THE EFFECTS OF PARASITISM ON CONSUMER-DRIVEN NUTRIENT RECYCLING

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Abstract: The effects of parasitism on consumer-driven nutrient recycling

Charlotte Narr

Daphnia are keystone consumers in many pelagic ecosystems because of their central role in nutrient cycling. *Daphnia* are also frequently infected, and the parasites causing these infections may rival their hosts in their ability to regulate ecosystem processes. Therefore, parasitic exploitation of *Daphnia* may alter nutrient cycling in pelagic systems. This thesis integrates existing knowledge regarding the exploitation of *Daphnia magna* by 2 endoparasites to predict parasite-induced changes in the nutrient cycling of infected hosts and ecosystems. In chapter 1, I I contextualizing the integration of these themes by reviewing the development of the fields of elemental stoichiometry and parasitology. In chapter 2, we show how the bacterial parasite, *Pasteuria ramosa*, increased the nitrogen (N) and phosphorus (P) release rates of *D. magna* fed P-poor diets. We used a mass-balance nutrient release model to show that parasite-induced changes in host nutrient accumulation rates and diet-specific changes in host ingestion rates were responsible for the accelerated nutrient release rates that we observed. In chapter 3, we extended our examination of the nutrient mass balance of infected *D. magna* to include another parasite, the microsporidian *H. tvaerminnensis*. We found differences in the effects of these two parasites on host nutrient use as well as support for the hypothesis that parasite-induced changes in *Daphnia* N release are caused by the effects of infection on *Daphnia* fecundity. In chapter 4, we examined the relationship between P concentrations and the presence and prevalence of *H. tvaerminnensis* in rock pools along the Baltic Sea. We found that particulate P concentrations were negatively associated with the prevalence of this parasite, a result that is consistent with the increase in P

sequestration of *H. tvaerminnensis*-infected *Daphnia* that we observed in chapter 3. I discuss the potential implications of the work presented in chapters 2-4 for other parasite-host systems and ecosystems in chapter 5. Overall, the research presented here suggests that parasite-induced changes in host nutrient use may affect the availability of nutrients in the surrounding environment, and the magnitude of this effect may be linked to parasite-induced reductions in fecundity for many invertebrate hosts.

Keywords
Parasitism, Phosphorus, Nitrogen, Ingestion rates, Assimilation rates, Consumers, Nutrient recycling, Mass-balance, *Pasteuria ramosa*, *Hamiltosporidium tvaerminnensis*, Host exploitation strategy, Fecundity, Parasite load, Rock pools, Baltic Sea

Preface

My thesis is written in manuscript format, as each of my chapters has been (or will be) published in the peer-reviewed literature. Chapter 2 has been published in *Oecologia*, chapters 3 and 4 will be published in other journals. I am first author on each of these manuscripts, but they were all done in collaboration with my supervisor, Dr. Paul Frost, and Dr. Dieter Ebert was also a substantial contributor to chapter 4. Therefore, I have used "we" in the middle 3 chapters and "I" in the general introduction and discussion (chapters 1 and 5). Appendix A shows consent from *Oecologia* to reprint chapter 2.

Acknowledgements

I see no better format for my acknowledgements than a quick walk down memory lane, and I will organize my memories in roughly chronological order.

My master's advisor, Dr. Amy Krist, is solely responsible for my interest in both parasitology and ecological stoichiometry and her constant enthusiasm directed me to the lab of an academic hero, Dr. Paul Frost, in pursuit of this Ph.D. I imagine it was, in part, her letter of recommendation that earned me the Ontario Trillium scholarship upon admission to Trent, which made it possible for me to live comfortably and pay international tuition fees at a Canadian university. I am deeply indebted to the Ontario government and Trent University for this financial support over 4 years.

In the Frost and Xenopoulos labs I found an incredible array of machines and people on whom I could rely for data, ideas, and comfort. For their amalgamation and maintenance, I thank both Dr. Paul Frost and Dr. Maggie Xenopoulos, a truly dynamic academic duo. Maintaining *Daphnia* cultures is not a solitary job, and I am so grateful to Dr. Nicole Goulding, Clay Prater, Andrea Conine, Jade Laycock, and a handful of dedicated undergraduate students for helping with the day-to-day *Daphnia* maintenance and taking over when I left town to see friends and family.

Of course, I also found Dr. Paul Frost in the Frost lab, and I have him to thank for just about every aspect of this thesis. Summarizing his contribution to this research is an overwhelming task, so I will just highlight a few memories. Paul is a leading expert in many of the subjects in this thesis, and he added novel ideas and rigor throughout. But, despite his expertise, he always encouraged my independence and supported me in exploring new ideas and experimental designs. His door was always open, so he was always there to provide guidance or say "you can figure this one out", when I needed a

little push. And he consistently started his emails with “This is a great start!”, a sentence that greatly improved the digestion of pages of colored ink in track changes. I’m honestly not sure how I will publish papers in the future without Paul’s constructive criticism.

I am also extremely grateful to Dr. Dieter Ebert and Jürgen Hottinger for supplying me with the *Daphnia*-parasite host system used in all of my experiments. Their generosity in sharing these organisms is fueling research worldwide. While working on my 4th chapter, I benefited greatly from the creative and logistical direction provided by these two researchers as well as Dieter’s infectious enthusiasm for science. Dr. Guillaume Bastille-Rousseau catalyzed the transition of every one of these chapters from data to results with thoughtful suggestions and novel R code that enabled me to ask exactly the questions I felt were most important.

The field work in this thesis was heavily supported by The Walter and Andrée de Nottbeck Foundation. Without this support, I would not have had the opportunity to conduct this research at the Tvärminne Zoological Station in Finland. I am very grateful to the staff at the station, especially Jaana Koistinen who repeatedly dropped everything to find me the supplies I needed. I would also like to acknowledge the assistance of Dr. Isobel Booksmythe, Nina Gerber, and Dr. Peter Fields in the execution and enjoyment of field work at the Tvärminne Zoological Station.

Finally, my siblings provided many lively debates regarding the value of research to the general public, and offered glimpses of a different reality outside of the academic bubble in which I’ve been living. For the motivation to begin and finish this work, I thank my mother, Nancy Narr, who takes notes and asks questions during all my practice

presentations. And for the courage to push a little harder on the apparent limits of my project, I thank my father, Donald Narr, who gently insists that I be more assertive in everything I do.

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Chapter 1 - General introduction

> ***Thus the circulation of matter in nature must not only provide for the mere presence of certain substances on which the maintenance of life depends, but it must furnish them in suitable concentration and, generally, in available form. It must, therefore, in many cases include, as a definite step, a segregating or concentrating process as well as simple motion through a cycle.***
>
> - Alfred Lotka, 1925, *Elements of Physical Biology*

There can be a dramatic redirection of resources from host organisms into their parasites with up to 30% of host materials repurposed by the parasite (Hechinger 2009). This reallocation of resources from host to parasite tissue forms a segregating step in the movement of substances as they move through a host. How does this allocation of resources to the parasite alter the suitability and availability of "…certain substances on which the maintenance of life depends"(Lotka, 1925)? The studies included in this thesis explore changes in host nutrient cycling as a means to a first-level understanding of how parasites alter ecosystem level patterns in the distribution of nutrients. Because approximately 40% of all known species are parasitic (Dobson et al. 2008), it may be ambitious to try to produce ecosystem level predictions applicable to such a diverse group. In non-parasitic interactions, a clear picture of nutrient flows into one individual (e.g., a predator) requires the integration of information from many individuals (e.g., the prey) across a heterogeneous landscape. This makes it difficult to understand how nutrients move through the diet of the prey, through the prey, and into the predator. In contrast, the well-defined spatial boundaries of the parasite-host interaction enable us to study this interaction using basic laws of mass balance. Not only does the tight spatial scale of parasite-host interactions make it easier to study nutrient flows between interacting organisms, but it may also increase the value of studying these flows by

increasing the likelihood that parasites will respond to and influence host dietary nutrient availability.

Understanding the relationship between nutrients and parasites is important in anthropogenically altered landscapes where both nutrient and parasite dynamics are being modified at high rates. Observations of positive correlations between parasite abundance (i.e., parasite intensity and prevalence; Poulin 2007) and nutrient availability have raised concerns that anthropogenic nutrient deposition (via the application of fertilizers and burning of fossil fuels; Schlesinger & Bernhardt, 2013) may increase the risk of disease to wildlife (Johnson et al. 2007; Civitello et al. 2013). Conversely, human-driven changes in the geographic distribution of parasites (Torchin, Byers, & Huspeni, 2005, Krkosek et al., 2006) may lead to parasite-related changes in the availability of resources and nutrients in heavily parasitized environments (Bernot 2013; Caceres et al. 2014). Evidence for anthropogenic increases in nutrient availability dates back to 1900 (Hastings et al. 2009; Holtgrieve et al. 2011), and emerging infectious diseases have been on the rise since at least the 1940s (Jones et al. 2008). So, why has it taken a century to apply the law of conservation of matter (established in the 1700's) to an interaction defined by the closed flow of material from one organism to the next? Answering this question requires us to explore the development of both parasitology and ecology as scientific disciplines.

Stop the virulence! An extremely abbreviated history of the centuries-long battle between hosts and parasites

Human interest in disease developed out of necessity with a clear aim: to reduce or eliminate the virulence experienced by the host. For most of written history, this task

was inhibited by our inability to identify the causal agents of disease. Aristotle recognized parasites, but he considered them to be a symptom, rather than an agent, of disease. Even in 1824, the superintending surgeon of Bombay confidently argued that Guinea worms were lymphatic vessels of their hosts (Grant 1931).

In the same century (close to 200 years after the invention of the microscope), the work of Louis Pasteur, Robert Koch, and other scientists led to the formulation of Germ Theory, which established, for the first time, a causal link between disease and the living agents of disease. A few years after Pasteur demonstrated that spontaneous generation of life was impossible (and, by deduction, that disease could not generate parasites), Koch produced clear evidence that the causal agents of diseases like anthrax and tuberculosis were living organisms. He did so by satisfying a series of rules he had developed for identifying infectious disease agents (Koch's Postulates) that required that, among other things, the causal agent could be cultured in the lab. The publication of these postulates caused the study of what eventually became known as parasites to develop along two different paths: that taken by physicians who studied the transmission and virulence of parasites that satisfied Koch's postulates, and that taken by biologists who pieced together the sometimes very complex life cycles of parasites that did not satisfy Koch's postulates (Vickerman 2009). It was in this arduous task that biologists who studied disease remained engrossed as the study of evolution and ecosystems was being shaped by the work of researchers like Darwin and Haeckel.

In the early and middle 20th century, researchers from diverse disciplines became interested in the ecological and evolutionary roles of parasites. Geneticists like Biffen (1905), Moulton (1940), Johnson (1946), and Haldane (1949) pointed out that parasites

could drive host evolution (Lederberg 1999). The animal ecologist, Thomas Park, demonstrated the effect of infection by a protozoan parasite on the outcome of competition between 2 species of flour beetles (Park 1948), showing, for perhaps the first time, that the sublethal effects of parasites can shape communities. Even Alfred Lotka, the mathematician and chemist, applied laws from the physical sciences to the epidemiology of malaria (Lotka, 1925). However, perhaps because parasitism was not the primary focus of any of these researchers, these lines of inquiry that integrated parasites into our understanding of ecosystems were largely ignored until the 1970s and 80s (Hatcher and Dunn 2011).

Physical biology and the chemical constraints of aquatic ecosystems

Lotka's work on malaria may have been forgotten, but he left his mark on ecology when he applied physical laws to the function and evolution of ecosystems. He envisioned "the organism as a structured physico-chemical system" and, with incredible foresight, pointed out the potential for negative environmental effects of human modification of carbon (C), nitrogen (N), and phosphorus (P) cycles (Lotka, 1925). In 1942, the oceanographer Alfred Redfield observed that phytoplankton in the ocean could be treated as physico-chemical systems and that they also displayed extreme uniformity in their physico-chemical composition: for every 1 atom of P, phytoplankton contained approximately 16 atoms of N and 106 atoms of C. Furthermore, the chemical composition of phytoplankton was reflected in the chemical composition of the oceans. Redfield (1958) eventually reached the conclusion that it was the ocean's biota that determined the ocean's chemical concentrations and, in doing so, he connected the biology of ecosystems directly to geochemical cycles. Redfield's observations became

increasingly important after Schindler (1974) and others showed that variation in P input was responsible for variation in net primary production of freshwater ecosystems. Bio-available P was typically low in these ecosystems, but, as Redfield had determined for marine ecosystems, the biota (zooplankton) could dictate rates of nutrient turnover and net primary production (Porter 1976).

In 1986, as parasites were just beginning to be included in the study of community ecology, William Rieners took the relationship between nutrient cycles and biological systems a few steps further. He used the observations of Redfield and data compiled by Bowen (1979) on the elemental composition of many organisms to integrate the conventional framework of ecosystem energetics with laws describing the movement of matter (Reiners 1986). Rieners defined 9 axioms. He observed that there were "differences among species in the means and rates at which they can sequester limiting resources" (axiom 5), and that "biological effects on the availability and chemical form of elements are unique in kind and magnitude" (axiom 6). These axioms enabled him to propose 6 theorems regarding the distribution of elements in ecosystems. His fourth theorem is of particular relevance to us: "the world biota drives and regulates the biogeochemical cycles". However, it would be another 22 years before ecologists would demonstrate differences between infected and uninfected conspecifics in *the means and rates at which they can sequester limiting resources*, and begin to wonder how parasitism *drives and regulates biogeochemical cycles.*

Parasites and nutrient cycling: An emerging field

By the year 2000, parasitologists were presenting strong cases for the study of parasite ecology and evolution as a means of understanding parasite virulence and

transmission (Ewald 1994; Poulin 2007), and ecologists were arguing that information about parasites could improve our understanding of basic ecosystem properties (Lafferty 1997). At the same time, limnologists were demonstrating that continued net primary production in lakes is dependent on the recycling of P from organic to inorganic (bioavailable) forms and that the rate of this recycling could be altered by changing the N:P ratio of the tissues of the dominant grazer (Sterner et al. 1992). They realized that consumer tissue nutrient concentrations can affect ecosystem nutrient availability because the flux of nutrients from a consumer is driven, at least in part, by differences between the elemental composition of the consumer and its diet (Sterner 1990, Elser and Urabe 1999). So, because consumers largely maintain elemental homeostasis, they release nutrients that are ingested in excess of their specific requirements (Sterner and Elser, 2002). For example, when confronted with low dietary nitrogen (N) to phosphorus (P) ratios, a consumer should maintain elemental homeostasis by retaining N and releasing P. When high densities of consumers sequester the same limiting nutrient, this nutrient limitation can be transferred back to primary producers (Figure 1). In this way, the elemental composition of the dominant consumer in an ecosystem can determine which nutrient(s) limit(s) primary productivity (Sterner et al., 1992).

These studies motivated work that revealed differences in grazer body nutrient ratios caused by reproductive status (Ventura and Catalan 2005), life history characteristics (e.g., growth rate, Elser, Brien, Dobberfuhl, & Dowling, 2000), and, in 2008, parasitism (Frost et al. 2008a). The research presented in this thesis is an attempt to integrate these parasite-induced changes in host nutrient composition into our understanding of nutrient availability in freshwater ecosystems. In the second chapter,

this is done following the tradition of Lotka, by comparing the 'physico-chemical' structures of infected and uninfected individuals, and using stoichiometry to predict parasite-induced shifts in nutrient release.

In the past ten years, researchers have shown that parasites attain substantial biomass (Kuris et al. 2008) and comprise the majority of species in our ecosystems (Dobson et al. 2008). As a result, interest in integrating parasites into our understanding of ecosystem processes has increased greatly (McKenzie and Townsend 2007; Johnson et al. 2010b), but we lack an understanding of how ecosystems respond to different types of parasites. The third chapter examines aspects of the parasite-host interaction that are of interest to traditional parasitologists (i.e., parasite virulence and transmission) within a stoichiometric framework to explore how parasite type might influence host nutrient use. Specifically, we examined the relationships between parasite-induced or diet-induced changes in fecundity and spore load and multiple aspects of host nutrient use.

Despite the general expectation that parasite abundance will increase with increasing nutrient availability (Johnson et al. 2010b), few studies show correlations between parasites and nutrients in natural systems (but see, Johnson et al 2007; Civitello et al 2013). The lab experiments described in chapters 2 and 3 indicate that the effects of complex interactions between parasitism and host diet quality on host nutrient use might make it difficult to detect relationships between parasites and nutrients in nature. In the fourth chapter, we looked for empirical, field-based evidence that can be used to test the hypotheses generated from the experiment described in chapter 3. To do so, we examined correlations between the presence/prevalence of one of the parasites we examined *in vitro* and P availability in rock pools on islands in the Baltic Sea.

Ultimately, this thesis is informed by the traditions of both parasitology and basic ecology, and is intended to help forge a stronger link between them. The focal *D. magna*-endoparasite system is a classic model system for both parasitology (Ebert 2005) and freshwater nutrient cycling (Sterner and Elser 2002), and is a particularly good example of how easily these two traditions can inform one another. Since 2008, researchers have shown increasing interest in applying laws of mass-balance to parasite-host interactions (e.g. Aalto, Decaestecker, & Pulkkinen, 2015; Bernot, 2013). It is our hope that the patterns observed in this thesis will not only be of interest to the growing group of researchers that are specifically interested in the relationship between parasites and nutrients, but also to researchers focused on other aspects of disease and ecosystem processes. It is high time we put mass-balance principles to work in understanding the role of parasites in ecosystems.

Figure:

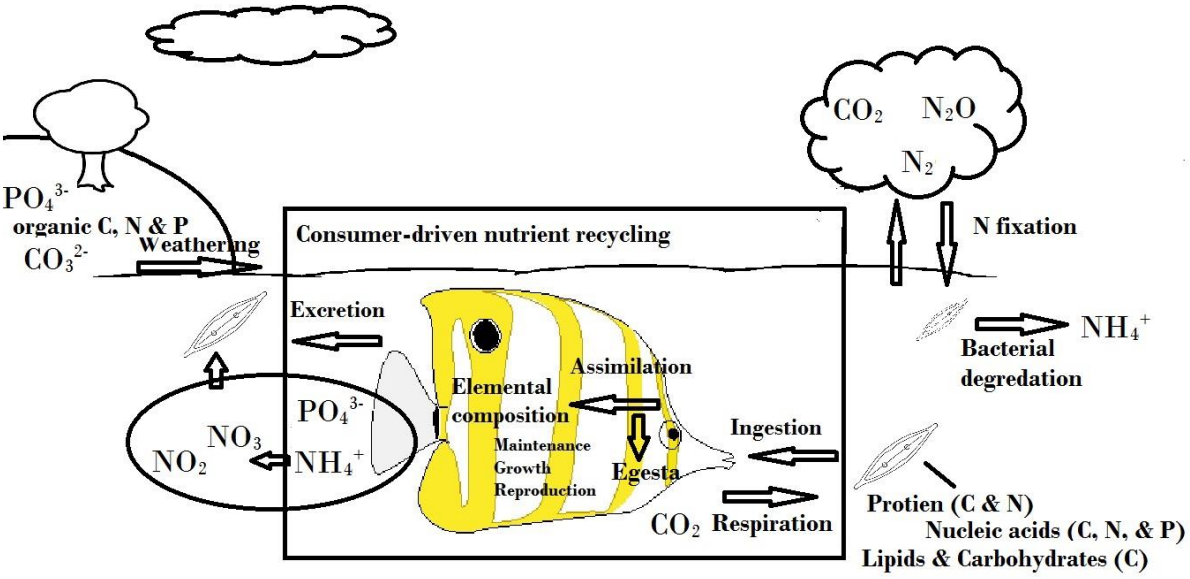

Figure 1: Simplified schematic of the major fluxes of C, N, and P through ecosystems and consumers.

Chapter 2 – Does infection tilt the scales? Disease effects on the mass balance of an invertebrate nutrient recycler

Charlotte F. Narr and Paul C. Frost

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Abstract

While parasites are increasingly recognized as important components of ecosystems, we currently know little about how they alter ecosystem nutrient availability via host-mediated nutrient cycling. Here we examined whether infection alters the flow of nutrients through hosts and whether such effects depend upon host diet quality. To do so, we compared the mass specific nutrient (i.e., nitrogen and phosphorus) release rates, ingestion rates, and elemental composition of uninfected *Daphnia* to those infected with a bacterial parasite, *P. ramosa*. N and P release rates were increased by infection when *Daphnia* were fed P-poor diets, but we found no effect of infection on the nutrient release of individuals fed P-rich diets. Calculations based on the first law of thermodynamics indicated that infection should increase the nutrient release rates of *Daphnia* by decreasing nutrient accumulation rates in host tissues. Although we found reduced nutrient accumulation rates in infected *Daphnia* fed all diets, this reduction did not increase the nutrient release rates of *Daphnia* fed the P-rich diet because infected *Daphnia* fed this diet ingested nutrients more slowly than uninfected hosts. Our results thus indicate that parasites can significantly alter the nutrient use of animal consumers, which could affect the availability of nutrients in heavily parasitized environments.

Key words: Phosphorus, Parasitism, Consumers, Nutrient recycling

Introduction

Parasites are increasingly recognized as important components of ecosystems (Kuris et al. 2008). Parasite epidemics can reduce the population size of their hosts which, in turn, alters foodwebs and ecosystems (Kohler and Wiley 1997; Wilmers et al. 2006). Even without reducing host density, parasites can affect the nature of consumer-primary producer interactions (Wood et al., 2007; Bernot and Lamberti, 2008) and modify the trophic transfer of nutrients and energy (Quested et al. 2003; Johnson et al. 2010; Grami et al. 2011). While efforts to incorporate entire parasite communities into ecosystem paradigms are proving fruitful (Lafferty et al. 2006; Amundsen et al. 2009), we still lack a basic framework for predicting how parasites affect ecosystem function through changes in host metabolism. Such a framework would be particularly useful in ecosystems where parasites can strongly mediate ecosystem processes (Thomas et al. 1999; Mouritsen and Poulin 2005).

Despite a growing recognition that parasites can sometimes infect and manipulate a substantial fraction of the consumers in an ecosystem (Kuris et al. 2008), their effects on the rate at which consumers recycle organically bound nutrients into their inorganic, bioavailable forms has received little previous study (Bernot 2013; Caceres et al. 2014). Parasitism-related changes in consumer nutrient recycling could result from disease-induced changes in the host including ingestion rate (Wood et al. 2007; Bernot & Lamberti 2008), digestive efficiency (Munger and Karasov 1989), and/or changes in tissue elemental composition (Forshay et al. 2008; Frost et al. 2008a). For example, increased feeding rates, lowered nutrient(?) assimilation rates, or the production of nutrient poor tissues should all increase rates of consumer-driven nutrient recycling (CNR) from infected individuals because, based on mass balance principles, nutrients

that the host ingests but does not assimilate and subsequently incorporate in its tissues will be released back into the environment. Such changes in nutrient recycling would alter the nutrient content and productivity of primary producers in ecosystems where consumer excretion acts as an important source of labile nutrients (Elser and Urabe 1999).

