LINKING HOST-PARASITE INTERACTIONS AND ECOSYSTEM PROCESSES WITH ENERGY AND ELEMENTS

by

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ABSTRACT OF THE DISSERTATION

Linking host-parasite interactions and ecosystem processes

with energy and elements

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Parasitism is an incredibly common consumer strategy, yet the connections between the host-parasite interactions that are ubiquitous in natural ecosystems and the large-scale ecological processes that shape these systems are poorly understood. In this dissertation, I explored the utility of energy (Chapter 1) and elements (Chapters 2-4) as conceptual currencies to link host-parasite interactions with ecosystem processes.

In Chapter 1, I took a first step toward understanding the ecosystem-scale energetics of parasitism by measuring the biomass density of all major consumer groups, including macroparasites infecting fish and macroinvertebrates, in streams of the New Jersey Pine Barrens. In contrast to prior studies that reported parasite biomass densities as high as those of major free-living groups in other types of aquatic ecosystems, parasites made up a very small fraction of total consumer biomass in Pine Barrens streams. I compiled data from similar studies using this approach and found that high variability in parasite biomass density within and

among aquatic ecosystem types is likely due to both methodological differences and strong effects of abiotic ecosystem characteristics on parasite biomass.

In Chapter 2, I began to explore the ecological stoichiometry of parasitism by measuring the elemental content of a diverse assemblage of macroparasites and asking whether taxonomy or traits predicted variation in organismal stoichiometry. Parasites varied more than ten-fold in N:P ratios across taxa, which likely indicates differences in the balance of N relative to P that may limit parasite growth. While taxonomic group was not related to variation in elemental content across parasite taxa, key functional traits predicted this variation in a manner consistent with stoichiometric theory. Variation in parasite organismal stoichiometry across taxa likely represents diversity in ecological function and response to changes in resource quality.

In Chapter 3, I used a stoichiometric framework to test the effects of environmental nutrient availability on host-parasite interactions and to describe the nutrient dynamics underlying these interactions. I conducted a laboratory experiment to test the effects of abiotic P concentrations on an acanthocephalan parasite and its isopod host, and I found that nutrient treatments did not alter the growth or stoichiometry of hosts or parasites. Across experimental treatments, infected isopods were lower in P content and more balanced with their dietary resources than uninfected isopods. Parasites obtained the largest body sizes when host P content exceeded that of parasite tissue, which may suggest that parasites are P limited in this system. Understanding these natural patterns in host-parasite

nutrient dynamics will aid predictions on the effects of basal resource quality on host-parasite interactions.

In Chapter 4, I explored the relationships between patterns of macroparasite infection and the ways in which hosts store and recycle nutrients. I sampled three populations of freshwater fish that were infected with diverse parasite communities and measured the body size, tissue stoichiometry, and excretion chemistry of individual hosts. Host body size was the best predictor of both nutrient storage and recycling among individuals, but infection was also related to host nutrients for several host-parasite species pairs. These results suggest that infection played a small role in creating heterogeneity in the storage and recycling of nutrients within the populations sampled, but these effects were highly variable across host-parasite species pairs.

These four chapters provide an introduction to several conceptual and empirical avenues by which host-parasite interactions can be linked to ecosystem processes. Both energy and elements are useful currencies to bridge the disciplinary gap between parasite ecology and ecosystem ecology, and opportunities to generate additional data and theory on this theme provide a promising research frontier.

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INTRODUCTION

Parasitism is an incredibly common consumer strategy, yet the connections between the diverse and abundant parasites that are ubiquitous in natural ecosystems and the large-scale ecological processes that shape these systems are poorly understood (Thomas et al. 2005, Ostfeld et al. 2008, Hatcher et al. 2012). This dissertation addresses two fundamental sets of questions on this theme. First, how do abiotic ecosystem characteristics influence the nature of host-parasite interactions, and how will these interactions respond to environmental change? Second, what roles do parasites play in the structure and function of ecosystems? I view the persistent lack of clarity on these topics as a phenomenon resulting from the absence of an appropriate conceptual framework to link large-scale ecosystem processes with microscopic organisms that are easily overlooked. In the following four chapters, I explore the utility of energy (Chapter 1) and elements (Chapters 2-4) as conceptual currencies to link host-parasite interactions with ecosystem processes.

One recent approach to estimating the functional importance of parasitism is to compare the densities of all parasitic and free-living organisms in an ecosystem using biomass as a common unit (Kuris et al. 2008). The distribution of biomass among taxa reflects the flow of energy and matter through ecosystems, so comparing the biomass pools of consumer groups has long been used as a simple proxy to describe relative, functional importance of groups of organisms (Lindeman 1942, Bar-On et al. 2018). Prior studies using this approach have reported parasite biomass densities as high as those of major free-living groups in aquatic ecosystems

(Kuris et al. 2008, Preston et al. 2013, Lagrue and Poulin 2015), which would suggest a functional importance of parasitism at the ecosystem scale.

In Chapter 1, I describe my seasonal measurements of free-living and parasitic consumer biomass density in streams of the New Jersey Pine Barrens. I also present a compilation of data from similar studies using this approach in aquatic ecosystems, review the methods used in these studies, and argue for a consistent and transparent methodology for future research. While this type of descriptive study provides a first step for evaluating relationships between parasitism and ecosystem properties, I emphasize that a more mechanistic approach is needed to link these topics.

Throughout the remainder of the dissertation, I explore the utility of ecological stoichiometry as a conceptual framework to mechanistically link host-parasite interactions with ecosystem processes, especially nutrient cycling. I argue that ecological stoichiometry provides a powerful framework to do just this, but that it has been underutilized in the context of parasite ecology and evolution. Ecological stoichiometry links organismal traits, species interactions, and ecosystem-level nutrient cycling by simplifying the biochemical complexity of organisms and their interactions into ratios of key chemical elements (most often carbon, nitrogen, and phosphorus) that are essential to all of life (Sterner and Elser 2002). The same elements that function as key components of biological macromolecules also limit the growth of individual organisms and populations, influence species interactions, and are important at the scale of nutrient cycling in ecosystems. For this reason, ecological stoichiometry has been identified as a unifying framework for biology

with the power to link processes from genes to ecosystems (Elser et al. 2000, Sterner and Elser 2002, Elser 2006).

This approach was developed through the seminal work of limnologists who demonstrated mechanistic links between zooplankton consumers, their algal resources, and ecosystem-level nutrient dynamics in lakes (Sterner and Elser 2002), and it has seen a recent expansion as ecologists apply and test this framework with fundamental and applied research in a variety of systems (Hessen et al. 2013). Despite this expansion, stoichiometric theory has rarely been extended to parasite ecology and evolution. My interest in bridging this gap is twofold. First, given the ubiquity of parasitism, it is essential to evaluate stoichiometric paradigms in the context of host-parasite interactions if this approach is to be called a unifying framework for biology. Second, stoichiometric theory has strong potential to contribute to the study of parasite ecology and evolution as a framework to link processes across scales.

Prior studies have demonstrated that environmental nutrient availability may influence the success of resource-limited parasites (Clasen and Elser 2007, Frost et al. 2008a, Bernot 2013) and that parasites may alter the rates and ratios at which their hosts store and recycle nutrients (Narr and Frost 2015, Mischler et al. 2016). Despite growing interest in the ecological stoichiometry of parasitism, few studies have measured the elemental content of parasites (but see Frost et al. 2008b, Bernot 2013), and none have done so for multiple parasite species. In Chapter 2, I report the range of variation in the organismal stoichiometry of a diverse assemblage of parasite taxa that I sampled from vertebrate and invertebrate

hosts in freshwater ecosystems of New Jersey. In addition to assessing the stoichiometric diversity of parasites across many species, I also test several hypotheses based on stoichiometric theory to determine whether taxonomic group or functional traits predict parasite elemental composition. Understanding the range of variation present in parasite organismal stoichiometry and the factors driving this variation will be useful for understanding interspecific differences in parasite nutritional demand, the magnitude of resource extraction from hosts, and the functional importance of parasitism to nutrient recycling.

In Chapter 3, I ask how environmental nutrient availability shapes infection patterns and what nutrient dynamics underlie host-parasite interactions. It is largely unknown how parasites and pathogens respond to changes in environmental nutrients (Smith 2007), a topic that is especially relevant in the context of widespread changes to nitrogen and phosphorus cycling caused by human activities (Carpenter et al. 1998, Smith and Schindler 2009). While there are many observational accounts of how nutrient enrichment impacts disease in human and wildlife hosts (McKenzie and Townsend 2007, Johnson et al. 2010, Budria 2017), these studies often lack a mechanistic description of how abiotic nutrient availability leads to changes in infection patterns. I present the results of a laboratory experiment that I conducted to test the effects of basal resource quality on host-parasite interactions and to assess naturally occurring patterns in nutrient dynamics between parasites and hosts.

Just as environmental nutrients have the potential to shape host parasite interactions, parasites may also mediate ecosystem nutrient cycling indirectly

through effects on their hosts (Narr and Frost 2015, Mischler et al. 2016). In Chapter 4, I explore the utility of a stoichiometric framework to describe the relationships between parasite infection and the rates and ratios at which hosts store and recycle nutrients. I sampled populations of freshwater fish that each serve as hosts to diverse parasite communities to test hypotheses about the relationships between infection, host body size, organismal stoichiometry, and excretion chemistry that occur in natural host populations.

Overall, these four chapters introduce several conceptual and empirical avenues by which host-parasite interactions can be linked to ecosystem processes. Both energy and elements are useful currencies to bridge the disciplinary gap between parasite ecology and ecosystem ecology, and opportunities to generate additional data and theory on this theme provide a promising research frontier.

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CHAPTER 1

LOW PARASITE BIOMASS IN OLIGOTROPHIC STREAMS DIFFERS FROM PREVIOUS ESTIMATES IN AQUATIC ECOSYSTEMS *

Abstract

Parasites may mediate ecosystem functioning through a number of direct and indirect mechanisms, but the importance of parasitism at the ecosystem scale is poorly understood. Measuring the density of free-living and parasitic consumers in units that are directly comparable provides a first step toward understanding the importance of parasitism to ecosystem processes. I sampled 2 streams in the New Jersey Pine Barrens seasonally for 1 y to measure the biomass density of all major consumer groups, including macroparasites infecting fish and macroinvertebrates. Parasites made up a small percentage of consumer biomass in Pine Barrens streams, representing just 0.00643 to 0.00733% of total consumer biomass annually. These low values contrast with higher estimates from other aquatic ecosystems, where parasite biomass exceeds that of some free-living consumers. The mean biomass densities of all consumer groups differed significantly between the 2 streams, perhaps because of stream characteristics, such as productivity or pH. Comparison of parasite biomass density in these 2 streams with that in 3 other types of aquatic ecosystems reveals substantial variation both within and among ecosystem types. Methodological differences among published studies complicate comparisons of

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parasite biomass across ecosystems. I reviewed the methods used in previous studies on parasite biomass and argue for a consistent and transparent method for future research. Comparing the biomass of free-living and parasitic consumers is a first step toward understanding the ecosystem-level importance of parasitism, but more work is needed to understand the specific mechanisms by which parasites influence ecosystem processes and the magnitude of parasite effects.

Introduction

Parasites are diverse and ubiquitous in nature, but their cryptic presence commonly leads to their omission from studies of the organization of communities and ecosystems (Thomas et al. 2005). Over the last decade, parasites have been recognized increasingly as an important functional group that plays an influential role in communities by altering the trophic interactions of hosts (Bernot and Lamberti 2008, Sato et al. 2012) and the structure of food webs (Lafferty et al. 2008). Despite these advances, many questions about the ecosystem-level consequences of parasitism remain unanswered (Ostfeld et al. 2008, Hatcher et al. 2012). For example, the degree to which parasites influence overall ecosystem energetics, productivity, or nutrient cycling and how this influence varies among systems are not known (Preston et al. 2016).

The distribution of biomass among taxa reflects the flow of energy and matter through ecosystems (Lindeman 1942), and comparing the relative biomass of parasitic and free-living organisms is one approach to approximating the functional importance of parasitism at the ecosystem scale. The first report of

ecosystem-level biomass distribution that included parasites was remarkable in that parasites accounted for large amounts of total consumer biomass in estuaries on the Pacific coast of North America. In these ecosystems, parasite biomass density exceeded the standing stock biomass of some free-living consumer groups, including the birds that serve as top predators (Kuris et al. 2008). The study did not address the mechanistic roles that parasites might play in these estuarine ecosystems, but the report of high parasite biomass density suggested that parasites might mediate substantial components of ecosystem functioning.

Comparative biomass studies across ecosystems very rarely include parasites, so whether high parasite biomass is common or specific to the type of system studied by Kuris et al. (2008) is not known. Subsequent studies of California ponds (Preston et al. 2013) and New Zealand lakes (Lagrue and Poulin 2015c) reported parasite biomass densities roughly one order of magnitude lower than what was estimated in the Pacific estuaries, but still exceeding the biomass of some free-living groups. In addition to differences in the overall density of parasite biomass among systems, these studies revealed substantial variation in parasite biomass distribution among various taxa and life-cycle stages. As data accumulate, they will provide opportunities to test hypotheses concerning relationships among ecosystem characteristics, constraints on parasite density, and the functional importance of parasitism at the ecosystem scale.

Previous studies on parasite biomass took place in relatively productive ecosystems (Kuris et al. 2008, Preston et al. 2013, Lagrue and Poulin 2015c). Parasite biomass density has not been reported for oligotrophic ecosystems, where

low nutrients, productivity, and energy flow often correspond to low overall consumer densities (Dodds and Whiles 2010). Low primary productivity may limit energy available to parasites feeding at higher trophic levels, and low host density may impede transmission and limit parasite habitat. However, parasites may still be functionally important in oligotrophic systems if they use large quantities of energy and nutrients or if they indirectly affect ecosystem processes by altering host biology.

I sampled macroscopic, free-living animals and their parasites in oligotrophic streams of the New Jersey Pine Barrens to describe biomass distribution patterns in these relatively simple food webs. Pine Barrens streams are characterized by low pH, dissolved nutrients, and primary productivity, but a water-quality gradient exists between 'pristine' examples of this stream type and those disturbed by watershed development (Zampella 1994, Zampella et al. 2001). The acidity of Pine Barrens streams leads to low densities of mollusks (Patrick et al. 1998), the hosts of much of the parasite biomass reported in prior studies (Kuris et al. 2008, Preston et al. 2013). Previous work in Pine Barrens streams indicates that parasite prevalence is high in fish (Hernandez et al. 2007), parasitism is important to food web structure (Hernandez and Sukhdeo 2008b), and parasitism can modulate ecosystem functioning indirectly through effects on host biology (Hernandez and Sukhdeo 2008a).

I conducted standardized, seasonal sampling of fish and macroinvertebrates to measure the standing stock biomass of all consumers in Pine Barrens streams. I dissected all free-living individuals collected to quantify the abundance and biomass

of their macroparasites. The conversion of these data to standing-stock biomass density estimates for stream ecosystems allowed me to make comparisons among taxa, streams, and seasons. I also compared parasite biomass density from this system to published values from several other aquatic ecosystems and evaluated the potential influence of sampling methods on apparent differences among ecosystems. Last, I assessed the potential for indirect effects of parasitism on ecosystem functioning in Pine Barrens streams by summarizing infection using ecological (host density) and parasitological (prevalence and intensity) variables.

Methods

Study area

I sampled 2 streams in the Mullica River basin of the New Jersey Pine Barrens from August 2013 to July 2014. The streams are small and have low flow rates, with a sandy substrate and high levels of allochthonous inputs. The streams are 7.7 km apart at the points sampled but differ in water quality because of differences in surrounding land use. Skit Branch (global positioning system coordinates: 39.767215 N, 74.676949 W) drains predominantly protected pine forest within Wharton State Forest and is characterized by low pH, conductivity, and dissolved nutrients. Muskingum Brook (39.817195 N, –74.737765 W) lies outside the preserved area and drains a mixture of agricultural, developed, and forested land. Relative to Skit Branch, Muskingum Brook has higher pH and dissolved nutrients (Morgan and Good 1988, Zampella et al. 2001).

Sampling protocol

The sampling area at each stream was a 100-m reach, split into five 20-m transects. To minimize disturbance of adjacent transects, I sampled downstream transects before moving upstream. I sampled macroinvertebrates every 3 mo by taking 1 Hess sample (0.1 m^2) in a haphazardly chosen area of the stream bed and 1 D-net sample (0.3 m^2) along the stream bank at each transect (Hauer and Resh 2006). I sampled fish monthly by seining continuously in each transect for 10 min (0.32-cm-mesh seine), and I collected every fish caught during this time. This sampling scheme led to the collection of 40 macroinvertebrate samples (10/season) and 60 fish samples (15/season) for each stream. I froze all samples on the day of collection and thawed them at a later date for processing.

Biomass measurements

I rinsed macroinvertebrate samples through a 500-μm sieve to remove fine benthic matter and scanned entire samples visually to remove all macroinvertebrates from stream detritus. I identified each free-living organism to species (fish) or family (macroinvertebrates) and then dissected all individuals to remove, identify, and count macroparasites present in all tissues. I identified all metazoan macroparasites to the lowest taxon possible based on morphology (Schell 1985, Hoffman 1999). I included all metazoan macroparasites detected in the samples. I did not quantify bacterial, fungal, and protozoan parasites. I did not count monogenean parasites infecting the gills of several fish species because detachment during sample freezing prevented reliable quantification of this group.

To measure fish biomass, I removed all parasites and intestinal contents, dried remaining tissues at 60°C for ≥3 d, and weighed each fish individually. For free-living invertebrate biomass, I used published length–mass regressions (Benke et al. 1999, Méthot et al. 2012) to estimate the dry mass of each individual from its measured length. For mollusks, this dry mass estimate included shell mass. I measured parasite biomass by pooling parasites on preweighed, fiberglass filters (Whatman GF/F), drying at 60°C, and weighing with a microbalance (±0.0001 mg). I calculated the mean individual mass of each parasite species and stage, then used this mean value for stream density estimates.

Biomass density estimates

I converted the dry mass values for fish, macroinvertebrates, and parasites collected at each stream, transect, and season to consumer biomass density (mg dry mass/m^2 of stream). Some key assumptions were necessary for this conversion. First, I used an estimated 5% fish capture rate and seasonal measurements of stream transect area to estimate the total dry mass of fish/stream transect for each season. I applied the same transformation to parasite stages infecting fish. The assumption of a 5% fish capture rate provided a conservative estimate of fish density and reflected the likelihood that many fish escaped through the ends of the transects during seining. For macroinvertebrates and their parasites, I calculated biomass density as the quotient of dry mass collected in each sample and sampler area. Last, I used bootstrapping (Manly 2007) to estimate the means and confidence limits of parasite contribution to total consumer biomass for each stream and

season.

Comparison with other systems

To compare the results of this study with those of 3 other aquatic systems where parasite biomass density has been measured, I compiled data on the mean biomass density of parasites (mg/m^2) and the total % consumer biomass made up of parasites from previously published studies (Kuris et al. 2008, Preston et al. 2013, Lagrue and Poulin 2015b).

Infection levels and biomass of parasitized animals

I classified free-living consumer biomass according to infection status to represent the overall portion of consumer biomass with the potential to be directly affected by parasitism. I grouped host individuals as infected (harboring ≥1 parasites) or uninfected, then calculated the biomass density for each group. I also calculated the prevalence (% hosts infected with ≥1 parasite individuals) and mean intensity (mean number of parasites/infected host) (Bush et al. 1997) for each host–parasite species pair in the data set to represent infection with traditional parasitological variables.

Results

Data set description

The data set produced from this sampling scheme contained temporal and spatial abundances of all free-living organisms >500 μm and their parasites (Table

1). Overall, I collected 13 fish species, 46 macroinvertebrate families (insects, crustaceans, and mollusks), and 9 macroparasite species (Table S1). Many parasite species were observed infecting multiple host species (Table 2), a pattern that reflects complex life cycles and low host specificity.

Biomass densities of free-living and parasitic consumers

All major groups of free-living consumers collected surpassed parasites in total biomass density (Fig. 1, Table S2). The mean biomass densities of parasites differed between streams: 0.16441 ± 0.027195 mg/m^2 (SE) in Muskingum Brook and 0.08572 ± 0.01878 mg/m^2 in Skit Branch. The overall % consumer dry mass made up of parasites did not differ between streams: 0.00733% (95% CI = 0.00462–0.01112) at Muskingum Brook and 0.00643% (95% CI = 0.00312–0.0117) at Skit Branch. All consumer groups fluctuated slightly in biomass density among seasons, but the total % consumer biomass composed of parasites did not show large seasonal variation (Fig. 2A, B).

Parasite biomass distribution

The distribution of parasite biomass among taxa varied considerably between streams (Fig. 3). All 4 major groups of macroparasites (trematodes, cestodes, acanthocephalans, and nematodes) contributed substantially to parasite biomass density at Muskingum Brook. Nematodes made up most of the parasite biomass at Skit Branch, where cestodes were entirely absent. In both streams, nearly all parasite biomass occurred in fish hosts, for which infection levels varied

among host–parasite species pairs but were generally high (Tables S3, S4). Consistently low infection levels in macroinvertebrate hosts (Table S5) corresponded to a low portion of overall parasite biomass contained in these hosts.

Comparing parasite biomass across aquatic ecosystems

The density of parasite biomass varied substantially within and among ecosystem types, and the values reported here for Pine Barrens streams are lower than those for any other system studied (Fig. 4A, B). Differences in sampling and reporting methods probably contributed to some of the observed differences in biomass density among systems. Wet mass was reported in 2 data sets (Kuris et al. 2008, Lagrue and Poulin 2015c), and dry mass was reported in 2 others (Preston et al. 2013, my study). One way to compare parasite biomass across systems while avoiding some methodological bias is to consider the % total consumer biomass made up of parasites (% parasite biomass; Fig. 4A). An alternative way to compare parasite biomass among systems is to express the spatial density of parasite biomass (biomass density; Fig. 4B). Both measures show that Pine Barrens streams have the lowest parasite biomass values of any system studied. The Pacific estuaries that were the original focus of this type of research surpass all other ecosystem types in both measures of parasite biomass.

Infection levels and biomass of parasitized animals

Free-living organisms harboring ≥1 macroparasites made up a large portion of biomass in both Pine Barrens streams (Fig. 5A, B). Parasitized individuals made up

29.234% (95% CI = 14.27–50.791) of free-living consumer biomass at Muskingum Brook and 21.476% (95% CI = 8.793–43.366) of free-living biomass at Skit Branch. The mass of parasitized fish exceeded that of unparasitized fish in both Muskingum Brook (82.474% fish biomass parasitized) and Skit Branch (71.08% fish biomass parasitized). All other consumer groups (insects, mollusks, and crustaceans) were higher in unparasitized biomass than in parasitized biomass at Muskingum Brook, and parasitized individuals of some groups were not collected at Skit Branch. Expressing host density according to infection status provides a summary of parasitized biomass in Pine Barrens streams, and infection prevalence (Tables S3–S5) and intensity (Tables S5–S7) provide additional information on each host–parasite pair.

Discussion

Parasite biomass density among ecosystems

Descriptive studies comparing the standing stock biomass densities of free-living and parasitic consumers provide a first step toward understanding the functional importance of parasitism to ecosystem processes. Food web studies including parasites describe the importance of parasitism to trophic interactions at the community level (Hernandez and Sukhdeo 2008b, Lafferty et al. 2008), but biomass studies provide an estimate for the proportion of energy and materials used by parasites at the ecosystem scale (Kuris et al. 2008). In Pine Barrens streams, parasites make up a very small portion of consumer biomass (0.00643–0.00733% of total consumer standing stock biomass annually; Fig. 1). Low parasite density was

consistent for both streams throughout the 4 seasons sampled (Fig. 2A, B).

The overall percentage of parasite biomass was very similar between streams, but the mean biomass density of parasites at Muskingum Brook was nearly double that of Skit Branch. All free-living consumer groups were similarly higher in biomass density in Muskingum Brook than in Skit Branch (Fig. 1). These substantial differences may relate to differences in the pH and overall productivity of the 2 ecosystems. The streams were sampled at points just a few kilometers from one another and are very similar in size and flow rate, but differences in surrounding land use contribute to substantial abiotic differences between the streams. Both streams are relatively oligotrophic, as is typical of the Pine Barrens, but agriculture and development surrounding Muskingum Brook have led to the elevation of its pH, conductivity, and dissolved nutrients relative to Skit Branch (Zampella 1994, Zampella et al. 2001). The effects of pH and productivity probably work in concert to limit consumer biomass in Pine Barrens streams, but the individual effects of these variables are unknown. For example, low pH at Skit Branch may limit the density and diversity of mollusks and isopods, acid-intolerant groups that are the obligate intermediate hosts for trematodes and acanthocephalans, respectively. Alternatively, if consumer and resource densities are coupled across space and time, parasites may track the densities of their host resources (Sonnenholzner et al. 2011, Lagrue and Poulin 2015a). Higher overall resource availability for the free-living community at Muskingum Brook may contribute to a dense and stable resource pool for parasites, thereby leading to a higher parasite density relative to Skit Branch.

A comparison of Pine Barrens streams to 3 other types of aquatic ecosystems

reveals substantial variation in the biomass density and total % consumer biomass made up of parasites within and among systems (Fig. 4A, B). These large differences among systems provide an interesting perspective on questions about ecosystem productivity and biomass density at various trophic levels. The importance of bottom-up mechanisms to the abundance of free-living consumers at various trophic levels is a classic issue in ecology (Lindeman 1942, Odum 1957), but parasitologists do not often take this perspective. How basal ecosystem productivity affects parasites feeding at various trophic levels in food webs generally is unknown, but some evidence exists that parasite density tends to increase when abiotic nutrients are added to aquatic ecosystems (McKenzie and Townsend 2007, Johnson et al. 2010b). A bottom-up explanation for the large differences in parasite biomass density among ecosystems illustrated in Fig. 4A, B may exist, but more work is needed to identify the patterns and mechanisms for such a relationship between primary productivity and parasite density.

The distribution of parasite biomass among hosts provides one explanation for the low parasite density values in Pine Barrens streams compared to other ecosystem types. In Pine Barrens streams, infection prevalence and intensity are very high among some fish hosts (Tables S3–S4, S6-S7), but consistently low among macroinvertebrates hosts (Table S5). As a consequence, nearly all parasite biomass in Pine Barrens streams occurs in fish hosts. This distribution differs from previously sampled ecosystems, where large portions of parasite biomass occurred as larval trematodes in molluskan intermediate hosts (Kuris et al. 2008, Preston et al. 2013, Lagrue and Poulin 2015c). Thus, the low densities of mollusks in Pine

Barrens streams and correspondingly low larval trematode densities are responsible for the stark differences in parasite biomass observed in my study relative to other systems.

Methodological differences among studies

Ecosystem properties probably drive much of the observed variation in parasite biomass densities among systems, but methodological differences may bias comparisons across studies. Many measurements, estimations, and assumptions are necessary to quantify the density of any aquatic organisms at the ecosystem scale. Authors of the 4 studies in which the density of aquatic parasites has been estimated have used a variety of methods, each of which may hinder cross-system comparisons.

