Why are invaders invasive?

Development of tools to understand the success and impact of invasive species

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

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Nigel Taylor developed the idea, conducted the experiments, analysed the data and wrote the manuscript. Alison Dunn formulated the idea, supervised the research and contributed to writing the manuscript. Caroline Liddell provided assistance in conducting the experiments.

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Abstract

Biological invasions are a major facet of anthropogenic global change, with severe negative environmental and socioecological impacts. Effective and efficient management of biological invasions requires a mechanistic understanding of the factors driving invasion success and impact. I investigate three factors likely to have broad relevance in explaining success and impact of alien invaders: resource use, behaviour and propagule pressure.

Alien decapod and amphipod crustaceans may have different patterns of trophic resource use to native analogues. Through quantification of functional responses and food 'choice', I highlight an exceptionally large predatory impact of alien *Eriocheir sinensis* on invertebrate prey, relative to both native and alien crayfish. Through similar methods, I suggest the larger size of alien *Dikerogammarus villosus* relative to native *Gammarus pulex* could facilitate higher predatory impacts on fish eggs and larvae.

I quantify personality traits (boldness, exploration, activity, sociability and voracity) of invasive and native decapod crustaceans in the laboratory. Invasive *E. sinensis* and *Pacifastacus leniusculus* were bolder than European *Austropotamobius pallipes*. Boldness may a common trait of successful, high-impact invaders. I provide the first evidence of personality (consistent within-individual behaviours) in these decapods, but find no evidence that it drives dispersal in signal crayfish. Comparisons of core and invasion-front populations of *P. leniusculus* suggest its spread is driven by density rather than behaviour.

Using experimental invasions of ciliate protists into laboratory microcosms, I provide quantitative data to show how propagule pressure – the number of introduced organisms and introduction events – can increase invasion success (rate and population density) and invader impact.

In general, resource use, behaviour and propagule pressure all have potential to predict the identity, impact and dynamics of successful invaders and thus inform management strategies. Having measured metabolism alongside these other factors, I propose that metabolic rate could provide another readily-measurable, general predictor of invasion success and impact.

List of Abbreviations

AAS	Absolute aerobic scope
AIC	Akaike's information criterion
ANOVA	Analysis of variance
CE	Common Era
cmax	Maximum carapace dimension
CPUE	Catch per unit effort
df	Degrees of freedom
DO	Dissolved oxygen
FR	Functional response
GLM	Generalised linear model
LM	Linear model
MAM	Minimum adequate model
$\dot{M}O_2$	Rate of oxygen consumption
MMR	Maximum metabolic rate
MR	Metabolic rate
NNSS	Non-Native Species Secretariat
PCA	Principal component analysis
PVC	Polyvinyl chloride
RMR	Routine metabolic rate
SE	Standard error
SMR	Standard metabolic rate

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Chapter 1

Introduction: the problem of biological invasions

"They [biological invasions] are so frequent nowadays in every continent and island, and even in the oceans, that we need to understand what is causing them and try to arrive at some general viewpoint about the whole business."

Charles S. Elton (1958)

1.1. Outline

Alien species are one of the leading threats to global biodiversity and ecosystem function (Sala et al. 2000; Millennium Ecosystem Assessment 2005). Biological invasions are the process by which alien species are introduced, established and thrive outside their native range. Effective prediction of invasions and management of alien species relies on a mechanistic understanding of the processes that facilitate successful invasions and the factors that contribute to large impacts of invaders. This thesis investigates some key mechanisms related to invasion success and impact, using invasive Crustacea and representative protists as model systems. I aim to provide specific information about success and impact in these systems, whilst providing case studies to inform more general understanding and potential tools for assessing invasion success and impact.

This Chapter sets this work in context. First, I introduce the concept of biological invasions and outline why they are of research interest: invasions can have negative impacts, but also offer insights into ecological patterns and processes. Second, I explain options for management of biological invasions and how these could be improved by a mechanistic understanding. I then outline existing mechanistic hypotheses for invasion success and impact, and identify three foci for this thesis: propagule pressure, behavioural traits and resource use. I briefly introduce the study systems I use to investigate these mechanisms, and finally expand on the aim and structure of the thesis.

1.2. The Anthropocene and biological invasions

Human activity is having strong, pervasive and inter-related effects on the Earth's environment (Vitousek et al. 1997). Current rapid climate change is associated with anthropogenic emissions of CO_2 and other greenhouse gases (IPCC 2014). The Earth's oceans contain over five trillion floating plastic particles, with large debris causing a physical hazard for marine life and small

particles acting as a carrier for toxic chemicals into the food web (Eriksen et al. 2014). As humans move themselves and cargo around the world, at an ever increasing rate and on an expanding diversity of routes, an increasing number and diversity of species are being introduced beyond their native range where they can have serious negative impacts (Hulme 2009). Extinction rates are so much greater than background that we are arguably experiencing a sixth mass extinction (Dirzo and Raven 2003; Barnosky et al. 2011). The combined effect of human activities on the environment is so great that the current geological epoch may be appropriately referred to as the Anthropocene (Crutzen and Stoermer 2000).

Biological invasions are one major facet of human-induced environmental change. Biological invasions are a process, involving the human-mediated transportation of organisms beyond their native range (wherein they are termed aliens), and the progression of these organisms through various stages towards being invasive (Fig. 1.1; Richardson et al. 2011). Over the last 60 years, increasing rates and diversifying routes of transport around the globe have led to a sharp increase in the number of alien introductions, and these are likely to continue into the foreseeable future (Vitousek et al. 1997; Hulme 2009; Rabitsch et al. 2016). Biological invasions are of interest because of the negative consequences they may have, but also as large-scale experiments in community ecology (section 1.2.2).

1.2.1 Terminology and the invasion process

There is much debate over terminology surrounding biological invasions, so the terminology and concepts used in this thesis are outlined in Fig. 1.1. An **alien** species (or more correctly, alien population of a species; Colautti and MacIsaac 2004) is one which has been transported by humans to an area in which it is not native (i.e. beyond its natural biogeographical limit). Some aliens are moved intentionally by their human chauffeurs (e.g. species utilised for agriculture, aquaculture, recreational or aesthetic purposes) whilst others travel incognito (e.g. hitchhikers in ballast water, in luggage, on recreational equipment or on transported produce).

Whilst some aliens may remain in captivity or cultivation (e.g. pets, crops, zoo animals), others are subsequently introduced into the wild, wherein they are either **casual** or **established**: the latter form self-sustaining populations whilst the former do not. Depending on the species/population and nature of the recipient environment, somewhere between 5 and 50% of introduced aliens successfully establish in the novel range (Williamson and Fitter 1996; Jeschke and Strayer 2005).

Ironically, the term **invasive** is one of the most misunderstood in invasion ecology. One definition of an invasive species is an alien that has spread over a large area in the wild, especially one that

forms multiple self-sustaining populations (Richardson et al. 2000; Blackburn et al. 2011). Again, a minority of species make the transition from established to invasive: probably somewhere between 5 and 50% (Williamson and Fitter 1996; Jeschke and Strayer 2005). Following this definition, a successful invasive species has crossed all barriers in the invasion process, having been introduced and then spreading beyond the area of first introduction (Fig. 1.1). Alternatively, invader success can be defined at any preliminary stage of the invasion process e.g. establishment success refers to an alien population successfully crossing the survival and reproduction barriers (Fig. 1.1), but not necessarily spreading. Different mechanisms might contribute to success at each stage, so it is often helpful to consider stage-by-stage success (as I do in Chapters 5 and 6).

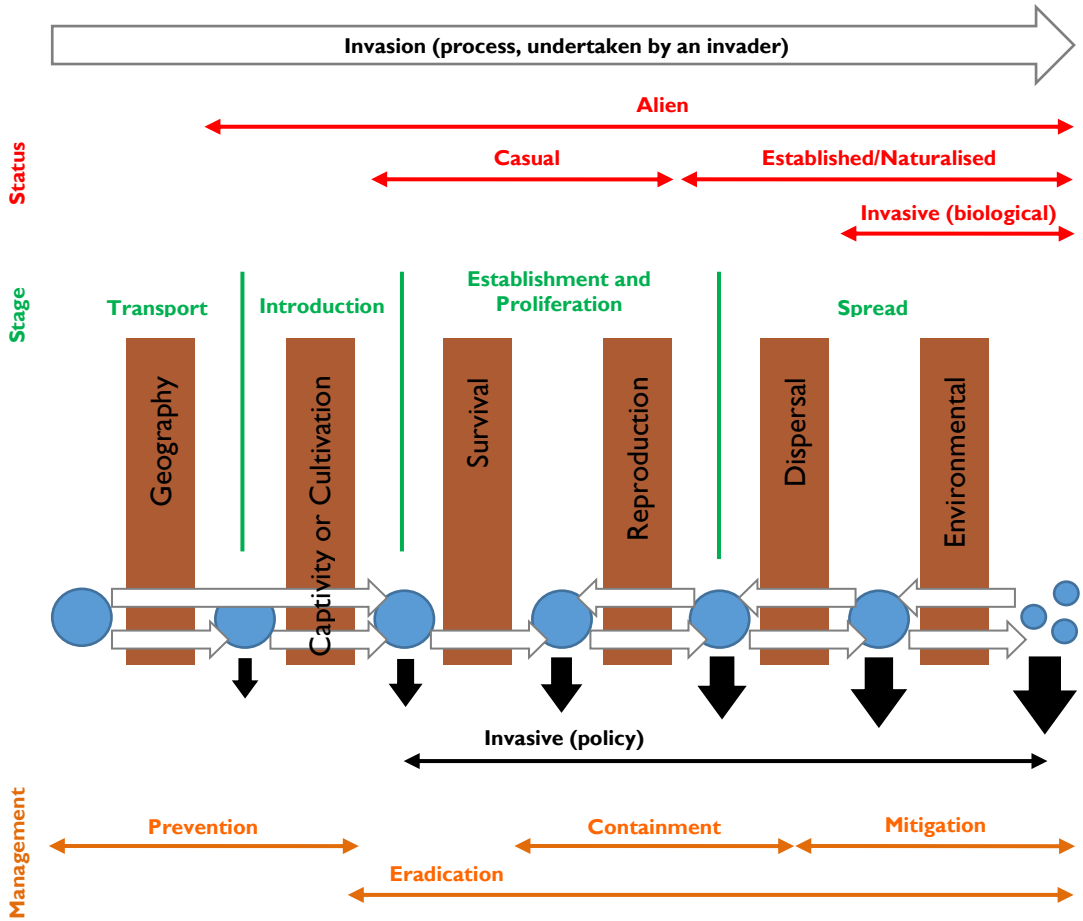


Figure 1.1 The invasion process, modified from Blackburn et al. (2011). Populations (*blue circles*) progress from stage to stage (*green*) by crossing barriers (*brown*). Terminology for populations at each stage is given in *red*. *White arrows* describe transitions of populations across barriers; note that these can occur in both directions (the status of a population can change over time), or that multiple barriers can be crossed at once (e.g. deliberately introduced populations cross the Geography and Captivity barriers in one go). Success can be considered as the crossing of any single barrier, but overall invasion success requires the crossing of all barriers to become invasive; failure to become invasive can therefore occur at any stage of the invasion process. ***Black arrows*** indicate impacts of alien populations. An invader at any stage can have impact, but impact is likely to increase in magnitude through the invasion process.

Other definitions consider population size to contribute to invasiveness. Thus, an alien species may be considered invasive if it locally abundant (in the wild) but *not* widespread (Colautti and MacIsaac 2004) or only if it is both locally abundant *and* widespread (Valéry et al. 2008). This is more than just a semantic issue, as different processes are likely to drive abundance and range (Colautti and MacIsaac 2004; Speek et al. 2011) and impact depends separately on both abundance and range (Parker et al. 1999). Here, I use 'invasive' to refer to spread only, but in recognition that abundance can be an important additional level of invasion success and modulator of impact, I include it in descriptions of invaders where appropriate and explicitly measure population size of protist invaders in Chapter 6.

Further confusion over the term 'invasive' arises because in a policy context, invasive species are defined by negative impacts (black arrows, Fig. 1.1). For example, the European Union defines an invasive alien species as one, "whose introduction or spread has been found to threaten or adversely impact upon biodiversity and related ecosystem services" (EU 2014). Impact is not necessarily contingent on invasiveness (in the biological sense): it can occur at any stage of the invasion process, and there are examples of invaders that have not spread but have a strong local impact (e.g. Amur clam *Potamocorbula amurensis* in San Francisco Bay; Ricciardi et al. 2013). Equally, invasiveness does not always predict impact. Ricciardi and Cohen (2007) found no association between impact on native taxa and establishment success or spread rate in vertebrate taxa. As a specific example, the Eurasian aster *Tragopogon dubius* is a widespread invasive species in North America but occurs at low densities so has minimal ecological impact. Its windborne seeds facilitate wide dispersal, but are palatable to rodents limiting local abundance (Pearson et al. 2012). However, there is likely to be much overlap between biologically invasive species and policy-defined invasive species. Impact can be considered as a combination of *per capita* effect (E), abundance (A) and range (R) (Parker et al. 1999) such that widespread invasive species (large R) – especially those that are also numerically dominant (high A) – will tend to have large impacts. Accordingly, Richardson et al. (2000) estimated that the majority (between 50 and 80%) of biologically invasive plant species have negative impacts.

Finally, I note that native species could also be considered invasive, in either the biological sense (widespread) or in a policy context (having a negative impact/being pests). This illustrates the point that the concept of alien species is biogeographical, whilst being invasive is an ecological or social concept (Valéry et al. 2009). Invasiveness is typically dependent on novelty – and that can stem from a change IN the environment in the case of native invasive (e.g. following land use change or in response to climate change) or a change OF environment, as in the case of alien species (Valéry et al. 2008). However, the focus of invasion ecology – and this thesis – is on alien invaders, because aliens are more likely to become biologically invasive or have negative impacts

(Simberloff et al. 2012; Hassan and Ricciardi 2014). In Section 1.4, I outline possible mechanistic explanations for the success and impact of alien species.

This thesis considers the question, "Why are invaders invasive?" from both the biological and policy perspectives. That is, I investigate both the success and impact of alien invaders. An invader is an organism, population or species at some point on the invasion process: it is the entity performing an invasion (Fig. 1.1). Taking 'invasive' in the biological sense, I investigate factors that allow invaders to enter and progress through various stages of the invasion process. In some cases (Chapter 4) I consider overall invasion success (transition through the entire invasion process to become fully invasive). Elsewhere, I define specific stages at which success is measured e.g. transport success and the influence of behaviour (section 1.4.2), spread of signal crayfish (Chapter 5) and establishment of protists (Chapter 6). Taking the policy definition of 'invasive', I investigate mechanisms that might explain (and allow us to predict) why some invaders have larger impacts than others (Chapters 2 and 3).

To clarify, herein I use the term 'invasive' in the biological context, referring to aliens that have spread from their original site of introduction (as in Fig. 1.1). An 'invader' is an entity undergoing an invasion. Invaders may or may not be fully invasive or damaging. To refer to species with impact, I use the terms 'high-impact' or 'damaging', rather than using the term invasive.

1.2.2 Why are biological invasions important?

Biological invasions are of great practical and scientific interest (Parker et al. 1999; Mack et al. 2000). With respect to the former, many biological invasions are associated with strong impacts which can be detrimental to humans. This applied aspect of invasive species research forms the primary focus of this thesis. Regardless of impact, invasions are of scientific interest as semi-natural experiments in species translocation and colonisation; this is a further, underlying theme of this thesis.

Impacts are defined as net changes relative to a non-invaded or pre-invasion situation. The projection of human values onto impacts leads to their perception as positive or negative (Pyšek et al. 2012). Some alien species can have positive impacts: wheat *Triticum* spp. and cattle *Bos taurus* provide food, plantation forests provide timber products and recreational opportunities, and zebra mussel *Dreissena polymorpha* filter feeding can improve water quality (Pimentel et al. 2001; McLaughlan and Aldridge 2013). However, a subset of aliens can be problematic. The proportion of aliens that have negative impacts is difficult to quantify – not all species have been assessed and not all impacts recorded, and it will vary between alien species and invaded

environments – but it is likely to be a minority (Simberloff 2011). Williamson and Fitter (1996) estimated that between 5 and 20% of established alien species have negative economic impacts. Of the freshwater aliens established in Europe or North America, Hassan and Ricciardi (2014) classify 5.8% and 9.1% respectively as pests (having negative socioeconomic impacts). Finally, *severe* negative impacts are caused by an even smaller subset of species: only 30% of alien bird species with known impacts on native taxa have a more than a minor impact (Evans et al. 2016). Although impacts can occur at any stage of the invasion process, their severity is likely to increase through the process as the abundance and/or range of the invader increases (Fig. 1.1).

This thesis is concerned with the ecological impacts of alien species. Alien species can have ecological impacts across all levels of biological organisation, from genes to ecosystems (Parker et al. 1999). Aliens can hybridise with native species, or induce rapid evolution (Mooney and Cleland 2001), are one of the major causes of vertebrate and plant extinctions (Bellard et al. 2016) and can negatively affect ecosystem structure, function and service provision (Charles and Dukes 2007; Pejchar and Mooney 2009; Piscart et al. 2011; Hänfling et al. 2011). Biological invasions are recognised as one of the leading threats to native biodiversity and ecosystem function worldwide (Sala et al. 2000; Millennium Ecosystem Assessment 2005). The success of a non-random subset of aliens is leading to the biotic homogenisation, whereby genetic, taxonomic and functional diversity is being eroded at a global scale (Rosenzweig 2001; Olden et al. 2004; Capinha et al. 2015). Alien species now constitute a substantial proportion of many floras and faunas: of the British vascular flora, 21% (442/2065 species) are aliens (Vitousek et al. 1996) as are 29% (31/107 species) of British freshwater macrofauna (Keller, zu Ermgassen and Aldridge 2009).

Alien invaders can also have substantial socioeconomic impacts, which may or may not be related to ecological impacts. Social impacts of invaders include health problems such as the introduction of mosquito disease vectors to the Galapagos Islands (Bataille et al. 2009), and impacts on cultural heritage like the loss of local festivals centred on the native European crayfish *Austropotamobius pallipes* (Lodge et al. 2012). Economic costs associated with alien species arise from management (e.g. control, eradication and research) or impact (e.g. increased pest or predator activity, reduction in ecosystem service provision, higher prices) and can be substantial (Charles and Dukes 2007; Williams et al. 2010). Alien species are estimated to cost Great Britain £1.7bn *per annum* (Williams et al. 2010) – equivalent to an extra annual 'tax' of £26 *per capita*. Globally, the cost of alien species is in the region of US$1.4 trillion, or 5% of the global economy (Pimentel et al. 2001).

All biological invasions, regardless of impact, offer semi-natural experiments in species translocation and colonisation that can inform general ecological and evolutionary understanding (Shea and Chesson 2002; Sax et al. 2007; Catford et al. 2009). For example, successful establishment of alien populations provides strong evidence that biological communities are not saturated, suggesting local competition is of limited importance (relative to regional processes such as speciation and dispersal) in determining species richness at a site (Sax et al. 2007). Further, genetic change in invading populations indicates that evolution can be extremely rapid in natural situations (Mooney and Cleland 2001), whilst the spread of invaders provides useful data to refine models of dispersal (Hastings et al. 2005).

1.3 Management of biological invasions

The severe negative impacts of some alien species necessitates management, but to be efficient and effective this must be carefully planned. The identification of species, sites, pathways and vectors to manage will be aided by a mechanistic understanding of success and impact. Understanding the mechanisms driving success through the invasion process can also inform the timing and design of management actions.

1.3.1 Management through the invasion process

Management of biological invasions involves averting or slowing invasion or reducing the impacts of invaders at some stage of the invasion process. Management options vary along the invasion process (Fig. 1.1; Sakai et al. 2001; EU 2014; Dunn and Hatcher 2015). Earlier interventions are likely to be more successful and, for damaging invaders, cost effective: prevention is better than cure (Bax et al. 2001; Caffrey et al. 2014). Unsurprisingly, much existing legislation on biological invasions has a strong focus on minimising the number and diversity of aliens transported and introduced to a novel range (Reaser et al. 2007; DEFRA 2015). Introductions can be prevented by good biosecurity such as import/export regulations, quarantines at international borders or mid-ocean ballast water exchange to expose coastal/estuarine hitchhikers to lethal high salinities. Having been ratified by a sufficient number of states in September 2016, the International Maritime Organisation's Ballast Water Management Treaty will come into force in September 2017 (IMO 2016). Introductions through escape from captivity or cultivation can be prevented by secure housing (e.g. for animals in zoos, or fish in aquaculture), education of the pet-buying or gardening public to discourage release and/or legislative control, such as the UK Wildlife and Countryside Act 1981 (Simberloff et al. 2005).

Post-introduction, management options for single alien populations shift to containment and eradication. Containment involves restricting the range of the alien population through good

biosecurity by users of the contaminated environment. Public users of contaminated environments for recreation can be an important vector for invaders (Anderson et al. 2015), so biosecurity campaigns targeted towards the public (e.g. Check Clean Dry; GB NNSS 2016a) are an important aspect of containment. Containment may also involve the eradication of satellite populations in the novel range to prevent spread, by mechanical or chemical means or with biological control agents. Ideally, the original alien population will also be eradicated. These eradications are more likely to be successful and are more cost effective if done soon after the alien population is established, so surveillance, early detection and rapid response to introductions are commonly employed. For example, in Great Britain the Non-Native Species Secretariat (NNSS) has a dynamic list of 'Alert' species (GB NNSS 2016b) – including (as of September 2016) the killer and demon shrimp *Dikerogammarus villosus* and *D. haemobaphes*, and the Asian hornet *Vespa velutina* – for which it encourages reports of sightings by the public and has rapid response protocols to eradicate incipient satellite (shrimp) or pioneer (hornet) populations.

Eradication of fully invasive species, especially when they are also abundant, is difficult. Extreme biological, physical or chemical control methods (e.g. biocide treatment, flaming, dewatering, manual removal) have varying levels of success, can require large investments of time and money, and can have negative side effects (Aldridge et al. 2015). An alternative strategy is mitigation of impact e.g. creation of refuges for species threatened by an invasion. For white-clawed crayfish threatened by signal crayfish invasions in Great Britain, Ark Sites are being established in isolated lotic stretches and lentic bodies, beyond the reach of the invading crayfish (Peay 2009).

1.3.2 What to manage: risk assessment

Resources for management are limited. We cannot, for example, assign every potential invader as an Alert species or continuously monitor every potential site which may be invaded. Therefore, we must focus management efforts where the risk of impact is greatest to give the maximum return on investment into management. In this vein, the 2014 European Union Regulation on invasive alien species explicitly states that, "management measures should be proportional to the impact on the environment" (EU 2014).

Such prioritisation relies on risk assessment, in which risk is a product of the likelihood of an event and severity of the consequences (NRC 2002). In the context of biological invasions, risk assessment is concerned with the likelihood of an invader crossing invasion barriers (Fig. 1.1) and the severity of its impact. In addition to scientific evidence, value judgements will come into play when assessing the severity of impact (Pyšek et al. 2012; Kumschick et al. 2012). Risk assessments can be applied to vectors (mode of introduction), pathways (source of introduced

organisms), sites (recipient locations) or species. Risk assessments may be conducted for potentially damaging invaders, prior to their introduction, to identify targets for preventative management, or can be conducted following introduction to allocate resources for containment or eradication (Parker et al. 1999; Andersen et al. 2004; Kumschick et al. 2012).

The risk posed by individual species can be assessed using scoring systems based on their characteristics and/or history of invasion success and impact. As an early example, the Australian Weed Risk Assessment (Pheloung et al. 1999) evaluates plant species for importation based on 49 questions regarding their attributes and impacts. High-scoring plants (with 'high' being defined against a training set of known weeds and non-weeds) are deemed too great a risk to import. More recently, Roy et al. (2014) used expert opinion to score likely success and impact of potential invaders to Great Britain, identifying species that might be the focus of heightened surveillance efforts to prevent introduction. In addition, scoring systems can be used to rank the impacts of established invaders to prioritise their management (Nentwig et al. 2009; Kumschick et al. 2012; Blackburn et al. 2014). Laverty et al. (2015b) applied the Generic Impact Scoring System to aquatic macroinvertebrates, identifying species likely to have a high impact in the field (*Eriocheir sinensis*, *Dreissena polymorpha* and *Pacifastacus leniusculus*) as well as knowledge gaps for 12 species with no known impact. Data in Chapters 2-5 could contribute to such risk assessments for specific taxa, as well as informing criteria used in generalised risk assessments.

Vectors, pathways and sites are often considered together in risk assessments based on socioeconomic and environmental factors. For example, Chan et al. (2013) focussed on the ballast water vector and used environmental data to identify certain ports (e.g. Churchill, Manitoba) and pathways (from coastal domestic sources) associated with the greatest risk of invasion – based on environmental similarity between recipient and donor regions, and the number of alien individuals likely to be introduced (propagule pressure). The identity of species transported also feeds into pathway risk assessments such as that of Chan et al. (2013): pathways that transport high impact invaders are higher priorities for management. Meanwhile, Gallardo and Aldridge (2013) identified areas of the British Isles most at risk of invasion by high-impact aquatic plants and animals based on a combination of climatic, geological and land-use variables with socioeconomic variables such as distance to ports and the Human Influence Index. Thus, they implicitly considered the interaction between pathways and sites (environmental suitability) and exposure to relevant vectors for the focal species.

Clearly, all of these risk assessments rely on some existing knowledge of the factors leading to invasion success or impact. However, this tends to be correlative rather than mechanistic (e.g. climate matching, history of success or impact in the focal species or close relative) or even

shaped by expert judgement (e.g. horizon scanning exercises). Our ability to prioritise alien species for management would be improved by more detailed information for specific species, and a better mechanistic understanding of invasion success and impact that would allow prediction and generalisation (see Section 1.4).

1.3.3 How to manage: designing effective strategies

Management strategies for alien species can be informed by explicit experimentation: trying different strategies and seeing what works (Peay et al. 2006; Aldridge et al. 2015). However, an understanding of the mechanisms underlying invasion and impact can inform management techniques and strategies *a priori* (Suarez et al. 1999; Byers et al. 2002).

A mechanistic understanding of invasions can be used to reduce the success of alien species. For example, models based on demographic parameters can be interrogated to identify the "Achilles heel" of a particular alien species: the life stage that makes the greatest contribution to population persistence. In this manner, Sebert-Cuvillier et al. (2007) identified eradication of the juvenile bank of invasive American black cherry *Prunus serotina* as the most effective method of control. Similarly, Houghton et al. (2015) used demographic models to identify the optimal timing and combination of control strategies to limit signal crayfish populations. Knowing the mechanistic relationship between propagule pressure and success – for example whether the number of individuals introduced per event or number of events is most important, and whether there are any threshold effects – is important for determining how to manage pathways and vectors for alien introductions (Hulme et al. 2008).

Alternatively, mechanisms can be manipulated to mitigate impact. For example, if predation is demonstrated to be the main mechanistic driver of impact, then changing the behaviour of predators or their victims could offer a mitigation strategy – even if predator numbers can't be controlled within economic or biological reason (Sutherland 1998). Equally, if naiveté of native prey is the mechanism underlying impact (Cox and Lima 2006), then a potential management strategy would be to subsidise the survival of the native species for long enough to allow the evolution of appropriate responses. Simply, this could involve provision of additional refugia (Schlaepfer et al. 2005).

1.4. Mechanisms underlying invasion success and impact

In his seminal work on biological invasions, Charles Elton noted that, "we need to understand what is causing [biological invasions] and try to arrive at some general viewpoint about the whole business" (Elton 1958). A mechanistic understanding of the proximate factors leading to invasion

success and impact would allow sound predictions (Dick et al. 2014). Predictions, models or tools based on a deep mechanistic understanding are robust across contexts, and less sensitive to stochasticity and nonlinearity when extrapolating beyond observed conditions (Bolker 2008; Kearney and Porter 2009). Thus, a mechanistic understanding facilitates predictions for new and emerging invaders with no invasion history, and for extrapolating existing invasions into a rapidly changing future world (Kueffer et al. 2013).

Many mechanistic explanations have been proposed for the success of biological invaders and their ecological impact. Catford et al. (2009) and Ricciardi et al. (2013) provide overviews of these mechanistic hypotheses, which are summarised and synthesised in Table 1.1. This table highlights that similar mechanisms are likely to contribute to both success and impact. The mechanisms describe how abundance (A), range (R) or *per capita* effect (E) of an alien species may be increased. Invasion success is defined and favoured by these factors (cf. Fig. 1.1), just as an increase in these factors is associated with a larger impact (Parker et al. 1999). However, the success and impact of alien species are not *necessarily* correlated and may be driven by different mechanisms (Ricciardi and Cohen 2007; Speek et al. 2011; Pyšek et al. 2012).

Overlap between the mechanistic hypotheses in Table 1.1 is reflected by grouping into general themes: introduction effort, species traits, distinctiveness of the invader, new associations formed in the invaded range, and abiotic characteristics of the invaded range. Specific hypotheses vary the details within these themes. The hypotheses can also be categorised by four fundamental drivers of success and impact (or their interactions): (P) propagule pressure (the number of individuals introduced), (B_I) the biology of the invading organism, (B_C) the biology of the recipient community and (A) the abiotic conditions recipient environment (Ricciardi 2003; Hayes and Barry 2007; Catford et al. 2009; Ricciardi et al. 2011; Pyšek et al. 2012). These drivers are important because the success and impact of any particular invasion depends on all four being accommodating, if not favourable (Catford et al. 2009). A neat analogy of this concept is the lock-key model of Heger and Trepl (2003), whereby invasion success depends on how well the invading organism (B_I) fits into the recipient environment (B_C and A). Similarly, Reaser et al. (2007) liken propagule pressure (P) to straws that can break a camel's back, but the number of straws that will do so depends on the length and weight of each straw (B_I) and the age, health and other cargo of the camel (B_C and A). These four drivers thus provide a general framework for understanding biological invasions.

Table 1.1 Overview of mechanistic hypotheses that may explain invasion success and impact, adapted from Catford et al. (2009) and Ricciardi et al. (2013). Hypotheses are grouped into *Themes* based on similarities in proposed mechanisms. *B* – mechanism can involve behavioural interactions; *R* – mechanism can involve resource use.

Theme	Key driver(s)[1]	Link to success and impact[2]	Specific hypothesis	Explanation of hypothesis	Key References	B	R
Introduction effort	P * B_I	High supply of individuals (in terms of propagule size or number) combats environmental and demographic stochasticity. Genetic diversity is increased.	Propagule pressure	The number of individuals per introduction event and/or number of introduction events increases initial invader population size and/or diversity.	Lockwood et al. (2005); Colautti et al. (2006)	✓	
			Colonisation pressure/ Sampling	The greater the number of species introduced, the higher the chance of one being a successful and/or high impact invader.	Crawley et al. (1999); Lockwood et al. (2009)	✓	✓
Species traits	B_I B_I * B_C B_I * P	Characteristics and traits of the alien species facilitate introduction or allow alien to outcompete natives, reach high abundance and/or have high *per capita* impact.	Ideal weed	Some traits show consistent correlation with invasion success or impact (e.g. fecundity, trophic position, resource use, aggression, body size, phenotypic flexibility, genome size, growth rate, consumption rate)	Elton (1958); Baker and Stebbins (1965); Ehrlich (1986); Rejmánek and Richardson (1996); Chapple et al. (2012)	✓	✓
			Trophic position	Predatory interactions tend to drive especially strong effects. Omnivory (flexible feeding) may favour success and broaden impact.	Elton (1958); Moyle and Light (1996); Dick et al. (2014)	✓	✓
			Adaptation	Invader is pre-adapted to recipient environment, or adapts post-introduction.	Duncan and Williams (2002); Lee and Gelembiuk (2008)	✓	✓
Distinctiveness of invader	B_I * B_C	Alien species are functionally distinct from species in the recipient community. They occupy an unfilled niche, within which competition is minimal and impact is unprecedented; or present challenges to which the native community is not adapted, giving the alien an advantage in ecological interactions.	Limiting similarity/ Empty niche	Invaders are not similar to species in the novel community, so fill an empty niche.	Darwin (1859); MacArthur and Levins (1967); Vitousek (1990); Cleland (2011)	✓	✓
			Novel weapons	Invader has novel behavioural, chemical or physical weapons to which resident species cannot respond appropriately	Callaway and Ridenour (2004)	✓	✓
			Evolutionary naiveté	Resident species have not evolved with the invader, so lack appropriate responses to the alien archetype.	Diamond and Case (1986); Cox and Lima (2006)	✓	✓
			Phylogenetic distinctiveness	Phylogenetic distinctiveness is a proxy for trait distinctiveness. Novel taxa to a community have greater success and impact.	Ricciardi and Atkinson (2004)	✓	✓

Theme	Key driver(s)[1]	Link to success and impact[2]	Specific hypothesis	Explanation of hypothesis	Key References	B	R
New associations	$B_I * P$ $B_I * B_C$	Changes in species interactions in the new environment. Invader benefits (abundance and virility) from increased positive interactions, reduced negative interactions, or increased negative effects on resident species.	Enemy release	Enemies from invader's native range are not present in novel range. Predators and parasites are not sampled and introduced with the invader.	Keane and Crawley (2002); Colautti et al. (2004)	✓	✓
			Enemy reduction	Similar to enemy release, but with a reduction of enemies rather than complete loss.	Colautti et al. (2004)	✓	✓
			Enemy of my enemy	Invader accumulates generalist pathogens (from its native and/or novel range), which affect its fitness but affect resident species more.	Colautti et al. (2004); Eppinga et al. (2006)	✓	✓
			Enemy inversion	Natural enemies are introduced with the invader, but their effect is lost (or reverses) in the novel environment.	Colautti et al. (2004)	✓	✓
			Evolution of increased competitive ability	Release from enemies frees up resources which can be allocated to growth and reproduction (greater competitive ability) or adaptation to the novel environment.	Blossey and Notzold (1995)	✓	✓
			Specialist-Generalist	Success and impact greatest when enemies in the novel range are specialists (do not harm invader) but mutualists are generalists (benefit invader).	Callaway et al. (2004); Sax et al. (2007)	✓	✓
			Invasional meltdown	Existing aliens benefit a novel invader, perhaps by restoring beneficial interactions from the native range.	Simberloff and Holle (1999)	✓	✓
			Biotic indirect effects	Invader is involved in indirect interactions in novel range, which can aid its establishment or mediate impact.	Callaway et al. (2004); White et al. (2006)	✓	✓
Abiotic environment	$A * B_I$	Abiotic conditions (perhaps at a certain point in space or time) are favourable for invasion to occur, and for the invader to reach high abundance.	Opportunity windows/ Environmental heterogeneity	Niche availability is dynamic in space and time. Aliens can invade empty niches when and where they become available.	Johnstone (1986); Shea and Chesson (2002); Levine and Rees (2004); Melbourne et al. (2007)		✓

Theme	Key driver(s)[1]	Link to success and impact[2]	Specific hypothesis	Explanation of hypothesis	Key References	B	R
			Environmental matching/ Habitat filtering	Aliens are better able to establish, and have large impacts sooner, in environments similar to their native range.	Weiher and Keddy (1995); Williamson (1996); Kestrup and Ricciardi (2009)	✓	✓
			Disturbance	Disturbance increases resource availability, reduces competition from residents, or shifts conditions towards those that favour the alien over the natives.	Sher and Hyatt (1999); MacDougall and Turkington (2005)	✓	✓
			Fluctuating resources/ Increased resource availability	Resources are fully utilised under normal conditions. When resources become available, they can be exploited by a novel alien to establish, or used by established aliens to boost vigour.	Sher and Hyatt (1999); Davis et al. (2000)	✓	✓
			Dynamic equilibrium	Disturbance and productivity interact to determine invasion success and impact. They may be favoured in low disturbance-low productivity systems, or high disturbance-high productivity systems.	Huston (1979, 2004)	✓	✓

[1] Following terminology of Catford et al. (2009). A - abiotic conditions in the recipient environment; B_C - biotic characteristics of the recipient community; B_I - biotic characteristics of the alien organism; P - propagule pressure.

[2] Hypotheses explain success if they benefit the invader directly (e.g. abundance, vigour, free up resources) or harm resident species which in turn would benefit the invader. Hypotheses may directly explain impact (e.g. Evolutionary Naiveté), or indirectly explain impact if it arises through increasing abundance or range above the minimum needed for successful invasion.

Note the inverse of these hypotheses can explain invasion failure or low impact, and these 'inverse hypotheses' may have their own names. For example:
- Low genetic diversity (from low Propagule Pressure) can lead to **Increased Susceptibility** of an invader to novel enemies (Colautti et al. 2004)
- In the absence of Disturbance of Environmental Heterogeneity, **Biotic Resistance** (presence of natural enemies in the novel range) can inhibit invasion (Levine et al. 2004; Alpert 2006)
- Under the **Specialist-Generalist** hypothesis, specialist mutualists and generalist enemies in the novel range can inhibit invasion (Callaway et al. 2004; Sax et al. 2007)
- Loss of associations through the invasion process (cf. enemy release) can inhibit invasion if those associations benefitted the invader (**Missed Mutualisms**; Mitchell et al. 2006; Alpert 2006)

This being said, primary research into invasion mechanisms involves examining the specific, discrete hypotheses within this conceptual framework. Individual factors of interest can be varied whilst others are controlled or accounted for (Barney and Whitlow 2008; Catford et al. 2009). Although specific factors might not be able to explain all variance in invasion success, and may be neither necessary nor sufficient in any single invasion, we can identify factors that are generally associated with an *increased probability* of success and impact (Rejmánek and Richardson 1996; Heger and Trepl 2003; Pyšek et al. 2012; Gaertner et al. 2014). Knowledge of whether and how specific factors affect invasion success or impact can be incorporated into holistic decision-making tools e.g. decision trees (Kolar and Lodge 2002) or models (Gallardo and Aldridge 2013).

This thesis focusses on three factors contributing to invasion success and impact: propagule pressure, behaviour and resource use – introduced in more detail below. Behaviour and resource use both fall into the 'species traits' theme. Thus, the focal factors of this thesis are amongst the simplest themes likely to be applicable to most if not all invasions (Colautti et al. 2006; Catford et al. 2009). Further, the behavioural and resource use traits have broad relevance in that they may contribute to most of the specific invasion hypotheses in Table 1.1. They mediate the response of individuals to environmental conditions (A) as well as interaction with the novel community (B_C).

1.4.1 Propagule pressure

Propagule pressure is a measure of the number of alien individuals introduced to a novel area. It is a combination of the number of introduction attempts (propagule number) and the number of individuals introduced in each attempt (propagule size) (Lockwood et al. 2005).

Propagule pressure is emerging as a consistent predictor of establishment success (Kolar and Lodge 2001; Hayes and Barry 2007), based on numerous empirical studies (Beirne 1975; Grevstad 1999; Lockwood et al. 2005; Simberloff 2009; Britton and Gozlan 2013). High propagule pressure can increase establishment success simply by introducing a greater number of individuals. In fluctuating environments, a large number of propagules can replace nascent populations lost to environmental stochasticity (Simberloff 2009), or increase the chance that propagules are introduced at a time when conditions are suitable for establishment (Haccou and Iwasa 1996). In more stable environments, a large propagule size is important to overcome demographic problems of small populations, such as demographic stochasticity (random fluctuations in reproductive output or sex ratio) or Allee effects (reduction of individual fitness at small population sizes; Taylor and Hastings 2005; Skarpaas and Økland 2009), to ensure the nascent population persists.

Above the threshold for establishment, higher propagule pressures can increase the size (von Holle and Simberloff 2005) and growth rate (Grevstad 1999) of an invading population. Faster growing, larger populations are more likely to spread across the landscape to become fully invasive (Duncan et al. 1999; Bowler and Benton 2005). Similarly, larger, more widespread populations tend to have stronger impacts (Parker et al. 1999). Fast-growing alien populations, fuelled by high propagule pressures, can also exert a larger impact because they allow native species less time to adapt to the novel invader or recover from disturbance that facilitated invasion (Ricciardi et al. 2011). Finally, high propagule pressures may contribute to impact simply by sustaining an alien population where it would not otherwise establish (Gonzalez et al. 2008). In Chapter 6 I detail an experimental investigation of the effect of propagule size and number on invasion success and impact in protist microcosms.

In addition to the effect of numbers alone, higher propagule pressures may be associated with a genetic rescue effect: an increased input of genetic material to the alien population. This may increase establishment success by introducing a genotype preadapted to the novel range, or may increase genetic variation within the population to provide the raw material for evolution and selection of new, successful genetic combinations. Greater genetic diversity may also contribute to increased abundance and range expansion, and consequently impact (Ricciardi et al. 2011). For example, since 1817 alien green crabs *Carcinus maenas* have been found on the eastern seaboard of North America. It is thought that in the 1980s, subsequent introductions from the native range in Europe to the edge of the novel range in Canada increased the genetic diversity of the crabs at the invasion front. As a result, *C. maenas* was able to expand its range (and spatial extent of its impact) into cooler, more northerly waters (Roman 2006).

However, increased propagule pressure may not always increase invasion success or impact. In some cases, there may be a saturating relationship between propagule pressure and success or impact, such that introducing any more individuals has no further effect. Moreover, increasing propagule pressure could in fact decrease invasion success if it swamps the establishing alien population with poorly adapted genotypes from the native range (Holt et al. 2005), or increases the chance that parasites are also introduced from the native range (Torchin et al. 2003).

Although there is strong evidence that propagule pressure increases establishment success, we have a poorer understanding of how it affects other aspects of the invasion process such as spread, and how it contributes to impact. Many studies of propagule pressure are based on introductions to the field and so are unavoidably confounded by other factors that affect invasion success (biology of the invading organism, and conditions in the invaded habitat). Further, our mechanistic understanding of propagule pressure is limited (Blackburn et al. 2015). For example,

we don't understand the quantitative relationship between propagule pressure and success (the dose-response curve) (Lockwood et al. 2005), the relative importance of propagule size and number (Wittmann et al. 2014), and how propagule pressure might interact with invader and recipient-environment biology (Lockwood et al. 2005; Zayed et al. 2007; Chapple et al. 2012; Duncan 2016).

1.4.2 Behaviour

Behaviour – the responses, movements and actions of an animal – could be a fundamentally important driver of success and impact in animal invasions (Holway and Suarez 1999; Catford et al. 2009). First, many hypotheses for success and impact depend on interactions between an alien species and its environment (Table 1.1), and those interactions are mediated by behaviour e.g. competition, predation, mutualism, habitat choice and activity patterns. The ability to exploit an empty niche may depend on behaviour, behavioural traits may be novel weapons, and the nature and intensity of parasite acquisition (in the native or novel range) may be related to activity levels, space use (Boyer et al. 2010) or interactions with conspecifics (Aplin et al. 2013). Second, behaviour may provide a proxy measurement of other traits that could affect invasion success, such as fecundity and competitiveness, since all these traits may be linked in a 'pace of life' syndrome (Ricklefs and Wikelski 2002). Third, behaviour may modify other drivers of success and impact, for example if propagule pressure is associated with boldness or exploration behaviour, since these traits increase the likelihood of uptake in transport vectors (Chapple et al. 2011; Chapple et al. 2012). Fourth, there is empirical evidence that behavioural traits can explain residual variation in invasion success and impact that is not explained by propagule pressure (Lockwood et al. 2005; Catford et al. 2009). The inclusion of behavioural traits in models improves predictions of establishment success (Sol et al. 2002; Suarez et al. 2005). Practically, small propagules might succeed where behavioural traits negate the problems of small population sizes. In the Argentine ant *Linepithema humile*, behavioural plasticity in terms of reduced intraspecific aggression facilitates the establishment of even small (10 worker) propagules (Sagata and Lester 2009).

Behaviour can affect invasion success across all stages of the invasion process (Fig. 1.1) – although different behaviours may be favoured at different stages. Bold and exploratory species are most likely to proceed through the transport and introduction stages of unintentional introductions, as these behaviours favour uptake by transport vectors (freight, cargo and personal effects) and entry into the new environment. Accordingly, the delicate skink *Lampropholis delciata*, which is a successful invasive species outside of its native Australia, is more exploratory than the non-invasive garden skink *L. guichenoti* (Chapple et al. 2011). Other behaviours such as

flexibility in food choice, or sociality for thermoregulation, may facilitate survival during transit (Chapple et al. 2012). However, there is trade-off in that boldness and exploration may increase the chance of detection during transportation or immediately after introduction, when an alien population is most vulnerable to control (Section 1.3.1; Chapple et al. 2012).

A different set of behavioural traits might facilitate establishment success. Aggressive species may be better able to outcompete incumbent natives, as in the case of the western bluebird *Sialia mexicana* replacing the incumbent mountain bluebird *S. currucoides* in the north west USA (Duckworth and Badyaev 2007). Behavioural plasticity may provide an initial solution to the challenges posed by a novel environment. Bird species with large brains (for a given body mass) and a higher frequency of foraging innovation have higher establishment success (Sol et al. 2002). At low propagule pressures, establishment may depend upon behaviours that mitigate Allee effects such as sociality, mate recognition and low dispersal tendency (Blackburn et al. 2009; Sinclair and Arnott 2016).

Spread of an alien population can be human-mediated or 'natural'. Where humans act as a dispersal vector, spread in the novel range can be thought of as a series of mini invasions from a beachhead (e.g. mitten crab *E. sinensis* transport from China to Europe and then to North America; Hänfling et al. 2002). In this case, bold and exploratory behaviours that favoured initial uptake will also favour subsequent spread. Natural dispersal is also clearly related to behaviour because it involves movement of individuals (Phillips and Suarez 2012). Qualitatively, species with behaviours that permit cheap, easy dispersal are likely to spread rapidly – this includes flight (in birds and insects) and passive dispersal (in pathogens and parasites) (Phillips and Suarez 2012). However, the relationship between behaviours that vary continuously – the level of boldness, or aggression, for example – and dispersal may be highly context-dependent. Where there are functionally similar organisms to displace, high levels of aggression may be important for spread (Duckworth and Badyaev 2007). Where predation pressure is high, shyer species may disperse more rapidly while bold species ignore predators and stay put (Cote et al. 2010a; Cote et al. 2011). In Chapter 5, I investigate how behavioural traits might influence natural, within-catchment dispersal of invasive signal crayfish.

Behaviour may influence the impact of biological invaders. Qualitatively, negative ecological impacts are most commonly caused by alien species showing predatory behaviour (Sax and Gaines 2008), and the novel behaviours and strategies of alien predators can exaggerate their impact relative to native predators (Salo et al. 2007). Quantitatively, behavioural traits such as boldness and exploration can influence the total amount of food consumed *per capita*, as well as the pattern of resource use. For example, bold sticklebacks *Gasterosteus aculeatus* consume more

food than shyer conspecifics (Ward et al. 2004), as do exploratory largemouth bass *Micropterus salmoides* relative to less exploratory conspecifics (Nannini et al. 2012). Exploratory bass, however, are more selective, with mosquito prey forming a greater proportion of their diet than for less exploratory conspecifics (Nannini et al. 2012). In Chapter 4, I explicitly examine the potential functional link between behaviour and consumption in decapod Crustacea. Finally, I note that behaviour can affect the impact of groups of alien organisms, by generating locally high abundance (e.g. aggregation) or modulating *per capita* effects at high density. In some species, interference between conspecifics reduces *per capita* effects in groups (e.g. killer shrimp *Dikerogammarus villosus*; Médoc et al. 2015) whilst in others behavioural correlations can maintain *per capita* impacts despite high densities (e.g. signal crayfish *Pacifastacus leniusculus*; Pintor et al. 2009). These examples are important when interpreting the results of Chapters 2 and 3 in particular.

In addition to this classical view that compares average behavioural traits between species (Tinbergen 1963; Sol et al. 2002; Rehage and Sih 2004; Sih et al. 2012), it is now recognised that the behaviour of individuals within the species, and variation amongst those individuals, can have important ecological implications, including for invasion success and impact. This is especially the case where individual behaviours are consistent across situations (conditions and stimuli around an animal e.g. different levels of predation risk, or different times of day) and time (e.g. from one week to the next) – that is, the individuals have personalities. Personalities may arise as alternative behavioural strategies, constrained by limited plasticity within individuals (Dall et al. 2004; Sih et al. 2004)

The ecological implications of personalities stem from the fact that they create structured behavioural variation, which means a single species occupies multiple parts of functional space: a single species effectively functions as multiple species (Wolf and Weissing 2012; Sih et al. 2012). This can increase (a) establishment success, as each behavioural type buffers fluctuations in the others, (b) dispersal success, as certain extreme behavioural types initiate the invasion front for others to follow (Cote et al. 2010b), and (c) impact, because different behavioural types utilise different resource bases. This broadens the impact of the invader by expanding its niche (Shea and Chesson 2002) but also intensifies impact, as the species can reach a greater abundance with less intraspecific competition (Fogarty et al. 2011; Phillips and Suarez 2012; Wolf and Weissing 2012). As an empirical example, Koester et al. (2016) suggest that intraspecific diet variation of *D. villosus*, inferred from stable isotope analyses, could contribute to its invasion success and broad impact. The existence of multiple behavioural types within a population also offers a solution to the need for different behavioural traits at different invasion stages – as a whole, the population possesses all the necessary traits (Wolf and Weissing 2012; Chapple et al. 2012).

Although personalities have been studied in a wide range of invertebrate taxa, our knowledge of invertebrate personalities remains limited relative to the diversity and numerical dominance of invertebrate taxa (Gherardi et al. 2012; Mather and Logue 2013). We also have a limited empirical knowledge of how behaviour influences invasion success, founded on speculation or few case studies (Chapple et al. 2012) that may be inconsistent (e.g. Hudina et al. 2014). Consequently, no comprehensive list of behaviours that generally affect invasion success and impact has been compiled (Phillips and Suarez 2012), although as argued above this could prove to be a useful predictive tool.

1.4.3 Resource use

Resource use is emerging as another major driver of success and impact of biological invaders. Although habitat or space can be considered a resource whose utilisation can affect invasion dynamics (e.g. Beggel et al. 2016), here I focus on depletion of a trophic resource: consumption of a primary producer by a herbivore, consumption of prey by a predator, nutrient depletion by a plant or mortality of hosts in the case of pathogens. In this sense, resource use describes some of the most fundamental ecological interactions. Resource use is also fundamental to the majority of invasion hypotheses in Table 1, for instance defining whether invaders are specialists or generalists, the niches they can exploit, their response to disturbance and how competitive they are (Dick et al. 2014). Thus, resource use is likely to contribute to success and impact in many invasions (examples given below) – but not all (Lagrue et al. 2014; Ercoli et al. 2015a).

Resource use may be intimately related to invasion success. Overall, successful invasive species have a higher rate of resource consumption than native analogues (Dick et al. submitted; McKnight et al. 2016). Species with a high rate of resource consumption are able to fuel rapid growth rates and early reproductive maturity, facilitating establishment and spread. High rates of nitrogen uptake in the invasive grass *Andropogon gayanus* fuel growth rates up to ten times those of native competitors – especially important to gain a pre-emptive advantage when recovering from fire damage (Rossiter-Rachor et al. 2009). In California, alien lumbricid worms *Aporrectodea trapezoides* have a stronger negative effect on food resources than native congeners, facilitating a greater relative growth rate when resources are abundant (Winsome et al. 2006). Alternatively, invaders might be efficient resource-users, allowing them to persist in poorer quality environments than natives (Funk and Vitousek 2007). In such environments, rapid resource use may actually be a disadvantage: low-resource environments cannot support the voracious alien *A. trapezoides* (Winsome et al. 2006). Successful invaders could also be flexible in their resource use, allowing them to establish and proliferate even in the absence of their preferred resource. For example, establishment of rose-ringed parakeets *Psitticula krameri* may

be facilitated by opportunistic use of bird feeders (Clergeau and Vergnes 2011), and strong inter-population variation in niche breadth may contribute to the invasive success of *D. villosus* (Koester et al. 2016). Across bird families, dietary breadth is a significant (if weak) predictor of invasion success (Cassey 2002).

Impact may similarly be defined by invader resource use. When a native species is the resource (e.g. as prey for a predator), consumption rate and impact are clearly linked. Following signal crayfish invasion, macroinvertebrate community composition changes and taxa such as Hirudinea, Gastropoda, Trichoptera and Ephemeroptera decline, and direct predation is likely a causal factor (Mathers et al. 2016). Clearly, when the affected native species are commercially exploited or are an important food resource, this ecological impact translates into a socioeconomic one (e.g. decline in tilapiine fishery in the African Great Lakes following Nile perch *Lates niloticus*; Ogutu-Ohwayo 1990).

Alternatively, invaders can have an impact if they consume the same resource as native species i.e. there is competition. Rapid consumption of a shared resource by a strongly competitive invader (with a low R*; Tilman 1982) could negatively impact native competitors. For example, high rates of resource use by alien *A. trapezoides* may lead to competitive exclusion of native *A. marmoratus*, as resources are driven to levels lower than those which support *A. marmoratus* growth (Winsome et al. 2006). Similarly, invasive zebra mussels *Dreissena polymorpha* consume phyto- and microzooplankton at a greater rate than native bivalves in the Hudson River, and this reduced food density has negative effects on populations of competing macrozooplankton and unionid bivalves (Strayer et al. 1999). Such indirect effects of invader consumption are likely to cascade even further, affecting the structure and function of entire ecosystems.

Impact may also depend on the qualitative patterns of resource use (Ehrlich 1986; Shea and Chesson 2002). A broad diet in an invader means multiple species will be directly impacted. This could reduce the impact on any one food species (the food species share the burden of predation), or intensify impacts as the population of the predator is sustained when any single food source is depleted or unavailable. The ecosystem impact of an invader could also be defined by its dietary pattern. Crayfish predation on invertebrate detritivores can reduce leaf litter decomposition rates in a trophic cascade, but when crayfish also directly consume leaf material this cascade is reduced to a "trickle" (Jackson et al. 2014).

In addition to its likely generality in explaining invasion success and impact, resource use has great potential as a predictive tool for invasions because it is quick and easy to measure. It can give reliable results even when context-dependencies are ignored, or can be adapted to explicitly

include context dependencies (e.g. environmental conditions or resource species). Resource consumption is best quantified as a functional response (FR) – the relationship between availability of a resource and consumption of that resource (Holling 1959) – because this avoids errors associated with choosing a single starting resource density (Dick et al. 2014). There is growing support for the use of FRs to understand and predict invasive species' impacts (Haddaway et al. 2012; Dick et al. 2013; Dodd et al. 2014; Paterson et al. 2014). In Chapters 2 and 3, I use the FR methodology to predict the impacts of invasive Crustacea, whilst using existing knowledge of impact to assess the efficacy of FRs as predictive tools. These Chapters also provide case studies of whether invaders do consume more resources than natives.

1.5 Study systems

In this section, I outline the study systems used in this thesis: decapod Crustacea, amphipod Crustacea, and protists. The Crustacea were chosen to allow comparison of important invasive (widespread and abundant) alien species to resident analogues, and thus answer questions of applied significance. Protists were used in Chapter 6 to investigate propagule pressure: a major driver of invasions that could not be assessed using relatively large Crustacea with long lifespans.