The elemental quality of a consumer's diet could alter the effect of parasites on the flow of nutrients through consumers by changing the prevalence of infected individuals in a population or the physiology of parasite-host interactions (Frost et al. 2008a; Zalewski et al. 2011). In both theoretical and field studies, high levels of nutrients and primary production increase the prevalence of parasites in populations (Lafferty and Holt 2003; Johnson et al. 2007), which indicates that high quality diets may amplify the effects of parasitism on nutrient cycling through populations. High quality diets can also increase the load and decrease the virulence of parasites in individual hosts (Frost et al. 2008b). The effect of diet quality on parasite load could be to produce changes in host nutrient recycling if, for example, the physical burden imposed by the parasite prevents the host from obtaining food (sensu Hall et al. 2007). Likewise, diet-induced changes in parasite virulence (e.g. host castration) may affect the recycling of nutrients through hosts via changes in the metabolic and nutritional demands of the host reproductive system (Wood et al. 2007; Lettini and Sukhdeo 2010). Interactive effects of diet and parasitism on host physiology could result in a strong influence of parasites on host nutrient recycling in high nutrient conditions but very little influence in nutrient poor conditions or vice versa. At present, our limited understanding of how diet quality influences the physiology of parasite-host interactions precludes formulation of more precise

predictions regarding the effect of parasites on the cycling of nutrients through consumer hosts.

Here, we test the hypothesis that parasites affect the rates at which nutrients are ingested and released by their host animal and that the nature and magnitude of this effect depends on the food quality available to the host. We tested this hypothesis using an experimental system consisting of an invertebrate host and its bacterial parasite. We used a mass-balance model, derived from first principles, to explore the physiological mechanisms that account for changes in host nutrient excretion in our experiments. In this way, we hope to show how parasitism can affect the rate of nutrient sequestration in individual hosts, and to examine the utility of adopting a mass-balance model to explore the potential mechanisms of this change. Our findings should be particularly relevant to human-altered landscapes where the acceleration of nutrient transport into biological systems (Vitousek et al. 1997) and changes in the prevalence and distribution of parasites (Telfer et al. 2005; Torchin et al. 2005) enhance the urgency of predicting feedbacks between parasitism and nutrient cycling.

Methods

Host-parasite system

The aquatic crustacean, *Daphnia magna* (or water flea), is frequently used as a model system for studies of both producer-grazer and parasite-host interactions (Sterner and Elser 2002; Ebert 2005). *D. magna* is cyclically parthenogenetic and typically only produces males when conditions are stressful. However, the clone used in our experiment (OER-3-3) was collected from ephemeral rock pools along the Baltic Sea approximately 8 years prior to our experiment and produces males regularly (Ebert, personal communication). *Pasteuria ramosa* is a bacterial endoparasite of *D. magna* that is strictly

horizontally transmitted by spores released from the cadavers of dead hosts (Ebert et al., 1996). *P. ramosa* enters its host via ingestion and resides in the hemolymph (Duneau et al. 2011). Approximately 14 days post-infection, the infected host is larger than uninfected conspecifics, has been castrated by it and appears darkly pigmented (Ebert et al. 1996). The *P. ramosa* isolate used in this experiment originated from our host clone (Ebert, personal communication).

Experimental procedure.

Neonates (< 24 hrs old) were collected from the 2nd-5th broods of *D. magna* mothers maintained under conditions of high food quantity. The first brood was not used in order to increase the number of individuals available for the experiment. The neonates were grown individually in 20 mL (days 0-6) and 40 mL (days 6-25) of *Daphnia* COMBO, a standardized freshwater medium that consists of deionized water and an assortment of dissolved nutrients that was developed to support the culturing of zooplankton (Kilham et al. 1998). To ensure *Daphnia* were not limited by food quantity, every other day individuals were fed 2 mg $C*L^{-1}$ (day 0, 2), 4 mg $C*L^{-1}$ (day 4) and 8 mg $C*L^{-1}$ (day 6-28) of a diet of the alga, *Scenedesmus obliquus*. After day 6, animals were transferred to new media every 4 days and their neonates were removed every other day. To create diets that varied in P-content, we created 3 algal monocultures: one with high, one with medium, and one with low phosphorus concentrations by diluting each culture at a rate of 0.5, 0.25, and 0.15 (the fraction of the culture replaced) per day with media enriched by 10, 0.9, and 0.4 mg $P*L^{-1}$, respectively. We then mixed algae derived from these different cultures to create 3 diets with C:P ratios of 100, 300, and 700. We verified the elemental content of the diets after mixing the algal cultures by measuring the

particulate C, N, and P of the mixed algal solution on an Elementar Vario EL III (C and N) and spectrophotometer using the ascorbic acid method (APHA 1992)after digestion in potassium persulfate (P, Table 1).

Individuals in the "infected" treatment were exposed to a high dose of *Pasteuria ramosa* spores (~75,000*(individual *Daphnia)*$^{-1}$) from day 0 until day 6 by adding a solution of homogenized infected *D. magna* to neonate tubes. To verify spore dose, we counted spores in a homogenized solution of infected *Daphnia* using a hemocytometer and then diluted this solution to achieve the appropriate dose. Individuals in the "uninfected" treatment were exposed to the same dose of homogenized uninfected *D. magna*. We assessed the effect of infection and diet treatment on *Daphnia* fecundity by recording the daily number of offspring produced by 20 individual *Daphnia* from each treatment. On day 16 of our experiment, we visually verified the infection status of every individual based on the presence of dark pigmentation and lack of eggs.

Nutrient excretion measurements

To measure rates of nutrient excretion from 18 day old *Daphnia,* we held animals without food for 60 minutes and measured the increase in dissolved nutrient concentrations in their chambers. Groups of 4 *Daphnia* fed high and intermediate quality diets and 11 *Daphnia* fed low quality diets were rinsed in N and P free COMBO 3 times, and then pipetted into 30 ml of N and P free COMBO. We measured the nutrient release rates of 8 replicate chambers per diet for infected and uninfected treatments. More *Daphnia* per chamber were required to measure the low quality treatment because these individuals were significantly smaller and release P more slowly than those raised on high quality diets. To examine the possibility that nutrients in animal growth chambers

were transferred into excretion chambers along with the *Daphnia*, baseline nutrient levels were estimated by pipetting individuals into 2 chambers per treatment and then immediately removing them. As nutrient concentrations in these chambers were detectable (average $\mu g\ P*L^{-1} = 2.0$ and $\mu g\ NH_4*L^{-1} = 9.5$), we subtracted these concentrations from the total post-excretion nutrient concentrations. We did not detect differences in these baseline nutrient levels among the different treatments. After 60 minutes, we removed all *Daphnia* from the excretion chamber and measured soluble reactive P concentrations in the excretion water using the molybdate blue-ascorbic acid method on a spectrophotometer (APHA 1992). NH_4 concentrations in the excreted water were measured using the phenol hypochlorite method (Solórzano 1969). We dried all of the *Daphnia* from the excretion experiment to obtain the total dry mass of *Daphnia* in each excretion chamber and for subsequent analysis of their C and N content on an elemental analyzer. The body P content of these *Daphnia* was measured on a spectrophotometer using the molybdate-blue-ascorbic acid method after persulfate digestion. We could not remove the parasite spores from these tissues, so, for infected individuals, our estimates of nutrient content reflect the combined nutrient contents of host and parasite.

Ingestion measurements

On day 18 of the experiment, we estimated feeding rates of *Daphnia* by calculating the change in algal density before and after groups of 3 animals from a single treatment were allowed to feed for approximately 1.2 hrs. For these measurements, three *Daphnia* were placed in ~5 ml grazing chambers (6 chambers per treatment) with 8 mg $C*L^{-1}$ of their assigned algal diet treatment. After this grazing period, *Daphnia* were

removed with a pipette, dried for 24 hours and weighed. One mL samples of the initial and grazed algal solution were preserved with Lugol's solution and the density of algal cells in each was estimated by counting them in four 12 μl aliquots on a hemocytometer. We converted the number of algal cells consumed in each chamber to moles of algal C consumed over the incubation period by multiplying the change in algal density by the amount of C per algal cell. This number was divided by the mass of the individual to yield the ingestion rate in moles of C per mg of *Daphnia* dry weight. We calculated algal carbon separately for each diet by measuring the amount of carbon in the concentrated algal diet (via an elemental analyzer) and dividing the mass of algal C initially made available in each chamber by the initial concentration of algal cells (estimated from ungrazed samples of the algal solution).

Statistical analyses

We conducted multiple 2-way ANOVAs to examine the effect of diet and infection status on *Daphnia* nutrient release rates, ingestion rates, body % nutrient content, and body nutrient ratios. We used Student's t-test for planned comparisons of these parameters for infected and uninfected individuals within each diet treatment. We adjusted the alpha level for our planned comparisons using the Dunn- Šidák correction (adjusted $\alpha = 0.017$). To satisfy the assumptions of normality and equal variances for these tests, we ran these tests on the square root of the N and P excretion rates and body C:P ratios, the log of the ingestion rate and body %P data, and the % C data raised to the 10^{th} power. Because we were unable to satisfy the assumption of normality for our fecundity data and the %P data within the 100 diet treatment, we used a Kruskal-Wallis rank sum test to determine if diet affected the fecundity of uninfected individuals, and we

used Mann-Whitney U tests (with the normal approximation to account for ties) for planned comparison of fecundity and body %P of individuals fed the 100 diet treatment.

Nutrient release model and parameterization.

We used mass-balance principles to estimate the effect of infection on the N and P release from *D. magna* and the relative influence of disease-induced changes in host ingestion, nutrient assimilation, and elemental composition on N and P release. Our two element mass-balance model was originally formulated to estimate *Daphnia* threshold elemental ratios (Sterner 1997) and has been revised and extended several times (Frost and Elser 2002; Frost et al. 2004; Rothlisberger et al. 2008). The model assumes that nutrients are incorporated into *Daphnia* tissue in tightly constrained ratios (Sterner 1997). When there is a mismatch between the N:P ratio of *Daphnia* tissues and the N:P ratio of their diet, nutrient assimilation and release become a function of the nutrient in least supply (Frost et al. 2004). To determine which nutrient this is, we compared the diet N:P ratio to an estimate of the threshold N:P ratio for *Daphnia* growth, where the animal switches from N to P limitation (L). Because L is dependent on the elemental composition of *Daphnia*, we calculated this ratio separately for each of the diet-infection combination using our observed estimates of *Daphnia* elemental composition and literature-derived assimilation efficiencies:

$$L = Q_{N:P}(A_P(A_N)^{-1}) \quad (1)$$

All parameters are defined in Table 2. For N-limited *Daphnia* ($L < f_{N:P}$), the rate of both N and P accumulation in tissues is based on the rate of N ingestion and assimilation. Therefore, release rates (N_{Ex} and P_{Ex}) can be calculated using the equations:

$$N_{Ex} = I_C f_{N:C}(1-A_N) \quad (2)$$

$$P_{Ex} = I_C(f_{P:C} - f_{N:C}A_N Q_{P:N}) \quad (3)$$

We rearranged equation 2 to estimate A_N for N-limited individuals from our observed estimates of each parameter:

$$A_N = 1-(N_{Ex}(I_C f_{N:C})^{-1}) \quad (4)$$

Conversely, the excretion rates of P-limited *Daphnia* could be calculated from the equations:

$$N_{Ex} = I_C(f_{N:C} - f_{P:C}A_P Q_{N:P}) \quad (5)$$

$$P_{Ex} = I_C f_{P:C}(1-A_p) \quad (6).$$

And we used our parameter estimates to calculate A_p using the equation:

$$A_p = 1-(P_{Ex}(I_C f_{P:C})^{-1}) \quad (7)$$

Because we had multiple, independent observations for each parameter in equations 4 and 7, we calculated A_N (or A_p) for each permutation of N_{Ex} (or P_{Ex}), I_C, and $f_{N:C}$ (or $f_{P:C}$). We used the model outputs for each infection-diet treatment combination to calculate the mean and confidence interval for A_N and A_p of each treatment. Ninety five percent confidence intervals were calculated by multiplying the standard error around the mean by 1.96.

To examine the relative influence of each physiological parameter on host nutrient release, we first calculated nutrient release rates for uninfected *Daphnia* from each diet treatment by parameterizing equations 2, 3, 5, and 6 with each permutation of values for uninfected *Daphnia* fed each diet. Then, we recalculated nutrient excretion by substituting the values from infected individuals fed each treatment for: 1) I_C alone and 2) I_C and $Q_{N:P}$ into each permutation. We examined the effect of infection on nutrient excretion for each of these simulations by calculating the proportional effect of infection

on nutrient release that was predicted from each set of parameters and then graphically comparing these predicted proportional differences with the proportional difference in nutrient excretion that we observed in our experiments. We calculated the proportional difference in nutrient excretion by subtracting our uninfected model predictions from the infected model predictions and then dividing them by the excretion rates that predicted by the uninfected model. We obtained confidence intervals around the proportional differences predicted from each permutation within each diet by multiplying the standard error of these differences by 1.96. Because these intervals were extremely small for our predicted differences in nutrient release, we do not display the standard errors in the figure.

Results

Nutrient excretion

We found a marginally significant interaction between food P and infection on N release (Table 3). In contrast, diet and infection both significantly affected the release rates of P from *Daphnia* (Fig. 2, Table 3). *Daphnia* fed P-poor diets released significantly less (~93%) P than those fed P-rich diets and, across all diet treatments, infected individuals released more P than uninfected *Daphnia*. Pairwise comparisons between infected and uninfected individuals fed each diet indicate that the effect of infection on both N and P release primarily reflected elevated nutrient release of infected *Daphnia* fed P-poor diets (Fig. 2, Table 4). While the N:P ratio of excreta increased four-fold with increases in dietary C:P ratios, this ratio was not affected by animal infection state (Table 3).

Nutrient ingestion and accumulation rates and body tissue ratios

We quantified diet and infection-induced changes in *Daphnia* food ingestion, nutrient accumulation rates and body tissue ratios to assess whether they potentially contributed to the changes we observed in nutrient release. We found a significant interaction between diet C:P and infection status on mass-specific ingestion rates (Table 3, Fig. 3). Planned comparisons indicate that this interaction was driven by significant (adjusted $\alpha = 0.017$) infection-induced reductions in ingestion rates of individuals fed the P-rich diet ($t_{(d.f.)} = -6.80_{(10)}$, $p << 0.001$), but not those fed the intermediate or P-poor diets (respectively, $t_{(d.f.)} = 2.34_{(10)}$, $p = 0.041$; $t_{(d.f.)} = -0.42_{(10)}$, $p = 0.68$). Our calculations indicate that, within an infection treatment, P was accumulated more efficiently by *Daphnia* fed P-poor diets than those fed intermediate P diets (Fig. 4). Within each diet, infection significantly reduced *Daphnia* nutrient accumulation efficiencies (Fig. 4).

P-poor diets increased body %C but reduced the %N and %P of *Daphnia* dry mass (Table 3, Fig. 5). Likewise, the C:N, C:P, and N:P ratios of *Daphnia* body tissues increased by 8%, 55%, and 42%, respectively, as diet C:P increased from 100 to 700 (Table 3, Fig. 5). Infection, alone, significantly increased the %C and N of *Daphnia* dry mass by approximately 1% and 3%, respectively, and reduced the C:N ratio of *Daphnia* dry mass by approximately2% (Table 3, Fig. 5). A significant interaction between diet C:P ratio and infection on *Daphnia* %P was driven by infection-induced decreases in the body %P of *Daphnia* fed intermediate C:P diets ($t_{(d.f.)} = -8{,}82_{(5)}$, $p < 0.001$). Likewise, interactions between diet and infection on body C:P and N:P ratios were explained by increases in these body ratios of *Daphnia* fed the P-rich diet (respectively, $t_{(d.f.)} = 9.37_{(5)}$, $p < 0.001$; $t_{(d.f.)} = 6.56_{(5)}$, p 0.0012) and intermediate P diet (respectively, $t_{(d.f.)} = 8.00_{(5)}$, $p < 0.001$; $t_{(d.f.)} = 8.03_{(5)}$, $p < 0.001$).

Infection characteristics

On day 17, 97% of individuals exposed to the spore dose possessed the characteristic red pigmentation and empty brood sac of *P. ramosa*-infected individuals. Individuals that were exposed to the spore dose but did not become infected were not used in our analyses. None of the individuals in the infected treatment for whom we recorded daily fecundity had reproduced by day 16, indicating that all of these individuals were successfully infected. The P-poor diet reduced the number of offspring produced by uninfected animals (*Kruskal-Wallis chi-squared*$_{(d.f.)}$ = 41.04$_{(2)}$, p <<0.001). Uninfected individuals fed the 700 C:P diet had produced significantly fewer offspring by day 16 than those fed the 100 and 300 C:P diets (respectively, w= 400, p << 0.001; w= 400, p < 0.001), but we were unable to detect a difference between the fecundities of individuals fed the 100 and 300 C:P diets (w = 129.5, p = 0.058).

Mass-balance model results

When we used the mass-balance model to predict the effects on nutrient excretion from the infection-induced changes in ingestion rate and elemental composition that we experimentally observed, we found that our model predictions were inconsistent with our observations. Specifically, parameterizing our model with the reduced food ingestion rates that we measured for infected individuals fed P-rich and intermediate P diets lead us to predict that infection would lower nutrient release rates (Fig. 6). However, our experimental results were inconsistent with this predicted effect of infection on the nutrient excretion rates of *Daphnia* fed these diets. Incorporating into the model the infection-induced changes in body N:P ratios that we measured did not improve the match between our model results and our experimental observations. In our mass-

balance model, the effect of infection on body N:P ratios would reverse the effects of changes in ingestion rate on P release of *Daphnia* fed P-rich diets and result in a net increase in the excretion rates of infected *Daphnia* fed this diet (Fig. 6). Conversely, the effect of infection on the body N:P ratios of *Daphni*a fed intermediate P diets were predicted by our model to further reduce the N release of infected individuals (Fig. 6). Neither of these effects were seen in our experimental data (Fig. 2). Infection-induced changes in ingestion and body N:P ratios were predicted to have very little effect on the nutrient release of individuals fed P-poor diets given that infection did not drastically alter the ingestion or body N:P ratios of these individuals. However, our experiments indicate that infection significantly increased the nutrient excretion rates of *Daphnia* fed this diet (Fig. 2). The discrepancy between the effect of infection on nutrient release that we predicted and observed appears to be the result of large reductions in the N and P accumulation rates of infected individuals; we calculated that *P. ramosa* reduced N accumulation by approximately 16% when *Daphnia* were fed P rich diets, and *P. ramosa* reduced P accumulation by 34% and 10% when *Daphnia* were fed diets with C:P ratios of 300 and 700, respectively (Fig. 4).

Discussion

Consumer populations can affect ecosystems by acting as sources and sinks for nutrients (Andersen 1997; Elser and Urabe 1999; Sterner and Elser 2002). Here we demonstrate how the rate that nutrients are recycled by an invertebrate consumer from their organically bound form to their inorganic and bioavailable form is altered by parasitism. We found interactive effects between infection and food quality on host physiological processes, which yielded reductions in the rate organically bound nutrients were ingested by *Daphnia* fed high quality diets and increases in the rate inorganic

nutrients were excreted by *Daphnia* fed poor quality diets. Our study contributes to the emerging paradigm that parasites can affect ecosystem structure and function by demonstrating how first principles can be used to investigate the mechanisms underlying parasite effects on nutrient cycling.

We examined the proximate physiological changes responsible for *P. ramosa*-induced shifts in *Daphnia* nutrient recycling with experiments and a mass-balance nutrient release model. Our model results indicate that parasite-induced changes in nutrient ingestion and assimilation exert more control over host nutrient metabolism than do changes in body nutrient ratios. Infection by *P. ramosa* decreased the ingestion rates of *Daphnia* fed high and intermediate P diets. If this were the only effect of infection on *Daphnia* physiology, nutrient release rates from diseased animals should have declined proportionally with this effect. However, consistent with previous work (Frost et al. 2008a), we also observed effects of infection on the elemental composition of *Daphnia* body tissues. Specifically, we found that *Daphnia* N:P ratios were increased by infection in the same diet treatments for which we found parasite-induced declines in ingestion rate. These changes in body stoichiometry reflect the combined nutrient content of parasite and host (because we could not separate the two) and have been accounted for by the accumulation of spores within the body of the host and the lack of N-rich eggs in castrated hosts (Frost et. al., 2008a). This altered body N:P ratio should counteract the effects of reduced ingestion rates on P release for individuals fed P rich diets and amplify the effects of reduced ingestion rates on N release for individuals fed intermediate P diets. Consequently, when infection-induced changes in ingestion rate and body N:P ratios are both incorporated into the model, decreased N and increased P release rates are

predicted from infected hosts fed P-rich diets and decreased N and P release rates are predicted from hosts fed intermediate P diets. However, our experimental data revealed no effect of infection on release rates of individuals fed P rich or intermediate P diets, which is a discrepancy that, based on mass-balance principles, must result from lower accumulation efficiencies in infected animals (Fig. 4). Nor did we find an effect of infection on the N:P ratio of *Daphnia* excreta. In light of the elevated body N:P ratios of *Daphnia* fed diets with high or intermediate P content, this result suggests that infection reduced the P assimilation efficiency of these *Daphnia* much more than their N assimilation efficiency. Unfortunately, mass-balance principles do not enable us to calculate the efficiency of non-limiting nutrient accumulation in host tissues (because nutrient release is only constrained by the nutrient that limits individual production), so we were unable to verify this possibility. Altogether, our results show that the nutrient metabolism of *Daphnia* is strongly affected by infection, but how these physiological changes affect nutrient recycling depends on the stoichiometric quality of the host's diet: because nutrients were ingested more slowly by infected *Daphnia* fed high and intermediate quality diets, these *Daphnia* recycled nutrients at the same rate as their uninfected conspecifics even though *P. ramosa* increased the flow of nutrients from *Daphnia* via reductions in host nutrient accumulation efficiencies.

