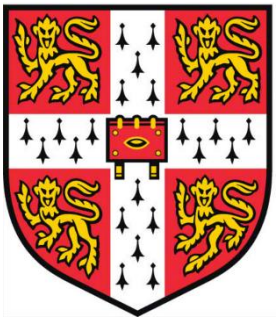
Parasites in freshwater mussels: community ecology and conservation



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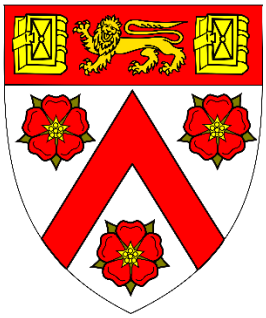

This thesis is submitted for the degree of

Doctor of Philosophy

Department of Zoology

September 2021

Preface

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the preface and specified in the text. This thesis is not substantially the same as any work that has already been submitted before for any degree or other qualification except as declared in the preface and specified in the text.

This thesis does not exceed the prescribed word limit of 60,000 words for the Degree Committee for Biology.

Parasites in freshwater mussels: community ecology and conservation

Summary

Parasites can be studied with respect to their spatial distribution, abundance and diversity (a parasite-centric view), or with respect to their effects on host individuals, populations, communities and the wider ecosystem (a host-centric view). The former contributes to understandings of what drives parasite community structure across scales, while the latter furthers knowledge on how the conservation of host species or the functioning of ecosystems may be influenced by those parasite communities. In this thesis, I study both perspectives using a previously unexplored system: the ecosystem-engineering freshwater mussels (Unionida) and their macroparasites. As such, this thesis has three broad aims: to characterise knowledge to date on unionid mussel parasite communities and develop tools to further knowledge in this area; to analyse the drivers of parasite community assembly in their hosts; and to evaluate the implications of parasitism on freshwater mussel individuals, populations and the ecosystems that the mussels modify.

In Chapter 2, I provide a review of all unionid-parasite records from Europe and North America to date, comprising 1476 records and at least 188 unique parasitic or endosymbiotic species. However, 53% of mussel species have no records, and few observations record the effects of the parasites, highlighting key research gaps that need to be filled. Chapters 3 and 4 provide novel methods to study the effect of digenean trematodes, a common and important class of parasite in freshwater mussels. Chapter 3 describes an efficient and reproducible method of accurately quantifying trematode infection in the gonad of freshwater mussels; this has been challenging due to the asexual growth of this parasite group and inability to count individuals. This method allows researchers to move past subjective judgements of infection intensity. Further, Chapter 4 describes a rapid way of non-destructively assessing trematode infection without killing the mussel, allowing even highly endangered bivalve species to be sampled for parasites.

In Chapter 5, I analyse the drivers of parasite community structure in a single mussel species (*Anodonta anatina*) at a single site across a full year, and show that parasite assembly was influenced by a combination of environmental, host-level and within-host factors. Specifically, the time of year sampled, in addition to host size and host gravid status,

influenced both the prevalence and intensity of the parasite communities inside individual hosts. Allowing for these factors also enables parasite-parasite interactions to be detected, showing that the distributions of individual freshwater mussel parasites are not independent and cannot be considered in isolation. Chapter 6 extends this approach by studying parasite communities across multiple host mussel species (*A. anatina* and *Unio pictorum*) and sites, and demonstrates that variation between sites and between host species is greater than expected, highlighting the operation of both abiotic and biotic filters on freshwater mussel parasites. Parasite-parasite interactions were once again detected, but only after accounting for site-level patterns of parasite prevalence and variation in the infection rates of individual hosts, highlighting the importance of considering interplay among ecological scales when characterising patterns in community ecology.

Chapter 7 quantifies the effect of parasitic trematodes, mites and invasive zebra mussels (*Dreissena polymorpha*) on the reproductive capacity of *A. anatina*. Rather than just focus on individuals, I estimate the reduction in population-level reproductive capacity caused by parasites across multiple sites, showing that parasites alone reduce potential reproductive output by up to 13%, even at low prevalences. Chapter 8 demonstrates the role that trematodes and bitterling fish (*Rhodeus amarus*) embryos play in altering the filtration capacity of *A. anatina* and *U. pictorum*, and how this scales to the ecosystem level. Using a combination of field surveys, field experiments, laboratory experiments and ecological modelling I show that these parasites alter the time taken for mussel communities to filter the Old West River (Cambridgeshire, UK) by up to 50%, a statistic that depends on parasite and host community composition as well as the suspended particle concentration. Finally, Chapter 9 explores how parasites may affect the success of captive breeding or translocation programs for endangered freshwater mussels (and for endangered species generally), and how a failure to consider parasites and disease in these programs may amplify the spread of harmful pathogens to already threatened populations or species.

While this thesis emphasises the possible implications of parasites for host individuals, populations and their ecosystems, it also introduces unionid mussels as a tractable system to further our knowledge of parasite community assembly across ecological scales. Both approaches are important to advance understandings in the ecological role of parasites in the context of global environmental change.

Acknowledgements

I am indebted to my supervisor, Professor David Aldridge. David has been an incredibly supportive and engaged supervisor in difficult circumstances, showing as much concern for the mental health of those in his lab as their academic health. He has consistently treated me as a colleague rather than a student, allowed me to pursue some of my more wild ideas (as well as contributing a fair share of his own), displayed an infectious interest for natural history and ecology in general, always encouraged me to strive for the absolute best, shared his nice wine, and remained calm when I locked the keys in the car during a thunderstorm. I left every meeting with him feeling uplifted and positive, both about the direction of this thesis and of my decision to pursue a PhD. If I ever reach a similar position, I would wish to be as he has been to me.

Coming to Cambridge would not have been possible without the generous contribution of the Woolf Fisher Trust. Their financial support, alongside their ongoing check-ins, were incredibly important and much appreciated. I also benefited from the Department of Zoology's Hitchcock Fund.

Many people contributed their time to help expediate the extensive field sampling and laboratory work required: Arlie McCarthy, Isobel Ollard, Sam Reynolds, Sebastian Dunne, Christine Ellis, Camilla Campanati, James Vereycken, Jasmine Yang, Miranda Johnstone, and Lorraine Archer from Plant Sciences, who grew algae for me at very short notice. I am also very grateful for the Aldridge lab group for their support, and interesting and useful discussions over the last three years. In particular, Sam and Isobel were incredibly important in the development of Chapters 8 and 9, and Arlie was an R role model and troubleshooter as well as an all-round consistent source of encouragement. I am also appreciative of my advisors, Dr Edgar Turner and Professor William Sutherland, for their positive and constructive feedback throughout the thesis, and in particular for their encouragement to think carefully about the community dynamics of parasites.

Outside of the academic sphere, many friends helped me to always see the bigger picture – Nathanael Walker-Hale, Stephanie McGimpsey, Tadhg O'Keefe, Hikaru Seki, Leib Celnick, José María Arroyo Nieto, Tez Clark, Kasia Warburton, George Mather, Giulia Bellato, and

Kyle Frohna from Trinity always kept me amused and distracted with board games, dinners, as well as interesting (and frequently inane) conversation.

Living on the other side of the world from your family is always difficult, especially in a pandemic. Talitha Kearey essentially became my and Miriam's in-person family for 18 months, and her cooking, friendship, constant supportive presence, and dubious Great Western Trail tactics helped me more than I can say. I am also incredibly grateful to Martha Leyton and Martin Shovel for providing (and continuing to provide) a home away from home throughout my PhD.

When I began this journey I did not expect to be ending it with a fiancée as well as a degree, but I know which one I value more. Miriam Shovel has been everything to me in this journey, lifting me up at every step while reminding me how good life can be. I hope she knows that she has been and is more than I could ever express in the front material of a thesis.

Finally, this thesis is the product of 27 years of love and support from Hannah, Alan and Belinda Brian. I hope it makes you as proud as I am to call you my family.

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Publications

Each of the eight substantive chapters (2-9) are presented as a research paper, six of which have been accepted for publication. All chapters were undertaken in collaboration with others; in each case, I was the lead author, and the contribution from each co-author is detailed below.

Chapter 2:

Brian, J. I., & Aldridge, D. C. (2019). Endosymbionts: An overlooked threat in the conservation of freshwater mussels?. *Biological Conservation*, **237**, 155-165.

J.I.B and D.C.A. conceived the idea. J.I.B. carried out the literature review, analysed data and drafted the manuscript. D.C.A. edited the manuscript.

Chapter 3:

Brian, J. I., & Aldridge, D. C. (2020). An efficient photograph-based quantitative method for assessing castrating trematode parasites in bivalve molluscs. *Parasitology*, **147**(12), 1375-1380.

J.I.B and D.C.A. conceived the idea and carried out sampling. J.I.B. carried out laboratory work, analysed the data and drafted the manuscript. D.C.A. edited the manuscript.

Chapter 4:

Brian, J. I., & Aldridge, D. C. (2021a). A rapid, non-destructive method for sampling castrating parasites in endangered bivalve molluscs. *Aquatic Conservation: Marine and Freshwater Ecosystems*, **31**(3), 729-735.

J.I.B and D.C.A. conceived the idea and carried out sampling. J.I.B. carried out laboratory work, analysed the data and drafted the manuscript. D.C.A. edited the manuscript.

Chapter 5:

Brian, J. I., & Aldridge, D. C. (2021b). Abundance data applied to a novel model invertebrate host shed new light on parasite community assembly in nature. *Journal of Animal Ecology*, **90**(5), 1096-1108.

J.I.B. and D.C.A. designed the study and carried out sampling; J.I.B. conducted all laboratory work, analysed the data and wrote the first draft of the manuscript; J.I.B. and D.C.A. contributed substantially to revisions.

Chapter 6:

Brian, J. I., & Aldridge, D. C., *in prep*. Factors at multiple scales drive parasite community structure.

J.I.B and D.C.A. conceived the idea and carried out sampling. J.I.B. carried out laboratory work, analysed the data and drafted the manuscript. D.C.A. edited the manuscript.

Chapter 7:

Brian, J. I., Dunne, S. E., Ellis, C. L. & Aldridge, D. C. (2021b). Population-level effects of parasitism on a freshwater ecosystem engineer, the unionid mussel *Anodonta anatina*. *Freshwater Biology*, **66**(12), 2240-2250.

J.I.B. and D.C.A. conceived the idea. All authors carried out sampling. S.E.D. and C.L.E. carried out laboratory work. J.I.B. supervised lab work, analysed the data and drafted the manuscript. D.C.A. edited the manuscript.

Chapter 8:

Brian, J. I., Reynolds, S. A., & Aldridge, D. C., *in prep.* Parasitism dramatically alters the ecosystem services provided by freshwater mussels.

J.I.B. and D.C.A. conceived of and designed the study, and carried out the field components. J.I.B. and S.A.R. carried out the laboratory experiments. J.I.B. designed the ecosystem models and analysed and interpreted the data, with input from S.A.R. J.I.B. drafted the manuscript. D.C.A. edited the manuscript.

Chapter 9:

Brian, J. I., Ollard, I. S., & Aldridge, D. C. (2021a). Don't move a mussel? Parasite and disease risk in conservation action. *Conservation Letters*, **14**(4), e12799.

J.I.B. and D.C.A. conceived the idea. I.S.O. carried out the literature review and wrote section 9.2. J.I.B. produced figures and tables and wrote the rest of the manuscript. D.C.A. edited the manuscript.

Chapter 1: General Introduction

Parasitism is a common and ecologically important evolutionary strategy, with parasites often playing a central role in the functioning of ecosystems (Hudson et al. 2006). For example, parasites contribute to and stabilise food webs globally (Lafferty et al. 2008; Poulin et al. 2013): a recent study showed that, of 21,956 established links in a kelp forest web, over 55% involved parasites (Morton et al. 2021). This is facilitated by their significant biomass: in Oregon streams, parasite biomass is greater than that of all aquatic insects combined (Preston et al. 2021), and in Californian estuaries it exceeds that of bird biomass (Kuris et al. 2008). This biomass exerts influence both by generating an 'extended phenotype' through parasitised organisms (*sensu* Dawkins 1982), but also as free-living biomass in transmission stages that provide an important but often overlooked food source (Morley 2012; McKee et al. 2020). Further, parasites are able to regulate host populations (Tompkins & Begon 1999), facilitating the coexistence of multiple species (Strona 2015). However, parasites can also have significant negative effects at the individual, population and community level, and in extreme cases can lead to local population extinction (Katsanevakis et al. 2019) or the global extinction of certain species (Daszak et al. 2000).

In this era of global change, communities are being assembled and dissembled at increasing rates (Pandolfi et al. 2020). There is strong evidence that parasite communities are no exception: some parasite species are spreading unpredictably (Gillis-Germitsch et al. 2020), while other communities have collapsed (Sitko & Heneberg 2020). Indeed, the extinction risk for parasites is becoming increasingly appreciated (Strona 2015; Carlson et al. 2020a). Given the diversity of influences that parasites may have, it is important to understand the composition of parasite communities, and what factors influence their assembly. This will allow for more reliable predictions on how they may change in future, as well as facilitating a more nuanced understanding of their effects on host individuals, populations and communities (Wood & Johnson 2015). These goals require host-parasite interactions to be considered from both community ecology and conservation perspectives.

In this brief general introduction, I first provide an overview of parasite community assembly, the subsequent impacts that parasites can have on individuals, populations and communities, and how these two sets of processes vary with ecological scale. I then introduce freshwater mussels and their parasites as a tractable and ecologically important, but

previously unexplored, study system to investigate both the community ecology of parasites and the effects on their hosts. I finally discuss the overarching aims of this thesis and explain how each chapter contributes to those aims.

1.1. Factors at multiple scales can drive parasite community composition

Understanding how factors at different scales influence parasite distributions is key to predicting parasite community structure, how these parasite communities may change when environments or host distributions are altered, and what the effects of parasites may be on their hosts (Johnson et al. 2015; Moir & Brennan 2020). Like free-living communities (and indeed, even more so), parasite communities are hierarchical: individual parasites live inside a single host organism, which itself is part of a wider population, community and metacommunity. General theories of community assembly are therefore applicable to parasites, and *vice-versa* (Dallas & Presley 2014). Using the 'PAB' framework of Catford et al. (2009), successful infection of a host relies sequentially on dispersal to a certain site and host within that site, appropriate abiotic conditions (which includes characteristics of both the site and the host species), and a suitable biotic environment (encompassing both the host-parasite interface, and interactions with coinfecting parasites) (Fig. 1.1a – d) .

The first filter determining whether a host individual is infected is whether the parasite is present at the location where the potential host is found (Fig. 1.1a). This initially depends on dispersal to the site, which frequently relies on host dispersal: in the case of multi-host parasites, parasite distributions are often determined by the range of their most mobile hosts (Paterson et al. 2019). However, free-living stages of parasitic organisms can also be an important dispersal mechanism, especially in aquatic environments (Zimmer et al. 2009). Temporal context is frequently vital, with some parasites only present at certain times of year due to the cyclical nature of life histories (Olori et al. 2018). Following successful dispersal, persistence at a given site may depend on site-specific factors such as temperature or pH, as well as the spatial distribution of appropriate hosts (Coen & Bishop 2015; McDevitt-Galles et al. 2018; Aalto et al. 2020). Hosts are 'appropriate' or 'inappropriate' at multiple scales: at the host community level, some species are parasitised and others are not (Fig. 1.1b), which may be a product of co-evolutionary history (Blasco-Costa et al. 2021), or characteristics of those species such as longevity or diet breadth (Dallas & Presley 2014). At the population level (within a single species), filters include characteristics like host weight (Morris et al.

2019), size (Bolnick et al. 2020a), sex (Christe et al. 2007), age (Nielsen et al. 2020) or genotype (Sallinen et al. 2020), which influence which individuals are infected (Fig. 1.1c). Horizontally transmitted parasites can also spread between individuals (Chantrey et al. 2014), either from the same species or different species. Therefore, individual infection may also depend on close neighbours, with some hosts acting as reservoirs (Streicker et al. 2013).

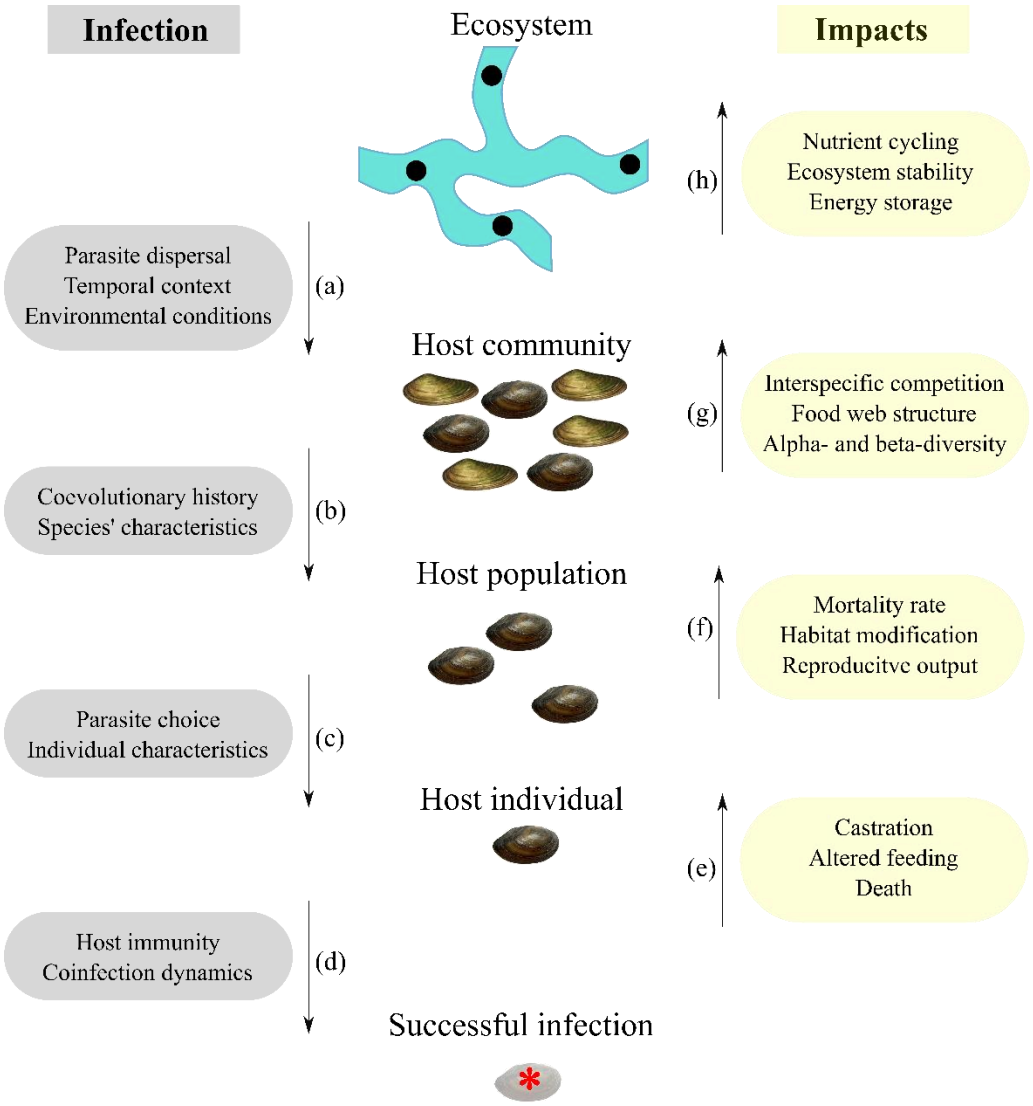


Figure 1.1: Both parasite infection (a – d) and parasite impacts (e – h) are dependent on processes occurring at multiple scales. Conceptually we cycle from part (a) to (d) in order for infection to be successful, and then from part (e) to (h) to see the consequences of parasitism across scales. See text for further details. The example visualized here is for mussels in freshwater systems (see section 1.3), but the concept applies across all host-parasite scenarios.

Once an individual has been infected (and not cleared *via* host immunity), competition or facilitation between parasites can alter the likelihood of that infection being successful (Fig. 1.1d). This interaction can be mediated by the host's immune system: one parasite may alter

the immune profile of the host that can either encourage (Magalhães et al. 2015; Eidelman et al. 2019) or inhibit (Izhar & Ben-Ami 2015; Halliday et al. 2018) other parasites. Parasites may also interact directly through resource competition, either host-wide or within certain tissues (Ferrari et al. 2009; Wilcox et al. 2018), and some parasites can consume others within the host (Esch & Fernandez 1994; Hopkins et al. 2016). There can also be apparent interactions mediated outside the host, with some parasites able to detect previously infected hosts and avoid coinfection (Allan et al. 2009). Such parasite interactions may also be highly context-dependent: 'priority effects' can influence outcomes of competition depending on which parasite arrives first (Clay et al. 2020; Halliday et al. 2020a), while in other cases the order of arrival has no effect (Rynkiewicz et al. 2019). While parasite interactions are typically studied at a scale of one parasite interacting with one other parasite, there is increasing evidence that host-wide parasite networks need to be characterised to fully understand coinfection patterns (Griffiths et al. 2014; Beechler et al. 2019). A holistic approach is required when considering parasite communities (Serrano & Millán 2014; Hoarau et al. 2020): for example, the presence of one parasite can alter wider parasite community dynamics (Beechler et al. 2019), and so the drivers of that parasite's distribution may indirectly influence others.

Despite the broad framework of assembly across scales being well-understood (Fig. 1.1a – d), there are few generalities that can be drawn. Some studies find that site-specific factors are the most important overall factor determining parasite communities in individuals (McDevitt-Galles et al. 2018), while others showed that host characteristics are the most important (Dallas & Presley 2014). In some systems, coinfection and interactions between parasites are the clearest predictors of infection (Telfer et al. 2010), while in others there are no interactions between parasites at all (Olori et al. 2018; Sallinen et al. 2020). However, studies do not typically take into account all the scales discussed here: for example, there may be detectable parasite-parasite interactions once site-level parasite prevalences are accounted for, but ignoring the effect of site masks this interactive signal (see Chapter 6). Therefore, while it is possibly true that individual parasite communities are highly idiosyncratic and unpredictable (Poulin 2019), there may also be generalities that can be determined with an appropriate study system or framework that incorporates scale-dependency (Bolnick et al. 2020b).

Generalities are also difficult to draw due to different data types employed in parasitological studies: parasite records are frequently limited to presence-absence data, despite abundances likely being a more responsive measure to many of the factors discussed here. While in some cases parasite prevalences are suitable and may even be preferable (Krasnov et al. 2021), general theory suggests that presence-absence data is inferior to abundance data especially for predicting interactions between species (Blanchet et al. 2020), and parasite-specific modelling shows that varying abundances can alter the strength of or even reverse the direction of parasite-parasite interactions (Fenton 2013). A careful consideration of parasite abundances is therefore also required.

1.2. The impacts of parasites across scales

Scale-dependency and parasite abundance is also important when considering the consequences of parasite infection. While parasites only directly affect individual hosts, the severity of which may depend on the intensity of infection, the subsequent effects can scale to higher levels of ecological organisation (Fig. 1.1e – h).

At the most extreme individual level, parasites can lead to host death (Fig. 1.1e): for example, 'squirrelpox' led to a severe decline of red squirrels in the UK (Tompkins et al. 2003; Chantrey et al. 2014). Parasites can also inhibit host reproduction to varying degrees, ranging from reduced fecundity (Albery et al. 2021) to complete castration (Cichy et al. 2017). Host processes such as metabolic (Nadler et al. 2021) or feeding (Haddaway et al. 2012) rates are also affected by parasites at the level of individual hosts. Further, such effects may only be observed under particular environmental conditions. For example, the trematode *Rhipidocotyle campanula* typically castrates its bivalve host, but in more anoxic conditions, infected individuals also suffered much higher death rates (Jokela et al. 2005). Nutrient limitation can induce a similar effect, with parasite-infected *Daphnia magna* suffering reduced biomass after being fed a phosphorus-limited diet compared to uninfected hosts (Pulkkinen et al. 2014). These studies further emphasise that understanding environmental drivers of parasite (and host) communities, as well as how parasites affect their hosts, is crucial in predicting the causes and consequences of parasitism in a changing world.

The observed effects at an individual level can scale to the host population (Fig. 1.1f), community (Fig. 1.1g) and ecosystem level (Fig. 1.1h). Some parasites regulate host

populations through their individual-level reductions in host fecundity; this may be particularly important for invasive non-native species or those expanding their geographic range (Bojko et al. 2020). Through altering population sizes or host behaviour, parasites have the potential to alter the environment those hosts exist in, especially if the hosts have ecosystem engineering capabilities. For example, following mass mortality of an abundant tube-building amphipod due to parasites, both the topography and sediment characteristics of an intertidal mudflat changed (Mouritsen et al. 1998). Non-fatal effects can have similar outcomes: a parasite causing snails to consume less macroalgae on rocky shores stimulated a change in both macroalgal cover and diversity, with downstream consequences for other species on the shore such as increased space competition for barnacle and blue mussel recruits (Wood et al. 2007). Parasites can also lower the biodiversity of host communities, through direct death (Bojko et al. 2020) or through harming the competitive ability of one species relative to another, known as 'cryptic virulence' (Prenter et al. 2004; Dunn et al. 2012). Recent experimental evidence supports the conclusion that parasites can rapidly alter the relative abundances of host species (Friesen et al. 2020). At a host metacommunity/ecosystem level, parasites may significantly alter nutrient storage and flow. Aside from the direct links in food webs (Morton et al. 2021), parasite-induced death affects ecosystem-level nutrient pools (Borer et al. 2021). Parasites can also alter the rate at which hosts excrete carbon to the environment (Mischler et al. 2016), thus altering nutrient cycles at the ecosystem level (Fischhoff et al. 2020). In summary, parasitism may have powerful and far-reaching consequences for hosts, communities and the wider ecosystems in which they exist.

Theory in the area of parasite impacts is well-developed, particularly in how parasites may mediate biological invasions (e.g. Prenter et al. 2004; Dunn et al. 2012). However, tangible examples of the effects of parasites at higher scales, especially at the ecosystem level, remain rare (Fischhoff et al. 2020). Therefore, despite broad hypotheses and general understandings about the bi-directional links between parasite presence and ecological scale (Fig. 1.1), knowledge about parasite communities lags behind that of free-living communities (Budischak et al. 2016; Poulin 2017). I argue that knowledge on the parasites of invertebrate communities are particularly underdeveloped but could provide further insights into parasite assembly and impacts, and that freshwater mussels provide one such community.

Patterns of parasite community assembly are 'messy', and as such it is difficult to produce general laws (Poulin 2007). The goal of producing generalisations about parasite community composition may be inhibited by a focus on vertebrates, which some have argued are "strange" study organisms thanks to the diverse and idiosyncratic range of behaviour they display (Webster & Rutz 2020). Even putting aside this point, it is unlikely that the complete nature of host-parasite interactions can be captured by focusing on less than 5% of Earth's biodiversity. Despite this, invertebrates are severely neglected in parasite studies (Wilson et al. 2015), with one exception being snail-trematode interactions (e.g. Esch & Fernandez 1994; Schwelm et al. 2020). For example, a recent study looking at the global diversity of helminth parasites explicitly excludes invertebrates as hosts (Carlson et al. 2020b), despite invertebrates being important definitive hosts for some trematodes such as aspidogastreans (Alves et al. 2015). Further, nearly all trematodes require molluscs as their first intermediate hosts (Schwelm et al. 2020), so understanding drivers of parasite distributions in molluscan hosts should be a high priority. In particular, freshwater environments are understudied (Adlard et al. 2015); in most cases, we lack a baseline for what a normal parasite fauna looks like in these environments, and thus cannot begin to predict the impacts of environmental change or the potential for parasite or disease outbreaks (Coen & Bishop 2015).

1.3. Unionid mussels as a study system for host-parasite interactions

Freshwater mussels in the order Unionida (henceforth generally referred to as 'unionid mussels') provide a candidate system to expand our knowledge on parasite community ecology. Unionids are ecosystem engineers, providing a range of services. Their shells provide attachment for epibionts as well as refuge from predation for a range of organisms (Vaughn & Hakenkamp 2001; Gutiérrez et al. 2003). Unionid mussels can filter up to 55 L day^{-1} (Tankersley & Dimock 1993), contributing to water clarity and influencing sedimentation rates (Chowdhury et al. 2016). Through this extensive filtering they affect nutrient regimes in freshwater ecosystems (Vaughn & Hakenkamp 2001; Hoellein et al. 2017), and in general are associated with more biodiverse environments (Aldridge et al. 2007; Chowdhury et al. 2016). Given their ecosystem-level effects, studying the effects of their parasites can shed light on the influence of parasites on the wider host environment. Mussels are static, abundant, widespread and easily sampled, making them ideal model systems for addressing fundamental questions about host-parasite interactions and the

consequences of this at the level of individuals, populations, communities and ecosystems (Fig. 1.1e – h).

Unionid mussels are also highly imperiled: globally 33% are threatened (Böhm et al. 2020), a figure rising to 70% in North America (Lopes-Lima et al. 2018). While some of their threats are known, such as pollution, eutrophication and habitat loss (Lopes-Lima et al. 2017), there are also increasing reports of enigmatic declines (Lydeard et al. 2004; Haag 2019) which have even captured media attention (e.g. Holden 2019; Renault 2020). It is possible that parasites or diseases are implicated in these declines, especially given that little is known about unionid parasites (Ferreira-Rodríguez et al. 2019), and the pathogenicity of those parasites that are known is not well established (Grizzle & Brunner 2009). The fact that other taxa, such as fish populations, in the same location as mussels experiencing these declines are unaffected (Sanchez Gonzalez et al. 2021), also suggests a mussel-specific biotic driver as opposed to broader environmental factors. Parasites may become more prevalent, or have more deleterious effects, through: (a) changing environmental conditions; (b) the breeding or translocation of freshwater mussels (e.g. Thomas et al. 2010), which could spread disease (Strayer et al. 2019), or (c) spillover or spillback (*sensu* Kelly et al. 2009) from invasive freshwater mussels that can host the same or different parasites (Mastitsky & Veres 2010; Cichy et al. 2016). In each of these cases, we need to understand the structure of parasite communities in order to accurately predict the outcomes for vulnerable hosts. This invites a close study on the drivers of unionid mussel parasite communities across scales (Fig. 1.1a – d). However, to date, parasite communities in unionids have not been considered, with the few researchers who have targeted freshwater mussel parasites focusing on specific parasite groups, such as trematodes (e.g. Taskinen et al. 1991, 1994, 1997) or mites (e.g. Edwards and Dimock 1995a, 1995b; Edwards & Vidrine 2006). The broader ecological context of unionid parasitism (i.e. Fig. 1) is unclear, though recent interest in more community-minded approaches to freshwater mussel parasites (Richard et al. 2020; Taskinen et al. 2021) highlights the timeliness of this approach.

In summary, researching parasites in freshwater mussels is significant for two reasons. It provides the opportunity to study parasite community ecology (including possible ecosystem consequences) in an understudied system, and it also informs the conservation of an imperiled host group. It is along these two complementary lines that the direction of this thesis lies.

1.4. Major mussel parasite groups

Parasites of unionid mussels are incompletely characterised (Grizzle & Brunner 2009; see Chapter 2), though the broad parasite groups are known. Here, I provide a brief introduction to the major known parasites of freshwater mussels as a reference point for the following chapters.

1.4.1. Trematodes

Multiple types of trematode use mussels as first intermediate, second intermediate, or definitive hosts. The most common of these (and those that are considered the most in this thesis) are bucephalid trematodes (e.g. *Rhipidocotyle* spp.) (Jokela et al. 1993; Gustafson et al. 2005). These have a three-host life cycle and use mussels as a first intermediate host (Fig. 1.2a). Upon a miracidia (hatched from an egg produced by an adult trematode) infecting a mussel, it typically migrates to the gonad of the host mussel and rapidly grows asexually, producing long branching sporocysts that themselves produce cercariae (mobile infective stages). In spring and summer, these cercariae are released into the environment where they will infect a fish and encyst into metacercariae. If the host fish is eaten by a larger predatory fish, the metacercariae excyst and develop into adult trematodes, which sexually produce eggs to release into the environment with fish faeces. Host fish species vary from trematode to trematode; for *Rhipidocotyle campanula* (the most common trematode in this thesis), the second intermediate host is the common roach *Rutilus rutilus*, and the definitive host is the perch *Perca fluviatilis* (Taskinen et al. 1991).

Unionid mussels are also targeted by gorgoderid trematodes (e.g. *Phyllodistomum* spp.) (Kudlai & Yanovich 2013). These trematodes only have a two-stage life cycle, with cercariae developing directly into metacercariae inside the sporocysts. These sporocysts, which are found in the gills of host mussels rather than the gonad, are released from the host and float to the surface of the water, where they are consumed by fish (Molloy et al. 1997).

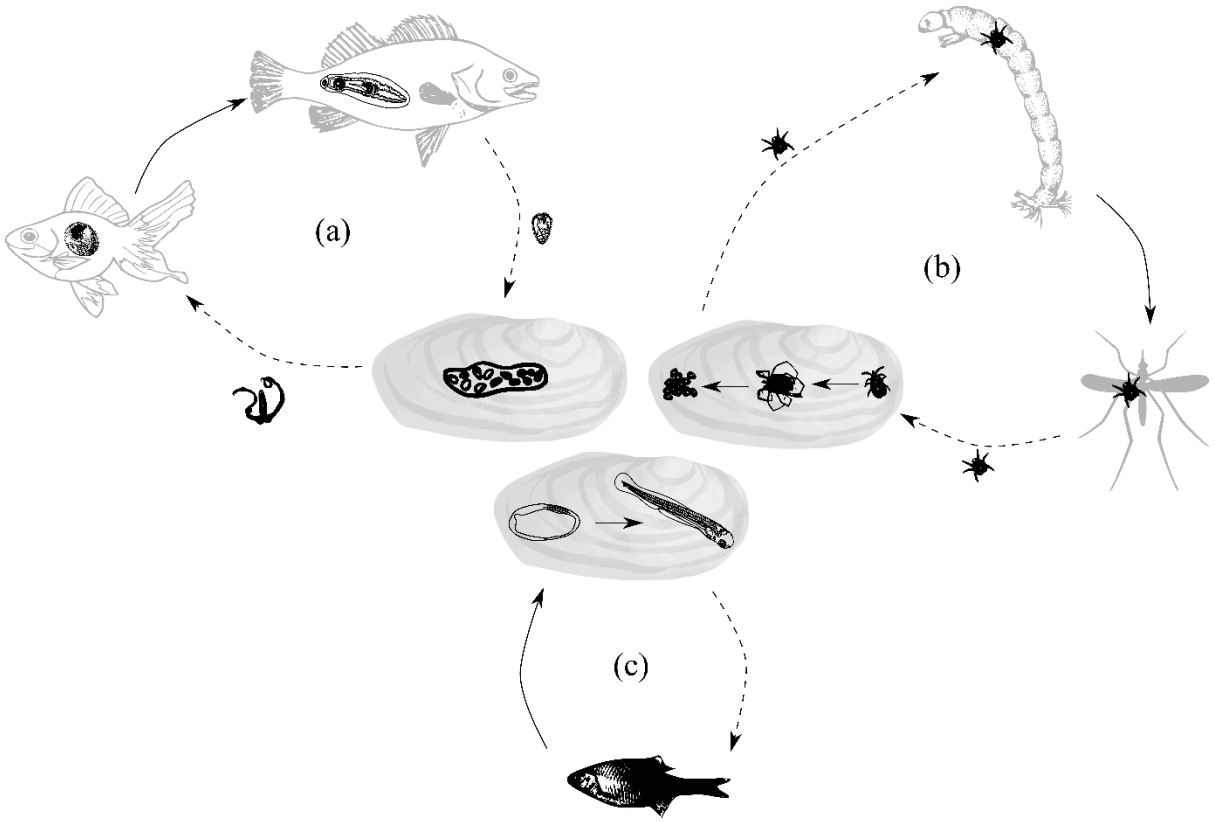


Figure 1.2: Parasite life cycles of (a) Bucephalid trematodes; (b) Unionicolid mites; (c) Bitterling fish. In all cases, parasite life history stages are shown in black, and hosts shown in grey. Dashed lines indicate transmission through the environment, while solid lines indicate direct transmission (e.g. direct consumption, life-history transformation or egg-laying). See text for further details.

In contrast to bucephalid and gorgoderid trematodes which use unionid mussels as first intermediate hosts, echinostomatid trematodes (e.g. *Echinoparyphium* spp.) use mussels as a second intermediate host (Marszewska & Cichy 2015). They follow a similar life cycle as bucephalid trematodes (Figure 1.2a), but use snails as their first intermediate host, bivalves as their second intermediate host and waterfowl as their definitive hosts (Molloy et al. 1997).

Finally, unionid mussels also act as definitive hosts to aspidogastrean trematodes (e.g. *Aspidogaster conchicola*) (Gangloff et al. 2008). These trematodes only require single hosts for development, and can develop and breed successfully using freshwater bivalves alone (Alves et al. 2015).

1.4.2. Mites

Unionicolid mites are a highly diverse parasite group, with high levels of specificity – in many cases, a particular mite is found only associated with one or two mussel species

(Edwards & Vidrine 2020). Mites also display a complex multi-host life cycle (Fig. 1.2b). Adult mites lay eggs in the mantle or gills of mussels, which hatch to larvae. These larvae leave the mussel and are parasitic on chironomid larvae, which they locate in the water column (Jones 1965; Edwards & Dimock 1995). When the chironomid larvae transform into adults, the mites remain attached and are therefore aerially dispersed while feeding on host haemolymph (Edwards & Dimock 1995). The larval mites drop off adult chironomids, and the mite larvae then reinfect freshwater mussels in either the mantle or the gills, going through several nymphal stages before becoming adults (Edwards 2014).

This general life cycle holds for all mussel mites, but the obligate nature varies: some mites spend their entire nymphal and adult lives in unionid mussels, while others are predominantly free-living and only return to mussels to lay eggs and go through initial larval development (e.g. *Unionicola intermedia*) (Baker 1988).

1.4.3. Bitterling fishes

Bitterling fishes (*Rhodeus* spp.) obligately parasitise freshwater mussels to fertilise and develop their eggs (Fig. 1.2c). In late spring to early summer (typically May – July), females deposit unfertilised eggs in the gills of mussels through an extended ovipositor, which a male will fertilise by ejecting sperm over the inhalant siphon of the mussel (Smith et al. 2004). Once fertilised, bitterling embryos move through several developmental stages in the gills, lasting approximately one month (Aldridge 1999b), before emerging as developed fish.

1.4.4. Other parasites

Many other phyla are found in freshwater mussels. Ciliates are commonly observed, with mussels frequently having multiple species (e.g. *Conchopthirus* sp., *Trichodina* sp.) in the mantle and gills (Chittick et al. 2001); thousands of ciliates have previously been observed in a single mussel (Antipa & Small 1971). Unionid mussels also host leeches in facultative or obligate associations (Bolotov et al. 2019), as well as more infrequent observations of taxa like dragonfly larvae (Levine et al. 2009), copepods (Saarinen & Taskinen 2003), viruses (Richard et al. 2020) and bacteria (Mioduchowska et al. 2020).

As highlighted previously, the pathogenicity of many of these 'parasites' (and indeed, of trematodes, mites and bitterling), is not well understood (Grizzle & Brunner 2009). It may be more appropriate to call them endosymbionts rather than parasites, especially for taxa that that have been observed only infrequently. Understanding the nature of all possible host-parasite interactions in freshwater mussels is therefore a vital step in informing the ecology of freshwater mussels and their ecosystems more generally, something this thesis takes steps towards. While acknowledging this current deficiency, the large number of potential macroparasites present, which often exceed those of vertebrates and is another advantage of using invertebrates (Wilson et al. 2015), provides ample scope to explore the community ecology and conservation implications of parasitism in unionid mussels.

1.5. Aims of the thesis

This thesis has three broad aims, which are addressed by specific chapters. However, all chapters are complementary and feed strongly into each other and into each aim.

Aim 1 is to characterise knowledge to date on the parasites of freshwater mussels and develop tools to further this knowledge, and is addressed by Chapters 2, 3 and 4. Chapter 2 provides a review of all unionid mussel-endosymbiont interactions recorded in Europe and North America as at January 2019. This chapter highlights knowledge gaps and areas of conservation concern and provides recommendations for further research, which drives later chapters. Chapters 3 and 4 describe novel methods that can be used to better characterise both the presence and intensity of infection of trematodes in freshwater mussels. These methods are used extensively in the rest of the thesis.

Aim 2 is to analyse the drivers of parasite community assembly in freshwater mussels, and is addressed by Chapters 5 and 6. Chapter 5 studies the drivers of parasite communities at a single site and in a single mussel species through the course of one year. Chapter 6 broadens this scope by analysing parasite communities across multiple host species and sites. These two chapters combine to address the influence of scale in the assembly of parasite communities (Fig 1.1a – d). In addition, both chapters look outwards and consider what parasite community ecology, and community ecology in general, can learn from this study system.

Aim 3 is to assess the implications of parasitism on the conservation of unionid mussels. This aim is introduced in Chapter 2, and then more explicitly addressed in Chapters 7, 8 and 9. Chapter 7 explores the impact of multiple parasites on the reproductive output of both individuals and populations, and how this may vary among host populations (Fig. 1.1e – f). Chapter 8 shows how two different parasites differentially influence the filtration capacity of freshwater mussels, and incorporates parasite choice and parasite-parasite interactions to demonstrate how this effect scales to the ecosystem level (Fig. 1.1h). Finally, Chapter 9 considers the risks posed by parasites and diseases when taking conservation action, and how freshwater mussel translocations and captive breeding programs could promote rather than ameliorate mussel declines by spreading pathogens between populations and species (Fig. 1.1f – g).

All substantive chapters (2-9) are structured as independent publications. As such, there is minor overlap between the content of the introductions, though in each case it is presented in a way that emphasises its relevance to the chapter. As each publication was written in collaboration with others, the pronoun ‘we’ is used throughout.

Chapter 2: Endosymbionts: an overlooked threat in the conservation of freshwater mussels?

Abstract

Endosymbionts can often have profound impacts on the growth, reproduction and survivorship of their hosts. Freshwater unionid mussels (Unionida) are important ecosystem engineers, and one of the most globally imperilled taxa, yet evidence concerning their endosymbiotic fauna remains patchy. Further, endosymbionts are not considered in an IUCN assessment for any unionid mussel. Here, we conduct the first literature review of all endosymbionts of the 16 extant European and 279 extant North American unionids, in addition to the four most significant invasive bivalves in Europe. There were 1476 host-endosymbiont records from 239 different studies over a 168-year period, documenting at least 188 unique endosymbiont taxa. However, study effort was uneven in its distribution, with 53% of unionid species (n=157) having no endosymbiont records. Eighty-eight percent of all hosts are considered under-sampled, including 99% of Endangered or Critically Endangered mussels. This is of significant concern given that when the effects of endosymbionts were examined, 72% showed potentially negative effects on their host, including complete castration in the case of digenean trematodes. However, only a small number of endosymbionts have had their effects quantified. Bipartite network analyses revealed invasive mussels may be competent for native parasites. This leads to the potential for parasite spillback, with conservation implications for vulnerable native species. Recommendations for future work include greater sampling of sympatric native and invasive populations (including non-destructive sampling of endangered species) and experimental manipulation of host-endosymbiont communities. This will facilitate better conservation outcomes for this crucial group of ecosystem engineers.

Key words: *Dreissena*, endosymbiont, enemy release, invasion, parasite, unionid

2.1. Introduction

Parasites are a ubiquitous feature of ecosystems, and their important ecological role is now acknowledged. They are crucial for the maintenance of ecosystem health (reviewed in Hudson et al. 2006), and their ecological role in mediating apparent competition and

sustaining intraguild predation can facilitate co-existence among competing hosts (Hatcher et al. 2006, 2014). On a broader scale, parasites can also influence the evolutionary trajectory of their hosts (Dargent et al. 2016), and can even lead to the maintenance of sex in populations that would otherwise be predicted to reproduce exclusively asexually (Lively 1987). In addition, multiple interacting parasites may show emergent effects that could not be predicted in isolation (Ferrari et al. 2009). This has been shown to significantly affect conservation efforts on species like voles (Telfer et al. 2010) and buffalo (Ezenwa & Jolles 2015). Novel or previously unreported parasites can also cause significant declines of economically or ecologically vital species (e.g. Meeus et al. 2011; Rowley et al. 2013). Despite the important implications for conservation and management, parasite communities of certain host taxa remain unexplored.

Parasitism is extremely common among bivalves. It is frequently reported in both freshwater and marine ecosystems, even from extreme environments like hydrothermal vents (Ward et al. 2004). In many cases, parasites have been shown to severely impact bivalve populations, such as in the spread of the haplosporidian MSX in Chesapeake Bay oysters which produced annual mortality rates of up to 60% in the early 1960s (Andrews 1966). More recently, Aegean Sea fan mussel populations have experienced up to 100% mortality from the sudden spread of a parasite of unknown origin (Katsanevakis et al. 2019). Parasites have even been implicated in the extinction of bivalve families throughout evolutionary history (Ozanne & Harries 2002). Therefore, understanding the interactions between bivalves and their parasites is vital for assessing population trends and species health.

There is evidence that freshwater mussels (order Unionida) experience negative consequences as a result of parasitism. For example, digenean trematodes utilise freshwater mussels as first or second intermediate hosts in their complex life cycle, with sporocysts, cercariae or metacercariae found parasitizing gonad tissue (Fig. 1.1; see Molloy et al. 1997 for summaries of trematode life cycles). By targeting the gonad of unionids, trematodes redirect host reproductive energy for their own resources, and hence maximise energetic exploitation whilst maintaining host survival (Jokela et al. 1993; Taskinen et al. 1997). In extreme cases, trematodes have been observed to reduce gonad tissue by 90% (Jokela et al. 2005), or induce complete castration in unionids (Molloy et al. 1996; Walker 2017). In addition, unionicolid mites live on the gills or mantle of freshwater mussels. Their presence may come at an energetic cost to the mussel (Gangloff et al. 2008), and explicit gill damage

has been recorded for unionids in Australia and the United States (Fisher et al. 2000; Walker 2017). Over broad scales, these interactions have the possibility to significantly affect the conservation outcomes of freshwater mussels.

The conservation of freshwater mussels is of high priority given the influence they exert on their environment. Their hard shells provide a substratum for attachment of other organisms, as well as a refuge from predation or adverse environmental conditions (Vaughn & Hakenkamp 2001; Gutiérrez et al. 2003). Through burrowing, unionids oxygenate the sediment, and release trapped nutrients back into the water column (Vaughn & Hakenkamp 2001). Their collective filtration can significantly alter the nutrient regime in streams and lakes they inhabit (Hoellein et al. 2017). As important ecosystem engineers, unionids support diverse communities (Aldridge et al. 2007; Chowdhury et al. 2016). Unionids are also among the most imperilled bivalves, with 45% of species being near-threatened, threatened or extinct (Lopes-Lima et al. 2018), thanks to a broad range of threats (reviewed in Lopes-Lima et al. 2017). Combined with the potential negative effects of parasitism, the ecological benefits gained from freshwater mussels would appear to be under significant risk.

The recent and rapid global spread of invasive bivalves (Aldridge et al. 2004; Sousa et al. 2014) presents another potential threat for the transmission of existing and novel endoparasites to unionid populations. Parasite spillover occurs when invasive species pass on their own parasites to native taxa, while spillback can occur when native parasites infect the invaders, potentially increase in density thanks to an additional host, and hence have an emergent deleterious effect on native populations (Kelly et al. 2009). These mechanisms have the potential to severely harm native populations, but assessing the risk requires a thorough understanding of shared parasitism (or the potential for it) between native and invasive taxa. For unionid mussels and sympatric invasive bivalves, such information has not previously been collated. In addition, invasive bivalves may be introduced as larvae or experience post-establishment bottleneck effects, and hence lose most of their own parasites. This ‘enemy release’ can then lead to disproportionate success in their invaded range (Torchin et al. 2003).

Despite the range of risks outlined above, a complete characterisation of the parasites of unionids remains absent. Records are scattered and patchy (Grizzle & Brunner 2009), which makes a systematic understanding of parasites and their effects extremely difficult. Previous reviews of unionid-parasite interactions only consider broad parasite categories (Grizzle &

Brunner 2009), or focus on a small subset of hosts (Molloy et al. 1997; Carella et al. 2016) or parasites (Alves et al. 2015). In addition, it is not clear how the presence of invasive species may influence parasite communities in native unionids. A recent review (Ferreira-Rodriguez et al. 2019) has noted that in general, there are very few studies available to assess the importance of parasitism. Given the important ecological role that unionids play in freshwater systems worldwide, understanding factors that may affect population and species success should be a priority.

The aims of this review were therefore two-fold. The first was to generate a comprehensive list of all parasite records for European and North American unionid mussels. These two localities were chosen as they constitute the majority of freshwater mussel studies (Lopes-Lima et al. 2018). This serves to highlight the wide extent of host-parasite relationships in this group, the research gaps that need to be addressed, and the implications for freshwater mussel conservation. The second aim of this review was to also generate a comprehensive list of all parasite records for key invasive bivalves in Europe; this exercise was limited to European studies to facilitate complete cross-referencing of parasite records between native and invasive species (the volume of native North American species would preclude this approach). This allows an assessment of potential spillover and spillback, and therefore contributes to an understanding of how non-native species may indirectly influence the conservation of native species.

2.2. Methods

2.2.1. Scope and terminology

The first aim of this study was to summarise all parasite records for native unionid mussels in North America and Europe. There is continuing uncertainty regarding unionid taxonomy; for the purposes of this review, the lists of Williams et al. 2017 (279 extant species, North America) and Lopes-Lima et al. 2017 (16 extant species, Europe) were considered authoritative. These species will be referred to as ‘native unionids’ throughout the study. The second aim of this study was to also document parasite records for the four most widespread invasive bivalves in Europe only (*Corbicula* sp., *Dreissena polymorpha, D. rostriformis bugensis, Sinanodonta woodiana*), and to use these records to assess the possibility for enemy release, parasite spillover and parasite spillback. These species will be referred to as ‘invasive

bivalves,' though it should be noted that as the dreissenids are native to the Ponto-Caspian region, some of the studies recorded include these species in their native range. Only Europe was chosen for the second part of the review, as the number of host species in North America would have precluded the use of bipartite networks for in-depth analysis of invasive-native interactions.

Because there is little to no evidence regarding the pathogenicity of most of the 'parasites' recorded, they will henceforth be referred to as endosymbionts, a term which allows for a full range of interactions, from parasitism to mutualism (Douglas 2008). The term 'endosymbiont' is used very broadly in this review, and incorporates records of taxa such as nematodes which are largely free-living but occasionally find their way into the mantle cavity of mussels. This decision was taken to ensure all possible interactions are highlighted, given these groups have occasionally been shown to have an effect on other endosymbionts (see section 2.4).

To provide an initial overview on the threats that unionids face, and to contextualise parasitism within this, all 295 native unionids were searched on the global IUCN Red List database (www.iucnredlist.org). Current status was noted, and the number and identity of threat categories were recorded for each species. In addition, for those species that had Threat Category 8 ('Invasive and other problematic species, genes & diseases'), the identity of the threatening species was noted, in the cases where it was listed.

2.2.2. Literature search and processing

The number of European host species meant they could be individually searched for using a systematic review methodology. Using the Web of Science and Google Scholar databases, every possible combination of {'Unio', 'Anodonta', 'Margaritifera', 'Pseudanodonta', 'Potomida', 'Microcondylaea', 'Dreissena', 'Corbicula', 'Sinanodonta', 'mussel', 'zebra', 'quagga'} and {'parasit*', 'trematod*', 'mite', 'Unionicol*', 'buceph*', 'aspidogast*', 'phyllodist*', 'cili*', 'chirono*', 'endosymbio*'} was searched from October 2018 to January 2019. These search terms were chosen based on preliminary searches of the literature. North American host-endosymbiont records were found using combinations of {'unionid', 'mussel'} and the endosymbiotic search terms listed above, also in the Google Scholar and Web of Science databases over the same period. Following those searches,

reference lists of papers were also checked for relevant studies that had not been found in the initial searches. In particular, the reviews of Molloy et al. (1997), Grizzle and Brunner (2009) and Alves et al. (2015) were informative in finding literature. In many cases, papers reported unionid species that are no longer recognised (e.g. they have been synonymised with other species, subsequently assigned to new genera etc.); the World Register of Marine Species database (which includes freshwater species), in addition to Williams et al. (2017) and Lopes-Lima et al. (2017), were used to assign the record to the updated species.

Papers were added to the database (Appendix A1 Part 1, Tables A1.2 and A1.3) if they met *all* of the following criteria:

(a) they contained reports from a European or North American country (i.e. USA or Canada);
(b) they identified one of the 299 potential host organisms to at least genus level;
(c) they listed at least one endosymbiont found naturally in the host, at any taxonomic classification level. Laboratory studies that reported experimental infection of a host with a certain endosymbiont were excluded, unless they demonstrated the association was also found under natural conditions;
(d) they appeared in a peer-reviewed journal or book. Government reports, conference proceedings and theses (both Master's and Doctoral dissertations) were excluded; this decision was taken to avoid potential 'double-recordings' (e.g. in many cases it was evident that the same records appeared in a thesis or conference presentation and a subsequent journal article);
(e) they were written in the English language. This decision was taken to avoid potential bias against non-English languages that may be harder or not possible to translate with current resources, though it should be noted that there is a body of non-English literature concerning the endosymbionts of dreissenids from Eastern Europe.

In several cases there were references to potentially relevant records that were not accessible to the authors of this review. All steps were taken to access these papers, including emailing the original authors where possible. In total, eight potentially relevant papers were not able to be assessed. In the interest of transparency, these papers appear in Table A1.1, alongside all other literature encountered that met criteria (a) but failed at least one of the criteria (b) to (e). In addition, literature on bitterling (fish that lay their eggs inside unionid mussels) were not

included, as these have already been subject to comprehensive review and study (e.g. Smith 2017; Smith et al. 2004).

In this review we utilise the following terms. A *novel* host-endosymbiont record refers to the first time an association between a particular host and a particular symbiont is recorded. A *unique* endosymbiont record refers to a taxonomically distinct endosymbiont (e.g. a mussel with a particular trematode and a particular mite would have two unique endosymbionts). For the purposes of simplicity, the word 'record' is used as a qualitative rather than a quantitative term; for example, a study that finds a single mite species inside 50 conspecific mussels constitutes one record, not 50 records (as it only documents a single unique host-endosymbiont relationship). Each endosymbiont recording was classified according to whether it was found inside a native species, an invasive species in its native range, or an invasive species in its invaded range. The richness of endosymbiont taxa (i.e. the total number of unique endosymbionts in a given host) was calculated in the most conservative manner possible. For a given host, if one study reported '*Echinoparyphium recurvatum*' (a digenean trematode), and another study reported '*Echinoparyphium* sp.', this would only count as one unique endosymbiont overall, to account for the fact that '*Echinoparyphium* sp.' may have in fact been *E. recurvatum*. This yielded a minimum endosymbiont richness per host.

2.2.3. Data analysis: assessment of sampling effort

An estimate for how thoroughly unionid hosts have been sampled was made by combining endosymbiont reports from Europe and North America. This approach is acceptable as it was shown that the frequency of records among different hosts was drawn from the same underlying distribution for North America and Europe (discretized Kolmogorov-Smirnov test, $D = 0.091$, $p > 0.05$; Conover 1972, implemented in `dgof` package). Invasive species were excluded. For a given host *x*, the proportion of records that documented a novel endosymbiont was calculated (i.e. [minimum endosymbiont richness of *x*]/[total records for *x*]). These data were plotted against the total number of records per host, which generated a negative power curve describing the relationship between sampling effort and novel endosymbiont records for a given host. This curve was used to calculate how many records are required before it becomes probabilistically unlikely that a new record would document a novel endosymbiont. Hosts that failed to reach this number of records (i.e. hosts whose

current sampling effort predicts that each new record would still yield a novel endosymbiont) are considered ‘under-sampled.’

To further explore the phenomenon of ‘under-sampling’, host unionids were separated into three broad categories based on their international IUCN Red List classification.

1. No Data (ND): Hosts that are either Data Deficient or lack an IUCN entry. N = 90
2. Stable Populations (SP): Least Concern or Near Threatened. N = 105
3. At Risk (AR): Vulnerable, Endangered or Critically Endangered. N = 100

These categories have the advantage of being reasonably numerically balanced. Differences in the mean number of records per host in each of the three categories was assessed using negative binomial regression (Generalized Linear Model, log link) using the package `MASS` (Venables & Ripley 2002). This included hosts with no records, and is intended to assess differences in study effort among the different groups. This model was shown to be an appropriate fit for the data (comparison of residual deviance to a χ^2_{292} distribution, null hypothesis of model fitting data, $p = 0.916$).

2.2.4. Data analysis: invasive and native records in Europe

Endosymbionts in native unionids and invasive bivalves in Europe were investigated with the creation of two bipartite network graphs using the package `bipartite` (Dormann et al. 2009). One network was created using records from countries considered the potential native range of invasive dreissenids (Russia, Belarus, Ukraine; Brown & Stepien 2010), while the other was created using data from all other European countries. To test whether these networks contained a structure different from that predicted by random data, three indices describing the networks were computed from within the `bipartite` package: Connectance, Links per Species, and NODF (a measure of nestedness). Null models were then created for each of the two networks (`nullmodel` function, option “r2dtable”, N = 1000) and the same indices computed to create a null distribution to compare the observed values to.

To visualise the relative distributions and overlap of sampling effort for invasive bivalves and native unionids across Europe, two geographic heat maps were created in QGIS v3.2.3 (QGIS Development Team 2018).

In addition, the mean number of endosymbiont records per host was compared for invasive bivalves in their native range, invasive bivalves in their invasive range, and native European unionids, using negative binomial regression. The model was shown to be an appropriate fit for the data (comparison of residual deviance to a χ^2_{89} distribution, null hypothesis of model fitting data, $p = 0.954$). Because of unevenness in study effort between the different groups (i.e. some species, particularly *D. polymorpha*, have been the subject of many more studies), these calculations were executed on a per-study basis. Therefore, the averages presented in Fig. 2.7 represent the number of unique endosymbionts recorded in the group *per study* (as opposed to the total number of unique symbionts recorded in a given group across all studies of that group); this accounts for the differential study effort between species. This is in contrast to the negative binomial regression analysis from section 2.2.3, which compares the total number of records among different groups. To account for the fact that many of the bivalves contain records that may not be 'true' endosymbionts (insect larvae, nematodes, human bacteria, oligochaetes, amoebae), this analysis was repeated with these taxonomic groups excluded (Appendix A1 Part 2).

2.2.5. Data analysis: effects of endosymbionts

To explore the potential effects of the endosymbionts recorded, all studies were categorized as to whether they explicitly investigated the effect of the endosymbiont on the host bivalve and, if so, the identity of the endosymbiont and the direction of the interaction (positive, negative or neutral/no effect).

Unless otherwise specified, all the analyses described were conducted in R v3.5.1 (R Core Team 2018).

2.3. Results

2.3.1. Summary of literature

A summary of data from the IUCN Global Red List reveals that parasitism has not been considered as a threat to freshwater mussels in Europe and North America. Parasites and diseases would be classed under the IUCN 'Invasive species' category. While 18% of species have this category listed as a threat (Fig. 2.1a), parasites are not mentioned in any recordings

(Fig. 2.1b). However, 46% of this threat category is represented by the three invasive bivalve genera considered in this study (*Dreissena*, *Corbicula*, *Sinanodonta*; Fig. 2.1b).

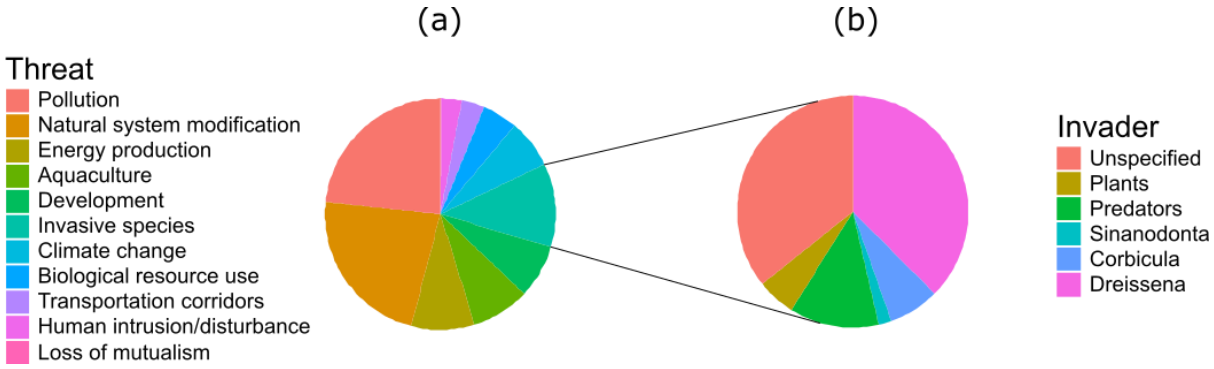


Figure 2.1: Threats to unionid mussels in Europe and North America. Data compiled from searches of each native unionid in Europe and North America in the IUCN Global Red List. (a) Distribution of all threats (scaled to total 100, as many species are faced by multiple threats). (b) Distribution of threats within the 'Invasive and other problematic species, genes and diseases' IUCN category.

In total, the literature search generated 239 studies (150 North America, 89 Europe) that described 1476 separate host-endosymbiont records (1220 North America, Table A1.2; 256 Europe, Table A1.3). Of the 299 potential hosts in this study, 142 had at least one endosymbiotic record (6/16 native European unionids, 3/4 invasive bivalves in Europe, 133/279 native North American unionids). These records encompass a minimum of 60 unique endosymbiont taxa in Europe, and 135 unique endosymbiont taxa in North America, representing the most comprehensive lists to date of host-endosymbiont records in unionid mussels.

Intuitively, there is a strong relationship between study effort and the number of novel host-endosymbiont associations recorded (Fig. 2.2). There was a peak in North American studies from 1970 – 1994 (Fig. 2.2a), corresponding to a spike in the number of novel records (Fig. 2.2c). However, study effort has since steadily decreased, with a subsequent tailing-off in the number of new host-endosymbiont associations recorded. A similar trend is seen in Europe, though there is a marked difference between native and invasive species. Studies and recordings of invaders has accelerated since the late 1990s (Figs. 2.2b, 2.2d), while studies on native species reached an early plateau, before experiencing a slight increase in the last five years (Fig. 2.2d).

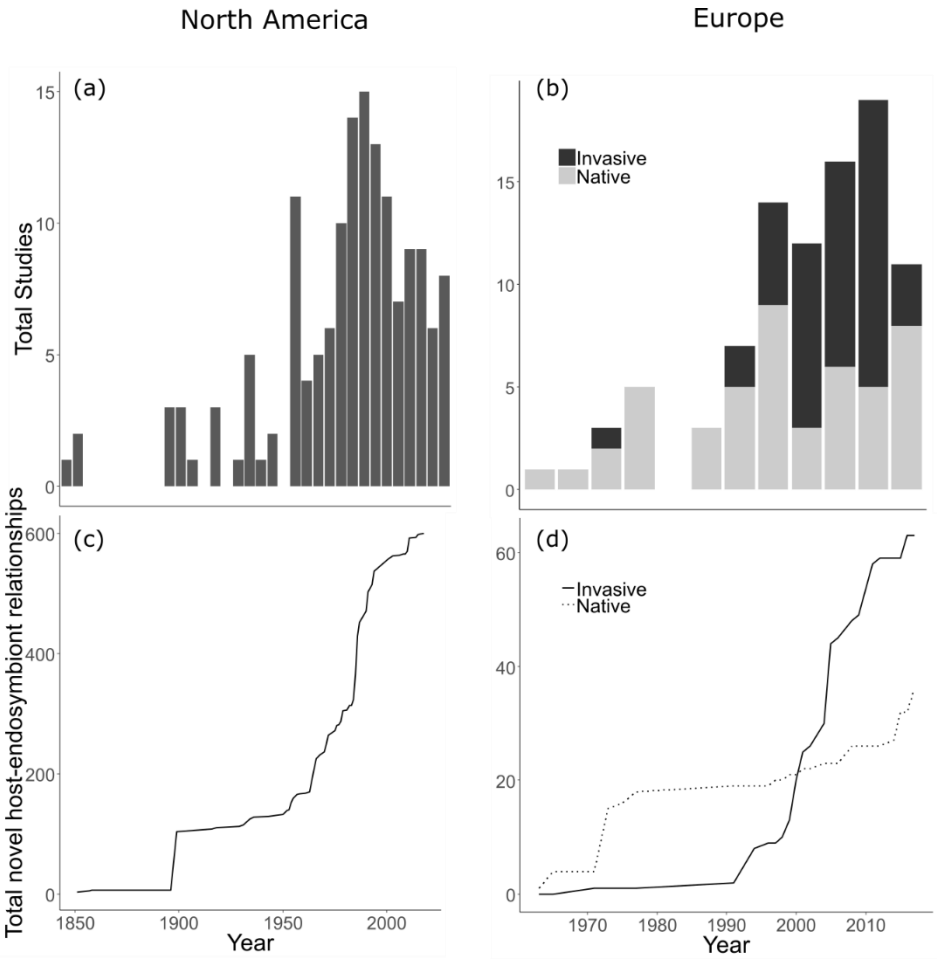


Figure 2.2: Relationships between study effort and cumulative novel host-parasite recordings. Data compiled from Web of Science and Google Scholar searches. (a) The total number of studies per five-year period (beginning 1850) on endosymbionts in native North American unionids. (b) The total number of studies per five-year period (beginning 1960) for both invasive bivalves and native unionids in Europe. (c) The cumulative number of novel host-endosymbiont relationships for native North American unionids (using the same time scale as (a) but as a continuous variable). (d) The cumulative number of novel host-endosymbiont relationships for both invasive bivalves and native unionids in Europe (using the same time scale as (b) but as a continuous variable). Note that for (a) and (b), the final year bin (2015-2019) represents a slightly shorter time period as publications post-January 2019 are not included.

2.3.2. Assessment of sampling effort

Endosymbiont records were highly unevenly distributed among host species. For example, 78% of all records in Europe come from the native *A. anatina* and invasive *D. polymorpha* (Table A1.3), while the unionid genera *Cyclonaias*, *Lampsilis* and *Pyganodon* account for 30% of North American records, despite only representing 15% of possible host species (Table A1.2). In some cases, European studies also document where parasites were searched for and found to be absent. These results are summarised in Table A1.4. The species with no

host records did not appear there either, which indicates they have never been examined for endosymbionts (or, if they have, it has not been published). Fig. 2.3 shows the variation in number of host records, and further emphasises that the number of unique endosymbionts recorded is strongly linked with study effort on a per-species basis. The power relationship can be described by the simple equation:

$y = 0.973x^{-0.269}$ (1),

where x is the number of records for a given host, and y is the proportion of those records that document a novel parasite type.

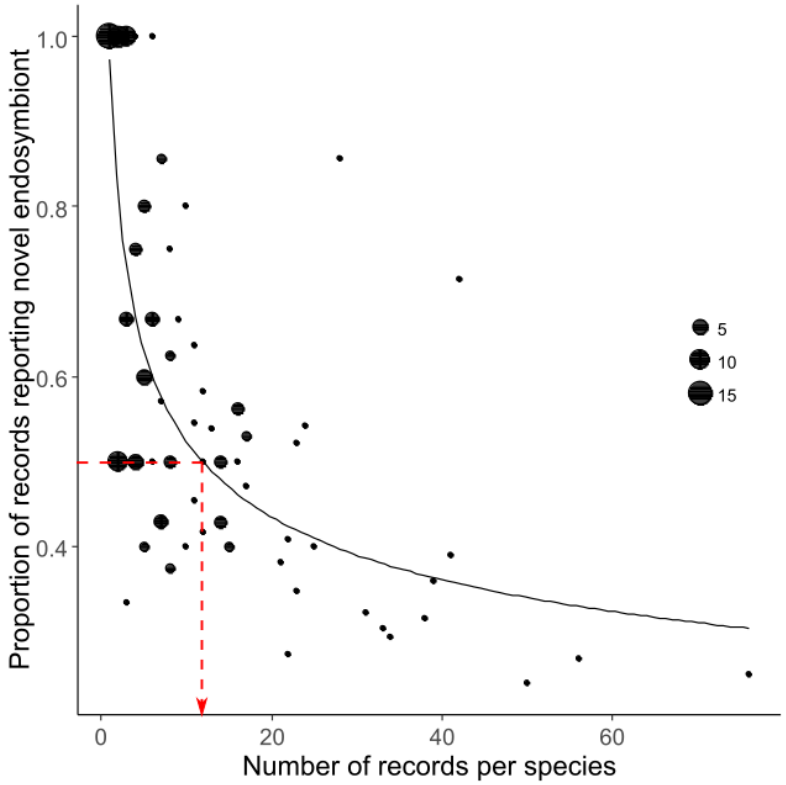


Figure 2.3: Relationship between number of records for each host species in the study, and the proportion of those records that document a novel endosymbiont, with the fitted power relationship in black (Eq. 1). The dashed line (y = 0.5) indicates the point at which it is equally likely that a new endosymbiont record does or does not report a novel association, and indicates with an arrow the sampling effort required to reach this point (x = 11.88). Dot size corresponds to the total number of records that occupy that point. Hosts with zero records (n = 157) are not included.

Setting y as 0.5 in Eq. 1 and solving for *x* reveals that if a single host is sampled less than 11.88 (i.e. 12) times, there is a higher chance than not (> 50%) that each new recording will document a novel parasite type for that host. Only 36 hosts of the 295 in the study have 12 or more records; therefore for 88% of freshwater unionids in North America and Europe it is

statistically likely that a new record would document a novel endosymbiont, and these species can be considered under-sampled. Further, the proportion of species under-sampled in each IUCN risk category (ND, SP, AR) was highly variable. 76.2% of SP hosts are considered under-sampled, in contrast to 87.8% of ND hosts, and 99% of AR hosts. This is supported statistically by the negative binomial regression model. The average number of endosymbiont records per host was significantly different between the three risk categories (Overall model deviance = 63.83; d.f. = 2, 292; $p < 0.0001$; Fig. 2.4). Unionids in the AR category have 5 times and 12 times fewer records than ND and SP unionids, respectively.

