Parasite ecology in freshwater wetlands: Consequences for hosts, communities and ecosystems

Daniel Lucas Preston
University of Colorado at Boulder, daniel.preston@colorado.edu

Follow this and additional works at: https://scholar.colorado.edu/ebio_gradetds
Part of the Ecology and Evolutionary Biology Commons, and the Parasitology Commons

Recommended Citation
https://scholar.colorado.edu/ebio_gradetds/66

This Dissertation is brought to you for free and open access by Ecology & Evolutionary Biology at CU Scholar. It has been accepted for inclusion in Ecology & Evolutionary Biology Graduate Theses & Dissertations by an authorized administrator of CU Scholar. For more information, please contact cuscholaradmin@colorado.edu.
PARASITE ECOLOGY IN FRESHWATER WETLANDS:

CONSEQUENCES FOR HOSTS, COMMUNITIES AND ECOSYSTEMS

by

Daniel Lucas Preston

H. B.S. Oregon State University, 2008

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Ecology and Evolutionary Biology

2014
This thesis entitled:
Parasite ecology in freshwater wetlands: Consequences for hosts, communities and ecosystems
written by Daniel L. Preston
has been approved by the Department of Ecology and Evolutionary Biology

________________________________________
Pieter Johnson

________________________________________
Sharon Collinge

________________________________________
Valerie McKenzie

________________________________________
Yuri Springer

________________________________________
Alan Townsend

The final copy of this thesis has been examined by the signatories and we find that both the content and the form meet acceptable presentation standards of scholarly work in the above mentioned discipline.
Parasites can exert effects on multiple levels of ecological organizations, including individuals, populations, communities, and whole ecosystems. Historically, however, most research on wildlife diseases has rarely integrated across ecological scales. I used a combination of laboratory experiments and field surveys to examine how parasites in freshwater wetlands affect individual host behavior, food web structure, and ecosystem processes. First, I explored how individual behavioral responses to parasites compare to well-known behavioral responses to predators. In both the laboratory and an outdoor mesocosm experiment, I found no evidence that tadpoles alter their behavior to reduce the risk of infection prior to direct contact with parasites, despite showing strong responses to predation risk. After infection, however, tadpoles reduced activity in predictable ways based on traits of the host, the parasite, and their interaction. Such behavioral changes likely have consequences for community interactions including, competition, predation and parasite transmission. I then examined the roles of parasites...
and other complex life cycle organisms in a freshwater food web. I specifically asked how variation in the resolution of nodes - from taxonomic species to life stages - affects the observed role of parasites in the web. I found that parasites were prominent within the food web and were involved in 45% of the >1000 total links. Importantly, most web metrics were sensitive to node resolution, and in particular, the effects of parasites on connectance and nestedness were reversed when nodes were disaggregated. Extending these results to explore how parasites affect energy flow, I quantified the contributions of trematode parasites to the animal biomass and production in three wetland ecosystems. I found that trematode biomass exceeded that of virtually all invertebrate groups and that trematode production was comparable to many published estimated of free-living invertebrate production. Lastly, to enhance links between disease ecology and ecosystem science, I review our current understanding of how parasites can affect ecosystem structure and function, including underlying mechanisms, a review of case studies, conceptual predictions, and priorities for future research. Taken together, my findings indicate that parasites can play prominent roles in the structure and functioning of freshwater ecosystems.
DEDICATION

This thesis is dedicated to my parents for encouraging my interests in biology.
ACKNOWLEDGEMENTS

My advisor, Piet Johnson, has played a prominent underlying role in the vast majority of my successes in graduate school. His guidance has prepared me to be successful in academia and for that I am grateful. I also thank my dissertation committee – Valerie McKenzie, Sharon Collinge, Alan Townsend and Yuri Springer – who provided valuable guidance, advice and resources throughout my dissertation. Many graduate students and post-docs in the Johnson Lab – Sara Paul, Katie Richgels, Sarah Orlofske, Joseph Mihaljevic, Max Joseph, Travis McDevit-Gales, Brett Goodman, Bethany Hoye and Jason Hoverman – were great colleagues and friends throughout the past five years. I hope we can work together in the future. A number of undergraduate students were instrumental in my research. I want to sincerely thank Clara Boland, Hayden Hedman, Ewelina Pena, Nora Brown, Gerardo Hidalgo-Cuellar, Evan Esfahani, Margaret Summerside, Oscar Goodwin, Sarah Palmer, Taylor Hayes and Michelle Baragona for their hard work and positive attitudes in the lab and field. Four summers of my graduate career where spent at the Hopland Research and Extension Center in Mendocino County, California. I couldn’t have asked for a better place to work, in large part due to the amazing people who work there, especially Bob Keiffer, Steven Poor, Bill Marston, Shane Feirer and Amber Shrum. Jeremy Henderson and Miranda Welsh made it a fun and exciting place to live. I was fortunate to be involved in the National Science Foundation GK-12 Program and I thank Jessica Feld, Lesley Smith, Bill Bowman and the teachers and students at Crest View Elementary for making the experience a uniquely memorable one. I want to acknowledge the National Science Foundation, the Achievement Rewards for College Scientist Program, the National Geographic Society,
the Society for Wetland Scientists, and the United States Geological Survey for funding during my dissertation. The University of Colorado has also been generous and I want to thank the Department of Ecology and Evolutionary Biology, the Graduate School, the Museum of Natural History, the Bioscience Undergraduate Skills and Research Training program, the Howard Hughes Medical Institute fellowship program, and the Undergraduate Research Opportunities Program for funding.
CONTENTS

CHAPTER

I. Ecological consequences of parasitism ................................................. 1
   Effects on individuals to ecosystems .................................................. 1
   Organization of the dissertation ....................................................... 7

II. Natural enemy ecology: comparing the effects of predation risk, infection risk and disease on host behavior .................................................. 10
   Abstract ............................................................................................. 10
   Introduction ......................................................................................... 11
   Methods ............................................................................................. 15
   Results ............................................................................................... 21
   Discussion ......................................................................................... 25
   Appendix A ....................................................................................... 33

III. Complex life cycles in a pond food web: effects of life stage structure and parasites on network properties, trophic positions and the fit of a probabilistic niche model .................................................. 41
   Abstract ............................................................................................. 41
   Introduction ......................................................................................... 42
   Methods ............................................................................................. 46
   Results ............................................................................................... 51
   Discussion ......................................................................................... 60
   Appendix B ....................................................................................... 68

IV. Biomass and productivity of trematode parasites in pond ecosystems .......... 71
   Abstract ............................................................................................. 71
   Introduction ......................................................................................... 72
   Methods ............................................................................................. 76
   Results ............................................................................................... 82
   Discussion ......................................................................................... 87
   Appendix C ....................................................................................... 91

V. Disease ecology meets ecosystem science: where we have been and where to go next .................................................................................. 96
   Abstract ............................................................................................. 96
   Introduction ......................................................................................... 97
   Ecosystem ecology: a role for parasites? .............................................. 101
   Ecosystem structure .......................................................................... 103
   Biogeochemical cycles ..................................................................... 108
   Ecosystem energetics ........................................................................ 111
Temporal ecosystem dynamics.................................................................115
Future research: when can parasites be ignored? ..................................120
Conclusion ...............................................................................................125
Appendix D...............................................................................................126

VI. Summary and Conclusions ..................................................................130
Parasite ecology in freshwater wetlands ..................................................130
Future directions .....................................................................................134
Conclusion ...............................................................................................137

REFERENCES ............................................................................................138
TABLES

Table
3.1  Food web properties and probabilistic niche model (PNM) results for three versions of the Quick Pond food web, each with and without parasites .................................................................53

5.1  Examples of parasites affecting the structure and function of ecosystems ..................................................................................................................107

5.2  Traits of the parasite, the host, their interaction, and the ecosystem that are predicted to influence the ecosystem effects of parasites and disease ........................................................................................................122

A.1  Mean snout-vent lengths of amphibian larvae used in behavior experiments ..................................................................................................................34

B.1  Food web properties for web versions with parasites but lacking concomitant links .................................................................................................................69

B.2  Summary metrics for each of the four sub-webs and the total taxonomic species ................................................................................................................70

C.1  Aquatic free-living organisms detected at three California ponds...........93 -94

C.2  Length-to-dry mass regression equations for snails and three amphibian taxa ....................................................................................................................94

C.3  Proportional trematode biomass within infected Helisoma trivolvis snails .....................................................................................................................................94-95

C.4  Infection prevalence of five trematodes in second intermediate hosts from three California ponds .................................................................95

D.1  Empirical studies of parasites affecting ecosystems that were used to generate figure 5.2 ................................................................................................................128-129
FIGURES

Figure

2.1 Natural enemies and their hosts or prey.................................................................16

2.2 Amphibian activity levels after exposure to chemical cues from natural enemies in the laboratory and an outdoor mesocosm experiment ...........................................................................................................22

2.3 Activity levels of *Pseudacris regilla* tadpoles before and after exposure to multiple trematode species .................................................................24

2.4 The effects of tadpole size and and exposure intensity on relative activity and escape distance from a simulated predator ........................................26

3.1 Degree distributions and trophic vulnerability and generality of parasites and free-living organisms in three versions of the Quick Pond food web ........................................................................................................56

3.2 Frequency distributions of nodes at different trophic positions within the three versions of the Quick Pond food web ........................................58

3.3 Changes in trophic level with node disaggregation for organisms with complex life cycles and for predators with direct life cycles ...............59

3.4 The observed links in the three versions of the Quick Pond food web ..................61

4.1 Prevalence of trematode infections in *Helisoma trivolvis* snails from three California ponds ........................................................................................................................83

4.2 Biomass of free-living taxa and aquatic life stages of trematode parasites from three ponds in California .................................................................85

4.3 Production of biomass by trematode cercariae ......................................................86

5.1 Empirical literature search results quantifying the disconnect between disease ecology and ecosystem science .................................................99

5.2 Patterns in existing work on ecosystems and parasites ......................................100

5.3 Mechanisms through which parasites can affect ecosystem properties ...........103
A.1  Laboratory enclosures used in the pre-infection behavior experiments ...............35
A.2  Outdoor mesocosms used in the pre-infection behavior experiments ...............36
Chapter 1

Ecological Consequences of Parasitism


The ecological interactions of parasites are often challenging to observe; many live their lives secretively, in intimate contact with their host but invisible to the outside world. With some notable exceptions, parasites also tend to be very small. It may be easy to assume that because parasites are generally small and inconspicuous, they play less important roles in ecology than do their free-living counterparts. Yet advances in disease ecology have revealed that parasites are not only ecologically important, but they can sometimes exert effects that equal or surpass those of free-living species in shaping community structure and ecosystem properties. In fact, parasitism is more common than traditional predation as a consumer lifestyle (De Meeûs and Renaud 2002) and arguably represents the most widespread life-history strategy in nature (Price 1980). Parasites also influence host behavior and fitness, and can regulate host population sizes, sometimes with profound effects on trophic interactions, food webs, competition, biodiversity and keystone species. In this chapter I first review some of the ways in which parasites can affect community and ecosystem ecology. I then briefly outline the subsequent chapters of my dissertation.

Effects on individuals to ecosystems

Parasitism, Competition and Biodiversity – Parasites can influence biodiversity when they alter the outcome of competitive interactions between host species, a
phenomenon termed parasite-mediated competition (Price et al. 1986). In some cases, this occurs when a tolerant host species amplifies a parasite's abundance, causing an indirect negative effect on a second, less tolerant host species. For example, the displacement of red squirrels by grey squirrels in Britain may have been facilitated by a parapox virus (Tompkins et al. 2003). The virus infects both species, but native red squirrels are highly susceptible, whereas invasive grey squirrels experience relatively minor effects. In this case, a microparasite has probably facilitated a biological invasion, thereby reducing local biodiversity by eliminating populations of one host species.

Parasites can also positively contribute to biodiversity by allowing a competitively inferior species to coexist with a dominant species. For example, *Anolis gingivinus* outcompetes *Anolis wattsi* everywhere on the Caribbean island of St. Maarten except the isolated interior of the island. Both lizards host a malarial parasite, *Plasmodium azurophilum*, but the two lizards co-occur only where *A. gingivinus* is heavily parasitized. This suggests that malaria reduces the competitive ability of the dominant lizard, thereby allowing the competitively inferior lizard to coexist (Schall 1992). A similar outcome in a very different system occurs with the pathogenic soil oomycete, *Pythium* and its plant hosts. The presence of a particular plant can change the composition of the local soil community such that the growth of that species is diminished, and other colonizing species are given a competitive advantage, which ultimately increases overall plant biodiversity (Mills and Bever 1998).

Parasites also influence biodiversity through the direct regulation of host populations. Even though parasites can cause disease, they rarely cause extinctions because pathogen transmission is usually reduced at low host densities. However,
important exceptions can occur, particularly in cases when pathogens invade naïve host populations or when reservoir hosts allow parasites to persist despite low host densities. The emergence of the amphibian fungal pathogen, *Batrachochytrium dendrobatidis* (Bd), for example, represents a case of a parasite causing extirpations and perhaps extinctions on a global scale (Kilpatrick et al. 2010). Collectively, the examples described here illustrate how parasites may have opposing net effects on biodiversity, which depend on the context of the parasite-host relationship (e.g. whether host populations are naïve and whether parasite transmission is density-dependent), and on whether parasites most negatively affect competitively dominant or competitively inferior species in a community.

*Parasitism and Trophic Interactions* – Parasites can function as both predators and prey. Parasites that feed on hosts engage in a special type of predation (Raffel et al. 2008). Alternatively, parasites can also serve as important sources of prey. For example, predators on islands in the Gulf of California, including lizards, scorpions and spiders, are one- to two orders of magnitude more abundant on islands with sea bird colonies because they feed on bird ectoparasites (Polis and Hurd 1996). Predators also inadvertently consume parasites during the consumption of infected hosts (Johnson et al. 2010). When macroparasites are relatively large, such as nematodes in the gut of vertebrate hosts, the contributions of parasites to the diet of predators can be significant. The roles of parasites as predators and prey suggest that considerable amounts of energy may directly flow through parasites in food webs, despite their small size and cryptic nature.

In some cases, predation can serve as a vehicle of transmission, allowing a parasite with a complex life cycle to move from one host to another. Parasites that infect
new hosts via trophic transmission frequently alter their host's behavior or morphology in ways that increase predation risk, thereby aiding the parasite in reaching the next host in its life cycle (Poulin et al. 2005). For example, estuarine killifish infected with the trematode *Euhaplorchis californiensis* exhibit erratic swimming behavior that ultimately makes them up to 30 times more susceptible to bird definitive hosts (Lafferty and Morris 1996). Another trematode, *Ribeiroia ondatrae*, causes amphibians to develop severe limb deformities, including extra or missing limbs (Johnson et al. 1999), which impair the hosts's ability to jump and swim and presumably make them more susceptible to predation by bird definitive hosts. The roles of parasites in predator-prey interactions are rarely obvious, yet they may influence the outcome of trophic interactions at the community scale.

*Parasitism, Food Webs and Ecosystem Energetics* – Considering the prominent roles played by parasites in trophic interactions, we might expect parasites to strongly influence food web characteristics. Recent efforts to include parasite in food webs have revealed sharp changes in metrics of topological food webs, including species richness, the total number of links, food chain length (the number of trophic levels in a web) and connectance (the ratio of observed links among species to realized links; Lafferty et al. 2008). In a salt marsh food web in California, parasites were involved in 78% of all links and increased estimates of connectance by 93%, which may have implications for web stability (Lafferty et al. 2006). Incorporating parasites into food webs also reveals that mid-trophic levels - rather than the lowest trophic levels - are most susceptible to natural enemies because this group is at risk from both predators and trophically transmitted parasites. Integrating parasites into food webs also suggests that the classical Eltonian
pyramid (Elton 1927) may need to be revised; if parasites feed at a trophic level above their hosts, parasites would occupy the pinnacle of this new pyramid (Sukhdeo and Hernandez 2005), which would be a significant departure from the traditional placement of top predators at the peak of the food chain.

For decades, parasites were omitted from food web ecology based on the assumption that they contributed negligible biomass to ecosystems. Measuring biomass, or productivity (change in biomass over time) allows us to quantify contributions of organisms to ecosystem energetics. However, when parasite biomass was actually measured on an ecosystem scale, the results challenged the notion that parasites are unimportant in ecosystem energy flow. In some estuarine systems, the biomass of parasites is comparable to that of top predators (Kuris et al. 2008). Yearly productivity of trematode parasites, for example, was higher than the biomass of birds. Similarly, the estimated biomass of plant fungal foliar pathogens in the Northeastern US was comparable to that of herbivores in experimental grassland plots (Mitchell 2003). In fact, top-down control by fungal pathogens was more important than herbivory in predicting grass biomass. These studies suggest that parasites can contribute significantly to ecosystem energetics and exert strong control over the biomass of producers. While much remains to be learned about the roles of parasites in food webs, the classical approach of omitting parasites from food web ecology could lead to serious gaps in our understanding.

*Parasitism, Keystone Species and Ecosystem Structure* – The effects of parasitism on ecological communities can be particularly pronounced when the hosts are keystone or dominant species with important functions in an ecosystem. For example, *Diadema*
urchins in the Caribbean experienced a massive die-off associated with microbial pathogens, eliminating the keystone roles of urchins as grazers and bioeroders on coral reefs (Lessios 1988). Reefs in affected regions became overgrown with algae that displaced mature corals and prevented new coral settlement. In the most extreme examples, algae cover jumped from near 1% prior to the disease outbreak, to 95% in the following two years (Lessios 1988). Nearly twenty years later, Diadema populations recovered on some Jamaican reefs and a shift back to a coral-dominated ecosystem has begun (Edmunds and Carpenter 2001).

A second example involves the introduction and subsequent removal of a viral disease called rinderpest in African ungulates. The virus was introduced to native ungulates from domestic livestock in 1890 and spread throughout the African continent within 10 years, reducing populations of buffalo and wildebeest by 80% in some regions. Vaccination of domestic cattle began in the 1950s (Plowright and Taylor 1967) and virtually eliminated the disease in wild African ungulates by 1968. The release of herbivores from parasite control had dramatic cascading effects on the ecosystem; populations of top carnivores, including lions and hyenas increased and productivity of grasses decreased (Sinclair 1979; Thomas et al. 2005). These examples of parasite introductions and removals provide a rare glimpse of how ecosystem structure can be dramatically altered when parasites regulate populations of functionally important host species.

The prominent roles of parasites in food webs, competitive interactions, biodiversity patterns and the regulation of keystone species make it clear that parasites contribute to structuring communities and ecosystems. Yet, we have only begun to
disentangle the complex ecological roles played by parasites. Patterns of increased disease emergence in wildlife, with potential implications for human health, make it an especially relevant time to further integrate parasitism into community ecology and improve our understanding of the roles of parasites in nature.

**Organization of the dissertation**

In this dissertation I examine the ecological consequences of parasitism at multiple levels of ecological organization, including individual hosts, communities, and ecosystems. I first consider the ways in which trematode parasites affect the behavior of amphibian intermediate hosts (Chapter II). I specifically ask how infection risk, prior to direct parasite contact, compares to predation risk, prior to direct predator contact. I also address the host behavioral consequences after parasite infection and compare these changes to effects prior to contact with natural enemies. I use a combination of experiments to understand factors relating to the host, the parasite, and their interaction that drive variation in behavioral responses.

In the next chapter I explore the ways in which parasites affect the structure of an empirical food web from a California wetland (Chapter III). In this chapter, I consider how the way nodes in the web are designated affects the role of parasites in the web. Integrating parasites into food webs is challenging in part because parasites have complex life cycles where they interact with a different suite of species at each life stage. Incorporating parasites as taxonomic species therefore collapses this variability that occurs across life stages. For this reason, I generated multiple food webs that use either life stages or taxonomic species for organisms with complex life cycles. Using these
different approaches has important consequences for the patterns that are observed in the web.

Extending my results on the roles of parasites in food webs, I then consider their contributions to animals biomass and energy flow in the same wetlands (Chapter IV). In this chapter, I quantify the biomass of all of the most abundant aquatic animals (amphibians, invertebrates and zooplankton) and compare their standing biomass to that of the aquatic life stages of trematodes. I also conduct measurements to calculate the yearly productivity of free-swimming larval stages. This comparison is important because although freshwater ecology has a rich history of quantifying the biomass and production of animals to understand their roles in energy flow, there have been no studies to incorporate parasites into freshwater energy flow.

In the subsequent chapter (Chapter V) I use a series of literature searches to explore how parasites can affect ecosystem structure and function. I first quantify the traditional divide between ecosystem science and disease ecology and then consider the mechanisms through which parasites can influence large-scale processes such as ecosystem structure, energy flow, biogeochemical cycles and temporal ecosystem dynamics. I use the literature searches to review existing empirical case studies and then generate predictions about how traits of the host, the parasites and their interactions can be used to predict the ecosystem consequences of parasitism and disease. Lastly, I identify a range of future research priorities, including ecosystem experiments and the incorporation of parasites into models of ecosystem function.

The final chapter (Chapter VI) summarizes the primary conclusions from the aforementioned studies and identifies several key areas for future research that will help
extend and generalize the effects of parasites in ecosystems. Primary areas of future work include understanding how parasites can affect biogeochemical cycles, the integration of disease ecology and ecological stoichiometry, and the relationship between parasitism, disease and ecosystem stability.
CHAPTER 2

NATURAL ENEMY ECOLOGY: COMPARING THE EFFECTS OF PREDATION RISK, INFECTION RISK AND DISEASE ON HOST BEHAVIOR


Abstract

Growing interest in unifying the field of natural enemy ecology has revealed similarities between predation and parasitism. In parallel with predation, parasite infection – and even the threat of infection – can alter host traits and indirectly affect community interactions. Nonetheless, few studies have considered multiple mechanisms of natural enemy-induced behavioral alteration in parallel (e.g., effects before and after enemy contact) or the factors that drive variation in behavioral responses. We first evaluated how the threat of infection by a virulent trematode (*Ribeiroia ondatrae*) compared to the well studied risk of predation in triggering inducible defenses in amphibian hosts, prior to direct contact with either enemy. We then evaluated five separate factors that influenced the magnitude of parasite-induced behavioral changes after successful transmission. In both the laboratory and an outdoor mesocosm experiment, we found no evidence that tadpoles of two species (*Pseudacris regilla* and *Anaxyrus boreas*) altered their activity levels in response to chemical cues from uninfected host snails, trematode-infected snails, or from conspecifics actively becoming infected. In contrast, tadpoles sharply reduced their activity in response to lethal predation risks posed by caged dragonfly larvae. After infection, however, *Ribeiroia* caused strong decreases in host activity and escape distance that correlated positively with infection intensity and negatively with host size and developmental stage. Five days after infection with a one-time pulse exposure, hosts
recovered to near-normal activity levels. Hosts exposed to a chronic daily exposure of equal intensity, however, continued to decrease activity. Unlike *Ribeiroia*, two less virulent trematodes had no detectable effects on host behavior. Our results highlight key distinctions between predation and parasitism. The contrasting effects prior to enemy contact may stem from the fact that unlike predation, the consequences of macroparasite infection are intensity-dependent and unpredictable. In contrast, the strong changes in host behavior after infection are more similar to nonconsumptive predator effects in terms of their potential influences on host fitness and community interactions.

**Introduction**

Animals in nature exist in complex communities in which they must concurrently defend against multiple natural enemies, including parasites and predators. Recognition of this ‘natural enemy ecology’ has spurred growing interest in the ecological similarities of host/parasite and predator/prey interactions (Lafferty and Kuris 2002; Raffel et al. 2008; Kortet et al. 2010). Both parasites and predators have potential to control the densities and alter the individual traits of hosts or prey (Raffel et al. 2008). Such effects of parasites and predators can occur at multiple phases of an interaction, including before, during, and after physical contact with an enemy, and can lead to qualitatively similar density- and trait-mediated indirect effects on other community members (Schmitz et al. 2004; Sih et al. 2012).

Despite the need to integrate research on predation and parasitism, ecologists have historically studied host/parasite and predator/prey interactions in isolation, rarely using the same context to evaluate the effects of both natural enemies. Most prior research on
inducible defenses against predators has focused on nonconsumptive effects, in which the presence of a predator alters prey traits before direct contact and a traditional predator/prey interaction has occurred (Preisser et al. 2005; Peckarsky et al. 2008). In contrast, most research on inducible defenses against parasites has focused on the consequences of infection after contact between parasite and host (e.g., behavioral changes and immune responses; but see Moore 2002). This disparity in approaches likely stems from the fact that, aside from certain sublethal predators, a successful predation event typically involves death of the prey, such that most defensive strategies emphasize preventing a predator encounter. Indeed, the presence of non-fatal cues has been posited as a prerequisite for inducible defenses to be favored over constitutive defenses (Harvell 1990). For most parasites, however, hosts have ample opportunities for defensive action during and after the initial infection event. During infection hosts can utilize behaviors that limit infection success (e.g., grooming; Clayton 1991) and after infection they can rely on immune responses to eliminate parasites (Frost 1999). Ultimately, linking our understanding of inducible defenses across multiple types of trophic interactions will require considering how existing theory – including environmental cues, phenotypic trade-offs, and plasticity – applies to both predation and parasitism.

A comprehensive understanding of the similarities and differences between predation and parasitism requires examining the effects of both natural enemies at multiple phases of the interaction (e.g., before and after parasite infection) and an exploration of factors that drive variation in responses. Relatively few studies have examined the ability of host species to modify their behavior or other traits to reduce disease risk prior to parasite contact (Kiesecker et al. 1999; Behringer et al. 2006;
Fritzsche and Allan 2012). Consequently, few generalities have emerged with regard to how and when hosts will react to infection risk. Because behavioral modifications that mitigate risks from natural enemies are costly (Loose and Dawidowicz 1994; Downes 2001), the behavioral responses to threats from distinct natural enemies are predicted to vary with the severity of the risk imposed (McCarthy and Fisher 2000; Ferrari et al. 2009). Similarly, after an encounter has occurred, the consequences should vary with traits of the enemy (e.g. virulence), traits of the individual being attacked (e.g., host tolerance), and the dynamics of their interactions (e.g., timing of exposure and infection intensity) (Rohr et al. 2010; Johnson et al. 2011; Johnson et al. 2012). Comparisons between the effects of predators and parasites can therefore enhance understanding of how these traits may lead to trade-offs in the responses of individuals.