Why then did *P. ramosa* reduce the ingestion rates of *Daphnia* fed some diets, but not others? One explanation for the effect of *P. ramosa* on *Daphnia* ingestion rates is that castration altered the energetic and material demands of hosts (Wood et al. 2007; Lettini and Sukhdeo 2010). This explanation is consistent with our observation that P-poor diets caused significant declines in the fecundity of uninfected *Daphnia.* Likewise, the size-

specific reproductive investment rate (in terms of C) for uninfected *Daphnia* fed P poor diets is smaller than that for uninfected *Daphnia* fed P rich diets (Urabe and Sterner 2001). As a result of this effect of diet quality on the fecundities of uninfected individuals, low quality diets also decrease the effect of sterilization on host physiology (i.e. the relative difference between the reproductive status of uninfected and infected individuals, Frost et al. 2008b). Given these links between reproduction and diet quality, we would expect infection-induced changes in ingestion rate to be amplified by high quality diets. We observed this pattern in our results. In theory, other castrating parasites that reduce host nutrient assimilation rates might induce similar patterns in host ingestion and nutrient release rates in response to a variety of food quality gradients. For example, the relationship between sterilization and diet quality may explain observations that castrating trematodes exert a stronger effect on host nutrient excretion when their snail hosts are fed diets with high N:P ratios (Bernot 2013). However, the limited extent of the literature on parasite-induced changes in host nutrient release precludes our ability to evaluate this hypothesis in additional parasite systems.

The diet-specific changes in *Daphnia* nutrient use that we observed raise the possibility that infection by *P. ramosa* may indirectly stimulate primary production via changes in *Daphnia* grazing pressure and nutrient recycling. We found that infection by *P. ramosa* altered *D. magna* ingestion rates when *Daphnia* were fed high quality diets and, when *Daphnia* were fed P-poor diets, infected *Daphnia* released nutrients more quickly than their uninfected conspecifics. *Daphnia* often thrive in P-rich ecosystems and can be constrained by poor food quality in in P-poor ecosystems (Elser et al. 2001; Urabe et al. 2002). When *Daphnia* abundance is high, primary production can be constrained by

grazing pressure; conversely, when *Daphnia* biomass is low, primary production is often nutrient limited due to low levels of nutrient recycling (Carpenter and Kitchell 1984; Bergquist and Carpenter 1986). Our observations do not take into account the effect of *P. ramosa* on host density (which can be devastating, e.g. Capaul and Ebert 2003) and solely reflect the response of one (susceptible) host clone to a single parasite isolate. Given the high level of virulence in this parasite-host system, this individual-level response may be an extreme case of parasite-induced change in host nutrient use. Furthermore, the reduced spore loads of *Daphnia* fed P-poor diets indicate that *P. ramosa* prevalence may decline with declining diet quality, making our observations of *Daphnia* fed low P diets less relevant in natural conditions. Nevertheless, *P. ramosa* is capable of maintaining prevalences near 100% for multiple weeks in natural ecosystems (Duncan et al. 2006). If the effects we observed of *P. ramosa* on individual host ingestion and nutrient release rates translate into population-wide changes, primary production may be increased by infection-induced reductions in grazing pressure in high P ecosystems and by increased nutrient release in low P ecosystems.

Understanding feedbacks between parasitism and nutrient cycling is an important challenge in complex, human-altered landscapes. Parasitism may also alter ecosystem nutrient availability via many pathways that we did not explore (e.g. via the production of transmission stages that serve as dietary supplements for other organisms (Grami et al., 2011) and behavioral modifications that increase predation rates on infected individuals (Johnson et al., 2010). In addition to diet, many factors (e.g., temperature, maternal nutrition and exposure to the parasite) may alter the physiological responses of *D. magna* to infection by *P. ramosa* (Vale & Little 2009; Frost *et al.* 2010; Schlotz, Ebert & Martin-

Creuzburg 2013). Each of these factors could further alter host nutrient release through changes in host physiology, and this natural variation highlights the need for an improved understanding of the proximate and ultimate drivers of the symptoms of disease. Our study demonstrates the utility of applying first principles to explore hypotheses regarding these drivers in a well-studied parasite-host system. Moreover, our results indicate that the manipulation of parasite abundance and distributions (both intentional and accidental) have the potential to mitigate or exacerbate the negative effects of nutrient loading in aquatic ecosystems.

Acknowledgments

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Tables and Figures

Table 1: Mean$_{(SD)}$ for C:N, C:P, N:P and %C, P, and N of algal diets (identified by their desired C:P ratio). Values represent the post-mixing composition of diets fed to animals throughout the experiment.

Diet	C:N	C:P	N:P	%C	%N	%P
100	6.4$_{(0.3)}$	86.7$_{(6.9)}$	12.7$_{(1.6)}$	46.0$_{(0.5)}$	8.5$_{(0.4)}$	1.5$_{(0.2)}$
300	7.2$_{(0.6)}$	277.7$_{(35.8)}$	37.3$_{(6.3)}$	48.1$_{(0.6)}$	7.9$_{(0.7)}$	0.5$_{(0.1)}$
700	8.7$_{(0.6)}$	617.2$_{(76.2)}$	68.8$_{(10.3)}$	48.5$_{(0.6)}$	6.6$_{(0.4)}$	0.2$_{(0.03)}$

Table 2: Parameter abbreviations and units for the mass-balance nutrient release model.

Parameter	***Abbreviation***	***Unit***
N excretion rate	N_{Ex}	$\mu mol\ N\ x\ \mu mol\ C_{Daphnia}^{-1}\ x\ day^{-1}$
P excretion rate	P_{Ex}	$\mu mol\ P\ x\ \mu mol\ C_{Daphnia}^{-1}\ x\ day^{-1}$
Ingestion rate	I_C	$\mu mol\ C_{Algae}\ x\ \mu mol\ C_{Daphnia}^{-1}\ x\ day^{-1}$
Food P:C	$F_{P:C}$	$\mu mol\ N: \mu mol\ C$
Food N:C	$F_{N:C}$	$\mu mol\ P: \mu mol\ C$
C accumulation efficiency	A_C	*None*
N accumulation efficiency	A_N	*None*
P accumulation efficiency	A_P	*None*
N:P body content	$Q_{N:P}$	$\mu mol\ N\ x\ \mu mol\ P^{-1}$

Table 3: Results of 2-way ANOVAs for Daphnia *physiological parameters by diet and infection status. Significant values are in bold and marginal values are starred.*

		Food C:P ratio	**Infection**	**Food x Infection**
NH_4 Release	F-ratio	$0.044_{(2,42)}$	$1.92_{(1,42)}$	$2.59_{(2,42)}$
	P-value	0.96	0.17	0.088*
SRP Release	F-ratio	$73.124_{(2,44)}$	$7.935_{(1,44)}$	$1.905_{(2,44)}$
	P-value	**$<2x10^{-16}$**	**0.0072**	0.162
N:P Release	F-ratio	$107.41_{(2, 42)}$	$1.31_{(1,42)}$	$6.80x10^{-3}$ $_{(2,42)}$
	P-value	**$<2x10^{-16}$**	2.59	0.99
Ingestion rate	F-ratio	69.09 $_{(2,32)}$	16.91 $_{(1,32)}$	$5.89_{(2,32)}$
	P-value	**$2.44 x10^{-12}$**	**$2.55 x10^{-4}$**	**0.0070**
Body %C	F-ratio	$7.36_{(2,44)}$	$5.08_{(1,44)}$	$2.438_{(2,44)}$
	P-value	**$1.74x10^{-3}$**	**0.029**	0.010
Body %N	F-ratio	$22.98_{(2,44)}$	$20.06_{(1,44)}$	$0.93_{(2,44)}$
	P-value	**$1.47x10^{-7}$**	**$5.28x10^{-5}$**	0.40
Body %P	F-ratio	$82.72_{(2,17)}$	$22.39_{(1,17)}$	$34.86_{(2,17)}$
	P-value	**$1.73x10^{-9}$**	**$1.93x10^{-4}$**	**$2.30x10^{-6}$**
Body C:N	F-ratio	$68.98_{(2,44)}$	$12.61_{(1,44)}$	$0.33_{(2,44)}$
	P-value	**$2.73x10^{-14}$**	**$9.29x10^{-4}$**	0.72
Body C:P	F-ratio	$114.81_{(2,17)}$	$27.43_{(1,17)}$	$14.71_{(2,17)}$
	P-value	**$1.34x10^{-10}$**	**$6.69x10^{-5}$**	**$2.91x10^{-4}$**
Body N:P	F-ratio	$57.59_{(2,17)}$	$22.19_{(1,17)}$	$10.46_{(2, 17)}$
	P-value	**$2.69x10^{-8}$**	**$2.02 x10^{-4}$**	**$1.75x10^{-3}$**

Table 4: Results of Student's two sample t-tests comparing nutrient release rates of infected vs uninfected Daphnia *within each diet treatment. Significant values (after Dunn-Šidák correction: α = 0.017) are in bold.*

Nutrient	Diet C:P ratio	$t_{(d.f.)}$	*p*
N	100	$-0.95_{(14)}$	0.36
	300	$0.55_{(12)}$	0.59
	700	$-2.96_{(14)}$	**0.010**
P	100	$-0.17_{(14)}$	0.87
	300	$1.76_{(12)}$	0.10
	700	$2.73_{(14)}$	**0.016**

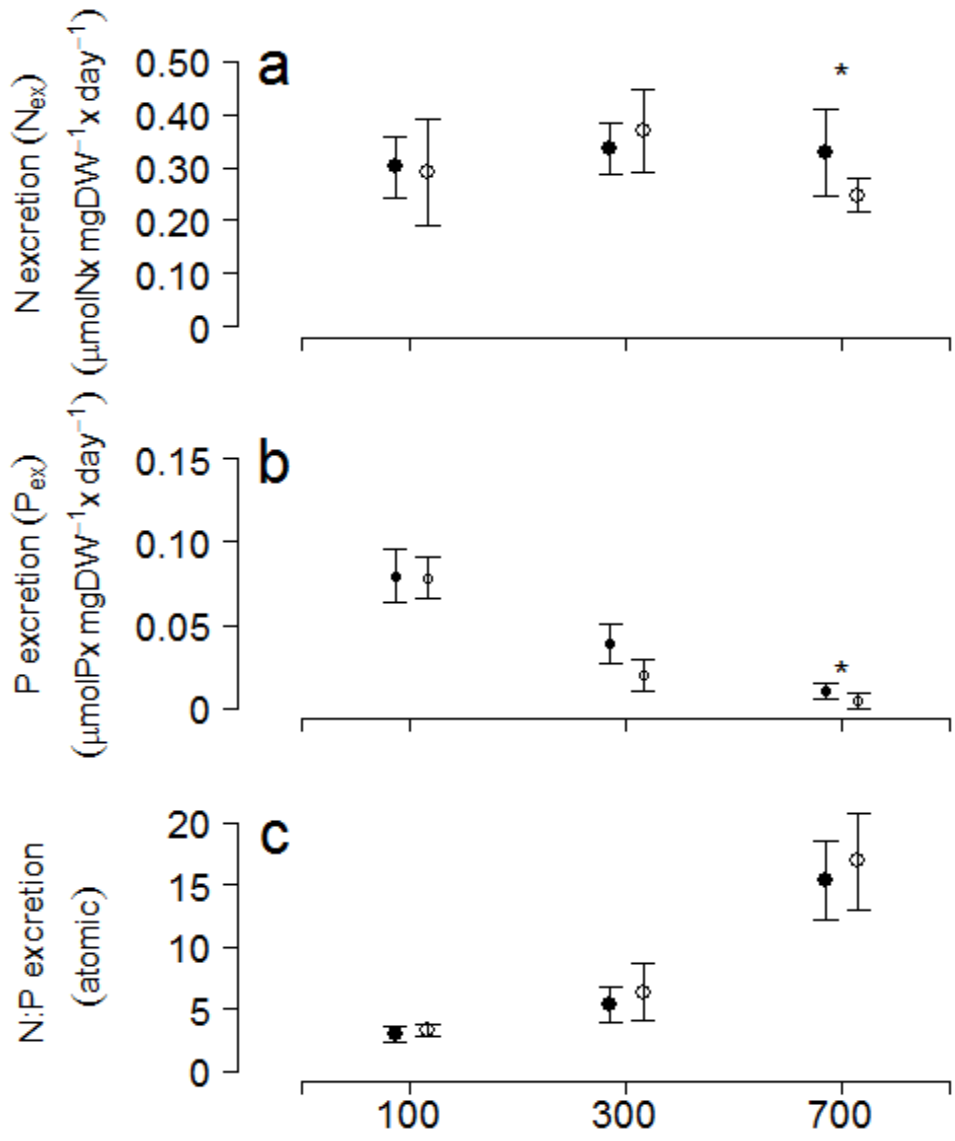

Figure 2: N (µmol N(mgDW*day)$^{-1}$) and P (µmol P*(mgDW*day)$^{-1}$) excretion rates and molar N:P release ratios for* Pasteuria ramosa*-infected (filled circles) and uninfected (open circles)* Daphnia magna *provided algal food of different C:P ratios (n = 46). Planned t-tests revealed significant differences (adjusted α = 0.017) in N and P release rates between infected and uninfected* Daphnia *fed the C:P = 700 diet treatment (N release:* $t_{(d.f.)} = -2.96_{(14)}$, $p = 0.010$; *P release:* $t_{(d.f.)} = 2.73_{(14)}$, $p = 0.016$). *Data are shown as mean ± 95% confidence intervals*

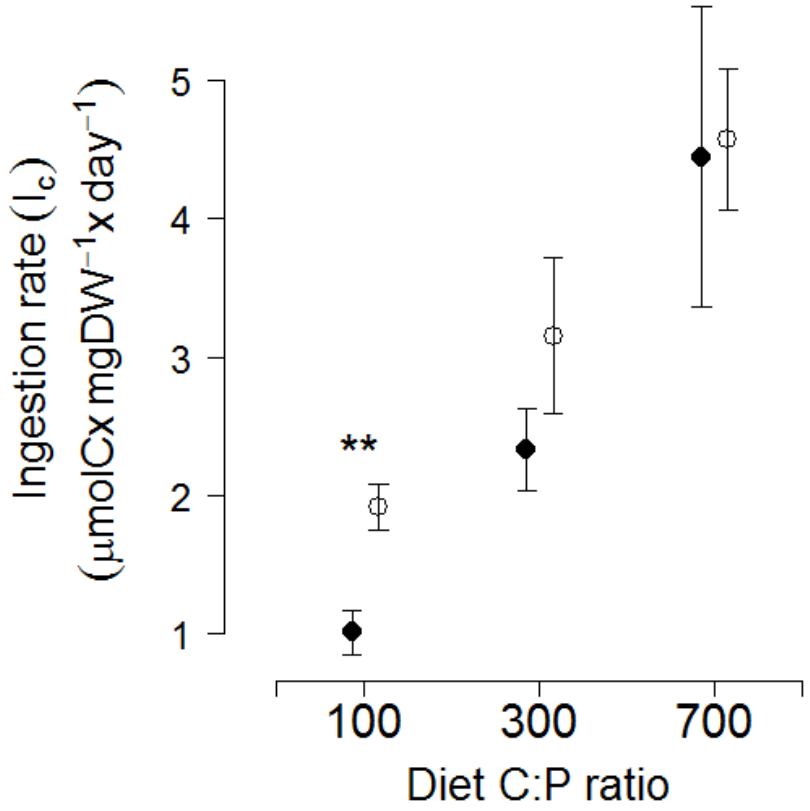

Figure 3: Ingestion rates (µmol C(mgDW*day)$^{-1}$) for* Pasteuria*-infected (filled circles) and uninfected (open circles)* Daphnia *provided algal food of different C:P ratios (n = 36). An interaction between diet quality and infection status was driven by significant (adjusted α = 0.017) infection-induced reductions in the ingestion rates of individuals fed the P-rich diet (*$t_{(d.f.)} = -6.80_{(10)}$, $p << 0.001$*), but not those fed the intermediate or P-poor diets (respectively,* $t_{(d.f.)} = 2.34_{(10)}$, $p = 0.041$; $t_{(d.f.)} = -0.42_{(10)}$, $p = 0.68$*). Data are shown as mean ± 95% confidence intervals*

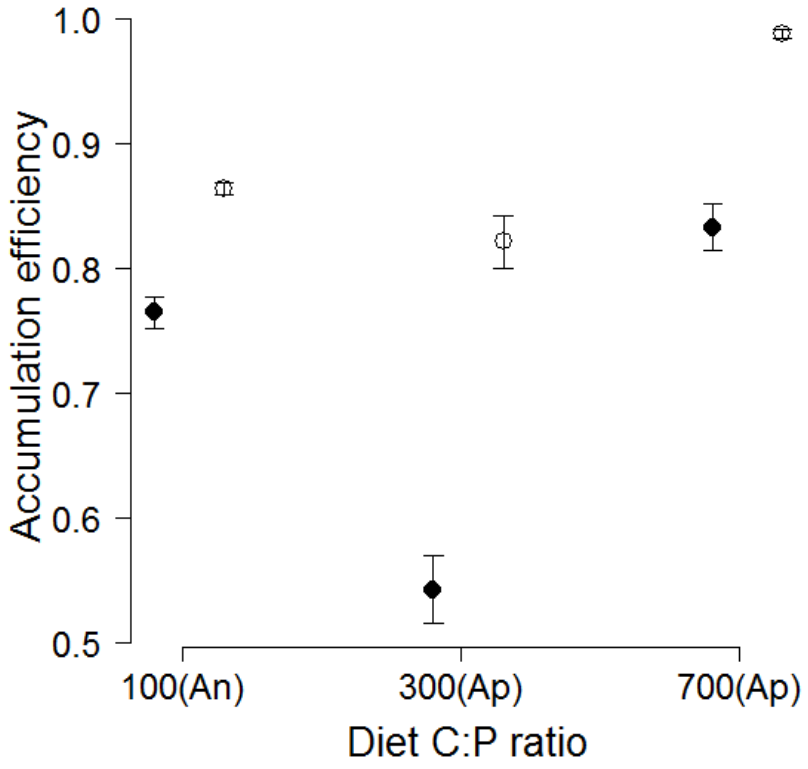

Figure 4: Calculated accumulation efficiencies for the limiting nutrient of P. ramosa *infected (filled circles) and uninfected (open circles)* Daphnia *fed diets of varying C:P ratios. The limiting nutrient for which accumulation rates are calculated is indicated in parentheses next to the diet C:P ratios (e.g.* A_N *or* A_P*). Shown are 95% confidence intervals calculated as SE x 1.96*

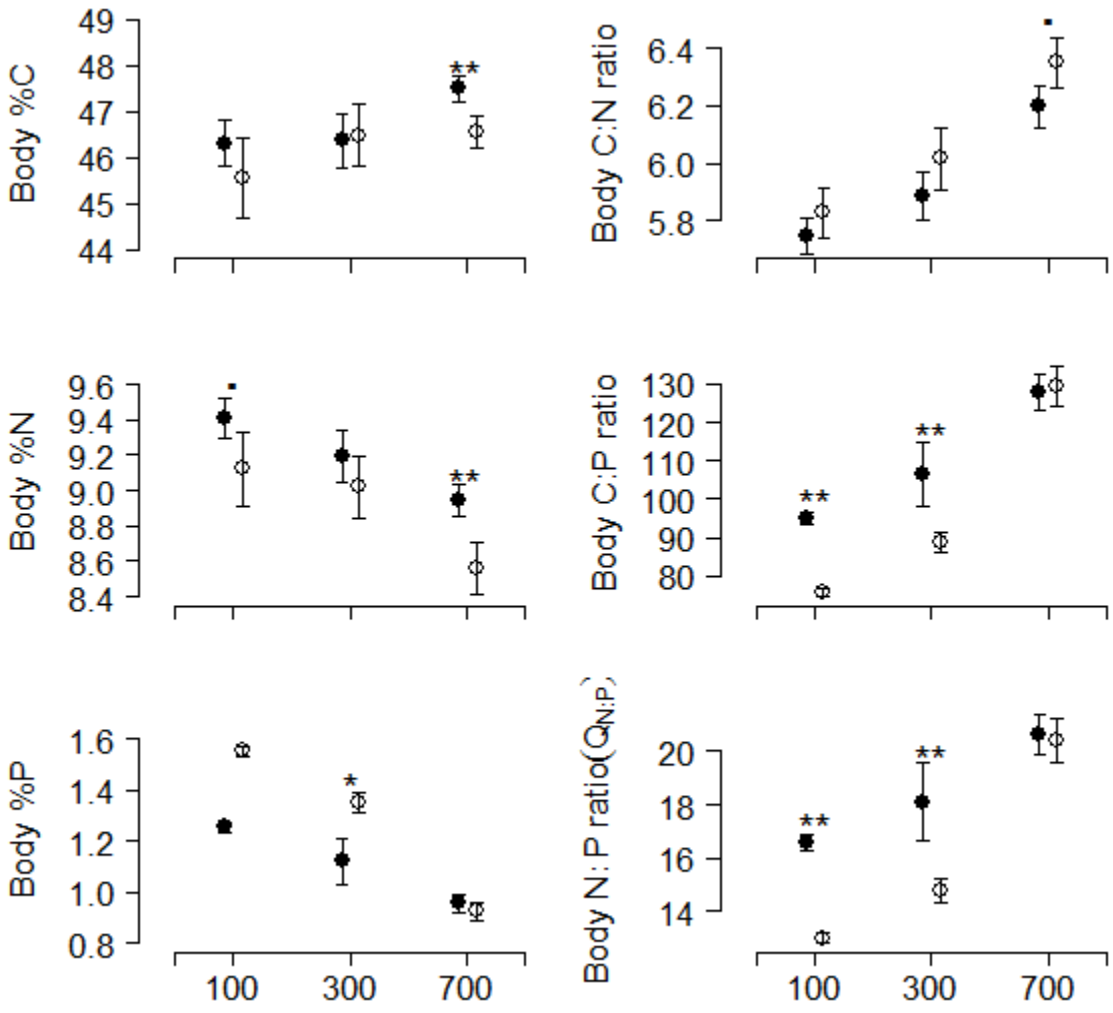

Figure 5: Body % C, N, and P and molar C:N:P ratios for Pasteuria*-infected (filled circles) and uninfected (open circles)* Daphnia *provided algal food of different C:P ratios (n = 21). We did not separate parasite and host tissues, so the nutrient content of infected* Daphnia *represents the combined nutrient contents of both parasite and host. P-poor diets increased body %C but reduced the %N and %P of* Daphnia *dry mass (Table 3). The C:N, C:P, and N:P ratios of* Daphnia *body tissues were elevated by high diet C:P ratios (Table 3). Infection increased the %C and N of* Daphnia *dry mass and reduced the C:N ratio of* Daphnia *dry mass (Table 3). A significant interaction between diet C:P ratio*

and infection on Daphnia *%P was driven by infection-induced decreases in the body %P of* Daphnia *fed intermediate C:P diets (*$t_{(d.f.)} = -8{,}82_{(5)}$, $p < 0.001$*). Likewise, interactions between diet and infection on body C:P and N:P ratios were explained by significant (adjusted* $\alpha = 0.017$*) increases in these body ratios of* Daphnia *fed the P-rich diet (respectively,* $t_{(d.f.)} = 9.37_{(5)}$, $p < 0.001$; $t_{(d.f.)} = 6.56_{(5)}$, p *0.0012) and intermediate P diet (respectively,* $t_{(d.f.)} = 8.00_{(5)}$, $p < 0.001$; $t_{(d.f.)} = 8.03_{(5)}$, $p < 0.001$*). Data are shown as mean ± 95% confidence intervals.*