One major difference among studies is the method used to estimate the mean individual mass of each parasite taxon and stage, which is then multiplied by population size to scale up to the level of community biomass. To obtain individual parasite mass, investigators have used biovolume estimates (approximating the geometric volume of parasite taxa and multiplying by an expected wet mass density, often 1 g/mL) (Lagrue and Poulin 2015c), direct measurements of parasite mass with a microbalance (Preston et al. 2013), or a combination of the 2 approaches (Kuris et al. 2008). Biovolume estimation carries the assumptions that geometric volumes accurately represent parasite shapes (e.g., cylinders for nematodes and spheres for metacercariae) and that the wet mass density of parasites is consistent across taxa and life-cycle stages. Lambden and Johnson (2013) tested these

assumptions by comparing biovolume estimates with empirical mass measurements for 5 trematode species. A parasite density estimate of 1.1 g/mL fit the data fairly well, but parasite species varied considerably in their % water mass. Thus, use of a constant density value for a diverse parasite assemblage introduces error into biomass estimates, which can have large repercussions when scaled up to the level of community or ecosystem. Direct measurement of parasite mass, as used in my study, removes several assumptions associated with the biovolume approach.

A related issue is the use of wet mass (Kuris et al. 2008, Lagrue and Poulin 2015c) rather than dry mass (Preston et al. 2013, my study) to represent parasitic and free-living taxa. This difference in methods across studies prevents unbiased comparisons among ecosystem types because water mass is included in only some estimates (Fig. 4A, B). Within a system, the use of wet mass to compare biomass density across diverse taxa is also problematic because organism density varies widely (e.g., soft-bodied parasitic taxa vs arthropods and vertebrates with substantial skeletal mass). Measuring dry mass requires only marginally more effort than measuring wet mass, and dry mass more accurately reflects the energetic and material requirements of organisms. For these reasons, I suggest that direct measurements of dry mass be used in future studies attempting to quantify the ecosystem-scale importance of parasitism.

The conversion of individual mass and field-collection data to the simple, ecosystem-scale density estimates presented in these studies necessitates several key assumptions, many of which have not been thoroughly described in past research. In my study, the densities of fish and their parasites were estimated by

assuming a constant capture rate of 5% and scaling by the stream reach area measured on each sampling date. Because I collected every free-living consumer sampled for dissection, I left the ends of each transect open during seining to avoid catching all fish present. The 5% capture estimate reflects the likelihood that many fish escaped the transects during seining, and it provides an arbitrary, conservative estimate of the true density of the fish community. The choice of this capture estimate necessarily biases the comparison of densities across taxa. However, because nearly all of the parasites observed in my study occurred in fish hosts, this assumption does not significantly affect the relative proportions of fish and parasite biomass, nor does it affect the relative proportions of parasitized and unparasitized biomass within taxa. Using higher fish capture rates would decrease estimates of parasite biomass density and % total consumer biomass made up of parasites. Therefore, the density transformation represents an untested assumption, but it does not change the qualitative results of my study. Regardless of the estimate used, parasites in Pine Barrens streams are several orders of magnitude lower in density than any free-living consumer groups, a result that differs substantially from those of prior studies (Kuris et al. 2008, Preston et al. 2013). Similarly, the percentage of total consumer biomass made up of parasites in Pine Barrens streams remains several orders of magnitude lower than the values reported for any other type of ecosystem.

Density estimates for macroinvertebrates and their parasites include the assumption that haphazardly chosen sampling sites were representative of each 20-m transect, ignoring habitat heterogeneity within these areas. The use of published

length–mass regressions to estimate macroinvertebrate dry mass is another potential source of error in my study because length–mass relationships vary depending upon geographical location and ecosystem properties (Benke et al. 1999, Méthot et al. 2012).

Despite the difficulties of estimating consumer biomass density at the ecosystem scale, direct comparisons of the densities of parasitic and free-living organisms are valuable. Beyond their utility in assessing the potential influence of parasitism at the ecosystem scale, these data have been used to empirically test broad concepts, such as the metabolic theory of ecology (Hechinger et al. 2011) and other ecological power laws (Lagrue et al. 2015). Novel data sets from additional study systems will yield greater insights into the ecosystem-level implications of parasitism, but they must be accompanied by methods and assumptions that are carefully chosen and explicitly described.

Ecosystem implications of parasite biomass density

Descriptive biomass studies are a useful first step toward understanding the ecosystem-scale implications of parasitism because the relative biomass of different taxa can be viewed as a proxy for their energetic and material needs. However, the relationship between standing stock biomass and overall biomass production often is not proportional across taxa, so simple biomass measurements may not accurately represent relative differences in the energetic and material needs of different taxa (Dodds and Whiles 2010). This issue has been addressed for free-living macroinvertebrates, where the rate of secondary production is commonly

estimated from measurements of population density and size structure (Benke and Huryn 2006).

Simple methods to estimate production from standing stock biomass have not been developed for parasites, whose cryptic presence and complex life cycles create an empirical challenge for quantifying biomass production at the ecosystem scale. Trematodes in their molluskan hosts often have very high cercarial release rates (Kuris et al. 2008, Thieltges et al. 2008, Preston et al. 2013), and this type of measurement must be expanded for additional life-cycle stages and parasite taxa if we are to understand the full energetic and material costs of parasitism at the ecosystem scale.

The high reproductive output of many parasites is an evolutionary life-history response to the uncertainties of transmission. A relatively small proportion of larvae or eggs released are likely to establish in a new host (Poulin 2006). Unsuccessfully transmitted propagules are lost from the perspective of parasite populations, but they represent a potentially substantial flow of energy and nutrients to other ecosystem components. Once parasite propagules exit a host, they become part of the free-living food web (Morley 2012), where they may become prey for free-living organisms (Johnson et al. 2010a, Thieltges et al. 2013), die and decompose, or enter another ecosystem as allocthonous inputs. Measuring the rates at which parasites contribute to any of these ecological functions is methodologically difficult, but will be essential to mechanistically link parasite production with ecosystem processes. Development of an ecosystem-scale framework that includes parasite production energetics would allow a mechanistic

assessment of the importance of parasitism to ecosystem functioning.

Parasites mediate ecosystem functioning indirectly when their effects on host biology cascade to ecosystem-level processes (Hatcher et al. 2012). Parasite-induced shifts in host ecology may be density mediated (e.g., changing host population dynamics by altering fecundity; Lafferty and Kuris 2009) or trait-mediated (e.g., altering an ecologically relevant host behavior; Sato et al. 2012). At the community level, the functional importance of a parasite population may be disproportionately greater via these cascading effects than its abundance or biomass, consistent with the idea of a keystone species (Paine 1969, Hatcher et al. 2014).

In Pine Barrens streams, the parasite *Acanthocephalus tahlequahensis* reaches up to 40% prevalence in its intermediate host, the isopod *Caecidotea communis* (REP, unpublished data). *Caecidotea communis* is the dominant shredder in Pine Barrens streams, where the breakdown of allocthonous leaf litter is essential to ecosystem energetics. Isopods reduce their rate of detritus shredding when infected with *A. tahlequahensis*, and this reduction has potential to affect overall ecosystem functioning (Hernandez and Sukhdeo 2008a). *Caecidotea communis* and *A. tahlequahensis* occurred at low densities in my study. However, given the ecological function of *C. communis* and the effect on this function by *A. tahlequahensis,* this example illustrates the potential for parasites to have a disproportionally large effect on ecosystem functioning relative to their small size and low densities.

The indirect effects of other parasitic species on the ecological functioning of

their hosts have not been studied in Pine Barrens streams, but with 20 to 30% of free-living biomass composed of parasitized animals, ample opportunities exist for parasites to mediate ecosystem functioning through their hosts. This statement is especially true for parasites of fish because 71.08 and 82.474% of fish mass is parasitized at Skit Branch and Muskingum Brook, respectively (Fig. 5A, B). Whether and how parasites affect the ecology of fish hosts are generally unknown, though some dramatic examples exist (Gilbert and Granath 2003, Lafferty 2008). Given the importance of fish to many freshwater ecosystem functions (Carpenter et al. 1985, Vanni 2002), the indirect effects of fish parasites on ecosystem processes deserve attention in future research.

Conclusions

Comparative studies that estimate the densities of parasitic and free-living organisms at the ecosystem-scale are useful for trying to understand the ecological function of parasitism (Kuris et al. 2008, Preston et al. 2013) and for testing general ecological theories (Hechinger et al. 2011, Lagrue et al. 2015). However, caution is necessary when generalizing results from past studies or making comparisons among ecosystems. Previous estimates of very high parasite biomass density in some aquatic ecosystems are not universal. Variation in parasite biomass density within and among ecosystem types suggests that ecosystem properties may lead to differences in available energy for parasites, but the patterns and mechanisms are not yet known. Even at low densities, numerous direct and indirect mechanisms exist by which parasites may mediate ecosystem processes, such as productivity,

decomposition, and nutrient cycling (Hatcher et al. 2012, Preston et al. 2016). Additional empirical data involving a greater diversity of parasite taxa, ecosystem types, and ecological processes must be coupled with the development of a theoretical framework to understand the importance of parasitism at the ecosystem scale.

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Chapter 1 Tables and Figures

Table 1.1. Summary of free-living and parasitic consumer taxa collected from two streams in the New Jersey Pine Barrens. For fish and parasites, number of taxa represents species. For insects, crustaceans, and molluscs, number of taxa represents families. Individual larval trematodes in their first intermediate hosts are not included in these counts because they reproduce clonally within the host. Instead, all the clonal parasites in one host are combined as one "individual" for this table.

	Muskingum Brook		Skit Branch	
Consumer group	Number of taxa	Number of individuals	Number of taxa	Number of individuals
Insects	35	6,091	33	2,987
Fish	10	358	8	192
Crustaceans	2	40	1	7
Molluscs	3	348	1	6
Parasites	9	2,362	7	1,621

Table 1.2. Host-parasite associations observed in Pine Barrens streams. Full names of fish hosts are given in Table S1.1.

Parasite taxon	Phylum	Stage	Host species
Acanthocephalus tahlequahensis	Acanthocephala	Cystacanth	*Caecidotea communis*
Acanthocephalus tahlequahensis	Acanthocephala	Adult	*A. sayanus, E. chaetodon, E. obesus, E. oblongus, E. fusiforme, L. gibbosus, U. pygmaea*
Neoechinorhynchus cylindratus	Acanthocephala	Cystacanth	*E. obesus, E. fusiforme, L. gibbosus, L. macrochirus, N. chalybaes, U. pygmaea*
Neoechinorhynchus cylindratus	Acanthocephala	Adult	*E. americanus, E. niger*
Ascaridida 1	Nematoda	Adult	*A. sayanus, E. obesus, E. americanus, U. pygmaea*
Larval nematode 1	Nematoda	Larva	*A. pomotis, A. sayanus, E. chaetodon, E. obesus, E. americanus, E. fusiforme, L. gibbosus, N. chalybaes, N. gyrinus, U. pygmaea*
Larval nematode 2	Nematoda	Larva	*A. pomotis, A. sayanus, E. chaetodon, E. obesus, E. americanus, E. niger, E. fusiforme, L. gibbosus, L. macrochirus, U. pygmaea*
Crepidostomum isostomum	Platyhelminthes	Redia	Sphaeriidae
Crepidostomum isostomum	Platyhelminthes	Metacercaria	Polycentropodidae
Crepidostomum isostomum	Platyhelminthes	Adult	*A. sayanus, E. americanus, U. pygmaea, E. fusiforme*
Phyllodistomum pearsei	Platyhelminthes	Redia	Sphaeriidae
Phyllodistomum pearsei	Platyhelminthes	Adult	*A. sayanus, E. obesus, U. pygmaea*
Posthodiplostomum minimum	Platyhelminthes	Metacercaria	*E. obesus, L. gibbosus, L. macrochirus*
Proteocephalus ambloplitis	Platyhelminthes	Plerocercoid	*N. chalybaes*

Figure 1.1. Mean biomass density (± 1 SE) of free-living and parasitic consumers in Pine Barrens streams.

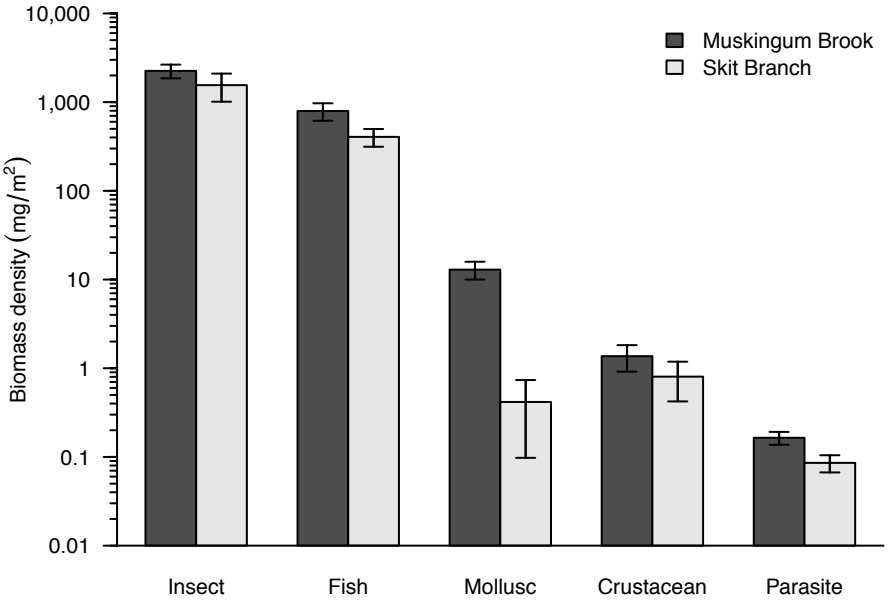


Figure 1.2. Mean seasonal biomass density (± 1 SE) of free-living and parasitic consumers in Muskingum Brook (A) and Skit Branch (B).

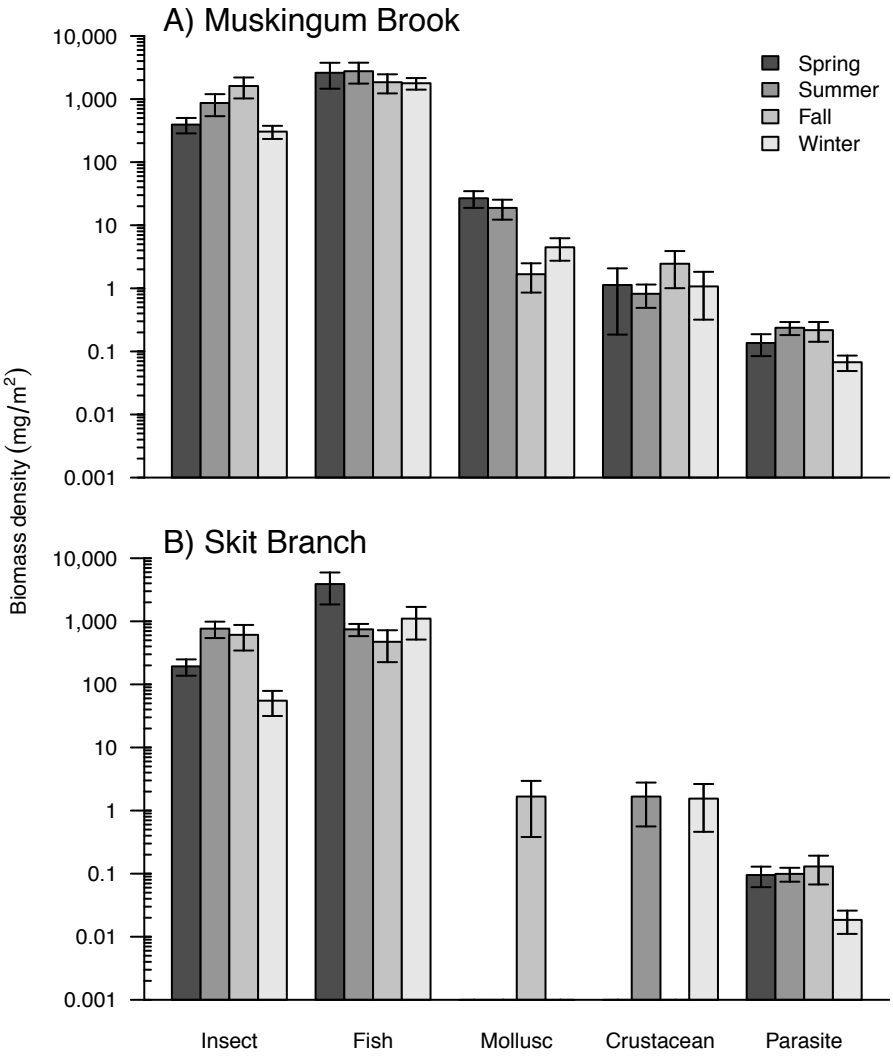


Figure 1.3. Mean biomass density (± 1 SE) for four major parasite groups in two Pine Barrens streams.

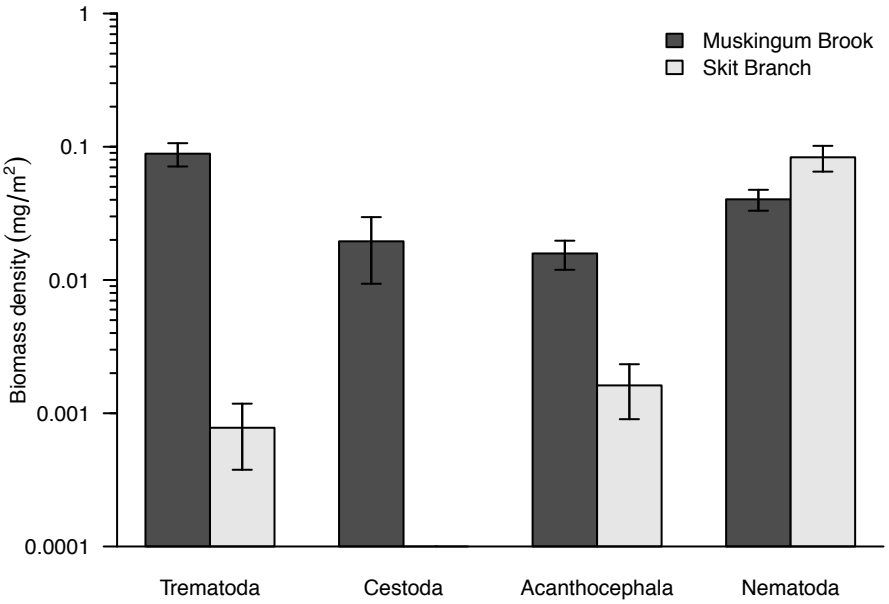


Figure 1.4. Overall percentage of consumer biomass composed of parasites (A) and mean biomass density of parasites (B) in the 4 aquatic ecosystems for which comparative biomass studies have been completed. Data are from Pacific estuaries (Kuris *et al.* 2008), California ponds (Preston *et al.* 2013), New Zealand lakes (Lagrue and Poulin 2015b), and New Jersey streams (present study). Data for the California ponds only reflect trematode biomass, while values from all other systems represent the combined biomass of all macroparasite taxa detected. Percent parasite data (A) were not readily available for the California ponds study. Note that data for the Pacific estuaries and NZ lakes reflect parasite wet mass estimated from a biovolume approach, while data on the CA ponds and NJ streams represent direct measurements of dry mass.

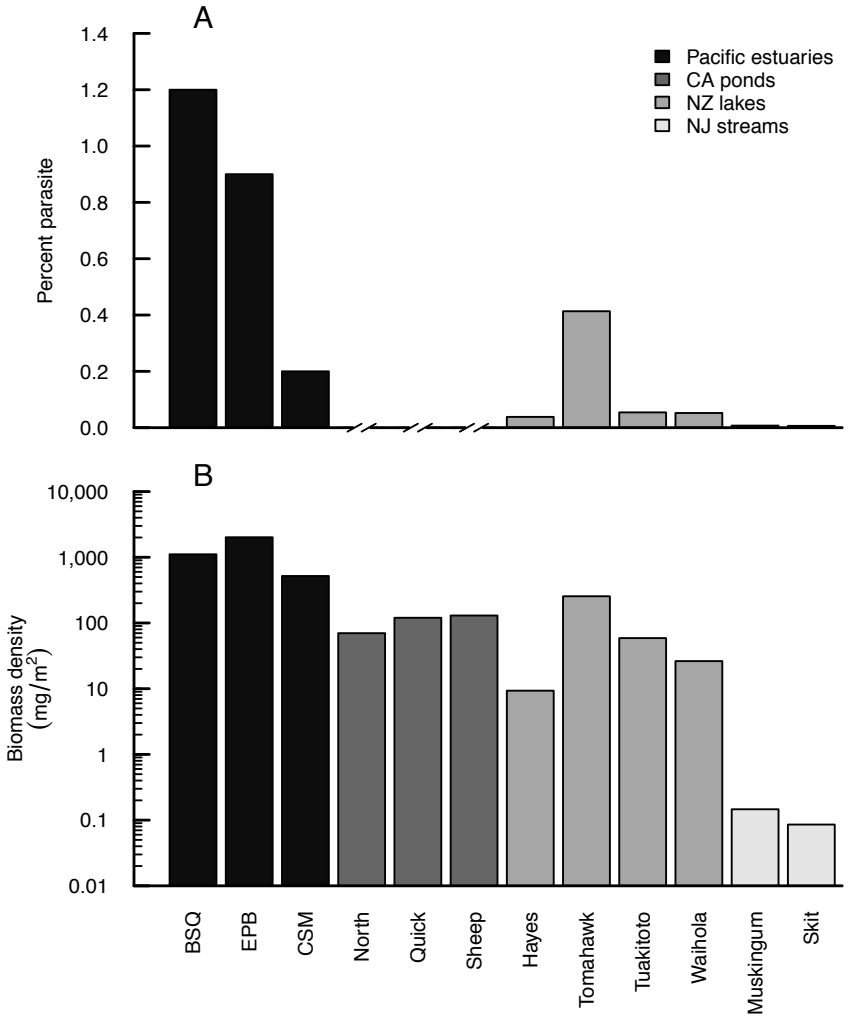
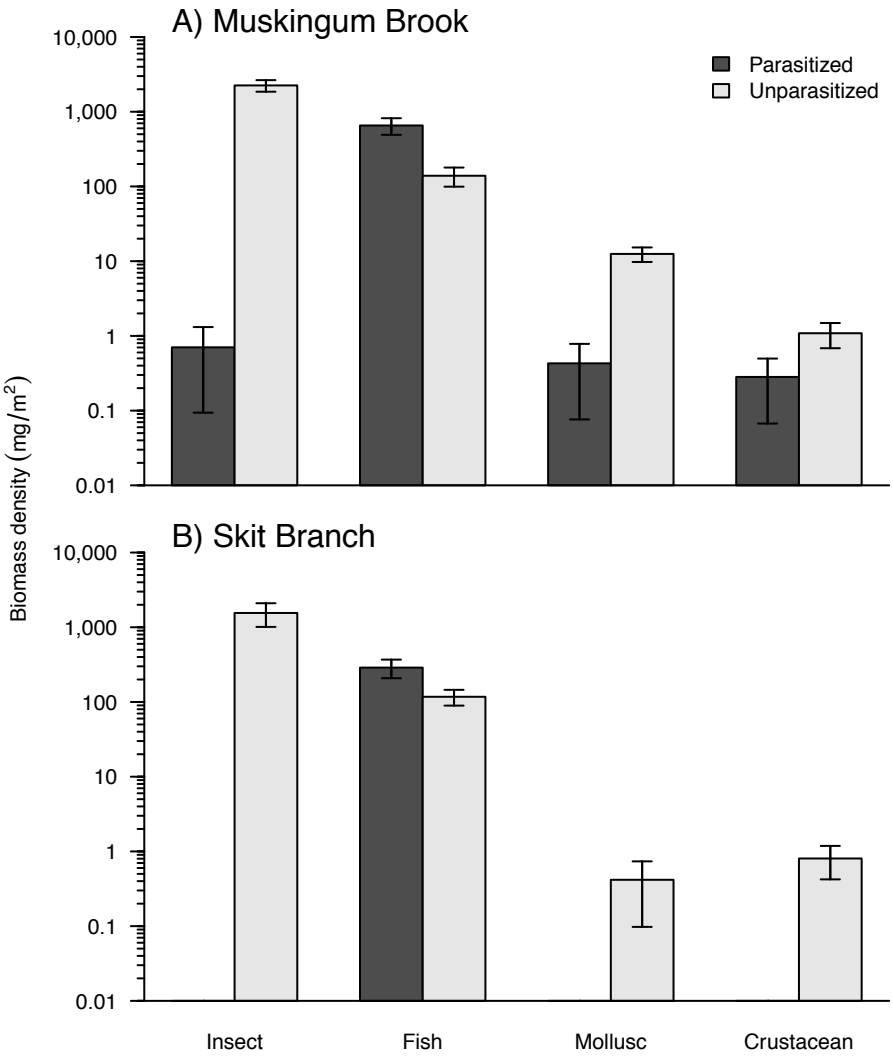


Figure 1.5. Mean biomass density (± 1 SE) of free-living consumers divided by infection status for Muskingum Brook (A) and Skit Branch (B). The biomass of parasitized consumers does not include the mass of their parasites.



Chapter 1 Appendix

Table S1.1. Free-living and parasitic taxa sampled in two Pine Barrens streams. 'X' indicates presence.

Taxon	Group	Muskingum Brook	Skit Branch
Acantharchus pomotis	Fish		X
Acanthocephalus tahlequahensis	Parasite	X	X
Aeshnidae	Insect	X	X
Aphredoderus sayanus	Fish	X	X
Ascaridida 1	Parasite	X	X
Asellidae	Crustacean	X	X
Baetidae	Insect	X	
Brachycentridae	Insect	X	X
Calopterygidae	Insect	X	X
Ceratopogonidae	Insect	X	X
Chironomidae	Insect	X	X
Coenagrionidae	Insect	X	X
Cordulegastridae	Insect	X	X
Corduliidae	Insect	X	
Corixidae	Insect	X	
Corydalidae	Insect	X	X
Crepidostomum isostomum	Parasite	X	X
Dytiscidae	Insect	X	X
Elmidae	Insect	X	X
Empididae	Insect	X	X
Enneacanthus chaetodon	Fish		X
Enneacanthus obesus	Fish	X	X
Ephemerellidae	Insect	X	X
Erimyzon oblongus	Fish	X	
Esox americanus	Fish	X	X
Esox niger	Fish	X	
Etheostoma fusiforme	Fish	X	X
Gammaridae	Crustacean	X	X
Glossosomatidae	Insect	X	X
Gomphidae	Insect	X	X
Gyrinidae	Insect	X	X
Haliplidae	Insect	X	
Heptageniidae	Insect	X	X
Hydrophilidae	Insect	X	X
Hydropsychidae	Insect	X	X

Hydroptilidae	Insect	X	X
Larval nematode 1	Parasite	X	X
Larval nematode 2	Parasite	X	X
Lepomis gibbosus	Fish	X	
Lepomis macrochirus	Fish	X	
Leptoceridae	Insect	X	X
Leuctridae	Insect		X
Libellulidae	Insect		X
Limnephilidae	Insect		X
Macromiidae	Insect		X
Molannidae	Insect	X	X
Nemouridae	Insect	X	X
Neoechinorhynchus cylindratus	Parasite	X	X
Notonectidae	Insect	X	
Notropis chalybaeus	Fish	X	
Noturus gyrinus	Fish		X
Odontoceridae	Insect	X	
Perlodidae	Insect		X
Phryganeidae	Insect	X	X
Phyllodistomum pearsei	Parasite	X	X
Physidae	Mollusc	X	
Planorbidae	Mollusc	X	
Polycentropodidae	Insect	X	X
Posthodiplostomum minimum	Parasite	X	
Proteocephalus ambloplitis	Parasite	X	
Pyralidae	Insect	X	
Sialidae	Insect	X	X
Simuliidae	Insect	X	X
Sphaeriidae	Mollusc	X	X
Tabanidae	Insect		X
Tipulidae	Insect	X	X
Umbra pygmaea	Fish	X	X
Veliidae	Insect	X	

Table S1.2. Mean biomass density and standard error for all major consumer groups in two Pine Barrens streams across all seasons and stream transects.

