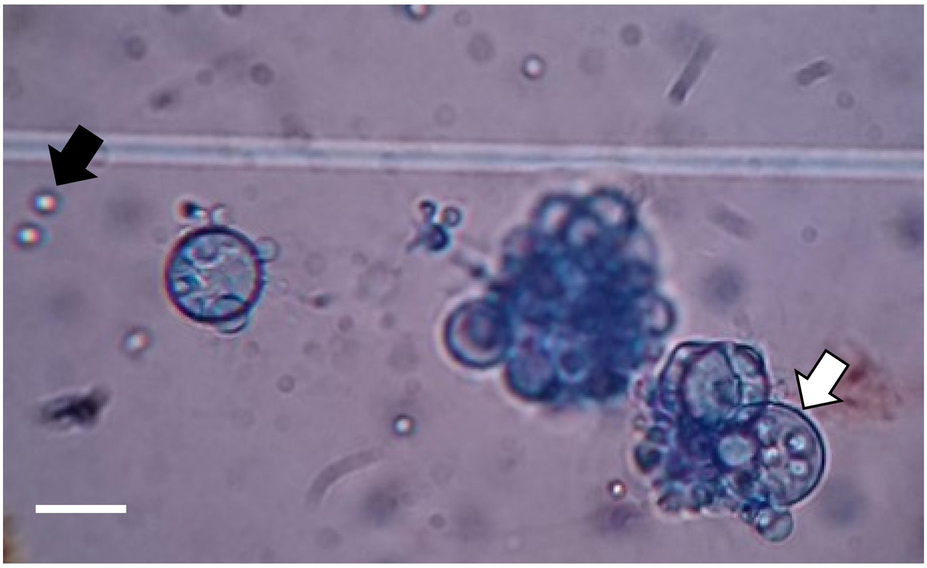

Figure 5.1. *Batrachochytrium dendrobatidis* cultured on tryptone/gelatin hydrolysate/lactose (TGhL)-broth, showing motile zoospores (black arrow) and maturing zoosporangia (white arrow), stained with Trypan Blue dye. Scale bar = 20µm.

5.3.4 Collection and Maintenance of Study Organisms - Amphipods

In October 2018, *Dikerogammarus villosus* were collected from Grafham Water Reservoir, Cambridgeshire (52º17′31.2″N 0º19′23.6″W), removed from submerged artificial substrates and transported to the laboratory in cool boxes, containing constantly aerated site-sourced water. In the laboratory, amphipods were maintained in aerated communal tanks containing dechlorinated tap water and were provided with a diet of conditioned sycamore leaves (*Acer pseudoplatanus*), *ad libitum*. Communal tanks were maintained at 14ºC, under a 12 h:12 h light:dark photoperiod, for a period of 48 h prior to use.

Amphipods were categorised into different body size classes by eye, with intermediate-sized individuals (mean ± SEM weight = 62.431 ± 1.267mg) selected for use in experimentation. Both male and female *D. villosus* were used in experimental trials, although ovigerous (egg-bearing) females were excluded. Amphipods exhibiting visibly apparent signs of other parasitic infections were excluded. Amphipods selected for use in experimental trials were transferred to an incubator and the ambient temperature was increased from 14ºC to 20ºC, under the same photoperiod as previously described. Transitioning to the higher thermal regime was gradual (1ºC per 2 h), ensuring amphipods did not experience thermal shock. Amphipods were maintained at 20ºC for a period of 120 h before being used in experimentation, thereby providing adequate time for animals to acclimate to the new thermal regime. Amphipods were fed conditioned sycamore leaves *ad libitum* throughout.

5.3.5 Experimental Infection Study

Experimental exposures were conducted at 20°C under a 12 h:12 h light:dark regime, and were carried out in petri dishes (diameter = 90mm, depth = 16mm) containing 30ml of autoclaved dechlorinated tap water and a single sterilised glass bead for shelter. One hundred amphipods were individually exposed to either the Bd^{+} or Bd^{-} (control) inoculum (n = 50 amphipods per treatment) for three consecutive 72 h exposures. Prior to the introduction of amphipod specimens (one amphipod per petri dish), and the commencement of experimental exposures, each petri dish received 100µl of either the Bd^{+} (zoospore concentration ~600 zsp μl^{-1}) or Bd^{-} (TGhL culture broth only) inoculum; with inoculations performed under aseptic conditions. During exposures, individual amphipods were provided with two 9mm discs of conditioned sycamore leaf for food. Leaf discs had previously been autoclaved to kill any bacteria which may be present, thus preventing any bacterial blooming from occurring, whilst also maintaining any biofilms already present on the leaf discs. At the end of exposures, individuals were transferred to fresh petri dishes containing the next round of inoculations. Exposures were repeated over three consecutive 72 h periods, with petri dishes, water and food replaced at the conclusion of each exposure period.

Once experimental exposures had concluded, amphipods were rinsed three times, using autoclaved dechlorinated tap water to remove any residual media retained from the Bd^{+} or Bd^{-} inocula, before being individually housed in sterile petri dishes containing 40ml dechlorinated tap water, and a single sterilised glass bead for shelter. Amphipods were provided with five 9mm leaf discs as food, with additional leaf discs provided as required, up to a maximum of eight discs per week. Housing, substrates, food and water were replaced weekly for a period of up to four weeks. Each week, a subset of amphipods (n = 10) were euthanised, dissected and their tissues prepared for molecular diagnostics and histology. The first subset of 10 individuals were killed and dissected immediately following the post-exposure "triple-wash" (i.e. T = 0 days post-exposure), with a further 10 individuals dispatched each week, for four weeks.

To prepare tissues, amphipods were euthanised via prolonged exposure to carbonated water, typically used as an anaesthetic (Cooper, 2011). Amphipods were independently stored in carbonated water until individuals no longer responded to external stimulation; at which point individuals were considered dead. Once confirmed dead, individuals were rinsed three times using distilled water, before the pleon was dissected (Figure 5.2). The pleon, containing sections of the hindgut, cuticle and gill lamellae, were stored at -20°C in preparation for DNA extraction and subsequent molecular diagnostics. Structures were selected for screening based on previous literature in which *Bd* was detected in crustacean hosts (see McMahon et al., 2013). The remainder of each animal was prepared for histological analysis; initially stored in Davidson's freshwater fixative for a period of 24 h before being transferred into 70% ethanol.

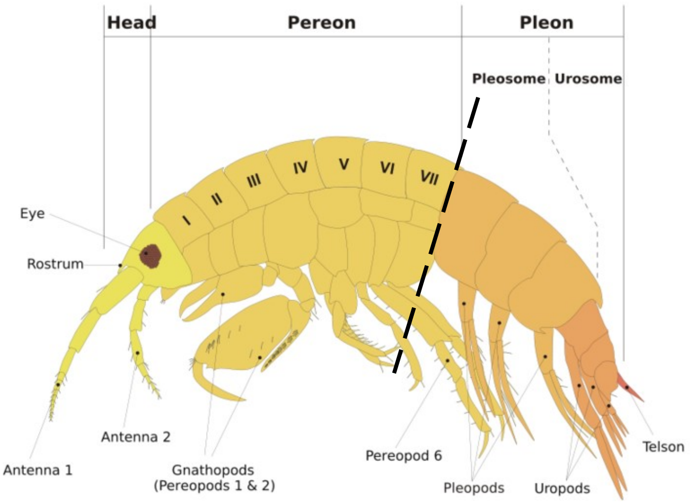

Figure 5.2. Schematic of amphipod body plan, indicating where tissue samples were excised (dashed line), to be tested for the presence of *Bd* DNA via molecular screening (i.e. diagnostic qPCR).
Image created by Hans Hillewaert.

Over the course of this four-week period a number of individuals moulted, observed in both the *Bd*$^+$ and *Bd*$^-$ exposure treatments (n = 27 and 28 individuals, respectively). For individuals in the *Bd*$^+$ exposure treatment, moults were removed from the environment within 24 h of moulting, washed three times and frozen in preparation for molecular screening. Although the same was attempted for individuals which moulted in the *Bd*$^-$ exposure treatment, subjects appeared to readily consume moults soon after shedding the old cuticle (all 28 moults consumed). Conversely, the majority of moults generated by individuals in the *Bd*$^+$ exposure treatment were not ingested (n = 22 moults; Fisher's exact test, $p < 0.001$), instead persisting in the environment. As such, only moults produced by individuals belonging to the *Bd*$^+$ treatment were subjected to molecular diagnostics.

5.3.6 Experimental Mortality Study

Dikerogammarus villosus selected for use in the experimental mortality study were subjected to the same exposure and maintenance protocols as those individuals in the infection study (see Section 5.3.5), with 60 individuals exposed to either the *Bd*$^+$ or *Bd*$^-$ (control) inoculum (n = 30 amphipods per treatment) for three consecutive 72 h periods. Individuals were then monitored daily for a period of four weeks. Monitoring was carried out at 14:00 each day to ensure consistency in the recording of observations. Animals which died were removed, triple-washed and dissected, using the same protocol as described above. As with the experimental infection study, any moults produced by subjects of the *Bd*$^+$ exposure treatment were removed (n = 13 out of 18 moults), triple-washed, and frozen in preparation for molecular diagnostics. After four weeks, the remaining live amphipods were also killed and screened for *Bd*.

5.3.7 Detection of *Bd* – Molecular Diagnostics

Frozen samples (tissues and moults) were transported to the Zoological Society of London (ZSL) for molecular diagnostic screening for the presence of *Bd* DNA. DNA extraction and the application of quantitative polymerase chain reactions (i.e. qPCR) were performed by ZSL technical staff using the RACE protocol (**R**isk **A**ssessment of **C**hytridiomycosis to **E**uropean amphibian biodiversity; see Appendix 5, Section A5.1 for protocol details). For each amphipod sample tested, diluted 1/120 to avoid inhibition, qPCR reactions were carried out in duplicate.

Infection burden was defined as the mean number of zoospore genomic equivalents (GE), averaged across both duplicated reactions, per µl of extract tested (result multiplied by 120). GE was determined using four standards, containing known concentrations of *Bd* DNA (0.1, 1, 10, 100 *Bd* GE), which were conducted alongside samples, in addition to one negative control (i.e. sterile water). A positive control consisting of amphipod host primers was not used. A sample was assigned a positive score (i.e. *Bd* DNA detected) if duplicate reactions were successfully amplified, and a mean GE ≥ 0.1 was estimated (see Clare et al., 2016). For samples where detection of *Bd* DNA was uncertain in the initial duplicate reactions (n = 5 samples), additional repeats were performed.

5.3.8 Detection of *Bd* – Histological Analysis

Amphipods which returned a positive signal for *Bd* DNA were subjected to subsequent histological analyses, as were five negative controls, in order to visually detect the presence of fungal structures. Tissues, previously fixed and then stored in 70% ethanol were processed (see Appendix 5, Table A5.2 for protocol) and embedded in paraffin wax blocks. Transverse sections were removed from embedded tissues using a microtome, at a thickness of 3-4µm, and mounted on glass viewing slides. Mounted sections were stained using standard Haemotoxylin & Eosin (H & E), using a modified protocol suitable for staining amphipod tissues (see Appendix 5, Table A5.3 for protocol). Sections were viewed using a compound light microscope, observed under oil immersion at a magnification of 1000x (100x objective). Exterior and interior structures (e.g. cuticle and digestive tract respectively) were examined for the presence of zoospores or encysted reproductive zoosporangia. For any observed structures which appeared to reflect known *Bd* morphologies, life-stages were determined based on previously published descriptions of *Bd* reproductive lifecycles (Van Rooij et al., 2015).

5.3.9 Statistical Analysis

All statistical analyses were conducted using the statistics package ‘R’ (version 3.5.1; R Core Team, 2018), performed through the RStudio interface, with significance level (α) set at 0.05. Confidence intervals (CI), presented hereafter, were calculated at the 95% level using the Wilson score interval method (*binom::binom.confint*; version 1.1-1; Dorai-Raj, 2014).

For both infection and mortality experiments, prevalence (i.e. the proportion of amphipods which returned a positive signal for *Bd* DNA), and moulting occurrence (i.e. the proportion of amphipods which did/did not moult), were independently analysed with respect to exposure treatment (i.e. Bd^+/Bd^- inoculum), using Fisher's exact probability tests. To determine whether there was a relationship between infection and the occurrence of moulting, Fisher's exact probability tests were also applied to amphipods belonging to the experimental exposure treatment (i.e. Bd^+ inoculum).

For the infection experiment, prevalence – recorded for amphipods which were exposed to the Bd^+ inoculum only – was also assessed with respect to time since exposure (five sampling points over a four-week period), and moult status (un/moulted) using logistic regression analysis. However, due to perfect separation (i.e. binary response variable causing a complete separation of predictor variables; see Zorn, 2005), as evidenced by abnormally high coefficient standard errors with regards to sampling point, a penalised logistic regression approach was implemented (*logistf::logistf*, version 1.24; Heinze and Ploner, 2018). Utilising the Firth penalised logistic regression model (see Firth, 1993), the standard log-likelihood function $l(\beta)$, generated whilst calculating regression coefficients (i.e. β), was modified by introducing a penalty term $\prod(\beta)$: $l^*(\beta) = l(\beta) + \prod(\beta)$. This penalty term reduces estimation bias, causing estimated coefficients to shrink, thereby controlling for model separation (Doerken et al., 2019).

For the mortality experiment, survival data was analysed using the '*survival*' package (*coxph* function, version 3.2-3; Therneau, 2020) and the '*survminer'* package (*survfit* function, version 0.4.8; Kassambara et al., 2020). Survival curves were fitted, using the Kaplan-Meier survivorship function (*survminer::survfit*), to describe the effect of exposure treatment upon amphipod survival. A Cox proportional hazard model (*survival::coxph*) was used to examine the effects of exposure treatment and infection status in determining survival probability. Prior to analysis, data pertaining to three amphipods, belonging to the Bd^- exposure treatment, were removed from the dataset due to deaths occurring during experimental exposures.

For moults which returned a positive signal for the presence of *Bd* DNA, non-parametric Spearman's rank correlation analysis was applied to examine the relationship between infection burden and the timing of moulting events (days post exposure). Infection burden (i.e. estimated GE) in tissue samples and in moults was also compared using a non-parametric Mann-Whitney U test. Data were analysed independently, for each experimental study.

5.4 Results

5.4.1 Experimental Infection Study

Of the 50 amphipods exposed to the Bd^{+} inoculum, qPCR revealed seven individuals (14%, 95% CI 7 – 26%) which returned a positive signal for the presence of *Bd* DNA in tissues, indicating potential infections. Based on scoring criteria (i.e. GE $\geq$ 0.1), all seven individuals were deemed "infected", with a mean estimation of 3.285 GE per µl (range: 0.967 – 7.920 GE per µl). No amphipods screened from the Bd^{-} exposure treatment (i.e. control) tested positive for *Bd* DNA. As such, infection prevalence was significantly higher in the experimental exposure treatment, than in the controls (Fisher's exact test, $p = 0.012$). There was no significant difference in the proportion of amphipods which moulted following repeated exposure to either the Bd^{+} inoculum (54%, 95% CI 40 – 67%), or the Bd^{-} inoculum (56%, 95% CI 42 – 69%; Fisher's exact test, $p > 0.05$). However, moulting did appear to have a significant effect on prevalence, when regarding those amphipods sampled in the Bd^{+} exposure treatment (Fisher's exact test, $p = 0.007$). Of the 54% of amphipods which moulted following exposures, all individuals tested negative for *Bd*-infections, whereas ~30% of individuals which had not moulted tested positive.

When analysed in combination with time since exposure (days post exposure; DPE), moulting status still had a significant effect on prevalence, measured in amphipods exposed to the Bd^{+} inoculum (Firth penalised logistic regression; $\chi^2 = 5.652$, $p = 0.017$). Prevalence was also significantly affected by time (Firth penalised logistic regression; $\chi^2 = 4.483$, $p = 0.034$; Figure 5.3). Immediately following exposures (i.e. 0 days post exposure; DPE), *Bd* DNA was detected in 30% (95% CI 11 – 60%) of amphipods screened (3/10), and in 40% (95% CI 17 – 69%) of amphipods screened during the second sampling period (7 DPE). However, *Bd* detection was zero for those individuals which were sampled at 14, 21, and 28 days post exposure. Similarly, estimated GE was highest in amphipods sampled immediately after the termination of experimental exposures (mean = 1.294 GE per µl; range = 2.143 – 7.920 GE per µl; Figure 5.4), decreasing slightly in those individuals sampled at 7 DPE (mean = 1.005 GE per µl; range = 0.967 – 3.840 GE per µl). No *Bd* DNA was detected in individuals screened at 14, 21, and 28 days post exposure.

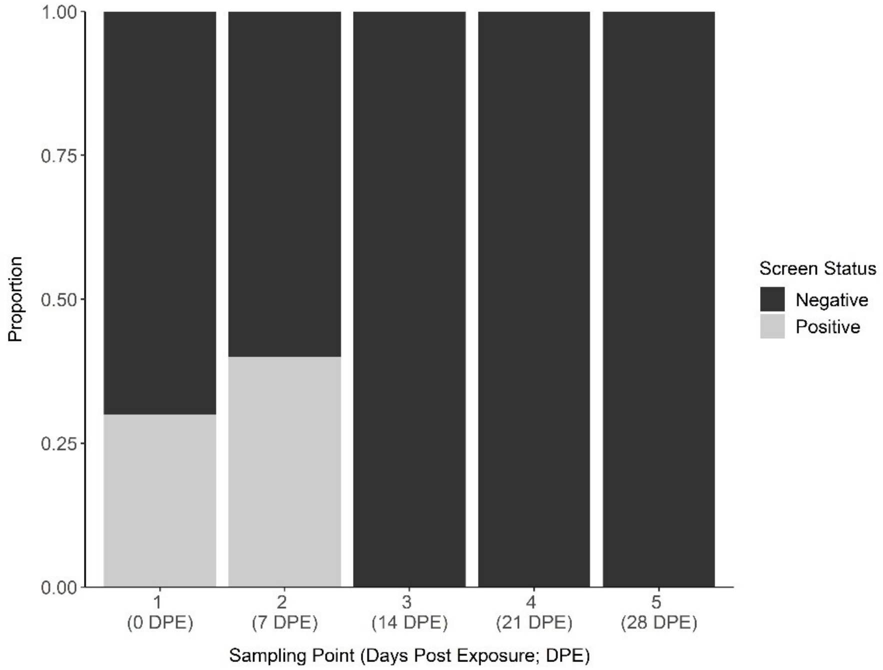

Figure 5.3. Proportion of *D. villosus* for which a positive (light grey) or negative (dark grey) signal for *Bd* DNA was obtained via diagnostic qPCR, following repeated exposures to the Bd^{+} inoculum, containing viable *Bd* zoospores. Amphipods were sampled at five time points (days post exposure; DPE).

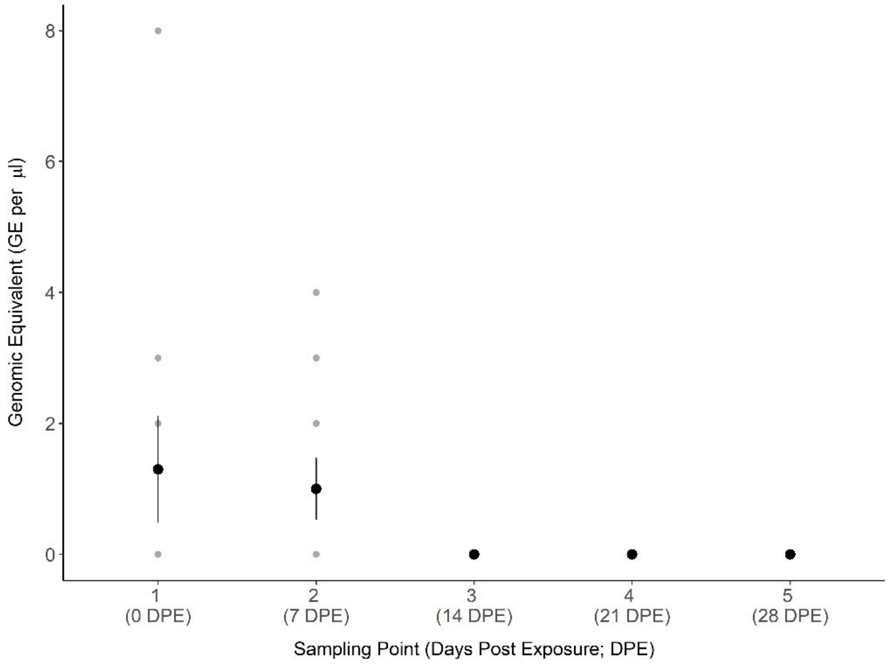

Figure 5.4. Raw (light grey filled circles) and mean (+ SEM; black filled circles) estimations of genomic equivalents (i.e. fungal burden; GE per µl), measured in amphipods sampled from the experimental infection experiment. Estimates obtained via qPCR. Sampling conducted weekly, with n = 10 amphipods, sampled at five sampling points (days post exposure; DPE).

Over the course of the experimental infection study, 22 moults were collected and screened. Of these 22 moults, 21 returned a positive signal for the presence of *Bd* DNA, with a mean burden of 17.520 GE per µl (range = 1.680 – 56.280 GE per µl); although one moult was collected during the exposure phase, and thus disregarded from analysis. All moults which yielded a positive signal were collected from individuals which subsequently tested negative for *Bd* DNA, based on tissue screening. Compared to infection burdens estimated for amphipod tissues, GE estimates were significantly higher in moults (W = 120, p = 0.004; Figure 5.5). However, there was no correlation between estimated GE, recorded in moults, and the time at which moulting occurred post-exposure (r_s = -0.282, p = 0.220).