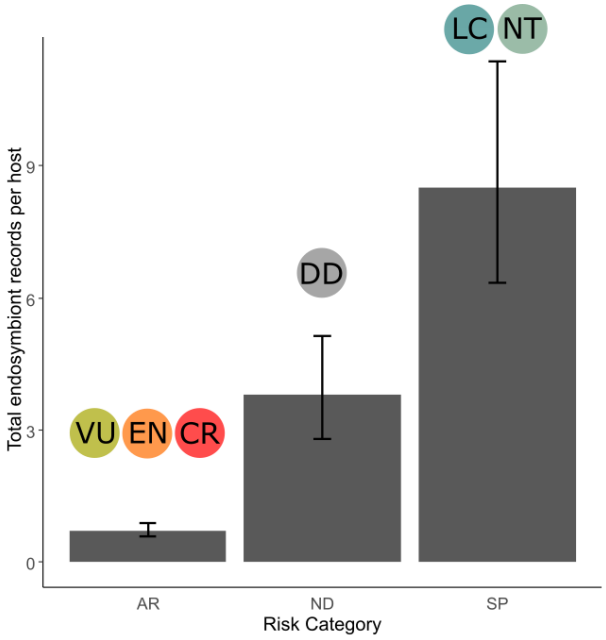


Figure 2.4: Total endosymbiont records per possible host species (found in this review) for each IUCN risk category (mean ± SE). AR = At Risk (Vulnerable, Endangered, Critically Endangered IUCN designation). ND = No Data (Data Deficient IUCN designation, or not listed in IUCN database). SP = Stable Population (Least Concern, Near Threatened IUCN designation). Note unevenness of error bars due to back-transformation from logarithmic estimates.

2.3.3. Invasive and native bivalves in Europe

Of the 89 European studies documented, 45 studied native unionids, 41 studied invasive bivalves, while just 3 investigated both. Bipartite host-endosymbiont maps were created to show records in both the native range of invasive dreissenids (Fig. 2.5a) and for the rest of Europe (Fig. 2.5b).

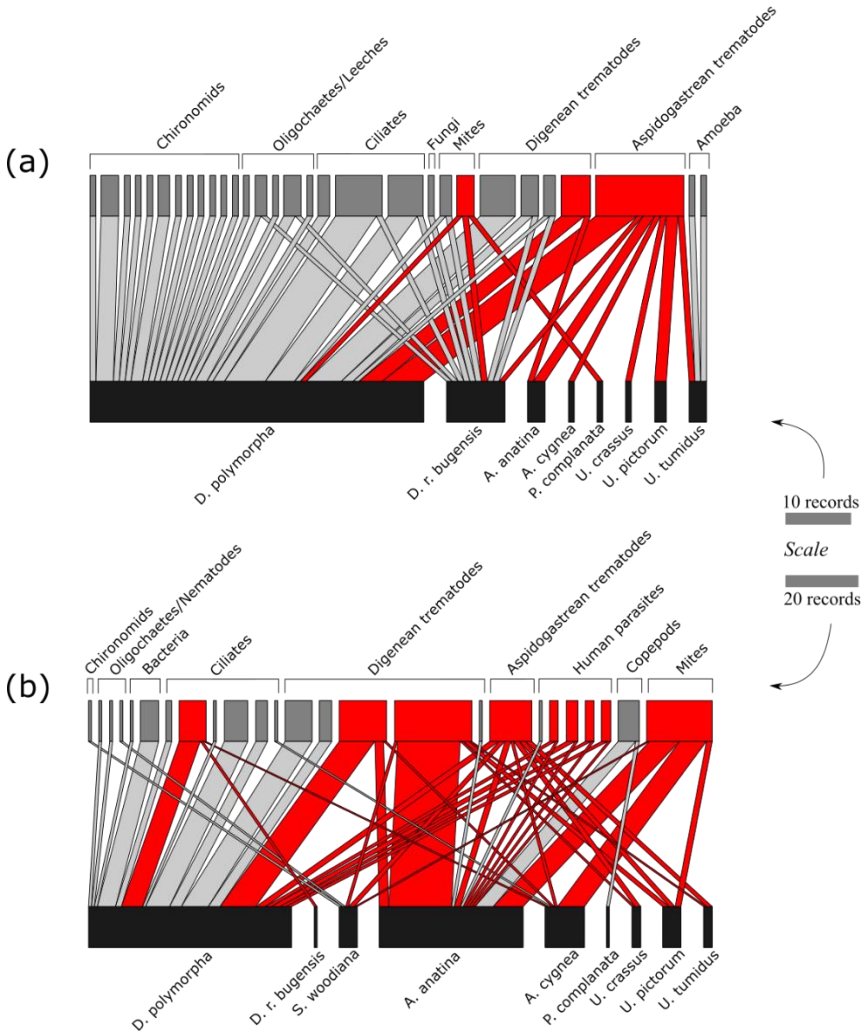


Figure 2.5: Bipartite host-endosymbiont networks for (a) records from Belarus, Russia, and Ukraine, and (b) records from Croatia, England, Finland, France, Germany, Ireland, Lithuania, The Netherlands, Poland, Romania, Spain and Sweden. Each endosymbiont box (upper row of each network) represents a genus, with broad taxonomic designations indicated by square brackets, while each host box (lower row of each network) represents a species. Widths of boxes and linking parallelograms are proportional to the number of records. Endosymbiont boxes and links in red indicate genera that are shared between native unionids and invasive bivalves. See Table A1.3 for full records.

The bipartite networks reveal significant overlap in parasite communities between native and invasive species, with three genera being shared in the native range of invasive dreissenids, and eight across the rest of Europe. There are still broad host-parasite clusters, with several chironomids and ciliates showing high specificity for the invasive *D. polymorpha*. This is reflected in host-endosymbiont relationships being more structured than would be expected under a null model (Fig. 2.5b shows significantly lower Connectance and Links per Species and higher NODF than random data; $p < 0.0001$ in all cases). However, mites and both aspidogastrean and digenean trematodes display some overlap between native unionids and

invasive bivalves in both networks. This is particularly significant given there is little overlap in the sampling of native unionids and invasive bivalves over broad geographic scales (Fig. 2.6). Only six countries have records for both native and invasive species (Russia, Lithuania, Poland, The Netherlands, Ireland, Ukraine), and it is clear that bivalve endosymbionts have not been assessed for most European countries (70% with no records).

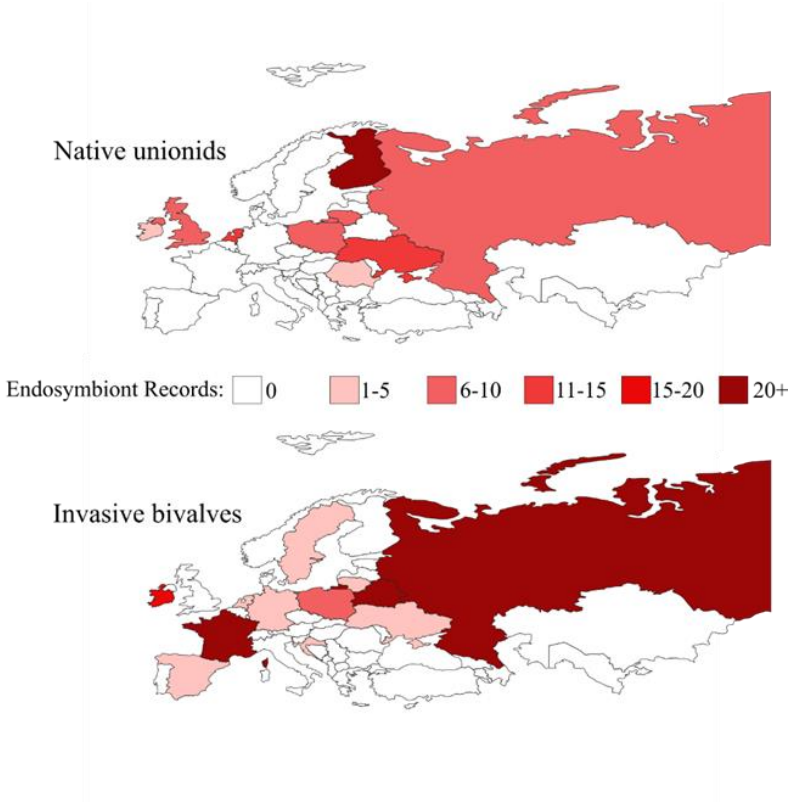


Figure 2.6: Total number of endosymbiotic records in native unionids and invasive bivalves per country, for both native species (top) and invasive species (bottom).

Table 2.1: The number of records that investigated the effect of different endosymbiont categories on the host, and the direction of that effect. Note that several studies investigated the effect of more than one endosymbiont category, so the 60 records are drawn from 48 different studies.

Endosymbiont category	Records	Positive	Neutral/no effect	Negative
Digenean trematodes	30	0	8	22
Aspidogastrean trematodes	9	0	1	8
Ciliates	7	0	4	3
Mites	7	0	2	5
Bacteria/haplosporidians	4	0	1	3
Insect larvae	2	0	0	2
Amoeba	1	0	1	0
TOTAL	**60**	**0**	**17**	**43**

Finally, the mean number of unique endosymbionts per host differed significantly between invasive bivalves and native unionids (Overall model deviance = 35.56; d.f. = 2, 89; $p < 0.0001$). This result can be broken down further by examining the terms in the model in more detail. There was no significant difference between the number of unique endosymbiont records per host between invasive bivalves in their native and invasive range ($z = 0.86$, $p = 0.390$), but there were 2.8 times fewer unique records per host in native unionids ($z = -4.68$, $p < 0.0001$; Fig. 2.7). Because this looks at the number of unique endosymbionts reported in a single study (and is then subsequently averaged across all studies), this is independent of total study effort on a given species. However, it should be noted that the higher total number of studies on *D. polymorpha* does mean that this does increase the chance of finding novel endosymbionts in this species. As the analysis with potentially non-endosymbiotic groups removed displayed identical statistical significance and direction of effects (Appendix A1 Part 2), the complete results are presented here as they represent the most comprehensive investigation of the data.

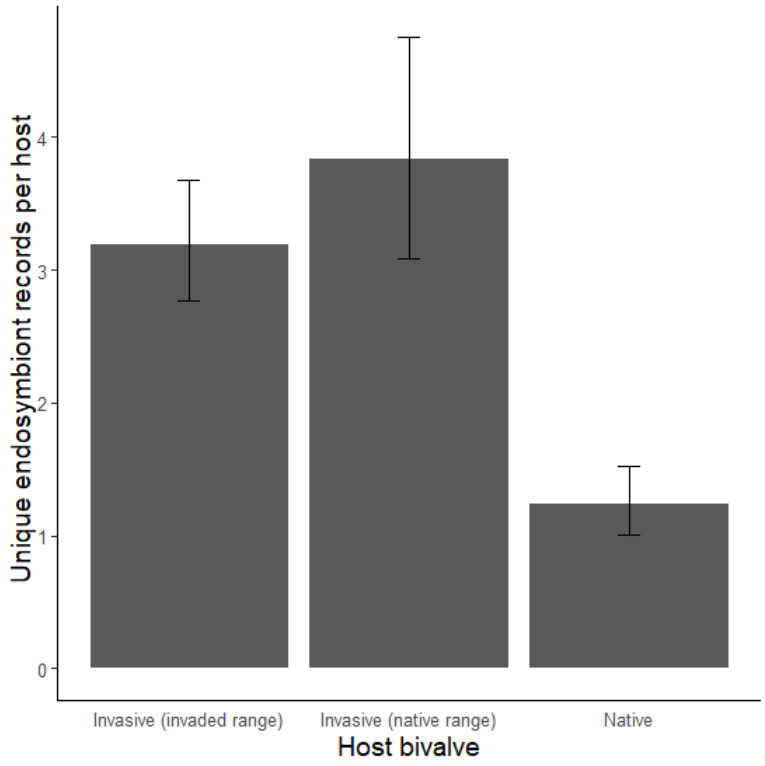


Figure 2.7: Unique endosymbiont records per host in a given study for each host category (invasive bivalves in invaded or native range, native unionids) (mean ± SE). Note unevenness of error bars due to back-transformation from logarithmic estimates.

2.3.4. Effect of endosymbionts

In total, just 20% of studies investigated an effect of the endosymbionts recorded (23/89 Europe, 25/150 North America). In addition, of the 48 studies that document an effect, only four were demonstrated experimentally, with the other 44 being purely observational. Results are summarised in Table 2.1, which shows that no symbionts are recorded as having a positive effect on their host, while 71.6% have a negative effect.

2.4. Discussion

A review of the literature in North America and Europe has revealed at least six significant issues regarding freshwater bivalves and their suite of endosymbionts:

1. more than fifty percent of hosts have never been assessed for endosymbionts, and current trends do not indicate this will be improved in the near future (Fig. 2.2);
2. only 12% of native unionids can be considered adequately investigated (Fig. 2.3);
3. the endosymbiont communities of the most endangered unionid hosts are the least understood (Fig. 2.4);
4. the effects of most endosymbiont taxa recorded remain unquantified, and those that have been evaluated show largely negative effects (Table 2.1);
5. there is minimal overlap between sampling of native and invasive hosts, yet there is still evidence of shared endosymbionts (Figs. 2.5, 2.6);
6. invasive bivalves still possess large parasite communities in their invaded range (Fig. 2.7).

A holistic overview of these issues suggests that endosymbionts may have a significant yet severely under-appreciated influence on the conservation of native unionids.

2.4.1. Sampling imbalances

It is clear from this review that an understanding of host-endosymbiont patterns in unionid mussels remains incomplete. Only 47% of possible hosts have one or more endosymbiont records, a lack of sampling that disproportionately affects species with a high level of extinction risk (Fig. 2.4). Indeed, only 25% of At Risk (AR) hosts have any records at all. This is intuitive, for several reasons. Because they are by definition rare or declining, they will be encountered less often and hence there is less opportunity for documenting

endosymbiotic communities. In addition, searching for endosymbionts in mussels is typically destructive and involves dissection or histopathological analysis of key tissues such as the gill and gonad, an approach potentially untenable for critically endangered species. However, there are non-invasive methods of investigation, such as repeatedly flushing the mantle cavity to dislodge resident mites (Davids et al. 1988), monitoring mussels in the laboratory for evidence of trematode cercarial release (Choo & Taskinen 2015) or taking small samples of gonadal fluid to examine for trematode life-history stages (Galbraith & Vaughn 2011; Zieritz & Aldridge 2011).

There also sampling imbalances evident for invasive bivalves. Zebra mussels (*D. polymorpha*) comprise 89% of all records for invasive species, and they drive the spike observed in Fig. 2.2d. This coincides with the recognition of invasions of this species in the late 1990s in the United States, Ireland and Spain (Aldridge et al. 2004). However, this focus on zebra mussels has meant that endosymbionts of other species have been neglected. Despite being spread across Europe, there are no studies at all on *Corbicula* sp. in Europe. This is particularly concerning, as elsewhere in the world it has been shown to host *Unionicola* mites (Abdel-Gaber et al. 2018), in addition to both aspidogastrean and echinostomatid trematodes (Karatayev et al. 2012; Alves et al. 2015). This suggests *Corbicula* sp. could be a significant source, or viable alternate host for many endosymbionts.

2.4.2. Effects of endosymbionts

Many of the endosymbionts recorded have been assumed to be largely commensal in nature, especially mites (e.g. Edwards 2014) and ciliates (e.g. Burlakova et al. 2006). However, Table 2.1 shows that when these relationships have been explicitly investigated, a large majority of them are revealed to have a negative effect on the host, especially mites and trematodes (see also Jokela et al. 1993; Taskinen 1998a; Fisher et al. 2000; Gangloff et al. 2008; Walker 2017). In addition, the endosymbionts that have been investigated represent a small minority of the ~190 unique endosymbiont taxa recorded in this study. Particular absences include an understanding of nematodes and annelids. These have often thought to be 'incidental' invaders, accidentally entering the mantle cavity from the periphyton (Reid et al. 2012). However, Conn et al. (1994) report 61% of *D. polymorpha* hosting nematodes from the St Lawrence River, and they were the most common endosymbiont from a study in Sweden (Mastitsky et al. 2008). The frequency with which this relationship occurs suggests

there may be a previously unexplored positive or negative relationship between host and symbiont that remains unexplored. In general, further work is required to ascertain the nature of many of observed associations, given the diverse range of combinations (Figs. 2.2c, 2.2d).

When the effects have been explored, they are largely focused on the scale of an individual mussel, and population- and species-level effects have not been considered (but see Taskinen & Valtonen 1995). Many of the records in Table 2.1, which largely demonstrate negative effects, are only based from single-mussel observations (e.g. an observation that a mite consumed gill tissue), and the possible implications for mussel populations remains unquantified. This is aptly demonstrated by Fig. 2.1, which shows parasites are not considered in IUCN assessments to threaten any native unionid populations, despite often having very high prevalence, such as 80% of *Lampsilis radiata* in Maryland, USA being infected with castrating trematodes (Kat 1983). Therefore, there may be significant effects over broad scales. For example, the European digenean *Rhipidocotyle campanula* destroys on average 90% of gonad tissue in mussels it infects (Jokela et al. 2005). If this (or similar) trematodes are cryptically present in a population, estimates of fecundity based on density or size of mussels may overpredict the reproductive capacity of the population. In addition, there are now captive breeding and reintroduction programs for some endangered mussels (e.g. *Margaritifera margaritifera*, Thomas et al. 2010). If there is no available information on harmful endosymbiotic communities, they may be reintroduced into an area with high parasite loads and thus render the action far less efficient than it otherwise may have been (see Chapter 9). Furthermore, restocking of depleted populations with hatchery-reared mussels may need to consider the risk of introducing harmful endosymbionts into recipient communities, given stock populations can often experience high parasite densities (e.g. Meeus et al. 2011). While the effects (if not the extent) of castrating trematodes on populations may be predicted, further work is required on the other endosymbiont groups listed in Table 2.1 to appreciate the population-level effects.

Interactions between endosymbionts and the environment are very common (Johnson et al. 2015). An infection that appears to be under control can suddenly explode with an environmental driver, as was the case with the oyster parasite *Perkinsus marinus* (Ford 1996). There is the potential for this to occur in unionids. For example, trematode cercarial release is induced by temperature, and temperature also increases the volume of release (Choo & Taskinen 2015). Therefore, climatic warming has the potential to increase digenean infection

rates in unionids. In addition, different macro-parasites can themselves interact inside hosts. For example, the mite *Unionicola ypsilophora* is strongly territorial and defends a harem of females from heterospecific competitors, competitively excluding *U. intermedia* from *A. cygnea* (Davids et al. 1988). Competition can occur between trematodes, and whole dominance hierarchies have been established for trematode larvae inside a host (Esch & Fernandez 1994). Taxonomically diverse endosymbionts may also interact; for example, oligochaetes have been shown to consume cercariae in snails, and significantly lessen trematode infection rates in the host (Hopkins et al. 2016). This emphasises the value of the broad approach taken to the term 'endosymbiont' in this review, as it highlights the fact that the oligochaetes, nematodes and related taxa may have a functional impact on bivalve conservation that is important to consider. In general, effects may not be able to be predicted by studying one endosymbiont in isolation; the wider endosymbiotic community, in addition to the environment, needs to be taken into consideration. Given the current state of under-sampling, the potential for endosymbiotic interactions and implications on host conservation is currently not assessable.

Finally, some endosymbionts may have broader implications. Both native and invasive bivalves are competent for microbes that are pathogenic to humans (Fig. 2.5, Table A1.3); as they remain viable and can be concentrated by the mussels (Graczyk et al. 2004), they fitted our broad definition of endosymbiosis and were hence included in this review. High mussel densities could therefore increase the threat of water-borne disease in humans, something that is of increasing concern globally (Pandey et al. 2014).

2.4.3. Role of invasive bivalves

The assembly of all relevant literature has brought to light the fact that the endosymbiotic communities of native and invasive bivalves are not completely disparate, which leads to the potential for parasite spillback. There are several genera shared between the two groups, in both the invasive and native ranges of invaders (Fig. 2.5), despite the fact that only 3.5% of European studies have sampled both native and invasive species together, and there are only six countries where they have both been studied. Therefore, there is potentially much more overlap to be explored. In some cases, it should be acknowledged that the overlap comes from a single record, such as in the case of the digenean trematode *B. polymorphus* infecting *Anodonta* sp. (Pavluchenko & Yermoshyna 2017), which is widely accepted to only infect

dreissenids (Molloy et al. 1997). Singular records may be vulnerable to mis-identifications, and could potentially inflate the amount of overlap demonstrated. However, other congruences can be considered unambiguous, such as the multiple recordings of *Phyllodistomum* sp. in both *D. polymorpha* and *A. anatina*, and the photographic evidence from Cichy et al. (2016) of the invasive unionid *S. woodiana* hosting multiple parasites found in native bivalves. In several cases, this has challenged previous assertions. For example, the evidence that *S. woodiana* can take up *Unionicola* (Cichy et al. 2016) suggests that these mites are not wholly host-specific, as was previously thought (e.g. Edwards & Labhart 2000). In general, three notable endosymbiont categories with significant overlap between native unionids and invasive bivalves are aspidogastrean trematodes, digenean trematodes, and mites (Fig. 2.5). These taxonomic categories have also been shown to generate predominantly negative effects in their hosts (Table 2.1). Typically, such negative effects would serve to stabilise endosymbiont populations. If endosymbionts are deleterious to the extent that they prevent reproduction of their host, they will lose the population density required for their successful transmission, and are hence regulated in repeatable cycles (Lafferty & Harvell 2014). However, invasive bivalves may disrupt such regulation. By acting as a viable alternative host, they release endosymbionts from the pressure of density-dependent transmission and hence the parasite can increase in number (see Mastitsky & Veres 2010). Therefore, the increasing spread of invasive bivalves across Europe, that are competent for a range of deleterious endosymbionts, may have a significant indirect negative effect on native unionid populations. This could be true even if endosymbionts rarely infect invaders. For example, the trematode *Aspidogaster conchicola*, which features in multiple European *Anodonta* and *Unio* species, can also infect *D. polymorpha*, but at very low prevalence (e.g. 2.7%, Toews et al. 1993). This particular value suggests that a trematode occurs once in every 37 *D. polymorpha*. Given that single *A. anatina* have been observed to have up to 34 *D. polymorpha* living on them (Sousa et al. 2011), even this small infection frequency could mean a large proportion of native mussels are in close contact with competent invaders.

In contrast, there is no evidence for parasite spillover from this review. Aside from the *B. polymorphus* record mentioned above, there are no records of endosymbionts native to invasive species that appear in unionids. In particular, dreissenids host several other digenean trematodes, as well as a wide diversity of ciliate species, that were never observed in native populations. Early work from Raabe (1950) stated that in many years of fieldwork, ciliates native to *Dreissena* sp. were never observed within unionids, a trend which is still borne out

today. Therefore, a large majority of dressenid parasites appear highly specific to this genus, or even to the species (*D. polymorpha*, *D. r. bugensis*) within it. Further work in foreign languages, which have not been reviewed comprehensively for this study, also suggests that native European unionids do not become infected with dreissenid endosymbionts. However, the evidence that they may host endosymbionts that are also found in natives suggests varying levels of specificity between endosymbiont groups. Further, invasive endosymbionts may not be competent in native hosts (or *vice versa*), but may still attempt infection (for example, an invasive trematode miracidia may penetrate a native host, but then die). This would represent a 'wasted' infection, and therefore lessen the parasite burden on invaders, further facilitating their success. Hence, the relative success of natives and invaders could still be mediated by endosymbionts, even in the absence of a successful persistent infection. Understanding the degree of specificity within different parasites should be a future priority to fully understand the influence of endosymbionts on the interaction between invasive and native species.

This review also provides the opportunity for an assessment of enemy release. Enemy release has been accepted as a key concept in parasitological invasion literature (Torchin et al. 2003). Despite its popularity, evidence remains equivocal from freshwater and related systems, with opposite results found by the studies of Troost (2010) and Karatayev et al. (2012). The present study supports the fact that there has not been a significant loss of parasites in invaders. While endosymbiont richness was 1.2 times lower in the invasive range (Fig. 2.7), this difference was non-significant and is nowhere the 8.5 times decrease reported for invasive species from the United States (Karatayev et al. 2012). The comparatively small reduction in 'enemies' is suggestive of adult introductions of invasive species through Europe, or that there have been repeated introductions (or both). The presence of host-specific ciliates found in zebra mussels from Ireland (Burlakova et al. 2006) and Sweden (Mastitsky et al. 2008) has been taken as evidence they have been introduced as adults, and therefore further supports this conclusion. This has two significant conservation implications. First, if invasive bivalves are bringing largely intact endosymbiotic communities with them, there is significant potential for introductions of novel endosymbionts into vulnerable native populations. Second, the vectors of invasive larvae versus those of adult hosts can be different; this review suggests that, at least in Europe, the vectors of adults should be targeted to prevent the spread of dreissenid mussels. However, this does not preclude the possibility that dreissenids are also being spread through larval stages also.

2.4.4. Recommendations

Based on our review of the literature, we make the following recommendations to ensure positive conservation outcomes for unionid mussels:

(a) Significant effort should be invested in sampling the endosymbiont communities of unionids, particularly those with no or few records. Endangered species should be non-invasively sampled and returned, using the techniques mentioned in section 2.4.1 and Chapter 4.

(b) The effects of specific endosymbionts should be investigated, using experimental techniques to verify observational patterns. Particular efforts should be placed on how infection alters the reproductive potential of mussels, as current data on population- and species-level effects are largely absent.

(c) Interactions between endosymbionts should be studied experimentally, for example by attempting to infect a mussel with one endosymbiont, in the presence or absence of a second endosymbiont. This will be an important first step in appreciating community-level effects between multiple macroparasites inside a host, and better reflect biological reality.

(d) The potential influence of invasive bivalves should be established. An initial positive step will be to conduct further observational studies of endosymbionts in sympatric native and invasive populations. Experimental work should then be conducted to verify the competence of invasive endosymbionts in native hosts, and vice versa.

(e) The implications of parasite transmission in any introduction or translocation scheme requires serious attention, as there is the risk that efforts to bolster vulnerable populations may unwittingly be contributing to declines.

Further, we suggest that freshwater mussels are just one group that currently have severely uncharacterised endosymbiont communities, and that the failure to consider endosymbionts may have negative conservation implications for such groups. These recommendations will hence be widely applicable to a variety of study systems. In general, endosymbionts have been shown to be crucial in food webs (Poulin et al. 2013), ecosystem stability (Pederson & Fenton 2007), and as indicators of ecosystem health (Rynkiewicz et al. 2015). Therefore, only by characterising endosymbionts and their effects can unionid mussels, and other important groups, be appropriately conserved to maintain their vital ecological functions.

Chapter 3: An efficient photograph-based quantitative method for assessing castrating trematode parasites in bivalve molluscs

Abstract

Parasitic castration of bivalves by trematodes is common, and may significantly reduce the reproductive capacity of ecologically important species. Understanding the intensity of infection is desirable, as it can indicate the time that has passed since infection, and influence the host's physiological and reproductive response. In addition, it is useful to know the developmental stage of the trematode, to understand trematode population trends and reproductive success. However, most existing methods (e.g. visually estimating the degree of infection) to assess intensity are approximate only and not reproducible. Here, we present a method to accurately quantify the percentage of bivalve gonad filled with digenean trematode tissue, based on small squashes of gonad tissue rapidly photographed under light microscopy. A maximum of 15 photographs is required to determine the percentage of the whole gonad occupied by trematodes with a minimum of 90% confidence, with smaller mussels requiring fewer. In addition, the stage of trematode infection can be assessed because full sporocysts, spent sporocysts and free cercariae are clearly distinguishable. While variation exists in the distribution of trematodes in gonad tissue, and thus in the estimate of percentage of the gonad filled with trematodes, this method represents a marked improvement on current coarse assessments of infection which typically focus on binary presence/absence measures. This technique can be used to facilitate a more sophisticated understanding of host-parasite interactions in bivalves, and can inform the conservation and reproductive biology of environmentally crucial species.

Key words: *Anodonta*, bivalve, castration, cercariae, gonad, sporocyst

3.1. Introduction

Parasites face a classic trade-off between maximising host exploitation and maintaining host longevity. A proposed evolutionary solution is castration of the host, which allows for complete redirection of host reproductive energy towards the parasite, whilst facilitating continued host survival (Baudoin 1975; Lafferty & Kuris 2009). Castration has a wide range of potential consequences on the host, including timing and success of reproduction, changes

in distribution and overall energy allocation dynamics (Lafferty & Kuris 2009), which can influence population-level reproductive output (Fredensborg et al. 2005). The type of consequences will depend on a range of factors including prevalence (what proportion of the population is infected), intensity (how many parasites, or how much parasite tissue, is present in a single individual), and infection biases such as age, sex and size. Parasitic castration is evident throughout the animal kingdom, but is particularly common in fish (e.g. Jobling & Tyler 2003) and molluscs (e.g. Averbuj & Cremonte, 2010; Choubisa & Sheikh, 2013; Yee-Duarte et al. 2017).

The castration of molluscs, and in particular bivalves, is valuable and important to study for three reasons. First, bivalve population dynamics are of specific concern given they often fulfil important ecosystem engineering functions through filter-feeding, burrowing and providing a hard substrate to increase niche complexity (Sousa et al. 2009; Lopes-Lima et al. 2017). For example, through their filtering they can modify nearby water chemistry (Ninokawa et al. 2020), act as important nutrient cyclers (Atkinson et al. 2010) and form nutrient hotspots (Atkinson & Vaughn 2015). As a result, their presence is associated with increased species richness in both marine and freshwater ecosystems (Aldridge et al. 2007; Borthagaray & Carranza 2007; Chowdhury et al. 2016). Second, bivalves include some of the most globally imperilled taxa (Bogan 1993; Smith et al. 2006; Lopes-Lima et al. 2018) and effective conservation programmes may benefit from understanding drivers of reduced fecundity. Third, bivalves are often found at high density, vary in possible resource availability to parasites in predictable and easily measurable ways (e.g. size, sex, gravidity) and are amenable to simple manipulation. These qualities make them a potential model system to study the individual, population-level, and evolutionary effects of parasitic castration.

Digenean trematodes are common castrating parasites of bivalves, which utilise them as a first intermediate host (see Chapter 2). Digeneans fill the gonad with asexually-reproducing sporocysts, rediae or both, which produce cercariae that are eventually released to infect the next host. This continued asexual growth eventually leads to castration of the bivalve host. These infections are chronic, and can last for life (Taskinen et al. 1997). Digeneans may also infect bivalves as a second intermediate host in the form of metacercariae. However, understanding the effects of digenean castration on bivalves is currently hindered by an

inability to reliably quantify the level of infection, as the asexual branching growth of sporocysts means there are no specific ‘individuals’ that can be counted.

Evidence suggests that to understand the population-level effects of infection, quantitative data are required. For example, previous research indicates that being castrated (or not) and being infected (or not) should not be treated as binary variables. Taskinen and Valtonen (1995) demonstrated that 18.5% of mussels could still reproduce when infected with sporocyst tissue (though the level of infection for each mussel was not recorded). This suggests that mussels can still reproduce at some level of infection, and that sporocysts need to fill a certain proportion of the gonad before the bivalve is castrated. They further demonstrated that an increased quantity of sporocysts led to reduced number of hosts eggs being produced by females. Other studies have also suggested that an increased volume of trematode tissue can lead to more cercariae being produced (e.g. Hay et al. 2005; Thieltges et al. 2008), showing that infection intensity may have consequences for both host and parasite populations. However, most studies examining castrating parasites in bivalves use a small portion of the gonad, and either record infection status as a binary yes/no (e.g. Valderrama et al. 2004; Baudrimont et al. 2006; Zieritz & Aldridge 2011; Marszewska & Cichy 2015), or provide granular assessments of infection such as ‘low’ or ‘high’ (e.g. Taskinen et al. 1994, 1997; Yanovich 2015). Binary records of infection may not be enough to capture the nuances of host-parasite dynamics, while the granular assessments of intensity are highly subjective and not reproducible, hampering efforts to compare population impacts between studies and locations. A better metric would potentially be a measure of intensity of infection, such as the percentage of the gonad filled with trematode tissue. Such an explicit quantitative measure is likely to correlate directly with the amount of tissue available for mussel and parasite reproduction. In addition, more comprehensive methods such as histology are time-consuming, require a high level of skill, and may still lack objectivity unless a clear quantitative procedure is followed.

In this paper, we present a rapid photograph-based method to quantify the infection level within the gonad of a mollusc. It provides a reproducible measure of the percentage of the gonad filled, and can capture within-host trematode dynamics, by distinguishing between full sporocysts, spent sporocysts and free cercariae. While this technique is exemplified with unionid mussels, we see it as applicable to all marine and freshwater bivalves.

3.2. Methods

3.2.1. Study site and collection

The duck mussel *Anodonta anatina* (Linnaeus 1758) is a common unionid with a pan-European distribution (Lopes-Lima et al. 2017). We collected samples of this mussel on a monthly basis between January and September from the Old West River at Stretham (52.3343° N, 0.2243° E), part of the River Great Ouse system (UK). Exploratory dissections had revealed a proportion of these mussels to be infected with the digenean trematode *Rhipidocotyle campanula* (Dujardin 1845); this trematode produces cercariae from asexual sporocysts. We collected mussels by hand from near the bank, and transported them back to the laboratory in 10 L buckets filled with river water. In the laboratory, we held mussels at 8 °C under aeration, for a maximum of 72 h before assessments of trematode infection. Prior to assessment, we rinsed mussels under cold fresh water while holding the valves gently shut to remove any organisms on the shells, and measured maximum length to the nearest 0.5 mm with Vernier callipers.

3.2.2. Quantification of trematode infection

We sacrificed and dissected mussels by inserting a scalpel between the valves and slicing the posterior and anterior adductor muscles. We gently removed the visceral mass by cutting the connective tissue at each end, and sliced it open using a single cut of a scalpel at the posterior end.

To confirm that a subsample of the gonad will approximate the true percentage of the gonad filled with trematode tissue, we quantified the entire gonad of 10 mussels of variable size. We repeatedly removed ~40 mg samples of gonad tissue with tweezers, and pressed individual samples gently between two glass microscope slides to create a squash ~10 mm in diameter. While this method describes the technique for bivalves, this could equally be applied to the isolated gonad of any mollusc. We repeated this until there was no more gonad tissue left in the visceral mass. We inspected each glass microscope slide thoroughly at 40× magnification using a GXM-L3200 compound microscope. Where present, we captured photos of trematode material in .tif format using a HiChrome-S camera attached to the microscope and the software GX Capture 8.5 (loaded on a standard Windows PC). Each photograph captured

an area of 1630 × 917 μm. We took three photographs for each squash (i.e. a single 40 mg sample), resulting in n photographs (n ranged between 18 and 48, depending on the size of the mussel).

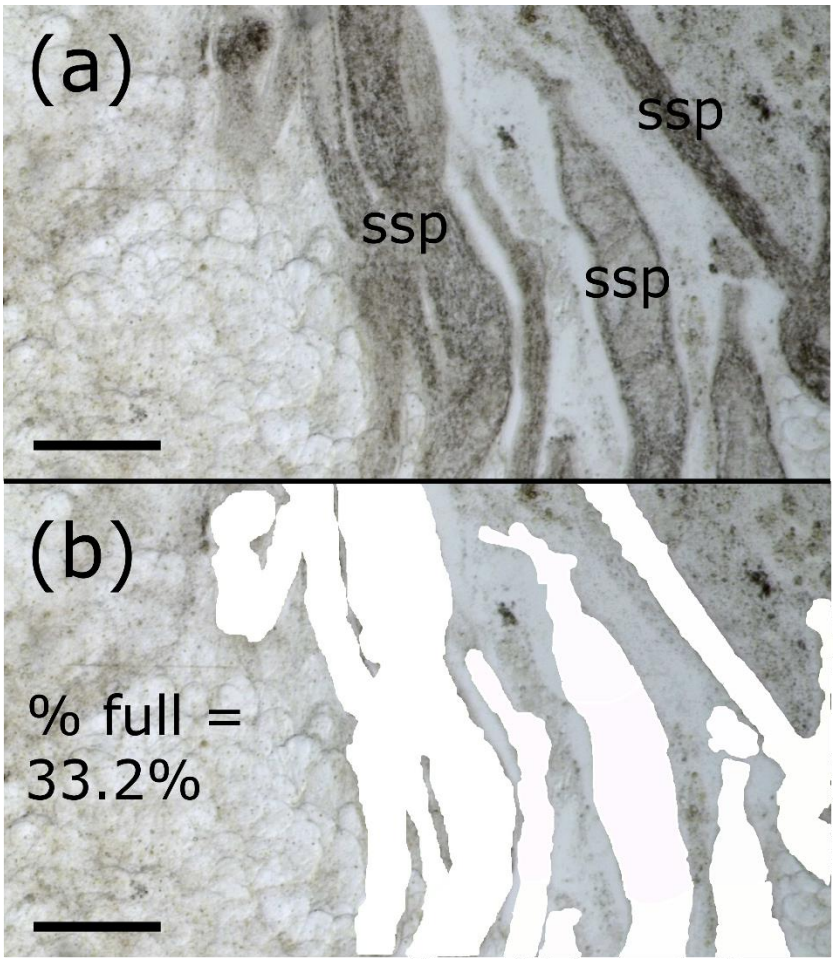


Figure 3.1: Tracing and measuring procedure for a single photograph. Scale bars = 250 μM. (a) An example photo, showing spent sporocysts (ssp). (b) The traced sporocysts and their cumulative area (white), with the corresponding percentage of picture filled with sporocyst.

Based on the extensive dissections and subsampling of the 10 mussels we concluded that assessing 12 photographs from an individual mussel generally provided reliable data on infestation levels (see Results and Discussion). Therefore, for all other mussels, we removed four replicate samples of gonad tissue with tweezers (~40 mg each), and took three photos per sample from a random point in the squash. We took each sample from a different area of the gonad, namely the dorsal and ventral areas on each side of the slice. We loaded each .tif image into ImageJ 2.0.0, and carefully traced around the digenean tissue (sporocysts and cercariae) using the 'Freehand selection' tracing tool. Using the 'Measure' function in ImageJ, we calculated the total area of each trace, and hence the percentage of the photo that

contained trematode material (Fig. 3.1). We averaged this value over all 12 replicate photos, to give the mean percentage of infected gonad for each mussel. This procedure was done for all mussels to confirm the method's appropriateness throughout the year, when the proportion of sporocysts and cercariae may change.

3.2.3. Statistical analysis

All statistical analyses were executed in R v3.5.1 (R Core Team 2018). To confirm that a subsample of the gonad approximated the true percentage of the gonad filled with trematode tissue, we analysed the 10 mussels that had their entire gonad quantified (using `n` photographs) in the following fashion. We calculated the percentage of the gonad filled with trematode for each photo, averaged all photos to the give the true mean percentage of gonad filled for each mussel, and then calculated an associated 99% confidence interval. Subsequently for each mussel, we took a random subsample of photographs without replacement (beginning with 2 photographs, then 3, up to `n` photographs), and calculated the mean percentage of photo filled with trematode. We repeated this procedure 1000 times for each number of photographs, and calculated the proportion of those replicates which had the estimated mean falling inside the 99% confidence interval of the true mean. To understand how this was affected by mussel size, we compared the number of photographs required to have 90% of replicates approximating the true mean with the length of the mussel using linear regression. We checked and confirmed assumptions of linearity, normality and homoscedasticity of residuals using Q-Q and residuals versus predicted values plots.

3.3. Results

Overall, trematode infections of varying intensity were observed in 72 mussels (17.1% of all mussels examined), based on the inspections of the squashes from the gonad dissections (min: 0.2% of gonad filled with trematode tissue; max: 78.1%). Photographs of these squashes produced high-quality images throughout the year that were used to calculate the percentage area of the gonad filled with trematode tissue, and accurately characterise the developmental stages of trematode infection (Fig. 3.2).

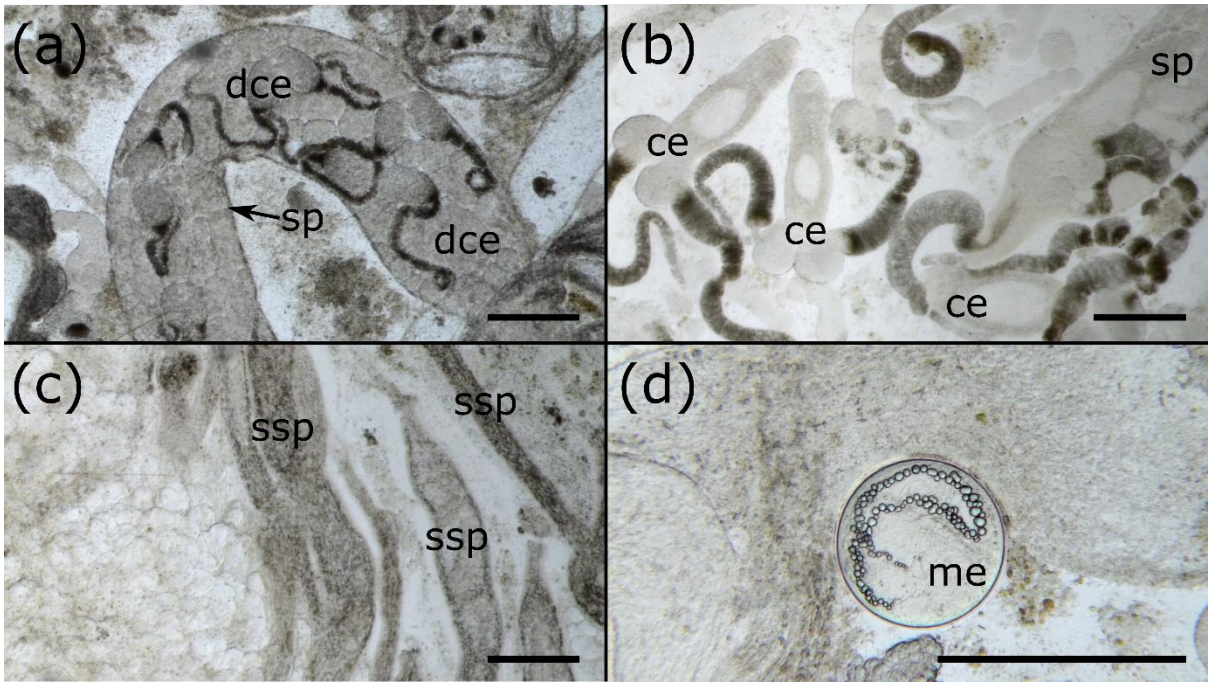


Figure 3.2: Different developmental stages of digenean trematodes occupying *A. anatina*, as captured by photography. All scale bars 250 μM. (a) Full sporocyst (sp) with developing cercariae (dce) inside. (b) Free cercariae (ce), ready to be released and infect a second intermediate host. (c) 'Spent' sporocysts (ssp), having released their cercariae. (d) Metacercariae (me) of echinostomatid trematodes were also occasionally observed, utilising the mussel as a second intermediate host. Small bubbles within the metacercaria are excretory vacuoles.

The amount of trematode tissue present varied between photographs, and shows the utility of replication of photographs for a single mussel. Additionally, as the number of photographs increased, success at estimating the true mean increased rapidly (Fig. 3.3). Fig. 3.3a shows that the number of photographs required to reliably estimate the true mean is dependent on the size of the mussel, with smaller mussels approaching the true mean faster. This trend is confirmed by Fig 3.3b, which demonstrates the significance of this relationship ($R^2 = 0.67$, $p = 0.004$). However, all mussels had at least 90% of replications approximating the true mean after 15 photographs (Fig. 3.3b), and Fig. 3.3 suggests that for the mean mussel size in this study (65mm), 10 photographs are appropriate to estimate the true mean.

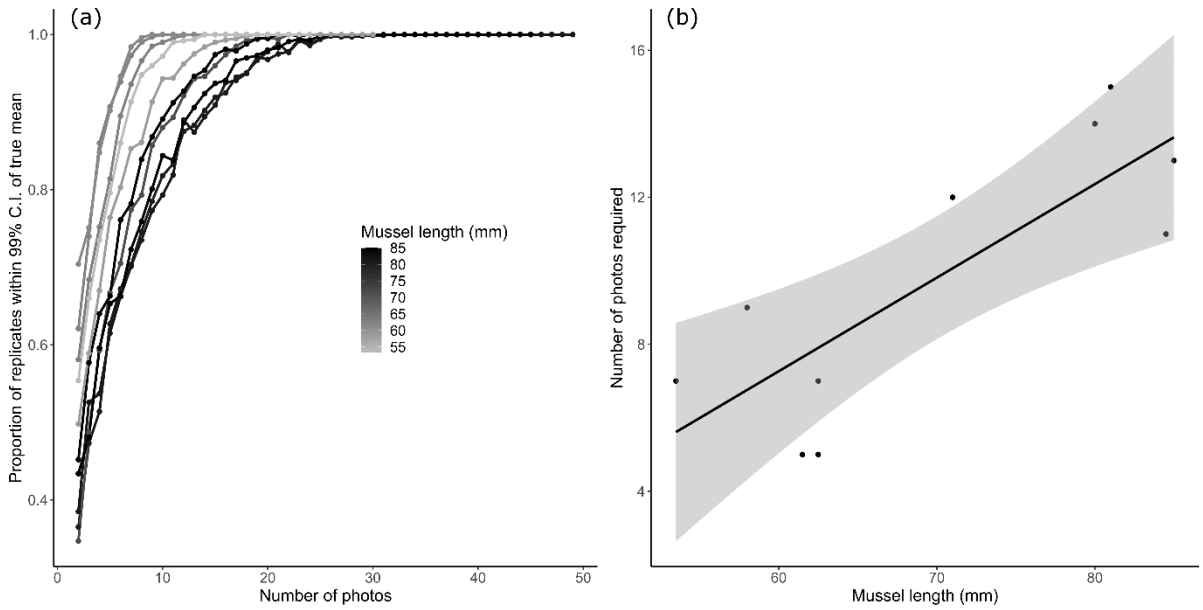


Figure 3.3: Accuracy of the method for mussels of variable size. (a) Relationship between the number of photographs taken (each point represents 1000 random samples of that number of photographs, from the pool of all possible photographs), and the proportion of those 1000 replicates where the estimated mean trematode percentage fell within the 99% confidence interval of the true mean trematode percentage. Each mussel (n = 10) is plotted separately, and shaded according to its length in mm. (b) Linear regression between the length of the mussel, and the number of photos required to have 90% of the replicates approximating the true mean. Shading denotes the 95% confidence interval of the fitted line.

3.4. Discussion

3.4.1. Recommendations

A gonad squash technique has been described for the quantitative assessment of trematode infection in bivalve gonads. This method is fast and simple to use, and provides clear evidence for the presence of trematode infection, in addition to the developmental stage of the trematodes (Fig. 3.2). Furthermore, we have demonstrated that small sub-samples of the gonad rapidly capture the pattern in the whole gonad (Fig. 3.3). We therefore recommend that:

1. the number of photographs taken is determined by the size of the bivalve;
2. the developmental stage of the trematode is reported in addition to the infection intensity;
3. the photographs are stored in an appropriate digital repository;

4. future studies of parasitic castration in bivalves utilise this method to quantitatively assess infection.

The rationale behind these recommendations is briefly discussed below.

3.4.2. Assessing method success

There is a high level of variation evident between photographs within a single mussel. However, variation is not unexpected, given the previously observed uneven distribution of trematode infection within bivalve gonads (Taskinen et al. 1997). However, Fig. 3.3 shows that for an average mussel 65mm long, 10 photographs will reliably estimate the true mean percentage of the gonad filled with trematode tissue. To balance time considerations against predictive power, we have recommended basing the number of photographs on the size of the bivalve, but we note that for all except the largest mussels, a conservative 12 photographs may be an appropriate number to analyse, as this linear relationship could vary between species. While there may still be variation present around estimates, the presented method represents a significant improvement on current quantitative efforts, where intensity is generally classed into a maximum of three categories (e.g. ‘low’, ‘medium’, ‘high’). This technique has an additional advantage in that it is reproducible, assessable by other researchers (e.g. photos can be made publicly available for inspection) and therefore can be used to compare infection prevalence and intensity across multiple studies executed by a diverse range of researchers.

We consider our method to stand up favourably to alternative procedures. Histological examination of gonadal cross-sections has been used to determine the intensity of infection in bivalve-trematode systems (e.g. Lajtner et al. 2008; Ceuta & Boehs 2012). However, we see cross-sections as problematic. As histology uses very thin cross-sections, the resulting image for analysis is reliant on the orientation of the sporocyst in the plane that the section was taken. A transverse section of sporocyst would give a very low area, while a longitudinal section would give a very large area, even though both represent a single sporocyst. Our technique, which takes 3D samples and then gently compresses them, provides a more natural approximation of how much of the gonad is represented by trematode tissue. In addition, it is a lot less labour-intensive than histology, which requires setting of material in paraffin, sectioning and staining (Laruelle et al. 2002). In contrast, our technique can produce images

within five minutes of the mussel being dissected, ready to be analysed at the researcher's convenience, thus increasing efficiency. Estimating the proportion of mollusc biomass contributed by trematodes has also been used to quantify infection, by separating host and parasite tissue (e.g. Preston et al. 2013). However, it can be very difficult to separate host and parasite tissue (Kuris et al. 2008), leading to estimates of parasite mass based on cross-sections of host tissue (e.g. Hechinger et al. 2008), a method that has the potential to result in similar errors to those associated with histology. In addition, weighing such light quantities can be a long and complex procedure (e.g. see Lambden & Johnson 2013). Therefore, the method presented here represents a simpler procedure with at least as much accuracy. There is the potential for the analysis of photographs to be further automated, through the training of computer algorithms to recognise sporocyst tissue. Automation was met with difficulties in the present study (see Appendix A2 and Fig. A2.1), but provides a potential avenue for future development.

3.4.3. Utility of the technique in improving parasite studies

Future studies investigating the effect of parasitism on bivalves could benefit from the method presented in this paper. Currently, when the effects of parasitism on a quantitative variable (e.g. gene expression, phenoloxidase activity, or host fecundity) are explored, parasitic state is often defined simply as 'parasitized' or 'unparasitized' (e.g. Valderrama et al. 2004; Baudrimont et al. 2006; Magalhães et al. 2017). This approach does not accord with evolutionary theory which predicts that at initial infection and low parasite intensity, hosts may invest heavily in reproduction and defence, while at high intensity they will direct resources elsewhere (Hurd 2001; Lafferty & Kuris, 2009). In short, a bivalve that is uninfected and a bivalve that has 5% of their gonad filled are likely to be more physiologically similar than two infected bivalves that have 5% and 95% of their gonad filled, respectively. This point is also supported by experimental data and field observations (Sorensen & Minchella 2001; Munoz et al. 2006). However, studies that explicitly quantify the effect of infection intensity on host or parasite dynamics are rare, and methods like the one presented in this paper should be used wherever possible. We believe it is erroneous to treat parasitism as a binary variable, as infection intensity instead represents a continuum, with variable host responses predicted along it (Kabat 1986). In general, increased parasitic intensity may lead to increased host mortality (Mouritsen & Poulin 2002), making its characterisation crucial to understand the outcomes of parasitism for both individual hosts

and host populations. The technique described in this paper provides a simple way of assessing the level of infection, not just whether it is present or not, and therefore facilitates a more nuanced characterisation of host responses to parasite infection. In addition, the clear ability of the method to distinguish between developmental stages of the trematode should be taken advantage of to understand parasite development both within and between host individuals.

In summary, the method presented facilitates the rapid and realistic quantification of trematode infection in bivalve gonads. Given the important ecological role of bivalves, their conservation status, and their potential as a model host-parasite system, we strongly advocate for the use of this technique to enhance biological understanding in future observational or experimental studies.

Chapter 4: A rapid, non-destructive method for sampling castrating parasites in endangered bivalve molluscs

Abstract

Bivalves are important ecosystem engineers, and there is emerging evidence that many species are afflicted with castrating parasites. Understanding the prevalence of these largely overlooked parasites is crucial in understanding the fundamental biology of bivalves, informing conservation efforts, and providing a wider understanding of host-parasite dynamics. Current techniques to assess the presence of parasites are destructive, making them untenable for endangered or protected populations. Here, we present a non-destructive method of sampling bivalve molluscs (*Anodonta anatina*) to detect castrating trematodes. Gonadal fluid is removed with a hypodermic needle from bivalves *in situ* and analysed in a laboratory setting without removing the mollusc from the field; this sampling mechanism has previously been shown to not harm the mollusc. A single 50 μL sample is sufficient to detect both the presence and developmental stage of the trematode with greater than 95% reliability, with all but the lightest infections visible. We recommend that this technique be used to enhance knowledge on host-parasite dynamics in bivalves, and inform sensible conservation for threatened species.

Key words: conservation evaluation, invertebrates, littoral, monitoring, ocean, river

4.1. Introduction

Bivalve molluscs function as important ecosystem engineers in marine and freshwater ecosystems. Their bioturbation through burrowing increases oxygen content (Vaughn & Hakenkamp 2001), and their shells provide substrate for epibionts and shelter for small benthic organisms (Gutiérrez et al. 2003; Gribben et al. 2009). In addition, they filter large volumes of water (Tankersley & Dimock 1993), increasing nutrient deposition and improving water quality (Hoellein et al. 2017). These roles contribute to stable, highly diverse communities of associated taxa (Aldridge et al. 2007; Spooner et al. 2013; Chowdhury et al. 2016), making the protection of bivalves a significant priority for the wider conservation of aquatic ecosystems.

There is emerging evidence that bivalve populations can be devastated by pathogens (e.g. Katsanevakis et al. 2019), and parasites affect all levels of bivalve ecology (Coen & Bishop 2015). Castrating parasites are of particular concern for endangered or vulnerable populations, as they have a direct impact on the reproductive capacity of an individual (Taskinen & Valtonen 1995; Taskinen et al. 1997). In addition, they can make their host more vulnerable to other threats through altering physiological processes (Baudrimont et al. 2006). Both marine and freshwater bivalves are commonly afflicted by castrating parasites (Averbuj & Cremonte 2010; Chapter 2), and many bivalves are also endangered (see Lopes-Lima et al. 2018). Therefore, assessments of castrating parasites in endangered populations are highly desirable, facilitating an understanding of the full range of threats facing bivalves and how best to conserve them in future (Chittick et al. 2001). For example, failing to recognise the presence of a cryptic castrating parasite could lead to over-estimates of fecundity and therefore under-predict the necessity of conservation steps for a population.

Typically, assessing whether or not individuals are infected with castrating parasites requires destructive practices, such as squashing gonad tissue between plates or histological analysis. This has resulted in a significant under-studying of endangered or protected species (see Chapter 2). When they have been studied, it has only been in very small numbers, limiting the ability to detect parasites (e.g. Chittick et al. 2001). However, previous studies have shown that sex of mussels can be accurately determined by a non-destructive removal of gonadal fluid with a fine-gauge needle (Saha & Layzer 2008; Tsakiris et al. 2016); such a technique has also been shown to recover trematode tissue (Galbraith & Vaughn 2011; Zieritz & Aldridge 2011). This potentially provides a way to non-destructively sample parasites in the gonad of vulnerable populations. However, it has not been demonstrated that this will reliably recover trematode tissue from an infected mussel.

In addition to sexing, non-destructive methods have also been developed for sampling mantle tissue (Berg et al. 1995) and larval mussels from female gills (Beaver et al. 2019). This reveals both a recognition that non-destructive methods are desirable, and the need to characterise population trends in freshwater mussels, given their generally threatened conservation status and important ecosystem engineering effects. Here, we present a qualitative method for the non-destructive sampling of endangered or protected species. This method adapts the previously developed framework for sexing mussels, and reliably

identifies bivalves infected with castrating parasites. While exemplified with a freshwater unionid mussel, this technique could be applied to any freshwater or marine bivalve.

4.2. Methods

4.2.1. Study species and collection

The duck mussel *Anodonta anatina* (Linnaeus 1758) was sampled from the Old West River at Stretham (52.3343° N, 0.2243°E), a lower reach of the River Great Ouse (UK). Previous dissections (Chapters 3, 5) had revealed this population of mussels to host the trematode *Rhipidocotyle campanula* (Dujardin 1845). This parasite utilises the mussel as a first intermediate host, with asexually-spreading sporocysts producing cercariae, a process which rapidly induces castration in the host mussel (Taskinen et al. 1997). Cercariae are released from the mussel to infect the second intermediate host, most frequently the common roach *Rutilus rutilus*. Over 30 definitive hosts have been recorded (Strona et al. 2013), though commonly it infects the perch *Perca fluviatilis* (Taskinen et al. 1991; Gibson et al. 1992). However, research has not yet been done on specific fish hosts in the River Great Ouse.

Sixty mussels were collected in each of May, June and July 2019. Mussels were transported to the laboratory in 10 L buckets containing river water, and held at 8 °C with constant aeration, for up to 72 hours before analysis. Directly before analysis, mussels were rinsed in cold fresh water while holding the valves shut gently, to remove any organisms on the shells.

4.2.2. Gonadal fluid extraction

An extraction of fluid with a fine-gauge needle was used to non-destructively sample the gonad of all 180 mussels. This extraction used a procedure modified from Tsakiris et al. (2016) which was originally developed to assess gamete production. The shell of a mussel was opened on the ventral side by inserting the tip of a blunt scalpel and twisting gently, to provide a small gap that a finger could be inserted into to hold the mussel open. An 18-gauge hypodermic needle attached to a syringe was used to take up a small amount of tap water (~50 μL), and the needle was then inserted into the gap in the shell, through the foot and into the posterior side of the visceral mass where the gonads are located (verified through previous exploratory dissection) (Fig. 4.1). The syringe was gently depressed to insert the

water, and suction was then applied, in conjunction with moving the end of the needle slowly back and forth within the gonad, to loosen fluid. The water insertion was necessary as often the gonadal fluid by itself was too viscous to remove. A small amount of fluid (~100 – 200 μL) was extracted and placed in 300 μL of 10% buffered formalin. These samples were refrigerated at 4 °C and examined the following week. The needle was changed between each mussel to ensure sterility. Previous work (Saha & Layzer 2008; Tsakiris et al. 2016) has shown that long-term mussel survival is not compromised by the insertion of a needle.

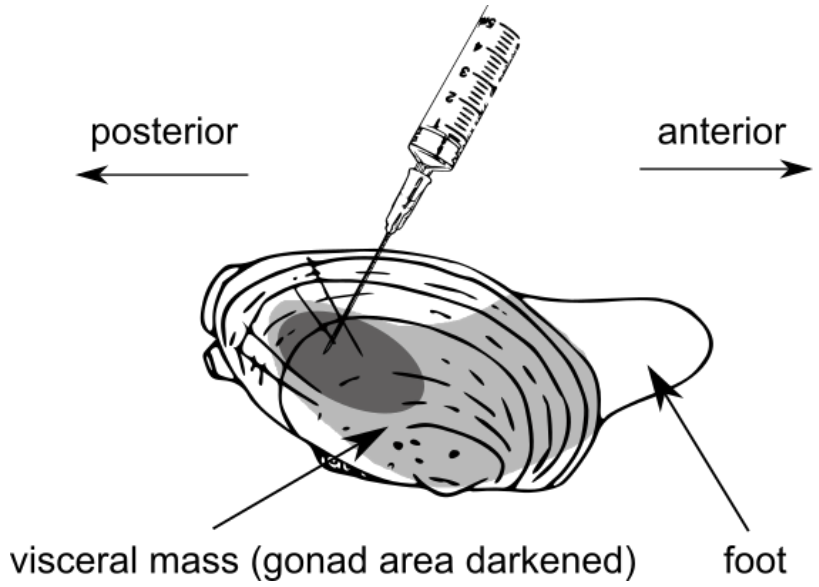


Figure 4.1: The mussel is gently opened with a blunt scalpel, and a hypodermic needle is inserted between the two valves to remove gonadal fluid from the posterior end of the visceral mass.

Following the removal of gonadal fluid, the mussel was sacrificed by cutting the posterior and anterior adductor muscles and the visceral mass was removed. The gonad was then examined for trematodes and, if present, the percentage of the gonad filled with trematodes was calculated, through squashing gonad samples between two slides and calculating the mean trematode area in replicate photographs (Chapter 3).

4.2.3. Microscopy and analysis

Repeated 50 μL subsamples (8-10) of formalin-fixed gonadal fluid from each mussel were placed on a glass microscope slide with a pipette, and inspected thoroughly at 40× magnification using a GXM-L3200 compound microscope. Photographs were also taken to record the trematode material (where present). Images (1630 × 917 μm) were captured in .tif

format using a HiChrome-S camera attached to the microscope and the software GX Capture 8.5.

To assess the success of the needle procedure (as a binary of successful/not successful), the formalin-stored gonadal fluid samples were cross-referenced with the results of the full gonad dissection (which definitively diagnosed trematode infection). Success rate of the needle procedure was expressed as a percentage (number of fluid samples that recovered trematode material, divided by the total number of dissections that revealed trematode infection, multiplied by 100), and a 1-sample proportions test was executed in R v.3.6.3 (R Core Team 2020) to verify the effectiveness of the procedure.

4.3. Results

Of the 180 mussels that were sampled between May and July, 29 were revealed to have trematodes by the full gonad dissection. The success rate of the gonadal fluid extraction to detect trematodes was 96.6% (with the 95% confidence interval being 82.2% - 99.9%), i.e. in 28 of the 29 cases, trematode material was recovered and identified using the non-destructive technique. Therefore, the probability of successfully diagnosing infection was clearly higher than would be predicted by chance ($p < 0.0001$). The only sample which was unsuccessful was from a very light infection in May, where a single small sporocyst was observed in the full gonad dissection (area of gonad covered by trematode tissue: 0.24%). In contrast, the technique was successful for all other infection intensities, which ranged from 3.1% to 62.8% of the gonad filled with trematode tissue (31.8% ± 19.2%, mean ± s.d.). Success did not depend on the overall level of trematode prevalence in the population (Fig. 4.2). In all cases where trematode material was recovered from the gonadal fluid, the first 50 µL examined was sufficient to determine the presence or absence of trematode infection. In addition, no false positives were observed (i.e. observing supposed trematode material despite gonad dissection showing the mussel to be uninfected; Fig. 4.2).

Further, the developmental stage of the trematode was also evident from the first 50 µL sample of the gonadal fluid extraction. In all cases where free cercariae were observed in the full gonad dissection, these were also observed in the samples extracted by needle (Fig. 4.3b). Large sporocyst sections and sporocyst fragments were frequently observed (Fig. 4.3).

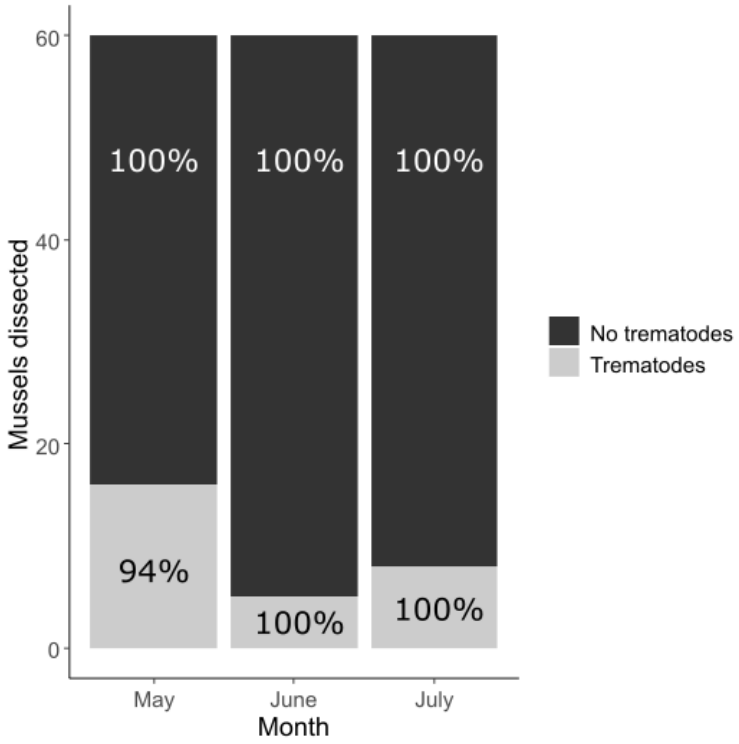


Figure 4.2: Summary of 180 mussels assessed using needle procedure across three months. Percentages within bars indicate the success rate of the needle procedure to characterise the mussel as parasitised or not (with 'true' status being determined by full dissection).

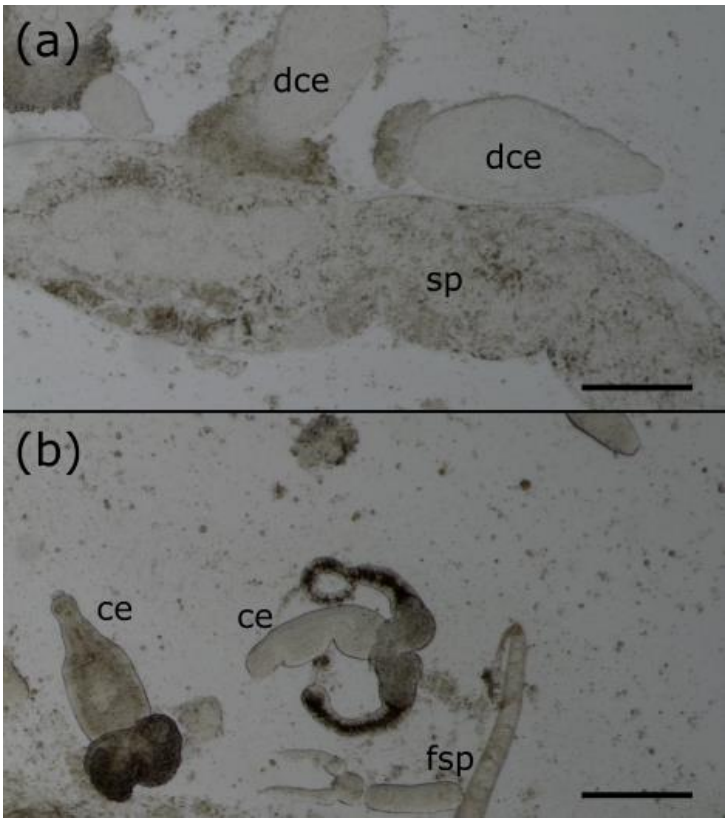


Figure 4.3: Examples of non-destructive fluid extractions from the gonad of *A. anatina*. Both scale bars 250 μM. (a) Large sporocyst section (sp), in addition to developing cercariae (dce). (b) Developed cercariae (ce) and sporocyst fragments (fsp).

4.4. Discussion

4.4.1. Utility of the technique

This technique has been shown to successfully detect trematode infection in bivalve gonads, in addition to identifying the developmental stage of the trematode. We therefore recommend that this technique is used in the field, in particular for the following scenarios:

1. In studies examining the population structure or decline of vulnerable or endangered bivalve molluscs;
2. For testing the source and recipient populations of any translocations of bivalve species, to avoid the spread of potentially harmful castrators;
3. For the rapid *in situ* assessment of infection for any bivalve mollusc.

Microcentrifuge tubes can be prepared with formalin before entering the field, and then transported back for analysis once the gonadal fluid has been added, thus allowing for rapid *in situ* sampling of threatened populations. Once the formalin-preserved gonadal fluid has been returned to the laboratory, only a single 50 μL sample of that fluid needs to be examined to confirm trematode presence.

This technique has advantages over other non-destructive assessments of trematode infection. Previously, non-destructive assessment has been achieved through monitoring of cercarial release in the laboratory (e.g. Taskinen 1998a; Choo & Taskinen 2015; Magalhães et al. 2017). However, recent work (Magalhães et al. 2017, 2019) has shown that this results in estimates of infection rates between five and 10 times less than reality, as determined by destructively dissecting the gonad. There is the possibility for cercarial monitoring to reach high accuracy: at elevated temperatures, Choo and Taskinen (2015) showed this technique could reliably diagnose infection. However, using ambient temperature, only one quarter of infected mussels were successfully identified. In contrast, our method reliably detects trematode infection and development stage in all but very low levels of infection, and does not necessitate removing organisms from the field for an extended period of time. In addition, cercarial release is frequently seasonal (Taskinen et al. 1994) and therefore estimates of infection using cercarial release can only be used at certain times of the year. The needle

technique can be utilised year-round, and can accurately predict whether sporocysts, cercariae or both are present (Figs 3.2, 3.3).

This method is exemplified using the trematode *R. campanula*. This trematode has previously been shown to specifically target the gonad with reasonably heavy infections (Taskinen et al. 1994, 1997). In contrast, the same authors showed that the closely-related *R. fennica* can target other organs and may not always be found in the gonad; when it is, infection intensity is typically lower. Therefore, using *R. campanula* could potentially over-predict the success of this technique, relative to other trematodes. However, our results show that this method can detect infection at intensities as low as 3% of the gonad filled with trematode tissue. Further, while infection in other organs could have detrimental health consequences, it is unlikely to have the immediate population-level impact that castration does. Therefore, we believe our method is appropriate in a conservation context for assessing trematode infection, regardless of trematode species, though we acknowledge that the method has only been tested on one such species.

While this method is non-destructive, there is the potential for the health of the mussel to be compromised through the insertion of a needle. However, the authors of the needle procedures for gamete assessment have conducted long-term monitoring of needled individuals, and shown that bivalves suffer no ill effects from the procedure, or any future loss in gamete production (Saha & Layzer, 2008; Tsakiris et al. 2016). Therefore, this technique should be appropriate for the monitoring of trematode infection in vulnerable populations.

4.4.2. Potential conservation applications

The non-destructive extraction of gonadal fluid provides a particularly useful way of generating large sample sizes to assess parasite impacts on endangered species. Interestingly, there is also the potential for it to be used on preserved historical museum samples, to understand how prevalence may have changed through time, and to identify possible drivers of temporal patterns in parasitism. These questions could have real management implications: with the increasing trend of translocation schemes to bolster endangered populations (e.g. Thomas et al. 2010), castrating parasites could potentially be transported between populations, which may have a range of unpredictable effects (Northover et al. 2018) and

could compromise conservation actions (see Chapter 9). Examining gonadal fluid provides a rapid and non-lethal way of assessing the possibility of inadvertently introducing trematodes to a naïve population or *vice versa*, and could improve long-term outcomes for species. Further, genetic analysis on material extracted using this method could be used for population genetic studies of trematodes, as markers for *R. campanula* and related species have previously been developed and applied (Petkevičiūtė et al. 2014).

This technique can also be used to improve investigations on the effect of trematodes utilising molluscs as first intermediate hosts. While many studies have assessed the effect of metacercariae on molluscs experimentally (molluscs as the second intermediate host, e.g. Stier, Drent & Thieltges 2015; O'Connell-Milne et al. 2016), few studies do the same for trematode sporocysts and cercariae, because miricidia (the infective stage) are exceptionally difficult to successfully isolate and introduce into molluscs (see Richardson 1990). Therefore, studies comparing the effect of parasitized and unparasitized individuals are largely observational, and may erroneously conflate cause and effect. For example, studies that record lower growth rates among individuals that, at the end of the monitoring period are revealed to be infected with sporocysts, may conclude that the trematode infection hampered growth (e.g. Taskinen 1998b). However, perhaps those individuals were lower-quality initially (and therefore they would have suffered reduced growth anyway), and as a correlate they were disproportionately vulnerable to infection. Understanding infection status at the start of the experiment facilitates a clearer disentangling of the effects of parasitism versus other effects, and allows a more accurate characterisation of host-parasite interactions and outcomes through the period of the experiment. In addition, utilising the method described in this paper allows a clear assignment of individuals into different treatment groups (for example, being able to assess the impact of an additional environmental stressor on parasitized and unparasitized mussels). While experimental infection is the ideal scenario, our method allows increased rigour in studies of parasitic castration.