1.5.1 Freshwater Crustacea

Despite covering just 1% of the Earth's surface, fresh water ecosystems are incredibly important. They contain approximately 10% of all known species (Strayer and Dudgeon 2010) and provide numerous ecosystem services (Aylward et al. 2005). However, they are also amongst the ecosystems most threatened by human activity (WWF 2014). Along with land use- and climate change, biological invasions are one of the leading threats to freshwater biodiversity (Sala et al. 2000). The incidence of invasions in fresh waters is particularly high. Intense human activity around fresh waters facilitates high propagule pressure and the introduction of many different species; unique vectors such as ballast water facilitate indiscriminate and massive transport of propagules; and the connectedness of waterways facilitates efficient dispersal between habitats and basins (Sala et al. 2000). In addition, the impact of aliens in fresh waters is probably strong relative to the marine or terrestrial realms since resident biota are more isolated and thus evolutionarily naïve to novel invader archetypes (Cox and Lima 2006). However, our knowledge of the impacts of fresh water invaders is limited owing to research bias towards terrestrial biomes (Lowry et al. 2013).

Amongst invaders of fresh waters, crustaceans – and especially decapods – are some of the most important in terms of distribution and impact (Karatayev et al. 2009; Strayer 2010). In Great Britain, crustaceans constitute approximately 18% of the established alien freshwater species, or

24% of animals (Keller et al. 2009). Moreover, the range (and therefore impact) of invasive aquatic invertebrates is likely to increase further as the climate changes in the next 100 years (Bellard et al. 2013). A mechanistic understanding of success and impact of invasive Crustacea is thus particularly important, but severely lacking. Here, I focus on three species of invasive freshwater Crustacea that are abundant, widespread and amongst the most damaging in Europe (Laverty et al. 2015b) and the world (Lowe et al. 2004) – although their impacts remain to be fully quantified and understood.

1.5.1.1 Signal Crayfish Pacifastacus leniusculus *(Dana, 1852)*

Figure 1.2 Adult signal crayfish *Pacifastacus leniusculus*. Carapace length approximately 80mm. Image credit: Trevor Renals, published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

The signal crayfish *Pacifastacus leniusculus* is an astacid crayfish native to western North America. It was introduced deliberately to Sweden in 1959 and 1960 to boost crayfish stocks following decline of the native *Astacus astacus*, and from there has spread across Europe through a combination of intentional stocking, unintentional transport and autogenic spread (Souty-Grosset et al. 2006). It is now the most widely distributed alien crayfish in Europe, being established in 29 countries (Kouba et al. 2014), and has also been introduced to Japan (Souty-Grosset et al. 2006). It has been proposed that the autogenic spread of signal crayfish is driven by periodic dispersal of individuals from high-density populations (Peay and Rogers 1999; Hudina et al. 2014). In Chapter 5 I further investigate the behavioural mechanisms that might drive this spread.

The success and impact of signal crayfish in Europe has been facilitated by a favourable combination of propagule pressure, environmental characteristics and the biology of the crayfish (Section 1.4), with introduction effort and climate similarity to the native range being especially strong predictors of signal crayfish distribution (Capinha et al. 2013). Intense and widespread introductions in Sweden and Great Britain, owing to the commercial value of signal crayfish as a food product, likely contributed to the success of crayfish in these countries (Henttonen and Huner 1999). Other biological characteristics of the invading crayfish have helped it to overcome any biotic resistance and outcompete natives: diet and habitat flexibility, high fecundity, aggression, mate recognition ability and ability to reach high densities (Holdich et al. 2014; Tricarico and

Aquiloni 2016). The signal crayfish is also a vector for the crayfish plague fungus, *Aphanomyces astaci*, which has little pathogenic effect on signal crayfish, but is lethal to European crayfish such as *Astacus astacus* and *Austropotamobius pallipes*. Spillover of *A. astaci* to European crayfish contributes to rapid replacement by *P. leniusculus* (Dunn et al. 2008; Holdich et al. 2014).

Once established, signal crayfish are difficult to eradicate. The highest success rates are associated with extreme management such as biocide treatment or infilling of lentic systems (Peay et al. 2006). Population control, containment and mitigation of impact can be achieved with high intensity trapping (Moorhouse and Macdonald 2011; Moorhouse et al. 2014). Other eradication and mitigation options include male sterilisation, pheromone control, electrocution, emerging biological control agents (bacteria, viruses and fungi; Stebbing et al. 2012). Given the difficulty and expense of managing established crayfish populations, the prevention of new introductions through legislative control and biosecurity is highly desirable.

The ecological impacts of invasive *P. leniusculus* are complex. Crayfish are opportunistic omnivores, so *P. leniusculus* likely has impacts across multiple trophic levels through direct consumption, competition and trophic cascades. Native fish and crayfish may suffer due to competition with *P. leniusculus* for food (Wood et al. 2016) and shelter (Griffiths et al. 2004; Dunn et al. 2008). Direct consumption of macroinvertebrate prey can reduce abundance, biomass and alpha diversity, with soft-bodied or slow-moving prey (e.g. chironomids, Hirudinea and Gastropoda) being particularly vulnerable (Stenroth and Nyström 2003; McCarthy et al. 2006; Crawford et al. 2006; Hayes 2012; Mathers et al. 2016). Predation, in combination with competition and spillover of disease (*A. astaci*), has contributed to the replacement of native European crayfish by *P. leniusculus*, with potential changes in ecosystem function and service provision. However, the economic impact of native species loss may be minimal if the invader yields its own commercial fishery (Lodge et al. 2012). Changes in macroinvertebrate populations can have indirect effects: typically positive effects on primary production (Lodge et al. 1994) and negative effects on leaf litter breakdown, although crayfish detritivory can somewhat mitigate the latter effect (Jackson et al. 2014).

P. leniusculus also alters the physical ecosystem. Burrowing activity can reduce the integrity of river banks, inducing collapse (Holdich and Pöckl 2007). Movement and digging by crayfish alters the shape and structure of stream beds, and increases sediment transport and turbidity (Johnson et al. 2011; Rice et al. 2014). Increased sediment load, in combination with consumption and non-consumptive shredding, can reduce macrophyte density: a direct impact on flora with indirect impacts on animals that depend on plants for shelter (Twardochleb et al. 2013).

We do not fully understand the ecological impacts of signal crayfish. Context dependencies yield idiosyncratic results in field and mesocosm studies, and few studies directly compare the impact of native and alien crayfish. Changes in invertebrate communities could just reflect the addition of a large decapod predator, and it could be that signal crayfish are effectively a functional substitute for the native crayfish they replace (Ercoli et al. 2015a; James et al. 2015). In Chapter 2, I use FR and switching experiments to quantitatively compare predation by *P. leniusculus* on a range of macroinvertebrates (amphipods, chironomids and gastropods) to predation by European *Austropotamobius pallipes*.

1.5.1.2 Chinese mitten crab Eriocheir sinensis *H. Milne Edwards, 1853*

Figure 1.3 Sub-adult Chinese mitten crab *Eriocheir sinensis*. Carapace width approximately 30mm. In juveniles of this size, the 'fur' on the chelae that gives the crabs their common name is poorly developed. Image credit: Christian Fischer, published under a Creative Commons license https://creativecommons.org/licenses/by-sa/3.0/.

The mitten crab *Eriocheir sinensis* is native to the eastern and northern coasts of China, but has been introduced to western Asia, the USA and Europe. The principal established populations are in western Europe and on the west coast of the USA, in San Francisco Bay (Dittel and Epifanio 2009). The likely vector of introduction is ballast water, which may contain adults or juveniles, but since the crab is a Chinese delicacy deliberate introductions are also a possibility (Cohen and Carlton 1997). *E. sinensis* is catadramous: it migrates upstream in fresh waters as it matures, but returns to the sea to breed (Dittel and Epifanio 2009). Although this has interesting ramifications for impact (multiple ecosystems may be affected, and migrations represent transport of a large amount of biomass between systems), in this thesis I focus on the fresh water stages in comparison with fresh water crayfish.

High propagule pressure likely contributes to successful invasion and impact of mitten crabs. *E. sinensis* has a marine larval stage, which provides access to ballast water as a vector. In this way, large numbers of propagules can be transported over large distances. Abiotic conditions in the novel range are a crucial determinant of success and restrict the range of mitten crabs. Introduced crabs require access to water of high enough salinity for reproduction, which probably prevents

establishment in the Baltic Sea and the Mississippi Basin, despite records of introduced individuals (Rudnick et al. 2000; Ojaveer et al. 2007). Similarly, short estuary flushing times and cool sea waters (below the threshold for larval survival) limit the distribution of *E. sinensis* in the Pacific Northwest of North America (Hanson and Sytsma 2007). Traits of *E. sinensis* that could contribute to its success include high fecundity (multiple broods in each reproductive season of up to 1 million eggs; Panning 1939); its ontogenetic upstream migration which limits intraspecific competition; its competitive dominance (Gilbey et al. 2008); and its flexible, omnivorous diet (Rosewarne et al. 2016). Interestingly, the closely related *E. japonica* is not recognised as successful invader so could provide a comparator for clarifying invader traits (Dittel and Epifanio 2009) although that is beyond the scope of this thesis.

Research into control of mitten crabs is limited. Fyke nets are the most effective method for trapping crabs and controlling an established population, with possible economic benefits as the catch can be sold (Clark 2011). A combination of legislation – both international (IMO 2016) and local – and public education will minimise the risk of new introductions.

The ecological impacts of *E. sinensis* are less well understood than those of *P. leniusculus*. *E. sinensis* is also an opportunistic omnivore, so its impacts are probably similar to those of *P. leniusculus*. Gut content analyses, stable isotope analyses and mesocosm experiments indicate *E. sinensis* will consume plants, detritus and animals – especially slow-moving or soft-bodied ones – and this consumption can lead to changes in populations and community structure (Yu and Jiang 2005; Rudnick and Resh 2005; Czerniejewski et al. 2010; Rosewarne et al. 2016). However, *E. sinensis* also appears better able to handle more active prey such as amphipods (Mills et al. 2016) which is reflected in field diets (Rosewarne et al. 2016).

Competition is another mechanism by which mitten crabs can negatively impact resident taxa. *E. sinensis* is a better competitor for space than native British *Carcinus maenas* (Gilbey et al. 2008) and may outcompete introduced signal crayfish for shelter (Rudnick et al. 2000). Other impacts of *E. sinensis* include destabilisation of river banks through burrowing activity (a particular problem on Chiswick Eyot, London; pers. obs.; Clark 2011), and clogging of infrastructure (e.g. fishing nets, fish collection facilities, power station cooling water intakes), especially during mass breeding migrations (Panning 1939; Veldhuizen and Stanish 1999).

In Chapter 2, I use laboratory experiments to provide a mechanistic understanding of the predatory impacts of *E. sinensis* on a range of macroinvertebrate taxa. Predation by *E. sinensis* is compared to predation by native and invasive crayfish: functionally similar species that may exist together

(or replace each other through competition or predation) in fresh waters. Behavioural influences on success and impact of *E. sinensis* are explored in Chapter 4.

1.5.1.3 Killer shrimp Dikerogammarus villosus *(Sowinsky, 1894)*

Figure 1.4 Killer shrimp *Dikerogammarus villosus*. Body length approximately 20mm. The tail cones are characteristic of this genus. Image credit: NOAA, published under a Creative Commons license https://creativecommons.org/licenses/by-sa/2.0/.

Dikerogammarus villosus is an amphipod crustacean native to the Ponto-Caspian region of south-eastern Europe. It has spread north-westerly through Europe through the network of rivers and canals that now forms a continuous channel through the continent (Bij de Vaate et al. 2002; Rewicz et al. 2014). *D. villosus* crossed the English Channel and was detected in Grafham Water, Cambridgeshire, in 2009 (MacNeil et al. 2010). Spread through Europe has likely been facilitated by accidental transport on commercial or recreational equipment, and may be assisted by natural vectors such as birds. Further, accidental intercontinental transport (e.g. to the American Great Lakes) is now highly likely (Pagnucco et al. 2014).

Behavioural traits are likely to be a key driver of the success and impact of *D. villosus*. Arguably, *D. villosus* possesses a suite of traits that make it the "perfect invader" (Rewicz et al. 2014): fast growth, early sexual maturity, a long breeding season and flexible feeding behaviour. Furthermore, *D. villosus* shows low levels of activity and low exploratory tendencies (Truhlar and Aldridge 2014) as well as an ability to remain attached to substrates and survive out of water for up to 15 days (Fielding 2011; Bacela-Spychalska et al. 2013a): behaviours that contribute to effective passive transport on human vectors (e.g. boat hulls, recreational equipment). Consequently, successful *D. villosus* invasions tend to occur in waters at transport or recreational hubs – where propagule pressure is highest (Bacela-Spychalska et al. 2013a). *D. villosus* may also benefit from release from the negative fitness effects of natural enemies following invasion bottlenecks (Arundell et al. 2015), or from co-invasion with other species from its native Ponto-Caspian range (Devin et al. 2003; Beggel et al. 2016).

Again, the best management strategy for *D. villosus* invasions is prevention. A range of chemical treatments and hot (45ºC) water have been shown to be effective in killing *D. villosus* in quick biosecurity procedures (Stebbing et al. 2011). Knowledge of physical, chemical and biological eradication methods is limited but research is ongoing (Stebbing et al. 2012a; Aldridge et al. 2015).

D. villosus has been nicknamed the 'killer shrimp' in accord with its tendency to rapidly consume a wide range of macroinvertebrate taxa in the laboratory (Dick et al. 2002; Platvoet et al. 2009; Dodd et al. 2014) and evidence from stable isotope analyses in the field (van Riel et al. 2006). Dominance of *D. villosus* in invaded ecosystems, at the expense of native amphipods, could strongly affect ecosystem functioning. In particular, leaf litter processing may be reduced up to 11-fold (Piscart et al. 2011; MacNeil et al. 2011; Boeker and Geist 2015) – although this relationship is context-dependent and may be reversed in extreme water temperatures (Truhlar et al. 2014).

Predatory behaviour is likely to contribute to the strong impacts of *D. villosus* on macroinvertebrate abundance and biomass in the field, especially isopods, tubificids and resident amphipods (Dick and Platvoet 2000; Dick et al. 2002; Kley and Maier 2003; Josens et al. 2005; Gergs and Rothhaupt 2015). There is potential for *D. villosus* to cause analogous declines in fish populations of conservation or commercial concern, but this is poorly quantified. In Chapter 3, I compare predation of fish eggs and larvae by *D. villosus* to predation by an amphipod native to Great Britain, *Gammarus pulex* (L. 1758). Maximum predatory impact is quantified using FRs, and electivity experiments are used to assess predation in the presence of alternative foods.

1.5.1.4 Analogues

The success and impact of alien species can be understood through comparisons with native or resident analogues, with the precise question answered depending upon the choice of analogue (van Kleunen et al. 2010; Dick et al. 2014). As an analogue for the invasive decapods, I used the white-clawed crayfish *Austropotamobius pallipes* (Lereboullet, 1858). *A. pallipes* is native to mainland Europe and is considered native to Great Britain following the IUCN threshold of presence in the wild before 1500. However, it is not clear whether populations of *A. pallipes* in Great Britain are a result of natural migration or historical anthropogenic introduction circa 1500 CE (Holdich et al. 2009). In any case, it is important to compare the *impact* of alien decapods to the incumbent to understand how communities and ecosystems might change following invasion. Meanwhile, traits conferring invasion *success* can be inferred through comparisons between

successful invaders and a rare and declining resident species (van Kleunen et al. 2010): *A. pallipes* is listed as Endangered on the IUCN Red List (Füreder et al. 2010).

In contrast, the native analogue for alien amphipods in Britain is *Gammarus pulex* (L. 1758). *G. pulex* is widespread and common in British fresh waters, and is a successful invader elsewhere, displacing native amphipod populations in Ireland (Kelly et al. 2006). Thus, comparisons between *G. pulex* and invasive amphipods in Britain would reveal traits that make the novel invaders so dominantly invasive, but cannot necessarily reveal general determinants of invasion success at a global scale (van Kleunen et al. 2010). Equally, assessments of relative impact are made with Great Britain as the focus.

1.5.2 Ciliated protists

In order to investigate the role of propagule pressure in invasion success and impact – an important factor that could not be ignored – I used a distinct study system. Manipulation of protist populations in the laboratory allows (a) controlled experimentation, as opposed to correlative investigations of propagule pressure where invasion success is confounded with multiple other variables (Cassey et al. 2004); (b) observation of invasion dynamics over long time scales from the perspective of the organisms involved i.e. tens to hundreds of generations; and (c) replication of identical systems to identify patterns without the context-dependencies associated with invasions in the field (Warren et al. 2006). Results from laboratory microcosm experiments may not generalise to other systems, but the advantages they offer make them a useful complement to other approaches (e.g. field experiments and observations) and on other organisms (metazoan animals and multicellular plants) (Warren et al. 2006). However, understanding the dynamics of microbial invasions themselves is useful, given the likely prevalence of microbial invasions and their negative impacts (Gillis and Chalifour 2010; Litchman 2010; Acosta et al. 2015). Understanding such invasions has wide-ranging implications from disease control to biocontrol, biofertilisation and probiotic use (Hatcher et al. 2012; Mallon et al. 2015).

In Chapter 6 I use two species of ciliated protist, *Blepharisma japonicum* Suzuki 1954 and *Colpidium striatum* (Stokes), as reciprocal invaders to quantify the influence of propagule pressure on invasion success and impact. These species are not known to be damaging invasive species in the wild, but rather serve as exemplar organisms introduced to a novel community.

Both *B. japonicum* (*Blepharisma*) and *C. striatum* (*Colpidium*) will feed upon bacteria, but phenotypic plasticity in *Blepharisma* allows it to obtain larger sizes and prey upon smaller protists, such as *Colpidium* or conspecifics, in suitable conditions (Giese 1938). Thus, although

the two species can coexist in a stable community (Warren et al. 2003), this coexistence depends on nutrient enrichment (Diehl and Feissel 2000; Diehl and Feissel 2001). At low enrichment levels, *Colpidium* outcompetes *Blepharisma* but at high enrichment levels there are spare resources, unused by *Colpidium*, which allow *Blepharisma* to establish and then prey upon and exclude *Colpidium*. Meanwhile, at intermediate enrichment levels and moderate propagule pressures (c. 30 cells), establishment success of each protist in a population of the other is variable: *Colpidium* exhibits rapid positive growth when invading *Blepharisma*, whilst *Blepharisma* variously grows or declines when introduced to populations of *Colpidium*, and does so slowly (Law et al. 2000). Together, these characteristics provide a good system to investigate the role of propagule pressure in invasion success, which should lead to variable outcomes.

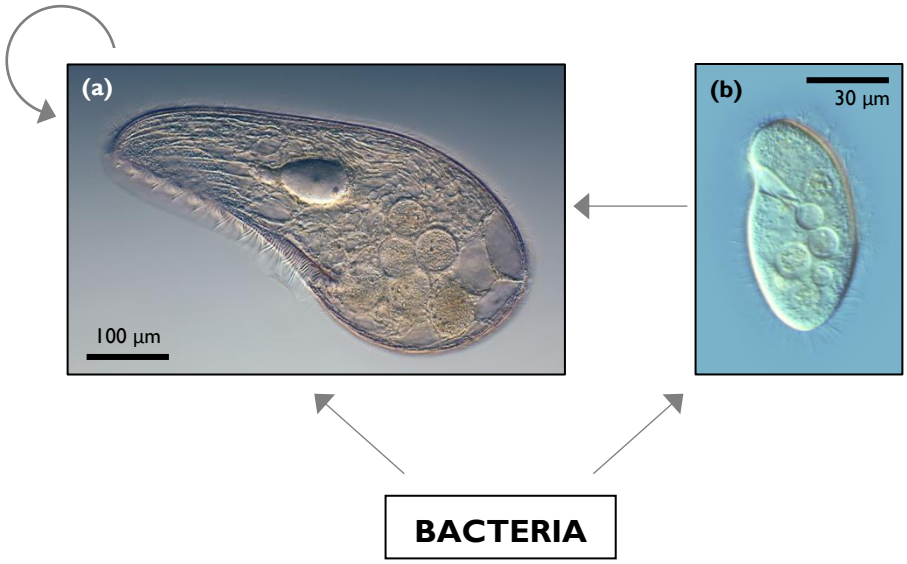


Figure 1.5 Simple food web in microcosm experiments (Chapter 6) **(a)** *Blepharisma japonicum*, a large omnivorous ciliate. Approximate length 500µm. **(b)** *Colpidium* sp, a small bactivorous ciliate. Approximate length 80µm. Arrows show flow of nutrients. Both protists consume bacteria, whilst *Blepharisma* acts as an intraguild predator by preying upon *Colpidium*. Large morphs of *Blepharisma* can also be cannibalistic.

Image credits: *Blepharisma* Frank Fox, published under a Creative Commons license https://creativecommons.org/licenses/by-sa/3.0/de/deed.en; *Colpidium* Proyecto Agua, published under a Creative Commons license with some rights reserved https://creativecommons.org/licenses/by-nc-sa/2.0/.

1.6. Research aims and thesis plan

In this thesis, I investigate in detail three major factors that can affect invasion success and impact as outlined above – propagule pressure, behaviour and resource use. These factors could provide a conceptual basis for tools to understand and predict invasions. Figure 1.6 gives an overview of the thesis Chapters and how they relate to the major factors. I focus on success and impact in

study systems of applied interest. Understanding the success and impact of specific alien taxa is important in itself, for instance in informing management (Kumschick and Richardson 2013). Even if an invader cannot be managed, it is important to understand how it is changing its recipient, novel ecosystem. In addition, it is anticipated that this thesis also provides case studies for an understanding of the invasion process in general. Amongst much context-dependency, there may be common factors driving success or impact across invasion scenarios (Kolar and Lodge 2001; Hayes and Barry 2007). Identifying these factors, and understanding any common relationships with invasions, would allow their use to make generalised predictions and risk assessments when information for specific taxa is not available (Kumschick and Richardson 2013; Thomsen et al. 2014).

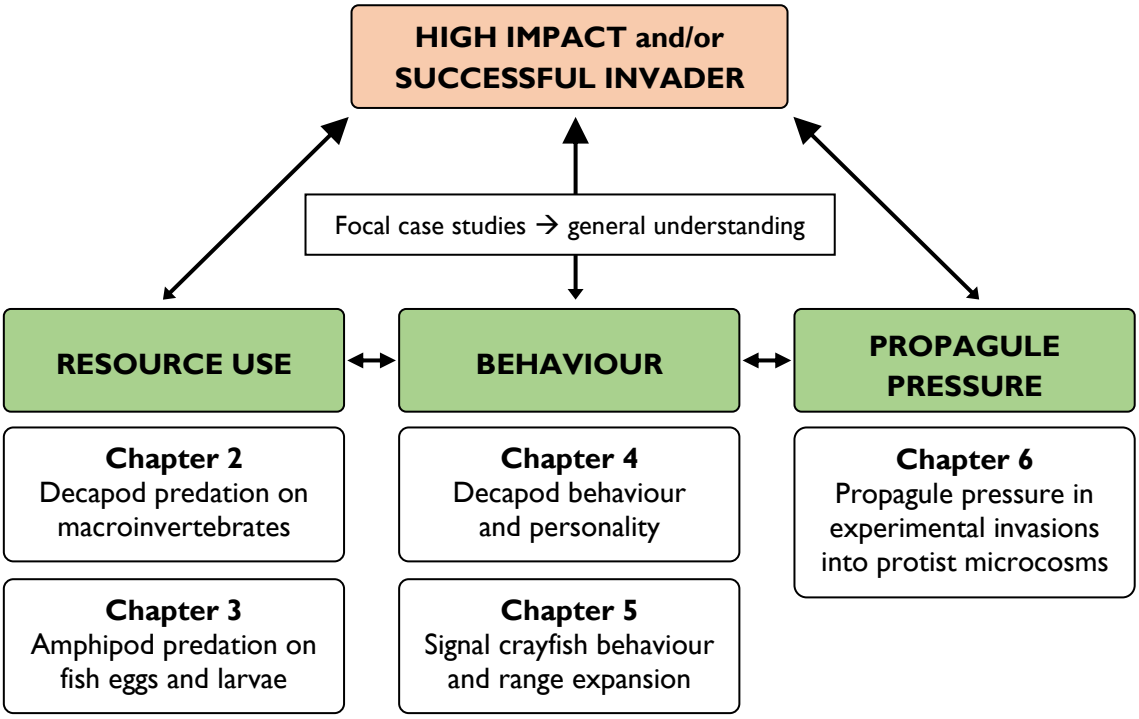


Figure 1.6 Overview of thesis chapters (*white boxes*) in the context of three major factors (*green boxes*) that may contribute to invasion success and impact. *Large upwards arrows* reflect the focal questions of this thesis, with implications for management of alien species: do these factors influence invasion success and impact, and if so, how? *Small reverse arrows* indicate that invasion and impact can feed back and influence driving factors e.g. an abundant invader will offer greater propagule pressure for new introductions. *Arrows between factors* indicate that they can influence each other e.g. propagule pressure may depend on behaviour (active and exploratory species more likely to be entrained in transport vectors), whilst the size of an initial propagule may affect behaviour of individuals within that propagule.

In Chapters 2 and 3, I compare resource consumption between alien invaders and native species. I apply tools (functional responses, electivity experiments and switching experiments) to understand the relative impacts of these species, and compare my results to known impacts to contribute to the development of these tools. Functional responses (FRs) are emerging as a reliable tool to make quantitative assessments of invader impacts (Dick et al. 2014), but require further verification with empirical data. Diet breadth, as measured in electivity and switching experiments, can also affect the impact of consumers (Ehrlich 1986; Shea and Chesson 2002). In Chapter 2, I compare the predatory impacts of native European *A. pallipes* and the aliens *P. leniusculus* and *E. sinensis* on three different macroinvertebrate prey (amphipod, chironomid and gastropod). In Chapter 3, I compare the predatory impacts and diet composition of alien *D. villosus* and native *G. pulex*, with fish eggs and larvae as the focal prey. In Chapter 2, I also measure metabolic rates as a potential underlying mechanistic explanation, and predictive tool, for differences in resource consumption.

In Chapters 4 and 5, I quantify more general behaviours of successful, damaging invasive species. Behavioural traits may help to explain success and impact, whilst behavioural assays could provide simple tools to rapidly assess invasion and impact risk. In Chapter 4, I compare behavioural traits (boldness, activity and exploration) in the declining European crayfish *A. pallipes* and the successful invaders *P. leniusculus* and *E. sinensis*, to explore associations between behaviour and success/impact across species. In Chapter 5, I focus on the invasive signal crayfish and examine if and how behavioural traits might drive dispersal dynamics and contribute to spread. I compare the behaviour of crayfish from established populations and those on the invasion front in three rivers. In this Chapter, I also quantify correlations between behaviour and metabolism – which, in Chapter 7, I combine with similar data from Chapter 2 to suggest a general metabolic explanation for invasions. In both Chapters 4 and 5, I quantify consistency of individual behaviour (personality), which is poorly understood in invertebrates (Mather and Logue 2013) but can have important implications for invasion success and impact (Wolf and Weissing 2012).

Chapter 6 switches focus, manipulating propagule pressure in protist invasions into laboratory microcosms. This experimental approach allows close examination of the mechanisms that relate propagule pressure to invasion success and invader impact, which are poorly understood (Lockwood et al. 2005; Blackburn et al. 2015). Specifically, I consider a wide range of propagule pressures, their interaction with nutrient enrichment and robustness of patterns across different invader-resident combinations.

Chapter 2

Predatory impacts of freshwater decapod Crustacea: predicted by functional responses and explained by differences in metabolic rate

Abstract

With an ever increasing number of alien species around the globe, there is a pressing need to understand and predict the impacts of invaders. Here, I compare the predatory impacts of three freshwater decapod crustaceans: invasive alien mitten crabs *Eriocheir sinensis*, invasive alien signal crayfish *Pacifastacus leniusculus* and the analogous European *Austropotamobius pallipes*. I quantify predatory functional responses (the relationship between prey density and consumption by predators) on three macroinvertebrate prey of differing morphologies and physical defence, and examine the potential of each predator to switch between prey items as their relative availability changes. I also measure oxygen consumption as a proxy for metabolic rates, which could provide a mechanistic explanation for differences in predation.

The overall pattern of FRs was consistent across prey species. *E. sinensis* had the highest attack rate (a) and maximum feeding rate ($1/hT$), and these parameters were higher for *P. leniusculus* than *A. pallipes*. The magnitude and significance of these differences was variable, however. *E. sinensis* had an exceptionally high FR on soft-bodied prey (up to 3.0 times that of the crayfish), but a similar FR to the crayfish on hard-shelled gastropod prey. The maximum feeding rate of *P. leniusculus* was only significantly greater than that of *A. pallipes* on chironomid larvae prey. The direction and size of these differences are concordant with impact predictions based on other methodologies. Further, they may be related to differences in activity: *E. sinensis* had a greater mass-corrected routine metabolic rate than *P. leniusculus*, which in turn consumed more oxygen than *A. pallipes*. Standard metabolic rate did not differ between the decapods. There was no evidence for switching by any of the decapod species, although this may have been difficult to detect given the strong null electivity of all predators towards *D. villosus*.

My data suggest *E. sinensis* could have a very strong predatory impact, even relative to the other invader *P. leniusculus*. Impacts of *P. leniusculus* may be driven by body size or abundance more than *per capita* effects. Relative FR magnitude is dependent on prey type and matches existing knowledge of invader impacts, supporting the use of FRs for quantitative, prey-specific impact predictions.

2.1 Introduction

As a consequence of globalisation and the breakdown of biogeographic barriers, alien species are prevalent in the modern world. Alien species have been transported by humans to biogeographical regions beyond their native range. A subset of aliens have negative economic and ecological impacts, including being one of the major drivers of biodiversity loss and affecting ecosystem service provision (Sala et al. 2000; Pejchar and Mooney 2009). Strategic management of ecologically damaging alien species is written into international (e.g. SCBD 1992; EU 2014) and national (e.g. DEFRA 2015) policy, but informed management decisions must be based on evidence of impact. All else being equal, limited management resources should be directed towards the most damaging, or potentially most damaging, invaders (Ricciardi 2003; Lodge et al. 2012; Kumschick et al. 2012; Kumschick and Richardson 2013).

Resource use is a key driver of success and impact in alien species, such that comparisons of resource use can further understanding and prediction of success and impact (Catford et al. 2009; Dick et al. 2014). Resource consumption by predators should be a particular focus: predators can have strong impacts on prey populations (Salo et al. 2010), especially when the predator is an alien and can exploit naivety of native prey (Cox and Lima 2006; Salo et al. 2007). Thus, deleterious ecological impacts of alien species are often driven by predation (Davis 2003; Sax and Gaines 2008). As well as affecting prey populations directly, alien predators can have indirect impacts on community structure and ecosystem functioning (Paine 1966; Balčiūnas and Lawler 1995; Baum and Worm 2009; Jackson et al. 2014).

Specifically, the impact of an alien predator can be determined by the magnitude of predation on any particular prey type and the range of resources used (Grosholz 2005; Salo et al. 2007; Dick et al. 2013). Predatory interactions can be described by a functional response (FR) – the relationship between prey density and predation rate (Solomon 1949) – with both the height and shape of the FR curve being important. High FR curves reflect high rates of resource consumption and therefore high *per capita* impacts of a particular predator on a particular prey species (Dick et al. 2013; MacNeil et al. 2013b; Alexander et al. 2014; Dodd et al. 2014; Rosewarne et al. 2016). Asymptotically-declining Type II FRs are likely to be associated with the most severe impacts on prey because predation pressure remains high even at low prey densities (Murdoch and Oaten 1975; Juliano 2001). In contrast, sigmoid Type III FR curves are associated with a reduction in predation pressure as a prey type becomes rare. That is, rare prey are attacked disproportionately less often than would be expected based on their relative abundance, leading to a low density refuge from predation (Murdoch 1969). This switching behaviour stabilises predator-prey dynamics, maintaining populations of prey at moderate densities. In the context of invasions,

switching may spread predatory impact across multiple prey species and temper the impact on any single prey species.

Fresh waters are particularly susceptible to alien introductions (Richter et al. 1997; Sala et al. 2000), and the impacts of alien predators in fresh water are especially strong (Cox and Lima 2006). Decapod crustaceans are amongst the most widely-distributed and high-impact invaders of fresh waters (Karatayev et al. 2009; Strayer 2010) and, as flexible omnivores, impart impacts through predatory behaviour. Globally, two of the most successful and damaging alien decapods are the signal crayfish *Pacifastacus leniusculus* and the Chinese mitten crab *Eriocheir sinensis*. Both species are biologically invasive, having spread across a large area outside their native range and reaching high densities, and both are thought to have serious ecological or economic impacts (Lowe et al. 2004). *P. leniusculus* is native to North America, but has been introduced and become an pest across much of Europe (Souty-Grosset et al. 2006). Following transport from its native range in the north-western Pacific, *E. sinensis* has been recorded in multiple sites around the world, with key established populations on the west coast of the USA and in Europe (Gollasch 1999; Dittel and Epifanio 2009).

The white-clawed crayfish *Austropotamobius pallipes* is native to Europe and has long been the only decapod crustacean in the fresh waters of Great Britain (Holdich et al. 2009). The distribution of *A. pallipes* in Britain has reduced substantially in association with the spread of *P. leniusculus* since 1976 (Imhoff et al. 2011). More recent advancement of *E. sinensis* populations has created zones of overlap with *P. leniusculus* (Rosewarne et al. 2016), and sympatry between *E. sinensis* and *A. pallipes* is also possible (Clark et al. 1998). It is important to understand the relative ecological impacts of these species to inform risk assessments and management decisions, or simply to understand how communities might change as the invaders replace or coexist with an incumbent decapod – whether that is *A. pallipes* or one of the other invaders. Invasion by *P. leniusculus* can change community structure, through a combination of competition, disease transmission and resource consumption (Crawford et al. 2006; Dunn et al. 2008; Twardochleb et al. 2013; Ercoli et al. 2015b; Mathers et al. 2016). Evidence from mesocosms and field manipulations suggests *E. sinensis* may cause similar declines in macroinvertebrate populations through predation (Yu and Jiang 2005; Rudnick and Resh 2005; Rosewarne et al. 2016). However, our knowledge of these predatory impacts and their underlying mechanisms remains incomplete, especially for *E. sinensis* (Veldhuizen and Stanish 1999; Rosewarne et al. 2016).

Here, I aim to assess the relative predatory impacts of native and alien decapod crustaceans: *A. pallipes*, *P. leniusculus* and *E. sinensis*. First, I compare laboratory-derived functional responses between the three predator species on three prey types of differing morphology and behaviour (an

amphipod crustacean, chironomid larvae and a gastropod mollusc). Predatory impacts may vary among prey species (Moustahfid et al. 2010; Dodd et al. 2014) such that assessing functional responses across a variety of prey species is important (Dick et al. 2014). Second, I examine the tendency of the predators to switch between the similar-sized gastropod and amphipod when presented at varying relative densities. I hypothesise that the alien species will have higher FR curves than *A. pallipes* based on current evidence of impact, and will show a greater tendency to switch between prey since diet flexibility may be a common trait of successful invasive species (Moyle and Light 1996). Third, I investigate possible mechanistic explanations for differences in resource consumption by comparing metabolic rates (derived from oxygen consumption rates) between the three decapod species. Metabolic rates and food consumption should be positively associated: high MRs should necessitate and/or facilitate high consumption rates (Careau et al. 2008; Biro and Stamps 2010).

2.2 Methods

2.2.1 Experimental animals and husbandry

Decapods were collected from established populations between 2013 and 2015. *A. pallipes* were collected from Adel Beck, Leeds, West Yorkshire (lat 53°52'N, long 1°35'W) under license from Natural England (#20131266 and #20144477). *P. leniusculus* were collected from Fenay Beck, Huddersfield, West Yorkshire (lat 53°39'N, long 1°44'W). *E. sinensis* were collected under agreement with the Port of London Authority from the River Thames, Chiswick, London (lat 51°29'N, long 0°15'W). All animals were collected by hand by searching refugia. The three experiments (FR, switching and oxygen consumption) were run at different times on different batches of animals (but all three species were tested simultaneously within each experiment).

Decapods were kept in a controlled environment room in the University of Leeds, at 14 ± 0.2ºC and 12:12h light:dark cycle. Stock tanks were communal by species, contained aerated aged tap water and excess PVC piping (10 cm length, 5 cm diameter,) as shelter. Stock tanks were maintained on a diet of Hikari® Crab Cuisine™ pellets and dried leaf litter (abscised *Acer pseudoplatanus* L. leaves) *ad libitum*. Animals were held for at least two weeks in the laboratory before use in experiments in order to monitor their moulting status, allow for acclimation to laboratory conditions and reduce the influence of any wild environmental cues (e.g. tidal cycles for *E. sinensis*; Gilbey et al. 2008).

Decapods used in experiments were sub- or young-adults (Brewis and Bowler 1982; Rudnick et al. 2003; Haddaway et al. 2012). Across all experiments, mean ± SE masses of decapods were: *A. pallipes* 10.6 ± 0.4 g; *P. leniusculus* 10.5 ± 0.3 g and *E. sinensis* 12.7 ± 0.4 g. Mean ± SE

maximum carapace dimensions (cmax; carapace length for crayfish and width for crabs) of decapods were: *A. pallipes* 32.3 ± 0.4 mm; *P. leniusculus* 32.7 ± 0.3 mm and *E. sinensis* 31.0 ± 0.3 mm. Both mass and body dimensions can affect predatory impact (Nilsson and Brönmark 2000; Rall et al. 2012). Therefore, I used *E. sinensis* that were slightly heavier and had a shorter cmax than the crayfish to account for different body plans of crabs and crayfish. In this way, within each experiment decapods were matched by 'body size' (Table 2.1): the first principal component from a principal component analysis on body mass and cmax of all individual decapods used in experiments, which explained 88.6% of the variance in these parameters. Rarefaction of data sets to match body mass within experiments yielded similar results (see Appendices 2.2 and 2.4).

Table 2.1 Statistical comparisons of body size of decapod species used in each experiment. Body size derived from PCA of mass and maximum carapace dimension (cmax). For FR experiments, each usage of an animal contributes its body size to the data set (so size data are weighted for the number of times a predator was used).

Experiment	**Kruskal Wallis χ^2**	**df**	***p***
FR (Amphipod prey)	1.135	2	0.567
FR (Chironomid larva prey)	1.400	2	0.497
FR (Gastropod prey)	0.796	2	0.672

Experiment	**ANOVA *F***	**df**	***p***
Switching	0.078	2,92	0.925
Metabolism	0.122	2,27	0.885

Animals of both sexes were used in experiments. I do not anticipate that the mixture of sexes influenced my results given that behaviour, including feeding, is frequently reported to be similar for both sexes (Chapter 4; Briffa et al. 2008; Brodin and Drotz 2014; Webster et al. 2015); sample sizes were too small to make meaningful comparisons between sexes in the present study. Decapods were monitored before and after experiments: no decapods moulted within a week of use in any experiment. All decapods used were in good condition (all limbs intact, no injuries to body) and free of visible parasites (Souty-Grosset et al. 2006).

For feeding experiments, three different prey species were used, chosen to represent differing motility and physical defence. *Dikerogammarus villosus* were collected from Grafham Water, Cambridgeshire (lat 52°17'N, long 0°19'W). *Bithynia tentaculata* were sourced from laboratory stocks, originating from various ponds and canals around Leeds. Chironomid larvae were sourced

from a pet retailer in Leeds. The decapod predators used in this experiment are known to consume prey in these orders (Hymanson et al. 1999; Rosewarne et al. 2013; Rosewarne et al. 2016), and preliminary observations confirmed consumption of these specific taxa. Because prey size can affect functional responses (Streams 1994), prey items were size-matched by eye. Throughout the experiment, subsamples of prey items were selected and measured to check consistency in size (Table 2.2).

2.2.2 Functional response experiments

2.2.2.1 Experimental design

Crayfish and crabs were isolated in individual plastic tanks (23 cm length, 15 cm width, 8 cm depth) with translucent white lids and sides covered in black plastic to minimise visual disturbance. Each tank received a constant flow of air through an air stone, and contained one black PVC shelter (10 cm length, 5 cm diameter). Isolated animals were fed a standardised diet (four Hikari® Crab Cuisine™ pellets every other day) for at least one week before trials began. Each animal was fed 48 hours before a trial began and then starved (by changing the water) 24 hours before a trial began.

One hour before each trail began, separate experimental tanks were set up containing three litres of aged tap water, approximately 150 glass stones (1 cm diameter) and a designated number of prey animals (Table 2.2). Prey densities were concentrated at the lower end of the range to allow better identification of FR shape, but were extended far enough such that all FR curves approached an asymptote. The stones provided habitat structure: a more realistic scenario in which to assess predatory behaviour, and a factor that can alter the shape of functional responses (Alexander et al. 2012). The one hour acclimation period, without a predator, allowed prey to settle and find refuge.

Table 2.2 Sizes and densities of prey supplied to predators in functional response experiments. Mean lengths and masses estimated from a random sample of 30 prey items across replicate runs. *E. sinensis* and *P. leniusculus* were also supplied with chironomid larvae at densities of 140, 300 and 800.

Prey Type	Length (mm) ± SE	Wet mass (mg) ± SE	Densities (prey.tank^{-1})
Amphipod	16.34 ± 2.98	46.84 ± 8.55	2, 5, 8, 12, 16, 25, 40, 80, 130, 180, 230, 280
Chironomid larva	8.72 ± 0.22	2.77 ± 0.17	2, 5, 8, 12, 16, 25, 40, 80, 220, 400, 600, 1200
Gastropod	9.44 ± 0.10	52.77 ± 1.56	2, 4, 8, 12, 16, 25, 40, 80, 150, 250

Experiments were run in the same controlled environment room as the stock tanks at 14 ± 0.2°C and with a 12:12h light:dark cycle. Trials were initiated by transferring decapods from their home tanks to their respective experimental tanks. Predators were left to consume prey for 24 hours, after which the tank was destructively sampled and remaining prey enumerated. I distinguished live prey, dead but complete prey, and identifiable parts of prey. Consumption was calculated as the number of prey supplied minus all remaining flesh (whole and damaged prey). Killing was defined as prey that had been wholly or partially consumed, as opposed to dead but undamaged prey assumed to reflect background mortality. Controls, to check prey survival, were three replicate tanks (per prey type per density, excluding 1200 chironomids) without a predator.

Predators were re-used up to eight times at different prey densities (Haddaway et al. 2012; Rosewarne et al. 2016) until each prey density/predator combination was replicated five (*B. tentaculata* prey) or six times (chironomid and *D. villosus* prey). Re-use was a constraint enforced by the use of *A. pallipes*, because the use of fresh individuals of this Endangered species for every trial would have been irresponsible and legally impossible. Uneaten and uninjured prey items were also re-used. Between uses, prey were returned to communal tanks and predators were fed a standard food ration (four Crab Cuisine™ pellets) and rested for 48 hours. Across replicate trials for any individual, the order of presentation of prey densities was randomised. Within each experimental block (carried out over two consecutive days), all predator species – prey density combinations were tested once. Together, randomised order of presentation but blocking replicates by time controlled for any potential changes in predator or prey behaviour over time and with experience.

For logistical reasons and because of seasonal prey availability, each prey item was tested over a one to two month period at different times of year (*D. villosus* Nov-Dec, chironomids Jan-Feb; *B. tentaculata* Jun-Jul). However, because I permitted experimental animals to acclimate to constant laboratory conditions, I believe that seasonal influences on the results are minimal. Moreover, the primary comparisons made are between predator species on each prey item, rather than between prey items. To reduce bias in allocating prey (e.g. size of prey chosen), prey items were counted out blind (the experimenter was unaware of the predator species that would receive those prey items).

2.2.2.2 Statistical methods

I conducted all FR analyses using number of prey consumed (based on total amount of flesh eaten, including complete and approximate partial consumption) and number of prey killed (all deaths caused by predation i.e. excluding undamaged prey assumed to represent background mortality)

as response variables. Consumption is the response variable of interest when comparing predator physiology, but when considering impact on prey populations, killing is the relevant response variable. Where partial consumption of prey is common, killing and consumption can be decoupled (Dick et al. 2002).

For each predator-prey combination, FR type was determined with a proportional consumption curve, describing the relationship between prey density and the proportion of prey consumed and defined by a GLM with linear and quadratic terms. Due to overdispersion, these models were fit with a quasibinomial error distribution. Significantly negative linear (first order) terms are suggestive of Type II FRs, whilst significantly positive quadratic (second order) terms indicate Type III FRs (Trexler et al. 1988; Juliano 2001). Where significance of terms gave ambiguous results (i.e. negative but non-significant first order terms), Type I and Type II fits to the data were compared using Akaike's Information Criterion (AIC), lower values of which indicate a better fit of the model to the data (Paterson et al. 2014).

Since FRs were Type II, I modelled the curves by maximum likelihood estimation, using Rogers' random predator equation (Equation 2.1, Rogers 1972) within the R package *frair* (Pritchard 2016).

$$N_e = N_O\left(1 - e^{a(N_e h - \mathrm{T})}\right) \qquad [2.1]$$

where N_e is the number of prey consumed or killed, N_0 is the initial density of prey, a is the attack constant, h is the handling time and T is the total time available for predation (24 hours). This model is appropriate for Type II FRs when prey are not replaced and thus deplete over the course of an experiment (Juliano 2001). To make Rogers' random predator equation solvable, *frair* employs a modified version of Equation 2.1 with the *Lambert W* function (Equation 2.2).

$$N_e = N_O - \mathrm{lambert}W\left(\frac{ahN_0 e^{-a(\mathrm{T} - N_0 h)}}{ah}\right) \qquad [2.2]$$

To visualise variability around the fitted curves, 95% BCa confidence intervals were drawn from bootstrap populations generated from the original data (*frair::frair_boot*; n = 1999).

Parameter estimates for a and h, the two essential parameters of the Rogers random predator equation, were obtained from fitted FR curves for each predator-prey combination and then compared using indicator variables (*frair::frair_compare*; Paterson et al. 2014; Pritchard 2016). An implicit function containing the indicator variables is generated (Equation 2.3).

$$0 = N_O - N_O^{\{[a+D_a(j)]\ \{h+D_h(j)]\ (N_e)-T\}\}} - N_e \qquad [2.3]$$

where *j* is an indicator variable, given a value of 0 for one base species and 1 for the comparator species. The parameters *Da* and *Dh* estimate the differences between the species for the *a* and *h* parameters respectively. Thus, each parameter is significantly different between populations if the corresponding *D* value is significantly different from 0 (Juliano 2001). Because multiple pairwise comparisons were made, significance was considered after Holm-Bonferroni correction of *p* values (Holm 1979).

2.2.3 Potential for switching

2.2.3.1 Experimental design

The potential for predators to switch between alternative prey items depending on their density was investigated by presenting predators with *D. villosus* and *B. tentaculata* at a range of relative abundances. These prey items were chosen because individual prey items are roughly similar in size (Table 2.2) and will not prey upon each other.

Switching experiments followed the same protocol as FR experiments (isolation and feeding, settlement of prey items in tanks with habitat structure, same temperature and light regime), except that two prey types were presented simultaneously at a fixed starting density. 280 individual prey were added to tanks at a ratio of 0.15:0.85, 0.35:0.65, 0.50:0.50, 0.65:0.35 or 0.85:0.15 (n = 5 at each density for *A. pallipes*, n = 6 for *P. leniusculus* and n = 8 for *E. sinensis*). The extreme values (0.15:0.85) were chosen to ensure some individuals of both prey species remained in tanks at the end of every trial. As a further difference to the FR experiments, three and two days before use in the experiment, each animal was allowed to feed on 10 *D. villosus* and 10 *B. tentaculata*. Only individuals that consumed each prey type were used in switching experiments, such that all individuals had recent experience feeding on both prey types and were actively feeding – and individuals were only used once. As for FR experiments, following a feeding period of 24h tanks were destructively sampled, remaining prey enumerated, and consumption and killing calculated. Five controls, with no decapod predator, were run at the intermediate density (140 *D. villosus* and 140 *B. tentaculata*) to check prey survival.

2.2.3.2 Statistical methods

First, total consumption of prey items (number of individuals) was compared between decapod species, using a quasipoisson GLM and post-hoc Tukey contrasts with Holm-Bonferroni adjustment of *p* values (*multcomp::glht*; Hothorn et al. 2016).

Switching is defined as a change in electivity towards prey types as their relative densities change (Murdoch 1969). That is, as relative prey densities change they are consumed more (or less) than would be expected based on consumption when they are equally common.

To test for switching by each predator species, I used χ^2 tests (with Yates' continuity correction; R function *prop.test*) to compare the observed population proportion of *D. villosus* in the diet (P_{Dv}) at each relative prey density to the expected proportion, obtained from Equation 2.4 (Murdoch 1969). If the proportion of *D. villosus* in the diet is lower than expected when *D. villosus* is rare, but higher than expected when *D. villosus* is common, then switching will have occurred.

$$P_{Dv} = \frac{cF_{Dv}}{1 - F_{Dv} + cF_{Dv}} \qquad [2.4]$$

where F_{Dv} is the proportion of *D. villosus* in the food available, and *c* is electivity towards *D. villosus* (Equation 2.5).

$$c = \frac{N_{Dv}}{N_{Bt}} \qquad [2.5]$$

where *N* is the number of prey consumed (with subscripts *Dv* and *Bt* referring to *D. villosus* and *B. tentaculata* respectively) when prey items are equally available. *A. pallipes* did not consume any *B. tentaculata* in this situation, so a value of $N_{Bt} = 1$ was used to allow calculation of *c*. I describe *c* as electivity rather than preference, as it does not necessarily depend on a behavioural 'choice' by the predator (Murdoch 1969; Underwood et al. 2004).

Equation 2.4 assumes that absolute and relative prey densities do not change over time. This is a reasonable assumption for my data. The high prey densities ensured that in 84% of trials ≤ 20% of the prey were consumed (and in 99% of trials < 30% of prey were consumed), and wide spacing of relative prey densities meant that final relative densities never became more extreme than adjacent starting densities. Note that *c* is estimated from sample data, so there is variation around this estimate that is not incorporated into the χ^2 tests. This increases the Type I error rate of the χ^2 tests. However, given limited significance in the results this does not affect my conclusions.

Analyses were also carried out using prey killed as the response variable. These yielded identical results to analyses based on prey consumption, and are not presented in detail in the main text (see Appendix 2.3 for more details).

2.2.4 Metabolic rates

2.2.4.1 Experimental design

As a potential mechanistic explanation for differences in prey consumption, I estimated aerobic metabolic rates (MR) from rates of oxygen consumption ($\dot{M}O_2$). Metabolism and foraging are theoretically linked by the common currency of energy: metabolic rate is the rate of energy conversion within an organism, whilst foraging provides fuel for this process (Biro and Stamps 2010). I measured both standard metabolic rate (SMR; energy consumption under minimal functional activity i.e. associated with the idling cost of the individual's metabolism) and routine metabolic rate (RMR; energy consumption incorporating SMR and all other spontaneous activity; Cech and Brauner 2011) because these test subtly different hypotheses. High foraging rates may be necessitated by a high SMR and/or needed to fuel activity that contributes to RMR.

Oxygen consumption ($\dot{M}O_2$) of individual animals was measured in a custom made, intermittent-flow respirometer (Fig. 2.1), set up following Quetin (1983) and Svendsen et al. (2015). The respirometer chamber was a 505 ml PVC food storage container, which could be clipped shut to maintain an airtight seal. The chamber contained a magnetic stir bar to ensure mixing of water during measurements (Rodgers et al. 2016), a hard plastic mesh to suspend the crayfish above the stir bar and a PVC shelter (6 cm length, 4.5 cm diameter,) to minimise stress. An optical dissolved oxygen (DO) probe (YSI ProODO, YSI Incorporated, OH) was inserted into the chamber through a rubber seal, with extensions of the plastic mesh separating this from the crayfish to prevent damage to the sensor cap. One piece of inflow silicone tubing (3 mm internal diameter) connected the chamber to a flush pump (Sacem BIP 4w) *via* an air trap, whilst another length of tubing provided an outflow. The chamber and attachments were submerged in a water bath, which was constantly aerated and contained a combined filter/ultraviolet light (All Pond Solutions, Middlesex, UK) which ensured water in the bath was continually homogenised and minimised microbial growth. The entire setup was housed in an incubator with identical temperature (14.0 ± 0.3ºC) and photoperiod (12:12h) as the controlled environment room. Housing in a separate incubator completely removed subjects from any visual or acoustic disturbance during measurement of oxygen consumption.

Prior to measurement, hard-shelled (intermoult) animals were isolated for one week and fed in a set schedule, including a 48h starvation period prior to measurement to empty the gut and minimise the influence of digestive processes on metabolic rate. An individual animal was transferred to the respirometer chamber at 8pm, ensuring the animal remained under water to avoid introduction of any air bubbles. This gave the animal a five hour acclimation period in the respirometer, during which the flush pump was on continually to provide fresh oxygenated water.

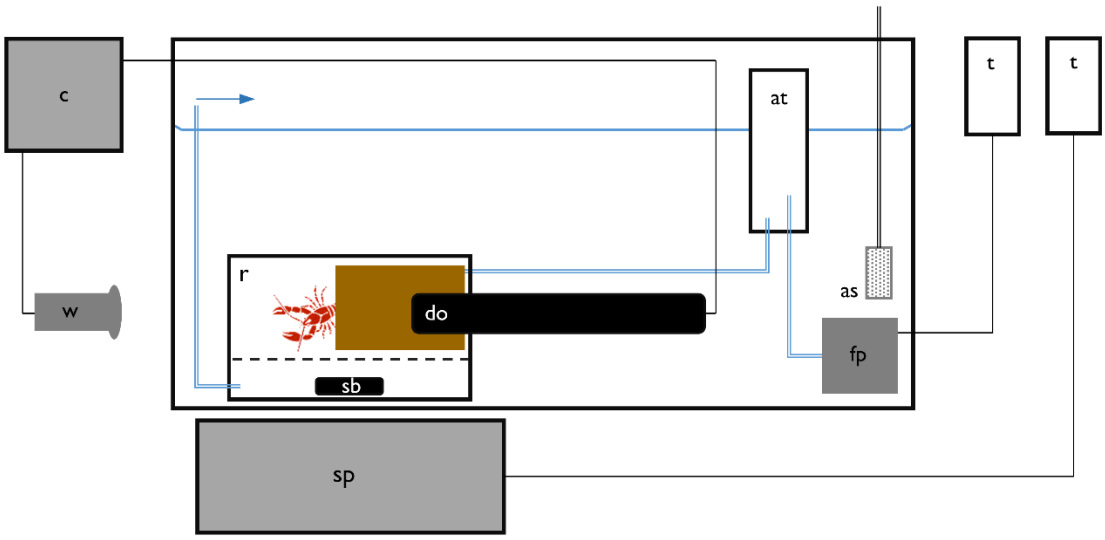


Figure 2.1 Respirometry apparatus. *as* – air stone; *at* – air trap; *c* – computer for logging data; *do* – optical dissolved oxygen probe; *fp* – flush pump; *r* – respirometer, containing crayfish and shelter above a plastic mesh; *sb* – magnetic stir bar; *sp* – magnetic stir plate; *t* – electronic timers to control flush pump and stir plate; *w* – webcam. *Double blue lines* – 3 mm diameter silicone tubing; *solid black lines* – electronic cables; *solid blue line* – water level in holding tank. Filter/ultraviolet steriliser also present in holding tank and in continual operation (but not shown on diagram for simplicity).