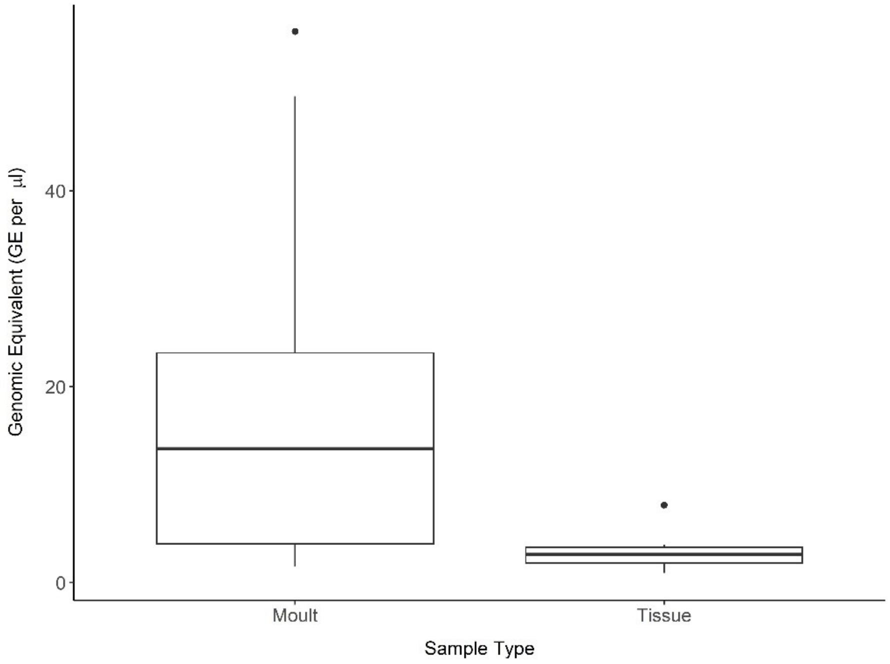

Figure 5.5. Genomic equivalents (GE per µl) estimated in amphipod moults and tissues, taken from *D. villosus* specimens in the experimental infection study, following repeated exposures to the Bd^+ inoculum.

5.4.2 Experimental Mortality Study

As with the infection study, a higher infection prevalence was recorded in amphipods belonging to the experimental exposure treatment than in the controls (Fisher's exact test, p = 0.017). Of the 30 amphipods exposed to the Bd^+ inoculum, *Bd* DNA was detected in four individuals (13.33%, 95% CI 5 – 30%) (mean = 5.686 GE per µl; range = 0.164 – 9.888 GE per µl), up to 24 days after exposures were conducted. By comparison, *Bd* DNA was not detected in any amphipods sampled from the control treatment. Comparisons between exposure treatments revealed there to be no statistically significant difference in moulting occurrence, with moulting occurring in 60% of amphipods in the experimental exposure treatment and 37% of amphipods in the control treatment (Fisher's exact test, p

= 0.121, 95% CI 42 – 75% and 22 – 54%, respectively). For those amphipods exposed to the *Bd*$^{+}$ inoculum, moulting appeared to have a significant effect on prevalence (Fisher's exact test, p = 0.023). Of the 60% of amphipods which moulted following exposures, all individuals tested negative for *Bd*-infections, whereas ~33% of individuals which had not moulted tested positive.

When comparing survival data, recorded for amphipods in both exposure treatments, the Cox proportional hazard model revealed there to be no significant effect by treatment (p = 0.739) in determining survival probability (Figure 5.6; *Bd*$^{+}$ treatment = 60% mortality, 95% CI 42 – 75%; *Bd*$^{-}$ treatment = 55.56% mortality, 95% CI 37 – 72%). There was a significant effect of infection status on mortality (p =0.011), with all four individuals infected with *Bd* having died during experimental trials, whereas those individuals which tested negative for *Bd* showed lower mortality overall (53.85%, 95% CI 35 – 71%). Of the 12 individuals which shed *Bd*-positive moults, yet tested negative for *Bd*-infections themselves, seven died during experimental trials (58.33%, 95% CI 32 – 81%).

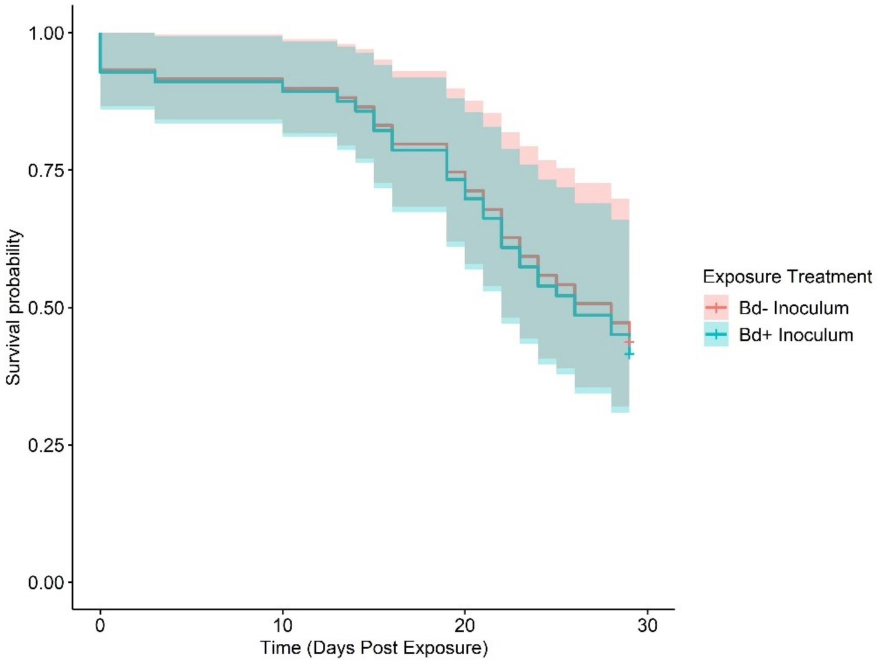

Figure 5.6. Survival curves generated for *D. villosus* examined in the experimental mortality study, following exposures to the *Bd*$^{+}$ (blue line and shading) or *Bd*$^{-}$ (red line and shading) inoculum. The x-axis (Time) is the number of days the specimen survived post-exposure, and the y-axis (Survival probability) identifies the proportional decrease in population size over time. Shaded areas denote 95% confidence intervals.

As with the experimental infection study, a number of moults were collected from amphipods and subjected to molecular screening. Thirteen moults were screened for the presence of *Bd* DNA, of which 12 returned a positive signal, with a mean estimated GE of 17.520 GE per µl (range = 4.080 – 47.040 GE per µl), although one moult was collected during the exposure phase, and thus omitted from analysis. As with the infection experiment, all moults which tested positive for *Bd* DNA were taken from amphipods which subsequently tested negative for *Bd*-infections, based on tissue screening. Estimated GE, recorded in moults, did not differ significantly from estimates generated for amphipod tissues (W = 37, p = 0.056; Figure 5.7). Additionally, analysis confirmed there to be no significant correlation between estimated GE, recorded in moults, and the time between exposures and moulting ($r_s = 0.511$, p = 0.089).

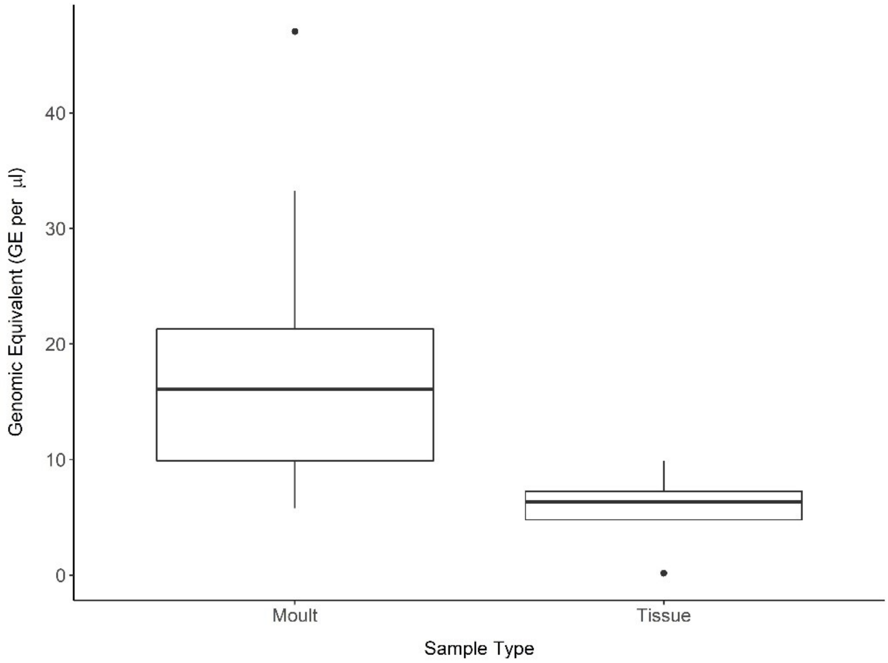

Figure 5.7. Genomic equivalents (GE per µl) estimated in amphipod moults and tissues, taken from *D. villosus* specimens in the experimental mortality study, following repeated exposures to the Bd^{+} inoculum.

5.4.3 Detection of *Bd* – Histological Analysis

Examination of tissue sections, removed from amphipods which tested positive for *Bd* DNA, revealed a number of fungal-type structures that resemble *Bd* (Figure 5.8a-d), found associating near to, or directly with the exterior surface of the cuticle (Figure 5.8b, d), as well as the gill lamellae (Figure 5.8a, c). There was no evidence of fungal structures in muscles, the digestive tract, digestive glands, haemocoel or the hepatopancreas.

Fungal structures, ranging from 1.407 – 8.706µm in diameter (mean = 3.814µm), appeared non-hyphal in nature, spherical/ovoid in shape, and morphologically consistent with zoosporangia at different stages of maturation, as recorded in other crustacean hosts (e.g. *P. clarkii*; see McMahon et al., 2013), but also supported by independent assessments conducted by clinical pathologists at the IOZ. The surface of these structures appeared smooth, with relatively large numbers of smaller entities situated internally – believed to be developing zoospores (e.g. Figure 5.8a). Visual inspection of more than 50 sections, taken from amphipods which returned the greatest estimations of *Bd*-GE, did not definitively identify motile zoospores, or the presence of empty zoosporangia; although some potential structures were found (see Figure 5.8c).

Structures found interacting with the surface of the cuticle, and/or gill lamellae, appeared to be attached directly to the integument, as indicated by their continued presence following the triple-wash procedure. Those structures found proximate to the host cuticle were also assumed to have originally been attached to the cuticle, likely becoming dislodged during tissue fixation, tissue processing, or tissue sectioning. Some structures were observed as independent entities (e.g. Figure 5.8a, b), whilst others were found in colonial arrangements (Figure 5.8c, d). Comparisons between structures associating with the exterior cuticle, and with the gill lamellae/swimmerets, showed slight variability in the size and density of structures, observed between these two localities. Structures found near or on the gill lamellae/swimmerets were often larger (mean = 4.194µm diameter; range = 1.934 – 8.706µm), when compared to those associated with the cuticle (mean = 3.223µm diameter; range = 1.407 – 5.977µm). Conversely, fungal structures found near to or on the cuticle were frequently present at slightly higher numbers (range = 4 – 7 objects per field of view), than those associating with the gill lamellae/swimmerets (1 – 6 objects per field of view).

In contrast, I saw no evidence of any fungal structures in, or on, tissues removed from amphipods exposed to the *Bd*$^{-}$ (control) inoculum (Figure 5.9a-c). Comparisons between infected and uninfected host tissues also revealed no evidence of pathologies associated with fungal infections, or any obvious signs of an induced immune response – either in the localised area of infection (i.e. cuticle/gill lamellae), or within the surrounding tissues.

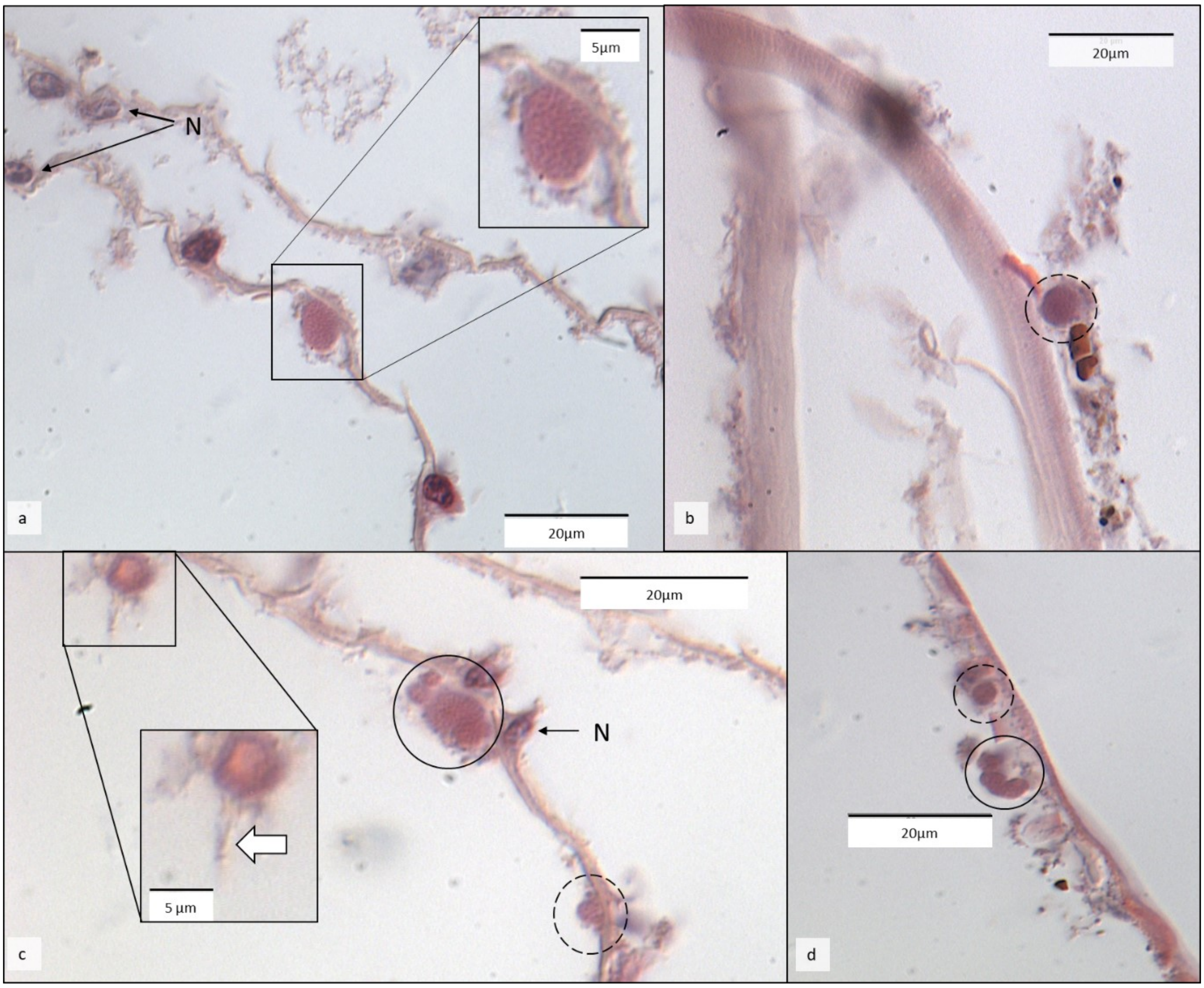

Figure 5.8. Transverse histological sections of tissues removed from *Dikerogammarus villosus* experimentally infected with *Batrachochytrium dendrobatidis* (*Bd*) following repeated exposures to inoculants containing infectious *Bd* zoospores (i.e. Bd^{+} inoculum). (a) A single non-hyphal fungal structure, identified as a maturing *Bd* zoosporangium, interacting with the surface of the gill lamellae of the amphipod host (box). When viewed under greater magnification (inset box), fungal structure appeared to contain a large number of internalised substructures; thought to be developing zoospores. (b) A single zoosporangium interacting with the surface of the amphipod host cuticle (dashed circle). (c) Zoosporangia interacting with the surface of the gill lamellae, arranged either independently (dashed circle), or in a colony (solid circle), along with an alternative structure which may be an empty zoosporangium (box); as determined by the presence of possible discharge tubule when viewed under higher magnification (inset box; white arrow). (d) Zoosporangia interacting with the surface of the amphipod host cuticle, arranged either independently (dashed circle), or in a colony (solid circle). Histology images are sections stained with haemotoxylin and eosin. Host nuclei (N) are indicated using black arrows.

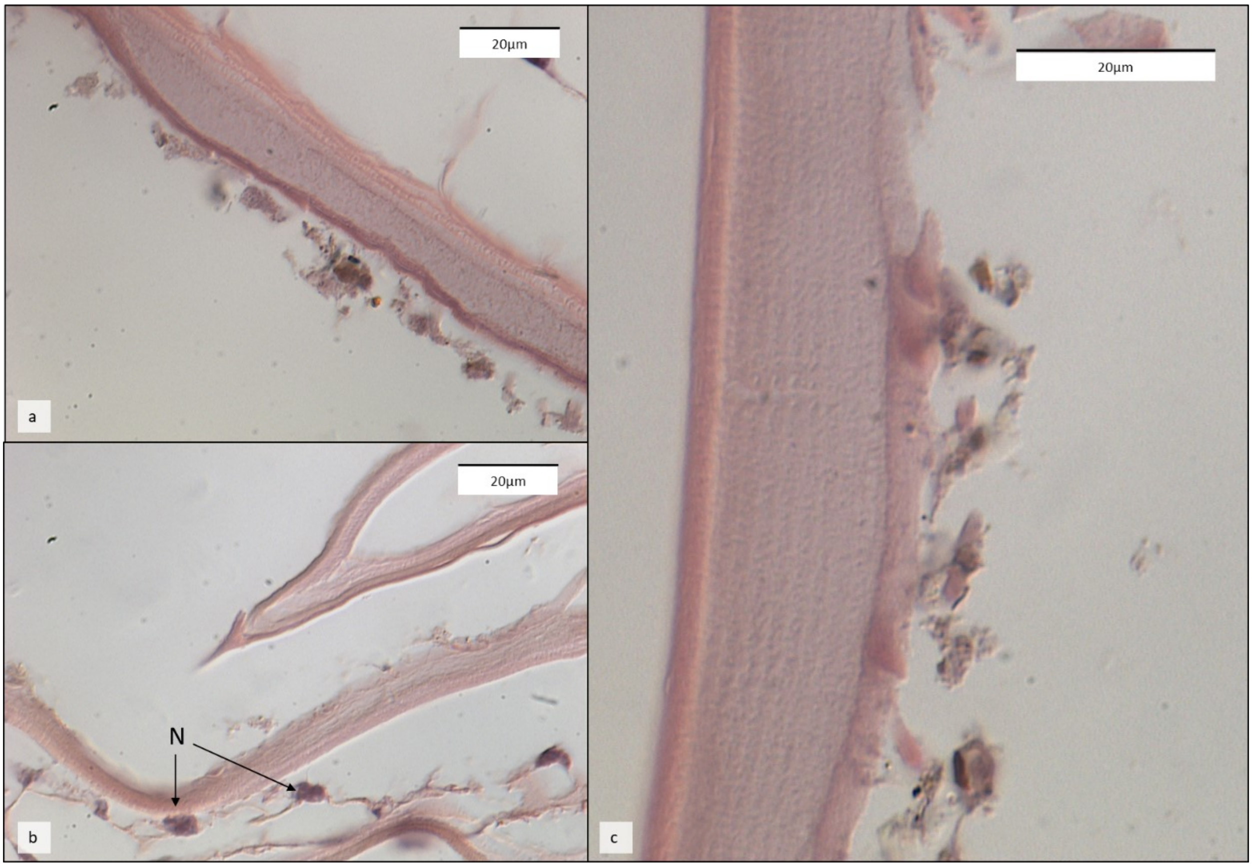

Figure 5.9. Transverse histological sections of tissues removed from uninfected *Dikerogammarus villosus* sampled from the control treatment; repeatedly exposed to inoculants without infectious *Bd* zoospores (i.e. *Bd*$^{-}$ inoculum). (a-c) sections of the amphipod host cuticle, free of any apparent fungal structures. Histology images are sections stained with haematoxylin and eosin. Host nuclei (N) are indicated using black arrows.