Amphibians provide a useful study system to examine parallels in how animals respond to distinct natural enemies. The inducible defenses of amphibian larvae towards predators have been extensively documented and provide a useful point of comparison to threats from other classes of natural enemies. Many amphibian species react predictably to chemical cues signaling predation risk by altering their morphology and reducing activity levels (e.g., Van Buskirk and Relyea 1998; Relyea 2001). To date, however, few studies have examined how amphibians respond to infection risk prior to parasite contact. Cues from infected conspecifics (Kiesecker et al. 1999) and from infectious parasite stages (Kiesecker and Skelly 2000; Rohr et al. 2009) have been suggested to elicit behavioral responses in amphibian larvae, although the mechanisms are not entirely clear. More studies have demonstrated that amphibians will react to infectious stages on initial physical contact in an effort to dislodge parasites and reduce infection success. Free-
swimming larval trematode parasites, for instance, elicit strong behavioral responses in amphibian hosts in the form of evasive tail movements and short bursts of activity (Taylor et al. 2004; Daly and Johnson 2011). The majority of studies have focused on the behavioral responses of amphibian larvae after successful parasite infection (e.g., Lefcort and Blaustein 1995; Parris et al. 2006; Venesky et al. 2009; Han et al. 2011). In general, these prior experiments have aimed to determine whether infection has an effect on behavior, rather than decomposing factors underlying variation in host responses.

Our goals were to 1) compare the effects of predators and parasites on amphibian larvae prior to direct contact with either natural enemy and 2) evaluate the factors driving variation in host responses after infection, including both host and parasite characteristics. We combined outdoor mesocosm and laboratory experiments to compare the behavioral inducible defenses of amphibian larvae in response to a lethal predator (*Anax junius* dragonfly larvae) and a highly virulent trematode (*Ribeiroia ondatrae*). In both study venues, we included treatments that allowed us to separate potent effects from snails (i.e., first intermediate hosts), trematodes themselves, and conspecific amphibians actively becoming infected. We followed up the pre-infection studies with laboratory experiments to test how parasite identity, infection intensity, time since exposure, exposure duration (pulse vs. chronic), and host developmental stage influenced host behavioral outcomes after parasite infection. We hypothesized that in response to infection risk, amphibian larvae would increase their activity levels to avoid free-swimming parasite stages (Taylor et al. 2004; Rohr et al. 2009). After successful infection, we predicted that trematode parasites would cause decreases in host activity.
and that host behaviors would vary with the virulence of the parasite, the timing and duration of exposure and the developmental stage of hosts (Johnson et al. 2011).

Methods

Study system – Our experiments involved Pacific chorus frogs (*Pseudacris regilla*), western toads (*Anaxyrus boreas*), predatory green darner dragonfly larvae (*Anax junius*), rams horn snails (*Helisoma trivolvis*), and three species of trematode parasites (*Ribeiroia ondatrae*, *Echinostoma trivolvis* and *Alaria* sp. 2) (Fig. 2.1). Amphibian egg masses and invertebrates were collected from ponds in Mendocino County, California, USA. The trematodes have complex life cycles in which they reproduce asexually within rams horn snails, produce free-swimming larval stages (cercariae) that actively seek amphibian hosts, and then are trophically transmitted into predatory bird or mammal definitive hosts (Fried and Graczyk 1997). To acquire trematode parasites, we screened field-collected rams horn snails for infection using standardized methods (see Johnson and Hartson 2009). Table A.1 in the appendix contains average body sizes of amphibians used in all experiments.

Pre-infection behavior experiments – We conducted complementary laboratory- and outdoor mesocosm experiments to compare the responses of amphibian larvae to chemical cues signaling a threat from predation by *Anax* or infection by *Ribeiroia*. Both experiments consisted of the following five treatments that were each replicated five times: 1) controls without chemical cues from predators or parasites 2) exposure to
chemical cues from an uninfected snail 3) exposure to chemical cues from a snail infected with *Ribeiroia* 4) exposure to chemical cues from an infected snail in the presence of a tadpole host, and 5) exposure to chemical cues from a dragonfly larva that was fed tadpoles *ad libitum*. We included treatments with uninfected snails to test for possible effects of snail cues alone. The treatment containing an infected snail with a tadpole, combined with the treatment containing an infected snail alone, allowed us to distinguish possible effects of cues coming from trematodes versus cues from tadpoles becoming infected.

In the laboratory study, our experimental units consisted of plastic tubs (41 x 29 x 17 cm) containing 12 liters of water. In the center of each tub, we attached a cage enclosed in 35 μm Nitex mesh that was designed to allow passage of chemical cues into
the water without predators or parasites contacting focal tadpoles within the tub (Fig. A.1). Dissections of hosts at the end of the experiment (n = 3 per replicate) confirmed that cercariae were unable to pass through cages. Each experimental unit contained 15 randomly assigned *P. regilla* (see appendix A for additional experimental details).

The outdoor mesocosm experiment extended our results from the laboratory study by including a second species of amphibian, the western toad (*Anaxyrus boreas*), and by examining natural enemy interactions in a more realistic setting. The mesocosms consisted of covered 378 L livestock watering tanks containing sand, algae, zooplankton and 15 tadpoles each of *P. regilla* and *A. boreas* (see Preston et al. 2012 and appendix A for details on mesocosm methods). We placed two floating cages covered with 35 μm Nitex mesh into each mesocosm. In the treatments with dragonfly predators or infected snails housed with tadpoles, we maintained one tadpole of each amphibian species within each of the two cages in every mesocosm. This approach ensured that tadpoles were always receiving cues from conspecifics being preyed on or infected (see appendix A for more details).

Our primary response variables included tadpole activity levels (lab and mesocosm) and position in the water column (lab only). The activity data were collected in the same manner in the lab and mesocosm experiments; on each day an observer recorded the number of tadpoles that were moving within each replicate tub or mesocosm. The behavioral observations were repeated five times per replicate per day in the laboratory study and fifteen times per replicate per day in the mesocosm experiment (see appendix A for additional details). In both experiments, we recorded two days of behavioral data before the introduction of chemical cues and eight days after the
introduction of chemical cues. On the seven days after chemical cue exposure in the lab study, we also recorded the number of tadpoles that were on the surface of the water (< 5 cm below the surface, again taking five repeated observation per replicate). At the end of both studies, we recorded the survival and wet mass of all individuals.

Post-infection behavior experiments – We conducted four related laboratory experiments to examine how traits of trematode parasites and of *Pseudacris regilla* tadpoles influence host behavioral responses after successful transmission. Unlike the pre-infection studies, the post-infection experiments were conducted with individual tadpoles as replicates (n =10 per treatment). These experiments were conducted with similar designs (excluding the escape distance experiment; see below). Tadpoles were infected and maintained within containers of 750 mL water and the primary response variable was tadpole activity. Each tadpole was observed 30 times per day to quantify activity (yes/no data based on whether each tadpole was active or inactive; see appendix A for additional details). At the conclusion of all four experiments, tadpoles were euthanized with an overdose of MS-222 and the number of successfully encysting *Ribeiroia* parasites was quantified using standard methods (Johnson and Hartson 2009; Johnson, Kellermanns and Bowerman 2011).

In the first experiment, we assessed the role of parasite identity in determining how infection alters behavior by exposing tadpoles to 40 cercariae from one of three trematode species (*Ribeiroia ondatrae*, Echinostoma trivolvis or Alaria sp.), or to water without cercariae (see appendix A for exposure methods). We then quantified tadpole activity levels one day before and one day after infection at 14:00 hrs as described above. In the second experiment, we examined the effects of infection intensity and elapsed time
since exposure on host behavior. We exposed tadpoles to 0, 5, 10, 20, 30, or 40 *Ribeiroia* cercariae administered in a one-time pulse exposure, or 40 cercariae administered in a chronic exposure over four days (i.e., ten cercariae per day). This design allowed us to compare the effects of a pulse exposure, common in experimental designs, with a low-level chronic exposure that is more similar to how animals are exposed to parasites in nature. We then quantified activity levels for four days before infection and for five days after exposure in the pulse infection treatment. In the chronic exposure treatment we quantified activity for four days before infection and up until one day after the last exposure (all at 08:00 hrs).

In the third experiment we examined how *Ribeiroia* infection intensity altered the escape distance of *Pseudacris regilla* tadpoles in response to a simulated predator. We exposed tadpoles to 0, 5, 10 or 20 *Ribeiroia* cercariae. One day after infection, tadpoles were placed into an aquatic track (1 m x 8 cm x 8 cm) and gently touched by a wooden stick, initiating a flight response. The starting and ending position of each tadpole was recorded and each individual was used in three trials with a 20 min rest period between runs (see appendix A). Lastly, in the fourth experiment we examined the effects of tadpole size and developmental stage on host behavior by exposing 40 individually housed tadpoles that varied in Gosner stage (Gosner 1960) and snout-vent length (5 to 12 mm) to 25 *Ribeiroia* cercariae. We collected egg masses at different times to create the gradient of sizes. Tadpole activity levels were quantified one day before and one day after infection at 0:800 hrs as described above.

**Analyses** – For the pre-infection experiments, we used generalized linear mixed effects models (GLMMs) with a Poisson error distribution and a log link function to
analyze data on the number of active tadpoles per observation in both the laboratory and mesocosm studies (Zuur et al. 2009). We focused our analysis only on the data from after the introduction of chemical cues and we included a fixed effect of treatment and random effects of observation date and of experimental unit (plastic tub or mesocosm) (see appendix A for additional details on analyses).

For the post-infection behavior experiments, we utilized GLMMs with a binomial error distribution and a logit link function when the response variable was individual tadpole activity (yes/no) (Warton and Hui 2011). In all post-infection analyses we specified individual tadpole host as a random effect. In the parasite identity experiment, we specified trematode species as a fixed effect and we focused our analysis on the data from one day after parasite exposure. In the experiment in which we varied parasite exposure intensity and monitored behavior over time, we specified a GLMM with fixed effects of day, parasite dosage, and their interaction, and again focused on the data from after tadpoles were exposed to parasites. For the escape distance experiment we generated a model with a Gaussian error distribution that included a fixed effect of dosage to predict distance travelled of each tadpole (log transformed to improve normality). Because we ran three trials for each tadpole, we included a random effect of trial nested within tadpole. Lastly, in the experiment varying tadpole size and development stage, we used a model with a fixed effect of tadpole size (snout-vent length), a fixed effect of experimental period (before or after exposure) and an interaction between tadpole size and experimental period. All mixed effects models were run using the lme4 package in R (R Core Team 2013).
Results

Pre-infection behavior experiments – *Pseudacris regilla* in the laboratory enclosures strongly decreased activity in response to dragonfly chemical cues during the first five days after the establishment of treatments (Fig. 2.2a; GLMM, \( z = -7.548, p < 0.001 \)). There were also fewer tadpoles at the water surface within enclosures containing caged dragonflies relative to control treatments (GLMM, \( z = -3.207, p = 0.001 \)). However, there were no significant effects of uninfected snails, infected snails, or infected snails + tadpoles on either *P. regilla* activity or the number of *P. regilla* at the water surface (all \( p \) values > 0.3). *Pseudacris regilla* survival and wet mass at the conclusion of the study did not differ among treatments.

Supporting our results from the laboratory study, both *P. regilla* and *A. boreas* in outdoor mesocosms responded to caged dragonfly predators, but did not react to cues from uninfected snails, infected snails, or infected snails caged with tadpole hosts (Fig. 2.2b, 2.2c). Relative to control treatments, the presence of dragonflies reduced activity of both *P. regilla* (GLMM, \( z = -3.900, p < 0.001 \)) and *A. boreas* (GLMM, \( z = -6.183, p < 0.001 \)). There were no other significant effects of the other treatments on tadpole activity (all \( p \) values > 0.15). As with the laboratory study, behavioral changes were strongest in the days immediately following the establishment of treatments and then decreased over the duration of the study (Fig. 2.2b, 2.2c). Lastly, there were no differences in the wet mass or survival of either tadpole species between any of the treatments. Survival averaged 96% for *A. boreas* and 99% for *P. regilla* across all mesocosms.

Post-infection behavior experiments - After successful transmission, *Ribeiroia* caused decreases in host activity levels that varied with characteristics of both the
Figure 2.2. The relative difference in the mean number of active Pacific chorus frog (*Pseudacris regilla*) and western toad (*Anaxyrus boreas*) tadpoles between control treatments and treatments exposed to cues from predatory dragonfly larvae, uninfected snails, trematode-infected snails, and infected snails with amphibians in either the laboratory (a) or in an outdoor mesocosm experiment (b and c). Only tadpoles exposed to dragonfly cues (grey boxes) showed different activity levels from controls (dashed horizontal lines). The vertical dashed lines indicate the time at which chemical cues were introduced to all treatments.

parasite exposure and the tadpole host. One day after infection, *Ribeiroia*-exposed tadpoles decreased activity by over five-fold relative to unexposed control tadpoles (Fig. 2.3a; GLMM, $z = -5.898, p < 0.001$). In contrast, exposure to an identical dosage of
*Echinostoma* or *Alaria* infectious stages had no effect on host activity one day after exposure (Fig. 2.3a; GLMM, *Echinostoma*, \( z = 0.946, p = 0.344 \); *Alaria*, \( z = 0.654, p = 0.513 \)).

Both the magnitude of activity reduction and the time required for hosts to regain normal activity levels were influenced by the dosage of *Ribeiroia* administered (Fig. 2.3b). At high dosages, the magnitude of reduction in activity level depended on time-since-exposure, such that infection initially induced a strong reduction in activity that subsequently weakened over the next four days (Fig. 2.3b; GLMM, day x dosage, \( z = 8.930, p < 0.001 \)). The initial effect of infection increased with parasite dosage; exposure to five or ten cercariae resulted in small reductions in host activity one day after exposure, whereas dosages of 20 or greater cercariae induced a three fold decrease in host activity. Five days after exposure, infected tadpoles were still 10 to 30% less active than controls, indicating that recovery of normal activity was not complete even at the lowest dosages (Fig. 2.3b). Importantly, the timing of host recovery after infection also depended on whether parasites were administered as a one-time pulse exposure or as a daily chronic exposure. On day five, tadpoles receiving a chronic daily exposure of ten cercariae over four days were over 60% less active than unexposed controls and 40% less active than hosts receiving a pulse exposure of 40 parasites on day one (Fig. 2.3b; GLMM, pulse vs. chronic, \( z = 2.062, p = 0.0392 \)).

In parallel with the effects on host activity, increasing exposure also correlated strongly with decreases in escape distance from a simulated predator (Fig. 2.4a). Exposure to 20 *Ribeiroia* cercariae caused an 80% decrease in escape distance relative to unexposed controls, whereas five cercariae caused only a 20% decrease (LME, dosage,
Figure 2.3. Box plots showing activity levels of *Pseudacris regilla* tadpoles before and one day after infection by one of three species of trematode parasites (*Echinostoma trivolvis*, *Alaria* sp 2. or *Ribeiroia ondatrae*). Time active on the y-axis represents the average proportion that individual tadpoles were active out of 30 observations (a). Activity levels of *Pseudacris regilla* tadpoles one and five days after a pulse exposure to *Ribeiroia ondatrae* (0 to 40 cercariae) or a chronic exposure of 10 cercariae administered nightly for four nights (the far right column labeled “chronic”) (b). In all box plots, the upper and lower hinges correspond to the first and third quartiles, the horizontal line shows the median, the whiskers extend to the highest and lowest values within 1.5 times the interquartile range, and outliers are shown as solid points.

$t = -6.201, p < 0.001$). Furthermore, dosage was a strong predictor of the number of successfully infecting parasites per host at the end of both the dosage experiment ($r^2 = 0.88$) and the escape distance experiment ($r^2 = 0.82$), giving us the same results whether we used dosage or infection intensity as the independent variable (see appendix A).
The magnitude of reductions in activity one day after infection was mediated by the size and stage of the tadpole hosts (Fig. 2.4b). Although smaller hosts were slightly less active than larger individuals prior to infection, this effect became much stronger one day after infection, such that small individuals decreased activity by around 50% while larger individuals were unaffected by infection or increased in activity (GLMM, snout-vent length x experimental period, z = -6.592, p < 0.001). Similar results were obtained using developmental stage, rather than snout-vent length, as the predictor variable (GLMM, stage x experimental period, z = -5.484, p < 0.001). The effects of host size and developmental stage were not driven by differences in the number of encysting parasites, as there was no relationship between tadpole snout-vent length and Ribeiroia infection success two days after exposure (F_{1,48} = 9.23, r^2 = 0.06, p = 0.126).

Discussion

Parasites can alter the behavior of their hosts at multiple phases of the host-parasite interaction, including before, during and after infection (Moore 2002). Here, we find that prior to parasite transmission, hosts did not respond to the threat of infection from a virulent parasite. Thus, in contrast to the well-documented inducible defenses to predation risk (Relyea 2001), tadpoles did not exhibit phenotypic plasticity in response to infection risk. These results suggest that the mechanisms used to avoid or minimize threats from different classes of natural enemies have evolved along different trajectories within our study system; in contrast to defenses used to minimize predation risk, amphibian hosts may have few options to minimize trematode infection prior to direct contact with infective stages. However, nine hours after successful transmission, infected
Figure 2.4. The escape distance swam by *Pseudacris regilla* tadpoles in response to a simulated predator one day after exposure to 0 to 20 *R. ondatrae* cercariae. The y-axis represents the log transformed mean of three trials per tadpole and the y-axis shows the numbers of successfully infecting parasites at the end of the experiment (a). The effects of tadpole size (snout-vent length) on the relative change in activity levels of *Pseaudacris regilla* tadpoles from one day before to one day after exposure to 25 *Ribeiroia ondatrae* cercariae (b).

hosts strongly reduced activity levels and escape distances from a simulated predator.

The magnitudes of the changes in activity after infection were predictably influenced by multiple factors including parasite identity, infection intensity, time-since-exposure, exposure duration (pulse vs. chronic), and host developmental stage. These findings indicate that the behavioral consequences of infection, while variable in time and space,
are predictable outcomes that depend on multiple traits of the parasite, the host, and the dynamics of their interaction.

A growing body of research has aimed to test for ecological similarities between predation and parasitism, including the roles of distinct natural enemies in the ‘ecology of fear’. Our results add to this conceptual development in several key ways. As predicted, the presence of dragonflies induced the formation of behavioral defenses in larval amphibians, which are known to reduce the risk of predation (Lawler 1989; Relyea 2001). In contrast, the same host species did not show behavioral changes in response to multiple types of chemical cues from parasites. Our experiments included treatments that tested for host responses from multiple ecologically relevant cues that could signal infection risk, including cues from infected host snails, infectious free-swimming parasite stages, and conspecific amphibian hosts actively becoming infected. None of these cues elicited defenses in tadpole hosts. Moreover, this result was consistent between a simplified laboratory experiment with one host species (Pseudacris regilla) and a more realistic outdoor mesocosm experiment with two host species (Pseudacris regilla and Anaxyrus boreas).

The lack of a response to infection risk contrasts with several previous studies involving amphibian hosts. Two studies have shown some ability of tadpoles to behaviorally react to threats from trematodes, potentially before parasite contact (Kiesecker and Skelly 2000; Rohr et al. 2009). In both studies, however, the exact mechanism of parasite avoidance was not entirely clear. In the more recent study, Echinostoma trivolvis cercariae were contained within 75 μm Nitex mesh to prevent direct contact with tadpoles (Rohr et al. 2009). However, Ribeiroia ondatrae cercariae,
which can be twice as large as *Echinostoma trivolvis* cercariae (Preston et al. 2013), are able to pass through 63 μm Nitex mesh (D. Preston pers. obs) suggesting that parasites could have been directly contacting hosts in the prior study and necessitating the use of smaller mesh sizes (Lunde et al. 2012; Marino et al. 2013). More broadly, there is some evidence that certain hosts are able to detect waterborne cues signaling infection risk. Caribbean spiny lobsters, for example, avoid sharing dens with conspecifics that are infected with a lethal virus (Behringer et al. 2006) and bullfrog tadpoles avoid conspecifics infected with a pathogenic yeast (Kiesecker et al. 1999). Whether such host responses are elicited by cues from the parasites or the infected conspecifics remains unclear. In our experiments, it is somewhat surprising that conspecifics becoming infected did not elicit host responses. *Ribeiroia* trematodes cause severe tissue damage and hemorrhaging (Johnson et al. 2004) and most species of tadpoles readily react to alarm cues from injured conspecifics (Chivers and Smith 1998; Schoeppner and Relyea 2005). We note, however, the possibility that tadpoles may have reacted to cues signalling *Ribeiroia* infection risk at night but not during daylight. Chemical cues from cercariae released at night might have weakened by the time behavioral observations were made each day, although cues from infected conspecifics were likely present continuously. Furthermore, host responses to cues other than chemicals (e.g., water vibrations from cercariae) should also be investigated in the future.

For the induction of inducible defenses against natural enemies to enhance individual fitness, the potential benefits gained must outweigh the costs. Individuals expressing morphological and/or behavioral defenses to specific natural enemies sometimes perform poorly at other vital functions including foraging, growth, seeking
mates and responding to risks from disparate threats (Harvell 1990; Van Buskirk 2000; Relyea and Auld 2004). In the case of predation, these costs are commonly offset by the benefits of avoiding a predation event. This trade-off may be less predictable in the case of parasite infections, particularly involving macroparasites. While predator attacks are typically fatal for prey, the consequences of macroparasite infection are intensity dependent such that the ultimate risk depends on the number of infection events, rather than merely the presence of parasites. Furthermore, animal hosts are equipped with a variety of behavioral and physiological mechanisms to reduce or repair the consequences of infection after parasite contact. Macroparasite infection risk can be reduced through grooming behaviors or parasite avoidance strategies that are triggered by tactile cues coming directly from infective stages (Clayton 1991; Mooring et al. 2004; Taylor et al. 2004). Additionally, immune responses provide a further line of defense after successful infection. These responses to infection after parasite contact are costly (Lochmiller and Deerenberg 2000) and may make it inefficient for hosts to invest heavily in anti-parasite strategies both before and after infection.

In contrast to the lack of behavioral response to disease risk before infection, amphibian hosts exhibited strong reductions in activity levels after successful infection. These changes in behavior, which were observed >9 hrs after parasite exposure, are distinct from the adaptive parasite avoidance behaviors tadpoles exhibit when they are first contacted by infectious cercariae (i.e., evasive tail movements and rapid bursts of swimming; Taylor et al. 2004; Daly and Johnson 2011). The host behaviors observed here occurred later in the host/parasite interaction and the magnitude of these effects varied with traits of the parasite and the host. Infection by Ribeiroia strongly reduced
host activity (>80%) whereas two other trematodes (Alaria sp. 2 and Echinostoma trivolvis) administered at the same exposure intensity did not alter host behavior relative to controls. These differences are most likely due to variation in virulence, some of which is associated with differences in the size and/or mode of entry of invading cercariae (Orlofske et al. 2009; Rohr et al. 2009; Johnson and Hoverman 2012; Preston et al. 2013). Unlike the two less virulent parasites, Ribeiroia cercariae use proteolytic enzymes to penetrate second intermediate hosts and cause hemorrhaging and tissue damage upon entry (Johnson et al. 2004). The behavioral outcomes of Ribeiroia infection also depended strongly on both the exposure dosage and the timing of infection. A dosage of 20 to 40 cercariae led to a three-fold decrease in host activity 24 hrs after infection, whereas smaller dosages had minimal effects on host behavior. Importantly, such effects were reversible after a one-time pulse exposure, but persisted over time during a daily chronic exposure. In nature, chronic low-level exposures are perhaps most realistic, suggesting that long term host behavioral changes may be common.

In addition to parasite identity and exposure dynamics, host traits influenced the behavioral outcome of infection. We found a strong correlation between host size and behavioral changes, where smaller hosts experienced the largest changes in activity level after infection. This result is likely due to the fact that the area of tissue damage is larger relative to the tadpole's body in smaller individuals, although we note that tadpole immune systems also change considerably over the course of development, which can limit infection success. Our results are consistent with prior work that indicates host tolerance varies over the course of host growth and development (Sollid et al. 2003; Rohr et al. 2010). In a previous study, Pseudacris regilla tadpoles over the same range of
developmental stages used in our experiment experienced an increase in survival from 55% in the smallest size class to 100% in the largest size class after *Ribeiroia* exposure (Johnson et al. 2011). This result parallels the size-dependent changes in behavioral effects observed here and in both studies these changes in activity and mortality can be attributed to differences in tolerance (the ability of a host to limit pathology) rather than resistance (the ability of a host to limit infection success) (Raberg et al. 2009). These findings indicate that environments that favor rapid host growth will narrow the window in which infection is likely to cause significant decreases in host activity.

The observed changes in behavior likely have consequences for host and parasite fitness. In general, parasite-induced changes in host behavior can be adaptive for the parasite, adaptive for the host, or simply side-effects associated with pathology (Poulin 2010). While we cannot rule out that the observed changes are somehow adaptive for the parasite, it seems more likely that they stem from side-effects of pathology and/or adaptive host responses. In the simplest explanation, injuries caused by the penetration of cercariae may lead to reduced muscle function, which impairs movement and reduces host activity. Alternatively, lethargy is consistent with adaptive host ‘sickness behaviors’ that allow hosts to divert resources towards fighting infection or repairing damage (e.g., immune responses; Hart 1988; Adelman and Martin 2009). While such responses are generally associated with microparasite infections (Martin et al. 2008; Llewellyn et al. 2011), it seems plausible that they could apply to our observed results with macroparasites. Furthermore, if the changes in host behavior were adaptive for the parasite, they would be strongest when the parasites are infectious to the next host in the life cycle (> 24 hrs after encysting in tadpoles). We found the opposite pattern, where the
host behavioral changes were strongest before the parasite was infectious, supporting the idea that the observed changes are not a case of parasite manipulation.