After this acclimation period, measurements of oxygen consumption were taken automatically for 20 minutes (crabs) or 30 minutes (crayfish) within 50 minute cycles. Mitten crabs were allocated a shorter measurement phase than crayfish because pilot measurements suggested oxygen consumption by the crabs could be much higher than the crayfish so they were allocated a shorter measurement phase than the crayfish. These measurement phase durations ensured oxygen pressures in the respirometer never dropped below 80% but R^2 values would remain high (≥ 0.88) even when oxygen consumption was low.

At the start of a cycle, the magnetic stir bar was switched on (by an electronic timer). After a 2 minute wait phase, the flush pump was switched off (by electronic timer) and the measurement phase began, during which time the respirometer was effectively a closed system (Svendsen et al. 2016). During the measurement phase, animals were also recorded by webcam (Logitech Pro 9000 and Webcam XP 5 software). After the 20 or 30 minute measurement phase, the stir bar was switched off and flush pump switched back on to replenish the respirometer with oxygenated water. This flush phase lasted 28 minutes for crabs and 18 for crayfish such that overall cycle duration remained the same, but always allowed [DO] to return to equilibrium. Throughout the cycles, temperature- and pressure-compensated [DO] (mg O_2 L^{-1}) and temperature (^oC) were continually logged every 20 seconds via YSI's Data Manager Software. Nine cycles were completed for each animal in each of the dark and light phases.

Due to equipment limitations, only one individual could be measured per day (*n A. pallipes* = 8, *n P. leniusculus* = 12 and *n E. sinensis* = 10). The order in which individuals of each species were tested was randomised to remove any confounding temporal effects. Animals were monitored for two weeks after measurement of $\dot{M}O_2$: no animals moulted or died during this period.

2.2.4.2 Statistical methods

For each animal, $\dot{M}O_2$ curves for each measurement period were split by eye into linear sections, and a least-squares regression line (with $R^2 \geq 0.88$) fit to each section in Microsoft Excel. $\dot{M}O_2$ for each section was calculated according to Equation 2.6, suitable for closed-system respirometers (Myles-Gonzalez et al. 2015; Svendsen et al. 2016):

$$\dot{M}O_2 = m \times (V_t - V_c) \times 3600 \qquad [2.6]$$

where $\dot{M}O_2$ is the weighted average oxygen consumption (mg O_2 h^{-1}), m is the gradient of the linear decline in oxygen concentration (mg O_2 L^{-1} s^{-1}), V_t is the total volume of the respirometer chamber (0.505 L) and V_c is the volume of each individual crayfish (determined by displacement immediately after $\dot{M}O_2$ measurement). $\dot{M}O_2$ was uncorrected for background respiration, as controls (respirometer without decapod present) indicated this was negligible.

The lowest recorded $\dot{M}O_2$, across all sections, was taken as an estimate of SMR. Webcam recordings were used to verify that this coincided with a period of minimal activity. For one mitten crab, no SMR was recorded as the animal was always active during measurement. Two mitten crabs showed negligible $\dot{M}O_2$ over a 2-3 minute section. These measurements were not considered as estimates of SMR, but outliers reflecting anaerobic respiration (e.g. Chabot et al. 2016), and were therefore excluded from analyses.

RMR was estimated as a weighted average of $\dot{M}O_2$, values for all measured sections, separately for the light and dark phases (Equation 2.7):

$$RMR = \sum_{s=1}^{n} \dot{M}O_{2\,(s)} \times \frac{t_s}{T} \qquad [2.7]$$

where $\dot{M}O_{2\,(s)}$ is the oxygen consumption rate for section s, t_s is the duration of section s, and T is the total duration of all sections. In this way, RMR incorporates periods of activity as well as periods of rest.

Metabolism and oxygen consumption rate are strongly mass-dependent (Cech and Brauner 2011). Animals used for $\dot{M}O_2$ measurements that were matched by overall body size (Table 2.1) differed significantly in mass (mean ± SE masses *A. pallipes* 12.1 ± 1.0 g, *P. leniusculus* 11.6 ± 0.4 g, *E. sinensis* 14.3 ± 0.4 g; ANOVA for difference in mass between species $F_{2,27} = 5.85$, $p = 0.008$). To account for these differences in mass, MR data were adjusted to a mass of 13 g (close to the mean for all species) using Equation 2.8 (adapted from Cech and Brauner 2011):

$$MR_{(13\text{g})} = MR \times \left(\frac{13}{mass}\right)^{b} \qquad [2.8]$$

where *mass* is the mass of the individual animal (g) and *b* is the scaling exponent for MR against mass. Ideally, *b* would be species- and rate-specific (i.e. idiosyncratic to SMR and RMR for each species) but such data do not exist. Instead, I take $b = 0.71$ as the best estimate based on the field metabolic rate of *Orconectes rusticus* crayfish (McFeeters et al. 2011).

Mass-corrected MRs (equation 2.8) were compared between species using ANOVA and post-hoc Tukey contrasts with Holm-Bonferroni adjustment of *p* values (*multcomp::glht*). Within species, diurnal and nocturnal MRs were compared using paired *t* tests.

2.3 Results

2.3.1 Functional responses

Prey survivorship in the presence of decapods was significantly lower than survivorship in control treatments (*Dikerogammarus* 97.1% control vs. 75.9% experimental, chironomids 94.5% vs. 37.5%, *Bithynia* 97.4% vs. 83.6%; χ^2 tests for these overall proportions and for each decapod species separately all $p < 0.001$). Thus, I infer that the decapods were acting as predators (not just scavenging dead prey) in the experimental arenas. Further, predation was directly observed in separate tanks. Although vertical migration to evade decapod predation is a defensive behavioural strategy in some gastropods (Haddaway et al. 2014), *B. tentaculata* remained underwater and predominantly among the benthic habitat structure in the present experiments.

Using consumption of prey as the response variable, the functional responses for all predator-prey combinations were best described by a Type II curve, levelling off to an asymptote. In most binomial GLMs of proportional deaths against prey density, the second order term was significantly negative (Table 2.3). Where this was not the case, lower AIC values for Type II fits compared to Type I fits indicated that the former were more appropriate.

Table 2.3 Parameter estimates and significance levels, from second order logistic regression of the proportion of prey consumed by decapod predators against initial prey density. Quasibinomial errors were used due to overdispersion. ϕ – dispersion parameter for GLM; N_0 – first order term; N^2_0 – second order term.

Prey	Decapod	ϕ	Intercept	*p*	N_0	*p*	N^2_0	*p*	Type
Amphipod	*A. pallipes*	2.64	−0.227	0.180	−0.018	< 0.001	3.733×10^{-5}	< 0.001	II
	P. leniusculus	3.03	0.891	< 0.001	−0.029	< 0.001	6.810×10^{-5}	< 0.001	II
	E. sinensis	4.02	2.216	< 0.001	−0.026	< 0.001	5.202×10^{-5}	< 0.001	II
Chironomid	*A. pallipes*	18.05	2.444	< 0.001	−0.007	< 0.001	3.760×10^{-6}	< 0.001	II
	P. leniusculus	25.90	3.122	< 0.001	−0.007	< 0.001	2.471×10^{-6}	< 0.001	II
	E. sinensis	29.54	3.565	< 0.001	−0.002	0.169	-1.032×10^{-6}	0.311	II[a]
Gastropod	*A. pallipes*	2.32	−1.240	< 0.001	−0.009	0.049	9.588×10^{-6}	0.545	II[a]
	P. leniusculus	2.05	0.646	0.001	−0.011	0.003	1.296×10^{-5}	0.322	II
	E. sinensis	5.11	0.945	< 0.001	−0.029	< 0.001	6.195×10^{-5}	0.002	II

[a] Type II fit deemed to be most appropriate by comparing AIC values for Type I and Type II fits. AIC for Type II fits were lower.

Across all prey items, *E. sinensis* had a significantly greater attack rate than both crayfish species (*z* tests, $p \leq 0.012$ for all comparisons): at least 2.2 times that of *A. pallipes* on all prey types, and between 1.2 (on chironomids) and 4.1 (on *Bithynia*) times that of *P. leniusculus* (Table 2.4). In addition, the attack rate of *P. leniusculus* was at least 1.7 times greater than that of *A. pallipes* on all prey items, and always significantly greater (*z* tests, $p \leq 0.007$ for all comparisons). Higher attack coefficients are manifested as higher FR curves (greater predation rates) at low prey densities (Fig. 2.2).

E. sinensis tended to have a high maximum feeding rate (1/*h*T; Table 2.4) on all prey items, by virtue of its short handling time. The maximum feeding rate of *E. sinensis* was significantly higher than the maximum feeding rate of both crayfish species when *D. villosus* or chironomid larvae were prey: at least 2.9 times higher on *D. villosus* and at least 1.9 times higher on chironomid larvae (*z* tests, $p < 0.001$ for all comparisons; Table 2.5). With *B. tentaculata* as prey, *E. sinensis* had a higher FR than *A. pallipes* (but not significantly so; $z = 1.49$, $p = 0.136$) but a similar maximum feeding rate to *P. leniusculus* ($z = -0.02$, $p = 0.984$). Considering the two crayfish species, *P. leniusculus* tended to have a higher maximum feeding rate than *A. pallipes* on all prey items (1.03 times higher on *D. villosus*, 1.1 times higher on chironomid larvae and 1.3 times higher on *B. tentaculata*), but only significantly so on chironomid larvae ($z = 6.39$, $p < 0.001$).

Table 2.4 Estimates of functional response parameters for decapod predators consuming each of three macroinvertebrate prey species, extracted from Rogers' random predator equation fitted to data in the *frair* package (Pritchard 2016). *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); 1/*h*T – maximum feeding rate (prey.day^{-1}), where T = time in days; *SE* – standard error. *Diff* – within each prey item and for each parameter, different letters in this column indicate significantly different parameters (after Holm-Bonferroni correction for multiple comparisons).

Prey	Decapod	*a*	SE	Diff	*h*	SE	1/*h*T	Diff
Amphipod	*A. pallipes*	0.721	0.082	a	0.042	0.003	23.9	a
	P. leniusculus	1.905	0.195	b	0.041	0.002	24.5	a
	E. sinensis	2.529	0.154	c	0.014	< 0.001	71.7	b
Chironomid	*A. pallipes*	2.444	0.088	A	3.298×10^{-3}	6.332×10^{-5}	303.2	A
	P. leniusculus	4.382	0.130	B	2.888×10^{-3}	3.610×10^{-5}	346.3	B
	E. sinensis	5.456	< 0.001	C	1.546×10^{-3}	1.284×10^{-5}	647.0	C
Gastropod	*A. pallipes*	0.298	0.043	α	0.057	0.007	17.6	α
	P. leniusculus	0.494	0.058	β	0.045	0.004	22.1	α
	E. sinensis	2.006	0.227	γ	0.045	0.003	22.1	α

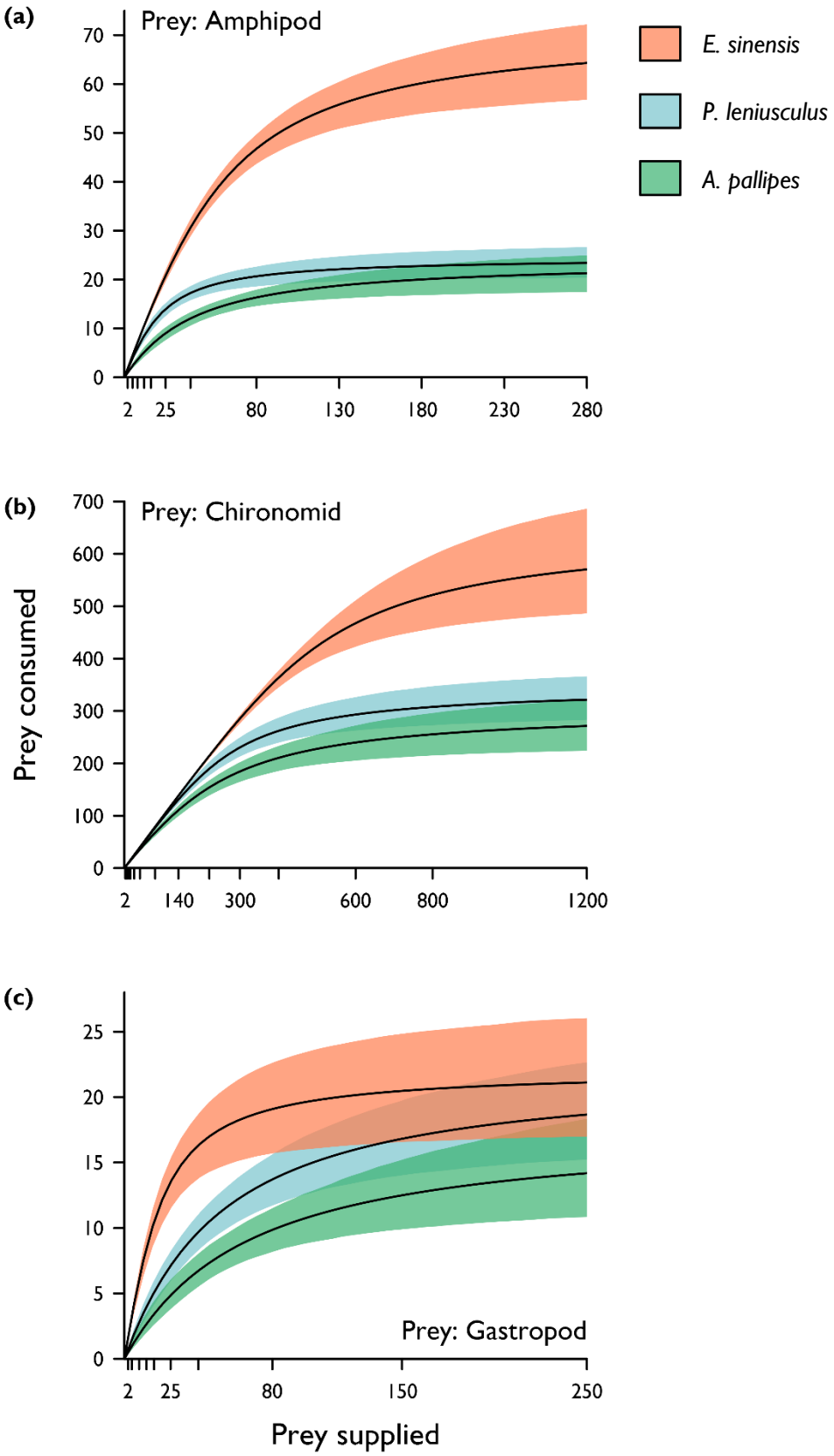


Figure 2.2 Functional response curves of *A. pallipes* (green), *P. leniusculus* (blue) and *E. sinensis* (orange) on **(a)** *D. villosus* **(b)** Chironomid larvae **(c)** *B. tentaculata*. Curves were fitted in *frair* using Rogers' random predator equation. *Shaded areas* show 95% bootstrapped BCa confidence intervals for each curve.

Table 2.5 Comparison of functional response parameter estimates for decapod predator consumption of macroinvertebrate prey, based on analysis using indicator variables in the *frair* package (Pritchard 2016). Raw *p* values are presented; significant differences (α = 0.05) after Holm-Bonferroni correction within each prey group are indicated in bold. *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); *D* – difference; *SE* – standard error.

Prey	Base Group	Comparison		Estimate (*Da* or *Dh*)	SE	*z*	*p*
Amphipod	*A. pallipes*	*P. leniusculus*	*a*	1.184	0.211	5.600	< **0.001**
			h	< 0.001	0.003	−0.317	0.751
	P. leniusculus	*E. sinensis*	*a*	0.625	0.248	2.517	**0.012**
			h	−0.027	0.002	−15.061	< **0.001**
	A. pallipes	*E. sinensis*	*a*	1.808	0.174	10.402	< **0.001**
			h	−0.028	0.003	−10.730	< **0.001**
Chironomid	*A. pallipes*	*P. leniusculus*	*a*	1.938	0.037	53.070	< **0.001**
			h	< 0.001	< 0.001	−6.393	< **0.001**
	P. leniusculus	*E. sinensis*	*a*	1.076	0.039	27.454	< **0.001**
			h	−0.001	< 0.001	−39.922	< **0.001**
	A. pallipes	*E. sinensis*	*a*	3.187	0.036	87.429	< **0.001**
			h	−0.002	< 0.001	−28.601	< **0.001**
Gastropod	*A. pallipes*	*P. leniusculus*	*a*	0.196	0.072	2.703	**0.007**
			h	0.012	0.008	−1.380	0.167
	P. leniusculus	*E. sinensis*	*a*	1.514	0.235	6.446	< **0.001**
			h	< 0.001	0.005	0.021	0.984
	A. pallipes	*E. sinensis*	*a*	1.710	0.232	7.382	< **0.001**
			h	−0.011	0.008	−1.489	0.136

Table 2.6 Comparison of attack rates (*a*) and maximum feeding rates (1/*h*T) for decapod predation on macroinvertebrates, using prey consumed or prey killed as the response variable.

Prey	Decapod	Attack rate *a*			Maximum feeding rate 1/*h*T		
		Prey consumed	Prey killed	killed/ consumed	Prey consumed	Prey killed	killed/ consumed
Amphipod	*A. pallipes*	0.721	0.735	1.02	23.9	25.0	1.05
	P. leniusculus	1.905	1.878	0.99	24.5	25.2	1.03
	E. sinensis	2.529	2.487	0.98	71.7	77.1	1.08
Chironomid	*A. pallipes*	2.444	2.457	1.01	303.2	304.8	1.01
	P. leniusculus	4.382	4.373	1.00	346.3	347.3	1.00
	E. sinensis	5.456	5.450	1.00	647.0	648.3	1.00
Gastropod	*A. pallipes*	0.298	0.292	0.98	17.6	18.5	1.05
	P. leniusculus	0.494	0.482	0.98	22.1	23.5	1.06
	E. sinensis	2.006	1.972	0.98	22.1	23.5	1.06

The death of prey that are not subsequently consumed will also have implications for prey populations in the wild. Analyses generating FR curves using all prey killed were not both qualitatively and quantitatively similar to the analyses presented (see Appendix 2.1 for full analyses): all curves were Type II, significant differences between attack rates and handling times were as for Table 2.4 and attack rates were within 2% of those based on consumption (Table 2.6). Maximum killing rates were always greater than maximum consumption rates (Table 2.6), indicating some partial consumption of prey. Partial consumption was more frequent on amphipod and gastropod prey (maximum killing rates up to 1.08 times maximum consumption rates) than on chironomid larvae (maximum killing rates no more than 1.01 times maximum consumption rates (Table 2.6).

2.3.2 Switching

Mortality in controls, containing 140 of each prey animal, was low (*D. villosus* 3.2% and *B. tentaculata* 0.3%), indicating that inter- and intra-specific predation by prey animals was minimal. As for FR experiments, I therefore assume decapods were acting as predators rather than scavengers.

In the switching experiments, *E. sinensis* consumed significantly more food in total (across all relative densities mean ± SE individuals consumed = 50.3 ± 3.2) than *P. leniusculus* (18.1 ± 1.7) and *A. pallipes* (18.6 ± 1.1) (Tukey adjusted $p < 0.001$ for both). The crayfish species did not differ in the number of prey items they consumed overall (Tukey adjusted $p = 0.883$).

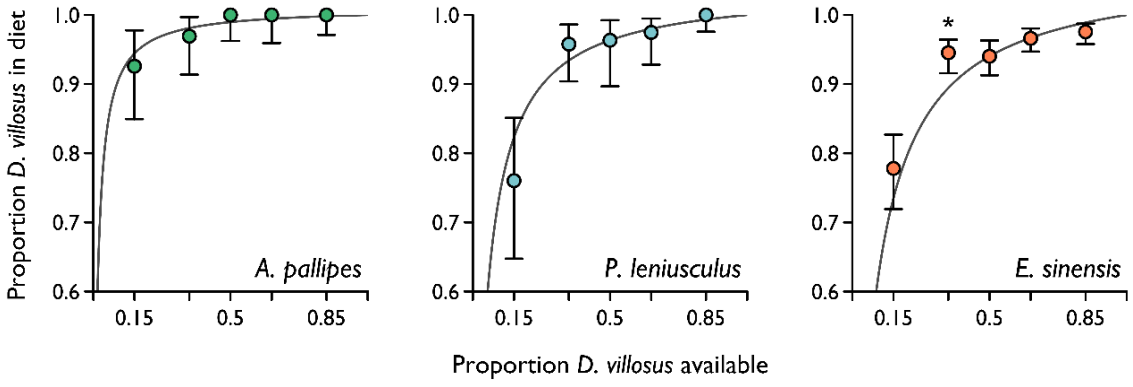


Figure 2.3 Proportion of *D. villosus* in the diet of decapod predators at varying relative densities of *D. villosus* to *B. tentaculata*. At all relative densities, total prey density was fixed at 280.tank^{-1}. Note that the y axes begin at 0.6. *Points* are population proportions with 95% binomial confidence intervals. *Curves* are expected proportions in the absence of preference, based on consumption when prey types are equally available. *Asterisk* indicates significant deviation from null hypothesis (binomial tests; without correction for multiple testing).

All decapods showed strong electivity towards *D. villosus* when both prey types were equally common: *D. villosus* formed a significantly greater proportion of the diet than would be expected under random feeding (*A. pallipes* $c = 96.0$; *P. leniusculus* $c = 26.3$; *E. sinensis* $c = 15.7$; binomial tests of null $P_{Dv} = 0.5$, $p < 0.001$ for all three predator species). When electivity $\neq 0$, the null hypothesis for switching (Equation 2.4) yields a non-linear curve on a plot of P_{Dv} against relative prey density (Fig. 2.3). Observed P_{Dv} only differed from expected P_{Dv} for *E. sinensis* at a relative density of 0.35 ($\chi^2 = 5.64$, df = 1, $p = 0.018$). Thus, there was no consistent deviation of observed P_{Dv} from expected P_{Dv} (Fig. 2.3), indicating an absence of switching in any decapod predator: electivity towards *D. villosus* was maintained at all relative prey densities.

2.3.3 Metabolic rates

There was no difference in mass-adjusted SMR between the three decapod species (Fig. 2.4a; ANOVA $F_{2,26} = 0.02$, $p = 0.980$). Mean ± SE SMRs for 13g animals were *A. pallipes* 0.35 ± 0.05 mg O_2 hr^{-1}, *P. leniusculus* 0.34 ± 0.03 mg O_2 hr^{-1}, *E. sinensis* 0.34 ± 0.04 mg O_2 hr^{-1}.

The RMR of the alien species was significantly higher at night than during the day (*E. sinensis* paired $t = 3.10$, df = 9, $p = 0.013$; *P. leniusculus* $t = 4.85$, df = 11, $p < 0.001$, whilst the RMR of *A. pallipes* was marginally higher during the day than at night ($t = -2.01$, df = 7, $p = 0.084$).

In contrast to SMR, mass-adjusted RMR did differ between species, both during the day (ANOVA $F_{2,27} = 8.52$, $p = 0.001$) and at night (ANOVA $F_{2,27} = 18.79$, $p < 0.001$). During the day, *E. sinensis* had a higher RMR than both crayfish species (Fig. 2.4b; 1.8 times higher than *A. pallipes*, Tukey

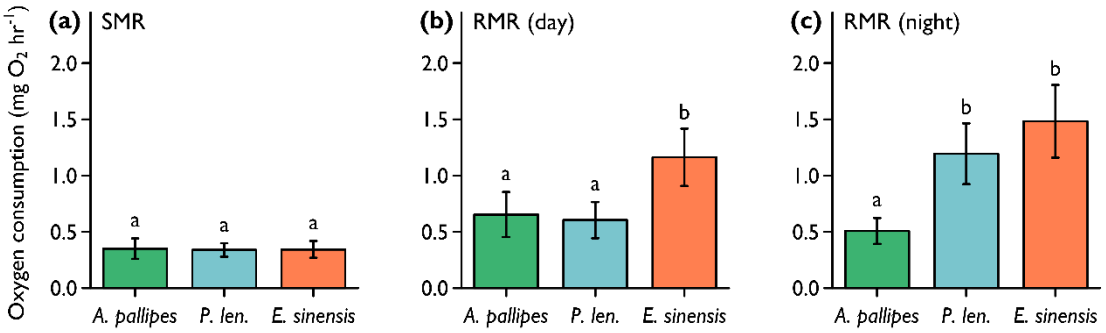


Figure 2.4 Mass-adjusted (to 13 g) oxygen consumption rates of decapod crustaceans, as proxies for metabolic rates. **(a)** Standard metabolic rate (SMR): the lowest recorded $\dot{M}O_2$ associated with minimal activity **(b)** diurnal routine metabolic rate (RMR): a weighted average of all $\dot{M}O_2$ measurements during the light phase and **(c)** nocturnal RMR: a weighted average of all $\dot{M}O_2$ measurements during the dark phase. *Letters* indicate significant differences based on Tukey contrasts with Holm-Bonferroni correction of *p* values. *Bars* show means ± 2 SE. *P. len.* – *Pacifastacus leniusculus*.

adjusted $p = 0.009$; and 1.9 times higher than *P. leniusculus*, Tukey adjusted $p = 0.002$), whilst RMR did not differ between the crayfish (Tukey adjusted $p = 0.689$). However, at night the RMR of *E. sinensis* was not significantly higher than that of *P. leniusculus* (Fig. 2.4c; Tukey adjusted $p = 0.158$), but both invaders had a higher RMR than *A. pallipes* (Tukey adjusted $p < 0.001$ for both comparisons).

2.4 Discussion

This Chapter highlights an exceptionally high predatory capacity of alien *E. sinensis*, especially on softer-bodied prey. Alien *P. leniusculus* had attack rates and maximum feeding rates that were consistently higher than those of *A. pallipes* across all prey types. Further, my data suggest the impact of the alien predators on any single prey type may not be tempered by more flexible diet choices. Higher predation rates of the invaders are associated with higher RMRs (but not SMRs), suggesting differences in predation could be explained by differences in activity levels.

My FR curves indicate that *E. sinensis* is a voracious predator with the potential to have strong impacts on prey populations in invaded rivers. Attack rates and maximum feeding rates of *E. sinensis* are higher than, or as high as, those of signal crayfish across a range of macroinvertebrate prey of differing motility and physical defence, in accord with previous FRs derived with *G. pulex* as prey (Rosewarne et al. 2016). My data reveal the magnitude of these differences may be higher than previously reported, with maximum feeding rates of *E. sinensis* as much as 2.9 times those of an equally-sized alien crayfish, and attack rates as much as 4.1 times those of alien crayfish.

Meanwhile, maximum feeding rates on gastropod prey were similar between crayfish and crabs. Crayfish are particularly adept at consuming gastropods with their large crushing chelae, and accordingly gastropod populations are amongst the most strongly affected by crayfish (Lodge et al. 1994; McCarthy et al. 2006; Twardochleb et al. 2013). Meanwhile, *E. sinensis* is relatively poor at handling gastropods – especially those with thick shells like *B. tentaculata* – with shorter, narrower chelae than crayfish for a given body size (pers. obs.; Bertness and Cunningham 1981; Mills et al. 2016). Thus, the low maximum feeding rates of *E. sinensis* on *B. tentaculata* are likely to be limited by physical handling time (extracting flesh from snail shells) rather than digestion time or motivation to feed (Jeschke et al. 2002; Mills et al. 2016), and implies that crabs feeding on gastropods were not satiated at the end of experiments.

The wider implications of predation on macroinvertebrates by *E. sinensis* are complex. Trophic cascades (e.g. reduction in processing of leaf litter) could flow from this predation, but they could be mitigated by crab omnivory (Jackson et al. 2014). Impacts also depend on the balance between

direct biotic interactions (this Chapter) and habitat-mediated effects such as sediment resuspension (Harvey et al. 2011; Gallardo et al. 2016). Intense predation by *E. sinensis* could provide biotic resistance, especially to Ponto-Caspian amphipods such as *D. villosus* and *D. haemobaphes* (Twardochleb et al. 2012; MacNeil et al. 2013b), but the high biomass of these invaders could equally facilitate crab invasion (invasional meltdown; Simberloff and von Holle 1999).

Considering the crayfish species, my FR data suggest the *per capita* impact of the invader *P. leniusculus* consistently exceeds that of *A. pallipes* on a range of prey types, although the magnitude of this difference is relatively small. Again, this is in accord with studies using just *G. pulex* as prey (Haddaway et al. 2012; Rosewarne et al. 2016). Attack rates of *P. leniusculus* were significantly higher than *A. pallipes* on all prey items reflecting a steeper initial rise of the FR curve – even with the constraints on the curves at low densities imposed by my non-replacement design (Dick et al. 2014). High attack rates are associated with higher predation pressures at low prey densities, at which prey populations will be most vulnerable to additional mortality (Murdoch and Oaten 1975; Juliano 2001). *P. leniusculus* also had a higher FR curve (maximum feeding rate) than *A. pallipes* on all prey items, although this difference was not significant on amphipod prey and only marginally so on *B. tentaculata*. High inter-individual variability associated with variation in body size would have reduced my power to detect significant differences. The magnitude of difference in maximum feeding rates was relatively small (up to 1.3 times higher in *P. leniusculus*), although the largest difference was on *Bithynia*, corroborating observations that *P. leniusculus* is particularly adept amongst crayfish at handling thick-shelled snails (Olden et al. 2009). Overall, the higher FRs associated with a damaging invader relative to a native analogue are concordant with the general pattern emerging from FR studies in invasion biology (Dick et al. 2014), although my data caution that these differences may not always be large or significant.

Interestingly, the magnitudes of FR differences corroborate relative impact predicted by other methods. The FR of *E. sinensis* was especially high on *D. villosus* as prey, and alien *E. sinensis* consume amphipods more readily in the field (based on stable isotope analysis) and in laboratory choice trials, and have a greater impact on mesocosm populations of amphipods, compared to *P. leniusculus* (Czerniejewski et al. 2010; Rosewarne et al. 2016). *E. sinensis* also possesses behavioural and morphological traits that assist in the capture of motile prey (Mills et al. 2016). In contrast, mobile taxa such as amphipods are amongst the least affected by crayfish predation in experimental manipulations (Twardochleb et al. 2013) and in the field (Mathers et al. 2016). *E. sinensis* also consumed chironomid larvae at a greater rate than the crayfish, matching the high prevalence of these – and other similar vermiform prey, for which I assume chironomid larvae

are an approximate proxy – in mitten crab diets (Rudnick and Resh 2005; Czerniejewski et al. 2010).

Switching – a disproportionate change in the contributions of prey items to predator diets as the relative abundance of prey varies – was not observed in any of the predator species. The proportion of *D. villosus* and *B. tentaculata* in predator diets matched null expectations, assuming no switching. This implies that in the field, predation pressure on a single prey type could be maintained even when it becomes rare, potentially leading to local prey extinction (Murdoch and Oaten 1975). Interestingly, *E. sinensis* showed a tendency towards negative prey switching i.e. *higher* than expected proportional consumption of *D. villosus* when it is the less abundant prey. This was more obvious for smaller crabs (when crabs and crayfish were matched by mass rather than body size; Appendix 2.4), explained by the larger increase in gastropod than amphipod handling time in smaller crabs (Appendix 2.1). Negative switching would increase predation pressure on amphipods even when rare – matching observations of a large contribution of amphipods to *E. sinensis* diet in the field (Rosewarne et al. 2016). I note that the absence of switching in the present study may be related to the very strong null electivity towards *D. villosus* ($c \geq 15.7$). Experiments with prey items that are more similar in defence and handling time may reveal different patterns. Generally, diet flexibility or generalism may be trait associated with successful invasive species (Moyle and Light 1996; Cassey 2002). Previous feeding experiments suggest *P. leniusculus* and *E. sinensis* consume a wider range of prey types than *A. pallipes* (Haddaway et al. 2012; Rosewarne et al. 2016) and the latter is more hesitant to feed on novel prey (Gherardi et al. 2001).

Metabolic rates were measured as a potential mechanistic explanation for differences in feeding rates. Mass-adjusted SMR did not differ between the decapod species, suggesting a similar-sized metabolic engine with a similar idling cost in all species (Biro and Stamps 2010). Thus, intrinsic differences in metabolic machinery make little contribution to differences in feeding rates. Note *E. sinensis* were slightly heavier than the crayfish when matched by overall body size, meaning the SMR of *E. sinensis* would be slightly higher than that of the crayfish on average, owing to allometric scaling of MR with body size (Brown et al. 2004). However, the difference in mass was small (crabs around 1.2 times heavier than crayfish, thus with an MR approximately $1.2^{0.71}$ times greater) relative to the difference in feeding rate (at least 1.9 times on amphipods and chironomids), leaving much of the difference in feeding rate unexplained. Further, when decapods were matched by body mass, large differences in feeding rates remained (Appendix 2.2).

In contrast with SMR, there were large differences in RMR between the decapod species. *E. sinensis* had a greater mass-adjusted RMR than the crayfish species, and *P. leniusculus* had a

greater RMR than *A. pallipes* at night. Inspection of webcam recordings indicated that periods of high RMR were associated with activity in the respirometer. Further, the higher RMR of *E. sinensis* and *P. leniusculus* at night is consistent with their known nocturnal activity (Styrishave et al. 2007; Gilbey et al. 2008). The interspecific differences in activity and RMR match the rank order of differences in feeding rate and make biological sense (Careau et al. 2008; Rall et al. 2012). Together, the RMR and FR data indicate a positive association between the supporting traits of activity, RMR and feeding rate across species. These traits are likely linked through feedbacks: a species that is more active and operates at a high RMR both needs (in order to fuel the metabolic engine) and is able (via increased encounter rates with prey) to feed at a higher rate. In turn, this higher feeding rate fuels the higher RMR.

FRs are an emerging methodology for impact assessment (Dick et al. 2014). My data support the use of FRs as a simple, cost-effective tool for rapid assessment of invader impacts, and provide guidance on how they may be used. At one level, comparative FRs on a single prey type can be used to rapidly score impact potential given that similar conclusions regarding relative FR shape and height are drawn for all prey types. At another level, because my FRs were sensitive to prey type in accord with predictions from other methodologies, my data support the use of FRs to make specific predictions about magnitude of impact on different prey groups. Analogously, Dick et al. (2013) found the relative heights of lab-derived FRs to predict field impacts in the invasive shrimp *Hemimysis anomala*, and Dodd et al. (2014) found that FR heights and shapes corroborated known field impacts of *D. villosus* on macroinvertebrate prey. Additionally, given that successful invasive and alien species tend to have higher resource consumption rates than native analogues (McKnight et al. 2016), I encourage the use of FRs to predict invasion success as well as impact (e.g. Xu et al. 2016).

However, I acknowledge limitations in the FR approach (Dick et al. 2014). Extrapolation to success and impact in the field must be done with caution given (a) the simplicity of the experimental system (b) only predators of a single size were used and (c) predators were tested in isolation rather than in groups. In natural scenarios, total consumption is likely to be reduced as the decapods will allocate more of their time budget to other activities such as predator avoidance and interactions with conspecifics. More complex natural habitats can increase predator-free space, altering the height and shape of FRs (Barrios-O'Neill et al. 2015). When a greater diversity of food types is available, consumption of any single prey type is likely to decrease, with patterns of consumption depending on the habitat, activity and relative densities of prey. Finally, different sized individuals might have different impacts in the field, both in terms of amount and pattern of *per capita* resource consumption (Chapter 3). Still, my FRs allow a fair comparison to be made

between species to estimate relative impacts: for a given, representative size class, what is the *maximum* potential impact on prey?

In natural situations, predators will occur in groups. Local impacts are likely to be positively related to predator population densities, in addition to *per capita* effects measured by FRs (Parkyn et al. 1997; Parker et al. 1999; Ricciardi 2003). Alien *P. leniusculus* can reach much higher densities than *A. pallipes* in similar systems: summer surveys in riffles with cobble/pebble substrate yielded *P. leniusculus* densities of $14.m^{-2}$ (Guan 2000), compared to *A. pallipes* densities of around $4.m^{-2}$ (Demers et al. 2003). Alien *E. sinensis* can also reach high population densities relative to native decapods (Rudnick et al. 2003; Gherardi et al. 2011). These differences in density could augment the differences in *per capita* feeding rate identified in this Chapter. However, the increase will not necessarily be additive. Interactions between conspecifics ('multiple predator effects') can reduce *per capita* effects at high density (Soluk 1993; Médoc et al. 2015). In *P. leniusculus, per capita* consumption of chironomid larvae and zebra mussels is reduced at high conspecific densities – although behavioural correlations between aggression and foraging activity can maintain *per capita* feeding rates on gastropods (Pintor et al. 2009; zu Ermgassen and Aldridge 2010). Measurement of population rather than individual impacts would address the problems of density, interference and body size that are not incorporated in individual FRs (Barney et al. 2013), although this adds complexity to a predictive tool whose simplicity and rapidity is a virtue. Moreover, the balance of evidence suggests predictions based on individual FRs generally reflect field impacts and so are robust to these complexities (Dick et al. in press).

Quantitative evidence of alien species' impacts is important for making robust decisions about their management (Kumschick et al. 2012). My data provide such evidence for two important (widespread and abundant) decapod crustacean invaders in Great Britain. *E. sinensis* and *P. leniusculus* had consistently high predatory impacts on a range of macroinvertebrate prey relative to the impact of *A. pallipes*, associated with differences in routine metabolic rate. The difference in *per capita* impact between the crayfish species is relatively small, suggesting impacts of *P. leniusculus* may be driven more by differences in abundance or body size. The *per capita* impact of *E. sinensis* is exceptionally high on soft-bodied prey, highlighting predation as a mechanism by which mitten crabs could cause large impacts.

Chapter 3

Predation of fish eggs and larvae by invasive alien and native amphipods: size matters

Abstract

Alien predators can have dramatic impacts on invaded communities. Extreme declines in macroinvertebrate populations often follow killer shrimp (*Dikerogammarus villosus*) invasions. There are concerns over similar impacts on fish through predation of eggs and larvae, but these remain poorly quantified.

I compare the predatory impact of invasive alien and native amphipods (*D. villosus* and *Gammarus pulex* respectively) on fish eggs and larvae (ghost carp *Cyprinus carpio* and brown trout *Salmo trutta*) in the laboratory. I use size-matched amphipods, as well as larger *D. villosus* reflecting natural sizes. I quantify functional responses, and electivity amongst eggs or larvae and alternative food items (invertebrate, plant and decaying leaf).

D. villosus, especially large individuals, were more likely than *G. pulex* to kill trout larvae. However, the magnitude of predation was low (seldom more than one larva killed over 48 hours). Trout eggs were very rarely killed. In contrast, carp eggs and larvae were readily killed and consumed by all amphipod groups. Large *D. villosus* had maximum feeding rates 1.6 to 2.0 times higher than the smaller amphipods, whose functional responses did not differ. In electivity experiments with carp eggs, large *D. villosus* consumed the most eggs and the most food in total. However, in experiments with larvae, consumption did not differ between amphipod groups.

Overall, my data suggest *D. villosus* will have a greater predatory impact on fish populations than *G. pulex*, primarily due to its larger size. Higher invader abundance could amplify this difference. The additional predatory pressure could reduce recruitment into fish populations.

3.1 Introduction

Alien species continue to have negative impacts on populations, communities and ecosystems across the globe (Strayer 2010; Simberloff et al. 2013; Gallardo et al. 2016). One important mechanism behind these impacts is predation (Ross 1991; Davis 2003; Sax and Gaines 2008; Blackburn et al. 2014). Predation is a fundamental ecological interaction with the capacity to shape and structure natural communities (Thorp 1986; Case and Bolger 1991; Wellborn et al.

1996; Jackson et al. 2001). Owing to factors such naivety in prey populations (Case and Bolger 1991; Cox and Lima 2006), release from natural enemies (Roy et al. 2011) or intrinsic behavioural characteristics (Weis 2010), damaging alien predators frequently consume prey more rapidly than analogous native species and thus have stronger effects on resident prey populations (Dick et al. 2014; Dick et al. submitted).

Invasions by alien species are one of the primary threats to freshwater biodiversity, reflecting the globally extensive but locally intensive use of fresh waters by humans (Richter et al. 1997; Sala 2000; Light and Marchetti 2007). Moreover, introduced predators in freshwaters have particularly severe impacts relative to those in terrestrial or marine systems (Sala et al. 2000; Cox and Lima 2006). For example, fish populations – many of great commercial or biological importance – frequently decline following invasion as a result of predation. All life stages are vulnerable, from adults (e.g. Lawrie 1970; Ogutu-Ohwayo 1990; Ruzycki et al. 2003) to young fish (e.g. Garman and Nielsen 1982; Lemly 1985) to eggs and larvae (e.g. Meffe 1985; Ruzycki et al. 2003).

Predation is probably the biggest single cause of fish egg and larval mortality (Bailey and Houde 1989; Houde 2002). Consequently, it can have particularly strong effects on populations, greatly influencing recruitment of even the most fecund fish (Köster and Möllmann 2000; Bajer et al. 2012). For example, in experimental ponds, egg predation by *Orconectes virilis* decreased or completely prevented recruitment of pumpkinseed (*Lepomis gibbosus*) and bluegill (*L. macrochirus*) sunfish respectively (Dorn and Mittelbach 2004). Meanwhile, in the Upper Mississippi River Basin, egg predation by *L. macrochirus* drastically reduces carp recruitment, providing local biotic resistance to invasion by carp where the predator is present (Bajer et al. 2012). Vulnerability to predation is conferred by the aggregated distribution and limited mobility of fish eggs and larvae (Hassell 1978; McGurk 1986). Moreover, their small size makes them accessible to a wide range of predators, including macroinvertebrates such as Trichoptera, Plecoptera and Crustacea (Zuromska 1967 cited in Paling 1968; Fox 1978; Mills 1981; Brown and Diamond 1984).

The amphipod crustacean *Dikerogammarus villosus* is a potentially devastating, invasive alien predator of fish eggs and larvae. *D. villosus* is native to the Ponto-Caspian region, but has spread north-west through the river and canal network of Europe to form multiple self-sustaining populations, and can reach locally high densities (Bij de Vaate et al. 2002; Gallardo et al. 2012; Rewicz et al. 2014). *D. villosus* also threatens to invade elsewhere (e.g. the American Great Lakes; Pagnucco et al. 2014). Evidence implicates *D. villosus* as a voracious predator, earning it the 'killer shrimp' title, special attention as an 'alert' species in Great Britain, and a listing as one of the 100 worst invaders in Europe (www.europe-aliens.org).

Invasion by *D. villosus* frequently coincides with the decline or extinction of resident benthic macroinvertebrates such as isopods, tubificids and amphipods (Dick and Platvoet 2000; Dick et al. 2002; Kley and Maier 2003; Josens et al. 2005; Boets et al. 2010; MacNeil et al. 2013a; Dodd et al. 2014; Gergs and Rothhaupt 2015). Thus, once established *D. villosus* typically dominates the macroinvertebrate community in both number and biomass (Josens et al. 2005; van Riel et al. 2006). Trophic links and ecosystem functions can also be transformed by the invader (Dick et al. 2002; Piscart et al. 2011; MacNeil et al. 2011; Boeker and Geist 2015). Predation by *D. villosus* may be an important mechanism behind these changes. In the laboratory, *D. villosus* will consume a wide range of animal prey, including aquatic bugs, leeches, isopods, juvenile crayfish, chironomid larvae, odonate larvae, ephemeropteran larvae and even other amphipods (Dick and Platvoet 2000; Platvoet et al. 2009; Boets et al. 2010; MacNeil et al. 2013a). Stable isotope and fatty acid analyses suggest predatory tendencies tend to be retained in the field (van Riel et al. 2006; Maazouzi et al. 2007; but see Hellmann et al. 2015).

D. villosus will also prey upon fish eggs and larvae, raising concerns about its potential to cause analogous declines in fish populations. *D. villosus* will kill and eat *Cottus perifretum* eggs and larvae in the laboratory and has been found with damaged *C. perifretum* eggs in the field (Platvoet et al. 2009). Further, Casellato et al. (2007) showed that *D. villosus* will consume *Coregonum lavaretus* eggs preferentially over other animal prey. However, these experiments produce few quantitative data for few species of fish, and do not compare impacts with native species. Comprehensive and objective data on invader impacts, ideally relative to native species, are vital to understand how invaders might change ecosystems and as a basis for management decisions (Byers et al. 2002; NRC 2002; Kumschick et al. 2012; Dick et al. 2013; Dick et al. 2014).

Using laboratory experiments, I compare predatory impacts of invasive *D. villosus* and an analogue native to Great Britain (although invasive elsewhere), *Gammarus pulex*. As prey, I used the early life stages of salmonid and coarse (i.e. non-salmonid) fish. I use size-matched amphipods to examine intrinsic differences between species as well as large *D. villosus* to reflect natural differences in amphipod size: both species identity and body size can be critical aspects of predator-prey interactions (Bailey and Houde 1989; Luecke et al. 1990; Miller et al. 1992; Woodward et al. 2005; Rall et al. 2012; Anderson et al. 2016). I quantify amphipod predation on fish eggs and larvae (a) as functional responses (FRs), a fundamental measure of resource use with the potential to predict impacts in the field (Dick et al. 2013; 2014) and (b) in the presence of alternative foods to examine differences in electivity, which can also influence predator impacts (Grosholz 2005; Dodd et al. 2014). Finally, I discuss the results of these experiments in the context of potential impacts on fish populations.

Since damaging alien species tend to consume resources at faster rates than native analogues (Dick et al. 2014), I predict that *D. villosus* will have a higher FR and consume more food in electivity experiments than size-matched *G. pulex*. I also predict larger *D. villosus* will consume more food than the smaller amphipods in both FR and electivity experiments (Woodward et al. 2005; Maier et al. 2011; Rall et al. 2012). In electivity experiments, I predict that *D. villosus* will show a stronger tendency than *G. pulex* to consume fish eggs and larvae given the known predatory tendencies of the invader (e.g. van Riel et al. 2006).

3.2 Methods

3.2.1 Experimental organisms

3.2.1.1 Fish eggs and larvae

Fish were a representative salmonid (native brown trout *S. trutta* L. 1758) and coarse fish (non-native ghost carp *Cyprinus carpio* L. 1758). These were chosen to represent two contrasting sizes of freshwater fish propagule (Table 3.1; Teletchea and Fontaine 2010), the two main types of freshwater fishery in the UK (Mawle and Peirson 2009) and the most speciose European fish families (Freyhof and Brooks 2011).

Live trout eggs were sourced from a commercial hatchery in Grassington, UK in January and kept in aerated, aged and circulating tap water in incubators at 7.0 ± 0.2ºC (range) and under a 9:15h light:dark cycle. Live carp eggs were sourced from a commercial hatchery in Nottingham, UK in early May and kept in aerated, aged and circulating tap water in a controlled-temperature (CT) room at a temperature of 13.9 ± 0.1ºC (range) and under a 12:12h light:dark cycle. Temperatures and light regimes were chosen to match typical development conditions for each fish (Alabaster and Lloyd 1982). Tap water was aged (at the same temperature as the eggs) through aeration for 24h. Egg and larval stock tanks were cleaned daily. Conditions facilitated high survival and hatch rates. Larvae were only kept and used when recently-hatched and relying on yolk sacs for nutrition (Teletchea and Fontaine 2010), thus falling outside the remit of the UK Animals (Scientific Procedures) Act (1986). Mean sizes of eggs and larvae (Table 3.1) were typical for salmonids and coarse fish (Teletchea and Fontaine 2010).

3.2.1.2 Amphipods

Amphipods were collected from established populations in 2014 and 2015. *G. pulex* were kick-sampled from a stream in Golden Acre Park, Leeds (lat 53°52'N, long 1°36'W) and *D. villosus* sampled from artificial substrates in Grafham Water, Cambridgeshire (lat 52°17'N, long 0°19'W). Each species was transported to Leeds in insulated boxes and maintained in the laboratory on a

diet of stream-conditioned *Acer pseudoplatanus* L. leaves (which were readily consumed). Amphipods were kept in aerated, aged tap water under the same light and temperature regime as fish eggs and larvae for at least one week before use in experiments, and in single-sex tanks for at least 72h before use.

Only male amphipods were used in experiments to avoid potential variation in predatory impact with breeding status in females, and control for the fact that male *D. villosus* may be more predatory than females (Dick and Platvoet 2000; Kinzler and Maier 2003). Males were identified by precopulatory pairing (*G. pulex*) or presence of genital papillae and absence of oostegites (*D. villosus*). All amphipods were free of obvious visual parasites that may affect behaviour (Dick et al. 2010; Bacela-Spychalska et al. 2013b). Amphipods were only used once in each experiment (i.e. combination of fish species, developmental stage and experimental design) but were re-used between experiments within fish species. Re-used amphipods always had at least 24h to recover in communal tanks, and all amphipods had the same level of experience with prey at the start of each experiment.

Table 3.1 Length and mass of fish eggs and larvae used in experiments. $n = 24$, except for trout eggs $n = 10$. Carp larvae were measured after killing in 70% ethanol.

Fish	Stage	Length (mm) ± SE	Mass (mg) ± SE
Carp	Egg	1.92 ± 0.01	3.81 ± 0.07
	Larva	5.69 ± 0.07	1.32 ± 0.06
Trout	Egg	5.04 ± 0.05	70.60 ± 1.51
	Larva	15.37 ± 0.24	65.60 ± 1.46

Following Dodd et al. (2014) and in recognition of the larger size of *D. villosus* (pers. obs.; Pinkster 1970; Nesemann et al. 1995; Kinzler et al. 2009) amphipods were divided into three size groups: large *G. pulex,* intermediate *D. villosus* and large *D. villosus*. Amphipods were size-matched by eye prior to experiments, keeping handling and stress to a minimum. On termination of experiments, amphipods were weighed (live, blotted dry) and photographed (in curved natural resting state), with length subsequently measured as a curved line from rostrum tip to telson tip in ImageJ (Rasband 1997-2016). Datasets for all experiments were rarefied using post-experiment body size parameters to ensure size-matching between large *G. pulex* and intermediate *D. villosus*, thus allowing comparison of intrinsic differences in the species' predatory impact. Meanwhile, large *D. villosus* were significantly longer and heavier than intermediate *D. villosus* and large *G.*

pulex in all experiments, enabling quantification of differences in predation rate associated with the larger size of the invader. Mean lengths and masses of amphipod groups used in each experiment, and statistical comparisons, are given in Appendix 3.1. Mean sizes (± SE) across all experiments were: large *G. pulex* length 16.54 ± 0.08 mm, mass 46.95 ± 0.57 mg; intermediate *D. villosus* 16.79 ± 0.11 mm, 48.81 ± 0.70 mg; and large *D. villosus* 22.12 ± 0.09 mm, 106.72 ± 1.12 mg.

3.2.2 Functional response experiments

3.2.2.1 Experimental design

Four separate experiments were run in which amphipods were presented with a single prey type (carp eggs or larvae, or trout eggs or larvae) in varying densities – one experiment for each prey type. The aim of these experiments was to quantify predator FRs, modelling the relationship between resource use and availability (Holling 1959; Dick et al. 2013). This methodology for comparing alien and native species' impacts is becoming widely adopted and is accumulating supporting evidence (Haddaway et al. 2012; Dick et al. 2013; Alexander et al. 2014; Paterson et al. 2014; Dick et al. 2014).

Individual amphipods were starved for 24h, in clear plastic arenas (87 mm diameter, 50 mm depth) with approximately 200 ml of aged tap water and a single glass bead (20 mm diameter, 9 mm height) as substrate to prevent perpetual swimming. Starved amphipods were then transferred to experimental arenas, identical to starvation conditions but containing a known number of prey items (1, 2, 3, 5, 8, 10, 15, 25, 35, 50 or 80 carp eggs; 1, 2, 3, 5, 8, 12, 25 or 50 carp larvae; or 1, 3, 5, 8, 12, 16, 25, 35 or 50 trout eggs or larvae). Egg membrane strength (Zotin 1958) and larval swimming ability (Fuiman 2002) change over time, but I only selected eggs that were robust on handling, only used larvae > 12h (carp) or > 24h (trout) old, and observed no obvious changes in larval swimming ability over the time course of the experiments. Furthermore, treatments (amphipod group x density combinations) were blocked by day within each experiment to control for any temporal variation in prey (and predator) condition. Within each block, arenas were randomly arranged in space. Controls (without an amphipod) were run at all prey densities to check prey survival in the absence of predators. Controls were interspersed spatially and temporally with experimental arenas.

Arenas were placed in incubators with temperature and light regimes identical to those used to keep stock eggs and larvae: 13.9 ± 0.1°C (range) with 12:12h light:dark cycle for carp, and 7.0 ± 0.2°C (range) with 9:15h light:dark cycle for trout. Temperatures were within the range at which both amphipod species will feed (Sutcliffe et al. 1981; van der Velde et al. 2009; Maier et al.

2011). Each amphipod was allowed to feed for a set period: 24h on carp eggs or larvae, or 48h on trout because preliminary experiments indicated that predation rates on trout were much lower.

At the end of this experimental period, amphipods were removed and remaining alive, dead and damaged prey (body parts) enumerated. For each damaged prey item, the amount of flesh remaining was estimated by eye, to the nearest 10%. Consumption was calculated as the number of prey supplied minus all remaining flesh (whole and damaged prey). Deaths due to predation were defined as prey that had been wholly or partially consumed, as opposed to dead but undamaged prey assumed to reflect background mortality ($\leq$ 3.2% in all experiments). The number of partially consumed larvae was estimated from remaining body parts, assuming that if body parts may have originated from a single individual (e.g. a tail and a head) then they did so.

Used amphipods were isolated, fed with conditioned *A. pseudoplatanus* leaves and monitored for 24h. Any individuals that moulted or died in this period were excluded from the dataset. Following rarefaction to ensure size-matching, data were retained for at least four replicates at all prey densities and at least five replicates (and up to eight) for densities of five or more.

3.2.2.2 Statistical methods

All statistical analyses were carried out in R version 3.2.1 (R Core Team 2015).

For the experiments with carp eggs and larvae, predation was sufficient to construct and compare FR curves. Analyses were carried out using number of prey consumed (rounded to the nearest whole prey) or number of prey killed as response variables, but for carp prey I present only the former in the main text (a) to be consistent with analyses of electivity experiments and (b) because partial consumption was rare, so consumption was closely associated with number of prey killed and thus a reasonable basis for predicting population impacts. If frequent, partial consumption could decouple this consumption-impact relationship (Dick et al. 2002).