5.5 Discussion

Mathematical epidemiological modelling has shown that increased pathogenic virulence demonstrated by *Bd*, and its ability to persist in the environment, even in the absence of suitable amphibian hosts, is linked to the generalist nature of *Bd* (Mitchell et al., 2008; Briggs et al., 2010); capable of saprophytic growth (Johnson and Speare, 2003), or subsisting as a parasite of alternative hosts, including crustaceans (Kilburn et al., 2011; Garmyn et al., 2012; McMahon et al., 2013; Hanlon et al., 2017; Liew et al., 2017). In this chapter, I explored the potential for the invasive amphipod crustacean *D. villosus* to act as an alternative non-amphibian carrier of *Bd*, and to examine how infections might develop within amphipod hosts and affect host survival. Through the application of advanced molecular diagnostics, *Bd* DNA was detected in a number of amphipods following repeated exposures to infectious *Bd* zoospores, indicating possible infections. Histological analysis revealed a number of visibly apparent non-hyphal structures, thought to be developing zoosporangia, which were situated external of host animals, observed associating proximate to and/or directly upon the exterior surface of the amphipod cuticle, as well as the gill lamellae. Examinations of infected regions, as well as the surrounding tissues, revealed no evidence of pathology, or of any induced immune responses (e.g. melanisation; see Bojko et al., 2017). Inspection of internal tissues and organs revealed no evidence of similar fungal structures, nor of any associated pathologies and/or immune responses. Interestingly, *Bd* was also detected on a number of moults, taken from amphipods which subsequently tested negative for *Bd*-infections.

A typical characteristic of *Bd* infections in both amphibian and non-amphibian vertebrate hosts is the colonisation of keratinised regions of the host integument (Piotrowski et al., 2004; Garmyn et al., 2012; Liew et al., 2017). In susceptible amphibian species – as well as in some fishes – infections usually manifest intracellularly, with maturing zoosporangia developing throughout the outer epidermal layers (Van Rooij et al., 2015; Liew et al., 2017). In contrast, studies concerning alternative non-amphibian vertebrate hosts (birds and reptiles) have shown *Bd* growth to be epibiotic, with colonisation limited to the exterior surface of the epidermis (Longcore et al., 1999; Garmyn et al., 2012). Epibiotic growth has also been reported in some asymptomatic amphibians, such as invasive *X. laevis* (e.g. Van Rooij et al., 2012).

In this study, *Bd* demonstrated similar patterns of epibiotic infection with respect to amphipod crustaceans, with fungal structures found to interact directly with the cuticle of *D. villosus*, as well as gill lamellae. Despite the cuticle being structurally and/or compositionally dissimilar to vertebrate epidermis (see Roer and Dillaman, 1984; Chuong et al., 2002), *Bd* appeared capable of persisting as an epibiont upon the exterior surface of amphipod host cuticle, indicating possible compatibility with components of the crustacean integument. A major component of amphipod cuticle is chitin, a structurally important polysaccharide present in the outer layers of the exoskeleton (Halcrow, 2001; Havemann et al., 2008; Kaya et al., 2013; Trevisan et al., 2014). Whilst keratin is generally considered a main source of nutrition for *Bd* (see Voyles et al., 2011), chitinous materials can also be an effective growth media, with *Bd* having been shown to grow and persist upon the carapace and gastrointestinal (GI) tract of freshwater decapods (see McMahon et al., 2013; Oficialdegui et al., 2019). It is still unclear as to the exact mechanism by which *Bd* zoospores adhere to chitinous materials, but it may be linked to specific fungal proteins. For example, CBM18, a chitin-binding modular protein found in *Bd*, may enable chytrid to bind to chitinous structures, such as crustacean integument (Van Rooij et al., 2015), and may even protect *Bd* from host immunity (Abramyan and Stajich, 2012).

Here, associations observed between fungal structures and the cuticle of *D. villosus* would suggest that amphipod integument may also serve as an appropriate material to be utilised by chytrid. However, as only a limited range of fungal life stages were observed, further investigation is required to determine whether chytrid metabolises chitin within amphipod cuticle, thereby facilitating parasitic fungal growth, or is simply utilising the integument as a surface for attachment. Regardless, this study indicates an ability for *D. villosus* to act as a possible host for chytrid, identifying the amphipod cuticle as a potentially important component of fungal-host dynamics.

In amphipods, the cuticle also forms an integral part of the digestive system, lining the interior surface of the foregut (i.e. oesophagus) and hindgut (Johnston et al., 2004). Despite providing a potential opportunity for *Bd* to infiltrate the digestive tract (see McMahon et al., 2013), there was no evidence of fungal structures in associated tissues, indicating an inability for the chytrid fungus to colonise the digestive system of amphipods. Whilst this may be indicative of host immunity, an absence of fungal structures may also be attributed to the presence of commensal micro-organisms, situated within the digestive tract of *D. villosus* (see Bojko et al., 2013; Bojko and Ovcharenko, 2019), which may have prevented *Bd* colonisation (see Harris et al., 2009; Liew et al., 2017). However, further research is needed to explore the potential effects of commensal microbiomes in amphipods.

Infections by *Bd* were detected in a number of amphipods, sampled within approximately three weeks of exposure to infectious *Bd* zoospores. Prevalence and intensity of infections were relatively low when compared to alternative host species – such as decapods (see McMahon et al., 2013) and *Bd*-susceptible amphibians (see Searle et al., 2011; Ellison et al., 2014) – but were comparable to estimates recorded in more *Bd*-tolerant host species (e.g. *X. laevis*; see Tinsley et al., 2015a). Although each individual amphipod was only screened once, the declining prevalence in groups of amphipods screened over time provided some evidence to suggest that *Bd*-infections may be transient, with amphipods appearing to clear infections during experimental trials. Clearance of chytrid appeared to coincide with moulting events, as evidenced by an apparent absence of detectable *Bd* DNA in all amphipods which moulted post-exposure. However, molecular screening of shed cuticles revealed a large proportion of exuviae to be carrying *Bd*, with each moult bearing a relatively high burden of chytrid, on average, when compared to infected amphipod tissues samples.

In arthropods, moulting (i.e. ecdysis) is a natural physiological process typically associated with growth and development (Trevisan et al., 2014), but is also an efficacious immune defence which can be induced prematurely as a means of eliminating pathogenic agents (Laufer et al., 2005; Duneau and Ebert, 2012). Amphibians also possess the ability to moult, regularly shedding skin to renew epidermal integrity and functionality (Wells, 2013), and to also regulate cutaneous microbial abundance (Cramp et al., 2014). In species susceptible to *Bd* infection, moulting by amphibians has been shown to reduce fungal burden, and in some cases, clear infections completely (Ohmer et al., 2017). Within the context of the current study, it may be possible that amphipod moulting might fulfil a similar immune function, allowing *D. villosus* to effectively remove chytrid from host surfaces, whilst fungal structures remain upon discarded exuviae instead.

The detection of *Bd* DNA upon the moults of *D. villosus* would suggest the potential for chytrid to persist upon amphipod cuticle, even in the absence of live hosts. Despite being considered as a primarily parasitic fungus, *Bd* demonstrates the ability to adopt a saprophytic life history, capable of growing and persisting upon dead, organic materials, including birds feathers, arthropod exoskeletons, and the skin of amphibians, reptiles and birds (Longcore et al., 1999; Johnson and Speare, 2003; Johnson and Speare, 2005; Garmyn et al., 2012; McMahon et al., 2013). Amphipod exuviae may also serve as a vector by which *Bd* could persist in the environment; although without histological analysis of discarded cuticle, it is uncertain whether chytrid is able to proliferate upon amphipod moults. With evidence to suggest that *Bd* can persist in the environment for up to three months in the absence of natural amphibian hosts (Johnson and Speare, 2005), amphipod moults may act as an effective environmental reservoir upon which *Bd* might survive, until more suitable hosts arrive.

Examination of tissues removed from amphipods which returned a positive signal for *Bd* DNA, revealed a distinct lack of pathologies when compared to individuals examined from the control treatment. Despite observations of fungal structures associating with gill lamellae, amphipods appeared to be asymptomatic, displaying little evidence of *Bd*-induced pathological damage; possibly due to the transient nature of amphipod-chytrid dynamics. By comparison, previous studies concerning the pathogenesis of *Bd*-infections in both amphibian and non-amphibian hosts have described pathological symptoms of varying degrees of severity, including acute skin malformations in susceptible amphibian hosts (e.g. Berger et al., 1998; Voyles et al., 2009), fin erosion and muscle degeneration in fish (Liew et al., 2017), and recessed gills in decapods (McMahon et al., 2013); all of which have been associated with increased host mortality. Although analysis showed mortality to be strongly associated with infection status, the number of infected hosts was small (n = 4), so it cannot be determined whether *Bd*-infections were the main cause of amphipod deaths, based on the available data. Another possibility to consider is that the apparent association between infection status and amphipod mortality might also be the product of reverse causality. Rather than *Bd*-infections being the cause of mortality in all four individuals, it may be that those individuals were infected because they died due to another cause. If those individuals had survived for longer, they might have had the opportunity to clear infections via moulting – as evidenced in a number of amphipod specimens.

Whilst the ability for crustaceans to be infected by *Bd* has previously been reported in freshwater decapods (McMahon et al., 2013; Brannelly et al., 2015; Oficialdegui et al., 2019), this is the first empirical study indicating the potential for freshwater amphipod crustaceans to act as carriers. Several other studies have previously been published, suggesting the possibility for *Bd* to naturally, and/or experimentally, infect several species of feral, and farmed, freshwater shrimp (Order: Decapoda; Rowley et al., 2006; Paulraj et al., 2016); although these studies were subsequently retracted due to an inability to replicate original findings through repeated experimentation (Rowley et al., 2007), or criticised/questioned due to the incorrect identification of fungal pathogens (Pessier et al., 2017). Here, I present molecular diagnostic data, indicating the presence of *Bd* DNA in amphipod tissues, supplemented by histological analysis, highlighting non-hyphal fungal structures; consistent both in morphology and locality to *Bd* zoosporangia, as reported in previous, widely accepted literature (McMahon et al., 2013). My findings were also independently assessed by several clinical pathologist. However, in light of Rowley et al. (2006) and Paulraj et al. (2016), I acknowledge that the data presented in this chapter must be considered as preliminary, and that further research is required to validate these findings, and to ascertain the importance of freshwater amphipods, especially invaders, in the host-pathogen relationship of *Bd* and amphibians.

Nevertheless, the findings of the current study suggest that *Bd* can effectively colonise the cuticle of *D. villosus*, persisting upon the chitinous integument as an epibiotic aquatic fungus. Amphipods colonised by chytrid appeared to exhibit transient, asymptomatic low-level fungal burdens. Prevalence and infection loads were comparable to that of invasive amphibians, such as *X. laevis* and *R. catesbeiana*; both of which are implicated in the global spread of *Bd* (Hanselmann et al., 2004; Weldon et al., 2004; Garner et al., 2006; Fisher and Garner, 2007). Infections were detected in amphipods screened several weeks after exposures, although there was also some evidence to suggest the potential for *D. villosus* to clear infections via moulting; after which *Bd* did not re-colonise amphipods post-moult. Chytrid was also found to persist upon amphipod exuviae, although it is uncertain whether these fungal structures remained viable.

As a potential carrier of *Bd*, *D. villosus* may enable chytrid to persist in the environment, even in the absence of natural amphibian hosts. As a high-impact invader, considered to be one of more than 100 worst invaders in Europe (Nentwig et al., 2018), *D. villosus* might also act as an effective disease vector, capable of transmitting *Bd* between geographic regions. The dispersal of *D. villosus* throughout most of Europe, including the UK (Rewicz et al., 2015), has been attributed to the movement of contaminated ballast water (Bruijs et al., 2001), and recreational water-sports equipment (Anderson et al., 2014; Smith et al., 2020). Within invaded regions, *D. villosus* typically exist at high densities (Haas et al., 2002; Lods-Crozet and Reymond, 2006; van Riel et al., 2006; MacNeil et al., 2010; Tricarico et al., 2010; Gallardo et al., 2012a; Lods-Crozet, 2014; Borza et al., 2017), which may facilitate increased intraspecific transmission; thereby promoting a strong presence of chytrid within the environment (Young et al., 2017). The presence of *Bd* within invaded field regions might further be enhanced by the ability for *D. villosus* to shed chytrid via moulting. Persistence of *Bd* on amphipod moults might provide a potential vector by which chytrid may spread. For example, exuviae are typically consumed by crustaceans in order to reclaim nutrients and minerals (Swift, 1992; Steel, 1993; Buřič et al., 2016). If colonised by pathogenic agents, ingestion of exuviae may lead to infection (Roth and Iversen, 1971). With studies having shown that up to 50% of fungal spores can survive ingestion (Duffy, 2009; but see Searle et al., 2013), one might posit that the consumption of moults, colonised by *Bd*, may provide an alternative route of infection, although the apparent avoidance of *Bd*-infected moults by post-ecdysial amphipods might also indicate a possible strategy, developed to prevent reinfection via the consumption of their own exuviae.

As a potential carrier of *Bd*, it is as yet undetermined whether *D. villosus* is capable of transmitting chytrid to vulnerable amphibians; a mechanism which has previously been demonstrated by another invasive crustacean – *P. clarkii* (McMahon et al., 2013). If possible, the transmission of *Bd* may likely be facilitated by direct interactions between amphipods and amphibians (Daszak et al., 2000), with laboratory studies having recently identified the potential for *D. villosus* to predate upon native UK amphibians, consuming larvae at much higher rates when compared to native analogues (Chapter 2).

In conclusion, this study provides novel evidence, identifying the highly invasive *D. villosus* as a potential asymptomatic carrier for the highly virulent amphibian chytrid fungus.

Chapter 6

General Discussion

6.1 – Thesis Aims

Throughout this thesis I investigated the potential for the invasive freshwater amphipod *Dikerogammarus villosus* to impact upon UK anurans; directly as a predator of early life-stages, and indirectly as a possible carrier of the amphibian chytrid fungus *Batrachochytrium dendrobatidis* (*Bd*). In this final chapter I will summarise my main findings and discuss how they increase our understanding regarding the potential ecological impact of this high-impact invader within invaded freshwater communities. I will also discuss how these findings fit into the wider context of biological invasions and global amphibian declines.

6.2 – Direct Predatory Impact of Invasive *D. villosus*

Determining the ecological impact of invasive alien species has often been achieved by assessing key functional traits and behaviours, such as resource use (see Dick et al., 2014). The predatory impact of invasive *D. villosus* towards native macroinvertebrates is well established (e.g. Dick et al., 2002; Platvoet et al., 2009c), with invasions often leading to reductions in community abundance and diversity (van Riel et al., 2006; MacNeil et al., 2013a). When compared to native amphipod species, invasive *D. villosus* can consume prey at a much higher rate (e.g. Bollache et al., 2008; Dodd et al., 2014); a pattern also seen towards early stage fish, with predicted implications for aquaculture (Taylor and Dunn, 2017). In this thesis I present evidence to suggest that predation can also extend to amphibians. Given that invasive alien predators are regarded as one of several primary drivers of global amphibian declines (reviewed in Nunes et al., 2019), these findings indicate that *D. villosus* might also have the potential to contribute to these declines.

6.2.1 – Predation of Early-Stage Amphibians Confirmed

In this thesis, I provide empirical evidence confirming the ability for *D. villosus* to consume freshly hatched larvae (Chapters 2, 3 and 4). As a previously undocumented predator-prey dynamic (but see Fries and Tesch, 1965; Hudgens and Harbert, 2019), these findings expand upon our current knowledge regarding the potential dietary breadth of *D. villosus*, and suggest that predation of freshwater vertebrates can extend to other aquatic taxa, not just the embryos and larvae of non-/salmonid and gobiid fishes (e.g. Casellato et al., 2007; Platvoet et al., 2009c; Taylor and Dunn, 2017).

Data presented in Chapter 2 confirmed that predation of early stage anurans may be a generalised response in amphipods, with consumption confirmed in both invasive *D. villosus* and native *Gammarus pulex*. Through the application of comparative FR methodologies, both amphipod species were found to exhibit Type II FRs, indicating potentially destabilising predator-prey dynamics (Hassell, 1978; Dick et al., 2014). Comparatively, invasive *D. villosus* may likely exert a greater predatory impact than native *G. pulex* on larval amphibians, as a result of its naturally larger body size, rather than any inherent difference in the capability of each species. Indeed, large-bodied *D. villosus* were significantly better able to predate upon *R. temporaria* larvae – consuming up to seven times more anuran prey than native amphipods. Conversely, size-matched native and invasive amphipods demonstrated generally similar predatory impacts.

By utilising the relative impact potential metric (RIP), incorporating estimations of both predatory functional response and numerical response proxies (i.e. population abundance; Dick et al., 2017), I was able to discern even greater discrepancies in the predicted ecological impact of native and invasive amphipods. Indeed, marked differences in the relative abundance – total and demographic – of invasive *D. villosus* and native *G. pulex* populations, present in British and European freshwater field sites, translated into substantially higher estimations of invader impact potential towards *R. temporaria* larvae. Despite size-matched *D. villosus* demonstrating similar maximum feeding rates as native *G. pulex*, significantly higher abundance estimations for this invasive demographic group produced an RIP score 15 times higher than that of native amphipods. Despite similarities in the relative abundance of large-bodied *D. villosus* and *G. pulex*, significantly higher maximum feeding rates recorded in substantially larger invaders generated an RIP score 6 times higher than that of native *G. pulex*. Consistent with previous examples in which the RIP metric was applied whilst assessing the relative potential impact of invasive *D. villosus* and other invaders (see Dick et al., 2017), the findings presented in Chapter 2 predict that invasive *D. villosus* may impose a significantly greater ecological impact towards larval anurans when compared to native amphipods, likely driven by numerical superiority and higher *per capita* feeding rates.

Ecological impacts imposed because of superior invader feeding rates, are likely to be exacerbated in amphibian populations which lack sufficient eco-evolutionary experience, either with *D. villosus* or with functionally similar native predators (Dick et al., 2014; Saul and Jeschke, 2015). Given the rate at which *D. villosus* has spread throughout Western Europe (see Rewicz et al., 2014), and differences in invader morphology (e.g. body size) and behaviour (e.g. hunting strategy), when compared to native amphipod species (see Dodd et al., 2014; Truhlar and Aldridge, 2015), it may be possible that *D. villosus* is regarded as a relatively novel amphipod predator within invaded regions. As such, larval *R. temporaria* populations might be eco-evolutionarily naïve, incapable of effectively recognising or responding to this invasive amphipod, and therefore more susceptible to predation, and possible extirpation (Sih et al., 2010; Saul and Jeschke, 2015).

Differential predatory pressures imposed by invasive *D. villosus* and native *G. pulex* also extended to other amphibian species, with predation of invasive *Xenopus laevis* embryos recorded. Predation was recorded in *D. villosus* only, with *per capita* predation rates shown to also increase as a function of invader body size, whereas native *G. pulex* demonstrated an apparent aversion to *X. laevis* embryos in general. Having identified the potential for *D. villosus* to consume the early life stages of several anuran species, I believe that further research is required to determine the breadth of amphibian species adversely affected by this invasive amphipod. This is of particular importance for Biodiversity Action Plan (BAP) species, such as the Great Crested Newt (*Triturus cristatus*), for which monitoring, conservation and protection from invasive alien species is vitally important (e.g. The Conservation of Habitats and Species Regulations, 2017; Grillas et al., 2018). By realising the potential predatory threat that *D. villosus* may pose towards amphibians in general, these findings may contribute towards informing upon future monitoring, mitigation and/or conservation strategies (Falaschi et al., 2020).

6.2.2 – Conspecific Interactions are likely to be a Strong Predictor of Ecological Impact

Data presented in Chapter 3 would suggest that interactions amongst conspecific invasive predators may likely modify the predatory success of individual *D. villosus* when attempting to consume anuran larvae; thereby altering predictions concerning the ecological impact of this invader.

Through manipulation of both native *R. temporaria* larvae and invasive *D. villosus* densities, *per capita* predation was found to be contingent upon the intensity of interactions between conspecific predators, as well as the relative availability of larval anurans per amphipod predator; as evidenced by the expression of a Type II ratio-dependent FR, but also several predator-dependent FR variants which exhibited ratio-dependent tendencies. Ratio- and/or predator-dependence would suggest that, whilst present at higher densities, individual *D. villosus* are likely to interact with conspecifics more frequently as the relative availability of *R. temporaria* larvae decreases, thereby promoting the emergence of mutual interference (see Arditi and Ginzburg, 2012). With evidence of a density-dependent increase in the overall *per capita* handling times of *D. villosus*, interactions amongst conspecific predators are likely to be interferential, with individuals spending a greater proportion of time interacting with other predators, rather than feeding on anuran larvae. As such, mutual interference amongst conspecific *D. villosus* is predicted to cause an overall reduction in the *per capita* feeding success of this invasive amphipod – potentially conveying a less destabilising effect towards larval anurans (Arditi and Ginzburg, 2012; Médoc et al., 2015).

The expression of ratio dependence, and the associated emergence of mutual interference, is consistent with previous literature, having been reported across both terrestrial and aquatic systems (reviewed in Arditi and Ginzburg, 2012; but also Spataro et al., 2012; Hebblewhite, 2013; Zimmermann et al., 2015; Prokopenko et al., 2017). Ratio-dependence has also been reported in some amphipod species, including *D. villosus* (Médoc et al., 2013, 2015), as well as odonates – a common, yet effective predator of larval amphibians (Hossie and Murray, 2016). In both latter cases, ratio-dependence, and the development of mutual interference, was largely determined by spatial dynamics, of conspecific predators (Médoc et al., 2015), but also amphibian prey (Hossie and Murray, 2016). In Chapter 3, analysis of amphipod spatial arrangement revealed similar distribution patterns, with *D. villosus* found to aggregate with conspecifics over time, as the number of predators increased within experimental systems. Gregariousness was also strongly linked to changes in prey availability, with increasing rates of prey depletion, occurring at higher predator densities, causing *D. villosus* to become more aggregated over time. When considered in conjunction with the expression of ratio dependence, but also predator dependence, by *D. villosus*, it is likely that conspecific aggregation, in response to increasing restrictions in the availability of larval anuran prey, may lead to the emergence of mutual interference (Arditi and Ginzburg, 2012; Médoc et al., 2015). Despite previous predictions of a significantly greater relative potential impact imposed by invasive *D. villosus*, as a function of differential *per capita* predation and abundance (Dick et al., 2017; Dickey et al., 2020; Chapter 2), these findings would suggest that the *per capita* predatory efficiency of this invasive freshwater amphipod towards larval anurans might actually decrease with increasing predator abundance, depending on the relative availability of prey.