Understanding the community level effects of changes in host behavior – whether adaptive or otherwise – remains an important challenge in disease ecology (Lefèvre et al. 2009; Hawley and Altizer 2011). The increased lethargy observed in our study will likely have consequences for host competitive ability, predation risk and subsequent infection dynamics (Lefcort and Blaustein 1995). Many amphibian predators rely on visual cues, such that inactive tadpoles are less susceptible to predation (Lawler 1989). However, our simulated predator experiment indicated that the escape distance of tadpoles is reduced with increasing infection intensity. For predators that actively pursue their prey, this could result in increased predation rates. Host behavioral modifications can also shape parasite transmission and subsequent host infections. Within our system, reduced activity levels make amphibian larvae more susceptible to trematode infection (Thiemann and Wassersug 2000; Szuroczki and Richardson 2012). In this instance, host lethargy may lead to feedbacks that enhance infection and contribute to parasite aggregation (Johnson and Hoverman 2014) and the skewed distribution of parasite infections in natural populations (i.e., 20% of hosts harbor 80% of the parasites), as well as the occurrence of superspreading individuals that contribute disproportionally to disease transmission (Lloyd-Smith et al. 2005; Paull et al. 2011). The net effect of reductions in activity levels on *Ribeiroia* transmission, however, is difficult to predict because *Ribeiroia* must be trophically transmitted from amphibians to its definitive hosts. If reductions in activity levels reduce predation rates by definitive hosts on infected tadpoles, they may reduce net transmission. Ultimately, the net effect of infection-induced behaviors on both predation
rates and subsequent host infection will likely depend on the environmental context and the community composition in which the host-parasite interaction is embedded (Marino et al. 2013; Marino and Werner 2013; Orlofske et al. 2014).

Our results illustrate that despite some similarities, predation and parasitism can elicit disparate responses that depend on traits of the natural enemy and the individual being attacked. Interestingly, the changes in host activity after infection observed here show parallels to the behavioral changes often associated with nonconsumptive predator effects (i.e., reduced activity). In this regard, the sublethal effects of predators on prey prior to direct contact may be most similar to the ecological consequences of parasite infection (rather than fear responses prior to parasite contact). These results underscore the need to consider similarities and differences between the ecology of distinct natural enemies at multiple phases of their interactions, which in turn will foster a more comprehensive understanding of natural enemy ecology.

Appendix A

*Pre-infection behavior experiments* – In the pre-infection laboratory experiment, fifteen tadpoles were placed into each replicate enclosure that contained a cage within the center (see Fig. A1). Enclosures were surrounded with brown paper to minimize the potential for tadpoles to respond to visual cues from the observer. The cages consisted of plastic cups (20 cm tall by 11 cm diameter) that were open to the air on top and affixed to the center of each enclosure with silicone glue (see Fig. A1). Each cage had three
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pseudacris regilla</th>
<th>Anaxyrus boreas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>snout-vent length, mm (SE)</td>
<td>snout-vent length, mm (SE)</td>
</tr>
<tr>
<td>Pre-infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td>7.7 (.07)</td>
<td>-</td>
</tr>
<tr>
<td>Mesocosm</td>
<td>10.2 (0.06)</td>
<td>12.9 (0.13)</td>
</tr>
<tr>
<td>Post-infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple trematodes</td>
<td>5.6 (0.08)</td>
<td>-</td>
</tr>
<tr>
<td>Dosage and recovery</td>
<td>8.4 (0.09)</td>
<td>-</td>
</tr>
<tr>
<td>Escape distance</td>
<td>4.3 (0.05)</td>
<td>-</td>
</tr>
<tr>
<td>Tadpole size</td>
<td>see Fig. 3b</td>
<td>-</td>
</tr>
</tbody>
</table>

Table A.1. Mean snout-vent lengths of amphibian larvae used in all experiments. Amphibians were measured at the conclusion of each study.

openings (~ 8 x 5 cm) that were covered in 35 μm Nitex mesh. The mesh prevented parasites and predators from reaching tadpoles in the enclosure, but allowed the passage of chemical cues from inside of the cups.

_Pseudacris regilla_ tadpoles used in the laboratory experiment were mixed from ~15 egg clutches that hatched in captivity. Prior to and during the laboratory experiments, tadpoles from all studies were maintained at ~20°C and were fed ground flake fish food and spirulina _ad libitum_. Prior to the experiments (~1 to 3 weeks post-hatching), tadpoles were housed in groups of ~50 individuals within 53 L plastic enclosures. At the onset of each experiment, tadpoles were size-matched (i.e., very large or small individuals were removed) and then were randomly assigned to replicates. Snails and tadpoles within mesh cages in the pre-infection laboratory study were fed solid food made from fish flakes,
Figure A1. Replicate enclosures in the pre-infection laboratory experiment. Each cup has openings covered in mesh that emit chemical cues into the surrounding water while also preventing natural enemies from reaching tadpoles in the enclosures. Photo by Dan Preston.

spirulina and agar. Equal amounts of food were placed in the control and dragonfly cages to account for chemical cues coming from food within cages. The tubs were arrayed in a randomized block design and we rotated their position and conducted water changes approximately every third day.

Mesocosm methods – The mesocosms consisted of 378 L livestock watering tanks that were filled with well water and 6 kg of silica sand as substrate (Fig. A.2). We covered the mesocosms with mesh screen lids (1.5 mm) to prevent unintended colonization by insects. On May 16th, we added 30 g of rabbit chow and 250 mL of periphyton suspended in natural pond water as a source of nutrients and algae inoculum, respectively. Periphyton was collected by shaking aquatic plants into a bucket of pond water. Between May 18th and May 31st we added a total of 750 mL of concentrated
Figure A2. Outdoor mesocosms at the Hopland Research and Extension Center in Mendocino County, California that were used to examine the effects of chemical cues from predators and parasites on amphibian behavior. Photo by Dan Preston.

zooplankton from a local wetland (primarily calanoid copepods and cladocerans). On June 3rd and June 4th we introduced 15 *P. regilla* tadpoles and 15 *A. boreas* tadpoles that had been previously collected as embryos in Mendocino County, California. As with the laboratory studies, tadpoles were mixed from multiple egg masses. Prior to the start of the experiment, tadpoles were maintained in the laboratory as described above.

The cages used in the mesocosm experiment consisted of plastic 16 oz cups that were allowed to float freely on surface of the water. The cups were 12 cm tall by 9 cm wide at the top. We affixed a piece of 35 μm Nitex mesh to the opening of each cup using
a secure rubber band (no holes were made in the cups, in contrast to the cages in the laboratory experiment). This allowed us to access the cages to check on the dragonflies, snails and tadpoles that were held within. The cages naturally floated upside down within the mesocosms (i.e., the mesh was facing down into the tank allowing the passage of chemical cues). Into each cage we also placed a 10 cm length of periphyton-covered cattail stem (Typha sp.) that provided a food source to snails and tadpoles and perching surface for dragonfly larvae. The cages were checked every other day throughout the experiment and deceased tadpoles were replaced as necessary.

*Pre-infection response variable measurement* – The behavioral observations for the pre-infection experiments were conducted at one time each day, beginning at 08:00 hrs in the laboratory study and at 12:00 hrs in the mesocosm study. Each replicate was observed five times per day in the laboratory study and fifteen times per day in the mesocosm study. In both experiments, the order of observations was reversed between consecutive rounds (i.e., the first replicate observed became the last replicate observed on the subsequent observation round; this approach aimed to remove potential effects of observation time). Observing each replicate took approximately 5 seconds in the laboratory experiment and 15 seconds in the mesocosm experiment. Changes in movement status within the observation period did not affect the final count of active tadpoles because each tadpole was counted at only one specific time within each observation period and therefore could only be moving or not moving.

*Post-infection behavior experiments* – For three of the four post-infection experiments, individual *Pseudacris regilla* tadpoles were maintained in containers with 750 mL of water and fed as described above. Tadpoles were infected with trematodes by
isolating cercariae from field-collected infected snails within 50 mL vials. Cercariae were removed from the 50 mL vials using a glass pipet, counted under a dissecting microscope, and administered to individually housed tadpoles according to the dosage of each treatment. Tadpoles in treatments that were not infected received a sham dosage of water that did not contain cercariae. Because cercariae are short lived (< 24 hrs) and the peak time of trematode emergence from snails varies between the species used, we conducted infections at 14:00 hrs for *Echinostoma* and *Alaria* and at 23:00 hrs for *Ribeiroia* (see also Johnson and Hartson 2009; Johnson et al. 2011 for exposure protocols).

The response data collected on tadpoles in three of the four experiments consisted of observations of tadpole activity levels (yes/no data based on whether each individual was moving or not). Each replicate was observed one after another until every replicate had been observed 30 times (as opposed to observing each replicate 30 times in a row and then moving on to the next replicate). The order of observations was reversed between consecutive rounds (i.e., the first replicate observed became the last replicate observed on the subsequent observation round). Each observation on the single tadpole per replicate was instantaneous (< 1 second), so the total amount of time elapsed between observations on the same tadpole varied with the number of replicates in the experiment (from 40 tadpoles in experiments one and four to 70 tadpoles in experiment three). We estimated that the amount of time between observations on the same tadpole ranged from two to four minutes for the three experiments.

In the escape distance experiment, the track was made of white PVC plastic. It held approximately 2 L of water and the water was not changed between trials. A tape
with markings every one cm was affixed to the laboratory bench along the length of the track. The final position of each tadpole in the track was determined visually and was recorded in a laboratory notebook after each trial run.

**Analyses** – For the pre-infection experiments, we initially determined that there were no significant differences in the number of active tadpoles across treatments prior to the introduction of chemical cues and therefore focused our analysis on the data from after the introduction of chemical cues (days three through ten in both the mesocosm and laboratory experiments). Our GLMMs included a fixed effect of treatment and random effects of observation date and of experimental unit (plastic tub or mesocosm). The random effect of experimental unit accounted for non-independence of multiple observations made on each replicate (i.e., five observations per day in the laboratory study and 15 observations per day in the mesocosm study). The random effect of date accounted for correlated daily variation in activity levels due to environmental conditions (e.g., temperature). We analyzed the data on the number of tadpoles at the surface of the water from the laboratory study in the same manner as tadpole activity.

In all post-infection analyses we specified individual tadpole host as a random effect to account for non-independence of multiple observations made on the same tadpole on the same date. All of the details for the analyses of the first three experiments are provided in the main text. For the fourth experiment, in which we varied tadpole size and development stage, we detected significant differences in tadpole activity levels one day prior to exposure because small tadpoles were less active than larger individuals. To account for this pre-existing difference, we created a model with a fixed effect of tadpole size (snout-vent length), a fixed effect of experimental period (before or after exposure)
and an interaction between tadpole size and experimental period. If tadpole size mediated the magnitude of behavioral responses to infection we expected to see an interaction between experimental period and snout-vent length, where the effect of snout-vent length differed from pre- to post *Ribeiroia* exposure (see Results).

The analyses presented in the main text for the post-infection experiments used parasite dosage as a predictor variable. Because we necropsied all of the amphibians at the end of the experiments, we were also able to examine whether using infection intensity (i.e., the number of successful cysts per host) altered any of our conclusions from the two studies in which we varied parasite dosage. We found that infection intensity closely mirrored the parasite dosages administered in both the dosage and recovery experiment ($r^2 = 0.88$) and the escape distance experiment ($r^2 = 0.82$). In the dosage experiment, the number of successfully encysting parasites at the conclusion of the study was a negative predictor of host activity one day after exposure (GLMM, $z = -2.997$, $p = 0.002$). Similarly, infection intensity was also a strong negative predictor of distance travelled in the escape distance experiment (GLMM, $z = -7.241$, $p < 0.001$).
CHAPTER III

COMPLEX LIFE CYCLES IN A POND FOOD WEB: EFFECTS OF LIFE STAGE STRUCTURE AND PARASITES ON NETWORK PROPERTIES, TROPHIC POSITIONS AND THE FIT OF A PROBABILISTIC NICHE MODEL


Abstract

Most food webs use taxonomic- or trophic species as building blocks, thereby collapsing variability in feeding linkages that occurs during the growth and development of individuals. This issue is particularly relevant to integrating parasites into food webs because parasites often undergo extreme ontogenetic niche shifts. Here, we used three versions of a freshwater pond food web with varying levels of node resolution (from taxonomic species to life stages) to examine how complex life cycles and parasites alter web properties, the perceived trophic position of organisms, and the fit of a probabilistic niche model. Consistent with prior studies, parasites increased most measures of web complexity in the taxonomic species web; however, when nodes were disaggregated into life stages, the effects of parasites on several network properties (e.g., connectance and nestedness) were reversed, due in part to the lower trophic generality of parasite life stages relative to free-living life stages. Disaggregation also reduced the trophic level of organisms with either complex- or direct life cycles and was particularly useful when including predation on parasites, which can inflate trophic positions when life stages are collapsed. Contrary to predictions, disaggregation decreased network intervality and did not enhance the fit of a probabilistic niche model to the food webs with parasites.
Although the most useful level of biological organization in food webs will vary with the questions of interest, our results suggest that disaggregating species-level nodes may refine our perception of how parasites and other complex life cycle organisms influence ecological networks.

**Introduction**

Twenty-five years ago, ecologists began to argue that the ecological patterns obtained from food webs were only as valid as the methods and data used to construct the webs (Paine 1988; Polis 1991). At the time, most available food webs significantly underrepresented existing biodiversity and there were large inconsistencies in the resolution of nodes and links (Dunne 2006). Importantly, the realization that poor data sets could result in artifactual food web patterns motivated efforts to comprehensively understand how discrepancies in methodology altered observed patterns (Winemiller 1989; Martinez 1991) and initiated a movement to improve the quality of food-web data sets (Cohen et al. 1993).

Despite considerable improvements in food-web data, most webs do not fully capture the dynamic nature of feeding interactions. Currently, the majority of existing food webs are constructed using taxonomic species as nodes, often with the exception of basal groups that are aggregations of taxonomic species (Thompson and Townsend 2000; Ings et al. 2009). This approach to characterizing feeding interactions combines a large amount of variation in trophic interactions among individuals (e.g., owing to ontogenetic stage, body size, sex, reproductive status, or time-of-year) into an approximation at the species level (Woodward 2007; Rudolf 2008). Species with complex life cycles have
perhaps the most dynamic trophic interactions because they often exploit distinct niches throughout the course of growth and development, sometimes involving dramatic shifts in both predators and prey from one life stage to another (Wilbur 1980; Werner and Gilliam 1984). The effects of disaggregating complex life cycle species on the structure and dynamics of food webs have not been well studied; however, one of the first empirical assessments indicated that incorporating life stage structure increased the vulnerability of food webs to secondary extinctions (Rudolf and Lafferty 2011). This important result came about largely because many organisms that are represented in food webs as dietary generalists at the species level are in fact ontogenetic specialists at the life stage level, making them highly sensitive to secondary extinction.

Understanding the implications of complex life cycles on food web structure is particularly relevant to the integration of parasites into food web theory because many parasites undergo extreme ontogenetic niche shifts during development (Lafferty et al. 2008). For example, some trematodes in pond ecosystems must sequentially infect snails, amphibians, and predatory birds to complete their life cycle (Fried and Graczyk 1997). In between the three parasitic life stages are two free-swimming, non-feeding stages that can serve as prey to other species (Johnson et al. 2010). The prevalent view of nodes as taxonomic species requires grouping these five life stages together into a node that appears as a dietary generalist feeding on multiple trophic levels (from snails to herons) and is susceptible to an immense diversity of predators (from zooplankton to coyotes). Nonetheless, most prior food web studies to examine the roles of parasites have utilized taxonomic- or trophic species as nodes (e.g., Thompson et al. 2005; Lafferty et al. 2006; Hernandez and Sukhdeo 2008; Amundsen et al. 2009; Dunne et al. 2013; but see Huxham...
et al. 1995). These studies leave open the question of whether life stage structure alters our understanding of how parasites and other complex life cycle species influence most aspects of food webs.

Disaggregating species level nodes is predicted to alter food webs in a variety of ways. Measures of web complexity, such as connectance and linkage density, can change in unpredictable ways due to disaggregation because it is mathematically possible for the ratio of links to nodes to either increase or decrease with network size. Such changes will depend on the biology of the organisms in the web, including factors such as diet breadth and predator vulnerability of the taxonomic species relative to life stages. Additionally, incorporating life stages will lead to inherent increases in web size and may cause changes due to the scale dependence of some web properties alone (Schoener 1989; Martinez 1993, 1994). Life stages may also be more appropriate than taxonomic species for particular types of food-web analyses. For instance, considerations of trophic levels present a unique challenge for food webs that incorporate parasites or other organisms with complex life cycles. Using traditional indices (e.g., Williams and Martinez 2004), the trophic position of a parasite represented as a taxonomic species would be designated based on all of the hosts used within its life cycle and would likely be overestimated relative to the actual trophic level of each life stage. This problem becomes compounded if predators feed on parasite life stages, which is a prominent link type in most webs with parasites (Thieltges et al. 2013).

Disaggregating species level nodes into life stages has also been suggested as one way to improve the fit of recent food-web models to empirical food-web data sets. The niche model (Williams and Martinez 2000) and probabilistic niche model (Williams et al.
2010, Williams & Purves 2011) assume that each organism in the food web feeds along a contiguous or near contiguous feeding interval. Complex life cycle organisms, and particularly parasites, may challenge this assumption at the species level. The distinct niche shifts of most complex life cycle organisms, in terms of both resources and predators, probably result in less contiguous niches than direct life cycle species (Dunne et al. 2013). Parasites are also smaller than their resources and often feed on different hosts that vary greatly in body size and trophic position, with may contribute to decreases in the fit of niche models to food webs that include parasites (Dunne et al. 2013). Consequently, life stage structure may improve the fit of niche models if life stages have a more contiguous feeding interval than taxonomic species.

Our goals in the present study were to examine how node disaggregation influenced the role of parasites, the trophic positions of organisms, and the fit of a probabilistic niche model to an empirical pond food web. To accomplish these aims, we analyzed three versions of a food web from a freshwater pond, each with and without parasites. Version one included taxonomic species for most nodes, excluding some aggregated basal nodes, and is referred to as the "taxonomic species web"; version two included disaggregated nodes only for species that display distinct ontogenetic diet shifts and is referred to as the "niche shift web"; version three included disaggregated nodes for all species with complex life cycles for which we had diet information for each life stage and is referred to as the "life stage web". We predicted that the effects of parasites on food web properties would be sensitive to how nodes were defined in the web due to differences in trophic generality and vulnerability between life stages and species. We also predicted that trophic level analyses would become more accurate when conducted
on food webs with life stage structure because life stages often undergo shifts in diet and many predators specialize on life stages rather than taxonomic species. Lastly, we hypothesized that life stage structure would improve the fit of the probabilistic niche model by making the feeding niches of nodes in the web more contiguous.

Methods

Study Site - The food web was constructed for Quick Pond within Alameda County in the San Francisco Bay Area of California, USA. The pond is at an elevation of 435 m and is surrounded by grassland and mixed oak habitat that is lightly grazed by domestic sheep during the summer. Quick Pond has a permanent hydroperiod, an early summer surface area of ~2,200 m², and a maximum depth of ~2.5 m. In June of 2009, nutrient concentrations in the pond measured 53 ug/L total dissolved phosphorus and 1779 ug/L total dissolved nitrogen.

Food-Web Construction - The complete food-web data set is available online and can be found in Preston et al. (2012). The food web includes detailed information on each node, such as classification, lifestyle, consumer strategy, mobility, residency and body size/biomass information for abundant organisms. It also includes information on each link, including link type (e.g., detritivory, predation, parasitism, etc.) and link evidence (e.g., direct observation, modeled from a similar system, or literature citations when information came from published studies). The species list of free-living organisms came from data collected between 2009 and 2011 and is based primarily on direct observations using visual encounter surveys, dip-net and seine-net sweeps, stove-pipe samples, and zooplankton tows (see also Preston et al. 2013). A few less common invertebrate taxa
were added based on surveys at the same site made by Lunde and Resh (2012). Parasites were added into the food web based on evidence from host dissections and in a few instances, from evidence in the literature (<10% of species). When one parasite life stage was detected, we assumed the other life stages were present. Our parasite quantification likely undersampled certain groups, including phytoplankton, parasites of zooplankton, and non-trematode parasites of birds and mammals (see Preston et al. 2012 for a complete list of undersampled groups and aggregated nodes and appendix B for more details on web construction).

Our analyses involved three different versions of the Quick Pond food web, each of which we analyzed with and without parasites. For consistency with other work (Dunne et al. 2013), we also conducted analyses on versions of the web that included parasites but lacked concomitant links (i.e., links that occur when a parasite is consumed by a predator feeding on infected prey; Johnson et al. 2010). All web versions can be extracted from the published data set. We created three versions of the web (the taxonomic species web, niche shift web, and life stage web) to independently examine the effects of both parasites and of incorporating complex life cycles on web structure. The taxonomic species web is the most aggregated version and every node is either a single taxonomic species, or in some instances an aggregation of trophically similar species (aggregation of certain poorly resolved nodes [e.g., phytoplankton, bacteria] is common in most previously published food webs; Thompson and Townsend 2000). The other two web versions contained nodes that were disaggregated according to life stage characteristics of species with complex life cycles. In the niche shift web, we disaggregated nodes into life stages only when the species in question underwent distinct
ontogenetic diet shifts during the course of development. We quantified the degree of ontogenetic diet shift \( p \) for each node with a complex life cycle following Rudolf and Lafferty (2011):

\[
p = \frac{\sum_{i,j=1}^{S} (k_{ij} \cap w_{ij})}{S}
\]

where \( S \) is the number of life stages within a node, \( k_{ij} \) is the number of shared resources used between life stages \( i \) and \( j \), and \( w_{ij} \) is the number of unique resources used between life stages \( i \) and \( j \). This index ranges from \( p = 0 \), for an ontogenetic specialist with zero dietary overlap between life stages, to \( p = 1 \), for an ontogenetic generalist with complete dietary overlap between stages. In the niche shift web, we only divided nodes into life stages when \( p < 0.5 \) for that specific node. This restricted the disaggregation of nodes to only species that showed a high degree of dietary specialization between life stages. Finally, in the life stage web we included disaggregated nodes of all complex life cycle species for which we had detailed life stage information, regardless of whether they underwent significant niche shifts between life stages.

**Food-Web Properties** - We calculated the number of nodes (\( S \)), number of links (\( L \)), linkage density (\( D \)), connectance (\( C \)), degree distributions, nestedness, and clustering coefficients for each version of the Quick Pond food web (i.e., the taxonomic species web, the niche shift web, and the life stage web, each without parasites, with parasites but no concomitant links, and with parasites and concomitant links). Linkage density quantifies the average number of links per node \( (D = L/S) \) and connectance quantifies the proportion of observed links out of the total number of links possible \( (C = L/S^2) \). Both connectance and linkage density are measures of web complexity and much research has examined how they are related to important network properties such as stability (e.g.,
May 1973; Cohen and Briand 1984; Dunne et al. 2002). We also examined the degree distributions of both free-living and parasitic nodes within each web to examine how the number of links per node (i.e., node degree) changes with node aggregation and the addition of parasites to the web. Average node degree is also equal to the sum of trophic generality (the number of links a node makes with resources) and trophic vulnerability (the number of links predators make with a node) (Schoener 1989). We calculated these values separately for both free-living organisms and their parasites. The aforementioned web properties were calculated with Network3D (Yoon et al. 2004) and the Cheddar package in R (Hudson et al. 2013). We also calculated nestedness, which is the degree to which specialists interact with nested subsets of the species interacting with generalists (Ings et al. 2009). Using the program ANINHADO (Guimaraes and Guimaraes 2006), we calculated the nestedness metric based on overlap and decreasing fill (NODF; Ulrich et al. 2009), as well as relative nestedness (N*; Bascompte et al. 2003). Lastly, we calculated clustering coefficients using the networkx package in Python (Hagberg et al. 2008). Clustering coefficients measure the fraction of connected link pairs that are both connected to an adjacent node (Dunne et al. 2002). For consistency with prior studies, we also calculated web properties for the four subwebs within the main food web (Lafferty et al. 2006). Additional information on analyses and results pertaining to the subwebs is provided in the ESM.

_Trophic Levels and Omnivory_ - We calculated short-weighted trophic level for each node, which is equal to the average of a node’s shortest trophic level (1 + the shortest chain to a basal taxon) and its prey-averaged trophic level (1 + the mean trophic level of the consumer’s resources) (Williams and Martinez 2004). We used these values
to obtain a mean trophic level per web and to investigate changes in trophic levels due to
disaggregation that occurred for organisms with complex life cycles and for organisms
with direct life cycles. For organisms with complex life cycles, we compared the trophic
level of the species in the taxonomic species web with the corresponding life stages in the
life stage web. We calculated larval trophic positions as the mean of all larvae when a
species had multiple larval stages. We also compared the trophic level of direct life cycle
species between the taxonomic web and the life stage web to examine effects of prey
disaggregation. We used the trophic level designations to calculate omnivory, which is
the fraction of species feeding on multiple trophic levels (defined as having a trophic
position that is not within 0.05 of an integer value; Thompson et al. 2007). For
calculations of trophic level and omnivory, we grouped non-feeding life stages of
heterotrophs (e.g., some adult flies and larval parasite stages) within their previous
feeding life stage. This adjustment was made because non-feeding life stages would
otherwise be assigned a trophic level of zero, equivalent to a basal taxon, despite the fact
that they are heterotrophs. We decided to not remove the non-feeding life stages for these
analyses because they frequently have a completely different suite of predators than other
stages and this adds valuable information to the web.

Probabilistic Niche Model and Intervality – We examined the fit of the
probabilistic niche model to all of the aforementioned web versions. The probabilistic
niche model uses the links in the food web to infer latent niche roles and probabilistically
interval feeding ranges (Williams et al. 2010; Williams & Purves 2011). The model was
recently applied to seven food webs with parasites, using trophic species as nodes (Dunne
et al. 2013) and can be used to accurately detect and reproduce structure in food webs
(Williams & Purves 2011). The goodness of fit is determined by the fraction of links predicted correctly, $f_L$, calculated as the average probability of existence assigned to observed links, when the number of expected links is close to the number of observed links. We used Markov Chain Monte Carlo to search for a maximum likelihood solution to the model (after Williams et al. 2010; Williams & Purves 2011).