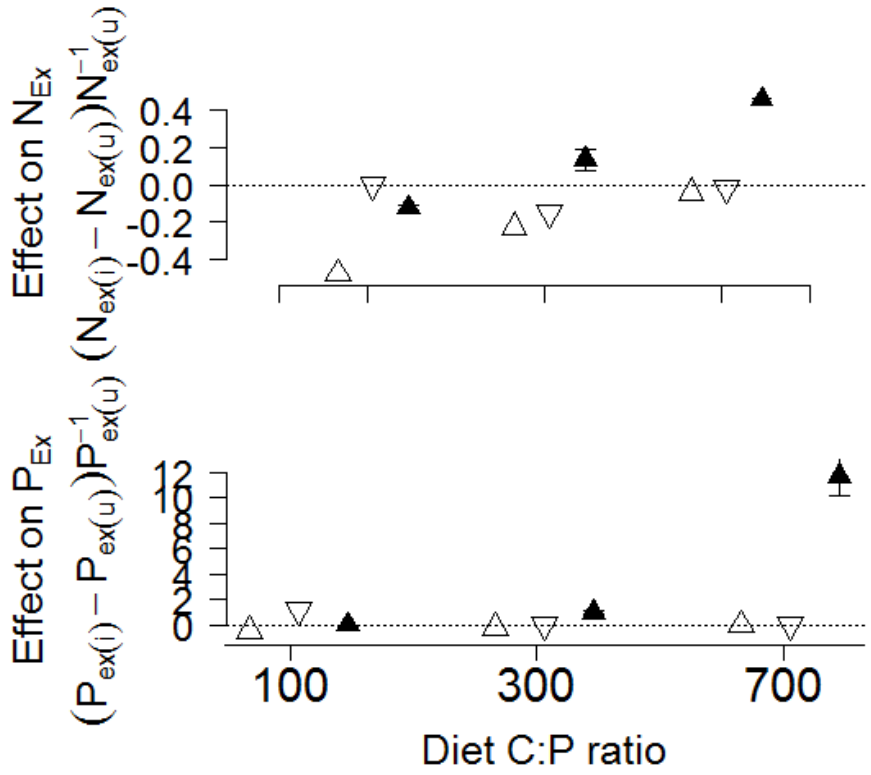

Figure 6: Proportional effect of P. ramosa *infection on* D. magna *N (A) and P (B) excretion rates as a function of* Daphnia *diet C:P ratio. We calculated these effects by subtracting the nutrient excretion rates that were predicted from parameters measured on uninfected individuals from the nutrient excretion rates that were predicted from parameters measured on uninfected individuals and then deviding this predicted difference in excretion by the predicted excretion rates of uninfected individuals. Shown are predictions based on the observed effect of* P. ramosa *on* Daphnia *ingestion rate (open, right-side up triangles), and the effects of both ingestion rate and body N:P ratio (open, upside down triangles). Observed changes in nutrient excretion rate (mean ± 95% confidence intervals) are shown in black for comparison.*

Chapter 3 - From host exploitation to excretion: Parasite type affects host nutrient recycling

Charlotte F. Narr, Paul, C. Frost

Abstract

Parasites can change the nutrient balance of their animal hosts by altering rates of ingestion, assimilation efficiencies, and biomass production. Such changes could alter the availability of nutrients in the environment by changing consumer-driven nutrient recycling. Because the effects of parasitism on host nutrient use are mediated by host physiology, they may depend on the type of parasitic infection and the diet quality of the host. We tested the hypothesis that parasite type and diet quality (i.e., food C:P ratio) affect nutrient release rates by measuring these rates in uninfected *Daphnia* and conspecifics infected by two different species of microparasites after they were fed algae with a gradient of C:P ratios. We found that infection type affected host N and P release rates, but only when *Daphnia* were fed a high-P diet. We also found that diet C:P ratio affected *Daphnia* P release rates, but evidence for this effect was stronger in some infection treatments than others. To improve our understanding of the changes in host physiology that were associated with these diverse effects, we further examined whether two separate aspects of host exploitation (i.e., parasite-induced reductions in host fecundity and parasite load) could account for variation in *Daphnia* nutrient use caused by our infection and diet treatments. These comparisons showed that variation in N release rates and body C:N ratios was best accounted for by variation in *Daphnia* fecundity (relative to variation in *Daphnia* infection status, diet quality, and spore load) regardless of whether we compared individuals across the infection or dietary treatments.

In addition, variation in the P excretion and ingestion rates of *Daphnia* fed the same diet was described better by fecundity than infection status or parasite load. Our results suggest that the potential for feedbacks between host nutrient use and parasitism varies in response to parasite type as well as host diet quality. Furthermore, parasite virulence may be a valuable indicator of the magnitude of some parasite-induced changes in host nutrient use.

Keywords: Fecundity, *Daphnia*, Nutrient recycling, Parasitism, Host exploitation

Introduction

Parasites are increasingly recognized for their potential to substantially affect foodwebs and ecosystem processes (Lafferty et al. 2006; Kuris et al. 2008; Grami et al. 2011). These effects can be mediated by parasite-induced changes in host population dynamics and/or changes in the physiology of individual hosts (Hatcher and Dunn 2011). In the latter case, the functional role of a parasite within an ecosystem is defined by its prevalence, the symptoms of its host, and the ecological role of host populations. High prevalence and strong disease effects have been documented for a range of consumer taxa including many that are known for their contributions to nutrient cycling (Duncan et al. 2006; Wood et al. 2007; Bernot and Lamberti 2008). Recent evidence indicates that parasitism can affect the rate at which consumers recycle nutrients (Bernot 2013; Narr and Frost, 2015), but our understanding of these parasite-induced changes in nutrient release remains limited to relatively few host-parasite pairs.

Hosts exploited by different types of parasites might recycle nutrients at different rates if they exhibit different symptoms or levels of virulence. One way that we can distinguish (albeit roughly) between the symptoms of hosts based on characteristics of their parasites is to consider the parasite's exploitation strategy. For example, many parasites of invertebrates reduce host fecundity by their diversion of energetic and material resources (Lafferty and Kuris 2009). This strategy can drastically change host physiology including, at its extreme, castration, and may alter the energetic demands, ingestion rates, and elemental composition of hosts (Wood et al. 2007; Frost et al. 2008a). Parasite exploitation strategy can also influence parasite load, which is another potential source of variation in nutrient release among hosts infected by different parasite types. From a physiological perspective, this relationship between parasite load and nutrient

release may be mediated by declines in host ingestion rate (Hall & Sivars 2007) or shifts in body nutrient ratios (Forshay et al. 2008; Frost et al. 2008a) caused by the accumulation of parasites within the host. Parasite load could also signify physiological stress/damage and thereby be associated with changes in nutrient demand.

Each of these changes could cause individuals infected by different parasite species to recycle nutrients at different rates than uninfected individuals. However, because the intensity of symptoms and level of virulence experienced by hosts is, in large part, context dependent (Scholthof 2007), it may be useful to consider these symptoms across environmental gradients that are relevant to nutrient recycling. The availability of nutrients in an individual's diet influences consumer-driven nutrient recycling directly and indirectly via many of the same pathways potentially altered by parasitic infections (e.g., via changes in nutrient assimilation, ingestion, and incorporation rates; Andersen 1997; Frost, Xenopoulos & Larson 2004). Such effects of host diet also appear to extend to parasite-host interactions themselves via many pathways, including effects on parasite virulence (Vale et al. 2011), parasite load (Frost et al. 2008b), and inter-specific parasite interactions (Lange et al. 2014).

Here we compared two *D. magna*-parasite systems to test the hypothesis that parasite type affects host nutrient recycling. We expected to observe differences between the nutrient recycling rates of uninfected *Daphnia*, those infected with the bacterial parasite *Pasteuria ramosa*, and those infected with the microsporidian *Hamiltosporidium tvaerminnensis*. The exploitation strategies of *P. ramosa* and *H. tvaerminnensis* are different: *P. ramosa* is transmitted horizontally after castrating its host, while *H. tvaerminnensis* is transmitted both vertically and horizontally and only rarely castrates its

host. Therefore, we expected that *P. ramosa* would have a stronger effect on host nutrient recycling rates than the less virulent *H. tvaerminnensis*. We further examined the relationship between host nutrient release rates and parasite load and parasite-induced reductions in host fecundity. Because of the potentially tight relationship between both of these aspects of host exploitation and host physiology, we expected that both parasite load and *Daphnia* fecundity would be associated with gradients in *Daphnia* N and P release as well as gradients in other aspects of host physiology that determine nutrient excretion rates (e.g. ingestion rates and body elemental composition). The relationship between these more general aspects of host exploitation and changes in host nutrient use may prove useful in understanding parasite-host systems beyond those examined in this study.

We compared the ability of each of these three variables (i.e. infection status, fecundity, and spore load) to account for variation in *Daphnia* nutrient release rates, nutrient ingestion rates, and body nutrient composition. In addition, we conducted the same comparisons on uninfected and infected *Daphnia* fed diets with high, intermediate, and low levels of phosphorus. This allowed us to assess whether differences in nutrient use between daphnids infected with the two types of parasites varied across a nutritional gradient. Finally, we compared the ability of diet, fecundity, and spore load to account for variation in nutrient use within an infection type. This comparison assessed the generality of the relationships we observed by exploring the possibility that a diet-induced gradient in fecundity or spore load can describe the same changes in *Daphnia* nutrient use as a parasite-induced gradient in fecundity or spore load.

Methods

Study system

The water flea, *Daphnia magna*, is a cyclically parthenogenetic aquatic crustacean that is frequently used in studies of producer-grazer and parasite-host interactions (Sterner and Elser 2002; Ebert 2005). The clone used in our experiment (OER-3-3) was collected from ephemeral rock pools along the Baltic Sea approximately 8 years prior to our experiment (Ebert, personal communication). *Pasteuria ramosa* is a bacterial endoparasite of *D. magna* that is horizontally transmitted in the form of spores released from the cadavers of dead hosts (Ebert et al. 1996). *P. ramosa* enters its host via ingestion and resides within the hemolymph (Duneau et al. 2011). Approximately 14 days post-infection, the infected animal is larger than uninfected conspecifics, has been castrated and appears darkly pigmented (Ebert et al., 1996). *Hamiltosporidium tvaerminnensis* is a microsporidium that is transmitted both horizontally and vertically and resides within the adipose tissue, ovaries, and hypodermis of its *D. magna* host (Haag et al. 2011). *H. tvaerminnens*is typically reduces host fecundity by approximately 20%, but rarely castrates it (Bieger and Ebert 2009). Both of the parasite isolates used in this experiment were originally collected inside our host clone (Ebert, personal communication).

Experimental procedure.

Neonates (< 24 hrs old) were collected from the 3rd-5th broods of *Daphnia magna* mothers maintained under conditions of high food quality and quantity. They were grown in groups of 8 in 160 mL (days 0-6) and 320 mL (days 6-25) of *Daphnia* COMBO (Kilham et al. 1998). Every other day, *Daphnia* were fed 2 mg C/L (days 0 and 2), 4 mg C/L (day 4) and 8 mg C/L (days 6-18) of a diet of the alga, *Scenedesmus obliquus* per individual. After day 6, animals were transferred to new media every 4 days and their neonates were removed and counted every other day. To create diets that varied

in P-content, we manipulated the %P of continuously grown cultures of *S. obliquus* by spiking the algal medium with known amounts of NaH_2PO_4. We then mixed algae derived from these different cultures to create 3 diets with C:P ratios of approximately 100, 300, and 700. We verified the elemental content of the diets after mixing the algal cultures (Table 5).

Neonates with the vertically transmitted *H. tvaerminnensis* infection were obtained from the 3-5th broods of *D. magna* infected with the parasite. Two generations prior to the experiment, these brood mothers were taken as neonates from our stock uninfected brood moms and horizontally infected by exposure to the crushed bodies of *H. tvaerminnensis*-infected *D. magna*. Transmission of this parasite to offspring is nearly 100%. Individuals in the *P. ramosa* treatment were exposed to a high dose of spores (~75,000*individual^{-1}) from day 0 until day 6 by adding a solution of homogenized infected *D. magna* to neonate jars. Individuals in the uninfected and *H. tvaerminnensis* infection treatments were exposed to the same dose of homogenized uninfected *D. magna*. Infection status was verified visually (for *P. ramosa*, this was verified by the presence of dark pigmentation and the lack of eggs, for *H. tvaerminnensis*, this was verified by the presence of opaque white material in the brood cavity) on day 18.

Nutrient excretion measurements.

We measured rates of nutrient excretion from 20 day old *Daphnia* because, at this age, all infected individuals exhibited the symptoms of disease that we expected to affect nutrient release. To do this, we held animals without food for 60 minutes and measured the increase in dissolved nutrient concentrations in their chambers over that time. We measured excretion on groups of *Daphnia* taken from one (or, for low quality diets, 2)

experimental units. In each excretion chamber, we placed a minimum of 4 and maximum of 6 *Daphnia* fed the P rich diet, 7 *Daphnia* fed the intermediate P diet, and a minimum of 9 and maximum of 11 *Daphnia* fed the P-poor diet. More individuals were used from the lower P diet treatments in order to maintain a relatively constant biomass of *Daphnia* in each chamber regardless of diet treatment. *Daphnia* were rinsed in N and P free COMBO 3 times, and then pipetted into approximately 11.5 ml of N and P free COMBO per mg of *Daphnia* dry mass. Dry mass was estimated based on the average dry mass of individuals fed the same diet in a previous experiment (Chapter 2). To examine the possibility that nutrients in animal growth chambers were transferred into excretion chambers along with the *Daphnia*, baseline nutrient levels were estimated by pipetting individuals into 2 chambers per treatment and then immediately removing them. As nutrient concentrations in these chambers were detectable (average $\mu g\ P*L^{-1} = 1.1$ and $\mu g\ NH_4*L^{-1} = 20.7$), we subtracted these concentrations from the total post-excretion nutrient concentrations. After 60 minutes, we removed all *Daphnia* from the excretion chamber and measured soluble reactive P concentrations in the excretion water using the molybdate blue-ascorbic acid method on a spectrophotometer (APHA 1992). Ammonium concentrations in the excreted water were measured using the phenol hypochlorite method (Solórzano 1969). We dried all of the *Daphnia* used in our excretion experiment to obtain the total dry mass of *Daphnia* in each chamber and for subsequent analysis of their C and N content on an elemental analyzer (Vario EL; Elemental). The body P content of these *Daphnia* was measured on a spectrophotometer using the molybdate blue-ascorbic acid method after persulfate digestion.

Ingestion Measurements.

Prior to the excretion experiment, we estimated the feeding rates of the same groups of *Daphnia* by calculating the change in algal density before and after groups of animals from a single treatment were fed for approximately 1 hr. For these measurements, *Daphnia* were placed in a chamber with 0.83 ml of COMBO per mg of *Daphnia* dry mass and given 8 mg $C*L^{-1}$ of the algal diet on which they had been raised. After this grazing period, *Daphnia* were removed with a pipette and placed in their excretion chambers. One ml samples of the initial and grazed algal solution were preserved with Lugol's solution and the density of algal cells was estimated by counting four 12 μl aliquots on a hemocytometer. We measured the C content of a subsample of the ungrazed algal mixture on an elemental analyzer in order to determine the mass of C in each algal cell. This mass was used to convert the change in algal biomass into C specific ingestion rates.

Statistical Analyses

To determine if infection type and diet affected *Daphnia* nutrient release, we conducted nested ANOVAs for N and P release rates where infection was nested within diet treatment and then another set of nested ANOVAs for the same rates where diet was nested within infection type. Nesting within diet enabled us to determine if the effects of infection were consistent across diet treatments, while nesting within infection enabled us to determine if the effects of diet C:P ratio were consistent across infection types. If an ANOVA indicated significant effects of either of these treatments, we conducted planned t-tests to determine which treatments were driving the effect. We used the Dunn-Šidák correction to adjust our alpha for each of the 9 t-test comparisons we conducted for each nutrient use parameter. This adjusted alpha was 0.005.

To improve our understanding of the effects of infection and diet quality on nutrient release, we further examined whether *Daphnia* fecundity or parasite load could be used to describe the variation in *Daphnia* nutrient use caused by our treatments. To do so, we compared our ability to describe variation in nutrient release rates of *Daphnia* using the treatment that caused the variation (i.e. infection type or diet C:P ratio), fecundity, and spore load. Mass-balance principles require that changes in nutrient release must result from changes in nutrient ingestion rate, assimilation rate, or tissue content. Therefore, to develop a better understanding of the relationship between these descriptive variables and nutrient release, we also compared the ability of each of these 4 models to describe variation in ingestion rates and body elemental content. As in the nested ANOVA described above, we nested each of our predictor variables within diet treatment to examine variation caused by our infection treatment, and we nested each of our predictor variables within infection type to examine variation caused by our diet treatment.

Because our fecundity and spore load data were on very different scales, we standardized these data to a mean of 0 and SD of 0.5 (Gelman 2008) prior to model selection to facilitate our ability to compare their relationships with the dependent variables. Uninfected individuals were excluded from models in which spore load was included as a variable in order to minimize the likelihood that large variations in uninfected individuals (with a spore load of zero) created spurious significant relationships. This exclusion reduced our sample size from 52 to 32 and made our estimate of the effect of spore load more conservative by reducing the potential spread of

the parameter. We present coefficients for models with delta AICc < 4 because we considered these models to have the most support.

Results

Infection and diet effects on nutrient release.

When we compared *Daphnia* N release rates across infection types, we found that they responded to infection (nested within diet treatment). Planned t-tests indicated that *P. ramosa* infected individuals released N more quickly than uninfected individuals when *Daphnia* were fed the high P diet treatment (Fig 7). Comparison of *Daphnia* N release rates across diets indicated that these rates were only marginally affected by diet C:P ratio (nested within infection treatment).

Like N release rates, *Daphnia* P release rates also responded to infection (nested within diet treatment), and this effect was significant when *Daphnia* were fed the high P diet treatment. Planned t-tests indicated that *P. ramosa*-infected individuals released P faster than both uninfected individuals and those infected with *H. tvaerminnensis* in this diet treatment (Fig 7). P release responded to diet C:P (nested within infection treatment) as well: in all infection types, P release was elevated when *Daphnia* were fed a C:P ratio of 100 relative to those fed a C:P ratio of 400 (Fig 7). *P. ramosa*-infected individuals also released P faster when they were fed a diet C:P of 100 relative to 700.

Describing infection and diet effects on nutrient release

To improve our understanding of these diverse effects of infection and diet quality on nutrient release, we further examined whether *Daphnia* fecundity or parasite load could be used to describe the variation in *Daphnia* nutrient use caused by our treatments. Parasitism caused significant declines in host fecundity ($f = 43.11_{(8, 43)}$; $p < 2.2\text{x}10^{-16}$, Fig 8). We found that variation in N release rates of *Daphnia* was described better by this

variation in fecundity (nested within diet treatment, shown as 'Fecund in diet' in Table 6) than infection status (nested in diet, shown as 'Infection in diet' in Table 6), spore load (nested in diet, not shown in Table 6), or the null model (shown as 'Null' in Table 6). A 55% increase in fecundity was associated with a decline in C-specific N release of 6.6 $x10^{-3}$ mol per day per mg C for animals fed the 100 C:P diet treatment and 6.4 $x10^{-3}$ mol per day per mg C for animals fed the 300 C:P diet (Table 8, Fig 9). When we examined variation in N release *within* infection type (i.e. variation caused by our diet treatment), we found that fecundity was also the best at describing this variation (shown as 'Fecund in Infection' in Table 7). For uninfected animals, a 55% increase in fecundity (nested within infection type) was associated with a drop in N release of 5.9 $x10^{-3}$ mol per day per mg C (Table 9, Fig 9).

Like variation in N release rates, we found that variation in P release rates of *Daphnia* was described better by fecundity (nested within diet treatment) than infection status (nested in diet), spore load (nested in diet), or the null model (Table 6). For animals fed C:P ratios of 100, a 55% increase in fecundity was associated with a decline in C-specific P release of $3.6x10^{-4}$ mol per day per mg C (Table 8, Fig 9). However, variation in P release across diets was described better by diet (nested in infection type) than fecundity (nested in infection type), spore load (nested in infected type), or the null model (Table 7). Consistent with the results of the nested ANOVA described above, the negative relationship between diet C:P ratio and P release was strongest for *P. ramosa* infected individuals (Table 8, Fig 70).

Describing variation in proximate physiological drivers of nutrient release

We also compared the ability of the same set of models to describe variation in more proximate drivers of nutrient release (i.e. *Daphnia* ingestion rate or body elemental content). Like variation in N and P release rates, variation in ingestion rates was described better by fecundity (nested within diet treatment) than infection status (nested in diet), spore load (nested in diet), or the null model (Table 6). However, unlike variation in N and P release rates, the relationship between fecundity and ingestion rate was strongest in the low and intermediate P diet treatments (Table 8). Eliminating the fecundity of the average uninfected *Daphnia* fed a P-poor diet (~10 offspring in 16 days) was associated with a 17% decline in ingestion rate. Likewise, castrating an uninfected *Daphnia* fed an intermediate P diet was associated with a reduction in its ingestion rate by 33%. Variation in ingestion rate *within* an infection type was best described by a positive relationship between ingestion rate and diet C:P ratio (nested within infection type). This relationship was strongest for uninfected animals (Table 9).

Variation in Daphnia body C:N ratio across infection types was described better by both fecundity (nested within diet) and infection (nested in diet) than spore load (nest in diet) or the null model (Table 6). These two top models indicate that the C:N ratio of *Daphnia* fed all diets was decreased by fecundity and increased by *P. ramosa* (Table 8, Figs 3 and 4). If we split this C:N ratio into its component parts, we see that body %C is best described by variation in infection type (nested within diet; Table 6), and positively associated with *P. ramosa* regardless of *Daphnia* diet treatment (Table 8, Fig 70). Conversely, variation in *Daphnia* %N is best described by fecundity (nested within diet, Table 6), and the negative association between *Daphnia* %N and fecundity is evident in all diet treatments (Table 8, Fig 9). Based on this relationship, castration of individuals

fed diets with C:P ratios of 100, 400, and 700 was associated with a reduction in %N of these animals by 13, 9, and 2% respectively (Table 8, Fig 9).

Variation in body C:N ratio across diet treatments was described better by fecundity (nested within infection) than diet (within infection), spore load (within infection), or the null model. However, if we reduce this ratio into its components, we find that variation in *Daphnia* %C across diets was best explained by infection (which is not very informative, because this is the intercept of the model) and variation in *Daphnia* %N was best explained by diet C:P ratio (Table 7). Diet C:P ratio was inversely correlated with body %N regardless of *Daphnia* infection status (Table 9, Fig 70).