	Muskingum Brook		**Skit Branch**	
	Mean biomass density (mg/m^2)	**SEM**	**Mean biomass density (mg/m^2)**	**SEM**
Insects	2251.5	396.45	1554	543.04
Fish	794.17	176.68	405.83	91.786
Molluscs	12.939	2.9375	0.417	0.3193
Crustaceans	1.3681	0.45336	0.80423	0.38151
Parasites	0.16441	0.027195	0.085722	0.018781

Table S1.3. Infection prevalence for all fish sampled at Muskingum Brook and their parasites. All seasons are combined. N is the number of fish collected of each species.

Fish host	**N**	*A. tahlequahensis (adult)*	*A. tahlequahensis (cystacanth)*	*N. cylindratus (adult)*	*N. cylindratus (cystacanth)*	Ascaridida 1 (adult)	Larval nematode 1	Larval nematode 2	*C. isostomum (adult)*	*P. pearsei (adult)*	*P. minimum (metacercaria)*	*P. ambloplitis (plerocercoid)*
A. sayanus	70	47.14	4.29	0	0	12.86	7.14	37.14	61.43	71.43	0	0
E. obesus	5	0	0	0	60	0	0	80	0	0	100	0
E. oblongus	1	100	0	0	0	0	0	0	0	0	0	0
E. americanus	11	0	0	9.09	0	0	0	0	9.09	0	0	0
E. niger	2	0	0	50	0	0	0	50	0	0	0	0
E. fusiforme	12	16.67	0	0	16.67	0	8.33	33.33	0	0	0	0
L. gibbosus	9	11.11	0	0	100	0	11.11	100	0	0	100	0
L. macrochirus	6	0	0	0	66.67	0	0	50	0	0	100	0
N. chalybaes	6	0	0	0	16.67	0	33.33	0	0	0	0	66.67
U. pygmaea	236	2.54	0	0	0.42	0	8.90	40.25	8.90	22.88	0	0

Table S1.4. Infection prevalence for all fish sampled at Skit Branch and their parasites. All seasons are combined. N is the number of fish collected of each species.

Fish host	**N**	*A. tahlequahensis (adult)*	*A. tahlequahensis (cystacanth)*	*N. cylindratus (adult)*	*N. cylindratus (cystacanth)*	Ascaridida 1 (adult)	Larval nematode 1	Larval nematode 2	*C. isostomum (adult)*	*P. pearsei (adult)*	*P. minimum (metacercaria)*	*P. ambloplitis (plerocercoid)*
A. pomotis	4	0	0	0	0	0	25	50	0	0	0	0
A. sayanus	5	0	0	0	0	0	0	20	20	0	0	0
E. chaetodon	3	33.33	0	0	0	0	100	66.66	0	0	0	0
E. obesus	108	1.85	0	0	0.93	6.48	19.44	65.74	0	1.85	0	0
E. americanus	12	0	0	0	0	8.33	8.33	16.67	0	0	0	0
E. fusiforme	5	0	0	0	0	0	0	20	20	0	0	0
N. gyrinus	2	0	0	0	0	0	50	0	0	0	0	0
U. pygmaea	53	1.89	0	0	0	1.89	1.89	54.72	1.89	1.89	0	0

Table S1.5. Mean infection intensity (mean number of parasites per infected host) for all fish sampled at Muskingum Brook. Numbers in parentheses are standard deviations; (-) indicates that only one fish was infected by a parasite species. Empty cells indicate that a host-parasite interaction was not observed in this stream. X indicates that parasites were present in one sample but the number of individuals was not recorded.

Fish host	*A. tahlequahensis (adult)*	*A. tahlequahensis (cystacanth)*	*N. cylindratus (adult)*	*N. cylindratus (cystacanth)*	Ascaridida 1 (adult)	Larval nematode 1	Larval nematode 2	*C. isostomum (adult)*	*P. pearsei (adult)*	*P. minimum (metacercaria)*	*P. ambloplitis (plerocercoid)*
A. sayanus	6.97 (9.01)	1 (1)			7.11 (5.30)	3 (2.92)	1.92 (0.93)	7.67 (16.10)	5.04 (4.42)		
E. obesus				2 (1)			11 (7.30)			19.6 (21.45)	
E. oblongus	1 (-)										
E. americanus			1 (-)					1 (-)			
E. niger			2 (-)				X				
E. fusiforme	1.5 (0.71)			2 (1.41)		1 (-)	15.75 (13.45)				
L. gibbosus	9 (-)			8.67 (3.74)		1 (-)	19.11 (12.57)			38.33 (61.79)	
L. macrochirus				2 (1.15)			2.67 (2.08)			25.17 (21.23)	
N. chalybaes				1 (-)		1.5 (0.71)					1.5 (0.58)
U. pygmaea	2.83 (2.71)			1 (-)		2 (2.59)	2.07 (1.58)	1.43 (0.75)	2.22 (1.48)		

Table S1.6. Mean infection intensity (mean number of parasites per infected host) for all fish sampled at Skit Branch. Numbers in parentheses are standard deviations; (-) indicates that only one fish was infected by a parasite species. Empty cells indicate that a host-parasite interaction was not observed in this stream.

Fish host	*A. tahlequahensis (adult)*	*A. tahlequahensis (cystacanth)*	*N. cylindratus (adult)*	*N. cylindratus (cystacanth)*	Ascaridida 1 (adult)	Larval nematode 1	Larval nematode 2	*C. isostomum (adult)*	*P. pearsei (adult)*	*P. minimum (metacercaria)*	*P. ambloplitis (plerocercoid)*
A. pomotis						2 (-)	6.50 (7.78)				
A. sayanus							1 (-)	6 (-)			
E. chaetodon	1 (-)					3.67 (3.79)	22 (12.73)				
E. obesus	3.5 (3.54)			1 (-)	1.57 (0.53)	2.38 (1.83)	14.70 (15.44)		1.5 (0.71)		
E. americanus					3 (-)	188 (-)	14 (11.31)				
E. fusiforme							1 (-)	30 (-)			
N. gyrinus						40 (-)					
U. pygmaea	1 (-)				1 (-)	2 (-)	5.48 (5.46)	1 (-)	2 (-)		

Table S1.7. Infection prevalence for all macroinvertebrates sampled at Muskingum Brook and their parasites. All seasons are combined. N is the number of macroinvertebrates collected of each taxon. *A. tahlequahensis* cystacanths and *C. isostomum* metacercariae were observed only at an intensity of 1 (1 worm per infected host). Intensity was not calculated for trematodes in their first intermediate hosts because individual parasites were not counted. Infected macroinvertebrates were not collected from Skit Branch.

Host	**N**	**Parasite**	**Prevalence**
Asellidae	29	*Acanthocephalus tahlequahensis* (cystacanth)	10.34
Polycentropodidae	175	*Crepidostomum isostomum* (metacercaria)	1.14
Sphaeriidae	334	*Crepidostomum isostomum* (redia)	0.6
Sphaeriidae	334	*Phyllodistomum pearsei* (redia)	0.9

CHAPTER 2

ALLOMETRIC AND TRAIT-BASED PATTERNS IN PARASITE STOICHIOMETRY

Abstract

Parasites interact with the cycling of N and P by responding to environmental nutrient availability and by altering host nutrient recycling function. Ecological stoichiometry provides a framework to quantify the exchange of multiple elements in consumer-resource interactions, though this perspective has rarely been applied to parasite-host systems. Measuring the elemental composition of parasite tissues and identifying factors related to variation among species is useful for understanding interspecific differences in parasite nutritional demand, the magnitude of resource extraction from hosts, and the functional importance of parasitism to nutrient recycling. In this study, we measured the elemental content (%C, N, and P) and ratios (C:N, C:P, N:P) of a diverse assemblage of parasitic helminths to ask whether taxonomy or traits were related to stoichiometric variation among species. We sampled 27 macroparasite taxa, spanning 4 phyla, from vertebrate and invertebrate hosts inhabiting freshwater ecosystems in New Jersey. Macroparasites vary widely in elemental content, exhibiting 4.7-fold variation in %N, 4.6-fold variation in %P, and 11.5-fold variation in N:P. Across all species, parasite %P scaled negatively and C:P scaled positively with body size. Similar relationships between parasite P content and body size occur at the phylum level and within individual species. The allometric scaling of P across species supports

the growth rate hypothesis, which predicts that smaller taxa require more P to support relatively higher growth rates. Life cycle stage is related to %N and C:N, with non-reproductive parasite stages lower in %N and higher in C:N than actively reproducing parasites. Parasite phylum, functional feeding group, and trophic level did not explain stoichiometric variation among species. This project is the first to document variation in the organismal stoichiometry of parasites, and the wide variation that we describe is of central importance to understanding relationships between parasitism and nutrient cycling.

Introduction

Parasites interact with the cycling of elements such as N and P through several mechanisms. Environmental nutrient availability may influence the success of resource-limited parasites (Frost, Ebert & Smith 2008a; Bernot 2013), and parasites may alter the rates and ratios at which their hosts store and recycle nutrients (Narr & Frost 2015; Mischler *et al.* 2016). While data on these topics are available for only a few host-parasite species pairs, they suggest species-specific variation in the interactions between parasitism and nutrient cycling (Aalto, Ketola & Pulkkinen 2014; Narr & Frost 2016).

Ecological stoichiometry provides a framework to study the balance of multiple chemical elements across levels of biological organization (Sterner & Elser 2002), though this approach has been applied to host-parasite systems infrequently (Aalto, Decaestecker & Pulkkinen 2015). Under a stoichiometric framework,

consumers are represented by the ratios of elements composing their tissues, and this organismal stoichiometry is an important determinant of nutritional requirements and nutrient recycling function (Sterner & Elser 2002).

Despite growing interest in the ecological stoichiometry of parasitism, few studies have measured the elemental content of parasites (but see Frost et al. 2008b, Bernot 2013), and none have done so for multiple parasite species. Measuring the range of variation in the organismal stoichiometry of diverse parasite taxa and assessing the factors related to this variation are necessary to understand interspecific differences in parasite nutritional demand, the magnitude of resource extraction from hosts, and the functional importance of parasitism to nutrient recycling.

Here we present the first comparative study to report variation in organismal stoichiometry across many macroparasite taxa. We measured the elemental content (%C, N, and P) and molar ratios (C:N, C:P, N:P) of a diverse assemblage of parasitic helminths, then asked whether taxonomy and functional traits were related to stoichiometric variation among species. Prior studies on groups of related, free-living animal species have identified that phylogeny and several key traits correspond to interspecific differences in organismal stoichiometry (e.g. Fagan et al. 2002, Woods et al. 2004, Hendrixson et al. 2007, González et al. 2011). These comparative studies have only been completed for a small number of animal groups, mostly zooplankton, fish, and insects. Parasites make up a large portion of animal diversity (Poulin & Morand 2000; Dobson *et al.* 2008), so identifying factors related to parasite stoichiometry will be useful for

assessing the generality of these patterns for metazoans. We evaluated each of the following variables as potential correlates of parasite stoichiometry.

1) Body size. The growth rate hypothesis predicts that fast-growing organisms require large amounts of ribosomal RNA for protein synthesis, which corresponds to high total organismal P content (Elser *et al.* 1996, 2003). Given the commonly observed negative allometry of specific growth rate with body size across metazoan taxa (Peters 1983), it is also expected that body size and P content will be inversely related for animals that lack P-rich structural investments in bone (Gillooly *et al.* 2005). Relationships between body size and invertebrate stoichiometry have been observed in benthic macroinvertebrates (Cross *et al.* 2003) and terrestrial arthropods (Woods *et al.* 2004; González *et al.* 2011). We tested the hypothesis that across parasite taxa, P content is negatively related to body size, while C:P and N:P are positively related to body size. We also predicted that these relationships would occur among actively growing parasite taxa, but not among encysted larvae.

2) Taxonomic group. Organismal stoichiometry is a product of evolution, and elemental content is one aspect of an organism's phenotype (Kay *et al.* 2005; Leal, Seehausen & Matthews 2017). With this perspective, it follows that groups of related organisms may show similar patterns of structural or metabolic nutrient allocation. Phylogenetic or taxonomic affiliation is related to stoichiometric variation in free-living groups including fish (Hendrixson et al. 2007), benthic macroinvertebrates (Cross *et al.* 2003; Evans-White, Stelzer & Lamberti 2005), and terrestrial arthropods (Fagan *et al.* 2002; Woods *et al.* 2004; González *et al.* 2011).

We sampled macroparasites from four phyla (Acanthocephala, Annelida, Nematoda, and Platyhelminthes) and tested the hypothesis that differences in parasite stoichiometry correspond to phylum.

3) Life cycle stage. Some free-living organisms with distinct developmental stages exhibit ontogenetic variation in organismal stoichiometry (Villar-Argaiz, Medina-Sánchez & Carrillo 2002; Kay, Rostampour & Sterner 2006; Back *et al.* 2008), which may correspond to differences in growth rate or morphology. Many macroparasites have complex life cycles with two or more distinct stages that differ in morphology, feeding strategy, host species, infection site, and trophic level (Combes 2005). Similar life cycle stages across parasite species share traits that may therefore correspond to patterns in organismal stoichiometry. We tested the hypothesis that differences in parasite stoichiometry correspond to life cycle stage. We predicted that stages involved in active growth and reproduction would be higher in N and P than encysted parasites to support protein synthesis.

4) Functional feeding group. Feeding mode corresponds to stoichiometric variation for some free-living invertebrates (Cross *et al.* 2003; Evans-White *et al.* 2005). Analogous to the categorization of benthic macroinvertebrates by functional feeding group (Cummins 1973), parasites use feeding strategies that do not always align with broad taxonomic placement. For example, tapeworms and acanthocephalans occupy two distantly related phyla and evolved parasitic lifestyles independently, but both groups feed absorptively in the intestine of vertebrate hosts as adults. In contrast, all trematodes share a relatively recent common ancestor but employ diverse feeding styles and occupy many infection sites within hosts (Combes

2005). We tested the hypothesis that differences in parasite stoichiometry correspond to functional feeding group.

5) Trophic level. At the community scale, C:P and C:N ratios are generally expected to decrease with increasing trophic level as carbon is lost through heterotroph metabolism, effectively pooling P and N in upper trophic levels (Sterner *et al.* 1998; Boersma *et al.* 2008). Within groups of related organisms, trophic level is an important determinant of organismal stoichiometry in freshwater fish (Hendrixson *et al.* 2007) and terrestrial arthropods (Fagan *et al.* 2002; González *et al.* 2011; Lemoine, Giery & Burkepile 2014). Parasites occupy variable trophic levels depending upon host trophic position, and parasites with complex life cycles occupy multiple trophic levels during an individual lifespan. We tested the hypothesis that parasite %N and %P are positively related to trophic level, and C:N and C:P are negatively related to trophic level.

The aim of this study was to assess broad, interspecific patterns in parasite stoichiometry, but there is also significant potential for intraspecific variation in parasite stoichiometry. Our dataset allowed preliminary analyses of within-species variability for several species, and we tested for intraspecific differences in parasite stoichiometry related to individual body size and life cycle stage.

Materials and Methods

Sample collection

We sampled macroparasites infecting vertebrate and invertebrate hosts from freshwater ecosystems in New Jersey. Upon collection, we euthanized hosts and,

when possible, removed parasites via dissection on the day of collection. In some cases, we euthanized and immediately froze hosts to allow time for dissection at a later date. The New Jersey Division of Fish and Wildlife donated frozen Alewives (*Alosa pseudoharengus*) and Great Blue Herons (*Ardea herodias*). We thawed frozen hosts just prior to dissection to minimize time between thawing and parasite removal. For parasite taxa with large body sizes, we collected individuals in microcentrifuge tubes. For taxa with small body sizes, we pooled parasites by taxon onto ashed, pre-weighed GF/F filters (Whatman). We dried all parasite tissues at 60° C for a minimum of 48 hours, weighed them with a microbalance, and stored them in a desiccator until elemental analysis.

We subsampled parasites for morphological identification and prepared specimens using heat fixation, dehydration in an alcohol series, and staining. Since freezing may obscure parasite morphology, we sampled fresh specimens for identification when possible. When only frozen specimens were available for identification and for parasites that are not easily identifiable by morphology (e.g. some larvae), we identified parasites to the lowest taxon possible. We selected parasite taxa that represent wide variation in taxonomic placement and functional traits, though the practicalities of elemental analysis required that a taxon be either large or common to obtain enough biomass for replicated analysis. For several species with complex life cycles, we followed the common practice of treating distinct developmental stages as separate 'taxa' to reflect the differences in morphology, body size, feeding mode, and host identity that occur within a life cycle (e.g. Hechinger *et al.* 2011; Lagrue, Poulin & Cohen 2015).

We calculated mean body size for each taxon using direct dry mass measurements. For life cycle stage, we grouped taxa into three major stages: adults, reproductively active larvae (trematode sporocysts and rediae), and non-reproductive larvae (trematode metacercariae, cestode plerocercoids, acanthocephalan cystacanths, and larval nematodes). We grouped taxa into four functional feedings groups: absorbers, blood suckers, grazers (parasites feeding directly on host tissue), and encysted (presumed non-feeding). We assigned numeric values for parasite trophic level (2, 3, or 4° consumer) based on community observations. We placed parasites that feed absorptively on host dietary material in the same trophic level as their hosts, and we placed parasites with all other feeding strategies one trophic level higher than their hosts. Table 1 summarizes all parasite taxa collected, including their hosts, collection locations, and traits analyzed.

Elemental analysis

We measured C and N with a Carlo Erba NA 1500 Series 2 elemental analyzer (Verardo, Froelich & McIntyre 1990), and we measured P on a spectrophotometer with the molybdate method following combustion at 500° C and digestion in acid (Solorzano & Sharp 1980). For each taxon, we analyzed 3 to 12 replicates for each of both C and N or P (Table 1). For small taxa on filters, we analyzed the entire pooled sample as one measurement of C and N or P. For large taxa in microcentrifuge tubes, we ground each individual parasite with a mortar and pestle and subsampled for elemental analysis. We use the terms 'P content' and '%P' (or C or N) to describe the

percentage dry mass of each element. We calculated all elemental ratios using molar concentrations, and we use the term 'C:N' (or C:P or N:P) to describe these ratios.

Statistical analysis

We calculated the mean and standard error of elemental content (%C, %N, and %P) for each taxon or group across replicates. For elemental ratios (C:N, C:P, and N:P), we estimated ratio means and standard errors with bootstrapping to account for variation in the measurement of multiple elements across replicates (Manly 2007). We used these mean values as our response variables when testing for relationships between parasite stoichiometry and all other variables (body size, phylum, life cycle stage, functional feeding group, and trophic level).

We used ordinary least-squares regressions on $\log_{10}$-transformed data to assess relationships between stoichiometric variables and body size, and we used analysis of covariance (ANCOVA) to determine whether these relationships varied among phyla. We also ran separate regressions after grouping taxa as 'active' (those sampled during a life cycle stage in which they feed) or 'encysted' (those sampled during a life cycle stage in which they are assumed not to feed). We used ANCOVAs to determine the effects of categorical variables (phylum, life cycle stage, functional feeding group, and trophic level) on stoichiometric response variables while accounting for differences in body size. We evaluated significant differences among treatment groups using Tukey's HSD multiple comparison test ($\alpha = 0.05$).

Finally, we evaluated drivers of intraspecific parasite stoichiometry for several species. We tested for relationships between stoichiometric variables and

individual body size using ordinary least-squares regressions, and we tested for differences between larval and adult forms of individual species using Student's t-tests. We conducted all statistical analyses and generated graphics using R version 3.2.3 (R Core Team 2015).

Results

The mean elemental content of macroparasites sampled varied widely: 35 to 61% for %C, 3 to 13% for %N, and 0.44 to 2.0% for %P (Figure 1). Mean elemental ratios were similarly variable among species, ranging from 3.5 to 15.7 for C:N, 18.9 to 108.0 for C:P, and 1.65 to 18.9 for N:P (Fig. 2). Across all taxa, there was no relationship between mean %N and %P (r^2 = 0.00532, p = 0.72), nor between C:N and C:P (r^2 = 0.000686, p = 0.9). Similarly, these measures of N and P were not related within individual phyla.

Across all taxa, mean %P was negatively related to body size (r^2 = 0.4197, p = 0.0003), and C:P was positively related to body size (r^2 = 0.3551, p = 0.001) (Fig. 3, Table 2). ANCOVA results indicated that these %P-body size and C:P-body size relationships occurred independently of phylum (Table S1). Mean %C, %N, C:N, and N:P were not related to body size across all species (Table 2), although ANCOVA results indicated that N:P-body size scaling was phylum-dependent (Table S1). Within individual phyla, N:P scaled positively with body size for Acanthocephala and Nematoda, and N:P scaled negatively with body size for Platyhelminthes (Fig. 3, Table 2).

Across all taxa actively feeding taxa, %P scaled negatively with body size (r^2 = 0.5613, p = 0.0003), and both C:P (r^2 = 0.5416, p = 0.0005) and N:P (r^2 = 0.3817, p = 0.0063) scaled positively with body size. For the taxa that we sampled as encysted larvae, there were no relationships between stoichiometric variables and body size (Table 2).

Grouping taxa by phylum did not explain any variation in stoichiometric variables after accounting for differences in body size (Table S1). Grouping taxa by life cycle stage and accounting for body size revealed that stage corresponds to differences in %N ($F_{2,21}$ = 4.577, p = 0.0224) and C:N ($F_{2,21}$ = 4.104, p = 0.0313), (Table S2). Encysted parasites were lower in %N and higher in C:N than other life cycle stages (Fig. 4). Grouping taxa by functional feeding group (Sup. Fig. S1) and trophic level (Sup. Fig. S2) did not explain any variation in stoichiometric variables after accounting for differences in body size (Sup. Tables S3-S4).

While the main purpose of this study was to characterize interspecific variation in parasite stoichiometry, we also opportunistically tested for intraspecific variation within several taxa in our dataset. %P scaled with individual body size for several large parasite species that could be analyzed at the individual level, and the parameters of this relationship varied substantially among species (Sup. Fig. S3, Sup. Table S5). Three additional species for which we sampled both larval and adult forms revealed that many stoichiometric variables also differed intraspecifically between life cycle stages. Overall, adults had higher %N and lower C:N than larvae. All three species also varied in P (%P, C:P, and/or N:P) between stages, but there was no consistent pattern across species (Sup. Fig. S4).

Discussion

Our results highlight substantial interspecific variation in elemental composition across macroparasite species, and we identified several traits that correspond to differences in parasite stoichiometry. %P scaled negatively with body size, whereas C:P and N:P scaled positively with body size. Differences in %N and C:N corresponded to life cycle stage, with encysted taxa characterized by lower %N and higher C:N than actively feeding parasites. Phylum, functional feeding group, and trophic level were not related to any stoichiometric variables after accounting for the effects of body size.

Stoichiometric variation among parasite taxa

Parasites exhibited 4.7-fold variation in mean %N and 4.6-fold variation in mean %P, while %C was relatively constant with only 1.8-fold variation across species (Fig. 1). Parasites also varied in elemental ratios across species, with 4.4-fold variation in C:N, 5.7-fold variation in C:P, and 11.5-fold variation in N:P (Fig. 2). Our measured values for elemental content in parasites are similar to those observed in free-living invertebrates (e.g. Cross et al. 2003, González et al. 2011), with the exception of several larval parasites in our dataset that are very low in %N (<4%). Across all species, we observed no relationships between %N and %P or between C:N and C:P. This result differs from the close coupling of N and P that has been observed for several other invertebrate groups such as zooplankton (Sterner & Hessen 1994) and *Drosophila* (Jaenike & Markow 2003). While strong correlations

between N and P support the intriguing hypothesis that the ratios of proteins to nucleic acids are constrained across taxa (Elser *et al.* 2000b; Loladze & Elser 2011), our data do not fit this pattern.

Importance of taxonomy and phylogeny

Contrary to our prediction that phylum would be a major determinant of parasite stoichiometry, we observed no differences in any stoichiometric variables among phyla after accounting for parasite body size. This result is surprising because taxonomic or phylogenetic affiliation are important to stoichiometric variation in free-living invertebrates (Fagan *et al.* 2002; Cross *et al.* 2003; Woods *et al.* 2004; Evans-White *et al.* 2005; González *et al.* 2011). In addition to explaining stoichiometric variation, phylogenetic relatedness can also lead to incorrect observations that life history variables are linked to elemental composition. For example, the application of phylogenetic correction (Felsenstein 1985; Pagel & Harvey 1988) to comparative analyses of stoichiometric variation has negated the significance of traits such as body size (González *et al.* 2011) and trophic level (Hendrixson *et al.* 2007) that appeared to be related to stoichiometric traits before correction.

We acknowledge the importance of phylogenetic correction in comparative studies, but we chose not to apply such a correction to our data for two reasons. First, we sampled parasites from four phyla. These species are linked by a common lifestyle, not by close phylogenetic relatedness. Prior studies that have used phylogenetic correction did so for groups of organisms that were closely related

(relative to our dataset), and for which phylogenies were more readily available. Trying to fit all of the parasite species we sampled onto a single phylogeny using published data on parasite systematics would result in a coarse approximation of relatedness, one that we do not think would add rigor to our analysis. The second reason for our choice is that dividing parasites by phylum does not explain variation in any stoichiometric variables after correcting for body size. Taxonomic affiliation at this broad scale does not appear to be an important determinant of parasite stoichiometry, so it is unlikely that phylogenetic correction at this level would change the outcome of our analyses. While it would be somewhat more feasible to apply phylogenetic correction within phyla and then assess the importance of life history traits to stoichiometry at this scale, the size of our dataset (2-13 taxa per phylum) does not allow rigorous tests of hypotheses within phyla.

Our choice to treat separate life cycle stages within one species as distinct 'taxa' is a related issue. However, for the three species in which we sampled more than one stage, our observations that conspecific stages varied both in life history traits (Table 1) and in many stoichiometric variables (Sup. Fig. S5) supported our decision to treat these stages separately in interspecific analyses.

Allometric scaling of parasite stoichiometry across taxa

Parasite body size was strongly linked to several stoichiometric variables across all taxa sampled, within phyla, and within several individual species (Fig. 3, Sup. Fig. S3). The strong relationships between several measures of parasite P (%P, C:P, and N:P) and body size support the growth rate hypothesis. Small parasite taxa

have higher P on average than larger taxa, which likely corresponds to high levels of ribosomal RNA needed to support rapid growth rates (Elser *et al.* 1996; Sterner & Elser 2002). These trends are consistent for taxa we sampled at actively feeding life cycle stages (adults in their definitive hosts and trematodes in their first intermediate hosts) but not for encysted parasites, supporting the idea that P allometry relates to the elemental costs of growth. The growth rate hypothesis predicts a relationship between body size and N:P in particular because this ratio reflects a balance of proteins to nucleic acids that is important to growing organisms (Sterner & Elser 2002). We observed this relationship within three phyla, but the differences in response among phyla caused the overall model including all taxa to be non-significant. Acanthocephala and Nematoda exhibited the positive N:P-body size allometry predicted by the growth rate hypothesis, but N:P scaled negatively with body size for Platyhelminthes. Like free-living invertebrates that vary in P allometry across various taxonomic groups (Cross *et al.* 2003; Woods *et al.* 2004; González *et al.* 2011), parasites do not have a uniform pattern for the N:P-body size relationship. Factors driving differences in P allometry across free-living and parasitic invertebrates are unknown, and additional work is needed to identify the patterns and mechanisms associated with this variation. For macroparasites, the allometric scaling of the stoichiometric variables %P, C:P, and N:P adds to a suite of variables, including abundance, metabolic rate, and fecundity, that commonly scale with body size across species (e.g. Von Brand 1979; Skorping, Read & Keymer 1991; Morand 1996; Arneberg, Skorping & Read 1998).