To aid in the interpretation of the probabilistic niche model results, we also calculated intervality directly (Stouffer et al. 2006, Zook et al. 2011). Interval food webs are defined either by contiguous diets or contiguous predators, where an optimal latent ordering of species can be constructed such that each species’ diet (or each species’ predators) are consecutive within the ordering. Intervality is measured as the number of gaps (non-links) in those intervals, given some optimal ordering of species. Each missing species from the interval is counted as a single gap. The probabilistic niche model seeks to embrace the “quasi-intervality” commonly found in food webs (Stouffer et al. 2006), and so more flexibly captures this structure by allowing for gaps peripheral to the main interval of each species. We calculated intervality separately as consumer gaps and diet range gaps using the Cheddar package in R (Hudson et al. 2013).

**Results**

*Food-Web Versions* - Complex life cycles and ontogenetic diet shifts were prevalent within the Quick Pond food web. The taxonomic species web included 63 nodes made up of 5 basal groups, 43 free-living species and 15 parasites. Twenty of the 43 free-living nodes and 12 of the 15 parasite nodes were organisms with complex life cycles for which we had feeding information for each life stage. The free-living
organisms with complex life cycles were primarily insects and amphibians. The parasites with complex life cycles were primarily protists and helminth worms that obligately infect multiple host species. Disaggregating the complex life cycle organisms with distinct diet shifts at each life stage (i.e., the niche shift web with \( p < 0.5 \) for each node) resulted in an increase in network size to 105 nodes, of which 42 were parasite life stages. Disaggregating the nodes further, such that all life stages became a distinct node (the life stage web) increased the total number of nodes to 113. The entire increase in node count between the niche shift web and the life stage web was the result of disaggregating free-living insects and amphibians that did not undergo distinct diet shifts between life stages (all of the parasites with complex life cycles underwent distinct diet shifts; i.e., \( p < 0.5 \)).

**Food-Web Properties** - Disaggregating the nodes of the web led to obvious increases in the number of links (from 1088 to 1432 to 1905, respectively); however, this effect did not lead to consistently positive or negative changes in most web metrics. For connectance, linkage density, clustering coefficients and nestedness, the lowest metric was associated with the niche shift web, which has an intermediate number of nodes (Table 3.1). This pattern was consistent whether we included concomitant links or not (Table B.1). Disaggregating the nodes from the taxonomic species web to the niche shift web resulted in a decrease of connectance from 0.27 to 0.13 (Table 3.1). Disaggregating the nodes further resulted in a connectance of 0.15, reflecting the higher node degree of the added life stages in the life stage web (i.e., dietary generalists with complex life cycles). Linkage density showed the largest decrease in the niche shift web because only species with high resources specificity were disaggregated in this web (Table 3.1; linkage density = 17.3, 13.6, and 16.9 for the three webs). Clustering coefficients and nestedness
<table>
<thead>
<tr>
<th></th>
<th>Taxonomic Species Web</th>
<th>Niche Shift Web</th>
<th>Life Stage Web</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- Parasites</td>
<td>+ Parasites</td>
<td>- Parasites</td>
</tr>
<tr>
<td>Number of Nodes</td>
<td>48</td>
<td>63</td>
<td>63</td>
</tr>
<tr>
<td>Observed Links</td>
<td>597</td>
<td>1088</td>
<td>714</td>
</tr>
<tr>
<td>Linkage Density</td>
<td>12.4</td>
<td>17.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Connectance</td>
<td>0.26</td>
<td>0.27</td>
<td>0.18</td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>0.63</td>
<td>0.63</td>
<td>0.45</td>
</tr>
<tr>
<td>Nestedness (N*)</td>
<td>1.3</td>
<td>1.8</td>
<td>0.6</td>
</tr>
<tr>
<td>Nestedness (NODF)</td>
<td>40.4</td>
<td>50.0</td>
<td>29.1</td>
</tr>
<tr>
<td>Mean Trophic Level</td>
<td>1.8</td>
<td>4.4 (5.1)</td>
<td>1.8</td>
</tr>
<tr>
<td>Fraction Omnivores</td>
<td>0.71</td>
<td>0.83 (0.88)</td>
<td>0.8</td>
</tr>
<tr>
<td>Mean Consumer Gaps</td>
<td>4.1</td>
<td>6.8</td>
<td>4.6</td>
</tr>
<tr>
<td>Mean Diet Range Gaps</td>
<td>3.9</td>
<td>6.8</td>
<td>6.1</td>
</tr>
<tr>
<td>PNM Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_L$</td>
<td>0.73</td>
<td>0.66</td>
<td>0.62</td>
</tr>
<tr>
<td>Expected links</td>
<td>606</td>
<td>1101</td>
<td>732</td>
</tr>
<tr>
<td>Observed links</td>
<td>597</td>
<td>1088</td>
<td>714</td>
</tr>
</tbody>
</table>

Table 3.1. Food web properties and probabilistic niche model (PNM) results for three versions of the Quick Pond food web, each with and without parasites. The numbers for mean trophic level and fraction omnivores that are outside of parentheses apply to versions of the food web without concomitant predation links and the numbers inside of parentheses apply to versions of the food web with concomitant predation links. See the appendix B for additional results for web versions that lack concomitant predation links. $f_L$ indicates the fraction of links predicted correctly by the probabilistic niche model.
followed a similar pattern as connectance and linkage density where the lowest value was obtained in the niche shift web (Table 3.1). This finding is consistent with the idea that clustering coefficients tend to increase with node degree (Dunne 2006) and nestedness generally increases with connectance (Thebault and Fontaine 2010). All webs displayed significantly nested patterns of interactions relative to null matrices, regardless of life stage structure or whether parasites and concomitant links were included.

The effects of parasites on web structure were influenced by the treatment of complex life cycles within the web. Adding parasites and concomitant links into the webs increased the number of links by 82% (taxonomic species web), 100% (niche shift web), and 84% (life stage web) (Table 3.1). Of the increase in link number, 40% was due to concomitant links in the taxonomic species web, 37% was due to concomitant links in the niche shift web and 32% was due to concomitant links in the life stage web (Table B.1). In the taxonomic species web, inclusion of parasites increased connectance slightly from 0.26 to 0.27 and this effect was primarily driven by the high connectance of the predator-parasite subweb (Table B.2). In the niche shift and life stage webs, however, connectance decreased with inclusion of parasites from 0.18 to 0.13 and from 0.21 to 0.15, respectively (Table 3.1). The decrease was slightly stronger in the webs lacking concomitant links (Table B.1). Nestedness demonstrated a similar pattern, in which inclusion of parasites increased nestedness in the taxonomic species web, but decreased it slightly in the disaggregated niche shift and life stage webs (Table 3.1). Unlike connectance and nestedness, linkage density increased in all web versions with the inclusion of parasites, except for the two disaggregated webs that lacked concomitant links (Table B.1). The magnitude of increase in the taxonomic species web (39%) was
~2x larger than the increases in the niche shift web (20%) and the life stage web (15%) (Table 3.1). Parasites increased clustering coefficients in the two webs with life stage structure and concomitant links (Table 3.1), but decreased clustering coefficients in the same webs lacking concomitant links (Table B.1). Concomitant links from a predator to a parasite, in combination with a host parasite link, represent a form of clustering, so this result is not surprising.

Examining the degree distributions (i.e., the number of incoming and outgoing links per node) helped explain the contrasting effects of parasites on web structure across the three web versions (Fig. 3.1). In the taxonomic species web with parasites and concomitant links, the average node degrees of free-living organisms ($k = 34.8$, SE = 2.7) and of parasites ($k = 33.8$, SE = 2.8) were similar and inclusion of parasites into the web raised the overall node degree (all nodes in the web) by nearly 10 links per node (from 24.9 [SE = 2.0] to 34.5 [SE = 2.2]). For the niche shift web, in contrast, the average node degree of free-living organisms ($k = 33.8$, SE = 2.6) was nearly twice that of parasites ($k = 17.5$, SE = 1.3) and inclusion of parasites increased the overall node degree of the web by less than 5 links per node. The pattern in the life stage web was similar to that observed in the niche shift web. The changes in node degree were caused by differences in trophic generality and vulnerability between free-living organisms and parasites. In the taxonomic species web, trophic generality of parasites was over 3x lower than it was for free-living organisms while vulnerability was nearly 2x higher (Fig. 3.1). In the niche shift and life stage webs, vulnerability of parasites and free-living nodes was roughly equivalent but trophic generality was over 5x lower (Fig. 3.1). This result reflects the higher resource specificity of parasite life stages relative to free-living life stages.
Unsurprisingly, the inclusion of parasites caused a large decrease in average node degree in the webs lacking concomitant links. The trophic vulnerability of parasites was reduced by 50% in the life stage web, 64% in the niche shift web and 70% in the life stage web.
when concomitant links were removed. Similarly, the trophic generality of free-living species was reduced by 20%, 30% and 30%, in the three web versions when concomitant links were removed.

Trophic Levels and Omnivory - The inclusion of parasites caused increases in mean trophic level and omnivory in all web versions whether concomitant links were included or not; however the magnitude of change depended on how life stages were treated in the web (Table B.1). In the taxonomic species web, the inclusion of parasites caused the frequency distribution of trophic positions to shift to higher values than in the disaggregated webs (Fig. 3.2). In the taxonomic species web with parasites but lacking concomitant links, mean trophic level increased from 1.8 to 4.4 with the inclusion of parasites, whereas in the niche shift and life stage webs the increases were smaller (1.8 to 3.6 and 1.9 to 3.7, respectively; Table 3.1). These increases indicate that the trophic position of parasites themselves and predation on free-living parasite stages (as opposed to only concomitant predation) are important in increasing the trophic levels in the web. As expected due to elevated rates of predation on parasites, the increases in trophic level with parasite inclusion were greater in the taxonomic species and life stage webs that included concomitant links (although concomitant links had no effect on mean trophic level in the niche shift web; Table 3.1). Omnivory showed the greatest increase with the addition of parasites in the life stage web. The changes in trophic level and omnivory reflect the assignment of trophic positions to species relative to corresponding life stages. For invertebrates, amphibians and parasites, the species-level node in the taxonomic species web was always assigned a higher trophic position than the larval and adult nodes in the disaggregated webs (Fig. 3.3). Direct life cycle organisms followed a similar
Figure 3.2. Frequency distributions of nodes at different trophic positions within the three versions of the Quick Pond food web: (a,b) the taxonomic species web, (c,d) the niche shift web, and (e,f) the life stage web. The left panels show each web without parasites and the right panels show each web with parasites. Trophic positions were calculated using the equation for short-weighted trophic level. A trophic level of zero represents basal non-feeding nodes (e.g., primary producers), a trophic level of one represents herbivores, and a trophic level above two represents predators. Concomitant predation links have been omitted from the web versions used in this figure.
Figure 3.3. Changes in trophic level with node disaggregation for organisms with (a) complex life cycles (invertebrates, amphibians and parasites) and (b) for predators with direct life cycles (reptiles, birds and mammals). In panel a, the three bars represent the species-level node from the taxonomic species web (black bars) and the larval and adult nodes from the life stage web (light and dark grey bars). In panel b, the two bars represent the species level nodes from the taxonomic species web (black bar) and the life stage web (light grey bar). Concomitant predation links have been omitted from the webs used in this figure. All bars are means (+SE).

pattern because the trophic position of their prey decreased with node disaggregation. For amphibians and parasites, the adult stages fed at a higher trophic position than the larvae, but there was no difference for invertebrate stages (Fig. 3.3).

**Probabilistic Niche Model and Intervality** - The fit of the probabilistic niche model, as measured by the fraction of links predicted correctly, decreased in all web versions with the inclusion of parasites (Table 3.1). Model fit also decreased with disaggregation from the taxonomic species web to the niche shift web, but then increased slightly from the niche shift web to the life stage web. Webs that included concomitant links universally resulted in better fit than webs without concomitant links (Table B.1).
Niche structure can be approximately assessed through the direct intervality measures. Including parasites decreased intervality (i.e., increased gaps) in terms of both contiguous consumer ranges and contiguous diet ranges for all versions of the food web, consistent with the niche model fit results (Table 3.1). The magnitude of these changes for consumer gaps was largest in the two disaggregated webs (i.e. these have more gaps with parasites). For diet range gaps, the magnitude of change with parasite inclusion was largest in the taxonomic species web. Visualizations of the trophic niche space occupied by organisms in the food web show an increase in the number of gaps and a decrease in contiguous feeding niches with web disaggregation (Fig. 3.4). Although some contiguous feeding ranges appear in the niche shift web and life stage web, particularly for parasites, there is a large increase in the number of peripheral links that lie outside contiguous feeding intervals (Fig. 3.4). This effect may be due to the increase in the number of available prey nodes with disaggregation (i.e., predators have a wider range of suitable prey items leading to a less contiguous feeding interval). This result may also partly be due to the scale dependence of model fit (Dunne et al. 2013).

Discussion

Organisms with complex life cycles present unique challenges in food-web studies due to the wide variation in feeding interactions that occur during their growth and development. Using life stages as nodes in food webs may add valuable information, yet the consequences of utilizing this approach are not obvious. Here we examined how the disaggregation of species level nodes influences the perceived role of parasites in a pond food web, with particular emphasis on the analysis of trophic positions and the fit of
Figure 3.4. The observed links of the three versions of the Quick Pond food web, with each consumer node $i$ ordered by latent feeding position ($c_i$) and each resource node $j$ ordered by latent niche position ($n_j$): (a,b) the taxonomic species web, (c,d) the niche shift web, and (e,f) the life stage web. The left panels show each web without parasites and the right panels show each web with parasites. Green dots indicate resource links for free-living consumers and orange dots indicate resource links for parasite consumers.
a probabilistic niche model to the empirical web. Our main findings were that 1) the
effects of parasites on web properties were highly sensitive to the level of node
aggregation, 2) disaggregation of species level nodes into life stages provided more
accurate analyses of trophic positions in food webs with parasites and other complex life
cycle organisms, and 3) node disaggregation did not improve the fit of the probabilistic
niche model to the food web with parasites, contrary to predictions. Although the unique
aspects of our food web must be considered in extending these results to other systems
(e.g., the high proportion of species with complex life cycles), our results nonetheless
highlight the need to carefully consider whether the types of nodes used in a food web are
best suited to addressing the ecological questions of interest.

Incorporating parasites as taxonomic species increased most structural metrics
associated with web complexity, largely consistent with findings from previous studies
(Lafferty et al. 2006; Hernandez and Sukhdeo 2008; Amundsen et al. 2009; Dunne et al.
2013). Parasite inclusion led to an 82% increase in the total number of links and
concurrent increases in connectance, linkage density and nestedness in the taxonomic
species web that included concomitant links. While it is often assumed that changes in
food web properties with the inclusion of parasite are due to unique aspects of parasite
biology, it is important to distinguish between unique effects of parasites and generic
effects driven by increases in network size (Dunne et al. 2013). To date, the most unique
effects of parasites in food webs have been associated with robustness (Rudolf and
Lafferty 2011; Lafferty 2012), the relative frequency of certain motifs, and the contiguity
of feeding intervals (Dunne et al. 2013). Our results for most web properties within the
taxonomic species web (e.g., connectance) are largely consistent with expected changes due to network size.

In contrast to the taxonomic species web, the addition of parasites to the disaggregated webs altered web properties in ways that were not consistent with scale dependence. When we disaggregated species level nodes to capture variability in feeding relationships between life stages, the effects of parasite inclusion on connectance and nestedness were reversed relative to the taxonomic species web. Analyses of the degree distributions suggested that this result was associated with differences in the generality and vulnerability of parasite life stages relative to free-living life stages. Life stages of parasites had a higher vulnerability to predators but a much lower trophic generality (i.e., high host specificity) at the life stage level compared to free-living species. These differences effectively equalized the average node degree (i.e., the number of incoming and outgoing links) for free-living organisms and parasites within the taxonomic species web. In contrast, within the two disaggregated webs, the reduced generality of parasite life stages led them to have a much lower average node degree relative to free-living organisms. Despite increases in linkage density, the number of observed links in these web versions did not increase in proportion with the number of possible links added by parasite life stages, leading to a decrease in connectance with the inclusion of parasites. Similarly, the inclusion of parasites increased nestedness in the taxonomic species web but decreased nestedness in the disaggregated webs. This finding is consistent with recent results demonstrating that connectance has a strong positive relationship with nestedness (Thebault and Fontaine 2010). It is possible that disaggregation may also obscure nested
structure because networks composed of specialists tend to show greater nestedness than networks rich in generalists (Lima et al. 2012).

Disaggregating nodes into life stages was particularly useful for analyses involving trophic positions and omnivory in the food web. Organisms with distinct life stages, and particularly parasites, feed at varying trophic levels throughout development. The traditional approach of grouping these life stages led to an overestimation of the trophic level of the species relative to its individual life stages. In some cases, the trophic position of a taxonomic species was two to three trophic positions above the larval trophic level, and in all cases the species level trophic position exceeded that of both the larval and adult stages in the disaggregated food webs. This likely overestimated the trophic position in the taxonomic species food web that included parasites. In the life stage web lacking concomitant predation links, 23% of the remaining links still involved predation on parasites, primarily representing cases of predation on free-living parasite stages. In the case of complex life cycle parasites, most of these predator-parasite links occur with life stages of parasites that feed on a relatively low trophic level (e.g., predation on trematode cercariae; Orlofske et al. 2012). The net effect of the overestimation of parasite trophic positions, coupled with a large proportion of predator-parasite links, serves to overestimate the mean trophic level of the entire food web. In web version with concomitant links, the number of predator-parasite links was 36% of the total and the mean trophic level of the web increased to 5.1, indicating that concomitant links further compound this problem when they are included. We verified this interpretation by conducting the analyses of trophic position on a version of the food web without links between predators and parasites, and indeed the overestimation found
in the taxonomic species web with parasites was eliminated. This same scenario (i.e., overestimation of the trophic position of complex life cycle species) is likely with all complex life cycle organisms and provides one impetus to consider using life stage structure for questions related to trophic levels and omnivory. We also note, however, that our findings may be sensitive to the measure of trophic position that is utilized (Williams and Martinez 2004).

Contrary to predictions, the fit of a probabilistic niche model to the empirical webs with parasites did not improve as a result of disaggregating species level nodes. Niche models are based on the assumption that food webs are interval and organisms feed within a relatively contiguous feeding range (Williams et al. 2010). It has been posited that parasites, due to their complex life cycles and potential for host specificity, may reduce the intervality of food webs because they have a broad feeding niche with many gaps or secondary feeding niches (Dunne et al. 2013). As a result, we predicted that disaggregating species level nodes into life stages would improve the fit of the probabilistic niche model because each life stage is likely to have a more narrow niche breadth than the species as a whole. Our results, however, did not support this hypothesis, as model fit decreased with node disaggregation and the inclusion of parasites decreased model fit in all three food webs by a similar amount. Two possible explanations, which are not mutually exclusive, may underlie these findings. First, Dunne et al. (2013) found that fit of the probabilistic niche model tends to decrease with increasing food-web size. Disaggregating species level nodes inherently increases web size, so this mechanism may contribute to the decrease in model fit. Second, the contiguous-diet intervality metrics show that the number of gaps increased with disaggregation, whether parasites were
included or not, which likely makes it more difficult for the model to fit probabilistically- contiguous intervals for consumers. The increase in diet range gaps, which increased more with disaggregation than consumer gaps, probably reflects the increase in prey availability due to life stage structure.

Describing the structural characteristics of food webs has been of interest because of hypothesized relationships between structure and measures of network stability, such as robustness, persistence, and resilience (Ives and Carpenter 2007; Rooney and McCann 2012). An important question within the field of disease ecology is whether parasites have a net positive or negative effect on network stability. Analyses of past webs have indicated that parasites increase certain metrics (e.g., connectance) associated with network stability (Lafferty et al. 2006; Hernandez and Sukhdeo 2008; Amundsen et al. 2009) and engage in many weak interactions, which have been suggested to further enhance stability (McCann et al. 1998). Yet parasites also posses traits associated with network instability; complex life cycle parasites are highly susceptible to secondary extinctions (Lafferty and Kuris 2009; Chen et al. 2011; Lafferty 2012) and diseases occasionally induce extinctions of their hosts directly (e.g., Skerratt et al. 2007). Our results contribute to this discussion by highlighting how the effects of parasites on connectance and nestedness are sensitive to the way nodes are defined, underscoring the need to carefully consider how web construction alters perceived outcomes. While the role of connectance in web stability is still a topic of debate, there is increasing evidence that high levels of nestedness can destabilize food webs (Thebault and Fontaine 2010). Understanding how parasites influence network stability is a promising avenue for future theoretical and empirical work.
The observed differences in the three versions of the Quick Pond food web emphasize the need to carefully consider which level of biological organization (e.g., trophic species, taxonomic species, life stages) is best tailored to the aims of a particular web analysis. Cohen et al. (1993) made the recommendation that taxonomic species level nodes should be disaggregated into more resolved units when a change in diet occurs with increasing size or life stage. This is essentially our approach in the niche shift web, where ontogenetic stages are only divided when there is a significant diet shift between stages. This approach has the advantage of incorporating valuable information on the variation in predators and prey throughout development and does not lead to redundancy in links if the only species disaggregated are those that show shifts in predators and/or diet (a criticism of disaggregating nodes based on life stage alone; Huxham et al. 1995). In our analyses, disaggregating nodes into life stages made assigning trophic levels and calculating omnivory more accurate, particularly for food webs with parasites. However, care must be taken in comparing food webs with and without life stages because disaggregating species level nodes leads to numerous inherent changes in web structure, as observed here and elsewhere (Pimm and Rice 1987; Rudolf and Lafferty 2011). One drawback of separating species into life stages is that different stages are effectively treated as separate populations even though the success of one stage is tightly coupled to the success of the previous stage. For web analyses that include quantitative population metrics, this drawback may offset any advantages of dividing taxonomic species into life stages. Clearly defining life stages or ontogenetic niche shifts can also be challenging across the diversity of taxa included in food webs. Here, we incorporated a somewhat arbitrary delineation in diet between life stages in the niche shift web. More precise
definitions of niche shifts that are readily applicable in a food-web context would be useful.

As the field of food-web ecology advances in new directions, two promising avenues involve integrating multiple types of species interactions (e.g., mutualism, parasitism, predation) into the same network (e.g., van Veen et al. 2008; Fontaine et al. 2011) and examining how variability in linkages beyond the species level influences network structure and function (Ings et al. 2009). By considering individual life stages in a food web that incorporates both predator-prey and host-parasite interactions, our study highlights how network properties and the observed roles of functional groups are sensitive to the ‘rules’ used in building the network. Further analyses of food webs from other ecosystems are needed to test whether these patterns are generally applicable across other systems. Understanding how subtleties in food-web construction influence observed patterns in feeding interactions will be useful in examining a wide range of food-web questions, including responses of networks to ecosystem change (Tylianakis et al. 2008) and the movement towards more quantitative webs that incorporate measures of interaction strength and energy fluxes (Cohen et al. 2003; Berlow et al. 2004).

Appendix B

*Inclusion of parasites into the food web* - Parasites were added into the food webs following the methods outlined in Lafferty et al. (2006). In brief, the web consists of a matrix containing four subwebs (predator-prey, parasite-host, predator-parasite and parasite-parasite interactions, respectively). The predator-prey and parasite-host subwebs are straightforward and have been included in many previous webs. The predator-parasite
<table>
<thead>
<tr>
<th></th>
<th>Taxonomic Species Web</th>
<th>Niche Shift Web</th>
<th>Life Stage Web</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Nodes</td>
<td>63</td>
<td>105</td>
<td>113</td>
</tr>
<tr>
<td>Observed Links</td>
<td>891</td>
<td>984</td>
<td>1317</td>
</tr>
<tr>
<td>Linkage Density</td>
<td>14.1</td>
<td>10.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Connectance</td>
<td>0.22 (0.29)</td>
<td>0.09 (0.15)</td>
<td>0.10 (0.16)</td>
</tr>
<tr>
<td>Clustering Coefficient</td>
<td>0.57</td>
<td>0.49</td>
<td>0.54</td>
</tr>
<tr>
<td>Nestedness (N*)</td>
<td>1.3</td>
<td>0.5</td>
<td>0.7</td>
</tr>
<tr>
<td>Nestedness (NODF)</td>
<td>37.5</td>
<td>17.2</td>
<td>20.1</td>
</tr>
<tr>
<td>Mean Trophic Level</td>
<td>4.4</td>
<td>3.6</td>
<td>3.7</td>
</tr>
<tr>
<td>Fraction Omnivores</td>
<td>0.83</td>
<td>0.91</td>
<td>0.92</td>
</tr>
<tr>
<td>Mean Consumer Gaps</td>
<td>7.2</td>
<td>6.4</td>
<td>7.7</td>
</tr>
<tr>
<td>Mean Diet Range Gaps</td>
<td>7.6</td>
<td>6.5</td>
<td>7.7</td>
</tr>
<tr>
<td>PNM Results</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$f_i$</td>
<td>0.60</td>
<td>0.54</td>
<td>0.51</td>
</tr>
<tr>
<td>Expected links</td>
<td>896</td>
<td>1083</td>
<td>1403</td>
</tr>
<tr>
<td>Observed links</td>
<td>891</td>
<td>1055</td>
<td>1409</td>
</tr>
</tbody>
</table>

Table B.1. Food web properties for web versions with parasites but lacking concomitant links. Note that connectance is represented as both the traditional measure (outside parentheses) and as adjusted connectance (inside parentheses).

subweb represents cases where parasites are eaten concurrently with infected prey (concomitant predation; Johnson et al. 2010) or the free-living stages of parasites are directly consumed by predators (e.g., Kaplan et al. 2009; Orlofske et al. 2012). The parasite-parasite subweb represents trophic interactions between parasites, and in our web involves only trematode larval stages that prey on one another inside of snail hosts with dual infections (Esch and Fernandez 1994).