To determine FR type, the relationship between proportional consumption of prey and prey density was modelled using second order logistic regression with quasibinomial error distributions to account for overdispersion (Crawley 2007). The sign and significance of the coefficients indicate FR type (Trexler et al. 1988; Juliano 2001).

Then, FRs were modelled using Rogers' random predator equation (Equation 3.1, Rogers 1972), appropriate because FRs were Type II and prey were not replaced over the course of the experiments (Juliano 2001).

$$N_e = N_O\left(1 - e^{a(N_e h - \mathrm{T})}\right) \qquad [3.1]$$

where N_e is the number of prey eaten, N_0 is the initial density of prey, a is the attack coefficient, h is the handling time and T is the total time available for predation (days). Modelling was performed in the R package *frair* (Pritchard 2016) which utilises a modified version of Equation 3.1 with an additional *Lambert W* function to make the equation solvable (Equation 3.2).

$$N_e = N_O - \mathrm{lambert}W\left(\frac{ahN_0 e^{-a(\mathrm{T} - N_0 h)}}{ah}\right) \qquad [3.2]$$

Curves were bootstrapped to visualise variability (n = 1999), and the parameters a and h compared between amphipod groups (within each prey type) and prey types (within amphipod groups) using indicator variables (function *frair_compare*; Juliano 2001; Paterson et al. 2014; see also Section 2.2.3).

Incidence of partial consumption of carp larvae (whether individual amphipods partially consumed any carp larvae) was analysed with respect to prey density and amphipod group using a generalised linear model (GLM) with binomial errors. Then, considering just amphipods that exhibited partial consumption, the number and proportion of partially consumed larvae were analysed with respect to prey density and amphipod group using GLMs, with quasipoisson and quasibinomial errors respectively. To identify significant explanatory variables, GLMs were simplified to minimum adequate models (MAMs) following Crawley (2007), discarding terms whose exclusion from the model did not significantly increase deviance. χ^2 tests of significance were employed for binomial models, and F tests of significance for models involving quasi-likelihood.

In FR experiments with trout eggs, negligible levels of predation precluded statistical analysis. In FR experiments with trout larvae, levels of predation were too low to fit FR curves. Instead, incidence of predation (whether individual amphipods killed any larvae) was analysed with respect to prey density and amphipod group using a GLM with binomial errors, simplified as above (Crawley 2007). Then, amongst the amphipods that killed larvae, the magnitude of predation (number of larvae killed) and incidence of partial consumption were analysed with respect to prey density and amphipod group through simplification of quasipoisson and binomial GLMs respectively. Finally, the amount of flesh consumed by predators was compared between amphipod groups using Kruskal Wallis tests with post-hoc Dunn tests (package *dunn.test*; Dinno 2016) and Holm-Bonferroni adjustment of p values (Holm 1979).

3.2.3 Electivity experiments

3.2.3.1 Experimental design

Predatory impact also depends on electivity: the relative proportions of food types in a consumer's diet compared with the relative proportions available (Ivlev 1961; Underwood et al. 2004). Electivity is a similar concept to preference, but does not imply behavioural choices by the consumer that were unquantified in this study. Here, I quantified amphipod electivity in two experiments – one involving carp eggs with three alternative food types, and one involving carp larvae with three alternative food types – with particular focus on the tendency of amphipods to consume eggs and larvae in the presence of alternative foods.

Alternative food types were selected based on likely coincidence with carp eggs and larvae, and on prior knowledge of consumption by gammarids (Eichenberger and Weilenmann 1982; MacNeil et al. 1997; Platvoet et al. 2009). Plants were fresh, live *Ranunculus aquatilis* L. (ordered online). Leaves were *A. pseudoplatanus* leaf discs, 1 cm diameter (leaves collected from Woodhouse Ridge, Leeds, lat 53°52'N, long 1°36'W, and conditioned in stream water for three months). Invertebrates were *Asellus aquaticus* (L. 1758) isopods (collected from Woodhouse Ridge, Leeds).

Arenas were set up containing 180 ml of aged tap water, fifteen glass beads (20 mm diameter, 9 mm height) to provide habitat structure, and four food types: 10 carp eggs or larvae, plus 3-5 leaf discs, 1-3 *R. aquatilis* sections and 2-3 live *A. aquaticus*. Most food types were presented in approximately equal masses (range 34-47 mg across all arenas but < 10% variation in mass between food types within each arena). However, because of their small size (Table 3.1), adding a similar mass of carp larvae would have made them unrealistically abundant. Larvae were also too fragile to weigh prior to experiments. Thus, 10 carp larvae were added to each arena, to match the number of eggs presented in prior experiments with eggs. Food was generally provided in excess (< 30% total mass was consumed and no individual food type completely was consumed, except for larvae in four of twelve arenas containing *G. pulex*).

Individual amphipods (starved for 24h as for FR experiments) were transferred to experimental arenas and allowed to feed for 24h. Environmental conditions in incubators were the same as for carp stocks: 13.9 ± 0.1°C (range) with 12:12h light:dark cycle. Within each experiment, treatments (amphipod groups) were blocked by day, and within each block arenas were randomly arranged in space. Controls (arenas with four food types but no amphipod, to quantify prey survival and autogenic change in food masses) were interspersed spatially and temporally with experimental arenas.

At the end of the feeding period, amphipods were removed from their arena. Remaining food items were counted and, except for larvae, weighed to the nearest mg. For larvae, approximate initial and final masses were back-calculated from the mean mass of a separate sample of larvae (Table 3.1). Used amphipods were monitored for 24h as for FR experiments. Data for amphipods that died or moulted in this period were removed, leaving a final data set with 9 to 15 replicates for each amphipod group in each experiment.

3.2.3.2 Statistical methods

A small amount of autogenic change was observed in food choice controls (mean ± SE change in mass: carp eggs −0.3 ± 0.4 mg; leaf discs −1.8 ± 0.4 mg; *R. aquatilis* +1.7 ± 0.3 mg; *A. aquaticus* −1.9 ± 0.7 mg; carp larvae not weighed). Thus, true consumption was calculated by adjusting masses consumed in the presence of an amphipod by the change in mass in their absence (Haddaway et al. 2012).

First, the mass of eggs, larvae and all food consumed in each experiment were compared between amphipod groups. Where residuals were normal (after log transformation where necessary), ANOVA and post-hoc Tukey HSD tests were used to compare means. Zeros in the *G. pulex* egg consumption data rendered parametric tests unsuitable, so egg consumption was compared using a Kruskal Wallis test and post-hoc Dunn tests (Dinno 2016) with step-down Holm-Bonferroni adjustment of *p* values (Holm 1979).

Second, within each experiment and amphipod group, compositional analysis was used to detect non-random feeding and rank food items by their contribution to amphipod diet. Although originally proposed as a method to compare habitat usage, compositional analysis can equally be applied to diets (Aebischer et al. 1993; Strain et al. 2014).

The diet composition of each individual amphipod was summarised as the percentage contribution of each food type (fish, leaf, plant or invertebrate) to total mass consumed. Availability was defined as the percentage mass of each food presented (analyses assuming equal availability in the larvae experiments generated identical rankings; Appendix 3.3). These data were analysed the R package *adehabitatHS* (Calenge 2015), which first converts the percentages into log-ratios, making data for each food group linearly independent and allowing the use of standard statistical methods (Aitchison 1986). To facilitate calculation of log-ratios, zeros were replaced with a small value (for my data 0.01% was appropriate, being two orders of magnitude below the smallest measured percentage; Aebischer et al. 1993). Then, across all individuals in each amphipod group, MANOVA compared food consumption to availability, testing the null hypothesis of random

food consumption using Wilks' lambda (Λ). Significance was determined by randomisation (n = 1999). Following a significant MANOVA, an electivity ranking was generated based on differences between consumption and availability (as log-ratios) for each pair of food types. Mean differences across individuals were used to rank food types in order of importance to amphipod diet, with significant rankings identified by randomisation (n = 1999, which generated stable ranking matrices).

3.3 Results

3.3.1 Functional response experiments

3.3.1.1 Predation of carp eggs and larvae

In experimental arenas, mortality of carp eggs (21.3%) and carp larvae (50.4%) was significantly greater than mortality in controls (0.0% and 3.2% respectively; Fisher's exact tests $p < 0.001$ for both), implying that amphipods were acting as predators rather than scavengers. Amphipods were also directly observed to prey upon live eggs and larvae. However, there was variation in predation rate between individuals, including some intermediate *D. villosus* and large *G. pulex* that consumed nothing even when presented with prey at the highest densities.

FRs of all amphipod groups on both carp eggs and larvae were Type II (logistic regression first order coefficients significantly negative; Fig. 3.1, Table 3.2). Large *D. villosus* had a significantly shorter handling time on both eggs and larvae than the smaller amphipods, which did not differ in their handling time (Tables 3.3 and 3.4). By inference, large *D. villosus* had a significantly higher maximum feeding rate ($1/hT$) on both carp eggs (12.3 day^{-1}) and carp larvae (15.6 day^{-1}) than the smaller amphipods (6.2 and 8.6 day^{-1} respectively for intermediate *D. villosus*, and 7.5 and 9.4 day^{-1} for *G. pulex*). The attack coefficient on eggs or larvae did not differ between the three amphipod groups (Tables 3.3 and 3.4).

Every amphipod group had a significantly higher attack coefficient on carp larvae than on eggs (Table 3.3). Handling times were also shorter on larvae than on eggs, but only significantly so for *D. villosus* (indicator variable comparisons on eggs as base and larvae as comparator: *G. pulex* difference in attack coefficient (Da) = 2.14, p = 0.023, difference in handling time (Dh) = −0.03, p = 0.114; intermediate *D. villosus* Da = 2.44, p = 0.009, Dh = −0.05, p = 0.017; large *D. villosus* Da = 2.41, $p < 0.001$, Dh = −0.02, p = 0.027).

Table 3.2 Parameter estimates and significance levels, from second order logistic regression of the proportion of prey eaten against initial prey density, for three amphipod groups feeding on carp eggs or larvae. Quasibinomial errors were used due to overdispersion. ϕ – dispersion parameter for GLM; N_0 – first order term; N^2_0 – second order term.

Prey	**Amphipod group**	**ϕ**	**Intercept**	***p***	**N_0**	***p***	**N^2_0**	***p***	**Type**
Carp eggs	*G. pulex*	3.975	0.471	0.260	−0.067	0.006	3.536×10^{-4}	0.185	II
	Inter. *D. villosus*	1.371	0.238	0.337	−0.067	< 0.001	3.921×10^{-4}	0.016	II
	Large *D. villosus*	1.923	1.157	< 0.001	−0.079	< 0.001	5.323×10^{-4}	<0.001	II
Carp larvae	*G. pulex*	2.318	1.675	0.003	−0.107	0.018	8.428×10^{-4}	0.239	II
	Inter. *D. villosus*	1.929	2.312	< 0.001	−0.176	< 0.001	1.956×10^{-3}	0.008	II
	Large *D. villosus*	1.185	3.147	< 0.001	−0.156	< 0.001	1.484×10^{-3}	0.006	II

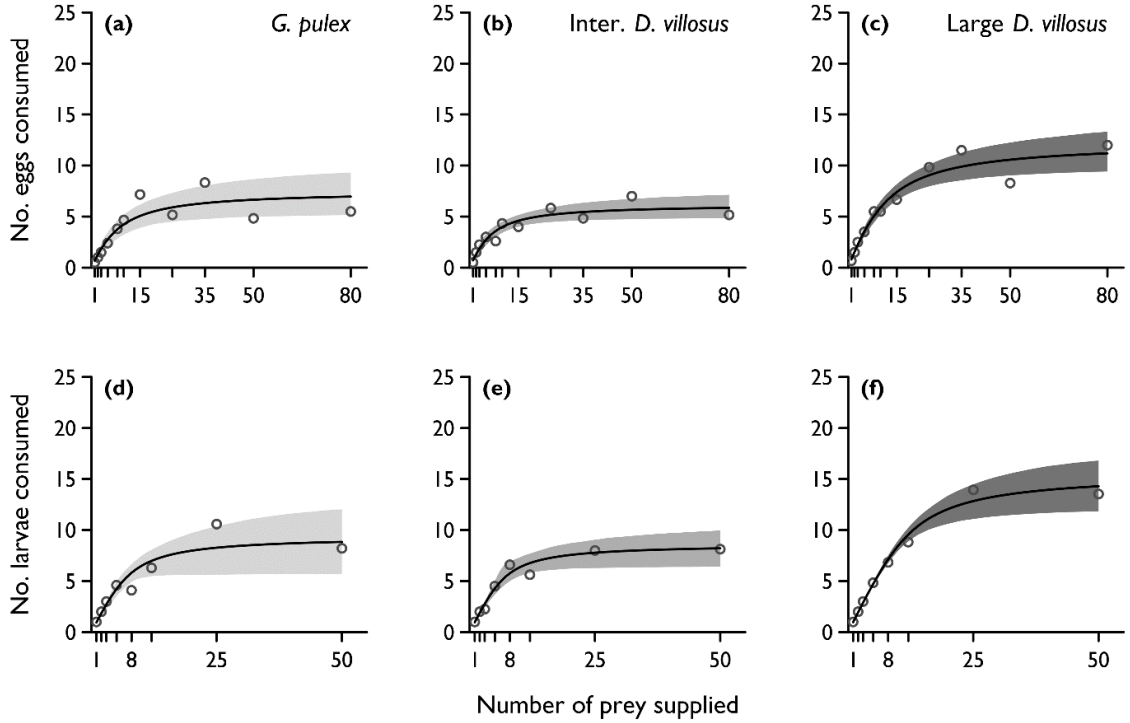


Figure 3.1 Rogers Type II functional responses of amphipods on carp eggs (upper three panels) or carp larvae (lower three panels). Predators are *Gammarus pulex* **(a,d)**, intermediate *Dikerogammarus villosus* **(b,e)** and large *D. villosus* **(c,f)**. *Open circles* are means at each density supplied ($n \geq 4$ for all prey densities and $n \geq 6$ for prey densities of ten or above). *Shaded regions* are approximate 95% confidence intervals for functional response curves based on 1999 bootstraps.

Table 3.3 Functional response parameter estimates for three amphipod groups on carp eggs or carp larvae as prey, extracted from Rogers' random predator equation fitted to data in the *frair* package (Pritchard 2016). *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); 1/*h*T – maximum feeding rate (prey.day^{-1}), where T = time in days; *SE* – standard error. *Diff* – within each prey item and for each parameter, different letters in this column indicate significantly different parameters (after Holm-Bonferroni correction for multiple comparisons).

Prey	Amphipod Group	*a*	SE	Diff.	*h*	SE	1/*h*T	Diff.
Carp eggs	*G. pulex*	1.269	0.232	a	0.133	0.012	7.5	a
	Inter. *D. villosus*	1.419	0.343	a	0.162	0.016	6.2	a
	Large *D. villosus*	1.710	0.239	a	0.081	0.006	12.3	b
Carp larvae	*G. pulex*	3.410	0.910	A	0.107	0.012	9.4	A
	Inter. *D. villosus*	3.861	0.869	A	0.116	0.010	8.6	A
	Large *D. villosus*	4.115	0.638	A	0.064	0.004	15.6	B

Table 3.4 Comparison of functional response parameter estimates for three amphipod groups on carp eggs or carp larvae as prey, based on analysis using indicator variables in the *frair* package (Pritchard 2016). Significant differences ($\alpha = 0.05$) are indicated in bold. *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); *D* – difference; *SE* – standard error.

Prey	Base Group	Comparison		Estimate (*Da* or *Dh*)	SE	*z*	*p*
Carp eggs	Inter. *D. villosus*	*G. pulex*	*a*	−0.151	0.414	−0.365	0.715
			h	−0.028	0.020	−1.408	0.159
	Inter. *D. villosus*	Large *D. villosus*	*a*	0.290	0.418	0.694	0.488
			h	−0.080	0.171	−4.689	< **0.001**
	Large *D. villosus*	*G. pulex*	*a*	−0.441	0.333	−1.324	0.186
			h	0.052	0.014	3.839	< **0.001**
Carp larvae	Inter. *D. villosus*	*G. pulex*	*a*	−0.451	1.258	−0.358	0.720
			h	−0.009	0.016	−0.598	0.550
	Inter. *D. villosus*	Large *D. villosus*	*a*	0.251	1.079	0.233	0.816
			h	−0.052	0.011	−4.532	< **0.001**
	Large *D. villosus*	*G. pulex*	*a*	−0.709	1.110	−0.639	0.523
			h	0.042	0.013	3.321	< **0.001**

Carp eggs were always completely consumed. Partial consumption of carp larvae was exhibited by individuals within all amphipod groups, but was rare and low in magnitude: only 34% of amphipods partially consumed larvae, and amongst these the number of partially consumed larvae was low (mode = 1, median = 2, range 1-6). The incidence of partial consumption did not differ between amphipod groups (not retained in MAM) but was positively associated with prey density (binomial GLM $n = 133$, $\phi = 1.134$, $\text{Deviance}_{1,131} = 58.33$, $p < 0.001$).

Amongst amphipods that partially consumed larvae, the number of partially consumed larvae increased with prey density with marginal significance (quasipoisson GLM $n = 45$, $\phi = 0.69$, $\text{Deviance}_{1,43} = 2.55$, $p = 0.061$) whilst proportional partial consumption significantly decreased with increasing prey density (quasibinomial GLM $n = 45$, $\phi = 0.59$, $\text{Deviance}_{1,43} = 21.62$, $p < 0.001$). Neither the number nor proportion of available larvae that were partially consumed differed between amphipod groups (not retained in MAMs). The similarity in partial consumption between amphipod groups, in addition to its rarity and low magnitude, means it did not decouple predatory consumption from killing and likely population impact: separate analyses of prey killed reveal identical patterns to analyses of prey consumed (Appendix 3.2).

3.3.1.2 Predation of trout eggs and larvae

In experimental arenas, mortality of trout larvae was low (4.5%), but exceeded mortality in controls (2.2%; Fisher's exact test $p = 0.022$) implying that amphipods were preying upon trout larvae. As further evidence of predation, live but damaged larvae were observed in some arenas at the end of experiments, and in separate arenas amphipods were directly observed to prey upon live trout larvae.

Only 3 of 53 *G. pulex*, 12 of 52 intermediate *D. villosus* and 40 of 54 large *D. villosus* preyed upon trout larvae. This incidence of predation did not depend on prey density (not retained in MAM) but significantly differed between amphipod groups (Fig. 3.2; binomial GLM $n = 159$, $\phi = 1.02$, $\text{Deviance}_{2,156} = 64.03$, $p < 0.001$). Large *D. villosus* were more likely to kill trout larvae than intermediate *D. villosus* ($z = 4.98$, $p < 0.001$), which in turn were more likely do so than *G. pulex* ($z = 2.37$, $p = 0.018$). Amongst the amphipods that preyed upon trout larvae, the magnitude of predation was low (mode and median number of larvae killed = 1, maximum = 2), although this did not differ between amphipod groups or depend on prey density (neither explanatory variable retained in MAM).

Partial consumption of killed larvae was frequent, but with no evidence of differing incidence across amphipod groups or prey densities (neither explanatory variable retained in MAM). Of the larvae attacked by intermediate *D. villosus*, 86% were partially consumed, compared to 70% of larvae attacked by large *D. villosus* and 67% of larvae attacked by *G. pulex*. The high incidence of partial consumption decoupled killing from feeding. Thus, despite no difference between amphipod groups in number of prey *killed*, amphipod groups differed in the amount of larval flesh they *consumed* (Kruskal Wallis $\chi^2 = 7.25$, df = 2, $p = 0.027$). Large *D. villosus* consumed a greater amount of the larvae they killed (median 0.80 larvae, interquartile range 0.50) than intermediate *D. villosus* (median 0.25, interquartile range 0.33; Dunn test adjusted $p = 0.015$). Consumption by *G. pulex* was not significantly different to consumption by either size class of *D. villosus*, but this is influenced by the small sample size for *G. pulex* (three individuals consumed 0.2, 0.2 and 1.0 larvae respectively).

Incidence of predation on trout eggs was even lower than on trout larvae. Trout eggs were completely consumed by only 3 of 152 amphipods: two large *D. villosus* and one *G. pulex*. Burst eggs were occasionally observed in tanks at the end of experiments and some of the openings appeared to have been nibbled. However, I make no further analysis of this damage (a) because it occurred rarely, (b) a very small proportion (c. 5%) of each damaged egg was apparently consumed and (c) because bursting did not occur any more frequently in tanks with amphipods

(0.6% of eggs burst) compared to control tanks (0.9%; Fisher's exact test $p = 0.529$), so initial bursting (and death) of the egg is unlikely to have been caused by the amphipods.

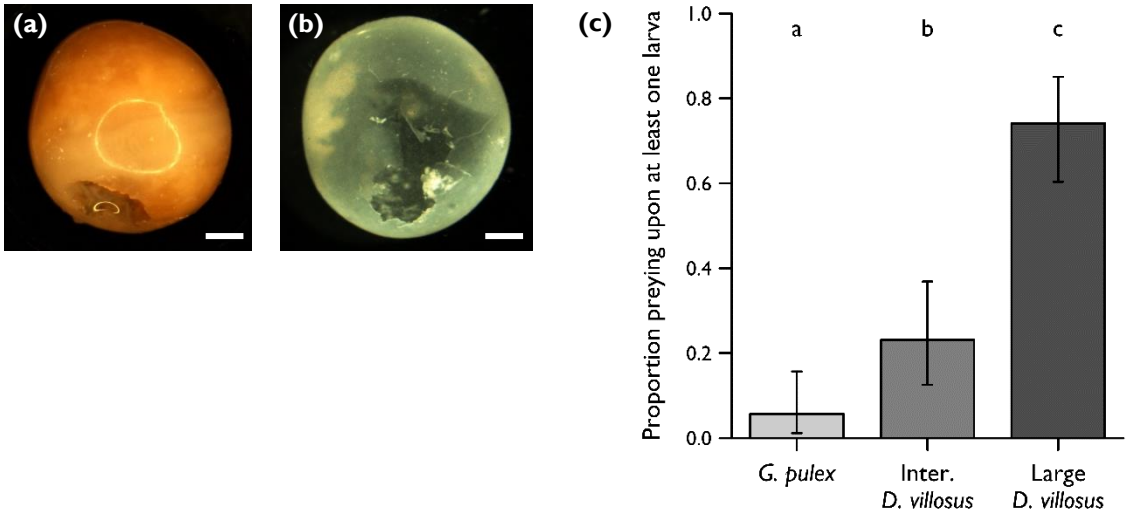


Figure 3.2 Consumption and predation of trout eggs and larvae by amphipods. Trout eggs rarely died in the presence of amphipods (overall 0.6% of eggs burst). Damage to some eggs was observed: most eggs that had burst had been nibbled around the opening **(a)**, and interior of three eggs was completely consumed **(b)**. *Scale bars* (white) approximately 30 μm. Trout larvae were more frequently killed: **(c)** shows proportion of each amphipod group that preyed upon trout larvae in functional response experiments (*n G. pulex* = 53, *n* intermediate *D. villosus* = 52, *n* large *D. villosus* = 54). *Error bars* are 95% Clopper-Pearson confidence intervals. *Letters* indicate significant differences based on a binomial GLM.

3.3.2 Electivity experiments

In electivity experiments, consumption of eggs and larvae was assumed to reflect amphipod predation because mortality in control arenas was very low (eggs 0.8%, larvae 0.0%) and no partial consumption of eggs or larvae was observed in experimental arenas. Mortality of *A. aquaticus* in control arenas was also low (3.4%).

In electivity experiments involving carp eggs, the amphipod groups consumed different masses of eggs (Fig. 3.3a; Kruskal Wallis $\chi^2 = 15.20$, df = 2, $p < 0.001$). *D. villosus* consumed a greater mass of eggs than size-matched *G. pulex* (Dunn test adjusted $p = 0.020$) and large *D. villosus*

consumed a greater mass of eggs than intermediate *D. villosus* (Dunn test adjusted $p = 0.035$). This is partially explained by differences in overall consumption (Fig. 3.3b; ANOVA $F_{2,36} = 13.05$, $p < 0.001$). Large *D. villosus* ate more food in total than intermediate *D. villosus* (Tukey HSD $p = 0.004$) and *G. pulex* (Tukey HSD $p < 0.001$). The size-matched amphipods did not differ in the amount of food consumed (Tukey HSD $p = 0.157$) although there was a tendency for *D. villosus* to consume more (Fig. 3.3b).

Amongst considerable inter-individual variation in diet composition, each amphipod group overall fed non-randomly in electivity experiments involving eggs (Fig. 3.4 a-c; *G. pulex* Wilks' $\Lambda = 0.52$, $p = 0.046$; intermediate *D. villosus* $\Lambda = 0.26$, $p = 0.002$; large *D. villosus* $\Lambda = 0.06$, $p = 0.007$). Eggs made the greatest contribution to *D. villosus* diet (Table 3.5), reflecting the fact that most individuals consumed eggs (100% of large *D. villosus* and 93% of intermediate *D. villosus*) and eggs made up the majority of *D. villosus* diet, on average (58% of large and 50% of intermediate). *Large D. villosus* supplemented egg predation with herbivory (plant material was consumed by all individuals but in small amounts) or predation on *A. aquaticus* (making a large contribution to individual diet but for only 56% of individuals). Intermediate *D. villosus* supplemented egg predation with detritivory: leaf material was consumed by 73% of individuals and made up 25% of the diet on average. In contrast, leaf material was at the top of the electivity ranking for *G. pulex*, being consumed by 87% of individuals and constituting 47% of the diet on average. Unlike *D. villosus*, the native amphipods did not consume eggs significantly more or less than any other food item (Table 3.5). Only 54% of *G. pulex* individuals consumed eggs, and eggs constituted on average 30% of *G. pulex* diet.

When carp larvae were presented as one of the food options, feeding by the three amphipod groups was remarkably similar. There was no difference in the mean mass of larvae consumed by predators in each group (Fig. 3.3c; ANOVA $F_{2,32} = 2.32$, $p = 0.115$) or in the log-transformed mean mass of all food consumed (Fig. 3.3d; ANOVA $F_{2,32} = 0.45$, $p = 0.639$).

Again, each amphipod group fed non-randomly in electivity experiments with larvae as prey (Fig. 3.4 d-f; *G. pulex* $\Lambda = 0.04$, $p = 0.001$; intermediate *D. villosus* $\Lambda = 0.07$, $p = 0.001$; large *D. villosus* $\Lambda = 0.03$, $p = 0.001$). Larvae made the greatest contribution to the diet of all amphipod groups (Table 3.5): all amphipods consumed larvae and larvae formed the greatest proportion of diets, especially for *G. pulex* (on average 78% *G. pulex* diet was carp larvae, compared to 60% for intermediate *D. villosus* and 66% for large *D. villosus*). The amphipod groups differed in the food they consumed to supplement larval predation. For example, large *D. villosus* tended to consume plant and invertebrate material as above, whilst *G. pulex* consumed leaf and plant material and avoided *A. aquaticus* (Fig. 3.4 d and f).

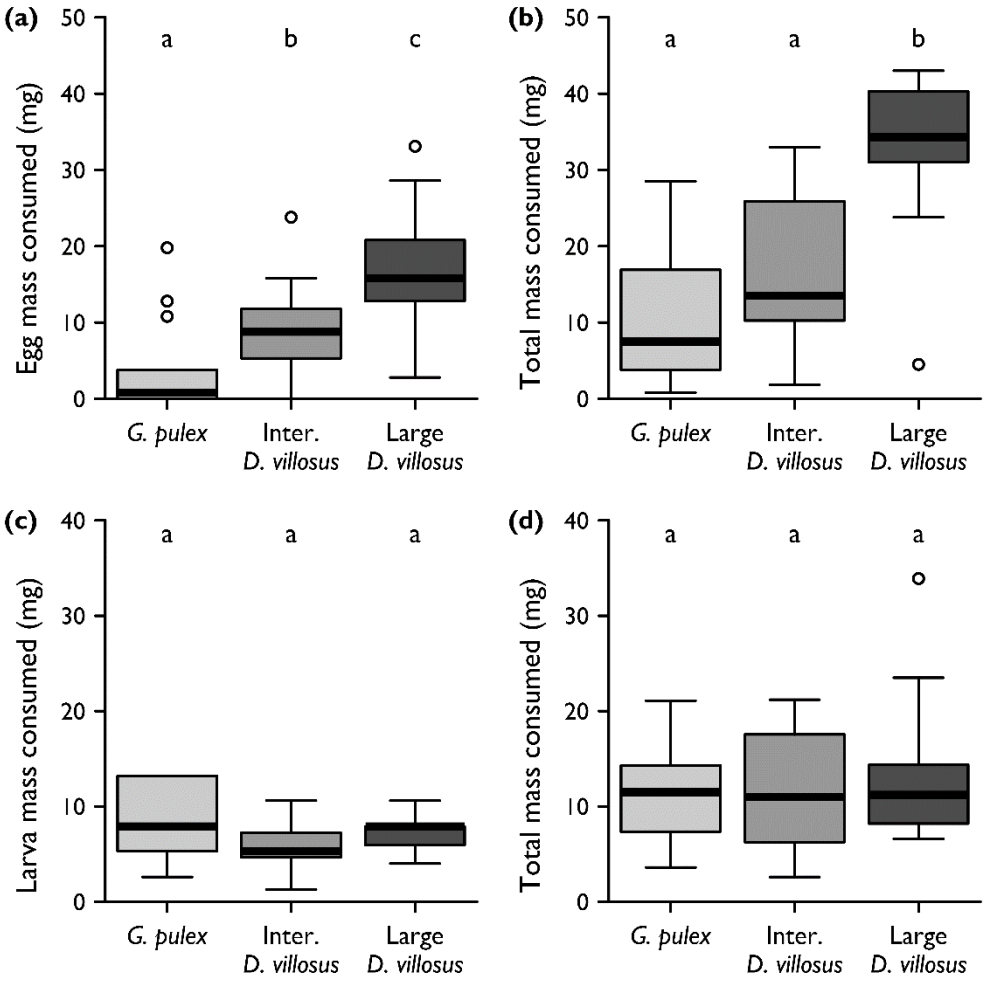


Figure 3.3 Average food consumption by each amphipod group used in electivity experiments involving carp eggs **(a-b)** or carp larvae **(c-d)**. Panels on left **(a,c)** show consumption of the focal fish prey, whilst panels on the right **(b,d)** show total consumption of all food types combined. Masses are adjusted for autogenic change. *Boxes* show medians and interquartile range, *whiskers* show data range excluding outliers, *circles* are outliers. *Letters* above boxes indicate significant differences based on Tukey HSD or Dunn post-hoc tests, as appropriate to each data set. $n \geq 9$ for all boxes: precise samples sizes are given in Fig. 3.4.

Table 3.5 Ranking of food types by contribution to amphipod diet, based on a comparison of percentage consumption to percentage availability (Aebischer et al. 1993; Calenge 2015). Full ranking matrices are given in Appendix 3.3. Eggs or larvae were presented alongside the other food items in separate experiments.

Experiment	Contribution Rankings					
	G. pulex		Intermediate *D. villosus*		Large *D. villosus*	
Eggs	leaf	a	egg	a	egg	a
	egg	ab	leaf	ab	plant	b
	plant	b	plant	bc	invertebrate	abc
	invertebrate	b	invertebrate	c	leaf	c
Larvae	larva	a	larva	a	larva	a
	leaf	b	plant	b	plant	b
	plant	b	leaf	b	invertebrate	bc
	invertebrate	c	invertebrate	b	leaf	c

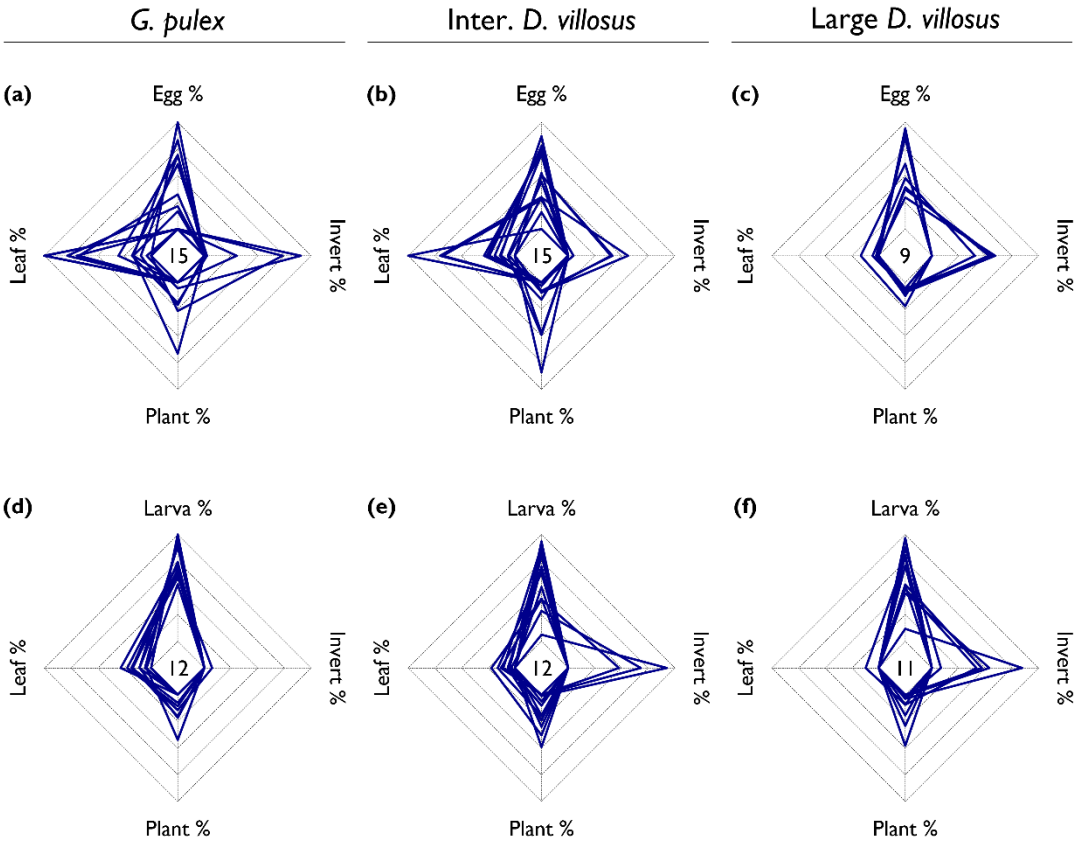


Figure 3.4 Radar plots representing the diet compositions of amphipods in electivity experiments involving carp eggs **(a-c)** or carp larvae **(d-f)**. For each experiment-amphipod combination, *n* is given in the centre of the respective plot. The diet of each individual amphipod is represented by a *dark blue polygon*, with each vertex representing the percentage of each of the four food types in the diet of that amphipod; note that some polygons overlap. Plots constructed in R package *fmsb* (Nakazawa 2015).

3.4 Discussion

The 'killer shrimp' *D. villosus* is spreading across Europe with significant ecological impacts, including declines in resident macroinvertebrate populations attributed to predation by the invader (Dick and Platvoet 2000; Josens et al. 2005; van Riel et al. 2006; MacNeil et al. 2013a). Since *D. villosus* has been observed to feed upon fish eggs and larvae, there is concern over its potential impact on biologically and commercially important fish populations. One major contributor to impact is *per capita* effect (Parker et al. 1999) and my data suggest the invasive alien *D. villosus* will have a greater *per capita* effect than British native *G. pulex* on fish populations as a predator of eggs and larvae. However, this is more a reflection of the larger size of the invader (pers. obs.; Pinkster 1970; Nesemann et al. 1995) than any intrinsic interspecific difference in predation. Relative to the smaller amphipods, large *D. villosus* showed (a) a greater consumption of food *per se* (b) a greater tendency to consume animal prey, including fish eggs and larvae, and (c) greater ability to prey upon larger fish eggs and larvae.

Large amphipods consume food (of a given size) at a greater rate than small amphipods. In FR experiments, maximum feeding rates of large *D. villosus* were 1.6 and 1.7 times greater than *G. pulex* on carp eggs and larvae respectively, and 2.0 and 1.8 times greater than intermediate *D. villosus* (Table 3.3). These differences reflect the shorter handling times of large *D. villosus* on both prey types. In experiments with trout larvae, large *D. villosus* also consumed a greater mass of the trout larvae they killed than did intermediate *D. villosus*. In electivity experiments with carp eggs, large *D. villosus* consumed the most eggs and the most food in total: median 4.6 times more food than *G. pulex* and 2.5 times more food than intermediate *D. villosus* (Fig. 3.3a-b).

Anomalously, in electivity experiments with carp larvae, large *D. villosus* consumed a similar mass of food and larvae to the smaller amphipods (Fig. 3.3c-d). The low consumption of larvae probably reflects an interaction between predator size, prey type and substrate. The largest amphipods are less able to manoeuvre through interstitial spaces, but motile prey can make best use of these spaces to evade predation (Barrios-O'Neill et al. 2015). However, it is not clear why low consumption of larvae should be associated with low overall consumption i.e. why large *D. villosus* did not consume other food items in larger quantities to compensate.

The generally positive association between size and resource consumption is in accord with previous empirical work with amphipods (Maier et al. 2011; Dodd et al. 2014) and, given the predator-prey body size ratios in the present experiment, more general theoretical work (Brose 2010; Rall et al. 2012). Metabolic rate scales positively with size (Kleiber 1932). This fundamental physiological difference must be balanced by higher consumption rates in larger

amphipods, facilitated by morphological differences such as larger mouthparts and a larger gut volume which decrease the time needed to subdue, ingest and digest prey of a given size (Brose 2010; Vucic-Pestic et al. 2010). The similarity of attack coefficients across all three amphipod groups suggests that such physiological and morphological factors, rather than behavioural ones, determine the higher feeding rate of large *D. villosus*. However, I acknowledge that the lack of differentiation in attack coefficients could be an artefact of the non-replacement design of my FR experiments (Dick et al. 2014).

As well as consuming more *per se*, large amphipods are more predatory than smaller amphipods. Whilst all amphipod groups were omnivorous in electivity experiments, in accord with MacNeil et al. (1997) and with potential fitness benefits (Cruz-Rivera and Hay 2000), animal prey tended to make a greater contribution to the diet of large *D. villosus*. It was the only amphipod group for which eggs and larvae were consumed significantly more than all other food types, and for which invertebrates (*A. aquaticus*) were not rooted at the bottom of the diet-contribution rankings (Table 3.5). Size-based dietary shifts in *D. villosus* are also apparent in the field, with stable isotope analyses indicating a tendency for large individuals to be more predatory (van Riel et al. 2006; Koester et al. 2016). It is likely that this predatory tendency will be directed towards fish eggs and larvae in the field, given the tendency of *D. villosus* to consume eggs over alternative prey (this Chapter; Casellato et al. 2007) and general electivity towards benthic prey (Dodd et al. 2014).

Larger predators are also able to capture and kill larger prey than small predators (Elton 1927; Woodward et al. 2005; Brose 2010). By virtue of their size and associated massive mouthparts, large *D. villosus* are better equipped to kill large prey. *D. villosus* can therefore have a greater impact on fish species with large eggs and larvae, such as salmonids – which were almost invulnerable to *G. pulex* predation in my experiments (Fig. 3.2). Further, the ability to feed on larger prey could intensify the impact of *D. villosus* on any given fish species in the field, given that it will be able to prey upon fish larvae for a longer period: it will take larvae longer to grow to a size that is invulnerable to *D. villosus* predation.

Meanwhile, size-matched *D. villosus* and *G. pulex* had similar predatory impacts. Neither could prey upon trout eggs, they consumed similar a similar mass of carp larvae in electivity experiments, and incidence and magnitude of partial consumption were comparable between the species. Most strikingly, FRs on both carp eggs and larvae did not differ between the size-matched amphipods – in terms of shape, attack coefficients, handling times or maximum feeding rates. Type II FRs are consistent with published amphipod FRs on invertebrate prey (Bollache et al. 2008; Alexander et al. 2012; Dodd et al. 2014; Médoc et al. 2015). The similarity of FR parameters probably reflects the nature of the prey (Moustahfid et al. 2010). Carp eggs and larvae

are relatively soft, and predation rates of size-matched *D. villosus* and *G. pulex* tend to be similar on soft-bodied prey e.g. chironomid larvae (Krisp and Maier 2005; Dodd et al. 2014). Pronounced differences between feeding rates occur when the prey is relatively tough e.g. *A. aquaticus* (Bollache et al. 2008; Dodd et al. 2014).

There were, however, two subtle differences between the size-matched amphipod species. Both are associated with a higher predatory impact of *D. villosus*, complementing its size-based impact, but are smaller in magnitude than differences related to size, so are likely to play a much smaller role in dictating impacts in the field. First, *D. villosus* was more likely than *G. pulex* to prey upon trout larvae, perhaps because its long gnathopods aid handling of large prey (Mayer et al. 2009) or its higher glycogen reserves facilitate high speed attacks to counter defensive burst swimming (Maazouzi et al. 2011). Secondly, *G. pulex* consumed fewer carp eggs than *D. villosus* in electivity experiments. *G. pulex* may be less able to crush or puncture egg capsules than *D. villosus*, and thus rejects eggs in favour of soft decaying leaves – but does not face this issue with softer carp larvae. Alternatively, the presence of habitat structure could have interfered with the detection of static carp eggs, but not motile larvae, by *G. pulex*.

In my experiments, coarse fish eggs and larvae were much more vulnerable to predation by amphipods than salmonid eggs and larvae. Whilst carp eggs were readily consumed, trout eggs were almost completely invulnerable to amphipod predation and few amphipods, of any size, killed more than one trout larva over 48 hours (Fig. 3.2). These differences in predation could reflect differences in prey size, defensive mechanisms, and/or temperature. Trout eggs and larvae are larger than those of carp. Consequently, predator-prey body size ratios of amphipods to salmonid larvae are very low (e.g. 0.45 for large *D. villosus* and trout larvae) and at these ratios attack rates are low and handling times long (Luecke et al. 1990; Brose 2010; Rall et al. 2012). Each individual salmonid larva also presents a large mass of food to be processed, meaning they will take a long time to consume and fewer individual larvae will be needed to induce predator satiation. In addition, trout eggs and larvae are both more physically defended than their coarse counterparts. Trout larvae are strong burst swimmers, assisting them to evade capture (Fuiman 2002). Trout eggs possess a thick, tough outer casing (chorion) to protect them from mechanical damage when buried in redds (Zotin 1958), but the chorion could also provide an important defensive mechanism against biological enemies such as fungal diseases (Songe et al. 2016) and invertebrate egg predators (this Chapter). Finally, the difference in predatory impact may also reflect differences in temperature. I conducted my experiments in temperatures around which trout (7^{o}C) and carp (14^{o}C) eggs develop in the field (Alabaster and Lloyd 1982). As ectotherms, amphipod metabolism and activity – including predation – will likely be reduced at lower temperatures (Sutcliffe et al. 1981; van der Velde et al. 2009; Maier et al. 2011).

Low *per capita* predation rates on trout larvae do not necessarily negate the potential for substantial mortality in the field. Daily predation will accumulate over the long development period of salmonid eggs and larvae (Teletchea and Fontaine 2010), and salmonids have a relatively small reproductive output (Winemiller and Rose 1992), which increases the importance of each individual larva to the population. Predation by *D. villosus* is likely to have the greatest effect on salmonids that lay their eggs in or around lentic environments (e.g. lake trout *Salvelinus namaycush* in North America; Claramunt et al. 2005) rather than those that breed in fast-flowing upland streams that are less favoured by *D. villosus* (Boets et al. 2010).

In addition to its higher *per capita* effect by virtue of its large size, the impact of *D. villosus* in the field may be further magnified by its abundance (Parker et al. 1999; Ricciardi 2003). *D. villosus* reaches locally high densities (up to 10,000 m^{-2}; van Riel et al. 2006) which may exceed those of other amphipods in comparable systems. In the River Meuse, for example, invading *D. villosus* accumulates to higher densities (200-500 individuals per artificial substrate) than the previous native-naturalised community (50-120 individuals per substrate), of which *G. pulex* was part (Josens et al. 2005). This conforms to the general pattern of damaging alien species reaching higher densities in aquatic systems, on average, than native analogues (Hansen at el. 2013). Although *per capita* effects may increase nonadditively with density as a result of interference between conspecifics (Hassell 1978; Médoc et al. 2015), increased densities will be associated with increased impact provided this multiple predator effect is not antagonistic. Moreover, the larger size of *D. villosus* means more individuals within the population will exceed the (unquantified) size threshold at which amphipods can feed on fish eggs and larvae (cf. Mills 1981). Consequently, a greater proportion of individuals within *D. villosus* populations will be acting as predators – so differential abundance of *predators* will be even greater than apparent from a comparison of total abundance.

It is possible that the high density and biomass of *D. villosus* could somewhat offset its negative effects as a predator. It has been suggested that this invasive amphipod will provide a plentiful food resource for fish that traverse the predatory gauntlet (Luecke et al. 1990) to reach adulthood, perhaps boosting survival and fecundity (Kelleher et al. 1998; Madgwick and Aldridge 2011; Brandner et al. 2013a; Czarnecka et al. 2014). However, the higher density of *D. villosus* could just compensate for its lower quality and profitability as prey (Arbaciauskas et al. 2010; Błońska et al. 2015) and so provide little additional benefit to fish populations.

On balance, the high *per capita* effect and high density of *D. villosus* indicate it may have a stronger negative impact on fish populations, through predation of eggs and larvae, than the native *G. pulex* it is likely to replace (Dick and Platvoet 2000) – although this impact is context-

dependent and could vary in space and time (Ricciardi 2003). Where *D. villosus* imposes even a small additive increase in mortality, recruitment into fish populations could be significantly reduced. In fish, small changes in the slope of the survivorship curve in the early life stages can coarsely control a cohort's abundance later in life (Bagenal and Braum 1968; Houde 2002). In this context, both coarse fish and salmonid populations could be negatively affected by *D. villosus* invasion: in both cases, the predatory impact of *D. villosus* is *greater* than that of native *G. pulex*. Reduced recruitment could be particularly detrimental to populations of the 37% of European freshwater fish species that are already threatened (Freyhof and Brooks 2011). Furthermore, reduced recruitment to populations exploited by anglers could negatively impact this economically and socially valuable activity (Mawle and Peirson 2009; Brown et al. 2012). Although some commercial fish populations are maintained entirely by stocking of post-larval fish and will be unaffected by amphipod predation, populations that depend at least partly on natural recruitment could be suffer under the additional mortality imposed by *D. villosus*. Fish densities will be reduced or supplementary stocking, and its associated expenditure, must be increased to compensate.

Understanding and management of alien species will be improved by the availability of quantitative evidence of their impacts (NRC 2002; Sutherland et al. 2004; Kumschick et al. 2012). My laboratory experiments contribute to this evidence for *D. villosus*, suggesting this invader will have a greater negative impact on fish populations than native *G. pulex* through predation on eggs and larvae. The higher *per capita* impact of *D. villosus* on fish is primarily due to its larger body size. Thus, in this system – and for predicting the impacts of alien species in general – size matters.

Chapter 4

Behaviour and personality in invasive and endangered decapod Crustacea

Abstract

Animal behaviour plays a critical role in mediating ecological interactions. Evidence of personality in animals (consistent behaviour within individuals) is growing, and this can have significant implications for ecological and evolutionary processes. Thus, understanding variation in animal behaviour both within and between species is critical for understanding and managing populations of alien or endangered species.

Here, I quantify the behaviour of three decapod crustacean species from British populations: the Endangered white-clawed crayfish *Austropotamobius pallipes*, the invasive signal crayfish *Pacifastacus leniusculus* and the invasive Chinese mitten crab *Eriocheir sinensis*. I ran sub-adult individuals through a set sequence of behavioural assays to quantify activity, exploration, boldness (in the presence or absence of food) and foraging voracity, with each individual being tested twice. I then (a) compared the average and variance in behaviour of each species in each assay and (b) tested for the presence of personalities within species.

Interspecific comparisons of average behaviours suggests the invasive decapods were generally bolder than *A. pallipes*. *E. sinensis* was especially bold relative to the crayfish species, less active, less exploratory and more voracious. All species showed similar variance in behaviour. These interspecific patterns could be related to the invasion process and impact of the invasive decapods, and further investigation of behaviour of invasive species could aid our understanding of invader success and impact.

Personalities were present in all three species, with consistent behaviours in at least three of the five behavioural assays. Foraging voracity (number of prey consumed) was particularly consistent across time, with Pearson correlations between the first and second tests exceeding 0.52 in all three species. Evidence for behavioural syndromes (correlations between personality axes) was less comprehensive, but my data did indicate voracity-activity syndromes in the crayfish, a boldness-exploration syndrome in *A. pallipes*, an activity-exploration syndrome and a voracity-boldness syndrome in *E. sinensis*. I discuss how a consideration of personalities may aid management of both endangered and alien species.

4.1 Introduction

The study of animal behaviour is a key part of understanding ecological dynamics (Sutherland 1996; Sih et al. 2012). Behavioural interactions between individuals mediate the processes that structure populations and communities, from competition to predation, migration, disease and symbiosis (Begon et al. 2006; Réale et al. 2007).

The ecological role of behaviour can be studied at different levels: among species, among populations of a species or among individuals within a population (Réale et al. 2007). Traditionally, ethologists have focussed on comparisons of the average behaviour of species or populations; linked to their ecology by natural selection (Tinbergen 1963). This traditional approach has value in understanding the contemporary challenge of alien species: organisms that have been transported by humans beyond their natural range, wherein they can have large ecological and socioeconomic impacts (Parker et al. 1999). Various combinations of comparisons – between alien, native, non-invasive and invasive species – can yield insights into the mechanisms driving the impact of alien species, and driving success at various stages of the invasion process (van Kleunen et al. 2010; Blackburn et al. 2011). For example, alien species that successfully establish may have a greater capacity for innovation and problem solving than those that fail to establish (Sol et al. 2002; Lefebvre et al. 2004), whilst aliens that successfully spread to become invasive may possess greater dispersal tendencies, on average, than aliens or natives that are not invasive (Rehage and Sih 2004).

More recently, it has been recognised that individual variation in behaviour can modify ecological dynamics (Wolf and Weissing 2012; Sih et al. 2012). Just as individuals may vary in traits such as size, colour or physiology (Bolnick et al. 2011), they can vary along behavioural axes, including boldness, exploration, activity, sociability and aggression (Réale et al. 2007). When an individual's position is relatively consistent across time or contexts (conditions and stimuli surrounding an animal when it exhibits a behaviour; Stamps and Groothuis 2010), that individual is said to have a 'personality' or 'behavioural type'. Personalities have been documented in a wide range of taxa, from fish (Cote et al. 2010b; Byrnes and Brown 2016) to lizards (Chapple et al. 2011), arthropods (Briffa et al. 2016) and sea anemones (Briffa and Greenaway 2011). Behaviours on each axis may be correlated in behavioural syndromes – such that the boldest individuals are also most exploratory, for example (Sih et al. 2004; Wolf and Weissing 2012).

There are numerous hypothesised implications of personalities and behavioural syndromes for ecology and evolution (Wolf and Weissing 2012; Sih et al. 2012). For example, dispersal rates can depend on the mixture of personalities in a population. Dispersers can be a non-random subset

of the population. Association of other behavioural traits with dispersal tendency can then influence the ecological role and impact of this dispersing population (Cote et al. 2010a; Juette et al. 2014). Further, personality variation may aid population establishment and persistence, essentially because mixture of behavioural types can have a buffering effect at the population level: stochastic fluctuations, responses to environmental change and resilience to perturbation average out across the various behavioural types (Bolnick et al. 2011; Wolf and Weissing 2012).

Because of these intimate links to population establishment, persistence and spread, behaviour is being increasingly incorporated into both invasion (Chapple et al. 2012; Weis and Sol 2016) and conservation (Berger-Tal et al. 2011; Greggor et al. 2016) biology. For conservation biologists, re-establishment and persistence of threatened species is a fundamental goal. A major goal of invasion biology is to understand the success of introduced populations, including how they establish and spread, with the ultimate aim of predicting and preventing future invasions. Behaviour has further pervasive links with invasion biology, both before and after establishment: it can influence the initial stages of the invasion process like the uptake of species to transport vectors (Chapple et al. 2011) and determine the range and intensity of impact of alien species (Juette et al. 2014; Dick et al. 2014).

Decapod crustaceans command attention from both invasion and conservation biologists. Decapod crustaceans are important components of freshwater food webs, acting as keystone predators and principal processors of energy and materials (Momot 1995), but some are highly threatened. The extinction risk faced by freshwater decapod crustaceans is on a par with that faced by freshwater vertebrates (Collen et al. 2014), and it is estimated that between one third and one half of the world's crayfish are threatened with population decline or extinction (Taylor 2002). A principal threat to these native populations is introduced, alien crayfish (Taylor et al. 2007). Alien crayfish typically have broad ecological impacts in invaded ecosystems – causing declines of other macroinvertebrates, altering nutrient cycling, sediment dynamics and ecomorphology – as well as negative economic impacts such as reductions of fishing catches (Holdich 1999; James et al. 2015). More generally, Crustacea are overrepresented as alien species relative to their frequency as native species (Karatayev et al. 2009).

The signal crayfish *Pacifastacus leniusculus* is a particularly successful and high-impact invasive species in European waters. *P. leniusculus* was introduced from the USA to Sweden in 1960 to boost commercial crayfish stocks but has become the most widespread alien crayfish in Europe (Souty-Grosset et al. 2006). Through its feeding behaviour *P. leniusculus* can cause significant changes to community structure (Crawford et al. 2006; Mathers et al. 2016). Populations of resident crayfish, such as *Austropotamobius pallipes* in Great Britain, are commonly extirpated

following *P. leniusculus* invasion through a combination of competition for refuges, direct predation of juveniles or post-moult individuals and spillover of the fungal crayfish plague *Aphanomyces astaci* (Souty-Grosset et al. 2006; Bubb et al. 2006; Dunn et al. 2008).

The Chinese mitten crab *Eriocheir sinensis* is another decapod invader of global concern. It is a successful invasive species, having formed multiple established populations beyond its native range, notably on the west coast of the USA and in Europe (Gollasch 1999; Dittel and Epifanio 2009). *E. sinensis* is also listed as one of the world's most damaging invasive species (Lowe et al. 2004). *E. sinensis* can, like *P. leniusculus*, alter macroinvertebrate communities through its predatory behaviour (Rosewarne et al. 2016), with further problems caused by its tendency to burrow into river banks and aggressive interactions with both resident decapods and recreational water users (Veldhuizen and Stanish 1999). Given that *E. sinensis* and *P. leniusculus* can occur in similar habitats, and both are spreading in Great Britain, it is likely that they will meet and coexist with, or exclude, each other in the near future (Rosewarne et al. 2016).