Within invaded field systems, further dampening of *per capita* invader effects may be expected, given the tendency for *D. villosus* to exist at high densities (e.g. van Riel et al., 2006). In such situations, if interference amongst conspecific predators becomes too intense, overcompensating for density-dependent limitations in *per capita* prey availability, it could theoretically lead to the extirpation of invaders from a system (Arditi et al., 2004); although, given the generalist nature of *D. villosus* (e.g. Platvoet et al., 2009c), and historical reports of field impacts (e.g. van Riel et al., 2006; Dodd et al., 2014), this may be unlikely. It might also be possible that any reduction to *per capita* feeding rates, caused by strong conspecific interference, may ultimately be negated by amphipod abundance, with *D. villosus* imposing a significantly higher total potential impact as a function of greater numerical response (Dick et al., 2017; Dickey et al., 2020). Conversely, in situations whereby invasive *D. villosus* exist at lower densities, ecological impacts imposed by this invasive amphipod predator may actually be greater-than-expected. For example, during the early stages of biological invasions, a period in which invader abundance is typically lower, native populations may often suffer from higher rates of IAS-induced decline, when compared to later stages of the invasion process. During these initial stages of invasion, native populations may, on average, experience declines of up to 20% as invader abundance begins to rise (Bradley, B. et al., 2019); a trend likely attributable to the combined effects of both native and invasive predators prior to native replacement (Dickey et al., 2020), native prey naivety due to a lack of eco-evolutionary experience with invaders or functionally similar native congeners (Sih et al., 2010), invader enemy release (Blumenthal, 2006), increased consumption linked to high fecundity – thereby enabling invaders to colonise novel regions (Colautti et al., 2017) – as well as low level interference amongst predators as a function of greater *per capita* prey availability (Arditi and Ginzburg, 2012). As such, larval anurans might be expected to experience similar rates of decline, particularly during the early stages of invasion. Depending on the *per capita* availability of larval anurans, and their distribution throughout freshwater systems, FRs expressed by invasive *D. villosus* may be expected to transition from ratio- or predator dependence to prey dependence (Arditi and Ginzburg, 2012), conveying a greater *per capita* consumptive effect towards *R. temporaria* larvae seasonally. However, further research is needed to determine how predatory pressures imposed by *D. villosus* may affect amphibian populations in the field, especially as invasions progress and the dynamics of native and invasive predator populations change.

Nevertheless, these findings confirm that interactions between conspecific predators are likely to be an important predictor of *per capita* invader behaviours and impacts. As such, density-dependent effects of predator abundance, and the potential emergence of mutual interference, should be incorporated when predicting the ecological impact of invaders.

6.2.3 – Preferential Feeding by *D. villosus* may have Wider Implications for Invaded Communities

Data presented in Chapter 4 indicates that *per capita* predatory impacts imposed by invasive *D. villosus* towards larval anurans may also be determined by the presence and/or relative abundance of alternative freshwater organisms.

As a generalist predator, *D. villosus* has demonstrated indiscriminate feeding when multiple prey species are available (Dodd et al., 2014); a behaviour which has also been reported in other invasive crustaceans (e.g. Haddaway et al., 2012), and may be attributed to the uniform availability of prey species (Murdoch and Oaten, 1975; Tschanz et al., 2007). However, when presented with *R. temporaria* larvae, *A. aquaticus* and *Chironomus spp.* larvae in equal numbers, individual *D. villosus* were found to exhibit strong preferential feeding behaviours, consuming macroinvertebrate prey species in significantly greater quantities, when compared to anuran larvae. *Chironomus spp.* larvae were the most heavily predated prey species, consumed in significantly greater numbers, followed by *A. aquaticus* and then *R. temporaria* larvae. Native *G. pulex* demonstrated a similar prey choice hierarchy although, when compared to invasive *D. villosus*, native amphipods consumed a significantly higher number of dipteran larvae only, whereas invaders consumed substantially more isopodan and anuran prey as well, consuming a significantly greater number of prey overall.

Whilst in the presence of multiple prey species, predator feeding patterns are predicted to shift as the proportional availability of prey varies, with predators expected to feed upon more common prey, thereby limiting predatory pressures imposed upon rarer species. Frequency-dependent prey-switching amongst multiple prey species is expected to convey a more stabilising predator-prey dynamic, promoting the persistence of larger, more complex communities (van Leeuwen et al., 2013). As such, prey-switching behaviours often coincide with the expression of a Type III FR by consumers (Oaten and Murdoch, 1975). However, when provided with *R. temporaria* larvae, in combination with either *A. aquaticus* or *Chironomus spp.* larvae at varying relative densities, invasive *D. villosus* were found to exhibit a strong continued predatory preference for alternative macroinvertebrate prey species, regardless of proportional availability. Such differential feeding patterns towards macroinvertebrate and anuran prey were reflected by multiple prey species FR modelling (MSFR). Despite Type II FRs suggesting a potentially destabilising predator-prey dynamic towards vertebrate and invertebrate prey (Price et al., 2011), invasive *D. villosus* were predicted to have a far greater predatory impact towards alternative macroinvertebrate prey populations, potentially leading to their extinction (see van Leeuwen et al., 2007). As such, these findings would suggest that predation of larval anurans may be less likely than previously anticipated (Chapter 2).

It remains unclear as to the exact reason why *D. villosus* might demonstrate limited preference towards anuran larvae; despite larvae often representing a highly valued resource for many vertebrate and invertebrate predators (Alford et al., 2013). The observed preference for macroinvertebrate prey over larval amphibians may be attributed to differential cost-benefits associated with hunting amphibian larvae, when compared to alternative prey types (Eubanks and Denno, 2000), or possibly due to prey species demonstrating differential responses to predation pressures (Lima et al., 2003). Markedly lower selectivity may also be due to the novelty of anuran larvae as a potential prey for invasive *D. villosus*. Grafham Water, the site from which invasive *D. villosus* were sampled, is not a breeding site typically used by amphibians (see Beebee and Griffiths, 2001) – although breeding amphibians have previously been recorded in the reservoir itself, as well as the surrounding freshwaters (Anglian Water, pers. comm.; National Biodiversity Network, 2017) – whereas *Chironomus spp.* larvae and *A. aquaticus* have both previously been reported as present (Anglian Water, pers. comm.; Dodd et al., 2014). As such, *D. villosus* may likely lack the necessary experience required to recognise, develop and implement an effective hunting strategy against anuran larvae (Paradise and Stamp, 1991). However, over time such experience may be acquired, possibly as a result of social learning (see Damas-Moreira et al., 2018; Hämäläinen et al., 2020), thereby enabling *D. villosus* to become a more effective invasive predator (Schmidt et al., 2012); although this in turn might alter ecological predictions, with predator learning associated with the expression of a potentially stabilising Type III FR (see Holling, 1965; Hassell et al., 1977). As such, further research is required to determine how predatory pressures towards larval amphibians may be affected by predator learning.

Within invaded regions, community-level impacts imposed by invasive *D. villosus* through predation are likely to be determined by the composition of invaded freshwater communities (e.g. Kondoh, 2003; Romanuk et al., 2009; Loeuille, 2010), but may also be dependent upon the timing of invasions (Bradley, B. et al., 2019). For example, invaded communities often experience significant declines in richness, diversity and abundance during the early stages of the invasion process, when invaders are typically present at lower densities. The magnitude of declines might also intensify depending on the trophic position of invaders (Bradley, B. et al., 2019); for which *D. villosus* can be positioned quite highly (van Riel et al., 2006; Bacela-Spychalska and Van Der Velde, 2013; but see Hellmann et al., 2015).

Intense predation of macroinvertebrate prey species is likely to have a dramatic effect on freshwater communities (e.g. MacNeil et al., 2013a). If functionally important (e.g. keystone shredders), the extirpation of macroinvertebrates such as *A. aquaticus*, may impair vital ecosystem functioning (e.g. nutrient cycling), impacting upon communities via a cascade of secondary extinction events, occurring across multiple trophic levels (MacNeil et al., 2011; Piscart et al., 2011; Dodd et al., 2014; Boeker and Geist, 2015; Kenna et al., 2017; but also seeTruhlar et al., 2014; Worischka et al., 2018). Whilst predation may have a limited direct effect on larval anurans, changes to macroinvertebrate assemblages and/or nutrient dynamics by *D. villosus* may dramatically impact upon the seasonal recruitment of amphibians indirectly, potentially affecting not only herbivorous grazing/detritivorous larval species (e.g. *R. temporaria*; Nyström et al., 2001), but also more carnivorous/omnivorous species (e.g. *R. sylvatica*; Petranka and Kennedy, 1999). However, further investigation is needed to determine how post-invasional changes to native community food webs by *D. villosus*, as a result of selective feeding, altered nutrient dynamics, trophic cascades, and/or sequential extinctions, might affect larval amphibian communities (Lurgi et al., 2014; Frost et al., 2019).

One aspect of predation which was not examined in the current thesis, but which may have considerable implications for the fitness and/or survival of larval amphibians, is the potential effects of unsuccessful or partial predation events. Although regarded as a voracious invasive predator, *D. villosus* demonstrates a tendency for attacking prey items without consuming them, often biting and then releasing prey, leaving them to subsequently die (Dick et al., 2002). Throughout my research, I observed similar behaviours towards larval anurans, with *D. villosus* found biting either the tail or headbody of recently hatched larvae. Larvae which experienced tail injuries were often found alive, depending on the severity of injuries sustained, whereas those individuals which incurred injuries to the headbody typically died during experimental trials. Non-lethal tail injuries inflicted by invasive predators have been shown to have an adverse non-consumptive effect upon larval morphology, impacting upon individual fitness, performance and ultimately survival (Nunes et al., 2010). Non-consumptive effects imposed by invasive predators can also alter prey behaviours, contributing to the "landscape of fear" (see Laundre et al., 2010). Depending on the level of naivety, native prey species may recognise invaders – if sufficiently experienced with the invader, or functionally similar native predators – or may develop recognition over the course of multiple generations (Anton et al., 2020). With adequate experience, native prey species may acquire the ability to discern cues released by invasive predators, but also injured and killed conspecific prey, to perceive the potential risk of an invader, and to develop adaptive anti-predator behavioural responses; often at the expense of typical behaviours (e.g. foraging), and therefore individual fitness (Sih et al., 2010; Nunes et al., 2019). When compared to the negative effects of direct predation, non-consumptive effects have been shown to impose a strong – if not stronger – effect on prey populations overall, particularly within aquatic ecosystems (Preisser et al., 2005; Werner and Peacor, 2006). With further research, I believe that invasive *D. villosus* might also demonstrate the capacity to impose strong non-consumptive effects upon larval amphibians, thereby enhancing the overall impact of amphipod predation towards amphibian populations (Sih et al., 2010). Non-/consumptive effects exerted by *D. villosus* might also generate a potentially additive effect whilst in the presence of alternative predatory species (i.e. emergent multiple predator effect (MPE); see Barrios-O'Neill et al., 2014a), particularly other invaders (Beggel et al., 2016).

Regardless, the findings presented in Chapter 4 confirm that alternative prey can mediate the predatory impact of invasive *D. villosus* towards larval anurans, with evidence of strong selective feeding behaviours having potential implications when determining individual- and/or community-level invader impacts. As an established concept of classic ecological theory (see Murdoch and Oaten, 1975), incorporating preferential feeding behaviours into invasion ecology predictions is vital, especially when assessing the impact of invasive generalist predators (Snyder and Evans, 2006).

6.2.4 – The Efficacy and Adaptability of the Functional Response Approach

Whilst outside the principal scope of this thesis, I provide several case studies highlighting the applicability of FR methodologies, as a means of deriving quantifiable predictions concerning invader impacts. Beyond the traditional 'prey-dependent' FR approach, frequently utilised across invasion ecology literature to examine the potential impact of invasive alien consumers – often in response to specific abiotic and/or biotic context-dependencies (see Appendix 1, Table A1.1) – I demonstrate how FR methodologies can be adapted to consider the implications of complex predatory behavioural responses.

Having identified a previously unexplored predator-prey dynamic, FR methodologies effectively identified the potential for invasive *D. villosus* to impose strong top-down effects upon the early life-stages of several UK anuran species, with the possibility of generating a significantly greater total relative impact when compared to native amphipods (Chapter 2). By adapting classic FR methodologies, I was able to demonstrate that interferential interactions between conspecific predators may likely have a strong effect on predicted *per capita* effects, obtaining quantifiable estimates of such interactions in the process (Chapter 3). Modifications to FR theory enabled me to consider preferential feeding behaviours and revealed that strong selectivity, exhibited by *D. villosus* towards alternative prey species is likely to reduce *per capita* impacts towards larval anurans (Chapter 4). With additional experimental work, the approaches described within this thesis could be further modified to incorporate additional context-dependencies when analysing invader functional responses. By considering the effect of factors such as parasitism (e.g. Iltis et al., 2018), habitat complexity (e.g. Barrios-O'Neill et al., 2015), climate change (e.g. Pellan et al., 2016), and heterospecific competition and/or predation (e.g. Barrios-O'Neill et al., 2014a; Wasserman et al., 2016), the FR approach could allow more accurate predictions, concerning the potential ecological impact of invasive *D. villosus*, to be obtained, providing a more accurate description of amphipod-amphibian interactions, under more ecologically realistic conditions (Dick et al., 2014). Moreover, through the application of metrics (e.g. RIP), such context-dependent effects may alter population-level invader impacts (Dick et al., 2017; Dickey et al., 2020).

However, having obtained predictions concerning the potential ecological impact of *D. villosus* towards larval anurans, further research would still be required in order to determine whether these predictions are consistent with field impacts (Dick et al., 2014). Using comparative observation-based studies (e.g. Before-After-Control-Impact (BACI) conducted between invaded and uninvaded field systems (e.g. Kumschick et al., 2015), or alternatively experimental field mesocosms (e.g. Rosewarne et al., 2016; Fincham, 2018), in conjunction with stable isotope and/or gut content analyses (e.g. Pacioglu et al., 2019), would confirm whether *D. villosus* poses a potential threat to larval amphibians within invaded regions.

6.3 – Indirect Impact of Invasive *D. villosus* as Potential Reservoir for *Batrachochytrium dendrobatidis*

In Chapter 6 I provide preliminary empirical evidence to suggest that freshwater amphipod crustaceans may be suitable carriers of the amphibian chytrid fungus *Bd*. Following exposures to *Bd*-contaminated water, invasive *D. villosus* were found to acquire infections, localised to the exterior surface of the chitinous cuticle; a finding which has also been demonstrated with invasive decapod crustaceans (see McMahon et al., 2013). Individual amphipods were able to maintain infections for a period of several weeks before subsequently clearing them. Clearance of *Bd*-infections appeared to coincide with moulting events, with amphipods appearing to shed fungal bodies along with the contaminated cuticle. Having moulted, amphipods were free of infections, whereas *Bd* remained present upon the discarded exuviae. For those individuals which remained infected, examinations of tissues confirmed these individuals were asymptomatic, free of any apparent pathologies.

Given the ease with which invasive *D. villosus* has effectively spread throughout most of Western Europe, disseminating passively via human-mediated vectors (Casellato et al., 2007; Anderson et al., 2014; Smith et al., 2020), but also potentially by natural vectors (e.g. birds; see Gallardo et al., 2012a), the possibility for this invasive amphipod to act as an alternative, non-amphibian carrier of *Bd* may have far-reaching implications for amphibian conservation (Brannelly et al., 2015; Brannelly et al., 2018; Oficialdegui et al., 2019). As such, further research is necessary to determine the true extent by which *D. villosus* might facilitate the spread and/or persistence of *Bd* within the environment.

Whilst the findings presented in Chapter 6 indicate the possibility for *D. villosus* to acquire infections through exposure to *Bd*-contaminated water, alternative routes of infection may also exist; but are yet to be verified. Having identified the potential for *D. villosus* to predate upon anuran larvae (Chapters 2, 3 and 4), infections may be acquired through direct contact with infected larvae (Piotrowski et al., 2004). Regarded as an effective detritivore capable of feeding upon dead plant and animal matter (see Dick et al., 2002; Mayer et al., 2008; Worischka et al., 2018), *D. villosus* may also become infected whilst scavenging upon infected carrion (Bacela-Spychalska et al., 2012; Bosch et al., 2015), but also contaminated amphipod moults (Buřič et al., 2016). Given the propensity for *D. villosus* to aggregate with conspecifics whilst attempting to access limited shared prey resources (Médoc et al., 2015; Chapter 3), interactions with infected amphipod and/or larvae may cause *Bd*-infections to proliferate amongst amphipod groupings, leading to a rapid, dramatic increase in both prevalence and infection loads within established populations (Bacela-Spychalska et al., 2012; Courtois et al., 2017).

Further research is required to ascertain if transmission of this fungal pathogen between amphipod and amphibian hosts is possible – as a result of either proximate or direct contact with infected hosts (e.g. McMahon et al., 2013), or by interacting with contaminated exuviae in the environment (Cramp et al., 2014). In doing so, this would confirm a potentially complex host-pathogen dynamic, spanning multiple host taxa. Having identified *D. villosus* as a possible carrier of *Bd*, I believe that field populations of *D. villosus* should also be screened in order to evaluate whether *Bd*-infections occur in the field, and to determine the potential prevalence within established populations (e.g. McMahon et al., 2013; Brannelly et al., 2015; Oficialdegui et al., 2019). With *D. villosus* typically existing at high densities within invaded regions (e.g. van Riel et al., 2006), prevalence within established populations may be expected to be relatively high (Bacela-Spychalska et al., 2012), with populations functioning as potentially sizeable environmental reservoirs (Oficialdegui et al., 2019). If confirmed, then the predicted unintentional dispersal of *D. villosus* in Great Britain (Gallardo et al., 2012a; Gallardo and Aldridge, 2020), passively facilitated by human-mediated vectors such as angling equipment (Anderson et al., 2014; Smith et al., 2020), might inadvertently mediate the spread of *Bd*. Given that anglers may often frequent sites which are used by amphibians as annual breeding sites (pers. obs.), the introduction of *D. villosus* into these sites could result in localised *Bd* hotspots, thereby impacting upon the recruitment of amphibians; although further research is required to determine whether *D. villosus* might subsist in such environments. Moreover, with predictions indicating introduction of *D. villosus* into North America (Ricciardi and Rasmussen, 1998; Kramer et al., 2017), the ability for this invader to act as a carrier of *Bd*, could have implications for global amphibian conservation.

Future research should also extend to consider the potential for this invasive amphipod to act as a potential carrier of alternative pathogens, such as *Ranaviruses*; a group of virulent, globally present iridoviruses, which are capable of infecting vertebrate and invertebrate hosts, transmitted primarily through predation, necrophagy or cannibalism (reviewed in Gray et al., 2009; Chinchar et al., 2017).

Host potential might also extend to *Batrachochytrium salamandrivorans*, although historically this chytrid fungal species typically demonstrates a more specialised host range (i.e. urodeles), with very little evidence to suggest potential non-amphibian hosts (reviewed in Yap et al., 2017). Regardless, by addressing the possibility for *D. villosus* to maintain various amphibian pathogens, these findings would allow us to garner a far greater understanding about the underlying mechanisms surrounding disease-mediated global amphibian declines and the role that invasive non-amphibian hosts may have in driving these declines, and would also likely contribute to future mitigation and/or conservation strategies (e.g. biosecurity; see Woodhams et al., 2011; Scheele et al., 2014; Garner et al., 2016; Thomas et al., 2019).

6.4 – Implications of *D. villosus* invasions for Amphibians

Throughout this thesis I provide evidence to suggest that invasive *D. villosus* has the potential to negatively affect amphibian populations. *Dikerogammarus villosus* may function as an effective predator of vulnerable early life stages, exhibiting the capacity to impact directly upon the seasonal recruitment of amphibians within invaded freshwater systems; although the intensity of predatory pressures imposed by this invader is likely to be dictated by ecological context. *Dikerogammarus villosus* may also act as an alternative, non-amphibian carrier of *Bd*, demonstrating the potential to facilitate the persistence of the chytrid fungus within the aquatic environment, as an epibiont present on the cuticle of live amphipod hosts, or as a saprobe present on discarded exuviae.

Having identified *D. villosus* as a potential predator of early stage amphibians, as well as a possible carrier of *Bd*, it is vital that further research be conducted in order to determine the full extent by which this invasive freshwater amphipod might impact upon amphibian populations. As a previously undetermined dynamic it is still unclear whether interactions between invasive amphipods, native amphibians and/or pathogenic agents occur within current invaded regions. It is unlikely that sufficient historical data exists to confirm current predictions concerning the direct and/or direct impact of invasive *D. villosus*; despite potential overlaps between amphipod and amphibian populations within some invaded regions, both in the UK (Anglian Water, pers. comms.; National Biodiversity Network, 2017), and mainland Europe (e.g. Hesselschwerdt et al., 2008; Uehlinger et al., 2009; Koester et al., 2016; Haubrock et al., 2019). However, given the rapid expansion of *D. villosus* throughout Europe (reviewed in Rewicz et al., 2014), and its predicted propagation in Great Britain (see Gallardo et al., 2012a; Gallardo and Aldridge, 2020), this high-impact invasive freshwater amphipod may represent a prospective, yet overlooked, driver of amphibian declines.