**Analyses of the four subwebs** - To facilitate comparisons with prior studies examining the effects of parasites on food-web properties, we also calculated the number of nodes, number of links, linkage density, connectance, and nestedness for the predator-prey, parasite-host, predator-parasite and parasite-parasite subwebs within the taxonomic species web including parasites. Unlike the total food webs, the parasite-host and predator-parasite subwebs are not symmetrical and connectance is defined as $C =$
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total Food Web</th>
<th>Predator-Prey</th>
<th>Parasite-Host</th>
<th>Predator-Parasite</th>
<th>Parasite-Parasite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species Richness ($S$)</td>
<td>63</td>
<td>48</td>
<td>63</td>
<td>63</td>
<td>15</td>
</tr>
<tr>
<td>Potential Links ($L_p$)</td>
<td>3969</td>
<td>2304</td>
<td>720</td>
<td>720</td>
<td>225</td>
</tr>
<tr>
<td>Observed Links ($L$)</td>
<td>1088</td>
<td>597</td>
<td>87</td>
<td>388</td>
<td>16</td>
</tr>
<tr>
<td>Linkage Density ($L/S$)</td>
<td>17.3</td>
<td>12.4</td>
<td>1.4</td>
<td>6.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Connectance ($C$)</td>
<td>27.4</td>
<td>25.9</td>
<td>12.1</td>
<td>53.9</td>
<td>7.1</td>
</tr>
<tr>
<td>Nestedness ($N^*$)</td>
<td>2.1</td>
<td>1.3</td>
<td>0.2</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>Nestedness (NODF)</td>
<td>49.9</td>
<td>40.3</td>
<td>14.3</td>
<td>61.5</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Table B.2. Summary metrics for each of the four sub-webs and the total taxonomic species web.

$L/(F^*P)$, where $F$ is the number of free-living species and $P$ is the number of parasites (Lafferty et al. 2006).

*Food webs lacking concomitant links* - For consistency with other work (Dunne et al. 2013), we present results from analyses conducted on versions of the web that included parasites but lacked concomitant predation links. In these webs, connectance was calculated as both the traditional measure ($C = L/S^2$), where $L$ is the number of links and $S$ is the total number of nodes in the web, and as adjusted connectance ($C = L/(F*P)$); Lafferty et al. 2006), where $F$ is the number of free-living species. The adjusted connectance measure slightly overestimates connectance in these web versions because it excludes all predator-parasite links, whereas only concomitant predation links are actually omitted in the food web.
Chapter IV

Biomass and Productivity of Trematode Parasites in Pond Ecosystems


Abstract

Ecologists often measure the biomass and productivity of organisms to understand the importance of populations and communities in the flow of energy through ecosystems. Despite the central role of such studies in the advancement of freshwater ecology, there has been little effort to incorporate parasites into studies of freshwater energy flow. This omission is particularly important considering the roles that parasites sometimes play in shaping community structure and ecosystem processes. Using quantitative surveys and dissections of over 1,600 aquatic invertebrate and amphibian hosts, we calculated the ecosystem-level biomass and productivity of trematode parasites alongside the biomass of free-living aquatic organisms in three freshwater ponds in California, USA. Snails and amphibian larvae, which are both important intermediate trematode hosts, dominated the dry biomass of free-living organisms across ponds (snails = 3.2 g m$^{-2}$; amphibians = 3.1 g m$^{-2}$). An average of 33.5% of mature snails were infected with one of six trematode taxa, amounting to a density of 13 infected snails m$^{-2}$ of pond substrate. Between 18% and 33% of the combined host/parasite biomass within each infected snail consisted of larval trematode tissue, which collectively accounted for 87% of the total trematode biomass within the three ponds. Mid-summer trematode dry biomass averaged 0.10 g m$^{-2}$, which was equal to or greater than that of the most abundant insect orders (coleoptera = 0.10 g m$^{-2}$).
m², odonata = 0.08 g m⁻², hemiptera = 0.07 g m⁻² and ephemeroptera = 0.03 g m⁻²). On average, each trematode taxon produced between 14 and 1,660 free-swimming larvae (cercariae) infected snail⁻¹ 24 hrs⁻¹ in mid-summer. Given that infected snails release cercariae for three to four months a year, the pond trematode communities produced an average of 153 mg m⁻² yr⁻¹ of dry cercarial biomass (range = 70 to 220 mg m⁻² yr⁻¹). Our results suggest that a significant amount of energy moves through trematode parasites in freshwater pond ecosystems, and that their contributions to ecosystem energetics may exceed those of many free-living taxa known to play key roles in structuring aquatic communities.

**Introduction**

Quantifying the biomass and production of populations, communities and entire trophic levels has been a central tool in understanding how energy moves through ecosystems (Odum 1971). The early use of such methods led to fundamental ecological concepts including the designation of trophic levels, the corresponding decrease in trophic-level biomass with increasing trophic position, and the efficiency of energy transfer between consumers and resources (Elton 1927; Lindeman 1942). More recently, biomass and production measurements have proven useful in studies of biomass turnover (i.e., ratios of production to biomass [P/B]; Waters 1977), in constructing food webs with quantified interaction strengths (e.g., Hall et al. 2000) and as metrics of population success (Benke and Huryn 2010). Throughout the development of the field of production ecology, freshwater lakes, ponds and streams have proven especially useful as model systems (e.g., Lindeman 1942; Odum 1957) and most methods of quantifying secondary
production have been pioneered with aquatic macroinvertebrates (Benke 1984; Downing and Rigler 1984).

The rich literature on freshwater ecosystem energetics has largely omitted the roles of infectious agents, despite the increasingly recognized importance of parasites in ecosystem structure and function (Loreau et al. 2005; Tompkins et al. 2010). Despite being cryptic and small in size, parasites are involved in a disproportionately high number of species interactions in some ecosystems (Lafferty et al. 2006; Lafferty et al. 2008) and comprise a large component of biodiversity (Hudson et al. 2006). These findings suggest parasites can play important roles in trophic interactions and energy flow and several studies have shown that parasites can alter primary and secondary production through population-level effects on their hosts. For example, plant foliar fungal pathogens can exert strong top-down control on primary production, sometimes surpassing the impacts of herbivores in certain grassland ecosystems (Mitchell 2003). Animal parasites can also alter ecosystem energetics through a variety of indirect mechanisms including population reduction of herbivores that control primary production (Sinclair 1979), alteration of secondary production through reduced fecundity or growth of hosts (Hurd 2001), behavioral modification of habitat-forming species leading to changes in local production (Mouritsen and Poulin 2005; Mouritsen and Poulin 2006), and manipulation of host behavior resulting in increased energetic subsidies from terrestrial to aquatic ecosystems (Sato et al. 2011; Sato et al. 2012). Until recently, however, the direct contributions of parasites to ecosystem energetics have either been ignored or assumed to be minimal due to their small size (Marcogliese and Cone 1997).
The first study to empirically assess the direct role of parasites alongside free-living organisms in ecosystem energetics found that parasite biomass in three Pacific Coast estuaries was surprisingly high; indeed, parasite biomass exceeded that of the top bird predators and was comparable to fishes and many invertebrate groups (Kuris et al. 2008). Trematodes were particularly important, with a biomass density that was one to five times larger than the other parasite groups and an annual production of free-swimming larval stages (cercariae) that was three to ten times greater than the winter bird biomass. These striking results indicate that a large amount of energy can move through parasites, and they may have important direct effects on ecosystem dynamics. Nonetheless, the generality of these findings remains an open question. If estuaries are exceptionally rich in parasites relative to other ecosystems, the findings of Kuris et al. (2008) may represent a 'special case,' where parasites play an uncommon role in ecosystem energetics.

In the present study we focus on trematode parasites within freshwater pond ecosystems. Ponds are common model systems in studies of food webs and energetics (Warren 1989; Wilbur 1997), providing an extensive ecological framework for the integration of parasites. Small lakes and ponds also represent the most abundant type of inland waters (Downing et al. 2006) and are of importance to the surrounding terrestrial landscape due to the movement of nutrients and biomass between water and land (Polis et al. 2004). We focus our estimates of parasite biomass and productivity on trematodes, which are known to play important ecological roles; by virtue of their complex life cycles, trematodes influence interactions between a large number of organisms from multiple trophic levels (Fried and Graczyk 1997). Furthermore, trematodes can alter the
growth, reproduction, feeding behavior, predation risk, morphology and survival of their hosts, leading to significant consequences for host populations and communities (Lafferty and Morris 1996; Poulin et al. 2005; Hatcher et al. 2006). We also suspected that trematodes would play the largest direct role of all parasites in pond ecosystem energetics because infection prevalence can be high in many host species and because trematodes consume a large proportion of host body mass in intermediate hosts such as snails (Esch and Fernandez 1994; Hechinger et al. 2009).

Our specific aims were to quantify the biomass and productivity of trematode parasites alongside the biomass of the most abundant free-living taxa in three ponds in California, USA. We utilized quantitative field surveys to characterize the composition and density of free-living aquatic organisms > 1mm (excluding some inadequately sampled invertebrates living within the pond substrate) and combined measurements of body size with length-to-mass regressions to calculate mid-summer biomass of the most abundant taxa. To quantify trematode biomass, we targeted macroinvertebrate and amphibian hosts of aquatic trematode life stages for dissections (>1,600 individuals) and measured the biomass of trematode life stages in the laboratory. Lastly, we measured the in situ release of free-swimming larval trematode stages and used data on infection prevalence and host densities to estimate annual cercarial productivity. We made several predictions about the importance of trematodes in the flow of energy in pond ecosystems relative to previously studied marine systems. First, we predicted that the biomass of trematodes would vary significantly between the pond ecosystems as a result of changes in the prevalence of trematode infection and the density of suitable hosts, which in turn are linked to environmental characteristics (e.g., pond hydroperiod; Hoverman et al.)
Second, we predicted that the standing biomass of trematodes in the pond ecosystems would be comparable or greater than the biomass of trematodes in estuarine systems because snail hosts are able utilize the entire pond habitat and the infection prevalence of snail hosts can attain exceptionally high levels in ponds (Esch and Fernandez 1994). We suspected that this effect would outweigh the fact that estuarine snail hosts (e.g., *Cerithidea californica*) are larger at maturity and can attain higher densities than freshwater pond snails (Fingerut et al. 2003). Lastly, we predicted that environmental characteristics that differ between freshwater ponds and estuaries would lead to changes in the timing of trematode productivity between the two ecosystems. Two of our three study sites become completely dry in late summer, presenting a challenging environment in which most organisms (including trematode hosts) are highly productive in the spring and summer when they can grow and reproduce rapidly prior to the onset of unfavorable conditions (Plante and Downing 1989). We predicted that this environment would lead pond trematodes to be highly productive over a short time period when conditions are favorable for parasite transmission.

**Methods**

*Study system* – We estimated free-living and trematode parasite biomass in three ponds (Quick, North and Sheep) within Alameda County in the San Francisco Bay Area of California, USA. The local ecoregion surrounding our study sites consists of oak woodland and grassland. All three ponds lie between 360 and 440 m in elevation and have surface areas in early summer of 2,234 m² (Quick), 404 m² (North) and 145 m² (Sheep). In 2009, Sheep Pond dried on July 1st, North Pond dried in mid-August, while
Quick Pond has a permanent hydropedium. The pond shorelines are completely vegetated by emergent plants including *Juncus*, *Typha* and *Scirpus* and none of the ponds support fish. At the beginning of the summer, total dissolved nitrogen in the three ponds ranged between 686 and 1,150 μg L⁻¹ and total dissolved phosphorus ranged between 64 and 81 μg L⁻¹.

In these study systems, many trematodes utilize rams horn snails (*Helisoma trivolvis*) as first intermediate hosts, larval amphibians and/or aquatic invertebrates as second intermediate hosts, and birds, mammals or adult amphibians as definitive hosts (Fried and Graczyk 1997). Trematode eggs hatch into miracidia, which seek suitable snail hosts wherein they develop into rediae or sporocysts, which in turn reproduce asexually and generate free-swimming cercariae. Cercariae are short-lived (<24 hrs) and either actively penetrate, or are consumed by, downstream hosts. Cercariae form metacercariae or mesocercariae within second intermediate hosts, which are trophically transmitted into definitive hosts, wherein they develop into mature worms, reproduce sexually, and lay eggs. Eggs are passed through the feces of definitive hosts into water bodies, completing the life cycle.

*Biomass estimates for free-living taxa* – Between May and July of 2009, we sampled Quick, North and Sheep Ponds biweekly using a stovepipe sampler (53 cm diameter x 74 cm height) to characterize the macroinvertebrate and larval amphibian communities. Stovepipe samplers are effective at quantifying invertebrate densities and performed well when compared with seven other macroinvertebrate sampling methods in heavily vegetated wetlands (Turner and Trexler 1997). The total number of samples per pond was adjusted to pond size (Sheep: n = 10; North: n = 15; Quick: n = 23) and the
location of samples was randomly selected by using a number generator and a diagram of each pond with an overlaid numbered grid. This method allowed us to capture potential spatial variability in invertebrate communities by sampling all vegetation types within each pond. After pushing the stovepipe sampler through the water column into the pond substrate, we used a D-net (1.4 mm mesh, 2600 cm$^2$ opening) to remove larval amphibians and macroinvertebrates until five consecutive sweeps yielded zero additional organisms. All of the organisms removed from each sample were transferred into a sorting tray, identified, counted, measured (nearest mm), and released. When identification in the field was not possible, macroinvertebrates where preserved and identified using Merritt et al. (2008). Most taxa were identified to genus or species; however, some were identified only to family (Table C.1). In addition, several invertebrate groups were detected but not sampled thoroughly enough to provide an accurate biomass estimate, including benthic invertebrates that were found within the substrate (i.e., some chironomid midges and annelid worms) and zooplankton $< 1$ mm (see Table C.1 for a full list of free-living aquatic taxa and whether each was included in biomass estimates). Given that the biomass of aquatic organisms changes considerably over the course of a year due to growth, reproduction, mortality, and metamorphosis/emergence, our estimates should be considered ‘snap-shots’ of mid-summer biomass.

The dry biomass of free-living invertebrates was estimated by converting the density and size-distribution of each taxon into a biomass density (g m$^{-2}$) using published length-to-dry mass regressions for the same or closely related species (Anderson et al. 1998; Benke et al. 1999; Hall et al. 2006). We developed our own regressions for
Helisoma trivolvis snails and the amphibian larvae (Table C.2). For snails, we report the dry tissue mass excluding the shell in all results and figures (as per Kuris et al. 2008).

Trematode quantification – We identified the trematode community (six taxa) infecting the obligate first intermediate snail hosts (H. trivolvis) within our study sites using keys based on morphological characters (Gibson et al. 2002; Jones et al. 2005; Bray et al. 2008), followed by additional molecular analyses (Orlofske et al., unpublished data). We targeted downstream hosts for dissection by determining the life cycle of each trematode taxon from the literature (Bosma 1934; Macy et al. 1960; Lang 1968; Kanev et al. 1995; Johnson et al. 2004; Bolek and Janovy 2008). The aquatic free-living taxa we identified as trematode hosts included H. trivolvis snails, and ten potential second intermediate hosts that included 4 species of pond-breeding amphibians (Rana draytonii, Pseudacris regilla, Anaxyrus [=Bufo] boreas and Taricha torosa), two dragonflies (Anax junius and Tramea sp.), two damselflies (Lestes and Coenagrion spp.), and California clam shrimp (Cyzicus californicus). To determine snail infection prevalence, we dissected 414 H. trivolvis from Quick Pond, 395 from North Pond and 327 from Sheep Pond, which ranged in size from 2 to 16 mm shell length. We dissected a subset of amphibian and invertebrate second intermediate hosts from each pond to quantify metacercariae and mesocercariae (n > 30 per amphibian species; n > 20 per invertebrate species). One amphibian host, the California red-legged frog (R. draytonii) was omitted from parasite quantification because it is listed under the US Endangered Species Act. Adult trematodes, which use mammals, birds and adult amphibians as definitive hosts in our study systems, were not quantified due to the ethical and logistical challenges associated
with dissecting these taxa and because the definitive hosts are primarily terrestrial, thus representing transient members of the pond food web.

**Biomass and productivity estimates for trematodes** – We dissected infected *H. trivolvis* snails in the laboratory to obtain estimates of the average proportion of the total snail host/trematode biomass that consisted of trematode rediae or sporocysts (5 to 9 field-collected snails for each trematode taxon; Table C.3). The trematode tissue and snail tissue were separated under a dissecting microscope and individually dried (60°C for 24 hrs) and weighed on a microbalance using pre-weighed aluminum tins, and compared against ‘blank’ tins without tissue to ensure accuracy between measurements. We obtained estimates of the mass of cercariae from each trematode taxa by directly measuring the dry mass and volume of cercariae from two species and calculating a general cercaria density (μg/μm³) that was applied to volume measurements of cercariae from the other four trematode taxa (see appendix C for details). We directly measured the dry mass of metacercariae or mesocercariae from five of the trematode taxa and extrapolated the mass of the sixth taxon in the same manner as with cercariae (appendix C).

To quantify trematode productivity (the change in biomass over time), we measured output of cercariae for all six trematode taxa at four times during the summer and combined these data with the estimates of taxon-specific cercariae mass. Cercarial output was obtained by isolating snails (~150 per time point) inside of capped 50 mL conical vials maintained in situ within Quick Pond to ensure a normal range of environmental conditions during cercarial release. Vials were filled with pond water and suspended at a depth of 30 cm for 24 hrs, after which we counted the number of cercariae
released from infected snails under a dissecting microscope. To ensure that pond water in the vials did not contain cercariae prior to the addition of snails, we deployed 5 extra vials on each occasion to which we did not add snails. Cercariae were never found in these vials.

Our estimates of total trematode standing biomass were calculated as the sum of the rediae/sporocyst biomass in snails, the short-lived daily biomass of cercariae released from snails, and the metacercariae/mesocercariae biomass in second intermediate hosts. To calculate the rediae/sporocyst biomass in snails, we multiplied species-specific snail infection prevalence by the density of snails > 8 mm and the average species-specific proportional trematode biomass within each infected snail. Our snail infection data indicated that snails < 8 mm were rarely infected (< 1% prevalence), so we omitted snails under this size from our infection prevalence estimates and applied an average prevalence for all snails > 8 mm (this size class was generally 8 to 12 mm, as very few snails were > 12 mm). The standing biomass of cercariae was calculated as the species-specific density of infected snails multiplied by the species-specific average daily output of cercariae and the biomass of each cercaria. The metacercariae/mesocercariae biomass in second intermediate hosts (amphibians and invertebrates) was calculated as the density of infected hosts multiplied by the estimated biomass of metacercariae or mesocercariae.

Our equation for cercarial productivity included the species-specific density of infected snails multiplied by the species-specific average daily output of cercariae, the estimated biomass of each cercaria, and the duration of the year over which we assumed infected snails were releasing cercariae. Because previous research suggests that infected snails from our study sites are not releasing cercariae during the winter, after most second
intermediate hosts have emerged (Preston et al. unpublished data), we made the conservative assumption that trematodes only released cercariae for four months year\(^1\) in the ponds with the longest hydroperiods (Quick and North) and for three months year\(^1\) in Sheep Pond, which has a shorter hydroperiod. We also assumed that cercarial release rates were relatively constant during this time period, which is an assumption supported by data on the numbers of cercariae released at three time points (May, June and July) for two trematode taxa within our study systems (Paul et al. unpublished data).

Results

Infection prevalence – We detected six trematode taxa within \(H.\ trivolvis\) snails at Quick Pond, including \(Ribeiroia\ ondatrae, Echinostoma\ trivolvis, Halipegus\ occidualis, Cephalogonimus\ americanus\), one trematode identified to the genus \(Alaria\), and another identified only as an amphistome. At Sheep and North Ponds we detected the same taxa, minus \(H.\ occidualis\). Total infection prevalence in first intermediate snails hosts (summed among trematode species) was similar between the three ponds, with infections detected in 33\% of mature \(H.\ trivolvis\) from Quick Pond, 32\% from Sheep Pond and 35\% from North Pond (Fig. 4.1). While the species composition of trematodes infecting snails was generally similar across ponds, the most prevalent trematode taxa varied: 48\% of infections at Quick were \(C.\ americanus\), 84\% of infections at Sheep were \(R.\ ondatrae\), and 78\% of infections at North were \(E.\ trivolvis\) (Fig. 4.1). We detected four of these trematodes (\(R.\ ondatrae, E.\ trivolvis, Alaria\ sp. and \(C.\ americanus\)) in larval amphibian second intermediate hosts (\(P.\ regilla, A.\ boreas\) and \(T.\ torosa\); see Table C.4 for a summary of infection prevalence of trematodes in second intermediate hosts from all
Figure 4.1. Prevalence of trematode infections in Helisoma trivolvis snails (n > 325 per pond) from three California ponds. *H. trivolvis* is an obligate first intermediate host of each trematode taxon. The legend shows six trematode taxa that were detected, which correspond to stacks in the bars. Infection prevalence is for snails >8 mm in shell length only, as smaller snails were rarely infected (<1% prevalence).

three ponds). One trematode (*H. occidualis*) was detected in invertebrate second intermediate hosts (damselfly and dragonfly larvae [*Anax, Tramea, Coenagrion* and *Lestes* spp.] and California clam shrimp [*Cyzicus californicus*]; Table C.2). The mean infection prevalence was 98% (SE = 0.9%) for *R. ondatrae* and 73% (SE = 10.7%) for *E. trivolvis* in the three amphibian taxa from all three ponds (Table C.4). *Alaria* sp. and *C. americanus* only infected the anuran tadpoles (*A. boreas* and *P. regilla*), at average prevalences of 43% (SE = 18%) and 68% (SE = 12%), respectively. The mean infection intensity in second intermediate hosts ranged from a low of 5 mesocercariae (*Alaria* sp.) per host to 35 metacercariae (*R. ondatrae*) per host in the three ponds. Trematode infections occurred in 53% of odonates and 3% of clam shrimp dissected from Quick, 10% of odonates from Sheep, and 8% of odonates from North (Table C.4).

*Free-living and trematode biomass* - We detected 31 taxa of free-living aquatic macroinvertebrates and amphibians, of which 24 were sampled adequately to obtain
reliable biomass estimates (Table C.1). Amphibian larvae (primarily *P. regilla*, *A. boreas* and *T. torosa*) and snails (*H. trivolvis*) dominated the standing crop animal biomass in all three ponds, with dry biomass densities of one to two orders of magnitude above the other macroinvertebrate groups (Fig. 4.2). Larval amphibian biomass ranged between 1.5 g m$^{-2}$ (North) to 5.1 g m$^{-2}$ (Sheep) with a mean across ponds of 3.1 g m$^{-2}$ (± 1.1 SE). Snail biomass was remarkably consistent between the three ponds and ranged between 3.0 g m$^{-2}$ (Quick) to 3.4 g m$^{-2}$ (Sheep), with a mean of 3.2 g m$^{-2}$ (± 0.1 SE). The insect orders with the largest biomass densities (Fig. 2) included coleoptera (0.10 g m$^{-2}$ ± 0.03 SE), odonata (0.09 g m$^{-2}$ ± 0.02 SE), hemiptera (0.07 g m$^{-2}$ ± 0.02 SE) and ephemeroptera 0.03 g m$^{-2}$ (± 0.01 SE). The biomass of the aquatic life stages of trematode parasites was comparable to, or greater than the insect orders (Fig. 4.2). Estimates of trematode biomass (all trematode life stages excluding adult worms) from within each pond were 0.07 g m$^{-2}$ for North Pond (95% CI = 0.05 to 0.10), 0.12 g m$^{-2}$ for Quick Pond (95% CI = 0.08 to 0.16) and 0.13 g m$^{-2}$ for Sheep Pond (95% CI = 0.08 to 0.18).

Of the total trematode biomass, the majority (87%) consisted of redial or sporocyst life stages within snail first intermediate hosts. Our laboratory dissections indicated that an average of 25% of the total host/parasite biomass of infected *H. trivolvis* snails consisted of larval trematode tissue (range = 18% to 33% for the six trematode taxa; see Table C.3 for sample sizes and variances). Within the three ponds, the biomass of larval trematodes within snails was 3.6x the combined biomass of cercariae and metacercariae in Sheep Pond, 8.6x in North Pond and 17.1x in Quick Pond. The trematode biomass within snails comprised 3.9% of the total snail tissue biomass in Quick Pond, 3.5% of the total snail biomass in Sheep Pond and 2.1% of the total snail...
biomass in North Pond (excluding snail shell mass). If we follow Kuris et al. (2008) in considering the entire infected snail (i.e., trematode plus castrated snail host tissue) as the parasite ‘extended phenotype’, the proportion of extended phenotype biomass relative to total snail biomass (infected and uninfected) increased to 17% for Quick Pond, 16% for Sheep Pond, and 11% for North Pond, which is 3x greater than the biomass of the largest macroinvertebrate group in each pond.

_Trematode productivity_ – The cercarial density of the two trematode taxa for which we directly measured dry biomass were similar (_R. ondatrae_ cercariae = 1.38 x 10^{-7} \mu g \mu m^{-3}; _E. trivolvis_ cercariae = 1.54 x 10^{-7} \mu g \mu m^{-3}). Applying an average tissue density of 1.46 x 10^{-7} \mu g \mu m^{-3} to volume measurements of the other four trematode taxa resulted in individual cercaria dry masses (Fig. 4.3a) that ranged between 3.5 x 10^{-5} mg (_Alaria_ sp.) to 0.006 mg (amphistome). The average number of cercariae released 24 hrs^{-1} was
Figure 4.3. The mean number of cercariae released infected snail\(^1\) 24 hrs\(^{-1}\) plotted against the estimated dry biomass of a single cercaria of each trematode taxon. Error bars for the number of cercariae released represent one standard error (a). Yearly cercarial production of six trematode taxa in three California ponds. Error bars show upper 95% confidence intervals (b).

Inversely related to cercarial size (Fig. 4.3a) and ranged between 14 (amphistome) and 1,660 (\textit{H. occidualis}). Assuming cercarial release for 3 to 4 mos and constant snail density and infection prevalence, the total annual production of cercariae (Fig. 4.3b) was estimated at 0.22 g m\(^{-2}\) yr\(^{-1}\) for Quick Pond (95% CI = 0.14 to 0.30), 0.17 g m\(^{-2}\) yr\(^{-1}\) for
Sheep Pond (95% CI = 0.08 to 0.26) and 0.07 g m$^{-2}$ yr$^{-1}$ for North Pond (95% CI = 0.04 to 0.10). The largest annual cercarial production was for *R. ondatrae* in Sheep Pond (156 mg m$^{-2}$ yr$^{-1}$) and *C. americanus* in Quick Pond (110 mg m$^{-2}$ yr$^{-1}$). Annual cercarial production estimates for the other trematodes ranged between 0.9 and 37 mg m$^{-2}$ yr$^{-1}$ (Fig. 4.3b).