Within a given diet treatment, body C:P ratio was described better by infection type (nested in diet) than fecundity (nested in diet), spore load (nested in diet) or the null model (Table 6). *P. ramosa* infection was associated with an increased C:P in *Daphnia* fed all diets (Table 8, Fig 70). When we break this ratio into its component parts, we see that both *Daphnia* %C and %P are both best described by infection status (nested within diet; Table 6). *P. ramosa*-infection was associated with increased %C of *Daphnia* fed all diets and decreased %P of *Daphnia* fed P-rich and intermediate P diets (Tables 4, Fig 70). *H. tvaerminnensis*-infection, on the other hand, was associated with increased %P of *Daphnia* fed the intermediate P diet (Table 8, Fig 70).

Variation in body C:P ratios was described better by diet C:P ratio (nested within an infection type) than fecundity (in infection), spore load (in infection), or the null model (Table 7). A positive relationship between body C:P ratio and diet C:P ratio was apparent among individuals of all infection types. Likewise, variation in body %P was

best described by diet C:P ratio (in infection) and negatively associated with this variable for *Daphnia* in all infection types (Table 9, Fig 70).

Within-diet variance in *Daphnia* body N:P ratio was better described by diet (the intercept) than any of the potential slopes (infection (within diet), fecundity (within diet), spore load (within diet), or the null model), indicating that none of our within-diet predictors described variation in body N:P well (Table 6). Within infection type variance was also best described by diet C:P ratio (Tables 3). Body N:P was positively associated with diet C:P in all infection types (Table 9).

Discussion

The type of parasite that infects populations may have important effects on ecosystems through alterations in host-mediated nutrient cycling, but distinguishing between the functional roles of different parasite species can be challenging. Here, we used an experimental approach to examine how the ecological role of a single host can be mediated by two different types of parasites along an ecologically relevant nutritional gradient. We found that host nutrient release rates responded to infection type in a diet-specific way, and that diet C:P ratio had a stronger effect on the nutrient release rates of some infection types than others. Specifically, we found that, when *Daphnia* were fed a P rich diet, *P. ramosa* infection increased N release rates relative to uninfected *Daphnia* (but not relative to *H. tvaerminnensis*-infected individuals). Diet C:P ratio only marginally influenced *Daphnia* N release. Similar to the effects of infection on N release, we found that P release rates of *P. ramosa* infected individuals fed the P-rich diet were elevated relative to both uninfected individuals and those infected with *H. tvaerminnensis* fed the same diet, but there was no effect of infection nor infection type on the nutrient

release rates of *Daphnia* fed low or intermediate P diets. As expected, Diet C:P ratio was negatively associated with P release in all infection treatments, but this effect was most dramatic for individuals infected by *P. ramosa*.

In our experiment, we directly manipulated *Daphnia* infection status and diet C:P ratios. These manipulations caused variation in *Daphnia* nutrient release that was accompanied by variation in *Daphnia* fecundities, ingestion rates, and body nutrient ratios. To improve our understanding of the complex effects of infection and diet quality on nutrient release, we further examined whether the variation that we observed in *Daphnia* fecundity and parasite load (two important metrics of host exploitation) could be used to describe the variation in *Daphnia* nutrient use caused by our treatments. We found that fecundity, but not parasite load, described variation in N and P release rates, ingestion rates, and body C:N ratios well. Below, we discuss the relationship between fecundity and nutrient use that we observed and consider its potential to inform predictions regarding the nutrient release of host in other parasite-host systems.

Our results suggest that reductions in fecundity may be a useful indicator of changes in nutrient release for infected and uninfected individuals alike. We suspect that the relationship between both parasite- and diet-induced reductions in *Daphnia* fecundity and increased N release was mediated by elevated body C:N ratios. Model selection indicated that fecundity described these trends in N release and C:N ratios best, regardless of whether variation in these rates was caused by infection or diet. Likewise, mass-balance principles indicate that increased body C:N storage leads to increased N release. The negative correlation between fecundity and *Daphnia* C:N body ratios is

readily accounted for by the accumulation of C-poor (and N-rich) eggs or embryos in the *Daphnia* brood sac (Berberovic 1990; Frost et al. 2008a).

While a negative relationship between fecundity and body C:N ratios has been documented in other field-collected crustaceans as well (Ventura and Catalan 2005), we are unaware of any efforts to link this relationship to ecosystem nutrient cycling. Our results illustrate the potential value of incorporating fecundity-associated shifts in individual nutrient use into our understanding of biogeochemical cycles. This relationship may be a particularly useful indicator of the effect of parasites on the nutrient storage by hosts in which egg mass contributes a substantial portion of their total biomass. Because castration, as an exploitation strategy, is thought to be favored in hosts that invest a large fraction of their resources in reproduction (Lafferty and Kuris 2009), parasite-induced reductions in fecundity may serve as a useful predictor for changes in the nutrient use of many hosts.

Despite this potential, our results suggest that the descriptive power of fecundity is affected by host diet. We suspect that fecundity described nutrient release rates poorly when *Daphnia* were fed the lower P diets because these diets lead to positive associations between fecundity and ingestion rates that altered the mass-balance of nutrient release. Increases in fecundity were associated with large increases in ingestion rate in the P-poor diet treatment and more moderate increases in the intermediate P diet. Mass-balance principles suggest that the relationship between fecundity and ingestion rates compensated for the relationship between fecundity and body C:N ratios so that we did not observe a correlation between fecundity and the N release rates of *Daphnia* fed low and intermediate P diets. Previously, we observed an effect of *P. ramosa* on the ingestion

rates of *Daphnia* fed P rich diets (Narr and Frost, 2015). The results of the present study are inconsistent with this diet-specific effect, but the relationship between the effect of parasitism on *Daphnia* ingestion rates and nutrient release remains consistent: diets in which parasitism induces large changes in ingestion rate appear to mitigate the effects of parasitism on nutrient release.

Our understanding of the drivers of parasite-driven changes in host nutrient use would benefit greatly from a better understanding of the relationship between fecundity and ingestion rate. Amongst both infected and uninfected individuals, fecundity and ingestion rates are often positively correlated (Hogg and Hurd, 1995), but cause and effect have not been established for this relationship. It is particularly difficult to establish the cause of infection-induced reductions in ingestion and fecundity; just activating the immune system of the host can reduce host fecundity, feeding, and metabolism (Ahmed et al. 2002; Zerofsky et al. 2005; Bashir-Tanoli and Tinsley 2014), suggesting that all three effects may simply be by-products of immune function, or that these changes may confer a selective advantage in the face of parasitism. Indeed, anorexia is sometimes (but not always) associated with increased survival of infected *Drosophila* (Ayres and Schneider 2009).

Our data suggest that infection-induced change in fecundity may be a useful metric to describe and potentially predict changes in nutrient release in other parasite-host systems. However, we note that the trends described by this metric are imprecise: on average, the descriptive variables retained in our top models accounted ~50% of the variation in ingestion, body nutrient ratios and nutrient release rates. Therefore, it is apparent that other changes in physiology also contribute to the variation in nutrient use

that we observed. Some of these physiological changes may be infection-specific. For example, although fecundity accounted for variation in ingestion rate best among the models we compared, it consistently overestimated the ingestion rates of *Daphnia* infected by *H. tvaerminnensis* (as evidenced by significantly smaller residuals in this treatment, data not shown), indicating that traits specific to individuals infected by this parasite caused them to ingest food more slowly than other *Daphnia*. Likewise, infection status explained diet-specific variation in *Daphnia* %C, %P, and C:P ratios best among the models we compared. Consistent with previous work in the same system (Frost et al 2008a, Chapter 2), *P. ramosa*-infected individuals possessed lower P content than their uninfected conspecifics in both the P rich and intermediate P diets, as well as elevated C content, and elevated C:P ratios in all diet treatments. Conversely, we show here, for the first time, that *H. tvaerminnensis* elevated the % P of *Daphnia* fed the intermediate P diet. The physiological mechanism for these parasite-specific changes in *Daphnia* nutrient storage remains unclear.

Our results indicate that P-rich ecosystems that support high rates of *Daphnia* fecundity could be most susceptible to infection related declines in both N and P release. This is consistent with other studies showing that P-rich daphnids can immobilize large amounts of P when they dominate zooplankton biomass (Andersen 1997). Adding parasites that reduce host reproduction to these P-rich ecosystems could, therefore, increase net release of N and P from hosts. This may be especially important for pelagic ecosystems during the summer when nutrient regeneration by zooplankton can comprise an important part of the nutrient dynamics (Crumpton and Wetzel 1982; Lehman and Sandgren 1985; Sterner 1986). Conversely, our data show that in P-poor ecosystems,

small decreases in fecundity caused by parasites are expected to be associated with large decreases in the ingestion rates of infected individuals. Thus, despite the fact that P-poor diets ameliorate the effect of parasites on fecundity, we found no evidence that this mitigated the effect of parasitism on host ingestion rates. Rather, the ingestion rates of *Daphnia* fed low quality diets appeared to be more sensitive to changes in fecundity than those fed high quality diets.

Tables and Figures

Table 5: Mean$_{(SD)}$ for C:N, C:P, N:P and %C, P, and N of algal diets (identified by their desired C:P ratio). Values represent the post-mixing composition of diets fed to animals throughout the experiment.

Diet	**C:N**	**C:P**	**N:P**	**%C**	**%N**	**%P**
100	6.1$_{(0.2)}$	75.0$_{(25.1)}$	12.2$_{(4.0)}$	42.5$_{(7.1)}$	8.1$_{(1.3)}$	1.5$_{(0.3)}$
400	6.8$_{(0.3)}$	311.8$_{(138.6)}$	46.0$_{(21.6)}$	46.0$_{(6.5)}$	7.7$_{(6.5)}$	0.4$_{(0.1)}$
700	8.3$_{(0.3)}$	653.4$_{(64.8)}$	78.7$_{(8.5)}$	45.5$_{(5.3)}$	6.4$_{(0.8)}$	0.2$_{(0.01)}$

*Table 6: Results of model selection for models explaining variation in nutrient release,ingestion rate, and body nutrient composition within each diet using second order Akaike's information criterion. The number of parameters (K), change in AIC_C compared to the best-ranked model (ΔAIC_C), Akaike model weights (*W*), and log likelihood estimate (*LL*) are provided. The best models ($\Delta AIC_C<4$) are shown in bold.*

Dependent variable	Predictors	K	ΔAIC_C	*W*	*LL*
N release Rate	**Fecund in Diet**	7	0.0	0.97	210.8
	Infection in Diet	10	7.7	0.02	211.4
	Diet	4	11.3	$3.4x10^{-3}$	201.3
	Null	2	12.3	$2.0x10^{-3}$	198.5
P release Rate	**Fecund in Diet**	7	0.0	0.97	361.1
	Diet	4	8.1	0.017	353.2
	Infection in Diet	10	8.2	0.016	361.5
	Null	2	37.3	$7.7x10^{-9}$	336.3
Ingestion Rate	**Fecund in Diet**	7	0.0	0.97	27.4
	Infection in Diet	10	7.4	0.024	28.1
	Diet	4	11.5	$3.2x10^{-3}$	17.8
	Null	2	31.9	$1.1x10^{-7}$	5.3
Body C	**Infection in Diet**	10	0.0	1.0	331.2
	Fecund in Diet	7	35.5	$2.0x10^{-8}$	309.1
	Diet	4	54.1	$1.8x10^{-12}$	295.9
	Null	2	68.0	$1.7x10^{-15}$	286.7
Body N	**Fecund in Diet**	7	0.0	0.98	165.6
	Infection in Diet	10	8.2	0.016	170.5
	Diet	4	27.2	$1.2x10^{-6}$	146.2
	Null	2	33.0	$6.6x10^{-8}$	140.4
Body P	**Infection in Diet**	10	0.0	1.0	461.9
	Fecund in Diet	7	20.6	$3.4x10^{-5}$	447.2
	Diet	4	25.8	$2.5x10^{-6}$	440.7
	Null	2	56.9	$4.3x10^{-13}$	422.8
Body C:N	**Infection in Diet**	10	0.0	0.88	13.5
	Fecund in Diet	7	3.9	0.12	2.5
	Null	2	19.0	$6.4x10^{-5}$	-13.8
	Diet	4	20.9	$2.6x10^{-5}$	-11.8
Body C:P	**Diet**	4	0.0	0.67	-83.8
	Infection in Diet	10	1.8	0.27	-69.0
	Fecund in Diet	7	4.8	0.06	-80.2

	Null	2	10.6	$3.3x10^{-3}$	-92.1
Body N:P	**Diet**	4	0.0	0.99	-37.8
	Fecund in Diet	7	9.4	$8.9x10^{-3}$	-36.4
	Null	2	13.8	$9.8x10^{-4}$	-47.6
	Infection in Diet	10	18.1	$1.2x10^{-4}$	-31.1

*Table 7: Results of model selection for models explaining variation in nutrient release,ingestion rate, and body nutrient composition within each infection type using second order Akaike's information criterion. The number of parameters (K), change in AIC_C compared to the best-ranked model (ΔAIC_C), Akaike model weights (*W*), and log likelihood estimate (*LL*) are provided. The best models ($\Delta AIC_C<4$) are shown in bold.*

Dependent variable	Predictors	K	ΔAIC_C	*W*	*LL*
N release Rate	**Fecund in Infection**	7	0.0	0.7	210.2
	Diet in Infection	7	3.3	0.1	208.5
	Infection	4	3.5	0.1	204.6
	Null	2	11.1	0.0	198.5
P release Rate	**Diet in Infection**	7	0.0	1.0	349.3
	Fecund in Infection	7	12.7	0.0	343.0
	Infection	4	13.2	0.0	338.9
	Null	2	13.7	0.0	336.3
Ingestion Rate	**Diet in Infection**	7	0.0	0.9	18.0
	Fecund in Infection	7	4.5	0.1	15.7
	Infection	4	9.4	0.0	9.4
	Null	2	13.1	0.0	5.3
Body C	**Infection**	4	0.0	0.9	126.4
	Diet in Infection	7	4.8	0.1	129.8
	Fecund in Infection	7	5.7	0.0	129.4
	Null	2	8.3	0.0	119.4
Body N	**Diet in Infection**	7	0.0	0.9	166.3
	Fecund in Infection	7	5.0	0.1	163.8
	Infection	4	27.1	0.0	146.9
	Null	2	34.4	0.0	140.4
Body P	**Diet in Infection**	7	0.0	1.0	461.1
	Fecund in Infection	7	24.7	0.0	448.7
	Infection	4	56.7	0.0	428.8
	Null	2	64.1	0.0	422.8
Body C:N	**Fecund in Infection**	7	0.0	0.9	14.7
	Diet in Infection	7	5.3	0.1	12.0
	Infection	4	18.3	0.0	-0.3
	Null	2	39.5	0.0	-13.8
Body C:P	**Diet in Infection**	7	0.0	1.0	-70.0
	Fecund in Infection	7	11.3	0.0	-75.6
	Infection	4	25.6	0.0	-88.8
	Null	2	26.3	0.0	-92.1
Body N:P	**Diet in Infection**	7	0.0	1.0	-32.4

Fecund in Infection	7	10.1	0.0	-37.4
Null	2	12.5	0.0	-47.6
Infection	4	15.7	0.0	-46.3

Table 8: Coefficients of predictor variables from models describing variation within each diet (across infection type) with delta AICc < 4 for molar, daily, C – specific N and P release rates, C-specific ingestion rates, and body nutrient ratios. More than one estimate for the intercept (diet) is provided when more than one model had a delta AICc < 4. Coefficients are shown in bold when 95% confidence intervals to do not overlap with zero (shown in Appendix B).

Predictor	N release	P release	Ingestion rate	Body C:N	Body C:P	Body N:P	Body %C	Body % N $_{(5,15)}$	Body % P
Diet C:P = 100									
Diet (intercept)	**0.013**	**9.8×10^{-4}**	**0.50**	**5.6** $*^1$ **6.2** $*^2$	**93**$*^1$ **86**$*^2$	**16**	**0.040**	**6.6×10^{-3}**	**4.4×10^{-4}**
Fecundity	**-0.0066**	**-3.6×10^{-4}**	-0.073	**-0.73**				**6.3×10^{-4}**	
P. ramosa				**0.89**	**18**		**1.3×10^{-3}**		**-7.3×10^{-5}**
H. tvaerminnensis				0.27	-1.5		1.7×10^{-4}		-3.4×10^{-5}
Diet C:P = 300									
Diet (intercept)	-0.0026	**-7.1×10^{-4}**	-0.068	0.20$*^1$ -0.026$*^2$	**23**$*^1$ **24**$*^2$	**3.1**	-3.3×10^{-4}	-7.2×10^{-5}	**-9.4×10^{-5}**
Fecundity	**-0.0064**		**0.20**	**-0.91**				**6.8×10^{-4}**	
P. ramosa				**0.94**	**24**		**0.0017**		**-4.4×10^{-5}**
H. tvaerminnensis				0.31	-9.4		-6.8×10^{-5}		**4.3×10^{-5}**
Diet C:P = 700									
Diet (intercept)	-0.0015	-5.4×10^{-4}	**0.63**	**0.53**$*^1$ -0.076$*^2$	**35**$*^1$ **38**$*^2$	**4.4**	**-0.0013**	-2.2×10^{-4}	**-1.4×10^{-4}**
Fecundity	-0.0086	-2.3×10^{-4}	**0.92**	**-1.0**				**8.3×10^{-4}**	
P. ramosa				**0.81**	**19**		**0.0016**		-3.0×10^{-5}
H. tvaerminnensis				0.36	-8.9		1.9×10^{-4}		3.3×10^{-5}

Superscripts:$*^1$ - intercept for fecundity model, $*^2$ – intercept for infection status model

Table 9: *Coefficients (and 95% C.I.s) of predictor variables from models describing variation within an infection type (across diet treatments) with delta AICc < 4 for molar, daily, C – specific N and P release rates, C-specific ingestion rates, and body nutrient ratios. More than one estimate for the intercept (diet) is provided when more than one model had a delta AICc < 4. Coefficients are shown in bold when 95% confidence intervals to do not overlap with zero (shown in Appendix B).*

Predictor	N release	P release	Ingestion rate	Body C:N	Body C:P	Body N:P	Body %C	Body % N	Body % P
Uninfected									
Infection status (intercept)	**0.0089**$*^{1}$ $4.3x10^{-3}*^{2}$ $0.011*^{3}$	**6.21**$x10^{-4}$	**3.**$45x10^{-1}$	**6.037**	**80.22**	**14.70**	**0.039**	**7.29**$x10^{-3}$	**4.63**$x10^{-4}$
Diet	**1.08x10^{-5}**	$-4.04x10^{-7}$	**6.43**$x10^{-4}$		**0.060**	**0.0079**		**-1.26**$x10^{-6}$	**-2.19**$x10^{-}$
Fecundity	**-0.006**			**-0.46**					
H. tvaerminnensis									
Infection status (intercept)	$0.0023*^{1}$ $5.76x10^{-3}*^{2}$ $0.00015*^{3}$	$1.25x10^{-4}$	$-2.47x10^{-3}$	0.065	-1.35	-0.46	0.00032	**-3.52x10^{-4}**	-5.019x10
Diet	$3.00x10^{-6}$	$-5.41x10^{-7}$	$1.96x10^{-4}$		**0.048**	0.0047		**-1.11x10^{-6}**	**-1.49x10^{-}**
Fecundity	-0.001			**-0.62**					
P. ramosa									
Infection status (intercept)	$0.0058*^{1}$ $9.91x10^{-3}*^{2}$ $0.0011*^{3}$	**5.86x10^{-4}**	$1.75x10^{-1}$	**-1.052**	**18.79**	0.73	**0.0016**	**-8.47x10^{-4}**	**-7.80x10^{-}**
Diet	$1.23x10^{-6}$	**-1.31x10^{-6}**	$-6.52x10^{-5}$		**0.064**	**0.0078**		**-8.14x10^{-7}**	**-1.55x10^{-}**
Fecundity	-0.0066			**-4.11**					

Superscripts:$*^{1}$ - intercept for infection status (intercept only) model, $*^{2}$ – intercept for diet model, $*^{3}$ – intercept for fecundity model.