Here, I investigate the behaviour of these three decapod crustacean species – Endangered European *A. pallipes*, invasive *P. leniusculus* and invasive *E. sinensis* – in British populations. First, I compare average behaviours of the three species in an attempt to provide mechanistic insights into their overall invasion or colonisation success, their impacts and their interactions with each other and the wider community (van Kleunen et al. 2010; Blackburn et al. 2011). I expect the invasive alien species to be bolder, more active, more exploratory and more voracious than the Endangered European crayfish (Juette et al. 2014). I also expect the invaders to show less variance in behaviour, having been through filters associated with the invasion process (Juette et al. 2014). Second, I aim to identify personalities and behavioural syndromes within each species. If present, these could have important implications for our understanding and management of these species in a conservation or invasion context. More generally, this study contributes to our understanding of personality in invertebrates, which is currently limited (Gherardi et al. 2012; Mather and Logue 2013).

4.2 Methods

4.2.1 Experimental animals and holding conditions

Animals were collected from established populations in 2013 and 2015. *A. pallipes* were collected from Adel Beck, Leeds, UK under license from Natural England (#20131266 and #20144477). *P. leniusculus* were collected from Fenay Beck, Huddersfield, UK. *E. sinensis* were collected from the River Thames at Chiswick, London, UK with permission from the Port of London Authority. All animals were collected by hand to avoid bias and reduced variation in personality

types associated with trapping (Biro and Dingemanse 2009). Unavoidably, animals were collected from different rivers, so it is possible that any differences in behaviour are influenced by different local environmental conditions.

Animals were transported to Leeds in aerated cool-boxes with source water and then transferred to single-species communal tanks in a controlled environment room at 15 ± 1°C and with 12:12h light:dark cycle. Tanks contained a shallow (5 mm) layer of gravel, excess PVC tubing (10 cm length, 5 cm diameter) as shelter, and aged aerated tap water. Animals were fed liberally with Hikari® Crab Cuisine™ three times weekly. Water was changed weekly.

Animals were kept in the controlled environment room for an acclimation period of one to three weeks before being used in behavioural assays: assumed to be long enough for acclimation to laboratory conditions but short enough such that measured behaviour reflects natural behaviour as closely as possible.

Decapods used in behavioural assays were healthy (with all limbs present and free from parasites on visual inspection; Souty-Grosset et al. 2006) and intermoult (hard-shelled; no decapods moulted within 1 week of data collection). Animals were size-matched across species by a combination of mass and maximum carapace dimension (cmax). As a carapace measurement, cmax was used instead of carapace length due to the contrasting body plans of crabs and crayfish; the former being wider than they are long whilst the latter are longer than they are wide. Further, *E. sinensis* tended to be heavier for a given cmax so I used crabs that had a slightly shorter carapace than the crayfish but weighed slightly more. Body mass and cmax were combined into a single measure of body size using PCA. The first principal component explained 96.2% of variation in body size and did not differ between species (ANOVA $F_{2,77} = 1.45$, $p = 0.240$). Crayfish carapace lengths ranged from 26.4 to 42.8 mm, masses 5.3 to 29.2 g. Crab carapace widths ranged from 25.0 to 41.9 mm, masses 6.2 to 31.2 g. Animals of both sexes were used, but this was accounted for in subsequent analyses and did not affect any of the measured behaviours. To facilitate identification, individuals were marked on the dorsal carapace with correction fluid.

Animals were re-used in further experiments before euthanasia by hypothermia. Despite being a protected species, *A. pallipes* were not returned to the wild because of the risk of contamination through being housed in the same facilities as *P. leniusculus*.

4.2.2 Behavioural assays

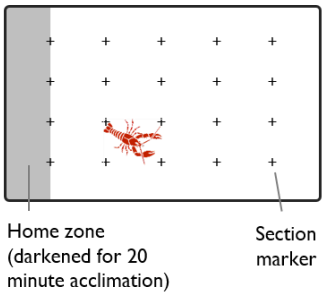
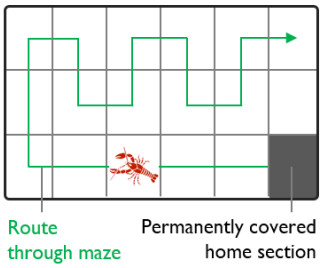
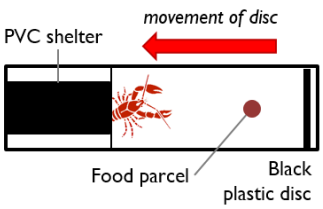
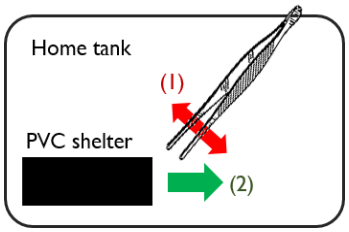
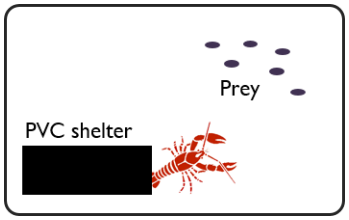
Animals were run through a set husbandry sequence, with a set order of behavioural assays interspersed with housing in individual 'home' tanks, and an identical feeding, cleaning and starvation schedule. Home tanks (23 cm length, 15 cm width, 8 cm depth) were constantly aerated, contained a PVC shelter and had their sides covered in black plastic to minimise visual disturbance (Pintor et al. 2008). For each animal, the entire husbandry sequence took 10 days.

Behavioural assays (Table 4.1) were designed following Réale et al. (2007) and Pintor and Sih (2008), to test major personality axes and with stimuli salient to the subjects. In brief, the assays and their associated measurements were (a) exploration: rate of movement through a novel maze, once animal had left sheltered home section (b) activity: number of sections covered in an open field, where a section is covered when the whole carapace of an animal is within the section (c) foraging voracity: number of prey items consumed in a given time (d) boldness 1: latency to emerge from a shelter and *feed* under predation risk and (e) boldness 2: latency to emerge from a shelter under predation risk, *without food present*. Where possible (i.e. in the activity, exploration and boldness 2 assays), behaviours were recorded by webcam (Logitech Webcam Pro 9000 with Webcam XP software) to minimise disturbance to animals.

Two tests for boldness were used to check that animals were indeed perceiving risk and not just responding to the presence of food in the first assay. The activity assay was unavoidably confounded with novelty because the open fields were too large (relative to available controlled-temperature space) to allow a prolonged familiarisation period. In the foraging voracity assay, prey consumption was checked every hour for the first seven hours after prey addition, and then again after 16 hours. Analyses were performed on consumption data after seven hours, when there was sufficient variation within and between species (i.e. most animals had consumed some, but few had consumed all, of the prey available).

To assess consistency of behaviour, each individual animal was run through each assay twice. Assays were run in the same sequence each time. The interval between the first and second runs of each assay was typically four days, except for the boldness 2 test for which both runs were run on the same day. The repeats of the open field test, maze test and boldness 2 test were run at different times of day (dawn and dusk for the former two, day and night for the latter), adding further situational variation to the temporal separation (Sih et al. 2004). Trails at dusk, dawn or night were run under red light.

Table 4.1 Overview of behavioural assays

Assay	Description	Diagram	Measurement
Activity Open Field (OF)	Large (72 x 50 x 20 cm) tank, divided into 30 sections (10 x 12 cm) with permanent marker to measure movement, Tank filled with water to 8 cm depth. Subject placed in darkened, home zone of tank for 20 minute acclimation period. Following acclimation, barrier is removed and subject free to move around entire open field for 20 minutes. Run 1 at dawn (approx. 1 hour before light phase); Run 2 at dusk (approx. 1 hour before dark phase).		Number of sections covered
Exploration Maze (MZ)	Large (80 x 50 x 10 cm) tank, physically divided into 18 equal-sized chambers. Subject isolated under darkness in first chamber for 20 minute acclimation period. Following acclimation, barrier is removed and subject free to emerge from shelter and move through maze for 20 minutes. Run 1 at dusk (approx. 1 hour before dark phase); Run 2 at dawn (approx. 1 hour before light phase).		Rate of exploration of chambers (number of sections covered/time in maze)
Boldness 1 with food (B1)	Animal placed in a 40 cm long drainpipe, with shelter glued in one end. After 48h familiarisation period, standard food ration (meat cat food and Crab Cuisine™ pellets in permeable pouch) added 15 cm from end of shelter. Predator simulated as black plastic disc moved towards animal at constant speed (6 $cm.s^{-1}$), then subject allowed to emerge at will. Trial terminated after 60 minutes. Always run 2 hours after onset of dark phase.		Latency to emerge from shelter
Boldness 2 without food (B2)	Animal picked up by sides of carapace, then trapped in 10 cm long home shelter (which it has lived with for at least 24 hours) for 5 minutes in home tank. Shelter uncapped, and danger simulated by rattling forceps in entrance of shelter 5 times (1). Tank left undisturbed, giving crayfish chance to emerge from shelter (2). Trial terminated after 20 minutes. Run 1 3 hours before dark phase; Run 2 1 hour after onset of dark phase.		Latency to emerge from shelter
Foraging Voracity (FV)	Subject placed in tank (23 x 15 x 8 cm), enclosed in a shelter (10 cm length, 5 cm diameter). Size-matched prey items added simultaneously. Prey and subject allowed 1 hour to acclimate separately in tank, then predator allowed to emerge from shelter at will. Always started in light phase. Trial covered 5 hours in light, then 3 hours in dark.		Number of prey consumed after 7 hours

The husbandry/assay sequence was followed in two separate years, with different groups of animals. In 2013 *E. sinensis* and *P. leniusculus* were tested in late August/September, whilst *A. pallipes* were tested slightly later in October/early November due to a delay in receiving the collection license. In 2015, all animals were tested in late June/July. Macroinvertebrate prey availability varied seasonally, such that in 2013 *E. sinensis* and *P. leniusculus* were presented with snails (*Lymnaea stagnalis*; 15-20 mm shell length) in the foraging voracity trials, whilst all other trials were run with *Asellus aquaticus* (6-9 mm body length).

4.2.3 Statistical analyses

All statistical analyses were performed in R version 3.2.1 (R Core Team 2015). When analysing voracity within species, I used data for the prey item for which sample size was largest i.e. *A. aquaticus* for *A. pallipes* but *L. stagnalis* for *P. leniusculus* and *E. sinensis*. Interspecific comparisons of foraging voracity were made using only the 2015 data (with *A. aquaticus* as prey) i.e. when all predator species were tested at the same time using the same prey species.

First, behaviours were compared between the decapod species. Mean behaviours in each assay were compared between species using Gaussian linear models. The averaged metrics had similar distributions as the scores from each individual run, suggesting they were reasonable summary variables. Initially, linear models were fit containing the focal response main effect 'species' (factor) and all potential confounding main effects 'sex' (factor), 'body size' (covariate, from PCA) or 'year' (factor). These were reduced to minimum adequate models, containing species and any significant confounds, by backwards stepwise procedures (Crawley 2007). I assessed the significance of the main effects using Type II tests (*drop1* with *F* tests), then tested for significant differences between species using Tukey HSD tests on the adjusted means for 'species'. Although confounds were reduced experimentally (by size matching species and running a similar number of animals in each year), this analytical procedure accounts for any remaining variation in behaviour owing to sex, body size or year/season (Packard and Boardman 1999; Darlington and Smulders 2001).

Second, within each species I tested for the presence of individual personalities: behaviours that are consistent within individuals across time and situations (Wolf and Weissing 2012). Raw data from each measurement were converted into a behavioural score that accounts for any influence of size, sex or year within species. Thus, behavioural scores were residuals from minimum adequate models, simplified from generalized linear models (GLMs) containing the main effects of size (cmax), sex and year (Table 4.2; Crawley 2007). Interactions were not included to avoid overfitting models. Model families were chosen as appropriate to each measurement; typically

Gaussian or negative binomial (on raw or transformed data), and occasionally quasipoisson (with quasi likelihood used to account for any overdispersion). Where no terms were retained in the minimum adequate model, implying none of the potential confounds significantly affected behaviour, the raw data were used as the behavioural score.

To identify personality, I calculated the correlation between the behavioural score from the first and second run of each assay (Stamps and Groothuis 2010; Truhlar and Aldridge 2014). The correlation coefficient quantifies the consistency of each individual's behaviour relative to the group mean. If data were, or could be transformed to, bivariate normality then I used Pearson correlation coefficients (r). Otherwise, I used non-parametric Kendall tau-b correlations (τ) which account for tied scores (Agresti 2012). I make no correction for multiple comparisons due to the exploratory nature of this analysis (Bender and Lange 2001), but acknowledge that some spurious correlations may arise by chance.

Third, I tested for the presence of behavioural syndromes (correlations between different behavioural traits) using Pearson or Kendall correlations between the average scores of individual animals in each assay (Truhlar and Aldridge 2014; Mazué et al. 2015).

4.3 Results

4.3.1 Interspecific comparisons

In all assays, the species differed in the average behaviour displayed (F tests $p < 0.001$). In most cases none of the candidate confounds significantly affected behaviour, the exceptions being activity which significantly differed between years ($F_{1,76} = 6.92$, $p = 0.010$) and foraging voracity which increased with body size ($F_{1,27} = 11.07$, $p = 0.003$).

Compared to the invasive species, *A. pallipes* were more active in the open field (Fig. 4.1a) and slower to emerge from shelter in the boldness 1 assay (in the presence of food; Fig. 4.1c) (Tukey HSD ps < 0.006). *A. pallipes* were also shyer than the invaders in the boldness 2 assay (without food; Fig. 4.1d), although only significantly shyer than *E. sinensis* ($p < 0.001$; vs *P. leniusculus* $p = 0.455$). *E. sinensis* was significantly less active ($p < 0.001$), less exploratory ($p < 0.001$) and bolder (boldness 2; $p < 0.001$) than both crayfish species. *E. sinensis* was also the most voracious species on average, although not significantly more so than both crayfish species. Differences in the average behaviour of the crayfish species were apparent in the open field test where *A. pallipes* was more active than *P. leniusculus* (Fig. 4.1a; $p < 0.001$), the boldness 1 assay (with food), where *A. pallipes* spent significantly longer hiding than *P. leniusculus* (Fig. 4.1d; $p = 0.002$), and the

foraging voracity test where *A. pallipes* consumed more isopods than *P. leniusculus* (Fig. 4.1e; $p < 0.001$).

4.3.2 Personalities

Behavioural scores were derived as residuals from GLMs, simplified to minimum adequate models from models containing all candidate confounds of size (cmax), sex and year as main effects. In most cases, these variables did not significantly affect behaviour so behavioural scores were simply the raw measurements. Raw behavioural measures were occasionally influenced by size and year (retained in minimum adequate models; Table 4.2), but never by sex, consistent with other studies of decapod behaviour (Briffa et al. 2008; Brodin and Drotz 2014). Behavioural differences between crayfish sexes tend to arise in reproductive animals (e.g. Mathews et al. 2009), whilst in the present study animals were non-reproductive (based on season of collection and visual assessment).

Individual consistency in behaviour was most apparent in *E. sinensis*, with significant correlations between repeat tests for exploration, activity, emergence latency in the absence of food, and

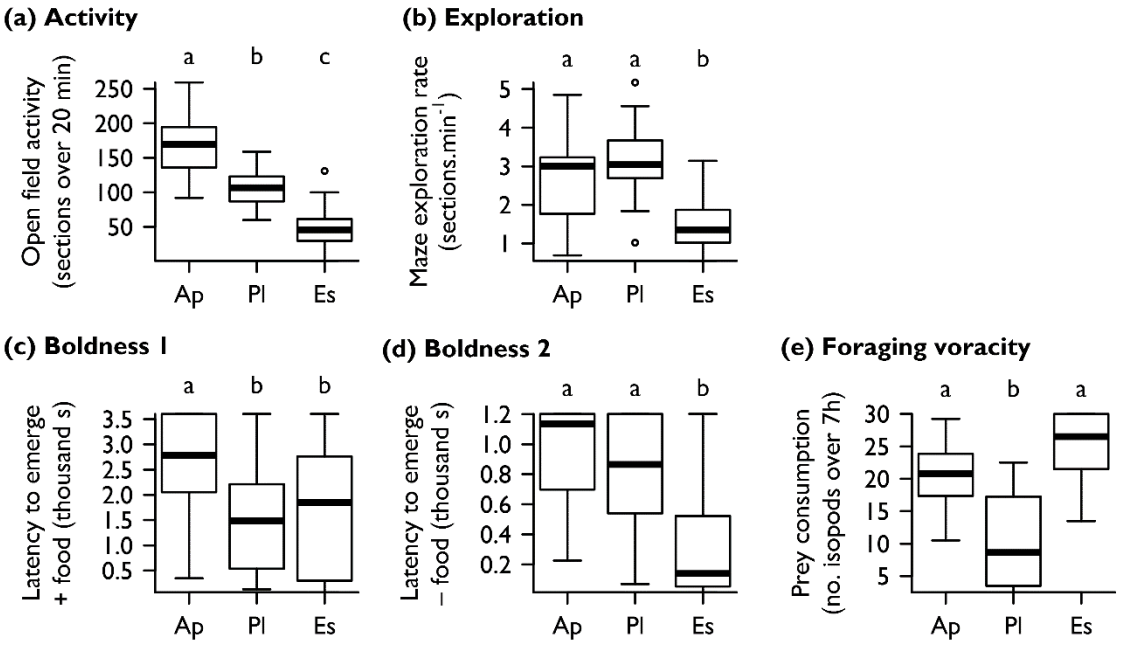


Figure 4.1 Comparison of behaviours between three species of decapod crustacean. *Ap – Austropotamobius pallipes*; *Es – Eriocheir sinensis*; *Pl – Pacifastacus leniusculus*. Data are raw values from each assay, averaged across both runs. For simplicity of plotting, confounds (year for activity, body size for voracity) are ignored, but these are accounted for in analyses. *Boxes* show medians and interquartile range, *whiskers* show data range excluding outliers, *circles* are outliers. *Letters* indicate significant differences between groups (based on Tukey HSD tests).

Table 4.2 Analysis of personality in three decapod crustacean species. **(a)** describes the model used to derive behavioural scores: the type of model and confounds retained in the minimum adequate model (from a starting maximal model containing cmax, sex and year). **(b)** describes the strength and significance of correlations between the first and second run of each assay. *p* values for correlations are uncorrected for multiple testing.

			(a) Derivation of behavioural score		(b) Consistency of behavioural score			
Species	**Personality axis**	**Assay/measurement**	**Model**	**Significant confounds**	**method**	***n***	**ρ or τ**	***p***
A. pallipes	Exploration	MZ rate of coverage	Quasipoisson	–	pearson	23	0.113	0.606
	Activity	OF sections	Gaussian	–	pearson	29	0.626	< **0.001**
	Boldness 1	Latency to emerge (+ food)	Negative binomial	–	kendall	29	0.327	**0.031**
	Boldness 2	Latency to emerge (− food)	Negative binomial	–	kendall	26	0.244	0.167
	Voracity	FV prey consumed	Gaussian	year (15 > 13) cmax (+)	pearson	29	0.537	**0.003**
P. leniusculus	Exploration	MZ rate of coverage	Gaussian	–	pearson	20	0.090	0.707
	Activity	OF sections	Gaussian	year (15 > 13)	pearson	25	0.260	0.209
	Boldness 1	Latency to emerge (+ food)	Gaussian	year (15 > 13)	pearson	25	0.432	**0.031**
	Boldness 2	Latency to emerge (− food)	Quasipoisson	–	kendall	25	0.373	**0.027**
	Voracity	FV prey consumed	Gaussian	cmax (+)	pearson	14	0.892	< **0.001**
E. sinensis	Exploration	MZ rate of coverage	Negative binomial	–	pearson	18	0.618	**0.006**
	Activity	OF sections	Quasipoisson	–	pearson	27	0.393	**0.043**
	Boldness 1	Latency to emerge (+ food)	Negative binomial	–	kendall	27	0.259	0.078
	Boldness 2	Latency to emerge (− food)	Gaussian	–	kendall	27	0.454	**0.001**
	Voracity	FV prey consumed	Gaussian	cmax (+)	pearson	18	0.528	**0.024**

foraging voracity (Table 4.2) with correlation coefficients of at least 0.393. In the crayfish, both behaviours involving food (foraging voracity and emergence latency in the presence of food) were consistent across trials. *P. leniusculus* also showed a consistent latency to emerge from a shelter in the absence of food, whilst *A. pallipes* showed consistent activity levels over time in the open field (Table 4.2).

I note that some of these correlations (where $p > 0.006$) would be rendered insignificant after Holm-Bonferroni correction for multiple comparisons. However, I avoid the use of multiple comparison correction and present these as results of an exploratory analysis.

4.3.3 Behavioural syndromes

Evidence of behavioural syndromes in all species was relatively weak: most behavioural scores were insignificantly correlated across assays. The strongest, significant correlations were (see also Table 4.3): a positive correlation between maze exploration rate and boldness in *A. pallipes* (faster explorers show a shorter latency to emerge; $\tau = -0.416$, $p = 0.016$); a positive correlation between open field exploration rate and voracity in *P. leniusculus* ($r = 0.564$, $p = 0.036$); a positive correlation between maze exploration rate and open field activity in *E. sinensis* ($r = 0.566$, $p = 0.014$); and also in *E. sinensis*, a negative correlation between latency to emerge from a shelter after a simulated predator attack and consumption of prey – but only when food was offered as an incentive to emerge from the shelter ($\tau = -0.436$, $p = 0.012$). Individuals that took longer to emerge from the shelter were less voracious predators.

I note that these correlations are not strongly significant, such that correction for multiple comparisons (e.g. Holm-Bonferroni) would render them insignificant. However, I avoid the use of multiple comparison correction and present these as results of an exploratory analysis.

4.4 Discussion

I observed temporal consistency of behaviour in *E. sinensis*, *P. leniusculus* and *A. pallipes*, which is indicative of personality. Amongst variation within species, individuals showed consistent behaviour across time and, for the maze, open field and boldness 2 tests, situations. *E. sinensis* in fact showed consistent behaviour in four of the five assays – for exploration, activity, voracity and boldness – with a marginally significant correlation in the other. *P. leniusculus* showed consistent voracity and boldness, whilst *A. pallipes* showed consistent activity in the open field as well as consistent voracity and boldness in the presence of food. All significant correlation coefficients exceeded 0.31, and generally exceeded the typical repeatability (0.37) in animal behaviour studies (Bell et al. 2009). Correlation coefficients were especially strong for the

Table 4.3 Correlations between mean behavioural scores of individual decapods in each assay, to identify behavioural syndromes. For each comparison, the correlation coefficient (r or τ) is given, followed by the p value and n data points. Raw p values are presented, with significant values (uncorrected for multiple testing) highlighted in **bold**.

A. pallipes	MZ rate	OF sections	FV prey	B1 LTE
OF sections	$r = 0.318$			*correlation*
	0.139			p *value*
	23			*sample size*
FV prey consumed (isopods)	$r = 0.359$	$r = 0.279$		
	0.092	0.212		
	23	28		
B1 latency to emerge ('shyness')	$r = -0.237$	$r = -0.217$	$r = -0.117$	
	0.122	0.117	0.398	
	23	28	28	
B2 latency to emerge ('shyness')	**$\tau = -0.416$**	$\tau = 0.000$	$\tau = -0.196$	$\tau = 0.103$
	0.016	1.000	0.197	0.511
	20	25	25	25

P. leniusculus	MZ rate	OF sections	FV prey	B1 LTE
OF sections	$r = -0.071$			*correlation*
	0.766			p *value*
	18			*sample size*
FV prey consumed (snails)	$r = 0.086$	**$r = 0.564$**		
	0.826	**0.036**		
	9	**14**		
B1 latency to emerge ('shyness')	$r = 0.025$	$r = -0.025$	$r = 0.220$	
	0.915	0.907	0.450	
	20	25	14	
B2 latency to emerge ('shyness')	$\tau = -0.137$	$\tau = 0.027$	$\tau = 0.214$	$\tau = 0.023$
	0.429	0.858	0.321	0.878
	19	24	13	25

E. sinensis	MZ rate	OF sections	FV prey	B1 LTE
OF sections	**$r = 0.566$**			*correlation*
	0.014			p *value*
	18			*sample size*
FV prey consumed (snails)	$r = 0.296$	$r = 0.355$		
	0.350	0.148		
	12	18		
B1 latency to emerge ('shyness')	$\tau = -0.153$	$\tau = -0.200$	**$\tau = -0.436$**	
	0.381	0.149	**0.012**	
	18	27	**18**	
B2 latency to emerge ('shyness')	$\tau = -0.026$	$\tau = 0.017$	$\tau = -0.105$	$\tau = 0.213$
	0.880	0.900	0.544	0.122
	18	27	18	27

voracity assay (0.528, 0.537 and 0.892; Table 4.2), suggesting feeding behaviour is particularly consistent within individuals.

My data provide the first evidence, to my knowledge, of consistent individual behaviours in *E. sinensis*, *A. pallipes* and *P. leniusculus*. Together, my data therefore add weight to the general existence of personalities in a wide range of animals, including invertebrates (Mather and Logue 2013), and that they should be taken into consideration when managing animal populations. Relocation or reintroduction of *A. pallipes* could benefit from explicit consideration of personality, for example ensuring a range of personality types are introduced to provide material for selection to act upon, rather than initiating a population with the most easily-trapped crayfish from source populations (Biro and Dingemanse 2009). Further, given the boldness-exploration syndrome in *A. pallipes*, these easily-trapped animals are likely to be more exploratory and consequently disperse (Fraser et al. 2001; Cote et al. 2010a), exacerbating Allee effects in small founder populations (Stephens et al. 1999). This could inhibit efforts to establish new populations of *A. pallipes* in 'ark sites', isolated from the threat of invading *P. leniusculus* (Peay 2009). Management of invasive species may similarly need to consider intraspecific behavioural variation. For example, because trapping invasive crayfish is likely to target the boldest subset of the population (Biro and Dingemanse 2009), a combination of eradication methods may be necessary to control crayfish populations.

Evidence for behavioural syndromes (correlations between personality axes within individuals) was less compelling, with few significant correlations between traits. In *A. pallipes*, I detected a boldness-exploration syndrome that has been previously reported in other (fish) species (Cote et al. 2010b; Mazué et al. 2015). In *P. leniusculus*, activity and voracity were positively correlated within individuals. Active individuals spend more time foraging, have more opportunity to encounter prey and thus can consume more (Pintor et al. 2008; Toscano and Griffen 2014). Metabolism could again provide a mechanistic explanation: individuals with a larger or more active metabolic engine would have more energy available for foraging, but would also need to consume more food in order to fuel their active metabolism (Biro and Stamps 2010).

In *E. sinensis*, exploration and activity were positively correlated, as were boldness and voracity (Table 4.3). I did not replicate a correlation between boldness and activity previously reported for invasive *E. sinensis* (Brodin and Drotz 2014), although the correlation between the boldness 1 and open field scores trended in agreement (Table 4.3). The significant correlations could be interpreted as behavioural syndromes. Alternatively, they could suggest that each pair of assays measured similar behaviours (Carter et al. 2013). For example, both movement through the maze and across the open field could have reflected activity of the crabs more than any exploration

behaviour, or the novelty inherent in the open field could have made this more of a test of exploration than activity (Réale et al. 2007; Decker and Griffen 2012). In the boldness 1 assay, *E. sinensis* may have responded to the food more than the simulated predator stimulus, effectively making this a test of voracity rather than boldness.

In fact, in no species were scores on the two boldness assays significantly correlated, indicating that they do not both measure the same aspect of decapod behaviour. Such concerns over exactly what behaviours are measured by each assay are of lesser importance when identifying personality: it is reasonable to assume that within a species, individuals responded to similar stimuli within each assay and there is individual consistency in that behaviour, although it may not be entirely clear what the behaviour is. I encourage further work to elucidate exactly what is being measured by laboratory behavioural assays, and caution that my behavioural labels (e.g. boldness) are simply one possible interpretation of the assays.

Comparing the average behaviours of the non-invasive European crayfish *A. pallipes* and invasive populations of *P. leniusculus* and *E. sinensis* reveals behaviours that may be associated with invasion success. *P. leniusculus* and *E. sinensis* were bolder than *A. pallipes*, generally emerging from a shelter more quickly after being scared by a simulated predator (Fig. 4.1). Boldness may be selected at various invasion stages. Boldness may favour initial uptake of invasive species, whether accidental or deliberate. Bold species and individuals are more likely to enter transport vectors, from traps for deliberate introduction (Biro and Dingemanse 2009) to ballast water tanks or recreational equipment for accidental transport (Cohen and Carlton 1997; Anderson et al. 2014). Individual and group-level boldness can also drive secondary spread of invasive species (Cote et al. 2010b; Cote et al. 2011). Boldness could also be related to stronger impacts (Juette et al. 2014): on prey (because feeding is less inhibited by predation threat), higher-order predators (boldness increases exposure to predation), and/or humans (e.g. interference with bait and clogging of infrastructure).

In addition, low levels of activity (as displayed by *E. sinensis* and *P. leniusculus* in the open field; Fig. 4.1) could favour successful invasion. Low activity levels may prevent detection in transport vectors and avoid excessive dispersal in establishing alien populations (Cote et al. 2010b; Chapple et al. 2012). However, I note that differences in activity between the species could reflect the context of the behavioural assay i.e. that activity was measured at dawn and dusk, which may not reflect the overall diel activity levels (Chapter 2; Barbaresi and Gherardi 2001; Styrishave et al. 2007; Gilbey et al. 2008). Further examination of personality traits at different times of day would be interesting and could be important for impact predictions e.g. prey taxa that are active at the same time as the decapod predators could be most vulnerable. Furthermore, activity may have

been influenced by novelty or fear in subjects placed in large experimental arenas (Walsh and Cummins 1976). Indeed, when enclosed in respirometers the crayfish species were, on average, much less active than *E. sinensis* (Chapter 2), in contrast to the results from the open field assay.

The differences in foraging voracity between species are also of interest, especially in that they are the most direct estimation of the impact of each species on prey populations. My data confirm previous findings of the high feeding rate of *E. sinensis* on macroinvertebrate prey (Chapter 2; Rosewarne et al. 2016). However, the relatively high feeding rate of *A. pallipes* was unexpected and in contrast to other work suggesting *P. leniusculus* is a more or equally voracious predator than *A. pallipes* (Chapter 2; Haddaway et al. 2012; Rosewarne et al. 2016), Further, Pintor et al. (2008) identified a boldness/voracity/activity syndrome across *P. leniusculus* populations such that if *P. leniusculus* are bolder on average (as in my experiments) I would also expect them to be more voracious. My unusual crayfish foraging results could be idiosyncratic to *Asellus* as prey, idiosyncratic to the season, or an artefact of the fixed prey density (30 individuals) presented to each predator. Quantifying functional responses across a range of prey densities would give more reliable results, for example revealing any crossover in consumption rates across prey densities (Dick et al. 2014), but was not practicable within the time constraints of the present experiment.

The behavioural profile of *E. sinensis* suggests individual crabs may have intense local impacts. *E. sinensis* was bold and voracious but not very active and exploratory, such that individual crabs may interact strongly with a relatively small area of their environment. In contrast, active and exploratory signal crayfish will have more diffuse impacts over a wider area. Obviously, the impact of *populations* of the invaders will depend on population density and the extent of the population in addition the behaviour of individuals. For example, the impact of *E. sinensis* populations can be spread over a vast area as a result of its catadromous lifestyle. Juveniles migrate up to 1400 km upstream from estuaries as they mature, and the adults return back to estuaries to breed (Panning 1939). Incidentally, individuals may undergo behavioural shifts associated with these migrations, such that the low activity observed in the present Chapter may be associated with the juvenile life stage tested.

My novel evidence for individual personalities in three species of decapod crustacean echoes growing calls for their utility in understanding, predicting and managing animal populations. Animal personalities can have important implications for population persistence, colonisation, dispersal, evolution and disease transmission (Wolf and Weissing 2012): factors that are critical in management of both endangered and alien species. My study also suggests that interspecific comparisons of behaviour, in particular boldness, could be useful for understanding the pathways, vectors, success and impacts of alien species.

Chapter 5

Variation in population characteristics, but not behaviour or physiology, along signal crayfish invasion gradients in upland rivers

Abstract

Understanding the causes and consequences of range expansion is fundamental ecological problem. Range expansion is particularly important for invasive alien species: they are defined by their ability to spread, and understanding this spread would provide an evidence base for their management. A useful framework for identifying traits associated with range expansion is to compare established populations with those at the invasion front.

Here, I compare established core populations of the invasive signal crayfish (*Pacifastacus leniusculus*) with front populations in three upland Yorkshire rivers. I conducted hand surveys to characterise population characteristics (density, sex ratio, injuries, body size), and used laboratory assays to quantify metabolic and behavioural traits (shyness-boldness, exploration, activity, sociability, foraging voracity).

I found consistent differences between core and front population characteristics, although these were not significant in all rivers. Front populations had lower crayfish densities and more male-biased sex ratios, and carapace lengths of crayfish were longer at invasion fronts. Behaviour and metabolism did not differ along the invasion gradient, with the exception of greater sociability of crayfish at the invasion front in the River Ure. My data are consistent with a social exclusion model for signal crayfish range expansion, whereby individuals (possibly subordinates) disperse on being forced out of high density established populations.

Because I measured traits in numbered individuals, I also tested for the presence of personalities and examined correlations between behaviour and metabolism. Behavioural traits were generally consistent within individuals over time, but given the lack of variation along the invasion gradient these personalities do not seem to influence range expansion dynamics. Standard and maximum metabolic rates were positively correlated with exploration (significantly) and shyness (marginally). Aerobic scope was significantly correlated with activity and sociability.

5.1 Introduction

The geographic range of many species is in a state of flux, as individuals and populations respond to changing environmental conditions or intrinsic growth (Parmesan et al. 1999; Holt 2003). Large-scale range shifts are a defining feature of alien species: species present in a geographic area to which they are not native, following human-mediated translocation (Blackburn et al. 2011). Alien species can have severe negative impacts: they are one of the leading drivers of biodiversity loss worldwide (Sala et al. 2000), degrade ecosystem service provision (Millennium Ecosystem Assessment 2005) and have substantial economic costs (Scalera et al. 2012).

Some alien species become biologically invasive upon their spread beyond the area of first introduction (Blackburn et al. 2011; Gurevitch et al. 2011). Understanding the causes, consequences and dynamics of this secondary spread is critical, providing a basis for prediction, management and intervention (Gherardi et al. 2011; Collin et al. 2013). Knowing if, where and how quickly alien species will become invasive (widespread) is important, because impacts are likely to increase in concert with range (Parker et al. 1999). The spread of alien species also provides a rare opportunity to study the process of dispersal over human timescales, and thus glean important general insights into range expansion (Kinlan and Hastings 2005).

Here, I focus on local secondary spread of alien species by autogenic dispersal of individuals, as opposed to anthropogenic translocations (e.g. Chapple et al. 2012) and/or large-scale expansion of species ranges (e.g. Capinha et al. 2011). A useful framework for studying this autogenic range expansion is to compare traits along an invasion gradient: comparing long-established, core populations with younger populations on the invasion front. At one level, characterising populations along invasion gradients reveals how impacts change over space and time (Phillips and Shine 2005; Iacarella et al. 2015) and can inform targeted management strategies based on the age or location of invasive populations (Hudina et al. 2012). At another level, such comparisons can allow inference about the drivers of dispersal, and ultimately better predictions of the spread of alien species. Characteristics and traits associated with dispersal should become overrepresented at the invasion front (Tracy et al. 2012; Canestrelli et al. 2016), reflecting differences among phenotypes in dispersal tendency (e.g. Cote et al. 2010b) and/or adaptive or plastic phenotypic responses to spatiotemporal changes in environmental characteristics along the invasion gradient (Burton et al. 2010; Phillips et al. 2010).

There are numerous examples of characteristics that are spatially sorted along invasion gradients, including population characteristics such as sex ratio (Stiver et al. 2007; Brandner et al. 2013b), morphological traits such as body size (Phillips and Shine 2005) and life history traits such as

reproductive investment and body condition (Lopez et al. 2012). Behavioural traits may be a particularly important mediators of range expansion (Estrada et al. 2016). Differences in aggression, sociability, shyness-boldness, activity, exploration and predatory behaviour have been linked to range expansion, either through comparisons along an invasion gradient or correlation with dispersal tendency (Cote and Clobert 2007; Chapman et al. 2011; Juette et al. 2014; Iacarella et al. 2015; Myles-Gonzalez et al. 2015), although the relationship is not consistent across species and contexts (Canestrelli et al. 2016). For example, greater aggression at the invasion front helps western bluebirds *Sialia mexicana* to outcompete congeners (Duckworth and Badyaev 2007), whilst in *Neolamprologus multifasciatus* cichlids it is the less aggressive females that are forced to disperse (Schradin and Lamprecht 2002). Importantly, *structured* behavioural variation within populations – individual personalities – can affect the dynamics and success of range expansion. Intra-population variation in personality traits can affect the speed and success of range expansion (Fogarty et al. 2011; Elliott and Cornell 2012), which may be driven by a limited subset of individuals with unusually high dispersal tendencies (Fraser et al. 2001; Cote et al. 2010a). Finally, physiological traits may vary along invasion gradients by virtue of their association with behaviour (Biro and Stamps 2010; Myles-Gonzalez et al. 2015), although this may not be the case if behaviour buffers selective pressures on physiology (Tracy et al. 2012).

The signal crayfish *Pacifastacus leniusculus* is a decapod crustacean, native to North America, but introduced for aquaculture purposes to Europe and Japan (Souty-Grosset et al. 2006) where it has become widespread and abundant (Kouba et al. 2014). Signal crayfish invasions are associated with changes in resident communities (Stenroth and Nyström 2003; Crawford et al. 2006; Peay et al. 2009; Mathers et al. 2016) and ecosystem processes (Moore et al. 2012; Harvey et al. 2014). In Great Britain, *P. leniusculus* is replacing the resident – and now Endangered (Füreder et al. 2010) – white-clawed crayfish *Austropotamobius pallipes*. *P. leniusculus* provides an ideal system for the study of local range expansion via spatiotemporal comparisons of traits along invasion gradients. Whilst initial introductions and large-scale translocations are a consequence (both intended and unintended) of human activity, the signal crayfish readily expands its local range by autogenic dispersal. Owing to concern from conservation biologists, the pattern of autogenic dispersal is well characterised in some river systems (e.g. Imhoff et al. 2011). Education and legislation against crayfish release in the UK (Wildlife and Countryside Act 1981) has limited anthropogenic dispersal, meaning spread within many river systems reflects 'natural' processes. Further, crayfish are amenable to behavioural (Moore 2005; Pintor et al. 2008) and physiological (Rutledge and Pritchard 1981; Rosewarne et al. 2015) study.

Field surveys and behavioural assays of adult signal crayfish in Croatian rivers indicated male-biased dispersal, and higher densities and greater aggression in older populations (Hudina et al.

2012; Hudina et al. 2015). However, the role of other behavioural and physiological traits in signal crayfish range expansion, and the potential for personality-dependent dispersal, remains unexplored. There are also few comparisons of traits along aquatic invasion gradients in organisms other than fish (but see Truhlar and Aldridge 2014), despite the prevalence and impact of biological invasions in aquatic systems (Sala et al. 2000; Strayer 2010; Moorhouse and Macdonald 2015).

Here, I make use of three reasonably well-characterised lotic invasions of signal crayfish *P. leniusculus* to compare population characteristics and traits between the established core and invasion front. Crayfish were collected from core and invasion front populations in three upland rivers in Yorkshire, UK (Bookill Gill Beck, the River Wharfe and the River Ure) and run through a series of behavioural and physiological assays in the laboratory. Specifically, I aim to test three main hypotheses (a) that population characteristics, behavioural traits and physiological traits will differ between core and invasion front populations (b) that the behaviour of individual crayfish will be consistent over time, indicative of personality (Stamps and Groothuis 2010) and (c) that there will be a correlation between physiology (metabolic rate) and behaviour, since they are linked by the common currency of energy (Biro and Stamps 2010).

5.2 Methods

5.2.1 Study sites and distribution surveys

The River Ure, River Wharfe and Bookill Gill Beck are all lotic water bodies, originating in the Pennine Hills (Yorkshire Dales National Park) at altitudes of over 400 m. The Wharfe and Ure are both tributaries of the Yorkshire Ouse which discharges on the east coast of England, whilst Bookill Gill Beck is a tributary of the River Ribble which flows westwards. Although the Wharfe and Bookill Gill Beck previously contained white-clawed crayfish *A. pallipes*, these are now absent from the immediate vicinity of the invasion front (Peay et al. 2009; Imhoff et al. 2011).

In each river, I identified a core population close to the original point of introduction: immediately downstream of Wildshare Plantation on Bookill Gill Beck, Grassington on the Wharfe, and West Tanfield on the Ure (Fig. 5.1). The population of signal crayfish in Bookill Gill Beck is thought to originate from a deliberate stocking of 4-12 crayfish in the upper reaches, around Wildshare Plantation, in 1995 (Peay et al. 2009). The Wharfe was colonised in 1990 from stocks introduced to adjacent fishing lakes in the previous decade. The point of entry into the main Wharfe was the confluence with White Beck at Kilnsey, but by 1995 signal crayfish were detected at Grassington (Peay and Rogers 1999). The Ure was also colonised by escapes from a stocked fishing lake, with crayfish first recorded at West Tanfield in 1997 (Bubb et al. 2005).

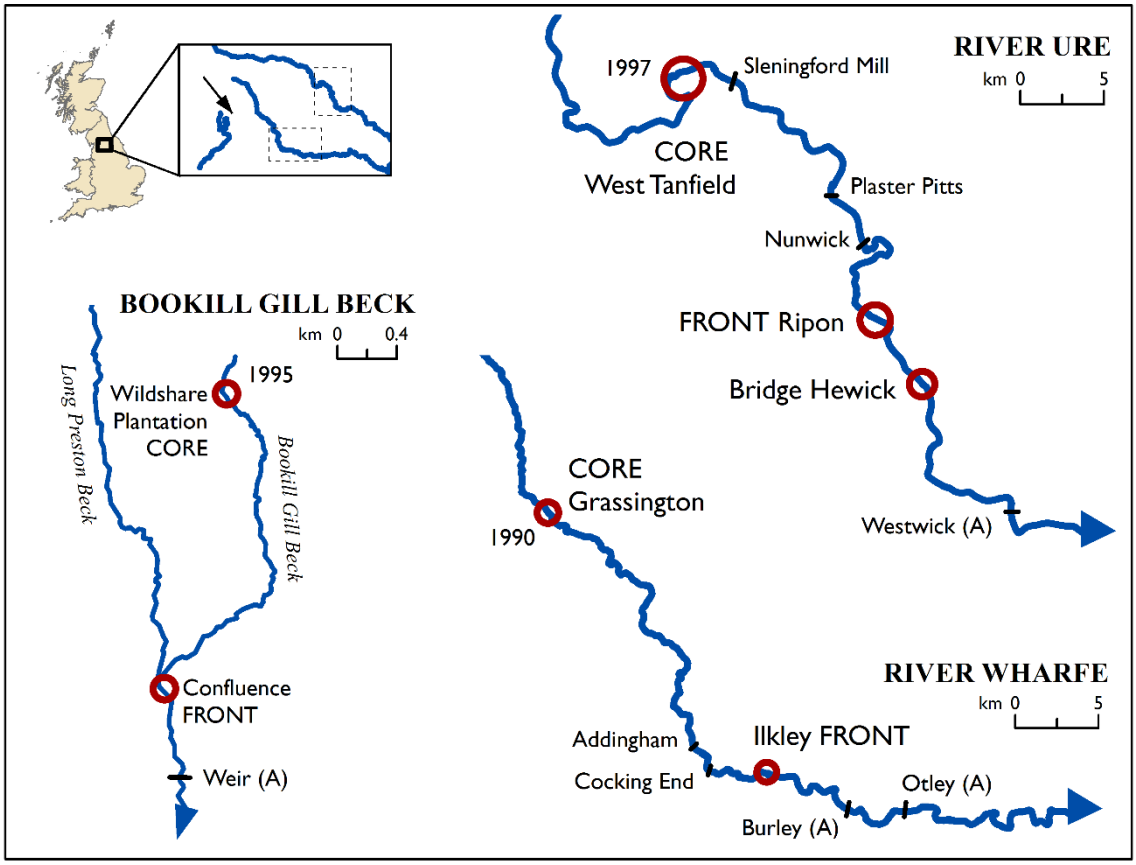


Figure 5.1 Location map of study sites. Inset shows rivers to scale, in the context of Great Britain. Arrow points to focal upper reaches of Bookill Gill Beck. On main figure red circles (not to scale) show core and front populations from which crayfish were collected for behavioural and physiological assays. (A) after a site name indicates site was searched but crayfish were absent. Dates give year and location of first detection of signal crayfish in each river. Blue arrows indicate direction of flow. Projection: OSGB36 National Grid.

To pinpoint the location of the invasion front, I carried out hand surveys at and beyond the predicted invasion front (based on survey data, published or from colleagues) between August and October 2015. Cobbles (65-256 mm by eye) and small boulders (< 400 mm) were turned and the exposed area searched for crayfish, working in an upstream direction. Underlying cobbles and pebbles were also removed and the area beneath searched, being counted as a separate refuge. Crayfish were caught if possible; crayfish that escaped, and their approximate size, were also recorded. Surveys were restricted to areas of low flow (glides or pools), identified by minimal surface disturbance. In Bookill Gill Beck, the entire width of the channel was searched. In the Wharfe and Ure, surveys were restricted to the margins (< 5 m from each bank) because the centre of the channel became too deep to survey, and the margins provide the preferred habitat of signal crayfish (Peay and Rogers 1999). Water depth in surveyed areas ranged from 5-50 cm. No white-clawed crayfish were encountered in any surveys.

In Bookill Gill Beck, the downstream spread of signal crayfish has been relatively slow, with the invasion front advancing only 0.1 km.yr^{-1} between 2007 and 2012. Signal crayfish were first recorded around the confluence of Bookill Gill Beck and Long Preston Beck in 2009, but remained at low density (< 0.4 crayfish.trap^{-1}) until 2012 (S. Peay pers. comm.). Hand surveys in 2015 found abundant signal crayfish in Long Preston Beck, immediately downstream of the confluence (54.0281, −2.2454; 0.21 crayfish.refuge^{-1}). The density declined to 0.05 crayfish.refuge^{-1} by Scalehaw Hill (54.0246, −2.2440) and no crayfish were found in 50 refuges above a weir further downstream (54.0221, −2.2432).

In the River Wharfe, signal crayfish have advanced downstream at a rate of between 1.3 and 2.5 km.yr^{-1} (Imhoff et al. 2011). In 2007 the leading downstream edge of the invasion in the Wharfe was estimated to be between Addingham and Cocking End, around 28 km from the original point of invasion. In 2009, a single juvenile crayfish was found 4 km further downstream in Ilkley (Imhoff et al. 2011). In 2015, hand surveys found signal crayfish immediately downstream of Ilkley (53.9311, −1.8056; 0.28 crayfish.refuge^{-1}), but found no crayfish in 50 refuges at Burley-in-Wharfedale (53.9120, −1.7341) or Otley (53.9105, −1.6834).

The spread of signal crayfish in the River Ure was documented by surveys in 2001 and 2003 (Bubb et al. 2005). Progression of the invasion front was slow (max 1.68 km.yr^{-1} downstream) and the crayfish were restricted to a 2 km reach around the outflow pipe from the fishing lakes (Bubb et al. 2005). Hand surveys in 2015 identified populations of signal crayfish at Ripon and Bridge Hewick at a similar density (0.16 crayfish.refuge^{-1}), but no crayfish in 50 refuges in the weir pool at Westwick (54.0973, −1.4576). Additional crayfish records between West Tanfield and Ripon suggest that these are part of one contiguous invasion rather than two separate populations. Crayfish were reported at Sleningford Mill around 2000 (Peay 2001), were found to be abundant at Plaster Pitts Farm (54.1727, −1.5309; 0.34 crayfish.refuge^{-1}), and have been recorded in electrofishing surveys as far as Nunwick (B. Morland, pers. comm. 2015).

5.2.2 Animal collection and population characterisation

Crayfish were collected from six focal populations: a core and front population in each of three rivers (Fig. 5.1; Fig. 5.2). In Bookill Gill Beck, the core population was at Wildshare and the front population just downstream of the confluence with Long Preston Beck. Herein I refer to the whole system as Bookill Gill Beck for simplicity. In the River Wharfe, the core population was at Grassington and the front population in Ilkley. In the River Ure, the core population was at West Tanfield and the front was a pooled sample, predominantly from Ripon but supplemented with individuals from Bridge Hewick (13% of total sample).

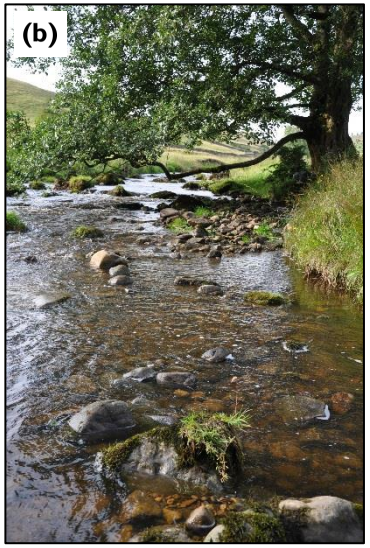
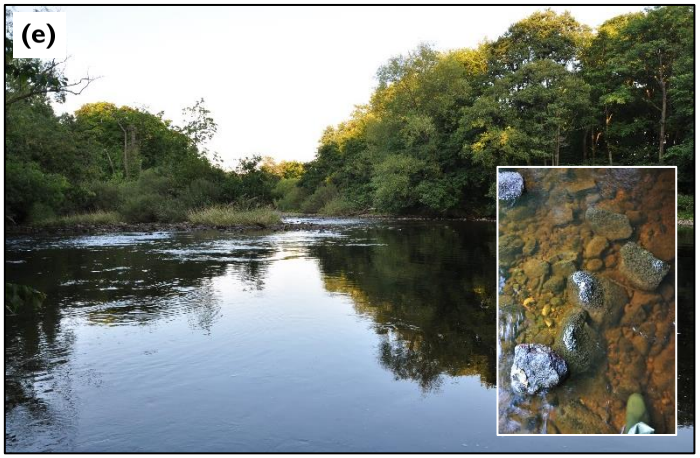


Figure 5.2 Sites from which crayfish were collected for behavioural studies. Images in the same column are from the same river; images on upper row are core populations and lower row are front populations. **(a)** Wildshare (Bookill Gill Beck core) **(b)** Confluence (Bookill Gill Beck front; this picture was taken in Long Preston Beck immediately downstream of the confluence with Bookill Gill Beck) **(c)** Grassington (Wharfe core) **(d)** Ilkley (Wharfe front) **(e)** West Tanfield (Ure core). Inset shows typical bed morphology in the Wharfe and Ure; note foot for scale. **(f)** Ripon (Ure front). In all cases, crayfish were collected from shallow marginal habitats (although at Wildshare this was effectively the entire channel width).



Core and front populations were identified through literature review and personal communications, and confirmed by manual surveys (Section 5.2.1). Front populations were sampled as far downstream from the core population as possible: signal crayfish populations did not exist in accessible sites further downstream (Wharfe/Ure), or were too small to provide reasonable sample sizes (Bookill Gill Beck). Thus although I may not have sampled right at the invasion front, my pairs of sites still compare long-established core populations and more recently-established populations, and such a comparison should bear the hallmarks of past range expansion dynamics (cf. Hudina et al. 2014). Downstream invasion fronts were assumed to reasonably reflect active dispersal because (a) at a small scale, downstream movements of signal crayfish are active rather than passive (Bubb et al. 2004) and (b) at a larger scale, downstream spread occurs gradually and a relatively constant rate (Bubb et al. 2005; Imhoff et al. 2011; S. Peay pers. comm.) rather than irregularly over time and space as would be expected were dispersal were passive, driven by e.g. drift in periods of high flow.

Individual crayfish were located following the hand search protocol described above. Hand searching allows for a more accurate description of the population than trapping, which shows strong sex-, size- and behaviour-related biases (Dorn et al. 2005; Biro and Dingemanse 2009; Price and Welch 2009). Hand collection of signal crayfish required no special permission from the UK Environment Agency, although they were aware of this activity. Barring c. 5% of encountered crayfish that escaped, all animals were collected and transported to the laboratory in cool boxes. Within 48h of collection, crayfish were measured (carapace length from tip of rostrum to base of carapace, and total length from tip of rostrum to base of uropods straightened against a rule, both to nearest 0.1 mm), weighed (blotted dry, to the nearest 0.1 g), sexed (presence/absence of gonopods) and assessed for injuries (missing legs or chelae).

5.2.3 Behavioural assays

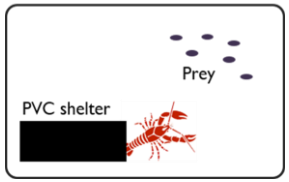
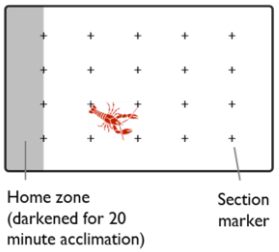
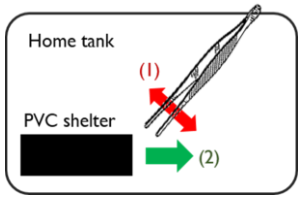
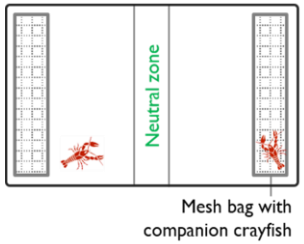
The most abundant size class of crayfish from each river was retained for behavioural experiments: carapace lengths Bookill Gill Beck 18.8-29.1 mm (median 22.0 mm), River Wharfe 23.4-36.2 mm, (median 28.5 mm), River Ure 24.4-39.9 mm (median 33.2 mm). There was no difference in size between crayfish used from core and front populations, from Bookill Gill Beck (carapace length $W_{25,27} = 337.0$, $p > 0.999$) or the River Ure (carapace length $t = 0.347$, df = 60.6, $p = 0.730$). For the River Wharfe, crayfish used from the invasion front (Ilkley) were significantly longer (carapace length $W_{18,26} = 98.0$, $p = 0.001$) and heavier (mass $W_{18,26} = 88.5$, $p < 0.001$) than those used from the core population (Grassington). Size variation between populations and amongst individuals is accounted for in statistical analyses.

Selected crayfish were housed in communal tanks according to source population (i.e. six tanks, one for each population). For each river, tanks for the core and front populations contained similar densities and sizes of crayfish. Each tank contained aged tap water to 15 cm depth, a thin layer of gravel and excess PVC shelters (10 cm length, 5 cm diameter), and was continually aerated. Each tank was fed with Hikari® Crab Cuisine™ *ad libitum* three times weekly, and water was changed once per week. Tanks were kept in a controlled environment room at 14 ± 0.2ºC (range) and 12:12h photoperiod. The photoperiod was shifted with respect to ambient to allow nocturnal behavioural measurements to be made during the working day: lights were off from 12 noon to 12 midnight.