A first step in conservation is to gather all appropriate information on a species (Margules & Pressey 2000). We argue this should include the parasite fauna of a species, especially when parasites that decrease host fecundity can have a major impact on the host population (McCallum & Dobson 1995). There are many examples of both endangered and non-endangered species suffering severe population reductions due to a parasite (e.g. Mouritsen et al. 1998; Zhang et al. 2008), including among molluscs (Katsanevakis et al. 2019). To predict

and potentially ameliorate such outcomes, parasites should be sampled as comprehensively as possible. Therefore, rapid and non-destructive methods such as the one presented here should be prioritised in future conservation assessments or interventions for both vulnerable and currently non-threatened species. This is particularly important in the face of environmental change. Choo and Taskinen (2015) showed that trematode cercarial release increases with temperature; such a mechanism has previously been implicated in the spread of a devastating oyster parasite (Ford 1996). Understanding parasite prevalence rates in current populations is crucial therefore to not only appreciate conservation risk in the present, but also to predict risk in future environmental scenarios.

The technique described in this note provides a rapid, reliable and non-destructive way of determining trematode presence, as well as the developmental stage of the trematode. We have shown that it compares favourably with currently existing methods, and have explored how it could be utilised to improve the understanding of mollusc-trematode interactions. Given the long-recognised enigmatic declines and threatened state of these important fauna (Bogan 1993; Lydeard et al. 2004; Smith et al. 2006), understanding internal biology whilst avoiding sacrificing mussels is a vital step in the conservation of these threatened species. We recommend that this simple technique is used to inform future conservation efforts.

Chapter 5: Abundance data applied to a novel model invertebrate host shed new light on parasite community assembly in nature

Abstract

Understanding how environmental drivers influence the assembly of parasite communities, in addition to how parasites may interact at an infracommunity level, are fundamental requirements for the study of parasite ecology. Knowledge of how parasite communities are assembled will help to predict the risk of parasitism for hosts, and model how parasite communities may change under variable conditions. However, studies frequently rely on presence-absence data and examine multiple host species or sites, metrics which may be too coarse to characterise nuanced within-host patterns. We utilised a novel host system, the freshwater mussel *Anodonta anatina*, to investigate the drivers of community structure and explore parasite interactions. In addition, we aimed to highlight consistencies and inconsistencies between presence-absence and abundance data. Our analysis incorporated 14 parasite taxa and 720 replicate infracommunities. Using redundancy analysis, a joint species distribution model and a Markov random fields approach, we modelled the impact of both host-level and environment-level characteristics on parasite structure, as well as parasite-parasite correlations after accounting for all other factors. This approach was repeated for both the presence and abundance of all parasites. We demonstrated that the regional species pool, individual host characteristics (mussel length and gravidity) and predicted parasite-parasite interactions are all important but to varying degrees across parasite species, suggesting that applying generalities to parasite community construction is too simplistic. Further, we showed that presence-absence data fails to capture important density-dependent effects of parasite load for parasites with high abundance, and in general performs poorly for high-intensity parasites. Host and parasite traits, as well as broader environmental factors, all contribute to parasite community structure, emphasising that an integrated approach is required to study community assembly. However, care must be taken with the data used to infer patterns, as presence-absence data may lead to incorrect ecological inference.

Key words: abundance, biotic interactions, freshwater mussel, infracommunity, Joint Species Distribution Modelling, Markov Random Fields, presence-absence, Redundancy Analysis

5.1. Introduction

Predicting the distribution and abundance of organisms is a central goal of ecology (Gravel et al. 2006). Because species do not exist in isolation, understanding how ecological communities are assembled and maintained has been and continues to be of large interest (e.g. Gleason 1927; Clements 1936; Diamond 1975; Tilman 1977; Hubbell 1997). Elucidating the factors determining community assembly is important for the management and conservation of species, and for predicting how communities may respond to disturbance such as establishment of an invasive species or large-scale environmental change (e.g. Davis et al. 2019).

Community assembly is governed by a series of discrete processes. Initially, the opportunity for individuals to enter a community is determined by the distribution of species and their relative frequencies in the metacommunity landscape, the regional species pool (Chase & Myers 2011). The likelihood of a species entering a community will then be determined by a combination of dispersal from that regional species pool, ecological selection and drift (Vellend 2010). Ecological selection occurs at the individual community level, and can broadly be divided into habitat suitability (abiotic factors) and species interactions (biotic factors) (Catford et al. 2009; Thompson et al. 2020). Despite this well-recognised framework, the relative importance of these factors and an understanding of the relationships between within-community and between-community dynamics remains under-developed (Thompson et al. 2020). In addition, community dynamics are regularly inferred from presence-absence data, which may poorly capture individual species responses (Blanchet et al. 2020); for example, one species may not preclude a second species from successfully establishing in a community, but could limit its abundance. Therefore, abundance data is crucial to capture the nuances of species responses and further our understanding of community assembly theory.

Individual organisms can also contain a community of parasitic or pathogenic taxa (Telfer et al. 2010; Rynkiewicz et al. 2015). This makes parasites an appealing and highly replicated system for understanding community dynamics, with hosts possessing a discrete parasite community (an ‘infracommunity’ in the parasite literature; Bush et al. 1997), and the host community representing the parasite metacommunity landscape (a ‘component community’). In addition, understanding the drivers of these communities is crucial (Budischak et al. 2016; Rynkiewicz et al. 2019) to enable predictions based on host and environmental characteristics

(Johnson et al. 2015). This allows an analysis of the possible effects of parasite communities on the host organism and the wider host population.

Assessing parasite community assembly and interactions is a challenge. Ideally, the whole suite of an organism's parasites should be studied to accurately map community patterns and understand the effect on the host (Vaumourin et al. 2015; Fountain-Jones et al. 2019). However, until recently, only one-to-one parasite interactions have been explored (Griffiths et al. 2014; Hellard et al. 2015). When entire parasite communities are considered, analysis frequently spans multiple host species and sites, which are often found as the greatest determinants of parasite community structure (e.g. Vidal-Martinez & Poulin 2003; Dallas & Presley 2014; Dallas et al. 2019). These studies incorporate multiple parasite component communities, making it difficult to disentangle factors driving infracommunity structure from those driving the size of regional species pools or dispersal between host communities (Thompson et al. 2020). As all parasite community data trends (among host species or sites) are driven by infracommunity-level interactions (Bush et al. 1997; Pederson & Fenton 2007), understanding influences on infracommunity structure is key to the study of parasite community ecology. However, there is still little research with this focus (but see Pilosof et al. 2015; Moss et al. 2020).

When parasite communities have been studied, investigations overwhelmingly focus on mammalian hosts, particularly rodents and ruminants (e.g. Watve & Sukumar 1995; Telfer et al. 2010; Pilosof et al. 2015; Dallas et al. 2019; see review in Ezenwa 2016). In contrast, invertebrates have been severely neglected in parasite community studies (Wilson et al. 2015). However, invertebrates provide several opportunities that mammals or vertebrates lack. The use of mammals generally necessitates the use of presence-absence data (Hellard et al. 2015), as destructive quantitative sampling is often untenable. This means that the key tenet of ecology - to understand distribution and *abundance* – can only ever be half-addressed. However, consideration of abundance is crucial, both for parasites' effects on hosts and also how the parasites may interact (Budischak et al. 2016; Rynkiewicz et al. 2019). Interaction strengths are an important component of community structure, and theoretical work has even shown that the direction of parasite interactions may reverse based on the abundance of one of the partners (Fenton 2013). In contrast to mammals, the relatively simple body plan of invertebrates can facilitate effective sampling of parasite load, rather than just their presence. In addition, invertebrates frequently harbour more macroparasites than their

vertebrate counterparts (Wilson et al. 2015), providing diverse communities to study. Finally, our understanding of parasite and disease ecology in invertebrates, and particularly aquatic invertebrates, lags behind terrestrial systems (Byers 2020), despite being a significant conservation threat (e.g. Katsanevakis et al. 2019). Therefore, further studies with a focus on aquatic invertebrates allows for development of parasite community assembly theory in this key group of organisms.

This paper introduces and takes advantage of one such invertebrate system. The duck mussel *Anodonta anatina* is a sessile unionid bivalve found commonly in freshwater environments around Europe. It is infected by a broad range of parasites from multiple phyla (Chapter 2), and it has clear discrete tissues (e.g. mantle, gills, gonad) that are each targeted by multiple parasites, analogous to niches in typical communities. Focusing on this species at a single site, we examine factors relating to the composition of replicate infracommunities, providing a sound theoretical basis for more extensive work across larger scales. Rather than focus on a subset of parasites, our analysis incorporates both presence-absence and abundance information for all parasites observed in *A. anatina* over the course of a full year's sampling. By modelling a range of potential environmental drivers and parasite-parasite interactions using both presence-absence and abundance frameworks, we show that different parasites respond to different factors. Further, we explicitly highlight the differences between abundance and presence-absence data, demonstrating that accounting for the abundance of parasites can lead to very different conclusions about environmental drivers and parasite interactions than if presence-absence data are used.

5.2. Methods

5.2.1. Study system and sampling

Unionid mussels host a broad range of parasites (Chapter 2). Common parasites include mites (e.g. *Unionicola intermedia*), which may infect the mantle as both eggs laid by adults, and as later larval and nymphal stages. There are also a broad range of trematodes, which utilise the mussels as first intermediate hosts in the form of sporocysts and cercariae (bucephalid trematodes e.g. *Rhipidocotyle campanula*), second intermediate hosts in the form of metacercariae (echinostomatid trematodes e.g. *Echinoparyphium recurvatum*), or as the only

hosts in adult form (aspidogastrean trematodes e.g. *Aspidogaster conchicola*). Bucephalid and echinostomatid trematodes occupy the gonad, and may castrate their hosts (Taskinen & Valtonen 1995). In addition, bucephalid infections are typically persistent and can last the lifetime of the host. Mussels host a range of ciliates in the mantle and gills, which can affect mussel reproduction (e.g. Lynn et al. 2018), and are also seasonally infected by bitterling fish (*Rhodeus amarus*) embryos, which are laid by female fish in the gills. Other taxa such as dorylaimid nematodes, chironomid larvae, oligochaete worms and amphipods can also be found inside the mantle of unionid mussels. We refer to all organisms in the present study as 'parasites', though it should be acknowledged that the pathogenicity of these latter taxa is not established. For full details about life history of parasites observed in the present study, see Appendix A3 Part 1.

Sixty individuals of the duck mussel *Anodonta anatina* (Linnaeus 1758) were sampled from the same location at the Old West River at Stretham (52.3343° N, 0.2243°E), a lower reach of the River Great Ouse (UK), at monthly intervals from February 2019 to February 2020 (12 collections, N = 720 mussels). Mussels were sampled by hand from the river margin. This sampling size (60) was selected as it gives a >95% chance of detecting parasites present in the mussel population at 5% prevalence (Grizzle & Brunner 2009). This monthly sampling did not affect the overall density of mussels present, as the time taken to collect 60 mussels did not vary from month to month. The size distribution of mussels was also unaffected by the sampling regime (63.9 ± 11.1 mm [overall mean ± 1 s.d.], see Fig. A3.1). Mussels were transported to the laboratory, and were dissected systematically to identify and count all parasites present (Appendix A3 Part 1; Figs. A3.2 – A3.8).

5.2.2. Analysis variables

Altogether, 14 parasite species were included in the analyses. As parasites may respond differently to environmental variables based on species-specific traits, we included two categorical parasite traits (Table 5.1): life history (whether the mussel is the only host, or whether the parasite utilises multiple hosts), and location (whether the parasite is present in the gills, mantle, or gonad of the mussel). These trait values were utilised in the Joint Species Distribution Modelling (see below).

Table 5.1: All parasites >1% prevalence identified across 720 *A. anatina*. To capture all possible interactions, species are included as multiple entries if they occur independently in multiple forms or in multiple host tissues (i.e. morphospecies, see Appendix A3 Part 1). Mean intensity was calculated only for those mussels with that particular parasite present (Bush et al. 1997). Intensity is a direct count of the number of that parasite in a given host, except for *R. campanula* in the gonad, which was measured as the percentage of the gonad filled with trematode tissue. Life history describes whether the mussel is the only host of the parasite (Single) or whether other hosts are utilised in the parasite life cycle (Multiple), while Location describes the location in the mussel that the parasite is found (mantle, gills or gonad). S. E. = standard error.

Parasite	Phylum, Class	Prevalence (%)	Mean intensity ± S. E. (min, max)	Life history	Location
Conchophthirus sp. (mantle)	Ciliophora, Oligohymenophorea	96.8	58.3 ± 2.4 (1, 559)	Single	Mantle
Conchophthirus sp. (gonad)	Ciliophora, Oligohymenophorea	30.0	3.8 ± 0.3 (1, 34)	Single	Gonad
Tetrahymena sp.	Ciliophora, Oligohymenophorea	68.9	4.7 ± 0.2 (1, 34)	Single	Gills
Unionicola intermedia (mites)	Arthropoda, Arachnida	67.6	16.3 ± 0.9 (1, 155)	Multiple	Mantle
Unionicola intermedia (eggs)	Arthropoda, Arachnida	73.1	15.4 ± 0.9 (1, 158)	Multiple	Mantle
Aspidogaster conchicola	Platyhelminthes, Trematoda	46.7	1.5 ± 0.04 (1, 5)	Single	Mantle
Rhipidocotyle campanula (gonad)	Platyhelminthes, Trematoda	16.1	31.7 ± 1.7 (1, 78)[a]	Multiple	Gonad
Rhipidocotyle campanula (gills)	Platyhelminthes, Trematoda	12.5	5.0 ± 0.4 (1, 16)	Multiple	Gills
Echinoparyphium recurvatum	Platyhelminthes, Trematoda	20.3	2.7 ± 0.3 (1, 38)	Multiple	Gonad
Rhodeus amarus	Chordata, Actinopterygii	3.1	1.5 ± 0.1 (1, 3)	Single	Gills
Chironominae	Arthropoda, Insecta	10.0	1.2 ± 0.05 (1, 3)	Single	Mantle
Dorylaimida	Nematoda, Enoplea	20.0	1.3 ± 0.05 (1, 4)	Single	Mantle
Tubifex tubifex	Annelida, Clitellata	1.7	1 ± 0 (1, 1)	Single	Mantle
Senticaudata	Arthropoda, Malacostraca	1.4	1 ± 0 (1, 1)	Single	Mantle

Analysis also incorporated five environmental factors as explanatory variables, in two broad categories. The first category of environmental variables ('dispersal influencers') are those which could affect a given parasite reaching the mussel. The first of these factors is month of the year (12 levels), which may influence the regional pool of parasite infective stages. The

second is whether invasive zebra mussels (*Dreissena polymorpha*) were attached to the host shell (yes/no), as this may interfere with the dispersal of parasite life history stages into the mussel. The second category of environmental variables ('habitat suitability') are characteristics of the individual host mussel: length (mm), gravid status (yes/no), and weight. Gravidity refers to the phenomenon of freshwater mussels brooding larval mussels for a period of time in specialist tubes (marsupia) in the outer demibranchs of their gills. A male/female designation was not made, as 94.3% of mussels displayed the female characteristic of marsupia in the outer demibranchs; hence there was likely a significant number of hermaphrodites. Mussel weight was regressed against length given the two variables covaried ($R^2 = 0.91$); the residuals from this model (in g) were used as the explanatory variable 'weight', to account for weight differences between mussels not explained by length. Therefore, our measured variables align closely with the described drivers of community assembly (regional species pool composition, dispersal, and habitat suitability), with species interactions subsequently being inferred from residual species associations after accounting for these variables (see below).

5.2.3. Statistical analysis

Modelling consisted of three complementary methods: Redundancy Analysis, Joint Species Distribution Modelling, and Markov Random Fields analysis. Research has shown that multiple approaches should be used to detect community drivers, as methods often have different sensitivities to environmental variables or the nature of the response data (Norberg et al. 2019; Ovaskainen et al. 2019). All analyses were carried out using R v.3.6.3 (R Core Team 2020). Redundancy Analysis was executed using the package `vegan` (Oksanen et al. 2019), Joint Species Distribution Modelling was executed with `Hmsc` (Tikhonov et al. 2019), while Markov Random Field modelling was executed with `MRFcov` (Clark et al. 2018). For all three, modelling utilised the following three matrices. (a) The matrix $\mathbf{Y_{AB}}$[1], which consists of abundance records for all 14 parasite types in 720 sampling units (individual mussels). This was used for the abundance (AB) models. (b) The matrix $\mathbf{Y_{PA}}$, in which $\mathbf{Y_{AB}}$ was modified to only contain zeroes and ones (i.e. records presence-absence only). This was used

[1] The matrices $\mathbf{Y_{AB}}$, **X** and **T** discussed in these methods, as well as code supporting these analyses, can be found at: Brian, J. I., & Aldridge, D. C. (2021). Data from: Abundance data applied to a novel model invertebrate host sheds new light on parasite community assembly in nature. Dryad Digital Repository, https://doi.org/10.5061/dryad.bnzs7h498

for the presence-absence (PA) models. (c) The environmental covariate matrix **X**, which consists of values for the five environmental variables for the corresponding mussels. All procedures outlined below were executed for both the $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$ matrices.

5.2.3.1. Redundancy analysis (RDA)

RDA (Rao 1964) is a form of constrained canonical ordination, and is the traditional method for examining multi-species responses to environmental variables (Tikhonov et al. 2019). Prior to analysis, $\mathbf{Y_{AB}}$ was Hellinger-transformed to make the resulting matrix Euclidean (necessary for RDA); of available transformations, this typically performs best (Blanchet et al. 2014). No transformation is necessary for $\mathbf{Y_{PA}}$ (Blanchet et al. 2014), though to confirm this an RDA was also run with a Hellinger-transformed presence-absence matrix; there was a <0.2% difference in explanatory power between the two. The results from the untransformed $\mathbf{Y_{PA}}$ are therefore presented.

A standard RDA of $\mathbf{Y_{AB}} \sim \mathbf{X}$ ($\mathbf{Y_{PA}} \sim \mathbf{X}$ respectively) with all default options was performed. Global significance of the model was tested using a permutation test with 999 permutations. To test whether both 'habitat suitability' and 'dispersal influencers' are important correlates of community structure, partial RDAs were executed. The three variables comprising habitat suitability were tested for significance, holding the two variables of dispersal influencers constant in the model, and *vice versa*. Finally, forward selection using AIC was executed to determine the specific variables contributing the significant results found from the tests above (see Results). If model AIC values differed by <2, the most parsimonious model was selected (Burnham & Anderson 2004). The explained variation in the global RDA model was then partitioned among those variables found significant by forward selection.

To check for multicollinearity, Variance Inflation Factors were calculated for each explanatory variable in **X** (and each level for categorical variables). All VIFs were <2, confirming an absence of linear dependencies.

5.2.3.2. Joint Species Distribution Modelling (JSDM)

JSDMs were implemented in the Hierarchical Modelling of Species Communities (HMSC) framework (Ovaskainen et al. 2017; Tikhonov et al. 2019); this framework performed the

best of any JSDM in extensive simulations (Norberg et al. 2019). This method uses Bayesian estimation to predict individual species' responses to environmental space in a unified framework, in addition to how species-specific traits influence these responses. Alongside the $\mathbf{Y_{AB}}$, $\mathbf{Y_{PA}}$ and **X** matrices described above, modelling also incorporated the trait matrix **T**, describing the values of the two traits for each parasite species (final two columns of Table 5.1). This matrix can be used to test for the possible influence of these traits on species' responses to **X**.

The model was constructed and run with default `Hmsc` priors (Tikhonov et al. 2019) using 2 MCMC chains, each of 300,000 samples, with the first third of each chain discarded as burn-in and the remainder thinned to every 200th sample. For $\mathbf{Y_{AB}}$, parasites were modelled using a log-normal Poisson distribution, except for *T. tubifex* and Senticaudata, which were modelled using probit regression given they had a maximum intensity of 1. For $\mathbf{Y_{PA}}$, all 14 parasites were modelled using probit regression. The fit and predictive power of the model was assessed through four-fold cross-validation, and model performance was quantified using Tjur's R^2 (Tjur 2009), SR^2 conditional on counts (Tikhonov et al. 2019), and area under the receiver operating characteristic (AUC). The variance in the model was partitioned according to the different environmental variables, to examine determinants of community structure.

5.2.3.3. Markov Random Fields (MRF) analysis

An MRF framework incorporating the covariate matrix **X** (a Conditional Random Fields analysis) was run to model parasite-parasite associations. This technique produces coefficients that facilitate direct comparison between the importance of biotic associations and environmental covariates, as well as allowing an exploration of how those biotic associations may vary across biological gradients (Clark et al. 2018).

To run this analysis, the input matrices were modified in the following ways: (a) The continuous variables length and weight in **X** were scaled to have mean 0 and standard deviation 1, as continuous variables are required to be on the same scale to ensure effective modelling; (b) the parasites *Conchophthirus* sp. in the mantle, *T. tubifex* and Senticaudata were removed from the $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$ matrices, as modelling cannot accommodate extremely high or low prevalences; (c) the morphospecies *U. intermedia* (eggs) and *U. intermedia* (mites), and *R. campanula* (gills) and *R. campanula* (gonad) were combined into single

columns in the $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$ matrices, as in exploratory modelling they showed extremely strong associations, the strength of which prevented effective visual presentation of other associations (see Fig. A3.12). However, this did not affect overall results.

The model was run with 10-fold cross-validation using the default settings in the `MRFcov` package. Parasites were modelled using the 'poisson' and 'binomial' options of the model function for the $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$ matrices, respectively. Model performance was also assessed with 10-fold cross-validation, with each 10-fold training run being repeated 10 times. The PA model was assessed using model sensitivity and specificity, while AB model performance was assessed by comparing model MSE and deviance to a null model without any environmental covariates. Note that model output consists of relative weightings of covariate importance, and so the proportions of variation discussed in the Results are calculated with respect the to variation explained by the model, not to the total variation in the data (in contrast to the JSDMs).

5.2.3.4. Differences between PA and AB models

To explore whether differences between the PA and AB models (see Results) were explainable by differential parasite abundances, parasites in the study were divided into two groups according to whether they occurred above median intensity (i.e. a high-abundance parasite) or below median intensity (i.e. a low-abundance parasite). For each parasite, we computed the differences between PA and AB model outputs in terms of variation explained (either by the environmental covariates for the JSDM modelling or by parasite associations for the MRF modelling), and compared these computed differences between the high-intensity and low-intensity groups using a Mann-Whitney U-test.

5.3. Results

In total, 14 different species or morphospecies of parasite were identified (Table 5.1). All 720 mussels had at least one parasite species, with a maximum of 10 (4.68 ± 1.61, mean $\pm$ s.d.; Fig. A3.9). Parasite prevalence and intensity varied both between parasite type (Table 5.1), and also throughout the year (Figs. A3.10, A3.11). To explore possible drivers of parasite community structure, three classes of analyses were executed: Redundancy Analysis, Joint

Species Distribution Modelling, and Markov Random Fields modelling, using both abundance (AB) and presence-absence (PA) models (the input matrices $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$, respectively).

5.3.1. Redundancy Analysis

The AB model was more successful than the PA model at explaining variation in the parasite matrix (R^2_{Adj} of 0.22 *vs*. 0.09); however, a global permutation test revealed that environmental covariates played a significant role in influencing the parasite community of a mussel in both cases ($p = 0.001$). All four partial RDAs (testing 'habitat suitability' factors holding 'dispersal influencers' constant, and *vice versa* for AB and PA models) were significant ($p = 0.001$ in all cases). The partial RDAs indicate that both mussel characteristics and dispersal influencers provide a valuable contribution to the model, even in the presence of the other group of factors. This was affirmed by forward selection, where environmental covariates from both these groups were selected in the final models. For both AB and PA models, the factors month, length and gravidity were included (Table 5.2), with month explaining the greatest proportion of variation, followed by mussel length and then gravid status.

Table 5.2: The overall percentage contribution of the environmental factors in explaining the variation of the $\mathbf{Y_{AB}}$ and $\mathbf{Y_{PA}}$ matrices. For the RDA analysis, where significance was able to be explicitly assessed, non-significant factors are denoted NS.

	Month	Length	Gravidity	Zebra	Weight	Random
AB Model (RDA)	15.4%	5.8%	0.9%	NS	NS	77.9%
PA Model (RDA)	5.0%	3.0%	0.9%	NS	NS	91.1%
AB Model (JSDM)	50.0%	11.7%	15.3%	3.3%	3.7%	16.0%
PA Model (JSDM)	48.3%	12.1%	8.6%	3.9%	2.4%	24.7%

5.3.2. Joint Species Distribution Modelling

Despite the high variation observed in both prevelance and intensity of different parasites between individual mussels and in different months, JSDMs generally performed well when predicting parasite communities. Both models performed significantly better than chance at predicting presences and absences (AUC mean ± σ: 0.70 ± 0.07 [AB]; 0.69 ± 0.08 [PA]), and both displayed a clear difference between mean fitted values for presences and absences

(Tjur's R^2: 0.062 ± 0.038 [AB]; 0.067 ± 0.052 [PA]). In addition, the AB model performed acceptably at ranking abundances (SR^2: 0.15 ± 0.13).

Analysis of the predicted posterior parameters showed the wide-ranging and variable effects the environmental factors had on the parasites (Fig. 5.1). Most parasite species were positively or negatively associated with multiple factors of importance. The predictions were highly consistent: with one exception (the square [*Conchophthirus* sp. mantle], [Month 3]), there were no direct contradictions between the AB and PA models. Often, both diagonals of the square were shaded (Fig. 5.1), highlighting the importance of that parameter for both models. The diverse range of effects in the 11 month parameters demonstrates how prevalence and intensity of parasitism varied through the year, often independently for different parasites. Parasites generally responded positively to increased mussel length, and

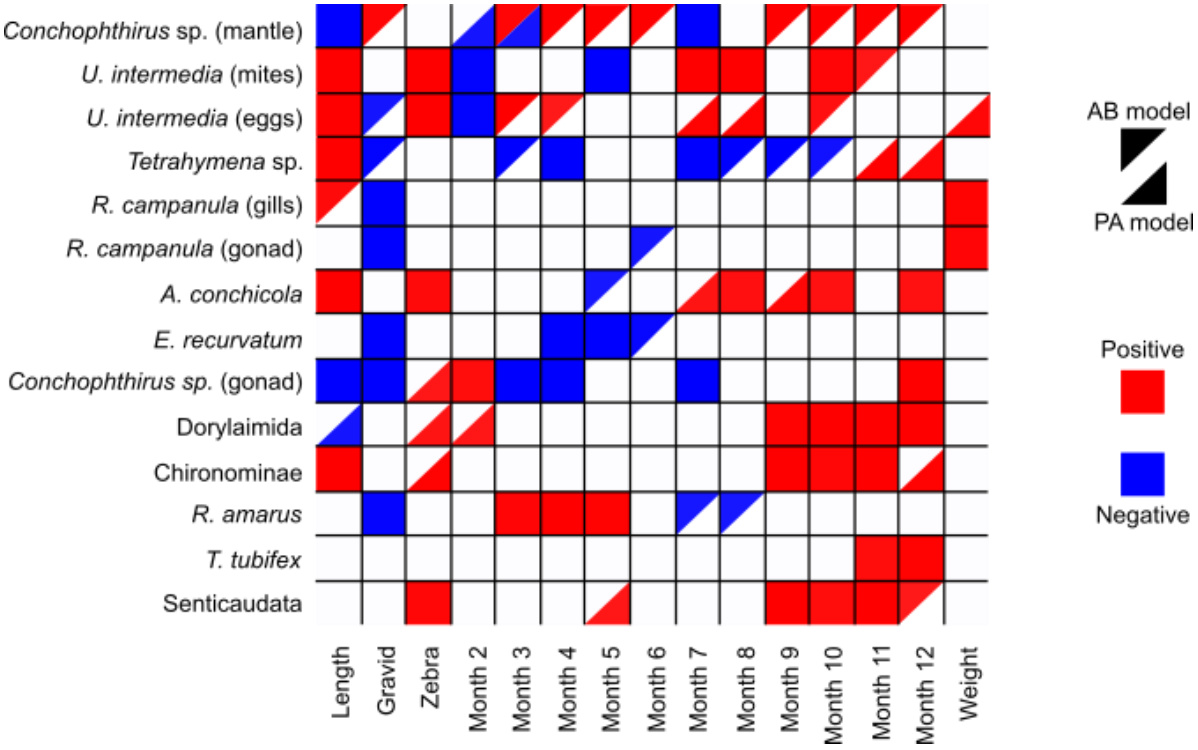


Figure 5.1: Matrix of β-parameters and their predicted impact on individual parasites, for the abundance (AB) and presence-absence (PA) models. Only effects with > 95% confidence are shown (rest in white). Red indicates positive parasite response to that particular parameter, with blue indicating negative. The upper diagonal of a given square denotes the AB model; the lower denotes the PA model. Note that parameter estimates for categorical factors (Gravid, Zebra and Month) are done with respect to a reference level, so the effect listed here as Gravid is comparing to non-gravid mussels, the factor listed as Zebra is comparing to mussels without zebra mussels, and all Month factors are comparing relative to Month 1. The uninformative Intercept parameter is excluded from the matrix.

negatively to the mussel being gravid, though there were exceptions. Mussel weight appeared to be a less important factor, but did have a positive impact on the castrating trematode *R. campanula*. The variation was then partitioned according to the different environmental covariates individually for each parasite (Fig. 5.2). Averages across all parasites are presented in Table 2. Overall, month was the most important environmental factor, accounting for roughly half the variation in the model (Table 5.2). Length and gravidity also explained a substantial portion of the variation in both models, though overall the PA model had a higher proportion of unexplained variation. However, the importance of these covariates was very different between parasite species (Fig. 5.2a). Further, there were some marked differences between AB and PA models for individual parasites (Fig. 5.2b). Particularly, *Conchophthirus* sp. (in the mantle) had more of its variation in abundance explained by month, and more of its variation in presence explained by mussel length. In contrast, the gill ciliate *Tetrahymena* sp. had much more of its variation in presence explained by month, while more variation in

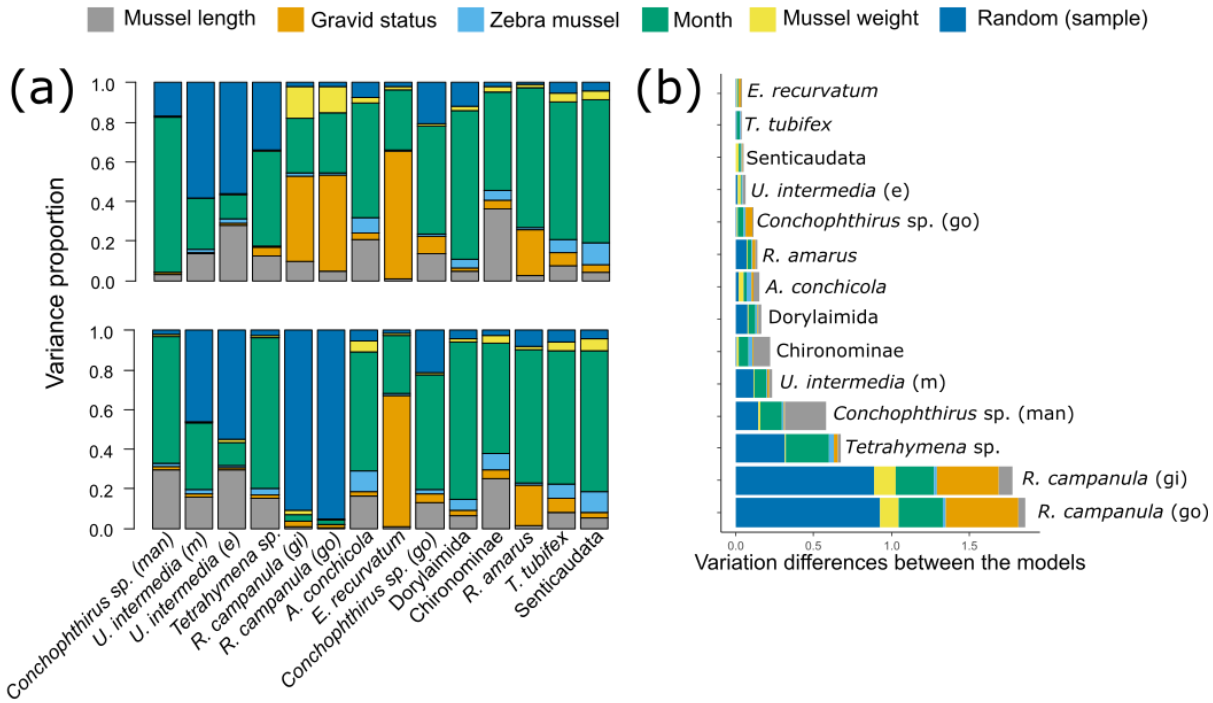


Figure 5.2: Variance partitioning based on posterior parameter estimates from the JSDM framework. (a) Variance partitioned individually for all 14 parasite species, for the AB model (top) and the PA model (bottom). The colour blue represents the random variation on an individual mussel level not explained by the environmental covariates. (b) Bar plot showing the cumulative differences between the AB and PA models for all 14 parasites. This was calculated as the absolute value of difference for each component of variance, and then visualised in the same colour scheme as (a) and ordered by total difference in variation. This highlights which parasite species had the greatest difference between the AB and PA models (towards the bottom of the chart), and which specific environmental covariates contributed to this difference. e=eggs; gi=gill; go=gonad; man=mantle; m=mites.

abundance was attributed to random variance. Finally, *R. campanula* (both in the gonad and gills) had a vast majority of their variation in presence attributed to random factors; when their abundance was considered, mussel weight and gravid status, in addition to month, became important.

Parasite traits played a role in the differing importance of environmental covariates to different parasites. On average, 42.1% (AB model) and 36.3% (PA model) of the variation between species' responses was explained by the trait matrix **T**. Particularly, parasite traits were very important in predicting a parasite's response to whether a mussel was gravid or not (average of 70% of the variation in response to gravidity explained by **T**, Table A3.1).

5.3.3. Markov Random Fields modelling

MRF model performance was sound. For the PA model, the combined mean value of sensitivity (mean ± σ: 0.64 ± 0.02) and specificity (0.90 ± 0.01) was >1.5, indicating good performance (Power et al. 2013). For the AB model, values of deviance (3241.9 ± 85.2) and MSE (170.9 ± 3.5) were significantly lower than the corresponding null model values of deviance (4658.7 ± 112.3) and MSE (210.3 ± 3.6), suggesting the model provided an acceptable fit to the data.

Even after accounting for all environmental covariates, there were multiple residual parasite associations observed, as estimated by the regression coefficients (Fig. 5.3). Of the variation explained by the model for each parasite species, 14.7% was attributed to associations with other parasites in the PA model, and 4.3% in the AB model, reflected by the much stronger correlation coefficients in the PA model; this suggests that possible interactions were more important in determining parasite presence than abundance. The odds of association (PA model) or relationships between abundances (AB model) did not change with any host characteristics: the parasite associations were consistent regardless of mussel length, gravid status or weight. In contrast, month occasionally influenced the strength of some associations, but did not affect direction. We also note that the estimated importance of the environmental covariates in the MRF framework matched that of the RDA and JSDM estimates (Table A3.2).

Negative parasite associations were more common than positive ones (11 of the 17 associations; Fig. 5.3). In particular, the bitterling *R. amarus* and the castrating trematode *R. campanula* had multiple negative associations in both models, both with each other and with other species in the gonad and gills (e.g. the trematode *E. recurvatum* that also occupies the gonad, and the ciliates *Conchophthirus* sp. and *Tetrahymena* sp. that occupy the gonad and gills respectively; Fig. 5.3). However, there were also positive associations observed between the ciliates *Conchophthirus* sp. and *Tetrahymena* sp., as well as between *Conchophthirus* sp. and *E. recurvatum*. While associations between parasites occupying the same tissue were common, 38% of potential interactions were between parasites occupying different mussel tissues.

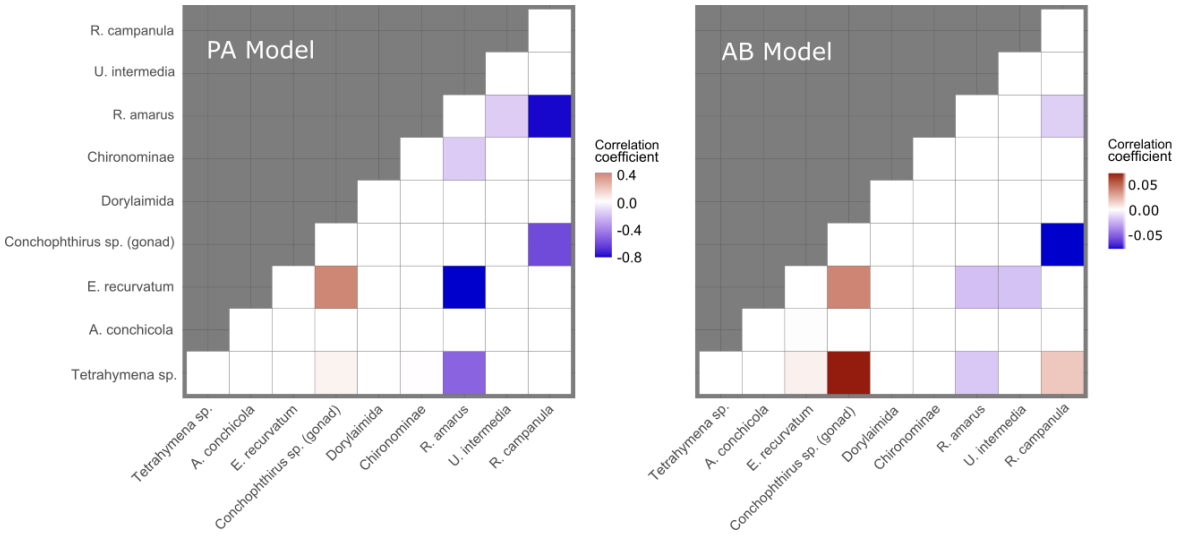


Figure 5.3: Residual parasite association matrices from the Markov Random Fields modelling. Only interactions with > 95% confidence are shown. Red indicates positive correlation, while blue represents negative correlation. Note the much stronger correlation coefficients for the PA model relative to the AB model.

5.3.4. Differences between PA and AB models

Some of our modelling results differed depending on whether abundance or presence-absence data was considered (Figs. 5.1, 5.2, 5.3). These differences depended on the relative intensity of the parasite in question (Fig. 5.4). Parasites that were above median intensity in our study had significantly larger differences between AB and PA models in the JSDM and MRF frameworks than did parasites below median intensity (Mann-Whitney, $U = 113.5$, $p = 0.004$). In short, high-abundance parasites had large differences in results between abundance and presence-absence models, while low-abundance parasites did not.

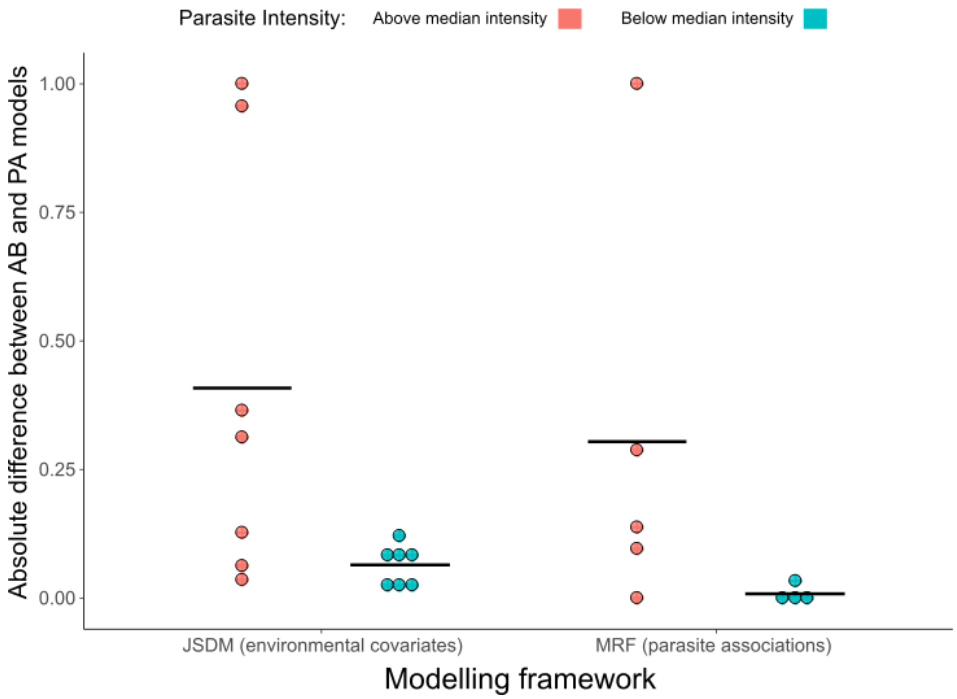


Figure 5.4: Differences between AB and PA models for both JSDM and MRF models (average differences shown by black lines), for parasite species above and below median intensity. Differences were scaled (by dividing all values by the largest difference in each case) to enable comparability on the same graph.

5.4. Discussion

This study modelled the response of freshwater mussel parasite communities to five environmental covariates (month, presence of zebra mussels; and mussel length, weight and gravid status), corresponding to the role of the regional species pool and dispersal, the importance of habitat suitability, and the significance of parasite-parasite associations after accounting for these factors. The total proportion of variation in the parasite matrix that was able to be explained varied between our three modelling frameworks (RDAs, JSDMs and MRF models). In particular, there was significantly more unexplained variation in the RDA results than the JSDMs (Table 5.2). This is likely due to two reasons: (a) higher parameterisation in the JSDMs than the RDAs; and (b) the absence of the trait matrix **T** from the RDAs, which was found to explain a significant amount of the parasite response to the environmental covariates (Table A3.1). However, all three modelling frameworks found that month explained a large proportion of variation in the parasite matrix, with mussel gravidity and length also important (Tables 5.2, A3.2; Figs. 5.1, 5.2); further, the MRF models

highlighted parasite associations as potentially playing an important role in parasite community assembly (Fig. 5.3), with up to 14% of the modellable variation in parasite species explained by other parasites. In addition, there were large differences observed between PA and AB models, with this difference significantly higher for high-intensity parasites (Fig. 5.4).

5.4.1. Presence-absence versus abundance data: implications for modelling

Overall predictions were similar between PA and AB models (Table 5.2). However, predictions for several parasite species individually had highly divergent outcomes between the two model sets (Fig. 5.2b), which was explained to a large extent by the mean intensity of the parasite species (Fig. 5.4). Importantly, there were no explicit contradictions between the two models (e.g. where one model finds a factor to be positively associated with a parasite, and another finds it to be negative), suggesting that model differences are being driven by varying sensitivity to different characteristics of the data, rather than necessarily displaying 'incorrect' information. This may still have severely negative consequences for predicting the impacts of parasitism on host populations. For example, the greatest difference between AB and PA models was observed for the castrating trematode *R. campanula* in the gills and gonad (Fig. 5.2b). Inspecting the PA model alone, *R. campanula* had little explanation for variation in its presence, leading to the conclusion that it has little relationship with the measured mussel characteristics. However, the AB model revealed a significant negative correlation with mussel gravidity (Figs. 5.1, 5.2a), likely reflective of the fact that a higher intensity of infection can lead to castration of the host mussel (Taskinen et al. 1997). In general, intensity of infection is crucial for determining outcomes on the host (Stjernman et al. 2008), making abundance data a vital part of predicting parasite impacts on host populations (Taskinen & Valtonen 1995). This study therefore highlights the importance of using abundance data to accurately characterise host-parasite interactions.

Previous research exploring whether presence-absence data is also useful in predicting abundances in ecological communities supports this conclusion. Joseph et al. (2006) showed that abundance data generally outperformed presence-absence data in assessing population declines and reductions in area of occupancy. However, for rare or cryptic species with low abundance or detectability, presence-absence measures performed as well as (and sometimes better than) abundance. Presence-absence data may therefore be appropriate for modelling

species with low abundance (where counts do not diverge far from binary presence/absence anyway), but species with higher abundance require explicit abundance measures. This is especially true for highly prevalent parasites, whose main source of variation across hosts will be in intensity rather than presence (e.g. see Glidden et al. 2019). Abundance data may therefore reflect habitat *quality* (i.e., how much of a particular parasite can a mussel support?), while presence-absence data reflects habitat *suitability* (i.e. can a mussel support that parasite?) (Gutiérrez et al. 2013). The more significant role of parasite associations in the PA model (Fig. 5.3) is therefore suggestive that parasite associations may be mainly exclusionary in nature (i.e. one parasite being present leads to another being absent), rather than modifying abundances. The biological basis for this is discussed further below.

Presence-absence data may still be useful for detecting broad prevalence trends and species' interactions, though recent theoretical work has demonstrated multiple issues with it (Blanchet et al. 2020). Sometimes, the same factors may govern both distribution and abundance, making presence-absence data a useful and simple way of assessing community trends (e.g. Gutiérrez et al. 2013). However, our work suggests that, for species with high abundance, presence-absence data may be misleading and lead to incorrect ecological inference, as we have demonstrated for *R. campanula*. Therefore, discussion on the environmental covariates below is focused on the AB results, though both PA and AB models are considered for parasite-parasite associations given the greater weight placed on these interactions in the PA model.

5.4.2. Factors driving parasite community assembly in A. anatina

The major driver of parasite community composition in *A. anatina* was month, which suggests that the regional species pool is an important determinant of parasite infracommunity structure. As most parasites (except the trematode *R. campanula*) do not show persistent infection, it makes sense that seasonal production of parasite infective stages would be a key driver of these communities (Rynkiewicz et al. 2019). For example, female bitterling (*R. amarus*) deposit their eggs in the gills of mussels between April and June, and the JDSM correspondingly found a positive effect of these three months for this species (Fig. 5.1). However, month can also reflect within-host parasite life cycles for species with persistent infection: *R. campanula* sporocysts occupy mussel gonads and gills year-round, but expand and spread to produce cercariae (to infect the next host in the life cycle) every year

from July to August before releasing them in September. This explains the spike and subsequent drop in intensity in this parasite (Fig. A3.10e), and why month is not important at all in explaining presence, but becomes important once abundance data is considered (Fig. 5.2a). Therefore, overall there is a significant temporal aspect to parasite community structure in mussels, which is partly explainable by parasite life history strategy (Table A3.1) that influences both the regional species pool and within-host reproduction. Importantly, different species show different monthly trends: there is not a consistent effect of month (Fig. 5.1). However, it may still be possible to predict parasite prevalence in a mussel community based on the time of year it was sampled, if detailed information on parasite life history traits is known.

The importance of considering parasite traits is also affirmed by the importance of mussel gravidity, which involves female mussels having gills filled with larvae in addition to a high proportion of eggs in their gonads. Correspondingly, gravidity was strongly negatively correlated with parasites occupying the gonad and gills (Figs. 5.1, 5.2a), suggesting that gravidity reduces the habitat suitability for parasites that utilise these niches of the mussel, likely through space or resource competition (for example, larval mussels require high oxygen levels). Considering individual mussel characteristics that influence resource availability could therefore also aid predictions regarding the type of parasites that may infect it. In this case, gravid mussels may experience reduced parasite pressure (or, conversely, mussels with high parasite loads may be less likely to become gravid). This also highlights the challenge of inferring cause and effect from correlative data, a hurdle typically yet to be overcome in parasite community ecology (but see e.g. Budischak et al. 2016).

Host length was also found to be a significant predictor of parasite community structure. In theory, larger mussels have a larger resource base, and generally parasites were positively correlated with increased length (Fig. 5.1). However, positive correlations between parasites and length may also reflect a greater cumulative chance of infection by a parasite, as length increases with age in all but the very oldest mussels (Lundquist et al. 2019). We suggest that age is a more likely explanation, as length-corrected weight (which would more accurately reflect mussel resources) was not found to be important, and parasite associations did not change with length, which may be expected if length was an important determinant of resources (e.g. there should be greater competition in smaller mussels due to fewer resources, see discussion in Hechinger et al. 2019). Finally, length could also represent a dispersal filter:

parasites such as *U. intermedia* and chironomids are more likely to fit through the siphons of larger mussels, reflecting greater importance of the length parameter in these species (Fig. 5.2a). Differentiating between these explanations ultimately requires experimental investigation (Fenton et al. 2014; Poulin 2019), though our results suggest that the variable 'length' may better reflect dispersal chance than habitat suitability in this and related systems.

Finally, the residual associations between parasites (Fig. 5.3) suggest that biotic interactions may also have an important structuring role, though the observed associations may be due to parasites responding in similar or opposite ways to an unmeasured environmental covariate (Blanchet et al. 2020). However, in some cases there is a clear biological basis for these associations. For example, *R. amarus* has a large number of interactions that are particularly strong in the PA model. Given ovipositing females show sensitivity to host quality (Mills & Reynolds 2002), they may detect lowered host quality due to the presence of other parasites and choose instead to oviposit in uninfected mussels. Space competition may also play an important role, with 62% of the interactions observed between parasites that can occupy the same tissue, such as the negative correlations in both the presences and abundances of *R. campanula* and *Conchophthirus* sp. in the gonad. Such relationships could also explain the positive interactions between parasites in different tissues, such as between the ciliates *Conchophthirus* sp. (gonad) and *Tetrahymena* sp. (gills). If *Conchophthirus* sp. competes in the gonad with *R. campanula*, which also infects the gills, this may have an indirect positive effect on *Tetrahymena* sp., or vice versa. While these remain hypotheses, the results demonstrate that biotic interactions may be an important structuring force for some parasites, especially in terms of the their presence.

Recent research has highlighted that multiple assembly rules can determine the structure of different community modules, both in free-living communities (e.g. Fournier et al. 2016) and parasite communities (e.g. Williamson et al. 2019). Our models support this idea by suggesting that the regional species pool, and ecological selection in terms of both habitat suitability and biotic interactions are important for structuring communities. Further, these factors are parasite-specific, which can be at least partly explained by parasite traits. However, the assembly of communities is commonly referred to as being driven by a consistent subset of factors (e.g. 'neutral' or 'niche-based' assembly, see Connolly et al. 2014; Mitchell et al. 2019). Such broad categorisations may oversimplify what structures a *community*, as it may be composed of different species all being influenced independently by

a range of factors to varying degrees. Overall, our results demonstrate that predicting parasite community structure requires an integrative approach, incorporating parasite and host traits alongside the wider environmental and temporal context (Hellard et al. 2015). Understanding such nuances provides a new and exciting challenge in parasite community ecology.

5.4.3. Conclusions

Parasite species that vary in intensity within a host population may have contrasting outcomes at both an individual host and population level (Hurd 2001; Lafferty & Kuris 2009); consequently, understanding the factors driving their abundance in individual hosts, in additional to their presence, is vital to predicting parasite effects. We strongly recommend that, where possible, abundance data is incorporated into studies of parasite community ecology, as it has the capacity to explain greater levels of variation in multiple analytical frameworks (Table 5.2) and better reflects population trends for high-intensity parasites. An increased emphasis on invertebrates may facilitate this, especially with the advent of new methods to accurately quantify infection in these hosts (e.g. Chapter 3). In addition, we have shown that detailed knowledge of parasite life histories is an important but often overlooked aspect of predicting community structure.

This paper has demonstrated the importance of infracommunity-level (parasite interactions, individual host characteristics) and component community-level (regional species pool) factors in determining parasite community structure, and how this varies between parasites. Given the complexity of this picture, which leaves aside multiple sites or host species, we show that data needs to be as nuanced and as fine-scale as possible to expand our understanding of parasite community ecology. Such understandings may also ultimately be translatable to free-living communities and metacommunities.

Chapter 6: Factors at multiple scales drive parasite community structure

Abstract

Ecological communities are hierarchical, meaning that factors acting at multiple scales can contribute to patterns of community structure. Parasites provide a natural system to explore this idea. We aimed to not only understand the relative contribution of multi-scale drivers in parasite community assembly, but also assess how patterns at one level may mask those occurring at another, using freshwater mussels and their complete parasite communities. We applied a Markov Random Fields model and assessed measures of turnover and nestedness for 420 replicate parasite infracommunities across two freshwater mussel host species, three sites and two time periods. We compared our results to simulations from four different ecologically relevant null models, which vary in their constraints on parasite prevalence and individual host infracommunity richness. We showed that turnover between sites (explaining 25% of variation in parasite distribution) and host species (41%) is greater than expected, and turnover between individual hosts is smaller than expected, even after accounting for parasite prevalence and patterns of an individual host's infracommunity richness. Further, parasite communities were significantly less nested than expected once parasite prevalence and host infracommunity richness were both accounted for, but more nested than expected otherwise, suggesting a degree of modularity at the within-host level that is masked if underlying characteristics of hosts and parasites are not taken into account. The Markov Random Fields model provided evidence for competitive within-host parasite interactions, providing a mechanism for the observed infracommunity modularity. An integrative approach that examines factors at multiple scales is necessary to understand the composition of ecological communities. Further, patterns at one level can mask ecologically important drivers at another if variation at higher scales is not accounted for, revealing a need to account for characteristics of the system under study.

Key words: β-diversity, community assembly, competition, infection, Markov Random Fields model, null model, prevalence, richness

6.1. Introduction

Ecological communities are inherently hierarchical, with individuals existing as members of populations, communities and metacommunities. However, generalities about the importance of drivers at different scales remains absent (Bolnick et al. 2020a). Parasites provide an opportunity to extend knowledge on the topic: a host individual represents a discrete and easily sampled community of parasites, the assembly of which is driven by factors operating at multiple levels. For example, key structuring forces such as dispersal or ecological selection (Vellend 2010) may operate on multiple scales: certain parasites may be excluded from host individuals, from a host species within a site, or from whole sites (Guégan et al. 2005; Bashey 2015; Mihaljevic et al. 2018). Such parasite distribution patterns may be driven by within-host competition (Chase & Myers 2011; Dallas et al. 2017a), the host species that are present at a given location (Mihaljevic et al. 2018) and broader characteristics of the environment in which those host species exist (Penczykowski et al. 2016). The temporal context is also an important determinant of parasite communities, with different parasite infracommunity structures observed in the same host population through time (Chapter 5). Finally, different parasites within a single infracommunity may not respond to these within-host, between-host and broader environmental factors in the same way (e.g. Snyman et al. 2020; Chapter 5), further complicating matters. This combination of complexity and the fact that parasite communities can be completely and reliably sampled makes parasites a tractable and highly replicable system to study community assembly.

β-diversity is an appealing way to address community ecology questions, because it highlights the extent of community differences between different sites or time periods (i.e. turnover), and therefore provides a starting point for the generation of hypotheses to explain the observed patterns. However, β-diversity can never be independent of α- and γ-diversity (Veech & Crist 2010a, 2010b). Therefore, comparisons of, for instance, β-diversity between host individuals, and between host species, may be driven by the inherent differences in total diversity (γ-diversity) at these scales, rather than by active drivers of ecological relevance (Kraft et al. 2011). In contrast, additive partitioning (Lande 1996; Crist et al. 2003; Gering et al. 2003) actively embraces the hierarchical nature of community ecology data, and allows the decomposition of total γ-diveristy among different scales: each scale in the hierarchy receives a portion of β-diversity that corresponds to the turnover at that scale. This facilitates

an examination of ecological levels at which assembly processes may be operating (Veech & Crist 2010b). Additive partitioning has been employed to explore patterns of community structure in a wide range of taxa, including plants (e.g. Marcilio-Silva et al. 2017), reptiles (e.g. Gao & Perry 2016) and macro-invertebrates (e.g. Ferreira et al. 2017; Kuznetsova & Saraeva 2018). However, it has rarely been applied to parasite community structure (but see Johnson et al. 2016; Moss et al. 2020). In addition to turnover, nestedness is another important component of β-diversity (Baselga 2010), and reflects the degree to which species-poor communities are subsets of richer ones. In contrast to turnover, which represents species replacement, nestedness reflects species absence, which can encompass processes such as local extinction or differential dispersal capabilities to a site (Baselga 2010). As richness *per se* may be an important metric of parasite pressure (Bordes & Morand 2009), understanding its turnover and degree of nestedness among scales allows possible impacts on host individuals and populations to be assessed.

The significance of observed additive partitions and nestedness within a dataset are typically assessed by comparison with repeated simulations of a null model, to ascertain whether the observed patterns differ from those expected at random. However, frequently only one null model is employed, with little transparency regarding the specific statistical characteristics of the model. There are two reasons this is undesirable. The first is mathematical. There are many different formulations of null models (e.g. having row or column totals constrained to the values for the observed incidence matrix, or both, or neither; see Strona et al. 2018), the appropriateness of which have been discussed for decades (Molina & Stone 2020). Null models that are too restrictive may reduce the sample space for the metric in question and bias the test (Gotelli & Ulrich 2012), while null models that are too loose may not reflect biological reality and often perform poorly in simulation studies (Ulrich & Gotelli 2007). In general, null models vary wildly in suitability (Ulrich & Gotelli 2010; Strona et al. 2018), and may lead to very different results. For one metric of community structure in Gotelli and Rohde (2002), using two different null models led to a 2-fold difference in the number of species deviating from random structure. Given the continued uncertainty (Molina & Stone 2020), it seems prudent to use multiple null models and compare their performance, thus increasing transparency in results. The second reason is that differences among null models may reveal important ecological processes (Crist et al. 2003). For example, Rynkiewicz et al. (2019) analysed the significance of nestedness in parasite communities. They found that the observed nestedness was significantly different from random when individual host richness

observed in the actual data was maintained in the null model, but not when parasite prevalence was maintained. They therefore suggested that nestedness patterns were driven by variable parasite prevalence in the community. Similarly, in an additive partitioning context, Belmaker et al. (2008) used multiple null models to show that high β-diversity among reef fish populations was driven entirely by site-specific coral species richness. Therefore, a careful implementation of alternative null models can shed light on ecological processes.

In this study, we analysed turnover (using additive partitioning) and nestedness (using the measure NODF [Nestedness based on Overlap and Decreasing Fill]) in the parasite communities of a novel model host system (unionid freshwater mussels) across two species, three sites and two time periods. This host system facilitates large sample sizes, allowing multiple factors to be investigated in tandem. In addition, mussels possess highly discrete tissues, allowing for comprehensive dissection to ensure data is reliably presence-absence rather than presence-only (Brotons et al. 2004). We compared our turnover and nestedness results to four different null models that vary in their constraints, from only total parasite count being constrained (least constrained null model) to both individual host infracommunity richness and parasite species prevalence being constrained (most constrained null model). Comparing our results to these models allows us to determine whether the observed patterns can be explained by parasite prevalence, host infracommunity richness, a combination of both, or whether additional factors must be invoked to explain parasite community structure. We note that an explicit statistical relationship can be derived between turnover and nestedness (see Baselga 2010; Podani et al. 2013). However, to our knowledge, these measures cannot account for the hierarchical nature of parasite community structure, and so we treated turnover and nestedness as two independent facets of β-diversity in the current study. Given the acknowledged importance of within-host interactions (Fountain-Jones et al. 2019), we confirmed and extended our results by implementing a Markov Random Fields model (Clark et al. 2018), which allowed us to examine the proportional impact of time, site and mussel species, as well as individual host-level characteristics and within-host parasite interactions. Our results demonstrate that parasite communities are structured by factors at a range of scales, from site and host community to within-host parasite interactions. Further, we show that a critical assessment of null models is both necessary and desirable to fully understand deviations from random community structure.

6.2. Methods

6.2.1. Sampling regime

We collected mussels from three sites in Cambridgeshire, UK: Brandon Creek (henceforth BC), King's Dyke (KD) and the Old West River at Stretham (OW), all of which are part of the Great Ouse river system (Fig. 6.1). Sampling incorporated two species: the duck mussel *Anodonta anatina* (Linnaeus 1758) and the painter's mussel *Unio pictorum* (Linnaeus 1758), both non-endangered unionid bivalves common throughout Europe (Lopes-Lima et al. 2017) that possess a broad range of parasites (Chapter 2). We sampled on two occasions: 7th May 2019 ("Visit 1"), and 7th November 2019 ("Visit 2").

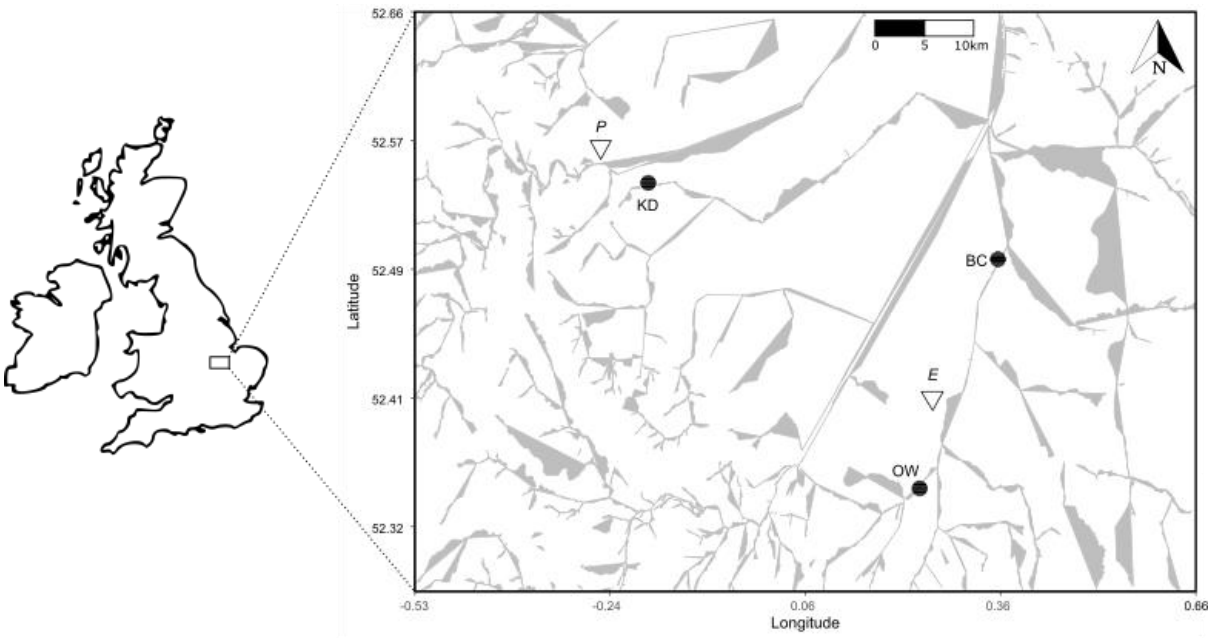


Figure 6.1: Map of the three sampling sites (black dots), where grey lines represent water bodies. BC = Brandon Creek; KD = King's Dyke; OW = Old West. For context, the population centres of Ely (E) and Peterborough (P) are also included (open triangles).

At all sites, mussels were sampled by hand from the river margin. In total across both visits, we collected 420 mussels (240 *A. anatina*, 180 *U. pictorum*). Because extended storage of live mussels in the laboratory could cause parasites to leave the host, or move between hosts, we immediately placed mussels in 75% ethanol upon sampling before transporting them back to the laboratory. The two species were not mixed at any point once being removed from the river. Exploratory dissection on both ethanol-stored and live mussels showed that the storage

of mussels in ethanol prior to analysis did not affect the detection of any parasites in the study, and it has previously been shown as an effective way of sampling mussel parasites (Conn et al. 2008).

6.2.2. Mussel dissection

In the laboratory, we sliced the anterior and posterior adductor muscles to open the mussel. We inspected all parts of the mussel in systematic fashion, and identified all parasites to the finest possible taxonomic resolution (see Appendix A4 Part 1). We inspected samples of mantle fluid (1 mL) under 40× magnification using a GXM-L3200 compound microscope to identify the presence of ciliates and nematodes. The mantle, gills and pericardial cavity of the mussel were inspected under a GXMMZS0745-T stereomicroscope at 16× magnification to identify further ciliates, mites, chironomids, bitterling (*Rhodeus amarus*) embryos and aspidogastrean trematodes. Finally, we squashed samples of gonad tissue between two glass microscope slides and studied them at 40× magnification (following Chapter 3) to identify digenean trematodes. For ciliates and digenean trematodes we only noted presence or absence, while for mites, chironomids, bitterling embryos, aspidogastrean trematodes, chironomids and nematodes we also counted the numbers of individuals. We measured the maximum length of all mussels (nearest 0.5 mm) with Vernier callipers, dried them to constant mass (nearest 0.001 g), and identified them as either male, non-gravid female or gravid female *via* inspection of their gill tissue (where gravidity refers to the phenomenon of female unionid mussels harbouring larval mussels in specialised water-tubes in their gills). For further details on life-history strategy and characteristics of all parasites, see Table A4.1, the additional information in Appendix A4 Part 1, as well as Appendix A3 Part 1.

6.2.3. Additive partitioning of diversity

All statistical procedures in this and the subsequent sections were executed in R v3.6.3 (R Core Team, 2020). To explore turnover in parasite richness across different scales, we implemented an additive partitioning approach (Lande 1996; Crist et al. 2003; Gering et al. 2003). We used this approach to explore parasite turnover (i.e. β-diversity) between individual hosts in a population, between the two host species within a site, and between the three sample sites (Fig. 6.2). We refer to the parasite community inside a single host

organism as the *infracommunity* (following Bush et al. 1997). As the response variable is parasite richness, this analysis utilised only presence-absence data (those parasites with recordings for intensity were reduced to a present/absent recording). In this framework, α_1 represents the individual parasite richness (infracommunity) of a single host within a population (with mean richness across all hosts $\bar{\alpha}_1$), α_2 represents the total parasite richness of a host population within a site (mean $\bar{\alpha}_2$), α_3 represents the total parasite richness of a given site (i.e. in the host community) (mean $\bar{\alpha}_3$), and γ represents the total parasite diversity of the region (i.e. in the host metacommunity) (Fig. 6.2). The relative importance of each spatial scale on parasite community structure can then be described using β-diversity, such that β_1 represents the average between-host variation (within a population), β_2 represents the average between-species variation (within a site), and β_3 represents the average between-site variation, where

$$\beta_1 = \bar{\alpha}_2 - \bar{\alpha}_1 \qquad (1)$$

$$\beta_2 = \bar{\alpha}_3 - \bar{\alpha}_2 \qquad (2)$$

$$\beta_3 = \gamma - \bar{\alpha}_3 \qquad (3)$$

and therefore

$$\gamma = \bar{\alpha}_1 + \beta_1 + \beta_2 + \beta_3 \qquad (4).$$

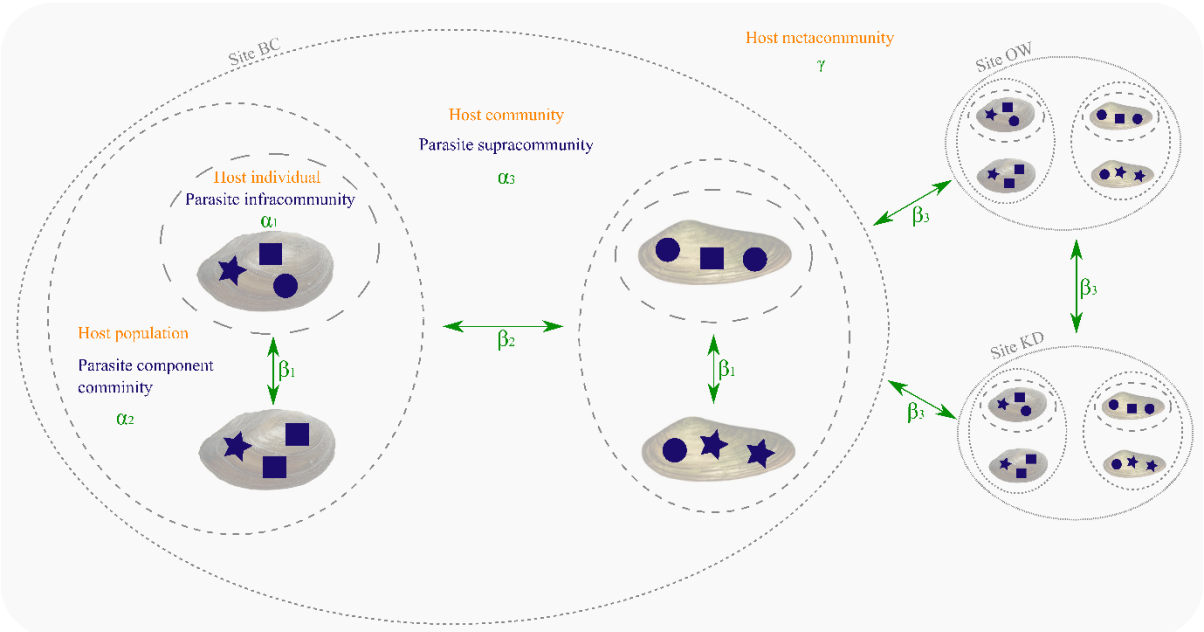
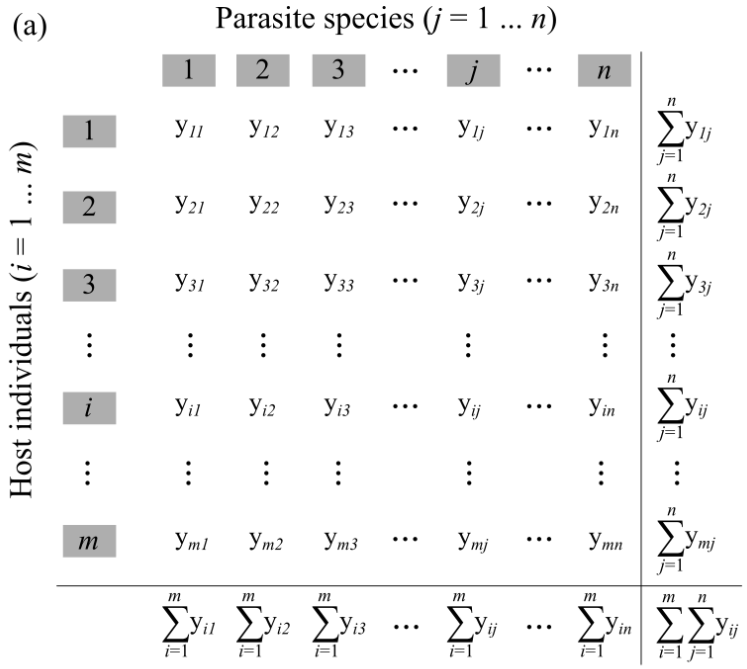


Figure 6.2: Terms used to describe host and parasite structure across scales. Terms in orange refer to host organisation; terms in blue refer to parasite organisation; terms in green refer to the division of diversity in the additive partitioning framework employed. Blue stars, squares and circles indicate different parasite species. Parasite terms are consistent with those of Bush et al. (1997).