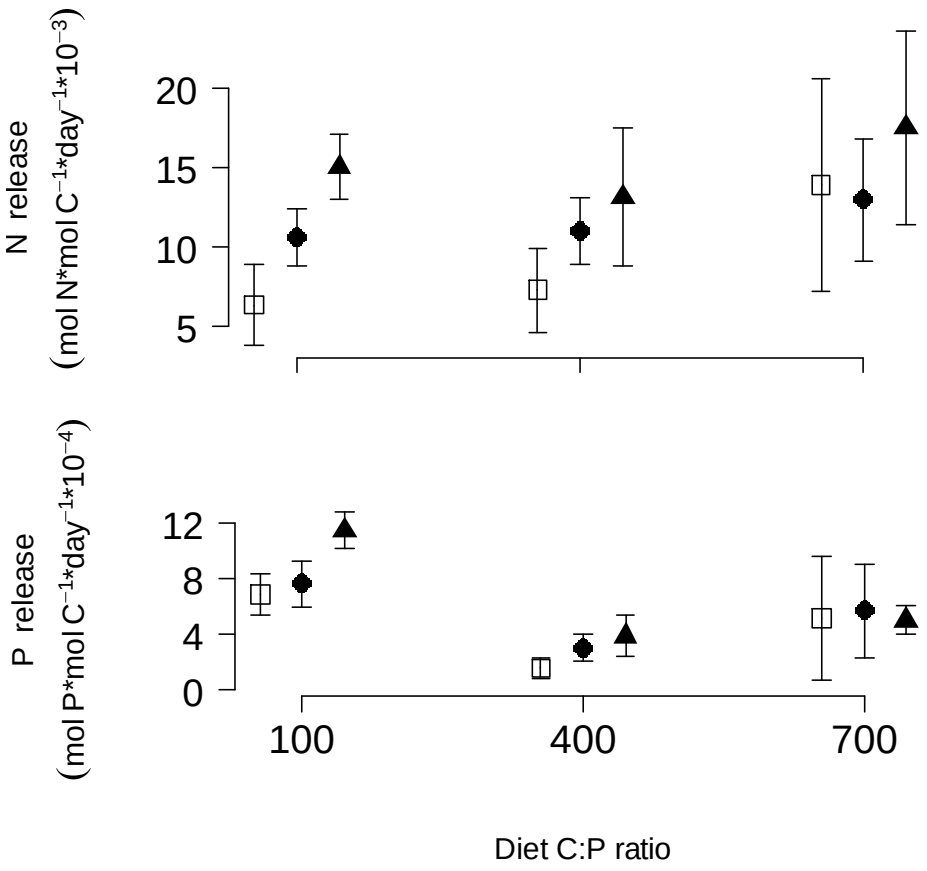

Figure 7: Daily C-specific molar N release (upper) and P release (lower) of uninfected D. magna *(open squares), those infected by* H. tvaerminnensis *(filled circles), and those infected by* P. ramosa *(filled triangles) as a function of diet C:P ratio.* Upper: *Within a diet treatment, infection affected* Daphnia *N release (*$f_{(d.f.)} = 3.39_{(6,43)}$, p = *0.008), with* P. ramosa *infected individuals releasing N more quickly than uninfected individuals when* Daphnia *were fed the high P diet treatment (*$t_{(d.f.)} = -5.25_{(12)}$, p = $2.030x10^{-4}$*). Within an infection treatment, diet C:P ratio had only a marginal effect on* Daphnia *N release (*$f_{(d.f.)} = 2.15_{(6,43)}$, p = *0.067).* Lower: *Infection (within diet) also affected* Daphnia *P release rates (*$f_{(d.f.)} = 2.66_{(6,43)}$, p = *0.028), with* P. ramosa *infected individuals releasing P faster*

than both uninfected individuals and those infected with H. tvaerminnensis *in the 100 C:P diet treatment (*$t_{(d.f.)} = -4.55_{(12)}$, p = $6.67x10^{-4}$*). Within a given infection treatment, diet C:P altered P release (*$f_{(d.f.)} = 9.902_{(6,43)}$, p = $7.48x10^{-7}$*). P release was elevated in all infection types when* Daphnia *were fed a C:P ratio of 100 relative to those fed a C:P ratio of 400 (Uninfected:* $t_{(d.f.)} = 6.29_{(12)}$, $p = 4.03x10^{-5}$*; H. tvaerminnensis:* $t_{(d.f.)} = 4.6629_{(10)}$ $p = 8.9x10^{-4}$*; P. ramosa:* $t = 7.5518_{(12)}$, $p = 6.75x10^{-6}$*).* P. ramosa*-infected individuals also released P faster when they were fed a diet C:P of 100 relative to 700 (*$t_{(d.f.)} = 5.84_{(8)}$, p = $3.89x10^{-4}$*). Error bars are 95% confidence intervals.*

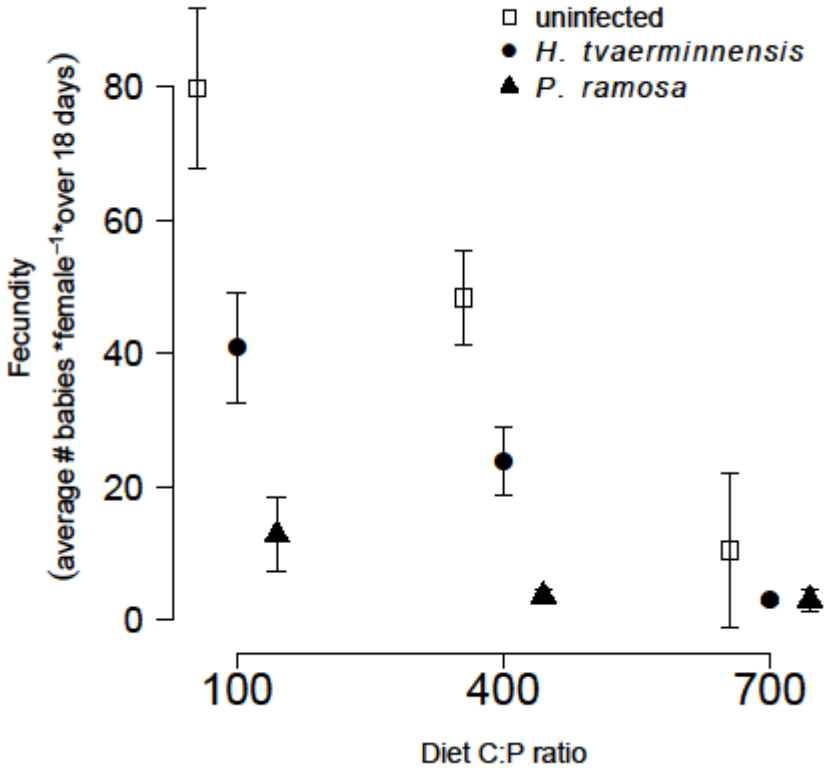

Figure 8: Average number of babies produced by individual females in each experimental unit of uninfected D. magna *(open squares), those infected by* H. tvaerminnensis *(filled circles), and those infected by* P. ramosa *(filled triangles) as a function of diet C:P ratio. Error bars are 95% confidence intervals.*

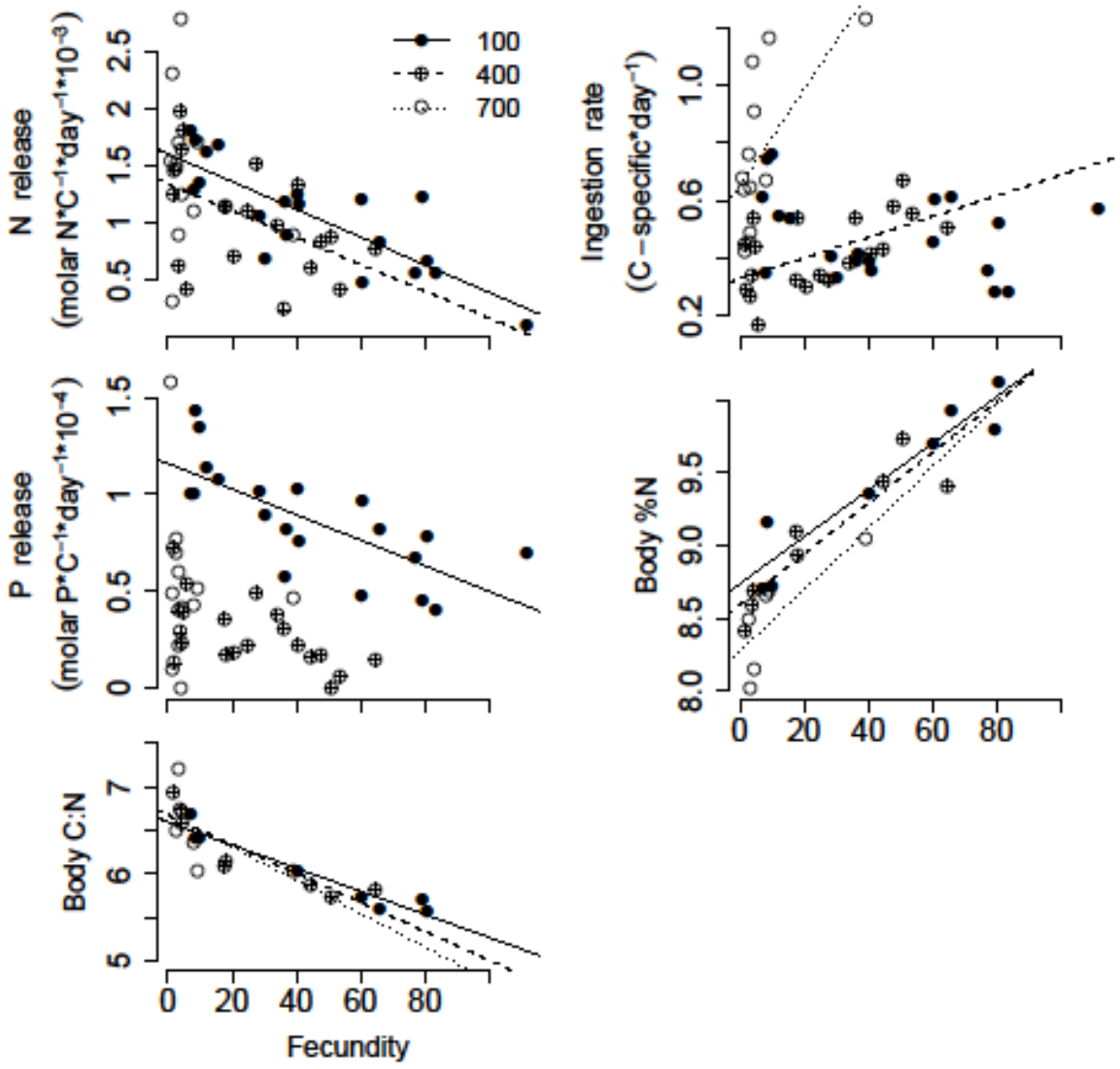

Figure 9: Daily C-specific molar N and P release of D. magna *fed varying food qualities as a function of fecundity. Fecundity estimates are the averaged cumulative reproductive outputs of 8-10 females in one experimental unit over the first 18 days of life. Trendlines are shown for diet treatments in which fecundity was considered a top predictor of nutrient release (delta AICc <4) using second order Akaike's information criteria.*

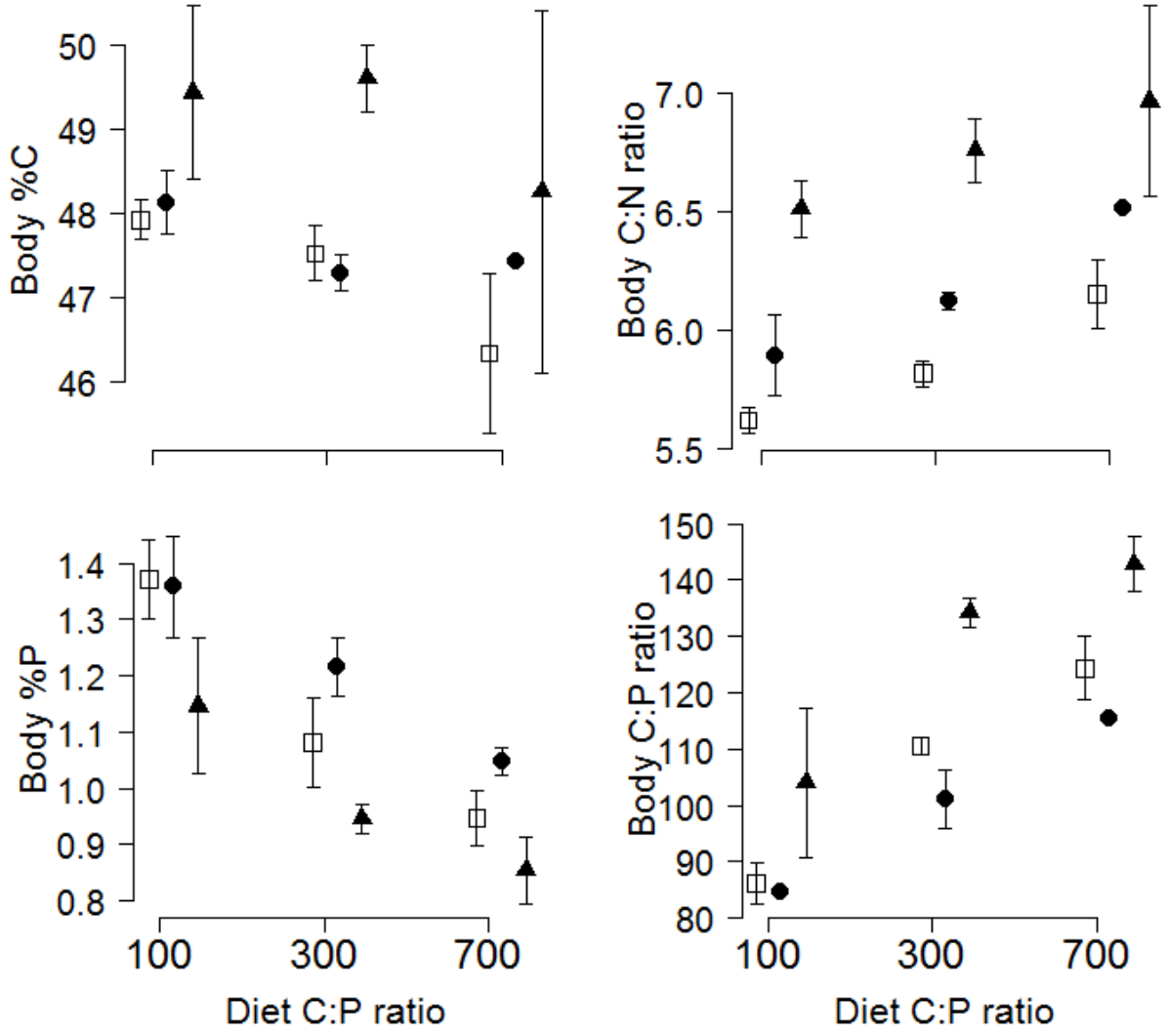

Figure 10: Mean ± standard error of uninfected D. magna *(open squares), those infected by* H. tvaerminnensis *(filled circles), and those infected by* P. ramosa *(filled triangles) for nutrient use parameters that were best explained by infection status (body % C, %P, and C:N and C:P ratios) for individuals fed a gradient of diet C:P ratio.*

Chapter 4 – Prevalence of a microsporidian parasite is elevated in rock pools with lower particulate P concentrations

Charlotte F. Narr, Dieter Ebert, Paul C. Frost

Abstract

Researchers are increasingly interested in understanding the relationship between nutrients and parasitism, but correlations between nutrient concentrations and parasite abundance in natural ecosystems have only rarely been documented. In general, parasite abundance is expected to increase with increasing nutrient concentrations, but experimental evidence indicates that parasites can alter host nutrient use in ways that could reduce the strength of this relationship. We examined the relationship between a microsporidium parasite of *D. magna* and P concentrations in rock pools along the Baltic Sea, a natural context that maximized our ability to detect parasite and nutrient driven gradients. We found a negative correlation between the prevalence of this parasite and particulate P concentrations in rock pools that was consistent with parasite-induced increases in host P sequestration observed in previous lab experiments. Although we cannot rule out the possibility that particulate P concentrations alter the prevalence of this parasite, the congruity between our field observations and previous experiments *in vitro* provides support for the hypothesis that parasites are capable of altering the availability of nutrients in the environment of their host. The potential effect of parasitism on nutrient availability may help to explain why correlations between parasite abundance and nutrient concentrations are not observed more frequently in natural ecosystems.

Keywords: Parasite prevalence, Phosphorus, Nutrients, *Daphnia*, *Hamiltosporidium tvaerminnensis*

Introduction

Whiles parasites are known to form a substantial fraction of ecosystem biomass (Kuris et al. 2008), incorporating these organisms into our understanding of ecosystem level processes remains challenging. As anthropogenic activities are altering the geographical distribution of parasites (Telfer et al. 2005; Torchin et al. 2005) and increasing the bioavailability of nutrients across the globe (Howarth et al. 1995; Galloway et al. 2004), there is a need to examine relationships between parasites and nutrient concentrations in diverse ecosystems (Johnson et al. 2010b). Parasite abundance is generally expected to respond positively to increases in nutrient availability for several reasons (McKenzie and Townsend 2007): parasite prevalence and load (intensity of infections) may positively associate with the availability of potential hosts (Arneberg et al. 1998; Johnson et al. 2007) and the population growth of hosts may be stimulated by nutrient inputs. In addition, increased nutrient availability can enhance host diet quality which in turn can result in higher parasite loads of individual hosts (Bruno et al. 2003; Frost et al. 2008b; Narr and Krist 2015). Therefore, if parasites are simply responding to the nutrient use of their hosts, we expect a positive relationship between nutrient availability and parasite abundance.

On the other hand, parasites do not only respond to host nutrient use, they may also manipulate it (Forshay et al. 2008; Frost et al. 2008a). This complicates straightforward predictions regarding the relationship between parasitism and nutrients because parasitic manipulation of host nutrient use may alter feedbacks between the host and the nutrients available in the environment of the host, with consequences for parasite within-host growth and parasite dynamics in the population (Chapter 2; Bernot 2013). For example, the reallocation of nutrients into hemiparasitic plant litter can stimulate the

growth of surrounding plants via increases in nutrient availability (Quested et al. 2003), and the reallocation of inedible diatom nutrients into smaller chytrid zoospores provides an important new source of nutrients for microzooplankton, increasing the trophic transfer efficiency of pelagic food webs (Grami et al. 2011). Likewise, trematodes can alter the grazing rates (Wood et al. 2007; Bernot and Lamberti 2008), elemental composition (Narr and Krist 2015), and nutrient release rates of their snail hosts (Bernot 2013). These shifts in grazing were responsible for changes in algal community composition (Wood et al. 2007) that could affect the availability of nutrients to hosts as well as other grazers. These relationships may be especially important for predicting the epidemiology of directly transmitted parasites (whose densities are particularly influenced by the densities of their hosts; Lagrue & Poulin 2015) in primary consumer hosts that occupy a central role in nutrient cycling (Andersen 1997). However, the effect of these changes in host nutrient storage and release as well as those observed in other parasite-host systems (e.g. parasites of *Daphnia*, Forshay *et al.* 2008; Frost *et al.* 2008b) on ecosystem nutrient availability have not been explored *in situ*. While experiments suggest that these feedbacks may be possible, it is unclear if the net outcome of these reciprocal forces produces a correlation between parasite prevalence and the nutritional quality of a host's diet. In other words, is nutrient concentration a predictor for parasite prevalence across populations?

Despite substantial (and growing) interest in the relationship between parasites and nutrient concentrations, detecting these trends *in situ* is challenging (Johnson *et al.* 2007; Civitello *et al.* 2013). This may be because relationships between nutrients and parasitism in natural ecosystems are obscured by noise from other biotic (e.g., predation

and competition, Duffy & Hall 2008; Decaestecker *et al.* 2014) and abiotic (e.g. urbanization or pesticide concentration; Kiesecker 2002; Bradley & Altizer 2007) effects on parasite-host interactions. Likewise, the availability and form of nutrients released from hosts is affected by water chemistry (Schlesinger and Bernhardt 2013). It may be difficult to detect patterns between host nutrient use and environmental nutrient availability if physical characteristics, such as pH, vary substantially among ecosystems. Here, we examine the relationship between parasitism and nutrients in a natural context that maximizes our ability to detect parasite and nutrient driven gradients.

We quantified the presence and prevalence of a directly transmitted parasite of *Daphnia magna,* a primary consumer that is frequently used to study producer-grazer and parasite-host interactions (Sterner & Elser 2002; Ebert 2005). We did so in rock pools on islands off the Finnish coast in the Baltic Sea, which naturally vary in their phosphorus levels. In addition to host diet (particulate) P concentrations, we measured a number of other physical parameters that could mediate the relationship between particulate P concentrations and parasite presence and prevalence (i.e. dissolved P concentrations, water pH and conductivity, algal chlorophyll a concentrations, and *Daphnia* densities).

We expected that if the parasitic microsporidium, *Hamiltosporidium tvaerminnensis,* of *Daphnia* responds positively to increases in P availability, we would find a positive correlation between *H. tvaerminnensis* prevalence and ambient P concentrations across pools because: 1) greater P should translate into better food quality for *Daphnia* and increased population densities and 2) *H. tvaerminnensis* is directly transmitted both vertically and horizontally so that the population dynamics of parasite and host are expected to be tightly associated. However, if this parasite alters the

availability of nutrients within the pools via parasite-induced changes in host nutrient use, we expected to detect a negative correlation between the prevalence of *H. tvaerminnensis* and ambient P concentrations in this system because: 1) *Daphnia* in these rock pools can achieve extremely high densities, making them important contributors to nutrient cycling within the pool, and 2) lab experiments indicate that *H. tvaerminnensis* increases the sequestration of P in *Daphnia* tissues.

Methods

Study system

In late July of 2015, we surveyed 28 rock pools on 7 skerry islands of the Baltic Sea near the Tvärminne Zoological Station in Southwest Finland (Appendix C). These pools are filled mainly by rain water and occasional inputs from the sea and provide natural gradients in many physical parameters including conductivity, pH, nutrient concentrations, and a variety of other abiotic and biotic variables (Pajunen and Pajunen 2007; Ebert et al. 2013). Nutrient inputs into the pools are generally thought to come from the sea or the feces of vertebrates (e.g. eider ducks, Ganning & Wulff 1969; Ebert *et al.* 2013).

The rock pools support metapopulations of 3 species of *Daphnia* (*D. magna*, *D. pulex,* and *D. longispina*). These species exhibit some degree of niche differentiation (Pajunen and Pajunen 2007), and are frequently infected by a diversity of parasites (Ebert et al. 2001). *H. tvaerminnensis* is a microsporidium parasite endemic to the skerry islands of the Baltic that resides within the adipose tissue, ovaries, and hypodermis of *D. magna* (Haag et al. 2011). Among pools dominated by *D. magna* in this area, *H. tvaerminnensis* achieves the highest endoparasite prevalence. On average about half of all *D. magna* populations harbour the parasite (Ebert *et al.* 2001), with prevalences of up to 100%

(Lass & Ebert 2006). Infections typically reduce host fecundity by approximately 20% (Bieger and Ebert 2009).

Sample collection and processing

We collected data from each pool on *D. magna* density and parasite prevalence. We estimated *D. magna* density by taking fifteen 250 ml samples along the length, depth, and perimeter of each pool, and then counting the number of individuals in 250 ml of a well-mixed subsample of these 15 samples. We separated these *Daphnia* into size classes using 0.25, 0.7, 1.0 and 1.25 mm mesh. In addition, we used a small hand net to collect up to 50 adult *Daphnia* (fewer *Daphnia* were collected from pools in which *Daphnia* densities were low) in order to estimate the prevalence of parasites (*H. tvaerminnensis, Spirobaccilus cienkowskii,* and *Larssonia sp.*). These *Daphnia* were kept in ADAM, fed *ad libitum* with *Scenedesmus obliquus* for 12 days, and then dissected and viewed under 200x phase contrast microscopy to identify infections. To prevent new infections from forming over the 12 days, *Daphnia* that died during this time were removed from the jars and dissected for parasite identification.

We also sampled the water column of each pool to analyze nutrient concentrations in the water and algae. These samples were filtered through acid-washed, 60 µm mesh in the field, kept cool, and then filtered onto 0.7 µm GF/F filters within 24 hours. The filters were either dried at 60 °C to estimate particulate dry mass or frozen for later analysis of chlorophyll A concentrations. The P content of both the particulate and dissolved fractions of each sample were analyzed using the molybdate blue-ascorbic acid method after persulfate digestion (APHA 1992). Chlorophyll A concentrations were estimated using spectrophotometry (Shimadzu UV-2501) according to (Arvola 1981) after

extraction in ethanol. We used a hand held pH meter to measure water conductivity and pH.

Statistical analyses

We focused our analyses on the presence and prevalence of *H. tvaerminnensis* in pools and did not include information regarding the much less common *Spirobaccilus* and *Larssonia* infections in our analyses. Therefore, we refer to pools with *H. tvaerminnenis* as "infected" and those without *H. tvaerminnensis* as "uninfected". To determine if uninfected and infected pools differed from each other, we looked for differences in chemical and biological parameters in both groups using Mann-Whitney U tests. We conducted this analysis for chlorophyll A concentration, conductivity, *D. magna* density, dissolved and particulate P concentration, and pH. To ensure that differences between uninfected and infected pools were not driven by island effects, we also compared the means of each variable after excluding islands without infected *D. magna* populations (this reduced our sample size to 11).