Life history and functional traits

Grouping parasites into broad life cycle stage categories, we found that non-reproductive parasite larvae are lower in %N and higher in C:N than reproductive stages (Fig 4). This was consistent with our prediction that actively growing and reproducing taxa would have elevated %N and reduced C:N relative to larvae that do not feed or reproduce, but contrary to our prediction that %P and C:P would differ among life cycle stages. We suggest that this pattern reflects differences in protein allocation among stages. Proteins are among the most N-rich of the major biomolecules, and they generally lack P. If the difference among stages were due to a difference in nucleic acid content, we would expect to see differences in both N and P content (Sterner & Elser 2002).

The other functional traits that we assessed, trophic level and functional feeding group, were not related to any stoichiometric variables after accounting for body size. Trophic level and functional feeding group are sometimes used interchangeably for free-living invertebrates, and both correspond to stoichiometric variation among species (Fagan *et al.* 2002; Cross *et al.* 2003; Evans-White *et al.* 2005; González *et al.* 2011). We considered these variables separately because they do not align neatly for parasites (e.g. an absorber may fall at many trophic levels depending on host trophic position). Both trophic level and functional feeding group represent the resource type on which parasites feed, and the non-significance of these variables in our analyses suggests that other factors drive parasite stoichiometry, regardless of resource quality.

Elemental variation within parasite taxa

Following the traditional expectation that most heterotrophs lack extensive intraspecific variation in organismal stoichiometry (Persson *et al.* 2010), we interpret broad differences in parasite stoichiometry as taxon-level variation among homeostatic organisms. However, our measurements of parasite stoichiometry may have been influenced by plastic responses particular to the ecosystems or hosts (in the case of generalist parasites) from which we sampled. The importance of bottom-up nutrient availability is of great importance to parasite ecology (Smith 2007; McKenzie & Townsend 2007; Budria 2017), but it is unknown whether parasites exhibit stoichiometric homeostasis or plasticity in response to variation in dietary resources. An ideal study would sample a diverse parasite assemblage from a single ecosystem to control for potential effects of ecosystem identity, but both the distribution of parasite species across our study system and our need for relatively high sample mass necessitated that we sample from many ecosystems to address our questions. Nonetheless, the potential that intraspecific variation has obscured our interspecific analyses is inherent in this type of study (El-Sabaawi *et al.* 2014).

Many factors may drive intraspecific variation in parasite stoichiometry, and we did not design our study with the intent of measuring intraspecific variation. Our opportunistic analysis of variation within several parasite species for which we had sufficient data revealed that the dominant patterns that we observed across species were consistent with intraspecific patterns. %P scaled allometrically with body size within several species, and adult parasites were higher in %N and lower in C:N than larval conspecifics. These findings support the idea that both body size and life cycle

stage are important drivers of parasite stoichiometry at multiple taxonomic scales. We emphasize that our findings are the first step to understanding broad patterns in parasite stoichiometry, and that further consideration of elemental composition within and among parasite taxa is warranted.

Parasitism and nutrient cycling

Broad variation in the elemental composition of parasites likely represents diversity in ecological function. As predicted by stoichiometric theory, variation in the elemental composition of free-living organisms corresponds to nutrient recycling function (Elser *et al.* 1988; Sterner, Elser & Hessen 1992; Vanni *et al.* 2002) and sensitivity to nutritional limitation (Elser *et al.* 2000a; Boersma *et al.* 2008). Extension of this theory to host-parasite systems will contribute to two active areas of research in parasite ecology.

First, ecological stoichiometry provides a mechanistic framework to study the importance of parasitism to ecosystem processes, a poorly understood topic (Ostfeld, Keesing & Eviner 2008). While describing pools of biomass for both free-living and parasitic consumers serves as one method to address this issue (Paseka 2017), stoichiometry provides a more mechanistic approach to measure the flux of energy and nutrients through host-parasite interactions. Second, while the response of parasites to changes in ecosystem-level nutrient cycling has received ample attention (Smith 2007; McKenzie & Townsend 2007; Budria 2017), most studies have not included a stoichiometric framework. Data from snail-trematode and zooplankton-bacteria systems demonstrate shifts in parasite production in response

to environmental nutrient ratios (Frost *et al.* 2008a; Bernot 2013). We predict that using a stoichiometric framework to study additional host-parasite systems will reveal diverse responses to bottom-up nutrient availability, corresponding to the wide variation in organismal stoichiometry we observed across parasite taxa. Macroparasites are diverse in both form and function, and organismal stoichiometry is one important aspect of this diversity.

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Chapter 2 Tables and Figures

Table 2.1. Summary of all parasite taxa collected, hosts and sampling locations, traits analyzed, and number replicate samples used for each elemental analysis. Numbers following host species correspond to collection sites: (1) Muskingum Brook, (2) Wesickaman Creek, (3) Farrington Lake, (4) Raritan North Branch, (5) Colliers Mills, (6) Mercer Lake, (7) Great Egg Harbor River, (8) Assunpink Lake, (9) Morin Detention Pond, (10) Passaic River, (11) Pequest Trout Hatchery, (12) Ten Mile Run, (13) Myrtle Creek.

Taxon	Phyl.	Stage	Host	Infection site	FFG	Troph Level	Mean mass	SEM mass	C:N (n)	P (n)
Acanthocephalus	Acan.	adult	*Aphredoderus sayanus*	gut	absorber	2	0.0887	0.0064	11	10
Acanthocephalus	Acan.	cystacanth	*Caecidotea communis* (2)	haemocoel	absorber	2	0.3311	0.0295	10	10
Leptorhynchoides thecatus	Acan.	adult	*Micropterus dolomieu* (4)	gut	absorber	3	0.2869	0.0248	12	10
Neoechinoryhnchus	Acan.	adult	*Catostomus commersonii*	gut	absorber	2	0.0167	0.0043	3	4
Neoechinorhynchus	Acan.	adult	*Micropterus dolomieu* (4)	gut	absorber	3	0.1348	0.0213	6	8
Neoechinorhynchus	Acan.	cystacanth	*Lepomis macrochirus*	liver	encysted	3	0.0223	0.001	7	7
Chaetogaster limnaei	Anne	adult	*Helisoma trivolvis* (9)	inside shell	grazer	2	0.0071	0.0007	3	3
Placobdella sp.	Anne	adult	*Chelydra serpentina* (4)	surface	blood	4	9.5391	1.987	10	10
Eustrongylides sp.	Nem	larva	*Fundulus diaphanus* (3)	body cavity	encysted	3	12.9577	2.5822	10	10
Hysterothylacium sp.	Nem	larva	*Alosa pseudoharengus*	body cavity	encysted	3	0.1749	0.0347	6	7
Nematoda1	Nem	larva	*Umbra pygmaea* (5)	body cavity	encysted	3	0.0334	0.0034	8	8
Philometra sp.	Nem	adult	Lepomis macrochirus (8)	body cavity	blood	3	3.1896	0.508	6	6
Philometra sp.	Nem	adult	*Lepomis macrochirus* (8)	eye	blood	3	2.3877	0.1823	10	10
Spinitectus sp.	Nem	adult	*Lepomis macrochirus*	gut	grazer	3	0.0048	0.001	5	5
Allocreadium commune	Plat.	adult	*Fundulus diaphanus* (6)	gut	grazer	3	0.0024	0.0006	5	4
Clinostomum marginatum	Plat.	metacerca	*Semotilus atromaculatus*	muscle	encysted	3	0.7321	0.0771	9	9
Crepidostomum isostomum	Plat.	adult	*Aphredoderus sayanus*	gut	grazer	3	0.0079	0.0015	4	3
Cyclophyllidea	Plat.	adult	*Oxyura jamaicensis* (3)	gut	absorber	3	0.7342	0.1819	6	5
Diplostomatidae	Plat.	adult	*Ardea herodias* (11)	gut	grazer	4	0.0047	0.0011	3	3
Gryporhynchidae	Plat.	adult	*Ardea herodias* (11)	gut	absorber	3	0.0227	0.0034	4	4
Microphallidae	Plat.	sporocyst	*Pleurocera virginica* (4)	gonad	grazer	2	0.0002	<0.0001	10	8
Philophthalmidae	Plat.	redia	*Pleurocera virginica* (4)	gonad	grazer	2	0.0031	0.0003	9	10
Phyllodistomum pearsei	Plat.	adult	*Aphredoderus sayanus*	urinary	grazer	3	0.0236	0.0056	8	9
Posthodiplostomum	Plat.	metacerca	*Lepomis auritus* (4)	liver	encysted	3	0.0302	0.0016	6	7
Proteocephalus ambloplitis	Plat.	plerocerco	*Perca flavescens* (3)	liver	absorber	3	0.1	0.0094	10	10
Strigeidae	Plat.	sporocyst	*Pleurocera virginica* (4)	foot	grazer	2	0.0103	0.0013	11	11
Uvulifer ambloplitis	Plat.	metacerca	*Perca flavescens* (3)	surface	encysted	3	0.0248	0.0032	9	7

Table 2.2. Scaling of parasite stoichiometry with body size across all taxa sampled and within individual phyla. The sample sizes, slopes (std. error), intercepts (std. error), r^2-values, p-values are indicated for the results of each ordinary least-squares regression performed on $\log_{10}$-transformed data. Bold text indicates statistically significant relationships ($p<0.05$).

		%C				%N				%P			
Group	**n**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**
All taxa	27	-0.007 (0.009)	1.677 (0.016)	0.0234	0.4461	-0.0182 (0.0305)	0.8893 (0.0512)	0.014	0.5565	**-0.088 (0.021)**	**-0.003 (0.035)**	**0.4197**	**0.0003**
Active	18	-0.004 (0.011)	1.692 (0.021)	0.0069	0.7426	0.004 (0.019)	0.981 (0.036)	0.0021	0.8562	**-0.073 (0.016)**	**0.04 (0.03)**	**0.5613**	**0.0003**
Encysted	9	-0.006 (0.021)	1.658 (0.025)	0.0107	0.7912	-0.01 (0.1)	0.775 (0.119)	0.0013	0.9261	-0.11 (0.07)	-0.068 (0.084)	0.258	0.1627
Acanthocephala	6	**-0.09 (0.018)**	**1.601 (0.021)**	**0.8623**	**0.0075**	-0.021 (0.066)	0.948 (0.078)	0.0244	0.7677	**-0.171 (0.034)**	**-0.076 (0.04)**	**0.8638**	**0.0073**
Nematoda	6	-0.016 (0.007)	1.689 (0.009)	0.5527	0.0903	0.017 (0.02)	0.941 (0.025)	0.1515	0.4456	-0.094 (0.046)	-0.066 (0.058)	0.5146	0.1085
Platyhelminthes	13	-0.011 (0.021)	1.652 (0.041)	0.0258	0.5999	**-0.139 (0.058)**	**0.605 (0.118)**	**0.3404**	**0.0363**	-0.014 (0.037)	0.147 (0.074)	0.0128	0.7132
		C:N				**C:P**				**N:P**			
Group	**n**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**
All taxa	27	0.011 (0.027)	0.791 (0.046)	0.0065	0.6898	**0.079 (0.021)**	**1.682 (0.036)**	**0.355**	**0.001**	0.069 (0.037)	0.895 (0.062)	0.1203	0.0763
Active	18	-0.007 (0.018)	0.715 (0.033)	0.0096	0.6982	**0.068 (0.016)**	**1.653 (0.03)**	**0.5422**	**0.0005**	**0.076 (0.024)**	**0.942 (0.045)**	**0.3836**	**0.0061**
Encysted	9	0.003 (0.091)	0.885 (0.109)	0.0002	0.971	0.102 (0.079)	1.729 (0.094)	0.1952	0.2337	0.099 (.139)	0.846 (0.166)	0.0668	0.502
Acanthocephala	6	-0.07 (0.066)	0.655 (0.077)	0.2231	0.3442	0.082 (0.038)	1.68 (0.045)	0.5393	0.0965	**0.151 (0.041)**	**1.026 (0.048)**	**0.7748**	**0.0207**
Nematoda	6	-0.031 (0.023)	0.75 (0.03)	0.3106	0.2506	0.076 (0.046)	1.756 (0.058)	0.4101	0.1707	**0.108 (0.038)**	**1.009 (0.048)**	**0.6694**	**0.0466**
Platyhelminthes	13	**0.129 (0.05)**	**1.051 (0.101)**	**0.3726**	**0.0267**	0.003 (0.038)	1.51 (0.076)	0.0005	0.9435	**-0.126 (0.057)**	**0.462 (0.114)**	**0.3094**	**0.0484**

Figure 2.1. Mean (± SE) elemental content of each taxon sampled. %C, %N, and %P represent percentage of total body dry mass. Where conspecific stages were sampled, (a), (l), and (i) denote adult, larval, and immature stages, respectively.

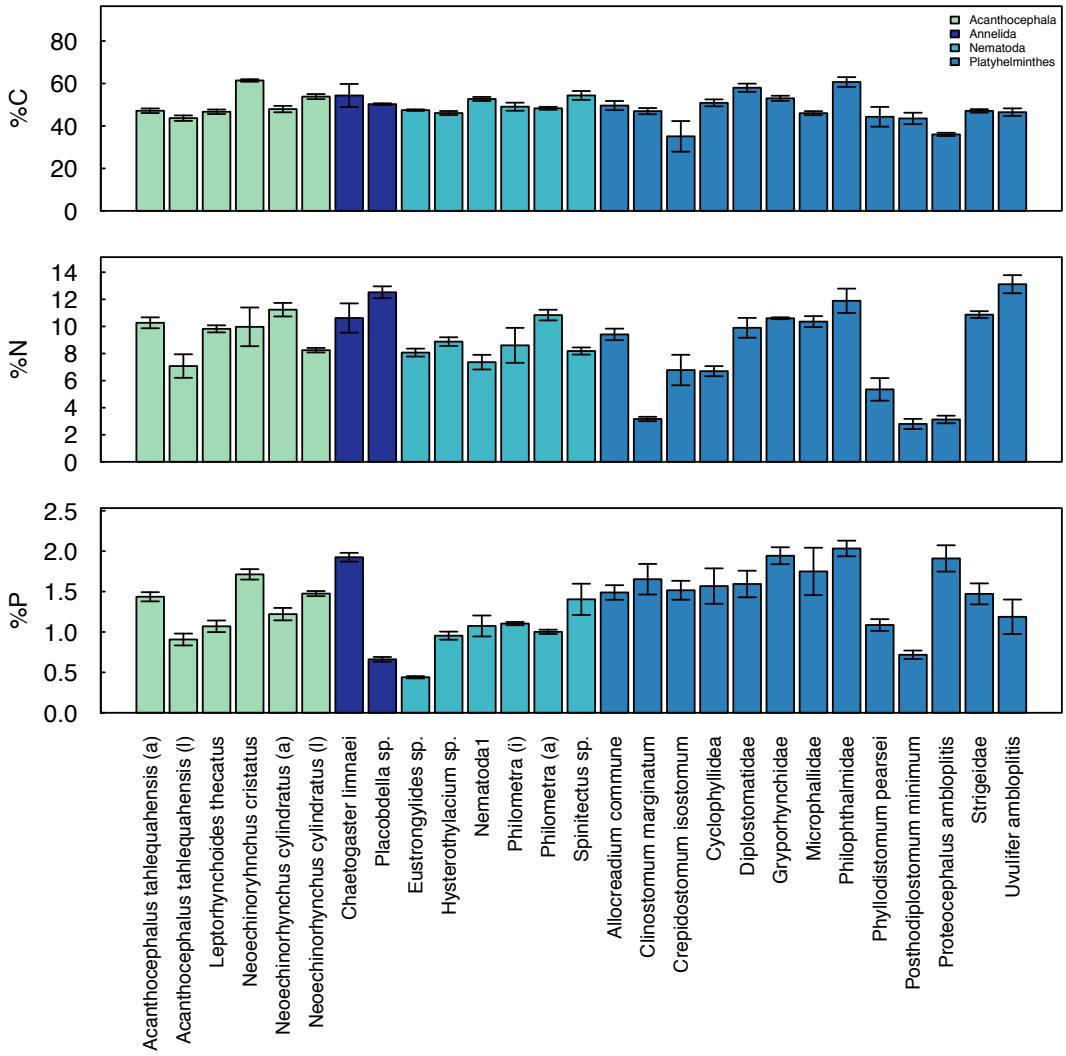


Figure 2.2. Mean (± SE) elemental ratios of each taxon sampled. C:N, C:P, and N:P represent molar ratios. Where conspecific stages were sampled, (a), (l), and (i) denote adult, larval, and immature stages, respectively.

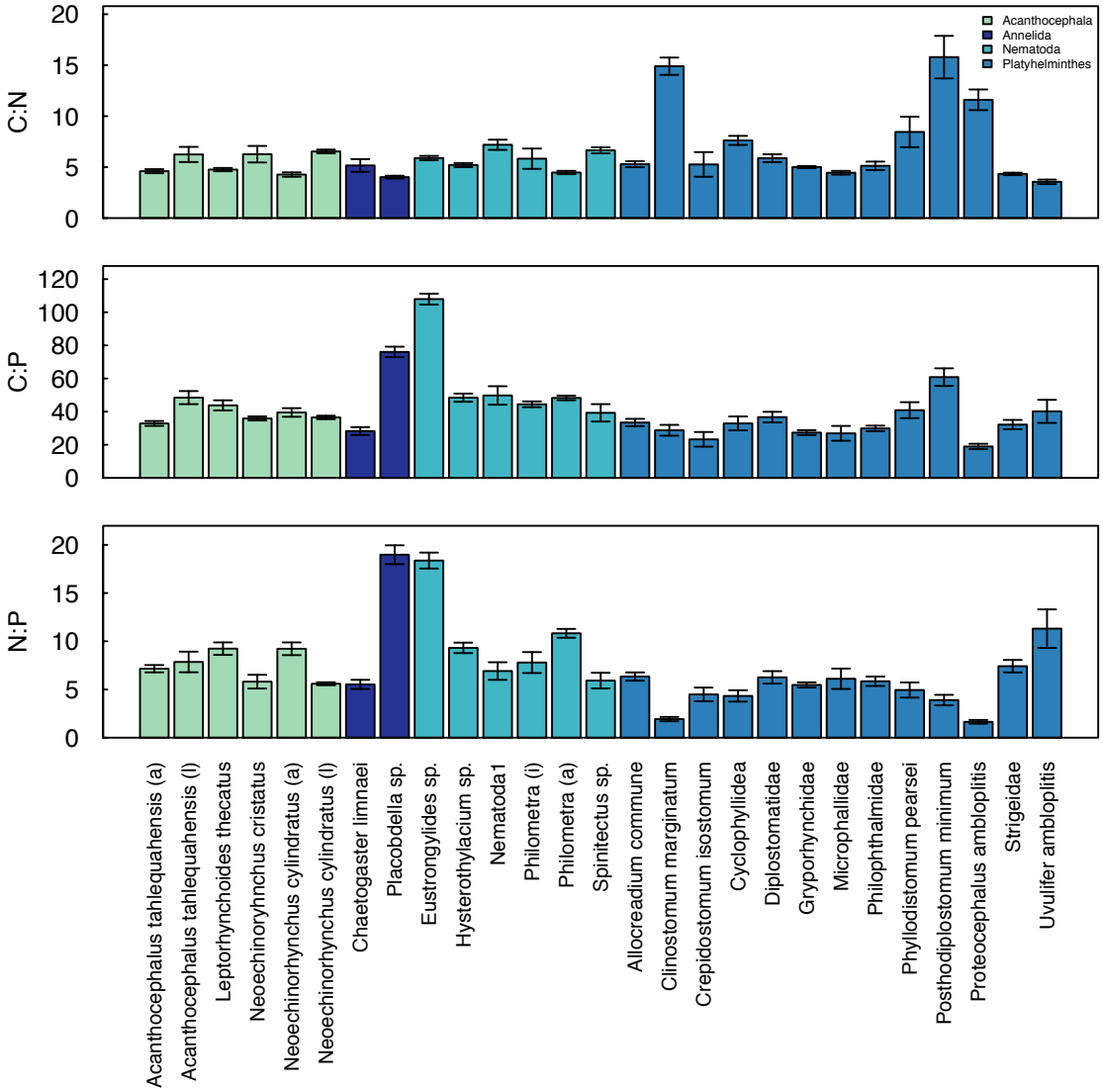


Figure 2.3. Regressions of stoichiometric variables (%P, C:P, and N:P) and body size (dry mass) for all species. Each point represents log-transformed means (± SE) for one taxon. Black regression lines represent significant relationships ($p<0.05$) when all taxa are included, and colored lines represent significant regressions for individual phyla. Correlation coefficients and p-values are given for regressions including all species, and all regression coefficients are given in Table 2.

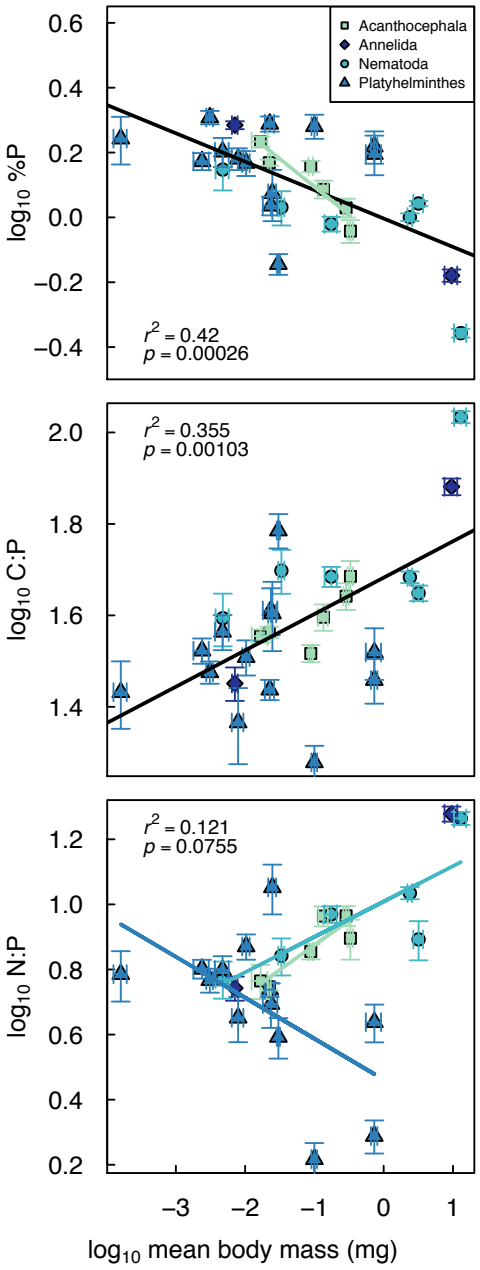
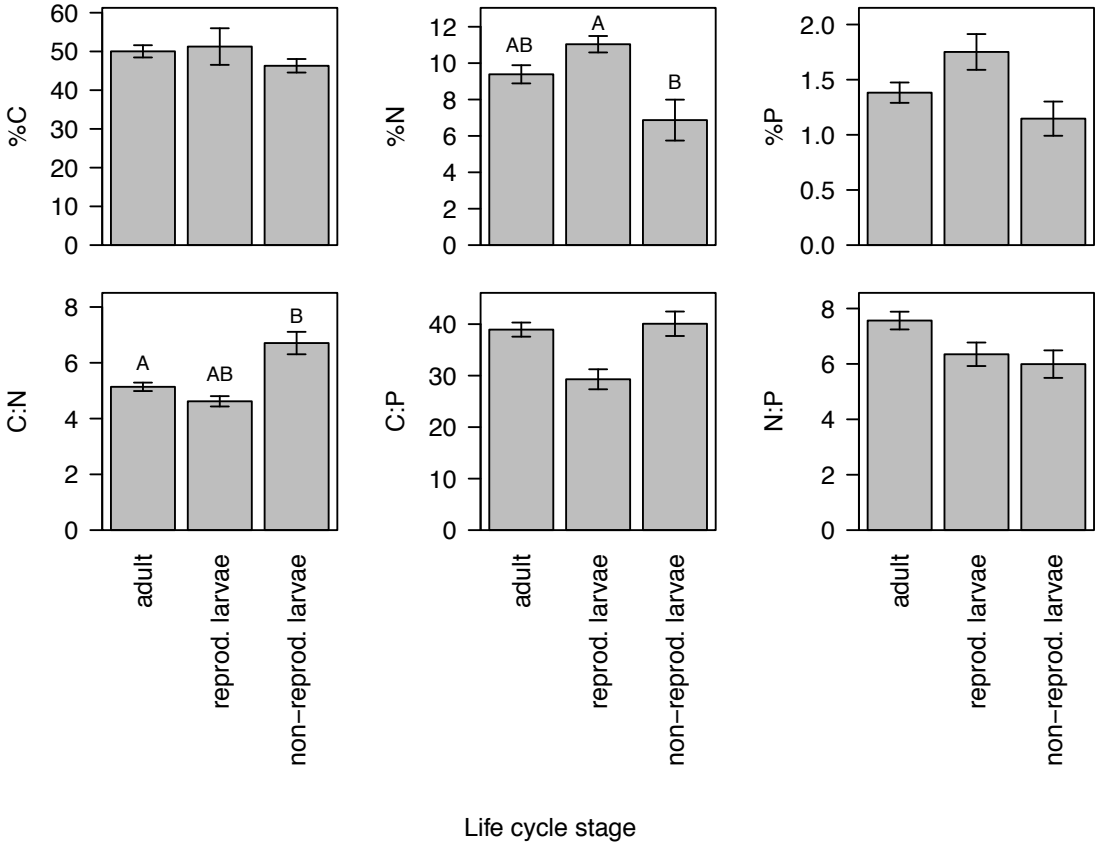


Figure 2.4. Mean values (± SE) of stoichiometric variables when taxa are grouped by life cycle stage. Adults are mature parasites sampled from their definitive hosts. Larval parasites were sampled from their intermediate hosts and are subcategorized here by reproductive activity. Letters above bars indicate differences among group means as determined by Tukey's HSD ($p<0.05$).



Chapter 2 Appendix

Supplementary Table S2.1. Results of ANCOVA for stoichiometric variables, using phylum as a factor and body size as a covariate. Bold text indicates statistically significant relationships ($p<0.05$).

Response variable	Source of variation	df	F-value	p-value
%C	body size	1	0.014	0.9083
	phylum	3	0.648	0.5938
	body size * phylum	3	1.067	0.3865
%N	body size	1	0.387	0.5412
	phylum	3	1.047	0.3945
	body size * phylum	3	1.449	0.2600
%P	**body size**	**1**	**17.359**	**0.0005**
	phylum	3	2.043	0.1419
	body size * phylum	3	2.171	0.1249
C:N	body size	1	0.446	0.5124
	phylum	3	0.937	0.4422
	body size * phylum	3	1.596	0.2234
C:P	**body size**	**1**	**71.849**	**<0.0001**
	phylum	3	1.734	0.1940
	body size * phylum	3	0.713	0.5562
N:P	**body size**	**1**	**67.556**	**<0.0001**
	phylum	3	1.896	0.1645
	body size * phylum	**3**	**3.131**	**0.0498**

Supplementary Table S2.2. Results of ANCOVA for stoichiometric variables, using life cycle stage as a factor and body size as a covariate. Bold text indicates statistically significant relationships ($p<0.05$).