**Discussion**

Our results indicate that trematode parasites can be prevalent members of pond communities with a large cumulative biomass. The aquatic life stages of trematodes had an ecosystem-level biomass that was comparable to the most abundant insect orders, including the beetles, damselflies and dragonflies, hemipterans, and mayflies. Across the three ponds, 87% of the total trematode biomass was composed of larval stages within snail first intermediate hosts. This result was due primarily to the high density of snail hosts (mean = 106 snails m$^{-2}$), the high trematode infection prevalence (33% of mature snails), and the large proportional trematode tissue mass per infected snail (17% to 33% across the six trematode taxa). Contrary to our prediction, the three ponds displayed remarkably similar densities of snail hosts and similar infection prevalence between sites. These results suggest that trematode biomass is linked to the biomass of suitable snail hosts, but may be less dependent on downstream hosts such as amphibians and larval insects, despite their high abundance and infection intensity. Furthermore, trematodes produced large numbers of short-lived, free-swimming larval stages, which amounted to an annual mean biomass across the three ponds of 153 mg m$^{-2}$. Collectively, our results underscore the potential roles of parasites in freshwater ecosystem energetics, and
provide further evidence that parasites have the potential to exert effects on ecosystem processes that are comparable to their free-living counterparts.

Our findings highlight both similarities and differences in the role of trematodes in the flow of energy through some freshwater and marine ecosystems. In the Pacific estuaries studied by Kuris et al. (2008), the biomass of parasites was dominated by trematodes, which use Cerithidea spp. snails as first intermediate hosts. Consistent with our results, trematode biomass in the three estuaries was comparable to, or exceeded that of many free-living groups. Although Kuris et al. (2008) reported trematode biomass in wet mass, if we are to assume a wet-to-dry mass conversion factor of 0.1 (Benke 1984), the dry biomass of trematodes in the three estuaries would average close to 0.1 g m\(^{-2}\), which is similar to the estimates from our ponds, which range between 0.07 to 0.13 g m\(^{-2}\). Similarly, converting the yearly cercarial productivity for the combined trematode communities in the three estuaries into dry mass yields estimates of about 0.1 to 0.4 g m\(^{-2}\) yr\(^{-1}\), which are slightly higher than our pond estimates (0.07 to 0.22 g m\(^{-2}\) yr\(^{-1}\)). The ratio of annual cercarial production to biomass of trematode life stages within snail hosts was about three in the estuaries, while in our pond systems it averaged two. The relative similarity of the production to biomass ratios of trematodes between the two different aquatic ecosystems is interesting considering the fact that in our study systems trematodes only produce cercariae for a few months during the summer. If the pond trematodes released cercariae for 12 mos year\(^{-1}\), our estimated ratios of cercarial production to biomass would increase to ~6.7, which is more than double the estimate from the estuaries. Additionally, the individual cercarial production per trematode taxon
within our study systems ranged between $8 \times 10^{-4}$ g m$^{-2}$ yr$^{-1}$ and 0.1 g m$^{-2}$ yr$^{-1}$, which are within the range of values reported for 18 marine trematode taxa (Thieltges et al. 2008).

The high biomass and productivity of trematodes suggest that parasites could play an important role as a prey resource in ponds. The fate of trematode cercariae biomass is not well known, but some (presumably small) fraction successfully infects downstream hosts, while the rest are eaten by predators or die within a short time and enter detrital food webs. The diversion of resources from snail reproduction into trematode reproduction presents a unique pathway of energy flow; in the absence of trematodes, snail secondary production remains embedded within benthic food webs, whereas the presence of trematodes producing free-swimming cercariae diverts some benthic production into the planktonic food web (Morley 2012). Studies from marine systems suggest that many predators feed on cercariae, which are nutrient-rich and poorly defended from predation (Kaplan et al. 2009). Our own laboratory studies indicate that a variety of organisms present in the study ponds, including damselflies and clam shrimp, feed readily on cercariae, potentially reducing disease risk to downstream hosts (Orlofske et al. 2012). Evidence from lake ecosystems also suggests an important role of parasites as prey. The free-living zoospores of chytrid fungi that infect phytoplankton are an abundant prey resource, perhaps shunting energy from inedible host phytoplankton species to planktivores via the consumption of zoospores (Kagami et al. 2007; Gleason et al. 2008). Food web analyses further support the prominent role of parasites as prey; a food web including parasites from one of our study sites (Quick Pond) included 1088 total links, of which 36% represented predation on parasites (Preston et al. 2012). This large number of predator-parasite links includes predation on free-living parasite stages.
(e.g., cercariae) as well as concomitant predation, when predators consume parasites alongside their infected hosts (Johnson et al. 2010; Johnson and Thielges 2010). Considering that every free-living host can become infected with multiple parasite species (Price 1980), it is not surprising that predator-parasite links can be as prevalent as traditional predator-prey links in some food webs (e.g., Lafferty et al. 2006; Amundsen et al. 2009). Measurements of parasite production in nature, alongside laboratory feeding studies and food web analyses, all suggest that parasites have the potential to function as important prey resources in diverse ecosystems.

The indirect consequences of parasitism on energy flow may be equally important as the direct roles of parasites as prey in pond ecosystems. For example, several of the trematodes within our study system reduce the fitness of amphibian hosts, which may make amphibian larvae more susceptible to predation, thereby strengthening trophic links between hosts and their free-living predators. *Ribeiroia ondatrae*, which was found in nearly 100% of the amphibian larvae at our study sites, causes limb abnormalities which reduce amphibian performance and likely increase predation susceptibility (Johnson et al. 1999; Goodman and Johnson 2011a, b). Similarly, *Echinostoma trivolvis*, which were found in close to 75% of all amphibian larvae, can cause edema and lethargy in amphibians when infection intensities are high, which again may lead to higher predation rates and reduced amphibian survival (Johnson and McKenzie 2008; Rohr et al. 2010). Whether such morphological and behavioral alterations, lead to community-level shifts in secondary production or the strength of trophic interactions remains to be tested. Nonetheless, parasite-induced mortality and host manipulation suggest that trematodes might play important indirect roles in trophic interactions, especially considering the
significant role of amphibians in linking aquatic and terrestrial food webs (Gibbons et al. 2006; Regester et al. 2006). Currently, one compelling example exists of parasites indirectly altering the strength of subsidies between aquatic and terrestrial ecosystems. In Japan, nematomorph worms cause cricket and grasshopper hosts to jump into streams, dramatically increasing the flow of energy from the forest to the stream, where the insects provide prey to trout (Sato et al. 2011; Sato et al. 2012).

Our results, alongside finding in marine ecosystems, provide the first steps towards a broader understanding of the direct contributions of parasites to ecosystem energetics. Ponds and small lakes are the most abundant type of inland waters globally, and more research is needed to evaluate the generality (or rarity) of our findings across other sites. Furthermore, the roles of parasites in energy flow within most terrestrial ecosystems, and many additional freshwater (e.g., streams, lakes) and marine systems (e.g., coral reefs, open ocean, sea floor) remains poorly understood. By showing that trematodes play significant roles in the energetics of pond ecosystems, our results help to advance the integration of parasitism into community- and ecosystem ecology. Future efforts aimed at understanding how parasite biomass alters the fate of nutrients and energy both within and across ecosystems are warranted and will provide valuable insights into the ecosystem-level roles of disease.

Appendix C

Trematode biomass measurements – We obtained direct mass measurements of *Ribeiroia ondatrae* and *Echinostoma trivolvis* cercariae by isolating field-collected snails within vials and collecting the free-swimming cercariae using a pipet. We filtered the
cercariae onto pre-dried/pre-weighed nylon filters that were weighed with a microbalance (13 filters of 100 cercariae for *R. ondatrae* and 20 filters of 50, 100 or 200 cercariae for *E. trivolvis*). Blank filters without cercariae were weighed alongside the samples to validate the method. We then generated a cercaria density (μg/μm³) based on the direct measurements of the mass and volume of *Ribeiroia ondatrae* and *Echinostoma trivolvis* cercariae. The average cercaria tissue density, which was very similar for both trematode species (see Results in main text), was then applied to volume estimates for the other four trematode taxa. The volume estimates were obtained from measurements of micrographs made using ImageJ software (Abramoff et al. 2004). Volume estimates were determined by converting the body and tail of each cercariae (n > 5 cercariae per trematode taxon) into geometric shapes with existing volume equations (Thieltges et al. 2008; Kuris et al. 2008).

Metacercaria and mesocercaria mass measurements were obtained by isolating trematodes from amphibian and snail hosts and weighing them on pre-dried/pre-weighed nylon filters in a manner similar to the cercariae described above. Sample sizes were as follows: seven replicates of 100 or 200 metacercariae for *Echinostoma trivolvis*; four replicates of 60 to 100 mesocercariae for *Alaria* sp.; five replicates of 30 metacercariae for the amphistome; three replicates of 50 or 70 metacercariae for *Cephalogonimus americanus*; and 19 replicates of 30 to 100 metacercariae for *Ribeiroia ondatrae*. For the sixth trematode having the smallest metacercariae (*Halipegus occidualis*), we achieved an estimated mass in a manner similar to the cercariae. We generated a metacercaria density from two of the taxa (*Ribeiroia ondatrae* and *Echinostoma trivolvis*) for which
we directly measured both mass and volume and applied this density to volume measurements of *H. occidualis* metacercariae.

<table>
<thead>
<tr>
<th>Group and Common Name</th>
<th>Level of Identification</th>
<th>Classification</th>
<th>Biomass</th>
<th>Dissection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oligochaeta</td>
<td>Genus</td>
<td><em>Dero</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oligochaete Worm</td>
<td>Family</td>
<td>Tubificidae</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Oligochaete Worm</td>
<td>Species</td>
<td><em>Chaetogaster limnaei</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Hirudinea</td>
<td>Genus</td>
<td><em>Erpobdella</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Copepoda</td>
<td>Genus</td>
<td><em>Macrocyclops</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Copepoda</td>
<td>Family</td>
<td>Diaptomidae</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cladocera</td>
<td>Genus</td>
<td><em>Simocephalus</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Cladocera</td>
<td>Genus</td>
<td><em>Simocephalus</em></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Amphipoda</td>
<td>Genus</td>
<td><em>Hyalella</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Ostracoda</td>
<td>Species</td>
<td><em>Cyzicus californicus</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Gastropoda</td>
<td>Species</td>
<td><em>Helisoma trivolvis</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Emphemeroptera</td>
<td>Genus</td>
<td><em>Callibaetis</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Coleoptera</td>
<td>Genus</td>
<td><em>Haliplus</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Genus</td>
<td><em>Notonecta</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Family</td>
<td>Gerridae</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Genus</td>
<td><em>Hesperocorixa</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Species</td>
<td><em>Lethocerus americanus</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Genus</td>
<td><em>Belostoma</em></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Diptera</td>
<td>Family</td>
<td>Chironomidae</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Odonata</td>
<td>Genus</td>
<td><em>Coenagrion</em></td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Spreadwing Damselfly  Genus  *Lestes*  Yes  Yes  
Saddlebag Dragonfly  Genus  *Tramea*  Yes  Yes  
Green Darner Dragonfly  Species  *Anax junius*  Yes  Yes  

**Amphibia**

California Newt  Species  *Taricha torosa*  Yes  Yes  
Western Toad  Species  *Anaxyrus boreas*  Yes  Yes  
Pacific Treefrog  Species  *Pseudacris regilla*  Yes  Yes  
California Red-legged Frog  Species  *Rana draytonii*  Yes  Yes  

Table C.1. Aquatic free-living organisms detected at three California ponds. The first three columns provide the group or common name, the level of identification, and the scientific classification for each taxon. The fourth column indicates whether or not we included that taxon in our biomass estimates and the fifth column indicates whether or not they were necropsied to quantify trematodes (only taxa that were known from the literature to be a host in the life cycle of the detected trematode species were necropsied). The group names in bold correspond to the taxa which appear in Figure 2 in the main text.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sample Size</th>
<th>Size Range (mm)</th>
<th>Regression Equation</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snail Host</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Helisoma trivolvis</em></td>
<td>110</td>
<td>2.1 - 23.8</td>
<td>DM = (10^{-4})L^{2.17}</td>
<td>0.77</td>
</tr>
<tr>
<td>Amphibian Hosts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudacris regilla</em></td>
<td>25</td>
<td>3.3 - 12.1</td>
<td>DM = (2 x 10^{-6})L^{3.32}</td>
<td>0.92</td>
</tr>
<tr>
<td><em>Anaxyrus boreas</em></td>
<td>25</td>
<td>5.2 - 17.0</td>
<td>DM = (4 x 10^{-6})L^{3.34}</td>
<td>0.96</td>
</tr>
<tr>
<td><em>Taricha torosa</em></td>
<td>47</td>
<td>9.6 - 23.5</td>
<td>DM = (2 x 10^{-7})L^{4.13}</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Table C.2. Length-to-dry mass regression equations for snails and three amphibian taxa. Regressions for all other taxa where obtained from the literature. The table includes the taxon name, the sample size for the regression, the size range of individuals used (mm), the regression equation, and the r² value of the regression. In the regression equations, DM = dry mass in grams, and L = length in mm (snout-vent length for amphibian larvae and shell length for snails).

<table>
<thead>
<tr>
<th>Trematode Taxon</th>
<th>Sample Size</th>
<th>% Trematode Tissue</th>
<th>Standard Error</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alaria sp.</em></td>
<td>8</td>
<td>33.2</td>
<td>3.2</td>
</tr>
<tr>
<td>Amphistome</td>
<td>9</td>
<td>18.9</td>
<td>1.8</td>
</tr>
<tr>
<td><em>Cephalogonimus americanus</em></td>
<td>5</td>
<td>30.4</td>
<td>2.5</td>
</tr>
<tr>
<td><em>Echinostoma trivolvis</em></td>
<td>8</td>
<td>17.7</td>
<td>1.6</td>
</tr>
<tr>
<td><em>Halipegus occidualis</em></td>
<td>7</td>
<td>30.6</td>
<td>2.4</td>
</tr>
<tr>
<td><em>Ribeiroia ondatrae</em></td>
<td>9</td>
<td>20.1</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table C.3. Proportional trematode biomass within infected *Helisoma trivolvis* snails for six trematode taxa that were detected at the three study ponds. The sample size indicates
the number of infected snail hosts that were dissected to quantify trematode and host dry tissue mass. The percentage trematode tissue indicates the proportion of the total host/parasite biomass that consisted of larval trematode tissue.

<table>
<thead>
<tr>
<th>Trematode Taxon</th>
<th>Quick Pond</th>
<th>Sheep Pond</th>
<th>North Pond</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>R. ondatrae</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. trivolvis</em></td>
<td>100</td>
<td>67.7</td>
<td>96.5</td>
</tr>
<tr>
<td><em>C. americanus</em></td>
<td>100</td>
<td>77.4</td>
<td>100</td>
</tr>
<tr>
<td><em>Alaria sp.</em></td>
<td>100</td>
<td>70.1</td>
<td>32.1</td>
</tr>
<tr>
<td><em>H. occidualis</em></td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
</tbody>
</table>

Table C.4. Infection prevalence (percent of the hosts infected out of the total number dissected) of five trematodes in second intermediate hosts from three California ponds (Quick, Sheep and North). We dissected at least 20 invertebrates and 30 amphibians per species per pond. The sixth trematode taxon detected (amphistome) was only found in snail first intermediate hosts and is not shown in the table. Dashes indicate that the trematode does not infect that host as part of its life cycle, while zeros indicate the trematode can infect that host, but was not detected. The suborders anisoptera and zygoptera both include two respective taxa that were grouped for infection prevalence estimates (anisoptera: *Anax junius* and *Tramea*; zygoptera: *Lestes* and *Coenagrion*; see Table C.1 for a full list of free-living taxa).
CHAPTER V

DISEASE ECOLOGY MEETS ECOSYSTEM SCIENCE: WHERE WE HAVE BEEN AND WHERE TO GO NEXT

Abstract

Disease ecology and ecosystem science have largely developed along independent pathways, in part due to differences in their scale of study. Indeed, research at the ecosystem level encompasses less than 2% of disease ecology publications and is uncommon relative to work on communities, populations or individual hosts. To help bridge these fields, we used empirical literature searches to explore links between parasitism and the structure and function of ecosystems. Our results indicate that parasites can sometimes play strong roles in shaping 1) the biotic and abiotic components of ecosystem structure, 2) biogeochemical cycles of water, carbon, nutrients and trace elements, 3) fluxes of biomass and energy, and 4) temporal ecosystem dynamics including invasibility, disturbance, succession and stability. Such effects have been documented within terrestrial, freshwater and marine ecosystems, and mechanistically, they include both indirect effects driven by changes in the density or traits of hosts, and direct effects driven by parasites themselves. We use these findings to identify traits of the ecosystem, the host, the parasite, and their interactions that will be useful in predicting when parasites and disease can have important ecosystem consequences.

Future research should include efforts to extend host-parasite studies across levels of ecological organization, ecosystem experiments to disentangle the ‘endemic’ roles of native parasites, and the integration of parasites and disease into conceptual models of ecosystem functioning.
Introduction

What is the most abundant organism in the earth’s oceans? Most people might respond with fish, zooplankton, phytoplankton, or perhaps bacteria. The answer, however, is viruses. Marine viruses of plankton can attain densities of $10^7$ to $10^{10}$ individuals l$^{-1}$ of sea water (Danovaro et al. 2011) and have been estimated to collectively contain as much carbon as ~75 million blue whales (Suttle 2005). Through their controls on bacteria and phytoplankton populations, viruses play fundamental roles in controlling cycles of carbon, nutrients and trace elements (Suttle 2007). Despite their potential to alter biogeochemistry on the global scale, viruses are generally omitted from ecosystem function and earth system models. In part this omission stems from the relatively recent recognition of their profound roles in marine ecosystems, but it is also representative of a broader pattern in which parasites are omitted from efforts to understand large-scale processes.

The fields of ecosystem science and disease ecology have traditionally experienced little overlap in their conceptual foci (Loreau et al. 2005; Tompkins et al. 2011). Ecosystem science seeks to understand the biotic and abiotic controls over the structure and functioning of ecosystems (Chapin and Matson 2011). Much research in this field aims to describe pools and fluxes of energy and matter, such as carbon, elemental nutrients and biomass. Most empirical and theoretical studies involve spatial scales ranging from a habitat patch to the biosphere. The nascent field of disease ecology, in contrast, focuses primarily on host-parasite interactions within individuals, populations, and more recently, communities (Collinge and Ray 2006). The disparate
levels of biological organization studied in ecosystem- and disease ecology, coupled with the fact that parasites are typically small and inconspicuous, has resulted in relatively few efforts to incorporate parasitism and disease into our broader understanding of ecosystems (Fig. 5.1). Indeed, an empirical literature search reveals that less than 2% of disease ecology publications involve work at the ecosystem level.

Despite their distinct foundations, recent movements seeking to integrate ecological research across scales has set the stage for rapid developments in our understanding of how parasites can affect large-scale processes. The last two and a half decades have seen a surge of research addressing how biodiversity, and the presence of individual species, can affect the functioning of whole ecosystems. Motivated by ongoing biodiversity losses, such studies have led to the consensus that biodiversity often affects the rates of fundamental ecosystem processes, such as biomass production and nutrient cycling (Hooper et al. 2005; Cardinale et al. 2012). Concurrently, disease ecologists have increasingly recognized the potential for parasites to control community structure and shape the outcome of species interactions; the percentage of disease research that focuses on community ecology has increased four-fold from 1980 to 2013 (Fig. 5.1). A few compelling cases that helped bridge the gap between community composition and ecosystem properties – such as rinderpest virus restructuring the Serengeti (Dobson and Hudson 1986), chestnut blight transforming hardwood forests (McCormick and Platt 1980), and microbial pathogens of sea urchins driving coral reef dynamics (Lessios 1988) – alerted ecologists to the hidden potential of parasites to influence whole ecosystems. Today, an increasing number of studies suggest that parasites can affect ecosystem structure and function in meaningful ways, yet to date, there have been relatively few
Figure 5.1. To quantify the disconnect between disease ecology and ecosystem science, we conducted literature searches in the Web of Science database that compared the number of disease ecology publications that focused on different levels of ecological organization (see appendix D for detailed search strings and methods). Using a search of title keywords with the terms ‘host’, ‘population’, ‘community’, or ‘ecosystem’, we found that from 1980 to 2013 the total number of disease ecology publications decreased sharply with increasing levels of organization (Fig. A). A more exhaustive search examining publication trends over time revealed that while the percentage of disease ecology research at the community level has increased steadily from 5% to 20% between 1980 and 2013, the percentage at the ecosystem level has remained under 2% (Fig. B). This comparison reveals the growing momentum in research linking community ecology and disease, but the relatively slower pace of new publications on ecosystems and disease.

Efforts to synthesize patterns in existing work, develop an underlying mechanistic framework, assess the generality of effects across ecosystems, or work towards a predictive capacity (but see Loreau et al. 2005; Eviner and Likens 2008).

Here, we aim to broadly link disease ecology with the core concepts in ecosystem science. We first consider potential mechanisms through which parasites can affect ecosystem structure and function. We then use a literature review to determine how commonly such mechanisms have been documented and the ecosystem properties that they affect (Fig. 5.2). We specifically review cases where parasites influence 1) the biotic
Figure 5.2. Using literature searches we identified 34 studies that examined the roles of parasites in shaping ecosystem structure and function (see appendix D for full citations). These studies have taken place in a diversity of ecosystem types including freshwater, marine and terrestrial ecosystems. The majority of the studies have involved ecosystem structure (32%) or biogechemistry (41%). Nearly half (47%) have been observational studies, with relatively fewer experiments or modelling approaches. Of the mechanisms studied, more than half (53%) have involved examples where parasites exert indirect effects by altering host densities. Relatively fewer studies have documented trait-mediated indirect effects (32%) or direct effects of parasites themselves (15%).

and abiotic components of ecosystem structure, 2) biogeochemical cycles of water, carbon, nutrients and trace elements, 3) the movement of energy and biomass, and 4) temporal ecosystem dynamics including stability, invasibility, disturbance and succession. Building from the empirical examples we generate predictions about how traits of the ecosystem, the parasite, the host, and their interactions can facilitate ecosystem effects. Because parasites are unlikely to be equally important in all ecosystems, a goal of our review is to consider when parasites must be incorporated into ecosystem studies, and alternatively, when they can be safely ignored. Lastly, we explore areas for future research that will strengthen the links between ecosystem science and disease ecology.
Ecosystem ecology: a role for parasites?

A large body of research has examined the ways in which free-living biodiversity effects ecosystem functioning (for reviews see Tilman 1997; Loreau et al. 2001; Hooper et al. 2005; Balvanera et al. 2006; Cardinale et al. 2012). One conceptual approach used in biodiversity-ecosystem functioning research hinges on identifying species traits that result in a unique or disproportionately large role of organisms in ecosystem function (Hooper et al. 2005). For instance, cyanobacteria can fix nitrogen from the atmosphere, certain insects pollinate specific plant species, and fungi are able to decompose organic matter. The abilities to perform these ecosystem processes – nitrification, pollination and decomposition – are associated with unique physiological, morphological or behavioral traits, known as functional effect traits (de Bello et al. 2010). Organisms can therefore play an important ecosystem role, even if they are not very common, because they possess unique functional traits. Alternatively, some species may be important simply because they are extremely abundant in the ecosystem. The species that play disproportionate ecosystem roles, by virtue of their functional traits and/or their abundance, are sometimes termed ‘dominant species’, ‘foundation species’, ‘keystone species’ or ‘ecosystem engineers’ (see Ellison et al. 2005 for definitions). If these organisms cannot be replaced by other functionally similar species, their presence becomes vital to the overall functioning of the ecosystem.

Extending the links between species traits and their functional roles to host-parasite interactions provides a useful launching point from which to understand how and when parasites will influence ecosystems. Parasites, by definition, possess a specific suite
of traits; all parasites live in or on a host species from which they gain resources for at least some portion of their life cycle (Price 1977; Lafferty and Kuris 2002). As a result, the most obvious way that parasites can influence ecosystems is through effects on their host. Such indirect effects of parasites can be broadly divided into two classes, which traditionally have been applied to predator-prey interactions: density-mediated effects and trait-mediated effects (Fig. 5.3) (Werner and Peacor 2003; Preisser et al. 2005).

Density-mediated indirect effects occur when parasites alter the density of a host species, which in turn alters other components of the ecosystem. The regulation of host density can occur through direct host mortality or changes in host reproductive rates due to side-effects of pathology or castration (Baudoin 1975; Anderson 1978; Scott and Dobson 1989). Trait-mediated indirect effects occur when a parasite alters a host trait, which in turn leads to effects on other components of an ecosystem. For example, many parasites alter the foraging rates of their hosts, which can cause indirect effects on primary producers (e.g., Wood et al. 2007). Whether or not parasites exert important indirect effects therefore depends largely on 1) the functional roles of their hosts within the ecosystem, and 2) the propensity of the parasite to change those roles by affecting host traits or host densities (Fig. 5.3).

In addition to indirect effects driven by changes in hosts, parasites can also exert direct effects on ecosystems, although such effects may be more difficult to detect (Fig. 5.3). Although parasites seemingly have few direct effects on the ecosystem around them, several recent studies have revealed that parasites can play direct ecosystem roles through the production of parasite biomass, which contributes to the movement of energy and matter through ecosystems (e.g., Kuris et al. 2008). This finding challenges the
assumption that parasites have a small ecosystem biomass, particularly for productive or large-bodied parasites, and also leads to the potential for parasites to play important roles in energy flow through predator-prey interactions (Johnson et al. 2010). Although more studies are needed to explore other direct pathways, these findings indicate that parasites can directly contribute to ecosystem processes, independent of effects driven by changes in their hosts. In the following sections we will explore how these mechanisms – indirect effects due to changes in hosts, and direct effects of parasites themselves – can affect ecosystem properties, including ecosystem structure, biogeochemical cycles, energy flow, and temporal ecosystem dynamics

**Ecosystem structure**
The structure of an ecosystem is defined both by its biota and its abiotic factors, including climate, topography, air, water, soil and rocks (Chapin and Matson 2011). Parasites can influence both components of ecosystem structure. First, parasites can directly control the composition and abundance of free-living species within an ecosystem. This topic lies primarily in the realm of community ecology (e.g., Collinge and Ray 2006; Hatcher and Dunn 2011 for reviews), but because ecosystem structure encompasses community composition, we briefly discuss it here. Secondly, parasites can exert effects on hosts that invoke feedbacks to the abiotic components of ecosystem structure, such as soil and water. The ecosystem consequences of such controls will be greatest when the affected free-living species serve as foundation species or ecosystem engineers that serve vital roles in creating the physical structure of the ecosystem and in stabilizing the local environment (Thomas et al. 1999).