We used logistic regression to determine if any of the habitat variables we measured were correlated with the prevalence of *H. tvaerminnensis* in pools. In order to avoid selecting predictors that prevent the parasite from establishing in a pool, pools without *H. tvaerminnensis* were not used in this analysis. We used second-order Akaike's information criteria to compare models that predicted infection prevalence as a function of each of the habitat predictors that we measured. In each model we included 'island' as a random intercept to account for similarities in prevalence among pools on the same island. We also included a random intercept "id" that distinguished between each pool in order to reduce overdispersion of our models. We then used second-order Akaike's

information criteria to determine which model best predicted variation in parasite prevalence. Because our predictors were on very different scales, we standardized each habitat predictor to a mean of 0 and SD of 0.5 prior to model selection (Gelman 2008) to facilitate our ability to compare their relationships with the prevalence of *H. tvaerminnensis* in pools. Graphical analysis of the leverage of the standardized Pearson's residuals indicated that one pool exerted a relatively high influence on the relationship between our habitat variables and parasite prevalence. However, removing this pool had no qualitative effect on our results (in terms of model selection of parameter estimates), and we could find no reason to question the integrity of our measurements on this pool. As a result, we decided to include this pool in our final data set.

Results

Presence of H. tvaerminnensis *in pools*

Pools in which *H. tvaerminnensis* infection was absent had higher pH than those in which it was present (W= 102, p= 0.0036, Fig 11). When we reduced our sample size to exclude pools on islands in which every pool we measured harbored the parasite, this difference remained significant ($t_{(d.f.)} = 2.53_{(9)}$, $p = 0.032$). We did not find significant differences in any of the other abiotic or biotic variables that we measured between pools that harbored *H. tvaerminnensis* infection and those that did not.

Prevalence of H. tvaerminnensis *in pools*

Particulate %P was our best predictor of *H. tvaerminnensis* prevalence in the pools we sampled that contained the parasite (Tables 10 and 11; Fig 12). Among pools that harbored this microsporidian, all of those that possessed particulate P concentrations less than 0.003 μg*ug dry weight^{-1} had prevalences of *H. tvaerminnensis* of 85% or greater. Above this concentration, parasite prevalence declined roughly linearly with

increases in particulate P concentration. The slopes of the other habitat variables and parasite prevalence were not different from zero (Table 11).

Discussion

Nutrient availability is generally thought to influence parasite dynamics (McKenzie and Townsend 2007; Johnson et al. 2010b). We found evidence that the prevalences of parasites in rock pools are linked to nutrient concentrations, but this effect is complicated by the pH of the water. Specifically, rock pools with higher pH values were less likely to harbor *Daphnia* with the microsporidian, *H. tvaerminnensis,* than were more acidic pools in a *Daphnia* metapopulation. Among those pools that harbored the infection, we found that high prevalences of *H. tvaerminnensis* were associated with decreased particulate P concentrations. Our data illustrate how changes in the mass-balance of host nutrients budgets observed in the lab (chapter 3) can be used to predict ecosystem level patterns in parasite and nutrient dynamics. Our study is one of only a few that have demonstrated a relationship between a nutrient and parasite prevalence in natural ecosystems (Johnson et al. 2007; Civitello et al. 2013). Of these studies, ours is the only one to show a negative association between parasitism and nutrients. Below, we discuss the mechanisms that might be driving the patterns in presence and prevalence that we observed and consider the potential for their application in other ecosystems.

Among the *D. magna* populations that we sampled, those pools with *H. tvaerminnensis* infections were, on average, significantly more acidic than those that did not harbor *H. tvaerminnensis* infections. It is surprising that we observed differences between infected and uninfected pools in pH but did not also observe differences in dissolved or particulate P concentrations, *Daphnia* density, or conductivity. We had expected that pH might mediate the relationship between P and parasitism because the

bioavailability of P is maximized at a pH of 7 (Schlesinger and Bernhardt 2013). As a result, it could be easier to detect a relationship between parasite-induced changes in *Daphnia* P use and parasitism in pools that possess a neutral pH. We had also anticipated that pH could be an indicator of how suitable the habitat is for *D. magna*. Water pH varies greatly among pools in this rock pool system, and is positively associated with the presence of *D. magna,* perhaps because *D. magna* has a higher salinity tolerance and requires more calcium than its congeners in the area (Pajunen and Pajunen 2007; Ebert et al. 2013). However, we did not find differences in P concentrations or *Daphnia* density between pools with and without *H. tvaerminnensis* infection, indicating that the relationship between pH and *H. tvaerminnensis* was not mediated by P availability or *D. magna* habitat suitability.

Instead we suspect that the relationship between *H. tvaerminnensis* presence and water pH was mediated by the length of time (number of years) the pool has been occupied by *D. magna*. Previous work indicates that the presence of *H. tvaerminnensis* in these rock pools is positively associated with the number of years a rock pool has consistently housed *D. magna* (Ebert et al. 2001). Although the uninfected pools we sampled possessed a higher pH than the Sea surrounding these islands (the pH of the Sea was, on average, 8.2 when we sampled the pools, while the average pH of uninfected pools was ~8.8), it is possible that elevated pH is indicative of pools that are more heavily influenced by salt inputs from the Baltic Sea. As a result, these pools may be more prone to extinction events caused by marine flooding. Ponds dominated by rainfall or overland water input tend to have lower pH (often with a pH < 7) and longer permanency. Therefore, flooding from the Baltic Sea may be simultaneously decreasing the likelihood

that the parasite will establish (due to the lack of hosts) and increasing the pH of these more ephemeral rock pools. If so, pH may be a useful indirect indicator of the likelihood of parasitism in similar rock pool environments, but the relationship would not prove useful in other more permanent pond settings. At present, the trends we have observed provide an incentive for more in-depth analyses of the relationship between pH and *H. tvaerminnensis* over larger spatial and temporal scales.

While the relationship between the presence of *H. tvaerminnensis* and pH is difficult to explain, the relationship between particulate P and *H. tvaerminnensis* prevalence may be explained by multiple potentially synergistic mechanisms. We find it likely that the relationship between particulate P and *H. tvaerminnensis* prevalence is mediated by elevated P sequestration in the tissues of infected *Daphnia*, elevated ingestion rates of *Daphnia* eating P poor food, or a combination of both. Experiments show increased sequestration of P in the tissues of *D. magna* infected by the microsporidium relative to uninfected conspecifics when *Daphnia* are fed low to intermediate P food (Chapter 3). These results are consistent with the pattern that we observed in rock pools: *H. tvaerminnensis* prevalence was negatively correlated with particulate P concentrations. It is reasonable to expect that changes in *Daphnia* body tissues could affect particulate nutrient concentrations in many of these pools where *Daphnia* achieved very high densities (and likely accounted for the majority of secondary productivity; Andersen 1997). Low P diets also increase the ingestion rates of both uninfected individuals and those infected by *H. tvaerminnensis* (Darchambeau & Thys 2005; chapter 3). Higher ingestion rates in pools with low particulate P concentrations could increase the likelihood of ingesting *H. tvaerminnensis* spores (but see

Penczykowski *et al.* 2014 for the opposite prediction). In that *H. tvaerminnensis* relies heavily on horizontal transmission during the planktonic phase of *Daphnia* population cycles (Lass and Ebert 2006), such an increase in ingestion rate could cause increased prevalence of infection.

Another potential explanation is that the negative relationship between *H. tvaerminnensis* and particulate P concentrations is caused by P-sensitive changes in competition between *H. tvaerminnensis*-infected and uninfected *D. magna*. *H. tvaerminnensis*–induced reductions in host fecundity reduce the competitive ability of *Daphnia* relative to uninfected *Daphnia* (Lass and Ebert 2006; Bieger and Ebert 2009). This effect is dramatic, capable of causing the extirpation of *H. tvaerminnensis* from pools during its host's planktonic phase if horizontal transmission is prevented. High diet P levels could enhance the competitive advantage of uninfected *Daphnia* in two ways: by increasing the virulence (i.e. decreasing the fecundity) of infected individuals, and/or by increasing the fecundity of uninfected individuals. *D. magna* fecundity can decline with increasing *H. tvaerminnensis* load, and host diet P is positively correlated with host spore load in another directly transmitted parasite of *D. magna* (Frost et al. 2008b). As a result, the high P diets that enhance the fecundity of uninfected *D. magna* (Sterner et al. 1993) may alternatively reduce the fecundity of infected *D. magna* causing an overall decline in the prevalence of infected individuals. However, given that a direct link between diet P and *H. tvaerminnensis* virulence has not been established, we cannot confidently conclude that these P-dependent shifts in competition explain the relationship we observed between particulate P concentrations and infection prevalence.

Overall, our results support the hypothesis that nutrient supply is linked to parasite-host interactions. While we found support for this hypothesis in this study system, this does not imply that similar correlations exist in other parasite-host systems or ecosystems. We deliberately maximized our ability to detect a relationship between parasitism and host diet nutrient concentrations by focusing on a parasite that varies greatly in prevalence and causes its already P-rich host to sequester even more P. Furthermore, we conducted this work in relatively simple habitats where the host is capable of dominating secondary production. However, we cautiously suggest that the negative correlation we observed may also exist in other parasite-host systems where parasites increase the P content of hosts capable of achieving high densities (e.g. trematodes in snails, Narr & Krist 2015). Regardless of the underlying mechanism, the negative relationship between *H. tvaerminnensis* prevalence and particulate P concentration runs counter to the logical expectation that parasite abundance increases with nutrient availability and its existence could help to explain the paucity of correlations between parasite abundance and nutrient concentrations in the literature.

Tables and Figures

Table 10: Results of model selection for models explaining variation in H. tvaerminnensis *prevalence among pools in which the parasite was present using second-order Akaike's information criterion (AICc). The number of parameters used in each model (K), change in* AIC_C *compared to the best-ranked model (*ΔAIC_C*), Akaike model weights (*W*), and log likelihood estimate (*LL*) are provided. Particulate phosphorus concentration was the best predictor of* H. tvaerminnensis *prevalence among the pools we sampled in which the parasite was present.*

Predictors	**K**	**ΔAIC$_C$**	***W***	***LL***
Particulate P	4	0	0.39	-56.22
Null	3	1.58	0.18	-58.56
Conductivity	4	1.67	0.17	-57.06
Chlorophyll A	4	2.55	0.11	-57.5
***Daphnia* density**	4	3.53	0.07	-57.99
pH	4	4.61	0.04	-58.53
Dissolved P	4	4.65	0.04	-58.55

Table 11: Coefficients, adjusted standard error, and upper and lower 95% confidence intervals of all predictor variables measured to describe H. tvaerminnensis *prevalence among pools in which the parasite was present. Effect sizes are standardized on 2 standard deviations. Coefficients are shown in bold when confidence intervals to do not overlap with zero. Of the habitat variables that we measured, only particulate phosphorus concentration was (negatively) correlated with* H. tvaerminnensis *prevalence.*

Predictor	**Units**	**Estimate**	**Adjusted SE**	**Lower CI**	**Upper CI**
Intercept	NA	**1.84**	0.92	0.04	3.64
Particulate P	μg x μg dry weight^{-1}	**-3.71**	1.41	-6.47	-0.94
Conductivity	mS x cm^{-1}	3.72	2.51	-1.20	8.64
Chlorophyll A	μg x L^{-1}	-1.63	1.19	-3.96	0.70
***Daphnia* density**	indivs x L^{-1}	-1.34	1.33	-3.95	1.28
pH	NA	0.35	1.54	-2.66	3.36
Dissolved P	μg x L^{-1}	0.20	1.29	-2.33	2.72

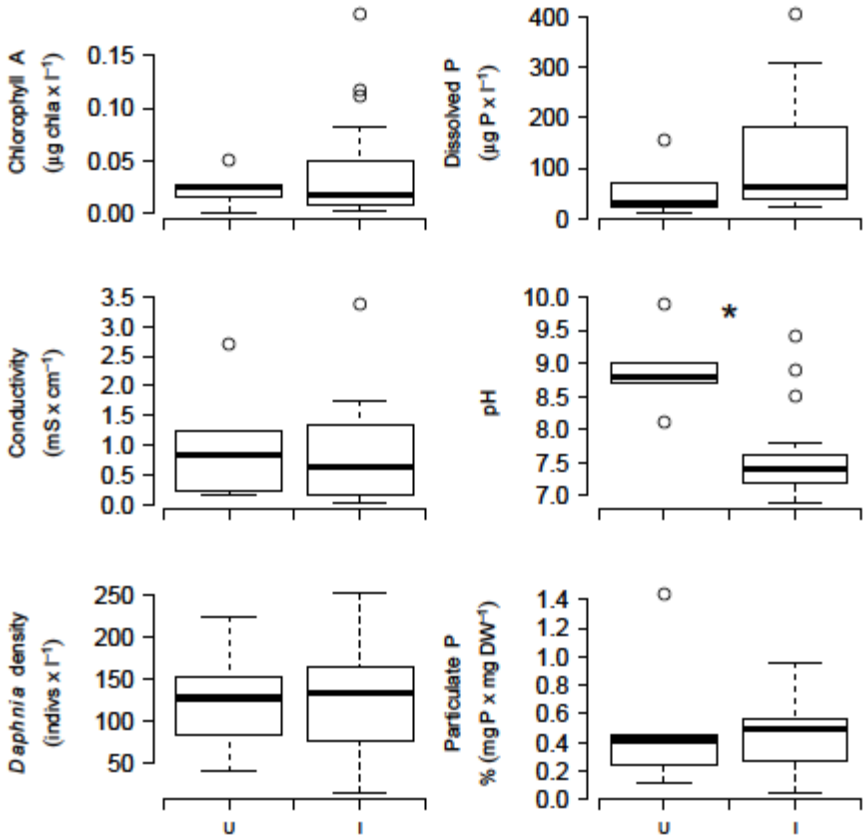

Figure 11: Box and whisker plots displaying chlorophyll A concentration, dissolved P concentration, conductivity, pH, D. magna *density, and particulate P concentration of pools where we did not detect the presence of the parasite* Hamiltosporidium tvaerminnensis *(U) and pools where the parasite was present (I). Pools without the infection were significantly more basic than those with the infection, but we did not detect any other differences between pools with and without the infection. The median, 25th and 75th percentiles and median values for each parameter are shown as the lower, upper, and thick black lines of the box. Outliers (>1.5 x the interquartile range above the upper quartile or below the lower quartile) are shown as open circles.*

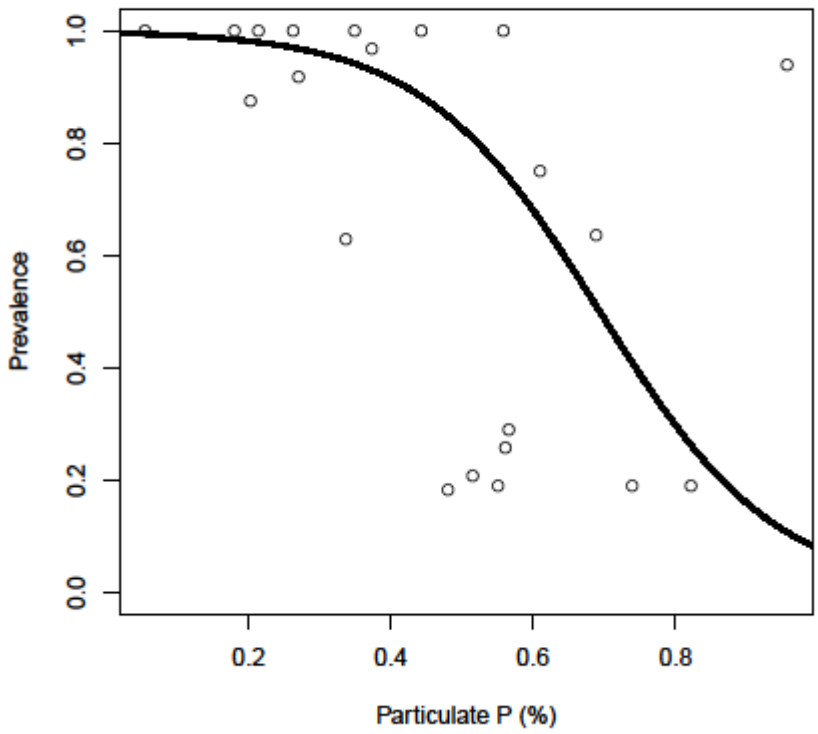

Figure 12: Prevalence of Hamiltosporidium tvaerminnensis *infection as a function of the particulate P concentrations in rock pools. The dark line is the predicted logistic regression line between prevalence and particulate P concentration.*

Chapter 5 – General Discussion

In the preceding chapters, I used basic principles of mass-balance to show how parasitism alters host nutrient excretion rates by changing host ingestion and assimilation rates and body nutrient composition. I explored the magnitude and direction of these changes under scenarios where the host was fed different diet C:P ratios and infected by one of two different parasites. These diet and parasite-specific effects were then compared to parasite-induced variation in fecundity and spore load in an attempt to provide more general predictions regarding the relationship between parasite type and host nutrient use. Finally, we examined correlations between parasite presence and prevalence and nutrient concentrations in a natural *Daphnia* metapopulation in the skerry islands of the Baltic Sea. Here I provide a summary of the patterns observed in each chapter, consider the implications of these findings for other parasite-host systems and ecosystems, and provide thoughts on future directions of this research on the stoichiometry of parasitism.

Summary of results

In the first experiment (chapter 2), we quantified the effects of a castrating, bacterial parasite, *P. ramosa,* and diet C:P ratio on individual *D. magna* excretion rates. We also examined the physiological drivers of these effects by measuring how this parasite influenced more proximate drivers of nutrient excretion (i.e. ingestion rates, body elemental composition, and nutrient assimilation rates). We showed that the effect of this parasite on N and P recycling rates was mediated by diet C:P ratio. Infected *Daphnia* fed P-rich diets ingested food more quickly but excreted nutrients at the same rate as uninfected *Daphnia* fed the same diet. Conversely, infected *Daphnia* fed P poor diets excreted both N and P more quickly but ingested nutrients at the same rate as uninfected

Daphnia fed the same diet. Furthermore, our calculations indicated that *P. ramosa* infection reduced nutrient accumulation rates in all hosts, regardless of the C:P ratio of their diets. Consistent with previous work (Frost et al. 2008a), we also observed diet-specific effects of *P. ramosa* on *Daphnia* body nutrient content. However, the results of a mass-balance nutrient release model indicated that these shifts in body nutrient content exerted less control over host nutrient excretion than parasite-induced changes in nutrient ingestion and assimilation.

We concluded that the diet-specific effect of infection on host ingestion rates dictated whether or not parasites altered host nutrient excretion rates. Regardless of the C:P ratio of the host's diet, *P. ramosa* increased the rate nutrients cycled through *Daphnia* via reductions in host nutrient accumulation efficiencies, but when *Daphnia* were fed high P diets, parasite-induced reductions in ingestion rates caused infected *Daphnia* to recycle nutrients at the same rate as their uninfected conspecifics. Because we suspected that these changes in ingestion could be due to the effect of castration on the energetic demands of hosts, we used an alternative study design to explore the relationship between fecundity and host nutrient use in chapter 3.

In the second experiment (chapter 3), we again quantified the nutrient ingestion rates, body elemental composition, and excretion rates of infected and uninfected *D. magna* fed diets of varying C:P ratios. However, in this experiment, in addition to infecting individuals with the horizontally transmitted *P. ramosa*, we infected some individuals with a second parasite, the microsporidium, *H. tvaerminnensis*. This microsporidium transmits both horizontally and vertically, and, instead of completely castrating its host (like *P. ramosa*), it produces an average reduction in *D. magna*

fecundity of approximately 20% (Bieger and Ebert 2009). We used gradients in fecundity and spore load produced by these two parasites as well as those produced by a dietary gradient to examine the effect of parasite type on host nutrient excretion. Then, we related the parasite-specific changes in nutrient excretion that we observed to the exploitation strategy (in terms of parasite-induced reductions in host fecundity and increases in spore load) of the parasite.

We found that parasite type does influence host nutrient use: *Daphnia* infected by *P. ramosa* released P more quickly and possessed a lower P content than those infected by *H. tvaerminnensis*. Differences in host nutrient use were mediated by diet C:P ratio, but we found that infected individuals released N and P more quickly than uninfected individuals when they were fed a P-rich diet (rather than the P poor diet, as observed in chapter 2). However, consistent with chapter 2, we found that the diet-specific reductions in host ingestion rates mitigated the effect of parasitism on host nutrient excretion rates. This experiment also showed that, among animals fed the same diet, parasite-driven reductions in fecundity were negatively associated with N and P excretion rates. A diet-driven gradient in fecundity also appeared to be tightly linked to variation in N release. We speculated that the relationship between reductions in *Daphnia* fecundity and increased N release was mediated by elevated body C:N ratios. However, because we were unable to directly manipulate host fecundities, it is unclear if changes in fecundity drove the altered nutrient release rates or if they were simply correlated with them.

In the 4th chapter, we tested to see if the patterns that we observed between the nutrient use of *H. tvaerminnensis*–infected individuals and host diet P content *in vitro* were consistent with those in natural rock pools along the Baltic Sea. Because this effect

is expected to be mediated by physical characteristics of the environment, we also measured the pH and conductivity of the pools. We found that rock pool pH was negatively associated with the presence of *H. tvaerminnensis* in *Daphnia* populations while the particulate P content of rock pool particulate matter was negatively associated with the prevalence of *H. tvaerminnensis*. We suspect that the relationship between the presence of the parasite in rock pools was unrelated to pool nutrient dynamics. We find it more likely that infection was observed less frequently in basic pools because marine flooding extirpates the *Daphnia* populations in these pools. This may concurrently increase pool pH and reduce the likelihood that the parasite can establish itself in recolonizing daphnid populations. We provide 3 different, but not mutually exclusive, hypotheses to explain the negative relationship between particulate P concentrations and the prevalence of *H. tvaerminnensis*: 1) increased P sequestration of infected individuals reduces the P that can be absorbed by algae and bacteria, 2) low P diets increase the ingestion rates of *Daphnia*, increasing the likelihood that they will ingest the spores, and 3) high P diets increase the competitive advantage of uninfected individuals over infected individuals by exacerbating differences in their fecundities. Future work in this ecosystem could disentangle the effects of each of these potential mechanisms.

Using stoichiometry to understand the relationship between nutrients and additional parasite-host systems

One of the benefits of adopting the perspective of Lotka (1925), and comparing infected and uninfected individuals as "structured physico-chemical systems", is an improved ability to predict patterns relating nutrients to parasitism in other parasite-host systems based on those we observed here. Given the paucity of literature on parasite-

induced changes in host excretion rates (I am only aware of the studies described in this dissertation and that of Bernot (2013)), the improved mechanistic understanding of these changes provided by the experiments described here is a substantial step forward in our ability to predict the relationship between parasitism and nutrients. The examples provided in the preceding text are dominated by *Daphnia*-parasite host systems because our understanding of consumer-driven nutrient cycling is historically rooted in pelagic ecosystems (Sterner and Elser 2002), and *Daphnia* parasite-host systems are extremely well studied (Ebert 2005). However, we can begin to produce predictions regarding the effects of additional parasites on the nutrient use of their hosts.