Response variable	Source of variation	df	F-value	p-value
%C	body size	1	0.013	0.9096
	stage	2	1.160	0.3328
	body size * stage	2	0.066	0.9367
%N	body size	1	0.448	0.5106
	stage	**2**	**4.577**	**0.0224**
	body size * stage	2	0.248	0.7829
%P	**body size**	**1**	**15.145**	**0.0008**
	stage	2	2.623	0.0962
	body size * stage	2	0.681	0.5171
C:N	body size	1	0.490	0.4918
	stage	**2**	**4.104**	**0.0313**
	body size * stage	2	0.008	0.9921
C:P	**body size**	**1**	**73.043**	**<0.0001**
	stage	2	2.619	0.0965
	body size * stage	2	0.270	0.7658
N:P	**body size**	**1**	**45.081**	**<0.0001**
	stage	2	0.189	0.8290
	body size * stage	2	0.683	0.5160

Supplementary Table S2.3. Results of ANCOVA for stoichiometric variables, using functional feeding group as a factor and body size as a covariate. Bold text indicates statistically significant relationships ($p<0.05$).

Response variable	Source of variation	df	F-value	p-value
%C	body size	1	0.012	0.9147
	feeding group	3	0.115	0.9503
	body size * feeding group	3	0.514	0.6775
%N	body size	1	0.375	0.5474
	feeding group	3	1.110	0.3696
	body size * feeding group	3	1.117	0.3671
%P	**body size**	**1**	**16.100**	**0.0007**
	feeding group	3	2.157	0.1267
	body size * feeding group	3	1.292	0.3058
C:N	body size	1	0.410	0.5295
	feeding group	3	1.548	0.2346
	body size * feeding group	3	0.278	0.8409
C:P	**body size**	**1**	**74.566**	**<0.0001**
	feeding group	3	2.451	0.0949
	body size * feeding group	3	0.328	0.8050
N:P	**body size**	**1**	**41.236**	**<0.0001**
	feeding group	3	0.247	0.8622
	body size * feeding group	3	0.354	0.7868

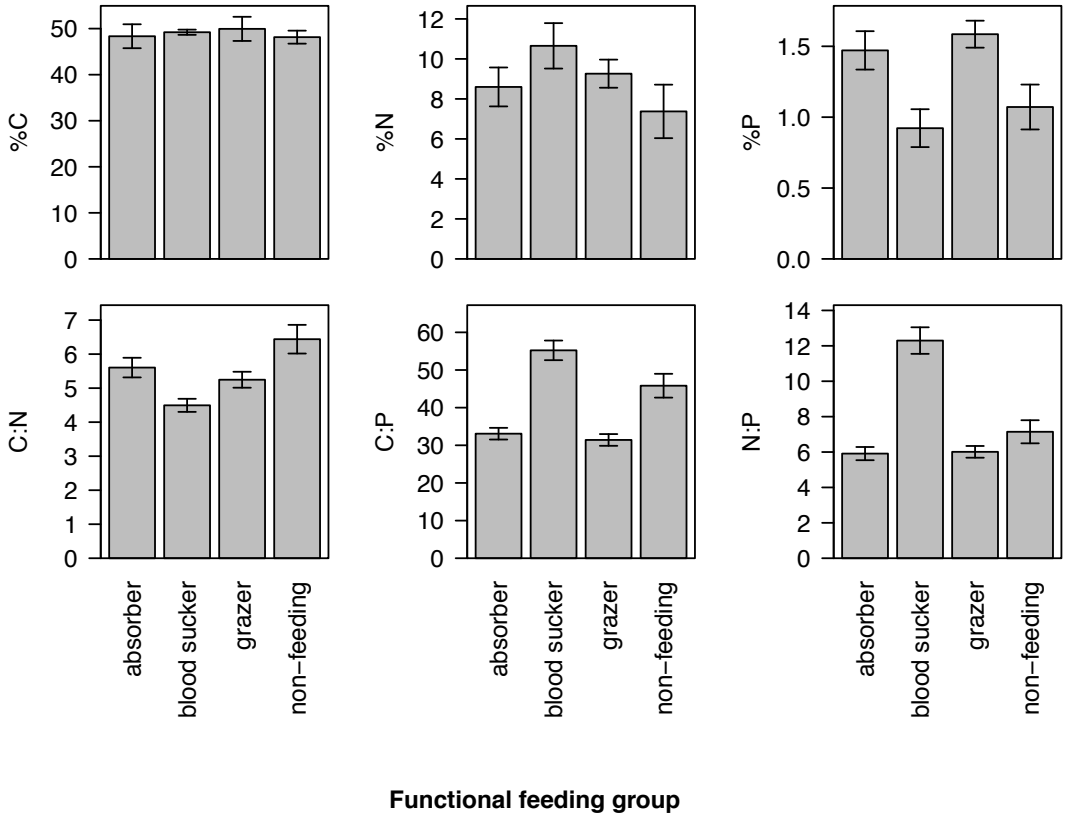
Supplemental Table S2.4. Results of ANCOVA for stoichiometric variables, using trophic level as a factor and body size as a covariate. Bold text indicates statistically significant relationships ($p<0.05$).

Response variable	Source of variation	df	F-value	p-value
%C	body size	1	0.013	0.9095
	trophic level	1	0.154	0.6979
	body size * trophic level	1	0.267	0.6103
%N	body size	1	0.390	0.5383
	trophic level	1	0.827	0.3726
	body size * trophic level	1	2.872	0.1036
%P	**body size**	**1**	**13.431**	**0.0013**
	trophic level	1	1.275	0.2705
	body size * trophic level	1	0.210	0.6514
C:N	body size	1	0.414	0.5265
	trophic level	1	0.910	0.3499
	body size * trophic level	1	0.772	0.3887
C:P	**body size**	**1**	**66.886**	**<0.0001**
	trophic level	1	0.144	0.7081
	body size * trophic level	1	1.377	0.2526
N:P	**body size**	**1**	**49.358**	**<0.0001**
	trophic level	1	0.005	0.9452
	body size * trophic level	1	1.897	0.1817

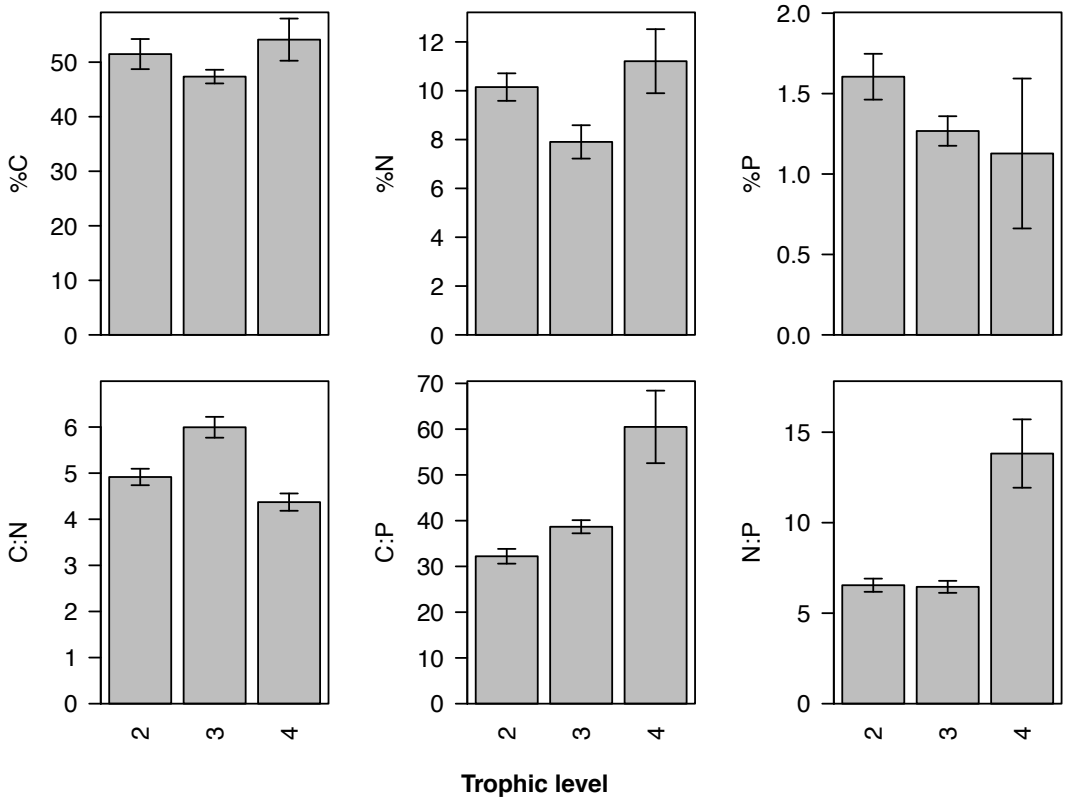
Supplemental Table S2.5. Scaling of parasite stoichiometry with body size within individual species. The sample sizes, slopes (std. error), intercepts (std. error), r^2-values, p-values are indicated for the results of each linear regression performed on $\log_{10}$-transformed data. Bold text indicates statistically significant relationships ($p<0.05$). Bracketed letters following species names indicate phylum: [A]=Annelida, [N]=Nematoda, [P]=Platyhelminthes.

		%C				**%N**				**%P**			
Species	**n**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**	**slope**	**int.**	**r^2**	**p**
Clinostomum marginatum [P]	9	-0.069 (0.075)	1.659 (0.018)	0.1094	0.3846	-0.0859 (0.1261)	0.4821 (0.0304)	0.0622	0.5175	**-0.551 (0.08)**	**0.134 (0.02)**	**0.8727**	**0.0002**
Eustrongylides sp. [N]	10	-0.007 (0.013)	1.684 (0.015)	0.0342	0.6089	0.1184 (0.0549)	0.7723 (0.0627)	0.3678	0.063	**-0.129 (0.033)**	**-0.215 (0.038)**	**0.6512**	**0.0048**
Placobdella sp. [A]	10	0.021 (0.01)	1.683 (0.009)	0.3503	0.0714	**0.1243 (0.0409)**	**0.9869 (0.0375)**	**0.536**	**0.0161**	**-0.151 (0.043)**	**-0.051 (0.04)**	**0.6057**	**0.008**
Philometra sp. (eye) [N]	10	0.014 (0.06)	1.681 (0.014)	0.0072	0.8154	-0.0682 (0.1526)	1.0468 (0.0361)	0.0244	0.6668	**0.218 (0.047)**	**-0.09 (0.021)**	**0.7267**	**0.0017**
Philometra sp. (body cavity) [N]	6	-0.085 (0.046)	1.711 (0.019)	0.4569	0.1405	-0.2331 (0.3605)	0.9477 (0.1449)	0.0946	0.5531	0.053 (0.049)	0.012 (0.03)	0.2264	0.3402

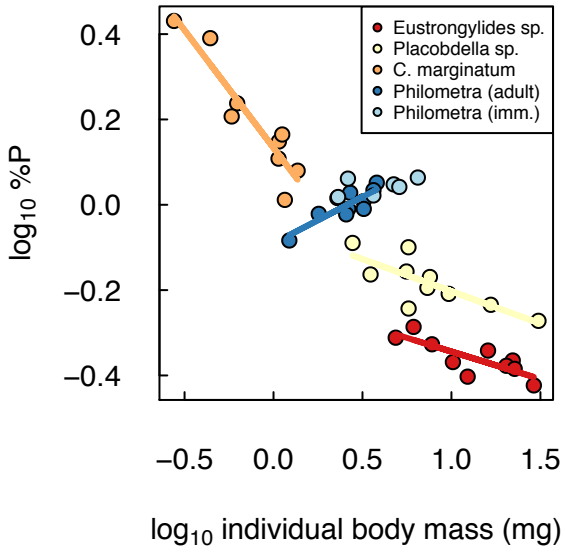
Supplementary Figure S2.1. Mean values (± SE) of stoichiometric variables when taxa are grouped by functional feeding group. ANCOVA results indicate that functional feeding group does not correspond to any stoichiometric variables after accounting for body size.



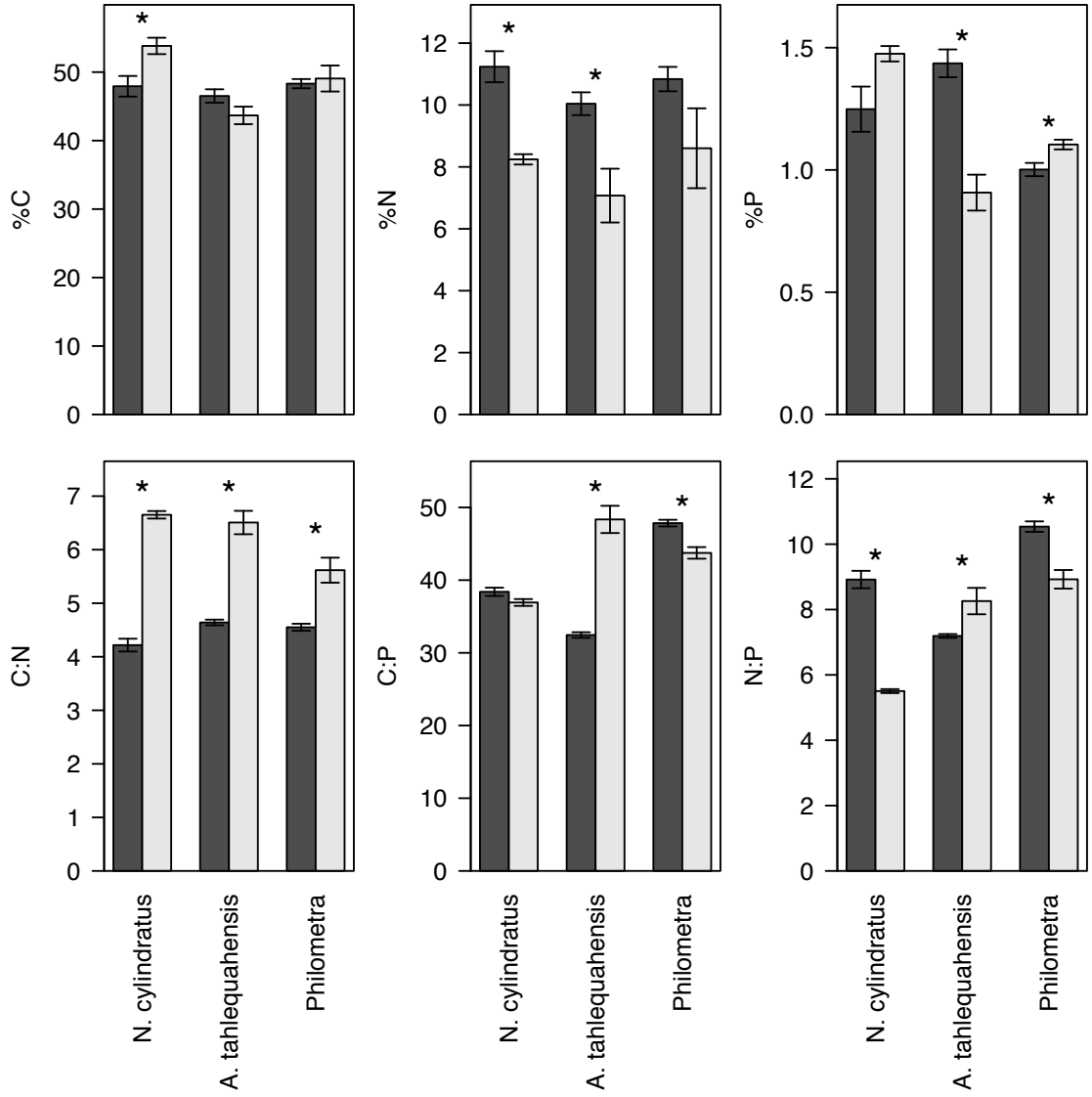
Supplementary Figure S2.2. Mean values (± SE) of stoichiometric variables when taxa are grouped by trophic level. X-axis values reflect numeric trophic level categories assigned to each parasite (2, 3, or 4° consumer) based on community observations. ANCOVA results indicate that trophic level does not correspond to any stoichiometric variables after accounting for body size.



Supplementary Figure S2.3. Regressions of %P against body mass for individual taxa. Each point represents the measured dry mass and %P content for one individual parasite. Regression lines are shown for species with a significant relationship ($p<0.05$) between individual %P and body size. Regression coefficients are given in Supplemental Table S3.



Supplementary Figure S2.4. Intraspecific comparisons of parasite stoichiometry between adult and larval forms. Dark bars indicate adults, and light bars indicate larval or immature worms. Asterisks above bars denote significant differences between stages (Student's t-test, $p<0.05$).



CHAPTER 3

RESOURCE QUALITY AND HOST-PARASITE NUTRIENT DYNAMICS IN AN ISOPOD-ACANTHOCEPHALAN INTERACTION

Abstract

Anthropogenic changes to the cycling of N and P are widespread in freshwater ecosystems and often lead to cascading effects through food webs due to the limiting roles of these elements in primary production and consumer growth. Little is known about how parasites and their interactions with hosts respond to these changes in environmental nutrients. Ecological stoichiometry provides a framework to describe consumer-resource nutrient dynamics and to link trophic interactions with ecosystem-level nutrient cycling. In this study, I extend stoichiometric theory to describe the balance of carbon, nitrogen, and phosphorus in host-parasite interactions while testing the effects of basal resource quality on these interactions. I conducted a laboratory experiment to test the effects of abiotic P concentrations on the detritivorous isopod, *Caecidotea communis*, and its larval acanthocephalan parasite, *Acanthocephalus tahlequahensis.* Experimental additions of dissolved P changed the elemental content of leaf litter and reduced the elemental imbalance between isopods and detritus. Despite these changes to basal resource quality, P treatment did not affect the survival, growth, or stoichiometry of hosts or parasites. This lack of effect may be due to the stoichiometric homeostasis of isopods or due to confounding variability in this system resulting from the use of field-collected isopods and natural acanthocephalan infections for the experiment.

While changes to basal resource quality had little effect on consumers in this experiment, the results provide insight into the naturally occurring nutrient dynamics of isopod-acanthocephalan interactions. Infected isopods were lower in P content and more balanced with detritus resources than uninfected isopods. Isopod P content scaled positively with growth rate as predicted by the growth rate hypothesis, but infection reduced the strength of this relationship. The effects of infection on host growth and stoichiometry may have important implications for host population dynamics and trophic interactions. Acanthocephalan P content scaled negatively with body size, a relationship also consistent with the growth rate hypothesis. While the P content of individual acanthocephalans tended to track the %P of individual hosts, the %P imbalance between parasite-host pairs was highly variable. This imbalance was an important predictor of final acanthocephalan body size, which suggests that parasite growth may be limited by host P content. Understanding the effects of parasite infection on host nutrient use and growth, as well as the nutritional demands of parasites, will aid predictions on the effects of basal resource quality on host-parasite interactions. The effects environmental N and P on these interactions is a conceptually important topic that warrants additional study, especially in in the context of human-induced changes to aquatic ecosystems.

Introduction

Nutrients drive the structure and function of communities and ecosystems from the bottom up, but it is largely unknown how parasites and pathogens respond to changes in basal nutrient availability (Smith 2007). This topic is especially relevant in the context of anthropogenic changes to the cycling of nitrogen and phosphorus, which occur globally and often have cascading effects through ecosystems due to the critical roles of these elements as limiting nutrients for both primary production and consumer growth (Carpenter et al. 1998, Smith and Schindler 2009). While there are many observational accounts of how nutrient enrichment impacts infectious disease in human and wildlife hosts (McKenzie and Townsend 2007, Johnson et al. 2010, Budria 2017), these studies often lack a mechanistic description of how abiotic nutrient availability leads to changes in infection patterns.

Ecological stoichiometry provides a framework to describe consumer-resource nutrient dynamics and to mechanistically link trophic interactions with ecosystem-level nutrient cycling (Sterner and Elser 2002), though this perspective has rarely been applied to host-parasite systems. Several past studies have used a stoichiometric framework to demonstrate that changes to abiotic N and P availability can cascade through food webs to change the quality of hosts as resources to parasites, leading to shifts in parasite growth and reproduction (Clasen and Elser 2007, Frost et al. 2008a, Bernot 2013).

Additional extensions of stoichiometric theory to the study of host-parasite interactions will provide greater insight into the mechanisms behind host-parasite

nutrient dynamics and the importance of basal resource quality to these interactions. For example, ecological stoichiometry predicts that imbalances in the elemental ratios between a consumer and its dietary resource will lead to two general outcomes. If the consumer is homeostatic with regard to body nutrients, then elemental imbalance should reduce its growth efficiency (Boersma and Elser 2006). Alternatively, consumers may deviate from strict elemental homeostasis to more closely match the chemical composition of their resources (Persson et al. 2010). In either scenario, the resulting changes to consumer body size or tissue stoichiometry would introduce variation in the resource quality available to macroparasites infecting the consumer. Additionally, the trophic interaction between a parasite and its host resource may also be elementally imbalanced, with similar potential consequences for parasite growth, homeostasis, and ultimately, fitness and transmission.

In this study, I describe the carbon, nitrogen, and phosphorus stoichiometry that occurs naturally in host-parasite interactions while testing the effects of basal resource elemental content on a host-parasite system. Specifically, I address the following four questions. (1) What are the causes and consequences of elemental imbalance in parasite-host and host-resource interactions, and do experimental alterations of basal resource ratios change these patterns? (2) Does resource quality determine the survival and growth of hosts and parasites? (3) Are hosts and parasites homeostatic or plastic in organismal stoichiometry when faced with variable resource quality? (4) Are the growth rate and P content of hosts and

parasites positively related, following predictions from the growth rate hypothesis (Elser et al. 1996)?

I addressed these questions using a freshwater detritivore, the isopod *Caecidotea communis*, and its larval acanthocephalan parasite, *Acanthocephalus tahlequahensis.* This host-parasite system is common in streams of the New Jersey Pine Barrens, where infection prevalence among mature isopods often exceeds 30% in early summer. *A. tahlequahensis* alters the feeding rate and energy budgets of infected individuals of *C. communis* in Pine Barrens streams (Hernandez and Sukhdeo 2008, Lettini and Sukhdeo 2010). The relatively large body size of *A. tahlequahensis* allowed me to measure the elemental content of individual parasites and assess interactions among resource quality, growth, and organismal stoichiometry for parasites and hosts.

Prior experiments with an acanthocephalan-rat system demonstrated that acanthocephalan survival, growth rate, and fecundity are driven by the carbohydrate composition of host diet (Nesheim et al. 1977, Keymer et al. 1983). For *A. tahlequahensis*, variation in host diet occurs through the quality of leaf litter detritus consumed by isopods. Pine Barrens streams vary extensively in concentrations of dissolved N and P across a land use gradient from areas of protected forest to those dominated by agriculture (Zampella et al. 2001). This abiotic variability likely corresponds to variation in the chemical composition of leaf litter in the streams when microbial communities on the surface of decomposing leaves take up abiotic nutrients and change the elemental ratios of detritus (Cross et al. 2003). Detritivores often experience high levels of elemental imbalance with

their carbon-rich resources (Cross et al. 2003, Evans-White and Halvorson 2017), so abiotic nutrient enrichment may alleviate potential nutrient limitation in the isopod-acanthocephalan system. *A. tahlequahensis* occupies the haemocoel of isopod hosts and feeds by absorbing host haemolymph. Some arthropods store excess dietary P in haemolymph (Woods et al. 2002), which may provide a link between host diet and the resource quality available to parasites in this system.

I created a basal resource quality gradient by incubating red maple leaves under varying concentrations of dissolved P, then feeding this detritus to *C. communis* individuals with and without *A. tahlequahensis* in a laboratory experiment. Following isopod incubation with treated detritus, I measured host and parasite survival, growth, elemental content, and the elemental imbalance between isopod-detritus and acanthocephalan-isopod interactions. My results provide novel insight into natural patterns in host-parasite nutrient dynamics and the effects of basal resource quality on these interactions.

Materials and Methods

Nutrient additions

I collected freshly fallen red maple leaves (*Acer rubrum*) from dry areas adjacent to Wesickaman Creek (Wharton State Forest, New Jersey) in autumn of 2016 and stored them in the laboratory until spring 2017. I prepared leaves for nutrient additions by cutting them into 3 cm squares and leaching them in DI water for 10 days. After leaching, I transferred leaves to glass beakers containing 900 mL DI water inoculated with 100 mL filtered stream water and enriched with a gradient

of P (as Na_2HPO_4) and N (as KNO_3). This incubation created four experimental P treatments: (1) control (no nutrients added), (2) Low P (50 µg P L^{-1} and 1 mg N L^{-1}), (3) Moderate P (100 µg P L^{-1} and 1 mg N L^{-1}), and (4) High P (500 µg P L^{-1} and 1 mg N L^{-1}). These values fall within the range of dissolved nutrient concentrations measured across Pine Barrens streams (Zampella et al. 2001). I incubated leaves in nutrient treatments for 23 days, moving leaves to fresh beakers of nutrient-enriched water weekly. At the end of the incubation period, I blotted each leaf square dry with a paper towel, measured wet mass, and placed them individually into isopod incubation vials. I subsampled leaves at the end of the nutrient incubation period and again at the end of the isopod incubation experiment to measure detritus elemental content on Days 0 and 15, respectively.

Isopod collection, sorting, and initial measurements

I collected isopods from Wesickaman Creek in June 2017 by taking dip net samples of benthic material, transferring this material to sieves, and removing adult isopods with forceps. In the lab, I sorted isopods by sex, excluding females from the experiment to control for potential stoichiometric differences related to isopod sex and to avoid the potentially confounding effects of pregnancy. I sorted male isopods by infection status with *Acanthocephalus tahlequahensis,* which is visible in infected isopods when viewed ventrally under a dissecting microscope. When selecting uninfected isopods to use in the experiment, I chose those that were large enough to sustain infection with *A. tahlequahensis* and assumed to have been born prior to spring 2017, based on size. I used 176 isopods for the experiment, half of which

were infected with *A. tahlequahensis.* I photographed each isopod used for the experiment under a dissecting microscope, then measured head capsule width as interocular distance using ImageJ (Schneider et al. 2012).

Isopod incubation

During the experiment, I incubated isopods individually in acid washed chambers constructed from polypropylene scintillation vials (20 mL) with fiberglass window screen covering two holes cut into the vial sides for water circulation. Each vial contained an individual isopod and one pre-weighed leaf square. I placed vials in aerated, 10 gallon, glass aquaria filled with equal volumes of well water and Wesickaman Creek water filtered through a 250 μm sieve. Each aquarium contained isopod vials containing leaves from only one P treatment in case leaf nutrients leached out into the water to affect neighboring vials. I used two aquaria per P treatment (8 aquaria total) with 22 isopods per aquarium (176 isopods total). Isopods infected with *A. tahlequahensis* were equally distributed among aquaria.