Crayfish were held in the controlled environment room for at least eight days prior to behavioural testing, to allow acclimation to photoperiod and temperature whilst minimising potential behavioural changes due to laboratory conditions. Following acclimation, crayfish were run through a defined sequence of behavioural assays, with one assay being run per day at a set time (detailed in Table 5.1). Crayfish selected for behavioural assays remained in good condition (both chelae and all legs present, no major injuries to the body). Crayfish behaviour changes close to moulting (Chang 1995), so I only selected crayfish that had not moulted since collection. Selected crayfish were individually marked on the carapace with correction fluid.

Briefly, the testing sequence consisted of (a) an isolation period of 24h, in an individual four-litre tank (23 cm length, 15 cm width, 8 cm depth) with a PVC shelter and aeration, and with sides covered in black plastic to minimise visual disturbance (b) a foraging voracity trial, in which crayfish were provided with 30 adult (body length 11-15 mm) *Gammarus pulex* (L. 1758) and the number of prey remaining after 8 and 22 hours was recorded (c) a maze exploration trial, where crayfish were free to explore series of interlinked plastic chambers (d) a boldness trial, in which I measured the latency of crayfish to emerge from a shelter after being scared and (e) a sociability/activity trial, in which I measured movement of a crayfish around a tank (number of times a central dividing line was crossed) and the time spent in the half of the tank with an enclosed conspecific. Crayfish were fed a set ration of supplementary food (four Crab Cuisine™ pellets) following the shyness-boldness assay.

Table 5.1 Schedule and details of behavioural assays

Day	Behaviour	Assay	Diagram	Time	Measurement
1-2	Foraging Voracity	Crayfish fed standard food ration for 24hrs, then starved for 24hrs. 30 adult *Gammarus pulex* counted out, starved for approx. 1 hour to partially clear gut. Gammarus added, with 4L of fresh water, to crayfish isolation tank (23 x 15x 8 cm), 1 hour before dark phase. Controls (30 *Gammarus* in a tank without crayfish, $n = 8$) were run simultaneously with experiments and yielded high survival of *Gammarus* (99.6%). Thus, deaths in the foraging voracity trials were attributed to crayfish predation.		Start 1100 (1 hr before dark phase). End 0900 the following day (3 hours before dark phase).	Prey remaining enumerated after 8 and 22 hours of feeding.
3	Exploration	80 x 50 x 10 cm maze, divided into 18 equal-sized chambers. Animal isolated under darkness in first chamber for 20 minutes acclimation period. Barrier removed, and movement around tank observed for 20 minutes.		1500 – 1900 (3-7 hours after onset of dark phase)	Latency to emerge from home zone, total time spent out of home zone, number of chambers covered. Converted to exploration rate (when out of home zone).
4	Shyness-Boldness	Animal picked up by sides of carapace, then trapped in 10 cm long home shelter (which it has lived with for at least 24 hours) for 5 minutes in home tank. Shelter uncapped, and danger simulated by rattling forceps in entrance of shelter 5 times (1). Tank left undisturbed, giving crayfish chance to emerge from shelter (2). Trial terminated after 20 minutes.		1500 – 1900 (3-7 hours after onset of dark phase)	Latency to emerge from shelter.
5	Sociability & Activity	Plastic tank (50 x 32 x 15 cm) filled with approx. 5L of water. Companion crayfish secured in 1 mm nylon mesh bag and control (empty) mesh bag placed at either end of a tank. Companion crayfish in good condition, matched for sex and size (carapace length within 10%) with focal crayfish. Focal crayfish secured in home shelter in centre of tank for 5 minutes, then gently tipped from shelter. Trial terminated after 20 minutes. Location of the companion crayfish with respect to the observer was randomised amongst crayfish and between trials.		2000-2300 (1-4 hours before onset of light phase)	Position of focal crayfish (with companion, away from companion, or neutral) and time of any change in position. Converted to sociability (time with companion) and activity (number of times neutral zone was crossed).

All behavioural assays were carried out under dim red light during the dark phase of the photoperiod, when signal crayfish are most active (Styrishave et al. 2007). Crayfish were directly observed, with the observer located in shadows to minimise disturbance to subjects. No obvious reaction to the observer was recorded. Clean, aged tap water was used for each assay and equipment thoroughly rinsed in tap water between use with different animals.

Following completion of the testing sequence crayfish were measured and weighed, then returned to communal tanks and checked daily. I excluded data collected within 10 days of an individual moulting. My final dataset contained behavioural measurements for 159 crayfish across the six populations, with a minimum of 18 from any one population.

A subset of individuals from each population (n = 128) was run through the testing sequence a second time, two to three weeks after the original assays, in order to assess individual consistency of behaviour over time.

5.2.4 Metabolism

5.2.4.1 Measurement

For a subset of crayfish from the River Ure (n = 42 from core and front populations combined), metabolic rate (MR) was estimated from oxygen consumption in a custom-built intermittent-flow respirometer (Chabot et al. 2016). Details of the respirometry setup and conditions are given in Chapter 2 (Section 2.2.5, Fig. 2.1) – although note that in the present study, temperatures varied slightly between SMR and MMR measurements (14.0 ± 0.2^{o}C range for SMR measurements; 14.2 ± 0.2^{o}C range for MMR measurements).

Estimates of standard metabolic rate (SMR) were derived from undisturbed crayfish exhibiting minimal movement, whilst maximum metabolic rate (MMR) was assessed after an exhaustive chase. Limited access to dissolved oxygen (DO) probes meant only one crayfish could be tested per day, and meant that these measurements had to be taken in the spring following the behavioural measurements. Given consistent holding conditions over the winter, I expect relative – if not absolute – MRs of the crayfish to have remained stable over this period. Huuskonen et al. (2014) determined that *P. leniusculus* MRs were repeatable (r = 0.67) over a period of approximately three months. Crayfish from each population (core and front) were tested on alternate days to account for any possible short-term temporal effects.

During the first eight hours of the light phase, nine respirometry cycles were run to measure metabolic rate of an undisturbed crayfish (Fig. 5.3). These were controlled by electronic timer

switches connected to the magnetic stir plate and flush pump. Each cycle was 50 minutes and consisted of (a) a 2 minute mixing phase, when the stir bar was first switched on (b) a 2 minute wait phase after the flush pump was switched off (c) a 26 minute measurement phase with the flush pump off and stir bar on, during which time the respirometer was effectively a closed system (Svendsen et al. 2016) and (d) a 20 minute flush phase, during which the flush pump was switched back on to replenish the respirometer with oxygenated water and the stir bar was switched off. The length of measurement phase and flush phase were refined during pilot experiments such that the former was long enough to give a decline in oxygen consumption with a reasonable R^2 value (> 0.90; Svendsen et al. 2015) and the latter long enough to return oxygen saturation back to equilibrium levels. Oxygen pressures in the respirometer remained above 80% in almost all (98%) measurements, and remained above 70% in the rest. The DO probe operated continually throughout the day, logging temperature- and pressure-compensated [DO] (mg O_2 L^{-1}) and temperature (^oC) every 20 seconds via YSI's Data Manager Software.

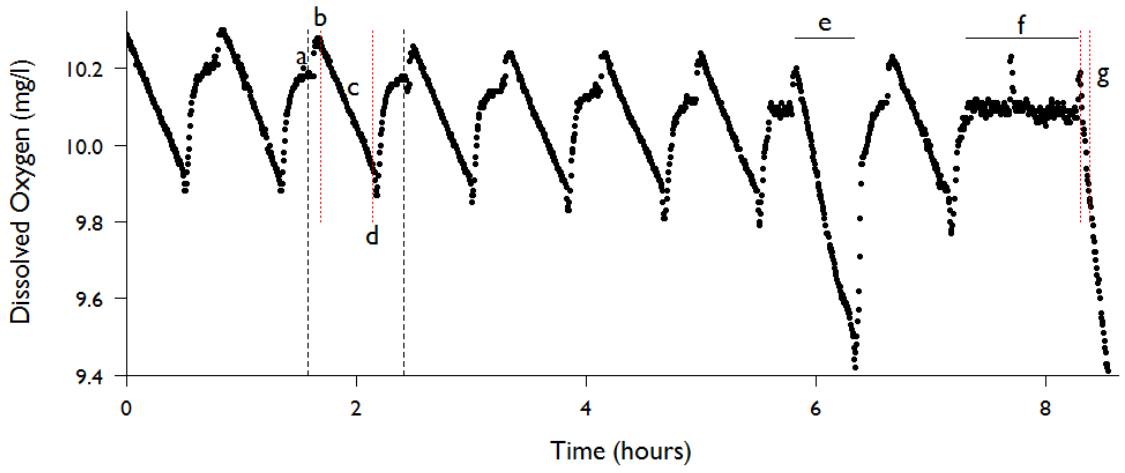


Figure 5.3 Oxygen concentration over time in the respirometer containing one crayfish (individual TM). *Vertical dashed black lines* delineate one complete cycle; *vertical dotted red lines* delineate one measurement phase. *a* – stir bar turned on, causing jump in [DO]; *b* – flush pump turned off, marking start of wait phase; *c* – measurement phase; *d* – flush pump turned on, marking start of flush phase and increase of [DO] to equilibrium; *e* – measurement phase in which crayfish was active (confirmed by webcam recordings); *f* – equilibrium [DO] whilst crayfish in separate tank undergoing exhaustive chase (peak in [DO] matches time when crayfish removed from respirometer allowing influx of saturated water); *g* – measurement phase for MMR.

Following SMR measurement, the crayfish was subjected to an exhaustive chase protocol to facilitate measurement of maximum metabolic rate (MMR), following Rosewarne et al. (2014) and Stoffels et al. (2016); see Appendix 5.1 for a schematic of the chase protocol. Exhaustive chases are a standard method for measuring MMR, especially for animals with poor sustained locomotor performance such as crayfish (Norin and Clark 2016). The crayfish was gently

removed from the respirometer and transferred to a plastic tray (400 x 250 mm) with rounded corners, with water to 4 cm depth. The crayfish was then exercised by chasing from behind with a pencil and gently grasping the chelae from the front to induce tail flipping. When the crayfish stopped tail-flipping, it was turned over to check its ability to self-right. A crayfish was deemed to be exhausted when it failed to self-right on three consecutive occasions (each separated by 30 seconds further exercise). At this point it was exercised for a final 30 seconds and transferred immediately back to the respirometer. Measurement of oxygen consumption began after a 1 minute wait phase to allow mixing of water in the respirometer, and lasted for 5 minutes. This measurement should quantify the maximum rate of oxygen uptake by the crayfish. Empirically, this period was associated with the steepest gradient of oxygen consumption.

Following measurement of MR, body mass and dimensions of each crayfish were recorded. Crayfish were then returned to communal tanks and checked daily. Data were removed for any individual that moulted within 10 days of measurement (to avoid the influence of physiological changes associated with moulting; Chang 1995; Huuskonen et al. 2014). Inspection of videos indicated three crayfish never completely settled in the respirometer during SMR measurements, so their data were also excluded.

5.2.4.2 Calculations

For each measurement phase, oxygen consumption was derived using Equation 5.1, applicable to closed-system respirometers such as ours during the measurement phase (Myles-Gonzalez et al. 2015; Svendsen et al. 2016).

$$\dot{M}O_2 = m \times (V_t - V_c) \times 3600 \qquad [5.1]$$

where $\dot{M}O_2$ is the rate of oxygen consumption (mg O_2 h^{-1}), m is the gradient of the linear decline in oxygen concentration during the measurement phase (mg O_2 L^{-1} s^{-1}), V_t is the total volume of the respirometer chamber (0.505 L) and V_c is the volume of each individual crayfish (determined by displacement immediately after MMR measurement).

Values of m were obtained by plotting oxygen concentration over time (Fig. 5.3), and fitting a least-squares regression line to the shallowest linear section of each measurement phase i.e. ignored parts of a measurement phase when a crayfish was active (most crayfish moved during at least one measurement phase). Data for each measurement phase were inspected graphically to identify the appropriate section for regression, implemented in Microsoft Excel. The section duration varied from 460s to the full 1600s. The R^2 of fitted lines was always > 0.90. $\dot{M}O_2$ was

uncorrected for background respiration as trials with a blank respirometer suggested this was negligible.

As an estimate for SMR, I took the single lowest $\dot{M}O_2$ value. This was never anomalously low: always > 85% of the next lowest $\dot{M}O_2$ measured without crayfish movement. I calculated absolute values for aerobic scope as MMR – SMR and, for the sake of completeness, factorial aerobic scope as MMR/SMR (Clark et al. 2013).

5.2.5 Statistical analyses

All statistical analyses were performed in R version 3.2.1 (R Core Team 2015).

First, to compare population characteristics between core and front populations, I excluded the young-of-year (CL < 10 mm) that could not be reliably caught, enumerated or sexed. CPUE, incidence of injury and sex ratios were compared between populations (within rivers) using χ^2 tests with Yates' correction for the single degree of freedom (R function *prop.test*). Sex ratios were also compared to equality using binomial tests (*binom.test*). The average size of crayfish caught was compared between populations using Wilcoxon rank sum tests (*wilcox.test*) due to the non-normal distribution of carapace lengths.

Second, to compare behaviour and metabolism between core and front populations in each river, I constructed linear models. Where possible, I fit general linear models (LMs) to raw or log-transformed data. Notably, for metabolic rate data both the response variable and mass were log transformed (Brown et al. 2004). If assumptions of LMs were not met after transformation, I fit generalised linear models (GLMs) instead: negative binomial for number of *Gammarus* remaining in Ure foraging voracity trials, and quasipoisson for activity data (dispersion parameters Bookill 1.79, Wharfe 2.87, Ure 2.88).

In these models, a single behaviour (from the first run of an assay) or metabolism measure was the response variable, with explanatory variables of population (core or front; factor), sex (factor) and body mass (covariate). Initially, I fit full models including all explanatory variables and their two-way interactions. These were simplified by backwards stepwise procedures, removing explanatory variables (other than population) that did not contribute significant explanatory power to the model i.e. their removal does not cause a significant increase in deviance of the model (Crawley 2007). The resulting simplified models therefore included population and any significant confounding main effects. Significance of each variable in the simplified models was tested by removing that variable, using the *drop 1* function with *F* tests or, for negative binomial

GLMs, χ^2 tests. This function tests each variable whilst leaving all others in the model (Type II sum of squares). In this way, I tested the significance of each variable whilst controlling for relevant confounding variables (Packard and Boardman 1999; Darlington and Smulders 2001; Garcia-Berthou 2001).

Data for some behavioural trials was censored. In the foraging voracity trial, some crayfish ate nothing or ate all the available prey. In the shyness-boldness and exploration trials, some crayfish did not leave their starting position within the time limit of the assay. In shyness-boldness trials, these individuals were allocated the maximum time. For maze exploration, data were missing for these individuals. For the shyness-boldness and foraging voracity assays, all individuals were included in statistical analyses because the proportion of individuals with censored data was typically small (< 0.15 within any population) or was accounted for using negative binomial GLMs. However, in the second run of the shyness-boldness assay for Bookill crayfish, the proportion of censored data rose to 0.30. In this case I ran analyses twice: once with and once without the censored data.

Third, two analyses assessed consistency of behaviours over time, in crayfish that were used in both runs of each assay. In one of these analyses, I assessed consistency of population mean behaviour across time using paired *t* tests on the scores of each crayfish in the first and second runs (or Wilcoxon signed rank tests when differences were not normally distributed). In the other analysis, I assessed consistency of individual crayfish behaviour across trials (i.e. personality) as the correlation between scores on the first and second runs. Correlations were typically Pearson's *r* where scores were normally distributed or could be transformed to normality, and otherwise Kendall's tau-b (τ). I correlated residual behavioural scores from simplified linear models containing population, sex or mass as significant explanatory variables (Boldsen et al. 2013). A positive residual indicates that an individual had a higher than expected behaviour or metabolism than expected given its body size, sex or source population. If none of these explanatory variables were significant, I correlated raw behavioural scores.

Fourth and finally, for crayfish from the Ure with MR measurements, correlations were calculated between each behaviour (from the first run of each assay) and MR. Again, residual scores were used when necessary to control for confounding variables, and Pearson's *r* was used for most correlations but Kendall's τ was employed for non-normal data.

5.3 Results

5.3.1 Core-front comparisons: population characteristics

Catch per unit effort (CPUE; number of crayfish per refuge) was higher in all core populations than front populations within the same river, although only significantly in Bookill Gill Beck and the River Ure (Table 5.2). Assuming CPUE accurately reflects crayfish density, crayfish were 2.1 times more abundant in the core population in the River Ure than at the invasion front, and 1.6 times more abundant in the core population in Bookill Gill Beck than at the invasion front.

Crayfish from core populations were more likely to be injured (missing ≥ 1 chela or leg) than crayfish from invasion fronts, although this difference was only significant in Bookill Gill Beck. Crayfish from the core population at Wildshare were 2.1 times more likely to be injured than those from the invasion front (Table 5.2).

Sex ratios within each population did not differ significantly from equality (binomial tests, $p > 0.154$), with the exception of Wildshare (Bookill core) where the sex ratio was significantly female-biased (ratio = 0.41, binomial test $p = 0.042$). Sex ratios tended to be more male-biased in front populations than core populations, but this difference was never significant (χ^2 tests, Table 5.2).

On average, crayfish from front populations were larger than those from core populations. This difference was significant in Bookill Gill Beck and the River Ure, with the same trend in the River Wharfe (Table 5.2). In the Ure, the difference was driven by larger males at the invasion front ($W_{48,57} = 1773$, $p = 0.009$) whilst females were similar in size along the invasion gradient ($W_{45,73} = 1884$, $p = 0.183$). In Bookill Gill Beck, however, females were larger in the front population ($W_{76,42} = 956$, $p < 0.001$) whilst males were similar in size along the gradient ($W_{52,29} = 595$, $p = 0.118$). In the Wharfe, both males and females were similar sizes in core and front populations, although there was a trend towards larger males at the front ($W_{35,29} = 378$, $p = 0.081$). These comparisons exclude young-of-year crayfish (CL < 10 mm). These were very rare in the habitats searched in the Wharfe and Ure (only one was caught or seen across both rivers). Young-of-year were more prevalent in Bookill Gill Beck, but their inclusion would only strengthen the size comparison: of the total catch, a greater percentage were young-of-year in the core population (28%) than at the invasion front (15%).

Table 5.2 Characteristics of core and front populations of signal crayfish in three Yorkshire rivers. All statistics calculated after young-of-year (carapace length < 10mm) were excluded. Data for Bookill Gill Beck are pooled from surveys on 21 Aug and 18 Sep, the River Wharfe 28-29 Sep, and the River Ure 9 Sep and 12 Oct. *Proportion injured* – proportion of crayfish with at least one leg or chela missing. 95% Confidence Intervals are exact intervals for proportions (R function *binom.test*) or percentile bootstrapped intervals (n = 1999) for carapace length. Bold p values, uncorrected for multiple testing, are significant at $\alpha = 0.05$.

River	Characteristic	Core		Front		Core-Front Comparison	
		Value	95% CI	Value	95% CI	Statistic	p
Bookill	Catch per unit effort (crayfish.refuge^{-1})	0.34	(0.29, 0.39)	0.21	(0.17, 0.26)	$\chi^2_{(1)} = 14.20$	< **0.001**
	Proportion injured	0.30	(0.22, 0.38)	0.14	(0.07, 0.24)	$\chi^2_{(1)} = 5.25$	**0.022**
	Sex ratio (proportion male)	0.41	(0.32, 0.50)	0.41	(0.29, 0.53)	$\chi^2_{(1)} = 0.00$	1.000
	Median carapace length (mm)	17.6	(16.0, 19.1)	21.2	(20.8, 22.0)	$W_{128,71} = 3062$	< **0.001**
Wharfe	Catch per unit effort (crayfish.refuge^{-1})	0.29	(0.24, 0.34)	0.28	(0.21, 0.35)	$\chi^2_{(1)} = 0.02$	0.904
	Proportion injured	0.14	(0.07, 0.23)	0.06	(0.01, 0.16)	$\chi^2_{(1)} = 1.39$	0.239
	Sex ratio (proportion male)	0.45	(0.34, 0.56)	0.57	(0.42, 0.70)	$\chi^2_{(1)} = 1.33$	0.250
	Median carapace length (mm)	24.6	(23.5, 29.1)	29.4	(27.9, 30.2)	$W_{78,51} = 1859$	0.531
Ure	Catch per unit effort (crayfish.refuge^{-1})	0.34	(0.29, 0.39)	0.16	(0.13, 0.19)	$\chi^2_{(1)} = 42.78$	< **0.001**
	Proportion injured	0.12	(0.07, 0.19)	0.10	(0.05, 0.18)	$\chi^2_{(1)} = 0.16$	0.690
	Sex ratio (proportion male)	0.44	(0.35, 0.53)	0.52	(0.41, 0.62)	$\chi^2_{(1)} = 1.02$	0.313
	Median carapace length (mm)	29.2	(27.3, 30.8)	32.5	(31.0, 33.6)	$W_{130,93} = 7309$	**0.008**

5.3.2 Core-front comparisons: behaviour

There was substantial variation between individual behaviour, but no difference between core and front populations in all but one case (Fig. 5.4; Table 5.3). Exceptionally, crayfish from the invasion front in the Ure at Ripon spent a significantly longer proportion of time with a conspecific (0.57) than did crayfish from the core population at West Tanfield (0.48) once the effect of mass was controlled for ($F_{1,53} = 7.44$, $p = 0.009$). However, the range of sociability was greater in the core population, with individuals spending between 16.3% and 79.8% of their time with a conspecific. There was a marginally significant difference in voracity between the core and front populations in Bookill Gill Beck ($F_{1,49} = 3.21$, $p = 0.080$), with crayfish from the core population tending to consume more than those from the front.

Interpopulation comparisons were made after controlling for the effect of the potential confounds of size and sex. Behaviour rarely differed between the sexes (Table 5.3), the exceptions being in the Ure where females were more active (median 17 line crosses, 95% bootstrapped CI = 13, 22) than males (median 14, 95% CI = 9, 16) and more voracious (median 2.2 *Gammarus* consumed per gram body mass in 8h, 95% CI = 1.9, 2.4) than males (median 1.7, 95% CI 1.4, 2.1). Body mass was positively related to foraging voracity in all rivers, with a linear relationship across the crayfish sizes used. Shyness increased with body size in crayfish from the Wharfe, and larger crayfish from the Ure were less sociable than smaller crayfish (Table 5.3).

5.3.3 Core-front comparisons: metabolism

Metabolic rates were measured for a subset of crayfish from the River Ure.

The logarithms of SMR and MMR and AAS were positively and linearly related to log body mass (exponents $b = 0.66$, 0.94 and 1.00 respectively). After correcting for differences in body mass using these exponents to divide MR by mass^b (Cech and Brauner 2011), substantial inter-individual variation was observed (Fig. 5.5). Mass-corrected SMRs varied more than two-fold from 3.8 to 8.7 mg O_2 $kg^{-0.66}$ h^{-1}. Mass-corrected MMRs ranged from 80.0 to 131.9 mg O_2 $kg^{-0.94}$ h^{-1}. Mass-corrected AAS varied from 87.2 to 148.4 mg O_2 $kg^{-1.00}$ h^{-1}. Factorial aerobic scopes varied from 3.6 to 8.1.

As for behaviour, metabolic rate and aerobic scope did not differ between the core and front populations after controlling for the influence of body mass within linear models (Table 5.4). However, there was a tendency for crayfish from the front population tended to higher metabolic rates (Fig. 5.5).

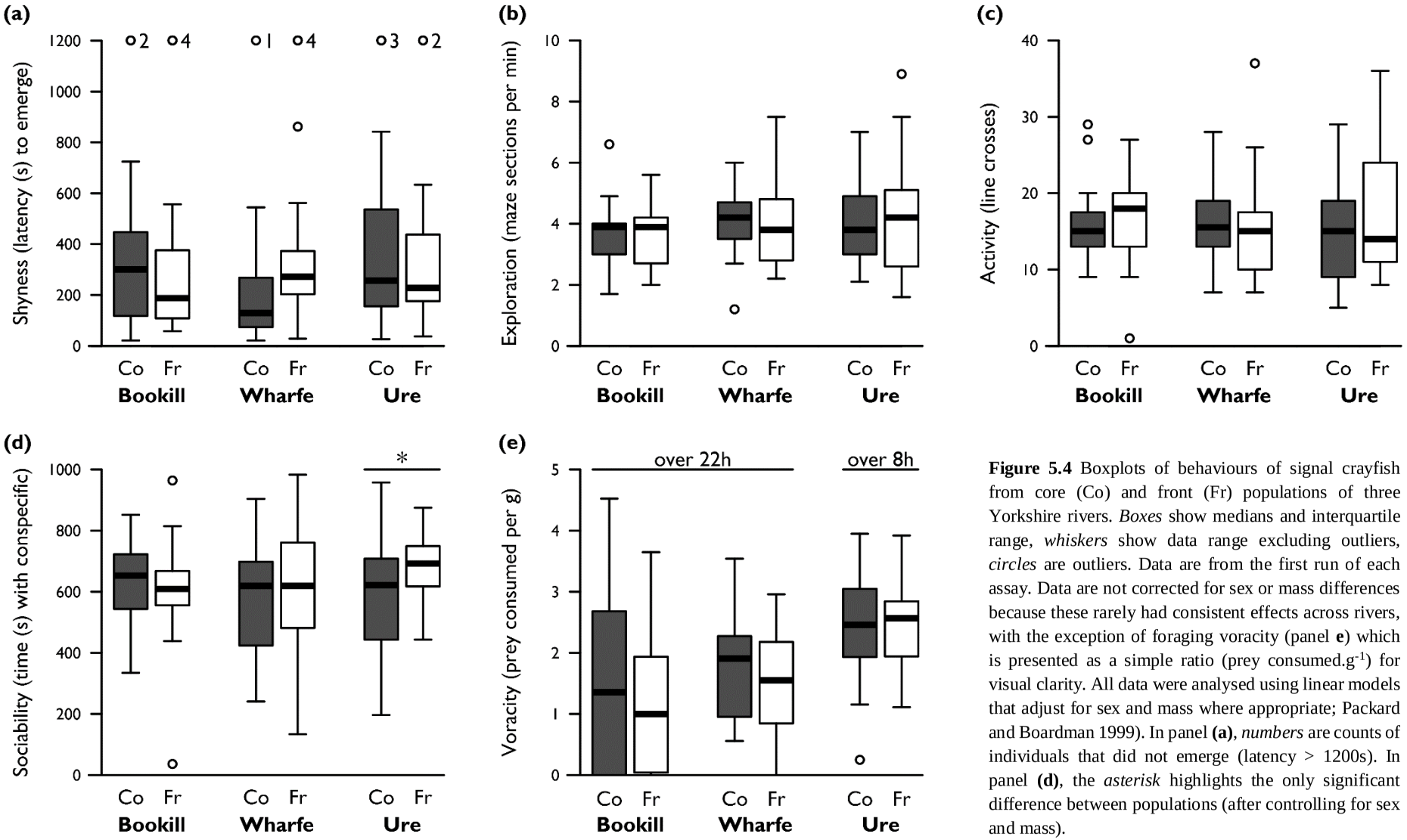


Figure 5.4 Boxplots of behaviours of signal crayfish from core (Co) and front (Fr) populations of three Yorkshire rivers. *Boxes* show medians and interquartile range, *whiskers* show data range excluding outliers, *circles* are outliers. Data are from the first run of each assay. Data are not corrected for sex or mass differences because these rarely had consistent effects across rivers, with the exception of foraging voracity (panel **e**) which is presented as a simple ratio (prey consumed.g^{-1}) for visual clarity. All data were analysed using linear models that adjust for sex and mass where appropriate; Packard and Boardman 1999). In panel **(a)**, *numbers* are counts of individuals that did not emerge (latency > 1200s). In panel **(d)**, the *asterisk* highlights the only significant difference between populations (after controlling for sex and mass).

Table 5.3 Linear models comparing behavioural traits between core and front populations of signal crayfish in three Yorkshire rivers. Models include population (core or front) and any other significant variables retained through backwards stepwise deletion. Significance refers to removal of each variable from the model, assessed using F or χ^2 tests as appropriate to model. For Ure Voracity, null deviance = 67.79, null df = 61. Bold p values are significant at $\alpha = 0.05$. Dev – residual deviance.

River	Behaviour	Model	Variable	Statistic (df)	*p*
Bookill	log Shyness	Gaussian	Population	$F_{1,50} = 0.03$	0.871
	log Exploration	Gaussian	Population	$F_{1,48} < 0.01$	0.978
	Activity	Quasipoisson	Population	$F_{1,49} = 0.17$	0.682
	Sociability	Gaussian	Population	$F_{1,49} = 0.04$	0.837
	Voracity	Gaussian	Population	$F_{1,49} = 3.21$	0.080
			Mass	$F_{1,49} = 21.82$	< **0.001**
Wharfe	log Shyness	Gaussian	Population	$F_{1,41} = 0.71$	0.405
			Mass	$F_{1,41} = 8.74$	**0.005**
	Exploration	Gaussian	Population	$F_{1,41} = 0.02$	0.901
	Activity	Quasipoisson	Population	$F_{1,40} = 0.38$	0.542
	Sociability	Gaussian	Population	$F_{1,40} = 0.13$	0.719
	Voracity	Gaussian	Population	$F_{1,41} = 2.56$	0.117
			Mass	$F_{1,41} = 30.64$	< **0.001**
Ure	log Shyness	Gaussian	Population	$F_{1,55} < 0.01$	0.973
	log Exploration	Gaussian	Population	$F_{1,56} < 0.01$	0.976
	Activity	Quasipoisson	Population	$F_{1,53} = 0.54$	0.465
			Sex	$F_{1,53} = 4.18$	**0.046**
	Sociability	Gaussian	Population	$F_{1,53} = 7.44$	**0.009**
			Mass	$F_{1,53} = 7.07$	**0.010**
	Voracity	Negative binomial	Population	$\text{Dev}_{1,58} = 68.49$	0.402
			Sex	$\text{Dev}_{1,58} = 72.59$	**0.028**
			Mass	$\text{Dev}_{1,58} = 99.77$	< **0.001**

Shyness – latency (s) to emerge from shelter after simulated attack; *Exploration* – rate of movement through maze; *Activity* – number of line crosses in bisected tank; *Sociability* – time (s) spent in half of tank with a conspecific over a 1200s trial; *Voracity* – number of *Gammarus* consumed after 22h (Bookill and Wharfe) or 8h (Ure).

Table 5.4 Linear models comparing metabolic traits of signal crayfish from core and front populations in the River Ure (metabolic rates were not measured for crayfish from Bookill Gill Beck or the River Wharfe). Models include population (core or front) and any other significant variables retained through backwards stepwise deletion. Significance derived from *F* tests, using R function *drop1*. Bold *p* values are significant at $\alpha = 0.05$.

River	Metabolic Trait	Parameter	Statistic (df)	*p*
Ure	log Standard MR	Population	$F_{1,34} = 0.45$	0.508
		log Mass	$F_{1,34} = 19.22$	< **0.001**
	log Max MR	Population	$F_{1,34} = 1.41$	0.244
		log Mass	$F_{1,34} = 117.00$	< **0.001**
	log Absolute aerobic scope	Population	$F_{1,34} = 1.06$	0.311
		log Mass	$F_{1,34} = 98.48$	< **0.001**
	Factorial aerobic scope	Population	$F_{1,35} = 0.005$	0.943

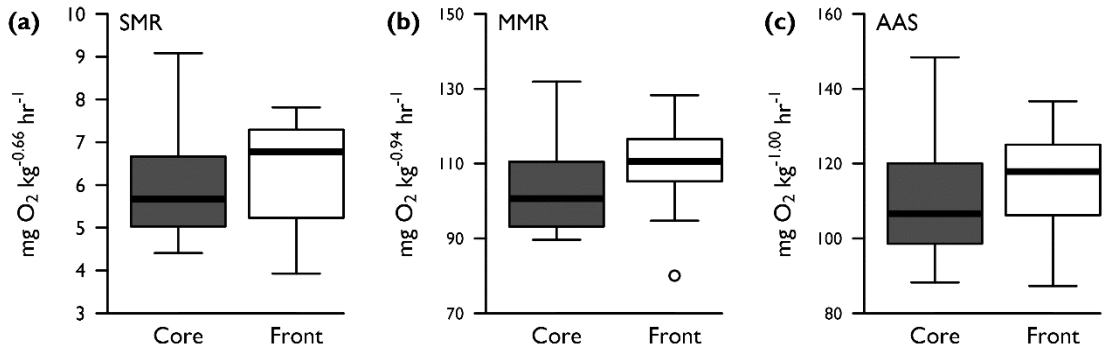


Figure 5.5 Boxplots of metabolic traits of signal crayfish from core (Co) and front (Fr) populations of the River Ure. *Boxes* show medians and interquartile range, *whiskers* show data range excluding outliers, *circles* are outliers. Data are corrected for differences in body mass using exponents derived from the empirical data.

5.3.4 Consistency of behaviour

A subset of crayfish were run through behavioural assays a second time to assess individual consistency. The overall pattern was of consistency across time (Tables 5.5 and 5.6).

Population average scores did not typically differ between runs (paired *t* tests or Wilcoxon signed rank tests; Table 5.5). However, Bookill crayfish were shyer (slower to emerge from their shelter) on the second run on the shyness-boldness test, driven by a higher proportion of individuals that

did not emerge on the second run (0.30 vs. 0.11 on the first run, Fisher exact test $p = 0.038$). Also, Ure crayfish were less voracious (consumed fewer *Gammarus*) on the second run of the foraging voracity test. There was a tendency for lower voracity on second runs in crayfish from the other two rivers, and marginally significant changes in exploration or activity in some rivers (Table 5.5).

Correlations between individuals' behavioural scores (raw scores, or residuals to control for confounding variables as necessary) were generally strong, positive and significant, indicating consistency of behaviour or individual personality. Activity and foraging voracity were significantly repeatable across runs for crayfish from all three rivers. Shyness was significantly repeatable for crayfish from the Ure and Wharfe, and significantly repeatable for Bookill crayfish that emerged within the time limit ($r = 0.44$, df = 29, $p = 0.012$ cf. Table 5.6 which includes all crayfish, including those that did not emerge and were allocated a censored maximum value). The correlation for Bookill crayfish is more sensitive to the inclusion of crayfish that didn't emerge in either trial, as this is 34% of the tested crayfish (in comparison to 17% of Ure crayfish and 11% of Wharfe crayfish). Exploration was significantly and strongly ($r \geq 0.59$) repeatable within the Ure and Wharfe, although the correlation was insignificant for crayfish from Bookill Gill Beck. Sociability was consistently inconsistent: crayfish from none of the three rivers showed significantly repeatable behaviour. Strangely, in all cases the correlation trended in a negative direction: crayfish that were the most social on the first trial were the least social on the second (Table 5.5).

5.3.5 Relationship between behaviour and physiology

I tested for associations between behaviour and metabolism by examining correlations between behaviour and metabolism within individual crayfish (Fig. 5.6; Table 5.7). Residual SMR (rSMR) and residual MMR (rMMR) were significantly and positively correlated with exploration behaviour ($r = 0.40$ and 0.42 respectively), and marginally negatively correlated with shyness ($r = -0.36$ for both). That is, crayfish with higher metabolic rates tended to explore more and emerge from shelters more quickly. rAAS was significantly and positively correlated with activity ($\tau = 0.22$) and sociability ($r = 0.43$). Foraging voracity was not significantly correlated with any metabolic trait. Note that none of these correlations remain significant following Holm-Bonferroni correction for multiple comparisons (Holm 1979).

There was an extremely strong positive correlation between rMMR and rAAS ($r = 0.94$, df = 35, $p < 0.001$) and a weaker positive correlation between rMMR and rSMR ($r = 0.34$, df = 35, $p = 0.037$). rSMR and rAAS were not correlated ($r = 0.01$, df = 35, $p = 0.953$).

Table 5.5 Temporal consistency of average behaviours of signal crayfish. Median raw scores from each run are given, with 95% percentile confidence intervals derived from bootstrapping (n = 1999). The difference between behaviours (raw scores) for repeat runs tested using paired t-tests or Wilcoxon signed rank tests. Bold p values are significant at $\alpha = 0.05$. *Shyness*, *Exploration*, *Activity*, *Sociability* and *Voracity* are as in legend to Table 5.3.

River	**Behaviour**	**Run 1**		**Run 2**		**Difference Run 1 v Run 2**	
		Median	**CI**	**Median**	**CI**	***Statistic***	***p***
Bookill	Shyness	200	(126, 343)	386	(178, 853)	$\mathbf{t_{46} = -2.45}$	**0.018**
	Exploration	3.9	(3.2, 4.0)	3.2	(2.90, 3.65)	$W_{45,45} = 635$	0.052
	Activity	16	(14, 18)	17	(14, 18)	$W_{47,47} = 565$	0.793
	Sociability	609	(567, 646)	608	(560, 655)	$t_{46} = 0.70$	0.490
	Voracity (22h)	11	(6.0, 13.0)	7.0	(5.0, 9.0)	$t_{46} = 1.87$	0.068
Wharfe	Shyness	268	(231, 330)	205	(156, 461)	$t_{26} = 0.84$	0.410
	Exploration	4.1	(3.2, 4.7)	3.8	(3.5, 4.55)	$t_{23} = -0.27$	0.793
	Activity	14	(12, 17)	17	(13, 19)	$t_{26} = -1.81$	0.082
	Sociability	620	(508, 675)	552	(503, 652)	$t_{26} = -0.01$	0.994
	Voracity (22h)	20	(13, 24)	15	(13, 19.5)	$t_{26} = 1.90$	0.069
Ure	Shyness	242	(202, 371)	246.5	(164, 414)	$t_{53} = 1.16$	0.250
	Exploration	3.8	(3.2, 4.65)	4.3	(4.0, 4.5)	$W_{52,52} = 472$	0.074
	Activity	15	(12.5, 18)	15	(14, 16)	$t_{53} = -1.40$	0.169
	Sociability	657	(614.5, 695)	575	(523.0, 629.5)	$t_{53} = 1.55$	0.127
	Voracity (8h)	24.8	(19.5, 27.5)	15.3	(13.0, 20.75)	$\mathbf{t_{56} = 5.56}$	**< 0.001**

Table 5.6 Temporal consistency of behaviour of individual signal crayfish. Correlation tested between scores on each run (residuals where appropriate; see main text), using Pearson's *r* or Kendall's tau-b (τ). Bold *p* values, uncorrected for multiple testing, are significant at α = 0.05. *Shyness*, *Exploration*, *Activity*, *Sociability* and *Voracity* are as in legend to Table 5.3.

River	Behaviour	Correlation Run 1 v Run 2			
		Statistic		df	*p*
Bookill	Shyness	τ	0.14	45	0.186
	Exploration	*r*	0.15	43	0.341
	Activity	*r*	0.39	45	**0.007**
	Sociability	τ	–0.08	45	0.425
	Voracity (22h)	τ	0.23	45	**0.025**
Wharfe	Shyness	*r*	0.55	25	**0.003**
	Exploration	*r*	0.60	22	**0.002**
	Activity	*r*	0.62	25	< **0.001**
	Sociability	*r*	–0.22	25	0.265
	Voracity (22h)	*r*	0.53	25	**0.004**
Ure	Shyness	*r*	0.34	52	**0.013**
	Exploration	*r*	0.59	50	< **0.001**
	Activity	τ	0.49	52	< **0.001**
	Sociability	*r*	–0.23	52	0.090
	Voracity (8h)	τ	0.57	52	< **0.001**

Table 5.7 Correlations between behaviour and metabolism in signal crayfish from the River Ure. Correlations were Pearson's *r* except for those involving Activity, which were Kendall's τ. Voracity transformed as 1 − voracity (i.e. number of prey remaining rather than consumed) to conform to negative binomial distribution for derivation of residuals. Bold *p* values, uncorrected for multiple testing, are significant at α = 0.05. Italicised *p* values are marginally significant (< 0.10). Shyness, Exploration, Activity, Sociability and Voracity are as in legend to Table 5.3.

Behaviour	**df**	**Standard MR (residual)**		**Maximum MR (residual)**		**Absolute AS (residual)**	
		r or τ	*p*	*r* or τ	*p*	*r* or τ	*p*
Shyness	26	*–0.36*	*0.060*	*–0.36*	*0.059*	–0.25	0.206
Exploration	26	**0.40**	**0.037**	**0.42**	**0.026**	0.31	0.104
Activity (residual)	26	0.06	0.635	*0.26*	*0.053*	**0.22**	**0.010**
Sociability (residual)	26	–0.28	0.146	0.29	0.128	**0.43**	**0.022**
1 − Voracity (residual)	26	–0.14	0.468	–0.15	0.442	–0.11	0.572

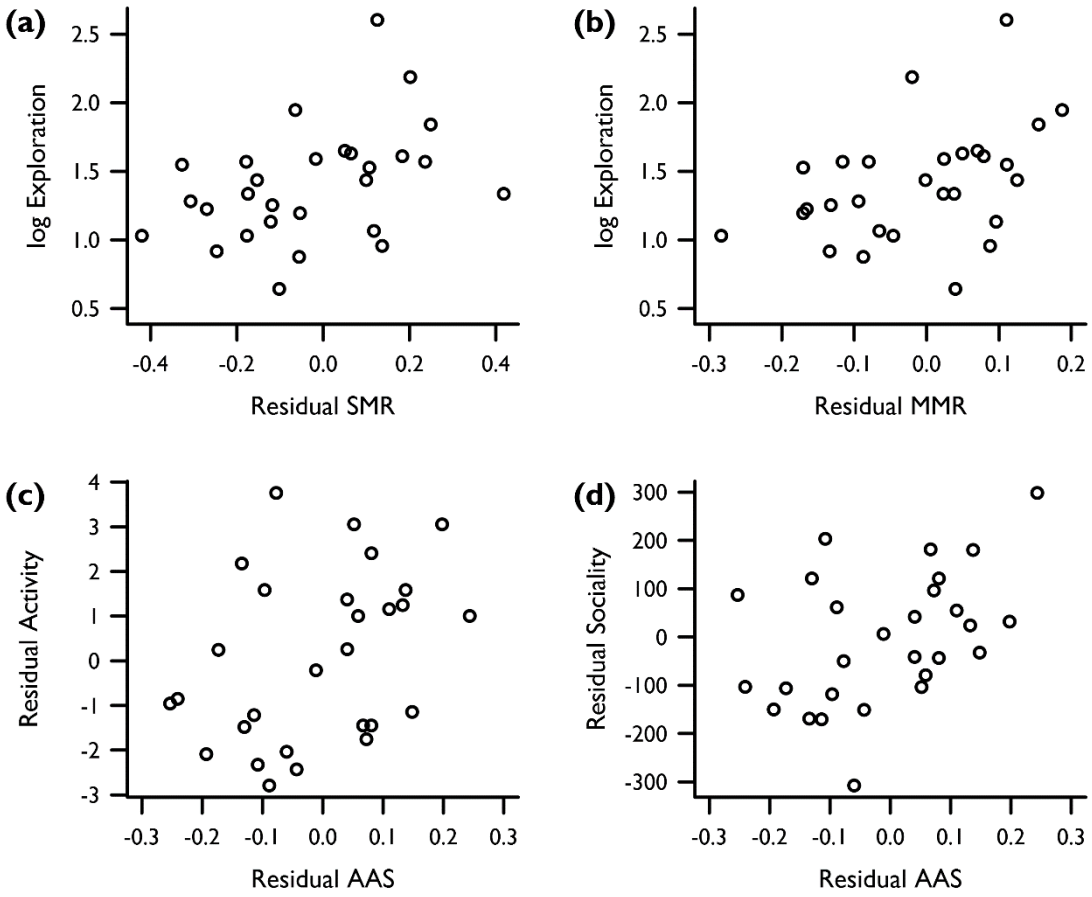


Figure 5.6 Significant correlations between behavioural and metabolic traits in signal crayfish from the River Ure. Where indicated in the axis labels, data are residuals from linear models containing significant predictors of population, sex and body mass. *SMR* – standard metabolic rate; *MMR* – maximum metabolic rate; *AAS* – absolute aerobic scope.

5.4 Discussion

5.4.1 Core-front comparisons

My primary hypothesis was concerned with comparing population characteristics, behavioural traits and physiological traits between core and invasion front populations. Traits associated with range expansion should be overrepresented towards the invasion front (Tracy et al. 2012; Juette et al. 2014). Whilst I found no clear association of behaviour or metabolism with invasion history, there were consistent differences between core and front population characteristics. Overall, these results suggest signal crayfish range expansion is driven by dispersal of individuals – possibly expulsion of subordinates – from high density populations.

Crayfish densities were higher in core than front populations. This is probably a consequence of the range expansion process, reflecting the fact that front populations have simply had less time to establish, rather than any significant environmental difference between the core and front in the study rivers (pers. obs.). However, this difference in density in itself becomes a significant ecological difference that could be causally linked to the observed variation in physical traits. Incidence of injury tended to be higher in core populations, and injuries are typically a consequence of aggressive interactions which increase with crayfish density (Savolainen et al. 2004). Meanwhile, at lower crayfish densities typical of invasion fronts, food resources are likely to be less fully exploited (Pintor et al. 2009; Raby et al. 2010) allowing crayfish to grow more rapidly and yielding the observed larger sizes.

The difference in population density along the invasion gradient could also be a key driver of dispersal. At high population densities, competitive interactions can reduce individual fitness and thus increase emigration propensity (Bowler and Benton 2005). In signal crayfish specifically, individuals move shorter distances in lower density populations, where competition for food and shelter is less intense (Moorhouse and Macdonald 2011). A density-driven model of dispersal would explain observations of peristaltic spread of signal crayfish, whereby range expansion appears to occur intermittently (Peay and Rogers 1999). Populations may need time to grow to high density before individuals are forced to disperse. My data suggest this individual dispersal is effectively random with respect to individual behaviour, metabolism and sex: there was almost no significant spatial sorting of these traits along an invasion gradient.

However, in the Croatian Rivers Mura and Drava, population density, sex ratios and aggressive behaviour differ along invasion gradients in a pattern that suggests dispersal from high density populations could be biased towards subordinate crayfish (Hudina et al. 2015). Dominant crayfish are better competitors for resources, such as shelter (Fero and Moore 2008) and food (Herberholz

et al. 2007). Where there is intense intraspecific competition as a result of high population density, subordinate crayfish may be forced to disperse away from the core population. Trends in my data are consistent with this model. First, sex ratios were more male-biased in front populations (but not significantly so). Male signal crayfish are subordinate to females (Peeke et al. 1995) and thus more likely to be expelled from a high density population. Second, crayfish from the invasion front on the Ure were more sociable than those from the core population. In clearwater crayfish *Orconectes propinquus*, nearest-neighbour distances between crayfish are positively related to dominance (Fero and Moore 2008). Thus, the fact that crayfish from Ripon (invasion front) spent more time close to a conspecific in the sociability assay than crayfish from Tanfield (core) could indicate they have a lower level of dominance.

A lack of behavioural differentiation along an invasion gradient has been reported in some other invasive species undergoing range expansion. For example, boldness and dispersal behaviour did not differ between core and invasion front populations of alien African Jewelfish in Florida (Lopez et al. 2012). Activity and boldness of round goby did not change along an invasion gradient in the Laurentian Great Lakes (Groen et al. 2012). The amphipod *Dikerogammarus villosus* displayed similar activity, boldness, exploration and sociability in long- and recently-established invasive populations in the Great Britain (Truhlar and Aldridge 2014). Although signal crayfish aggression differs between core and front populations in Croatian Rivers (Hudina et al. 2015), this could be a reflection of different selection pressures (such as population density, predation pressure or competition with resident heterospecific crayfish; Brown et al. 2005; Pintor et al. 2009; Hudina et al. 2013) rather than behaviour-dependent dispersal. Alternatively, behaviour may be a stronger driver of upstream dispersal (studied in Croatia) than downstream dispersal (studied in this Chapter): comparisons of upstream and downstream populations within rivers would be informative.

The similarity of crayfish populations along invasion gradients in British upland rivers suggest management strategies will be broadly effective across the entire gradient. However, strategies such as trapping could be especially effective at trapping large and male crayfish at the invasion front (Table 5.2; Price and Welch 2009) whilst male sterilisation (Aquiloni et al. 2009) would be most efficient in more female-biased core populations. The impact of signal crayfish is likely to be higher in established core populations by virtue of the higher population density, rather than any difference in *per capita* effects related to size, behaviour or metabolism (Parker et al. 1999; Pintor et al. 2009; Juette et al. 2014).

5.4.2 Consistency of behaviour

Generally, behaviour of individual signal crayfish was consistent over time, indicative of personality. Voracity, activity and shyness were significantly repeatable in all populations, whilst exploration was repeatable in two. Sociability – the tendency to spend time near a conspecific – was not repeatable in any population, with negative but non-significant correlations between scores on repeat tests. Sociability therefore appears to be a plastic trait in signal crayfish, which could therefore drive individual dispersal tendency in accord with the social dispersal model above, without generating spatiotemporal patterns in sociability along the invasion gradient.

Personality traits such as boldness, activity, exploration and voracity can affect invasion success and impact and have implications for management of alien species such as *P. leniusculus* (Juette et al. 2014). More generally, the possession of personality traits – and variation between individuals in these traits – can have important implications for ecological and evolutionary dynamics, including dispersal (Fogarty et al. 2011; Wolf and Weissing 2012; Sih et al. 2012). However, given the absence of variation in personality traits along the invasion gradient, my data do not support a model of personality-dependent dispersal in signal crayfish (Cote et al. 2010a). Explicit measurement of individual dispersal tendencies along with personality traits within individual crayfish would provide a further test of this conclusion (Fraser et al. 2001; Cote et al. 2010b).

5.4.3 Relationship between behaviour and physiology

Theoretically, metabolism and behaviour should be linked, to some extent, through the common currency of energy (Careau et al. 2008; Biro and Stamps 2010). Metabolism is the process of oxidising substrates to produce a net energy output, whilst activity and behaviour require energy or, in the case of foraging, acquire fuel for the metabolic engine.

SMR and MMR were positively correlated with exploration (significantly) and boldness (marginally). The correlation between metabolism and exploration is consistent with the performance model of Careau et al. (2008), whereby animals with a higher MR can generate more energy and thus exhibit greater movement. Exploration of the environment involves active movement. The marginal correlations between boldness and MR could be explained by subtly different logic, that animals with a high MR consume energy at a greater rate and therefore must procure food at a greater rate. Crayfish with a high MR cannot afford to be as cautious in waiting for predation risk to pass. Correlations between risk-taking behaviour and MR may have been more apparent had animals been starved for longer: in sea bass *Dicentrarchus labrax*, a metabolism-boldness correlation was only apparent after a week of starvation (Killen et al. 2012).

AAS was positively correlated with activity, almost by definition given that aerobic scope is the capacity of an organism to increase its rate of aerobic metabolism, and therefore sets its capacity for active behaviour (Fry 1947). MMR (but not SMR) was also marginally correlated with activity, suggesting this assay was energetically demanding and induced crayfish to work towards the upper end of their scope for activity. AAS was positively correlated with sociability. This is more challenging to interpret given that sociability is inconsistent within individuals over time (Section 4.2). In fact, the correlation between AAS and sociability becomes significantly negative ($r = -0.39$) if second round scores from the sociability assay are used. It is possible that metabolism is related to sociability through dominance. Social spacing of crayfish is related to dominance (Fero and Moore 2008) and AAS could be related to dominance by conferring advantages in territory acquisition or holding (Killen et al. 2014), given the high intensity of crayfish agonistic contests (Berry 2008). Clarifying the links between metabolic traits, dominance, aggression and sociability could enhance our mechanistic understanding of both invasive species dispersal and signal crayfish social dynamics. Generally, links between behaviour and metabolic traits, especially MMR and AAS, require further quantification (Biro and Stamps 2010; Metcalfe et al. 2016).

I anticipated that foraging voracity would be closely associated with metabolism, given that it is the means by which fuel for the metabolic engine is obtained (Biro and Stamps 2010). Observed correlations between voracity and metabolism were in support of this expectation, although not significant. The lack of significance could be related to *ad libitum* feeding in laboratory settings (Biro and Stamps 2010) meaning individuals had sufficient energy reserves to power their metabolic engine before their voracity was assayed and so the link between consumption and metabolism was weakened. Data censoring, whereby multiple individuals consumed all the available prey, could have reduced inter-individual variation in voracity and thus further limited my ability to detect a voracity-metabolism relationship.

5.4.4 Conclusion

Overall, I found no consistent significant differences between signal crayfish in core populations and those at the invasion front. Trends in my data support previous models of dispersal being driven by the exclusion of (subordinate) individuals from high-density established populations (Peay and Rogers 1999; Hudina et al. 2015). Population densities were generally higher in core populations, whilst sex ratios tended towards a male bias at the invasion front. My data suggest this dispersal may be associated with a temporary change in sociability, but is not apparently related to any personality or metabolic traits.

I provide evidence of correlations between metabolic traits (SMR, MMR and AAS) and behavioural traits within individuals. Further research to clarify these correlations and the mechanisms behind them would be valuable.

Core and front populations may differ in their impact as a result of differences in density, but not individual behaviour. Established and front populations will be susceptible to similar management techniques, but there is some scope for differential application of management along invasion gradients based on size and sex differences.

Chapter 6

Propagule pressure affects population size and impact, but not establishment success, of experimental invasions in protist microcosms

Abstract

The colonisation of new environments is an important ecological process, with applied relevance to conservation and invasion biology. For example, understanding the causes of successful and high impact invasions is important for informing management decisions. Propagule pressure (the total number of individuals introduced to a site) is emerging as a consistent correlate of establishment success in alien species, but we lack a full mechanistic understanding of the influence of propagule pressure on invasion success and impact. Here, I quantify the role of propagule pressure in experimental invasions into protist microcosms, which allow control and replication across temporal scales not achievable in the field.

In one set of experiments, I perform reciprocal invasions of *Blepharisma japonicum* (an omnivorous, intra guild predator) and *Colpidium striatum* (bactivorous, intra guild prey), alternating which species is the resident and which is the invader and varying the propagule size of the invader. I perform these invasions at two levels of enrichment to examine possible interactions between resource availability and the propagule pressure.

Propagule size has consistent positive effects on invader population density in the initial stages of invasions (before and at initial stable state). For *Blepharisma* invasions, time to establishment was also reduced by high propagule pressure. Establishment success was high in all microcosms, including those invaded by just one protist cell. Propagule pressure was also positively correlated with the impact of predatory *Blepharisma*: resident *Colpidium* densities were lower following invasion by large *Blepharisma* propagules. Nutrient enrichment did not affect the relationship between propagule pressure and invasion success, but had a significant effect on establishment time, and invader population density and extinction rate.

In a second experiment, total propagule pressure was fixed but I varied its components (propagule size and number) to investigate the relative importance of these two components. I could not distinguish propagule size or number as a more important driver of success or impact, which could be explained by the stability of my microcosms and asexual reproduction of protists.

My results support management strategies based on limiting propagule pressure, suggesting reductions in propagule size can provide consistent marginal benefits in terms of both success and impact. However, they also highlight the potential for microbial invasions to succeed from very small propagules.