*Parasites as drivers of community composition* – Parasites can control the presence of species within an ecosystem through multiple mechanisms, including facilitation, exclusion and extinction (Hatcher and Dunn 2011). Facilitation can occur when a parasite allows free-living species to become established within an ecosystem by suppressing competitively dominant species. In salt marsh plant communities, for example, parasitic plants (*Cuscuta salina*) reduce the dominance of their preferred host species (*Salicornia virginica*), allowing competitively inferior species to coexist, and thereby raising local species richness (Pennings and Callaway 1996). Exclusion can occur when a parasite prevents a highly susceptible immigrating species from becoming established or prevents species from coexisting due to apparent competition. Though challenging to demonstrate conclusively in nature, laboratory experiments (Bonsall and
Hassell 1997) and modeling approaches (Tompkins et al. 2000) indicate the potential for these mechanisms in simplified communities. Lastly, parasites can cause extirpations and extinctions of free-living species directly. While disease is often believed to be a rare cause of species extinction in nature (Smith et al. 2006), a variety of special cases, such as small host population sizes, frequency-dependent transmission, and generalist parasites with reservoir hosts, can increase the probability of a disease-driven extinction (De Castro and Bolker 2005). The wholesale loss of amphibian communities due to chytridiomycosis (Wake and Vredenburg 2008), the extinction of Hawaiian birds due to avian malaria (Atkinson and Samuel 2010), and the local extirpations of bat species due to white nose syndrome (Frick et al. 2010) present evidence for the capacity of parasites to restructure the biotic component of ecosystems by driving species declines.

**Parasites of dominant species** – The most profound parasite-induced changes to the biotic and abiotic components of ecosystem structure occur when affected hosts are foundation species that decline over a wide geographic area (Castello et al. 1995; Ellison et al. 2005). For instance, American chestnut was a dominant tree species in eastern North America before being devastated by the introduction of chestnut blight in the first half of the 20th century. Changes to the structure of terrestrial and aquatic ecosystems have likely ensued, including wide-scale alterations in species composition, decreases in soil carbon and nitrogen, changes in the quality of litter inputs to streams and alteration to stream channels due to the slow decomposition of chestnut wood relative to replacement species (Smock and MacGregor 1988; Paillet 2002; Rhoades 2007). Similar feedbacks to the abiotic components of ecosystem structure have likely occurred due to declines in at
least six pine species (*Pinus* spp.) associated white pine blister rust in western North America. (Table 5.1; Tomback and Resler 2007).

*Parasites of ecosystem engineers* – Parasites of animals can also strongly shape ecosystem structure when host species serve as ecosystem engineers. For example, the introduction of plague from Eurasia to the western United States around 1900 has led to local extinctions of prairie dog colonies (*Cynomys* spp.). Infected colonies often experience 100% mortality. Due to their burrowing and grazing activities prairie dogs play unique functional roles in grassland ecosystems; they directly alter soil characteristics, including soil turnover, porosity and nutrient content, and they also affect most of the biotic community including vegetation, invertebrates and vertebrates (Miller et al. 2000). After plague epizootics, the functional roles of prairie dogs are locally eliminated until recolonization occurs, which can take from two to over ten years (Table 5.1; Hartley et al. 1999). Further examples of parasites affecting the ecosystem engineering roles of hosts come from marine ecosystems (Mouritsen and Poulin 2002). For instance, trematode parasites of New Zealand cockles impair the burrowing ability of their hosts, leading to subsequent effects on the relative composition of attached limpets and anemones, as well changes to local species richness and secondary productivity (Mouritsen and Poulin 2002). Other changes can be driven by declines in the density of ecosystem engineers. Amphipod die-offs due to trematode infection removed the functional roles of amphipods as sediment stabilizers, leading to increases in sediment particle sizes and erosion rates (Mouritsen et al. 1998).
<table>
<thead>
<tr>
<th>Ecosystem Property</th>
<th>Ecosystem Type</th>
<th>Host/Parasite System</th>
<th>Mechanism</th>
<th>Ecosystem Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotic Structure</td>
<td>Alpine forest</td>
<td>Whitebark pine and blister rust</td>
<td>Host density regulation</td>
<td>Declines in whitebark pine have altered rates of soil erosion, snow melt and runoff (Tomback and Resler 2007)</td>
</tr>
<tr>
<td>Biotic Structure</td>
<td>Temperate forest</td>
<td>American chestnut and chestnut blight</td>
<td>Host density regulation</td>
<td>Extirpation of American chestnut has led to wholesale changes in forest composition (Paillet 2002)</td>
</tr>
<tr>
<td></td>
<td>Temperate grassland</td>
<td>Prairie dogs and plague</td>
<td>Host density regulation</td>
<td>Prairie dog colony extirpations lead to shifts in vegetation biomass and composition (Hartley et al. 1999)</td>
</tr>
<tr>
<td>Carbon Cycling</td>
<td>Open ocean</td>
<td>Marine phytoplankton and viruses</td>
<td>Host density regulation</td>
<td>Viruses alter the rates at which carbon sinks to the deep ocean and is lost to the air via respiration (Suttle 2007)</td>
</tr>
<tr>
<td>Nutrient Cycling</td>
<td>Temperate forest</td>
<td><em>Eucalyptus</em> trees and mistletoe</td>
<td>Direct effect of parasite</td>
<td>Mistletoe litterfall alters phosphorus and trace element cycling on the forest floor (March and Watson 2010)</td>
</tr>
<tr>
<td>Decomposition</td>
<td>Freshwater stream</td>
<td>Isopods and acanthocephalan worms</td>
<td>Trait-mediated effect</td>
<td>Acanthocephalan infection reduces rates of litter decomposition by isopod hosts (Hernandez and Sukhdeo 2008)</td>
</tr>
<tr>
<td>Primary Production</td>
<td>Freshwater stream</td>
<td>Caddisflies and microsporidian parasites</td>
<td>Host density regulation</td>
<td>Decreases in caddisfly abundance led to an order of magnitude increase in periphyton abundance (Kohler and Wiley 1997)</td>
</tr>
<tr>
<td></td>
<td>Temperate grassland</td>
<td>Grassland plants and fungal pathogens</td>
<td>Trait-mediated effect</td>
<td>Foliar fungal pathogens strongly reduced grassland primary production (Mitchell 2003)</td>
</tr>
<tr>
<td>Secondary Production</td>
<td>Marine estuary</td>
<td>Multiple taxa of hosts and parasites</td>
<td>Direct effect of parasites</td>
<td>Parasite biomass equals or exceeds many free-living groups, including birds (Kuris et al. 2008)</td>
</tr>
<tr>
<td></td>
<td>Freshwater pond</td>
<td>Snails and trematode worms</td>
<td>Direct effect of parasites</td>
<td>Trematode biomass equals or exceeds many free-living groups, including most insects (Preston et al. 2013)</td>
</tr>
<tr>
<td>Ecosystem Subsidies</td>
<td>Freshwater stream/temperate forest</td>
<td>Crickets and nematomorph worms</td>
<td>Trait-mediated effect</td>
<td>Nematomorphs increase energy flow from forest to stream, with consequences for aquatic food web (Sato et al. 2012)</td>
</tr>
<tr>
<td>Ecosystem Resilience</td>
<td>Coral reef</td>
<td>Sea urchins and microbial pathogens</td>
<td>Host density regulation</td>
<td>Extirpations of urchins facilitated macroalgal growth and eliminated coral recruitment (Lessios 1988)</td>
</tr>
</tbody>
</table>

Table 5.1. Examples of parasites affecting the structure and function of ecosystems.
**Biogeochemical cycles**

Biogeochemical cycles are quantified in terms of the pools (standing stocks) and fluxes (transport between pools) of elements within ecosystems. Host species including microbes, plants and animals can play fundamental roles in converting elements into biologically useable forms and in regulating pathways of cycling and pool sizes. As a result, parasites have potential to exert controls on biogeochemical cycles through multiple mechanisms.

*Viruses and global carbon cycling* – Viruses play a major role in ocean-wide biogeochemical cycles by causing mortality of heterotrophic bacteria and phytoplankton. Estimates of daily mortality rates range widely, but can approach 40% to 50% of the host population in surface waters under typical environmental conditions and can reach 100% during algae blooms (Fuhrman 1999; Suttle 2007). Because marine phytoplankton can account for as much as 40% of global primary production and plankton play a major role in the sequestration of carbon in the deep sea (Longhurst and Glen Harrison 1989; del Giorgio and Duarte 2002), high rates of virus-induced plankton mortality will have profound consequences for global cycles of carbon and nutrients (Table 5.1). Viral induced cell lysis releases virus particles and host cellular contents into the water column, through what has been termed the ‘viral shunt’ (Wilhelm and Suttle 1999). The specific fate and magnitude of such organic matter release remains a topic of uncertainty; nutrient rich particles from proteins and nucleic acids are utilized by heterotrophs, leading to increased losses of carbon to the atmosphere via respiration and the assimilation of limiting elements (e.g., iron) within the photic zone (Suttle 2007). The latter effect can lead to a positive feedback where limiting nutrients cycle through the base of the marine
food web, rather than moving to higher trophic levels (Fuhrman 1999). Furthermore, host lysis may alter the rates at which carbon and nutrients are exported to the deep ocean. By allowing the selective uptake of nutrient rich particles after cell lysis, viruses may increase the ratio of carbon to nutrients in organic matter that sinks to the deep sea. Marine viruses even have potential to alter climate patterns and cloud formation when sulfur compounds are released during the lysis of phytoplankton cells (see Danovaro et al. 2011 for a review).

Plant parasites and terrestrial nutrient cycling – In the terrestrial environment several model systems involving hemiparasitic plants – plants which usurp nutrients and water from their host, but also photosynthesize to some degree – have revealed the potential for parasites to accelerate rates of above- and below-ground nutrient cycling (Press and Phoenix 2005; Watson 2009). In Swedish heathlands, the hemiparaste Bartsia alpina accelerates rates of nutrient cycling and the growth of co-occurring uninfected plants because its leaf tissue and litter are enriched in nitrogen and decompose more rapidly relative to other members of the local plant community (Quested et al. 2002). Relatively similar effects occur due to mistletoe hemiparasites (Amyema miquelii) in Eucalyptus trees in Australian forests (Table 5.1; March and Watson 2010). In another model system, the presence of Rhinanthus hemiparasites decreased aboveground plant productivity but increased species richness and rates of nitrogen mineralization in experimental grassland communities (Bardgett et al. 2006). These changes were associated with an increase in the relative abundance of bacteria compared to fungi in the soil, an effect that was attributed to belowground inputs from the plant community to the soil (i.e., root turnover and root exudates).
Parasites of functionally important grazers and decomposers – Herbivores in aquatic and terrestrial environments play important roles in recycling nutrients within an ecosystem through grazing and excretion. This consumer driven nutrient recycling can sometimes provide a far greater portion of the bioavailable nutrients to primary producers than the quantity supplied by external inputs (Elser and Urabe 1999). The functional roles of consumers as nutrient recyclers can be altered through changes in rates of host nutrient excretion per individual or through changes in host densities. For instance, freshwater snails infected with the trematode *Trichobilharzia physellae* excrete more nitrogen but less phosphorus than uninfected hosts (Bernot 2013). In many freshwater ecosystems snails can exert strong controls on nutrient cycling, are the dominant grazers in terms of biomass, and can become infected by trematode parasites at a high prevalence, suggesting that individual-level effects of parasitism are likely to have consequences for nutrient cycling at the ecosystem scale (Hall Jr et al. 2003; Preston et al. 2013; Mischler et al. in revision).

Decomposition is another vital component of nutrient cycling and can be mediated by living organisms and their parasites (Gessner et al. 2010). Within stream ecosystems, for example, isopods that consume leaf litter play significant roles in decomposition and their rates of litter consumption are decreased by infection with an acanthocephalan parasite (Table 5.1; Hernandez and Sukhdeo 2008a). The magnitude of the effect varied across seasons, but approached a 47% reduction in the amount of detritus consumed when isopod densities were the highest. Similarly, in a study system involving a functionally important terrestrial decomposer, nematode infections reduced dung beetle feeding rates on monkey feces by 22 to 30% (Boze et al. 2012).
Ecosystem energetics

Energy is a fundamental currency that connects organisms within an ecosystem. One method to estimate the importance of populations in energy flow is to measure their change in biomass over time (i.e., productivity). For parasites to directly contribute to energy flow they must therefore accumulate a large biomass or be exceptionally productive on the ecosystem scale. Indirect contributions to energy flow can occur when parasites alter host productivity or control pathways of energy flow by influencing the strength of trophic interactions.

Direct and indirect controls on primary production – Empirical studies suggest that parasites can exert direct controls on primary production that may rival the bottom-up controls induced by nutrient limitation and the top-down controls of herbivores. In a three year grassland experiment, for instance, the exclusion of foliar fungal pathogens led to a 31% increases in overall plant biomass (Table 5.1; Mitchell 2003). This effect was driven by changes in root biomass, rather than aboveground biomass. Similarly strong controls on grassland primary production have been observed as a result of belowground fungal pathogens (Maron et al. 2011).

In addition to exerting direct controls on primary production, parasites can induce trophic cascades whereby they decrease the densities of functionally important grazing species and release producers from top-down control. For instance, the intentional introduction of the highly pathogenic *Myxoma* virus into populations of nonnative rabbits in England and Australia led to cascading effects on producers (Sumption and Flowerdew 1985). In both countries, rabbits had devastating effects on native vegetation and
agricultural crops. In England, the near eradication of rabbits after *Myxoma* introduction led to woodland regeneration of oak trees within grasslands, and ultimately fundamental changes in productivity and ecosystem structure that are evident today in the even-aged stands of trees in many regions of southern England (Dobson and Crawley 1994). Similarly rapid increases in tree recruitment have been documented in Australia after rabbit declines in the 1950’s (Cooke 1988).

Cascading effects of parasites on primary producers are not limited to the terrestrial environment. In freshwater streams in Michigan, USA, caddisflies (*Glossosoma nigrior*) play important roles in structuring communities of aquatic insects and in controlling production of benthic periphyton (Table 5.1). *Glossosoma* also experience periodic outbreaks of a lethal microsporidian (*Cougourdela* sp.) that exerts strong control on population densities (Kohler and Wiley 1992). In a well replicated (six streams) ecosystem level experiment, microsporidian outbreaks, on average, caused a 25 fold decrease in the density of *Glossosoma* caddisflies and a subsequent increase in periphyton abundance that approached an order of magnitude at some sites (Kohler and Wiley 1997).

*Direct contributions to secondary production* – The first study to empirically estimate the ecosystem-level contributions of parasite to energy flow found that in three Pacific Coast estuaries, parasite biomass exceeded that of top bird predators and was comparable to the biomass of fishes and many invertebrate groups (Kuris et al. 2008). Trematode worms with complex life cycles were prominent members of the parasite community from a biomass perspective. The annual production of free-swimming trematode larval stages that emerge from snail hosts was three to ten times greater than
the winter bird biomass within the three estuaries. Additional evidence from freshwater ecosystems, suggests these results are not unique. In three freshwater ponds in California, USA the biomass of trematode parasites exceeded that of most aquatic invertebrate insect groups, including predatory dragonflies that often exert top-down effects that can structure fishless aquatic communities (Preston et al. 2013). Furthermore, the estimated foliar fungal pathogen biomass in experimental grassland plots in Minnesota, USA (0.87 g m$^{-2}$) exceeded that of herbivorous insects (Mitchell 2003). Collectively, these results indicate that parasite production can directly contribute to the flow of energy through ecosystems in a previously overlooked way.

**Controls on ecosystem subsidies** – While often considered in isolation, ecosystems are intricately connected via subsidies of matter and energy (Polis et al. 2004). The movement of living organisms represents a common way in which seemingly discrete ecosystems are connected. Parasites can either strengthen or weaken the links between ecosystems depending in part on how they influence the movement patterns or survival of their hosts in different environments. In one compelling example, horsehair worms (Nematomorpha) in Japan manipulate the behavior of their insect hosts to increase the flow of energy from terrestrial ecosystems into streams. Infected crickets and grasshoppers were estimated to be 20 times more likely to enter a stream than uninfected conspecifics. As a result, infected insect hosts contributed up to 60% of the yearly caloric intake for native trout within the study stream (Sato et al. 2011). The effects of parasite-driven subsidies extended to other components of the ecosystem as well. In an experiment where inputs of terrestrial insects were manipulated, the addition of crickets to a stream reach resulted in a three-fold larger benthic invertebrate biomass relative to
stream reaches that excluded terrestrial invertebrates (Sato et al. 2012). This effect was attributed to the release of benthic invertebrates from trout predation and led to cascading effects on periphyton biomass, which was 50% lower in the presence of cricket subsidies. An increase in density of leaf shredding invertebrates also coincided with a 30% increase in leaf decomposition rates in the presence of cricket subsidies, suggesting that parasite manipulation led to not only changes in energy flow across ecosystem boundaries, but also fundamental alterations of aquatic ecosystem processes.

Other parasites are likely to limit the flow of energy between ecosystems, leading to a net recycling of energy and nutrients within an ecosystem rather than export to adjacent environments. For instance, biphasic amphibians with aquatic larval stages often export a vast amount of energy from the aquatic to the terrestrial environment during metamorphosis (Gibbons et al. 2006). Although not yet quantified in terms of energy flow, it is likely that amphibian parasites that induce mortality of embryos or aquatic larval stages would reduce the transfer of energy from the aquatic to terrestrial environment. This includes several emerging pathogens including water molds (*Saprolegnia* spp.) and iridoviruses (e.g., *Ranavirus* spp.) (Daszak et al. 2003).

*Parasites and trophic linkages* – Although interaction strengths have yet to be widely quantified in food webs that include parasites, existing studies hint at the potential for parasites to play common roles in energy transfer through ecosystems (for reviews on food webs and parasites see Sukhdeo and Hernandez 2005; Lafferty et al. 2008; Byers 2009; Dunne et al. 2013). Topological food webs from freshwater and marine ecosystems reveal that parasites are involved in a large number of trophic links within ecosystems. For example, parasites were involved in 78% of the links in a web from a Pacific Coast
estuary (Lafferty et al. 2006), 54% of the links in a web from the pelagic zone of a subartic lake (Amundsen et al. 2009), 45% of the links in a web from a freshwater pond (Preston et al. 2014), and 29% of the links in a web from freshwater stream (Hernandez and Sukhdeo 2008b). Within these webs, energy moves from hosts to parasites, but also from parasites to predators. Links from parasites to predators generally outnumber traditional host-parasite links and are most important to energy flow when parasites achieve a large biomass or when predators have evolved to feed exclusively on parasites (e.g., cleaner fish on coral reefs) (Johnson et al. 2010; Thieltges et al. 2013).

Even if parasites do not make substantial direct contributions to energy flow, they can still strongly alter the strengths of trophic interactions involving their host. Trophically transmitted parasites commonly manipulate the behavior of their host to enhance their transmission success (Lefèvre et al. 2009). For example, killifish parasitized by the trematode (*Euhapchloris californiensis*) display erratic swimming behavior and are estimated to be up to 30 times more susceptible to predation by bird hosts (Lafferty and Morris 1996), ultimately altering patterns of energy flow through trophic pathways.

**Temporal ecosystem dynamics**

Ecosystems change over time due to long-term environmental trends, such as climate change, and shorter-term events, such as fire or species introductions. Human alteration of the planet is fundamentally driving temporal ecosystem dynamics in new ways and parasites have potential to alter the type, frequency, and response of ecosystems to change.
Invasibility – Invasive species are widespread global drivers of ecosystem change and parasites have potential to either increase or decrease the susceptibility of ecosystems to invasion. The biotic resistance hypothesis states that natural enemies of an introduced species, including predators and parasites, can limit the ability of an invader to become established within the introduced range (Lockwood et al. 2009). Evidence for parasites preventing the establishment of an introduced species has thus far been equivocal, although this may stem in part from the fact that few failed invasions are documented. A meta-analysis indicates that biotic resistance from competitors and herbivores may be a stronger force in preventing plant invasions than resistance from pathogens (Levine et al. 2004). Alternatively, the enemy release hypothesis posits that a lack of co-evolved natural enemies within a species’ introduced range contributes to invasion success. Evidence for release from parasites has been found for invasive plants and animals. For instance, 26 taxonomically diverse animal species used in a meta-analysis had about half as many parasites in their introduced range as their native range (Torchin et al. 2003). Similarly, 473 plant species had 84% fewer fungal pathogens and 24% fewer viruses in their naturalized range relative to their native range (Mitchell and Power 2003). We also note that parasites themselves can act as invasive species and many of the most dramatic instances of parasites reorganizing the structure and function of an ecosystem come from the introduction of a novel parasite into a naïve host population that lacks a co-evolutionary history (e.g., amphibian chytridiomycosis, rinderpest virus and crayfish plague).

Disturbance and succession – Parasites and disease outbreaks can be thought of as disturbance events themselves or can alter the susceptibility of an ecosystem to other
types of disturbance. Introduced parasites that cause rapid host mortality to functionally important species over a wide geographic area are most likely to act as disturbance events (Eviner and Likens 2008). The most obvious examples come from pathogens of trees that cause disturbances to forest ecosystems, including chestnut blight, white pine blister rust disease, Dutch elm disease, butternut canker, beech bark disease and Port-Orford cedar root rot (Loo 2009). Such forest pathogens also have potential to alter the frequency and severity of other types of disturbance events, such as fires (Dickman and Cook 1989; Castello et al. 1995).

Epizootics in animals can also act as direct disturbance events. Perhaps the most highly cited example of parasites influencing ecosystem structure and function comes from the introduction and subsequent removal of rinderpest virus from African ungulates (reviewed in Dobson and Hudson 1986; Thomas et al. 2005). Around 1890, the virus was introduced from domestic livestock and spread rapidly throughout the African continent, leading to sharp declines in wildebeest and buffalo populations, changes in vegetation structure, increases in primary production, and decreases in the numbers of top predators. These cascading effects were later reversed when the virus was eradicated due to vaccinations of livestock, providing a rare glimpse into the ecosystem effects of a parasite after introduction and removal from the system.

After a disturbance event, parasites can influence the process of succession, particularly when they mediate competition between colonizing species. In European sand dunes, for example, colonization and succession follows a predictable pattern in terms of plant community structure, where a single clonal species generally dominates at one time. This pattern is mediated in large part by soil pathogens that are associated with
successional species (Van der Putten et al. 1993). Parasites have also been shown to play roles in controlling successional patterns in old growth Douglas fir forests (Holah et al. 1997) and in communities of freshwater phytoplankton (Van Donk 1989).

*Ecosystem stability* – A key characteristic of ecosystems is their ability to withstand change or return to their initial state after a disturbance. Stability measures include concepts such as resilience (the capacity of an ecosystem to return to an equilibrium state), resistance (the degree of variability in ecosystem responses after a disturbance), and robustness (the propensity for secondary extinctions after an initial species has been lost) (McCann 2000; Ives and Carpenter 2007; Rooney and McCann 2012). Unsurprisingly, the role of parasites in stabilizing or destabilizing ecosystems is complex and different conclusions have been reached depending on the approach and the type of stability in question.

Theoretical considerations of the relationship between stability and food web topology have generally indicated that parasites may enhance stability. When parasites are integrated into food webs they tend to increase measures of web complexity that have been associated with stability, such as connectance (Lafferty et al. 2006; Amundsen et al. 2009). Parasites also are likely to participate in numerous weak interactions, which has also been suggested to stabilize food webs (McCann et al. 1998). The most recent and thorough analysis of foods webs to include parasites, however, has indicated that the effects of parasites on most aspects of food web topology, including connectance, are largely consistent with changes that are expected with added species diversity, whether free-living or parasitic (Dunne et al. 2013). The way in which parasites are included in
food webs, for example as species or as life stages, may also alter conclusions regarding their effects on web metrics associated with stability (Preston et al. 2014).

Studies that consider robustness - the ability of species in a food web to persist after other species have gone extinct - suggest that parasites make food webs more susceptible to secondary extinctions and therefore might decrease stability. Parasites and other organisms with complex life cycles are extremely susceptible to secondary extinctions because, although they appear as generalists at the species level, they tend to be highly specialized in terms of resource use at the life stage level (Rudolf and Lafferty 2011). The presence of parasites may therefore decrease robustness and in turn, overall ecosystem stability. Furthermore, parasites can contribute to ecosystem instability when they induce population fluctuations in their hosts or they cause extinctions of their hosts directly.

A final mechanism through which parasites can influence the stability of ecosystems is through their effects on transitions between alternative stable states. Coral reef ecosystems, for example, can switch between a coral-dominated state and an algae dominated state (Nyström et al. 2000). On Caribbean coral reefs, parasites of a functionally important grazing species lowered ecosystem resilience by facilitating the switch from coral dominance to algae dominance. In the early 1980’s a combination of overfishing of herbivorous fish and a hurricane that reduced coral cover led to favorable conditions for algae dominance. At this time, the keystone grazers on the reef were sea urchins (*Diadema antillarium*), which play a critical role in facilitating the settlement of reef-building corals by removing macroalgae. When the functional role of urchins was removed due to an epizootic involving microbial pathogens, algae cover on some reefs
increased from 1% to 95%, effectively leading to a transition between stable states (Table 5.1; Lessios 1988). In the years since the outbreak urchin numbers have rebounded and many reefs have returned to coral dominance.

**Future research: when can parasites be ignored?**

Despite a growing appreciation of their ecological significance, parasites are still omitted from the majority of ecosystem-level studies. Bridging this gap will require a better ability to predict when parasites should play important ecosystem roles and new multidisciplinary approaches that allow scaling across different levels of organization (e.g., from host physiology to ecosystem process). These advances should ultimately aim to determine when we can ignore parasites, and conversely, when it is imperative to quantify their roles to gain a complete picture of ecosystem structure and function.

Indeed, not all parasites will be important at the ecosystem level and not all ecosystems will be significantly affected by parasitism and disease. Clarifying these distinctions will require new observational studies, field experiments, and modeling approaches that span a diversity of host/parasite systems and ecosystems in which they are embedded.