Mass measurements

After two weeks of incubations (Day 15), I removed each surviving isopod from its chamber, photographed it to measure final head capsule width, and dissected it to remove intestinal contents and acanthocephalans. I placed isopods in microcentrifuge tubes and acanthocephalans on ashed, preweighed GF/F filters, dried these materials at 60° C for 48 hours, and weighed them on a microbalance (Sartorius, ± 0.1 μg). I used dry mass (DM, mg) and final head capsule width (HCW,

cm) data from all surviving isopods to calculate a length-mass regression ($\log_{10}$DM = 2.572[$\log_{10}$HCW] + 2.963, R^2 = 0.679), then used this equation to estimate the dry mass of isopods on Day 0. I calculated isopod growth during the experiment as the change in isopod mass between Day 0 and Day 15. Because I could not measure acanthocephalan body mass without removing them from their hosts, I used final body size as a proxy for acanthocephalan growth. Hereafter, I use the term 'body size' to refer to individual dry mass (mg) of isopods and acanthocephalans.

Elemental analysis

I prepared leaves and isopods for elemental chemistry by grinding dry materials and subsampling for both C:N and P analyses. Due to biomass limitations, I analyzed whole acanthocephalans for P only. For all sample types, I measured C and N with a Carlo Erba NA 1500 Series 2 elemental analyzer (Verardo et al. 1990) and P on a spectrophotometer with the molybdate method following combustion at 500° C and digestion in acid (Solorzano and Sharp 1980). I use the term '%P' (or C or N) to describe the percentage dry mass of each element per dry mass of material. I calculated all elemental ratios using molar concentrations and use the term 'C:N' (or C:P or N:P) to describe these ratios.

Elemental calculations

I calculated the elemental imbalance for consumer-resource pairs (isopod-detritus and acanthocephalan-isopod) using the equation, $\text{imbalance}_x = \text{consumer}_x - \text{resource}_x$, where *X* represents elemental content (%P or N:P). For isopod-detritus

imbalance, I used individual measurements of isopod %P and N:P and mean values of detritus %P and N:P for each P treatment using leaves sampled on experiment Day 0. For acanthocephalan-isopod imbalance, I used individual measurements of %P for each parasite-host pair.

To compare the elemental content of infected isopods with and without parasite tissue, I used data on the dry mass and %P of individual acanthocephalans to estimate the %P, C:P, and N:P of each infected isopod combined with its corresponding parasite. Because I did not measure acanthocephalan %C or %N in this study, I used previously collected data on the mean elemental content of *A. tahlequahensis* (0.0364 moles C g^{-1} tissue, 0.00505 moles N g^{-1} tissue) collected from isopods at Wesickaman Creek (Paseka and Grunberg, in revision).

Statistical analysis

I analyzed data and generated graphics in R v.3.4.4 (R Core Team 2018). I used aquaria as replicates to test the effects of P treatment and infection status on isopod survival using analysis of variance (ANOVA). After conducting preliminary analyses to confirm that there was no effect of aquarium replicate on the growth or stoichiometry of isopods or acanthocephalans, I conducted subsequent analyses using individual hosts or parasites as replicates. I tested the effects of P treatment on detritus elemental content using a multivariate analysis of variance (MANOVA). I used a MANOVA to test the effects of P treatment and infection status on isopod-detritus elemental imbalance and an ANOVA to test the effects of P treatment and acanthocepahalan sex on acanthocephalan-isopod %P imbalance. I analyzed the

effects of initial body size, elemental imbalance, P treatment, and infection on isopod growth rate using an analysis of covariance (ANCOVA), and I used a multivariate analysis of covariance (MANCOVA) to determine the effects of growth rate, infection, and P treatment on isopod elemental content. Finally, I used ANCOVAs to determine the effects of host body size, P treatment, parasite sex, and elemental imbalance on parasite body size and the effects of body size, P treatment, sex, and host %P on parasite %P.

For all tests with multiple independent variables, I used Type III sums of squares to account for non-orthogonal design. I tested for differences among P treatments *post hoc* using Tukey's multiple comparison tests. Multivariate summary statistics from MANOVAs and MANCOVAs are presented in the text, while *F*-ratios and *p*-values from the accompanying univariate analyses are included in figure legends for more detailed interpretation. Figures also include trend lines resulting from simple linear regressions for significant covariate relationships from ANCOVAs and MANCOVAs.

Results

Enrichment of basal resources

Experimental P treatment resulted in changes to detritus P content (as %P, C:P, and N:P) both at the beginning of the experiment (Day 0, MANOVA, $F_{3,8} = 2.97$, $p = 0.03$) and the end of the experiment (Day 15, MANOVA, $F_{3,28} = 2.19$, $p = 0.03$). Leaves incubated with added P were higher in %P and lower in C:P and N:P than control leaves at the start of the experiment (Day 0), though different

concentrations of P addition treatments did not lead to significant differences in leaf elemental content among the experimental groups. Leaves sampled at the end of the experiment (Day 15) still differed in %P and C:P across P treatments, but not in N:P (Figure 3.1).

Elemental imbalance in consumer-resource interactions

P treatment determined the degree of %P and N:P imbalance between isopods and their detritus resources (MANOVA, $F_{3,32}$= 33.4, $p < 0.001$). All levels of P addition reduced isopod-detritus %P imbalance relative to the control, and isopod-detritus interactions were more balanced in N:P with each increasing level of P addition (Fig. 3.2). Infected isopods were more balanced with detritus resources than uninfected isopods in both %P and N:P ($F_{1,32}$ = 9.54, $p < 0.001$, Fig. 3.2). There was no interaction between P treatment and infection status ($F_{3,32}$= 1.04, p = 0.41).

The %P imbalance between acanthocephalans and isopods was highly variable (Fig. 3.3), and the direction of this imbalance (positive or negative) was unrelated to P treatment (ANOVA, $F_{3,14}$ = 1.35, p = 0.299), acanthocephalan sex ($F_{1,14}$ = 3.34, p = 0.089), and their interaction ($F_{2,14}$ = 0.114, p = 0.893).

Isopod survival, growth, and elemental content

Isopod survival was consistent across P treatments (ANOVA, $F_{3,11}$ = 0.581, p = 0.639). While the overall rate of survival for infected isopods tended to exceed the survival rate of uninfected isopods within each treatment, the effect of infection on survival was not statistically significant ($F_{1,11}$ = 3.60, p = 0.084, Fig. 3.4).

Isopod growth rate scaled negatively with initial body size (ANCOVA, $F_{1,29}$ = 4.50, p = 0.043) and positively with isopod-detritus %P imbalance ($F_{1,29}$ = 11.5, p = 0.002). There were no effects of infection status ($F_{1,29}$ = 2.81, p = 0.105) or P treatment ($F_{3,29}$ = 2.91, p = 0.051) on isopod growth rate (Figure 3.5). The effects of infection status did not interact with initial body size ($F_{1,29}$ = 2.54, p = 0.122) or %P imbalance ($F_{1,29}$ <0.001, p = 0.998).

Isopod elemental content (as %P, C:P, and N:P) was related to growth rate (MANCOVA, $F_{1,22}$ = 3.84, p = 0.026) and infection status ($F_{1,22}$ = 3.25, p = 0.044), with an interaction between the two terms ($F_{1,22}$ = 3.35, p = 0.04). Infected isopods were lower in %P and higher in C:P and N:P than uninfected isopods (Fig. 3.6). There was no effect of P treatment on isopod elemental content ($F_{3,22}$ = 0.957, p = 0.487, Fig. 3.6), and P treatment did not interact with any of the other predictors in the model.

After recalculating isopod %P, C:P, and N:P to include the mass and elemental content of acanthocephalans, uninfected isopods differed from both infected host tissue alone and infected host tissue combined with parasite tissue (MANCOVA, $F_{2,55}$= 10.6, p < 0.001), but infected hosts with and without parasite tissue added did not differ in elemental content (Sup. Fig. S3.1).

Acanthocephalan survival, body size, and elemental content

Acanthocephalan survival was measured as the survival of infected hosts, which occurred independently of P treatment (ANOVA, $F_{3,4}$ = 0.171, p = 0.911, Fig. 3.4). The maximum body size of acanthocephalans increased with each increasing level of P addition (Fig. 3.7), but there was no difference in the mean body size of

acanthocephalans among P treatment groups (ANCOVA, $F_{3,12} = 0.233$, $p = 0.871$). Female acanthocephalans were larger than male acanthocephalans ($F_{1,12} = 35.0$, $p < 0.001$). The %P imbalance between individual acanthocephalans and isopod hosts was related to acanthocephalan body size ($F_{1,12} = 41.7$, $p < 0.001$). There was an interaction between %P imbalance and acanthocephalan sex ($F_{1,12} = 10.9$, $p = 0.006$) such that female body size was inversely related to %P imbalance, and male body size was unrelated to %P imbalance (Fig. 3.7). Isopod body size did not predict acanthocephalan body size ($F_{1,12} = 0.008$, $p = 0.932$).

Acanthocephalan %P was related to body size (ANCOVA, $F_{1,12} = 18.371$, $p = 0.001$), but not to P treatment ($F_{3,12} = 1.37$, $p = 0.299$), sex ($F_{1,12} = 0.15$, $p = 0.705$), or isopod %P ($F_{1,12} = 0.409$, $p = 0.535$, Fig. 3.8). The effects of acanthocephalan sex did not interact with body size ($F_{1,12} = 0.403$, $p = 0.538$) or with isopod %P ($F_{1,12} = 0.639$, $p = 0.440$). However, among female acanthocephalans, %P scaled negatively with body size (linear regression, $R^2 = 0.791$, $p < 0.001$) and positively with isopod %P ($R^2 = 0.417$, $p = 0.032$).

Discussion

Basal resource quality and isopod-acanthocephalan interactions

Experimental P treatments substantially changed the elemental content of leaf litter and determined the degree of %P and N:P imbalance between isopod consumers and their detritus resources. Despite these basal shifts, P treatment did not affect the survival, growth, or elemental content of isopods or acanthocephalans. The elemental homeostasis of isopods and the invariance of isopod growth rate

across P treatments may reflect low sensitivity to changes in detrital resource quality, leading to consistent host resource quality for acanthocephalans despite environmental variability.

However, several aspects this experiment's design may also have masked the potential effects of basal resource quality on the isopod-acanthocephalan system. First, I used field-collected isopods that were naturally infected with acanthocephalans due to the intractability of maintaining the complex life cycle of *A. tahlequahensis* in the lab. In these collections, I could not control for isopod age, feeding history, or infection stage, any of which may have introduced heterogeneity in host and parasite size, growth rate, and elemental composition to the experiment. Second, I collected mature isopods in early summer, the season when *A. tahlequahensis* infection prevalence peaks and provides ample infected hosts for experiments. One drawback of this timing is that isopods have already reached adult size and may not have great needs of dietary nutrients to support continued growth. Additionally, isopod mortality during the experiment was high, reflecting the natural, post-reproduction mortality that occurs in the early summer for mature isopods. High mortality led to a low sample size of acanthocephalans within surviving isopod hosts and subsequent low statistical power in measurements of acanthocephalan body size and elemental content. I terminated the experiment after 16 days of isopod incubation to curtail the continued loss of isopods to natural mortality. Providing a longer period of time for basal nutrients to filter up through the detritus-isopod-acanthocephalan interaction may have yielded more insight into the effects of resource quality in this system.

Despite the experimental limitations of the acanthocephalan-isopod system for addressing the effects of basal nutrient ratios on host-parasite interactions, this study yields insight into several underlying patterns in host-parasite nutrient dynamics that occur in populations of naturally infected hosts. Understanding the effects of infection on host nutrient demand and growth, as well as the flexibility of parasite elemental content and its relationship to growth, will aid future work to understand the effects of environmental nutrients on host-parasite interactions and subsequent disease patterns.

Host stoichiometry, survival, and growth

Infected isopods were lower in P content than uninfected isopods (Fig 3.6), which led to reductions in the %P and N:P imbalance between isopod hosts and detritus resources (Fig. 3.2). While elementally balanced consumer-resource interactions should lead to optimal consumer growth efficiency (Boersma and Elser 2006), infected and uninfected isopods grew at similar rates over the course of the experiment (Fig. 3.5). Individuals of *C. communis* infected with *A. tahlequahensis* incur a substantial energetic cost of infection (Lettini and Sukhdeo 2010) that likely negates any potential gains in growth efficiency resulting from elemental balance with detrital resources. Contrary to expectations for the effect of consumer-resource imbalance on consumer growth, there was a weak, positive relationship between %P imbalance and isopod growth rate (Fig. 3.5). I interpret this relationship as an artifact of infection-based decreases in host P content (thus reducing isopod-

detritus elemental imbalance) combined with a non-significant decrease in growth rate for infected isopods.

Isopod P content was related to growth rate during the experiment, and infection changed the nature of this relationship (Fig 3.6). The growth rate hypothesis predicts that invertebrate P content will increase with growth rate due to the high P content of ribosomal RNA required for protein synthesis (Elser et al. 1996, 2003). The positive scaling of uninfected isopod %P with growth rate and the negative scaling of uninfected isopod N:P and C:P are consistent with these predictions. Infection reduced the strength of these relationships, such that the slopes of the %P and C:P relationships with growth rate were near zero (Fig 3.6). Infection-induced reductions in host P content and changes to the relationship between P content and growth rate may inhibit the growth and reproduction of individual hosts, with potential repercussions for host population dynamics.

Indirect effects of infection on host stoichiometry may also cascade to upper trophic levels through changes in the quality of hosts as prey. Infected isopods represent a lower quality resource to nutrient limited predators, even when accounting for the additional biomass and P contributed by acanthocephalans (Sup. Fig. S3.1). The fish that serve as definitive hosts to *A. tahlequahensis* therefore incur a two-fold cost in consuming infected isopods: an immediate, nutritional cost in consuming prey with reduced P content and the long term, energetic cost of infection.

Within each P treatment, infected isopods survived at a higher rate than uninfected isopods during the 16 day experimental period (Fig. 3.4), though this

result was not statistically significant across all treatments. This difference in survival is consistent with a pattern in Pine Barrens streams in the early summer in which the density of mature isopods drops as post-reproductive adults die, but the prevalence of infection increases as infected isopods apparently outlive uninfected isopods. This phenomenon is intuitive from the perspective of the acanthocephalan, which benefits from host mortality due to predation by a potential definitive host, but not due to natural senescence. The mechanism controlling this difference in survival and the potential role of the nutrient and energy costs of infection in creating this pattern are unknown.

Because I used field-collected isopods for this study, it is possible that infection is a consequence of low host P content, rather than a cause. P content may be linked to host immune function or other aspects of physiology that influence susceptibility to infection (Aalto et al. 2015), though these potential mechanisms lack empirical evidence. Until more information on the stoichiometry of host-parasite interactions becomes available, it is impossible to establish causality in relationships between infection and host nutrients. However, given evidence from experimental infections in another host-parasite system that demonstrate parasite effects on host elemental content (Frost et al. 2008, Narr and Frost 2016), I choose to interpret low P content in infected isopods as a consequence of parasite infection, rather than a cause. Acanthocephalans are well known for their effects on the behavior and ecology of intermediate hosts (Camp and Huizinga 1979, Moore 1983, Hernandez and Sukhdeo 2008). This study demonstrates that acanthocephalan

infection also alters the organismal stoichiometry and consumer-resource imbalance of isopod hosts.

Parasite stoichiometry, elemental balance with hosts, and implications for growth

Acanthocephalan %P content was highly variable among individuals and was primarily driven by body size (Fig. 3.8). Female acanthocephalan %P had a strong, negative relationship with body size. This negative scaling of parasite %P with body size is consistent with a similar trend across parasite species (Paseka and Grunberg, in revision). At both scales, this pattern supports the growth rate hypothesis, which predicts that smaller bodied organisms have relatively higher P demands compared to larger bodied organisms to support rapid growth (Elser et al. 2003, Gillooly et al. 2005).

Variation in acanthocephalan %P content also corresponds to high variability in the %P imbalance between individual parasites and isopod hosts (Fig. 3.3). Acanthocephalans were positively or negatively imbalanced with their hosts, and variation in %P imbalance was not related to acanthocephalan sex or experimental P treatment. While it is not clear what factors cause the degree of elemental imbalance in individual parasite-host pairs, an apparent consequence is that %P imbalance interacts with acanthocephalan sex to predict acanthocephalan body size (Fig. 3.7). Male acanthocephalans were smaller than females and varied little in body size, with no apparent effect of %P imbalance. Female acanthocephalans were larger than males overall and much more variable in body size, with a negative relationship between size and %P imbalance (Fig. 3.7). Contrary to the expectation

that acanthocephalans would reach maximum body size when balanced with hosts in elemental content (at a %P imbalance of zero), the largest acanthocephalan body sizes occurred at negative %P imbalance values. These negative values indicate that hosts were higher in %P than acanthocephalans; female acanthocephalans achieved the greatest body size when hosts provided ample P relative to parasite tissue. I speculate that the observed, negative relationship between female body size and elemental imbalance reflects P limitation of acanthocephalan growth. The dependence of this relationship on parasite sex likely reflects the differential growth requirements of male and female acanthocephalans. Female body size determines fecundity when mature worms begin producing eggs after mating in the intestine of a fish definitive host (Crompton and Nickol 1985). The importance of acanthocephalan male body size to reproductive success is unknown, but the low variability in size and lack of relationship with host resource quality may reflect a relative unimportance of male body size to fitness relative to females.

The nutrient requirements of female acanthocephalans may also be reflected in the weak, positive relationship in %P between individual host-parasite pairs (Fig. 3.8). While body size was the major determinant of acanthocephalan %P (and the only term that was significant in the ANCOVA for parasite %P), female acanthocephalans also appear to loosely track the %P of their hosts. If this relationship is causal, it suggests that acanthocephalans may not be strictly homeostatic in body stoichiometry, instead varying according to the nutrient content of their host resources. Little is known about parasite stoichiometric homeostasis, though prior work shows *A. tahlequahensis* varies in elemental content

depending on its life cycle stage (Paseka and Grunberg, in revision). It is important to note that attempts to quantify homeostasis using observational field studies often contain multiple confounding factors (Halvorson and Small 2016), so additional experimental work is needed to definitively measure the degree of stoichiometric homeostasis or flexibility exhibited by *A. tahlequahensis.* If stoichiometric flexibility facilitates the growth and fecundity of *A. tahlequahensis*, then assessing the generality of this plasticity and its consequences for parasite survival, growth, and reproduction will yield important insight into the effects of resource quality on infection patterns in nature.

Conclusions and future directions

Using a stoichiometric framework to study isopod-acanthocephalan nutrient dynamics yields insight into relationships between resource quality, growth, and organismal stoichiometry of parasites and hosts. In this system, infection lowered host P content, reduced the elemental imbalance between infected hosts and their dietary resources, and altered the relationship between host nutrients and growth rate. Parasite elemental content and parasite-host %P imbalance were highly variable, and parasites obtained the largest body sizes when host P content exceeded that of parasite tissue. Understanding these natural patterns in host-parasite nutrient dynamics will aid predictions on the effects of basal resource quality on host-parasite interactions.

This study was limited by the experimental tractability of the acanthocephalan-isopod system, and there is merit in additional experimental work

to identify the effects of environmental nutrients on host-parasite dynamics and the mechanisms controlling these interactions. An ideal system for this work would include hosts and parasites that can be reared in the lab, thereby eliminating the inherent variability of field-collected organisms. Extending this type of experiment to explore parasite reproduction, transmission, and subsequent infection patterns in host populations and communities will provide important context on the effects of environmental change to infectious disease. Several additional concepts from stoichiometric theory, such as homeostasis, relationships between organismal stoichiometry and growth, and threshold elemental ratios to more accurately predict consumer nutrient limitation have not been thoroughly explored in the context of parasitism. Exploring these topics will provide insight into the mechanisms behind host-parasite nutrient dynamics, and additional work on this theme will help to develop the power of stoichiometric theory to link parasitism with ecosystem processes.

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Chapter 3 Tables and Figures

Figure 3.1. Phosphorus content of *Acer rubrum* leaves at the start (Day 0) and end (Day 15) of the experiment. *F*-ratios and *p*-values correspond to the results of one-way ANOVAs for each elemental variable. Letters above bars indicate differences among group means as determined by Tukey's HSD tests ($p < 0.05$).

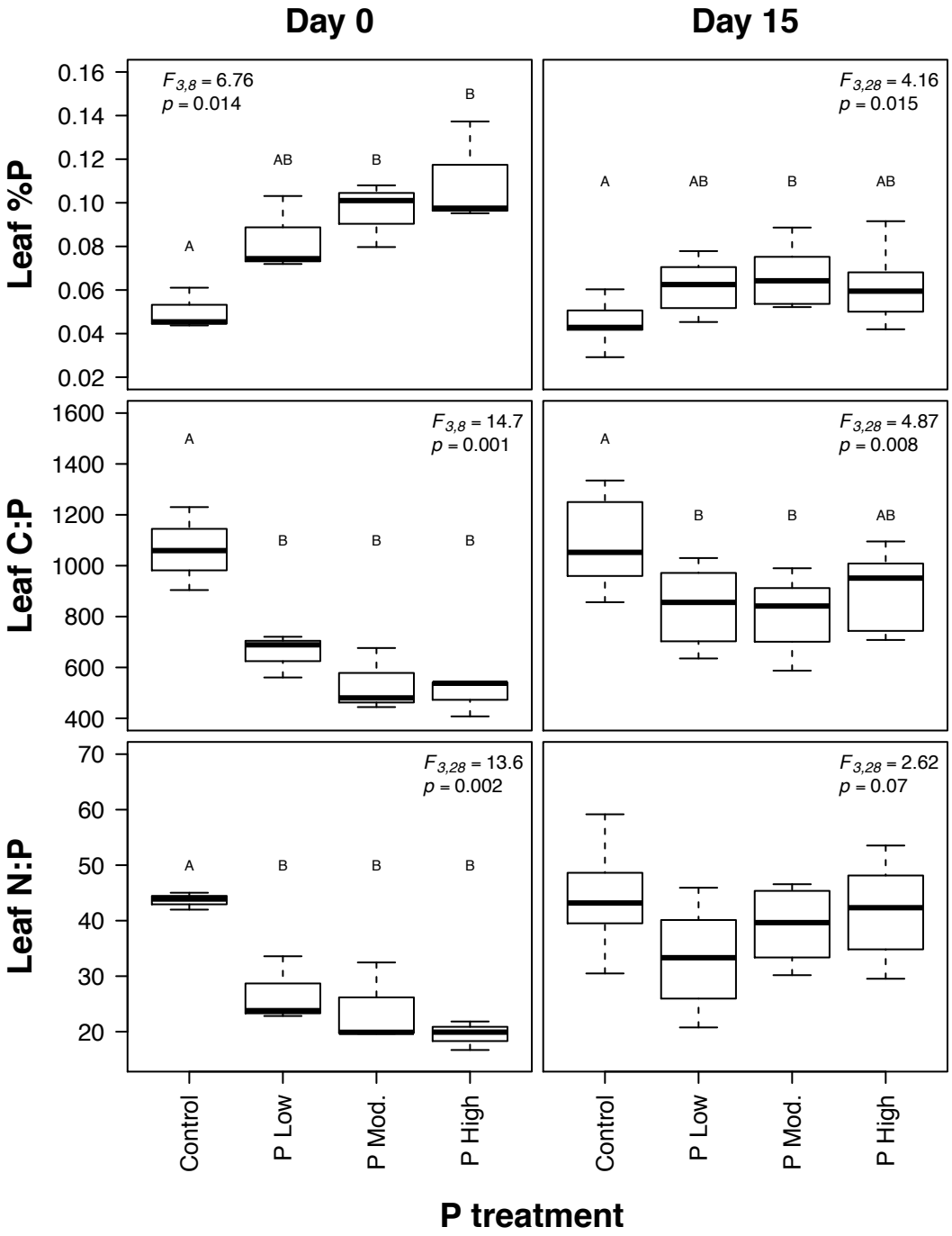


Figure 3.2. %P and N:P imbalance between isopods and detritus. *F*-ratios and *p*-values are univariate statistics corresponding to a MANOVA for the effects of P treatment and infection status on %P and N:P imbalance. Letters above bars indicate differences among group means as determined by Tukey's HSD tests ($p < 0.05$). The dashed line indicates an N:P imbalance of zero, where isopods and detrital resources would be equal in elemental content.

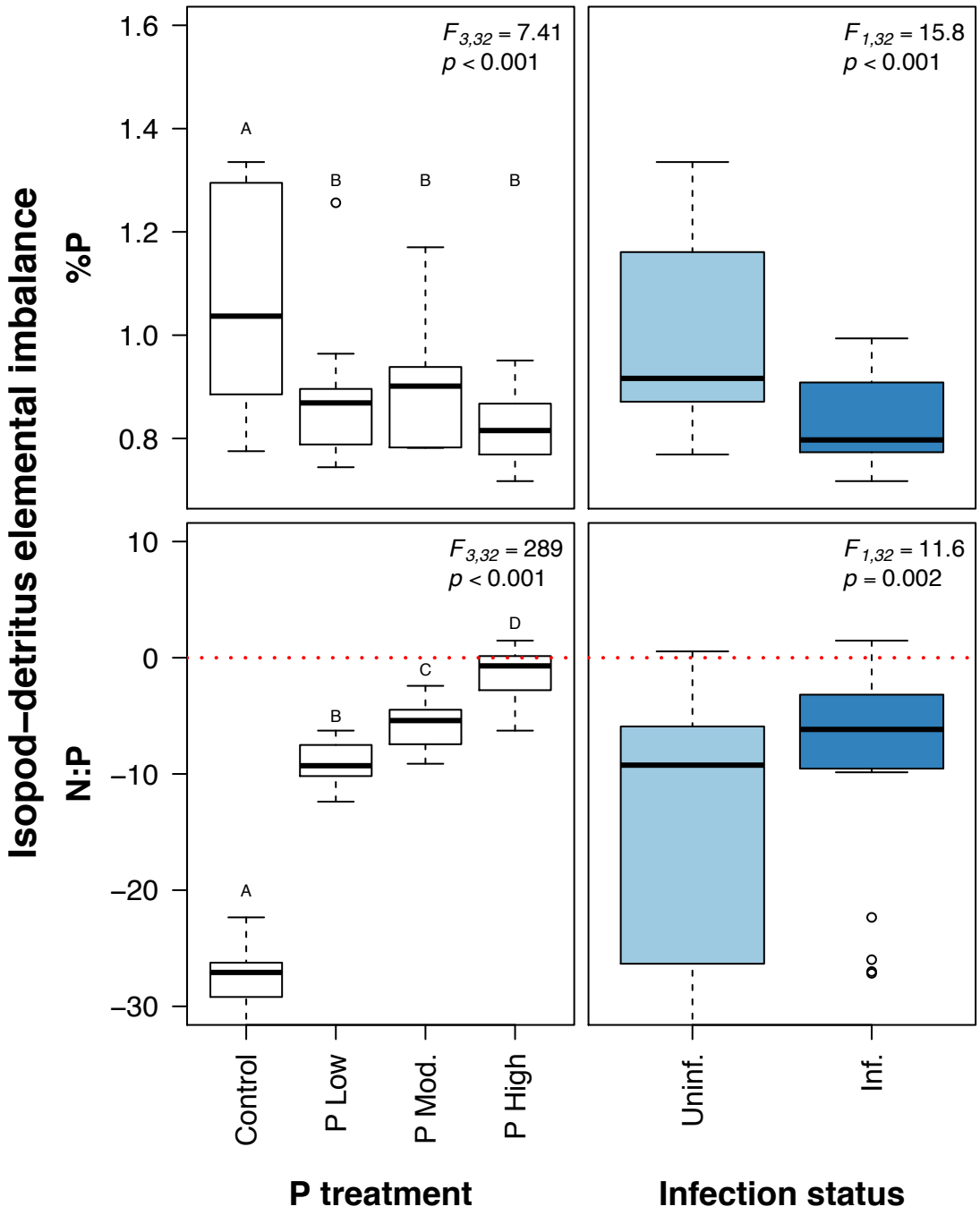


Figure 3.3. %P imbalance between acanthocephalans and isopods. *F*-ratios and *p*-values correspond to the results of a two-way ANOVA, with P treatment and acanthocephalan sex as factors. The dashed line indicates a %P imbalance of zero, where acanthocephalans are equal in %P to their isopod hosts.