6.1 Introduction

Alien species are organisms that exist outside their natural range (Blackburn et al. 2011). The introduction of alien species around the planet is leading to biotic homogenisation at the global scale (McKinney and Lockwood 1999; Olden et al. 2011) and alien invaders can have negative environmental and economic consequences locally (Williamson 1996; Jeschke and Strayer 2005). Management of alien species to prevent or mitigate these consequences is essential (Reaser et al. 2007; Kumschick et al. 2012). Effective management strategies must be based on an understanding of the factors that lead to successful invasions by alien species and their impacts. With this knowledge, we can prioritise pathways, habitats or species for control.

A multitude of hypotheses has been proposed to explain why some alien species successfully establish and spread in their novel range, and/or have an impact (Kolar and Lodge 2001; Barney and Whitlow 2008; Catford et al. 2009; Ricciardi et al. 2013). These tend to invoke characteristics of the invading organism or the recipient environment, or a combination of the two. An organism might possess certain traits that, all else being equal, predispose it to be a successful and damaging invader e.g. high fecundity (Baker and Stebbins 1965) or a high rate of resource consumption (Dick et al. 2014). Certain environments are more susceptible to invasion e.g. disturbed habitats with high resource availability (Davis et al. 2000) or with little biotic resistance to control invaders (D'Antonio et al. 2001). Invader and environmental traits may combine to facilitate invasion and impact, where habitat conditions in the new environment match the source (Kestrup and Ricciardi 2009; Ricciardi et al. 2013) or where the biota in the recipient environment is naïve to an invasive predator archetype (Cox and Lima 2006). However, few consistent predictors of invasion success and impact have emerged from this pool of hypotheses (Heger and Trepl 2003; Hayes and Barry 2007).

However, propagule pressure appears to offer one consistent predictor of successful, high impact invasions. Fundamentally, propagule pressure is the combination of the number of introduction attempts (propagule number) and the number of individuals introduced in each attempt (propagule size) (Williamson 1996; Lockwood et al. 2005; Colautti et al. 2006; Hayes and Barry 2007; Ricciardi et al. 2013; Blackburn et al. 2013). A high propagule pressure can increase establishment success by reducing the threats posed by demographic and environmental

stochasticity, and/or introducing genetic variability that both mitigates the genetic problems of breeding in small populations and facilitates adaptation to a new environment (Simberloff 2009). High propagule pressure may also increase the impact of alien species by facilitating higher abundance or range size: two factors associated with high impact (Parker et al. 1999; Ricciardi et al. 2011).

Whilst there is abundant evidence supporting the role of propagule pressure in driving establishment success (Grevstad 1999; Lockwood et al. 2005; Simberloff 2009), we have much less information about how propagule pressure affects other aspects of invasion success (e.g. population size and extinction rate; Ricciardi et al. 2013) and impact (Ricciardi et al. 2011). Even for the relationship between propagule pressure and establishment success, we lack a complete understanding of the underlying mechanisms (Blackburn et al. 2015) and it is these details that will facilitate design of more effective management strategies. For example, we lack understanding of species- and location-specific effects of propagule pressure, such as whether the effects of propagule pressure differ depending on the resources available in the recipient environment or the nature of ecological interactions with recipient species. Invasion success may be best explained by interactions between propagule pressure, invader traits and the recipient environment (Heger and Trepl 2003; Mata et al. 2013). Further, most studies consider propagule pressure as a composite variable despite theory suggesting propagule size and number may vary in their relative importance (Wittmann et al. 2014). The few studies that have broken propagule pressure down into size and number (Drake and Lodge 2006; Hedge et al. 2012; Britton and Gozlan 2013; Sinclair and Arnott 2016) have tended to release propagules into unrealistically "empty" environments. Finally, investigations of propagule pressure tend to be run across relatively short timescales relative to the generation time of the organisms involved, so we have little understanding of the long-term implications of propagule pressure.

Here, I address these issues with experimental invasions in protist microcosms. I use the ciliated protists *Blepharisma japonicum* (herein *Blepharisma*) and *Colpidium striatum* (herein *Colpidium*) as invaders (introduced to a community) and residents (in an established community). The small size (< 1 mm length) and short generation time (hours to days) of these protists allows for sufficient replication of invasions and observation of long-term invasion dynamics (Warren et al. 2006; Altermatt et al. 2015). As an experimental system, they also allow careful control of environmental conditions to test for the influence of propagule pressure in the absence of confounding factors – which can be a serious problem for interpreting long-term data from the field (Duncan 1997; Cassey et al. 2004). In addition to being a useful model system, understanding the dynamics of microbial invasions is important in its own right, given the likely

(but underreported) prevalence of microbial invasions and their negative impacts (Gillis and Chalifour 2010; Litchman 2010; Hatcher et al. 2012; Acosta et al. 2015).

I perform two sets of experimental invasions. In the first, I vary propagule size alone and measure the success of the invader and its impact on the resident protist. For these experiments, I used reciprocal combinations of *Blepharisma* and *Colpidium* as resident and invader, and use two different enrichment levels (nutrient concentration) to investigate species- or environment-specific effects of propagule pressure. My hypothesis was that larger propagule sizes would be associated with greater invasion success and larger impacts on the resident protist. In a second experiment, I investigated the relative roles of propagule size and number, varying each within a fixed total propagule pressure. This experiment focussed on just one invader-resident combination (*Blepharisma* invading *Colpidium*) and one enrichment level. For this experiment, I hypothesised that propagule size would be a more important factor in invasion success than propagule number: in the stable microcosms there is no need to compensate for environmental stochasticity with many separate introduction events (Wittmann et al. 2014). Although I frame my experiments in an invasion context, I note that they have wider applicability given that that colonisation is a process important across ecological spheres e.g. in community assembly (MacArthur and Wilson 1967) and in conservation biology (Caughley 1994; Seddon 2010).

6.2 Methods

6.2.1 Protist species and culture conditions

Founding stocks of *Blepharisma* and *Colpidium* were obtained from a commercial supplier (Sciento, Manchester, UK). The traits of these species are summarised in Table 6.1 and trophic relationships depicted in Fig. 1.5.

Colpidium is a smaller, faster-growing bacterivore and is a weak competitor for bacterial resources (Cadotte et al. 2006; Liess and Diehl 2006). *Blepharisma* is larger, slower-growing protist with more complicated trophic dynamics. It is generally a stronger competitor for bacteria, but is also morphologically plastic and can increase its cell size to facilitate predation (including occasional cannibalism; Giese 1973). Thus, *Blepharisma* acts as an intra-guild predator of *Colpidium*, but the population-level outcome of this interaction can depend upon the nutrient content (enrichment) of the culture medium (Morin 1999; Diehl and Feissel 2001). I therefore selected *Colpidium* and *Blepharisma* for use in my experiments because of the potential for different invasion outcomes depending on invader identity and enrichment, meaning I could assess the generality of the effect of propagule pressure on invasion success and impact. Using an intra-guild predator allowed incorporation of predatory interactions: other purely predatory

protists could not establish a monospecific resident population (by definition) and would likely exhaust prey too quickly to examine long-term interactions. Intra-guild predation also occurs frequently in natural systems, and is of importance for structuring communities (Polis et al. 1989).

Table 6.1 Comparative trait values for *Blepharisma* and *Colpidium*, taken from the literature. Values are for each genus under similar conditions, not the conditions used in the present experiments. Values are means ± standard errors, unless otherwise specified. *r* – density-independent, intrinsic rate of increase; *SD* – standard deviation.

Trait	*Blepharisma*	*Colpidium*	Reference
Size (μm)	470 ± 60 (SD)	80 ± 8 (SD)	Carrara et al. (2012)
Trophic guild	Omnivore Intra-guild predator	Bacterivore Intra-guild prey	Morin (1999) Diehl and Feissel (2001)
Growth rate (*r*, per day)	0.30 ± 0.06	3.84 ± 0.17	Fox and Morin (2001)
Competitive rank[a]	11.1	3.5	Cadotte et al. (2006)

[a] amongst 12 other protist species, where high value = high competitive ability

For each protist species, monobacterial stock cultures of 100 ml were maintained in 250 ml glass Schott bottles, with lids on but loosened to allow airflow. These cultures were comprised of (a) the supernatant from autoclaved protist pellet medium (b) four autoclaved organic wheat seeds to provide additional, slow release carbon and nutrients (c) a single species of bacterium (unidentified, but forming identical colonies on nutrient agar plates), transferred dry from a nutrient agar plate and (d) a single protist species: *Blepharisma* or *Colpidium*. Protist pellet medium was made by autoclaving ground dried plant material (protist pellets; Carolina Biological Supply, Burlington, NC) in bottled water (Pennine Vale spring water, Morrisons, UK) at 121°C for 20 minutes. Enrichment of stock media was 0.84 g protist pellet.L^{-1} (herein abbreviated to g.L^{-1}). Stock cultures were kept in a dark incubator (*Blepharisma* contains light-sensitive pigments and so grows best in the dark; Giese 1973) at a constant temperature of 20°C. To maintain stocks, 10% of the medium was replaced weekly and fresh stocks established every two months.

The original protist cultures supplied by Sciento contained a mixture of bacterial species. I used a single bacterial species in my stocks and experiments to simplify the food web within microcosms and aid reproducibility (Altermatt et al. 2015). The most abundant bacterium from the Sciento cultures was isolated as a single food source, and I verified that this bacterium alone supported growth of both protist species. Stocks were initially cleaned to remove unwanted bacterial species by washing protists in autoclaved protist pellet medium (by serial transfer with

micropipettes) and inoculating 10 cleaned cells into fresh medium containing my focal bacterium. This process was iterated up to three times until monobacterial cultures were obtained.

6.2.2 Experiment 1: propagule size manipulation

In the first set of experiments, I used *Blepharisma* and *Colpidium* as reciprocal invaders and residents. That is, one experiment involved adding *Colpidium* at a range of propagule sizes (1, 10, 30, 100 or 1750 cells) to established populations of *Blepharisma*. A second experiment involved adding *Blepharisma* at a range of propagule sizes (1, 5, 10, 30, 150) to established populations of *Colpidium*. Each of these combinations was carried out at high enrichment (1.68 $g.L^{-1}$ protist pellets with four wheat seeds per microcosm) and low enrichment (0.21 $g.L^{-1}$ protist pellets with one wheat seed). Although I did not quantify bacterial densities at these different enrichment levels, previous work has shown that similar variations produce significant differences in bacterial concentration (Balčiūnas and Lawler 1995; Morin 1999; Diehl and Feissel 2000).

I ran five replicates of each invader-enrichment-propagule size combination (giving 100 experimental microcosms in total). I also ran three controls to check survival of residents and invaders in the absence of each other, in case of extinction in experimental microcosms. These were three microcosms without residents at each invader-enrichment-propagule size combination, or three microcosms to which invaders were not added for each resident protist species (66 control microcosms in total).

Each establishing microcosm consisted of 24 ml of medium and wheat seed(s) in 50 ml polypropylene centrifuge tubes (Fisher Scientific, Loughborough, UK), with lids loosely secured to prevent contamination but allow gas exchange. The resident population in each microcosm was established as per stock cultures. Autoclaved supernatant (23 ml) was transferred to the microcosm tube with a sterile serological pipette, and wheat seed(s) added. When cool, 1 ml of bacteria suspension was added (from 24h cultures of bacteria in nutrient broth, subsequently washed with protist medium). After 24h for bacterial growth, 1 ml of medium from stock cultures was added to each microcosm. Microcosms were kept in a dark incubator at 20°C and protist densities in 6 random microcosms were counted every three days (*Blepharisma*) or every day (*Colpidium*). Once densities had stabilised (18 days for *Blepharisma*, 6 days for *Colpidium*), the invading protist was added.

On the day of invasion, all resident microcosms were pooled and realiquoted with a sterile serological pipette to equalise resident protist densities. Invading protists were added to each

microcosm by sterile micropipette (for propagule sizes of 30 or less) or by transferring a set volume of invader stock (for larger propagule sizes). To obtain the maximum propagule sizes, this volume was 1 ml. The number of protists in this volume was determined by counts immediately prior to invasion. For *Colpidium*, the propagule size of 100 was obtained by adding 57 µl of stock to each microcosm (0.057 ml x stock density 1750 *Colpidium*.ml^{-1}). To compensate for this addition of medium which slightly altered the enrichment level – stocks were grown at an enrichment level (0.84 g.L^{-1}) intermediate to the experimental enrichments (0.21 g.L^{-1} and 1.68 g.L^{-1}) – I topped up all microcosms to a final volume of 25 ml with filtered invader stock medium (filtered through 1.2 µm sterile Minisart® syringe filters; Sartorius, Göttingen, Germany).

Invaded microcosms were monitored for 60 days. Over the first 42 days, microcosms were inverted every three days and 1.25 ml of medium removed with a Nichiryo® EX pipette and sterile tip. Every six days, 2.5 ml of fresh medium was added. In this way, 10% of each microcosm was replaced every six days. Between days 42 and 60, microcosms were left undisturbed.

Data were obtained by counting protist densities (both resident and invader) in the removed medium every three days for the first 12 days, then every six days until day 42, with a final count on day 60. The removed medium was added to a 1 ml Sedgwick-Rafter counting chamber, in which protists were killed and stained using Lugol's Iodine (1% I w/v). To count *Blepharisma* I examined the whole 1 ml chamber. *Colpidium* densities were higher, so I counted approximately 200 cells, noted the volume containing this number of cells and multiplied up to a density per ml.

6.2.3 Experiment 2: propagule size and number manipulation

In a second experiment, I fixed total propagule pressure and varied propagule size and number within this overall propagule pressure. I focussed on a total propagule pressure of 16 cells at low enrichment levels. Based on results from the first experiment, a single propagule of 16 *Blepharisma* should comfortably establish. Propagule number was varied by splitting this single propagule over a 16 day period. Thus, treatments were (a) 16 cells introduced once, on day 1 (b) 8 cells introduced twice, on days 1 and 8 (c) 4 cells introduced four times, on days 1, 5, 9 and 13 (d) 2 cells introduced every other day over 16 days and (e) 1 cell introduced every day for 16 days. Eight replicates of each size-number combination were run, with eight controls to check survival in the absence of resident *Colpidium* and three controls to check *Colpidium* survival without invasion.

Following Sinclair and Arnott (2016), I performed an initial census after 8.5 days of average growth time (Table. 6.2). Thus, the single addition of 16 cells (treatment a) was counted after 8.5

days, treatment b was counted on day 13 (after 12 days of growth for the first propagule and 5 days of growth for the second propagule; average = 8.5 days), treatment c was counted on day 15.5 (14.5 + 10.5 + 6.5 + 2.5 days of growth for each propagule; average = 8.5 growth days), treatment d was counted on day 16.5 and treatment e counted on day 17.

Table 6.2 Overview of propagule additions and census times for propagule size-number manipulation (Experiment 2). *Tmnt* – treatment. *Numbers* indicate size of propagule (*Prop*) and the day on which it was introduced. *C* denotes census, initially performed after an average growth period of 8.5 days for each propagule.

Tmnt	Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	…	35
a	Prop	16								**C**										**C**
b	Prop	8							8					**C**						**C**
c	Prop	4				4				4				4			**C**			**C**
d	Prop	2		2		2		2		2		2		2		2		**C**		**C**
e	Prop	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	**C**		**C**

I also performed a final census after 35 actual days (Table 6.2). Based on results from the first experiment, this was sufficient time for treatment (e) to reach a stable state. Measurement of population density after a fixed time is relevant for propagule pressure-based management, for example in considering whether a certain management strategy will reduce the abundance of an invader after one year, or before some new legislation comes into force.

6.2.4 Statistical methods

All statistical analyses were performed in R version 3.2.1 (R Core Team 2015).

For the experiment manipulating propagule size, four response variables for invasion success were considered (a) establishment success, defined as an invader being present in at least two consecutive samples (b) time to establishment: time until the first sample in which an invader population, which subsequently established, was recorded; (c) invader density at various stages of the growth curve identified from inspection of graphical data: prior to reaching stable state, and at stable state; and (d) probability of extinction. As a response variable for invasion impact, I considered resident density at stable state.

Establishment success was high across all microcosms and was not analysed statistically. Extinction rate was analysed using Fisher's exact tests. Time to establishment and protist density variables were analysed using linear models, with explanatory variables of enrichment

(categorical, two levels) and propagule size (categorical with five levels for time response and continuous for density response).

For time to establishment, models were Poisson GLMs followed by Tukey Kramer contrasts to identify significant differences between factor levels (*multcomp::glht*; Hothorn et al. 2016). For density responses, models were ANOVAs. Densities were log10(x+1) transformed (Morin 1999; Li and Stevens 2012)**.** To calculate densities over defined time periods, I took the arithmetic mean of these log10(x+1) densities (which is equivalent to the log of the geometric mean, and thus accounts for temporal non-independence of density counts).

I started by fitting full models with the main effects and two-way interaction, then simplified models by stepwise deletion of terms that did not significantly increase the explanatory power of the model (using ANOVA to compare nested models with F or χ^2 tests of significance as appropriate; Crawley 2007). Significance of variables in MAMs was assessed using t statistics. Models were verified by inspection of residual plots. Where there was obvious non-linearity in the data, I tested for the significance of a squared propagule size term in the model.

In order to fit linear models, I followed Law et al. (2000) and Fox (2002) by excluding outliers: populations that failed to establish, went extinct or had anomalously high densities at the time point being analysed. This was never more than one microcosm from each invader-propagule size-enrichment combination, so the bias induced by removing outliers should be minimal (or at least much less than induced by including these highly influential points in the analysis).

For the experiment manipulating propagule size and number, the different size-number combinations were treated as a categorical explanatory variable (five levels). ANOVAs with post-hoc Tukey Kramer contrasts were used to compare the response variables of *Blepharisma* and *Colpidium* density (continuous) across these categories, at two time points (a) an average of 8.5 days after invasion by all propagules and (b) 35 actual days after initial invasion.

6.3 Results

6.3.1 Experiment 1: propagule size manipulation

Figures 6.1 and 6.2 provide an overview of population size over time in these experiments, with a general pattern of growth of the invader and decline in the resident cell density. These growth trajectories were used to identify phases of growth and stability for further analysis: an initial transient state when invader populations were increasing, followed by stable state(s) when population densities were relatively constant.

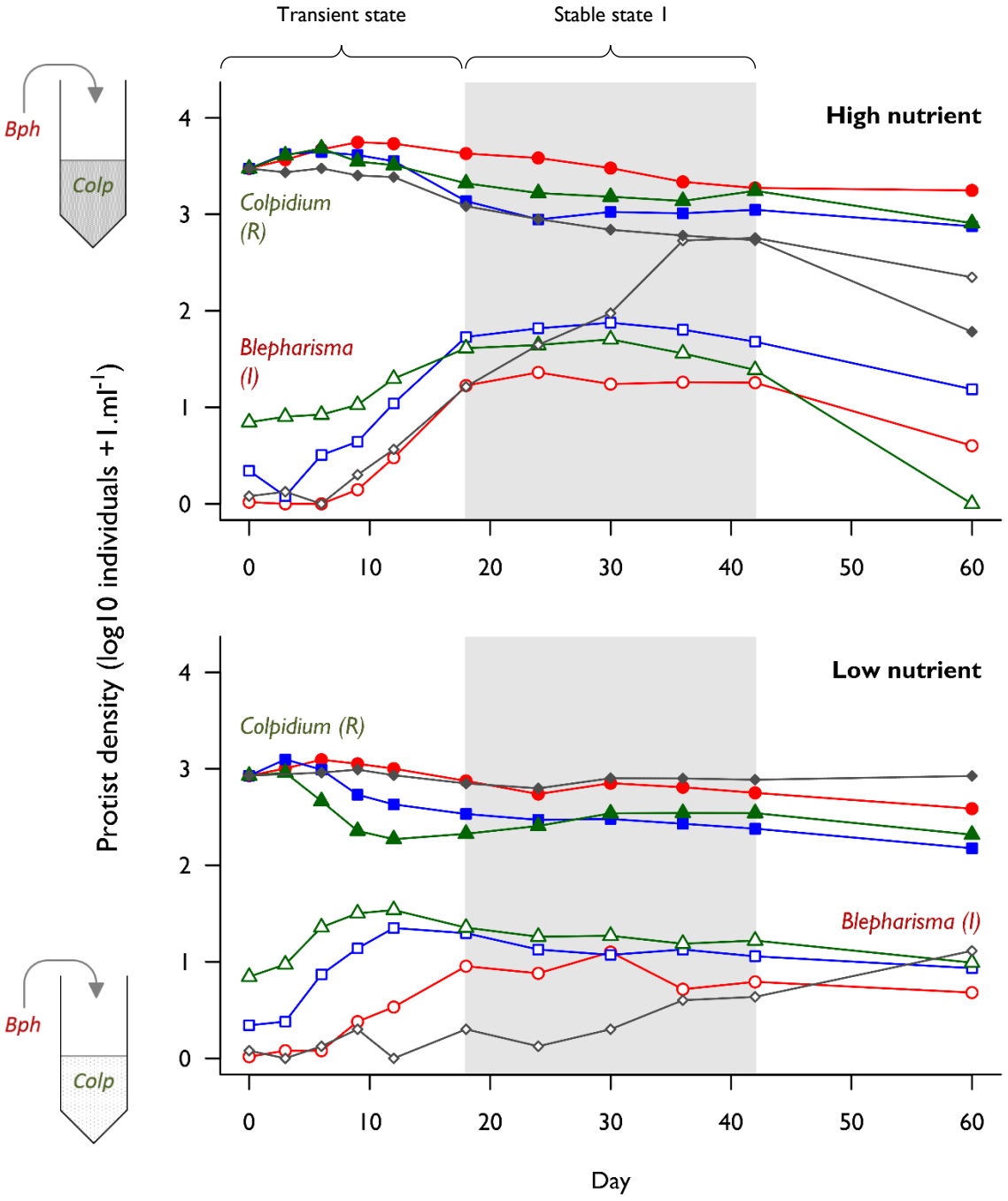


Figure 6.1 Overview of *Blepharisma* invasions. Mean densities of protists over time, where *Blepharisma* (*open symbols*) is the Invader and *Colpidium* (*filled symbols*) is the Resident.

Experimental invasions: *red circles* – 1 invader; *blue squares* – 30 invaders; *green triangles* – 150 invaders. For clarity, only three propagule size treatments are shown and standard error bars are omitted.

Control microcosms: *grey diamonds*. These are invasions of *Blepharisma* into bacteria (only propagule size of 5 cells shown), or resident *Colpidium* without invasion. *Shaded areas* identified, by eye, as stable state.

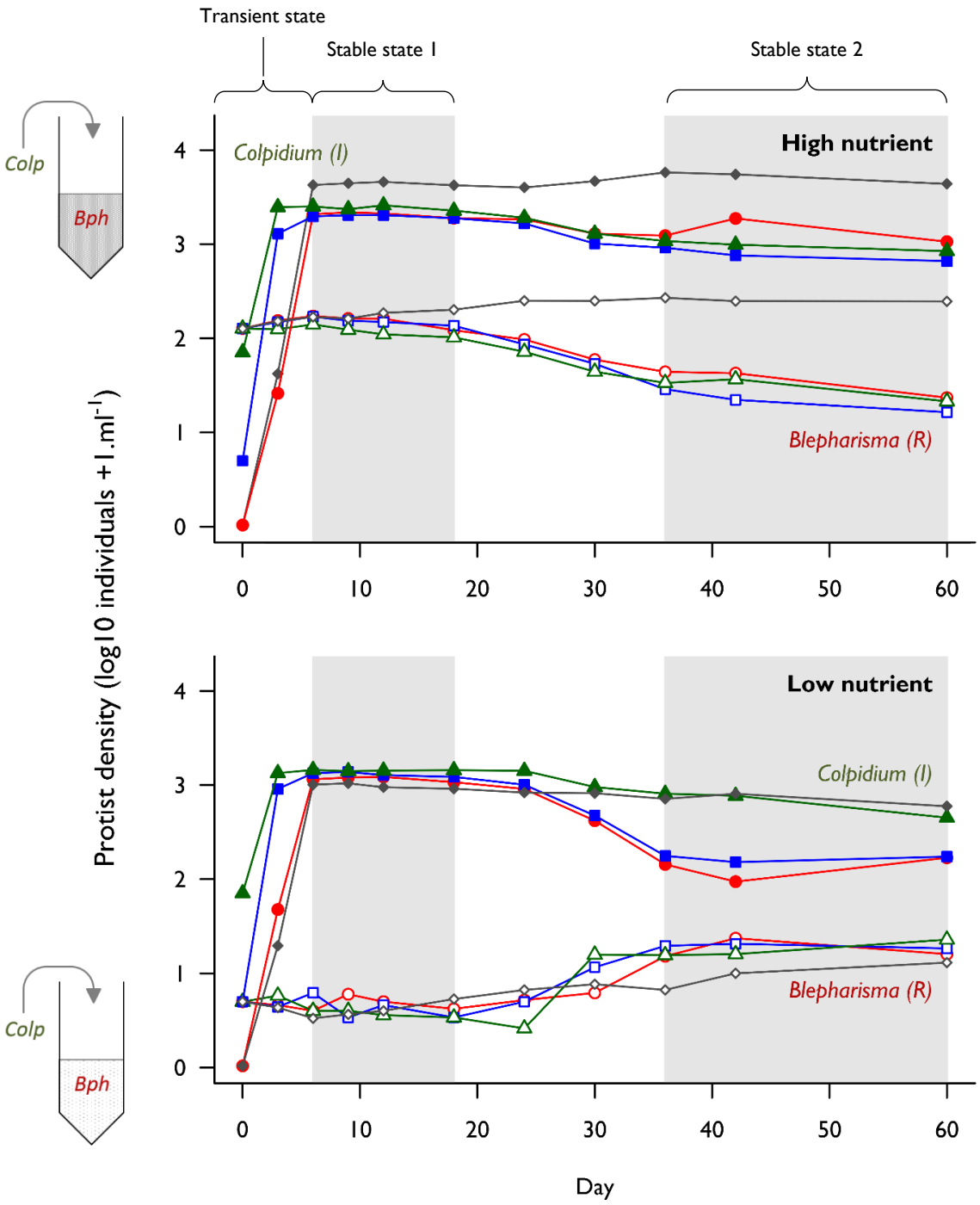


Figure 6.2 Overview of *Colpidium* invasions. Mean densities of protists over time, where *Colpidium (open symbols*) is the Invader and *Blepharisma* (*filled symbols*) is the Resident.

Experimental invasions: *red circles* – 1 invader; *blue squares* – 100 invaders; *green triangles* – 1750 invaders. For clarity, only three propagule size treatments are shown and standard error bars are omitted.

Control microcosms: *grey diamonds*. These are invasions of *Colpidium* into bacteria (only propagule size of 1 cell shown), or resident *Blepharisma* without invasion. *Shaded areas* identified, by eye, as stable states.

6.3.1.1 Establishment success

Invader establishment success was very high in all microcosms. All *Colpidium* invasions at all propagule sizes established, in both control and experimental microcosms. All *Blepharisma* invasions above the smallest propagule size successfully established. At the lowest propagule size, one of five *Blepharisma* invasions failed to establish in the low enrichment experimental microcosms (with *Colpidium* present). At both high and low enrichment, two of three control *Blepharisma* additions (microcosms with no *Colpidium* present) failed to establish.

6.3.1.2 Time to establishment

Colpidium invasions always established rapidly. All *Colpidium* populations had established by the first sampling occasion on day three. For *Blepharisma* invasions, time to establishment was more variable and depended upon both propagule size (Poisson GLM $n = 49$, $\chi^2 = 48.67$, df = 4, $p < 0.001$) and enrichment ($\chi^2 = 9.19$, df = 1, $p = 0.002$) but not the interaction between the two (full Poisson GLM $n = 49$, $\chi^2 = 3.32$, df = 4, $p = 0.505$). Larger propagules took less time to establish (Fig. 6.3). At high enrichment, for example, there were significant differences between (a) propagule sizes 1 and 10, 30 and 150 and (b) propagule size 5 and 150 (Tukey *p*s < 0.05). Establishment was also more rapid at low enrichment (Tukey $z = 3.00$, $p = 0.003$). For example, at low enrichment all invasions at a propagule size of 30 established within three days, but at high enrichment only one of five propagules of 30 *Blepharisma* established in three days (Fig. 6.3).

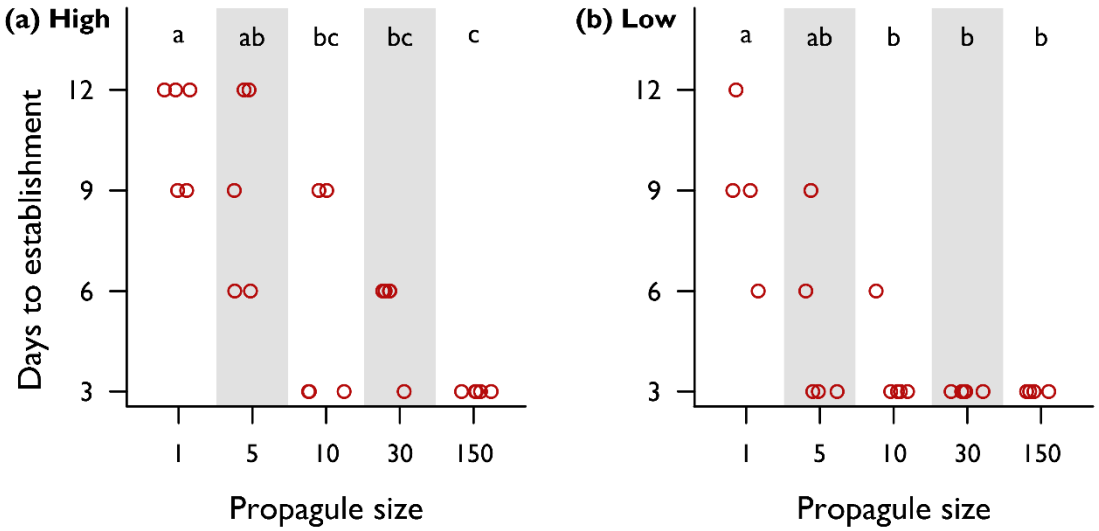


Figure 6.3 Establishment times for *Blepharisma* invading populations of *Colpidium* at **(a)** high enrichment and **(b)** low enrichment. *Points* show establishment times for single microcosms, jittered along the size axis for clarity. *Shaded regions* delineate size treatments. *Letters* indicate significantly different establishment times, based on Tukey Kramer post-hoc tests.

6.3.1.3 Invader density

No interactions between propagule size and enrichment were significant for any density response variables, so they were removed from all models. That is, the effect of propagule size was consistent across enrichment levels.

The density of invaders prior to steady state was significantly associated with propagule size (on day 3 for *Colpidium* and over the first 12 days for *Blepharisma*; Tables 6.3 and 6.4; Figs. 6.4 and 6.5). Then, at the initial stable state invader density was also dependent upon propagule size for both *Colpidium* and *Blepharisma*, although the effect was much weaker. For example, whilst every unit increase in log *Blepharisma* propagule size increased transient (pre stable state) density by 2.69 cells.ml^{-1} ($10^{0.431}$), the same increase in propagule size only increased stable-state density by 1.45 cells.ml^{-1} ($10^{0.160}$). Invading *Colpidium* settled to a second stable state between days 36 and 60, at which cell density was no longer dependent upon propagule size (rejected from model $t = 1.60$, $p = 0.116$).

Where propagule size was associated with invader density, there were typically marginal gains (i.e. every extra invader added increased population density), indicated by significant linear regression coefficients (Tables 6.3 and 6.4). However, these gains were diminishing for the transient density of *Colpidium* invaders. A linear model including a quadratic term was a significantly better fit for both enrichment levels (Fig. 6.5; ANOVA comparing models with and without quadratic term $F_{1,47} = 43.32$, $p < 0.001$). The benefit of the highest propagule size for the *Colpidium* population was less than would be expected based on the propagule size.

Both resident and invader protist density was typically higher at the higher enrichment level. However, in one case enrichment had no effect on invader density: pre-stabilisation densities of *Colpidium* did not differ between enrichment levels (Table 6.3). Further, in one case the relationship between enrichment and invader density was reversed. Transient densities of invading *Blepharisma* (mean of log densities days 3-12) were 2.1 times higher at low enrichment compared to high enrichment (Table 6.3, Fig. 6.4).

6.3.1.4 Resident density

I also analysed the influence of propagule size and enrichment on the density of the resident protist at stable state. The density of resident *Colpidium* was significantly related to both invader propagule size and enrichment, with larger *Blepharisma* propagules being associated with lower *Colpidium* densities (Table 6.3, Fig. 6.4). Resident *Colpidium* densities were also lower at low levels of enrichment. When *Colpidium* was the invader, its propagule size had no effect on the

stable state density of resident *Blepharisma* (Table 6.4, Fig. 6.5). Again, densities of the resident protist were dependent upon enrichment levels – although for *Blepharisma*, the magnitude of this difference was much smaller at the second stable state than at the first (Fig. 6.5).

Table 6.3 Analysis of *Blepharisma* invasions. Minimum adequate linear models relating density response variables to propagule size and enrichment explanatory variables. *SE* – standard error of mean. No interactions were significant.

Protist	**Response**	**Explanatory**	**Estimate**	**SE**	***t***	***p***
Blepharisma (invader)	Density pre stable state (days 3-12)	Propagule size	0.431	0.024	17.83	< 0.001
		Enrichment	−0.321	0.035	−9.18	< 0.001
	Density at stable state (days 18-42)	Propagule size	0.160	0.048	3.36	0.002
		Enrichment	0.332	0.068	4.92	< 0.001
Colpidium (resident)	Density at stable state (days 18-42)	Propagule size	−0.157	0.053	−2.97	0.005
		Enrichment	0.767	0.075	10.21	< 0.001

Table 6.4 Analysis of *Colpidium* invasions. Minimum adequate linear models relating density response variables to propagule size and enrichment explanatory variables. *SE* – standard error of mean. No interactions were significant. For transient density of *Colpidium*, initial examination of model indicated that inclusion of a quadratic term significantly improved the fit.

Protist	**Response**	**Explanatory**	**Estimate**	**SE**	***t***	***p***
Colpidium (invader)	Density pre stable state (day 3)	Propagule size	1.135	0.093	12.14	< 0.001
		Propagule size^2	−0.177	0.269	−6.58	< 0.001
	Density at first stable state (days 6-18)	Propagule size	0.018	0.006	3.24	0.002
		Enrichment	0.204	0.012	17.07	< 0.001
	Density at second stable state (days 36-60)	Enrichment	0.582	0.109	5.34	< 0.001
Blepharisma (resident)	Density at first stable state (days 6-18)	Enrichment	1.617	0.026	62.66	< 0.001
	Density at second stable state (days 36-60)	Enrichment	0.249	0.079	3.15	0.003

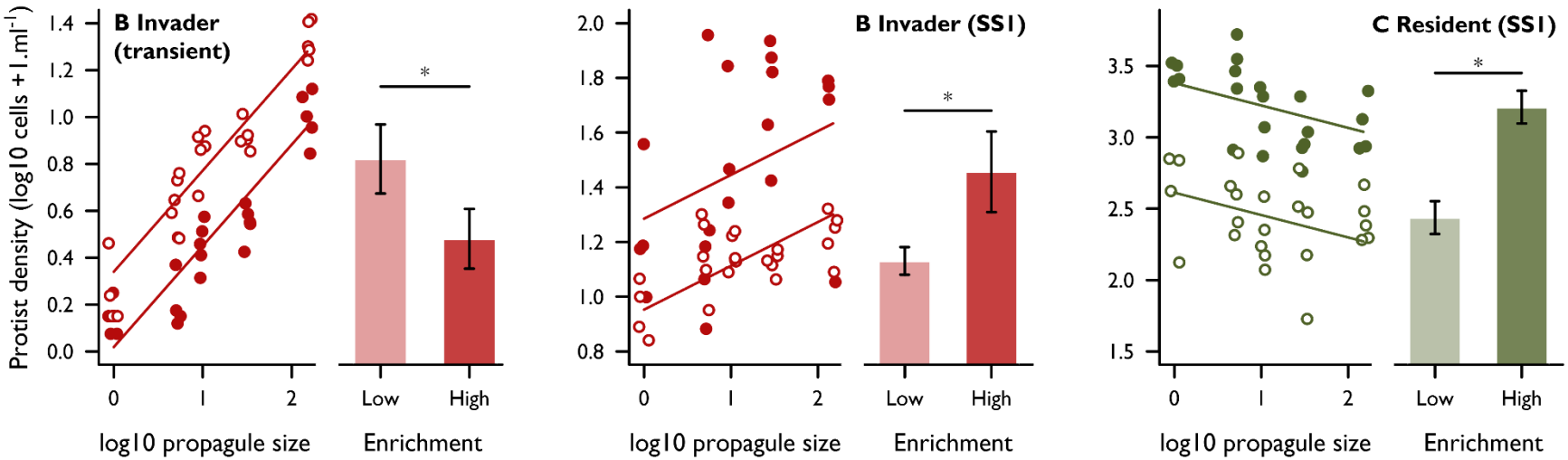


Figure 6.4 Details of *Blepharisma* invasions. Invader and resident protist densities at time periods throughout experimental invasions (cf. Fig. 6.1). Each graph corresponds to one response variable in Table 6.3. *Filled symbols* are for high enrichment treatments, *open symbols* for low enrichment treatments. *Lines* are predictions from minimum adequate models (Table 6.3). Two lines are plotted when there is a difference between enrichment treatments. Lines within each plot have the same gradient because no interactions were significant. *Bar plots* show mean protist density pooled across all propagule pressures ± 2 standard errors; *asterisks* indicate significant differences from linear models. Note that axis ranges differ between plots. *B – Blepharisma*; *C – Colpidium*; *SS* – stable state.

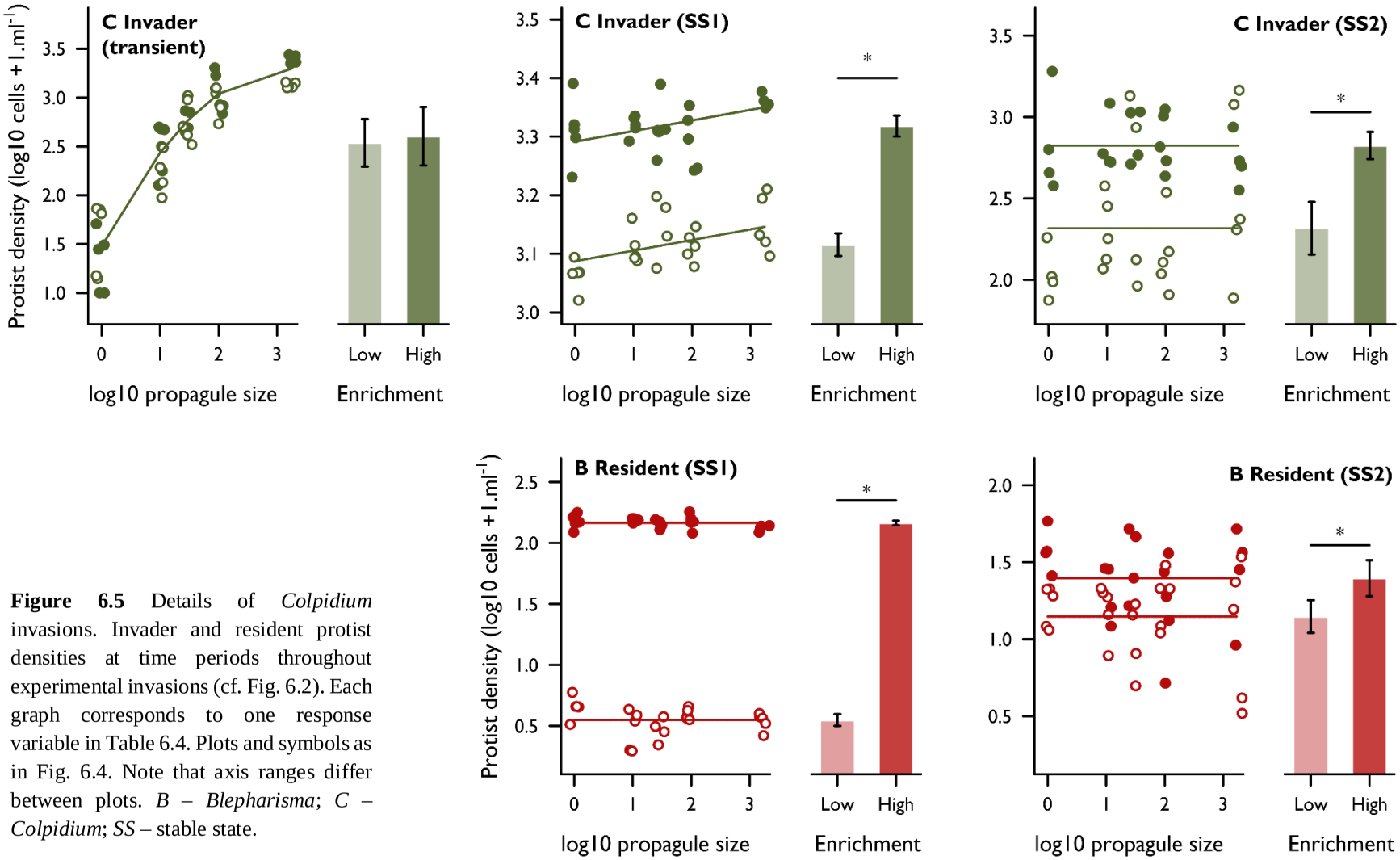


Figure 6.5 Details of *Colpidium* invasions. Invader and resident protist densities at time periods throughout experimental invasions (cf. Fig. 6.2). Each graph corresponds to one response variable in Table 6.4. Plots and symbols as in Fig. 6.4. Note that axis ranges differ between plots. *B* – *Blepharisma*; *C* – *Colpidium*; *SS* – stable state.

6.3.1.5 Extinction

Extinction is defined as the absence of protists at final census on day 60. Of the invader populations that established in control microcosms (containing a single species of protist), none went extinct.

In experimental microcosms invaded by *Colpidium* (Table 6.5b), extinction of either resident or invader was rare. The only observed extinction was of resident *Blepharisma* in one microcosm invaded by 10 *Colpidium*.

Table 6.5 Extinction of invader and resident protist populations **(a)** in experiments with *Blepharisma* as the invader and **(b)** in experiments with *Colpidium* as the invader. Extinction rates are presented as a fraction of the total established microcosms from which protists were absent by day 60 (final census). Treatments including extinctions are highlighted in bold.

(a)	**Protist**	**Enrichment**	**Propagule size**				
			1	**5**	**10**	**30**	**150**
	Blepharisma (invader)	High	**3/5**	**3/5**	**3/5**	**1/5**	**5/5**
		Low	**2/4**	0/5	0/5	0/5	0/5
	Colpidium (resident)	High	0/5	0/5	0/5	0/5	0/5
		Low	0/5	0/5	0/5	0/5	0/5

(b)	**Protist**	**Enrichment**	**Propagule size**				
			1	**10**	**30**	**100**	**1750**
	Colpidium (invader)	High	0/5	0/5	0/5	0/5	0/5
		Low	0/5	0/5	0/5	0/5	0/5
	Blepharisma (resident)	High	0/5	**1/5**	0/5	0/5	0/5
		Low	0/5	0/5	0/5	0/5	0/5

In microcosms invaded by *Blepharisma* (Table 6.5a), resident *Colpidium* never went extinct. At high enrichment levels, *Blepharisma* was absent from most microcosms on day 60, including all microcosms subject to the highest propagule pressure (150 invaders). However, I lacked sufficient replication of microcosms to demonstrate that propagule pressure was related to extinction probability (Fisher's exact test propagule size 150 vs all other propagule sizes $p = 0.061$). The odds of *Blepharisma* extinction in high enrichment microcosms were 16.5 times higher than in low enrichment microcosms (Fisher's exact test $p < 0.001$). At low enrichment levels, *Blepharisma* only went extinct in two microcosms in which it established following the lowest propagule pressure treatment.

6.3.2 Experiment 2: propagule size and number manipulation

In a separate experiment, I investigated the relative influence of propagule size and number in determining invasion success and impact. I added *Blepharisma* to resident *Colpidium* in different propagule size-number combinations. These additions were all carried out at the lower enrichment level (0.21 $g.L^{-1}$).

After a fixed growth period (average 8.5 growth days; Fig. 6.6a), protist densities were below those attained at stable state. There was significant variation in invader *Blepharisma* densities between size-number treatments (ANOVA $F_{4,35} = 2.76$, $p = 0.043$), although this was driven by a difference between the propagule size 2 and 16 treatments only (Tukey $p = 0.025$). However, there was a general hump-shaped pattern with higher densities attained under the less extreme combinations of size and number (Fig. 6.6a). The impact of invading *Blepharisma* was similar across different size-number treatments: resident *Colpidium* densities did not differ between treatments (ANOVA $F_{4,35} = 0.38$, $p = 0.825$).

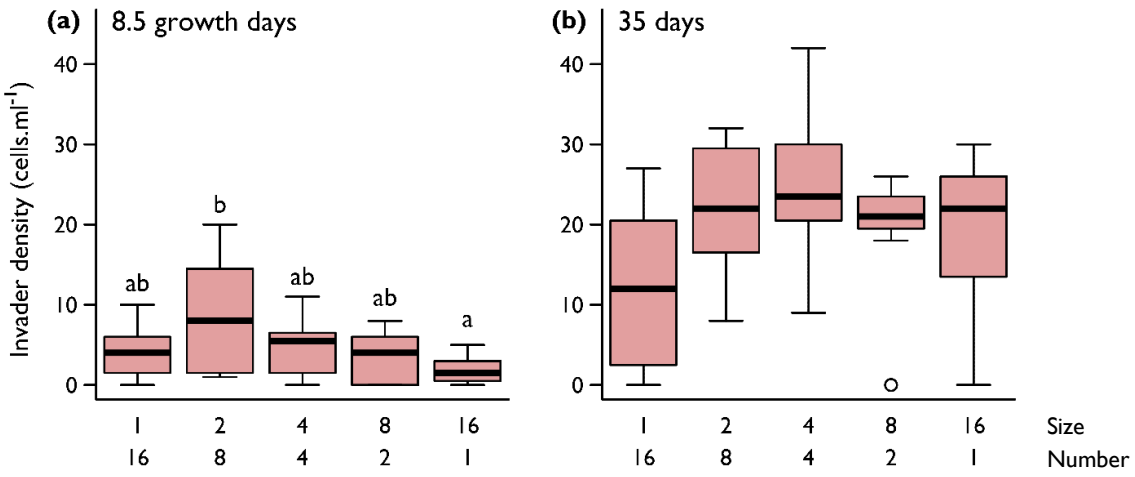


Figure 6.6 *Blepharisma* invader density after **(a)** 8.5 growth days and **(b)** 35 days, at varying combinations of propagule number and propagule size. All invasions were into established *Colpidium* in low enrichment medium. *Letters above boxes* indicate significant differences based on Tukey Kramer post-hoc tests. *Boxes* show medians and interquartile ranges, *whiskers* the range of the data excluding outliers, and *points* show outliers.

On day 35 (Fig. 6.6b), invading *Blepharisma* populations had reached a stable state at a similar level to the size-manipulation experiment (Experiment 1). Establishment success was high at all of the propagule size-number treatments, with no obvious difference between treatments. Only three of the 40 invasions into resident *Colpidium* failed to establish: one at each of the propagule

sizes 1, 8 and 16. I make no further analysis of this establishment success. Invader population density after 35 days also did not differ between size-number treatments, whether all microcosms were considered (ANOVA $F_{4,35} = 2.00$, $p = 0.115$) or just those that established (ANOVA $F_{4,32} = 1.62$, $p = 0.193$). However, *Blepharisma* density in the high number treatment (size 1: number 16) seemed to be lower than in the other treatments (Fig. 6.6b). With respect to impact, resident *Colpidium* densities did not differ significantly between treatments after 35 days (all microcosms ANOVA $F_{4,35} = 1.66$, $p = 0.181$; microcosms with established invader ANOVA $F_{4,32} = 1.54$, $p = 0.215$).

In sum, establishment success, invader population density and impact (resident population density) were similar whether small propagules were added multiple times, or a single large propagule was added once.

6.4 Discussion

In my experimental invasions into protist microcosms, high propagule pressure consistently increased invasion success, defined as time to establishment and invader population size. These results held irrespective of the identity and trophic guild of the invader and resident species, and irrespective of the enrichment level of the microcosm. The effect of propagule size was particularly evident in the initial stages of invasions. Coefficients describing the relationship between propagule size and invader density were largest before an initial stable state was reached, and for *Colpidium* invaders larger in the first than the second stable state. Similarly, larger propagule sizes facilitated faster establishment, with a prolonged lag-phase (time between introduction and establishment) only evident in the lowest propagule size *Blepharisma* invasions. Temporal resolution of my sampling was too coarse to detect a lag in *Colpidium* invasions. It is in this early stage of invasions that propagule pressure is likely to most critically affect invasion success, as population size (and associated demographic and genetic problems; Simberloff 2009) more strongly reflects the introduction of individuals than intrinsic population growth.

However, my data also indicate that the effects of propagule pressure – on both the invader and resident – can reach beyond the initial phase of the invasion. Propagule size was positively related to invader density at the initial stable state for both *Blepharisma* and *Colpidium*. Further, in invasions of *Blepharisma*, high propagule pressure was associated with lower densities of resident *Colpidium*, probably mediated by the higher density of the predatory protist. Predatory *Blepharisma* morphs were observed in microcosms, although they were rare (typically < 5% of the *Blepharisma* population, if present at all). In general, impacts of invasive species increase with abundance (Parker et al. 1999; Ricciardi 2003). I provide empirical data that link this impact

to propagule pressure, via abundance. Consequently, from a purely demographic perspective, management action to reduce propagule pressure could contribute to reduced impacts of alien species, even if it does not prevent establishment (Reaser et al. 2007).

At the range of propagule sizes I used, there were consistent marginal gains in most cases: every unit increase in propagule pressure was associated with an increase in invader population density. To paraphrase Lockwood et al. (2009), the more I introduced the more I got. The maximum propagule size I used was two (*Blepharisma*) or three (*Colpidium*) orders of magnitude below each species' carrying capacity in experimental microcosms. Perhaps diminishing returns would be obtained from larger propagules, as evidenced by the pre-stable state densities of *Colpidium*, but this may not be ecologically relevant if most invaders are introduced as small propagules relative to their carrying capacity. If consistent marginal gains are common in real invasions (as opposed to saturation), then management to reduce propagule pressure could still be beneficial in reducing abundance and impact, even if it does not prevent invasion completely.

Although it is commonly reported that propagule size increases establishment success (Lockwood et al. 2005; Simberloff 2009), I found no such relationship in my microcosms. There was no threshold propagule size that facilitated establishment, because the smallest propagule size (one cell) established in almost all microcosms. Success of small propagules in my experiments would have been favoured by the minimal environmental and demographic stochasticity, and the simple reproductive behaviour of protists (cf. Grevstad 1999; Sinclair and Arnott 2016). Conditions in my microcosms were stable (constant temperature and nutrients, minimal disturbance) and closely matched the source environment of the protists (except for slight variations in enrichment). Certain combinations of environmental conditions and invader biology can facilitate establishment from small propagules in the field (Zayed et al. 2007; Duncan 2016, but see King and Reed 2016). Further, establishment of very small propagules is likely when there are no Allee effects operating (Taylor and Hastings 2005; Drake and Lodge 2006). Both *Colpidium* and *Blepharisma* can reproduce asexually by binary fission (Giese 1973; Fox 2002), mitigating Allee effects even in a population consisting of a single cell.

Intra-guild predation can confer biotic resistance against invasion, especially when propagule pressure is low (Polis et al. 1989; Twardochleb et al. 2012), but this likely did not apply when *Colpidium* was invading *Blepharisma* in my microcosms. Larger predatory morphs were not present in *Blepharisma* stock cultures, but were induced by the presence of *Colpidium*. Low densities of invading *Colpidium* provided a weak stimulus, thus predatory morphs of resident *Blepharisma* were only observed 24 days after *Colpidium* invasions. (For comparison, when

Blepharisma were added to high density, established *Colpidium* populations, predatory morphs appeared within three days).

In a second experiment to compare the relative roles of propagule size and number on invasion success and impact, there were no consistent significant differences in establishment success, invader density or resident density between different combinations of propagule size and number. That is, invasion success was similar whether everything was introduced at once, or introductions were split over multiple smaller propagules. My microcosms were easy to invade, negating advantages of an increased propagule size or number (Simberloff 2009; Wittmann et al. 2014). Large propagules were not needed to overcome problems faced by small propagules: even the smallest propagules survived and reproduced (Experiment 1). The advantage of multiple small propagules replacing previous propagules wiped out by environmental stochasticity was also irrelevant in my microcosms. Further, given the homogeneity of the microcosms, there was no benefit to be gained from introducing multiple small propagules at different points in space. Invasion success of *Daphnia* into simple laboratory microcosms was similarly found to be more dependent on total propagule size (or 'immigration rate') than either size or number alone (Drake et al. 2005).

Although not the focus of this Chapter, my data provide some insight into the population dynamics of *Colpidium* and *Blepharisma*. *Colpidium* and *Blepharisma* may exclude or facilitate each other, depending on enrichment levels. Comparing my control microcosms (no resident protist) with experimental microcosms (resident present) (Fig. 6.1), *Colpidium* appeared to facilitate *Blepharisma* invasion at low enrichment levels and small propagule pressures, in line with theory and previous experiments (Lawler and Morin 1993; Diehl and Feissel 2000; Diehl and Feissel 2001). *Blepharisma* invasions into control microcosms failed, or were very slow, at the smallest propagule sizes, and populations were at lower density than those in experimental microcosms. In experimental microcosms, *Colpidium* provided an additional food source for *Blepharisma*; the latter had differentiated into predatory morphs within three days of addition to *Colpidium* cultures. Facilitation could be an important mechanism explaining the success of invaders, especially in 'invasional meltdown' scenarios (Simberloff and von Holle 1999; Adams et al. 2003) and in the gregarious settlement of marine invertebrates (Hedge et al. 2012). Protists could provide a useful experimental system to test this hypothesis. I did not observe competitive exclusion of either protist species, despite restricting the bacterial community to decrease the likelihood of trophic niche differentiation (Gonzalez et al. 1990; Thurman et al. 2010) and using enrichment levels previously associated with exclusion (Lawler and Morin 1993; Morin 1999). Observed extinctions in high enrichment microcosms probably reflect an accumulation of waste products and/or exhaustion of nutrients by the high-density populations (Kirk 1998; Fox 2007).

Spatial niche differentiation could have mediated coexistence. Further investigation of coexistence dynamics of these protists would be interesting.

In summary, my data provide evidence for a consistent relationship between overall propagule pressure and the success (abundance) of invading protist species, across enrichment levels and trophic guilds, with consistent marginal gains across a range of propagule pressures spanning two to three orders of magnitude. I also demonstrate a link between propagule pressure and the impact of a predatory invader on resident prey, likely to be mediated by the abundance of the invader. My data support proactive management based on reducing propagule pressure (Reaser et al. 2007) and suggest it may reduce (or delay) both success and impact of alien species. Equally, in conservation translocations or reintroductions, each marginal increase in propagule size will increase the chances of success.