*A traits-based approach to predicting ecosystem consequences of parasitism* – A traits-based approach has proven useful in working towards a predictive capacity in many disciplines of ecology (McGill et al. 2006). Our review of the literature suggests that whether parasites exert important effects on ecosystems will depend on a combination of host and parasite traits, the dynamics of their interaction, and the characteristics of the ecosystem (Table 5.2). Parasites that infect host species with important functional roles that cannot easily be replaced by other community members will *a priori* have the
greatest potential to alter ecosystem function. Therefore, the most important host traits are those that lead to important functional roles, and subsequently, the ability to maintain those functional roles after infection. For example, host tolerance is predicted to influence the magnitude of changes to a host’s functional role after infection, with more tolerant species showing a smaller change in functional roles. Conversely, the most important parasite traits will be those that strongly alter the ability of the host to perform its functional role, both at the individual and population level (Table 5.2). Transmission rates, infectivity, virulence and the specific type of host pathology elicited are all predicted to influence host functional roles through changes in host traits and host densities. For example, virulent parasites that cause high rates of host mortality or parasites that castrate their hosts will induce density-mediated effects, whereas parasites that manipulate host behavior can cause trait-mediated effects. Parasites that achieve a high ecosystem-level biomass are, thus far, the only types of parasites that can be predicted to exert direct effects on ecosystem properties (see Table 5.2). Future research should seek to identify which traits are most important in predicting when and where parasites will exert ecosystem level effects.

*Integrating across scales with observational studies* – Disease ecologists frequently examine the effects of a parasite on one or several host species, often using controlled exposures in an artificial setting. Response variables in these experiments include host survival and host traits that can influence functional roles (e.g., behavior, growth, fecundity, feeding rates). Scaling such studies to the ecosystem level requires integrating per-capita measurements from infected and uninfected individuals with observational field data that place that host’s roles into the wider context of ecosystem
function. This latter aim will generally require field data on host densities, infection prevalence and intensity, and the relative role of the host in the ecosystem property of interest (e.g., host nutrient excretion rates relative to other community members). As an
example, to fully understand the effects of a honeybee pathogen on rates of ecosystem pollination, a researcher must determine 1.) the per capita effects of the pathogen on bee pollination, in terms of either survival probability, changes in traits, or both, 2.) the host density, infection intensity and prevalence of the pathogen in the ecosystem, and 3.) the roles of the host species relative to other organisms in pollination within the ecosystem. Most studies accomplish the first aim, and occasionally the second, but rarely the third, which requires interdisciplinary expertise. Whenever possible, replicating observational studies across ecosystems can be informative in reaching generalizations and revealing whether certain systems represent ‘special cases’ or widespread phenomena. This may be feasible by simply measuring a selected group of variables across several replicate ecosystems (e.g., host density and infection prevalence).

Field experiments and endemic parasites – What happens when parasites are added to or removed from an ecosystem? Community ecology has a rich history of exclusion and addition experiments (e.g., Paine 1966), yet such approaches are not commonly employed in disease ecology research. Addition or exclusion experiments will be particularly valuable in disentangling the ubiquitous ecological roles of endemic parasites. To date, much of our understanding of ecosystem effects of parasites comes from the introduction of nonnative parasites into naïve host populations that result in dramatic epizootics (e.g., rinderpest). Such die-off events, which can be treated as ecosystem disturbances, tend to be obvious and relatively easy to quantify relatively to effects of endemic parasites (Eviner and Likens 2008). The endemic roles of parasites, however, are probably more common but more challenging to detect.
Disentangling the endemic roles of parasites can be accomplished through ecosystem-level experiments that either remove or add parasites and then evaluate the responses in ecosystem structure and function. Both introductions and removals of native parasites present unique challenges; parasite introductions must be carefully designed to avoid unintended consequences for non-target organisms while parasite removals are difficult to implement and replicate on the ecosystem scale. Similar experiments that involve the controlled introduction or removal of invasive species from an ecosystem provide a useful framework (e.g., Vredenburg 2004).

*Models of ecosystem function* – Dynamical models that are parameterized based on laboratory and field data can generalize and extend data from one or a few ecosystems and they can allow simulating the effects of a parasite under multiple scenarios, such as variation in characteristics of the hosts, the parasites, and the ecosystem. This flexibility allows addressing the important question of which conditions facilitate the strongest ecosystem effects of parasites. For example temperature, precipitation or nutrient availability may drive infection rates or changes in host densities, and such context-dependency can be revealed most practically with modeling approaches. Furthermore, models that can incorporate changes in parasite traits, such as transmission mode, basic reproductive rate ($R_0$), or virulence, will be useful in revealing which characteristics are most useful in predicting when ecosystem effects will occur (Table 5.2).

Energy fluxes and biogeochemical cycles are two topics that are well suited to integrating parasites into traditional ‘box and arrow’ models of ecosystem function. Accomplishing this aim will require more precise estimates of the effects of parasites on pools and fluxes of energy and matter over large spatial scales. Collecting such data has
rarely been achieved. For instance, fungi of phytoplankton in freshwaters are known to exert strong controls on plankton populations, yet they are not generally considering in models of freshwater primary production, nutrient cycling or carbon balance (Rasconi et al. 2011). A similar scenario exists for tree pathogens, which can alter most aspect of forest ecosystem function and even global processes such as carbon storage (Lovett et al. 2006), yet are generally omitted from ecosystem models.

Ideally, a multi-faceted approach will be best at revealing the ecosystem roles of parasites. A combination of laboratory studies to evaluate per-capita impacts, field data to scale-up results, controlled experiments to determine the magnitude of effects, and modeling approaches to generalize findings under different scenarios can provide the most compelling evidence for ecosystem effects of parasites and disease.

**Conclusion**

In 2005, Loreau et al. lamented that there had not been a single paper in the journal *Ecosystems* that included the words ‘parasite’, ‘parasitism’ or ‘parasitoid’ in its title, keywords or abstract (Loreau et al. 2005). In the short time since, five papers that involve parasites and disease have been published in *Ecosystems* between 2005 and 2013 (Connelly et al. 2008; Ruess et al. 2009; Lovett et al. 2010; Cobb et al. 2012; Whiles et al. 2012). Such progress has demonstrated that parasites can sometimes shape ecosystem structure, biogeochemical cycles, energy flow and temporal ecosystem dynamics. Current rates of environmental change, including the emergence of novel diseases, the movement of species around the globe, and ongoing extinctions further underscore the need to integrate parasites and disease into our understanding of ecosystems. Although research
in this area is still in its infancy, the unification of disease ecology and ecosystem science promises to mutually benefit both fields by enhancing our understanding of how ecosystems function.

Appendix D

Quantitative literature search methods – I conducted two distinct literature searches to quantify patterns linking research in disease ecology and ecosystem science (Fig. 5.1). The goal of the first search was to compare the total quantity of disease ecology research that has been conducted at different levels of ecological organization, including individual hosts, populations, communities, and ecosystems. The goal of the second search was to focus on time-trends at the two highest levels of ecological organization, communities and ecosystems. The overall approach was modified from Johnson and Paul 2011 and involved an advanced search of publications in the Ecology section of the ISI Web of Science search database. The search was restricted to the years 1980 to 2013 because there were no disease ecology publications at the ecosystem level prior to that date. In both searches, I first conducted a search for disease ecology publications using the follow search string for title keywords: TI=(parasit* OR disease* OR pathog* OR infect*) AND WC = (ecology). I then combined that search with four individual searches using titles keywords for each level of ecological organization: [TI = (host*) AND WC = (ecology)], [TI = (population*) AND WC = (ecology)], [TI = (communit*) AND WC = (ecology)], and [TI = (ecosystem*) AND WC = (ecology)]. The resulting number of publications for each of the four searches are shown in Figure 5.1a.
The approach for the second literature used a more exhaustive search string to examine trends in publications over time at the community and ecosystem levels. This search first involved the same search string for disease ecology publications that is provided above. To account for changes in the total number of disease ecology publications over time, I divided the number of publications at the community and ecosystem level per year by the total number of disease ecology publications per year. As such, the values used over time are percentages of each publication type out of the total number of disease ecology publications per year. To identify publications at the community level, I combined the disease search string with the following string:

\[
\text{TI}=(((\text{communit}^* \text{ OR assembl}^*) \text{ AND } (\text{ecolog}^* \text{ OR dynamic}^* \text{ OR change}^* \text{ OR structure}^* \text{ OR composition}^* \text{ OR interact}^* \text{ OR species} \text{ OR diversity} \text{ OR host} \text{ OR parasite} \text{ OR pathogen}) \text{ OR } (\text{food} \text{ AND web}^*) \text{ OR food-web}^* \text{ OR foodweb}^* \text{ OR (ecological AND network}^*) \text{ OR predat}^* \text{ OR prey} \text{ OR predator-prey} \text{ OR herbiv}^* \text{ OR trophic OR competit}^* \text{ OR facilitat}^* \text{ OR exclu}^* \text{ OR (species and interact}^*) \text{ OR co-infect}^* \text{ OR coinfect}^* \text{ OR transmi}^* \text{ OR (effect}^* \text{ AND (indirect OR direct OR trait-mediated)}) ) \text{ AND WC = (ecology). Similarly, for ecosystem level publications on disease, I then combined the disease string with the following string: TI}=((\text{ecosystem AND (function}^* \text{ OR process}^* \text{ OR propert}^* \text{ OR structure} \text{ OR stability} \text{ OR dynamic}^* \text{ OR service}^* \text{ OR engineer}^* \text{ OR disturbance}^* \text{ OR energ}^* \text{ OR change}^* \text{ OR diversity}) \text{ OR succession OR biogeochem}^* \text{ OR ((nutrient}^* \text{ OR nitrogen OR phosphorus OR carbon OR element}^*) \text{ AND (cycl}^* \text{ OR recycl}^* \text{ OR flux}^* \text{ OR dynamic}^*) ) \text{ OR decomposition OR (energ}^* \text{ AND (flow}^* \text{ OR flux}^* \text{ OR dynamic}^*) ) \text{ OR (product}^* \text{ AND (biomass OR primary OR OR}}
\]
secondary)) OR (change* AND (global OR environmental))) AND WC = (ecology). The results of this search are present in Figure 5.1b.

To identify patterns in published studies on the effects of parasites and disease on ecosystems, I selected publications from the above searches and combined them with additional selective searches using Google Scholar. I specifically examined the type of ecosystem (forest, freshwater, marine, grassland or other), the ecosystem property affected (biogeochemistry, ecosystem structure, energy flow, or temporal ecosystem dynamics), the approach used in the study (observational, experimental, modeling, or a combination), and the mechanism underlying ecosystem effects of parasites (density-mediated indirect, trait-mediated indirect or direct effect). The patterns observed are shown in Figure 5.2 and the list of 34 publications identified using this approach is given in Table D.1 and is also included within the References.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Ecosystem Type</th>
<th>Ecosystem Property</th>
<th>Mechanism</th>
<th>Venue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sumption and Flowerdew</td>
<td>1985</td>
<td>Forest</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Lessios</td>
<td>1988</td>
<td>Marine</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Suttle et al.</td>
<td>1990</td>
<td>Marine</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Experiment</td>
</tr>
<tr>
<td>Van der Putten et al.</td>
<td>1993</td>
<td>Sand dune</td>
<td>Dynamics</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Pennings and Callaway</td>
<td>1996</td>
<td>Marine</td>
<td>Structure</td>
<td>Indirect Trait</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Holah et al.</td>
<td>1997</td>
<td>Forest</td>
<td>Dynamics</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Kohler and Wiley</td>
<td>1997</td>
<td>Freshwater</td>
<td>Energy flow</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Thomas et al.</td>
<td>1998</td>
<td>Marine</td>
<td>Structure</td>
<td>Indirect Trait</td>
<td>Multiple</td>
</tr>
<tr>
<td>Mouritsen et al.</td>
<td>1998</td>
<td>Marine</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Quested et al.</td>
<td>2002</td>
<td>Alpine</td>
<td>Biogeochem</td>
<td>Direct</td>
<td>Multiple</td>
</tr>
<tr>
<td>Mitchell</td>
<td>2003</td>
<td>Grassland</td>
<td>Energy flow</td>
<td>Indirect Density</td>
<td>Experiment</td>
</tr>
<tr>
<td>Poorvin et al.</td>
<td>2004</td>
<td>Marine</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Experiment</td>
</tr>
<tr>
<td>Bardgett et al.</td>
<td>2006</td>
<td>Grassland</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Experiment</td>
</tr>
<tr>
<td>Borer et al.</td>
<td>2007</td>
<td>Grassland</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Modeling</td>
</tr>
<tr>
<td>Wood et al.</td>
<td>2007</td>
<td>Marine</td>
<td>Structure</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Connelly et al.</td>
<td>2008</td>
<td>Freshwater</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Multiple</td>
</tr>
<tr>
<td>Kuris et al.</td>
<td>2008</td>
<td>Marine</td>
<td>Energy flow</td>
<td>Direct</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Authors</td>
<td>Year</td>
<td>Ecosystem</td>
<td>Biogeochem</td>
<td>Trait Type</td>
<td>Method</td>
</tr>
<tr>
<td>------------------------------</td>
<td>------</td>
<td>-----------</td>
<td>------------</td>
<td>------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>Hernandez and Sukdeo</td>
<td>2008</td>
<td>Freshwater</td>
<td>Biogeochem</td>
<td>Indirect Trait</td>
<td>Multiple</td>
</tr>
<tr>
<td>Ruess et al.</td>
<td>2009</td>
<td>Forest</td>
<td>Biogeochem</td>
<td>Indirect Trait</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Holdo et al.</td>
<td>2009</td>
<td>Grassland</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Modeling</td>
</tr>
<tr>
<td>Lovett et al.</td>
<td>2010</td>
<td>Forest</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>March and Watson</td>
<td>2010</td>
<td>Forest</td>
<td>Biogeochem</td>
<td>Direct</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Sato et al.</td>
<td>2011</td>
<td>Freshwater</td>
<td>Energy flow</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Spasojevic and Suding</td>
<td>2011</td>
<td>Alpine</td>
<td>Biogeochem</td>
<td>Direct</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Maron et al.</td>
<td>2011</td>
<td>Grassland</td>
<td>Energy flow</td>
<td>Indirect Density</td>
<td>Experiment</td>
</tr>
<tr>
<td>Grimmett et al.</td>
<td>2012</td>
<td>Freshwater</td>
<td>Biogeochem</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Sato et al.</td>
<td>2012</td>
<td>Freshwater</td>
<td>Energy flow</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Mabuhay and Nakagoshi</td>
<td>2012</td>
<td>Forest</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Cobb et al.</td>
<td>2012</td>
<td>Forest</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Boze et al.</td>
<td>2012</td>
<td>Forest</td>
<td>Biogeochem</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Cobb et al.</td>
<td>2012</td>
<td>Forest</td>
<td>Structure</td>
<td>Indirect Density</td>
<td>Multiple</td>
</tr>
<tr>
<td>Preston et al.</td>
<td>2013</td>
<td>Freshwater</td>
<td>Energy flow</td>
<td>Direct</td>
<td>Observational Field</td>
</tr>
<tr>
<td>Bernot</td>
<td>2013</td>
<td>Freshwater</td>
<td>Biogeochem</td>
<td>Indirect Trait</td>
<td>Experiment</td>
</tr>
<tr>
<td>Whiles et al.</td>
<td>2013</td>
<td>Freshwater</td>
<td>Biogeochem</td>
<td>Indirect Density</td>
<td>Observational Field</td>
</tr>
</tbody>
</table>

Table D.1. Empirical studies of parasites affecting ecosystems that were used to generate figure 5.2.
CHAPTER VI

SUMMARY AND CONCLUSIONS

Historically, disease ecology research has rarely integrated across ecological scales, with most work occurring at the level of the individual or the population. As a result, many core areas of ecology have developed independently from disease research, leading to gaps in our understanding of how parasitism can affect ecosystems. The central aim of my dissertation was to understand ways in which parasites can affect the ecology of freshwater ecosystems across scales, from individuals to ecosystems. Using parasites within wetland ecosystems in northern California, I examined 1) the ways in which parasites affect host behavior, 2) the roles of parasites in trophic links within a food web, and 3) the contributions of parasites to animal biomass and productivity. I further assessed the evidence for parasites more broadly affecting the structure and function of ecosystems using a combination of empirical literature searches and a review of existing case studies. Collectively, my results reveal that the ecological roles of parasites can equal or surpass the importance of their free-living counterparts and that in many systems our understanding of key processes may be incomplete without considering parasitism and disease. In the sections that follow, I will review the key findings from the aforementioned studies and I will identify opportunities to extend and generalize this research in the future.

Parasite ecology in freshwater wetlands

To strengthen links between community ecology and disease research, I first examined how trematode parasites can affect the behavior of individual hosts, and
particularly how such responses compare to well-studied defenses associated with predators (Chapter II). Importantly, this study revealed several similarities, but also key differences, between the two types of interactions. Prior to direct, parasites elicited no inducible defenses in host amphibians, which contrasts with the consistent responses elicited by predators. After infection, however, the behavior of hosts changed in ways that were predictable based on characteristics of the host-parasite interaction. These post-infection behavioral changes closely paralleled the outcomes of inducible defenses to predators prior to contact and likely have similar ecological consequences. Future work should extend these findings to explore their importance to community interactions, including predation, competition, and parasite transmission. One promising way to do this is through the use of outdoor mesocosm experiments that quantify the strength of species interactions within communities that vary in the abundance of parasites. Several metrics exist for quantifying the strength of species interactions (e.g., Novak and Wooton 2008) and these indices would allow the rigorous testing of hypotheses regarding how parasitism affects competition and predation.

I next examined the roles of parasites in a topological pond food web. This work showed that parasites can be prominent players in trophic linkages, particularly links from predators to parasites (Chapter III). Parasites increased the number of links in the web by over 80%, and of those links, 79% were from predators to parasites. This appears to be a general finding in the few food webs that do include parasites to date (Thieltges et al. 2013). The overall effects of parasites on the topology of the web, however, were very sensitive to how the web was constructed (i.e., using either taxonomic species or life stages as nodes). Web metrics that have important implications for ecosystem dynamics,
such as connectance and nestedness, were influenced differently by parasites depending on how nodes in the web were defined. Whether this result also occurs in other webs remains to be seen, although it suggests that we should use caution in determining the overall effects of parasites on topological webs. Furthermore, it still remains debated as to which of the changes induced by parasites on topological food webs are truly unique effects of parasites, versus simply effects of adding additional species to the food web (Dunne et al. 2013). Many topological food web metrics are scale-dependent, meaning they change predictably with the size of the food web (i.e., the number of nodes).

Although this study indicated parasites participate in many trophic links within a wetland food web, but left it unclear whether such links have importance in the movement of energy though the food web. If relatively little energy moves through links involving parasites, their ecological importance in food webs may be equivocal.

To address just how much energy can move through trophic links involving parasites, I quantified the biomass and productivity of trematode worms within three wetlands, including the one for which I constructed the food web (Chapter IV). The study revealed that trematode parasite biomass can exceed that of the most abundant invertebrate groups, including beetles, mayflies, dragonflies and damselflies, true insects and crustaceans. Only the snails and amphibians, both important trematode hosts, had a higher standing biomass. Trematodes were also very productive, producing several hundred milligrams of new biomass per meter square per year. This is comparable to many invertebrate groups. These findings together indicate that there is potential for a large amount of energy to move along the links in the wetland food web that involve parasites. To date, the generality of such findings remains unclear; only three studies to
my knowledge have quantified parasite biomass at the ecosystem scale, and in all three of them, parasite biomass was significant (Mitchell 2003; Kuris et al. 2008; Preston et al. 2013). Knowing exactly where that energy derived from parasites ends up is another open question. The fraction of parasite productivity that successfully infects downstream hosts is probably very small. Other fates include detrital pools, where parasites may end up if the die as free-living stages, or energy that moves into predators. Laboratory trials suggest that many predators commonly consume trematode life stages (Orlofske et al. 2012). One elegant, though perhaps challenging, approach to answer this question involves tracing the fate of parasite biomass using an isotope label. This approach could be very informative if carried out in a real wetland. Furthermore, my study did not truly quantify the magnitude of energy flow along links involving parasites in the field. This would require very specific field data on predator-prey interactions in nature and could lead to more conclusive insights into the roles of parasites in energy flow.

Lastly, I explored the ways in which parasites can affect ecosystem structure and function using an empirical literature search and a review of case studies (Chapter V). My goals were to 1) quantify the disconnect between disease ecology and ecosystem science, 2) explore how parasites can be integrated into ecosystem science, 3) review evidence for the importance of their ecological roles, 4) work towards a predictive framework using traits of the host and parasite, and 5) identify future research needs. This approach revealed that research linking parasite ecology to ecosystem science is rare, with ecosystem level studies accounting for less than 2% of research on disease ecology. This disparity exists despite the fact that parasites can affect ecosystem structure, biogeochemical cycles, ecosystem energy flow, and temporal ecosystem dynamics within
freshwater, marine and terrestrial habitats. Mechanistically, existing studies indicate that parasites can contribute directly to ecosystem processes through the production of biomass, or indirectly by altering the density or traits of their hosts. The latter mechanism will be most important when host species play important functional roles. Taken together, this review indicates that although there is preliminary evidence for importance ecosystem roles of parasites, it remains unclear how general such affects are across systems.

**Future directions**

Three key areas for which I plan to pursue future research include 1) the effects of parasites on aquatic nutrient cycling, 2) the integration of host-parasite interactions and ecological stoichiometry, and 3) the roles of parasites in temporal ecosystem dynamics, including stability. Integrating parasites into ecosystem ecology requires knowledge of how parasites influence both energy flow and the movement of matter, which are the major conceptual foci of the field (Chapin and Matson 2011). While several chapters of my dissertation have examined the pathways and potential magnitudes of energy flow involving parasites, the roles of parasites in dynamic fluxes of matter, including nutrients, remain unknown in most ecosystems. Animals can play vital roles in nutrient cycling through the excretion of waste products containing nitrogen and phosphorus (reviewed in Vanni 2002), particularly in freshwaters where these elements often limit primary production (Elser et al. 2007). Freshwater snails, for example, can excrete up to two thirds of the nitrogen demand by primary producers in temperate streams (Hall et al. 2003) and can also exert strong controls on primary production through grazing (Liess
and Hillebrand 2004). In Chapter III, I found that aquatic snails can dominate the biomass of consumers in some wetlands in California, suggesting that they likely play key roles in consumer-driven nutrient recycling.

A promising area for future research involves understanding the ways in which trematode parasites and their hosts can affect nutrient cycling in wetlands. I have explored this question to date using a combination of mesocosm experiments and measurements of host traits in the laboratory. Two specific mechanisms exist through which parasites can affect nutrient cycling. The first mechanism involves density-mediated effects, whereby parasites control host density due to parasitic castration of infected individuals. I have recently completed a mesocosm experiment that has demonstrated this mechanism can occur, although it remains unclear how well the results will ‘scale-up’ to a real wetland and this remains an important question for future work. The second mechanism involves trait-mediated effects through which parasites alter per-capita rates of host nutrient excretion. Evidence for this second mechanism has been found in Physa sp. snails that excrete less phosphorus when they are infected by trematodes (Bernot 2013). I have ongoing research that aims to explore whether this is a general affect across other snail host-trematode systems and whether it has significant consequences for ecosystem level nutrient cycling. Lastly, modeling efforts will be very useful in understanding when such effects are most significant. Factors including host density, infection prevalence and nutrient inputs from external sources are all likely to moderate the importance of trematodes in nutrient cycling, making their effects variable across ecosystems. Biogeochemical models that allow varying these factors will be useful in understanding the context dependency of parasite effects on nutrient cycling.
Ecological stoichiometry seeks to understand the ecological consequences of mismatches in the elemental content between a resource and a consumer (Sterner and Elser 2002). These relationships have rarely been explored in the context of host-parasite interactions and provide an exciting area for future work (but see Frost et al. 2008). The elemental stoichiometry of parasites in relation to their hosts has potential to strongly influence parasite productivity and subsequent disease risk. This is a question that has importance for a wide range of host-parasite systems including both microparasites and macroparasites. The growth-rate hypothesis states that rapidly growing organisms require more phosphorus because they are producing large amount of RNA. Because parasites are fast growing and rapidly reproducing, they may have higher phosphorus demands than their hosts. Exploring this hypothesis can be accomplished with targeted measurements of host and parasite stoichiometry as well as controlled experiments that vary the resource quality of hosts.

An additional key question in disease ecology involves understanding the effects of parasites on ecosystem stability. To date, this question has been approached primarily from a topological food web perspective, which has yielded contrasting conclusions depending on the methods used (Rudolf and Lafferty 2011; Dunne et al. 2013). A more realistic way to address this question would involve a combination of experiments that involved the addition or removal of parasites and subsequent quantification of ecosystem stability metrics. This type of approach would be well suited to experiments at multiple spatial scales, including the laboratory microcosm, the outdoor mesocosm, and a real ecosystem.
Conclusion

Current trends in environmental change – including habitat loss, species extinctions, biological invasions, climate change, pollution, and overharvesting – all have potential to alter the ecological roles of parasites in complex ways. Understanding such changes will require baseline data on the roles of parasites in diverse ecosystems and a combination of synthetic approaches including field surveys, experiments, and modeling. In this dissertation, I examined how parasites in freshwater wetlands influence individual hosts, species interactions within a food web, and energy flow through the ecosystem. My results indicate that the ecological roles of parasites, despite being unseen, are important to the overall structure and functioning of the ecosystem. Further studies are needed to understand the generality of such effects across other systems and to predict exactly when and where parasites will play the most important ecological roles.


Lafferty, K.D. and Kuris, A.M. 2009. Parasites reduce food web robustness because they are sensitive to secondary extinctions as illustrated by an invasive estuarine snail. Philosophical Transactions of the Royal Society B 364: 1659-1663


Lunde, K.B., Resh, V.H. and Johnson, P.T.J. 2012. Using an ecosystem-level manipulation to understand host-parasite interactions and how they vary with study venue. Ecosphere 3: art84.


Sato, T., Watanabe, K., Kanaiwa, M., Niizuma, Y., Harada, Y. and Lafferty, K. D.