The `adipart` function in the `vegan` package (Oksanen et al. 2019) was used to carry out additive partitioning according to Equation 4. To assess the significance of the obtained diversity values, we compared the results to 10000 simulations using four different null models, which vary in the constraints they place on the null model matrix (Fig. 6.3). The EE model was implemented with the "r00" option (algorithm first developed by Atmar & Patterson 1995), the EF model with "c0" (Jonsson 2001), the FE model with "r0" (Patterson & Atmar 1986) and the FF model with "quasiswap" (Miklós & Podani 2004). We carried out the additive partitioning procedure separately for Visit 1 and Visit 2.

(a)



(b)

	EE	EF	FE	FF
$\sum_{j=1}^{n} y_{ij}$ maintained for each i = 1 ... m	NO	NO	YES	YES
$\sum_{i=1}^{m} y_{ij}$ maintained for each j = 1 ... n	NO	YES	NO	YES
$\sum_{i=1}^{m}\sum_{j=1}^{n} y_{ij}$ maintained for all i, j	YES	YES	YES	YES
Verbal interpretation	Total parasite count maintained	Parasite prevalences maintained	Individual host richness maintained	Individual host richness and parasite prevalences maintained

Figure 6.3: Mathematical description of the null models employed in the study. (a) The host-parasite incidence matrix, where each y_{ij} is limited to either 0 (absence) or 1 (presence). (b) Table showing the constraints on the four null models. Note that all four models also operate on the limitation that each y_{ij} can only take the values of 0 or 1. Null model names follow Ulrich and Gotelli (2007), where 'E' = equiprobable and 'F' = fixed. Therefore, EE is the least constrained (only overall sum matches observed matrix) and FF the most constrained (row totals and column totals both match observed matrix), while EF and FE are equally constrained but in opposite fashion (EF has column totals matching the observed matrix, FE has row totals matching the observed matrix).

In addition, we visually explored general patterns of parasite incidence across individuals, species and sites using a two-dimensional NMDS, also implemented in `vegan` using the Jaccard similarity index. The two-dimensional ordination was sufficient to appropriately visualise the data (stress = 0.109).

6.2.4. Nestedness

We calculated the nestedness of the observed parasite infracommunities (the tendency for species-poor communities to be subsets of richer communities) using NODF (Almeida-Neto et al. 2008), again using only presence-absence data. We compared the observed value to 10000 simulations of the same four null models (EE, EF, FE, FF) using the `oecosimu` function in `vegan`. In addition, we examined patterns of nestedness separately for each visit and for each species, using the same procedure outlined above.

6.2.5. Markov Random Fields modelling

We further explored the influence of visit, site, mussel species, host characteristics (length, sex, weight) and potential parasite interactions with a Conditional Random Fields (CRF) analysis (Clark et al. 2018). This is an extension of a Markov Random Fields model that incorporates a matrix of covariates, and facilitates partitioning of variance in community structure among environmental factors and parasite interactions. Our covariate matrix included the factors 'Visit' (Visit 1, Visit 2), 'Site' (BC, KD, OW), 'Species' (*A. anatina*, *U. pictorum*), as well as the mussel-specific characteristics 'Length' (in mm), 'Sex' (male, non-gravid female, gravid female) and 'Weight' (in g, residuals of a regression of weight against length, to account for differences in weight not due to length). The continuous variables Length and Weight were scaled to have mean 0 and standard deviation 1.

To account for parameter uncertainty, we fitted a bootstrapped-CRF to our data, using ten bootstraps and all other default settings in the `MRFcov` package (Clark et al. 2018). Model performance was assessed with 10-fold cross-validation, with each 10-fold training run being repeated 10 times. Combined sensitivity + specificity was 1.52, indicating good performance (Power et al. 2013). In order to allow direct comparison of results to our measures of turnover and nestedness (which used presence-absence data), we also only used presence-absence data

for our CRF. However, ignoring parasite abundance may have significant consequences on assessments of parasite community structure (Chapter 5), and so we carried out a similar analysis using a Joint Species Distribution Model (Tikhonov et al. 2019). This framework can incorporate presence-absence and intensity data, which allowed us to include abundances for parasites where we had such information (Table A4.1). For details on JSDM fitting, see Appendix A4 Part 1 and Table A4.2. Based on the results of our CRF, we carried out univariate tests on important variables to characterise the specific direction, size and significance of effects.

6.3. Results

In total there were 14 parasite groups identified (Table A4.1), with 2.91 ± 1.58 parasites per mussel (mean ± s.d.). Of the 14 parasites, two were specific to *A. anatina* and one was specific to *U. pictorum*, with 11 found in both hosts (Table A4.1). All 14 parasites were found in Visit 1, while 13 were present in the Visit 2 (absence of bitterling embryos).

6.3.1. Additive partitioning and nestedness results

Considering first the additive partitioning, regardless of the visit or the null model employed, results were consistent for the partitions β_1, β_2 and β_3 (Fig. 6.4). For all four null models and for both visits, the observed β_1 was significantly lower than null model predictions ($p < 0.001$, all cases), indicating that beta-diversity among individual hosts was less than what would be expected by chance. Similarly, the observed β_2 and β_3 values were all significantly higher than null model expectations ($p < 0.001$, all cases), highlighting that beta-diversity both between host species and sites was greater than expected by chance. However, the significance of the parameter α_1 was dependent on the visit and the null model employed. In Visit 1, the null model predictions were always identical, and matched the observed alpha-diversity ($p = 1$, all cases). In Visit 2, while the EF and FF null models always matched the observed alpha-diversity ($p = 1$ in both cases), the EE and FE null models predicted significantly lower diversity than what was observed ($p < 0.001$ in both cases).

While the choice of null model generally did not influence the overall result, there were clear differences between the four. The two null models that did not constrain parasite prevalence (EE, FE) gave very similar results, while likewise the two models that did constrain parasite

prevalence (EF, FF) were highly consistent in their predictions (Fig. 6.4). In particular, the EE and FE models resulted almost exclusively in predicting that both β_2 and β_3 were equal to zero.

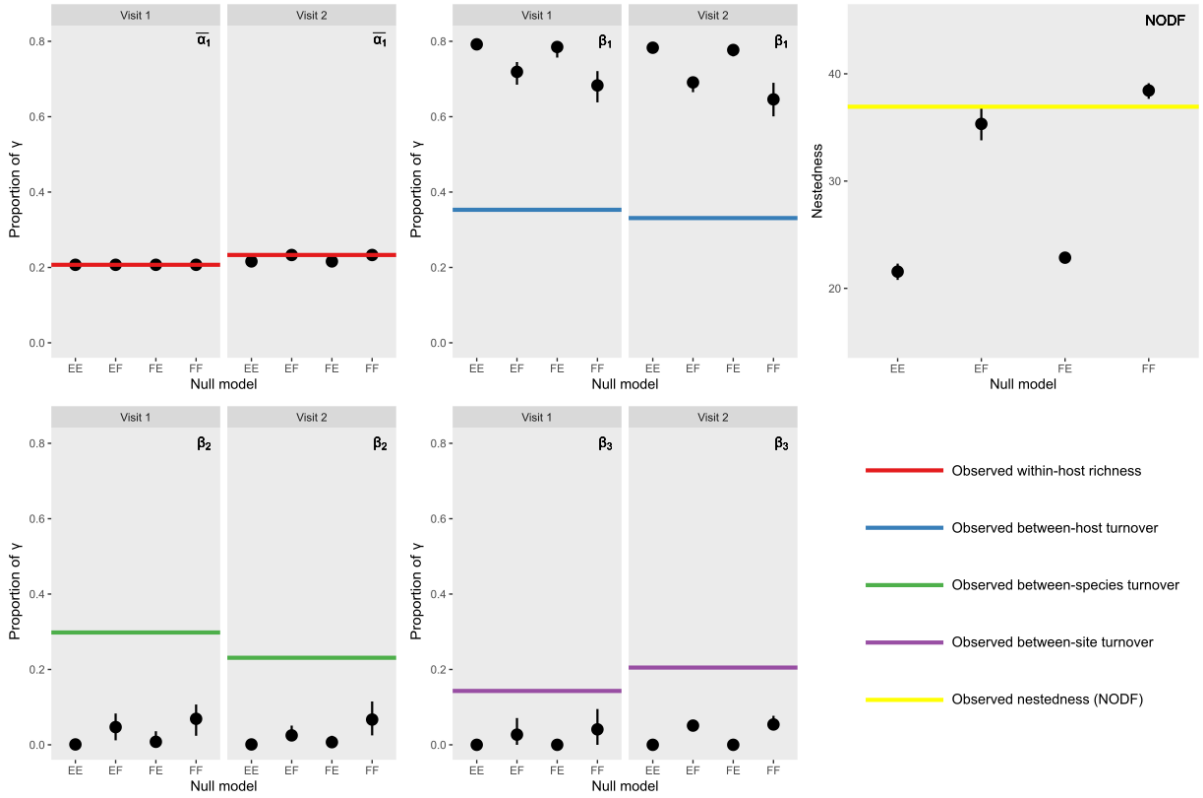


Figure 6.4: Results of additive partitioning of variance and nestedness for the observed data, and their comparison with the four null models. Actual observed values of turnover and nestedness represented by horizontal lines ($\bar{\alpha}_1$ = red; β_1 = blue; β_2 = green, β_3 = purple, NODF = yellow). The values for the 10000 null model simulations are visualized by black dots, with error bars indicating the extent of 95% of null model values. For the additive partitioning, separate panels are presented for Visit 1 (May) and Visit 2 (November).

In contrast, conclusions about observed nestedness depended on null model choice. When compared with EE, EF or FE models, the observed NODF (36.9) was greater than null model predictions ($p < 0.001$ [EE and FE]; $p = 0.037$ [EF]), indicating the within-host parasite communities (infracommunities) were more nested than expected by chance (Fig. 6.4). In contrast, when compared with the FF null model, which constrains the parasite prevalence and individual host richness to their observed values, actual nestedness was lower than expected by chance ($p < 0.001$; Fig. 6.4). These results were qualitatively consistent across both time periods, and for both host species separately.

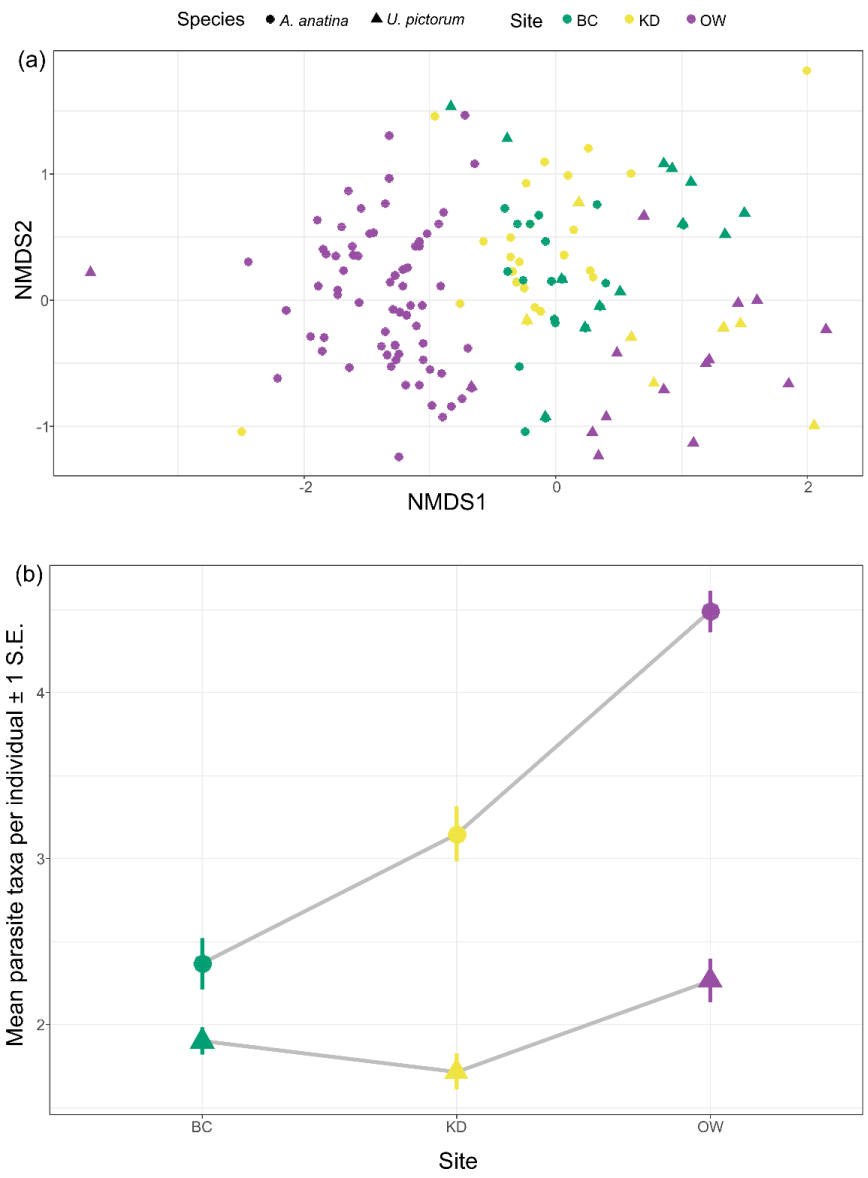


Figure 6.5: Patterns of parasite structure across host individuals, populations, species and sites. (a) NMDS visualization, where each point represents a parasite infracommunity. (b) Mean richness, with the associated standard error, of *A. anatina* (circles) and *U. pictorum* (triangles) at each of the three sites (BC = Brandon Creek; KD = King's Dyke; OW = Old West).

6.3.2. Conditional random fields modelling

CRF results support those above, particularly the additive partitioning results. Host species (*A. anatina* or *U. pictorum*) and site (BC, KD or OW) were the two most significant contributors to variation in community structure, explaining 40.5% (host species) and 25.1% (site) of the variation respectively, with clear clusters in the NMDS visualization (Fig. 6.5a). These results were highly consistent with those of the JSDM which incorporated parasite abundance, where 40.1% and 33.4% of the variation was explained by species and site

respectively (Fig. A4.3). *Post-hoc* univariate tests further explored role of host species and site (Fig. 6.5b). At each of the three sites, *A. anatina* individuals possessed a higher parasite richness than *U. pictorum* ($p = 0.027$ [BC], $p < 0.001$ [KD, OW]). However, this difference ranged from 1.3-fold (BC) to 2-fold (OW). Considering *U. pictorum* individuals only, BC and KD had similar richness ($p = 0.151$), while OW had 1.2-fold higher richness than BC ($p = 0.038$) and a 1.3-fold higher richness than KD ($p = 0.008$). Considering *A. anatina* individuals only, OW had a 1.4-fold higher richness than KD ($p < 0.001$), and a 1.9-fold higher richness than BC ($p < 0.001$). In addition, KD also had a 1.3-fold higher richness than BC ($p = 0.004$). Therefore, in general, individual mussels at OW had greater richness than the other two sites, but to a much greater extent in *A. anatina* than *U. pictorum* (Fig. 6.5b). However, all three sites had a similar total parasite richness (BC = 13; KD = 11; OW = 14).

In contrast to species and site effects, individual mussel characteristics (Length, Weight, Sex) had little effect, collectively explaining just 4% of the parasite community variance, though there was still a large amount of variation between host individuals of the same species at the same site (Fig. 6.5a). However, within-host parasite interactions explained 12.3% of the variance, suggesting that competition plays an important and detectable role even after taking into account site- and species-specific differences (Fig. 6.6). Of the 10 putative interactions, seven occurred between parasites occupying the same tissue of the mussel, of which five were negative (Fig. 6.6). Of the three interactions that occurred between parasites in different tissues, two were negative. In particular, the ciliates (*Conchophthirus* sp., *Tetrahymena* sp.1 and sp.2, and *Trichodina* sp.) had a range of negative correlations with other species occupying the gills and mantle of the mussel, such as the mites *U. intermedia* and *U. bonzi* and bitterling larvae *R. amarus*.

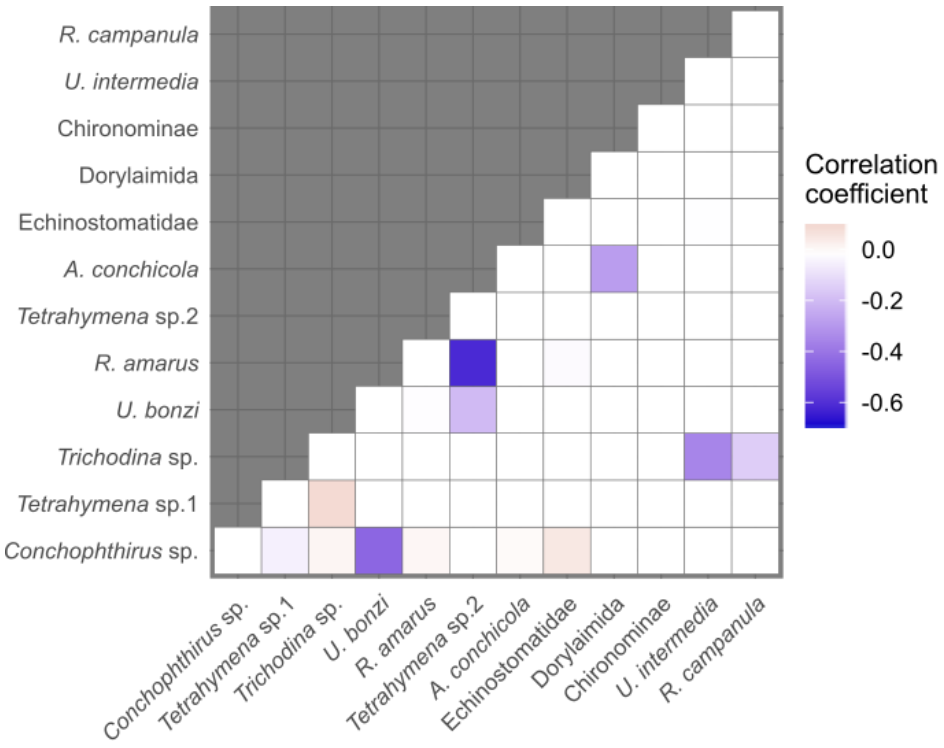


Figure 6.6: Mean correlation matrix for parasite taxa. Only interactions with >95% confidence are shown.

6.4. Discussion

This study compared observed turnover and nestedness to multiple null models, to explore facets of β-diversity across scales and draw conclusions about parasite community assembly. We also employed a Markov Random Fields model to extend these results and focus more closely on the factors governing host infection in this system.

6.4.1. Null model choice affects results

With respect to turnover (additive partitioning), the choice of null model did not affect the significance of the parameters β_1, β_2 and β_3, but it did for $\bar{\alpha}_1$ (Fig. 6.4). The reason for this was a time-specific difference in parasite richness, with the parasite *R. amarus* not observed in Visit 2 (i.e. $\gamma_{Visit1} = \gamma_{Global}$, but $\gamma_{Visit2} < \gamma_{Global}$). Because $\bar{\alpha}_1$ is expressed as a proportion of γ, in the null models each individual α_1 equals the randomized richness of that particular host, divided by the total parasite count. Therefore, as the total parasite count is maintained for all four null models (Fig. 6.3), the simulated $\bar{\alpha}_1$ value should always match the observed data

(i.e. while the α_1 of individual hosts will vary among simulations, their mean will always be consistent). However, in Visit 2, those null models that do not constrain parasite prevalences to the observed values fail to account for the fact that *R. amarus* is absent (prevalence = 0), and therefore the γ value for that visit is inflated relative to the actual data. This artificially lowers $\bar{\alpha}_1$ relative to the observed data for the models EE and FE (Fig. 6.4). Because additive partitioning is a zero-sum game, this in turn will artificially raise one or more β-parameters. This result is very interesting, as it highlights how the time-specificity of γ-diversity is instead observed at the α-diversity level inside this framework, with corresponding consequences for interpretations of β-diversity. This is consistent with previous work, which has shown that inferred differences in community richness and turnover are driven purely by γ-diversity differences (Kraft et al. 2011). While these differences can be accounted for in the current study, in studies with more time points, greater data complexity, or that only utilise one null model, one might state with confidence that within-host diversity was significantly larger than expected. This could lead, for instance, to inferences of parasite facilitation inside a host, when it is instead driven by the opposite end of the hierarchical scale (Chase & Myers 2011). Despite this incongruence at the α-diversity level, interpretations of β-diversity in terms of turnover were consistent regardless of which null model was used: between-host turnover was less than expected, while between-species and between-site turnover was greater than expected (Fig. 6.4).

Conversely, interpretations of β-diversity in terms of whether parasite infracommunities were considered significantly nested varied with null model selection. Comparisons of the observed nestedness to predictions from the EE, EF or FE null models suggest that infracommunities are significantly *more* nested than expected (Fig. 6.4); in contrast, comparing to the FF model, with both parasite prevalence and individual host richness constrained, suggests parasite infracommunities are significantly *less* nested than expected. Taken together with the additive partitioning results, we make the following four statements: (1) Turnover in parasite communities between host species, and between sites, is larger than expected; (2) This observed turnover cannot be explained by parasite prevalence or individual host richness; (3) Parasite infracommunties were more nested than expected, even when considering parasite prevalence or individual host richness in isolation; (4) Accounting for both parasite prevalence and individual host richness, infracommunities were less nested than expected. Below, we treat each statement in turn and consider their implications.

6.4.2. Statement 1: Turnover in parasite communities between host species, and between sites, is larger than expected.

This conclusion is consistent with previous work, which has found host species and location to be major determinants of parasite community composition (e.g. Vidal-Martinez & Poulin 2003; Dallas et al. 2019). This trend is expanded on by Fig. 6.5, which highlights similar parasite richness for individual *U. pictorum* hosts at all three sites, while being slightly elevated at OW. In contrast, *A. anatina* shows a clear increasing gradient from BC to OW. While overall site parasite richness was slightly higher at OW (14 parasites, vs. 11 [KD] and 13 [BC]), it is not to the same extent as the increase in individual *A. anatina* richness, suggesting that the interaction is driven by higher prevalences of parasites specific to *A. anatina* at KD and OW, such as *R. campanula*, the castrating trematode (Taskinen et al. 1997). This may have important functional consequences. Competition between hosts can be mediated by parasites (Hatcher et al. 2006, 2012), something which can vary on a population level (Reichard et al. 2015; Papkou et al. 2016). If this is the case, our results suggest there could be different outcomes of competition at BC (where *A. anatina* and *U. pictorum* individuals have similar parasite richness) and OW (where *A. anatina* individuals have on average twice as many parasites). Given the importance of unionid mussels as ecosystem engineers (Vaughn 2018), a distribution of parasite pressure that is uneven at both the site and host species level could have a significant effect on the wider environment (see Chapter 8), highlighting the need to consider parasite richness across sites and within host species.

6.4.3. Statement 2: This observed species and site turnover cannot be explained by parasite prevalence or individual host richness.

Even accounting for parasite prevalence or site-specific host richness patterns, null models predicted that β_2 and β_3 should be ~0 (Fig. 6.4); in other words, based on the prevalence of the different parasites in the study, and the mean infracommunity richness of all the hosts, it is statistically likely that all sites should have all parasites, and these parasites should be found in both host species. The fact that this is not observed is strongly suggestive of dispersal limitation at multiple scales (Guégan et al. 2005): parasites' dispersal ability to hosts is being mediated by both between-site differences, and subsequently by differences between host species within sites (i.e. host compatibility). Using the broad framework of Catford et al. (2009), successful infection is first reliant on propagule pressure, and then on

abiotic and biotic factors. Propagule pressure provides a valid explanation for the different prevalences between sites within a single species (Hoover & Brittingham 1993; Olori et al. 2018). Many of the parasites in our study system (trematodes and mites) require multiple hosts in their life cycle, and these additional hosts may exhibit differential abundance between the sites, thus limiting the ability of the parasite to complete its life cycle (Lafferty & Harvell 2014). Alternatively, there may be cryptic unmeasured environmental (abiotic) variation between the sites, which affects the transmission stages of the parasite (Tavares-Dias et al. 2014; Penczykowski et al. 2016). These are plausible non-mutually exclusive hypotheses to explain the mean richness and prevalence differences between sites (i.e. β_3), but fail to account for why *A. anatina* should have significantly higher richness than *U. pictorum* at all three sites (i.e. β_2) (Fig. 6.5b): a biotic filter is clearly operating. This raises the question of primary vs. secondary unsuitability (Grim et al. 2011): does *U. pictorum* have superior host defense, or are parasites preferentially infecting *A. anatina*? While from an observational standpoint these lead to the same outcome, it has important consequences. If it is the former explanation, attempted infections of *U. pictorum* are 'wasted', and this could actually help *A. anatina* through a diluting effect (Rigaud et al. 2010). In contrast, if parasites specifically target *A. anatina*, their fitness may be compromised relative to *U. pictorum*, and suffer in competitive interactions. Previous research has shown the importance of co-evolutionary history between hosts and parasites (Reichard et al. 2012; Feis et al. 2016); it is possible that *A. anatina* has a longer co-evolutionary history with the suite of parasites in the community which explains the closer association with them. While beyond the scope of the current study, the hypotheses developed here provide avenues of future research.

6.4.4. Statement 3: Parasite infracommunties were more nested than expected, even when considering parasite prevalence or richness in individual hosts in isolation.

Comparisons with three of the four null models (excluding FF) suggest that low-richness parasite infracommunities are nested subsets of richer infracommunities. Patterns of both parasite prevalence (Rynkiewicz et al. 2019) and richness in individual hosts (Poulin & Valtonen 2001) can contribute to such nestedness, and it appears both are acting here. Differential prevalences in a parasite community cause nestedness in intuitive fashion: assuming equivalent dispersal ability, highly prevalent parasites are likely to be found in many hosts, with progressively lower-prevalence parasites found in subsets of those hosts. Parasite prevalence is clearly contributing to nested patterns: allowing it to vary randomly led

to extremely low nestedness, while constraining it to the observed values led to much higher predicted nestedness (compare NODF of EE and EF models, Fig. 6.4). Similarly, individual host richness contributed to nestedness, though to a lesser extent: taking into account the observed host richness increased nestedness slightly (compare NODF of EE and FE models, Fig. 6.4) suggesting hosts with richer infracommunities were more likely to have parasites consistently absent from smaller infracommunities. This pattern can be caused by a range of factors (Baselga 2010) that are not immediately distinguishable in an observational context. However, host characteristics such as size have previously been shown to generate nestedness (Vidal-Martínez & Poulin 2003). Size reflects greater consumptive ability (= sampling ability of parasites in the environment) or age, which make progressively larger or older organisms more likely to host rarer parasites, thereby producing a pattern where the infracommunities of younger or smaller hosts are predictable subsets of larger ones. We suggest that host characteristics do have a role to play in our study system: while between-host turnover (β_1) was less than expected by chance, it still provided the single biggest contribution to γ-diversity in the study (~34% of γ; Figs. 6.4, 6.5a), and recent work has shown that, within a single site, mussel length and gravidity are both important in the construction of parasite infracommunities (Chapter 5). While the influence of site and species outweigh individual host characteristics (Fig. A4.3), their contribution should not necessarily be considered unimportant in the context of community construction across scales.

While both parasite prevalence and host richness contributed to the observed nestedness, nestedness was still greater than expected when considering these factors in isolation. However, after accounting for both of these factors together (model FF), infracommunities were less nested than expected by chance (Statement 4).

6.4.5. Statement 4: Accounting for both parasite prevalence and parasite richness of individual hosts, infracommunities were less nested than expected.

A pattern of anti-nestedness indicates that parasites are more dispersed than random among infracommunities (Poulin & Guégan 2000); in other words, infracommunities are more likely to be composed of discrete modules. In our study, this pattern was observed for both host species separately, which implies that this modularity is not solely caused by communities assembling differently in the two host species. Instead, it could reflect within-host parasite interactions (Fig. 6.6; see Bashey 2015; Clay et al. 2019). While the correlations could be

driven by an unmeasured environmental factor (Blanchet et al. 2020), 70% of the interactions were negative, and a majority of them occurred within the same host tissue (e.g. where both parasites were in the gills of the mussel), which aligns closely with previous work that showed predominantly negative interactions between parasites occupying the same tissue (Dallas et al. 2019). Further, interactions in this study system have been shown to be predominantly exclusionary (i.e. they limit the presence of parasites inside hosts, rather than their abundance; Chapter 5), which also supports the argument that within-host interactions contribute to infracommunity modularity. However, this pattern is only detected once parasite prevalence and host infracommunity richness are accounted for, demonstrating the importance of null model selection. While it has been previously found that factors influencing richness at the host level differ from those affecting the community level (Johnson et al. 2016), we suggest that the two levels could interact through mechanisms such as within-host competition.

6.4.6. Implications

This study makes two contributions to the broader fields of community ecology and biogeography. The first is that null model selection has real-world implications for the interpretation of community matrix data, but that differences in null models can be leveraged to understand nuances of the community in question. We encourage the critical use of null models in further work, especially with the development of recent algorithms that can explore variable degrees of constraint among row or column totals, rather than just being completely constrained or unconstrained (Strona et al. 2018).

The second contribution is that community composition is significantly driven by factors at all scales of organisation (Fig. 6.4); in this case, from between-site and between-species (Fig. 6.5b) to within-host (Fig. 6.5a, 6.6), and that an integrated approach is required to further understanding in community ecology. This conclusion has impacts for both understanding community and metacommunity assembly generally, but also for applications such as host and parasite conservation. Given the species- and site-dependency of parasitism, ignoring drivers of parasite community structure may jeopardise conservation action, especially for interventions such as captive breeding or translocation (see Chapter 9). By incorporating analyses of turnover and nestedness, we extend previous work which showed that drivers of community structure vary in importance across scales (Bolnick et al. 2020b; Moss et al.

2020) to show how parasite prevalence and richness of individual hosts influence the observed patterns. In sum, processes influencing community structure at one level may mask drivers at another. These results therefore further emphasise the need to understand what drives inherent commonness and rarity in populations, how propagule pressure (i.e. dispersal) varies with scale, and the transition from initial entry into communities to establishment.

Chapter 7: Population-level effects of parasitism on a freshwater ecosystem engineer, the unionid mussel *Anodonta anatina*

Abstract

Parasites can negatively affect hosts at individual, population and species-level scales. However, the link between individual- and population-level impacts is often poorly understood. In particular, the population-level response to parasitism may alter wider ecosystem dynamics if animals with ecosystem engineering capabilities are infected. Here, we examine the effects of parasitism on a freshwater ecosystem engineer, the unionid mussel *Anodonta anatina*, at two different sites. We study three common parasites: the digenean trematode *Rhipidocotyle campanula*, the unionicolid mite *Unionicola intermedia* and the ectoparasitic invasive zebra mussel *Dreissena polymorpha*. As well as demonstrating the individual-level effects of parasitism on the native host mussel, we construct a simple model to estimate the reduction in population-level reproductive output caused by parasites. We show that both infection prevalence and intensity were population-specific, with one site having over three times as many native mussels infected by trematodes and mites than the other, but over four times fewer mussels afflicted by invasive zebra mussels. Negative reproductive consequences for individual host mussels were documented as a result of parasitism, with trematodes causing castration at both sites. Mites were also correlated with a reduction in the viability of larval offspring (glochidia) by over 25%, but only at one site, suggesting some potential impacts of parasitism may be population-specific. The population-level model shows that parasitism alone reduces larval output of the two populations by 10% and 13% respectively. Our study takes the important step of scaling individual-level effects of parasitism to population-level processes, and highlights the influence that parasites may have in the population dynamics of unionid mussels. Given the ecosystem engineering capabilities of *A. anatina*, such effects may have important impacts on the wider biota. Even at relatively low prevalences, the observed effects of parasites on native mussel populations suggests that parasitism must be considered in the conservation of freshwater mussels, one of the world's most globally imperiled faunal groups. Further, understanding how the effects of parasitism on individual hosts scales to the ecosystem level is a crucial and unaddressed question in freshwater biology.

Key words: castration, ecosystem services, mite, trematode, zebra mussel

7.1. Introduction

Parasites are a ubiquitous feature of ecosystems (Lafferty et al. 2008), and host-parasite interactions show an extensive evolutionary history (Zhang et al. 2020). While parasites are crucial for the functioning of ecosystems (Hudson et al. 2006), they may affect individuals in a range of negative ways, such as reducing fecundity (Auld et al. 2012) and lowering body condition (Sánchez et al. 2018). The degree of this effect depends on the virulence of the parasite (Fig. A5.1). In turn, these individual-level effects can scale to the population level (Fig. A5.1), and may significantly impact the success of populations. There is evidence of this link from freshwater ecosystems; for example, the invasion success of the non-native amphipod *Dikerogammarus haemobaphes* is predicted to be limited by microsporidian parasites (Bojko et al. 2018), and there is a correlative link between fungal parasites and freshwater fish and amphibian declines (Rowley et al. 2013). In extreme cases, the impacts of parasitism on populations are observed as localised outbreaks with often devastating consequences (e.g. Katsanevakis et al. 2019). It is likely that parasite-induced population fitness differences are common to a less observable degree, but in general the link between individual impacts and population-level effects is poorly understood and requires further characterization (Wood & Johnson 2015).

The impacts of parasitism are particularly pertinent for populations of ecosystem engineers, as parasites could affect their influence on the environment (Dunn & Hatcher 2015). Freshwater mussels (Unionida) are one such ecosystem engineering group. Through their burrowing they increase oxygenation (Vaughn & Hakenkamp 2001), and their extensive filtering enhances water clarity and facilitates nutrient deposition (Howard & Cuffey 2006). They are associated with increased biodiversity in the rivers and lakes they inhabit (Aldridge et al. 2007; Chowdhury et al. 2016), and play a significant role in the healthy functioning of freshwater ecosystems. Unionid mussels are affected by a broad range of parasites (Chapter 2); however, few studies of parasitism in this group examine the impact on the hosts. Of the 237 studies reviewed in Chapter 2, which assessed all published host-parasite records for North American and European freshwater mussels, just 20% looked at the effects of parasitism, and these studies are largely focused at the level of the individual mussel. However, the negative fitness consequences of parasites on individual mussels may scale to population-level metrics of success, with subsequent impacts on the wider ecosystem.

The most commonly studied parasites of freshwater mussels are digenean trematodes, unionicolid mites (both endoparasites) and zebra mussels (an ectoparasite). The effects of trematodes are generally well-understood, causing significant reductions in reproductive output and potentially castrating their hosts (Jokela et al. 1993; Taskinen et al. 1994). However, the effects are generally reported at an individual level (e.g. is the mussel producing offspring or not), with no quantification of population-level effects (but see Taskinen & Valtonen 1995). The effects of unionicolid mites are less clear, with some studies reporting parasitic behaviour affecting the health of the host (e.g. Fisher et al. 2000) and others providing evidence for commensalism (e.g. McElwain et al. 2016). Previously reported parasitic behaviours of mites include consumption of gill tissue (Fisher et al. 2000; Walker 2017), thus interfering with larval brooding by females which use the gills as marsupia for their developing glochidia. However, similarly to trematodes, to our knowledge no studies have examined the population-level impacts of mite parasitism on mussels. Zebra mussels are invasive dreissenids that have spread from the Ponto-Caspian region of eastern Europe throughout North America and Europe (Aldridge et al. 2004). These attach to the shell of native mussels and intercept food particles drawn in by the underlying native mussel, thus providing a physical and physiological stress that can lower body condition (Sousa et al. 2011).

Freshwater mussels are in decline worldwide (Bogan 1993; Lydeard et al. 2004), and understanding their population viability and reproductive potential have been recently identified as key research priorities in their conservation (Ferreira-Rodriguez et al. 2019). Trematodes, mites and zebra mussels all have the potential to directly or indirectly interfere with mussel reproduction, making a quantification of their population-level impacts an essential yet poorly addressed concern (Jokela et al. 2005). Here, we study the freshwater unionid mussel *Anodonta anatina* to examine the effects of trematodes, mites and zebra mussels on host reproductive capacity. As well as studying parasite prevalence, intensity and observable individual-level effects, we develop a simple model to demonstrate significant population-level decreases in larval mussel production caused by parasites. Further, as site is a key predictor of parasite prevalence and abundance (Vidal-Martinez & Poulin 2003; Dallas et al. 2019; Chapter 6), we extend our approach by examining two hydrologically linked sites, which allows us to explore context-dependency in the impact of parasitism.

7.2. Methods

7.2.1. Study species and sampling

The non-endangered unionid duck mussel *Anodonta anatina* (Linnaeus 1758) was chosen as our study species as it is common throughout Europe (Lopes-Lima et al. 2017), and is infected with a broad range of parasites (Chapter 2, Chapter 5). We collected 60 mussels from each of two sites that are hydrologically connected but separated by approximately 20 kilometres: the River Great Ouse at Brandon Creek (52.5002° N, 0.3650° E; henceforth BC) and the Old West River at Stretham, a tributary of the Great Ouse (52.3343° N, 0.2243° E; henceforth OW) (see Fig. 6.1).

We sampled mussels by hand from the river margin, retaining mussels of all sizes until 60 had been collected at each site. Sampling of both sites took place on a single day (7th November 2019). We transported mussels back to the laboratory in the river water they were collected in, and held them in aerated water at ambient temperature (8° C) until subsequent dissection (maximum of 10 days until dissection). Transfer of parasites between individuals while being held is highly improbable, as all parasites considered were either encysted (mite larvae and eggs, zebra mussels) or require multiple hosts to complete their life cycle (trematodes) and so cannot be directly infected by conspecifics.

7.2.2. Dissection and parasite quantification

Prior to dissecting each native mussel, we removed and counted any invasive zebra mussels (*Dreissena polymorpha*) present on their shells. We then measured the maximum length of each native mussel to the nearest 0.1mm with Vernier callipers, before sacrificing mussels by slicing the posterior and anterior adductor muscles. The mantle of each *A. anatina* was inspected under a dissecting microscope to identify the presence of encysted larvae and eggs of the parasitic mite *Unionicola intermedia* (henceforth referred to as ‘mites’), and scored as a binary presence/absence. We then inspected the gonad of the mussel to identify and quantify infection with the digenean *Rhipidocotyle campanula* (henceforth referred to as ‘trematodes’), following Chapter 3. Briefly, we removed the visceral mass and made an incision ~1cm from the posterior end, then used forceps to remove samples of gonad tissue which we squashed between two glass microscope slides. These gonad squashes were

inspected under 40× magnification to identify trematode infection. If trematodes were present, we photographed these gonad squashes (12 replicate photographs in total) and used the program ImageJ to estimate the percentage of each mussel gonad filled with trematode tissue. In total, we therefore obtained presence-absence data for zebra mussels, mites and trematodes, in addition to a quantitative level of infection for zebra mussels and trematodes. While zebra mussels are themselves affected by parasites, a comprehensive review of invasive and native mussels from Europe and North America demonstrated that zebra mussels do not host *U. intermedia* or *R. campanula* (Chapter 2), and so zebra mussel parasites were not considered further in the present study.

Following identification of parasites and processing of gravid mussels (see below), we dried all mussel tissue to constant mass for 48 h and then weighed it to the nearest 0.001 g; we also weighed the dried shells for each mussel to the nearest 0.001 g.

7.2.3. Processing gravid mussels

For all mussels, we removed the right outer demibranch to determine the sex of the mussel and quantify gravidity if applicable. First, demibranchs were weighed to the nearest 0.001 g. Gravid mussels were immediately apparent by the swollen appearance of the demibranch, indicating the marsupia were filled with glochidia (larval mussels). For these mussels, we quantified glochidia viability. This involved breaking open the centre of the removed right demibranch using fine forceps and taking a sample of glochidia. These glochidia were gently mixed into water on a petri dish, and table salt (NaCl) was added to determine glochidial viability (following Bringolf et al. 2007). We examined the sample of glochidia under a microscope and the first 100 were counted. Glochidia which were snapping or which had closed were counted as viable, and any which remained open were counted as non-viable, with overall viability being expressed as a percentage.

For mussels that were not gravid, we gently teased apart the removed demibranch under a dissecting microscope to search for marsupia, the interlamellar tubes that bear glochidia. Mussels lacking marsupia were classed as male, while mussels possessing marsupia (in addition to those that were gravid) were classed as female/hermaphroditic.

7.2.4. Statistical analysis

All statistical analyses were executed in R v.3.6.3 (R Core Team, 2020). We utilised logistic regression (using a generalised linear model with a logit link) to explore significant factors explaining the presence or absence of parasites. We modelled the presence of each of the three parasites (zebra mussels, mites, trematodes) as the response variable in turn, and included site, mussel weight, mussel length, sex and the other two parasites as possible explanatory variables. Further, for zebra mussels and trematodes (which had quantitative data), we explored the influence of site and mussel length on the intensity of infection. For the site comparisons we used a non-parametric Kruskal-Wallis test, as assumptions of parametric tests could not be met using either raw or transformed data. For the trematode length analysis we used standard linear regression. For the zebra mussel length analysis we utilised standard linear regression on log-transformed data using site BC only, as there was a maximum of 2 zebra mussels on any given native mussel at OW (median = 0).

Next, we examined the impact of factors on native mussel weight, using three general linear models with a Gaussian link, which had tissue weight, shell weight and total weight as the response variable, respectively. Explanatory variables included were site, gravid status and the three parasites. Assumptions were checked and verified to have been met using normal Q-Q and residuals vs. fitted values plots. In addition, for these and the above analyses that included multiple explanatory variables, we verified an absence of multicollinearity by confirming the variance inflation factors were all <5.

Finally, we examined the effect of parasites on the reproductive capacity of native mussels. First, we utilised logistic regression with gravidity (yes/no) as the response variable, and site, mussel length and the three parasite types as possible explanatory variables. Next, we used non-parametric Kruskal-Wallis tests to look at the effect of zebra mussel and mite presence on glochidia viability. This was not attempted for trematodes, as no trematode-infected mussels were gravid (see Results).

In order to estimate parasite influence on the reproductive output of mussel populations, we incorporated results of all the above analyses into a model to predict glochidial output in the absence of parasitism, and in the presence of parasitism (i.e. the actual scenario). We chose to express reproductive output of the population in terms of glochidial production (in g) per 100

g of shell mass, as this measure does not require assumptions about total population size. The model described below was run four times, each with 1000 replicates: for OW with and without parasites, and for BC with and without parasites. For each site, we used the binomial 95% confidence intervals for both the proportion of gravid mussels and the proportion of mussels infected by trematodes to estimate the minimum and maximum number of mussels expected to be gravid at each site in the presence or absence of trematodes. Then, we sampled a random number of mussels from the population within those intervals (representing the pool of gravid mussels for that particular replicate), and summed their glochidial mass. The probability of any one mussel being selected in a given replicate was weighted according to their probability of being gravid, as larger mussels were more likely to be gravid. For mussels that were not gravid originally, their predicted glochidial mass was estimated using their length using site-specific linear equations, as glochidial mass was strongly correlated with length. The summed glochidial mass was multiplied by the average glochidial viability for that site, according to whether the run included the presence of parasites or not (as mites had a site-specific influence on glochidial viability, see Results). This yielded total mass of viable glochidia, which we then divided by the total shell weight of all 60 mussels (of either BC or OW mussels depending on the run). This produced an estimate for each replicate of the mass of viable glochidia per 100 g of shell weight. While this exercise used the sample size of 60 mussels at each site, assuming that our sample is representative of the overall mussel populations, this estimate is generalisable to the population as a whole, as it is independent of the actual number of mussels sampled. The overall results of these models were four means (averaged over the 1000 replicates) with associated 95% confidence intervals: viable glochidial mass per 100 g of shell weight for BC in the absence of parasitism; for BC in the presence of parasitism; for OW in the absence of parasitism; and for OW in the presence of parasitism. We also calculated the actual value of viable glochidial mass per 100 g shell mass for BC and OW in the study, and compared those values to the model results in the presence of parasitism, to confirm that our model gave realistic predictions. For full details of model specification and R code, see Appendix A5.

7.3. Results

7.3.1. Native mussel parameters

In total, 120 *A. anatina* were dissected across both sites. Using the presence or absence of marsupia in the outer demibranchs, three were identified as male (two at OW, one at BC) and 117 as either female or hermaphroditic (i.e. possessing the female trait of marsupia). There were no differences between the two sites in terms of length (overall length 69.7 ± 11.6 mm, mean ± s.d., $t_{118} = -1.02$, $p = 0.310$), total dried weight (8.4 ± 4.0 g, $t_{118} = -1.04$, $p = 0.194$) or the proportion of mussels that were gravid (45% of all mussels, $z = 0.27$, $p = 0.788$).

7.3.2. Factors influencing parasite distribution and abundance

Factors correlated with the presence or absence of parasites were investigated with logistic regression. Modelling showed that for both mites and zebra mussels, site had a significant relationship with the proportion of native mussels infected: there were on average 5.8 times more mussels infected with mites at OW ($z = 3.60$, $p < 0.001$), and 4.5 times more native mussels with attached zebra mussels at BC ($z = -4.93$, $p < 0.001$) (Fig. 7.1a). There were also 3 times as many native mussels infected by trematodes at OW than BC, though the lower total prevalences meant the sites were not statistically different ($z = 1.67$, $p = 0.095$, Fig. 7.1a). For the two parasites that had a quantitative measure taken (zebra mussels and trematodes), site was also a significant predictor when considering intensity of infection – the abundance of zebra mussels per native mussel was on average 40 times higher at BC ($\chi^2_1 = 87.85$, $p < 0.001$, Fig. 7.1b), and the infection intensity of trematodes was 2.2 times higher at BC ($\chi^2_1 = 6.23$, $p = 0.013$, Fig. 7.1c).

Native mussel length was not associated with the probability of infection with zebra mussels or trematodes, or the infection intensity of trematodes ($p > 0.05$, all cases). However, at BC native mussel length was significantly correlated with zebra mussel abundance ($t_{58} = 2.26$, $p = 0.028$, Fig. 7.2a), and was also correlated with the probability of infection with mites ($z = 2.42$, $p = 0.016$, Fig. 7.2b). For a given parasite, no other factors, including the presence of the other two parasites, were significantly related to patterns of distribution or abundance among sites or mussels.

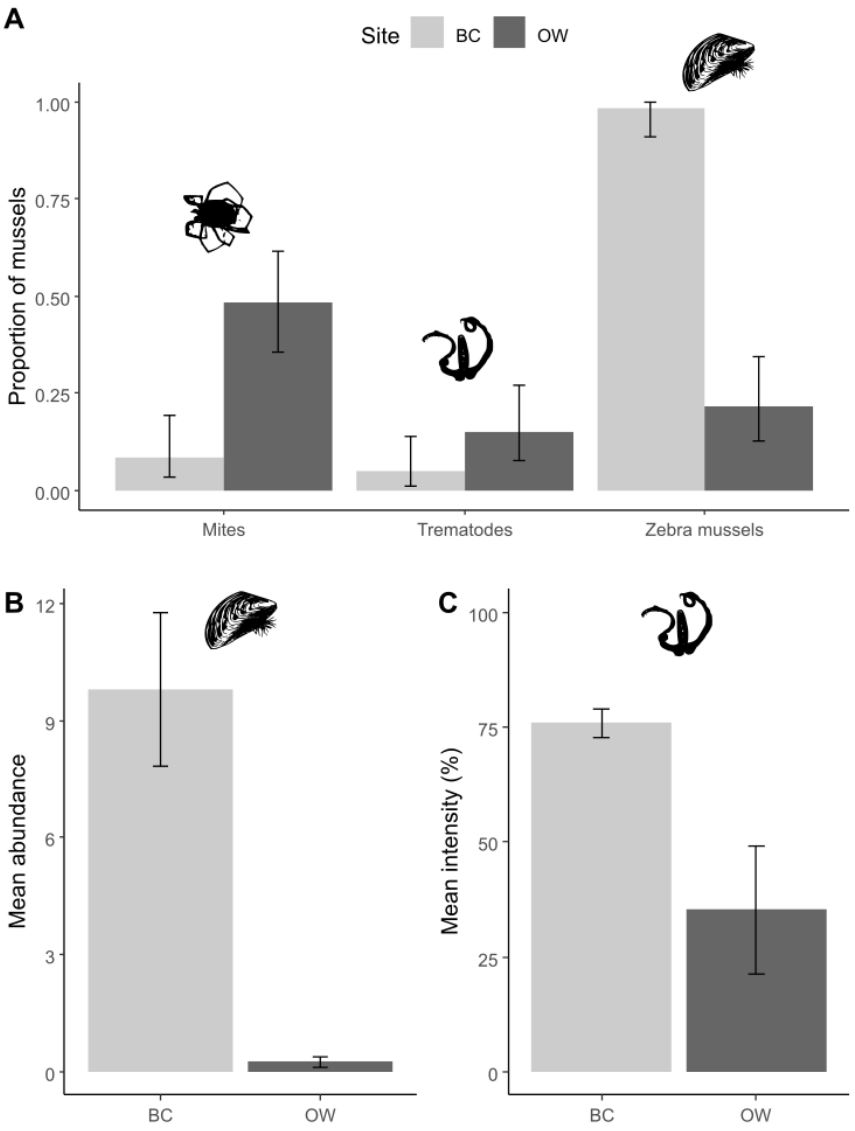


Figure 7.1: Comparisons of infection proportions and intensities between the sites Brandon Creek (BC, light grey) and Old West (OW, dark grey). (A) The proportion of mussels at each site infected with mites, trematodes and zebra mussels respectively. Error bars represent 95% binomial confidence intervals. (B) The mean abundance of zebra mussels (per native mussel) at BC and OW. Error bars represent standard 95% confidence intervals. (C) The average infection intensity (percentage of the gonad filled with trematode tissue, following Chapter 3) of mussels at BC and OW. Only infected mussels were included. Error bars represent standard 95% confidence intervals.

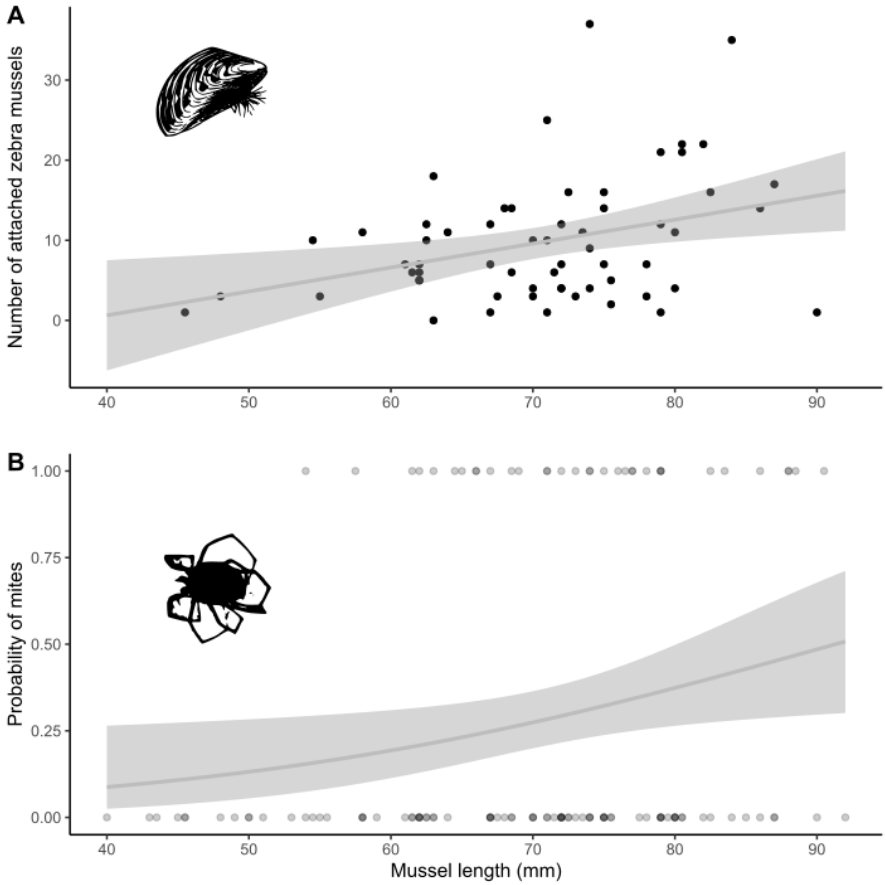


Figure 7.2: Relationship between native mussel length and parasite prevalence or abundance. Grey lines represent fitted relationships, with shading denoting the 95% confidence interval. (A) As native mussel length increased, the number of attached zebra mussels also increased. Only BC data were used, as a maximum of 2 zebra mussels were attached to any one native mussel at OW (median = 0). (B) As native mussel length increased, the probability of being infected with mites also increased.

7.3.3. Relationship between parasites and host population reproductive capacity

General linear models were used to explore the relationship between parasites and the weight of host mussels. After accounting for the effects of site and mussel gravidity (which increases tissue weight), parasites had no correlation with the weight of native mussel hosts, regardless of whether tissue weight, shell weight or combined weight was considered ($p > 0.05$, all cases).

Next, a logistic model was used to explore the factors determining whether or not a mussel was gravid. Larger mussels were significantly more likely to be gravid ($z = 3.49$, $p < 0.001$), and mussels infected with trematodes were significantly less likely to be gravid ($z = -0.02$, $p < 0.001$): indeed, no mussels with trematodes were observed to be gravid in either site (Fig.

7.3a). Mites and zebra mussels had no relationship with the likelihood of a native mussel being gravid ($p > 0.05$ in both cases).

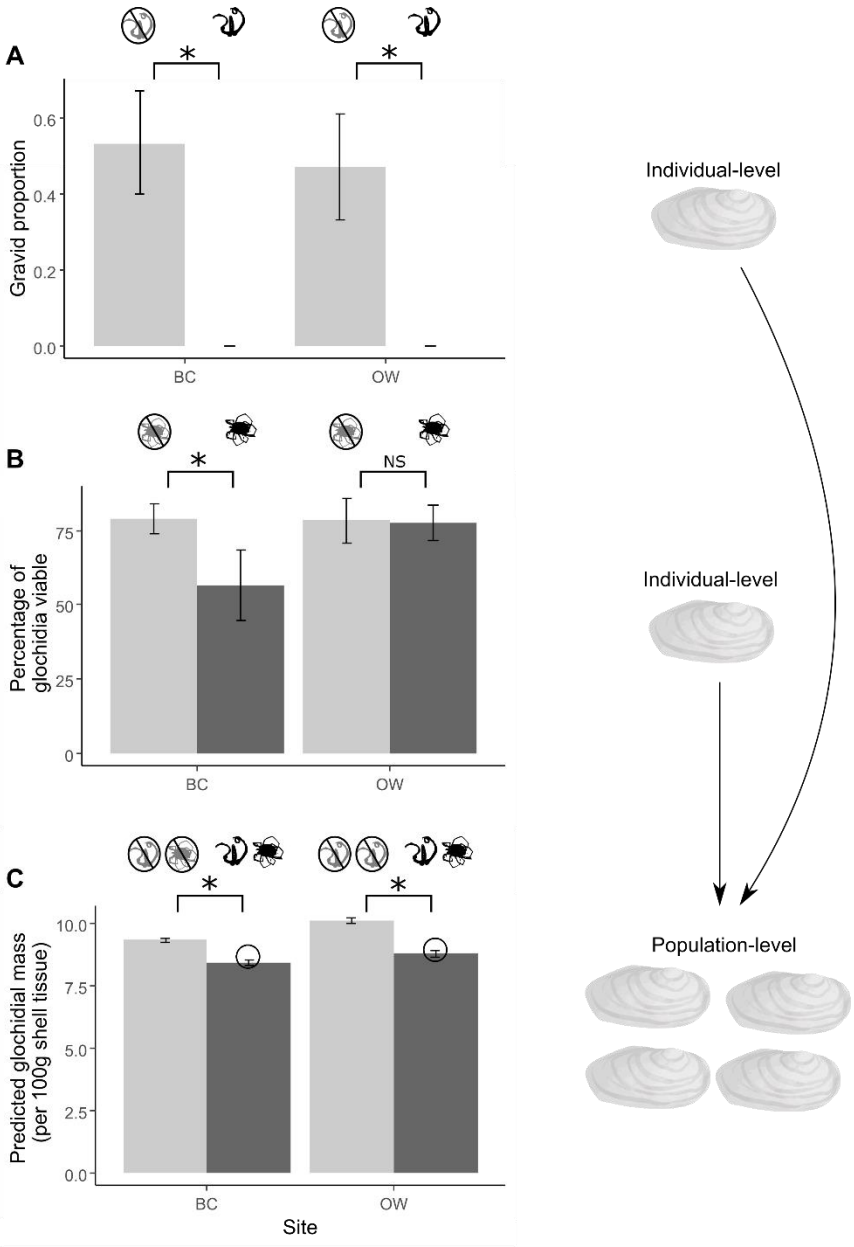


Figure 7.3: Impact of parasites (dark grey bars) on reproductive function of native mussels compared to non-parasitised mussels (light grey bars). All error bars represent 95% confidence intervals. A '*' indicates a statistically significant difference between groups, with 'NS' indicating differences are not significant. (A) Effect of trematode infection on the likelihood of an individual native mussel being gravid. (B) Effect of mite infection on the viability of glochidia produced by individual gravid mussels. (C) Results of the model predicting mean glochidial (in g) population output per 100 g of shell weight for native mussels in the presence of trematodes and mites, and in the absence of trematodes and mites. Open circles represent the true values at BC and OW, and are very similar to predictions in the presence of parasitism, as expected.

For gravid native mussels, the viability of glochidia was compared between mussels infected and not infected with zebra mussels, and between mussels infected and not infected with mites. This was not carried out for trematodes, as no mussels infected with trematodes were gravid. Zebra mussels were not associated with the viability of glochidia produced by native mussels, regardless of site ($p > 0.05$ in both cases). However, mite presence was correlated with a site-specific effect on glochidia viability; glochidia viability from mite-infected mussels at BC was significantly lower than non-infected mussels at BC (Kruskal-Wallis, $\chi^2_1=4.45$, $p = 0.035$), and glochidial viability was also lower than mite-infected mussels at OW ($\chi^2_1 = 5.14$, $p = 0.023$) (Fig. 7.3b). In contrast, there was no difference in glochidial viability between non-infected mussels at BC and OW ($\chi^2_1 = 0.17$, $p = 0.681$) or between infected and non-infected mussels at OW ($\chi^2_1 = 0.055$, $p = 0.815$) (Fig. 7.3b).

Finally, our model that incorporates the individual-level effects of trematodes (Fig. 7.3a) and mites (Fig. 7.3b) to predict population-level glochidial output revealed a significant reduction of 13.0% at OW and 9.6% at BC caused purely by the combined presence of these parasites (Fig. 7.3c). The actual values observed in the data aligned very closely with the predicted model values (Fig. 7.3c), suggesting the model constructed is applicable and reliable.

7.4. Discussion

In this paper, we have explored factors explaining the rate of parasitism and its consequences in a common unionid mussel, at both individual and population scales (Fig. A5.1), showing significant negative consequences for native mussel reproduction. In this discussion, we first consider our individual-level results in light of previous research, before considering the implications of our population-level findings for mussel conservation and ecosystem services.

7.4.1. The effect of parasitism on individuals

At an individual level, we found mussel length to be a significant predictor for the presence of mites, and for the number of zebra mussels at the site with high intensity (BC) (Fig. 7.2). Both these results are intuitive and support previously documented patterns. As hosts constitute a resource base, larger hosts represent a larger resource supply and are therefore preferentially infected by parasites with the ability to select hosts. The ability of mites to select hosts is well-established (e.g. Downes 1986, 1991), and previous studies found that

mites were more likely to infect larger mussel hosts (Dimock 1985; Wen et al. 2006), something borne out by our results (Fig. 7.2b). Similarly, larger native mussels also have a greater exposed shell area for attachment by zebra mussels (Sousa et al. 2011), in addition to the long lifespan of *A. anatina* (Aldridge 1999a) meaning that larger mussels will likely accumulate greater numbers of zebra mussels over time. As zebra mussels can settle on a range of substrates, at low abundance any effect of native mussel shell length may reflect this lifetime chance of infection rather than zebra mussels actively choosing larger mussels, which may explain why native mussel size was not correlated with the presence of zebra mussels, only their intensity (Fig. 7.2a). Given these patterns, we show that larger mussels are therefore at greater risk of parasitism from zebra mussels and mites. The population size structure of native mussel populations may therefore be important in predicting parasitism: sites with low incidence of recent recruitment will have predominantly larger individuals in the population and so may be disproportionately vulnerable to parasitism, which could further limit the production and recruitment of juvenile mussels.

Host mussel length was not a significant predictor for trematode presence or intensity, in contrast to the above. This is also different to previous studies, which reported an increased prevalence among larger mussels (e.g. Taskinen & Valtonen 1995; Müller et al. 2015). These studies hypothesised that trematodes preferentially infected larger mussels, or that larger mussels were older, and therefore had a greater lifetime chance of being infected. We did not detect this pattern, possibly because our sample size was too small to detect a significant effect. However, our results did align with previous research in showing a significant negative effect of *R. campanula* on the reproductive potential of their hosts, with no mussels observed as gravid at even low levels of infection (Fig. 7.3a). The eventual castrating effects of digenean trematodes are well-documented (e.g. Jokela et al 1993; Walker 2017), particularly for the virulent *R. campanula* (Taskinen et al. 1994; Müller et al. 2015), but these studies still record native mussels being able to reproduce at low infection intensities. We have also previously recorded gravid mussels at low infection intensities of *R. campanula* (Chapter 5), suggesting that understanding the degree of infection is as important as understanding its presence (Chapter 3; Fig. 7.1c). Nevertheless, our results highlight that even intermediate infection intensities are correlated with mussel castration, showing the need to understand the presence and distribution of this highly virulent parasite across individuals and populations.

The mite *U. intermedia* also showed a possible site-specific effect on the reproductive potential of their hosts, as this parasite was correlated with a 28% reduction in glochidial viability at BC relative to uninfected mussels (Fig. 7.3b). We acknowledge that these data are correlative only; it is possible that mussels inherently producing low-viability glochidia were also more susceptible to mite infection. Further, the mechanism for this reduction is not immediately apparent, given mite presence was observed as eggs and larval mites encysted in the mantle and therefore separated in space from the gills where glochidia are brooded. However, eggs and larvae signify the presence of adult mites, which are transient and return to mussels to produce offspring (Baker 1988) and may have previously imposed stress or damage on the native mussels. Adult *U. intermedia* have been observed feeding on gill tissue (Baker 1976, 1977), and in general mites of multiple species have been shown to damage gill tissue (Fisher et al. 2000; Walker 2017). This provides a plausible mechanism for reduced viability of host glochidia in the presence of mites, a trend which has also been previously reported (Gangloff et al. 2008).

7.4.2. The population-level implications of parasitism

We combined the negative reproductive effects of trematodes and mites in our model to show that parasitism correlates with a 9% to 13% reduction in population glochidial output of native mussels relative to predictions in the absence of parasitism (Fig. 7.3c). To our knowledge, this is the first study that demonstrates a reduction in population-level reproductive capacity caused by parasitism in freshwater mussel populations. While Taskinen & Valtonen (1995) did estimate the proportion of glochidia-bearing mussels in the population reduced by trematodes, this did not explicitly predict glochidial output, which may be influenced by other factors such as adult mussel size and glochidia viability patterns. We note that our predicted reduction in glochidial output does not necessarily translate to population impact: if recruitment is not glochidia-limited, a reduction in glochidial production may not matter to overall population success. However, glochidia are highly sensitive and suffer massive mortality (Jansen et al. 2001); in vulnerable or endangered populations, any significant reduction in the glochidial pool could have serious consequences. Even with the relatively low trematode and mite prevalence in the current study, especially at BC, we found a minimum reduction of 9.6% in glochidial production by the population. Other studies have reported much higher prevalences (e.g. Taskinen & Valtonen (1995) reported 32.3% of all studied *A. anatina* infected with *R. fennica*, which eventually castrates its host), which may

translate to dramatic reductions in reproductive potential. Recent years have seen a high number of enigmatic declines in freshwater mussels, many of which are characterised by a cessation of recruitment rather than by immediate death of all individuals (Haag 2019). This pattern of recruitment cessation is consistent with parasite-driven reductions in reproductive potential, something which is acknowledged to be an under-studied impact (Chapter 2; Ferreira-Rodríguez et al. 2019). Our demonstration that parasites are correlated with significant reductions in glochidial productivity, even at low prevalence, shows that parasites should be carefully considered in the conservation of threatened or endangered mussels.

The population-level impacts of parasitism may not just be limited directly to reproductive output; parasites also have the potential to influence the broader reproductive strategy of the population. For example, populations of the freshwater snail *Pisidium amnicum* become semelparous breeders under high levels of trematode parasitism (Rantanen et al. 1998). We found evidence for a similar influence in our study, with just 2.5% of the 120 mussels identified as male, in contrast to other reports for *A. anatina* of 47% (Zieritz & Aldridge 2011) and between 20% and 66% (Hinzmann et al. 2013). The high number of marsupia-bearing individuals means that an extraordinary female bias was present, or that there was a high number of hermaphrodites. While females and hermaphrodites were not separated in our study, hermaphroditism has previously been linked with trematode infection (Kat 1983; Walker 2017). As hermaphroditism increases the number of phenotypic females, this may help compensate for the loss of other larval-bearing mussels, especially as females may be targeted to a greater extent than males by trematodes (Taskinen & Valtonen 1995). Hermaphroditism is common in the genus *Anodonta* (Heard 1975), though in the UK it is mainly observed in *A. cygnea* (Zieritz & Aldridge 2011; Chase et al. 2018). However, the reproductive plasticity in this genus makes it possible that parasitism induces an alteration of population-level reproductive strategy, something consistent with the observed sexual bias in our study.

Our analysis of two sites also demonstrates that different populations may be affected differently, in terms of the prevalence and intensity of parasites, as well as their effects. For example, BC had a lower prevalence of mites and trematodes (by 5.8 and 3 times respectively; Fig. 7.1a) but a higher intensity of trematode infection (by 2.2 times; Fig. 7.1c) and a significant negative correlation between mite presence and larval viability (Fig. 7.3b). A potential explanation is that parasite populations only recently invaded BC mussels, and

host mussels at BC are therefore more evolutionarily naïve than OW mussels (i.e. 'naïve host syndrome', see Mastitsky et al. 2010; Lymbery et al. 2014). These results also agree with previous studies that individuals in different native mussel populations may respond differently to parasitism by trematodes (Jokela et al. 2005) and bitterling (*Rhodeus amarus*) fish (Reichard et al. 2015), though these studies do not consider the population-level outcomes of these differences. The differential effects of parasitism across populations, even across small spatial scales in interconnected waterways as in the current study, suggests that considerable caution is required when mussels are transported between populations, even across short distances within the same river (see Chapter 9).

While we did not detect a relationship between parasites at the individual host level, it is possible that they may interact at a host population level, further highlighting the need to link individual and population scales. Specifically, while zebra mussels did not alter the likelihood of a specific mussel hosting trematodes, there was a clear site-wide inverse relationship between the two, with BC mussels hosting high numbers of zebra mussels but few trematodes, and OW mussels hosting few zebra mussels but a higher number of trematodes (Fig. 7.1). As we only sampled two sites we were not able to statistically assess this trend, but it aligns closely with the results of Müller et al. (2015, 2021), who also showed, using multiple Polish lakes, that zebra mussels were not correlated with trematodes at the individual level but that their prevalences were inversely related at the population level. Given zebra mussels do not host *R. campanula* (Table A1.3), it may be that attempted trematode infections in *D. polymorpha* are 'wasted' (see Rigaud et al. 2010), which reduces the site-wide prevalence of trematodes. We recommend that further consideration is given to parasite interactions at the population level, as well as within hosts.

In our study, we have focused on how the effects of parasites on individual native mussels may influence population-level reproductive output. Such an effect may also cascade to the community and ecosystem level. Native mussels perform multiple ecosystem engineering roles, such as water filtration, bioturbation and nutrient deposition (Vaughn 2018). Therefore, any parasite-induced alteration in population outcomes will also affect the services provided by that population, thus changing the dynamics of whole ecosystems. Further, in our sites, *A. anatina* lives sympatrically with other unionid mussels such as *Unio pictorum* and *U. tumidus*, which are not affected to the same degree by trematodes in particular (Chapters 2, 6). The reproductive suppression and potential alteration in strategy (hermaphroditism)

experienced largely by *A. anatina* may also significantly alter community structure: for example, the composition of crustacean communities in freshwater systems can be altered by parasites differentially affecting its constituent species (Friesen et al. 2020). This will also shift ecosystem dynamics if the native mussel species differ in their contribution to ecosystem engineering processes.

To conclude, we have demonstrated a relationship between parasitism and reduced reproductive performance of individuals and populations of an important ecosystem engineer. We suggest that further research is required into the impacts of parasitism on freshwater mussels, especially at a population level. Documenting effects on individual mussels are valuable but may not scale to demographic impacts, while general statements on the effects of parasites may miss differences between populations. In general, the effects of parasites and pathogens on unionid mussel communities are poorly understood (Chapter 2; Ferreira-Rodríguez et al. 2019). While this paper takes a first step to exploring the implications of parasitism at a population level, future work is required to both establish clear causality for the effects of multiple parasites, and further demonstrate how parasites may influence host population dynamics. From there, the consequent impacts on ecosystem services deserve consideration.

Chapter 8: Parasitism dramatically alters the ecosystem services provided by freshwater mussels

Abstract

Parasites can indirectly affect ecosystem function by altering host phenotype, but the trait-mediated impacts of parasitism at an ecosystem level remain poorly characterised. We examined the effect of native (the trematode *Rhipidocotyle campanula*) and invasive (the bitterling fish *Rhodeus amarus*) parasites, and their interaction, on the clearance rates of unionid mussels, a dominant ecosystem-engineering group that modify freshwater ecosystems worldwide. We used a combination of field experiments, laboratory experiments and ecological simulations to demonstrate the phenotypic impact of parasites on two mussel species across an environmental gradient (suspended particle concentration), and extended this with host and parasite community data to demonstrate the consequences for a real-world ecosystem, the Old West River in Cambridgeshire, England. Both parasites altered the clearance rates of their hosts but in contrasting fashion: while *R. campanula* increased host clearance rates relative to uninfected conspecifics under all conditions, *R. amarus* supressed clearance rates at high suspended particle concentrations but elevated them otherwise. Both parasites displayed clear host preferences, and the invasive *R. amarus* avoided mussels infected with *R. campanula*. Given their disparate effects, the parasites' choices and interactions reversed the relative filtration capacity of the two host species under high *vs.* low concentrations of suspended particles, demonstrating how interactions between native and invasive parasites, as well as their individual effects, need to be considered. Overall, the time taken for the combined mussel community to filter their ecosystem changed by up to 50% in the presence of parasites. By incorporating multiple host species, as well as multiple parasite species and their interactions, we provide the most ecologically realistic evidence to date for the trait-mediated effects of parasites on ecosystem processes. Understanding parasite dynamics is central to understanding the ecosystem services provided by host species, especially in an era of global environmental change.