Chapter 7

General discussion: towards solving the problem of biological invasions

7.1 Thesis aim

The aim of this thesis was to investigate some key mechanisms related to invasion success and impact: propagule pressure, behaviour and resource use. It was anticipated that the outcomes would be both an increased understanding of the specific study systems used, and of invasion success and impact in general. This increased understanding could support the development of tools for predicting the success and impact of invasive species.

7.2 Species-specific lessons

The white-clawed crayfish *A. pallipes* is native to Europe and has long been established in Great Britain (Holdich et al. 2009), but is being replaced by the invading American signal crayfish *P. leniusculus* which is now the most abundant and widely distributed freshwater decapod crustacean in Great Britain (NBN 2016). The Chinese mitten crab *E. sinensis* is also invasive on a global and national scale, with British populations beginning to boom (Clark et al. 1998). It is important to understand the success and impact of these three species, to inform management decisions or, if control is not possible, to understand how fresh water ecosystems might change with the identity of the dominant decapod crustacean.

7.2.1 Mitten crabs Eriocheir sinensis

My data highlight *E. sinensis* as an invasive species with an extremely high ecological impact. *E. sinensis* does rank highly on risk assessments, being given the highest score of all freshwater invertebrate invaders assessed by Laverty et al. (2015b) and being listed as one of the 100 worst invaders in the world (Lowe et al. 2004), but these are largely influenced by socioeconomic impact and the ecological impact of *E. sinensis* remains poorly understood. Through deriving functional responses (FRs) of *E. sinensis* in the laboratory (Chapter 2), I demonstrate that this invasive crab is a highly damaging predator of a range of macroinvertebrates. It consistently had a higher FR than European *A. pallipes* on all macroinvertebrate prey tested, and an FR at least 1.9 times higher than that of another known damaging invader, *P. leniusculus*, on soft-bodied or fast-moving prey (chironomid larvae and *D. villosus*). Although Rosewarne et al. (2016) had previously demonstrated a high FR for *E. sinensis* on *G. pulex* prey, the magnitude of this impact

and the consistency across prey taxa, as demonstrated in Chapter 2, is unprecedented. Data on metabolic rates in Chapter 2 offer a novel mechanistic explanation for this pattern: *E. sinensis* consumes prey at a rapid rate to fuel an active lifestyle and high routine metabolic rate. Data on activity in Chapter 4 were inconsistent with this hypothesis: *E. sinensis* was actually the least active decapod at dawn and dusk. Diurnal patterns of activity in *E. sinensis* require further study, along with seasonal and ontogenetic patterns given the migratory lifestyle of this crab.

Per capita impacts of *E. sinensis* are likely to remain high in the field: this species is bold (Chapter 4) so sublethal effects of threats (such as predation) are likely to only weakly affect resource consumption by crabs. However, if this boldness is misplaced, novel predators in the invaded range (perhaps large mammals and birds) could control populations and mitigate impact. Meanwhile, migration of individual *E. sinensis* at they mature (up to 1400 km upstream; Panning 1939) will contribute to a broad geographical impact.

Given the potentially large ecological impact of *E. sinensis*, immediate control measures to limit its spread are recommended. Many environmentally-suitable river catchments remain to be invaded by *E. sinensis* (Herborg et al. 2007). Ballast water regulation, which will come into force in September 2017, is likely to help prevent new introductions on an international scale (Cohen and Carlton 1997; IMO 2016). On a local scale, spread can be limited by public education and good biosecurity practice. Spread through larval migration along coastlines is also possible and would be difficult to control, but the distribution and behaviour of larvae requires further study (Dittel and Epifanio 2009). Local control of mitten crab populations could be achieved through commercial fishing, although this is controversial as it could encourage further spread through deliberate introductions (Clark 2011). In addition, established populations of *E. sinensis* may have ecological benefits to counter their impact: by virtue of their high predation rates, they could offer stronger biotic resistance than resident decapods to new invaders, such as *D. villosus* (Twardochleb et al. 2012).

Further research into the impacts of *E. sinensis* in the field, using a combination of observation (Before-After-Control-Impact (BACI) studies, stable isotope analyses and gut content analyses) and experimental manipulation, would complement the laboratory experiments presented in this thesis (Kumschick et al. 2014). In 2014, I attempted a replicated Control-Impact study of *E. sinensis* in the East Anglian Fens, comparing macroinvertebrate communities in water bodies with and without crabs, but was unable to detect *E. sinensis* where it had previously been reported.

High rates of resource consumption and bold behaviour of *E. sinensis* could contribute to its invasive success, allowing it to outcompete resident decapods through both pre-emptive and

exploitative competition. There is some dietary overlap between mitten crabs and crayfish (Rudnick and Resh 2005; Rosewarne et al. 2016). However, a requirement for abundant food resources could limit its invasive success in resource-poor habitats (cf. *Blepharisma* struggling to invade control (*Colpidium*-free) low enrichment microcosms in Chapter 6).

7.2.2 Signal crayfish Pacifastacus leniusculus

My data also indicate that *P. leniusculus* has a higher rate of resource use than *A. pallipes*, but that the magnitude of this difference is small relative to the difference between crayfish and crabs. This broadly agrees with findings from previous work (Haddaway et al. 2012; Rosewarne et al. 2016) but again I extended this knowledge to a wider range of prey items and in the presence of habitat structure. By including habitat structure in my experiments, I demonstrate that the pattern of higher resource use is maintained in more realistic conditions. Furthermore, my data indicate that *P. leniusculus* is bolder than *A. pallipes* (Chapter 4) and is more active at night (Chapter 2), meaning higher predation rates in the laboratory are likely to translate into field situations. Predation by *P. leniusculus* likely contributes to its impact on macroinvertebrate populations in the field (Crawford et al. 2006; Mathers et al. 2016).

To improve our understanding of the impact of *P. leniusculus*, direct comparisons of field impact to that of *A. pallipes* are necessary. Given the relatively small difference in magnitude of resource use, it could be that *P. leniusculus* is effectively a functional replacement for *A. pallipes* (James et al. 2015). However, a greater local impact of the invasive alien could be driven by a higher abundance (Parker et al. 1999; Dick et al. in press). Crayfish densities can be highly context-dependent – between waterbodies, between habitat types within water bodies, between seasons and depending on size class considered – but in similar conditions *P. leniusculus* can reach densities ($14.m^{-2}$; Guan 2000) at least three times those of *A. pallipes* (c. $4.m^{-2}$; Demers et al. 2003). Studies to determine if the impact of *P. leniusculus* really is greater than resident *A. pallipes* are needed, and manipulation of species identity and density could tease apart the mechanisms.

In two separate studies (Chapters 4 and 5), I provide the first evidence that *P. leniusculus* show consistent, within-individual behaviour or personality. Previous work has only demonstrated inter-population behavioural consistency in *P. leniusculus* (Pintor et al. 2008). Against a backdrop of intra-species variation, personality can be a key driver of range expansion dynamics (Cote and Clobert 2007; Cote et al. 2010b). However, I found no evidence for personality-dependent dispersal in signal crayfish (Chapter 5). Instead, patterns of abundance and sex ratios supported a model of density-dependent dispersal, whereby subordinate individuals are forced out of a

population when it reaches high density (Peay and Rogers 1999; Hudina et al. 2015). An implication for management is that trapping of signal crayfish in established populations to reduce their density (e.g. Moorhouse et al. 2014) could slow the dispersal of individuals and spread of the population, even if the core population is not eradicated. An alternative strategy of eradicating nascent satellite populations is likely to be a constant battle against new dispersers.

I did not identify a clear role for behaviour in the success of signal crayfish invasions. Boldness may contribute to a competitive advantage over incumbent *A. pallipes* as foraging is less restricted in the invader. Behaviour may be more important in a group context. *P. leniusculus* maintains its high feeding rate despite conspecific interference at high density (Pintor et al. 2009). Alternatively, the dominance of *P. leniusculus* could be more simply related to (a) size (facilitating intraguild predation of the invader on *A. pallipes*; cf. Chapter 6) or (b) spillover of crayfish plague from the invader to the incumbent (Dunn et al. 2008).

7.2.3 Killer shrimp Dikerogammarus villosus

Predation by *D. villosus* has been implicated in reduced diversity and abundance of macroinvertebrates in invaded waters, but we have a poor understanding of the effects of *D. villosus* on vertebrate prey. Predation of fish eggs and larvae by *D. villosus* had been observed in laboratory situations (Casellato et al. 2007; Platvoet et al. 2009) but remained poorly quantified. Chapter 3 examined trophic resource use of invasive *Dikerogammarus villosus* with FR and electivity experiments, and fish eggs or larvae as the focal resource. I provide extensive quantitative data on the impact of *D. villosus* on both salmonid and coarse (non-salmonid) fish. *D. villosus* is likely to have a greater impact than the British native *G. pulex* on fish eggs and larvae, but this is because of its larger size rather than any intrinsic difference between the species. Whilst size-matched *D. villosus* and *G. pulex* had broadly similar predatory impacts, large *D. villosus* were better able to kill large salmonid larvae, and consumed more carp eggs and larvae than the smaller amphipods. These data, published in *Biological Invasions* (Taylor and Dunn 2016), provide quantitative evidence to contribute to risk assessments for this species, which could assist management decisions where it has already invaded (Europe) and horizon scanning exercises where it threatens to invade (North America; Pagnucco et al. 2014).

There is considerable uncertainty over the trophic position of *D. villosus*. Observations and early field studies suggested it was a voracious predator (Dick et al. 2002; van Riel et al. 2006; Platvoet et al. 2009), whilst more recent evidence points to a more omnivorous mode of feeding that may be highly context dependent (Hellmann et al. 2015; Koester et al. 2016). Although not the principal aim of Chapter 3, data from electivity experiments suggests *D. villosus* is omnivorous

but is more likely to be predatory than *G. pulex*, especially when large. Flexible omnivory – the ability to exploit a wide variety of food types – could contribute to the success of *D. villosus*. A varied diet can provide nutritional benefits (Cruz-Rivera and Hay 2000) as well as buffering shortages in any single food item.

7.2.4 Microbial invasions

Finally, Chapter 6 provides insight into microbial invasions, which are probably common but underreported, and can lead to large impacts (Gillis and Chalifour 2010; Litchman 2010; Acosta et al. 2015). Although the species used are not of interest as successful or damaging invaders in the wild, this Chapter suggests that microbial invaders can establish from very small propagules (at least in stable environments), due to a capacity for rapid, asexual reproduction. In terms of management, this means very strict biosecurity (perhaps impossibly so) would be needed to prevent microbial invasions.

7.3 General lessons: predictors of invasion success and impact

7.3.1 Propagule pressure

There is abundant evidence that propagule pressure is correlated with establishment success (Lockwood et al. 2005; Blackburn et al. 2015). However, much of this evidence comes from assumed correlates of propagule pressure (e.g. shipping traffic, length of roads, measures of general horticultural activity; Simberloff 2009) or analyses based on historical introduction data, mainly for birds (Cassey et al. 2004; Blackburn et al. 2009). There are few controlled, empirical, quantitative tests of the relationship between propagule pressure and invasion success, and such studies are necessary to inform a mechanistic understanding of the relationship. Further, relatively little is known about the effect of propagule pressure on aspects of invasions other than establishment probability – such as establishment rate, invader abundance and impact. The relative roles of propagule size and number on invasion success also remain poorly understood (Wittmann et al. 2014).

Data from my experiments with protist microcosms provide evidence for some important points with respect to propagule pressure. First, they show that establishment success does not always depend on propagule pressure: very small propagules can establish. This is also seen in some field invasions (Zayed et al. 2007; Duncan 2016), but is likely to depend upon a favourable combination of invader traits and environmental conditions. For example, environmental stochasticity may eliminate small propagules.

Second, my data provide evidence that increased propagule pressure can quantitatively affect other aspects of invasions beyond establishment success, including reducing lag times (cf. Grevstad 1999) and increasing invader abundance over many generations. Consequently, impact may be felt sooner and more strongly. Where every unit increase is associated with an increase in invader growth rate or abundance, management to reduce propagule pressure will have consistent marginal gains – even if introductions are not prevented completely. Although this relationship may break down if aliens are introduced in sufficient number, most introductions are likely to be small relative to an environment's carrying capacity, as in the microcosm experiments.

Third, I did not determine that propagule size or number alone was a more important driver of abundance. Generally, the overall product of propagule size and number may be a more important predictor of invasion success than either alone (Wittmann et al. 2014). Overall, propagule pressure could be a strong quantitative predictor of invasion success and impact, beyond its simple correlation with establishment success. A greater mechanistic understanding of the link between propagule pressure and success and impact could feed into more accurate and powerful predictive models.

Protist microcosms offer practical tools for investigating mechanistic hypotheses that would not be possible in field situations or with larger, longer-lived organisms (Warren et al. 2006; Altermatt et al. 2015). However, they require careful design (e.g. control and characterisation of the bacterial community) to ensure conclusions are meaningful and repeatable, and should be seen part of, not a replacement for, a wider toolkit – alongside field studies and mathematical modelling, for example (Benton et al. 2007).

7.3.2 Behaviour

Chapters 4 and 5 focussed on quantitative behavioural traits, such as boldness, exploration and activity, along which individuals may show consistent personalities (Réale et al. 2007). A combination of inter-individual variation but intra-individual consistency in such behaviours can have important ecological implications (Wolf and Weissing 2012) and a role for personality in invasion success or impact has been demonstrated in a growing list of taxa (Chapple et al. 2012; Juette et al. 2014). However, general patterns remain to be extracted.

In providing the first demonstration of individual personalities within *P. leniusculus*, *A. pallipes* and *E. sinensis*, I extend the number of taxa known to show personalities and support the idea that personalities may be widespread, even in 'simple' invertebrate animals (Gherardi et al. 2012; Mather and Logue 2013). However, the mechanisms by which personality drives invasion success

may be highly context-dependent. Processes that drive invasion in one taxon or in one environment might not do so in another. For example, despite evidence of personality dependent dispersal in fish (Cote et al. 2010b) and lizards (Cote and Clobert 2007), I found no evidence for it in signal crayfish. Thus, rather than attempting to use individual personality traits as general predictors of invasion success and impact, individual personality could be considered as part of a toolbox to understand the underlying mechanisms of (and inform management of) specific invasions.

Meanwhile, the average behaviour of species may have more use as a general predictive tool for invasion success and impact. In particular, my data add to evidence that suggests boldness – tolerance of risky situations (Réale et al. 2007) – may be a trait that is commonly associated with successful, high impact alien species. Monceau et al. (2014) demonstrated that the invasive wasp *Vespa velutina* is bolder on average than native *V. crabro* and suggested this may be associated with successful nest initiation in novel environments. Short and Petren (2008) found 'A' clones of the gecko *Lepidodactylus lugubris* to be bolder than the 'B' clones they displace, linked to a greater foraging ability in simple habitats. In mosquitofish, the mean boldness of laboratory populations influences individual dispersal tendency (Cote et al. 2011).

Overall, the behavioural assays in Chapter 4 are best interpreted as describing increased boldness in invasive *E. sinensis* and *P. leniusculus*, relative to declining European *A. pallipes*. However, further verification of the validity of these assays and/or testing of boldness using alternative assays would aid interpretation (Carter et al. 2013). Moreover, more information on the functional relevance of boldness in decapod crustaceans would clarify the link to invasion success and impact. For example, how does boldness relate to predation risk and prey consumption? Comparing data from Chapters 2 and 4 suggests that species-level boldness may be related to prey consumption, even in in the absence of obvious risk. *E. sinensis* was much bolder and consumed much more food than the crayfish in FR trials, whilst differences in both boldness and feeding rate between the crayfish were much smaller in magnitude.

If boldness is a consistent predictor of invasion success and impact, it could be used to screen potential invaders to prioritise preventative management, or even inform management strategies that are biased towards capturing bold species (e.g. trapping; Biro and Dingemanse 2009). However, like all trait-based predictors there will be some degree of context-dependency in the role of boldness in invasion success (Hayes and Barry 2007). In some environments or for some species, boldness may not be necessary for successful invasion. For example, the rapid and broad spread of *D. villosus* across Europe has probably been facilitated by passive transport on

recreational equipment (Bacela-Spychalska et al. 2013a), perhaps favoured by reduced boldness relative to native amphipods (Truhlar and Aldridge 2014).

7.3.3 Resource use

One particular aspect of behaviour that may have particular value as a predictive tool for invasion success and impact is resource use. This can be rapidly and easily quantified as a functional response, using laboratory experiments or field data (Moustahfid et al. 2010; Dick et al. 2014). There are a growing number of case studies demonstrating that high Type II FRs are associated with high impact invaders across a range of taxa and functional feeding groups (Rossiter-Rachor et al. 2009; Haddaway et al. 2012; Dodd et al. 2014; Laverty et al. 2015a; Rosewarne et al. 2016; Xu et al. 2016). Data in Chapter 2 adds to this pool of examples, with *a posteriori* comparisons to known impacts of decapod Crustacea in field and mesocosm situations suggesting the shape, rank order and magnitude of FRs are related to impacts on different prey taxa. The similarity of FRs between size-matched *G. pulex* and *D. villosus* in Chapter 3 could be a reflection of the fact that *G. pulex* is a successful invader with negative impacts outside of its native Great Britain, for example replacing native amphipods and altering community composition, richness and diversity in Irish rivers (Kelly et al. 2006). Thus, this comparison specifically investigates relative impact of *D. villosus* in Great Britain more than searching for general correlates of invasiveness and impact (van Kleunen et al. 2010).

Further explicit verification of the link between FRs and specific field impacts (rather than general impact, or *a posteriori* comparisons) is required. For example, information on impacts of *D. villosus* on fish populations in the field, either through experimental manipulation or using long-term monitoring data under a BACI design, could be used to test the predictions made in Chapter 3, and by inference the validity of the FR methodology.

Further lessons about using FRs as tools for predicting invasions can be learned from Chapters 2 and 3. First, when sympatric invasive and native organisms differ greatly in size, then these differences in size should be incorporated into FR experiments, rather than size-matching subjects as is commonly done. Only by explicitly considering size differences between *D. villosus* and *G. pulex* was I able to make realistic quantitative comparisons of the impact of the two amphipod species in Chapter 3. The comparisons made amongst decapod Crustacea in Chapter 2 are still informative given that the size distributions of *A. pallipes* and *P. leniusculus* substantially overlap with each other (Chapters 2 and 4), and indeed with the developmental stage of *E. sinensis* that may compete with or replace the crayfish in fresh waters (Veldhuizen and Stanish 1999). When comparing animals with different body plans, the appropriate measure on which to size-match

requires careful consideration. Is body mass, body length, length of certain body parts or some combination of these measures most important? Checking the robustness of analyses to the choice of size matching procedure is encouraged.

Second, impact predictions may benefit from combining FRs with abundance data. FRs measure *per capita* impacts, but the number of organisms exhibiting these impacts is also a crucial determinant of the overall impact of a population (Parker et al. 1999). Thus, although FRs may be similar between native and invasive alien species (*A. pallipes* vs. *P. leniusculus*, Chapter 2; size-matched *G. pulex* and *D. villosus*, Chapter 3), the impact of populations of these species may be vastly different. To this end, Dick et al. (in press) propose RIP (Relative Impact Potential) – a combination of individual FRs and population abundance – as a metric to compare impacts of alien and native species. However, this relies on robust comparative data on abundances, uncompromised by confounding variables, which are often lacking.

Third, I encourage a reasonable degree of replication (on the order of $n \geq 6$) at each prey density given the inherent variability in feeding data. Reasonable replication at high prey densities is necessary to obtain an accurate estimate of maximum feeding rates. To determine FR shapes, a high degree of replication at low prey densities is important. Data at low densities are crucial for distinguishing between Type II and Type III FRs, but are the most variable since each prey item consumed (or not) contributes to a large change in proportional mortality.

7.4 A metabolic explanation for invasions?

In Chapters 2 and 5, I measured metabolic rates of decapod crustaceans and compared them to feeding and general behaviours. Here, I argue that metabolism could be a general modulator of invasion success and impact, and thus a useful tool for predicting invasions.

An organism's metabolic rate is the rate at which it oxidises substrates to produce energy. Since energy is the common currency of life, fuelling biological processes at every level of organisation, metabolic rates are fundamentally important in ecology and evolution and determine the rate of almost all biological activities (Brown et al. 2004). Metabolic rates are strongly influenced by temperature and body size (Kleiber 1932; Brown et al. 2004). Thus, metabolic rates could explain differences in invasion success and impact based on these factors e.g. greater trophic impacts of large *D. villosus* in Chapter 3, or reduced amphipod feeding rates in cold water (Maier et al. 2011).

Moreover, there is much residual variation in metabolism unaccounted for by these parameters – with up to 6-fold differences between similar-sized, closely related species at a given temperature

(Brown et al. 2004; Careau et al. 2008) – and this could prove a useful predictor of success and impact of similar-sized species in similar conditions. Similar arguments may apply to predicting success and impact of *individual* invading organisms (Careau et al. 2008).

Metabolic rates may be involved in most, if not all, of the mechanistic hypotheses to explain invasion success and impact (Table 1.1 and Fig. 7.1). In particular, metabolic rates are closely linked to other species traits, from life history to behaviour and resource use. Theoretically, the allocation of limited resources amongst competing functions constrains individuals (and species) to possess a suite of linked traits, including metabolism, life-history traits and behaviour, somewhere along a fast-slow continuum (Ricklefs and Wikelski 2002; Réale et al. 2010). Generally, organisms with a fast pace of life may be more successful invaders (Baker and Stebbins 1965). They may be more likely to be taken up in transport vectors (propagule bias; Colautti et al. 2006) in large numbers (increasing propagule pressure), and will be better able to grow to large population sizes quickly. Fast organisms may also have larger impacts as they require more food to fuel their rapid, active lifestyle.

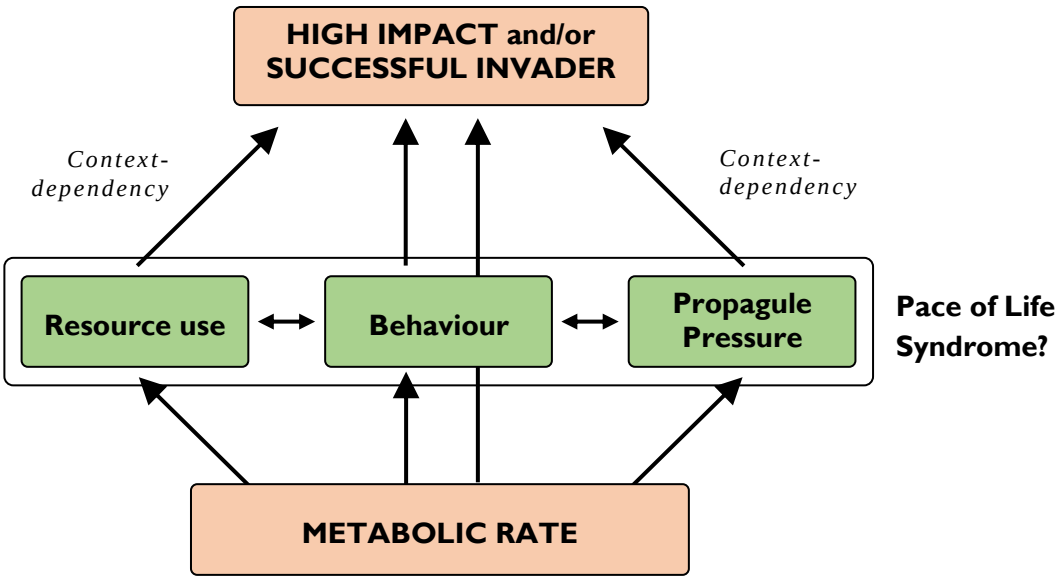


Figure 7.1 Could metabolic rate provide a common, mechanistic predictor of all invasions? It may do so, through links with resource use, behaviour and propagule pressure (amongst other proximate mechanisms of invasion success) – which may be linked in a pace of life syndrome (Réale et al. 2010). However, the sign of the relationship (whether high or low metabolic rate favours success and impact) is likely to be context-dependent.

In practice, many studies have demonstrated correlations between these pace-of-life traits. For example, in superb fairy-wrens *Malarus cyaneus*, risk taking behaviour is associated with residual reproductive value and survival probability in line with pace-of-life predictions (Hall et al. 2015). Importantly, metabolism seems to fit into pace-of-life syndromes, with correlations demonstrated between metabolic rate and behaviour in birds, mammals, fish and insects (Biro and Stamps 2010; Metcalfe et al. 2016). Interestingly, smaller mammalian species with higher mass-specific metabolic rates tend to produce faster (more 'active') sperm than larger mammalian species (Tourmente and Roldan 2015). In Chapter 5, metabolic traits of individual signal crayfish were correlated with active behaviours. In Chapter 2, differences between decapod species' average routine metabolic rate (RMR) mirrored differences in prey consumption. Thus, metabolic rate could provide a single trait that allows placement on the fast-slow continuum and consequently prediction of success or impact. The advantage of using metabolism as a proxy for other species traits is that it is now relatively quick and easy to measure, in a wide range of taxa and by non-invasive means – especially O_2 consumption (Chapters 2 and 5; Burton et al. 2011; Norin and Clark 2016).

As a trait, the influence of metabolic rate on invasion success and impact is likely to be context dependent. High metabolic rates may favour invasion of systems with abundant resources, for example after disturbance or where the invader occupies an unexploited niche (Table 1.1). In these cases, the invader can garner enough resources to fuel a high growth rate, early maturity and high fecundity that can confer competitive superiority over a native species (Baker and Stebbins 1965; performance model of Careau et al. 2008). However, where resources are less abundant, a low metabolic rate may favour successful invasion, as individuals have lower maintenance energy costs, so can survive when there is less energy available (Mueller and Diamond 2001) or can allocate more of the available energy to activity or growth (allocation model of Careau et al. 2008). Low metabolic rates may favour introduction success in accidental introductions, as individuals will be better able to survive long transport events with limited food (Anderson 1974), but in the different context of deliberate introduction this metabolic trait will be less important. Impact may be similarly context dependent if it is based on abundance, range or *per capita* effect (e.g. body size) (Parker et al. 1999). Still, all else being equal a high metabolic rate will be associated with high impact, because it necessitates (and/or facilitates) a high rate of resource consumption (Chapter 2).

Ultimately, metabolic rate may be most useful as a predictor of invasion success and impact in combination with other drivers: propagule pressure and the recipient environment (Section 1.4). In this regard, the best predictions of invasion success and impact will explicitly incorporate context-dependency. For example, integrating metabolic rate measurements with sea temperature

data allows for powerful predictions of alien species' distributions based on thermal habitat suitability: the most suitable environments have temperatures that maximise aerobic scope for activity (Marras et al. 2015). 'Thermal habitat suitability' thus explicitly characterises the match between an invader and the abiotic context of the invasion.

7.5 Concluding remarks

Biological invasions are, and are likely to remain, a major component of human induced environmental change with substantial negative impacts (Sala et al. 2000; Hulme 2009). We require tools to prioritise management of invasions and design effective strategies to mitigate the impacts of alien species. Arguably, the most reliable predictive tools will be based on a mechanistic understanding of invasion success and invader impact.

My data support the utility of propagule pressure and resource use as general and potentially quantitative predictors of invasion success and impact, whilst the role of behaviour appears to be more complex and idiosyncratic to individual invasions. As such, effective tools for predicting invasion success and impact could be based on propagule pressure (e.g. analysis of trade or tourism patterns, or species characteristics influencing uptake probability; Colautti et al. 2006) or resource use (e.g. functional responses). Metabolic rate may offer another general predictor of invasion success and impact.

Whilst these factors may be useful in predicting invasions *when all else is equal* (i.e. increases in these factors are generally associated with an *increased probability* of success or impact), such predictions will also be subject to much inter-site, inter-species, inter-population and temporal context dependency (Pyšek et al. 2012). Arguably, the ultimate aim of invasion ecology is a mechanistic understanding of these context-dependencies, which will facilitate truly powerful predictions (Kueffer et al. 2013).

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Appendices to Chapter 2

Appendix 2.1 Functional responses for decapods matched by body size: measurements and analyses using prey killed as response variable

cf. analyses in main text using prey consumed as response variable

Table A2.1 Sizes of animals used in each experiment (NB data for these animals were used in analyses in main text). *cmax* – maximum carapace dimension: carapace length for crayfish and carapace width for crabs. *Body size* derived from PCA of mass and cmax. Each usage of an animal contributes to the data set (so data are weighted for the number of times a predator was used).

Prey	Decapod	Wet mass (mg)	cmax (mm)	Body size	Difference in body size		
					Kruskal Wallis χ^2	df	*p*
		Mean ± SE	Mean ± SE	Mean ± SE			
Amphipod	*A. pallipes*	9.9 ± 0.4	31.0 ± 0.4	0.57 ± 0.19	1.13	2	0.567
	P. leniusculus	10.0 ± 0.3	32.1 ± 0.3	0.28 ± 0.16			
	E. sinensis	11.8 ± 0.4	30.2 ± 0.3	0.35 ± 0.18			
Chironomid	*A. pallipes*	10.8 ± 0.4	32.0 ± 0.3	0.11 ± 0.16	1.48	2	0.476
	P. leniusculus	10.2 ± 0.2	32.6 ± 0.2	0.10 ± 0.11			
	E. sinensis	12.1 ± 0.4	30.5 ± 0.3	0.19 ± 0.18			
Gastropod	*A. pallipes*	10.4 ± 0.3	33.0 ± 0.3	−0.02 ± 0.15	0.80	2	0.672
	P. leniusculus	10.1 ± 0.2	32.7 ± 0.2	0.10 ± 0.09			
	E. sinensis	12.3 ± 0.3	30.8 ± 0.3	0.07 ± 0.17			

Table A2.2 Parameter estimates and significance levels, from second order logistic regression of the proportion of prey *killed* by decapod predators against initial prey density. Quasibinomial errors were used due to overdispersion. ϕ – dispersion parameter for GLM; N_0 – first order term; N^2_0 – second order term.

Prey	Decapod	ϕ	Intercept	*p*	N_0	*p*	N^2_0	*p*	Type
Amphipod	*A. pallipes*	2.59	−0.194	0.245	−0.017	< 0.001	3.717×10^{-5}	< 0.001	II
	P. leniusculus	3.03	0.890	< 0.001	−0.029	< 0.001	6.620×10^{-5}	< 0.001	II
	E. sinensis	4.19	2.209	< 0.001	−0.025	< 0.001	4.899×10^{-5}	< 0.001	II
Chironomid	*A. pallipes*	17.82	2.461	< 0.001	−0.007	< 0.001	3.774×10^{-6}	< 0.001	II
	P. leniusculus	25.79	3.120	< 0.001	−0.007	< 0.001	2.457×10^{-6}	< 0.001	II
	E. sinensis	29.60	3.568	< 0.001	−0.002	0.168	-1.021×10^{-6}	0.317	II[a]
Gastropod	*A. pallipes*	2.30	−1.231	< 0.001	−0.009	0.041	1.129×10^{-5}	0.468	II[a]
	P. leniusculus	1.99	0.652	< 0.001	−0.011	0.003	1.327×10^{-5}	0.298	II
	E. sinensis	5.38	0.952	0.001	−0.028	< 0.001	5.943×10^{-5}	0.003	II

[a] Type II fit deemed to be most appropriate by comparing AIC values for Type I and Type II fits. AIC for Type II fits were lower.

Table A2.3 Estimates of functional response parameters for decapod predators on three macroinvertebrate prey species, with *prey killed* as the response variable, extracted from Rogers' random predator equation fitted to data in the *frair* package (Pritchard 2016). *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); 1/*h*T – maximum feeding rate (prey.day^{-1}), where T = time in days; *SE* – standard error. *Diff* – within each prey item and for each parameter, different letters in this column indicate significantly different parameters (after Holm-Bonferroni correction for multiple comparisons).

Prey	**Decapod**	***a***	**SE**	**Diff**	***h***	**SE**	**1/*h*T**	**Diff**
Amphipod	*A. pallipes*	0.735	0.082	a	0.040	0.002	25.0	a
	P. leniusculus	1.878	0.189	b	0.040	0.002	25.2	a
	E. sinensis	2.487	0.148	c	0.013	< 0.001	77.1	b
Chironomid	*A. pallipes*	2.457	0.088	A	3.281×10^{-3}	6.285×10^{-5}	304.8	A
	P. leniusculus	4.373	0.130	B	2.879×10^{-3}	3.600×10^{-5}	347.3	B
	E. sinensis	5.450	< 0.001	C	1.542×10^{-3}	1.282×10^{-5}	648.3	C
Gastropod	*A. pallipes*	0.292	0.042	α	0.054	0.007	18.5	α
	P. leniusculus	0.482	0.059	β	0.042	0.004	23.5	α
	E. sinensis	1.972	0.218	γ	0.043	0.002	23.5	α

Table A2.4 Comparison of functional response parameter estimates for decapod predation (*killing*) of macroinvertebrate prey, based on analysis using indicator variables in the *frair* package (Pritchard 2016). Raw *p* values are presented; significant differences ($\alpha = 0.05$) after Holm-Bonferroni correction within each prey group are indicated in bold. *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); *D* – difference; *SE* – standard error.

Prey	**Base Group**	**Comparison**		**Estimate (*Da* or *Dh*)**	**SE**	***z***	***p***
Amphipod	*A. pallipes*	*P. leniusculus*	*a*	1.143	0.206	5.550	**< 0.001**
			h	< 0.001	0.003	−0.098	0.922
	P. leniusculus	*E. sinensis*	*a*	0.609	0.240	2.540	**0.011**
			h	−0.027	0.002	−15.513	**< 0.001**
	A. pallipes	*E. sinensis*	*a*	1.751	0.169	10.379	**< 0.001**
			h	−0.027	0.002	−10.995	**< 0.001**
Chironomid	*A. pallipes*	*P. leniusculus*	*a*	1.918	0.037	52.458	**< 0.001**
			h	< 0.001	< 0.001	−6.334	**< 0.001**
	P. leniusculus	*E. sinensis*	*a*	1.079	0.039	27.533	**< 0.001**
			h	−0.001	< 0.001	−39.868	**< 0.001**
	A. pallipes	*E. sinensis*	*a*	2.994	0.035	84.573	**< 0.001**
			h	−0.002	< 0.001	−28.831	**< 0.001**
Gastropod	*A. pallipes*	*P. leniusculus*	*a*	0.190	0.070	2.730	**0.006**
			h	0.011	0.008	−1.428	0.153
	P. leniusculus	*E. sinensis*	*a*	1.490	0.225	6.614	**< 0.001**
			h	< 0.001	0.005	0.027	0.979
	A. pallipes	*E. sinensis*	*a*	1.679	0.222	7.561	**< 0.001**
			h	−0.011	0.007	−1.542	0.123

Appendix 2.2 Functional responses for decapods matched by body mass: measurements and analyses using prey consumed as response variable

cf. analyses in main text where decapod predators are matched by body size (a combination of mass and maximum carapace dimension). Data sets were rarefied to ensure matching by body mass, removing one replicate for each species at each density. Thus, these analyses are based on five replicates per predator species x prey species x density combination (compared to six replicates in the main text).

Table A2.5 Sizes of animals used in each experiment (NB data for these animals were used in analyses in main text). *cmax* – maximum carapace dimension: carapace length for crayfish and carapace width for crabs. *Body size* derived from PCA of mass and cmax. Each usage of an animal contributes to the data set (so data are weighted for the number of times a predator was used).

Prey	**Decapod**	**Wet mass (mg)**	**cmax (mm)**	**Body size**	**Difference in body mass**		
		Mean ± SE	**Mean ± SE**	**Mean ± SE**	**Kruskal Wallis χ^2**	**df**	***p***
Amphipod	*A. pallipes*	10.0 ± 0.4	31.1 ± 0.4	–	3.47	2	0.177
	P. leniusculus	10.5 ± 0.3	32.8 ± 0.3	–			
	E. sinensis	11.1 ± 0.3	29.8 ± 0.3	–			
Chironomid	*A. pallipes*	10.7 ± 0.4	32.0 ± 0.4	–	0.98	2	0.613
	P. leniusculus	10.4 ± 0.2	32.9 ± 0.3	–			
	E. sinensis	10.9 ± 0.3	29.6 ± 0.3	–			
Gastropod	*A. pallipes*	10.4 ± 0.3	32.9 ± 0.3	–	1.23	2	0.542
	P. leniusculus	10.4 ± 0.2	32.9 ± 0.2	–			
	E. sinensis	10.9 ± 0.3	29.3 ± 0.3	–			

Table A2.6 Parameter estimates and significance levels, from second order logistic regression of the proportion of prey consumed by decapod predators against initial prey density. Quasibinomial errors were used due to overdispersion. ϕ – dispersion parameter for GLM; N_0 – first order term; N^2_0 – second order term.

Prey	Decapod	ϕ	Intercept	*p*	N_0	*p*	N^2_0	*p*	Type
Amphipod	*A. pallipes*	2.60	−0.197	0.281	−0.018	< 0.001	3.965×10^{-5}	< 0.001	II
	P. leniusculus	2.91	0.851	< 0.001	−0.029	< 0.001	6.647×10^{-5}	< 0.001	II
	E. sinensis	3.30	1.964	< 0.001	−0.024	< 0.001	4.934×10^{-5}	< 0.001	II
Chironomid	*A. pallipes*	19.82	2.448	< 0.001	−0.007	< 0.001	3.773×10^{-6}	< 0.001	II
	P. leniusculus	21.34	3.413	< 0.001	−0.007	< 0.001	2.958×10^{-6}	< 0.001	II
	E. sinensis	32.93	3.578	< 0.001	−0.002	0.218	-9.723×10^{-7}	0.408	II[a]
Gastropod	*A. pallipes*	2.30	−1.240	< 0.001	−0.009	0.050	9.588×10^{-6}	0.545	II[a]
	P. leniusculus	1.86	0.535	0.004	−0.015	< 0.001	2.875×10^{-5}	0.027	II
	E. sinensis	5.67	0.449	0.115	−0.027	< 0.001	6.254×10^{-5}	0.004	II

[a] Type II fit deemed to be most appropriate by comparing AIC values for Type I and Type II fits. AIC for Type II fits were lower.

Table A2.7 Estimates of functional response parameters for decapod predators on three macroinvertebrate prey species, with *prey consumed* as the response variable, extracted from Rogers' random predator equation fitted to data in the *frair* package (Pritchard 2016). *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); 1/*h*T – maximum feeding rate (prey.day^{-1}), where T = time in days; *SE* – standard error. *Diff* – within each prey item and for each parameter, different letters in this column indicate significantly different parameters (after Holm-Bonferroni correction for multiple comparisons).

Prey	**Decapod**	***a***	**SE**	**Diff**	***h***	**SE**	**1/*h*T**	**Diff**
Amphipod	*A. pallipes*	0.725	0.090	a	0.042	0.003	23.7	a
	P. leniusculus	1.749	0.190	b	0.040	0.002	25.1	a
	E. sinensis	2.200	0.147	b	0.013	< 0.001	75.9	b
Chironomid	*A. pallipes*	2.409	0.095	A	3.211×10^{-3}	6.898×10^{-5}	311.4	A
	P. leniusculus	4.538	0.147	B	2.725×10^{-3}	3.675×10^{-5}	367.0	B
	E. sinensis	5.418	< 0.001	C	1.547×10^{-3}	1.413×10^{-5}	646.6	C
Gastropod	*A. pallipes*	0.298	0.043	α	0.057	0.007	17.6	α
	P. leniusculus	0.499	0.060	β	0.048	0.004	20.9	α
	E. sinensis	1.372	0.174	γ	0.052	0.003	19.3	α

Table A2.8 Comparison of functional response parameter estimates for decapod consumption of macroinvertebrate prey, based on analysis using indicator variables in the *frair* package (Pritchard 2016). Raw *p* values are presented; significant differences (α = 0.05) after Holm-Bonferroni correction within each prey group are indicated in bold. *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); *D* – difference; *SE* – standard error.

Prey	**Base Group**	**Comparison**		**Estimate (*Da* or *Dh*)**	**SE**	***z***	***p***
Amphipod	*A. pallipes*	*P. leniusculus*	*a*	1.025	0.210	4.876	< **0.001**
			h	−0.002	0.003	−0.698	0.485
	P. leniusculus	*E. sinensis*	*a*	0.450	0.241	1.872	0.061
			h	−0.027	0.002	−13.863	< **0.001**
	A. pallipes	*E. sinensis*	*a*	1.475	0.173	8.459	< **0.001**
			h	−0.029	0.003	−10.141	< **0.001**
Chironomid	*A. pallipes*	*P. leniusculus*	*a*	2.128	0.040	53.011	< **0.001**
			h	< 0.001	< 0.001	−7.041	< **0.001**
	P. leniusculus	*E. sinensis*	*a*	0.441	0.038	11.665	< **0.001**
			h	−0.001	< 0.001	−36.727	< **0.001**
	A. pallipes	*E. sinensis*	*a*	3.009	0.038	78.566	< **0.001**
			h	−0.002	< 0.001	−25.186	< **0.001**
Gastropod	*A. pallipes*	*P. leniusculus*	*a*	0.201	0.074	2.712	**0.007**
			h	0.009	0.008	−1.041	0.298
	P. leniusculus	*E. sinensis*	*a*	0.872	0.184	4.763	< **0.001**
			h	0.004	0.006	0.684	0.494
	A. pallipes	*E. sinensis*	*a*	1.074	0.179	5.989	< **0.001**
			h	−0.005	0.008	−0.619	0.536

Appendix 2.3 Switching analyses for decapods matched by body size: prey killed as response variable

cf. analyses in main text using prey consumed as response variable

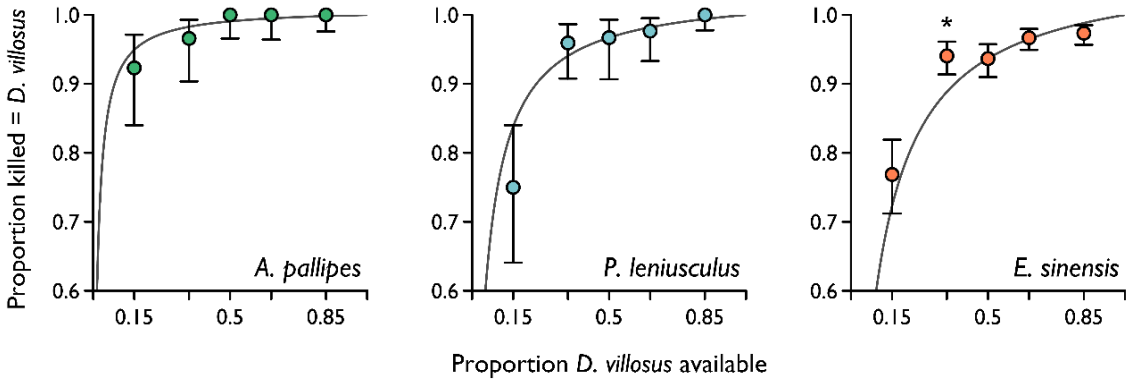

Figure A2.1 Proportion of *D. villosus* killed by decapod predators at varying relative densities of *D. villosus* to *B. tentaculata*. At all relative densities, total prey density was fixed at 280.tank^{-1}. Note that y axes begin at 0.6. *Points* are population proportions with 95% binomial confidence intervals. *Curves* are expected proportions in the absence of preference, based on killing when prey types are equally available. *Asterisk* indicates significant deviation from null hypothesis (binomial tests): $\chi^2 = 6.69$, df = 1, $p = 0.010$.

Appendix 2.4 Switching analyses for decapods matched by body mass: prey consumed as response variable

cf. analyses in main text where decapod predators are matched by body size (a combination of mass and maximum carapace dimension). Mean ± SE masses *A. pallipes* 10.6 ± 0.6 g, *P. leniusculus* 10.6 ± 0.6 g, *E. sinensis* 11.4 ± 0.4 g. ANOVA for difference in body mass of decapod species $F_{2,82} = 0.72$, $p = 0.492$. $n = 5$ for *A. pallipes* and $n = 6$ for *P. leniusculus* and *E. sinensis*.

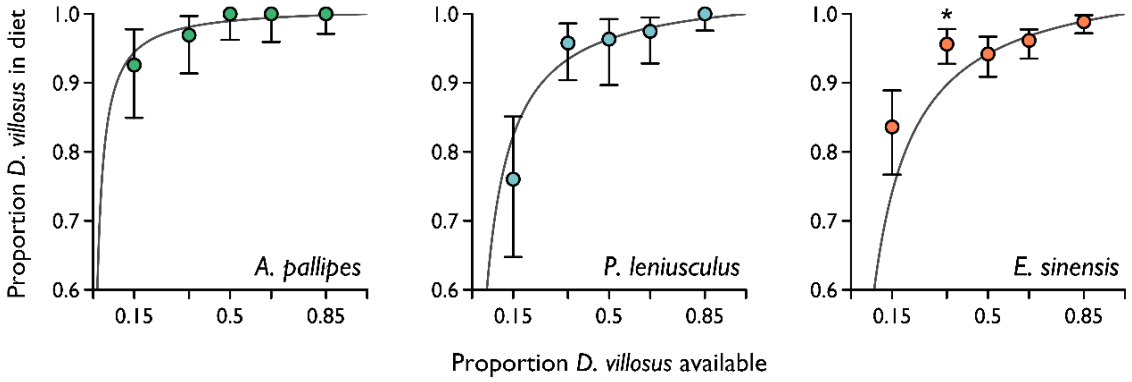

Figure A2.2 Proportion of *D. villosus* in the diet of decapod predators at varying relative densities of *D. villosus* to *B. tentaculata*. Symbols as for Fig. A2.1. Note that y axes begin at 0.6. *Asterisk* indicates significant deviation from null hypothesis (binomial tests): $\chi^2 = 6.92$, df = 1, $p = 0.009$.

Appendices to Chapter 3

Appendix 3.1 Amphipod sizes

Masses and lengths of amphipods used in each experiment (combination of experimental design, fish species and developmental stage in Table A3.1) were compared using ANOVAs. Length and mass were log-transformed where necessary to conform to model assumptions. Pairwise post-hoc comparisons were made using Tukey HSD tests.

ANOVAs for both length and mass were significant (ANOVA $p < 0.001$) for all experiments. Post-hoc tests confirmed that across all experiments, large *D. villosus* were significantly heavier and longer than both *G. pulex* and intermediate *D. villosus* (Tukey HSDs $p < 0.001$ for all tests). *G. pulex* and intermediate *D. villosus* did not differ in mass or length in any experiment (Tukey HSDs $p > 0.428$ in all tests except for comparison of length in carp larvae functional response experiment $p = 0.081$).

Table A3.1 Size (wet mass in mg and length in mm) of amphipods used in each experiment. Amphipods were blotted dry before measurement of mass; lengths are from rostrum tip to telson tip for amphipods in natural, curved resting state. In all experiments, large *D. villosus* is significantly larger than both large *G. pulex* and intermediate *D. villosus*, which in turn do not differ significantly in size.

Experimental Design	Prey sp.	Stage	Mean ± SE	Large *G. pulex*	Intermediate *D. villosus*	Large *D. villosus*
Functional Response	Carp	Eggs	Mass	52.3 ± 1.0	54.0 ± 1.2	105.0 ± 1.7
			Length	17.2 ± 0.1	17.5 ± 0.2	22.1 ± 0.1
		Larvae	Mass	51.7 ± 1.0	53.9 ± 1.3	106.7 ± 2.0
			Length	17.1 ± 0.1	17.6 ± 0.2	22.3 ± 0.2
	Trout	Eggs	Mass	41.4 ± 1.0	42.7 ± 1.2	109.5 ± 2.8
			Length	17.2 ± 0.1	17.5 ± 0.2	22.1 ± 0.1
		Larvae	Mass	41.4 ± 0.8	43.2 ± 1.2	106.1 ± 3.0
			Length	15.9 ± 0.1	15.9 ± 0.2	21.6 ± 0.3
Electivity	Carp	Eggs	Mass	49.0 ± 2.3	53.3 ± 3.4	102.1 ± 4.1
			Length	16.9 ± 0.3	17.3 ± 0.4	21.9 ± 0.3
		Larvae	Mass	49.4 ± 1.9	50.9 ± 3.5	110.8 ± 4.3
			Length	16.7 ± 0.3	16.2 ± 0.6	23.1 ± 0.4

I also compared a combined index of body size, derived from principal components analysis on log length and log mass, amongst amphipod groups in each experiment. The first principal component described between 96.7 and 98.6% of the variance in body size. Results based on this

first principal component confirmed the previous analysis on length and mass separately: large *D. villosus* were bigger than the other amphipod groups in all experiments (Tukey HSDs $p < 0.001$), whilst *G. pulex* and intermediate *D. villosus* did not differ in body size (Tukey HSDs $p > 0.518$ in all tests except for comparison of body size in carp egg experiment $p = 0.152$).

G. pulex and intermediate *D. villosus* were slightly larger in the carp experiments than the trout experiments, presumably due to seasonal differences in size structure of source amphipod populations (ANOVAs for amphipods used in egg experiments: *G. pulex* mass $F_{1,104} = 59.07$, $p < 0.001$ and length $F_{1,104} = 30.05$, $p < 0.001$; intermediate *D. villosus* mass $F_{1,106} = 42.22$, $p < 0.001$ and length $F_{1,106} = 19.20$, $p < 0.001$). Large *D. villosus* did not differ in size between seasons (ANOVAs for amphipods used in egg experiments: mass $F_{1,120} = 2.05$, $p = 0.155$ and length $F_{1,120} = 1.55$, $p = 0.216$) but this could reflect deliberate selection of similar-sized individuals rather than the actual maximum sizes within the population.

Appendix 3.2 Functional response analyses on larvae killed

In functional response experiments, some partial consumption of carp larvae was observed. When considering impacts of predators on prey populations, it is the number of prey killed (rather than consumed) which is important. If partial consumption is common relative to complete consumption, killing is less strongly related to satiation and the link between consumptive FRs and population impact is weakened (Dick et al. 2002). The carp larvae FR data were re-analysed using number of prey killed as response variables. The number of larvae killed was calculated as the number of larvae supplied minus the total number of live or dead but undamaged larvae remaining. These analyses yielded qualitatively identical and quantitatively similar results (Tables S3.1 to 3.3) to analyses based on prey consumption (see main paper). Thus, in this case consumptive FRs may provide a reasonable tool to infer impacts on prey populations.

Table A3.2 Parameter estimates and significance levels from second order logistic regression of the proportion of carp larvae killed against initial larval density, for three amphipod groups. Quasibinomial errors were used due to overdispersion. ϕ – dispersion parameter for GLM; N_0 – first order term; N^2_0 – second order term.

Amphipod Group	ϕ	Intercept	*p*	N_0	*p*	N^2_0	*p*	Type
G. pulex	2.273	1.661	0.002	−0.105	0.018	8.145×10^{-4}	0.243	II
Inter. *D. villosus*	2.001	2.265	<0.001	−0.169	< 0.001	1.852×10^{-3}	0.011	II
Large *D. villosus*	1.192	3.069	<0.001	−0.150	< 0.001	1.412×10^{-3}	0.007	II

Table A3.3 Functional response parameter estimates for three amphipod groups on carp larvae as prey, extracted from Rogers' random predator equation fitted to data in the *frair* package (Pritchard 2016). *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); 1/*h*T – maximum feeding rate (prey.day^{-1}), where T = time in days; *SE* – standard error.

Prey	Amphipod Group	*a*	SE	*h*	SE	1/*h*T
Carp larvae	*G. pulex*	3.424	0.854	0.100	0.011	10.0
	Inter. *D. villosus*	3.643	0.796	0.107	0.010	9.3
	Large *D. villosus*	3.757	0.539	0.058	0.004	17.4

Table A3.4 Comparison between functional response parameter estimates for three amphipod groups on carp larvae as prey, based on analysis using indicator variables in the *frair* package (Pritchard 2016). Significant differences (α = 0.05) are indicated in bold. *a* – attack coefficient; *h* – handling time (days.prey item^{-1}); *D* – difference; *SE* – standard error.

Prey	Base	Comparison		Estimate (*Da* or *Dh*)	SE	*z*	*p*
Carp larvae	Inter. *D. villosus*	*G. pulex*	*a*	−0.222	1.167	−0.190	0.850
			h	−0.007	0.014	−0.489	0.625
	Inter. *D. villosus*	Large *D. villosus*	*a*	0.114	0.962	0.118	0.906
			h	−0.050	0.011	−4.628	< **0.001**
	Large *D. villosus*	*G. pulex*	*a*	−0.333	1.010	−0.330	0.742
			h	0.042	0.012	3.654	< **0.001**

Appendix 3.3 Compositional analysis

Table A3.5 Ranking matrices generated by compositional analysis (Aebischer et al. 1993; Calenge 2015), ranking food types on their proportional contribution to amphipod diets. *Single symbol* (+ or -) indicates a food type is ranked above or below another food type respectively. *Triple symbols* (+++ or ---) indicate this ranking is significant. Matrices were generated by randomisation (with n = 1999 generating the stable matrices presented). Analyses (i) assuming equal availability of food types and (ii) using actual proportional masses available yielded identical matrices.

EGG experiments

CONSUMER: *G. pulex*

	leaf	egg	plant	invert
leaf	0	+	+++	+++
egg	-	0	+	+
plant	---	-	0	+
invert	---	-	-	0

CONSUMER: Inter. *D. villosus*

	egg	leaf	plant	invert
egg	0	+	+++	+++
leaf	-	0	+	+++
plant	---	-	0	+
invert	---	---	-	0

CONSUMER: Large *D. villosus*

	egg	plant	invert	leaf
egg	0	+++	+	+++
plant	---	0	+	+++
invert	-	-	0	+
leaf	---	---	-	0

LARVA expts

CONSUMER: *G. pulex*

	larva	leaf	plant	invert
larva	0	+++	+++	+++
leaf	---	0	+	+++
plant	---	-	0	+++
invert	---	---	---	0

CONSUMER: Inter. *D. villosus*

	larva	plant	leaf	invert
larva	0	+++	+++	+++
plant	---	0	+	+
leaf	---	-	0	+
invert	---	-	-	0

CONSUMER: Large *D. villosus*

	larva	leaf	plant	invert
larva	0	+++	+++	+++
leaf	---	0	---	-
plant	---	+++	0	+
invert	---	+	-	0

Table A3.6 MANOVA tests for non-random food consumption, assuming equal availability of food types. As for the analyses using actual availability of food types (presented in Chapter 3 and in Table A3.5), p values were generated by randomisation with n = 1999.

	G. pulex		**Inter. *D. villosus***		**Large *D. villosus***	
	Wilks' Λ	p	Wilks' Λ	p	Wilks' Λ	p
Experiments with eggs	0.518	0.049	0.261	0.004	0.070	0.006
Experiments with larvae	0.048	< 0.001	0.104	0.002	0.049	0.002

Appendix to Chapter 5

Appendix 5.1 Exhaustive chase protocol