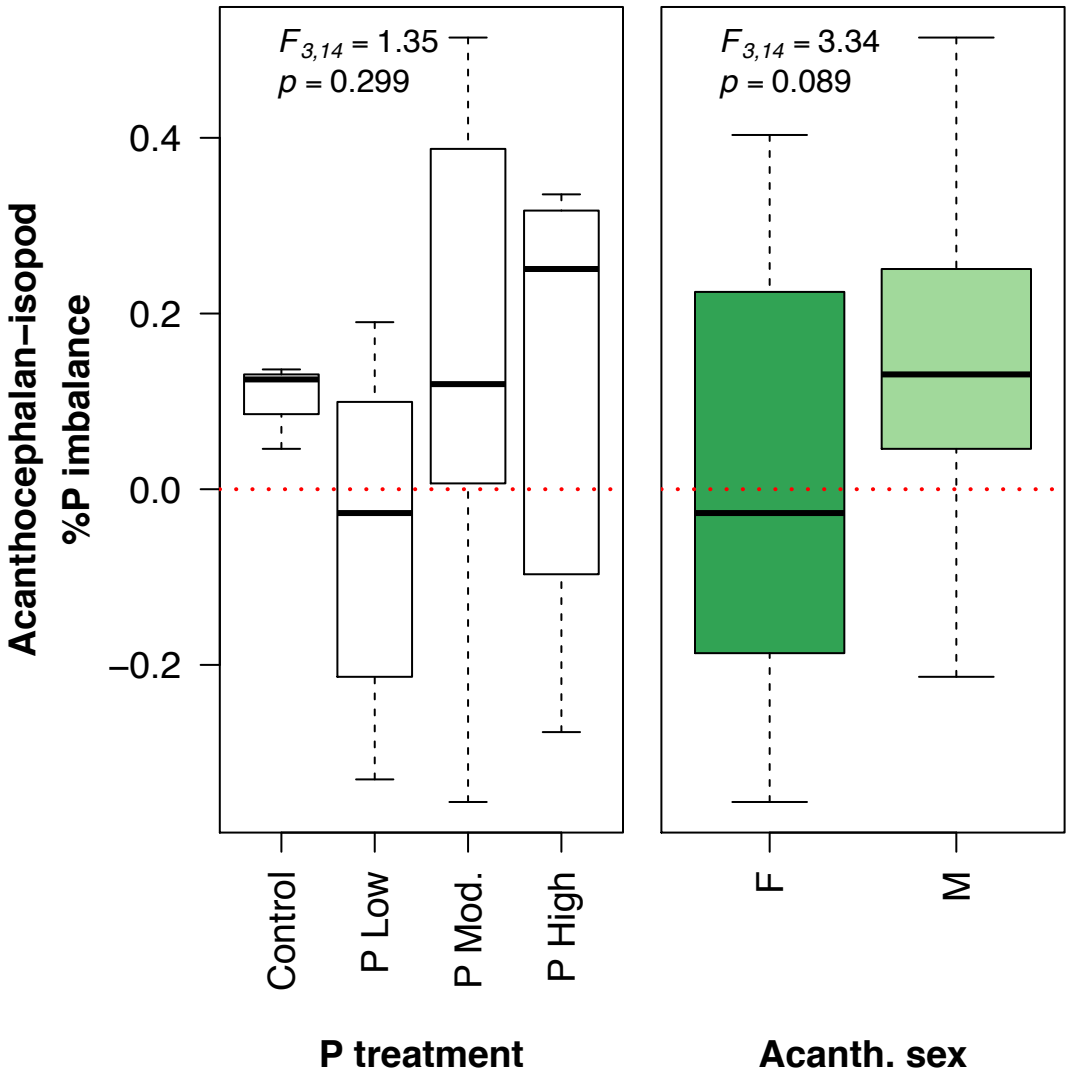


Figure 3.4. Isopod survival rate by P treatment and acanthocephalan infection status. The dashed line represents the overall survival rate across all treatments (32%). Bars show the mean survival rate of two aquaria per P treatment (±SE). The effects of P treatment (p = 0.639) and infection (p = 0.084) on isopod survival were not statistically significant.

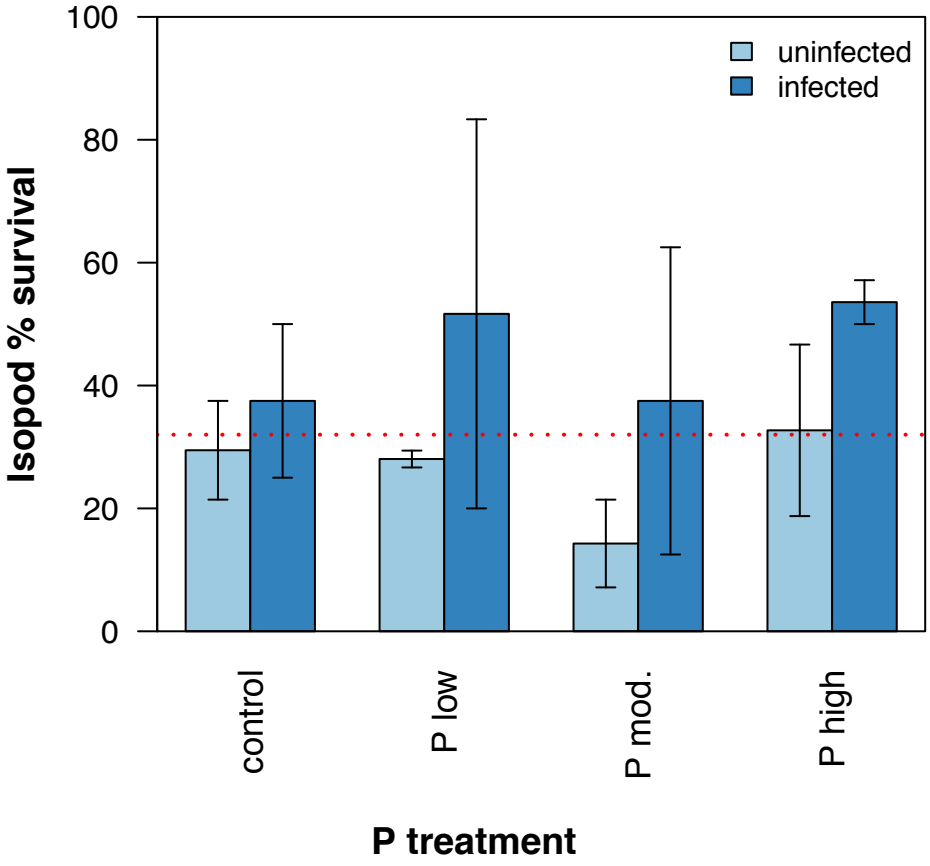


Figure 3.5. Isopod growth (dry mass gained) by initial mass, P treatment, infection status, and %P imbalance with detritus resources. *F*-ratios and *p*-values correspond to the results of ANCOVA, with initial mass and isopod-detritus %P imbalance as covariates and P treatment and infection status as factors. Trend lines show the results of simple linear regressions between growth and initial body size (R^2 = 0.202, p = 0.005) and growth and %P imbalance (R^2 = 0.107, p = 0.045), neither of which interacted with infection status.

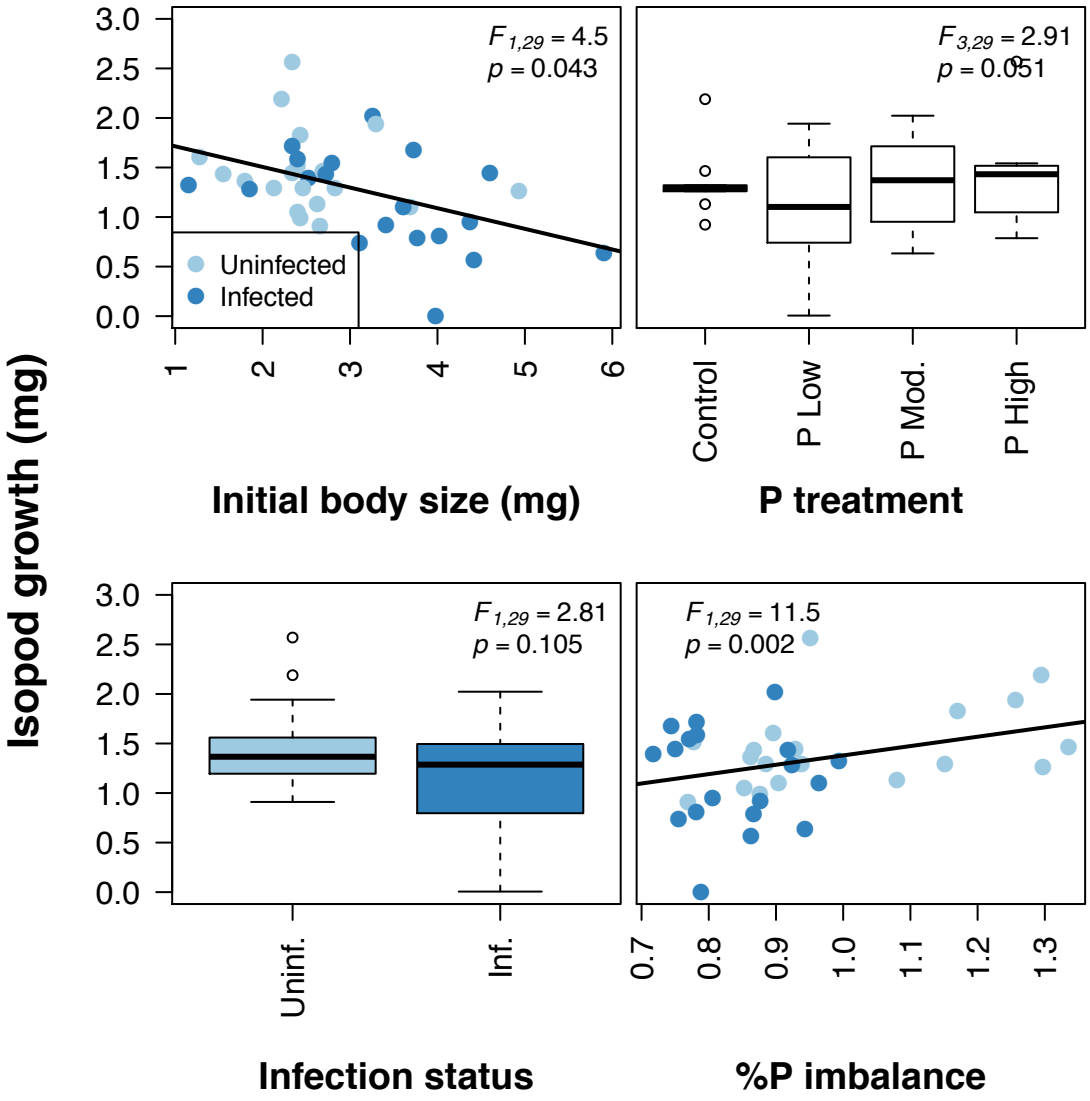


Figure 3.6. Isopod P content by growth rate, P treatment, and infection status. *F*-ratios and *p*-values are univariate statistics corresponding to a MANOVA including isopod %P, C:P, and N:P, with growth rate as a covariate and P treatment and infection status as factors. Regression lines represent significant interactions between growth and infection. Letters above bars indicate differences among group means as determined by Tukey's HSD tests ($p < 0.05$).

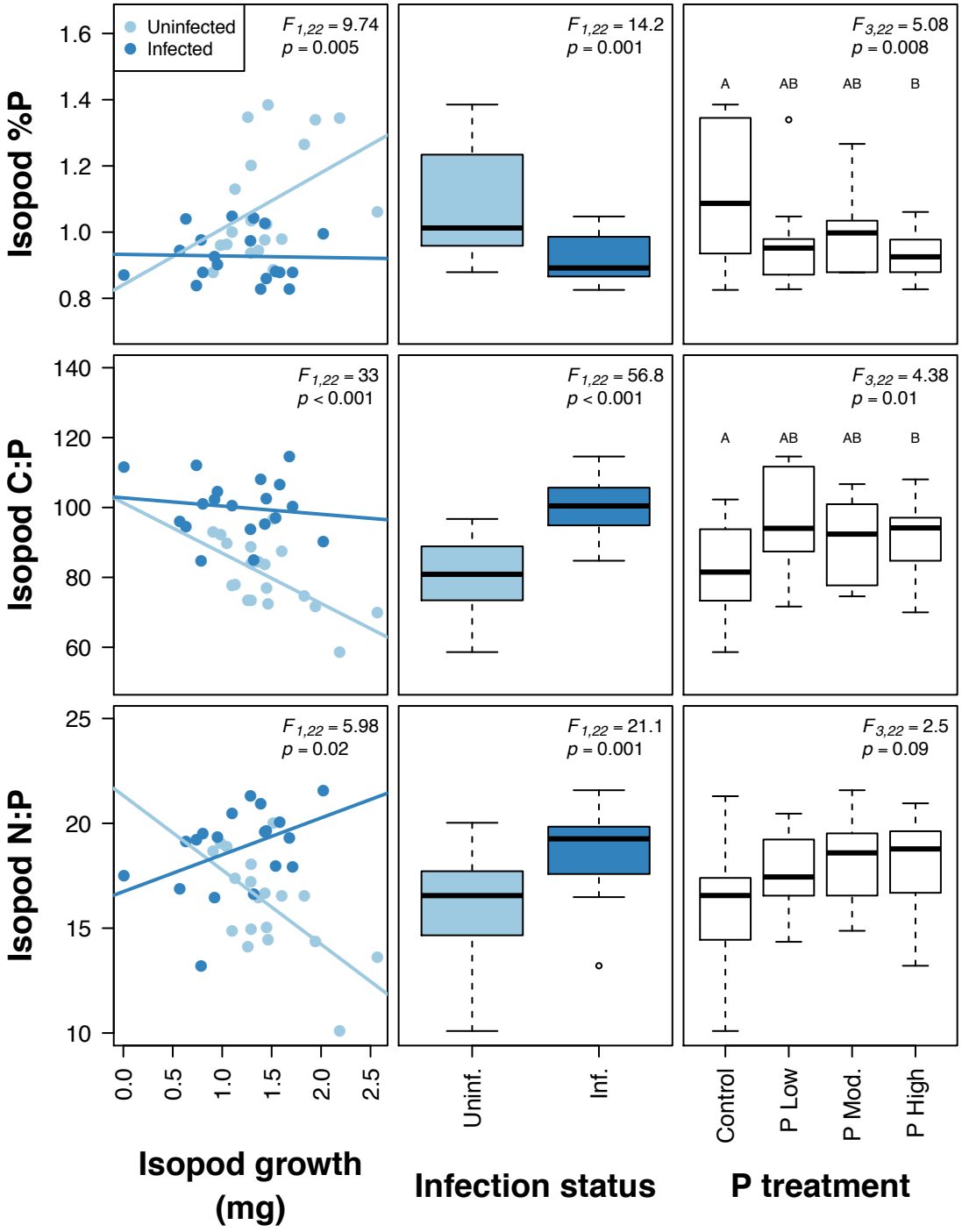


Figure 3.7. Final body size of acanthocephalans (dry mass, mg) by host body size, P treatment, acanthocephalan sex, and acanthocephalan-isopod %P imbalance. *F*-ratios and *p*-values correspond to ANCOVA results. Regression lines represent significant interactions between sex and %P imbalance.

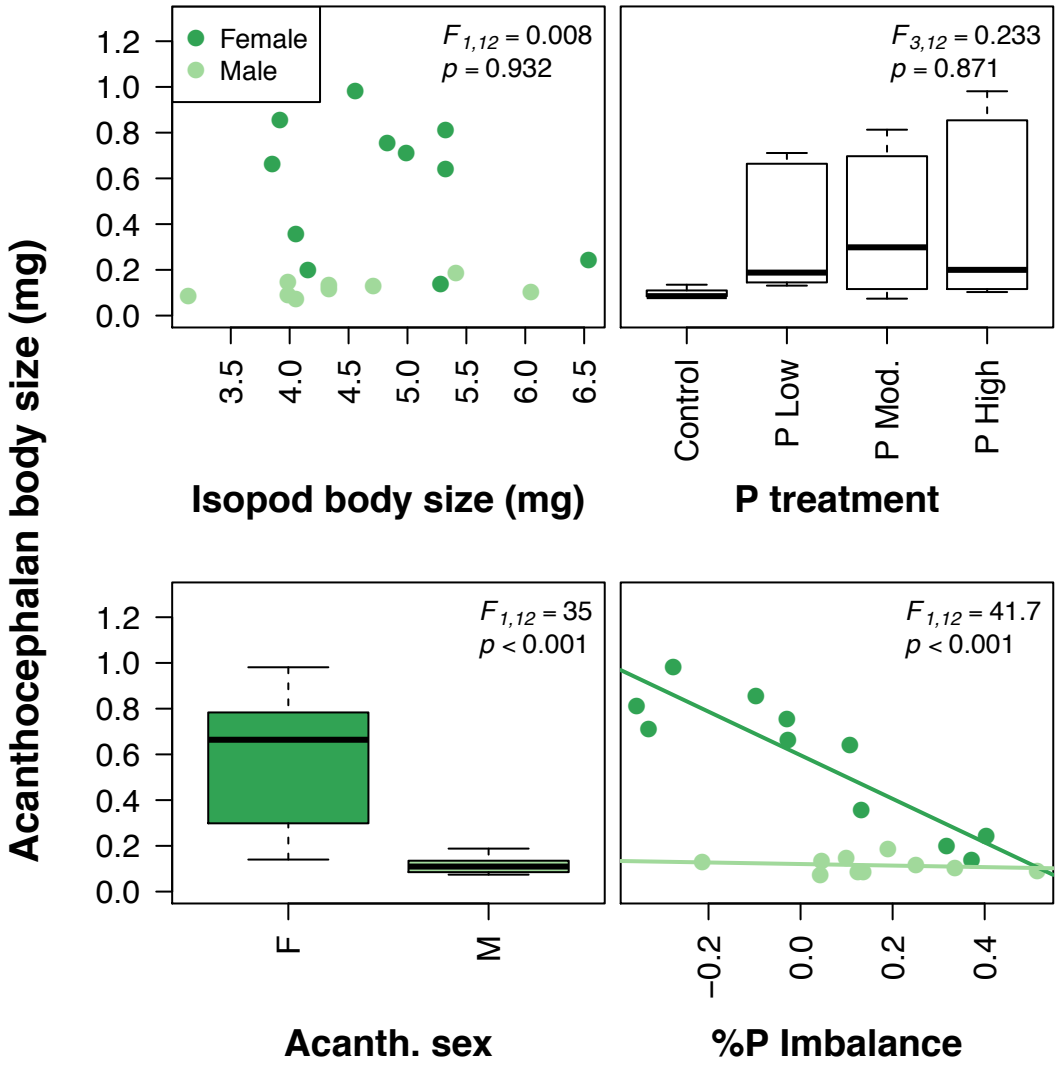
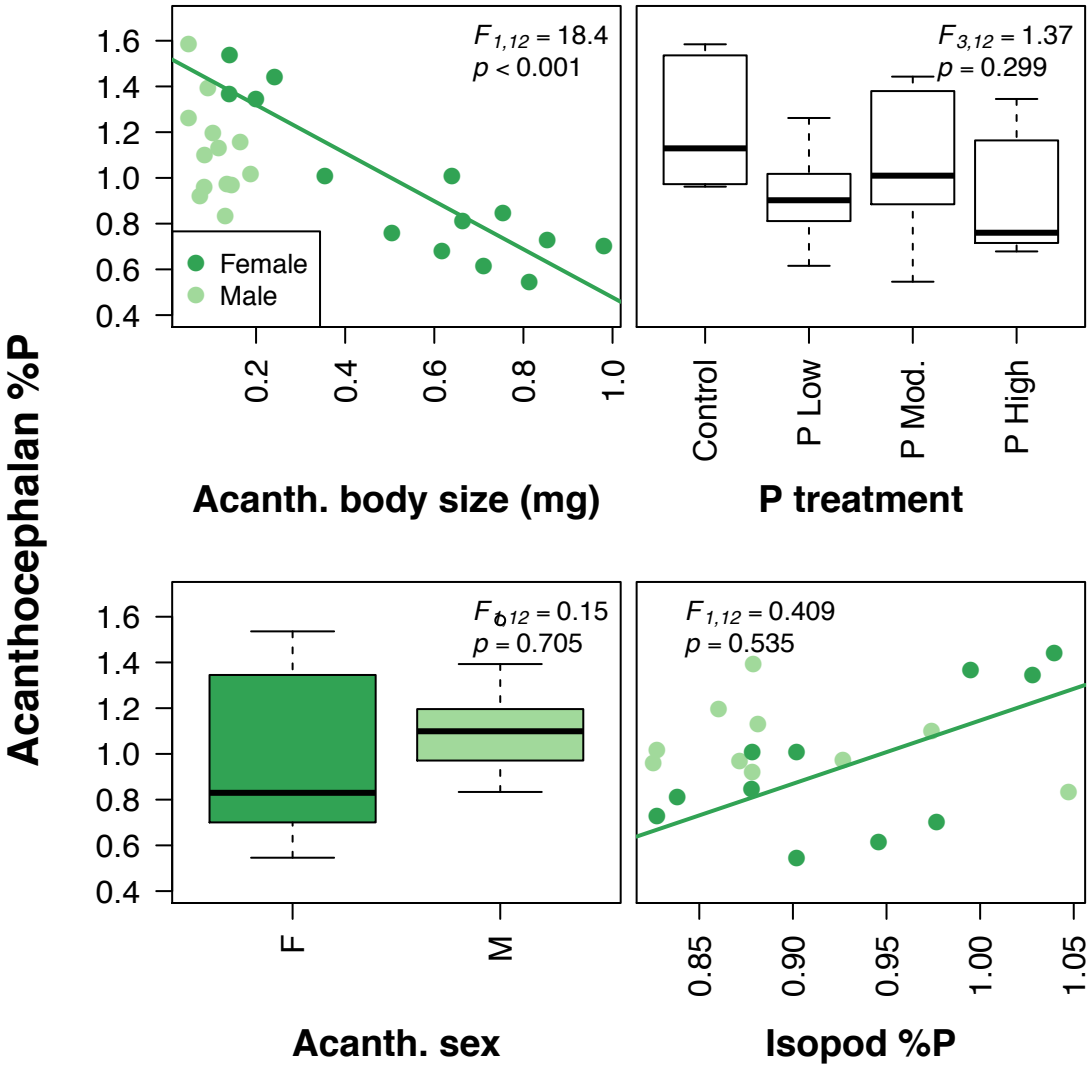
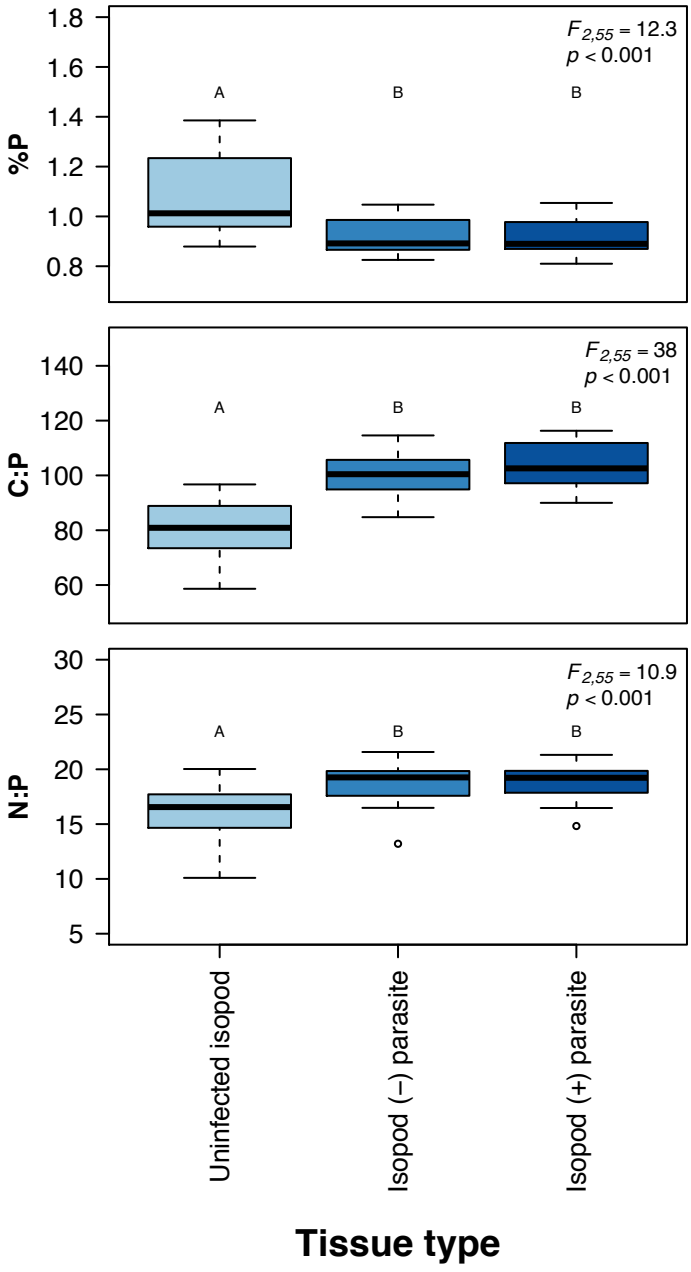


Figure 3.8. Acanthocephalan P content by body size, P treatment, and sex. *F*-ratios and *p*-values correspond to the results of ANCOVA, with body size as a covariate and P treatment and sex as factors. Trend lines show the results of simple linear regressions for female acanthocephalan %P and body size ($R^2 = 0.791$, $p < 0.001$) and for female acanthocephalan %P and isopod %P ($R^2 = 0.417$, $p = 0.032$).



Chapter 3 Appendix

Sup. Fig. S3.1. Isopod elemental content with and without acanthocephalan tissue. The categories "Uninfected isopod" and "Isopod (-) parasite" represent original measurements of isopod elemental content. The category "Isopod (+) parasite" represents the recalculation of isopod elemental content to include the mass and elemental content of acanthocephalans. *F*-ratios and *p*-values are univariate statistics corresponding to a MANOVA including isopod %P, C:P, and N:P. Letters above bars indicate differences among group means as determined by Tukey's HSD tests ($p < 0.05$).