Key words: clearance rate, community, ecosystem process, interaction, invasive, native, trait-mediated effect

8.1. Introduction

Parasites are an important and often underappreciated component of global ecosystems (Hudson et al. 2006; Lafferty et al. 2008; Carlson et al. 2020a). In an era of unprecedented global change, parasite abundances and distributions may be altered through multiple mechanisms. For example, increased temperatures are predicted to increase the prevalence of many parasites and diseases, such as helminths (Cohen et al. 2020). In addition, a general trend of biotic homogenisation facilitates parasite invasions (Olden et al. 2004); this can also be enhanced by environmental conditions and lead to outbreaks of previously low-abundance invaders (Spear et al. 2021). Host species may therefore encounter higher prevalences and intensities of native parasites, as well as increasing numbers of invasive parasites. The consequences of this redistribution of parasite pressure remains to be fully characterised. While the effects of parasites at an individual level are well-known for a broad range of host taxa (Sánchez et al. 2018), parasites may also affect ecosystem functioning (Wood & Johnson 2015) and can be considered as cryptic ecosystem engineers (Selbach & Mouritsen 2020). However, the impact of parasites on ecosystem-level processes is generally poorly understood and requires further work (Fischhoff et al. 2020).

Parasites affect ecosystems through three mechanisms: direct biomass effects, density-mediated effects and trait-mediated effects (Hatcher et al. 2006, 2014; Dunn et al. 2012; Fischhoff et al. 2020). Biomass effects occur when the parasite directly contributes significantly to the standing biomass of the ecosystem (e.g. Kuris et al. 2008; Preston et al. 2021) and, for example, acts as an important carbon or food source (Morley 2012). Density-mediated effects occur when parasites alter the mortality or reproductive rates of one or more host species, thus altering community structure or the total biomass of the system (e.g. Chantrey et al. 2014; Bojko et al. 2019, 2020; Friesen et al. 2020). Finally, trait-mediated effects occur when parasites alter the phenotype of their hosts, such as increasing metabolism and feeding rates (Dick et al. 2010; Nadler et al. 2021), bioturbation rate (Dairain et al. 2020) or nitrogen excretion rate (Mischler et al. 2016). For both density- and trait-mediated mechanisms, impacts are more likely to scale to the ecosystem level if hosts have strong engineering effects. Despite this sound framework, evidence for the different mechanisms is unevenly distributed. There is high support for density-mediated effects, with a large focus on how host death affects ecosystems (Coen & Bishop 2015; Borer et al. 2021). There is much less support for trait-mediated effects scaling to an ecosystem level (Fischhoff et al. 2020),

and studies that do support this mechanism are largely drawn from rocky shore and estuarine environments (e.g. Wood et al. 2007; Mouritsen & Poulin 2010; Dairain et al. 2020). In general, a recent review found that understanding the trait-mediated ecosystem-level effects of parasitism across a range of systems is highly underdeveloped and an important research frontier (Fischhoff et al. 2020).

Understanding the trait-mediated effects of parasitism requires consideration of two issues. The first is the underlying environmental conditions that the host phenotype exerts an effect on. For example, Fielding et al. (2003) found that infection with the acanthocephalan parasite *Echinorhyncus truttae* reduced the feeding rate of the amphipod *Gammarus pulex*, while Dick et al. (2010) found that infection increased feeding rate of the host in the same system. This discrepancy is explained by the fact that the first study only offered low prey densities to *G. pulex*, while the latter study offered a range of prey densities where elevated feeding could be observed (Dick et al. 2010). A functional response analysis is therefore desirable, to avoid drawing erroneous conclusions based only on a 'snapshot' assessment the conditions (Dick et al. 2014). Analysing the effect of parasites on host functional responses is also highly relevant given the changing global environmental conditions, as it effectively allows for comparison across a range of possible scenarios (e.g. Williams et al. 2019). However, few studies apply a functional response analysis to the trait-mediated impacts of parasitism, and those that have (e.g. Stier et al. 2015) do not consider the ecosystem-level implications of this.

The second issue requiring careful consideration in studying the trait-mediated effects of parasitism on ecosystem functioning is the role of parasite interactions and parasite choice. Most studies on the ecosystem-level effects of parasitism consider a one-host, one-parasite system, but this accords poorly with ecological reality. Hosts contain a range of parasites, which interact to facilitate or inhibit one another (e.g. Henrichs et al. 2016; Halliday et al. 2017; Clay et al. 2019; Sweeny et al. 2020). However, and possibly more significantly for trait-mediated effects, parasites can avoid coinfection altogether by preferentially infecting previously uninfected hosts (e.g. Mouritsen & Jensen 1997; Hopkins et al. 2016). Parasite choice can thus lead to different host individuals or different host species being infected by different parasites (Bashey 2015). Such a distribution of parasite pressure has the underappreciated corollary that more hosts in a community will be infected than expected by chance, and thus more hosts will exhibit the parasite-mediated phenotype, amplifying

ecosystem-level effects. A comprehensive understanding of the ecosystem effects of parasites thus requires understanding how the environmental conditions interact with parasite-mediated effects, and how those effects are distributed by multiple parasites within and between host species.

In this study, we leverage a two-host, two-parasite system to comprehensively explore how parasites affect ecosystems. Unionid mussels are prodigious filter-feeders in freshwater ecosystems worldwide, and so they significantly alter their environments (Tankersley & Dimock 1993; Vaughn 2018). They are also afflicted by a range of parasites which may interfere with this engineering service, though this has been hitherto unexplored (Chapter 2). As common techniques exist for examining the functional response and clearance rates of freshwater mussels (e.g. Kemp et al. 2018), and given the importance of this response in global freshwater ecosystems, this system provides an excellent and significant opportunity to further knowledge on the trait-mediated impacts of parasitism.

Digenean trematodes such as the native *Rhipidocotyle campanula* are commonly observed inside the host unionid mussel *Anodonta anatina*. While the castrating impacts of this parasite (and hence a possible density-mediated effect) are well documented (Chapter 7; Jokela et al. 1993; Gustafson et al. 2005), there is indirect evidence, such as shell morphology changes in infected mussels (Zieritz & Aldridge 2011), that trematodes may also affect the filtering phenotype of the host. In addition, both *A. anatina* and the sympatric *Unio pictorum* are infected by the invasive fish parasite *Rhodeus amarus*, which lays its embryos in the gills of freshwater mussels (Aldridge 1999b). *R. amarus* invaded the UK from the Ponto-Caspian region in the 20th century (Damme et al. 2007), and there is evidence the mussel hosts are still evolutionarily naïve (Reichard et al. 2006). Bitterling embryos compete with mussels for oxygen (Spence & Smith 2013; Methling et al. 2019) and deform the gills (Mills et al. 2005), and so could also affect the filtration rate of mussels. Bitterling are also highly discriminatory and can select hosts based on a variety of cues (Smith et al. 2001), and thus may avoid depositing embryos in hosts previously parasitised by trematodes (Chapter 5). This system therefore allows us to test the role of parasite choice, incorporating both native and invasive parasites, as well as parasite impacts on the ecosystem-level effects of mussels, using a combination of field surveys, field experiments, laboratory experiments and ecological simulations.

Scaling parasite effects to the ecosystem level requires integrating observational field data on host density and parasite prevalence with *per-capita* measurements of infected and uninfected mussels, and the relative role of the host in the ecosystem of interest (Preston et al. 2016). We therefore make and test four hypotheses: (1) Bitterling will avoid depositing embryos in *A. anatina* infected with trematodes; (2) Trematodes will reduce the clearance rate of *A. anatina*, its preferred host; (3) Bitterling embryos will reduce the clearance rate of *U. pictorum*, its preferred host; (4) Parasites alter the rate at which unionid mussels filter the river they live in. We test Hypothesis 1 with a field experiment, Hypotheses 2 and 3 with laboratory experiments, and Hypothesis 4 with a combination of field surveys and a simple ecological model. We show that parasites, through their impact on the host mussel trait of clearance rate, alter the speed at which a sampled river is filtered by up to 50%, but that this effect varies with host species and may even reverse based on the nutrient profile of the river.

8.2. Methods

We used a combination of field experiments, laboratory experiments and mathematical simulations to explore the interaction between trematodes, bitterling fish, their freshwater mussel hosts, and the ecosystem services that those mussels provide.

On the 7th of May 2019, we sampled 60 *A. anatina* and 30 *U. pictorum* from the Old West River (OW), the focal river of our study, at Stretham Old Engine (52.3343° N, 0.2243° E). We completely characterised the macroparasite communities of all sampled mussels, following procedures outlined in Appendices A3 Part 1 and A4 Part 1. Our sampling revealed that bitterling infection was mainly observed in *U. pictorum*, while trematode infection was only observed in *A. anatina* (see Results). Further, our sampling also suggested that coinfection between trematodes and bitterling was rare (Chapter 5). We therefore ran three experiments. The first was a field experiment, designed to test the hypothesis that bitterling fish avoid depositing embryos in trematode-infected *A. anatina*. The next two experiments were designed to test the effect of trematode infection on the clearance rate of *A. anatina*, and the effect of bitterling embryo infection on the clearance rate of *U. pictorum*, respectively. Finally, we combined the results of our experiments to generate a series of simulations, with the aim of investigating how the effects of parasitism influence the time taken for the freshwater mussel community to completely filter the ecosystem of OW.

8.2.1. Field experiment

To test Hypothesis 1 (bitterling fish avoid depositing embryos in *A. anatina* infected with trematodes), we carried out a choice experiment in the field, where naturally occurring bitterling populations were presented with replicate choices of trematode-infected or uninfected mussels.

8.2.1.1. Establishment

On the 14th of May 2021 we sampled 150 *A. anatina* from the River Great Ouse at King's Dyke (KD) (52.5397° N , -0.1753° E); this sampling location was selected as it has a high prevalence of the castrating trematode *R. campanula* (Chapter 6). We non-destructively assessed all mussels for trematode infection by extracting gonadal fluid using a hypodermic needle and examining it under a compound microscope (Chapter 4). Once all mussels had been assessed, 30 trematode-infected mussels were measured using Vernier calipers to the nearest 0.1 mm, and each was paired with a non-infected mussel of identical size (within 1 mm; average length difference between pairs of infected and non-infected mussels = 0.46 mm). This was to control for the potential effect of host size on bitterling choice. The average length of mussels (± S. E.) was 72.2 mm ± 0.97 mm.

We placed each pair of mussels inside a ceramic plant pot filled with river sediment (i.e. 30 pots in total), each with a diameter of 18 cm, and attached each mussel to the pot using ~30 cm of twine that was carefully superglued on to both the pot and the mussel shell, following zu Ermgassen and Aldridge (2010). Attaching mussels to pots with string does not affect mussel valve movements or opening. Pots were buried in the sediment with the top ~5 cm exposed in OW, at a water depth of ~1 m. Pots were placed at intervals of ~4 m along the river, to ensure that no two pots were likely to be within the same male bitterling territory (zu Ermgassen & Aldridge 2010). Pots were left for the natural bitterling population in the river to infect the experimental mussels for a period of one month, which maximises the chance of observing bitterling infection while minimising the possibility of deposited bitterling embryos fully developing and leaving the mussel before being observed (Aldridge 1999b). The experiment commenced on the 24th of May 2021, during the bitterling spawning season, and was terminated on the 24th of June 2021.

After the experiment ended, we recovered as many pots and mussels as possible, carrying out repeated sweeps of the experiment area. We returned all mussels to the laboratory and immediately dissected them, counting all bitterling embryos present in the gills and identifying their stage of development as 'a' to 'e' (following Aldridge 1999b), where 'a' is the least developed and 'e' the most developed. We also dissected the gonad of all experimental mussels, and verified via compound microscopy that in all cases our non-destructive diagnosis of mussels as trematode-infected or uninfected was accurate.

8.2.1.2. Analysis

We compared overall bitterling infection rates (i.e. initially leaving aside the paired nature of our experiment), in terms of both prevalence and average intensity, between trematode-infected mussels and uninfected mussels. In addition, for pairs where both mussels were successfully recovered, we carried out Fisher's exact test to assess independence in infection prevalence between trematode-infected mussels and uninfected mussels.

8.2.2. Clearance rate experiments

To test Hypotheses 2 and 3 (parasites will reduce the filtering capacity of freshwater mussels), we carried out two laboratory experiments. The first compared the filtration rates of *A. anatina* with and without trematodes, and the second compared the filtration rates of *U. pictorum* with and without bitterling embryos.

8.2.2.1. Experimental subjects

In total, 30 *A. anatina* (15 infected with trematodes, 15 uninfected) and 45 *U. pictorum* (15 infected with bitterling, 30 uninfected) were used in the filtration rate experiments. Infected and uninfected *A. anatina* were identified as described above, from the same population that was sampled at KD. Experimental *U. pictorum* subjects were collected from OW on the 24th of May 2021, and taken back to the laboratory to non-destructively assess bitterling infection. We carefully opened the valves of mussels by inserting and twisting a blunt scalpel, and then examined the mussel demibranchs for evidence of bitterling embryos with the aid of a rounded probe.

To calculate filtration rates we measured the change in concentration of algae cells between the start and end of the experiment, using Chlorophyll *a* concentration as a proxy for algal concentration. We used *Chlorella vulgaris* (strain CCAP 211/12) as our algae species, as this is readily consumed by both mussel species. *C. vulgaris* was grown using 3N-BBM+V medium and a 16:8 L:D cycle. We measured Chlorophyll *a* concentration using a handheld fluorometer (AquaFluor 8000-010) that was calibrated using serial dilutions of a Chlorophyll *a* analytical standard (Sigma Aldrich) of known concentration; the fit of the calibration curve was 97.8%.

8.2.2.2. Experimental procedures

The day before the experiments, we gently scrubbed the shells of all mussels to remove organic material and placed them in clean dechlorinated water overnight. The day of the experiment, buckets were filled to 500mL with varying amounts of clean dechlorinated water and *C. vulgaris* culture, to produce a range of Chlorophyll *a* concentrations (suspended particle concentration) between 20 and 160 μg/L. To start the experiment, mussels were placed in individual buckets and the Chlorophyll *a* concentration was immediately measured. Experiments were conducted in a constant temperature room matched to field conditions (19° C). To avoid location effects within the room, individual bucket location was randomised in terms of both algal concentration and whether the mussel was infected or uninfected with parasites. Buckets were aerated throughout the experiment, and therefore no adjustment was required for the settling rate of algae as this effectively resuspends particles (Kemp et al. 2018). Each mussel was allowed to filter for two hours, and then we measured the Chlorophyll *a* concentration again and ended the experiment.

All mussels were immediately dissected to confirm infection status and intensity. Bitterling intensities were calculated as the number of bitterling embryos per mussel; trematode intensities were calculated as the percentage of the gonad filled with trematode tissue (Chapter 3). Following these dissections, one *A. anatina* that was originally thought to be trematode-free was found to be trematode-infected, and three *U. pictorum* that were thought to be bitterling-infected were found to be uninfected; these mussels were therefore treated in the analyses below according to their updated infection status. After parasite diagnosis, all soft tissue from the mussels was removed, dried to constant mass (DW) and weighed to the nearest 0.0001 g.

8.2.2.3. Analysis

For each of the two experiments (*A. anatina* with or without trematodes; *U. pictorum* with or without bitterling) we analysed the consumption rate of mussels by fitting a generalised linear model (Gamma family, log link) to the response variable of Chlorophyll *a* (μg/L) consumed per g dry weight per hour. Independent variables were initial Chlorophyll *a* concentration (μg/L), infection status (infected/uninfected), and the interaction of these two factors.

We calculated clearance rates (mL of water filtered per g dry weight per hour) using Equation (1), following Kemp et al. (2018), where DW is the dry weight of mussels (to account for different sizes of mussel) and $C_{initial}$ and C_{final} are the starting and ending concentrations of Chlorophyll *a* (μg/L):

$$CR = \frac{500}{2DW} \ln\left(\frac{Cinitial}{Cfinal}\right) \qquad (1)$$

To confirm that the consumption rate of mussels followed a Type I functional response, we plotted the clearance rates for each mussel against the corresponding starting concentration of Chlorophyll *a*; the slope of this line should not be significantly different from zero (i.e. the clearance rate should be directly proportional to the amount of algae available to be consumed). We assessed departures from this assumption by fitting a Gamma-distributed generalised linear model (log link) of clearance rate against starting Chlorophyll *a* concentration, for both infected mussels and uninfected mussels.

8.2.3. Simulating the effects of parasitism at an ecosystem level

Using the results of our field and laboratory studies, we first parameterised a series of ecological simulations assessing how many days it would take mussel populations at OW to filter a 100 m long section of river. We plotted general solutions for a range of possible mussel densities (1 mussel per square metre to 50 mussels per square metre), and calculated actual estimates (separately) for populations of *A. anatina* and *U. pictorum* at their observed densities. The volume of water in a 100 m section of OW was calculated from the parameters of McIvor (2004). We make the simplifying assumption that the water in a given 100 m

section of river represents a discrete block, that can be completely filtered by mussels in a certain time frame, depending on the filtration capacity of individual mussels and their density.

Two sets of simulations were generated: one set assuming low Chlorophyll *a* concentrations (40 μg/L) were present in the river, and one set assuming high Chlorophyll *a* concentrations (120 μg/L) were present in the river. These two values of were chosen because they represent typical summer Chlorophyll *a* levels and highly eutrophic Chlorophyll *a* levels in English rivers, respectively (Neal et al. 2006). We ran our simulations at two values because the effect of bitterling depended on Chlorophyll *a* concentration (see Results). Each set of simulations considered four cases: an *A. anatina* population with trematodes at the observed prevalence; an *A. anatina* population with no trematode infection; a *U. pictorum* population with bitterling at the observed prevalence; and a *U. pictorum* population with no bitterling infection. Therefore, in total we simulated eight cases. Because the effects of parasites occur at an individual level, we did not average over populations, instead, our models explicitly accounted for individual-level variation.

Population-level clearance rates are typically expressed in terms of overall mussel biomass per square metre; it was not desirable or possible to do this here as parasite prevalences cannot be expressed in this fashion, and this would not take into account the variation among individual mussels. Instead, we calculated the average biomass of individual mussels at OW. In July 2021 we sampled 20 replicate 0.25m^2 quadrats at OW along the riverbank and measured all mussels found; this gave us average mussel densities in addition to size distributions of the populations. We used length to dry mass equations (parameterized using our May 2019 sampling where dry weights were calculated, following procedure of Coughlan et al. 2021) to work out the dry weight of an average mussel for both populations, and thus transformed our estimated average clearance rate from mL *per g dry weight* per hour, into mL *per mussel* per hour. This assumes that mussels of all sizes are equally likely to be infected by trematodes and bitterling; we have previously shown this assumption to be sound (Chapters 5, 7).

To give an estimate of the uncertainty of our predictions, we ran ten replicate simulations for each mussel density (1 m^{-2}, 2 m^{-2}, … , 50 m^{-2}) for each of the eight cases. We incorporated uncertainty and individual-level variation into our predictions in two ways. First, for those

cases that included parasites, we allowed the number of mussels infected with parasites to vary according to their binomial probabilities. For example, if the simulation involved a density of ten mussels and the observed parasite prevalence was 0.4, it would be most likely that four of the ten mussels were assigned as being parasitised; however, it is also possible that other numbers of mussels were assigned as being parasitised for that particular replicate, according to their declining binomial likelihood. This accounts for the fact we cannot know the true prevalence of parasites in the whole population, as our field sampling only provides an estimate. In line with our observations (and to allow us to parameterize all clearance rates using our laboratory experiments), we limited *A. anatina* to only be infected with *R. campanula*, and *U. pictorum* to only be infected with *R. amarus*. Second, for each mussel in each replicate, we allowed the simulated clearance rate for a particular mussel to be drawn from a uniform distribution where the minimum and maximum possible values were the lower and upper bound respectively of the 95% confidence interval for the estimated clearance rate. Each of the eight cases (*A. anatina* and *U. pictorum* populations, with and without parasites, at low or high Chlorophyll *a* concentrations) had its own 95% confidence interval, calculated using the results of the clearance rate experiments. This approach was used in favour of using the overall average clearance rate for each case, which would not take into account the variation observed in the clearance rates of individual mussels. For code supporting these models, see Appendix A6 Part 2.

Finally, we combined the known densities of *A. anatina* and *U. pictorum* and repeated the same procedure at a community level. These densities were estimated using the same 20 replicate quadrats used to estimate the mean biomass of mussels at OW. We thus had four simulated cases: the mussel community with associated parasites and without associated parasites, at environmental conditions of low and high Chlorophyll *a* concentration. The quadrats were exhaustively sampled, but it is possible there was a sampling bias making it more likely to detect larger mussels. We do not believe this will affect our results, as very small mussels will have correspondingly small filtration rates. In addition, our estimates do not include the contribution of *U. tumidus*, another freshwater mussel species also found at the site. This species does not host *R. campanula* (Chapter 2), and is only rarely parasitised by *R. amarus* relative to *A. anatina* and *U. pictorum* (Reynolds et al. 1997), and so the effect of parasites on its phenotype are expected to be extremely minor.

8.3. Results

8.3.1. Field observation and experiment

Sampling of mussels in May 2019 revealed that trematodes and bitterling displayed different infection patterns between *A. anatina* and *U. pictorum* host mussels. 27% of *A. anatina* were infected with trematodes, while no *U. pictorum* were infected with trematodes. In contrast, 66% of *U. pictorum* hosted bitterling embryos in their gills, compared to just 8% of *A. anatina*. In addition, there was no coinfection between trematode and bitterling parasites in *A. anatina*.

The field experiment in 2021 broadly supported Hypothesis 1 (bitterling fish avoid depositing embryos in *A. anatina* infected with trematodes). Unfortunately, recovery rates of the experimental mussels quite were low: despite recovering 28 of the 30 pots, only 28 of the 60 mussels were recovered, with only eight of the original 30 complete pairs recovered. We suspect that an unanticipated level of boat activity dislodged pots and caused the mussels to become unattached, as several pots were found on their sides.

Table 8.1 shows the overall results of the experiment: of the 28 recovered mussels, seven had bitterling infections, five of which were in mussels that did not have trematode infections. In total, 17 bitterling embryos were found in non-trematode-infected mussels (3.4 ± 0.78 bitterling per mussel, mean ± S. E.), in contrast to just 3 from trematode-infected mussels (1.5 ± 0.35 bitterling per mussel). However, the low sample size means that the proportion of mussels with bitterling infection did not differ between trematode-infected or non-trematode-infected mussels (Equality of proportions test, $p = 0.256$). All developmental stages of bitterling were observed in mussels that did not have trematodes (Table 8.1), including two mussels that had very late-stage bitterling embryos, suggesting they were infected soon after the experiment commenced.

Table 8.1: The number of bitterling embryos recovered from trematode-infected and non-infected mussels. Numbers refer to the total number of bitterling embryos of each stage observed; numbers in brackets indicate the number of mussels that those bitterling embryos were observed in.

	Bitterling development stage					
Trematodes?	a	b	c	d	e	Total
Yes	1 (1)	0	0	2 (1)	0	3 (2)
No	4 (3)	6 (3)	2 (2)	1 (1)	4 (2)	17 (5)

However, a slightly different pattern was observed when considering the eight recovered pairs in isolation. Here, results within the pair were always identical: either both trematode-infected and non-trematode-infected mussels had bitterling (2 pairs), or both did not (6 pairs). This suggests that occurrences of bitterling in trematode and non-trematode mussels are not independent (Fisher's exact test, $p = 0.036$). However, in both cases where both mussels in the pair had bitterling, the mussel that was uninfected with trematodes had a higher intensity of bitterling than the trematode-infected mussel. Therefore, the complete absence of coinfection in the observational field study could be a combination of both bitterling choice (Table 8.1), but also differential microhabitat use between bitterling and trematodes (see Discussion). However, it is clear from our study that coinfection between trematodes and bitterling is highly unlikely, with *A. anatina* disproportionately hosting trematodes and *U. pictorum* disproportionately hosting bitterling. Our results therefore focus on these discrete host-parasite combinations. In the following results, we distinguish between the 'consumption rate' (the raw amount of algae consumed by mussels *per* gram of dry weight *per* hour) and the 'clearance rate' (the amount of water filtered by mussels *per* gram of dry weight *per* hour).

8.3.2. Clearance rate experiments

Infection with trematodes significantly increased the consumption rate of algae by *A. anatina*, the opposite of the prediction made by Hypothesis 2 (Fig. 8.1). The shape of the consumption response was the same for trematode-infected and uninfected mussels (lack of interaction between infection status and starting Chlorophyll *a* concentration, $t_{24} = 1.339$, $p = 0.193$), but trematode-infected mussels showed a significantly higher consumption rate ($t_{24} = -2.434$, $p = 0.023$) (Fig. 8.1a). This higher consumption rate was weakly correlated with the intensity of trematode infection (Fig. A6.1), with higher intensities of infection having higher rates. The functional response was confirmed to be Type I: despite reasonably high variation, the slope of the clearance rate was not significantly different from zero when plotted against the starting concentration of algae for both trematode-infected ($t_{14} = -1.503$, $p = 0.155$) and uninfected ($t_{10} = -0.875$, $p = 0.402$) mussels, indicating the clearance rate was directly proportional to the amount of algae available (Fig. 8.1b). The mean clearance rate (95% confidence interval) for trematode-infected mussels was 407.2 (146.9 – 667.5) mL/g DW/hr, and the mean clearance rate for uninfected mussels was 159.4 (72.0 – 246.8) mL/g DW/hr.

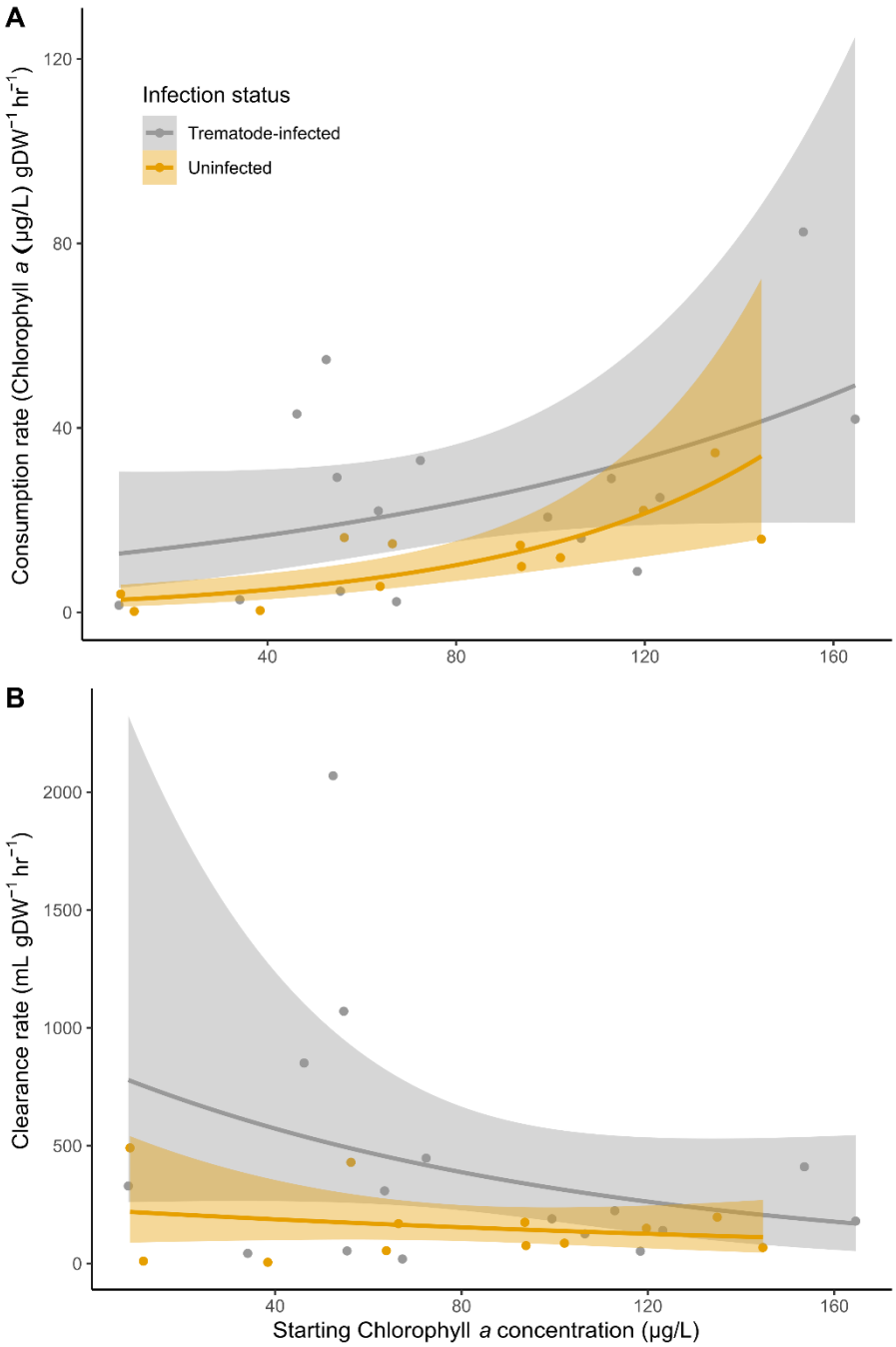


Figure 8.1: The functional filtering response of *Anodonta anatina* for trematode-infected (grey lines) and uninfected (yellow lines) mussels. Shaded regions are 95% confidence intervals around the means, which were fitted using a generalised linear model (Gamma family, log link). (A) The feeding rate of *A. anatina* (micrograms of Chlorophyll *a* consumed per litre per gram of mussel dry weight [DW] per hour) in response to different starting Chlorophyll *a* concentrations. (B) The clearance rate of *A. anatina* (millilitres per gram of dry weight per hour) in response to different starting Chlorophyll *a* concentrations.

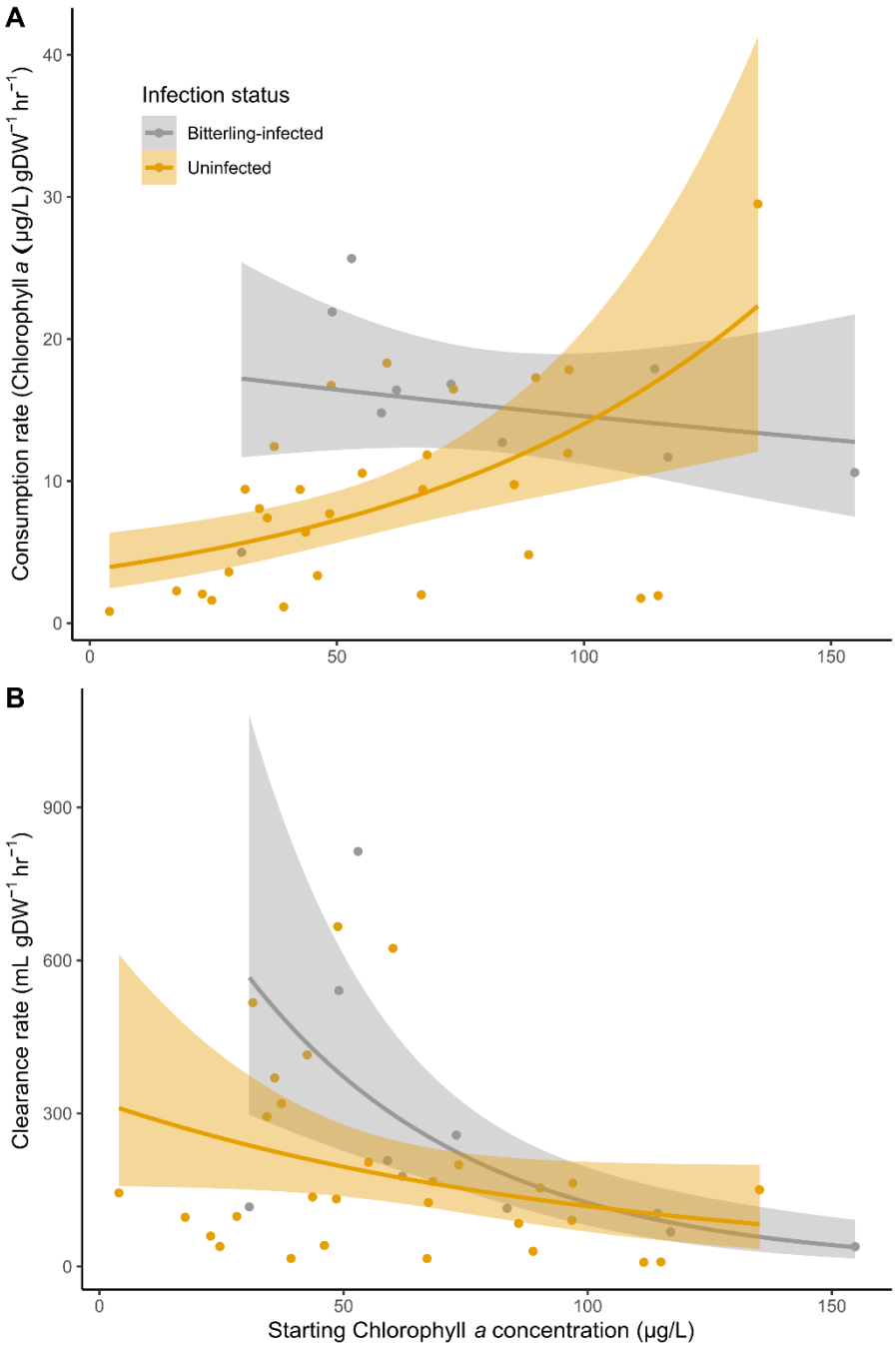


Figure 8.2: The functional filtering response of *Unio pictorum* for bitterling-infected (grey lines) and uninfected (yellow lines) mussels. Shaded regions are 95% confidence intervals around the means, which were fitted using a generalised linear model (Gamma family, log link). (A) The feeding rate of *U. pictorum* (micrograms of Chlorophyll *a* consumed per litre per gram of mussel dry weight [DW] per hour) in response to different starting Chlorophyll *a* concentrations. (B) The clearance rate of *U. pictorum* (millilitres per gram of dry weight per hour) in response to different starting Chlorophyll *a* concentrations.

In contrast, the effect of bitterling on the functional response of *U. pictorum* depended on the starting concentration of Chlorophyll *a*, as shown by a significant interaction between infection status and starting concentration (t_{35} = 2.473, p = 0.018) (Fig. 8.2a). At low concentrations, bitterling-infected *U. pictorum* showed much higher consumption rates than uninfected mussels, but this trend reversed at high concentrations. As with trematodes, this was weakly correlated with infection intensity (Fig. A6.2), with higher bitterling loads associated with higher consumption rates. Hypothesis 3 was therefore not supported at low concentrations, and was supported at high concentrations. This interaction is observable in the predicted clearance rates: while uninfected *U. pictorum* also demonstrated the expected Type I functional response (clearance rate slope not different from zero; t_{27} = -1.852, p = 0.075), the clearance rates of bitterling-infected *U. pictorum* significantly decreased with increasing Chlorophyll *a* concentration (t_8 = -4.035, p = 0.004) (Fig. 8.2b). The mean clearance rate (95% confidence interval) for uninfected *U. pictorum* was 185.1 (119.8 – 250.4) mL/g DW/hr, while the mean clearance rate for bitterling-infected *U. pictorum* varied with algal concentration.

8.3.3. Impact of parasites on clearance rates of populations

We extrapolated the results of our field and laboratory studies to predict the effect of parasites at an ecosystem level. First, we calculated the effect of parasites on mussel populations separately. Figure 8.3 shows how many days it would take either *A. anatina* or *U. pictorum* populations of varying densities to completely filter a 100 m section of the Old West River, for two different concentrations of Chlorophyll *a*: 40 μg L^{-1} (Fig. 8.3a) or 120 μg L^{-1} (Fig. 8.3b). Two broad scenarios were considered in each case: mussel populations with parasites present in their observed prevalences (grey lines), or mussel populations without parasites (yellow lines). Simulations were parameterised using data from the clearance rate experiments (see Methods).

The results support Hypothesis 4, that parasite infection alters the ecosystem engineering capacities of mussel populations. At low Chlorophyll *a* concentrations (Fig. 8.3a), both bitterling and trematodes decreased the number of days required for mussel populations to filter 100 m of river; in other words, they increased the ecosystem engineering capacity of mussels. At high Chlorophyll *a* concentrations (Fig. 8.3b), while trematodes still reduced the number of days taken for *A. anatina* populations to filter the river, bitterling increased the

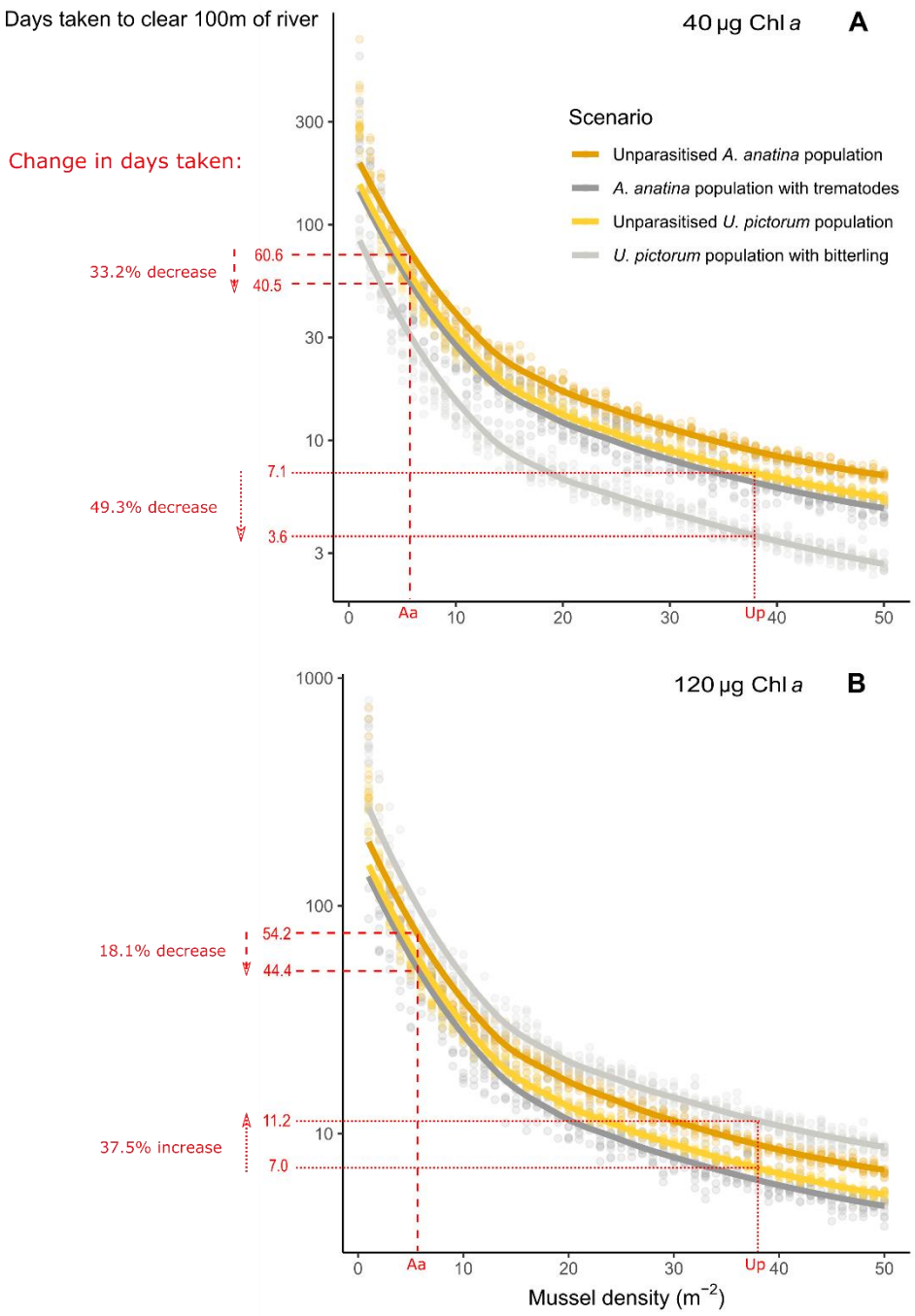


Figure 8.3: Simulated estimates for how long it would take different densities of *Anodonta anatina* or *Unio pictorum*, respectively, to completely filter a 100m section of the Old West River, at (A) low concentrations of Chlorophyll *a*, and (B) high concentrations of Chlorophyll *a*. Dots represent individual replicates for each case (10 replicate simulations per case per mussel density), with lines representing the average of those replicates. These simulations are based on clearance rate experiments and observed parasite prevalences (trematode prevalence of 0.27 in *A. anatina*; bitterling embryo prevalence of 0.66 in *U. pictorum*). Estimates are presented for the populations with parasites (i.e. the actual scenario), and compared with estimates for the populations in the absence of parasites. Differences in the days taken to filter the river volume between parastised and unparasitised populations are calculated and presented (red lines and text) for the actual densities of mussels present in the Old West River (*Anodonta anatina* = Aa, dashed lines, 6 mussels per square metre; *Unio pictorum* = Up, dotted lines, 38 mussels per square metre). Note the log-transformed y-axis.

time taken for *U. pictorum* to filter the same volume of water: they reduced the engineering capacity of this population. It is valuable to also compare between populations: for example, in Fig. 8.3a, parasitised *U. pictorum* are more efficient than parasitised *A. anatina* at filtering the river, but this trend reverses in Fig. 8.3b.

To provide a tangible example of the impact of parasites, we estimated the time taken to filter 100 m of OW for the observed densities of mussels present in the river (red lines and text, Fig. 8.3). *A. anatina* were present at a density of 6 m^{-2}, with an average length of 70.3 mm (estimated average dry weight = 1.46 g). *U. pictorum* were present at a density of 38 m^{-2}, with an average length of 60.5 mm (estimated average dry weight = 1.59 g). Using these densities as examples shows that parasites can stimulate up to a 50% difference in the time taken to filter a 100 m section of river, with a minimum of an 18.1% difference in the time taken.

Finally, we estimated the ecosystem effect of mussel parasites using the combined community density of mussel species present in OW (Fig. 8.4). At low Chlorophyll *a* concentrations, parasites halve the time taken for mussels to filter a 100 m section of river, but this trend reverses at high concentrations, where parasites increase the time taken to filter the same volume of water by 30%.

It is also important to note the possible impact of species-specific parasite distributions on the overall number of hosts infected by parasites. At the observed species-specific prevalences (with bitterling targeting *U. pictorum* and *A. anatina* uninfected with trematodes, and trematodes targeting *A. anatina*), and observed host densities, 62% of hosts have at least one parasite, compared with a null expectation of just 41% if parasite infection was random with respect to host identity and coinfection.

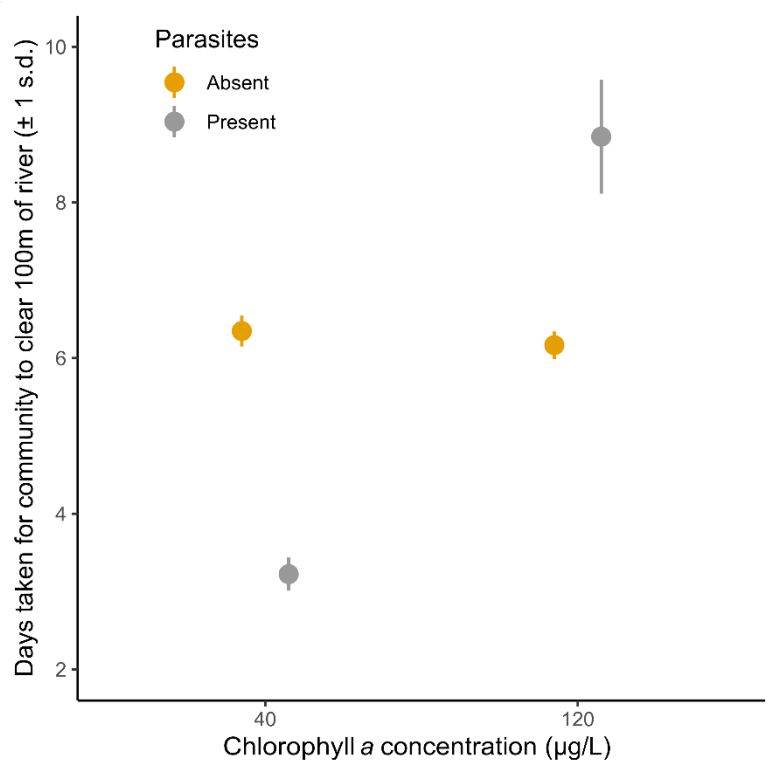


Figure 8.4: Days taken for the observed combined mussel community (*A. anatina* + *U. pictorum*) to filter a 100 m section of the Old West River, in the absence or presence of parasites, for low and high Chlorophyll *a* concentrations.

8.4. Discussion

There is a scarcity of experimental data exploring the effects of parasites on ecosystems (Fischhoff et al. 2020; Friesen et al. 2020), and generally for how processes at smaller scales affect those at larger scales (Wale & Duffy 2021). In this study, we filled this gap by integrating host densities, individual-level effects of parasitism and the distribution of both native and invasive parasites in a host community to demonstrate how parasites may influence ecosystem-level processes in a real-world system.

8.4.1. Parasites affect the clearance rates of freshwater mussels in diverse ways

The individual-level effect of parasites on mussel filtration rates did not accord with all our hypotheses. In Hypothesis 2, we predicted that trematodes would reduce the clearance rates of *A. anatina*, when in fact we observed increased clearance rates (Fig. 8.1). This is opposite to what has previously been observed for mussels (e.g. Stier et al. 2015). However, in the

system of Stier et al. (2015), trematode infection was focused on the gills and palps, the organs responsible for efficient filtration. In contrast, infection with *R. campanula* in our study is localized in the gonad, leaving the gills unaffected. Instead, *R. campanula* may induce oxygen competition, meaning the filtration rate is a product of both suspended particle availability and parasitism status, with parasitised mussels having a higher rate for a given concentration of Chlorophyll *a* (Fig. 8.1a). This is supported by MacLeod and Poulin (2016) who found that trematodes infecting the gonad in gastropods increased their host's oxygen uptake. Therefore, we suggest that trematodes could potentially enhance the filtration capacity of mussels, but that this effect is highly dependent on the host tissue of infection.

Our results did not fully support Hypothesis 3 either, that bitterling embryos would reduce the clearance rate of *U. pictorum*. At low Chlorophyll *a* concentrations, the clearance rates of infected mussels were elevated relative to uninfected mussels, but this trend reversed at high concentrations (Fig. 8.2). This result is likely due to a combination of oxygen competition and gill deformation. Bitterling embryos compete with mussels for oxygen (Smith et al. 2004; Spence & Smith 2013), and have been shown to increase oxygen consumption by mussels (Methling et al. 2018), so we would expect mussels to increase their filtration rates to meet elevated oxygen demands. However, bitterling also provide a physical stress by deforming the gills and disrupting their function (Mills et al. 2005; Methling et al. 2018), and so mussels may be unable to effectively increase their filtration rates to process higher concentrations of suspended particles. *U. pictorum*'s response to bitterling embryo parasitism is thus a product of both increased oxygen demand and reduced gill function. It should be acknowledged that female bitterling could have selected mussels to infect that *a priori* had a higher filtration rate (Mills & Reynolds 2002), rather than bitterling increasing clearance rates. However, previous work measuring O_2 capacity of individual mussels before and after bitterling infection definitively showed that bitterling increased oxygen demand (Methling et al. 2019), and therefore we suggest our results are the product of a bitterling-driven effect. In addition, though we have interpreted the impact of both trematode and bitterling parasitism as being caused by demands on the host (and is therefore a host-driven response), it is possible that these parasites could be manipulating the phenotype of the mussel in order to maximise their own resources. Given we know so little about freshwater mussel parasites (Chapter 2), this possibility cannot be excluded.

In contrast to Hypotheses 2 & 3, Hypothesis 1 (bitterling females will avoid depositing in trematode-infected *A. anatina*) was supported in terms of the direction of effects despite the low sample size, though there are possibly multiple mechanisms involved. We showed that it was more common for bitterling to deposit in uninfected rather than trematode-infected mussels, and that trematode-infected mussels hosted fewer bitterling embryos per mussel than uninfected ones (Table 8.1). This result is initially surprising, as we showed that trematodes actually increase filtration rate, something female bitterling have been shown to respond positively to (Mills & Reynolds 2002). However, bitterling also use oxygen concentration in exhalant water as a proximate cue for oviposition choice (Smith et al. 2001). If oxygen competition is high between mussels and trematodes as we have suggested, the rate of oxygen removal in the mussel will be high, leading to oxygen-poor water flowing from the exhalant siphon. Bitterling females may therefore avoid depositing in trematode-infected mussels, much like they also avoid mussels already parasitised by bitterling embryos (Smith 2017). This highlights the need to consider the sequential nature of parasite arrival, and how this can shape parasite distributions (and thus parasite impacts) at the community scale (Karvonen et al. 2019). However, we also observed that, within pairs of trematode-infected and uninfected mussels (which were spatially non-independent), infection likelihood was also not independent, with both mussels either being infected or uninfected with bitterling. Microhabitat may also therefore play a role in the non-random distribution of parasite pressure, and previous evidence supports the fact that bitterling prefer certain microhabitats (Reynolds et al. 1997). If bitterling microhabitat usage only weakly overlaps with a hypothetical trematode microhabitat, perhaps stimulated by hydrological factors limiting where microscopic trematode infective stages can reach, then this could also contribute to the observational field patterns. Microhabitat use has been demonstrated as important in determining the host range of other parasite species (e.g. Gobbin et al. 2021); we recommend that both active host choice by parasites, as well as nuanced environmental microhabitats, are considered in parasite distribution patterns and their subsequent ecosystem impacts.

Combining our field and laboratory results into an ecosystem model allowed us to provide clear support for Hypothesis 4, that parasites significantly alter the clearance rates of freshwater mussels: we observed that the time taken to filter the river may change by up to 50% relative to an uninfected host community. We first inspected the ecosystem impacts from the perspective of the two mussel populations separately (Fig. 8.3), which allowed us to compare the impact of parasites on one population's service provision relative to other

species in the community; this is a key and largely unaddressed step in scaling up parasite effects to the ecosystem level (Preston et al. 2016). Our results show the importance of this approach: at low Chlorophyll *a* concentrations, the clearance rate of the parasitised *U. pictorum* population is higher than the clearance rate of the parasitised *A. anatina* population (Fig. 8.3a), but this trend reverses at high Chlorophyll *a* concentrations (Fig. 8.3b). The overall ecosystem service provided by the mussel community is therefore a product of the underlying environmental conditions, parasite prevalences and host community composition. In our example, densities of *U. pictorum* are ~6.5 times higher than *A. anatina* and so the community-level impact of parasites closely follows the *U. pictorum* response, with the time taken to filter the river being 50% faster at low concentrations of suspended particles and 30% slower at high concentrations of suspended particles (Fig. 8.4). However, different proportions of host species would alter these conclusions. Future work should aim to incorporate how the whole-community ecosystem effect is influenced by host and parasite density and distribution, especially as host community composition is also a strong determinant of parasite community structure (Dallas & Presley 2014; Mihaljevic et al. 2018; Williamson et al. 2019).

8.4.2. Parasitism may affect ecosystem services globally

Our study supports and extends previous work which have demonstrated the trait-mediated effects of parasitism. Wood et al. (2007) convincingly showed that the parasite-altered feeding rate of a snail shifted macroalgal and invertebrate community composition; however, while they hypothesized about the potential ecosystem-level consequences of this shift they did not quantify it explicitly. Mischler et al. (2016) extended work in this area by quantifying how nitrogen flow in freshwater snails, and subsequent ecosystem-level nutrient cycling, was influenced by parasites, though they only parameterized models for hypothetical water volumes and averaged across snail populations, thus not allowing for sources of stochasticity. In contrast, we explicitly quantified the effect of parasitism on a real ecosystem; further, by considering the multiple species in the community (as opposed to a single host species, as in previous studies) we can more convincingly contextualise the effect of parasites at this level, and see how parasitism alters their relative contribution to an important ecosystem service. While our estimates do involve scaling up from the experimentally-quantified individual effects, by incorporating individual variation in our models we have shown our results to be robust to stochasticity. In addition, recent work has shown that scaling up to higher levels of

organisation tends to underestimate the magnitude of change (Orr et al. 2021). We therefore consider our results to be highly reliable, and they may even be conservative.

Interactions between invasive and native parasites are predicted to become much more common under global change scenarios (Olden et al. 2004). Recent evidence suggests that the spread of invasive species has not reached a saturation point, and may even be accelerating (Seebens et al. 2017). It is therefore vital to understand the nature of these interactions and how they may affect hosts. We have shown that invasive bitterling fish can avoid co-infection with trematodes (Table 8.1); while our sample size was small it does provide experimental support for previous extensive observational work (Chapter 5). Therefore, the native trematode *R. campanula* not only has a clear trait-mediated effect, but it also mediates parasite patterns by influencing ovipositing decisions in an invasive parasite, resulting in indirect consequences for ecosystem services. Such interactions, where native parasites alter the distribution or success of invasive parasites and *vice versa*, are likely to be common in this era of global change and must be included when considering the impact of parasites on the ecosystem services of their hosts.

Our results therefore have immediate and urgent implications, both for freshwater ecosystems and for other ecosystems globally. The filtration service of freshwater mussels is a key contributor to both less turbid water and nutrient deposition (Vaughn 2018), something that is increasingly important as waters become more eutrophic (Smith et al. 1999). However, our results suggest that, at least for the observed host community composition, parasitised populations will take over 30% longer to filter an equivalent volume of water under more eutrophic conditions, possibly lowering ecosystem health. This reduction in function will be exacerbated by unionid declines (Lopes-Lima et al. 2021), especially considering the fact that parasites influencing functionally important hosts are expected to have the greatest impacts (Preston et al. 2016). We have also left aside the possible density-mediated effect of parasitism; for example, trematodes can increase unionid mussel mortality (Jokela et al. 2005) and reduce population-level reproductive capacity (Chapter 7). In general, the effect of parasites on hosts and ecosystems may be wide-ranging but also context-dependent (Lange et al. 2014; Richard et al. 2021). As the global environment continues to change, and invasive species continue to spread, we have demonstrated that incorporating the trait-mediated impact of parasites is an essential step in predicting the shape of future ecosystem function.

Chapter 9: Don’t move a mussel? Parasite and disease risk in conservation action

Abstract

Freshwater mussels are one of the most endangered animal groups globally, making them a high conservation priority. Conservationists increasingly employ translocation or captive breeding procedures to support ailing populations, and the ecosystem engineering capabilities of mussels are being increasingly harnessed in bioremediation projects. However, there is little consideration of the risk of pathogen transmission when moving mussels from hatcheries or wild donor populations into new habitats. This is of significant concern as recent developments suggest parasites and diseases are highly prevalent and have contributed to several mass population-level die-offs. Here, we explicitly highlight the risks of pathogen spread in mussel translocations, explore how these risks are mediated, and provide recommendations for both research and action to avoid the inadvertent spread of virulent pathogens when conserving vulnerable mussel populations. While targeted at freshwater conservationists, this perspective has relevance for considering translocation-mediated disease and parasite spread in any study system.

Key words: captive breeding, freshwater, pathogen, prevalence, translocation, transmission, unionid

9.1. Introduction

Freshwater mussels (order Unionida, henceforth referred to as ‘unionids’) are globally distributed ecosystem engineers, playing a key role in many lentic and lotic freshwater ecosystems. Along with recycling and storing nutrients, they create structural habitat, modify the substrate and food webs, and provide a range of intangible cultural services (Vaughn 2018). However, unionids are also among the most endangered animal groups in the world; nearly 50% of species are threatened or near-threatened, rising to 70% in North America (Lopes-Lima et al. 2018). While many threats (such as pollution or natural system modification) are recognised, there have also been enigmatic declines with less obvious causes (Haag 2019), though disease has recently been proposed as a possible explanation (Carella et al. 2016; Richard et al. 2020).

The dire conservation status of unionids has spurred significant interest in captive breeding programs and translocations, to augment ailing populations, reintroduce mussels to an historic range or move them away from threats (Haag & Williams 2014; Strayer et al. 2019). Bioremediation projects also involve moving large numbers of unionids to exploit their ecosystem engineering capabilities (Sicuro et al. 2020). However, translocations may also move parasites or diseases (collectively, 'pathogens'), which can and has led to population- or species-level extinctions in other organisms (Daszak et al. 2000). Unionids host a range of pathogens (Grizzle & Brunner 2009; Chapter 2), though 88% of all European and North American mussels are predicted to be under-sampled in terms of their endosymbionts, and the pathogenicity for many of these symbionts remains unknown (Chapter 2). While we still lack substantial knowledge in this area, and use the term 'pathogen' loosely to refer to any endosymbiont that may have a negative effect, many organisms have been shown to harm unionids (Table A7.1), and while pathogen spread as a result of mussel conservation actions is beginning to be discussed (e.g. Waller & Cope 2019; Wolf et al. 2019), an explicit examination of risks and their mediators remains absent.

Some pathogens (e.g. bacteria, viruses, ciliates) complete their entire life cycle in mussels, and can transfer passively between mussels in the water column. Modern molecular techniques are revealing that these cryptic pathogens are much more common than previously realized (e.g. Carella et al. 2016; Goldberg et al. 2019; Richard et al. 2020). By bringing previously disparate populations together, translocations therefore may spread unrecognized disease agents through the landscape. Other pathogens (e.g. digenean trematodes, unionicolid mites, leeches) rely on a suite of intermediate and definitive hosts, leading to a diverse range of possible outcomes dependent on the ecosystem receiving the translocation. To ensure effective conservation, these outcomes and their associated risk factors must be clearly understood. In this perspective, we begin by outlining the scope of unionid translocations. We then define the associated pathogen-related risks, and provide research priorities and practical recommendations to ensure conservation actions do not unwittingly promote pathogen spread in vulnerable populations.

9.2. Scope of unionid translocations

We distinguish the source population (population from which mussels are taken) from the recipient population (existing population to which mussels are added) and the resultant population (total mussel population resulting from the translocation). In the case of captive breeding, the source and recipient populations can be the same.

A systematic literature review (Appendix A7) shows dramatic increases in both the numbers of papers reporting unionid translocations and the number of translocation events since the 1990s, with a recent tailing off attributable to a lag between a translocation and the publication reporting it (Fig. 9.1a). We also note that many translocations are not reported in the peer-reviewed literature (Haag & Williams 2014), suggesting the total number is likely to be higher. Nearly 45% of all translocations were motivated by restoration (Fig. 9.1b), though this was disproportionately driven by North American trends and motivation differed between continents (χ^2_{12}=97.0, p<0.001); in Europe, more translocations were for experimental purposes (e.g. exploring growth rates in different environments). Whether or not mussels were already present in the recipient ecosystem differed with the purpose of the translocation (Fig. 9.1c; χ^2_9=246, p<0.001), with restoration intuitively having the highest number of translocations where the recipient population had been extirpated. However, each purpose had at least 19% of translocations where there was an extant recipient population (overall mean 34%), and 35% did not report this information, leaving us unable to quantitatively assess the risk of pathogen spread. The incorporation of a pre-introduction quarantine varied with mussel presence in the recipient ecosystem (χ^2_6=108, p<0.001), with quarantine more likely when mussels were present (Fig. 9.1d). However, in total only 34% of translocations involved a quarantine stage.

Our review suggests that vulnerable populations may be slightly less at risk from translocated pathogens than stable populations. As expected, the threat status of translocated species varied with purpose (χ^2_{15}=236, p<0.001): restoration translocations (with a recipient population more commonly absent) involved the highest proportion of threatened unionids (47%), compared with experimental studies which generally used species categorised as Least Concern (Fig. 9.1e). Where threatened species were translocated, the distance moved between source and recipient population was shorter (mean 48km) than for stable species (mean=125km; t_{199}=4.11, p<0.001), potentially reducing the chance of pathogen transfer to

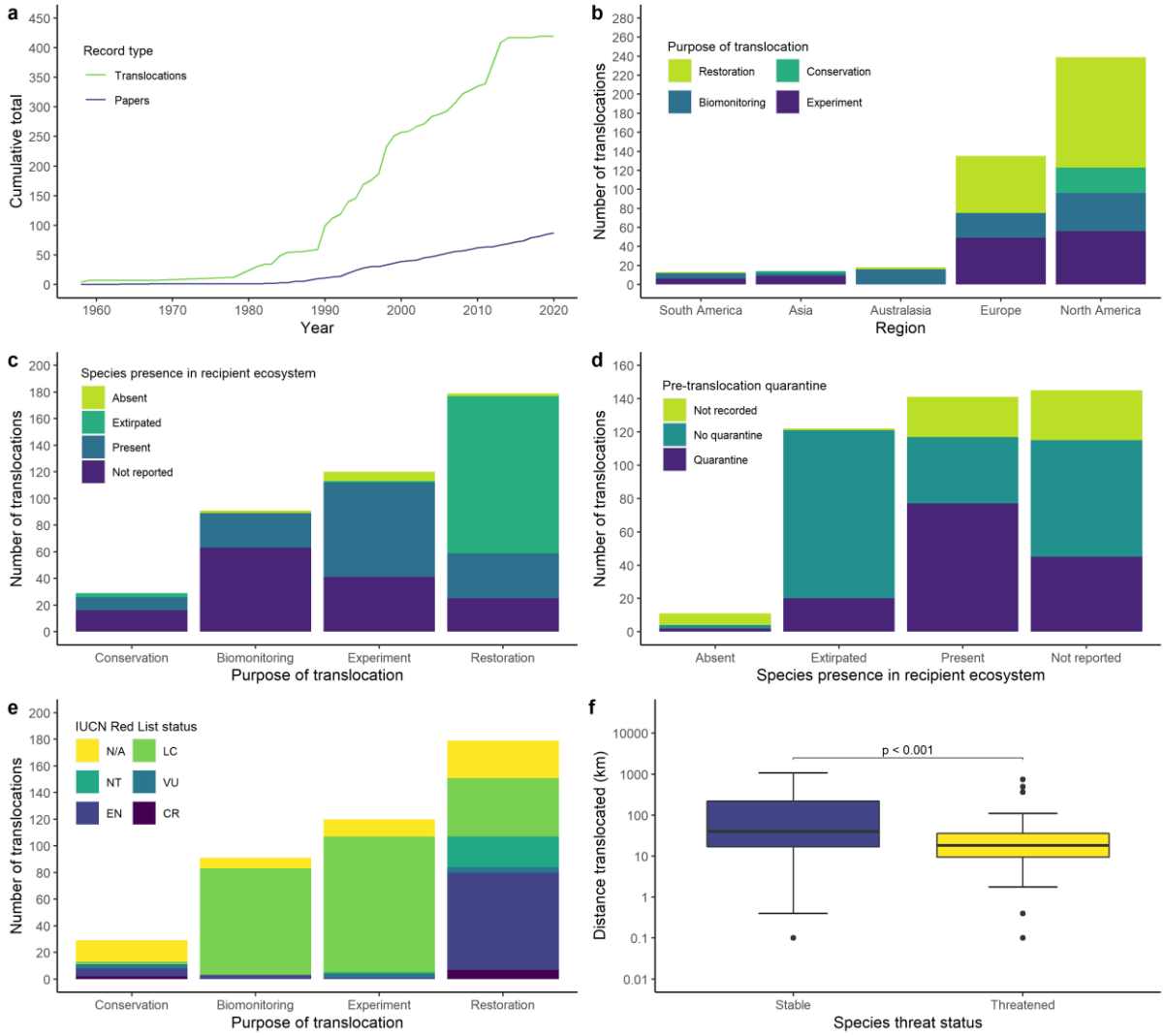


Figure 9.1: Results of a systematic search of the Web of Science database for literature describing unionid translocations. The dataset comprises 419 translocation events across 87 publications (see Appendix A7 for protocol and screening criteria). (a) Cumulative increase in translocation events and publications reporting them. Subsequent graphs use individual translocation events, of which several were often reported in a single publication. (b) The geographic distribution of translocations stratified by broad purpose category. Categories are restoration (supplementing or reestablishing a population), conservation (translocating a population specifically under threat, often due to construction), biomonitoring (generally to assess ambient concentrations of heavy metals or other pollutants), and experiment (other research for information-gathering rather than conservation directly). (c) The current and historical presence of the translocated species in the recipient ecosystem across different translocation purposes. (d) Presence or absence of a pre-translocation quarantine stage, grouped by species presence in recipient ecosystem. (e) Threat status of translocated mussels (according to IUCN Red List) across translocation purposes. (f) Euclidean distance between source and recipient site, compared across stable (Red List status LC or NT) and threatened (VU, EN, or CR) species.

immunologically naïve populations in these more vulnerable species (Fig. 9.1f, 9.2c). However, this may be offset by the fact that significantly higher number of mussels were moved per translocation for the purposes of restoration (mean=465) and conservation

(mean=2597) than for biomonitoring (mean=100) or experiments (mean=58; $F_{3,407}$=19.88, p<0.001), and significantly more mussels were moved per translocation when there was an extant recipient population (mean=438) than no recipient population (mean=325, $F_{3,407}$=5.13, p<0.01). In addition, currently stable populations could undergo pathogen-driven declines in future (see section 9.4), or act as an abundant reservoir for pathogens that could threaten more vulnerable populations or species.

Overall, translocations are common and widespread, though focused (in English-language literature) in North America and Europe. This is particularly concerning given the high percentage of those translocations with extant recipient populations. There is significant scope for pathogen spread between source and recipient populations; in the following sections, we explore the factors determining this outcome, and the implications for already vulnerable populations.

9.3. Determining the risk of pathogen spread

The risk of pathogen spread in translocations is determined by four key factors: pathogen prevalence, host population density, unionid immune capacity, and pathogen life-history (Fig. 9.2). These factors have not been considered for unionid mussels, so we use examples from other systems to illustrate their importance.

The first of these is prevalence: when taking mussels from the source population, the proportion of mussels infected (in addition to total number translocated) will determine the likelihood of transporting pathogens (Figs. 9.2a, 9.3). For example, the North American invasive amphipod *Crangonyx pseudogracilis* hosts a microsporidian pathogen in approximately 10% of individuals in its native range. In its invaded range, the microsporidian is either present at near 100% prevalence (e.g. in the UK, the Netherlands and France; Galbreath et al. 2010), or is completely absent (e.g. in Portugal; Banha et al. 2018). This may be because northern European invasive populations were established by an introduction of amphipods hosting this pathogen, while the Iberian population was established by pathogen-free amphipods (Banha et al. 2018). The median translocation in our review comprises 50 individuals; therefore a pathogen present in just 5% of individuals has a 92% chance of being transported to the recipient population in at least one mussel (Fig. 9.3). Given translocation

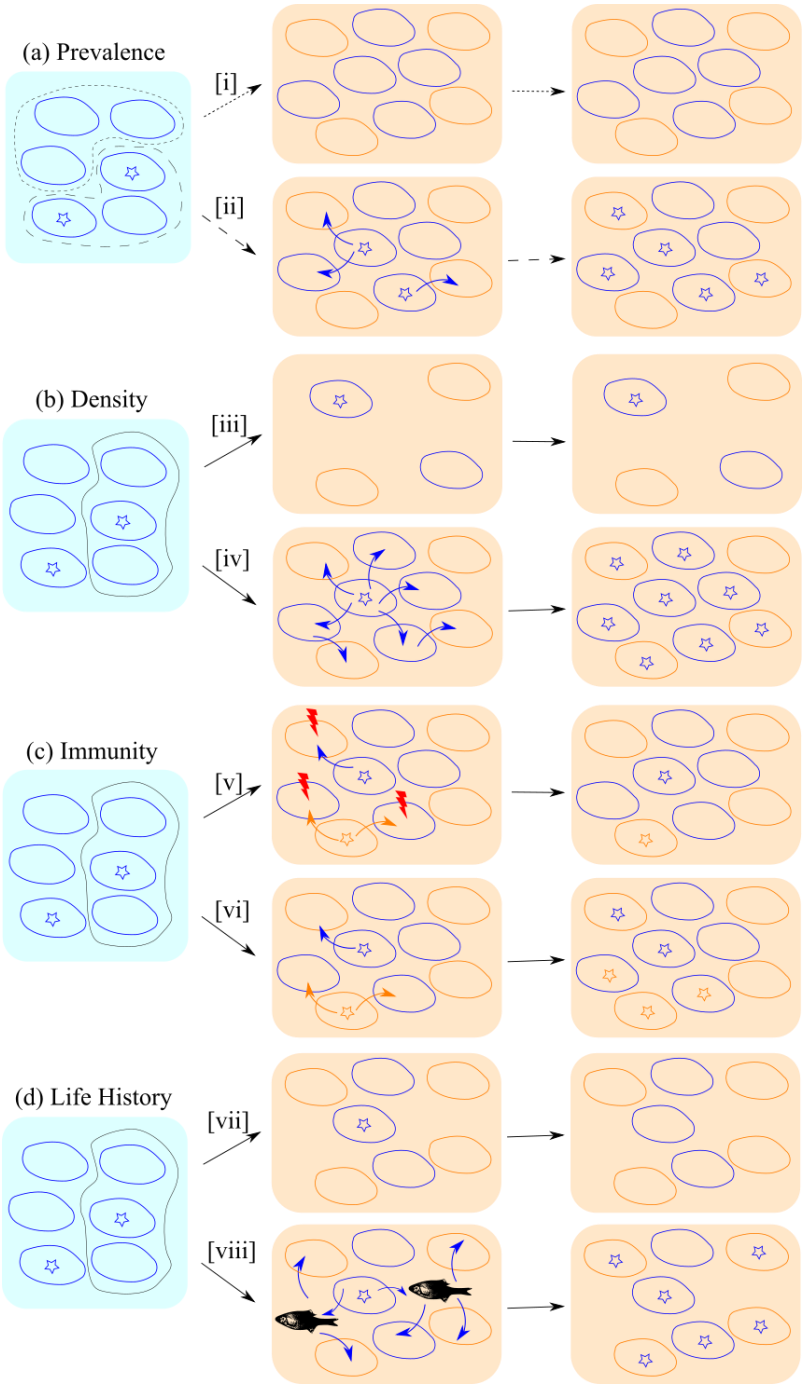


Figure 9.2: Determinants of risk when translocating individuals of a source population (blue mussels and boxes) to a recipient population (orange mussels and boxes), when pathogens originating in either the source (blue stars) or recipient (orange stars) population are involved. Coloured arrows indicate pathogen spread. (a) Pathogen prevalence in the source population will determine the chances of translocating pathogen-free mussels [i] or infected mussels [ii], which can spread in the resultant population. (b) A low-density resultant population [iii] may prevent rapid pathogen spread, while spread could be facilitated by high densities [iv]. (c) Non-naïve recipient populations that already have pathogens may have immunological resources (red lightning bolts) and vice versa, thus mediating disease [v], while naïve recipient populations may stimulate an outbreak in the resultant population [vi]. (d) For multi-host pathogens in the source population, if other obligate hosts are absent in the recipient ecosystem [vii] the pathogen cannot persist, but if those hosts are present, the pathogen can spread in the resultant population [viii].

sizes can often reach the thousands (e.g. Layzer & Scott 2006), there is high scope for moving and spreading even low-abundance pathogens.