In the absence of suitable long-term monitoring data, field-based mesocosm studies, conducted under a BACI design, could be used to test current predictions (Kumschick et al., 2015; Fincham, 2018). Greater understanding could also be gained through the application of gut content and/or stable isotope analyses, which have previously proven effective in determining the dietary composition of *D.*

villosus within the field (e.g. van Riel et al., 2006; Hellmann et al., 2015), including the detection of ingested fish prey (Marco Benucci, pers. comms.). If detected, the presence of amphibian prey matter in the diets of wild *D. villosus* would provide field data to support the predictions presented in Chapters 2, 3 and 4, but would also confirm a potential route for *Bd* transmission (e.g. Hamilton et al., 2012). Furthermore, I believe that screening established populations of *D. villosus* for the presence of *Bd* would also prove insightful in determining the possibility for this invasive amphipod to acquire infections naturally, whilst also monitoring for possible outbreaks (e.g. McMahon et al., 2013; Brannelly et al., 2015; Oficialdegui et al., 2019).

With additional evidence it may become prudent to consider the potential implications of *D. villosus* invasions towards UK amphibians when developing IAS management strategies. For example, *D. villosus* has previously been assessed under the Water Framework Directive (WFD; Gallardo et al., 2012a) – a management planning system designed to monitor, protect and improve the ecological health of "at-risk" aquatic systems (DEFRA, 2014). However, the WFD only takes into consideration aquatic plants, macroinvertebrates and fish when determining the ecological health of UK freshwaters (European Commission, 2011). Amphibians, despite being regarded as effective bioindicators of freshwater quality (Venturino et al., 2003; but see Beebee and Griffiths, 2005), are not considered under the WFD assessment (European Commission, 2011). Whilst all UK amphibian species are protected by some form of legislation (e.g. Wildlife & Countryside Act, 1981 and Conservation of Habitat and Species Regulations, 2017) – more so for UK-BAP species (Baker et al., 2011) – previous conservation efforts with regards to mitigating invader impacts have been relatively poor (Hoffmann et al., 2010). Following the global emergence of *Bd* (e.g. Olson et al., 2013), conservation efforts are on the rise (see Woodhams et al., 2011; Garner et al., 2016; Meredith et al., 2016; Hettyey et al., 2019; Fisher and Garner, 2020), including the development of European and/or international legislation (e.g. European Threat Abatement Plan; Fisher et al., 2012). As such, there are calls for the development of evidence-based conservation actions, as well as effective practical management strategies (Falaschi et al., 2020).

Having established the potential for *D. villosus* to impact upon native amphibian populations, it is important to monitor the spread of *D. villosus* throughout UK freshwaters. To date, approximately 300 sites routinely monitored by government organisations (e.g. the Environment Agency and Natural England; GB non-native species secretariat, 2020), with additional sites monitored by independent non-government societies (e.g. Anglers' Riverfly Monitoring Initiative; Brooks et al., 2019). However, greater monitoring should be applied to freshwater sites frequently used by breeding amphibians – particularly BAP species – including angling sites (pers. obs.; Smith et al., 2020), and Sites of Special Scientific Interest (SSSIs; Baker et al., 2011). To adequately allocate resources to prospective monitoring sites, predictive models should be implemented in order to determine priority locations. Beyond current models which have traditionally been used to map the potential spread of *D.*

villosus throughout the UK, based on bioclimatic suitability (e.g. Gallardo et al., 2012b), water chemistry, substrate type, hydrological connectivity and the presence of co-evolved invaders (e.g. zebra mussels; Gallardo et al., 2012b), future models should also consider potential overlap between amphipod and amphibian habitat suitability (e.g. Boets et al., 2014; Préau et al., 2020), as well as the projected movement of amphipods via human-mediated vectors (e.g. angling equipment; Smith et al., 2020). Conventional field monitoring methodologies (e.g. kick-sampling), when supplemented with non-invasive detection techniques, such as *environmental DNA* (eDNA; Blackman et al., 2017, 2018; Mauvisseau et al., 2019) and passive bioacoustic monitoring (e.g. Greenhalgh et al., 2020), would enable ecologists to detect the presence of *D. villosus* within "high-risk" amphibian breeding sites.

In addition to improved monitoring of priority habitats, practical management strategies must also consider greater efforts in preventing the spread of invaders (Falaschi et al., 2020). Legislatively, this might include stronger regulations concerning the intentional and/or accidental movement of species internationally (e.g. European Union (EU) Regulation no. 1143; European Commission, 2014; but also see Coughlan et al., 2020), especially those involved in the spread of pathogenic diseases (e.g. exotic amphibians; Auliya et al., 2016; Wombwell et al., 2016). Promoting better biosecurity practices would also aid in mitigating the spread of invasive species, both internationally (Black and Bartlett, 2020), but also nationally (Sebire et al., 2018), with procedures such as the application of hot water (e.g. Anderson et al., 2015b; Shannon et al., 2018), steam and commercial disinfectants (Bradbeer et al., 2020), having proven effective in killing invasive species, including *D. villosus*. Some biosecurity protocols, such as the application of chemical disinfectants, have also proven to be effective in controlling the spread of *Bd*; although this may not necessarily be consistent for all pathogenic agents (e.g. *B. salamandrivorans*; see Van Rooij et al., 2017).

6.5 – Concluding Remarks

Biological invasions are, and will likely continue to be, a major element of human-induced environmental change, predicted to continue increasing in frequency, causing substantial negative ecological impacts throughout the world (Hulme, 2009; Bellard et al., 2016a; Seebens et al., 2017; Blackburn et al., 2019; Sardain et al., 2019). As such, amphibians are likely to continue experiencing increasing rates of declines and extinctions, attributed to the movement of invasive predators, as well as carriers of deadly pathogenic diseases (Nunes et al., 2019; Fisher and Garner, 2020).

At the start of my doctoral research I set out to address a hypothetical, previously undocumented predator-prey interaction. Over the course of three years of research, I was able to identify the potential for the invasive killer shrimp *Dikerogammarus villosus* to negatively impact upon native UK anurans, both through the consumption of vulnerable early life-stages, and as a carrier of the amphibian chytrid fungus. *Dikerogammarus villosus* is regarded as a widespread high-impact invader, easily transported between regions (reviewed in Rewicz et al., 2014). Within invaded freshwaters, the risks imposed by *D. villosus* invasions, towards resident macroinvertebrate communities is well established (e.g. van Riel et al., 2006; MacNeil et al., 2013a), with more recent studies suggesting that these risks may also extend to some aquatic vertebrates (e.g. Taylor and Dunn, 2017). The findings I have provided in this thesis indicate that this high-impact invader may indeed be a ‘killer shrimp’, posing a credible threat to UK amphibians, with the potential to inflict long-term, adverse impacts upon already endangered species. As such, this small-bodied invasive freshwater amphipod might also function as a possible driver of amphibian declines.

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Appendices for Chapters

Appendix 1

Table A1.1. Summary of published literature concerning the application of functional response methodologies, used to determine the per capita effect of invasive alien consumers and biological control agents. Literature was compiled through a systematic review, conducted using Web of Science, Scopus, and Google Scholar. Terms applied to literature searchers include “Functional response” AND “Invasive alien species” OR “Invasive non-native species” OR “Invasive species” OR “Alien species” OR “Non-native species” OR “Exotic species” AND “Invasive” OR “Invader” AND “Ecological impact”

		Predator					
Reference	Ecosystem	Native	Invasive	Prey	Experimental design	Additional variables	Notes
(Dubs and Corkum, 1996)	Freshwater	Mottled sculpin *Cottus bairdi*	Round goby *Neogobious melanostomus*	Native amphipod *Gammarus spp.*	Single predator and various prey densities	No	Higher FR for the invasive predator
(Hooff and Bollens, 2004)	Marine		Copepod *Tortanus dextrilobatus*	Native calanoid *Acartia spp.* Invasive cyclopoid *Oithona davisae*	Single predator and various prey densities	Examined FR at two experimental temperature regimes (14ºC and 19ºC), representing two seasons.	Type II FRs identified for invasive predator, across both invasive prey species. Significant affect of temperature on feeding
(Mistri, 2004)	Marine		Mud crab *Dyspanopeus sayi*	Non-native Asian date mussel *Musculista senhousia*	Single predator and various prey densities	No	Type II FR identified – potentially destabilising to invasive *M. senhousia*.
(Ruscoe et al., 2005)	Terrestrial		House mouse *Mus musculus*	Native New Zealand beech seed *Nothofagus solandri* var. *cliffortioides* Native lepidopteran larvae *Tingena armigerella*	Single predator and various prey densities	Presence/absence of alternative lepidopteran prey – presented at two size classes (small/large) both at fixed densities (10 small/ 5 large)	Type II FR identified Maximum feeding rate reduced in presence of alternative lepidopteran prey, but FR remained Type II form.
(Bollache et al., 2008)	Freshwater	Amphipods *Gammarus duebeni* *G. roeseli* *G. pulex* (native in River Ouche, France)	Amphipod *Dikerogammarus villosus* *G. pulex* (invasive in River Lagan, N. Ireland)	Native isopod *Asellus aquaticus*	Single predator and various prey densities	No	Higher Type II FR for invasive *D. villosus*. FRs nearly identical for *G. pulex* within native and invasive ranges.
(Buhle and Ruesink, 2009)	Marine		Japanese drill *Ocinebrina inornate* Eastern drill *Urosalpinx cinerea*	Native Olympia oyster *Ostrea lurida* Invasive Pacific oyster *Crassostrea gigas*	Fully crossed experimental design in which the density of both oyster species (*O. lurida* and *C. gigas*), and *O. inornate* varied.	Field-based enclosure experiment to quantify the effects of direct interactions (competition and predation) and indirect interactions (facilitation) amongst *O. inornate*, *O. lurida* and *C.*	Type II FR for *O. inornate* towards both oyster species. Interspecific competition by *C. gigas* towards *O. lurida* reduced native oyster growth and survival.

						gigas on the survival of native *O. lurida*.	
(Dick et al., 2010)	Freshwater		Amphipod *G. pulex*	Native juvenile isopods *A. aquaticus*	Single predator and various prey densities.	Presence/absence of endoparasite (*Echinorhynchus truttae*)	Type II FR identified in both parasitized and unparasitized *G. pulex*. Higher FR identified in parasitized *G. pulex*, with infected predators consuming significantly more prey overall.
(Kestrup et al., 2011)	Freshwater	Amphipod *G. fasciatus*	Amphipod *Echinogammarus ischnus*	Amphipod Juveniles of opposing species	Single predator and various prey densities.	High/low water conductivity	Higher Type II FR for natives feeding on invasive juveniles, compared to reciprocal interaction. No influence of water conductivity.
(Jones et al., 2011)	Terrestrial		Stoat *Mustela erminea*	Invasive house mouse *Mus musculus* Invasive black rat *Rattus rattus*	Fitted relationship derived from gut content analysis of *M. erminea*, and abundance data collected for *M. musculus* and *R. rattus* in New Zealand. Minimum kill rate derived from feeding activity and estimated gut-passage time, recorded in captive stoats.	Considered how feeding habits shift in response to seasonal variability in the availability of different prey types. Considered two seasonal periods: spring – summer and autumn – winter.	Type II FR estimated for both prey types. Higher stoat-mouse FR curve recorded in autumn – winter period 11% higher than spring – summer period; indicating more specialist feeding habit during warmer months. FR curve generated for stoat – rat did not establish an asymptote, indicating continued increase in predation.
(Haddaway et al., 2012)	Freshwater	White-clawed crayfish *Austropotamobius pallipes*	American signal crayfish Pacifastacus leniusculus	Native amphipod *G. pulex*	Single predator and various prey densities.	*A. pallipes* infected/not infected with *Thelohania contejeani*.	Higher Type II FR for invasive predator. Infections with *T. contejeani* reduced FR of *A. pallipes*.
(Oyugi et al., 2012)	Freshwater		Common carp *Cyprinus carpio*	Dipteran larvae (maggots) Food pellets	Multiple predators (3 fish) and various prey densities	Experimental trials carried out at four different temperature regimes (16, 20, 24 and 28°C)	Strong thermal influence on foraging. Feeding rates optimum at >20°C. Highest feeding rates achieved at 24°C.
(Dick et al., 2013)	Freshwater	Mysid *Mysis salemaai* *M. diluviana*	Mysid *Hemimysis anomala*	Various zooplankton spp.	Single predator and various prey densities	No	Higher Type II FR for invasive predator. Lab results consistent with observed field impacts.
(MacNeil et al., 2013b)	Freshwater	Amphipod *Gammarus duebeni celticus*	Amphipod *Gammarus pulex*	Invasive amphipod *Crangonyx pseudogracilis*	Single predator and various prey densities	Presence/absence of complex habitat.	Significantly higher Type II FR demonstrated by invasive *G. pulex*, regardless of presence/absence of complex habitat.
(Alexander et al., 2014)	Freshwater	Cape kurper *Sandelia capensis* River goby *Glossobius marmoratus*	Largemouth bass *Micropteras salmoides* Sharptooth catfish *Clarias gariepinus*	Native Painted reed frog larvae *Hyperolius marmoratus*	Single predator and various prey densities	No	Higher Type II FR for invasive predator.

(Barrios-O'Neill et al., 2014a)	Freshwater	Mysid *M. salemaai*	Mysid *H. anomala*	Native cladocera *Daphnia magna*	Single predator/ groups of three conspecifics. Various prey densities	Presence/ absence of higher fish predator *Gasterosteus aculeatus*	Higher Type II FR for invasive predator. Reduced FR for native, but not for invasive whilst in the presence of *G. aculeatus*.
(Barrios-O'Neill et al., 2014b)	Freshwater	Mysid *M. salemaai*	Mysid *H. anomala*	Native cladocera *D. magna*	Single predator and various prey densities	*In situ* microcosm experiment, conducting FR trials at shallow and deep field site to reflect diurnal migration pattern of both predators and prey.	Context-dependent difference in FRs between two predators. Higher Type II FR for invasive *H. anomala* during the night, and especially in shallow surface waters.
(Dodd et al., 2014)	Freshwater	Amphipod *G. pulex*	Amphipod *D. villosus*	Native isopod *A. aquaticus* Native cladoceran *D. magna* Dipteran larvae *Chironomus spp*.	Single predator and various prey densities	With/without substrate. Two body sizes for invasive *D. villosus*; body-size matched with native *G. pulex* and significantly larger.	Higher Type II FR for invasive *D. villosus*, regardless of body size, and in both presence/absence of experimental habitat. Varying invasive – native difference in FR depending on supplied prey species. Lab results consistent with observed field impacts.
(Alexander et al., 2015a)	Freshwater		Largemouth bass *Micropterus salmoides*	Non-native guppies *Poecilia reticulata*	Single predator and various prey densities	Experimental trials conducted under four different habitat complexities (high, intermediate, low and no habitat)	Type II FRs identified across all habitat complexities. As habitat became less complex, attack rates and handling times significantly declined. Handling times decreased with habitat complexity.
(Alexander et al., 2015b)	Marine	Girdled Dogwhelk *Trochia cingulata*		Native Magellan mussel *Aulacomya atra* Invasive Mediterranean mussel *Mytilus galloprovincialis* Invasive Dwarf mussel *Semimytilus algosus*	Single predator and various prey densities	No	Type II FR generated by *T. cingulate* towards native and invasive mussel species. Consumed greater numbers of both invasive mussel species (*M. galloprovincialis* and *Semimytilus algosus*), when compared to native *A. atra*.
(Barrios-O'Neill et al., 2015)	Freshwater	Amphipod *G. pulex*	Amphipod *G. d. celticus*	Invasive corophiid *Chelicorophium curvispinum*	Single predator and various prey densities	Structural complexity of habitat manipulated, altering the volume of predator-free space. Three levels of predator-free space examined; low (i.e. absent), medium, high	Similar FRs identified between invasive *G. pulex* and native *G. d. celticus*. Type II FR identified for both predator species at low level of predator-free space, transitioning to stabilising Type III as complexity of habitat increased.
(Bovy et al., 2015)	Freshwater	Amphipod *G. pulex*	Amphipod *D. villosus* *D. haemobaphes*	Dipteran larvae *Chironomus spp.* Invasive amphipod *Chelicorophium curvispinum* with/without mud tube.	Single predator and various prey densities	No	Higher Type II FR for invasive *D. villosus* on both prey species. Equivalent FR between invasive *D. haemobaphes* and native *G. pulex* when feeding on native prey, but higher FR for *D. haemobaphes* when feeding on invasive prey – regardless of presence/absence of mud tube.

(Iacarella and Ricciardi, 2015)	Freshwater		Round goby *Neogobius melanostomus*	Native amphipod *Gammarus fasciatus* Mayfly nymphs *Stenonema spp.*	Single predator and various prey densities	Two dissolved ion concentrations assessed for both prey species; high and low concentrations of Calcium (Ca^{2+}). Each prey species tested independently under two acclimation regimes: 1) *G. fasciatus* with *N. melanostomus* acclimated to high- and low-Ca^{2+} concentrations. 2) *Stenonema spp* with *N. melanostomus* unacclimated to either high- or low-Ca^{2+} concentrations.	Type II FRs for invasive *N. melanostomus*, regardless of prey species, dissolved ion concentration, and acclimatory status. Acclimated gobies and *G. fasciatus* prey: Gobies acclimated to high-Ca^{2+} levels had the highest predatory impact when exposed to high-Ca^{2+} conditions, whereas fish exposed to low-Ca^{2+} levels had similarly lower predation rates, regardless of acclimation treatment. Unacclimated gobies and *Stenonema spp.*: Non-acclimated *N. melanostomus* had higher FR on mayfly nymphs at high-Ca^{2+} levels, than at low-Ca^{2+} levels.
(Iacarella et al., 2015a)	Freshwater	Mysid *M. diluviana*	Mysid *H. anomala*	Native cladocerans *Daphnia magna*	Single predator and various prey densities	Experimental trials conducted at six temperature regimes for *H. anomala* (5, 10, 15, 20, 24 and 28ºC) and three temperature regimes for *M. diluviana* (5, 10 and 15ºC).	Type II FRs for both native and invasive mysid species, regardless of temperature regime. Strong thermal influence on predatory efficiency – highest feeding rates measured near respective thermal optima. *H. anomala* had highest maximum feeding rate at 24ºC, and the lowest at 5, 15, and 28ºC. *M. diluviana* had higher maximum feeding rate at 10ºC, and lower feeding rate at 15ºC.
(Iacarella et al., 2015b)	Freshwater		Mysid *H. anomala*	Native cladocerans *Daphnia magna*	Single predator and various prey densities	Invasive *H. anomala* tested from two geographically separate populations present in Ireland. The first, a well-established population from a previous invasion, and the second, a recently expanded population, present at the invasion front.	Type II FRs identified in invasive *H. anomala*, regardless of population. *H. anomala* belonging to the invasion front had higher FR than well-established population, demonstrating higher attack rates – suggesting heightened ability to locate and capture prey.
(Laverty et al., 2015a)	Freshwater	Amphipod *G. d. celticus*	Amphipod *G. pulex*	Dipteran larvae *Simulium spp.* Native Ephemeropteran larvae *Baetis rhodani*	Single predator and various prey densities	Two dissolved oxygen concentrations, representative of anthropogenic changes in water quality.	Higher Type II FR for invasive predator on both prey species. Reduction of FR of both predators at lower oxygen concentrations, but of lower magnitude for invasive *G. pulex*, compared to native *G. d. celticus*.
(Paterson et al., 2015)	Freshwater	Amphipod *G. duebeni celticus*	Amphipod *G. pulex*	Native isopod *A. aquaticus* Dipteran larvae *Simulium spp.*	Single predator and various prey densities	Presence/absence of endoparasites (*Echinorhynchus truttae* for *G. pulex* and	Higher Type II FR for invasive *G. pulex* on *A. aquaticus* and *Simulium spp.* Context-dependency in results with *B. rhodani* – infection decreased predation