The abundance of literature on *D. magna* and its parasites enabled me to produce hypotheses relevant to an extremely broad class of parasites, castrators. For example, in chapter 3, we report a negative correlation between the C:N ratio of *Daphnia* body tissues and *Daphnia* fecundity. Mass-balance principles led us to speculate that this correlation could be responsible for the negative relationship we observed between *Daphnia* fecundity and N release rates. If so, we predict that other parasites that reduce host fecundity will also produce increases in host N release rates. However, previous work has shown that *Daphnia* eggs are rich in N, and comprise a large fraction of *Daphnia* biomass (Ventura and Catalan 2005; Frost et al. 2008a). Therefore, this prediction might more conservatively be applied only to those hosts who invest large fractions of their total N content into reproduction. Even so, our predictions should be applicable to many relationships where the host is parasitically castrated : castration, as an exploitation strategy, is thought to be favored by parasites of hosts that would otherwise invest a large

fraction of their resources in reproduction (e.g. many primary consumers, Lafferty and Kuris 2009).

In addition, the experiments described here provide hypotheses regarding the effect of parasite-induced changes in ingestion rate on host nutrient release. Altered ingestion rates have been observed in a wide variety of hosts ranging from livestock to isopods and snails (Hutchings et al. 1998; Wood et al. 2007; Bernot and Lamberti 2008; Lettini and Sukhdeo 2010). Mass-balance principles led us to conclude (in chapters 2 and 3) that parasite-induced changes in ingestion rate compensated for the effect of infection on other aspects of host nutrient use so that infected and uninfected individuals released nutrients at equal rates. These findings indicate that the effect of parasitism on nutrient release can be mitigated by compensating effects of parasitism on host ingestion rates. This leads me to hypothesize that, when parasite-induced changes in host ingestion rate are observed, it is these changes in ingestion (and not those of altered nutrient release rates) that have the strongest effect on the trophic levels below the host. Conversely, I suspect that, when parasite-induced changes in nutrient release rates are observed, the effect of parasite-induced changes in ingestion rate on lower trophic levels is minimal. Patterns as simple as this are rare in the parasite literature, but I argue that testing these hypotheses in a few of the parasite-host systems in which host ingestion rate is known to be altered is a logical next step in refining our understanding of host nutrient use.

Finally, the relationship between particulate P and *H. tvaerminnensis* that we reported in chapter 4 indicates that parasite-induced shifts in host elemental content observed *in vitro*, while potentially poor predictors of nutrient excretion rates (chapter 2), may be good predictors of the relationship between parasites and nutrients in natural

ecosystems. At present, there are only a handful of studies quantifying the effect of parasitism on host elemental composition, but the ease with which this data can be collected on small hosts enables us to test this hypothesis in a large array of parasite-host systems. Although we cannot confirm that the relationship observed in the rock pools was driven by parasite-induced changes in *Daphnia* P use, the congruence between our observations in the lab and the trends that we observed in nature is striking. In the lab, *H. tvaerminnensis*-infected *Daphnia* sequestered more P than uninfected *Daphnia* fed the same diet. In chapter 3 we found that rock pools with higher prevalences of *H. tvaerminnensis* were associated with lower concentrations of seston P (the diet of *Daphnia*). Parasite-induced shifts in elemental composition have been observed in other hosts capable of dominating secondary production as well (e.g. chytrids in *Daphnia*; Forshay *et al.* 2008, trematodes in snails Narr & Krist 2015), and these shifts could have similar consequences for ecosystem nutrient supplies.

Ecosystem implications

In general, it is expected that parasite abundance will be positively associated with ecosystem nutrient availability (McKenzie and Townsend 2007). However, *in situ* evidence of correlations between parasite abundance and nutrient concentrations are rare (but see Johnson *et al.* 2007; Civitello *et al.* 2013). The complex, diet-specific effects of parasitism on host nutrient use that we observed in chapters 2 and 3 may partly explain the shortage of unequivocal empirical evidence for these positive correlations. However, it is important to exercise caution when generalizing from trends observed in the lab to those expected in natural ecosystems. *P. ramosa* is capable of infecting 100% of a population of *D. magna* for multiple weeks (Duncan et al. 2006). Based on the diet-

specific effects we observed (chapter 2), we predicted that such high prevalence might stimulate primary production by reducing the grazing pressure of *Daphnia* in P-rich ecosystems and increasing the nutrient excretion rates of *Daphnia* in P-poor ecosystems (because we observed these effects when *Daphnia* were fed P rich and P poor diets, respectively). Conversely, in our second chapter, we observed that infection-induced reductions in fecundity were associated with reduced ingestion rates when *Daphnia* were fed P-poor diets and increased nutrient excretion rates when *Daphnia* were fed P-rich diets. Predicting the effect of this parasite on ecosystems requires that we understand why these effects were not consistent between our studies.

The effects observed in both chapters indicate that *P. ramosa* infected *Daphnia* will sequester fewer nutrients, but the mechanism by which this shift occurs (whether by changes in ingestion or excretion rates) can affect primary production. High *Daphnia* densities occur when *Daphnia* are fed P rich diets (Elser et al. 2001; Urabe et al. 2002). These high zooplankton densities are frequently associated with primary producers that are constrained by grazing pressure while low zooplankton densities are often associated with primary producers constrained by nutrient availability (Carpenter and Kitchell 1984; Bergquist and Carpenter 1986). Therefore, we would expect that declines in ingestion rate would have a greater effect on primary producers in nutrient rich environments and increased nutrient release would have a larger effect on primary producers in a nutrient poor environment (as indicated in chapter 2) than if the effects were observed in, respectively, nutrient poor and rich environments (as indicated in chapter 3). These discrepancies indicate a need for a more mechanistic understanding of the relationship between diet C:P ratio and parasite-induced changes in host ingestion rates and the

resulting interactions between grazers and their prey. Future work should incorporate the parasite-related mechanisms found here into detailed mass-balance models of grazers, their food sources, and environmental nutrient supply.

Despite my caution in extrapolating from lab experiments to predicted relationships in nature, I would again note the congruence between *H. tvaerminnensis*-induced increases in *Daphnia* P content (observed in chapter 3), and the negative relationship *H. tvaerminnensis* prevalence observed in a natural setting (chapter 4). This relationship is particularly exciting given the paucity of correlations between parasites and nutrient concentrations in the literature. However, the influence of *Daphnia* parasites on host nutrient use is a relatively extreme example of how parasites might influence ecosystem nutrient availability. When daphnids dominate the biomass of zooplankton, they are capable of immobilizing large amounts of P (Andersen 1997), and zooplankton nutrient regeneration is especially influential during the summer when algae is often nutrient limited (Crumpton and Wetzel 1982; Lehman and Sandgren 1985; Sterner 1986). Likewise, the rock pool ecosystem in which we observed correlations between parasite prevalence and particulate P levels are relatively simple ecosystems that I chose specifically to maximize the likelihood that we could detect a relationship between nutrient levels and parasite dynamics. Now that the presence of these correlations has been observed in a simple system, it is appropriate to begin looking for these patterns in more complex systems, so that we can develop an understanding of how these parasite-host interactions affect nutrient cycling and parasite dynamics on a broader scale.

Conclusion

The studies included in this dissertation provide a first-order understanding of how parasites might influence the nutrient distributions of ecosystems by changing host nutrient use. I have tried to integrate principles of aquatic nutrient cycling with parasitology. I began by applying basic laws of matter to a simple and well-studied parasite-host system. This framework lead me to consider how a parasite's host exploitation strategy influences host nutrient recycling rates, a question that was addressed by explicitly testing the hypothesis that parasite type affects host nutrient use. In my final study, I found that some of the parasite-induced changes in nutrient use that were observed in the lab could be used to predict the relationship between P concentrations in the diet of a host and the prevalence of parasitism in a natural ecosystem. Taken together, these studies demonstrate a coherent case-study of how 2 parasites respond to and manipulate the nutrient use of their consumer host. I've attempted to explore these effects with clear, testable hypotheses. It is my hope that this work will encourage parasitologists and ecologists alike to test these hypotheses in other parasite-host systems and ecosystems. Perhaps, in the future, we can shift our focus away from integrating these two fields, and instead, toward integrating our understanding of parasites into a holistic approach to managing nutrient cycles.

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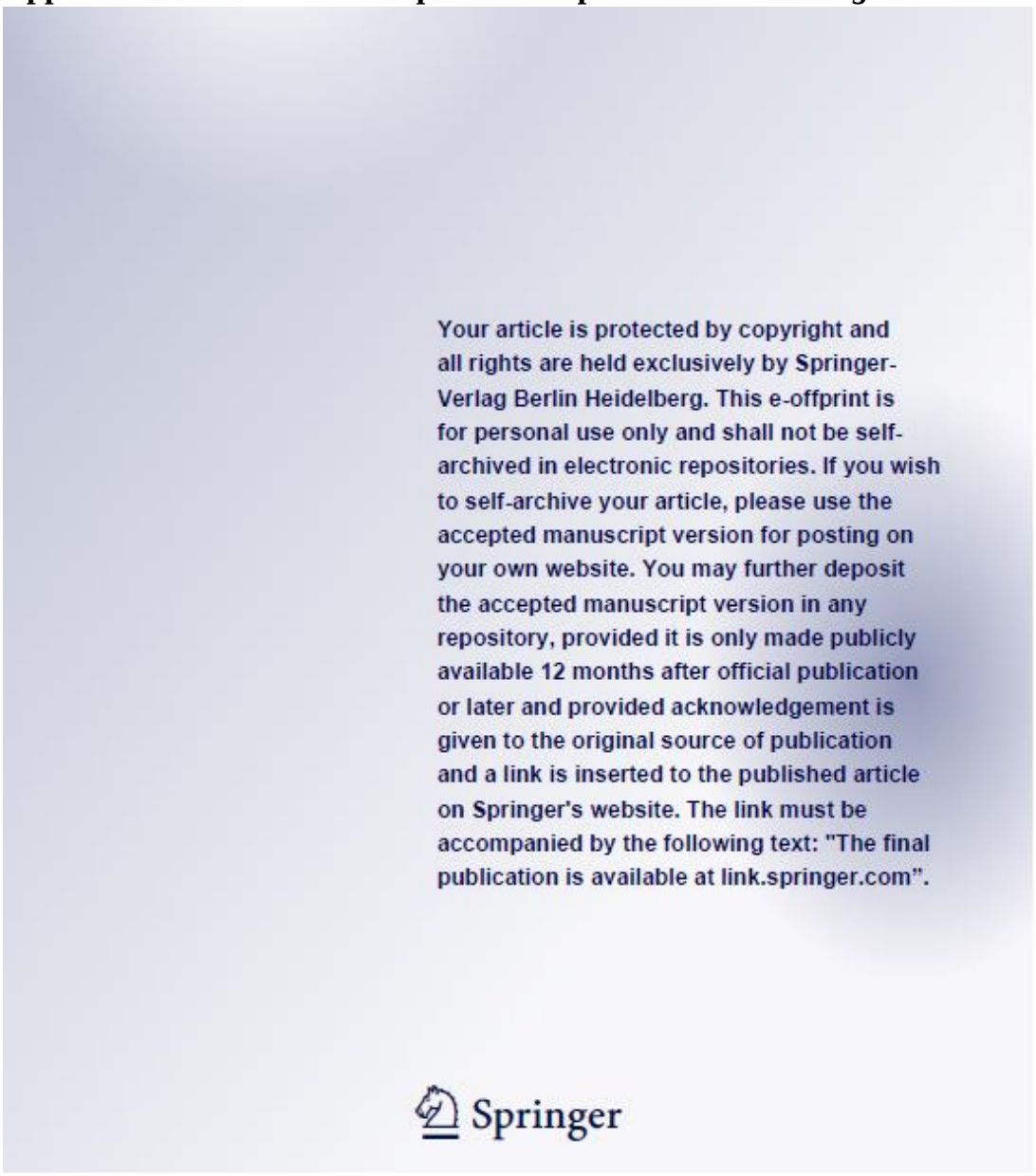
Appendices

Appendix A. Permission to reprint work published in *Oecologia*.



Appendix B. Supplementary material for chapter 2

Table 12: Coefficients (and 95% C.I.s) of predictor variables from models describing variation within diet treatments (across infection type) with delta AICc < 4 for molar, daily, C – specific N and P release rates, C-specific ingestion rates, and body nutrient ratios. More than one estimate for the intercept (diet) is provided when more than one model had a delta AICc < 4. Coefficients are shown in bold when 95% confidence intervals to do not overlap with zero (supplementary material).

Predictor	N release	P release	Ingestion rate	Body C:N	Body C:P	Body N:P	Body %C	Body % N $_{(5,15)}$	Body % P
Diet C:P = 100									
Diet (intercept)	**0.013** (0.010, 0.015)	**$9.8x10^{-4}$** ($8.5x10^{-4}$, $1.1x10^{-3}$)	**0.50** (0.42, 0.58)	**5.6** *[1] (5.4, 5.8) **6.2** *[2] (6.0, 6.4)	**93***[1] (82, 105) **86** *[2] (75, 97)	**16** (14, 17)	**0.040** (0.040, 0.040)	**$6.6x10^{-3}$** ($6.4x10^{-3}$, $6.7x10^{-3}$)	**$4.4x10^{-4}$** ($4.1x10^{-4}$, $4.7x10^{-4}$)
Fecundity	**-0.0066** (-0.010, -0.0023)	**$-3.6x10^{-4}$** ($-5.6x10^{-4}$, $-1.6x10^{-4}$)	-0.073 (-0.20, 0.051)	**-0.73** (1.1, -0.38)				**$6.3x10^{-4}$** ($4.2x10^{-4}$, $8.4x10^{-4}$)	
Infection: *P. ramosa*				**0.89** (0.59, 1.2)	**17.9** (2.7, 33)		**$1.3x10^{-3}$** ($7.6x10^{-4}$, $1.7x10^{-3}$)		**$-7.3x10^{-5}$** ($-1.2x10^{-4}$, $-3.0x10^{-5}$)
Infection: *H. tvaerminnensis*				0.27 (-0.06, 0.61)	-1.5 (-23, 20)		$1.7x10^{-4}$ ($-3.4x10^{-4}$, $6.8x10^{-4}$)		$-3.4x10^{-5}$ ($-7.8x10^{-5}$, $1.0x10^{-5}$)

Diet C:P = 300									
Diet (intercept)	-0.0026 (-0.0056, $5.0x10^{-4}$)	**$-7.1x10^{-4}$** ($-8.8x10^{-4}$, $-5.4x10^{-4}$)	-0.068 (-0.17, 0.036)	0.20 *[1] (-0.10, 0.50) -0.026 *[2] (-0.31, 0.26)	**23***[1] (8,39) **24** *[2] (9, 40)	**3.1** (1.5, 4.9)	$-3.3x10^{-4}$ ($-8.2x10^{-4}$, $1.6x10^{-4}$)	$-7.2x10^{-5}$ ($-2.4x10^{-4}$, $1.0x10^{-4}$)	**$-9.4x10^{-5}$** ($-1.4x10^{-4}$, $-5.1x10^{-5}$)
Fecundity	**-0.0064** (-0.012, $-8.3x10^{-4}$)	$-2.7x10^{-4}$ ($-5.8x10^{-4}$, $4.2x10^{-5}$)	**0.20** (0.0071, 0.39)	**-0.91** (-1.4, -0.45)				**$6.8x10^{-4}$** ($4.1x10^{-4}$, $9.6x10^{-4}$)	
Infection: *P. ramosa*				**0.94** (0.64, 1.2)	**24** (8.5, 39)		**0.0017** (0.0012, 0.0022)		**$-4.4x10^{-5}$** ($-8.6x10^{-5}$, $-1.2x10^{-6}$)
Infection: *H. tvaerminnensis*				0.31 (-0.031, 0.64)	-9.4 (-26, 7.6)		$-6.8x10^{-5}$ ($-5.8x10^{-4}$, $4.4x10^{-4}$)		**$4.3x10^{-5}$** ($8.5x10^{-6}$, $7.8x10^{-5}$)
Diet C:P = 700									
Diet (intercept)	-0.0015 (-0.008, 0.0051)	$-5.4x10^{-4}$ ($9.1x10^{-4}$, $-1.8x10^{-4}$)	**0.63** (0.40, 0.85)	**0.53** *[1] (0.23, 0.83) -0.076 *[2] (-0.50 0.35)	**35** *[1] (19, 52) **38** *[2] (23, 53)	**4.4** (2.6,6.3)	**-0.0013** (-0.0018,-$8.1x10^{-4}$)	$-2.2x10^{-4}$ ($-4.8x10^{-4}$, $3.3x10^{-5}$)	**$-1.4x10^{-4}$** ($-1.8x10^{-4}$, $-9.3x10^{-5}$)
Fecundity	-0.0086 (-0.023, 5.47)	$-2.3x10^{-4}$ (-0.0010, $5.6x10^{-4}$)	**0.92** (0.44, 1.4)	**-1.0** (-2.0, -0.10)				**$8.3x10^{-4}$** ($2.6x10^{-4}$, 0.0014)	

Infection: *P. ramosa*	**0.81** (0.48, 1.2)	**19** (1.5, 36)	**0.0016** (**(**9.4×10^{-4}, 0.0022)	-3.0×10^{-5} (-8.6×10^{-5}, 2.6×10^{-5})
Infection: *H. tvaerminnensis*	0.36 (-0.064, 0.79)	-8.9 (-30, 13)	1.9×10^{-4} (-4.6×10^{-4}, 8.4×10^{-4})	3.3×10^{-5} (-2.4×10^{-5}, 8.8×10^{-5})

Superscripts:$*^{1}$ - intercept for fecundity model, $*^{2}$ – intercept for infection status model

Table 13: Coefficients (and 95% C.I.s) of predictor variables from models describing variation within an infection type (across diet treatments) with delta AICc < 4 for molar, daily, C – specific N and P release rates, C-specific ingestion rates, and body nutrient ratios. More than one estimate for the intercept (diet) is provided when more than one model had a delta AICc < 4. Coefficients are shown in bold when 95% confidence intervals to do not overlap with zero.

Predictor	N release	P release	Ingestion rate	Body C:N	Body C:P	Body N:P	Body %C	Body % N	Body % P
Uninfected									
Infection status (intercept)	**0.0089**∗[1] (0.0067, 0.011)	**6.21**x10^{-4} (3.50x10^{-4}, 8.91 x10^{-4})	**0.345** (0.19, 0.50)	**6.037** (5.90, 6.17)	**80.22** (69.02, 91.43)	**14.70** (12.83, 16.57)	**0.039** (0.039, 0.040)	**7.29x10^{-3}** (7.08x10^{-3}, 7.49x10^{-3})	**4.63x10^{-4}** (4.36x10^{-} 4.89x10^{-4}
	4.3x10^{-3}∗[2] (2.6x10^{-4}, 8.4x10^{-3})								
	0.011∗[3] (0.0086, 0.013)								
Diet	**1.08x10^{-5}** (2.68x10^{-6}, 1.89x10^{-5})	-4.04x10^{-7} (-9.45x10^{-7} , 1.37x10^{-7})	**6.43x10^{-4}** (0.00033, 0.00096)		**0.060** (0.039, 0.082)	**0.0079** (0.0043, 0.012)		**-1.26x10^{-6}** (-1.66x10^{-6}, -8.58x10^{-7})	**-2.19x10^{-}** (-2.72x10 -1.66x10^{-}

Fecundity	**-0.006** (-0.010, -0.0022)			**-0.46** (-0.68, -0.24)					
H. tvaerminnensis									
Infection status (intercept)	0.0023 *[1] (-0.0011, 0.0056)	1.25x10-4 (-2.78x10^{-4}, 5.28x10^{-4})	-2.47x10^{-3} (-0.24, 0.23)	0.065 (-0.13, 0.26)	-1.35 (-23.50, 20.79)	-0.46 (-4.15, 3.24)	0.00032 (-0.00065, 0.0012)	**-3.52x10^{-4}** (-6.83x10^{-4}, -2.061x10^{-5})	-5.019x10 (-4.65x10 3.64x10^{-5}
	5.76x10^{-3} *[2] (-2.84x10^{-4}, 1.18x10^{-2})								
	0.00015*[3] (-.0032, 0.0035)								
Diet	3.00x10^{-6} (-6.82x10^{-6}, 1.28x10^{-5})	-5.41x10^{-7} (-1.20x10^{-6}, 1.14x10^{-7})	1.96x10^{-4} (-0.00019, 0.00058)		**0.048** (0.010, 0.086)	0.0047 (-0.0016, 0.011)		**-1.11x10^{-6}** (-1.68x10^{-6}, -5.42x10^{-7})	**-1.49x10** (-2.17x10 -8.17x10
Fecundity	-0.001 (-0.010, 0.0068)			**-0.62** (-0.99, -0.24)					
P. ramosa									
Infection status (intercept)	**0.0058***[1] (0.0025, 0.0090)	**5.86x10^{-4}** (1.98x10^{-4} 9.74x10^{-4})	0.175 (-0.052, 0.40)	**-1.052** (-2.03, -0.074)	**18.79** (2.82, 34.77)	0.73 (-1.93, 3.40)	**0.0016** (0.00077, 0.0025)	**-8.47x10^{-4}** (-1.14x10^{-3}, -5.53x10^{-4})	**-7.80x10** (-1.16x10 -3.99x10

	$9.91x10^{-3}$ *[2] ($4.09x10^{-3}$, $1.57x10^{-2}$)							
	0.0011 *[3] (-0.0068, 0.0091)							
Diet	$1.23x10^{-6}$ ($-8.16x10^{-6}$, $1.06x10^{-5}$)	**$-1.31x10^{-6}$** ($-1.94x10^{-6}$, $-6.83x10^{-7}$)	$-6.52x10^{-5}$ (-0.00043, 0.00030)		**0.064** (0.040, 0.088)	**0.0078** (0.0038, 0.012)	**$-8.14x10^{-7}$** ($-1.25x10^{-6}$, $-3.76x10^{-7}$)	**$-1.55x10^{-7}$** ($-2.16x10^{-6}$, $-9.30x10^{-7}$)
Fecundity	-0.0066 (-0.025, 0.012)			**-4.11** (-6.39, -1.82)				

Superscripts:*[1] - intercept for infection status (intercept only) model, *[2] – intercept for diet model, *[3] – intercept for fecundity model

Appendix C: Supplementary information for chapter 3.

Figure 13: Example of one of the rock pools sampled to quantify *Hamiltosporidium tvaerminnensis* prevalence among *Daphnia magna* populations near the Tvärminne Zoological Station along the Skerry islands of the Baltic Sea.