The second factor determining pathogen spread is the density of the resultant population (Fig. 9.2b). Creating a population with low densities limits the spread of pathogens, while high-density populations facilitate rapid transmission. Density is an important mediator of pathogen dynamics in natural populations (e.g. Lafferty 2004) and captively held organisms (Meeus et al. 2011). Therefore, unionid translocations and captive breeding programs, which artificially manipulate density, could stimulate previously cryptic or low-prevalence pathogens to spread rapidly.

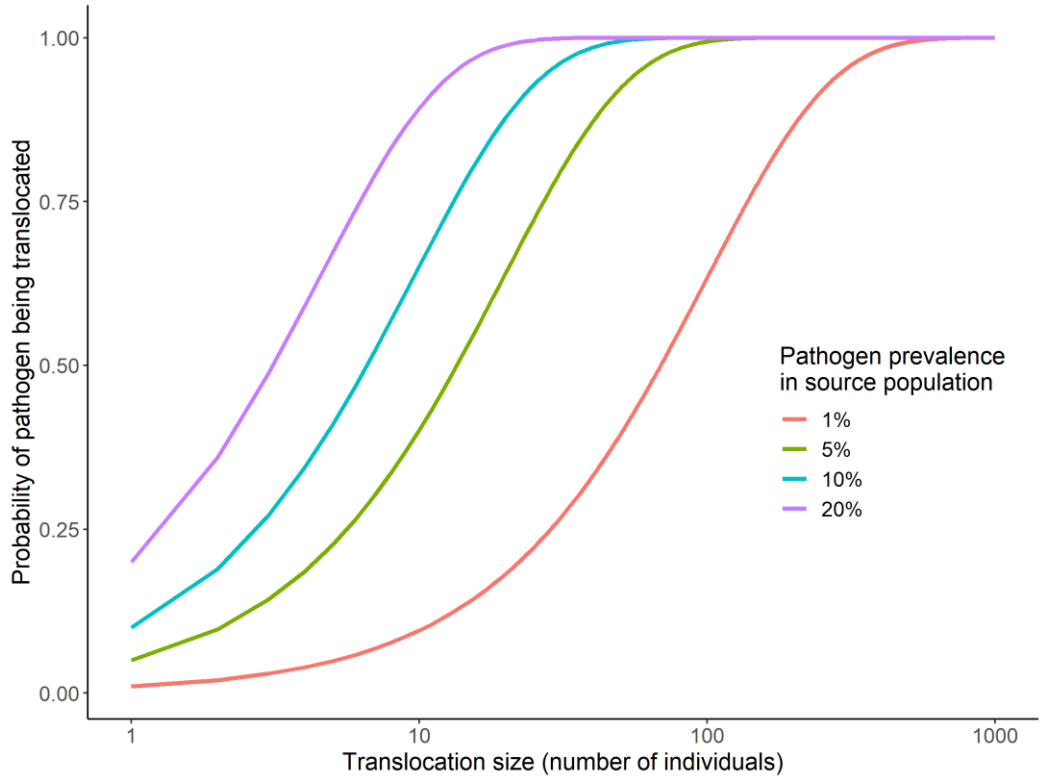


Figure 9.3: The probability of a pathogen being translocated from the source population along with a host mussel increases rapidly with translocation size and pathogen prevalence. Note log-transformed x-axis. Probabilities were calculated as $P(X \geq 1)$ (i.e., the probability of at least one translocated mussel being infected), where $X \sim \text{Binom}(n, p)$ with n representing the number of mussels translocated (1 to 1000) and p representing pathogen prevalence (0.01, 0.05, 0.1 or 0.2).

Host immunity plays a well-documented role in disease mediation (Fig. 9.2c). For example, an attempted translocation of endangered wolves in Yellowstone National Park failed due to immune naivety of the introduced wolves, which received parasites from local canines and experienced pack extinction (Almberg et al. 2012). Immune responses are poorly explored in unionids; though bivalves generally lack an adaptive immune system, they can mount an effective innate immune response against parasite attack (Munoz et al. 2006). Populations may be differentially adapted to pathogens, so understanding population connectivity and gene flow is key. This is particularly important if translocations involve moving endangered mussels between remnant populations that have been reproductively isolated for a long time.

Finally, the likelihood of pathogen spread is dependent on the pathogen's life history (Fig. 9.2d). Pathogens requiring a single host (e.g. bacteria, viruses, ciliates) could persist in mussel populations regardless of wider species assemblages, while pathogens that require multiple hosts (e.g. digenean trematodes, some unionicolid mites and leeches) will not persist unless their other hosts are also present. Host species often determine pathogen distribution patterns (e.g. Paterson et al. 2019), suggesting an ecosystem-wide perspective is required.

While these determinants of risk have intuitive application for direct translocations (i.e. a mussel being moved from one location to another), they also apply to increasingly popular captive breeding programs (Fig. 9.4). This process contains risks for source populations from which larval mussels are drawn (Fig. 9.4c), for juvenile mussels both in the facilities and introduced to the recipient population (Figs. 9.4e, 9.4g), and for the recipient population itself (Fig. 9.4h); the likelihood of these occurrences is determined by the processes outlined in Fig. 9.2. Due to close confinement and high densities, breeding facilities often act as reservoirs of disease, which is then spread wherever the organisms are distributed. For example, the spread of whirling disease in trout is almost exclusively driven by artificial rearing facilities (Bartholomew & Reno 2002), and the vulnerability of multiple marine bivalves to *Vibrio* spp. bacteria leads to frequent outbreaks and spread in shellfish hatcheries (e.g. Elston et al. 2008).

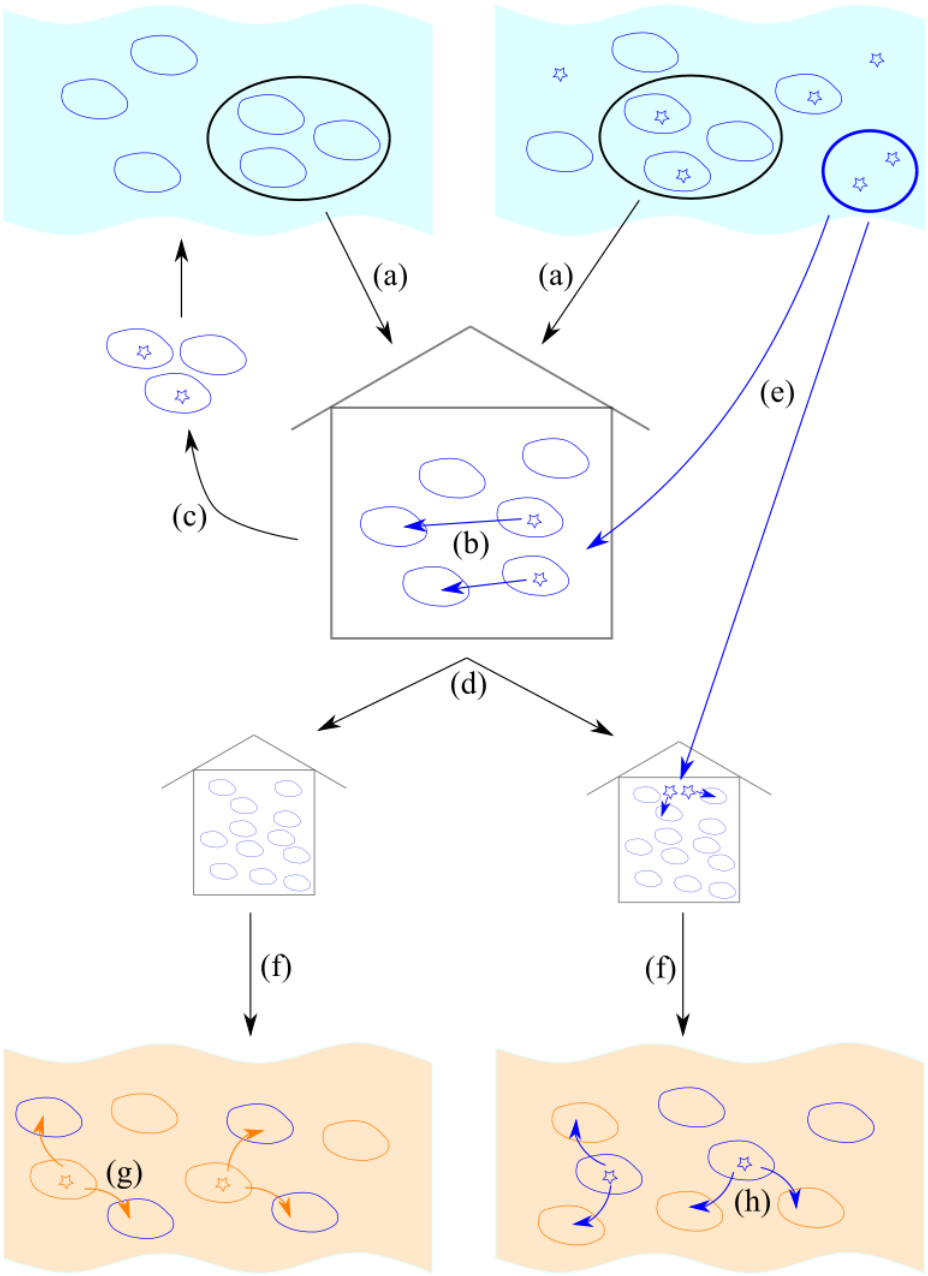


Figure 9.4: Pathogen-associated risks when moving captively-bred mussels. Buildings represent breeding facilities, black arrows indicate movement of mussels and colored arrows represent pathogen spread. Mussels, environments, and pathogens are colored according to Figure 9.2. Adult mussels may be collected from environments with or without pathogens to harvest glochidia (larval mussels brooded by the female) (a); if they are held in shared tanks or equipment is improperly cleaned, pathogens may spread between populations (b) and then transported back into previously unaffected populations via returning the adult mussels (c) sampled in the first step. In the process of both holding adults (a) and growing juveniles (d), water from source environments is frequently used, which may contain transmission stages of pathogens (e) and infect mussels. When mussels are placed in the environment after captive breeding (f), they may be naïve and suffer high infection rates from pathogens in the recipient environment (g), or contain pathogens themselves which may spread to vulnerable mussels in the recipient population (h). The likelihood of stages a, b, e, g, and h will depend on the processes outlined in Figure 9.2.

9.4. Outcomes of risk: Consequences for populations

We now consider the outcomes of these risks for unionid populations. Unionids possess a broad range of pathogenic fauna, including trematodes, mites, ciliates, nematodes, bacteria and viruses (Chapter 2; Table A7.1). There is also indirect evidence for parasitism by other taxa such as glossiphoniid leeches (Bolotov et al. 2019), highlighting the need for continued research in this area. Many pathogens have deleterious effects on unionid populations, including castration by bucephalid trematodes, and recent evidence of virally-driven mass mortality (Table A7.1). These pathogens may be shared between populations, depending on whether the pathogen is in the source population, recipient population, or both. Table 9.1 explores these potential outcomes and how they are mediated. Parasite prevalence emerges as a near-ubiquitous influence on the likelihood of pathogen sharing. Other risks depend on the type of pathogen considered: pathogens requiring multiple hosts may be less affected by the density of the resultant population as transmission is mediated by other host species, while the opposite is true for single-host pathogens. Further, Table 9.1 only considers outcomes for a *single* pathogen. However, mussels host multiple macro- and microparasites simultaneously (Chapters 5, 6; Richard et al. 2020), leading to a complex set of possible interactions. Consider again a median translocation size of 50 from a mussel population that now has two pathogens, both at a conservative 5% prevalence. Assuming they occur independently, the likelihood of at least one of those pathogens being translocated rises to 99.4%, a near-certainty. We suggest cryptic movement of pathogens is exceedingly common in freshwater mussel translocations.

In extreme cases, pathogens may lead to population collapse in bivalves (Katsanevakis et al. 2019; Richard et al. 2020). However, pathogens can significantly affect ecosystems even without complete collapse. Pathogens interact with other sublethal stressors to greatly enhance unionid mortality; for example, *Anodonta anatina* infected with the castrating trematode *Rhipidocotyle fennica* suffered significantly higher mortality than non-infected mussels in both anoxic and food-depleted environments, an effect not observed under normal environmental conditions (Jokela et al. 2005). Further, changing environmental conditions may stimulate a sudden outbreak. *Perkinsus marinus* was repeatedly introduced into various oyster populations where it remained undetected until it was stimulated to proliferate into an epizootic by extreme warming (Ford 1996). Environmental extremes are increasingly common, and may be related to die-offs of mussel fauna in recent decades (Strayer et al.

2019). Pathogens may also interact to worsen outcomes for vulnerable species. The Clinch River population of the unionid *Actinonaias pectorosa* suffered a mass die-off in 2016 hypothesized to be pathogen-related. Before the die-off, the prevalence of castrating parasites

Table 9.1: Possible outcomes of pathogen (including virus, bacteria and macroparasite) spread caused by translocation actions, where source population refers to the population that mussels are being removed from, recipient population refers to the existing population that translocated mussels are being added to, and the resultant population is the total mussel population resulting from the translocation. For simplicity, this table considers a single pathogen. (a) Major determinants of risk for pathogen spread, depending on whether the pathogen is present in the source population or the recipient population. (b) Possible outcomes of translocation with respect to pathogen spread. The first two columns specify whether the source and recipient populations respectively have a single-host pathogen (SHP; i.e. a pathogen that does not require another host in the life cycle), a multi-host pathogen (MHP) or no pathogens (NP) prior to translocation.

(a)

Origin of pathogen	Determinant of risk	Corresponding figure	Risk type
I: Source population	Prevalence in source pop	1a	1
	Vulnerability in recipient pop (i.e. immune naivety)	1c	2
	Presence and density of other hosts in pathogen life cycle in recipient ecosystem	1d	3
II: Recipient population	Vulnerability in source population (e.g. immune naivety)	1c	4
III: Either	Density of resultant population	1b	5

(b)

Source population	Recipient population	Possible outcome	Risks affecting outcome
SHP	No mussels	SHP to resultant pop	1
	NP	SHP outbreak in recipient pop	1, 2, 5
	SHP	SHP outbreak in resultant pop	5
	MHP	Both MHP and SHP in resultant pop	1, 2, 4
MHP	No mussels	MHP to resultant pop	1, 3
	NP	MHP outbreak in recipient pop	1, 2, 3
	SHP	Both MHP and SHP in resultant pop	1, 2, 3, 4
	MHP	MHP outbreak in resultant pop	3
NP	No mussels	No pathogen-associated risk	N/A
	NP	No pathogen-associated risk	N/A
	SHP	SHP outbreak in translocated source-pop mussels	4, 5
	MHP	MHP outbreak in translocated source-pop mussels	3, 4

was 12.5%; after the die-off it was 90% (Henley et al. 2019). Whether or not the castrators contributed to the die-off, their high subsequent prevalence significantly limits the capacity of the population to recover.

Overall, we believe such environment-pathogen or pathogen-pathogen interactions may become increasingly common as pathogen spread may be amplified by both translocation actions and environmental extremes. It is therefore crucial to limit their spread and carefully consider their role in conservation actions.

9.5. Recommendations

In this section we provide explicit policy recommendations, focused on two key areas: Research Recommendations (RR), and Action Recommendations (AR).

RR1: Understand parasite diversity and prevalence in both source and recipient populations.
The most important first step is to identify possible pathogens from a wide range of species and regions, and determine their pathogenicity. Over 85% of North American and European mussel species are considered under-sampled in terms of their pathogen fauna (Chapter 2), and our poor understanding of mussel pathogens is a key reason why many translocated mussels are not screened for diseases (Haag & Williams 2014). Different pathogens are found in different populations (e.g. Chittick et al. 2001; Chapter 6); translocations should ensure they are not spreading pathogens to new locations, which requires understanding pathogen geography and diversity. Within-population variation is also important: differences in filtering behaviour or sizes of individuals can influence parasite communities (Chapter 5). Assessing these factors may be difficult for endangered species, but recently developed non-destructive methods may help (e.g. Chapter 4).

RR2: Understand pathogen life histories.
This important determinant of risk has three key facets: how the pathogen responds to different host densities, how biotic and abiotic aspects of the habitat influence pathogen spread, and how pathogen exposure varies temporally (Chapter 5). This is particularly important when mussels cannot be screened for pathogens extensively: if we know how a pathogen spreads, or what times of year are important in its life-history, we can better predict the risk of it successfully establishing somewhere new, and how risk varies among scenarios.

For example, translocating mussels upstream in the same catchment area may pose a greater risk of infecting recipient populations than moving mussels downstream, where transmission stages are more likely to have travelled anyway. These factors will vary from pathogen to pathogen, precluding generalities and necessitating further study.

RR3: Understand immune responses of unionid mussels.
Very few published studies on unionid immune responses exist: this area should be explored further. The general principles we have discussed apply whether one considers pathogen transfer between the same species or different species. However, population- or species-specific immune adaptations may significantly influence the success of pathogen communities, and assessing variable resistance or tolerance to infection is an important part of understanding the risks of translocation. Additionally, RR2 and RR3 together will help in determining the dangers of pathogen spread for different translocation distances (Fig. 9.1f).

AR1: Only translocate when absolutely necessary.
This is not a novel recommendation (see Patterson et al. 2018, Strayer et al. 2019), as it is widely accepted that translocation is not a substitute for addressing the causes of decline. However, we bring a new context to this, especially given the high number of experimental translocations that have recipient populations (Figs. 9.1b, 9.1c). While experimental translocations are often useful to understand unionid biology, they should consider the risk of transporting pathogens to naïve populations. Regardless of purpose, poorly considered translocations contain significant scope for pathogen spread (Table 9.1), and may exacerbate rather than alleviate the significant threat to endangered populations. This is particularly pertinent as it appears that vulnerable populations (which are likely translocation targets, either as a source or recipient populations) after die-offs have high pathogen prevalence, which may have contributed to the die-off (Henley et al. 2019).

AR2: Quarantine translocated mussels, but tailor this to the pathogen of concern.
Quarantine procedures are well-established for avoiding the spread of zebra mussels (Patterson et al. 2018), but little consideration has been given to avoiding endoparasites or disease spread. These should be informed by RR1, to identify the possible pathogens of concern. For example, macroparasites such as trematodes and mites may require a long quarantine, to allow for life-history stages of these organisms to emerge as evidence of infection. However, bacterial or viral infections may remain cryptic; while a short quarantine

would allow for non-destructive tissue assessment and identification of potentially infected mussels, a long quarantine could facilitate their spread among mussels held together. Treating water in quarantine facilities with U.V. light may be effective at stopping bacterial or viral spread through the water (Schneider et al. 2009), but this cannot penetrate shells and kill pathogens *in situ*.

AR3: Where possible, consider introducing mussels as glochidia encysted on fishes.
The small size of glochidia (larval mussels) represents a significant barrier to vertical transmission, and to our knowledge they have no recorded pathogens, though this has not been studied in detail. This recommendation will not apply in some scenarios (e.g. moving an adult population faced with environmental degradation), but is an option for captive breeding programs, or for supplementing existing populations, though it does make assessing translocation success difficult. This strategy should carefully consider the risk of spreading pathogens of fish hosts (the vectors for glochidia), though this aspect has been evaluated elsewhere (e.g. Patterson et al. 2018). In addition, such a strategy will need to ensure pathogens are not extracted from the female mussel's gills along with the glochidia.

9.6. Conclusion

Understanding pathogen risk is a key factor in taking successful conservation action (Gross et al. 2000). In this policy perspective, we have demonstrated the scope of unionid translocations and explored the possible risks of pathogen spread between already highly threatened populations and species. Importantly, cryptic pathogens exist in mussel populations, the effects of which can be stimulated and exacerbated by environmental variation. Translocations, if not carefully considered, have immense scope to promote the spread of these pathogens. We acknowledge that our recommendations represent ideal best practice; however, we see them, and this perspective, as a key starting point in considering pathogens when acting to conserve unionid mussels.

Chapter 10: General Discussion

10.1. Chapter summaries

This thesis has studied the parasite communities of freshwater mussels, structured around three main aims: to characterise knowledge to date on freshwater mussel communities and develop tools to further this knowledge (Chapters 2, 3, 4); to analyse the drivers of parasite community structure (Chapters 5, 6); and to assess the influence of parasitism on the conservation of freshwater mussels and ecosystems (Chapters 7, 8, 9).

In Chapter 2, I reviewed all North American and European freshwater mussel-endosymbiont records, as well as parasite records for invasive bivalves in Europe. I showed that 53% of mussel species have no records at all, and 88% can be considered under-sampled. While studies examining the effects of parasitism were rare, of those that did, 72% of them recorded a negative effect on the host mussel, and none recorded a positive impact. In addition, the inclusion of invasive bivalves showed that while there is limited possibility for spillover to occur, spillback could be an important mechanism that influences native parasite loads.

In Chapters 3 and 4 I developed two simple methods to better characterise castrating trematode infection in freshwater mussels. Chapter 3 used photographs of gonad squashes to allow for an objective estimate of infection intensity by quantifying the percentage of gonad occupied by trematode tissue. Chapter 4 used a needle extraction of gonadal fluid to non-destructively identify whether or not hosts are infected; this has particular use for assessing the trematode communities of endangered bivalve species.

In Chapter 5, I turned my attention to the community assembly of parasites in the mussel *Anodonta anatina* at a single site over the course of a year. Using Joint Species Distribution Models (JSDMs) and Markov Random Fields (MRF) models I showed that the time of year, host length and host gravidity, and within-host parasite interactions were all important in determining parasite infracommunity structure. Incorporating parasite traits and abundance data allowed me to effectively contextualise these results to the underlying biology of the parasites.

Chapter 6 expanded on Chapter 5 by examining multiple host species (*A. anatina* and *Unio pictorum*) and multiple sites. Using variance partitioning and nestedness analyses across ecological scales (site, host species, host population, host individual) and comparing them to a variety of null models, I demonstrated that different sites and different host species supported very different parasite communities. However, within-host parasite interactions were still important once the site-specific parasite prevalences and parasite richness of individual hosts were accounted for, highlighting that processes at one scale need to be considered when searching for patterns at another.

In Chapter 7, I explored the effect of mites, trematodes and zebra mussels on the population-level reproductive capacity of *A. anatina*. Trematodes castrated their hosts at both the sites considered, while mites were correlated with a reduction in viability of mussel larvae (glochidia), but only at one site, suggesting that host-parasite relationships may need to be considered at a population level. Parasites reduced the population-level production of viable glochidia by 9.6% and 13% at the two sites – whether this scales to reducing long-term recruitment needs to be explored further.

Chapter 8 showed that bitterling fish embryos and trematodes both altered the clearance rates of their mussel hosts, but in contrasting fashion. At low concentrations of suspended particles, both bitterling embryos and trematodes increased host clearance rates, but this effect reversed for bitterling at high concentrations of suspended particles. I incorporated parasite prevalence and distribution, host community densities and river parameters to estimate the impact of these effects on a real-world ecosystem, and showed that parasites can change the rate at which host mussel communities filter the river by up to 50%.

Finally, in Chapter 9 I briefly explored the risks of captive breeding and translocation programs for spreading parasites and diseases among already threatened species. I showed that host density, parasite prevalence, host immunity and parasite life-history all require careful consideration when moving mussels, and that in general there needs to be more focus on diseases and parasites in freshwater mussel conservation programs.

This thesis has focused on the community ecology of parasites, and the conservation implications of parasitism for host individuals, populations and communities. It is important to note that the latter depends on the former: for example, the estimates of how many

glochidia a population can produce or how long it takes a mussel community to filter a body of water in the presence of parasitism would be completely different if the prevalences of the observed parasites were different. In addition, I highlighted in the General Introduction the important interplay between theories of free-living and parasite community structure. Therefore, this General Discussion is structured around how freshwater mussel parasites align with theories of community assembly, and what the implications are for the conservation of freshwater mussels. This approach allows me to highlight the contribution this thesis makes, and to centre my conclusions firmly in the wider ecological literature.

10.2. The deterministic-stochastic continuum in parasite community ecology

A major focus of this thesis has been on community assembly and structure. Chapters 2, 3 and 4 focused on the current lack of knowledge on the parasite communities of freshwater mussels and tools for how this could be improved; Chapters 5 and 6 analysed the processes contributing to freshwater mussel parasite community structure; while Chapters 7, 8, and 9 highlighted the consequences of parasite assembly and distribution for host populations, species and ecosystems. As such, I believe it is highly informative to first contextualise parasite community assembly in freshwater mussels to broader theories about community processes. Arguably the most significant of these is the relative role of stochastic *versus* deterministic processes in governing community assembly (Chase & Myers 2011; Johnson et al. 2015), of which niche and neutral theory is a high-profile example (Gravel et al. 2006).

Whether communities are structured predominantly by deterministic (i.e. niche-based) or stochastic (neutral) forces is a classic paradigm in community ecology (e.g. Hubbell 1997). Niche-based communities are driven by species' differential responses to environmental gradients, while neutral dynamics rely on the assumption that all species are equivalent and that the community space is saturated with individuals; the successful recruitment of any one individual into a community is down more to random chance. In practice, this means that neutral dynamics are more likely to be observed when species richness and dispersal are high (Gravel et al. 2006). Rather than binary categories, deterministic and stochastic dynamics are thought to be the ends of the same continuum (Gravel et al. 2006); where communities lie on that continuum may depend not only on demographic factors, but also the scale that the communities are studied at. It is especially interesting to think about this in terms of parasites, given the highly hierarchical nature of parasite communities. Scale can be both spatial and

temporal, and I consider both below. In both cases, I show that, while processes occurring at larger scales tend to be more deterministic (and hence explainable), small-scale dynamics are vital for understanding the impact of parasites, and that incorporating parasite traits demonstrates that even within-host trends can and should be incorporated into broader studies of host-parasite associations.

10.2.1. Spatial dynamics

A nuanced but critical point when studying hierarchical communities is that scale is a property of the observer, not the system (Fritsch et al. 2020). To explain what I mean and to highlight the significance of this idea, it is informative to directly compare the JSDM results modelling parasite prevalence between Chapter 5 (single host species, single site) and Chapter 6 (multiple host species, multiple sites). The amount of 'random' variation in parasite community structure remaining unexplained by the covariates or parasite-parasite interactions dropped from 24% to 8%. In Chapter 6, the additional variation explained was overwhelmingly driven by processes at larger scales, namely, the site and host species contrasts. Correspondingly, processes at smaller scales (i.e. host individual characteristics) explained less of the variation in Chapter 6, despite the overall proportion of variation explained increasing. For example, in Chapter 5, host length explained 12.1% of the variation in parasite infracommunity structure, but only 4% in Chapter 6 (similarly, in the MRF models, length went from explaining 3% to <1%). At an individual host level, it improbable to state that parasite community assembly suddenly became less random between Chapter 5 and 6, or that parasites suddenly became less responsive to host length. However, it appears our ability to explain total stochasticity significantly increased, because so much variation was observed between species and sites. In short, highly deterministic (explainable) differences between sites and between host species are observed when scaling up (Chapter 6); this reduces the overall unexplained portion of the variation. At a smaller scale (Chapter 5), we simultaneously see that host-level factors (host length, host gravidity) contribute more to explaining community variance, but that stochasticity has a more influential role (in terms of variation that cannot be explained by any of our covariates). However, it is important to note that this doesn't necessarily suggest that neutral dynamics are more important at smaller scales, as there may be fine-scale niche-based factors that we did not measure.

The dominance of deterministic factors at larger spatial scales is affirmed by König et al. (2021), who carried out JSDMs on identical community data but with increasing spatial grain. They found that JSDMs at coarse spatial grains explained more of the community variation, and attributed this to the greater influence of stochastic processes at smaller scales (König et al. 2021). This corresponds with general theory that deterministic processes are more important at coarser environmental scales (Chase & Myers 2011).

Interestingly, our results align closely with those of Moss et al. (2020), who also partitioned parasite variation among sites, host species and host individuals. Their results were qualitatively identical to Chapter 6: variation between species and sites was greater than expected. Therefore, the strongest structural forces occur at the largest scales, governing dispersal to and survival at sites, and dispersal to host species within those sites. Broadly, macroecological patterns of community ecology may therefore be repeatable in parasite communities, with deterministic factors dominant across larger scales. This has important implications for predicting risks of parasitism and disease for freshwater mussels. The focus in this thesis, and the focus of parasite community structure analyses generally (e.g. Dallas et al. 2019; Sallinen et al. 2020) has been to *explain* observed patterns: what could have caused what we have observed? However, JSDMs and similar tools can be used to *predict* community trends at sites with no previous knowledge of distributions (Wilkinson et al. 2021). If we have knowledge of environmental data and host community composition at various sites, it may be sound to draw conclusions about overall parasite prevalence at those sites. This could significantly enhance the ability of biologists to avoid the negative consequences described in Chapter 9, without having to carry out detailed dissections of mussels or handle potentially threatened species.

The general idea I am attempting to convey can be summarised as follows. First, it is both expected by theory and affirmed by this thesis that, over larger scales, the overall parasite (supra-)community structure appears more deterministically driven. This has clear benefits for predicting parasite patterns in unexplored host communities. However, a large proportion of variation in freshwater mussel communities being explained by site and species (Chapter 6) does not mean that host length, host gravid status or parasite interactions suddenly become less important: they are vital predictors of individual-based risk (Chapter 5). Chapters 7 and 8 demonstrate the importance of considering factors at the level of individual hosts.

Interactions between individuals at small scales are often masked by population-wide demographic trends at larger scales (Albery et al. 2020). The multi-faceted approach taken in Chapters 5 and 6 allowed me to account for environmental variation and differences between host species to detect host individual-level drivers of parasite community structure and parasite-parasite interactions; this approach of acknowledging local-scale drivers inside a metacommunity framework has been underused in ecology to date (Thompson et al. 2020). Chapters 7 and 8 show the importance of this approach. For example, in Chapter 7, mites are more likely to occur in larger individuals; these larger individuals are also more likely to produce more glochidia, and thus mites would have a greater than expected effect on the viability of glochidia at a population level. If only population-wide mite prevalence was considered, the impact of this parasite would be underestimated. Similarly, in Chapter 8, ignoring the role of parasite choice and trematode-bitterling interactions would lead to estimates of fewer mussels in the host community being infected than in reality. This in turn would underestimate the parasite-altered ecosystem effects of freshwater mussels. Therefore, when considering the conservation implications of parasitism for freshwater mussels or for any host, it is a trap to study parasite distributions purely at a macro-level and assume that individual host-level or within-host drivers are less important because they explain a smaller proportion of the observable variance. Interactions at smaller scales determine effects at larger scales (Chapters 7, 8).

10.2.2. Temporal dynamics

General theory suggests that patterns at smaller spatial scales are more stochastic, and patterns at larger spatial scales are more deterministic; the same is true for temporal dynamics. Over short time periods (days, months), population dynamics are frequently unstable and changeable; over years and decades, the same populations and species interactions are highly stable (CaraDonna et al. 2020; Ross et al. 2021). This stability has also been observed for parasite communities. While communities will naturally drift across very long time scales (Vellend 2010), across reasonably lengthy time periods (>10 years), parasite community structure remains relatively stable (e.g. Soares et al. 2014; Welicky et al. 2021), though global change will have the potential to disrupt this general rule (e.g. Sitko & Heneberg 2020). This is in contrast to the smaller scales observed in Chapter 5: monthly sampling showed that parasite prevalence and abundance was highly variable, with some parasites only present at certain times of year (Figs. A3.10, A3.11). In extreme cases, parasite

communities can vary on a time scale of hours; for example, ectoparasite communities on small mammals change on a daily basis as they drop on and off to obtain a bloodmeal (Krasnov et al. 2021).

However, once again, a higher level of stochasticity at smaller temporal scales does not mean that smaller temporal scales can be neglected. Integrating small-scale temporal variation with long-term population trends significantly improves predictions about species' distributions (Pérez-Navarro et al. 2021), and is important for understanding the broader influence of parasites on host populations. A major recommendation of Chapter 9 is for more extensive sampling of mussel host populations to avoid spreading parasites and diseases, affirming the general conclusion of Chapter 2 that many more host-parasite associations exist than have been currently observed. I suggest that the time of year this sampling occurs could have a major influence on conclusions, and that more comprehensive sampling across multiple time points is required: sampling in a single month may severely underestimate (or overestimate) the prevalence or abundance of parasites in the population. To fully appreciate parasite community dynamics, this needs to occur over multiple years. While both Chapters 5 & 6 sample at multiple time points, both are within the scope of a single year. Most of the observed parasites (mites, trematodes, bitterling) have previously well-characterised seasonality (e.g. Baker et al. 1992; Taskinen et al. 1997; Aldridge 1999), and so understanding their long-term population trends requires sampling over multiple years (Poulin 2019). This seasonality also suggests that in many cases, the 'stochasticity' that characterises smaller temporal scales may be predictable if the life-history characteristics of parasites are taken into account. A clear understanding of parasite temporal dynamics is also important to understand their vulnerability to environmental change, as it has recently been shown that being temporally rare makes species vulnerable to extinction, similarly to being spatially rare (Wilfahrt et al. 2021). This is particularly important given their impact on host individuals and ecosystems.

10.2.3. How do parasites fit the scale-dependent deterministic-stochastic dynamic?

I have argued that parasite communities generally fit the spatial and temporal patterns predicted by theory developed on free-living communities, but that dynamics at smaller scales are important and may determine outcomes across larger scales in a predictable manner. This is a potentially important difference with free-living communities, and may be

because parasite communities (at least in this thesis) violate a clear assumption of neutral theory: parasite species are not equal with respect to their demographic parameters (Poulin 2004). Instead, parasites show clear interspecific trait variability with respect to their mechanism of dispersal, host tissue occupied (Table 5.1), reproductive strategy (Fig. 1.1) and so on. This means they respond to the same factors in different ways (Chapter 5; see also Williamson et al. 2019; Snyman et al. 2020; Stuart et al. 2020), and it is difficult to class a parasite community as being definitively 'niche-structured' or 'neutral-structured' (see the wide variation in stochasticity between individual parasite species in Fig. 5.2). Trait variation is a vital but often overlooked aspect of community ecology (Kohli et al. 2021), and especially in the case of freshwater mussel parasites where such large variation exists between members of a single community. This is in contrast to communities where stochastic dynamics have been shown to dominate, such as tropical tree communities where all species disperse, grow and reproduce in functionally similar ways (Volkov et al. 2003).

To summarise this section of discussion, this thesis has affirmed the growing recognition of the importance of considering ecological scale (e.g. Chase et al. 2019; Albery et al. 2020; Fritsch et al. 2020; Thompson et al. 2020; König et al. 2021). Specifically, it is difficult to attribute patterns to deterministic or stochastic processes, as the proportion of 'randomness' in a system alters with the scale at which the system is observed. This is particularly relevant for freshwater mussel parasite communities, which show great diversity in life-history traits and therefore have the capacity to respond differentially to both coarse and fine-scale factors in nuanced fashion (Chapters 5 & 6), as well as interact with each other. These fine-scale processes may alter how host populations respond to parasitism (Chapter 7), how ecosystems are altered in accordance with parasite trends (Chapter 8), and how variation affects our ability to predict the risk of translocating infected individuals (Chapter 9). Most importantly, considering freshwater mussel parasites in the context of deterministic and stochastic processes shows that parasite community composition is predictable and deterministic across broad scales, in line with wider theory. However, deterministic processes are also highly important at smaller scales too, and incorporating interspecific trait variation (in terms of life-history characteristics, parasite choice and the density- and trait-mediated effects of parasitism) reduces perceived stochasticity and facilitates a more nuanced understanding of the causes and consequences of parasite community structure.

10.3. The interaction between parasites and host community richness

In this section, I focus more on the conservation of freshwater mussels by considering this thesis through the lens of the relationship between host and parasite richness. I first consider how host richness interacts with the prevalence and abundance of a focal parasite, before discussing the relationship between parasite community richness and host community richness.

10.3.1. The biodiversity-disease relationship

One of the most interesting results of this thesis is that different parasites are found in different hosts, or that the same parasite shows markedly different prevalences between hosts (Chapters 6, 8). In some cases, this is explainable. Female bitterling use a range of cues to select hosts (Smith et al. 2004) which explains their preferences, and there is high interspecific competition between mites, which leads to their species-specific distributions (Davids et al. 1988). However, in other cases such as digenean trematodes, the reason for host specificity is unclear. For example, there is no evidence that *U. pictorum* hosts any digenean trematodes (Chapters 2, 6), a source of long-standing confusion (e.g. Probert 1966). While there is some evidence that miracidia (Fig. 1.1) show active choice (Allan et al. 2009), it seems improbable that they completely avoid infecting *U. pictorum*, especially as *U. pictorum* is so much denser than other mussels at the main study site of this thesis, the Old West River. Therefore, digeneans such as *Rhipidocotyle campanula* are either unable to develop in *U. pictorum*, potentially due to a shorter coevolutionary history (Blasco-Costa et al. 2021), or *U. pictorum* shows a greater immune response than *A. anatina* and is able to rapidly clear infection. In either case, the interaction represents a 'wasted infection' for the trematode, and thus conditions exist for *U. pictorum* to dilute the infection risk of this trematode for *A. anatina* (Garrido et al. 2021).

In general, non-competent hosts may dilute the risk of parasitism or disease for competent hosts, and therefore more biodiverse communities can decrease the overall prevalence of a parasite in the community (Johnson et al. 2013; Fearon & Tibbetts 2021). This observation arises because abundant hosts (which are more likely to be observed in species-poor host communities) tend to host more parasites at higher prevalences (Vázquez et al. 2005; McCaffrey & Johnson 2017). As rarer and less competent hosts are added, the overall

potential for parasite transmission goes down, both through wasted infections but also through reductions in competent host density, which is a vital factor determining successful transmission (Chapter 9; Lafferty 2004; Poulin 2021).

The degree to which this diversity-disease relationship applies to freshwater mussels is important to understand for at least two reasons. First, it suggests that the overall prevalence of a parasite is a product not only of its focal host's distribution, but also of the distribution of non-competent hosts. Therefore, further sampling is required to not only understand what parasites are found in what hosts (Chapter 2), but also to be able to definitively state that a parasite is *not* found in a particular mussel species (e.g. Table A1.4). Second, it highlights the need to conserve freshwater mussel diversity as much as possible: trends of species loss in mussel communities may not only alter the net ecosystem services that mussels provide, but also amplify disease in the remaining species (see Halliday et al. 2020b for a meta-analysis supporting this general mechanism). This may potentially provide an explanation for the enigmatic declines experienced by freshwater mussels. For example, host-specific viruses of common mussel species (e.g. *Actinonaias pectorosa* in the Clinch River, USA; Jones et al. 2014) may have previously been diluted by non-competent mussel species. However, with local losses of some of these rarer species due to pollution and other factors (Ahlstedt et al. 2016), the dilution effect will be eroded, with the consequence being virally induced mass mortality in the focal host (Richard et al. 2020). In this thesis, it is impossible to assess the role of non-competent hosts due to the small number of sites and species studied. However, the evidence provided for host specificity in multiple parasites (Chapter 6) raises the possibility for the dilution effect to be operating, and therefore host community structure needs to be maintained to ensure balance in host-parasite interactions. Considerations of focal and non-focal hosts bring additional nuance to Chapter 9: translocations could amplify or dilute parasites and diseases depending on the relative competency of the translocated species and recipient community. In addition, specifically including non-competent hosts of parasites or diseases could improve refuge programs. This also highlights the importance of considering host richness at a population level: species may be lost from particular communities without going extinct globally (Chase et al. 2019), and so focus needs to be on maintaining community diversity in freshwater mussels, not just global diversity.

While the diversity-disease relationship is important for considering parasite pressure on host species, parasite choice can disrupt its predictions. For example, bitterling fish are highly

choosy parasites with females showing high discriminatory power regarding where to deposit their eggs (Chapter 8; Smith et al. 2000). Therefore, adding unfavoured host species to the community will not reduce the impact on preferred hosts, as bitterling will still select the focal host mussel. For a constant bitterling prevalence and total host density, increasing mussel diversity will actually increase the pressure on the preferred host species. This has been observed for temperate tree communities, with increases in host diversity leading to specific species being under greater parasitism pressure than expected (Berthelot et al. 2021). This re-emphasises the importance of understanding traits of freshwater mussel parasites, and the mechanisms by which parasites are absent from possible hosts (Shaw & Civitello 2021).

10.3.2. Parasite richness increases with host richness

In contrast to the prevalence of a focal parasite, which is predicted to decrease at high host community richness, overall parasite diversity is always predicted to increase with host diversity (Johnson et al. 2016). For example, there is a clear link between mussel diversity and freshwater mite diversity due to high host specificity (Tables A1.2, A1.3; Edwards & Vidrine 2020), supporting general conclusions that host community composition plays a major role in parasite community composition (Dallas & Presley 2014; Selbach et al. 2020). Highly diverse freshwater mussel communities will support many parasite species, all of which may have effects on host populations as well as the wider ecosystem. Chapter 8 dealt with a relatively 'simple' system involving just two parasites and their trait-mediated effects, which were demonstrated to be large. However, I suggest it is highly improbable that other parasites in the community have no effect; further, the parasites considered may also have long-term density-mediated effects (e.g. Chapter 7) that were not accounted for. The ecosystem services provided by diverse mussel communities may therefore be influenced in equally diverse ways by a large range of parasites. This makes the description of freshwater mussel communities a high priority.

The flipside of diverse host communities having diverse parasites is that species-poor host communities are also likely to have depauperate parasite communities. Parasites are as vulnerable to extinction as their hosts and indeed even more so, given transmission is unlikely to succeed below a certain density of viable hosts. Given the current failure to explore parasite communities in endangered mussels (Chapter 2), many parasites may already have gone extinct without our knowledge. An emphasis on parasite conservation is beginning

to gain traction (Lagrue 2017; Carlson et al. 2020a); especially given the potential diversity of ecosystem-level effects of mussel parasites, attention should be paid to their specificity and relationship with host diversity.

10.4. Future directions

The aims of this thesis have broadly been met, in terms of characterising knowledge to date on freshwater mussel parasite communities, analysing the drivers of parasite community structure, and exploring the effects of parasites across larger scales. However, this thesis has also raised many interesting questions that remain unanswered. Here, I highlight some of the most pressing. In the next paragraph I briefly reiterate key points that have arisen multiple times in this thesis, before developing three other points that are important going forward but have been given less consideration to date.

It should come as no surprise, particularly from Chapters 2 and 9, that further work needs to be done characterising the parasites of freshwater mussels. Even well-studied host-parasite networks are predicted to be missing a large proportion of links (Dallas et al. 2017b), and unionid parasite communities are certainly not well studied. Particular emphasis should go on microparasites (e.g. bacteria, viruses) and their interaction with macroparasites: such associations are highly influential in vertebrates (e.g. Graham 2008; Wuerthner et al. 2017; Clerc et al. 2019) and may also be important in this system. Once parasites have been identified, care should be taken to understand their life-history traits, as these influence their distribution, interactions with other parasites, and effects on hosts. Further, parasite abundance, and how it relates to parasite prevalence, should be incorporated wherever possible into community analyses (Brian & Aldridge 2021c). Many parasites vary temporally or spatially in abundance while showing little variation in prevalence (Podani et al. 2013), and their effects may also be dependent on their intensity inside individual hosts (Chapter 5). Together, parasite diversity, abundance and life-history represent foundational aspects of freshwater mussel parasite fauna that remain to be sufficiently described.

An important aspect of parasite community assembly is the role of propagule pressure, which determines the likelihood of a parasite reaching a certain site, as well reaching a host within that site, and the severity of infection (Chapters 5, 6; Catford et al. 2009; Stewart Merrill et al. 2021). In this thesis, ‘propagule pressure’ has been treated rather ambiguously, and has

been invoked as the causal mechanism for site-specific differences as well as the differences between sampling months. In reality, propagule pressure at these scales relies on several other factors that require future consideration. One is the role of environmental conditions, such as temperature, dissolved nutrients or pH, which can alter how well a parasite survives at a site (Bolnick et al. 2020b), in addition to how well it reproduces there (Choo & Taskinen 2015). While it is clear that the sites in this thesis differ in terms of suitability, and thus the likelihood of hosts within these sites being exposed to infective propagules also differs, it is not exactly clear *why* these sites differ. Integrating environmental conditions into studies of freshwater mussel parasites will help explain observed distributions, as well as aid in predicting parasitism in previously unexplored sites. A second piece of the propagule pressure puzzle that has been neglected is the role of other host species in parasite life cycles. Many freshwater mussel parasites require other hosts (e.g. chironomids for mites, fish for trematodes; Fig. 1.2), which are more mobile than mussels. Given the distribution of parasites is often determined by their most mobile host (Prugnolle et al. 2005; Paterson et al. 2019), propagule pressure will depend as much on the distribution and abundance of these other hosts as it will the distribution of mussels. Incorporating other hosts into unionid-parasite distribution studies is an important and interesting next step in understanding the community ecology of parasites.

Freshwater mussel immune systems, and how they may cope with parasites, has direct relevance for the diversity-disease relationship, as well as more generally understanding how mussels handle parasitism (Chapter 9). Invertebrates such as snails (Mitta et al. 2005) and oysters (Munoz et al. 2006) have previously shown an effective immune response to a parasite challenge, and trematodes can also disrupt immune cell-signaling in snails, suggesting well-developed Red Queen dynamics (Walker 2006). Further, environmental variation can disrupt immune processes and lead to disease outbreaks in bivalves (Mathai et al. 2020). It is likely that freshwater mussels can also mount an innate immune response, though this has been unexplored to date. Further work should study the capacity of freshwater mussels to mount an effective immune response, and how this alters infection prevalence across populations and communities.

Finally, the role of invasive bivalves requires more consideration. This has been touched on throughout the thesis, but not well-developed, because there is little capacity for spillover or spillback from *Dreissena polymorpha* (Chapter 2), the only invasive bivalve in the sites

studied, and it did not appear to play a major role in influencing parasite structure in native unionids (Chapter 5). However, its potential role at a site level (Chapter 7) makes it worthy of future consideration. In addition, other invasive freshwater bivalves, such as *Sinanodonta woodiana* which is rapidly spreading across Europe and outcompeting natives (Urbańska et al. 2019, 2021), is competent for native parasites and could amplify parasite loads (Chapter 2; Cichy et al. 2016). Other rapidly spreading invaders such as *Corbicula* sp. (Caffrey et al. 2016; Karaouzas et al. 2020), which remarkably has no parasite records at all from Europe and North America (Chapter 2, Tables A1.2 – A1.4), could potentially act as effective diluters of native parasites. The contribution of invasive bivalves to native parasite communities requires further disentangling, a point that becomes increasingly pertinent as they continue to spread.

10.5. Conclusion

Approaching freshwater mussel parasites from both parasite-centric and host-centric perspectives has shed light on how parasite communities are assembled in this system, and what the effects of parasites on freshwater mussels and the wider ecosystem may be. However, the two viewpoints are not independent: the conservation impacts of parasites are determined by parasite abundance, distribution and life-history characteristics, which in turn rely partly on the diversity, distribution and density of their hosts. The two key concepts in the title of this thesis, ‘community ecology’ and ‘conservation’ therefore apply equally to host and parasite. Taking such an integrated view of host-parasite interactions across scales will enhance the study of freshwater mussel ecology, as well as increase knowledge on the role of parasites in global ecosystems.

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Appendix A1: Appendices for Chapter 2

Part 1: Summary Tables

Table A1.1: Literature encountered in the searches that were not included in the final analysed database. Note that the intention is to increase transparency by providing evidence of studies that were excluded (see Methods section of Chapter 2), and is not intended as a comprehensive record of non-English records or theses on the topic.

Study	Notes/reason for exclusion
Antipa GA, Small EB (1971) A redescription of *Conchophthirus curtus* Engelmann, 1862 (Protozoa, Ciliatea). *J. Protozool*., 18, 491-503	Could not be accessed
Aristanov E (1986) Parasite fauna of the molluscs of the southern Aral Sea. In: Biological Resources of Aral, 155-168.	Non-English language
Aristanov E (1992) Role of *Dreissena polymorpha* Pallas in the life cycle of *Bucephalus polymorphus* Baer 1827. *Uzbekskii Biologicheskii Zhurnal*, 2, 75-76	Non-English language
Bates JM, van der Schalie H (1970) Ohio mussel fisheries investigation May 15, 1967 September 1, 1970, final report - part I mussel studies. Eastern Michigan University, Center for Aquatic Biology, Michigan	Report, not clear it has been peer-reviewed
Breitig G (1965) Beitrage zur Biologie, Verbreitung und Bekampfung von Dreissena polymorpha (Pall.) 1771 (Lamellibranchia). Master's thesis, Universitat Griefswald	Thesis; non-English language
Beedham GE (1965) A chironomid (Dipt.) larva associated with the lamellibranchiate mollusc, *Anodonta cygnea* L. *Entom. Mon. Mag*., 101, 142-143	Could not be accessed
Beedham GE (1971) The extrapallial cavity in *Anodonta cygnea* (L.) inhabited by an insect larva. *J. Conchol*., 26, 380-385	Could not be accessed
Benz GW, Curran S (1997) Results of an ongoing survey of metazoan symbionts of freshwater mussels (Unionidae) from Kentucky Lake, Tennessee. In: Proceedings of the Seventh Symposium on the Natural History of the Lower Tennessee and Cumberland River Valleys, pp. 39–66 (Scott AF, Hamilton SW, Chester EW, White DS, eds.). Clarksville, TN: Center for Field Biology, Austin Peay University	Conference proceeding
Bouttger K (1972) Biological and Ecological Studies on the Life Cycle of Freshwater-mites II. The Life Cycle of *Limnesia maculata* and *Unionicola crassipes*. *Internationale Revue der gesamten Hydrobiologie und Hydrographie*, 57, 263-319	Non-English language
Boyles JL (2004) An evaluation of adult freshwater mussels held in captivity at the White Sulphur Springs National Fish Hatchery, West Virginia. Master's thesis, Virginia Tech.	Thesis. Reports heavy digenean infection in *A. ligamentina*
Cable RM, Peters LE (1986) The cercaria of *Allocreadium ictaluri* Pearse (Digenea: Allocreadiidae). *The Journal of Parasitology*, 72, 369-371	*Allocreadium ictaluri* in *Lampsilis* sp., but only experimental infection
Calnan TR (1976) The systematics and distribution of metazoan parasites in the Unionidae from Navasota River, Texas. Master's thesis, Texas A and M University	Thesis
Chatton E (1949) Recherches sur les ciliés thigmotriches. I. *Arch Zool Exp Gén*, 86, 169-253	Non-English language

Chernogorenko MI, Boshko EG (1992) Parasite fauna of aquatic organisms of the Dnestr and Dnestr Liman. In: Hydrobiological Condition of the Dnestr and Its Reservoirs (Nesluzhenko VE, ed.). Kiev: Naukova Dunka Publishers, 321-329	Non-English language
Chernomaz TV (2001) Ciliary activity of cells of gill and leg glimmeral epithelium of Unionidae invaded by trematodes of *Aspidogaster conchicola* and *Bucephalus polymorphus*. *Parazitologiya*, 35, 443-448	Non-English language. Does have English abstract, but not clear which species are infested with which parasites
Ciapka P, Cichy A, Zbikowska E (2016) Preliminary studies on the occurrence of digenean trematodes in molluscs from the Konin lakes. *Folia Malacologica*, 24	Conference abstract
Coker RE, Shira AF, Clark HW, Howard AD (1921) Natural history and propagation of fresh-water mussels. US Government Printing Office	Government report, not peer-reviewed
Curry MG, Vidrine MF (1978) Two new freshwater clam hosts for the leach [sic] *Placobdella montifer* Moore and hypotheses for their association (abstract). *Proc. Louisiana Acad. Sci*., 41, 144	Conference abstract
Danford DW (1983) A survey of the aspidogastrean and hydracarine parasites of bivalue molluscs in Western West Virginia. Master's thesis, Marshall University	Thesis. Records are covered by Danford and Joy (1984) (see Table A1.2)
de Kinkelin P, Tuffery G, Leynaud G, Arrignon J (1968) Étude épizootiologique de la Bucéphalose larvaire a Bucephalus polymorphus, (Baer 1827) dans le peuplement piscicole du bassin de la Seine. *Rech. Vet.*, 1, 77-98	Non-English language
Dennis SD (1970) Pennsylvania mussel studies (June 1, 1968 - October 1, 1969). Center Aquat. Biol., East. Mich., Ypsilanti, Mich. 138pp	Report, not peer-reviewed
Dimock RV (1979) Behavioral ecology of the symbiotic water mite *Unionicola formosa*. *American Zoologist*, 19, 886	Conference abstract
Dobson R (1966) A survey of the parasitic Unionicolidae (Arachnida: Acarina) of the Apalachicolan faunal region of the southern United States. Master's thesis, Florida State University	Thesis
Dollfus RP (1958) Cours D' Helminthologie I. Trematodes, sous-classe Aspidogastrea. *Annales de Parasitologie Humaine et Comparée*, 33, 305-395	Non-English language
Dollfus RP (1959) Addenda à "Cours D'Helminthologie I. Trématodes, sous-classes Aspidogastrea". *Annales de Parasitologie Humaine et Comparée*, 33, 623-624	Non-English language
Downes BJ (1988). Coexistence in harlequin habitats: the organization of mite guilds inhabiting freshwater mussels. Ph.D. dissertation, Florida State University	Thesis
Downes BJ (1989) Host specificity, host location and dispersal: Experimental conclusions from freshwater mites (*Unionicola* spp.) parasitizing unionid mussels. *Parasitology*, 98, 189-196	Tests mites in lab experiments but no information regarding which species they were collected from
Downing JA (1999) Relationship between habitat characteristics and the extinction of lake mussels in Minnesota. A Final Report to the Minnesota Department of Natural Resources, FEt, (42-600), 4224	Government report, not peer-reviewed

Edwards DD (1993) Host specificity and reproductive isolation: Experimental evidence from the symbiotic water mite *Unionicola formosa.* Ph.D. dissertation, Wake Forest University	Thesis
Edwards DD, Vidrine MF (2006) Host specificity among *Unionicola* spp. (Acari: Unionicolidae) parasitizing freshwater mussels. *Journal of Parasitology*, 92, 977-983	Utilises the large database of Vidrine (1996) (this table); does not provide any novel records
Edwards DD, Vidrine MF (2013) Patterns of species richness among assemblages of Unionicola spp. (Acari: Unionicolidae) inhabiting freshwater mussels (Bivalvia: Unionoida) of North America. *Journal of Parasitology*, 99, 212-217	Utilises the large database of Vidrine (1996) (this table); does not provide any novel records
Fredericksen DW (1973) Biology of aspidobothrian trematodes. Ph.D. dissertation, Iowa State University	Thesis. Contains records of aspidogastrean trematodes
Fuller SLH (1974) Clams and mussels (Mollusca: Bivalvia). In: Pollution ecology of freshwater invertebrates (Hart CW, Fuller SLH, eds.), New York: Academic Press, 215-273	Found leeches (*Placobdella parasitica, P. montifera)* inside mussels but not specify what species
Golikova MN (1960) Ecological-parasitological study of the biocoenosis of some lakes in the Kaliningrad district. III. Parasitofauna of fish. *Vestn. Leningr. Univ. Biol*, 15, 110-121	Non-English language
Grizzle JM, Brunner CJ (2007) Assessment of current information available for detection, sampling, necropsy, and diagnosis of diseased mussels. Prepared for the Alabama Department of Conservation and Natural Resources Wildlife and Freshwater Fisheries Division, Montgomery, Alabama	Non-peer reviewed report, also functionally the same as Grizzle and Brunner (2009)
Hevers VJ (1980) Biologisch-okologische Untersuchungen zum Entwicklungszyklus der in Deutschland auftretenden *Unionicola*-Arten (Hydrachnellae, Acari). *Archiv fuir Hydrobiologie Supplement* 57, 324-373	Non-English language
Jenkinson JJ, Ahlstedt SA (1987) Mussel die-offs downstream from Pickwick Landing Dam, Tennessee River, 1985 and 1986. In: Proceedings of the Workshop on Die-Offs of Freshwater Mussels in the United States, pp. 29-38 (Neves RJ, ed.). U.S. Fish and Wildlife Service and Upper Mississippi River Conservation Committee	Workshop proceeding
Kelly HM (1926) A new host for the aspidogastrid trematode, *Cotylogaster occidentalis*. *The Proceedings of the Iowa Academy of Science*, 33, 339	Could not be accessed
Kidder GW (1934) Studies on the ciliates from fresh water mussels. II. The nuclei of *Conchophthirus anodontae* Stein, *C. curtus* Engl., and *C. magna* Kidder, during binary fission. *Biol. Bull*., 66, 286-303	Not original sampling, uses the same material described in Kidder 1934 (see Table A1.2)
Koubek P (1977) Occurrence of the trematode *Aspidogaster conchicola* Baer, 1827 and cercariae of *Bucephalus polymorphus* Baer, 1827 in our mussels (Czechoslovakia). *Folia Facultatis Scientiarum Naturalium Universitatis Purkynianae Brunensis (Helminthologicky Sbornik V),* 18, 47-53.	Only abstract in English; not clear it has been peer-reviewed
Kulczycka A (1939) Contributions to the study of larval trematode forms in the lamellibranchs near Warsaw. *C.R. Seances Soc. Sci. Lett. Varsovie Class IV*, 32, 80-82	Non-English language

Kupriianova-Shakhmatova RA (1965) Trematode fauna of molluscs in reservoirs of central Povolzh'e. *Rabot Gel'mint*, 40, 111-114	Non-English language
Liberty AJ (2004) An evaluation of the survival and growth of juvenile and adult freshwater mussels at the Aquatic Wildlife Conservation Center (AWCC), Marion, Virginia. Master's thesis, Virginia Tech	Thesis
Lyakhnovich VP, Karatayev AY, Antsipovich NN (1983) The effect of water temperature on the rate of infection of *Dreissena polymorpha* with larvae of *Phyllodistomum folium* Olfers in Lake Lukoml'skoe. *Biologiya Vnuttrennikh Vod Informatsionnyi Byulleten*, 58, 35-38	Non-English language
McDaniel JS, McDaniel SJ (1972) *Cotylaspis insignis* Leidy, 1857, from North Carolina mollusks. *Journal of the Elisha Mitchell Scientific Society*, 88, 205	Conference proceeding
Mellors PJ, Owen RW (1980) Some observations on the biology of a gasterostome digenetic fluke *Rhipidocotyle campanula* of fish in the River Aire, Yorkshire (Proceedings for the British Society for Parasitology Leeds, England). *Parasitology*, 81, XLVIII	Conference proceeding
Minyuk MY (2001) Aspidogasters – The parasites of unionids in the Zhitomir Polesye. *Parazitologiya*, 35, 552-555	Non-English language. Does have English abstract, but unclear what host species they refer to
Mitchell RD, Pitchford W (1953) On mites parasitizing *Anodonta* in England. *Journal of Conchology*, 23, 365-370	Could not be accessed
Monticelli FS (1892) *Cotylogaster michaelis* n. g., n. sp. e revisione degli Aspidobothridae. In: Taschenberg EOW (ed.), *Festschrift zum Siebenzigsten Geburtstage Rudolf Leuckarts dem Verchrten Jubilar Dargebracht von Seinen Dankbaren Schülern, & c. Leipzig*, 168-214	Non-English language
Moteka EN (2015) Shell morphology of the unionid mussels (*Anodonta anatina*, *Unio pictorum* and *U. tumidus*) in relation to gender and trematode parasitism. Master's thesis, University of Jyvaskyla	Thesis
Murray HD, Leonard AB (1962) Handbook of unionid mussels in Kansas (No. 594.141 MUR)	Government report, not peer-reviewed
Paterson CG, Macleod RK (1979) Observations of the life history of the water mite, *Unionicola formosa* (Acari: Hydrachnellae), *Canadian Journal of Zoology*, 57, 2047-2049	Laboratory study
Pavljuchenko OV (2006) *Aspidogaster conchicola* (Plathelminthes, Aspidogastrea) in unionid mussels (Mollusca, Bivalvia, Unionidae) of Ukraine. *Vestnik Zoologii*, 40, 333-340	Non-English language. Does have English abstract, but unclear what host species they refer to
Pekkarinen M (1993) Reproduction and condition of unionid mussels in the Vantaa River, South Finland. *Archiv für Hydrobiologie*, 127, 357-375	Could not be accessed
Proctor HC (1992) Mating and spermatophore morphology of water mites (Acari: Parasitengona). *Zoological journal of the Linnean Society*, 106, 341-384	Collected mites but does not specify from which species
Prosser RS, Lynn DH, Salerno J, Bennett J, Gillis PL (2018) The facultatively parasitic ciliated protozoan, *Tetrahymena glochidiophila* (Lynn, 2018), causes a reduction in viability of freshwater mussel glochidia. *Journal of invertebrate pathology*, 157, 25-31	Only experimental infection (not natural)

Raabe Z (1950) Recherches sur les ciliés Thigmotriches (Thigmotricha Ch. Lw.). V. Ciliés Thigmotriches du lac Balaton (Hongrie). *Ann. Univ. Mariae Curie-Sklodowska Sect. C Biol.*, 5, 197-215	Non-English language
Richardson SD (1990) Studies on the life-cycle of the digenetic trematode *Rhipidocotyle campanula* (Dujardin, 1845) (Gasterostomata: bucephalidae) with particular reference to the larval stages. Ph.D. dissertation, University of Leeds	Thesis
Roberts EA (1977) The behavior and ecology of the symbiotic water mite *Unionicola formosa*: I. Phototaxis II. Population structure. Ph.D. dissertation, Wake Forest University	Thesis
Robinson JL, Wetzel MJ, Tiemann JS (2017) Some Phoretic Associations of Macroinvertebrates on Transplanted Federally Endangered Freshwater Mussels. *Northeastern Naturalist*, 24, N29-N34	Reports a non-random selection of macroinvertebrates from the outside of mussel shells (i.e. not endosymbionts)
Seitner PG (1951) The life history of *Allocreadium ictaluri* Pearse, 1924 (Trematoda: Digenea). *J. Parasitol*., 37, 223-244	Describes metacercariae of the trematode *Allocreadium ictaluri* from unionids in Indiana, but provides no further details on species
Scholla MH, Hinman ML, Klaine SJ, Condor J (1987) Evaluation of a mussel die-off in the Tennessee River, Tennessee In: Proceedings of the Workshop on Die-Offs of Freshwater Mussels in the United States, pp. 144-151 (Neves RJ, ed.). U.S. Fish and Wildlife Service and Upper Mississippi River Conservation Committee	Workshop proceeding
Sinitsin DT (1901) An observation on the history of development of *Distomum folium* Olf. *Zoologischer Anzeiger*, 24, 689-694	Non-English language
Skryabin KI (1952) Trematodes of the subclass Aspidogastrea Faust et Tang, 1936. In: Skryabin KI (ed.), *Trematodes of Animals and Man. Osnovy Tremadodologii*, 5-149	Non-English language
Smirnova VA, Ibrasheva SI (1967) Larval trematodes from freshwater molluscs in the western Kazakhstan. *Trudy Instituta Zoologii Akademii Nauk Kazakhstoi SSR*, 27, 53-87	Non-English language
Stadnichenko AP, Anistratenko VV, Grabinskaya OV, Martynyuk OV, Miroshnichenko OA, Oleynik NG, Sergeychuk SA, Fasola OI (1994). The infection of unionid mussels (Mollusca: Bivalvia: Unionidae) with parthenites *Bucephalus polymorphus* (Trematoda) and effect of the parasites on the host heart activity. *Parazitologiya (St. Petersburg)*, 28, 124-130	Non-English language
Stalstedt J, Bergsten J, Ronquist F (2013) "Forms" of water mites (Acari: Hydrachnidia): intraspecific variation or valid species?. *Ecology and Evolution*, 3, 3415-3435	Collected mites free-swimming in the water column
Starliper CE (2001) The effect of depuration on transmission of *Aeromonas salmonicida* between the freshwater bivalve *Amblema plicata* and Arctic char. *J. Aquat. Anim. Health*, 13, 56-62	Only experimental infection (not natural)
Starliper CE (2005) Quarantine of *Aeromonas salmonicida*-harboring ebonyshell mussels (*Fusconaia ebena*) prevents transmission of the pathogen to brook trout (*Salvelinus fontinalis). J. Shellfish Res*., 24, 573-578	Only experimental infection (not natural)

Starliper CE (2008) Recovery of a fish pathogenic bacterium, *Aeromonas salmonicida*, from ebonyshell mussels *Fusconaia ebena* using nondestructive sample collection procedures. *Journal of Shellfish Research*, 27, 775-782	Only experimental infection (not natural)
Starliper CE, Villella R, Morrison P, Mathias J (1998) Studies on the bacterial flora of native freshwater bivalves from the Ohio River. *Biomedical Letters*, 58, 85-95	Could not be accessed
Starliper CE, Morrison P (2000) Bacterial pathogen contagion studies among freshwater bivalves and salmonid fishes. *J. Shellfish Res.*, 19, 251-258	Does not list host species of isolated bacteria
Starliper CE, Powell J, Garner J, Henley W (2007). An investigation of seasonal mussel dieoffs in the Tennessee River, Muscle Shoals, AL (Abstract), poster PO 38. In: Freshwater Mollusk Conservation Society Symposium, Little Rock, AK	Conference abstract
Taskinen J (1992) On the ecology of two *Rhipidocotyle* species (Digenea: Bucephalidae) from Finnish lakes. Ph.D. dissertation, University of Jyvaskyla	Thesis
Thiel PA (1987) Recent events in the mussel mortality problem on the upper Mississippi River. In: Proceedings of the Workshop on Die-Offs of Freshwater Mussels in the United States, pp. 66-75 (Neves RJ, ed.). U.S. Fish and Wildlife Service and Upper Mississippi River Conservation Committee	Workshop proceeding
Tuffery G (1978) Research on bucephalopsis due to B. polymorphus. *Bulletin de l'Acade'mie Veterinaire de France*, 51, 143-145	Non-English language
Tuzovskij PV, Semenchenko KA (2015) Water mites of the genus *Unionicola* Haldeman, 1842 (Acari, Hydrachnidia, Unionicolidae) in Russia. *Zootaxa*, 3919, 401-456	Collected mites free-swimming in the water column
Vidrine MF (1977) New host records for two water mites (Acarina: Unionicolidae) (Abstract). *Association of South Eastern Biologists Bulletin*, 24, 92	Conference abstract
Vidrine MF (1979) *Unionicola* (*Pentatax*) *fossulata* (Koenike, 1895) (Arthropoda: Acarina: Unionicolidae) in Eastern North American fresh-water mussels (Mollusca: Bivalvia: Unionacea: Unionidae: Lampsilinae: Lampsilini) (Abstract). *Proc. La. Acad. Sci*. 42, 84	Conference abstract
Vidrine MF (1979). Water-mite (Arthropoda: Unionicolidae) parasites of ambleminine freshwater mussels (Mollusca: Unionacea) in North America (Abstract). *Association of South Eastern Biologists Bulletin*, 26, 82	Conference abstract
Vidrine MF (1980) Systematics and coevolution of unionicolid water mites and their unionid mussel hosts in Eastern United States. Ph.D. dissertation, Louisiana State University	Thesis
Vidrine MF (1996) North American Najadicola and Unionicola: Systematics and coevolution. Gail Q. Vidrine Collectibles, Eunice, Louisiana, 146pp	Consists of a record of inspections of 22,231 mussels of 212 different species. Currently out of print. Significant overlap with the lead-author publications of MF Vidrine (Table A1.2)

Vidrine MF, Bereza DJ (1978) Some considerations and implications of host-specificity studies of unionicolid mite parasites on the systematics of some groups of North American unionacean fresh-water mussels. *Bull. Am. Malacol. Union, Inc. for 1977*, 85-86	Conference proceeding
Vidrine MF, Bereza DJ (1980). South American parasitic mite genus *Atacella* (Arthropoda: Acari: Unionicolidae) in North American freshwater mussels (Bivalvia: Unionoida: Unionacea and Mutelacea). *Bull. Am. Malacol. Union for 1979*, 50-52	Conference proceeding
Viets K (1956) Die Milben des Susswassers und des Meeres. Zweitter und Dritter Teil. Gustav Fischer Verlag, Jena. 870pp	Non-English language
Wallet M, Lambert A (1986) Enquête sur la répartition et l'évolution du parasitisme a Bucephalus polymorphus BAER, 1827 chez le mollusque Dreissena polymorpha dans le sud-est de la France. *Bull. Fr. Peche. Piscic*, 300, 19-24	Non-English language
Wasielewski L, Drozdowski A (1995) Anomalies of pericardial epithelium structure in *Anodonta cellensis* Schröter, 1779 (Bivalvia, Eulamellibranchia, Unionidae) caused by activity of *Aspidogaster conchicola* Baer, 1826 (Aspidogastrea). *Acta Universitatis Nicolai Copernici Biologia*, 49, 33-43	Non-English language
Wilson CB, Clark HW (1912a) The mussel fauna of the Maumee River. Bureau Fisheries Document No 757, Washington, 72 pp.	Government report, not peer-reviewed
Wilson CB, Clark HW (1912b) The mussel fauna of the Kankakee Basin. Bureau Fisheries Document No 758, Washington, 52 pp	Government report, not peer-reviewed
Wolcott RH (1898) On the North American species of the genus *Atax* (Fabr.) Bruz. *Transactions of the American Microscopical Society*, 20, 193-259	Lists lots of mites (*Atax*) but not clear what mussel genera he isolated them from
Woodhead AE (1931) The germ cell cycle in the trematode family Bucephalidae. *Transactions of the American Microscopical Society*, 50, 169-188.	Examines the same material as Woodhead 1929, 1930 (Table A1.2)
Yanovich LN, Stadnichenko AP (1997) Molluscs of the family Unionidae from the Central Polessye as intermediate hosts of the trematodes. *Parazitologiya (St. Petersburg)*, 31, 314-320	Non-English language. Does have an English summary, but not clear which parasites were found in which species
Yuryshynets VI (1999) Some aspects of interaction of *Unio tumidus* and *Unio pictorum* (Bivalvia, Unionidae) populations with their parasites and commensals. *Hydrobiological Journal*, 35, 119-122	Could not be accessed
Zdun VI (1965) Trematode larvae parasitizing dreissenids in the lower Danube. In: The Conference on Dreissenid Biology and the Protection of Hydroconstructions from Dreissena Growth, 14-15	Conference proceeding; non-English language
Zieritz A (2010) Variability, function and phylogenetic significance of unionoid shell characters. Ph.D. dissertation, University of Cambridge	Thesis

Table A1.2: Summary of all North American host-endosymbiont records reviewed in the study. Location is given to the scale of state (USA) or province (Canada) if samples from one or two states/provinces were taken, or is given as 'USA' or 'Canada' if sampling involved >3 states/provinces. Records are initially listed by host, arranged alphabetically. Within each host, parasites are arranged according to the following order (see 'Category' column): aspidogastrean trematodes [asp]; digenean trematodes [dig]; mites [mit]; ciliates [cil]; chironomids [chi]; nematodes [nem]; oligochaetes [oli]; leeches [lee]; protists [pro]; amoebae [amo]; fungi [fun]; bacteria [bac], other [oth]. Records are listed alphabetically by parasite within each of those groups, and then sequentially by year for each specific parasite.