				Native Ephemeropteran nymphs *B. rhodani*		*Pleistophora mulleri* for *G. d. celticus*). Presence/absence of higher fish predator *Salmo trutta* in fully factorial experimental design	by *G. pulex* and increased predation by *G. d. celticus*, only in the presence of *S. trutta*
(Gonçalves et al., 2016)	Freshwater		Red swamp crayfish *Procambararus clarkii*	Invasive zebra mussel *Dreissena polymorpha*	Single predator and various prey densities.	No	Type II FR identified.
(Pellan et al., 2016)	Freshwater		Amphipod *Dikerogammarus villosus* *Gammarus tigrinus*	Native cladocerans *Simocephalus exspinosus*	Single predator and various prey densities.	Experimental trials conducted at three temperature regimes (15, 20 and 25ºC)	Type II FRs identified for both invasive amphipod species, regardless of temperature regime. Increasing temperature caused an increase in attack rates by *G. tigrinus*, and a reduction in handling times in *D. villosus*.
(Rosewarne et al., 2016)	Freshwater	White-clawed crayfish *Austropotamobius pallipes*	Chinese mitten crab *Eriocheir sinensis* American signal crayfish *Pacifastacus leniusculus*	Native amphipod *Gammarus pulex*	Single predator and various prey densities.	No	Type II FRs identified for all native and invasive decapod species. Significantly higher Type II FR recorded for invasive *E. sinensis*, with maximum feeding rates 57% higher than native *A. pallipes*. No significant difference between invasive decapod species. FR demonstrated by invasive *P. leniusculus* statistically similar to native *A. pallipes*.
(Van Echelpoel et al., 2016)	Freshwater	Macrophyte *Lemna minor*	Macrophyte *Lemna minuta*	Nutrient concentrations Nitrogen Phosphorous	Fixed mass of macrophytes and various concentrations of nutrients.	No	Evidence of a decelerating increase in the rate of nutrient uptake, demonstrated by both macrophyte species, for both phosphorous and nitrogen – indicative of Type II response. No significant difference in FRs of native *L. minor* and invasive *L. minuta*.
(Wasserman et al., 2016)	Freshwater		Bluegill *Lepomis macrochirus* Southern mouthbrooder *Pseudocrenilabrus philander* Banded tilapia *Tilapia sparrmanii*	Non-native Mozambique tilapia *Oreochromis mossambicus*	Singe predator and/or pairs of predators and various prey densities	Considered potential intra- and interspecific competition by providing same prey densities to two competition treatments; two predators of same fish species, and two predators of different fish species: *L. macrochirus – P. philander* *L. macrochirus – T. sparrmanii* *P. philander – T. sparrmanii*	Type II FRs generated by all invasive fish species, regardless of presence/absence of conspecific or heterospecifics competitors. Comparisons between conspecific predator treatments revealed no difference by conspecific *L. macrochirus*, whereas there was a significant reduction in predation by conspecific *P. philander* and *T. sparrmanii*. Comparisons between heterospecifics predator treatments revealed strong reduction in predation for *P. philander* –

							T. sparrmanii and *L. macrochirus* – *T. sparrmanii* combinations, whilst *L. macrochirus* – *P. philander* combination demonstrated additive effect by both fish species.
(Xu et al., 2016a)	Terrestrial		Non-native parasitoid wasps *Eretmocerus hayati* *Encarsia sophia*	Invasive whitefly *Bemisia tabaci*	Singe predator and/or pairs of predators and various prey densities	Considered potential intra- and interspecific competition by providing same prey densities to two different competition treatments; two predators of the same parasitoid species, and two predators of different parasitoid species.	Type II FRs generated by both parasitoid species, regardless of presence/absence of conspecific or heterospecifics competitors. Potential interference between parasitoid species limited *per capita* attack rates and increased *per capita* handling times.
(Xu et al., 2016b)	Freshwater	Freshwater gastropod *Bellamya aeruginosa*	Golden apple snail *Pomacea canaliculata* Great ramshorn (Alien) *Planorbarius corneus*	Aquatic plants Water wisteria *Hydrophila difformis* Indian toothcup *Rotala indica* Water spinach *Ipomoea aquatica* Washington grass *Cabomba caroliniana*	Singe predator and various prey biomasses	No	Type II FRs generated by all native and invasive/alien gastropod species, regardless of prey type. Invasive *Po. canaliculata* had highest FR across all prey types, demonstrating highest attack rates, shortest handling times and highest maximum feeding rate. FRs demonstrated by alien *Pl. corneus* were consistently intermediate in intensity. Native *B. aeruginosa* demonstrated lowest FR across all, and did not differ significantly with *Pl. corneus*.
(Xu et al., 2016c)	Freshwater		Golden apple snail *Pomacea canaliculata*	Semi-/aquatic plants Chinese celery *Apium graveolens* Alligator weed *Alternanthera philoxeroides* Taro *Colocasia esculenta* Water hyacinth *Eichhornia crassipes* Pennywort *Hydrocotyle vulgaris* Sweet potato *Ipomoea batatas* Water spinach *Ipomoea aquatica* Lettuc *Lactuca sativa*	Singe predator and various prey biomasses	Experimental trials conducted at five temperature regimes (26, 28, 30, 32 and 34ºC)	Type II FRs generated by invasive *P. canaliculate* across all plant species, regardless of temperature regime. Strong *per capita* effect (i.e. higher attack rate and maximum feeding rate) by *P. canaliculate* towards plants with higher total nitrogen (N) content (e.g. *H. vulgaris*). Significant interaction between N and dry matter content (DMC). Variations in temperature did not significantly affect *per capita* consumption

				Parrotfeather *Myriophyllum aquaticum* Water lettuce *Pistia stratiotes*			
(Guo et al., 2017)	Freshwater	Common barbel *Barbus barbus* Chub *Squalius cephalus* Tench *Tinca tinca*	Common carp *Cyprinus carpio* Goldfish *Carassius auratus*	Dipteran larvae *Chironomus spp.* Native amphipod *Gammarus pulex*	Single predator and various prey densities.	No	Type II FRs identified for all native and invasive fish species. Higher Type II FR demonstrated by invasive *C. auratus* when compared to native *T. tinca*; significantly higher towards native *G. pulex*, yet similar towards *Chironomus spp.* larvae. Higher Type II FR demonstrated by native *T. tinca* towards both prey species, when compared to invasive *C. carpio*. FRs comparable between both fish species. Significantly higher Type II FR demonstrated by native *S. cephalus* towards both prey species, when compared to invasive *C. carpio*. Comparisons between invasive *C. carpio* and native *B. barbus* were context-dependent on prey species, with *C. carpio* exhibiting a significantly higher Type II FR towards native *G. pulex*. Native *B. barbus* demonstrated significantly higher Type II FR towards *Chironomus spp.* larvae, when compared to invasive *C. carpio*.
(Ingegno et al., 2017)	Terrestrial	Mirid bug *Dicyphus errans*		Alien arthropod pest species Poinsettia thrips *Echinothrips americanus* Greenhouse whitefly *Trialeurodes vaporariorum* Tomato borer *Tuta absoluta*	Single predator and various prey densities	No	Type II FRs identified across all prey species. Highest maximum feeding rates recorded towards *T. absoluta* (236 eggs per day), followed by *T. vaporariorum* (114 pupae per day), and *E. americanus* (62 adults per day).
(Laverty et al., 2017a)	Freshwater	European bullhead *Cottus gobio* Bitterling *Rhodeus amarus*	Round goby *Neogobius melanostomus* Asian topmouth gudgeon *Pseudorasbora parva*	Non-invasive amphipod *E. berilloni* Native isopod *A. aquaticus*	Single predator and various prey densities	No	

(Laverty et al., 2017b)	Freshwater		Amphipod *Gammarus pulex*	Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	Presence/absence of endoparasites (*Echinorhynchus truttae*). Experimental trials carried out at three temperature regimes (11, 16 and 20ºC)	Type II FRs identified, regardless of presence/absence of parasites and temperature regime. Infections associated with higher maximum feeding rates, which also increased with increasing temperature. Synergistic effect of parasitic infections and temperature.
(South et al., 2017)	Marine		Red lionfish *Pterois volitans*	Common ditch shrimp *Palaemon varians*	Single predator and various prey densities	Experimental trials conducted at two temperature regimes (22 and 26ºC), three light regimes (white, blue and red), and in the presence/absence of complex habitat (i.e. pipes/no pipes)	Type II FRs identified, regardless of temperature regime, light regime or presence/absence of complex habitat. Significantly higher Type II FR recorded at 26ºC. Type II FRs similar in presence/absence of complex habitat. Significantly higher Type II FRs recorded whilst under white and blue light – comparisons did not differ significantly between these two light regimes. Significantly higher attack rates under white light, yet no difference between handling times.
(Taylor and Dunn, 2017)	Freshwater	Amphipod *G. pulex*	Amphipod *D. villosus*	Native brown trout (eggs and larvae) *Salmo trutta* Alien ghost carp (eggs and larvae) *Cyprinus carpio*	Single predator and various prey densities	Two body sizes for invasive *D. villosus*; body-size matched with native *G. pulex* and significantly larger.	Type II FRs identified in all amphipod treatments towards embryos and larvae of *C. carpio*. Significantly higher Type II FRs by large *D. villosus* – significantly shorter handling times. No significant difference between size-matched native *G. pulex* and invasive *D. villosus* across *C. carpio* embryos and larvae. Negligible predation recorded towards *S. trutta* embryos and larvae.
(Cuthbert et al., 2018a)	Freshwater	Calanoid copepod *Lovenula raynerae*		Native mosquito larvae *Culex pipiens* complex	Single predator and various prey densities	Presence/absence of visual cues, chemical cues, or both cue types, provided by invasive mosquitofish (*Gambusia affinis*)	Type II FRs identified, regardless of presence/absence of visual cues, chemical cues, or both cue types. Cue types did not affect FR type or intensity
(Cuthbert et al., 2018b)	Freshwater	Amphipod *Gammarus duebeni celticus*		Native mayfly larvae *Baetis rhodani* Invasive amphipod *Crangonyx pseudogracilis*	Single predator and various prey densities	No	Type II FR identified for both prey types. Higher FR towards native *B. rhodani*, but not significantly different from FR for invasive *C. pseuogracilis*
(Cuthbert et al., 2018c)	Freshwater	Cyclopoid copepod *Macrocylops albidus*		Invasive mosquito larvae *Culex quinquefasciatus*	Single predator and various prey densities	Experimental trials conducted under three different temperature regimes (12, 16 and 20ºC)	Type II FRs identified across both predator species, regardless of temperature regime.

		Megacyclops viridis					Magnitude of FRs increased significantly with temperature, resulting in higher attack rates and shorter handling times. Attack rates higher in *M. albidus*, yet handling times and maximum feeding rates were similar between species, across temperature regimes.
(Cuthbert et al., 2018d)	Freshwater	Calanoid copepod *Lovenula raynerae*		Native mosquito larvae *Culex pipiens* complex	Single predator and various prey densities	Experimental trials conducted under three different water clarities: 0% = low clarity 50% = medium clarity 100% = high clarity	Type II FRs identified across all water clarities. *Per capita* predation unaffected by water clarity.
(Cuthbert et al., 2018e)	Freshwater	Cyclopoid copepod *Macrocyclops albidus* *Ma. fuscus* *Megacyclops viridis*		Invasive mosquito larvae *Culex quinquefasciatus*	Single predator and various prey densities	No	Type II FRs identified across all native copepod species. Highest Type II FR demonstrated by *Ma. albidus*, with shortest handling times and highest maximum feeding rate – indicating potential as effective biocontrol agent. *Ma. fuscus* exhibited highest attack rate.
(Dickey et al., 2018)	Freshwater		Yellow-bellied slider *Trachemys scripta scripta* Cumberland slider *T. s. troostii* Common musk turtle *Sternotherus odoratus* Eastern mud turtle *Kinosternon subrubrum*	Invasive amphipod *Gammarus pulex*	Single predator and various prey densities	No	Type II FRs identified across all predator species. Highest Type II FR demonstrated by *T. s. scripta*, followed by *T. s. troostii*, *K. subrubrum* and then *S. odoratus*. *T. s. troostii* had highest attack rates, lowest handling times and highest maximum feeding rates. *S. odoratus* had the lowest and *T. s. troostii* and *K. subrubrum* were intermediate, with *K. subrubrum* having a higher attack rate and handling times than *T. s. troostii*.
(Gebauer et al., 2018)	Freshwater		Round goby *Neogobius melanostomus* Western tubenose goby *Proterorhinus semilunaris*	Native common carp larvae *Cyprinus carpio*	Single predator and various prey densities	Experimental trials conducted at two temperature regimes (20 and 25ºC)	Type II FRs identified across both predator species, regardless of temperature regime. Marginally higher attack rates demonstrated by *P. semilunaris* at 20ºC. Handling times did not differ. Maximum feeding rate for *P. semilunaris* lower at 25ºC.
(Howard et al., 2018)	Marine	European green crab *Carcinus maenas*	European green crab *Carcinus maenas*	Native mussels *Mytilus trossulus* *M. edulis*	Single predator and various prey densities	Experimental trials conducted *in situ* across native and invaded regions	Type II FRs identified across all prey species, regardless of region.

		Native in Northern Ireland	Invasive in Canada (British Columbia and Nova Scotia) and South Africa	*M. galloprovincialis*			Attack rates, handling times and maximum feeding rates differed among regions. Higher attack rate in invaded regions. Lower handling times/higher maximum feeding rates in Canadian populations, than in South African and Northern Irish populations.
(Hoxha et al., 2018)	Marine	Ascidian *Ciona intestinalis*	Ascidian *Ciona robusta*	Algae *Isochrysis affinis galbana*	Single predator and various prey concentrations	No	Significantly higher Type I FR demonstrated by alien *C. robusta*, whereas native *C. intestinalis* exhibited Type II.
(Iacarella et al., 2018)	Freshwater		Amphipod *Gammarus pulex*	Dipteran larvae *Simulium spp*.	Single predator and various prey densities	No	Type II FR identified
(Iltis et al., 2018)	Freshwater	Amphipod *G. pulex*	Amphipod *D. villosus* (invasive) *E. berilloni* (non-invasive)	Native dipteran larvae *Chaoborus* spp.	Single predator and various prey densities	Presence/absence of endoparasite (*Cucumispora dikerogammari*) in invasive *D. villosus* only.	Type II FR identified for all predators, regardless of infection status of invasive predator. Higher FR by invasive *D. villosus*, when compared to *G. pulex* and *E. berilloni*. Infections increased invader FR, although this effect was not significant.
(Kemp and Aldridge, 2018)	Freshwater		Zebra mussel *Dreissena polymorpha* Quagga mussel *D. rostiformis*	Algae *Chlorella vulgaris*	Single predator and various prey concentrations	Experimental trials conducted at three temperature regimes (4, 12 and 24ºC)	Type I FRs identified in both species, regardless of temperature regime. No significant difference between species No difference between FRs between species at 4ºC and 12ºC. Significantly higher FR across both species between 4 and 24ºC, but not between species.
(Kemp et al., 2018)	Marine	Duck mussel *Anodonta anatina*	Gulf wedge clam *Rangia cuneata*	Algae *Nannochloropsis oculata*	Single predator and various prey concentrations	No	Type I FRs identified in both species. Significantly higher FR demonstrated by invasive *R. cuneate*.
(Médoc et al., 2018)	Freshwater	Amphipod *Gammarus pulex*	Amphipod *Dikerogammarus villosus*	Native cladocerans *Daphnia magna*	Single predator and various prey concentrations	Secondary food item (*Carpinus betulus* leaves) provided at a fixed density – corresponding to different relative densities, when compared to focal *D. magna*	Type II FRs identified across both predator species, regardless of relative abundance of alternative food type. When presented with focal prey type only, significantly higher Type II FR demonstrated by invasive *D. villosus*, when compared to native *G. pulex*. Presence of alternative food type at varying relative abundances caused invader FR to become depressed.

(Taylor and Dunn, 2018)	Freshwater	White-clawed crayfish *Austropotamobius pallipes*	Chinese mitten crab *Eriocheir sinensis* American signal crayfish *Pacifastacus leniusculus*	Native faucet snail *Bithynia tentaculate* Invasive killer shrimp *Dikerogammarus villosus* Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	No	Type II FRs identified across all predator species and for each prey species. Significantly higher Type II FR identified for *E. sinensis* towards *D. villosus* and *Chironomus spp.* larvae – predator FRs similar with respect to *B. tentaculate*. Attack rates and maximum feeding rates of *E. sinensis* up to 6.7 and 3.0 times greater than those of *A. pallipes* and *P. leniusculus*, respectively.
(Boets et al., 2019)	Freshwater	Topmouth gudeon *Pseudorasbora parva* Native to Japan	Topmouth gudeon *Pseudorasbora parva* Invasive in United Kingdom and Belgium	Native cladocerans (live prey) *Daphnia magna* Dipteran larvae (dead prey) *Chironomus spp.* larvae	Single predator and various prey densities	Experimental trials conducted using predators collected from several native and invaded regions → examined intra- and inter-continental differences between populations	Native regions: Type II FRs identified across both prey types. Invaded regions: Type II FR identified towards *Chironomus spp.* larvae, but Type III FR recorded for *D. magna*. Invasive populations consumed more *D. magna* prey when compared to native populations. No consistent trend observed between native and invasive populations whilst feeding on *Chironomus spp.* larvae.
(Britton et al., 2019)	Freshwater	Chub *Squalius cephalus* Common barbel *Barbus barbus*	Ide *Leuciscus idus*	Native Amphipod *Gammarus pulex* Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	No	Type II FRs identified across all predator species and for each prey species. Significantly higher attack rates maximum feeding rates demonstrated by *L. idus* towards both prey types, when compared to *B. barbus* → overlap at higher densities. Marginally higher Type II FR demonstrated by *S. cephalus* towards *G. pulex*, when compared to *L. idus* → overlap throughout. Consistent overlap between *S. cephalus* and *L. idus* when feeding on *Chironomus spp.* larvae.
(Bunke et al., 2019)	Freshwater	Amphipod *Gammarus duebeni celticus*	Amphipod *Gammarus pulex*	Conspecific juvenile amphipods (i.e. cannibalism experiment) or heterospecifics juvenile amphipods (i.e. intraguild predation)	Single predator and various prey densities	Presence/absence of endoparasite (*Pleistophora mulleri*; *G. d. celticus*) (*Echinorhynchus truttae*; *G. pulex*)	Type II FRs identified across both predator species, regardless of presence/absence of parasites, and the type of prey provided. Presence of *P. mulleri* infections caused an increase in IGP and cannibalism by *G. d. celticus*, with increased attack rates and reduced prey handling times. Presence of *E. truttae* infections did not alter IGP or cannibalism by *G. pulex*

(Crookes et al., 2019)	Terrestrial	Convergent ladybird *Hippodamia convergens*	Harlequin ladybird *Harmonia axyridis*	Native pea aphid *Acyrthosiphon pisum*	Single predator and various prey densities	No	Significantly higher Type II FR identified in invasive *H. axyridis*, when compared to native *H. convergens*.
(Cuthbert et al., 2019b)	Freshwater		Yellow-bellied slider *Trachemys scripta scripta* Cumberland slider *T. s. troostii* Common musk turtle *Sternotherus odoratus* Eastern mud turtle *Kinosternon subrubrum*	Invasive amphipod *Gammarus pulex* Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	Presence/absence of substrate	Type II FRs identified across all predator species and for each prey species. FRs typically higher for *T. s. scripta* and *T. s. troostii* when compared to *S. odoratus* and *K. subrubrum*. Modest reduction in magnitude of FRs in presence of substrate →most pronounced for *T. s. scripta* and *T. s. troostii*..
(Cuthbert et al., 2019c)	Freshwater	Calanoid copepod *Lovenula raynerae*		Native mosquito larvae *Culex pipiens* complex	Single predator and various prey densities	Three treatments of copepod predator tested – male, non-gravid female and gravid female	Type II FRs identified across all predator treatments. Significantly higher Type II FR demonstrated by gravid females – greater resource demand for offspring development. FRs by males and non-gravid females of similar magnitude.
(Cuthbert et al., 2019d)	Freshwater	Calanoid copepod *Lovenula raynerae* *Paradiaptomus lamellatus*		Native mosquito larvae *Culex pipiens* complex	Single of interspecific pairs of predators and various prey densities	Experimental trials conducted at two water depths (40 and 80ml)	Type II FRs identified across all predator treatments (single/interspecific pairs), regardless of depth. At shallow depths, *L. raynerae* demonstrated superior FR, when compared to *P. lamellatus*; with FRs diverging at intermediate prey densities. At greater depths, FRs similar between predator species, although predation by *L. raynerae* tended to be higher still. Regardless of depth, interspecific pairings appeared to demonstrate additive effect.
(Cuthbert et al., 2019e)	Freshwater	Cyclopoid copepod *Macrocylops albidus* *Ma. fuscus* *Megacyclops viridis*		Native mosquito larvae *Culex pipiens* complex Invasive mosquito larvae – Asian tiger mosquito *Aedes albopictus*	Single predator and single prey species at various densities	No	Type II FRs identified across all predator species and for each prey species. Higher Type II FR demonstrated by each predator species, towards invasive *A. albopictus*. *Ma. fuscus* consumed significantly greater numbers of prey overall, when compared to *Ma. albidus* and *Me. viridis*.
(Cuthbert et al., 2019f)	Freshwater	Notonectid *Anisops debilis*		Native mosquito larvae *Culex pipiens* complex	Single predator and various prey densities	Experimental trials conducted under three treatments of habitat	Type II FRs identified regardless of habitat complexity.