Species	Parasite	Category	Location	Reference
Actinonaias ligamentina	*Aspidogaster conchicola*[a]	asp	Ohio	Huehner and Etges 1981
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985[b]
	Cotylaspis insignis[a]	asp	Illinois	Kelly 1899
		asp	New York	Osborn 1905
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis[a]	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Bucephalus sp.[c]	dig	Illinois	Kelly 1899
	Bucephalidae[c]	dig	Kentucky	Moles and Layzer 2008
	Atax sp.[d]	mit	Illinois	Kelly 1899
	Najadicola ingens	mit	Missouri	Utterback 1916
	Unionicola abnormipes	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola bakeri	mit	Arkansas	Vidrine 1986b
		mit	Arkansas	Vidrine and Clark 1993
	Unionicola clarki	mit	Arkansas	Vidrine and Clark 1993
	Unionicola fossulata	mit	Ontario	Vidrine 1986b
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
		mit	Arkansas	Vidrine and Clark 1993
	Conchophthirus curtus	mit	Illinois	Antipa and Small 1971
	Conchophthirus sp.	mit	Illinois	Kelly 1899
	Ablabesmyia sp.[e]	chi	Tennessee	Roback et al. 1979
Actinonaias pectorosa	*Unionicola abnormipes*	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991

Alasmidonta heterodon	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
	Unionicola arcuata	mit	New Hampshire	Vidrine 1986a
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Alasmidonta marginata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cercaria micromyae[f]	dig	Michigan	Fischthal 1951
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola arcuata	mit	Arkansas	Vidrine 1986a
		mit	Arkansas	Vidrine and Clark 1993
	Unionicola bishopi	mit	Arkansas	Vidrine 1986a
		mit	Arkansas	Vidrine and Clark 1993
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Alasmidonta triangulata	*Unionicola formosa*	mit	North Carolina	Vidrine 1986a
Alasmidonta undulata	Bucephalidae[h]	dig	USA	Kat 1983
	Najadicola ingens	mit	New York	Baker 1982
	Unionicola arcuata	mit	New York	Baker 1982
		mit	USA, Canada	Vidrine 1986a
	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
Alasmidonta varicosa	*Cercaria tiogae*[f]	dig	New York	Fischthal 1954
	Unionicola arcuata	mit	USA	Vidrine 1986a
Alasmidonta viridis	*Cercaria honeyi*[f]	dig	Michigan	Fischthal 1951
	Unionicola arcuata	mit	USA, Canada	Vidrine 1986a
Amblema neislerii	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
		asp	Florida	Hendrix and Short 1965
Amblema plicata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Michigan	Stunkard 1917
		asp	Illinois	van Cleave and Williams 1943
		asp	Texas	Gentner and Hopkins 1966
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Texas	Gentner 1971
		asp	Texas	Flook and Ubelaker 1972
		asp	Oklahoma	Nelson et al. 1975

		asp	Louisiana	Vidrine and Causey 1975
		asp	Wisconsin	Williams 1978
		asp	West Virginia	Danford and Joy 1984
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
		asp	Manitoba	Carney 2015
	Cotylaspis insignis	asp	Ohio	Stromberg 1970
		asp	Texas	Gentner 1971
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Manitoba	Carney 2015
	Cercaria eriensis[f, g]	dig	Ohio	Coil 1954b
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Rhopalocercous gorgoderid[h]	dig	Texas	Flook and Ubelaker 1972
	"Sterilising trematodes"	dig	Alabama, Mississippi	Haag and Leann Staton 2003
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola amandita	mit	Tennessee	Mitchell and Lester 1965
		mit	Texas	Vidrine 1990
		mit	Tennessee	Vidrine and Wilson 1991
		mit	Arkansas	Vidrine and Clark 1993
		mit	Kentucky	Edwards et al. 2010
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola tupara	mit	Tennessee	Vidrine and Wilson 1991
		mit	Kentucky	Edwards et al. 2010
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
Anodonta californiensis	*Aspidogaster conchicola*	asp	Washington	Pauley and Becker 1968
		asp	USA	Hendrix et al. 1985
	Unionicola formosa	mit	Ontario	Mitchell 1957
	Conchophthirus anodontae	cil	Massachusetts	Kidder 1934
	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
Anodonta kennerlyi	*Unionicola conroyi*	mit	British Columbia	Vidrine 1986b

	Unionicola wolcotti	mit	British Columbia	Vidrine 1986c
Anodonta nuttalliana	*Unionicola conroyi*	mit	USA, Canada	Vidrine 1986b
	Unionicola wolcotti	mit	British Columbia	Vidrine 1986c
Anodonta oregonensis	*Aspidogaster conchicola*	asp	Washington	Pauley and Becker 1968
		asp	USA	Hendrix et al. 1985
	Unionicola conroyi	mit	Washington	Vidrine 1986b
Anodonta sp.	*Platyaspis anodontae*[i]	asp	New York	Osborn 1898
	Cotylaspis insignis	asp	Oklahoma	Fulhage 1954
Anodontoides ferussacianus	*Cotylaspis insignis*	asp	Michigan	Stunkard 1917
		asp	USA	Hendrix et al. 1985
	Cercaria honeyi[f]	dig	Michigan	Fischthal 1951
	Unionicola arcuata	mit	USA, Canada	Vidrine 1986a
	Unionicola dimocki	mit	Ontario	Vidrine 1986a
	Unionicola wolcotti	mit	Ohio	Vidrine 1986c
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
Anodontoides radiatus	*Unionicola arcuata*	mit	Alabama	Vidrine 1986a
		mit	Louisiana	Edwards et al. 2010
	Unionicola ernstingi	mit	Louisiana	Edwards et al. 2008
		mit	Louisiana	Ernsting et al. 2008
		mit	Louisiana	Edwards et al. 2010
Arcidens confragosus	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
	Bucephalus sp.	dig	Illinois	Kelly 1899
	"Sterilising trematodes"	dig	Alabama	Haggerty et al. 2011
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola belli	mit	USA	Vidrine 1986c
	Unionicola dimocki	mit	Kentucky	Vidrine 1986a
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Arcidens wheeleri	*Unionicola belli*	mit	USA	Vidrine 1986c
	Unionicola dimocki	mit	Oklahoma	Vidrine 1986a
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
Cyclonaias asperata	"Sterilising trematodes"	dig	Alabama, Mississippi	Haag and Leann Staton 2003
Cyclonaias houstonensis	*Aspidogaster conchicola*	asp	Texas	Gentner and Hopkins 1966
	Cotylaspis insignis	asp	Texas	Gentner and Hopkins 1966

		asp	USA	Hendrix et al. 1985
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
Cyclonaias infucata	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
		asp	Florida	Hendrix and Short 1965
	Lophotaspis interiora	asp	USA	Hendrix et al. 1985
Cyclonaias nodulata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
	Atax sp.	mit	Illinois	Kelly 1899
Cyclonaias pustulosa	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Illinois	van Cleave and Williams 1943
		asp	Texas	Gentner and Hopkins 1966
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Wisconsin	Williams 1978
		asp	West Virginia	Danford and Joy 1984
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Tennessee	Olson et al 2003
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Stunkard 1917
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	“Sterilising trematodes”	dig	Alabama, Mississippi	Haag and Leann Staton 2003

		dig	Oklahoma	Galbraith and Vaughn 2011
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola causeyae	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola vikitra	mit	Tennessee	Mitchell and Lester 1965
		mit	Texas	Vidrine 1990
		mit	Kentucky	Edwards et al. 2010
	Unionicola vikitrella	mit	USA	Vidrine 1987
		mit	Louisiana	Vidrine and Borsari 1994
	Ablabesmyia janta[e]	chi	Texas	Roback 1982
		chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Cyclonaias succissa	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Lophotaspis interiora[a]	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
Cyclonaias tuberculata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola abnormipes	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola causeyae	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola fossulata	mit	Michigan	Welsh 1931
	Unionicola sakantaka	mit	Tennessee	Mitchell and Lester 1965
		mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola vamana	mit	Tennessee	Vidrine and Wilson 1991
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Cyprogenia aberti	*Unionicola serrata*	mit	Arkansas	Vidrine and Clark 1993
Cyrtonaias tampicoensis	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985

	Cotylaspis insignis	asp	Texas	Gentner and Hopkins 1966
		asp	USA	Hendrix et al. 1985
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Phyllodistomum sp.[c]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Unionicola calani	mit	USA, Mexico	Vidrine 1986b
Disconaias fimbriata	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
Ellipsaria lineolata	*Aspidogaster conchicola*	asp	Tennessee	Hendrix 1968
		asp	USA	Hendrix et al. 1985
	Conchophthirus sp.	asp	Illinois	Kelly 1899
Elliptio arca	"Sterilising trematodes"	dig	Alabama, Mississippi	Haag and Leann Staton 2003
Elliptio arctata	*Aspidogaster conchicola*	asp	Alabama	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Alabama, Georgia	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Lophotaspis interiora	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
	Unionicola alleni	mit	USA	Vidrine 1987
Elliptio complanata	*Aspidogaster conchicola*	asp	?	Leidy 1851
		asp	Illinois	Kelly 1899
		asp	?	Leidy 1904
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Ontario	Ip et al. 1982
		asp	Ontario	Ip and Desser 1984a
		asp	Ontario	Ip and Desser 1984b
		asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Homalometron armatum[g]	dig	North Carolina	Chittick et al. 2001
	Homalometron armatum[g]	dig	North Carolina	Gustafson et al. 2005
	Atax sp.	mit	Illinois	Kelly 1899
	Najadicola ingens	mit	USA, Canada	Humes and Jamnback 1950
		mit	New Hampshire	Humes and Russell 1951
	Unionicola sp.	mit	New York	Jones and Baker 1984
	Conchopthirus anodontae	cil	Massachusetts	Kidder 1934

	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
		cil	USA [?]	Beers 1962
		cil	USA [?]	Beers 1963
	Conchopthirus magna	cil	Massachusetts	Kidder 1934
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Mantoscyphidia sp.	cil	North Carolina	Chittick et al. 2001
	Paratanytarsus sp.[e]	chi	Quebec	Ricciardi 1994
	Aeromonas hydrophila	bac	North Carolina	Chittick et al. 2001
	Baccillus sp.	bac	North Carolina	Chittick et al. 2001
	Enterobacter amnigenus	bac	North Carolina	Chittick et al. 2001
	Enterobacter cancerogenus	bac	North Carolina	Chittick et al. 2001
	Enterobacter cloacae	bac	North Carolina	Chittick et al. 2001
	Enterobacter intermedius	bac	North Carolina	Chittick et al. 2001
	Escherichia coli	bac	North Carolina	Chittick et al. 2001
	Escherichia hermanii	bac	North Carolina	Chittick et al. 2001
	Hafnia alvei	bac	North Carolina	Chittick et al. 2001
	Klebsiella pneumoniae	bac	North Carolina	Chittick et al. 2001
	Morganella morganii	bac	North Carolina	Chittick et al. 2001
	Pantoea agglomerans	bac	North Carolina	Chittick et al. 2001
	Proteus mirabilis	bac	North Carolina	Chittick et al. 2001
	Serratia marcescens	bac	North Carolina	Chittick et al. 2001
	Streptococcus (group D)	bac	North Carolina	Chittick et al. 2001
	Vibrio alginolyticus	bac	North Carolina	Chittick et al. 2001
	Vibrio fluvialis	bac	North Carolina	Chittick et al. 2001
	Asaccharolytic rod	bac	North Carolina	Chittick et al. 2001
Elliptio crassidens	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	Tennessee	Hendrix 1968
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Lophotaspis interiora	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
	Unionicola alleni	mit	USA	Vidrine 1987
Elliptio folliculata	*Cotylaspis insignis*	asp	Georgia	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985

Elliptio icterina	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola alleni	mit	USA	Vidrine 1987
Elliptio jayensis	*Cotylaspis insignis*	asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	USA	Hendrix et al. 1985
	Unionicola alleni	mit	USA	Vidrine 1987
Elliptio lanceolata	*Cotylaspis insignis*	asp	USA	Hendrix et al. 1985
Elliptoideus sloatianus	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
Epioblasma triquetra	*Aspidogaster conchicola*	asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
Eurynia dilatata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	Wisconsin	Williams 1978
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Bucephalus papillosus	dig	Michigan	Woodhead 1929
		dig	Michigan	Woodhead 1936
	Cercaria eriensis[g]	dig	Ohio	Coil 1954b
	Cercaria filicauda[f]	dig	Michigan	Fischthal 1951
	Rhipidocotyle septpapillata	dig	Michigan	Kniskern 1952
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola parkeri	mit	Arkansas	Vidrine 1987
		mit	Arkansas	Vidrine and Clark 1993
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola tupara	mit	Tennessee	Vidrine and Wilson 1991
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Fusconaia burkei	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Unionicola gowani	mit	USA	Vidrine 1987
Fusconaia cerina	"Sterilising trematodes"	dig	Alabama, Mississippi	Haag and Leann Staton 2003
	Unionicola serrata	mit	Louisiana	Edwards et al. 2010
Fusconaia chunii	*Najadicola ingens*	mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
	Unionicola parkeri	mit	USA	Vidrine 1987
		mit	Texas	Vidrine 1990

		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Ernsting et al. 2006
		mit	Louisiana	Edwards et al. 2010
		mit	Louisiana	Edwards et al. 2011
	Unionicola serrata	mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Fusconaia cuneolus	*Unionicola bogani*	mit	USA	Vidrine 1987
Fusconaia escambia	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
Fusconaia flava	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Wisconsin	Williams 1978
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
		asp	Manitoba	Carney 2015
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Manitoba	Carney 2015
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola bogani	mit	USA	Vidrine 1987
	Unionicola parkeri	mit	USA	Vidrine 1987
		mit	Tennessee	Vidrine and Wilson 1991
		mit	Arkansas	Vidrine and Clark 1993
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Ablabesmyia sp.[e]	chi	Oklahoma	Roback et al. 1979
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Fusconaia subrotunda	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985

Fusconaia sp.	*Phycoidella* sp.	oth	Lousiana	Roback 1979
Glebula rotundata	*Aspidogaster conchicola*	asp	Louisiana	Vidrine 1973[n]
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Louisiana	Vidrine 1973
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Gonidea angulata	*Aspidogaster conchicola*	asp	Washington	Pauley and Becker 1968
		asp	USA	Hendrix et al. 1985
Hamiota subangulata	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Lophotaspis interiora	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
Lampsilis cardium	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Illinois	van Cleave and Williams 1943
		asp	Ohio	Stromberg 1970
		asp	Ohio	Huehner and Etges 1981
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Manitoba	Carney 2015
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	West Virginia	Danford and Joy 1984
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Manitoba	Carney 2015
	Cotylogasteroides barrowi[j]	asp	Ohio	Huehner and Etges 1972
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Cercaria eriensis[g]	dig	Ohio	Coil 1953
		dig	Ohio	Coil 1954b

	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola abnormipes	mit	Arkansas	Vidrine and Clark 1993
	Unionicola hoesei	mit	Arkansas	Vidrine and Clark 1993
	Unionicola serrata	mit	Arkansas	Vidrine and Clark 1993
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
		cil	Illinois	Antipa 1977
Lampsilis cariosa	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
Lampsilis floridensis	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
	Cotylaspis insignis	asp	Florida, Georgia	Hendrix and Short 1965
Lampsilis fasciola	*Aspidogaster conchicola*	asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kofoid 1899
		asp	USA	Hendrix et al. 1985
Lampsilis higginsii	*Cotylaspis insignis*	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
Lampsilis hydiana	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Texas	Flook and Ubelaker 1972
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Lophotaspis interiora	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
	Bucephalus elegans	dig	Texas	Flook and Ubelaker 1972
	Najadicola ingens	mit	Texas	Vidrine 1990
	Unionicola abnormipes	mit	Texas	Vidrine 1990
		mit	Louisiana	Edwards et al. 2010
	Unionicola hoesei	mit	Texas	Vidrine 1990
		mit	Louisiana	Ernsting et al. 2014
		mit	Louisiana	Vidrine and Borsari 1994
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Lampsilis ovata	*Aspidogaster conchicola*	asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975

		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Lampsilis radiata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Kentucky	Rosen et al. 2016a
		asp	Kentucky	Rosen et al. 2016b
		asp	Kentucky	Rosen et al. 2017
	Cotylogaster occidentalis	asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Bucephalidae[h]	dig	USA	Kat 1983
	Atax sp.	mit	Illinois	Kelly 1899
	Najadicola ingens	mit	USA, Canada	Humes and Jamnback 1950
		mit	Quebec	Humes and Harris 1952
	Unionicola aculeata	mit	Indiana	Faust 1918
	Unionicola sp.	mit	USA	Welsh 1931
	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Paratanytarsus sp.[e]	chi	Quebec	Ricciardi 1994
Lampsilis reeveiana	*Cotylaspis insignis*	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
Lampsilis satura	*Unionicola abnormipes*	mit	Texas	Vidrine 1990
	Unionicola hoesei	mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Ernsting et al. 2014
	Unionicola serrata	mit	Texas	Vidrine 1990
Lampsilis siliquoidea	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970

asp	Oklahoma	Nelson et al. 1975	
asp	Louisiana	Vidrine and Causey 1975	
asp	Wisconsin	Williams 1978	
asp	Ohio	Huehner and Etges 1981	
asp	West Virginia	Danford and Joy 1984	
asp	USA	Hendrix et al. 1985	
Cotylaspis insignis	asp	Illinois	Kelly 1899
asp	New York	Osborn 1903	
asp	Ohio	Stromberg 1970	
asp	Oklahoma	Nelson et al. 1975	
asp	Louisiana	Vidrine and Causey 1975	
asp	West Virginia	Danford and Joy 1984	
asp	USA	Hendrix et al. 1985	
Cotylogaster occidentalis	asp	USA	Yamaguti 1963
asp	USA	Hendrix et al. 1985	
asp	Iowa	Fredericksen 1972	
asp	Iowa	Fredericksen 1978	
asp	Manitoba, North Dakota	Carney 2015	
Cotylogasteroides barrowi[j]	asp	Ohio	Huehner and Etges 1972
Cercaria basi[k]	dig	Michigan	Woodhead 1936
dig	?	Kniskern 1950	
Cercaria eriensis[g]	dig	Ohio	Coil 1954b
Cercaria lampsilae[f]	dig	Ohio	Coil 1954b
Cercaria pyriformoides[f]	dig	Michigan	Coil 1954a
Rhipidocotyle septpapillata	dig	Michigan	Kniskern 1952
Najadicola ingens	mit	Michigan	Mitchell 1955
Unionicola abnormipes	mit	Michigan	Mitchell 1955
Unionicola aculeata	mit	Michigan	Mitchell 1955
Unionicola fossulata	mit	Michigan	Mitchell 1955
mit	Michigan	Mitchell 1965	
mit	New York	Baker 1982	
mit	USA, Canada	Vidrine 1986b	
mit	Arkansas	Vidrine and Clark 1993	
Unionicola serrata	mit	Michigan	Mitchell 1955
Unionicola sp.	mit	New York	Jones and Baker 1984

	Conchophthirus curtus	cil	Iowa	Penn 1958
		cil	Illinois	Antipa and Small 1971
	Tetrahymena glochidophila	cil	Missouri	Lynn et al. 2018
	Fungal mat	fun	Michigan	Nichols et al. 2001
Lampsilis straminea	*Cotylaspis insignis*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Phycoidella sp.[e]	oth	Lousiana	Roback 1979
Lampsilis teres	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Florida	Hendrix and Short 1965
		asp	Louisiana	Vidrine 1973
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Louisiana	Curry and Vidrine 1976
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Florida	Hendrix and Short 1965
		asp	Texas	Gentner and Hopkins 1966
		asp	Texas	Flook and Ubelaker 1972
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Louisiana	Curry and Vidrine 1976
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Bucephalis elegans	dig	Texas	Flook and Ubelaker 1972
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Microcreadium parvum[g]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola abnormpies	mit	Louisiana	Vidrine 1973
		mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
	Unionicola hoesei	mit	Louisiana	Vidrine 1986b

		mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Ernsting et al. 2014
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Lasmigona complanata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	Ohio	Huehner and Etges 1981
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola belli	mit	Ohio	Vidrine 1986c
	Conchophthirus curtus	cil	Illinois	Kelly 1899
		cil	Illinois	Antipa and Small 1971
	Heterocinetopsis unionidarum	cil	Illinois	Antipa and Small 1971
Lasmigina compressa	*Unionicola arcuata*	mit	Canada	Vidrine 1986a
	Unionicola cooki	mit	Ontario	Vidrine 1986a
	Unionicola dimocki	mit	Canada	Vidrine 1986a
Lasmigona costata	*Aspidogaster conchicola*	asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Atax tumidus	mit	Missouri	Utterback 1916
	'Atax' sp.	mit	Illinois	Kelly 1899
	Unionicola belli	mit	Arkansas	Vidrine 1986c
	Unionicola clarki	mit	USA	Vidrine 1986c
		mit	Arkansas	Vidrine and Clark 1993
	Unionicola dimocki	mit	Canada	Vidrine 1986a
		mit	Tennessee	Vidrine and Wilson 1991
		mit	Arkansas	Vidrine and Clark 1993

	Unionicola smithae	mit	USA, Canada	Vidrine 1986c
		mit	Tennessee	Vidrine and Wilson 1991
		mit	Arkansas	Vidrine and Clark 1993
		mit	Arkansas	Edwards et al. 2010
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Chaetogaster sp.	oli	Illinois	Kelly 1899
Leptodea fragilis	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Missouri	Utterback 1916
		asp	Illinois	van Cleave and Williams 1943
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Illinois	Stunkard 1917
		asp	Louisiana	Vidrine and Causey 1975
		asp	Oklahoma	Nelson et al. 1975
		asp	USA	Hendrix et al. 1985
	Allocreadium ictaluri[g]	dig	Illinois	Hopkins 1934
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola serrata	mit	Louisiana	Vidrine and Borsari 1994
	Conchophtirus sp.	cil	Illinois	Kelly 1899
	Chaetogaster limnaei	oli	Iowa	Anderson and Holm 1987
Leptodea leptodon	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
	'Atax' sp.	mit	Illinois	Kelly 1899
Leptodea ochracea	*Cotylaspis insignis*	asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	USA	Hendrix et al. 1985

	'Atax' sp.	mit	Illinois	Kelly 1899
Ligumia nasuta	*Aspidogaster conchicola*	asp	?	Leidy 1851
		asp	Illinois	Kelly 1899
		asp	?	Leidy 1904
		asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Michigan	Fredericksen 1978
		asp	Michigan	Fredericksen 1980
		asp	USA	Hendrix et al. 1985
	Cercaria eriensis[g]	dig	Ohio	Coil 1954b
	Metacercaria quadraspinis[g]	dig	Ohio	Coil 1954b
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Ligumia recta	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix and Short 1965
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Michigan	LoVerde and Fredericksen 1978
		asp	USA	Hendrix et al. 1985
		asp	Manitoba	Carney 2015
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Cercaria eriensis[g]	dig	Ohio	Coil 1954b
	'Atax' sp.	mit	Illinois	Kelly 1899
	Unionicola abnormipes	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola gordoni	mit	USA	Vidrine 1987
	Unionicola serrata	mit	Tennessee	Vidrine and Wilson 1991
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Ligumia subrostrata	*Aspidogaster conchicola*	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Tennessee	Najarian 1955

		asp	Tennessee	Yamaguti 1963
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis reelfootensis[i]	asp	Tennessee	Najarian 1961
	Unionicola hoesei	mit	Texas	Vidrine 1990
	Unionicola sp.	mit	Tennessee	Najarian 1955
Medionidus conradicus	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Trematode (Bucephalidae?)[h]	dig	Virginia	Zale and Neves 1982
	Unionicola hendrixi	mit	USA	Vidrine 1987
		mit	Tennessee	Vidrine and Wilson 1991
Megalonaias nervosa	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Unionicola tupara	mit	Tennessee	Mitchell and Lester 1965
		mit	Tennessee	Vidrine and Wilson 1991
Obliquaria reflexa	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	'Atax' sp.	mit	Illinois	Kelly 1899
	Unionicola hoesei	mit	Texas	Vidrine 1990
	Unionicola megachela	mit	Texas	Vidrine 1990
	Unionicola vikitra	mit	USA	Vidrine 1987
		mit	Texas	Vidrine 1990
	Conchophthirus sp.	mit	Illinois	Kelly 1899
	Chaetogaster sp.	oli	Illinois	Kelly 1899
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Obovaria arkansasensis	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Louisiana	Vidrine and Borsari 1994

	Unionicola gailae	mit	Louisiana	Vidrine and Borsari 1994
Obovaria olivaria	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Illinois	van Cleave and Williams 1943
		asp	Tennessee	Hendrix 1968
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
	Unionicola ypsilophora[m]	mit	Iowa	Vidrine 1986a
Obovaria retusa	*Aspidogaster conchicola*	asp	Tennessee	Hendrix 1968
		asp	USA	Hendrix et al. 1985
Obovaria subrotunda	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
Obovaria unicolor	*Unionicola guilloryi*	mit	USA	Vidrine 1987
Plectomerus dombeyanus	*Aspidogaster conchicola*	asp	Louisiana	Vidrine 1973
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Unionicola megachela	mit	Iowa	Vidrine 1985a
		mit	Texas	Vidrine 1990
	Unionicola tupara	mit	Texas	Vidrine 1990
Plethobasus cyphyus	*Cotylaspis insignis*	asp	Tennessee	Hendrix 1968
		asp	USA	Hendrix et al. 1985
Pleurobema cordatum	*Aspidogaster conchicola*	asp	Tennessee	Hendrix 1968
		asp	West Virginia	Danford and Joy 1984
		asp	USA	Hendrix et al. 1985
Pleurobema pyriforme	*Unionicola gowani*	mit	USA	Vidrine 1987
Pleurobema riddellii	*Najadicola ingens*	mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
	Unionicola aculeata	mit	Texas	Vidrine 1990
	Unionicola gowani	mit	USA	Vidrine 1987
		mit	Texas	Vidrine 1990
		mit	Louisiana	Vidrine and Borsari 1994
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990

	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Pleurobema sintoxia	*Aspidogaster conchicola*	asp	Illinois	van Cleave and Williams 1943
		asp	Ohio	Stromberg 1970
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Conchophthirus curtus	asp	Illinois	Antipa and Small 1971
Pleurobema strodeanum	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
	Lophotaspis interiora	asp	Florida	Hendrix and Short 1972
		asp	USA	Hendrix et al. 1985
	Unionicola gowani	mit	USA	Vidrine 1987
Pleuronaia barnesiana	*Unionicola bogani*	mit	USA	Vidrine 1987
Popenais popeii	*Unionicola berezai*	mit	Texas	Vidrine 1985b
	Gomphus militaris[e]	oth	New Mexico	Levine et al. 2009
Potamilus alatus	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Wisconsin	Williams 1978
		asp	West Virginia	Danford and Joy 1984
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Manitoba, North Dakota	Carney 2015
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Manitoba	Carney 2015
	Atax stricta	mit	Missouri	Utterback 1916
	'Atax' sp.	mit	Illinois	Kelly 1899
	Unionicola australindistincta	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola fulleri	mit	Illinois	Vidrine 1986a
		mit	Tennessee	Vidrine and Wilson 1991

		mit	Indiana	Edwards et al. 2010
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
	Conchophthirus sp.	cil	Illinois	Kelly 1899
Potamilus amphichaenus	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
Potamilus ohiensis	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Wisconsin	Williams 1978
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
Potamilus purpuratus	*Aspidogaster conchicola*	asp	Oklahoma	Bailey and Tompkins 1971
		asp	Louisiana	Vidrine 1973
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Louisiana	Curry and Vidrine 1976
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Texas	Flook and Ubelaker 1972
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Bucephalis elegans	dig	Texas	Flook and Ubelaker 1972
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Unionicola australindistincta	mit	USA	Vidrine 1985a
	Unionicola fulleri	mit	Louisiana	Vidrine 1986a
	Unionicola hoesei	mit	Louisiana	Ernsting et al. 2014
	Unionicola serrata	mit	Texas	Vidrine 1990
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Unionicola sp.	mit	Louisiana	Curry and Vidrine 1976
	Mesanophrys cf. carcini	cil	Alabama	Pan 2016
	Parauronema cf. longum	cil	Alabama	Pan 2016
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976

Potamilus sp.	*Cotylaspis insignis*	asp	Oklahoma	Fulhage 1954
Ptychobranchus fasciolaris	*Aspidogaster conchicola*	asp	Ohio	Stromberg 1970
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Unionicola causeyae	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola hoesei	mit	Tennessee	Vidrine and Wilson 1991
Ptychobranchus occidentalis	*Unionicola abnormipes*	mit	Arkansas	Vidrine and Clark 1993
	Unionicola hoesei	mit	Arkansas	Vidrine and Clark 1993
	Unionicola serrata	mit	Arkansas	Vidrine and Clark 1993
Ptychobranchus subtentus	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
Pyganodon cataracta	*Aspidogaster conchicola*	asp	?	Leidy 1851
		asp	Illinois	Kelly 1899
		asp	?	Leidy 1904
		asp	Ohio	Huehner and Etges 1981
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	?	Leidy 1857
		asp	?	Leidy 1858
		asp	USA	Yamaguti 1963
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	USA	Hendrix et al. 1985
	Aspidogastridae	asp	Delaware	Curry 1977
	Rhopalocerca tardigrada[h]	dig	?	Leidy 1858
	Najadicola ingens	mit	USA, Canada	Humes and Jamnback 1950
		mit	Quebec	Humes and Harris 1952
	Unionicola formosa	mit	Ontario	Mitchell 1957
		mit	New Brunswick	Gordon et al. 1979
		mit	New York	Baker 1982
		mit	USA	Vidrine 1986a
		mit	North Carolina	Edwards and Dimock 1988
		mit	North Carolina	Edwards and Dimock 1995a
		mit	North Carolina	Edwards and Dimock 1995b
		mit	North Carolina	Edwards and Dimock 1997
		mit	North Carolina	Edwards et al. 1998
		mit	North Carolina	Fisher et al. 2000

		mit	North Carolina	Ernsting et al. 2006
		mit	North Carolina	Edwards et al. 2010
	Unionicola tumida	mit	New York	Baker 1982
	Unionicola wolcotti	mit	Michigan	Mitchell 1957
		mit	South Carolina	Vidrine 1986c
	Unionicola ypsilophora	mit	USA	Welsh 1930
		mit	USA	Welsh 1931
		mit	Canada	Vidrine 1986a
	Unionicola sp.	mit	New Hampshire	Humes and Russell 1951
		mit	Delaware	Curry 1977
	Baeoctenus bicolor	chi	New Brunswick	Gordon et al. 1978
	Paratanytarsus sp.	chi	Quebec	Ricciardi 1994
	Orthocladiinae	chi	New Brunswick	Gordon et al. 1978
	Placobdella montifera	lee	Delaware	Curry 1977
	Phycoidella sp.	oth	Lousiana	Roback 1979
Pyganodon gibbosa	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
Pyganodon grandis	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Illinois	Stunkard 1917
		asp	Texas	Gentner and Hopkins 1966
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Bailey and Tompkins 1971
		asp	Texas	Gentner 1971
		asp	Texas	Flook and Ubelaker 1972
		asp	Illinois	Hathaway 1972
		asp	Louisiana	Vidrine 1973
		asp	Oklahoma	Bailey and Rock 1975
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Wisconsin	Williams 1978
		asp	Illinois	Hathaway 1979
		asp	Ohio	Huehner and Etges 1981
		asp	West Virginia	Danford and Joy 1984

asp	Missouri	Huehner 1984	
asp	USA	Hendrix et al. 1985	
asp	Kentucky	Duobinis-Gray et al. 1991	
asp	Illinois	Williams 1942	
asp	Ohio	Huehner et al. 1989	
asp	Manitoba	Carney 2015	
Cotylaspis insignis	asp	Illinois	Kelly 1899
asp	Illinois	Kofoid 1899	
asp	New York	Osborn 1903	
asp	Missouri	Utterback 1916	
asp	Illinois	Stunkard 1917	
asp	Tennessee	Najarian 1955	
asp	Tennessee	Yamaguti 1963	
asp	Texas	Gentner and Hopkins 1966	
asp	Tennessee	Hendrix and Short 1965	
asp	Ohio	Stromberg 1970	
asp	Texas	Gentner 1971	
asp	Texas	Flook and Ubelaker 1972	
asp	Louisiana	Vidrine 1973	
asp	Oklahoma	Nelson et al. 1975	
asp	Louisiana	Vidrine and Causey 1975	
asp	West Virginia	Danford and Joy 1984	
asp	Minnesota	LoVerde and Fredericksen 1978	
asp	Missouri	Huehner 1984	
asp	USA	Hendrix et al. 1985	
asp	Alabama	Gangloff et al. 2008	
Cotylogaster occidentalis	asp	Michigan	Fredericksen 1978
asp	USA	Hendrix et al. 1985	
asp	Manitoba, North Dakota	Carney 2015	
Cotylaspis reelfootensis[i]	asp	Tennessee	Najarian 1961
Bucephalus sp.	dig	Illinois	Kelly 1899
Cercaria arqi[l]	dig	Michigan	Woodhead 1936
Cercaria anodontae[f]	dig	Michigan	Coil 1954a
Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
Rhipidocotyle septpapillata	dig	Michigan	Kniskern 1952

	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola crassipes laurentiana	mit	Louisiana	Vidrine et al. 1986
	Unionicola formosa	mit	Ontario	Mitchell 1957
		mit	USA	Vidrine 1986a
		mit	Indiana	Edwards et al. 1998
		mit	Indiana	Ernsting et al. 2006
	Unionicola furcula	mit	Wisconsin	Vidrine et al. 1986
	Unionicola mitchelli	mit	USA	Vidrine 1986c
	Unionicola smithae	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola wolcotti	mit	Michigan	Mitchell 1957
		mit	Arkansas	Vidrine 1986c
	Unionicola ypsilophora	mit	Iowa	Vidrine 1986a
	Unionicola sp.	mit	Tennessee	Najarian 1955
		mit	Louisiana	Curry and Vidrine 1976
		mit	New York	Jones and Baker 1984
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Unionicolidae	mit	Alabama	Gangloff et al. 2008
	Conchophthirus curtus	cil	Iowa	Penn 1958
		cil	Illinois	Antipa and Small 1971
		cil	Illinois	Antipa 1977
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Heterocinetopsis unionidarum	cil	Illinois	Antipa and Small 1971
		cil	Illinois	Antipa 1977
	Ablabesmyia sp.[e]	chi	Texas, Oklahoma	Roback et al. 1979
	Placobdella montifera	lee	Louisiana	Curry and Vidrine 1976
Pyganodon lacustris	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	?	Leidy 1857
		asp	?	Leidy 1858
		asp	USA	Hendrix et al. 1985
Quadrula apiculata	*Aspidogaster conchicola*	asp	Louisiana	Vidrine 1973
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966

Quadrula quadrula	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Texas	Gentner and Hopkins 1966
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Bailey and Tompkins 1971
		asp	Oklahoma	Nelson et al. 1975
		asp	Ohio	Huehner and Etges 1981
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
		asp	Manitoba	Carney 2015
	Cotylaspis insignis	asp	Texas	Gentner and Hopkins 1966
		asp	West Virginia	Danford and Joy 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Manitoba	Carney 2015
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola vamana	mit	Tennessee	Vidrine and Wilson 1991
	Unionicola vikitra	mit	USA	Vidrine 1987
		mit	Tennessee	Vidrine and Wilson 1991
	Unionicola spp.	mit	Texas	Flook and Ubelaker 1972
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Reginaia ebenus	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
		asp	Tennessee	Hendrix 1968
		asp	Wisconsin	Williams 1978
		asp	Kentucky	Duobinis-Gray et al. 1991
	Unionicola scutella	mit	Mississippi	Vidrine 1986b
	Aeromonas hydrophila	bac	Alabama	Starliper et al. 2011
	Aeromonas caviae	bac	Alabama	Starliper et al. 2011
	Aeromonas sobria	bac	Alabama	Starliper et al. 2011
	Aeromonas veronii	bac	Alabama	Starliper et al. 2011
	Aeromonas schubertii	bac	Alabama	Starliper et al. 2011
	Alcaligenes faecalis	bac	Alabama	Starliper et al. 2011
	Brevundimonas vesicularis	bac	Alabama	Starliper et al. 2011

	Burkholderia cepacia	bac	Alabama	Starliper et al. 2011
	Chromobacterium violaceum	bac	Alabama	Starliper et al. 2011
	Chryseobacterium	bac	Alabama	Starliper et al. 2011
	Comamonas testosteroni	bac	Alabama	Starliper et al. 2011
	Enterobacter cloacae	bac	Alabama	Starliper et al. 2011
	Hafnia alvei	bac	Alabama	Starliper et al. 2011
	Pleisomonas shigelloides	bac	Alabama	Starliper et al. 2011
	Pseudomonas alcaligenes	bac	Alabama	Starliper et al. 2011
	Pseudomonas fluorescens	bac	Alabama	Starliper et al. 2011
	Pseudomonas putida	bac	Alabama	Starliper et al. 2011
	Ralstonia pickettii	bac	Alabama	Starliper et al. 2011
	Serratia odorifera	bac	Alabama	Starliper et al. 2011
	Sphingobacterium multivorum	bac	Alabama	Starliper et al. 2011
	Sphingomonas paucimobilis	bac	Alabama	Starliper et al. 2011
	Yokonella regensburgei	bac	Alabama	Starliper et al. 2011
Strophitus connasaugaensis	*Aspidogaster conchicola*	asp	Alabama	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Alabama	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola sp.	mit	Alabama	McElwain et al. 2016
Strophitus subvexus	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Unionicola abnormpies	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola arcuata	mit	USA	Vidrine 1986a
	Unionicola dimocki	mit	USA	Vidrine 1986a
		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Ernsting et al. 2006
		mit	Louisiana	Edwards et al. 2010
	Unionicola formosa	mit	Louisiana	Vidrine 1986a
	Unionicola tumida	mit	Louisiana	Vidrine 1986a
		mit	Louisiana	Edwards et al. 2008
		mit	Louisiana	Ernsting et al. 2008
		mit	Louisiana	Edwards et al. 2010
Strophitus undulatus	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Missouri	Huehner 1984

		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Ohio	Stromberg 1970
		asp	West Virginia	Danford and Joy 1984
		asp	USA	Hendrix et al. 1985
	Cotylogaster occidentalis	asp	Manitoba	Carney 2015
	Cercaria catatonki[f]	dig	New York	Fischthal 1951
	Unionicola clarki	dig	USA	Vidrine 1986c
	Unionicola dimocki	dig	USA, Canada	Vidrine 1986a
		dig	Arkansas	Vidrine and Clark 1993
	Unionicola tumida	dig	USA	Vidrine 1986a
Theliderma cylindrica	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Unionicola sakantaka	mit	Tennessee	Vidrine and Wilson 1991
Theliderma metanevra	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix 1968
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola abnormipes	mit	Arkansas	Vidrine and Clark 1993
	Unionicola sakantaka	mit	Arkansas	Vidrine and Clark 1993
Toxolasma parvum	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Ohio	Stromberg 1970
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Najadicola ingens	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola causeyae	mit	USA	Vidrine 1985a
	Unionicola kavanaghi	mit	Louisiana	Vidrine 1987
		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Edwards et al. 2010
	Unionicola latipalpa	mit	USA	Vidrine 1985a

	Unionicola serrata	mit	Louisiana	Vidrine and Borsari 1994
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Chaetogaster sp.	oli	Illinois	Kelly 1899
Tritogonia verrucosa	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix 1968
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Bailey and Tompkins 1971
		asp	Louisiana	Vidrine 1973
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Wisconsin	Williams 1978
		asp	West Virginia	Danford and Joy 1984
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Kofoid 1899
		asp	Louisiana	Vidrine 1973
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Homalometron armatum[g]	dig	Texas	Gentner and Hopkins 1966
	Polylekithum ictaluri[g]	dig	Texas	Gentner and Hopkins 1966
	Najadicola ingens	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola serrata	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola vamana	mit	Tennessee	Mitchell and Lester 1965
		mit	USA	Vidrine 1987
		mit	Louisiana	Vidrine and Wilson 1991
		mit	Tennessee	Vidrine and Borsari 1994
		mit	Kentucky	Edwards et al. 2010
	Unionicola vikitra	mit	USA	Vidrine 1987
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Truncilla donaciformis	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899

		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Atax sp.	mit	Illinois	Kelly 1899
	Chaetogaster sp.	oli	Illinois	Kelly 1899
Truncilla truncata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	Wisconsin	Williams 1978
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kofoid 1899
		asp	Oklahoma	Nelson et al. 1975
		asp	USA	Hendrix et al. 1985
	Bucephalus sp.	dig	Illinois	Kelly 1899
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	'Atax' sp.	mit	Illinois	Kelly 1899
	Ablabesmyia sp.[e]	chi	Oklahoma	Roback et al. 1979
Uniomerus carolinianus	*Cotylaspis insignis*	asp	Florida, Georgia	Hendrix and Short 1965
Uniomerus declivis	*Najadicola ingens*	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola abnormipes	mit	Florida	Downes 1986
		mit	Florida	Downes 1991
	Unionicola fossulata	mit	Florida	Downes 1986
	Unionicola lasellai	mit	Florida	Downes 1990
		mit	Florida	Edwards and Labhart 2000
	Unionicola poundsi	mit	Florida	Downes 1991
		mit	Florida	Downes 1991
	Unionicola serrata	mit	Florida	Downes 1986
		mit	Florida	Downes 1991
		mit	Florida	Downes 1991
	Unionicola stricta	mit	Louisiana	Vidrine and Borsari 1994
Uniomerus tetralasmus	*Aspidogaster conchicola*	asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Tennessee	Najarian 1955

		asp	Tennessee	Yamaguti 1963
		asp	Louisiana	Vidrine and Causey 1975
		asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis reelfootensis[i]	asp	Tennessee	Najarian 1961
	Unionicola alleni	mit	USA	Vidrine 1987
	Unionicola lasellei	mit	Florida	Vidrine 1986b
	Unionicola stricta	mit	USA	Vidrine 1986b
	Unionicola sp.	mit	Louisiana	Vidrine 1973
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
Utterbackia imbecillis	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	Alabama, Florida	Hendrix and Short 1965
		asp	Ohio	Stromberg 1970
		asp	Illinois	Hathaway 1972
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	Wisconsin	Williams 1978
		asp	Illinois	Hathaway 1979
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Illinois	Stunkard 1917
		asp	Alabama, Florida	Hendrix and Short 1965
		asp	Ohio	Stromberg 1970
		asp	Oklahoma	Nelson et al. 1975
		asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola aculeata	mit	Indiana	Edwards et al. 2010
	Unionicola foili	mit	?	Edwards and Vidrine 1994
		mit	North Carolina	Edwards and Dimock 1995a
		mit	North Carolina	Edwards and Dimock 1995b
		mit	USA	Edwards and Dimock 1997
		mit	USA	Weiberg and Edwards 1997
		mit	?	Edwards et al. 1998
		mit	Indiana	Edwards 1999

		mit	Indiana	Edwards et al. 2002
		mit	Indiana	Edwards and Smith 2003
		mit	Indiana	Edwards 2004
		mit	Indiana	Ernsting et al. 2006
		mit	Indiana	Ernsting et al. 2009
		mit	Indiana	Edwards et al. 2010
	Unionicola formosa	mit	Louisiana	Vidrine 1973
		mit	South Carolina	Roberts et al. 1978
		mit	North/South Carolina	LaRochelle and Dimock 1981
		mit	North Carolina	del Portillo and Dimock 1982
		mit	North Carolina	Dimock 1983
		mit	North Carolina	Dimock 1985
		mit	Florida	Downes 1986
		mit	USA	Vidrine 1986a
		mit	North Carolina	Edwards and Dimock 1988
		mit	West Virginia	Joy and Hively 1990
		mit	?	Edwards and Dimock 1991
	Unionicola mitchelli	mit	Louisiana	Vidrine 1986c
	Unionicola tumida	mit	Louisiana	Vidrine 1986a
	Unionicola wolcotti	mit	South Carolina	Vidrine 1986c
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Ablabesmyia sp.[e]	chi	Florida	Roback et al. 1979
	Phycoidella sp.[e]	oth	Lousiana	Roback 1979
	Chaetogaster sp.	oli	Illinois	Kelly 1899
Utterbackia peggyae	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Unionicola formosa	mit	Florida	Vidrine 1986a
	Ablabesmyia sp.[e]	chi	Florida	Roback et al. 1979
Utterbackiana couperiana	*Cotylaspis insignis*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola formosa	mit	Florida	Vidrine 1986a
	Unionicola wolcotti	mit	South Carolina	Vidrine 1986c
Utterbackiana implicata	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985

	Unionicola formosa	mit	Ontario	Mitchell 1957
	Unionicola ypsilophora	mit	Rhode Island	Vidrine 1986a
	Conchophthirus curtus	cil	Massachusetts	Kidder 1934
	Baeoctenus bicolor[e]	chi	New Brunswick	Gordon et al. 1978
Utterbackiana suborbiculata	*Aspidogaster conchicola*	asp	Illinois	Kelly 1899
		asp	USA	Hendrix et al. 1985
		asp	Kentucky	Duobinis-Gray et al. 1991
	Cotylaspis insignis	asp	Illinois	Kelly 1899
		asp	Tennessee	Hendrix and Short 1965
		asp	USA	Hendrix et al.1985
		asp	Kentucky	Duobinis-Gray et al. 1991
	Conchophthirus curtus	cil	Illinois	Antipa and Small 1971
	Conchophthirus sp.	cil	Illinois	Kelly 1899
	Atax ypsilophorus	mit	Missouri	Utterback 1916
	Atax sp.	mit	Illinois	Kelly 1899
	Unionicola foili	mit	Indiana	Ernsting et al. 2006
		mit	Indiana	Edwards et al. 2010
	Unionicola formosa	mit	USA	Vidrine 1986a
		mit	?	Edwards et al. 1998
Venustaconcha ellipsiformis	*Aspidogaster conchicola*	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Missouri	Huehner 1984
		asp	USA	Hendrix et al. 1985
	"Macrocercus-like cercariae"	dig	Michigan	v.d. Schalie and v.d. Schalie 1963
Villosa amygdalum	*Unionicola poundsi*	mit	Florida	Vidrine 1986b
Villosa delumbis	*Cotylaspis insignis*	asp	USA	Hendrix et al. 1985
Villosa iris	*Aspidogaster conchicola*	asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	USA	Hendrix et al. 1985
	Bucephalus papillosus	dig	Michigan	Woodhead 1929
	Bucephalus elegans	dig	Michigan	Woodhead 1930
		dig	Michigan	Woodhead 1936
	Cercaria scioti[f]	dig	Michigan	Woodhead 1936
	"Trematode sporocysts"	dig	Virginia	Rogers et al. 2018
	Unionicola dimocki	mit	Arkansas	Vidrine and Clark 1993
	Unionicola hoesei	mit	Arkansas	Vidrine and Clark 1993

	Flavobacterium columnare	bac	Virginia	Starliper et al. 2008[n]
Villosa lienosa	*Aspidogaster conchicola*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida, Georgia	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Louisiana	Vidrine and Borsari 1994
	Unionicola gailae	mit	USA	Vidrine 1987
		mit	Texas	Vidrine 1990
		mit	Arkansas	Vidrine and Clark 1993
		mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Edwards et al. 2010
	Unionicola serrata	mit	Louisiana	Vidrine and Borsari 1994
		mit	Louisiana	Edwards et al. 2010
	Ablabesmyia janta[e]	chi	Texas	Vidrine 1990
	Ablabesmyia sp.[e]	chi	Texas	Roback et al. 1979
Villosa nebulosa	Trematode cercariae	dig	Virginia	Zale and Neves 1982
Villosa taeniata	*Unionicola hoesei*	mit	Tennessee	Vidrine and Wilson 1991
Villosa vanuxemensis	Trematode cercariae	dig	Virginia	Zale and Neves 1982
Villosa vibex	*Aspidogaster conchicola*	asp	Louisiana	Vidrine and Causey 1975
		asp	USA	Hendrix et al. 1985
	Cotylaspis insignis	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Florida	Downes 1986
	Unionicola fossulata	mit	Florida	Downes 1986
	Unionicola hoesei	mit	Louisiana	Edwards et al. 2010
	Phycoidella sp.[e]	oth	Lousiana	Roback 1979
Villosa villosa	*Cotylaspis insignis*	asp	Florida	Hendrix and Short 1965
		asp	USA	Hendrix et al. 1985
	Unionicola abnormipes	mit	Florida	Downes 1986
		mit	Florida	Downes 1991
		mit	Florida	Downes 1995
	Unionicola fossulata	mit	Florida	Downes 1986
	Unionicola poundsi	mit	Florida	Downes 1990
		mit	Florida	Downes 1991
		mit	Florida	Downes 1995

	mit	Florida	Edwards and Labhart 2000
Unionicola serrata	mit	Florida	Downes 1986
	mit	Florida	Downes 1991

[a]All aspidogastrid family, genus and species records are for adult trematodes

[b]These authors conducted a review of aspidogastrid-host relationships in North America, but also conducted extensive sampling in many different states across the USA. Only relationships found as part of their own sampling are included as records in this table (as much of the freshwater mussel literature they summarised also appears in primary form)

[c]All digenean family, genus and species records are for sporocysts/cercariae unless specified

[d]The genus *Atax* has been synonymised with *Unionicola*

[e]Larvae

[f]The genus *Cercaria* was applied haphazardly to cercarial stages of trematodes, leaving different scientific names for the different life history stages of trematodes. In several cases it is clear what cercarial designation corresponds to what adult species (notes k, l). However, in the remainder of cases it remains ambiguous as to what trematode species these cercarial names refer to

[g]Metacercarial cysts

[h]Cercariae/sporocysts

[i]Later synonymised with *Cotylaspis insignis*

[j]Later synonymised with *Cotlyogaster occidentalis*

[k]Later established as cercarial stage of *Rhipidocotyle septpapillata*

[l]Later established as cercarial stage of *Bucephalus pusillum*

[m]Mitchell (1957) expresses doubt that *Unionicola ypsilophora* exists in North America, and considers it to be an ambiguation of *U. formosa*. Vidrine (1986a) also expresses concern that they may be conspecific, with only the males being distinguishable, but maintains *U. ypsilophora* as a North American species. Records of this species should be treated with caution

[n]Other endosymbionts were also reported in this study, but could not be attributed to specific host species

Table A1.3: Summary of all European host-endosymbiont records reviewed in the study. Location is given to the scale of country if samples from one or two countries were taken, or is given as 'Europe' if sampling involved >3 countries. Records are initially listed by host, with invasive species listed before native species (arranged alphabetically within each sub-category). Within each host, parasites are arranged according to the following order (see 'Category' column): aspidogastrean trematodes [asp]; digenean trematodes [dig]; mites [mit]; copepods [cop]; ciliates [cil]; chironomids [chi]; nematodes [nem]; oligochaetes [oli]; leeches [lee]; protists [pro]; amoebae [amo]; fungi [fun]; bacteria [bac], other [oth]. Records are listed alphabetically by parasite within each of those groups, and then sequentially by year for each specific parasite.

Host species	Parasite	Category	Location	Reference
Dreissena polymorpha	*Aspidogaster limacoides*[a]	asp	Russia	Nagabina and Timofeeva 1971
		asp	Russia	Kuperman et al. 1994
		asp	Russia	Zhokhov and Kas'yanov 1995
		asp	Russia	Molloy et al. 1996
		asp	Russia	Pryanichnikova et al. 2011
		asp	Croatia	Lajtner 2012
	Aspidogaster sp.[a]	asp	Russia	Molloy et al. 1996
		asp	Belarus	Karatayev et al. 2000a
		asp	Europe	Laruelle et al. 2002
		asp	France	Minguez et al. 2011
	Bucephalus polymorphus[b]	dig	Russia	Kuperman et al. 1994
		dig	Russia	Molloy et al. 1996
		dig	Belarus	Karatayev et al. 2000a
		dig	Europe	Laruelle et al. 2002
		dig	Belarus, Lithuania	Stunžėnas et al. 2004
		dig	Croatia	Lajtner et al. 2008
		dig	Russia	Korsunenko et al. 2009
		dig	France	Minguez et al. 2011
		dig	Russia	Pryanichnikova et al. 2011
		dig	Croatia	Lajtner 2012
		dig	France	Minguez et al. 2012a
		dig	France	Minguez et al. 2012c
		dig	France	Minguez and Giambérini 2012
		dig	France	Minguez et al. 2013a
		dig	France	Minguez et al. 2013b
	Echinoparyphium recurvatum[c]	dig	Belarus	Mastitsky and Veres 2010

		dig	Russia	Pryanichnikova et al. 2011
		dig	Croatia	Lajtner 2012
	Echinoparyphium sp.[c]	dig	France	Minguez et al. 2011
		dig	France	Minguez and Giambérini 2012
		dig	France	Minguez et al. 2013b
	Echinostomatidae[c]	dig	Belarus	Karatayev et al. 2000a
		dig	Europe	Laruelle et al. 2002
		dig	Poland	Marszewska and Cichy 2015
	Neoacanthoparyphium echinatoides[a]	dig	Russia	Pryanichnikova et al. 2011
	Phyllodistomum angulatum[b]	dig	Russia	Kuperman et al. 1994
	Phyllodistomum folium[b]	dig	Europe	Laruelle et al. 2002
		dig	Belarus, Lithuania	Stunžėnas et al. 2004
		dig	Spain	Peribáñez et al. 2006
		dig	France	Minguez et al. 2011
		dig	Spain	Peribáñez et al. 2011
		dig	Russia	Pryanichnikova et al. 2011
		dig	France	Minguez and Giambérini 2012
		dig	France	Minguez et al. 2012a
		dig	France	Minguez et al. 2012c
		dig	France	Minguez et al. 2013a
		dig	France	Minguez et al. 2013b
	Phyllodistomum macrocotyle[b]	dig	The Netherlands	Kraak and Davids 1991
		dig	Europe	Petkevičiūtė et al. 2015
	Phyllodistomum sp.[b]	dig	Russia	Molloy et al. 1996
		dig	Belarus	Karatayev et al. 2000a
	Mideopsis orbicularis	mit	Russia	Pryanichnikova et al. 2011
	Unionicola sp.[d]	mit	Russia	Kuperman et al. 1994
	Mites (unidentified)	mit	Belarus	Karatayev et al. 2000a
		mit	Sweden	Mastitsky et al. 2008
	Ancistrumina limnica	cil	Europe	Laruelle et al. 1999
		cil	Belarus	Karatayev et al. 2000a
		cil	Belarus	Karatayev et al. 2003b
		cil	Ireland	Burlakova et al. 2006
	Conchophthirus acuminatus	cil	Belarus	Burlakova et al. 1998

		cil	Europe	Laruelle et al. 1999
		cil	Belarus	Karatayev et al. 2000a
		cil	Belarus, Ukraine	Karatayev et al. 2000b
		cil	Belarus	Karatayev et al. 2003a
		cil	Belarus	Karatayev et al. 2003b
		cil	Ireland	Burlakova et al. 2006
		cil	Ireland	Conn et al. 2008
		cil	Sweden	Mastitsky et al. 2008
		cil	France	Minguez et al. 2011
		cil	Russia	Pryanichnikova et al. 2011
		cil	Russia	Chuševė et al. 2012
		cil	France	Minguez and Giambérini 2012
		cil	France	Minguez et al. 2013b
	Hymenostomatida	cil	Russia	Molloy et al. 1996
		cil	Russia	Molloy et al. 1997
	Hypocomagalma dreissenae	cil	Europe	Laruelle et al. 1999
	Ophryoglena hemophaga	cil	The Netherlands	Molloy et al. 2005
		cil	Ireland	Burlakova et al. 2006
	Ophryoglena sp.	cil	Belarus	Karatayev et al. 2000a
		cil	Belarus	Karatayev et al. 2002
		cil	Belarus	Karatayev et al. 2003b
		cil	France	Minguez et al. 2011
		cil	Russia	Pryanichnikova et al. 2011
		cil	Russia	Chuševė et al. 2012
		cil	France	Minguez and Giambérini 2012
		cil	France	Minguez et al. 2012b
		cil	France	Minguez et al. 2012c
		cil	France	Minguez et al. 2013a
		cil	France	Minguez et al. 2013b
	Sphenophyra dreissenae	cil	Europe	Laruelle et al. 1999
		cil	France	Minguez et al. 2011
		cil	France	Minguez and Giambérini 2012
		cil	France	Minguez et al. 2013b
	Corynoneura sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005

	Cricotopus sp.1[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Cricotopus sp.2[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Cricotopus sp.3[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Cryptochironomus sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Eukieferiella sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Glyptotendipes sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Limnochironomus sp.1[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Limnochironomus sp.2[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Orthocladiinae sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Paratanytarsus sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Polypedilum sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Prodiamesa sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Tanytarsus sp.[e]	chi	Belarus	Mastitsky and Samoilenko 2005
	Chironomid sp.[e]	chi	Belarus	Karatayev et al. 2000a
		chi	Ireland	Burlakova et al. 2006
		chi	Sweden	Mastitsky et al. 2008
		chi	Russia	Pryanichnikova et al. 2011
	Chromadorina bioculata	nem	Sweden	Mastitsky et al. 2008
	Dorylaimus stagnalis	nem	Russia	Kuperman et al. 1994
	Laimydorus sp.	nem	Sweden	Mastitsky et al. 2008
	Nematodes (unidentified)	nem	Belarus	Karatayev et al. 2000a
		nem	Belarus	Karatayev et al. 2003b
		nem	Ireland	Burlakova et al. 2006
		nem	Russia	Pryanichnikova et al. 2011
		nem	Russia	Chuševė et al. 2012
	Chaetogaster limnaei	oli	Belarus	Karatayev et al. 2000a
	Oligochaetes (unidentified)	oli	Ireland	Burlakova et al. 2006
		oli	Russia	Pryanichnikova et al. 2011
	Caspiobdella fadejevi	lee	Russia	Kuperman et al. 1994
	Erpobdella octoculata	lee	Belarus	Karatayev et al. 2000a
	Helobdella stagnalis	lee	Russia	Kuperman et al. 1994
		lee	Belarus	Karatayev et al. 2000a
	Hyrudinea spp.	lee	Russia	Pryanichnikova et al. 2011
	Haplosporidium raabei	bac	Europe	Molloy et al. 2012

	Rickettsiales-like bacteria	bac	France	Minguez et al. 2011
		bac	France	Minguez and Giambérini 2012
		bac	France	Minguez et al. 2012b
		bac	France	Minguez et al. 2012c
		bac	France	Minguez et al. 2013a
		bac	France	Minguez et al. 2013b
	Cryptosporidium parvum	bac	Ireland	Graczyk et al. 2004
		bac	Ireland	Lucy et al. 2008
	Encephalitozoon hellem	bac	Ireland	Lucy et al. 2008
	Encephalitozoon intestinalis	bac	Ireland	Graczyk et al. 2004
		bac	Ireland	Lucy et al. 2008
	Enterocytozoon bieneusi	bac	Ireland	Graczyk et al. 2004
		bac	Ireland	Lucy et al. 2008
	Giardia lamblia	bac	Ireland	Graczyk et al. 2004
		bac	Ireland	Lucy et al. 2008
Dreissena r. bugensis	*Aspidogaster limacoides*	asp	Russia	Popova and Biochino 2001
	Echinoparyphium recurvatum	dig	Russia	Pryanichnikova et al. 2011
	Neoacanthoparyphium echinatoides	dig	Russia	Pryanichnikova et al. 2011
	Mideopsis orbicularis[d]	mit	Russia	Pryanichnikova et al. 2011
	Unionicola sp.[d]	mit	Russia	Popova and Biochino 2001
	Conchophthirus acuminatus	cil	Belarus, Ukraine	Karatayev et al. 2000b
		cil	Russia	Pryanichnikova et al. 2011
	Ophryoglena sp.	cil	Russia	Pryanichnikova et al. 2011
	Nematodes (unidentified)	nem	Russia	Pryanichnikova et al. 2011
	Caspiobdella fadejevi	lee	Russia	Popova and Biochino 2001
	Helobdella stagnalis	lee	Russia	Popova and Biochino 2001
	Acremonium sp.	fun	Russia	Popova and Biochino 2001
Sinanodonta woodiana	*Aspidogaster conchicola*	asp	Poland, Ukraine	Yuryshynets and Krasutska 2009
		asp	Ukraine	Yuryshynets 2010
	Rhipidocotyle campanula[b]	dig	Poland	Cichy et al. 2016
	Unionicola ypsilophora	mit	Poland	Cichy et al. 2016
	Glyptotendipes sp.	chi	Poland	Cichy et al. 2016
	Chaetogaster limnaei	oli	Poland	Cichy et al. 2016
Anodonta anatina	*Aspidogaster conchicola*	asp	The Netherlands	Bakker and Davids 1973

		asp	Lithuania	Petkeviciute 2001
		asp	Russia	Tolstenkov et al. 2010
		asp	Ukraine (?)	Pavluchenko and Yermoshyna 2017
	Cercaria duplicata[f]	dig	Europe	Petkevičiūtė et al. 2015
	Echinostomatidae	dig	Poland	Marszewska and Cichy 2015
	Phyllodistomum sp.	dig	Finland	Taskinen et al. 1991
		dig	Ukraine	Kudlai and Yanovich 2013
		dig	Poland	Marszewska and Cichy 2015
		dig	Poland	Müller et al. 2015
	Rhipidocotyle campanula	dig	Europe	Baturo 1977
		dig	Finland	Taskinen et al. 1991
		dig	Finland	Taskinen et al. 1994
		dig	Finland	Taskinen et al. 1997
		dig	Finland	Taskinen 1998a
		dig	Finland	Jokela et al. 2005
		dig	Europe	Petkevičiūtė et al. 2014
		dig	Poland	Marszewska and Cichy 2015
		dig	Poland	Müller et al. 2015
	Rhipidocotyle fennica	dig	Finland	Taskinen et al. 1991
		dig	Finland	Jokela et al. 1993
		dig	Finland	Taskinen et al. 1994
		dig	Finland	Taskinen and Valtonen 1995
		dig	Finland	Taskinen et al. 1997
		dig	Finland	Taskinen 1998a
		dig	Finland	Taskinen 1998b
		dig	Finland	Jokela et al. 2005
		dig	Europe	Petkevičiūtė et al. 2014
		dig	Lithuania	Stunžėnas et al. 2014
		dig	Finland	Choo and Taskinen 2015
	Rhipidocotyle sp.	dig	England	Zieritz and Aldridge 2011
	'Castrating trematodes'[b]	dig	Finland	Jokela 1996
		dig	Ukraine	Yanovich 2015
	Unionicola aculeata	mit	The Netherlands	Davids 1973
		mit	England	Baker 1988

	Unionicola intermedia	mit	The Netherlands	Davids 1973
		mit	England	Baker 1976
		mit	England	Baker 1977
		mit	The Netherlands	Dimock and Davids 1985
		mit	England	Baker 1988
		mit	The Netherlands	Davids et al. 1988
		mit	England	Baker et al. 1992
	Unionicola ypsilophora	mit	The Netherlands	Davids 1973
	Unionicola spp.	mit	?	Jones 1978
	Paraergasalis rylovi	cop	Russia	Chernysheva and Purasjoki 1991
		cop	Finland	Taskinen and Saarinen 1999
		cop	Finland	Saarinen and Taskinen 2003
		cop	Finland	Saarinen and Taskinen 2004
		cop	Finland	Saarinen and Taskinen 2005a
		cop	Finland	Saarinen and Taskinen 2005b
		cop	Finland	Taskinen and Saarinen 2006
	Blastocystis sp.[g]	bac	Poland	Słodkowicz-Kowalska et al. 2015
	Cryptosporidium sp.[g]	bac	Poland	Słodkowicz-Kowalska et al. 2015
	Encephalitozoon hellem	bac	Ireland	Lucy et al. 2008
	Enterocytozoon bienusi	bac	Ireland	Lucy et al. 2008
	Giardia lamblia	bac	Ireland	Lucy et al. 2008
Anodonta cygnea	*Aspidogaster conchicola*	asp	The Netherlands	Bakker and Davids 1973
		asp	Russia	Zhokhov and Gachina 1997
	Phyllodistomum elongatum	dig	Europe	Orecchia et al. 1975
	Unionicola aculeata	mit	The Netherlands	Davids 1973
		mit	England	Baker 1988
	Unionicola intermedia	mit	The Netherlands	Davids 1973
	Unionicola ypsilophora	mit	The Netherlands	Davids 1973
		mit	The Netherlands	Davids et al. 1988
		mit	The Netherlands	Dimock and Davids 1985
		mit	England	Baker 1988
		mit	England	Ernsting et al. 2006
	Unionicola sp.	mit	?	Jones 1978
	Conchophthirus anodontae	cil	“Scandinavia”	Fenchel 1965

	Trichodina unionis	cil	"Scandinavia"	Fenchel 1965
Anodonta sp.	*Aspidogaster conchicola*	asp	Ukraine (?)	Yuryshynets 1998
	Bucephalus polymorphus[h]	dig	Ukraine (?)	Pavluchenko and Yermoshyna 2017
	Unionicola ypsilophora[d]	mit	England	McLachlan et al. 1999
Pseudanodonta complanata	Unionicolidae	mit	Ukraine (?)	Pavluchenko and Yermoshyna 2017
	Paraergasalis rylovi	cop	Finland	Saarinen and Taskinen 2004
Unio crassus	*Aspidogaster antipai*	asp	Romania	Yamaguti 1963
	Aspidogaster conchicola	asp	Russia	Zhokhov and Gachina 1997
		asp	Lithuania	Petkevičiūtė 2001
	Rhipidocotyle campanula	dig	Lithuania	Petkevičiūtė et al. 2014
Unio pictorum	*Aspidogaster conchicola*	asp	The Netherlands	Bakker and Davids 1973
		asp	Russia	Zhokhov and Gachina 1997
		asp	Lithuania	Petkevičiūtė 2001
		asp	Ukraine (?)	Pavluchenko and Yermoshyna 2017
	Rhipidocotyle campanula	dig	Europe	Baturo 1977
		dig	Lithuania	Petkevičiūtė et al. 2014
	Unionicola bonzi	mit	The Netherlands	Davids 1973
		mit	The Netherlands	Dimock and Davids 1985
Unio tumidis	*Aspidogaster conchicola*	asp	Lithuania	Petkevičiūtė 2001
		asp	Poland	Marszewska and Cichy 2015
		asp	Ukraine	Pavluchenko and Yermoshyna 2017
	Rhipidocotyle campanula	dig	Poland	Marszewska and Cichy 2015
	Acanthamoeba sp.	amo	Ukraine	Patsyuk 2017
	Vahlkampfia sp.	amo	Ukraine	Patsyuk 2017
Unio sp.	*Aspidogaster conchicola*	asp	Ukraine (?)	Yuryshynets 1998
	Trichodina unionis	cil	Scandinavia	Fenchel 1965

[a]All aspidogastrid records are for adult trematodes

[b]All bucephalid records are for sporocysts/cercariae

[c]All echinostomatid records are for metacercarial cysts

[d]Eggs

[e]Larvae

[f]This recording is dubious, as UniProt currently lists this species as Digenea *incertae sedis*, and it is possibly an ambiguation of *Phyllodistomum* sp.

[g]Oocysts/cysts

[h]It is generally thought that *B. polymorphus* occurs exclusively in dreissenids. This does not preclude the validity of this recording, but it should be treated with caution

Table A1.4: Summary of all studies which specifically record an absence of a host-endosymbiont association when it was specifically looked for. Parasite categories and notes can be found associated with Table A1.3.

Species	Parasite	Category	Location	Reference
Dreissena polymorpha	*Rhipidocotyle campanula*[b]	asp	Europe	Baturo 1977
		asp	Poland	Marszewska and Cichy 2015
Anodonta anatina	*Aspidogaster conchicola*[a]	asp	Poland	Marszewska and Cichy 2015
	Phyllodistomum sp.[b]	dig	Ukraine	Kudlai and Yanovich 2013
Anodonta cygnea	Trematode cercariae/metacercariae	dig	Wales	Probert 1966
Pseudanodonta complanata	*Phyllodistomum* sp.[b]	dig	Ukraine	Kudlai and Yanovich 2013
	Rhipidocotyle sp.[b]	dig	Finland	Taskinen et al. 1991
Unio crassus	*Phyllodistomum* sp.[b]	dig	Ukraine	Kudlai and Yanovich 2013
Unio tumidis	Echinostomatidae[c]	dig	Poland	Marszewska and Cichy 2015
	Phyllodistomum sp.[b]	dig	Ukraine	Kudlai and Yanovich 2013
		dig	Poland	Marszewska and Cichy 2015
	Rhipidocotyle sp.[b]	dig	Finland	Taskinen et al. 1991
	Paraergasalis rylovi	cop	Finland	Saarinen and Taskinen 2004
	Blastocystis sp.[g]	bac	Poland	Słodkowicz-Kowalska et al. 2015
	Cryptosporidium sp.[g]	bac	Poland	Słodkowicz-Kowalska et al. 2015
Unio pictorum	*Aspidogaster conchicola*[a]	asp	Poland	Marszewska and Cichy 2015
	Echinostomatidae[c]	dig	Poland	Marszewska and Cichy 2015
	Phyllodistomum sp.[b]	dig	Ukraine	Kudlai and Yanovich 2013
		dig	Poland	Marszewska and Cichy 2015
	Rhipidocotyle sp.[b]	dig	Finland	Taskinen et al. 1991
	Rhipidocotyle campanula[b]	dig	Poland	Marszewska and Cichy 2015
	Trematode cercariae/metacercariae	dig	Wales	Probert 1966
	Paraergasalis rylovi	cop	Finland	Saarinen and Taskinen 2004

Part 2: Additional statistical results

This analysis has been conducted exactly as described in section 2.2.4, but removing those records that may not be considered 'true' endosymbionts (chironomid larvae, dragonfly larvae, oligochaetes, nematodes, fungi and human bacteria/diseases). Once again, the negative binomial regression model was a good fit for the data (comparison of residual deviance to a χ^2_{84} distribution, null hypothesis of model fitting data, $p = 0.981$). Results show that the statistical trends are exactly the same as those demonstrated in the main text. The mean number of endosymbionts per host differed significantly between invasive bivalves and native unionids (Overall model deviance = 59.46; d.f. = 2, 84; $p < 0.0001$). Examining the terms in the model in more detail, there was no significant difference between the number of endosymbiont records per host between invasive bivalves in their native and invasive range ($z = -0.61$, $p = 0.543$), but there were 2.8 times fewer records per host in native unionids ($z = -4.25$, $p < 0.0001$; Fig. A1.1). This supports the presentation of the full endosymbiont table data in the main body of the text.

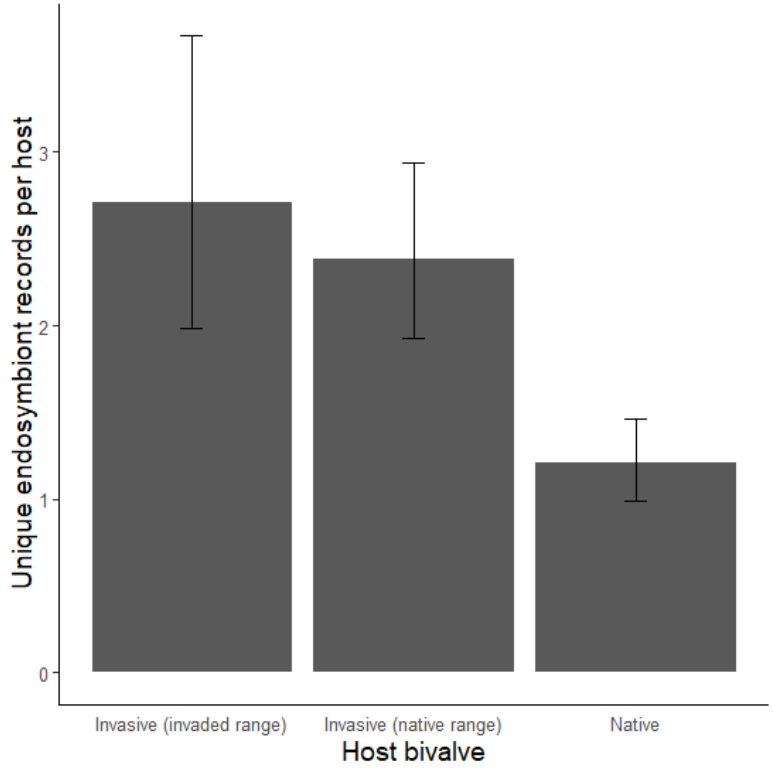

Figure A1.1: Unique endosymbiont records per host for each host category (invasive bivalves in invaded or native range, native unionids) (mean ± SE). Note unevenness of error bars due to back-transformation from logarithmic estimates.

Similarly, it is unlikely that North American results are influenced by records that may not be 'true' endosymbionts (chironomid larvae, dragonfly larvae, oligochaetes, nematodes, fungi and human bacteria/diseases). These records only compose 7.5% of all North American records (and 43% of these come from two studies); given the very large remaining sample size (1128 out of 1220 records), the trends presented can be considered reliable.

Appendix A2: Appendices for Chapter 3

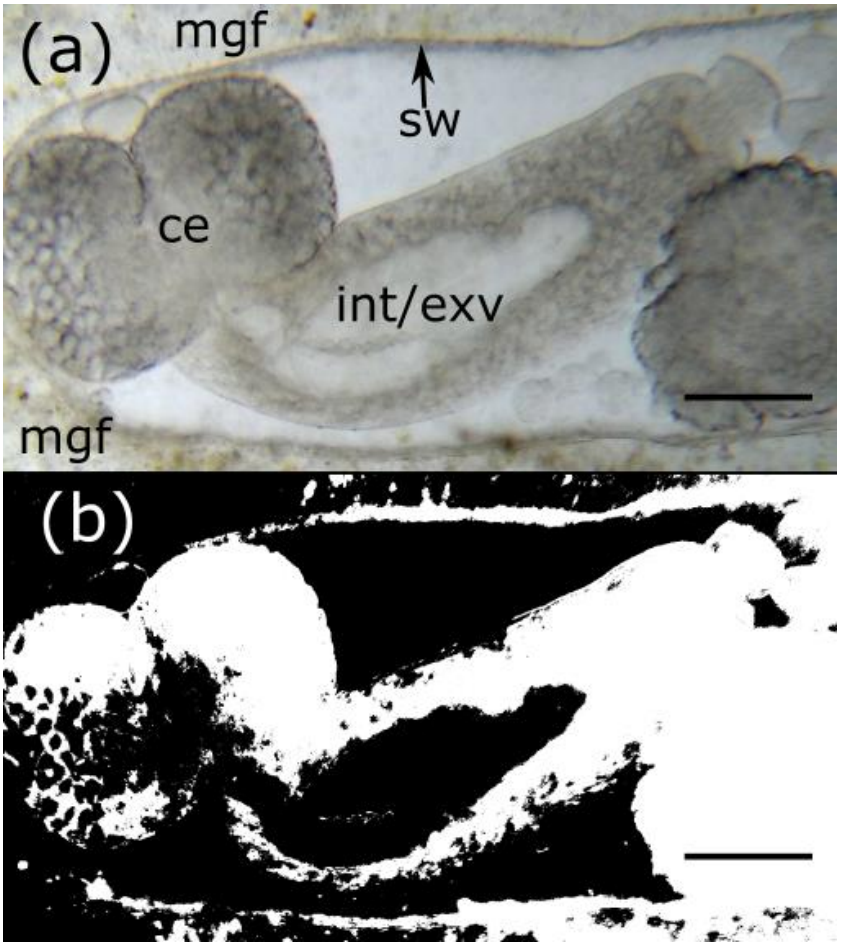

Figure A2.1: Demonstration of an issue with potential computer automation of sporocyst/cercariae recognition, using a close-up of the end of a sporocyst with a developing cercaria inside. Scale bars 250 μM. (a) The developing cercaria (ce) inside the sporocyst wall (sw), with the sporocyst residing in mussel gonadal fluid (mgf). Note the light-coloured developing intestine (int) and excretory vesicle (exv) inside the cercaria. (b) The results of a binary threshold classification of (a) in ImageJ.

Figure A2.1 shows that automation of the tracing and percentage filled procedure may be difficult. Everything inside the sporocyst wall (Fig. A2.1a) should be included as trematode tissue; however, the binary threshold mask (Fig. A2.1b) cannot distinguish between mussel gonadal fluid, the interior of the sporocyst, and intestine/excretory vesicle of the cercaria. Multiple different thresholding algorithms yielded similar results. While it is undoubtedly possible for automation of this procedure, it was beyond the remit of the current study.

Appendix A3: Appendices for Chapter 5

Part 1: Supplementary Methods

Mussel size through the sampling period

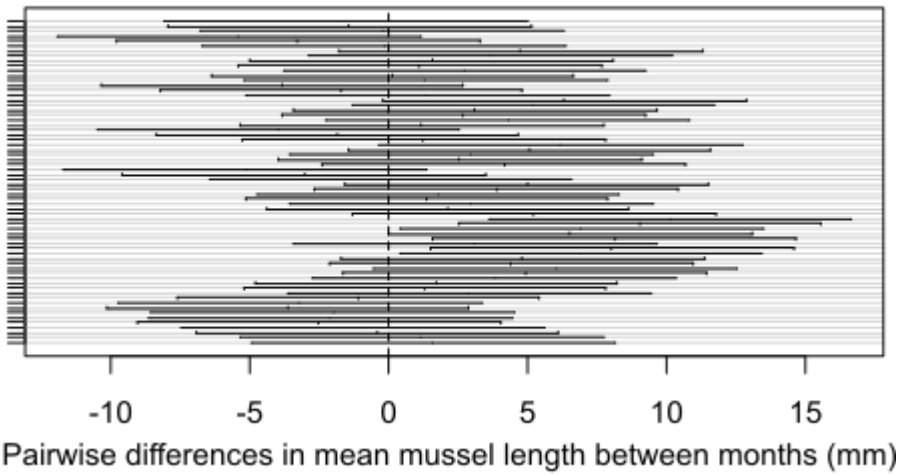

Figure A3.1: Pairwise differences (y-axis) between the mean mussel lengths for all 12 sampling months, beginning with all pairwise comparisons for the first month (top of y-axis) and moving progressively down. Black bars indicate a 95% C.I. for the difference between two months; note that 72 of the 78 C.I. overlap 0.

There was no evidence that mussel size changed significantly throughout the year, or that mean size was affected by our sampling. While there were differences between months ($F_{11, 108} = 4.17$, $p < 0.0001$), this was driven only by slightly smaller mussels in months 5 and 6 (Tukey post-hoc test, $p < 0.05$ for 6 of the 21 pairwise comparisons for these two months), consistent with timings of recruitment of new *A. anatina* into the population. Fig. A3.1 shows no clear pattern of sampling causing a consistent reduction in overall mussel size.

Mussel dissection and parasite isolation

Following collection, mussels were transported back to the laboratory in 10 L buckets in river water. In the laboratory, mussels were held at 8 °C under aeration, for a maximum of 72 h before dissection. Immediately prior to dissection, mussels were rinsed under cold fresh water while holding the valves gently shut to remove any organisms on the shells, and had their maximum length recorded to the nearest 0.5 mm with Vernier callipers.

All tissues of the mussel were inspected in systematic fashion. In all stages of dissection, both the presence and the number (i.e. abundance) of all parasites found were recorded, and subsequently identified to the finest possible taxonomic resolution.

Mussels were cut open by gently inserting a scalpel between the valves and slicing the posterior and anterior adductor mussels over a transparent petri dish, to allow all fluids from the mantle cavity to be collected. These fluids were then inspected under a GXMMZS0745-T stereomicroscope at 16× magnification. Following this, the mussel was placed under the stereomicroscope and the mantle and labial palps on both sides were systematically searched. The inner and outer demibranchs (gills) were then carefully removed individually, and the filaments gently teased apart under 16× magnification to record all the parasites present in the gills. The visceral mass was gently removed by cutting the connective tissue at each end, and was briefly placed aside, to expose the pericardial cavity, which was also dissected under 16× magnification. The visceral mass was then cut open with a scalpel at the posterior end where the gonads were located, and examined: samples of gonad tissue were removed with tweezers and pressed between two microscope slides to create a squash ~10 mm in diameter, to quantify infection (Chapter 3). These samples were inspected under a GXML3200 compound microscope at 40× magnification. In addition, each sample had three photos taken with a GXM HiChrome-S camera attached to the microscope to quantify infection with digenean trematodes.

The dissecting tray and all equipment were then washed before proceeding to the next mussel. Mussel shells were dried fully and weighed to the nearest 0.001 g, and mussel tissue was dried to constant mass (nearest 0.001 g).

Following all dissections, the water remaining in the transport and holding buckets was stirred thoroughly to agitate any sediment, and three 10 mL samples were taken from the bottom of each bucket and inspected under 16× magnification, to confirm that storage in the buckets did not induce parasites to leave the mussels and affect results. These samples contained large gammarid amphipods (which were commonly observed on the exterior of mussel shells) and very rarely oligochaetes and leeches, which were never observed inside mussels and were also likely present via exterior attachment. No mussel parasites were observed in these samples, which suggests the communities observed upon dissection were consistent with those that were present at collection.

Parasite identification

Broadly, the major parasite groups observed were ciliates, mites, aspidogastrean trematodes, bucephalid trematodes, echinostomatid trematodes, bitterling embryos, nematodes, chironomid larvae, oligochaetes, and amphipods. Identifications were made to the finest taxonomic resolution possible for each of these groups. As additional verification, all previous parasite records for this and closely-related mussel hosts were inspected (summarised in Chapter 2, Table A1.3), and significant deviations from previous records carefully checked.

Ciliates were isolated from the tissues they appeared in, stained with methyl-green pyronin (to highlight the nuclear material), then mounted and inspected under 400× magnification

following Foissner and Berger (1996). Two species of ciliates were clearly distinguished. The first was observed in the mantle (localised particularly on the labial palps), and was identified as *Conchophthirus* sp. (Fig. A3.2). The specific species is uncertain, as there are few explicit taxonomic keys available for unionid ciliates. One of the most useful is that of Kidder (1934); the mantle species observed here matches the life history strategy (with particular note of the location on the labial palps) of *C. anodontae*, but the location of the macronucleus and distribution of endoplasmic granules align very closely with that of *C. curtus*. Given this uncertainty, this mantle ciliate has been identified to genus only.

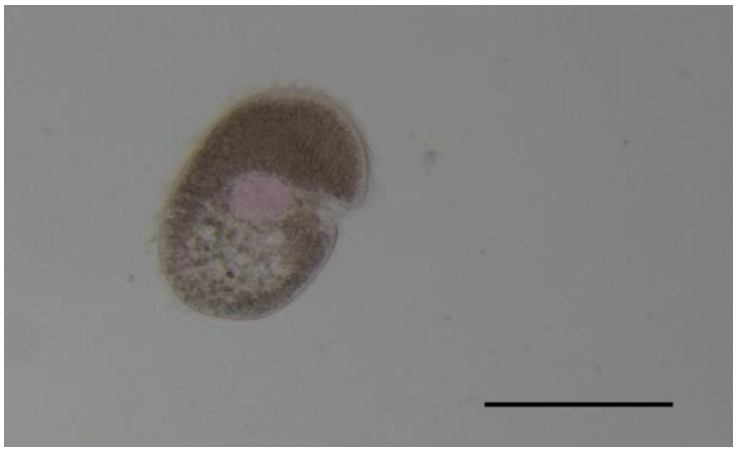

Figure A3.2: *Conchophthirus* sp. Note the macronucleus (pink). Scale bar 100 μM.

The second ciliate species was observed in the gills, and identified as *Tetrahymena* sp., possessing all the broad characteristics of this genus (Fig. A3.3). The macronucleus of this species proved difficult to stain, with no clear macronuclear region. However, there were small pink deposits through the cytoplasm, possibly representing the nuclei of ingested host cells (see Lynn et al. 2018). There appear to be no keys for the identification of *Tetrahymena* species, and so the classification was only done to genus level. Given that, to our knowledge, these are first ciliate records from *A. anatina* (see Table A1.3), it is possible and even likely that one or both are novel species.

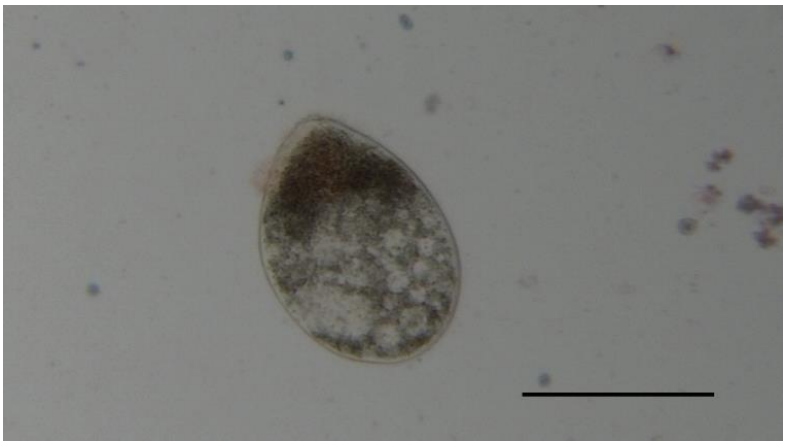

Figure A3.3: *Tetrahymena* sp. Note the absence of clear staining. Scale bar 150 μM.

The gonadal pore and leg segmentation of mites were inspected under 400× magnification and compared with the keys of Vidrine (1986), in addition to the ecological information of Baker (1988). Mite eggs were also observed; these were not identified directly, but inferred to be of the same species given the congruence of a linear pattern of occurrence on the mantle for both larval mites and mite eggs. Mites were identified as *Unionicola intermedia* (Fig. A3.4). The mites present were typically larval, as the adults are transient and do not spend extended periods of time in the mussel, but adults and later nymphal stages of the mites were also observed. Mites and mite eggs were typically on the mantle, though one nymphal stage was also occasionally observed on the gill margins.

Figure A3.4: *Unionicola intermedia* adult, from mantle cavity of *A. anatina*. Scale bar 750 μM.

Identification of: (a) aspidogastrean trematodes; (b) bucephalid trematodes; and (c) echinostomatid trematodes, was through consultation with (a) Huehner and Etges (1977), Schell (1985), Alves et al. (2015); (b) Taskinen et al. (1991), Gibson et al. (1992); and (c) Conn and Conn (1995), Molloy et al. (1997), Sohn (1998); respectively. Aspidogastrean trematodes were identified as *Aspidogaster conchicola* (Fig. A3.5). These have a simple life history with a single host (the mussel). Both juvenile and adult *A. conchicola* were observed in the mantle cavity under the visceral mass. Bucephalid trematodes were identified as *Rhipidocotyle campanula* (Fig. A3.6). These have a complex life cycle with three hosts; they utilise mussels as their first intermediate hosts (see Taskinen et al. 1997 for a summary of the life cycle), with sporocysts and cercariae present in the mussel gonad. These fill the gonad, and can induce complete castration in their mussel hosts. Cercariae are then released to infect the next intermediate host in the life cycle. Echinostomatid trematodes were identified as *Echinoparyphium recurvatum* (Fig. A3.7). These trematodes also have a complex life cycle with three hosts (see Molloy et al. 1997), and utilise the mussel as a second intermediate host. Metacercariae of this species were observed in the mussel gonad.

Figure A3.5: Juvenile *Aspidogaster conchicola*. Scale bar 250 μM.

Figure A3.6: *Rhipidocotyle campanula* cercariae. Scale bar 250 μM.

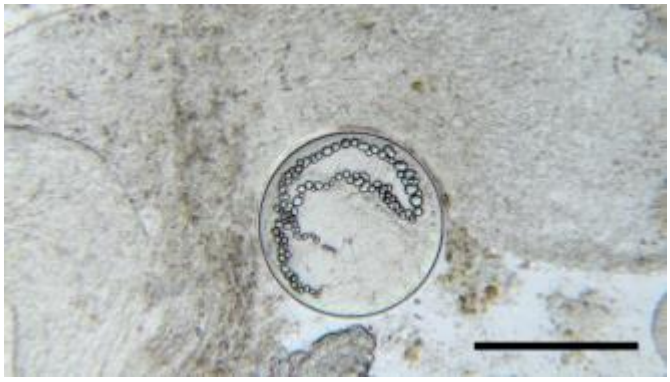

Figure A3.7: *Echinoparyphium recurvatum* metacercaria. Scale bar 150 μM.

Bitterling are freshwater fish that lay their eggs in the gills of freshwater mussels, where they constitute a stress both in terms of physical disfiguration and oxygen competition (Reichard et al. 2006; Methling et al. 2018). The bitterling embryos were readily identified as *Rhodeus amarus*, given this is the only sympatric fish with this life history strategy (Reynolds et al. 1997; Aldridge 1999b).

Chironomid larvae and nematodes were identified as far as possible by isolating them from the mussel mantle and inspecting their head structures under 40× (chironimids) or 400× (nematodes) magnification, respectively. In both cases, it is difficult to get fine taxonomic resolution, particularly for nematodes as it is likely that the nematodes observed inside mussels are larval (McElwain et al. 2019), and are therefore missing many of the diagnostic characteristics of adults. Literature consulted included Stewart and Loch (1973) and Pinder (1986) for chironomids, and Abebe et al. (2006), Gagarin and Gusakov (2016) and McElwain et al. (2019) for nematodes. Chironomids were classed as subfamily Chironominae, while nematodes were classed as order Dorylaimida (Fig. A3.8); it should be noted that in both of these cases, it is possible that there were multiple species within these groupings.

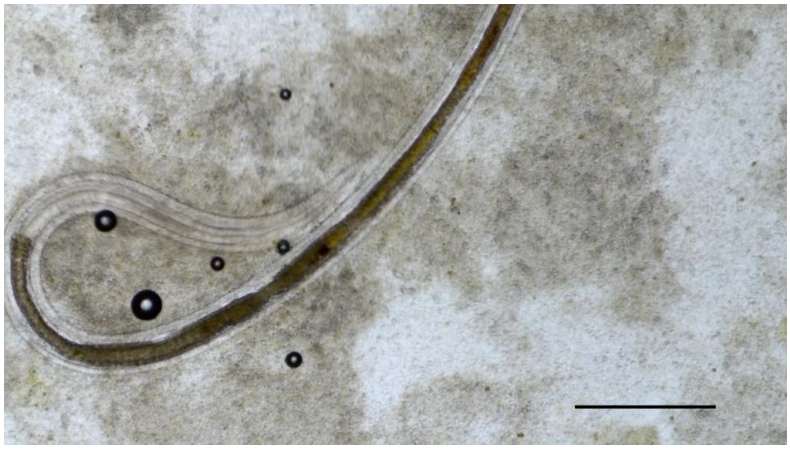

Figure A3.8: Head and start of body of a Dorylaimid nematode. Scale bar 300 μM.

All oligochaetes had the same appearance, which did not match the appearance of any previously recorded oligochaetes in the mantle of freshwater mussels (see Table A1.3). However, the oligochaetes seen were strikingly similar to *Tubifex tubifex* (compared through online taxonomic resources such as the Marine Life Information Network [marlin.ac.uk] and the World Register of Marine Species [www.marinespecies.org], both of which include freshwater species), and distribution patterns also match *T. tubifex,* and so the oligochaetes observed have been assigned this taxonomic classification.

Gammarid amphipods were also occasionally observed in the mantle. Given the freshwater environment in which these amphipods occurred, they have been assigned to the suborder Senticaudata (Lowry & Myers 2013), but further taxonomic classification was not attempted.

In addition to these parasites, several others were observed at frequencies <1%, including single observations of different leech species as well as echinostomatid and bucephalid trematodes. These were not included in the final parasite matrix, as models become increasingly difficult to fit with excess zeros (Ovaskainen et al. 2016; Lammel et al. 2018), and their extreme rarity suggests low importance (and predictive power) in mussel parasite communities.

Construction of the parasite matrix

In three cases, the same parasite species was observed in multiple locations in the mussel, or in different life-history stages. First, *Unionicola intermedia* was present as both mites and eggs in the mantle. Second, live *Conchophthirus* sp. was observed both in the mantle but also occasionally in the gonad. It is unclear how these ciliates ended up in the gonad; given their localisation on the labial palps, which coveys food to the mussel's mouth, they were perhaps accidentally transported into the visceral mass. Third, *Rhipidocotyle campanula* occurred both as sporocysts and cercariae in the gonad, but also as sporocysts running transversely through the gills. In all three of these cases, observations of different forms of the same parasite appear to be independent (for example, cases were observed where there was *R. campanula* present in the gonad but not gills, cases where it was in the gills but not the gonad, cases where both were observed, and cases where neither were observed).

As a result, we believe it is an over-simplification to conglomerate presence or abundance recordings into one category. For example, bitterling (who lay their embryos in the gills) may react negatively to *R. campanula* sporocysts in the gills, but sporocysts in the gonad may have no effect. Given the purpose of this study is to determine possible drivers of parasite community structure, important parasite-parasite interactions could be missed by failing to include life-history forms or different locations of the same parasite. The importance of not grouping potentially independent parasite types has been recently emphasised (Poulin 2019).

Therefore, the parasite matrix ($\mathbf{Y_{AB}}$) was constructed with 720 rows (sampling units, the mussels) and 14 columns, as follows: *Conchophthirus* sp. (mantle); *Conchophthirus* sp. (gonad); *Tetrahymena* sp.; *Unionicola intermedia* (mites); *Unionicola intermedia* (eggs); *Aspidogaster conchicola*; *Rhipodicotyle campanula* (gonad); *Rhipidocotyle campanula* (gills); *Echinoparyphium recurvatum*; *Rhodeus sericeus*; Chironominae; Dorylaimida; *Tubifex tubifex*; Senticaudata. See Table 5.1 (main text) for a summary of occurrences.

Part 2: Supplementary Results

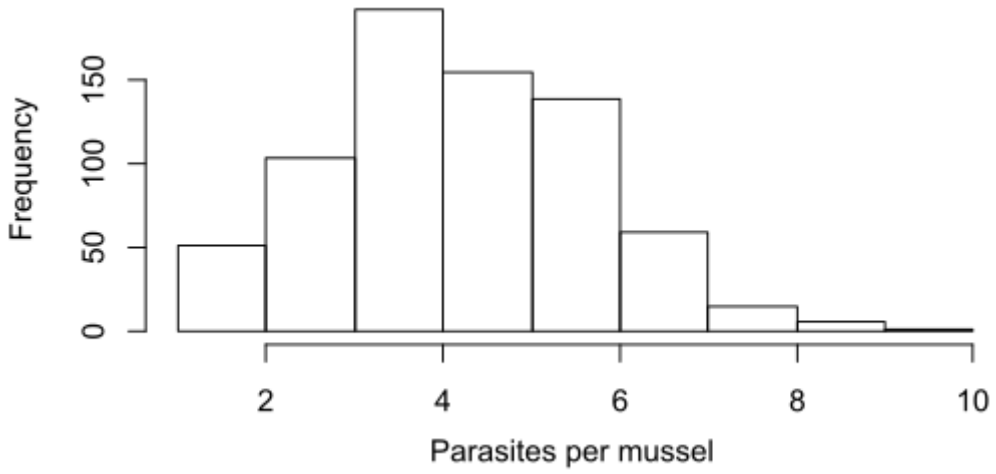

Figure A3.9: Histogram showing the distribution of parasite frequency counts per mussel.

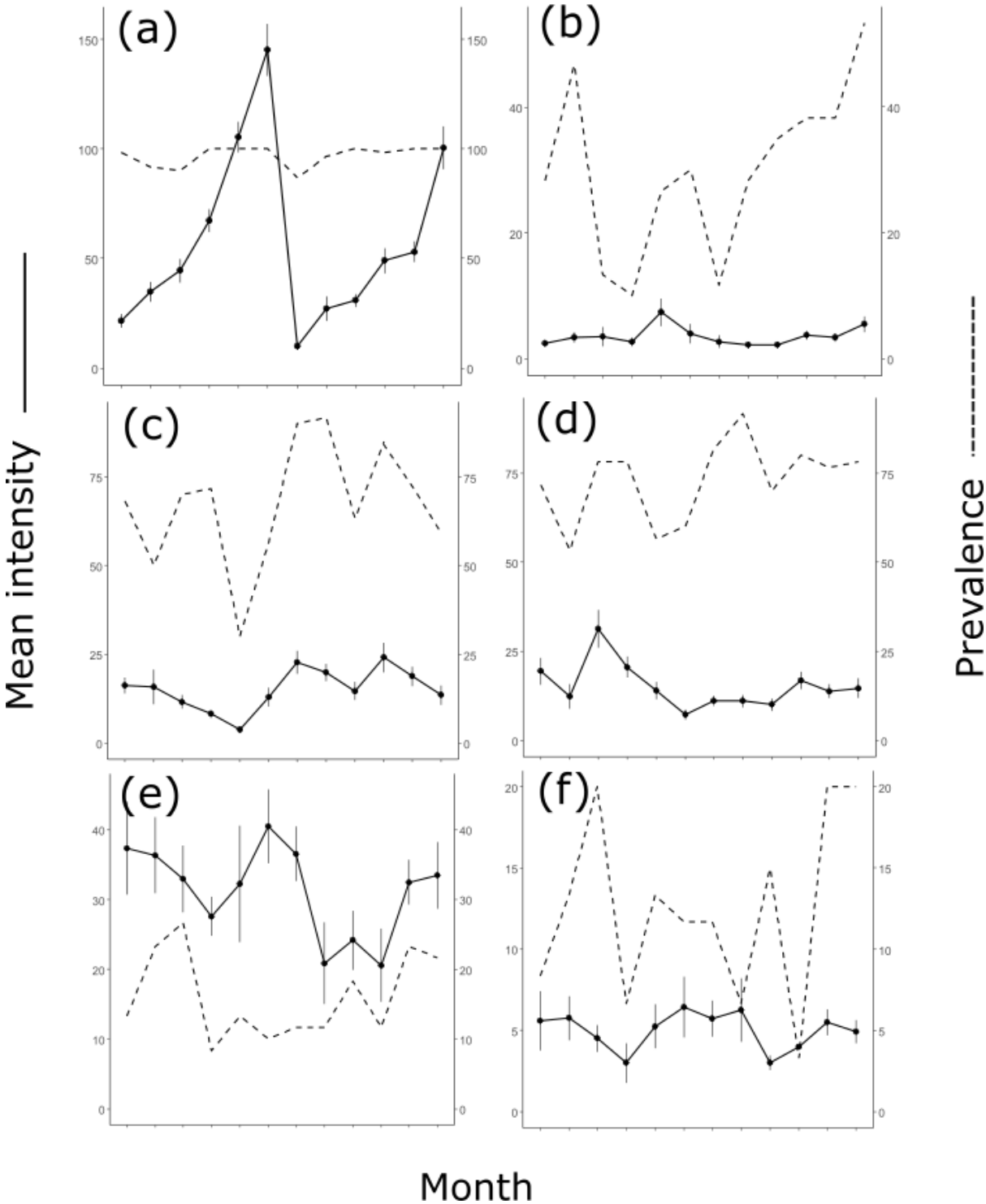

Figure A3.10: Mean intensity (± S. E.) and prevalence (as a percentage) for: (a) *Conchophthirus* sp. (mantle); (b) *Conchophthirus* sp. (gonad); (c) *U. intermedia* (mites); (d) *U. intermedia* (eggs); (e) *R. campanula* (gonad); and (f) *R. campanula* (gills). Note that each y-axis possesses a different scale.

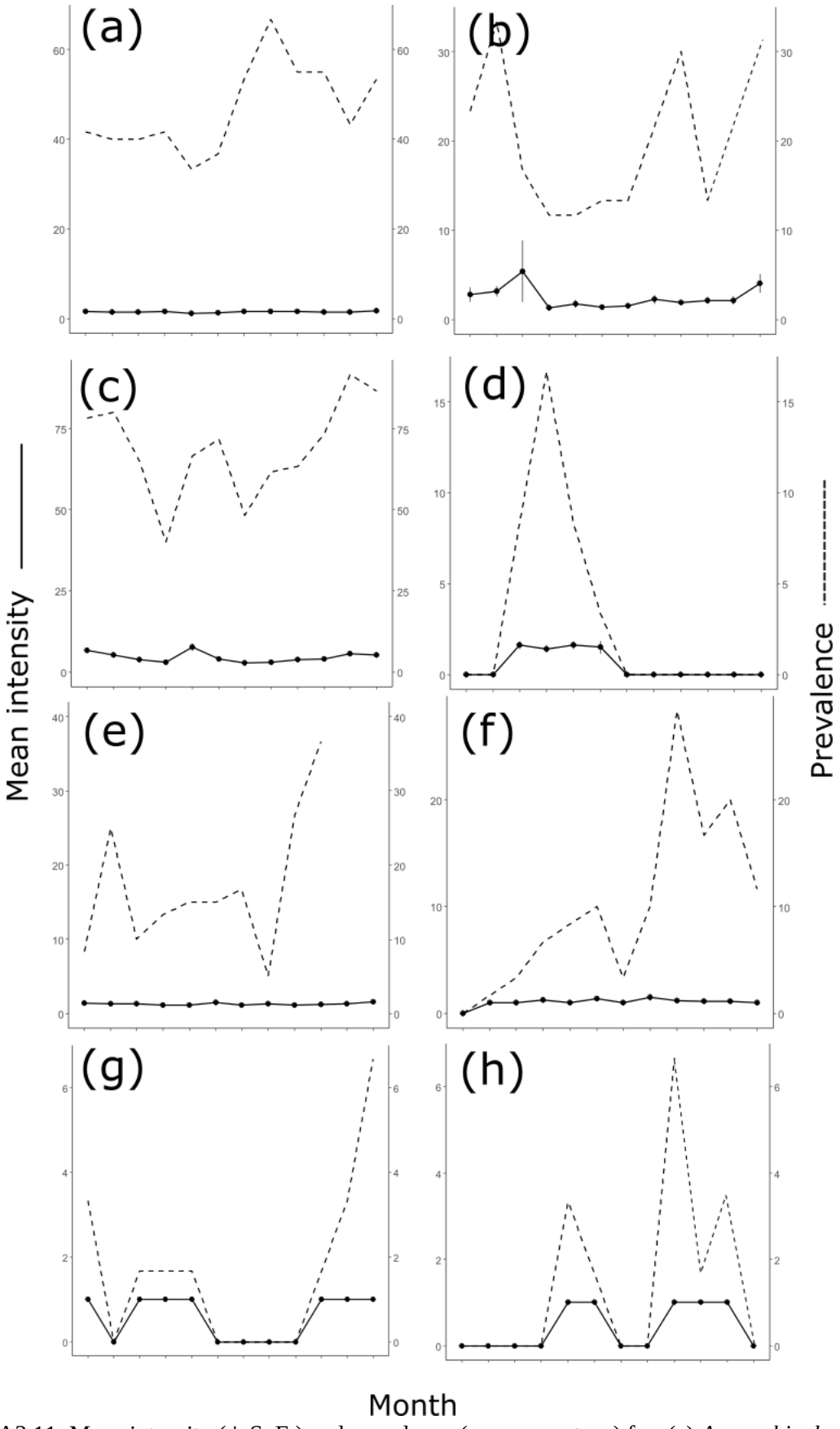

Figure A3.11: Mean intensity (± S. E.) and prevalence (as a percentage) for: (a) *A. conchicola*; (b) *E. recurvatum*; (c) *Tetrahymena* sp.; (d) *R. sericeus*; (e) Dorylaimida; (f) Chironominae; (g) *T. tubifex*; and (h) Senticaudata. Note that each y-axis possesses a different scale.

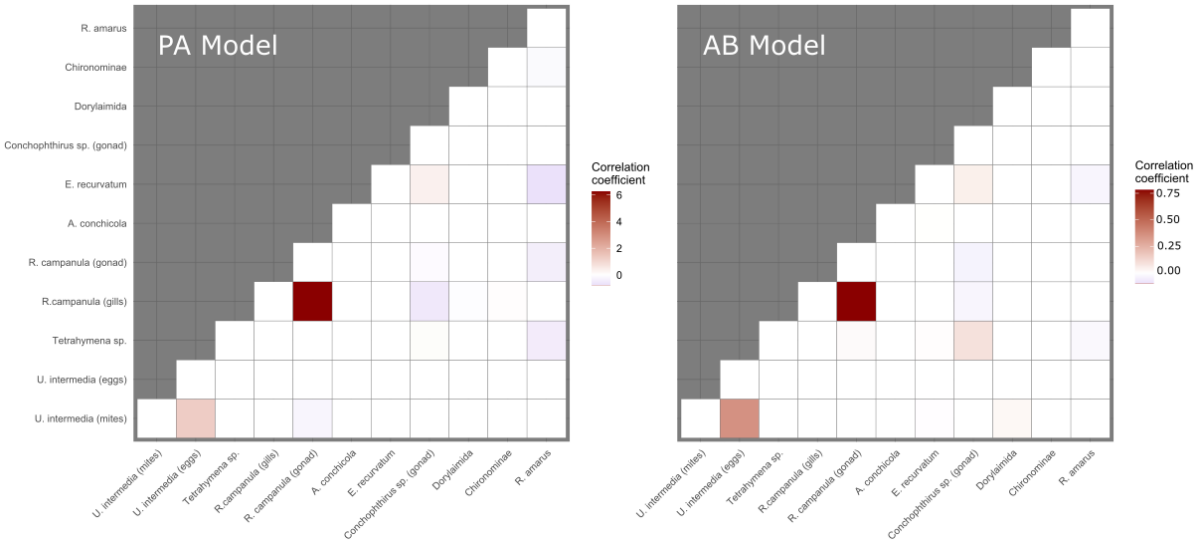

Figure A3.12: Matrices of residual parasite interactions associations extracted from the Markov Random Fields modelling, with the morphospecies maintained as separate entries. Only interactions with > 95% confidence are shown. Red indicates positive correlation, while blue represents negative correlation. Note the much stronger correlation coefficients for the PA model relative to the AB model. Also note the strong correlations between the two *U. intermedia* entries, and between the two *R. campanula* entries, the strength of which mask the (still significant) possible associations between other parasites.

Table A3.1: Exploring how much of the variation in parasite species' responses to individual environmental covariates can be explained by the trait matrix **T** (life history of the parasite, and location of the parasite in the mussel).

Environmental Covariate	AB model	PA model
Length	47.2%	38.9%
Gravid Status	75.4%	64.1%
Zebra mussel presence	29.9%	20.4%
Month	41.4%	36.6%
Weight	16.5%	21.7%
Average	**42.1%**	**36.3%**

Table A3.2: Predicted importance of environmental covariates in the MRF modelling framework.

Environmental Covariate	AB model	PA model
Month	65.3%	59.9%
Gravid Status	10.3%	8.7%
Length	8.1%	2.9%
Zebra mussel presence	0.9%	0%
Weight	0.3%	0%

The results from Table A3.2 support the conclusions drawn from the RDA and JSDM models, with month, mussel length and gravidity being the three most important factors.

Appendix A4: Appendices for Chapter 6

Part 1: Supplementary Methods

Parasite identification

Most of the parasites in the present study were identified according to the procedures outlined in Appendix A3 Part 1. This includes:

- *Conchophthirus* sp.
- *U. intermedia* (mites and eggs)
- *R. amarus*
- *Tetrahymena* sp.2 (note that in Chapter 5 and Appendix A3 this is referred to as '*Tetrahymena* sp.')
- *A. conchicola*
- *R. campanula* (in both the gill and gonad)
- Dorylaimida
- Chironominae
- Echinostomatidae (note that in Chapter 5 the only echinostomatid trematode found was *Echinoparyphium recurvatum*; however, the ethanol storage occasionally made it difficult to make out fine-scale features of the metacercariae in the gonad and therefore discrimination to species level was not possible in the present study – to be conservative, all trematode metacercariae were given the classification of Echinostomatidae).

The parasites below were identified for the first time in the present study.

The ciliates *Trichodina* sp. were commonly observed in the mantle (Fig. A4.1).

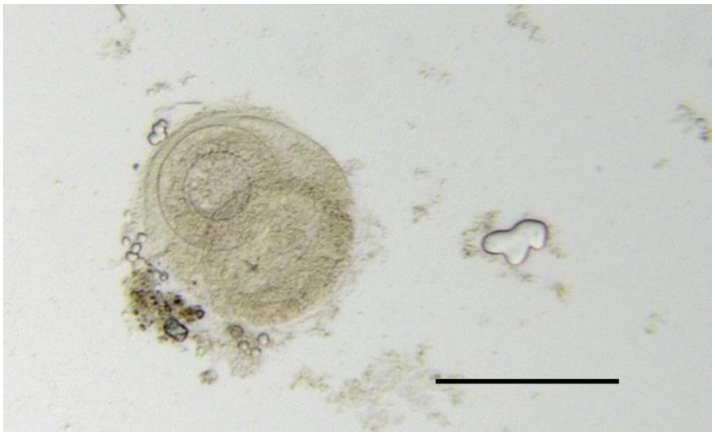

Figure A4.1: *Trichodina* sp. Scale bar 250 µM.

This was identified as genus *Trichodina* through consultation with Irwin et al. (2017) and Wiroonpan and Purivirojkul (2019). There is the possibility of this being *T. unionis*, given it has previously been observed as common within the hosts in this study (Fenchel 1965). However, given the absence of clear keys it has been conservatively identified as *Trichodina* sp.

The ciliates *Tetrahymena* sp.1 were also commonly observed in the mantle (Fig. A4.2).

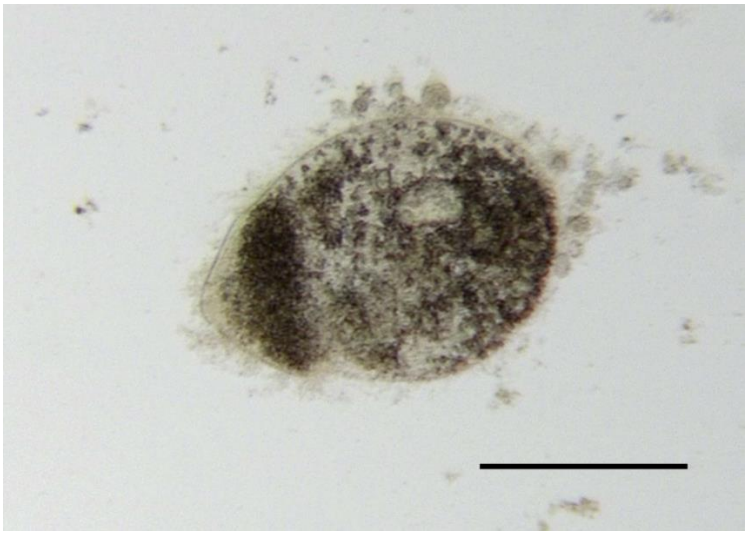

Figure A4.2: *Tetrahymena* sp.1. Scale bar 250 µM.

Identification of the genus *Tetrahymena* was done with reference to Martins et al. (2015) and Lynn et al. (2018). A lack of clear keys restricted the identification to genus only.

Finally, the mite *Unionicola bonzi* was identified through the ecological and descriptive information available in Davids (1973).

In two cases (*U. intermedia*, *R. campanula*) there are two separate entries in the parasite matrix. This is because they occurred in two different life-history stages (eggs and larval mites, *U. intermedia*) or in two distinct host tissues (gills and gonad, *R. campanula*). These occurrence of these entries did not appear to be obligate (e.g. there were instances in which *R. campanula* was present in the gills but not the gonad, instances in which it was present in the gonad but not the gills, and instances where it appeared in both). We argue that in both cases, the two forms cannot necessarily be assumed to be equivalent on their effects on the host, and therefore we have included both forms as separate entries in our parasite-host incidence matrix.

Joint Species Distribution Modelling

To further investigate drivers of community structure, we utilised a joint species distribution model, using the `Hmsc` package (Tikhonov et al. 2019). This uses a Bayesian framework to predict not only an individual's response to environmental space, but also species-species interactions after accounting for all environmental variables. As this package can accept both presence-absence and quantitative data in the same procedure, we utilised the intensity of infection where it was available. The same six variables as for the CRF (Visit, Site, Species, Length, Weight, Sex) were included in the model.

The model was constructed with the default `Hmsc` priors (Tikhonov et al. 2019) using 750,000 samples for each of 2 MCMC chains, with the first 250,000 samples discarded as

burn-in and the remainder thinned to every 500th sample. Parasites for which intensity data was available were modelled with a lognormal Poisson distribution, while parasites that only had presence-absence data available were modelled using probit regression. We confirmed convergence of beta and omega parameters, and further assessed model performance through four-fold cross-validation. Model performance was excellent (Table A4.2), suggesting predictions were reliable. We partitioned variance in the host parasite communities among the six explanatory variables.

Part 2: Supplementary Results

Table A4.1: All parasites found in the study, including a general description of life-history and the location they occupy in the host. Host species refers to the present study only, and does not preclude the occurrence of these parasites in other host species. Presence/absence indicates that only the occurrence of a parasite in a host was recorded, while Intensity indicates that the numbers of that parasite per host were recorded.

Parasite	General description and location in host	Host species	Presence/absence or intensity
Conchophthirus sp.	Ciliate that lives in the mantle; particularly associated with labial palps	Both	Presence/absence
Tetrahymena sp.1	Ciliate that lives in the mantle	Both	Presence/absence
Trichodina sp.	Ciliate that lives in the mantle	Both	Presence/absence
Unionicola intermedia (mites)	Larval mite that encysts in the mantle	Both (but very rarely in *U. pictorum*)	Intensity
Unionicola intermedia (eggs)	Mite eggs that also encyst in the mantle	Both (but very rarely in *U. pictorum*)	Intensity
Unionicola bonzi	Mite that occupy the gills	*U. pictorum* only	Intensity
Rhodeus amarus embryos	Embryos of bitterling fish that are deposited into the gills of the host mussel	Both	Intensity
Tetrahymena sp.2	Ciliate that lives in the gills	Both	Presence/absence
Aspidogaster conchicola	Aspidogastrean trematode that lives in the mantle, particularly under the pericardial cavity	Both (but very rarely in *U. pictorum*)	Intensity
Echinostomatidae	Echinostomatid trematode that encysts in the gonad as metacercariae	Both	Intensity
Rhipidocotyle campanula (gills)	Bucephalid trematode that produces long sporocysts running transversely through the host gills	*A. anatina* only	Presence/absence
Rhipidocotyle campanula (gonad)	Bucephalid trematode that produces long sporocysts and cercariae that occupies the gonad and castrates the host	*A. anatina* only	Presence/absence
Dorylaimida	Nematode that lives in the mantle	Both	Intensity
Chironominae	Chironomid larvae that live in the mantle	Both	Intensity

Table A4.2: Summary of Hmsc model performance. Tjur's R^2 measures the difference between mean fitted values between presences and absences; therefore a value and confidence limits >0 suggests the model performs significantly better than chance. AUC (Area Under the Curve) indicates the likelihood of successfully predicting a presence or an absence for a given parasite in a given host; therefore a value and confidence limits >0.5 suggests the model performs significantly better than chance at predicting presences and absences. The standard measures (first two rows, PA) are appropriate for parasites that only have presence/absence data; the next two rows (IN) provide the equivalent metric for intensity data. The final row refers to how well the model ranks abundances, can be interpreted as a traditional R^2, and is only used for intensity data.

Performance metric	Mean	Standard deviation
PA.Tjur's R^2	0.233	0.200
PA.AUC	0.822	0.057
IN.Tjur's R^2	0.449	0.211
IN.AUC	0.884	0.084
SR^2	0.460	0.186

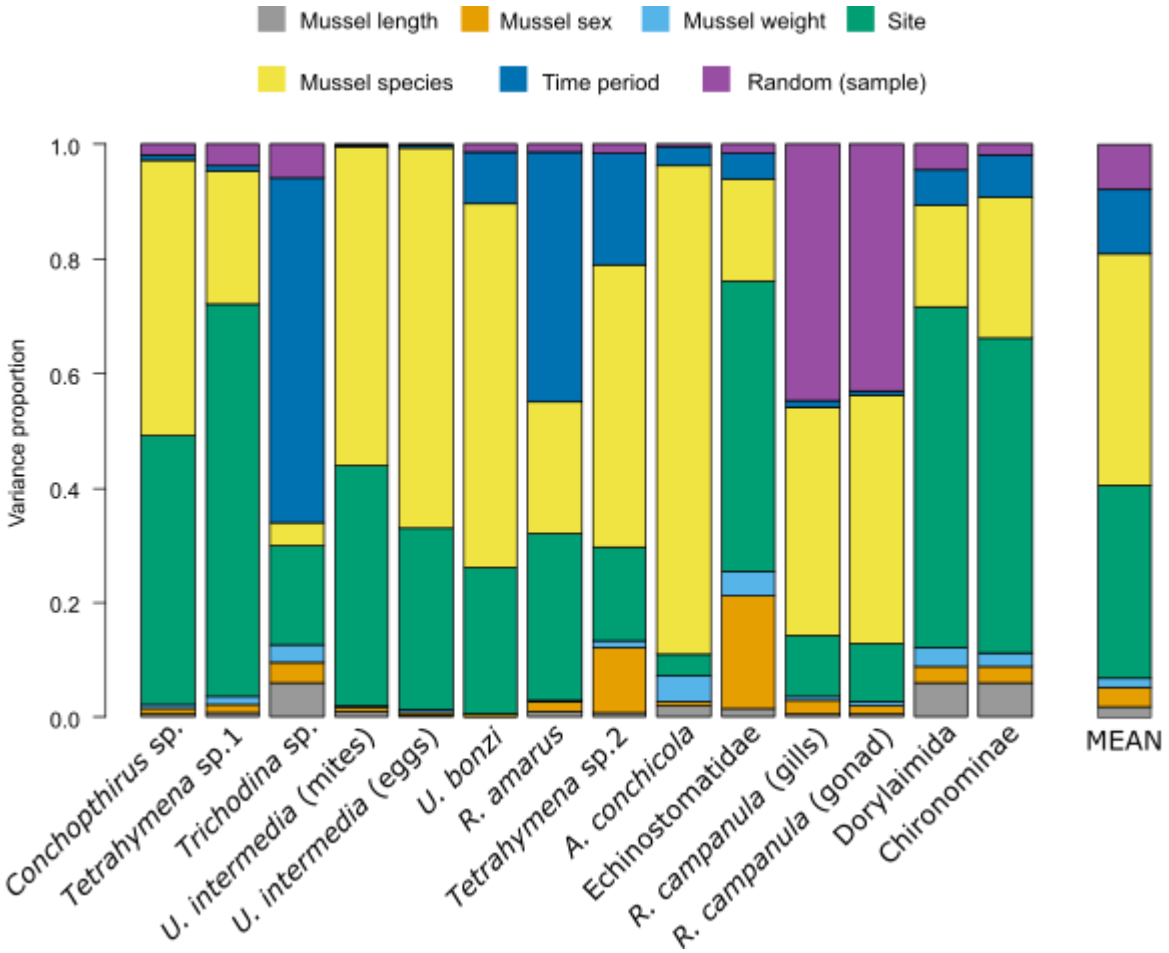

Figure A4.3: Variance partitioning results from the joint species distribution model. The total variation in presence/abundance of each parasite is partitioned among the six explanatory variables, in addition to random variation not attributable to any explanatory variable (purple). Overall mean is presented on far right.

Appendix A5: Appendices for Chapter 7

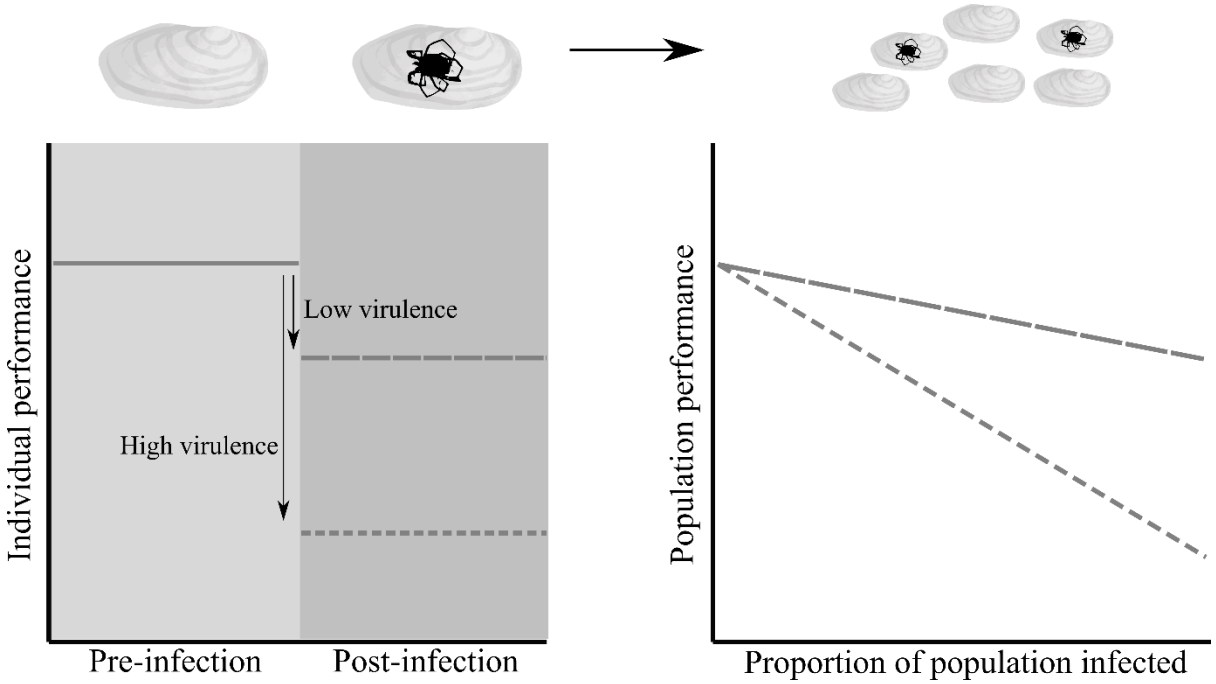

Figure A5.1: Parasite pressure affects performance of organisms (e.g. the reproductive output or filtering capacity of freshwater mussels) at the individual level (left), which scales to the population-level depending on the virulence and prevalence of that parasite (right).

In order to estimate parasite influence on the reproductive output of mussel populations (i.e. to link the left and right panels in Fig. A5.1), we incorporated results of all analyses into a model to predict glochidial output in the absence of parasitism, and in the presence of parasitism (i.e. the actual scenario). We chose to express reproductive output of the population in terms of glochidial production (in g) per 100 g of shell mass, as this measure does not require assumptions about total population size.

First, the proportion of mussels expected to be gravid at each site in the presence and absence of trematodes was calculated. It was assumed that mussels infected were trematodes were castrated (see Results). This utilised four proportions (each with associated 95% binomial confidence intervals, equations [1] – [4]). Note the probability of being gravid was calculated by excluding mussels identified as hosting trematodes, under our assumption that they are guaranteed to not be gravid.

$P_{OW,\ Gravid} = 0.47\ (0.33, 0.61);$ [1]

$P_{BC,\ Gravid} = 0.53\ (0.39, 0.66);$ [2]

$P_{OW,\ Trem} = 0.15\ (0.08, 0.27);$ [3]

$P_{BC,\ Trem} = 0.05\ (0.01, 0.14).$ [4]

From equations [1] and [2], the minimum and maximum number of mussels expected to be gravid at each site (out of the 60) was calculated. For the parasitism runs, equations [1] and [2] were applied to the number of mussels 'available' to be gravid by first working out the minimum and maximum number of mussels likely to be infected with trematodes (and thus castrated), using equations [3] and [4]. This yielded the following ranges of mussels predicted to be gravid (out of the total of 60 mussels):

$R_{OW,\ no\ trem} = 20 – 37;$ [5]

$R_{OW,\ trem} = 15 – 34;$ [6]

$R_{BC,\ no\ trem} = 24 – 40;$ [7]

$R_{BC,\ trem} = 20 – 39.$ [8]

Then, the model (further described below) was run four times, each with 1000 replicates: for OW with and without parasites, and for BC with and without parasites.

For each replicate, we sampled a random number of mussels from the intervals described in equations [5] – [8] (depending on the run), which represented the pool of gravid mussels in the population for that particular replicate. The probability of any one mussel being selected in a given replicate was weighted according to their probability of being gravid, as larger mussels were more likely to be gravid. Weights were assigned in site-specific fashion according to equations [9] and [10] and represent the relative probability of being gravid in a given replicate, where x = length:

$$P_{OWgravid} = \frac{e^{-7.905+0.113x}}{1+ e^{-7.905+0.113x}} \quad [9]$$

$$P_{BCgravid} = \frac{e^{-1.932+0.029x}}{1+ e^{-1.932+0.029x}} \quad [10]$$

Then, the glochidial mass of all mussels selected in a given replicate was summed. Because not all mussels had a measured glochidial mass (as not all mussels were gravid in the actual sample), mussels that were not gravid originally had their predicted glochidial mass calculated by site-specific length equations (equations [11] and [12], where x = length), as glochidial mass correlated strongly with length ($R^2 = 0.79$). While power relationships had a slightly stronger R^2 value, the relationships were modelled as linear, as power equations consistently under-predicted glochidial mass for mussels of intermediate size (where the majority of mussels that were not originally gravid lay).

$Glochmass_{OW} = 0.0707x - 3.4646$ [11]

$Glochmass_{BC} = 0.0839x - 4.3911$ [12]

For each replicate, the summed glochidial mass was multiplied by the average glochidial viability of that site; this varied depending on the run, as mites reduced glochidia viability at

BC (see Results). Therefore, for the BC run in the presence of parasites, summed glochidial mass was multiplied by 0.768; for the other three runs (BC with no parasites, and OW in the presence and absence of parasites), it was multiplied by 0.785. This yielded the total mass of viable glochidia, which we then divided by the total shell weight of all 60 mussels (of either BC or OW mussels depending on the run). This produced an estimate for each replicate of the mass of viable glochidia per 100 g of shell weight. While this exercise used the sample size of 60 mussels at each site, assuming that our sample is representative of the overall mussel populations, this estimate is generalisable to the population as a whole, as it is independent of the actual number of mussels sampled. In addition, this approach accounts for slight size differences between BC and OW mussels, though there were no significant differences observed (see Results).

The overall results of these models were four means (averaged over the 1000 replicates) with associated 95% confidence intervals: viable glochidial mass per 100 g of shell weight for BC in the absence of parasitism; for BC in the presence of parasitism; for OW in the absence of parasitism; and for OW in the presence of parasitism. We also calculated the actual value of viable glochidial mass per 100 g shell mass for BC and OW in the study, and compared those values to the model results in the presence of parasitism, to confirm that our model gave realistic predictions.

The model was achieved by running the following bespoke function four times in R, with the inputs adjusted according to the run (BC with parasites, BC without parasites, OW with parasites, OW without parasites), as below:

- `glocweight` is a vector of individual glochidia weights for each mussel, calculated using equations [11] and [12] where applicable;
- `gravidrange` is a vector of all numbers within the ranges specific by equations [5] – [8];
- `viability` is a single value, representing mean viability of glochidia;
- `shellweight` is a single value, representing the total shell weight of all mussels at the relevant site;
- `weighting` is a vector of weights determining the relative probability of each mussel being gravid, specified by equations [9] and [10].

Output is a vector of two values: the mean of the run (averaging over 1000 replicates), and the 95% confidence interval for that mean.

```
expectedglochidia <- function(glocweight, gravidrange,
                              viability, shellweight, weighting){

  reps <- replicate(1000, {
  n <- sample(gravidrange, 1)
  gravidmussels <- sample(glocweight, n, prob=weighting)
  viablegloch <- gravidmussels*viability
  viableglochweight <- sum(viablegloch)
  production <- viableglochweight/shellweight*100
  }
  )
  print(mean(reps))
  print(1.96*sd(reps)/sqrt(length(reps)))
}
```

Appendix A6: Appendices for Chapter 8

Part 1: Supplementary Results

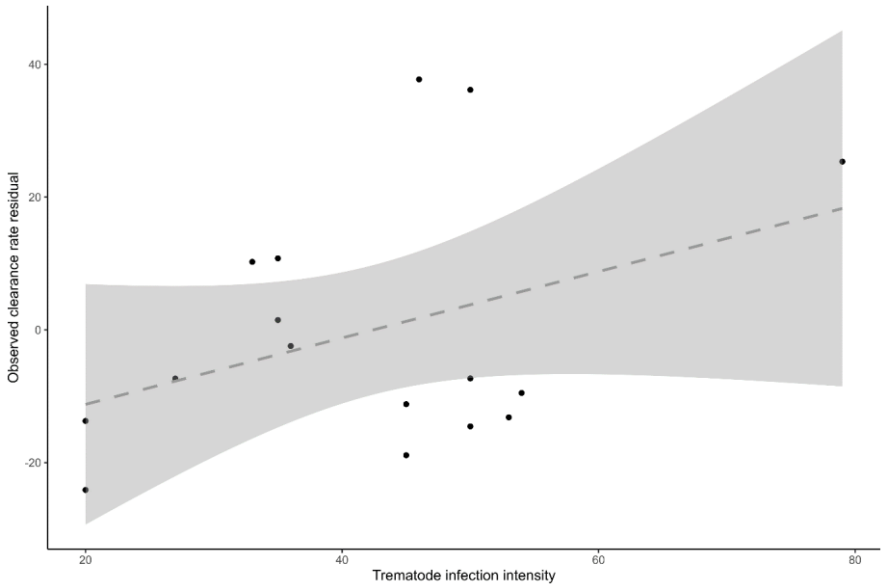

Figure A6.1: The intensity of trematode infection (percentage of gonad filled with trematode tissue) is weakly correlated with the clearance rate of infected *A. anatina* mussels: mussels with higher intensities of infection had higher clearance rates than predicted by the model ($R^2 = 0.15$, $p = 0.138$).

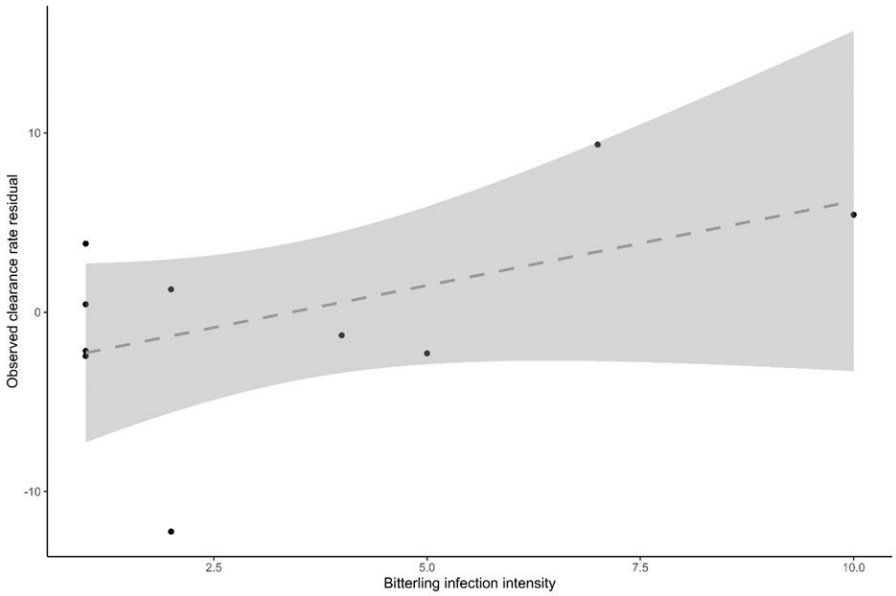

Figure A6.2: The intensity of bitterling infection is weakly correlated with the clearance rate of infected *U. pictorum* mussels: mussels with higher intensities of infection had higher clearance rates than predicted by the model ($R^2 = 0.25$, $p = 0.137$).

Part 2: Code to support ecosystem-level models

Modelling effect of parasites on the filtration level of populations and communities

The following function was written and run to estimate the effect of parasites on the freshwater mussel populations (i.e. Fig. 8.3, main text). This function was run 8 times, with the inputs adjusted according to the run (*U. pictorum* or *A. anatina*, with or without parasites, at 40 or 120 μg/L of Chlorophyll *a*) as below:

- `parasiteprevalence` is the prevalence of the parasite in the host population (either *R. amarus* or *R. campanula*). For the runs excluding parasites, this was set to '0'.
- `CIlowpara` is the lower bound of the 95% confidence interval for the clearance rate of host mussels (i.e. from Figs. 8.1b or 8.2b, depending on the run), when parasitised, at the relevant Chlorophyll *a* concentration. Ignored for runs where `parasiteprevalence` is zero.
- `CIhighpara` is the upper bound of the 95% confidence interval for the clearance rate of host mussels, when parasitised, at the relevant Chlorophyll *a* concentration. Ignored for runs where `parasiteprevalence` is zero.
- `CIlownopara` is the lower bound of the 95% confidence interval for the clearance rate of host mussels, when not parasitised, at the relevant Chlorophyll *a* concentration.
- `CIhighnopara` is the upper bound of the 95% confidence interval for the clearance rate of host mussels, when not parasitised, at the relevant Chlorophyll *a* concentration.

Output is a data frame with two columns: a column of the estimated time taken to filter 100 m of the Old West river, and a corresponding column listing the relevant population density that produced that estimate. Each population density (1 m^{-2}, 2 m^{-2}, … , 50 m^{-2}) had ten replicates. This code can also be modified to incorporate *U. pictorum* and *A. anatina* into a single calculation (i.e. Fig. 8.4).

```
ecosystemeffects <- function(parasiteprevalence, CIlowpara, CIhighpara,
                             CIlownopara, CIhighnopara){
  daystoclear <- NULL
  musseldensity <- NULL
for(n in 1:50){
musseldensity <- c(musseldensity, rep(n, 10))
daystoclear <- c(daystoclear, replicate(10, {
  #Work out how many mussels in a given sized sample have parasites
  prevalenceweight <- dbinom(0:n, size=n, prob=parasiteprevalence)
  numberwithparasites <- sample(0:n, 1, prob=prevalenceweight)
  noparasites <- n-numberwithparasites
  if(numberwithparasites != 0 && noparasites != 0)
  {
  #Calculate volume of water filtered by mussels with parasites
  parasitereps <- sum(replicate(numberwithparasites, {
    runif(1, CIlowpara, CIhighpara)
  }
  ))
  #Calculate volume of water filtered by mussels without parasites
  noparasitereps <- sum(replicate(noparasites, {
    runif(1, CIlownopara, CIhighnopara)
  }
  ))
  #totalclear is total volume of water (L) filtered per 1m^2 per hour
```

```
  totalclear <- parasitereps + noparasitereps
  #extrapolate to 100m long section of river (= 4000m^2) per day
  #There are 7600000L per 100m of river at OW
  #clearancerate is in units of days
  clearancerate <- 7600000/(totalclear*4000*24)
  }else if(numberwithparasites == 0){
    totalclear <- sum(replicate(n, {
      runif(1, CIlownopara, CIhighnopara)
    }
    ))
  clearancerate <- 7600000/(totalclear*4000*24)
  }else{
    totalclear <- sum(replicate(numberwithparasites, {
      runif(1, CIlowpara, CIhighpara)
    }
    ))
    clearancerate <- 7600000/(totalclear*4000*24)
  }
})
)
}
  finaldata <- data.frame(daystoclear, musseldensity)
  return(finaldata)
}
```

Appendix A7: Appendices for Chapter 9

Supplementary methods

We conducted a search for publications relating to the translocation of unionid mussels using the database Web of Science, on 15th September 2020. We used the following search terms:

> (freshwater mussel* OR unionid*) AND (translocat* OR relocat* OR transplant* OR introduc* OR establish*)

The initial search returned 711 results, from which one duplicate was removed. We screened the remaining 710 publications to exclude non-relevant results. We retained only results involving a translocation of unionid mussels between two waterbodies or sites within a waterbody, excluding those where the source or recipient site was an artificial or laboratory-based pond or tank or those where translocated mussels were lab-reared, rather than a natural ecosystem. Where the main subject of the paper was to describe one or more translocations originally reported elsewhere, this original publication was additionally included in the review where it had not already been returned by the literature search.

This screening yielded a final set of 87 publications which were included in the review. We then extracted the data listed below, where available, from each publication. The complete dataset can be found in the attached 'translocation_data.csv'.

All analyses reported in section 9.2 of were performed using functions available in Base R v3.6.2.

Information extracted:

Author(s)
Publication year
Translocation year – where reported this was the year in which translocations were carried out. If unavailable the year of publication was used as a substitute.
Purpose – purpose of the translocation, broadly categorised into the following four groups: Restoration (supplementing or (re-)establishing a population), Conservation (translocating a population specifically under threat, often due to construction), Biomonitoring (assessing ambient concentrations of heavy metals or other pollutants) or Experiment (other research for information-gathering rather than conservation directly).
Species – unionid species translocated, as reported by the publication
Updated species – where species nomenclature as reported by the publication is defunct or outdated, the currently accepted species name was recorded, using the MUSSEL Project database (http://mussel-project.uwsp.edu/db/index.html). This allowed standardised comparison between publications and reference to Red List category.
Threat code – species threat level according to IUCN Red List assessment. Recorded as LC (Least Concern), NT (Near Threatened), VU (Vulnerable), EN (Endangered) or CR (Critically Endangered), or N/A (either due to lack of a Red List assessment for the species, or because species was not reported by the publication).
Threat category – species threat level, grouped by Red List category. Recorded as either Stable (LC or NT), Threatened (VU, EN or CR) or No information (N/A).

Life stage – developmental stage of individuals at the time of translocation, recorded as Glochidia, Juvenile, Sub-adult or Adult.
Number – number of individuals translocated.
Country – country in which translocation activity (source and recipient locations) occurred. No instances were found of translocation across national borders.
Continent – continent in which translocation activity (source and recipient site) occurred.
Source – source location from which mussels for translocation were collected, reported as waterbody and identifying location where available.
Recipient – location to which translocated mussels were moved, reported as waterbody and identifying location where available.
Distance – as-the-crow-flies distance translocated (km) between source and recipient location, measured using Google Maps distance tool. Where available co-ordinates were used to pinpoint start and end location; in other cases, maps or descriptions provided were used to identify specific sites as accurately as possible.
Species presence – presence of the translocated species at the recipient site prior to translocation, based on pre-translocation surveys of species assemblage at the recipient site, if reported. Recorded as Present, Extirpated, Absent or No information.
Other species presence – Y/N. Recorded as 'Y' if at least one other unionid species was reported as present at the recipient site prior to translocation, or 'N' if surveys at the recipient site found no other species.
Water moved – Y/N. Recorded as 'Y' if water from the source site was reported to have been translocated to the recipient site along with the mussels, introducing a potential alternative route for pathogen or parasite transfer. Recorded as 'N' if description of methodology was sufficient to rule out transfer of water.
Pathogen check – Y/N. Recorded as 'Y' if any pre-translocation assessment was made of pathogen or parasite presence in the translocated individuals. Recorded as 'N' if description of methodology was sufficient to rule out pathogen assessment.
Quarantine – Y/N. Recorded as 'Y' if mussels were held in an enclosed environment separate from both source and recipient site for any length of time between collection from source site and installation at recipient site. Recorded as 'N' if description of methodology was sufficient to rule out quarantine.
Contained – Y/N. Recorded as 'Y' if translocated mussels at the recipient site were held in a way that prevented their dispersal, such as in a cage or net, or 'N' if mussels were placed directly into the waterbody with no enclosure.
Removed – Y/N. Recorded as 'Y' if translocated mussels were permanently removed from the recipient site some time after translocation, or 'N' if description of methodology was sufficient to suggest mussels were left permanently at the recipient site.
Survival – expressed as percentage of initially translocated mussels alive at the time of subsequent assessment. Where multiple assessments were made, the survival rate at the most recent survey (e.g. where longest time had elapsed after translocation) was used.
Follow up – number of days from date of initial translocation to date of either removal of the translocated individuals from the recipient site, or of assessment of survival/mortality rates at the recipient site. Recorded to the nearest day where specific dates were given, or as a multiple of 365 if years were reported instead. Where multiple follow-up dates were reported, the most recent follow up date (e.g. where longest time had elapsed after translocation) was used.

Table A7.1: Examples of negative impacts of different pathogens on unionid host individuals and populations. This table shows examples of each pathogen type and is not a comprehensive list.

Pathogen type	Pathogen species	Host species	Impact	Reference
Bucephalid trematodes	*Homalometron armatum*	*Elliptio complanata*	Castration via destruction of gonad tissue	1
	Rhipidocotyle campanula	*Anodonta anatina*	Castration via destruction of gonad tissue	2
	Rhipidocotyle fennica	*Anodonta anatina*	Castration via destruction of gonad tissue	2
Aspidogastrean trematodes	*Aspidogaster conchicola*	*Pyganodon grandis*	Disruption of host tissue via physical attachment, consumption of host tissue	3
	Cotylaspis insignis	*Pyganodon grandis*	Reduction in host glycogen stores	4
Unionicolid mites	*Najadicola ingens*	Multiple	Reduction in host gravidity	5
	Unionicola intermedia	*Anodonta anatina*	Host tissue damage, consumption of host tissue	6, 7
	Unionicola formosa	*Pyganodon cataracta*	Consumption of host tissue	8
	Unionicolidae	*Pyganodon grandis*	Reduction in host gravidity	4
Ciliates	*Tetrahymena glochidiophila*	*Lampsilis siliquoidea, L. cardium, L. fasciola*	Reduction in glochidia (larval mussel) viability	9, 10
Bacteria	*Aeromonas hydrophila*, others in low abundance	*Elliptio complanata*	Tissue lesions, digestive gland atrophy	11
	Yokenella regensburgei	Multiple	Correlatively linked with mass mortality event	12
Viruses	HcPV arenavirus	*Hyriopsis cumingii*	Mass mortality	13
	Clinch densovirus 1	*Actinonaias pectorosa*	Mass mortality	14

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