						complexity (low, medium and high)	FRs highest in low complexity treatment, driven by significantly higher attack rates, marginally lower handling times/higher maximum feeding rates. Attack rates decreased as habitat complexity increased. Handling times did not differ significantly.
(Cuthbert et al., 2019g)	Freshwater	Calanoid copepod *Lovenula raynerae*		Native mosquito larvae *Culex pipiens* complex	Single predator and various prey densities	Experimental trials conducted under three temperature regimes (13, 18 and 28ºC), and three salinity regimes (0.2, 4, 8 parts per trillion; ppt).	Type II FRs identified regardless of both temperature and salinity regime. Predation rates were generally higher as temperature increased, whereas increasing salinity reduced consumption. Interactions between environmental conditions: temperature effects were suppressed at higher salinities.
(Dalal et al., 2019)	Freshwater	Notonectid *Notonecta glauca*	Amphipod *Gammarus pulex*	Native mosquito larvae *Culex pipiens* complex	Single or intraspecific pairs of predators and various prey densities	*C. pipiens* larvae were offered at four instar stages (first, second, third or fourth larval instar), each varying in body size.	FR Types varied between predator species, predator treatment (i.e. single or pair) and the larval stage of prey. *N. glauca* generally exhibited Type II FRs, as singletons and whilst in pairs, across most larval prey stages; except for first larval instar prey in which a Type I FR was observed. Intraspecific pairs of *N. glauca* showed an additive effect on the consumption of second instar larvae, whilst no effect was observed towards first or third larval instars. Intraspecific predation of fourth instar larvae, showed potential reduction, resulting from antagonistic interactions. *G. pulex* generally exhibited Type III FR, as singletons and whilst in pairs, across most larval prey stages; except for fourth larval instars (single predator) and third larval instars (intraspecific pairs) where Type I FRs were recorded. Intraspecific pairs of *G. pulex* generally showed reduction in consumption of larval prey, across all larval instars. For second larval instars, evidence of antagonistic effects between predators was more apparent.
(Faria et al., 2019)	Freshwater	South American silver catfish	Channel catfish *Ictalurus punctatus*	Native blue tetra	Single predator and various prey densities	No	Higher Type II FR demonstrated by alien *I. punctatus*, with lower handling

		Rhamdia quelen		*Mimagoniates microlepis*			times/higher maximum feeding rates, when compared to native *R. quelen*.
(Fincham et al., 2019)	Terrestrial	Coccinellid Seven-spot ladybird *Coccinella septempunctata* Two-spot ladybird *Adalia bipunctata*	Coccinellid Harlequin ladybird *Harmonia axyridis*	Native grain amphid *Sitobion avenae*	Single predator and various prey densities	Presence/absence of entomopathogenic fungus *Beauveria bassiana* Compared unparasitized and parasitized adult and larval Coccinellid life-stages.	Type II FR identified for all predators, regardless of life-stage or infection status. Uninfected invasive *H. axyridis* displayed higher Type II FR, with larvae and adults consuming more prey overall. Context-dependency in results for infection status – reduced feeding efficiency of adult *H. axyridis* and *A. bipunctata*, yet increased efficiency of adult *C. septumpunctata*, and larval *H. axyridis* and *A. bipunctata*.
(Gebauer et al., 2019)	Freshwater		Round goby *Neogobius melanostomus* Western tubenose goby *Proterorhinus semilunaris*	Native common carp larvae *Cyprinus carpio*	Single predator and various prey densities	Experimental trials conducted under three habitat structures (sand, coarse gravel and coarse gravel with artificial plant).	Type II FRs identified for both predators, regardless of habitat structure. *Neogobius melanostomus* demonstrated significantly higher attack rates in sandy habitat. Handling times similar between predator species whilst in gravel and/or gravel with artificial plant habitats → similar *per capita* impact. Both predators demonstrated significantly lower handling times in sandy habitat.
(Hoxha et al., 2019)	Terrestrial	Gastropod White-lip globe *Mesodon thyroidus*	Gastropod Brown-lipped snail *Cepaea nemoralis*	Non-native flower Dandelion *Taraxacum officinale*	Single predator and various prey densities	No	Significantly higher Type II FR demonstrated by native *M. thyroidus*
(Joyce et al., 2019)	Marine	European green crab *Carcinus maenas* Common sea star *Asterias rubens*		Native blue mussel *Mytilus edulis* Invasive Pacific oyster *Crassostrea gigas*	Single predator and various prey densities	No	Native *A. rubens* demonstrated Type II FRs towards both native and invasive prey types, whereas *C. maenas* exhibited Type III FR towards both prey types. Both predators demonstrated significantly higher attack rates and maximum feeding rates towards native *M. edulis* when compared to invasive *C. gigas*.
(Mofu et al., 2019a)	Freshwater	River goby *Glossogobius callidus*	Mozambique tilapia *Oreochromis mossambicus* Western mosquitofish *Gambusia affinis*	Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	Experimental trials conducted under two temperature regimes (18 and 25°C).	Type II FRs identified across all predator species, regardless of temperature regime. Maximum feeding rates were consistently higher at 25°C, when

							compared to 18°C, whereas attack rates tended to be reduced. Non-native *O. mossambicus* had highest overall FR under both temperature regimes, followed by non-native *G. affiinis*, and finally native *G. callidus*.
(Mofu et al., 2019b)	Freshwater	River goby *Glossogobius callidus*	Western mosquitofish *Gambusia affinis*	Dipteran larvae *Chironomus spp.*	Singe predator and/or pairs of heterospecific predators and various prey densities	No	Type II FRs identified across both predator species, when present individually and in heterospecific pairs. Invasive *G. affinis* demonstrated significiantly higher FR in single predator trials. FR of native *G. callidus* was reduced in heterospecific pairings, whereas FR for invasive *G. affinis* was greatly enhanced.
(Mu et al., 2019)	Freshwater		Golden apple snail *Pomacea canaliculata*	Semi-/aquatic plants Alligator weed *Alternanthera philoxeroides* Water hyacinth *Eichhornia crassipes* Sweet potato *Ipomoea batatas* Parrot feather *Myriophyllum aquaticum* Water lettuce *Pistia stratiotes* Celery *Apium graveolens* Taro *Colocasia esculenta* Water spinach *Ipomoea aquatica* Pennywort *Hydrocotyle vulgaris* Lettuce *Lactuca sativa*	Single predator and various prey densities	First five plant species are evolutionarily novel, whilst the last five species are evolutionarily familiar. Experimental trials conducted under five temperature regimes (26, 28, 30, 32 and 34°C).	Type II FRs identified across all plant species, regardless of temperature regime. Invasive *P. canaliculate* demonstrated markedly higher Type II FR towards evolutionarily novel plant species. Feeding efficiencies were higher on evolutionarily familiar species which were more closely related to novel plant species. At higher temperatures, maximum feeding rates towards evolutionarily familiar plant species increased.
(Paton et al., 2019)	Freshwater	Northern logperch *Percina caprodes*	Round goby *Neogobius melanostomus*	Non-native amphipods *Hyalella azteca*	Single predator and various prey densities	Compared FRs of invasive *N. melanostomus*, sampled from high- and low-density sites. Compared FRs of invasive *N. melanostomus* sampled from low-density populations situated at the invasion front, and from a low-density established population.	Type II FRs identified across both native and invasive predator species, regardless of invader population. Generally higher Type II FR identified in invasive *N. melanostomus*, sampled from high-density populations → exhibiting significantly higher attack rates when compared to low-density populations.

						Compared FR of invasive *N. melanostomus*, sampled from the low-density invasion front, and the native logperch	FRs did not significantly differ between invasive *N. melanostomus*, sampled from the invasion front or from low-density established population. Invasive *N. melanostomus* demonstrated significantly higher Type II FR, when compared to native *P. caprodes*, with invaders exhibiting significantly lower handling times.
(South et al., 2019)	Freshwater		Red swamp crayfish *Procambarus clarkii*	Dipteran larvae *Chironomus spp.* Native cladocera *D. magna*	Single predator and various prey densities	Full factorial experimental design conducted under different types of substrate; no substrate (i.e. control), sand, and gravel.	Type II FR identified, regardless of prey species and substrate type. No difference in consumption of dipteran larvae under different substrate types. Consumption of cladocerans differed significantly under different substrate types; highest in absence of substrate and decreasing independently with increasing substrate complexity. Intermediate predation with sand, and low predation with gravel.
(Cuthbert et al., 2020b)	Freshwater	Notonectid *Anisops debilis*		Native mosquito larvae *Culex pipiens* complex Native cladocerans *Daphnia pulex*	Single predator and various densities of both the focal (*C. pipiens* larvae) and alternative (*D. pulex*) prey types, offered concurrently.	No	Type II FRs identified towards *C. pipiens* larvae, regardless of relative abundance of alternative *D. pulex* prey. Increasing the density of *D. pulex* significantly reduced the predatory impact of *A. debilis* towards *C. pipiens* larvae, characterised by lower attack rates, longer handling times and an overall decrease in maximum feeding rates.
(Cuthbert et al., 2020c)	Freshwater	Calanoid copepod *Paradiaptomus lamellatus*		Native mosquito larvae *Culex pipiens* complex	Singe predator and/or pairs of predators and various prey densities	No	Type II FRs identified for both single and paired predator treatments. Type II FR was higher for individual predators, whereas pairs exhibited an antagonistic reduction in *per capita* predation.
(Cuthbert et al., 2020d)	Freshwater		Bluegill *Lepomis macrochirus* Largemouth bass *Micropterus salmoides*	Non-native Mozambique tilapia *Oreochromis mossambicus*	Single predator and various prey densities	Experimental trials were conducted using three different size classes of predators and prey (small, medium and large).	Type II FRs were identified across both predator species, regardless of predator and/or prey size. *M. salmoides* a more effective predator when small or intermediate in size, when compared to *L. macrochirus* → No difference in *per capita* predation between large predators. Small prey most vulnerable overall, although differential attack rates among

							prey were emergent across predator sizes. Small *L. macrochirus* and *M. salmoides* exhibited higher attack rates towards small and intermediate prey, whilst larger predators exhibited greater attack rates towards large prey. Handling times increased with prey size, with small *L. macrochirus* exhibiting low feeding rates towards intermediate/large prey.
(Cuthbert et al., 2020e)	Freshwater	Calanoid copepod *Lovenula raynerae*		Native mosquito larvae *Culex pipiens* complex	Single and multiple predator treatments provided with prey at various densities	Experimental trials conducted using male and female copepods, under five predator treatments (1 male, 1 female, 2 females, 2 males, 1 male and 1 female)	Type II FRs identified across all predator treatments, with female copepods identified as being significantly more voracious than males when placed in multiple predator groups. Multiple predator effect was significantly negative, indicating a reduction in predation risk. Antagonistic interactions differed amongst predator-predator combinations and at different prey densities. Antagonism between females was prevalent at low prey densities, whereas antagonism amongst males was greater at higher prey densities.
(Cuthbert et al., 2020f)	Freshwater	Cyclopoid copepod (intermediate predator) *Macrocyclops albidus* Dipteran larvae (higher predator) *Chaoborus flavicans*		Native mosquito larvae *Culex pipiens* complex	Single and multiple predator treatments (three *M. albidus* or three *M. albidus* and one *C. flavicans*), provided with prey at various densities	No	Type II FRs identified across both predator species, as well as across both multiple predator treatments. Individually, *C. flavicans* consumed significantly more prey than *M. albidus* → significantly higher Type II FR. Overall, consumption of *C. pipiens* larvae increased with predator density (three *M. albidus*) and with richness (three *M. albidus* and one *C. flavicans*). Antagonistic and synergistic multiple predator effects between conspecific *M. albidus* not detected. Presence of *C. flavicans* did not effect intermediate predators → evidence of additive effect.
(Dalal et al., 2020)	Freshwater		Amphipod *Gammarus pulex*	Dipteran larvae *Chironomus spp.*	Single predator and various prey densities	Experimental trials conducted using adult female *G. pulex* across three states of reproduction (non-ovigerous,	Type II FR identified regardless of reproductive state of female amphipods. Attack rates highest in ovigerous females with immature-stage embryonic

						ovigerous with immature- or mature-stage embryonic broods)	broods, and significantly lower in females carrying mature-stage broods. Handling times were consistently lower, and hence maximum feeding rates higher, in brood-carrying females, compared to non-ovigerous females.
(DeRoy et al., 2020a)	Marine	Red grouper *Epinephelus morio* Graysby grouper *Cephalopholis cruentata*	Red lionfish *Pterois volitans*	Native pink shrimp *Penaeus duorarum*	Single predator and various prey densities	Experimental trials conducted with predators/prey having simultaneous access to heterogenous habitat patches (low, medium and high complexity).	Type II FRs identified across all predator species. FR and overall consumption rate of invasive *P. volitans* was intermediate when compared to native *E. morio* (higher), and *C. cruentata* (lower). *Pterois volitans* had highest attack rate, regardless of habitat type.
(DeRoy et al., 2020b)	Marine		Red lionfish *Pterois volitans*	Native pink shrimp *Penaeus duorarum*	Single predator and multiple predator (i.e. 2 or 4 predators) provided with various prey densities.	Experimental trials conducted with predators/prey having simultaneous access to heterogenous habitat patches (low, medium and high complexity).	Type II FRs identified across all predator treatments. Paired *P. volitans* had greatest *per capita* effect, with predation found to be additive. At highest predator density (four fish) antagonistic multiple predator effects caused greatest reduction in predation risk, resulting in lowest *per capita* effect. Heterogeneity of habitat did not have a significant effect on *per capita* predation
(Grimm et al., 2020)	Freshwater			Native amphipods *Gammarus pulex* (UK) *Gammarus fasciatus* (North America)			
(Haubrock et al., 2020)	Terrestrial		Mourning gecko *Lepidodactylus lugubris*	Native bean weevil *Acanthoscelides obtectus*	Single predator and various prey densities	Comparison between juvenile and adult predators. Experimental trials conducted under three temperature regimes (20, 23 and 26ºC).	Type II FRs identified, regardless of predator life-stage and temperature regime. Juvenile FR positive affected by temperature, with increased attack rates at higher temperatures. Handling times were shorter at higher temperatures, recorded in both juvenile and adult predators.
(Joyce et al., 2020)	Marine	European green crab *Carcinus maenas*		Native blue mussel *Mytilus edulis* Invasive Pacific oyster *Crassostrea gigas*	Single predator and various prey densities	Experimental trials conducted under two seasonal regimes (summer and winter) and under two levels of hydrological disturbance (high and low)	Type II FRs generally identified across both prey types, seasonal regimes and hydrological disturbance regimes; except towards invasive *C. gigas* and native *M. edulis* within the summer and low disturbance treatment (Type III),

							and native *M. edulis* within the winter and high disturbance treatment (Type III). Maximum feeding rates typically higher towards native *M. edulis*, when compared to invasive *C. gigas*. Handling times were lower during summer, when compared to winter. Hydrological disturbance level had no significant effect on predator handling times or maximum feeding rates.
(Khosa et al., 2020)	Freshwater		Largemouth bass *Micropterus salmoides* Florida bass *Micropterus floridanus*	Mosquito larvae *Culex spp.*	Single predator and various prey densities	Experimental trials conducted under three temperature regimes (18, 24 and 30ºC)	Type II FRs identified across both predator species, regardless of temperature regime. Handling times recorded in *M. salmoides* increased significantly with temperature, whereas for *M. floridanus* handling times decreased significantly. *Micropterus salmoides* demonstrated significantly higher maximum feeding rates at 18ºC. At 30ºC maximum feeding rates recorded in *M. floridanus* were significantly higher. No significant difference between predator species at 24ºC.
(Linzmaier and Jeschke, 2020)	Freshwater		Marbled crayfish (males only) *Procambarus virginalis* Spiny-cheek crayfish (male and female) *Faxonius limosus*	Invasive quagga mussel *Dreissena rostriformis* Invasive zebra mussel *D. polymorpha*	Single predator and various prey densities – comprising of four size-classes	Examined functional responses using behavioural assays, comparing activity, aggressiveness and boldness	Consistently higher Type II FR identified for male and female *F. limosus*, when compared to *P. virginalis*. Levels of activity, recorded for *F. limosus* and *P. virginalis*, did not significantly affect *per capita* consumption. For *P. virginalis*, increased aggression resulted in a higher overall Type II FR, caused by higher handling times, whereas FRs for *F. limosus* did not vary. For *F. limosus*, increased boldness translated into a higher Type II FR, resulting from significantly lower handling times, whereas FRs for *P. virginalis* did not differ.
(Mbedzi et al., 2020)	Freshwater		Banded tilapia (introduced) *Tilapia sparrmanii*	Microplastics (polyethylene)	Single predator and various prey densities	No	Type II FR identified
(Otturi et al., 2020)	Freshwater	Creole perch *Percichthys trucha*	Rainbow trout *Oncorhynchus mykiss*	Ephemeroptera	Single predator and various prey densities	Measured FR for native and invasive predators in mono-	Similar Type II FRs identified in both species in monospecific trials.

				Meridialaris chiloeensis nymphs		specific and multi-specific (paired) trials.	Invasive *O. mykiss* dominant in multispecific trials, causing reduction in *per capita* feeding in native *P. trucha*.
(Wilber et al., 2020)	Terrestrial		North American wild pigs *Sus scrofa*	Standard Crop Types Cereals Fruits and nuts Sugar Oilseeds Beverages and spices Roots and tubers Vegetables and melons Legumes Tobacco Other (cotton)	Used GPS data for individual predators, measuring crop usages and visitation rates, versus crop availability (i.e. FR).	Measured FR in relation to availability of non-agricultural resources. Also examined effect of sex, crop type.	Type III FR identified towards crop types – highly context dependent. Reduction in FR magnitude when non-agricultural resources were available – impact likely to be highly heterogenous. Significant effect of crop type and sex, with males spending 20% more time, and visiting crops 58% more often than females. Both males and females demonstrated different FRs depending on crop type.
(Xia et al., 2020)	Freshwater		Golden mussel *Limnoperna fortunei*	Algae *Chlorella vulgaris*	Single predator and various prey densities	No	Type I FR identified

Appendix 2

Table A2.1. List of reactants and their quantities used to make 1 x Modified Barth's Saline (MBS) solution (pH 7.8) up to a volume of 1 L.

Reagent	**Quantity (g)**	**Final Concentration (mM)**
NaCl	5.143	88
KCl	0.075	1
$MgSO_4$	0.120	1
HEPES	1.192	5
$NaHCO_3$	0.210	2.5
$CaCl_2$, dihydrate	0.103	0.7
Deionised Water	to 1 L	

Note: Adjust pH of final solution to 7.8 by adding 10 M of NaOH and autoclave solution to sterilise.
[Accessed from: http://cshprotocols.cshlp.org/content/2009/9/pdb.rec11946.full?text_only=true]

Table A2.2. List of freshwater field sites situated within West Yorkshire which were sampled for freshly deposited native *Rana temporaria* embryos.

Field Site	**Coordinates**
Nell Bank Environmental Study Centre, Ilkley	lat 53°56'00.2"N, long 1°48'26.0"W
Meanwood Park, Leeds	lat 53°50'23.0"N, long 1°34'34.8"W
Middleton Park, Leeds	lat 53°45'15.4"N, long 1°32'49.0"W
Farnley Hall Fishpond Local Nature Reserve, Leeds	lat 53°47'17.3"N, long 1°37'23.2"W

Table A2.3. The mean (± SEM) length and weight (i.e. body size) of amphipod groups used in each experimental system. Prior to experimentation, live amphipods were blotted dry before weight (mg) was measured; lengths (mm), taken from the tip of the rostrum to the base of the urosome for amphipods in curved, resting states, were measured from digital photographs. Across both experimental systems, 'large' *D. villosus* were significantly larger and heavier than both 'intermediate' *D. villosus* and 'large' *G. pulex*, which in turn did not significantly differ in size.

Prey Treatment	**Body parameter**	**'Large' *D. villosus***		**'Intermediate' *D. villosus***		**'Large' *G. pulex***	
		Mean	SE	Mean	SE	Mean	SE
Xenopus laevis embryos	Length	22.04	0.29	14.36	0.12	14.36	0.13
	Weight	165.03	3.21	44.31	1.09	46.49	1.34
Rana temporaria embryos	Length	23.85	0.23	17.49	0.17	16.44	0.15
	Weight	146.78	4.10	62.18	1.29	62.67	1.26
Rana temporaria larvae	Length	23.97	0.13	17.32	0.12	17.23	0.11
	Weight	137.41	2.34	61.01	0.88	60.12	0.74

Note: Length and weight data recorded for each amphipod group was compiled into a single dataset for all three experimental treatments (i.e. *X. laevis* embryos, *R. temporaria* embryos and *R. temporaria* larvae). Length and weight data was analysed using a non-parametric Kruskal-Wallis test, due to non-normal residuals, even following log-transformation. Pairwise *post-hoc* comparisons were conducted using the non-parametric Dunn's test, with Bonferroni adjusted p-values (*PMCMRplus::kwAllPairsDunnTest*, version 1.4.2; Pohlert, 2015). Non-parametric analysis indicated a statistically significant difference in the length ($\chi^2 = 293.60$, df = 2, $p < 0.001$) and weight ($\chi^2 = 293.02$, df = 2, $p < 0.001$) of the three amphipod size groups. *Post-hoc* tests confirmed that across all experimental treatments, large *D. villosus* were significantly longer and heavier than both intermediate *D. villosus* and *G. pulex* (Dunn test adjusted $p < 0.001$ for both tests). *Gammarus pulex* and intermediate *D. villosus* did not differ in length or weight (Dunn test adjusted $p > 0.05$ for both tests).

Table A2.4. Length (+ SEM) and approximate developmental stage of amphibian embryos and larvae used in experiments. n = 30, except for *R. temporaria* larvae n = 27. As amphibian embryos develop, vitelline jelly thickness varies (Roberts, 1951). Therefore, only embryos with complete vitelline membranes that were robust to manipulation were used to standardise prey life-stages. Similarly, throughout larval development, larval swimming capability also alters (Van Buskirk and McCollum, 2000). As such, only larvae between 12 h and 24 h post-hatching were selected.

Prey Type	**Stage**	**Length (mm)**	**SE**
X. laevis embryos	NF 10*	2.19	0.02
R. temporaria embryos	G 10**	7.83	0.16
R. temporaria larvae	G 20**	14.82	0.31

Length of embryos is diameter, including the vitelline jelly capsule. Length of larvae is taken from the anterior tip of the head/body to the posterior tip of the tail.

R. temporaria larvae were measured after killing in 70% ethanol

Note: NF = Nieuwkoop and Faber Stage (Nieuwkoop and Faber, 1994), G = Gosner Stage (Gosner, 1960).

Table A2.5 – List of British field sites sampled to obtain estimates of population abundance for native *Gammarus pulex* and invasive *Dikerogammarus villosus*. These sites predominantly comprised of rocky substrate, which is frequently favoured by *D. villosus* and *G. pulex* as suitable habitat (Elliott, 2005; MacNeil et al., 2010).

Amphipod Species	Field Site	County	Coordinates
G. pulex	Golden Acres Park	West Yorkshire	lat 53°52’07.3" N, long 1°35’19.1" W
	Adel Woods	West Yorkshire	lat 53°51’33.0" N, long 1°34’43.9" W
	Meanwood Valley Trail	West Yorkshire	lat 53°50'36.9" N, long 1°34'11.1" W
	Meanwood Park (Upper)	West Yorkshire	lat 53°50'23.8" N, long 1°34'35.3" W
	Meanwood Park (Lower)	West Yorkshire	lat 53°49'49.2" N, long 1°34'31.3" W
D. villosus	Valley Creek	Cambridgeshire	lat 52°17'26.8" N, long 0°19'43.7" W
	Grafham Water Fishing Lodge	Cambridgeshire	lat 52°17'28.8" N, long 0°19'28.1" W
	Gaynes Cove	Cambridgeshire	lat 52°17'07.3" N, long 0°17'44.0" W
	Grafham Water Visitors Centre	Cambridgeshire	lat 52°17'51.1" N, long 0°17'34.6" W
	Hedge End	Cambridgeshire	lat 52°18'18.2" N, long 0°18'15.6" W
	Hill Farm Bay	Cambridgeshire	lat 52°18'36.9" N, long 0°19'08.0" W

Table A2.6. Results of logistic regression of the proportion of prey consumed in each prey density treatment for each amphipod size and prey treatment. Analyses were conducted using a quasi-binomial error structure. A statistically significant negative first order term is indicative of a Type II FR (Juliano, 2001).

Prey treatment	Amphipod group	First Order Term – Estimate (±SE)	z	P
X. laevis embryos	'Intermediate' *D. villosus*	-0.041 (±0.004)	-8.547	< 0.001***
	'Large' *D. villosus*	-0.039 (±0.004)	-9.634	< 0.001***
R. temporaria larvae	'Large' *G. pulex*	-0.143 (±0.059)	-2.428	0.018*
	'Intermediate' *D. villosus*	-0.121 (±0.044)	-2.727	< 0.01**
	'Large' *D. villosus*	-0.112 (±0.028)	-3.967	< 0.001***

Asterisks indicate significance of P values; * = P < 0.05, ** = P < 0.01, and *** = P < 0.001.

Appendix 3

Table A3.1. List of freshwater field sites located in West Yorkshire sampled for native *Rana temporaria* embryos.

Field Site	**Coordinates**
Adel Woods, Leeds	lat 53°51'14.8"N, long 1°34'11.5"W
Meanwood Park, Leeds	lat 53°50'23.0"N, long 1°34'34.7"W
Lofthouse Colliery Nature Park, Leeds	lat 53°43'07.9"N, long 1°30'23.1"W
Shibden Park, Halifax	lat 53°43'37.3"N, long 1°50'27.2"W
Timble Ings, Otley	lat 53°58'16.8"N, long 1°45'45.5"W
Rodley Nature Park, Leeds	lat 53°49'27.3"N, long 1°38'49.0"W

Appendix 4

Table A4.1. List of freshwater field sites located in West Yorkshire sampled for native *Rana temporaria* embryos.

Field Site	**Coordinates**
Danefield Pond – Otley Chevin (W. Yorkshire)	lat 53°53'38.8"N, long 1°38'56.8"W
Yorkgate Quarry Pond – Otley Chevin (W. Yorkshire)	lat 53°53'48.4"N, long 1°40'20.8"W
Lofthouse Colliery Nature Park (W. Yorkshire)	lat 53°43'07.9"N, long 1°30'23.1"W
Shibden Park (W. Yorkshire)	lat 53°43'37.3"N, long 1°50'27.2"W
Timble Ings (N. Yorkshire)	lat 53°58'16.8"N, long 1°45'45.5"W
Cromwell Bottom Nature Reserve (W. Yorkshire)	lat 53°41'47.0"N, long 1°48'52.2"W

A4.2 – Description of the supplementary Type II van Leeuwen MSFR model, and the model fitting procedure applied to consumption data, recorded for invasive *D. villosus* when supplied with two experimental prey combinations (i.e. *Rana temporaria* larvae – *Asellus aquaticus*; *R. temporaria* larvae – *Chironomus* sp. larvae) at varying relative abundances (see Chapter 4; Section 4.3.3.2 for experimental methodology).

As stated in the main text above (see Section 4.3.3.2), alternative Type II MSFR models – referred to herein as the van Leeuwen MSFR models – were also applied to data pertaining to each experimental combination of prey species (*R. temporaria* larvae – *Chironomus* sp. larvae; *R. temporaria* larvae – *A. aquaticus*). As with the other MSFR model variants used, the van Leeuwen MSFR model incorporates predation of multiple prey species, providing the opportunity for prey-switching behaviours. However, unlike the other MSFR model variants, the van Leeuwen MSFR model explicitly accounts for prey-switching with the inclusion of an additional, quantifiable parameter:

$$f_i(N_i, N_j) = c_i N_i \sum_{j=1}^{n} s_{ij} c_j N_j \Bigg/ \sum_{j=1}^{n} c_j N_j \left(1 + \sum_{j=1}^{n} s_{ij} h_j c_j N_j\right)$$

With all notations as species-specific and n denoting the number of prey species consumed, N is initial prey density, c is the predator's base attack rate, h is the predator's handling time, and s_{ij} describes the similarity between prey species i and j. Prey-switching is based upon the behavioural assumption that predators tend to continue feeding on prey that are similar to previously consumed prey, whether it be similarities in morphology, behaviour, location, or habitat choice (van Leeuwen et al., 2013). For example, an $s_{ij} = 0.1$ suggests that a predator that has attacked prey species i is 10 times more likely to attack prey species i again than a predator that has attacked prey species j is to attack prey species i, and vice versa. If $s_{ij} = 1$, two prey species are deemed to be equivalent from the perspective of the predator, with no prey-switching taking place. If $s_{ij} > 1$, then a predator is expected to demonstrate negative prey-switching behaviours; consuming disproportionately less of the more abundant prey type (van Leeuwen et al., 2013). When $s_{ij} \geq 0$, base attack rate (c) becomes equivalent to classic attack rate (a), with $c_i s_{ij} \approx a_i$.

To explore the effects of prey-switching behaviours, in response to similarities amongst prey types and the relative abundance of each prey type, several MSFR models were fitted for each prey combination, using the van Leeuwen approach. The first model considered the probability of predation towards each prey type to be equal, by fixing s_{ij} = 1 (i.e. no prey-switching). The second MSFR model included s_{ij} as a free parameter to be estimated. As with the previous MSFR model variants, the van Leeuwen MSFR models were numerically solved using ODEs, thus integrating for prey depletion over experimental time. Best-fit parameter estimations were obtained via maximum likelihood estimations, and 95% confidence intervals were generated via non-parametric bootstrapping (n = 5000). Nested models (i.e. s_{ij} = 1) were compared to full models (i.e. estimated s_{ij}) via AICc.

Whilst the van Leeuwen MSFR model can be adapted to consider Type III responses, a primary assumption surrounding this modification is that the abundance of one prey type remains constant (see van Leeuwen, Jansen and Bright, 2007; van Leeuwen *et al.*, 2013). Given that the densities of prey types were experimentally varied across both prey combinations, analyses utilising the van Leeuwen MSFR model focussed solely upon examining Type II responses.

A4.3 – Results and outputs of supplementary Type II van Leeuwen MSFR models, fitted to consumption data recorded for invasive *D. villosus* when supplied with two experimental prey combinations (i.e. *Rana temporaria* larvae – *Asellus aquaticus*; *R. temporaria* larvae – *Chironomus* sp. larvae) at varying relative abundances (see Chapter 4; Section 4.3.3.2 for experimental methodology).

The van Leeuwen MSFR model variant, fitted with the similarity index (s_{ij}) provided as a free parameter to be estimated, suggested possible negative prey-switching behaviours demonstrated by *D. villosus* (s_{ij} > 1; see van Leeuwen *et al.*, 2013); with amphipods predicted to exhibit higher than expected proportional consumption of *A. aquaticus* (s_{ij} = 4.881) and *Chironomus* sp. larvae (s_{ij} = 2.258), whilst present as the least abundant prey types (Table S4.2). Compared to the nested models, whereby s_{ij} was fixed at 1 (i.e. no prey-switching), the *R. temporaria* larvae – *Chironomus* sp. larvae MSFR model performed better when s_{ij} was provided as a free parameter (AICc = 355.170 versus 350.564), whereas the *R. temporaria* larvae – *A. aquaticus* MSFR model did not differ statistically (AICc = 345.191 versus 345.211). This would suggest that, whilst estimates of s_{ij} suggest potential negative prey-switching, switching may actually have a minimal effect. However, when considered in conjunction with evidence of strong preferential feeding towards alternative macroinvertebrate prey types, regardless of proportional availability (see Table 4.2 – 4.3; Figure 4.2 – 4.3), it may be unlikely that estimated s_{ij} accurately represent predatory feeding preferences. Instead, the s_{ij} parameter(s) are likely to be overestimated, probably as a consequence of limited data for the number of FR parameters being estimated (Zhang et al., 2018; Novak and Stouffer, 2020).

When compared to the alternative Type III MSFR variant, the van Leeuwen MSFR models performed considerably better (Table S4.2 and 4.4). As with the Murdoch Type II MSFR model variant, the van Leeuwen MSFR models successfully demonstrated considerable differential predation between anuran and macroinvertebrate prey types. Attack rates were substantially higher towards *A. aquaticus* and *Chironomus* sp. larvae, with considerable separation of 95% confidence intervals when compared to predation of *R. temporaria* larvae. *Dikerogammarus villosus* also displayed lower handling times towards isopod and dipteran prey, although these did not differ significantly when compared to *R. temporaria* larvae. By inference, lower handling times towards macroinvertebrate prey types translated into greater maximal feeding rates towards *A. aquaticus* and *Chironomus* sp. larvae. FR curves produced via the van Leeuwen MSFR models predicted marked variation in the *per capita* predation of larval anurans and alternative macroinvertebrate prey types, with predation of *A. aquaticus* (Figure S4.1a), and *Chironomus* sp. larvae (Figure S4.1b), increasing at a steeper rate, before establishing a higher asymptote. Conversely, FR curves generated with respect to *R. temporaria* larvae indicated a shallow increase in *per capita* predation rates, before plateauing at a much lower level.

Table A4.2. Best-fit estimates for FR parameters, obtained using ODE-derived Type II (hyperbolic) MSFR models, regarding per capita consumption by invasive *D. villosus* towards several experimental prey combinations (*R. temporaria* larvae – *Chironomus sp.* larvae; *R. temporaria* larvae – *A. aquaticus*), when supplied at varying relative densities. Models were fitted with the similarity index (s_{ij}) fixed at 1 (i.e. no prey-switching) or provided as a free parameter to be estimated. Parameters were obtained using maximum likelihood estimations, and 95% confidence intervals generated via non-parametric bootstrapping (n = 5000). Models comparisons conducted using small sample corrected Akaike's information criterion (AICc).

Prey Combination	Prey Species	Type II MSFR Model (van Leeuwen et al., 2013)					Type II MSFR Model (van Leeuwen et al., 2013)				
		Parameter Estimate [95% CI]		s_{ij} (fixed)	*1/h*	AICc	Parameter Estimate [95% CI]		s_{ij} (estimated)	*1/h*	AICc
		a	*h*				*c*	*h*			
1	*R. temporaria* larvae	0.223 [0.133 – 0.348]	0.265 [0.148 – 0.360]	1	3.774	355.170	0.149 [0.089 – 0.263]	0.295 [0.141 – 0.403]	2.258	3.390	350.564
	Chironomus sp. larvae	3.843 [2.446 – 5.639]	0.156 [0.138 – 0.174]		6.410		3.348 [1.987 – 5.160]	0.151 [0.130 – 0.166]		6.623	
2	*R. temporaria* larvae	0.319 [0.194 – 0.496]	0.512 [0.314 – 0.692]	1	1.953	345.191	0.039 [0.023 – 0.073]	0.464 [0.118 – 0.731]	4.881	2.155	345.211
	A. aquaticus	1.566 [0.980 – 2.271]	0.348 [0.280 – 0.409]		2.874		0.677 [0.393 – 1.220]	0.188 [0.061 – 0.281]		5.319	

a = attack rate
h = handling time
1/h = maximum feeding rate
s_{ij} = similarity index between prey species i and j

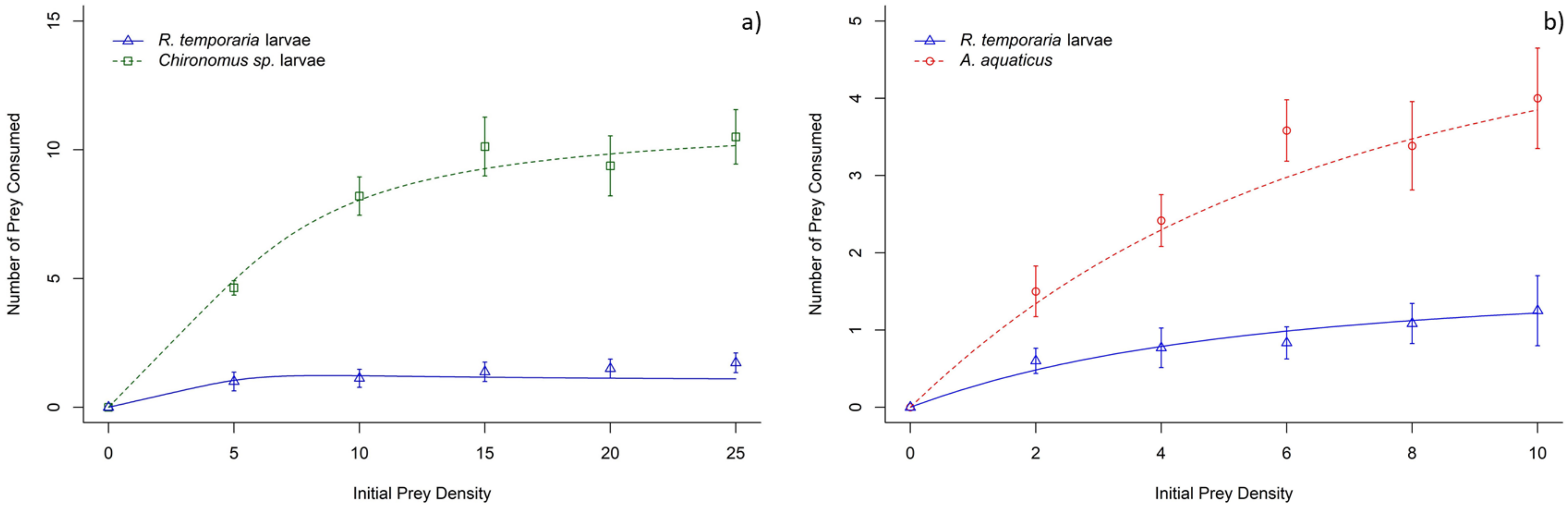

Figure A4.1. Type II multi-prey species functional response (MSFR) curves for per capita consumption by invasive *D. villosus* towards a) *R. temporaria* larvae (blue open triangles and solid line) and *Chironomus* sp. larvae (green open squares and dashed line), and b) *R. temporaria* larvae (blue open triangles and solid line) and *A. aquaticus* (red open circles and dashed line), when supplied at varying provisioning ratios. Curves obtained using an ODE-derived MSFR model (van Leeuwen et al., 2013), fitted via maximum likelihood estimations, with the similarity index (s_{ij}) provided as free parameter to be estimated. Points denote mean (± SEM) number of preys consumed.

Appendix 5

A5.1 – Protocol for DNA extraction and quantitative polymerase chain reactions (qPCR), performed by technical staff at the Zoological Society of London using the RACE protocol (**R**isk **A**ssessment of **C**hytridiomycosis to **E**uropean amphibian biodiversity; developed based on procedures previously published by Boyle *et al.*, 2004).

DNA extraction was conducted using 50µl Prepman Ultra. Samples were homogenised with 30mg of 0.5mm Zirconium/silica microbeads, using a bead beater for 45 seconds, and then centrifuged at 14500rpm for 30 seconds (repeated twice). Samples were placed in a water bath, maintained at 100°C, for 10 minutes, before centrifuging for a further 3 minutes, after which generated supernatant was extracted. To avoid inhibition, all extractions were diluted 1/120 prior to qPCR. Taqman Exogenous Internal Positive Control Reagent (IPCs) was added to reactions to assess inhibition by PCR, for which there was no inhibition detected.

Quantitative PCR analysis was conducted using a Master Mix (see Appendix 5, Table A5.1 for full list of reagents and quantities), containing the 29 base ITS1-3 Chytr (5'-CCT TGA TAT AAT ACA GTG TGC CAT ATG TC-3') and the 22 base 5.8S Chytr (5'-AGC CAA GAG ATC CGT TGT CAA A-3') primer sets, as well as the 15 base minor groove binder probe (Chytr MGB2; 5'-6FAM CGAGTCGAACAAAAT MGBNFQ-3'). These primers were developed for general amplification of *Bd* DNA (i.e. not strain-specific). For each sample tested, qPCR reactions were conducted in duplicate, with 5µl of the 1/120 diluted sample extract and 20µl of the Master Mix added to each reaction well. Reactions ran under the following amplification conditions; 50ºC (2 mins) and then 95ºC (10 mins), following by 50 cycles of 95ºC (15 seconds) and 60ºC (1 min).

Table A5.1. Reagents (and quantities) used to create Master Mix for quantitative polymerase chain reactions (qPCR), conducted using the Risk Assessment of Chytridiomycosis to European amphibian biodiversity (RACE) protocol. Quantities equate to one tissue sample, analysed in duplex (i.e. two wells).

Reagent	Quantity (µl)
Distilled Water (dH_2O)	9.875 (2 x 4.9375µl)
TaqMan Universal PCR Master Mix	25 (2 x 12.5µl)
Forward Primer	2.5 (2 x 1.25µl)
TaqMan MGB Probe	0.125 (2 x 0.0625µl)
Reverse Primer	2.5 (2 x 1.25µl)

Table A5.2. Protocol used to process amphipod tissues, previously fixed in Davidson’s Freshwater Fixative and stored in 70% ethanol. Protocol provided by the UK Centre for Ecology and Hydrology.

Step	Solution	Time (Mins)	Temperature
Step 1	70% Industrial Denatured Alcohol	30 Minutes	Ambient
Step 2	90% Industrial Denatured Alcohol	30 Minutes	Ambient
Step 3	Absolute Industrial Denatured Alcohol	30 Minutes	Ambient
Step 4	Absolute Industrial Denatured Alcohol	30 Minutes	Ambient
Step 5	Absolute Industrial Denatured Alcohol	30 Minutes	Ambient
Step 6	Absolute Industrial Denatured Alcohol	30 Minutes	Ambient
Step 7	Sub X Clearing Agent	40 Minutes	Ambient
Step 8	Sub X Clearing Agent	40 Minutes	Ambient
Step 9	Sub X Clearing Agent	40 Minutes	Ambient
Step 10	Sub X Clearing Agent	40 Minutes	Ambient
Step 11	Wax	45 Minutes	60°C
Step 12	Wax	45 Minutes	60°C
Step 13	Wax	50 Minutes	60°C

Table A5.3. Protocol used for histological staining (Haemotoxylin & Eosin), applied to processed amphipod tissues. Protocol provided by the UK Centre for Ecology and Hydrology.

Step	Solution	Time	Temperature
Step 1	Xylene Substitute	2 Minutes	Ambient
Step 2	Xylene Substitute	3 Minutes	Ambient
Step 3	Xylene Substitute	3 Minutes	Ambient
Step 4	Absolute Industrial Denatured Alcohol	3 Minutes	Ambient
Step 5	Absolute Industrial Denatured Alcohol	3 Minutes	Ambient
Step 6	Tap Water	6 Minutes	Ambient
Step 7	Haemotoxylin	3 Minutes	Ambient
Step 8	Tap Water	6 Minutes	Ambient
Step 9	Acid Alcohol	1 Seconds	Ambient
Step 10	Tap Water	6 Minutes	Ambient
Step 11	Alcoholic Eosin	3 Minutes	Ambient
Step 12	Tap Water	50 Seconds	Ambient
Step 13	70% Industrial Denatured Alcohol	30 Seconds	Ambient
Step 14	Absolute Industrial Denatured Alcohol	3 Minutes	Ambient
Step 15	Absolute Industrial Denatured Alcohol	3 Minutes	Ambient