CHAPTER 4

PARASITE-MEDIATED NUTRIENT CYCLING: VARIABLE EFFECTS OF INFECTION ON THE STORAGE AND RECYCLING OF NUTRIENTS BY FRESHWATER FISH

Abstract

The effects of parasitism on N and P cycling in aquatic ecosystems are poorly understood. This problem stems from both data scarcity and the lack of an appropriate conceptual framework to link host-parasite interactions with ecosystem processes. Ecological stoichiometry offers one potential framework to address this disconnect by linking organismal traits, species interactions, and ecosystem-level nutrient dynamics through elemental chemistry. I explored the utility of a stoichiometric framework to describe relationships between patterns of macroparasite infection and the storage and recycling of nutrients within host populations. I sampled three populations of freshwater fish that each serve as hosts to diverse parasite communities. For each individual host, I measured nutrient storage (dry mass %C, N, and P and molar ratios C:N, C:P, and N:P), recycling rates (excreted ammonium and soluble reactive phosphorus), body size, and parasite infection intensity. Infection was common in each population, with most individuals harboring two or more parasite species. However, infection levels, tissue chemistry, and excretion rates varied substantially among host individuals in each population. I used structural equation models to test multiple causal hypotheses about relationships between infection, host body size, and host nutrient storage and recycling. Body size was the most consistent predictor of host stoichiometry and

excretion chemistry across populations and mediated some relationships between host tissue stoichiometry and infection. Infection with several parasite species explained additional variance in host nutrients, but these relationships were highly variable across host-parasite species pairs and did not improve the overall predictive power of statistical models. Compared to laboratory studies that report clear effects of parasite infection on host tissue stoichiometry and excretion chemistry, the effects of infection on host nutrient cycling function are harder to identify in natural host populations that are coinfected with multiple parasite species. While a stoichiometric approach can contribute to our understanding of the links between parasitism and nutrient cycling, considering the diverse nature of host-parasite interactions is essential when evaluating these links.

Introduction

Parasites are ubiquitous in all natural systems and often have profound effects on the biology of individual hosts, host populations, and community dynamics (Lefèvre et al. 2009, Hatcher et al. 2012). These effects also correspond to functional importance of parasitism at the ecosystem scale, with parasites indirectly mediating processes such as productivity (Kohler and Wiley 1997, Sato et al. 2012), decomposition (Hernandez and Sukhdeo 2008), and nutrient cycling (Bernot 2013, Narr and Frost 2016) in some systems. Despite this evidence and considerable discussion on the topic of linking host-parasite interactions with ecosystem ecology (e.g., Ostfeld et al., 2008; Thomas et al., 2005), a general disconnect remains

between these research areas (Preston et al. 2016). This problem stems from both a scarcity of data and the lack of an appropriate conceptual framework to link ecological processes across scales and systems. No one framework is likely to bridge the gap between host-parasite interactions and all types of ecosystem functioning, but I posit that ecological stoichiometry and metabolic ecology provide a conceptual foundation to assess relationships between parasitism and nutrient cycling.

Consumers shape food webs and ecosystems not just by providing predation pressure from the top down, but also by excreting limiting nutrients that fuel ecosystems from the bottom up (Vanni 2002, Atkinson et al. 2016). Consumer-driven nutrient dynamics have a theoretical foundation in ecological stoichiometry, an approach that links organismal traits, consumer-resource interactions, and ecosystem-level nutrients through ratios of chemical elements that are essential to all of life (Sterner and Elser 2002, Hessen et al. 2013). Stoichiometric theory predicts that the balance between the elemental ratios of a consumer's body tissue and its diet will determine its nutrient recycling function (Elser and Urabe 1999). Empirically, animal body stoichiometry predicts excretion rates and ratios across species (Vanni et al., 2002), the effects of which can determine the nutrient limitation of entire ecosystems (Elser et al. 1988, Sterner et al. 1992, Allgeier et al. 2013).

The metabolic theory of ecology independently predicts that animal body size determines the rates of physiological processes, including nutrient excretion (Brown et al. 2004, Allen and Gillooly 2009). Two recent studies revealed that body size outperformed organismal stoichiometry in predicting animal nutrient excretion

across many species (Allgeier et al. 2015, Vanni and McIntyre 2016), indicating that an integration of metabolic ecology and ecological stoichiometry is warranted when considering the determinants of consumer driven nutrient dynamics at a broad scale. It is not well known how these two predictive frameworks function at a narrower scale, such as within species or populations. At this smaller scale, it is also important to consider how parasite infection may alter relationships among host body size, body stoichiometry, and nutrient recycling function.

Several prior studies have demonstrated that parasite infection may mediate host nutrient storage and recycling, thereby generating variation in nutrient dynamics among individuals in host populations. Evidence from snail-trematode and zooplankton-microparasite studies indicates that infection can alter host body stoichiometry (Frost et al. 2008, Mischler et al. 2016, Narr and Frost 2016) and the rates and ratios of nutrient excretion (Bernot 2013, Mischler et al. 2016, Narr and Frost 2016). While these studies provide a valuable introduction to parasite-mediated nutrient dynamics, they have generally been limited to a small number of host taxa (*Daphnia magna* and several snail species) infected with single species of parasites under laboratory conditions. Additional data are needed to determine whether parasites mediate nutrient dynamics in naturally infected host populations, especially in larger bodied hosts where coinfections with multiple parasite species are common.

Fish play important roles in freshwater nutrient dynamics (Vanni et al. 2013), and it is unknown whether parasite infection affects the storage and recycling of nutrients by fish hosts. Organismal stoichiometry and body size are

important predictors of excretion rate across fish species, consistent with predictions from both ecological stoichiometry and metabolic ecology (Vanni et al. 2002, Allgeier et al. 2015, Vanni and McIntyre 2016). These relationships have not been explored extensively within fish species, but phenotypic variation within a fish species may have important consequences for nutrient cycling function. For example, predation risk leads to changes in fish body stoichiometry and excretion rates within species (Dalton and Flecker 2014), and evolution in response to predation can lead to changes in fish phenotype and nutrient recycling that correspond to functional shifts in ecosystem processes (El-Sabaawi et al. 2015). While it is recognized that parasitized hosts may exhibit a functionally different phenotype than uninfected conspecifics (Lefèvre et al. 2009), this shift has not been explored in the context of consumer-driven nutrient dynamics.

I sampled three populations of freshwater fish that each harbor distinct parasite communities to assess potential relationships between macroparasite infection, host body size, and host nutrients (Fig. 4.1). For each host sampled, I measured nutrient storage (dry mass %C, N, and P and molar ratios C:N, C:P, and N:P), recycling rates (excreted ammonium [NH_4] and soluble reactive phosphorus [SRP]), body size, and the infection intensity of all parasite species present. Based on theory and evidence from both ecological stoichiometry and the metabolic theory of ecology, I hypothesized that fish tissue stoichiometry would scale with body size, and that body size and body stoichiometry would jointly determine the rate and elemental ratio of excreted nutrients (Fig. 4.1, solid lines). Based on evidence from prior studies that parasites alter host nutrients, I also hypothesized that parasite

infection would explain additional variation in host body stoichiometry and nutrient recycling (Fig. 4.1, dashed lines). Given the wide variation in infection sites, trophic strategies, and organismal stoichiometry of parasites infecting freshwater fish (Paseka and Grunberg, in revision), I predicted that relationships between infection and host nutrient variables would vary widely among parasite species. After evaluating univariate relationships among these variables within each population, I used structural equation models to evaluate multiple causal relationships among host nutrients, body size, and infection.

Methods

Field sampling

I selected three fish populations in which prior sampling indicated that (i) infection prevalence was high for several parasite species, and (ii) parasites species represented a range of infection sites, feeding strategies, and elemental composition (Paseka and Grunberg, in revision). I sampled 24 individuals from each of the following fish populations: *Lepomis macrochirus* from Myrtle Creek (Atlantic Co., NJ), *Fundulus diaphanus* from Mercer Lake (Mercer Country, NJ), and *Umbra pygmaea* from Muskingum Brook (Burlington County, NJ).

Prior to sampling, I acid washed all materials in the lab and rinsed with filtered stream or lake water (0.2 μm filter pore size) in the field. I collected fish with a seine net and placed them individually into incubation chambers. Each incubation chamber consisted of a plastic deli cup filled with 400 mL filtered stream or lake water. I placed chambers in plastic crates in shaded areas of the stream or

lake to maintain ambient water temperatures in the chambers. After introducing fish to the incubation chambers, I covered them with lids containing plastic airline tubing, which allowed water to be sampled from the chamber while minimizing disturbance to the fish. Following a 45 minute incubation period, I sampled water from each chamber, filtered samples immediately with ashed GF/F filters (Whatman), and froze water samples until analysis. For every six incubation chambers, I included one control chamber with no fish to determine baseline water chemistry from the lake or stream. After collecting excretion samples, I euthanized fish with MS-222, rinsed with them filtered water, and froze them until dissection.

Fish dissection and elemental analysis

I thawed fish individually in warm DI water just prior to dissection to minimize time between thawing and drying tissues. During dissection, I examined all fish tissues for parasites, removed and counted all macroparasites, and identified them by morphological characters (Hoffman 1999). Some larval nematodes could not be reliably identified to species, so they are represented by the lowest level of taxonomic identification possible with larval morphology (i.e., '*Eustrongylides*' and 'Nematoda'). Following traditional parasite ecology syntax (Bush et al. 1997), I report infection data as both prevalence (percentage of sampled hosts infected with a single parasite species) and intensity (the number of parasite individuals of one species infecting a single host).

I removed all dietary materials from fish intestines, dried fish tissues at 60° C for a minimum of 48 hours, and weighed them to obtain total body mass. I ground

each fish to a fine powder with a mortar and pestle, then subsampled ground material for elemental analysis. I used 2 mg subsamples to measure C and N with a Carlo Erba NA 1500 Series 2 elemental analyzer (Verardo et al. 1990), and I used 3-4 mg subsamples to measure P with the molybdate method following combustion at 500° C and digestion in acid (Solórzano and Sharp 1980). To account for potential error related to this subsampling, I analyzed two replicates from each fish for both CN and P. After determining that there was low variance in elemental replicates for each host, I calculated the mean values of each element for each fish to use in subsequent analyses. I report the results of elemental analyses as percentages of C, N, and P per unit of dry body mass (%C, %N, and %P) and as molar ratios (C:N, C:P, and N:P).

Expressions of host body size and stoichiometry

For each individual host, I calculated body size and body stoichiometry using two methods to separate two potential mechanisms by which parasites may alter these variables. To assess the potential that infection alters the elemental composition of host somatic tissues or body size through effects on host growth, I report the raw measurements of these variables following the removal of parasites by dissection. Parasites may also alter host stoichiometry through the accumulation of elementally distinct parasite biomass within the host (Frost et al. 2008). To assess this possibility, I used previously collected data on the mean body size and elemental content of the parasite species from these populations (Paseka and

Grunberg, in revision) to estimate the total body mass and elemental content of each host when parasites are included.

Excretion chemistry

Excretion samples were analyzed for NH_4 and SRP with a Lachat QuickChem 8500 auto-analyzer using phenol-hypochlorite (Solórzano 1969) and molybdenum-blue (Stainton et al. 1977) methods, respectively. I subtracted mean nutrient concentrations from control excretion chambers for each site from the values obtained from fish incubation chambers to determine the nutrient excretion rates of N and P (μg individual^{-1} hr^{-1}). I also calculated the molar N:P ratio of excretion for each individual fish. Hereafter, I refer to these variables as N excretion rate, P excretion rate, and excretion N:P.

Statistical analysis

I analyzed all data and generated graphics in R v.3.4.4 (R Core Team 2018). I conducted preliminary, univariate analyses to characterize relationships between fish body size, tissue stoichiometry, excretion chemistry, and infection. I used generalized linear models (GLMs) with Poisson or negative binomial error distributions to describe relationships between infection and host body size, and I used simple linear regressions to model all other relationships. To meet assumptions of normality for linear regression, I log10-transformed all excretion chemistry data prior to analysis. Preliminary analyses indicated that fish sex had no effects on body size, organismal stoichiometry, excretion, or infection (Sup. Table

S4.1), so sex was not included as a variable in analyses of relationships among these variables.

To address the network of hypothesized relationships among host body size, host body stoichiometry, nutrient recycling function, and infection (Fig. 1.), I used structural equation modeling (SEM) to test multiple causal hypotheses on these relationships (Grace 2006). In contrast to traditional SEM, which uses a global estimation method to simultaneously evaluate all paths in a model, I used a local estimation method (piecewise SEM) that evaluates the fit of component models for each response variable individually (Shipley 2009). Piecewise SEM was the appropriate choice for my analysis because it permits the inclusion of GLMs for variables with non-normal distributions and the use of smaller sample sizes than traditional SEM (Lefcheck 2016). Paths represented in a SEM are the regression coefficients corresponding to the component linear models or GLMs for each set of response variables and its predictors. Therefore, piecewise SEM is simply a collection simple and multiple regressions, with the added benefit of providing a graphical framework to represent a complex network of relationships, including direct and indirect effects among variables. Linking all models with the SEM framework also permits the use of Shipley's test of directed separation, which identifies any potentially influential paths that are missing from the model, uses Fischer's C statistic to evaluate overall model fit, and can be used to calculate Akaike's information criterion (AIC) values for model comparison (Shipley 2013).

For each fish population, I constructed and tested three SEMs using the R package 'piecewiseSEM' (Lefcheck 2016). I first constructed SEMs that omitted

parasite data (Model Set A) to test the hypothesis that fish tissue stoichiometry would scale with body size, and that body size and body stoichiometry would jointly determine the rate and elemental ratio of excreted nutrients. I included fish C:N, C:P, and N:P as one set of response variables with body size as a predictor, and I used N excretion rate, P excretion rate, and excretion N:P as a second set of response variables with body size and tissue N:P as predictors. I used Shipley's directed separation test to verify that no additional paths should be included in the initial models (e.g., links between fish C:P and excretion variables). I then used backwards model selection to remove non-significant paths by individually removing paths ranked by highest *p*-values. For each step of model simplification, I compared the initial model and the trimmed model with AIC values corrected for sample size (AICc) and considered a ΔAICc value of 2 or more to indicate a superior model (Burnham and Anderson 2002). I continued removing non-influential paths from the model as AICc values continued to decline until I reached the best-fitting model, in which all paths represented significant relationships between variables.
The resulting SEMs (Model Set A) represent the most parsimonious models to predict tissue stoichiometry and excretion chemistry within each fish population in the absence of infection data.

To test the hypothesis that parasite infection data would explain additional variation in host body stoichiometry and nutrient recycling, I constructed two additional SEMs for each fish population that included paths between infection and host nutrients (Model Sets B and C). In Model Set B, I used the sum of parasite intensity values for every parasite species infecting a host to obtain a 'total infection'

variable, which allowed me to test the hypothesis that total infection burden affects host nutrients. In Model Set C, I used intensity data for individual parasite species to test the prediction that diverse parasite species infecting a single host population would have different effects on host nutrients.

In each scenario, I used the most parsimonious model for each fish population in the absence of parasite data (Model Set A) and added paths to represent relationships between infection and host body size, body stoichiometry, and excretion. Given the model complexity that would result from including all parasite infection data in a SEM, I only included links between host nutrients and infection that were significant or marginally significant ($p < 0.07$) in univariate analyses (Sup. Tables S4.3-S4.5). Placing these links into the SEM framework allowed me to assess the role of host body size in mediating observed univariate relationships between infection and host nutrients. To achieve this goal, I did not perform further model selection on Model Sets A and B, instead retaining all paths between infection and host nutrients, regardless of statistical significance.

For all models, I report the R^2 values for each response variable, the regression coefficients for all paths included in the model, and *p*-values for each path. To facilitate the comparison of paths within and among models, I report standardized regression coefficients (the change in the response variable, in units of standard deviations, resulting from a change of one standard deviation in the predictor variable). To compare the relative fits of SEMs from Sets A-C for each host population, I calculated AIC and AICc values for each model using the method described by Shipley (2013). I also calculated separate AICc values for each

component model to directly compare the effects of different sets of predictors on each response variable.

Results

Infection was common in all fish populations sampled. Prevalence varied by host-parasite species pair and ranged from 50% to 100% (Fig. 4.2). Parasite community composition differed among host populations and represented variation in parasite taxonomic group, life cycle stage, infection site, and trophic strategy within hosts (Table 4.1). Despite the commonality of infection, there was substantial variation in parasite species composition and total parasite load among host individuals (Fig. 4.3). All *F. diaphanus* and *L. macrochirus* individuals sampled were infected with two or more parasite species. The majority of *U. pygmaea* individuals sampled were infected with one or two parasite species, but several individuals (3/24) were uninfected.

Fish hosts were also highly variable in both organismal stoichiometry (Fig. 4.4) and the rates and N:P ratios of excreted nutrients (Fig. 4.5) within each population sampled. Body size and tissue stoichiometry (%C, %N, %P, C:N, C:P, and N:P) did not differ between groups of hosts with and without parasite tissue added (Sup. Table S4.2).

Structural equation models constructed for each population provide a concise, visual representation of relationships among body size, nutrients, and infection (Fig. 4.6). Coefficients corresponding to each path linking variables in the models are simply the results of simple or multiple regressions for each response

variable (Table 4.2). While the graphical form of the results given in Fig. 4.6 and the regression results given in Table 4.2 provide a summary of the relationships analyzed in this study, additional tables and graphics representing univariate relationships between body size, infection, and nutrients are given in the Appendix.

In the models for each fish population constructed without parasite data (Model Set A), N excretion rate was positively related to body size in all three populations. N excretion rate was not related to tissue N:P independently of body size, and neither P excretion rate nor excretion N:P were related to body size or tissue N:P (Fig. 4.6, Table 4.2). Relationships between body size and tissue stoichiometry differed across populations. While tissue C:N, C:P, and N:P were all negatively related to body size for *F. diaphanus*, C:N was positively related to body size in *U. pygmaea,* and tissue stoichiometry was not related to body size in *L. macrochirus.*

Univariate analyses indicated that neither total infection nor infection with individual parasite species were related to host nutrient variables for *L. macrochirus* (Sup. Table S4.5), so no additional SEMs were constructed for this population. SEMs including total infection levels for *F. diaphanus* and *U. pygmaea* (Model Set B) indicate that univariate relationships observed between infection and host nutrients were mediated by body size (Table 4.2), as infection was not a significant term in any multiple regressions including body size as an additional predictor of nutrients.

SEMs including individual parasite species (Model Set C) indicated that infection with several species explained variation in host body stoichiometry independently of body size (Fig. 4.6). Tissue C:N and C:P of *Fundulus diaphanus* were

negatively related to the intensity of *Allocreadium*, an adult trematode that parasitizes the alimentary tract of the host. *F. diaphanus* C:N was positively related to the intensity of *Neoechinorhynchus*, a larval cestode that parasitizes the liver.

Other observed, univariate relationships between infection and host nutrients were mediated by the scaling of these variables with body size. *Eustrongylides* and Nematoda were included as predictors for host C:N in models for *F. diaphanus* and *U. pygmaea,* respectively (Fig 4.6), but multiple regressions including body size indicated that there were no relationships between infection with these species and host C:N (Table 4.2). Despite the lack of relationship between *Eustrongylides* and host tissue stoichiometry, there was a marginal, positive relationship ($p = 0.051$) between the P excretion rate of *F. diaphanus* and infection with *Eustrongylides* (a larval nematode that parasitizes the body cavity).

For *F. diaphanus* and *U. pygmaea*, several response variables were related to multiple sets of predictors (Table 4.3). While models including parasite variables resulted in the largest R^2 values among competing models, low ΔAICc values (< 3) for each set of competing models indicated that the addition of infection variables as predictors did not substantially improve the explanatory power of the models. Due to the low sample sizes used in these analyses, the model fit statistics describing entire SEMs (Table 4.4) offer limited interpretive value compared to the fit statistics obtained from component models (Table 4.3).

Discussion

General interpretation of structural equation and component models

Piecewise structural equation modeling is often used to circumvent small sample sizes when representing complex systems and can be completed as long as sample sizes are large enough to support component models for each response variable (Lefcheck 2016). However, the small size of my dataset relative to the complexity of some of the SEMs constructed calls into question the suitability of this dataset for the SEM approach. For this reason, I emphasize that the regression results from component models run for each response variable within the SEM framework (Table 4.2) provide greater interpretive power than assessments of the overall fit for each SEM. The path diagrams in Fig. 4.6 are included as a visual aid for interpreting the sets of relationships analyzed in this study.

Relationships between infection, host body size, and host nutrients

While macroparasite infection was nearly universal among hosts sampled from each population, infection variables were either unrelated or weakly related to host tissue stoichiometry and excretion chemistry, depending on the host-parasite species pair. Body size was the most consistent predictor of host nutrients across populations, and some univariate relationships between infection and host nutrients were driven by the scaling of these variables with size.

When all parasite species were grouped to represent the total infection level of each host individual (Model Set B), these infection variables were not significantly related to host stoichiometry or excretion chemistry. Analyzing parasite species separately (Model Set C) led to the identification of several relationships between infection and host nutrients that occurred independently of body size in *F.*

diaphanus, but not in *U. pygmaea* or *L. macrochirus* (Fig. 4.6, Table 4.2). While these relationships identify that there is a link between infection with several parasite species and *F. diaphanus* nutrients, the similarity of AICc values for competing models with and without infection predictors (Table 4.3) indicates that infection variables do not improve predictions over simpler models. It is valuable to recognize that infection contributes to nutrient heterogeneity among hosts in some populations, but the most parsimonious models to predict host tissue stoichiometry and excretion chemistry in this system do not include parasite terms.

My choice to interpret relationships between infection levels and host nutrients as the consequences of infection on hosts was based on evidence from other host-parasite systems that have documented these effects (Frost et al. 2008, Bernot 2013, Narr and Frost 2016). However, it is impossible to conclusively determine causality in a field-based study such as this one. Interpreting relationships between infection and host nutrients with the opposite causal assumption, that is, that variation in host stoichiometry drives differences in infection levels among hosts, is an equally intriguing concept. The effects of variable organismal stoichiometry on susceptibility to infection have not been explored, but evidence for such a relationship would provide interesting insight into the stoichiometry of disease susceptibility and the nutrient limitation of parasites within hosts.

Infection and ecosystem-level nutrient cycling

This study indicates that infection does little to alter host nutrient storage and recycling in three populations of freshwater fish that were heavily infected with macroparasites. These results contrast with those from laboratory studies that have consistently reported effects of infection on both the tissue stoichiometry and excretion chemistry of hosts (Frost et al. 2008, Bernot 2013, Narr and Frost 2016). This study was limited by the low numbers of fish sampled from each population, and it is possible that larger sample sizes would have helped to clarify some relationships between infection and host nutrients. However, it seems unlikely that additional sampling would substantially alter the overall patterns observed in these host populations.

Several factors may be responsible for the differences in results observed in this study compared to laboratory experiments. Experimental control of host age, body size, infection level, and feeding history likely helped to isolate the effects of infection in prior studies. Additionally, all prior studies used invertebrate hosts, where the biomass ratio of parasite to host tissue may have been much greater than in the fish hosts used here. Finally, coinfection with multiple parasite species was nearly universal among the hosts sampled here, which complicates interpretation of the patterns observed. For example, two parasite species infecting *F. diaphanus* had opposite relationships with host C:N, which may reflect differences in parasite infection site, feeding mode, or nutritional requirements within the same hosts.

While there is certainly great merit in conducting laboratory experiments to study the effects of infection on host nutrients in a controlled environment and to identify mechanisms leading to these effects, caution is necessary when generalizing

these responses for natural populations or for extending them across host-parasite systems. Beyond identifying when and how parasites alter host nutrient storage and recycling, it is also important to interpret these effects on the scale of ecosystem-level nutrient demands (Mischler et al. 2016). The importance of consumers to nutrient cycling at the ecosystem scale is highly context dependent (Atkinson et al. 2016), and considering consumer infection status adds an additional layer of complexity. Ecological stoichiometry provides a promising framework to link parasite nutritional demands, effects of infection on host nutrient dynamics, and the significance of these interactions to ecosystem processes. However, we are still far from understanding the effects of parasite infection on consumer-driven nutrient dynamics and how these relationships vary across a diversity of host-parasite interactions.

Acknowledgements

I thank Rita Grunberg for her assistance with field sampling and Darling Rojas for preparing fish samples for elemental analysis. Gary Taghon generously allowed me to complete all elemental analyses in his lab, and Ron Lauck completed water chemistry analyses. Krista Capps, Therese Frauendorf, and Dan Durston provided advice on sampling methods for excretion chemistry. Thanks to Peter Morin and members of the Morin Lab for comments that helped to improve this manuscript. Grants from the Rutgers University Ecology and Evolution Graduate Program and the New Jersey Water Resources Research Institute funded this

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Chapter 4 Tables and Figures

Figure 4.1. Hypothetical relationships between parasite infection and host traits (body size, body stoichiometry, and excretion chemistry) in populations of animal hosts. Solid arrows represent relationships among animal body size, nutrient storage, and nutrient recycling that are predicted by ecological stoichiometry (ES) and the metabolic theory of ecology (MTE). Dashed arrows represent hypothetical relationships between parasite infection and host nutrients that are tested in this study.

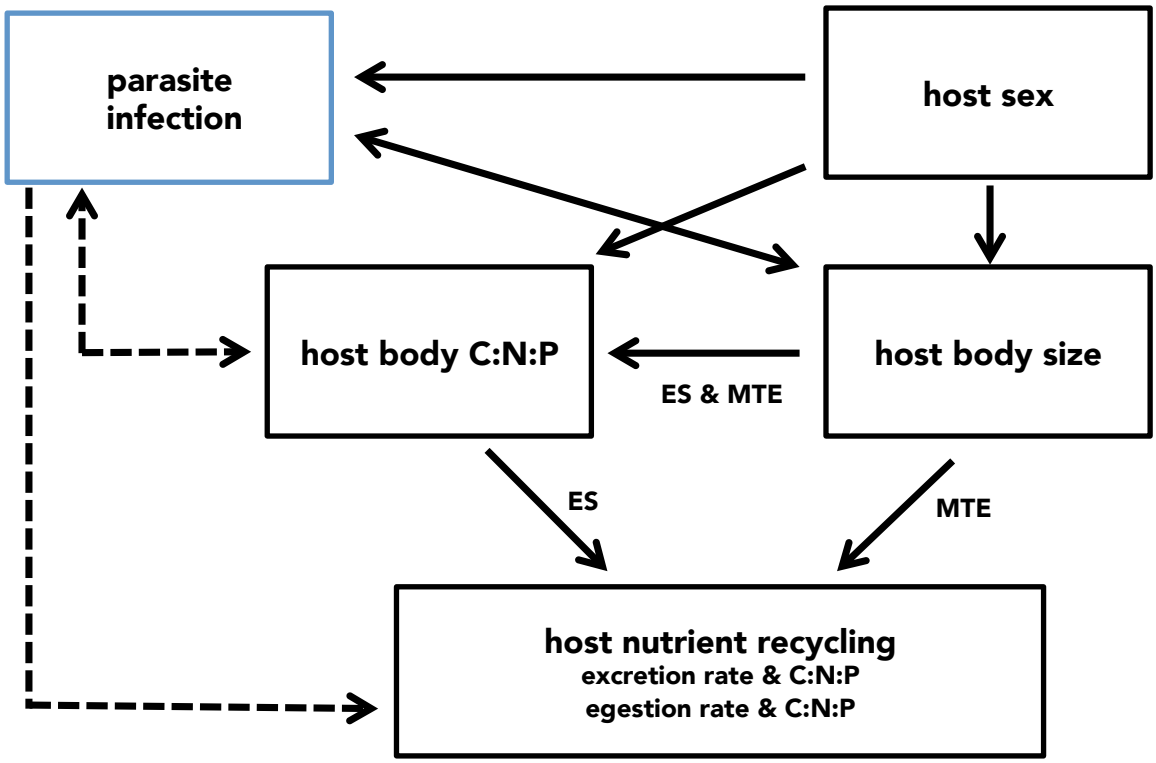


Figure 4.2. Infection prevalence in populations of *Fundulus diaphanus* (a), *Umbra pygmaea* (b), and *Lepomis macrochirus* (c). N=24 fish sampled from each population.

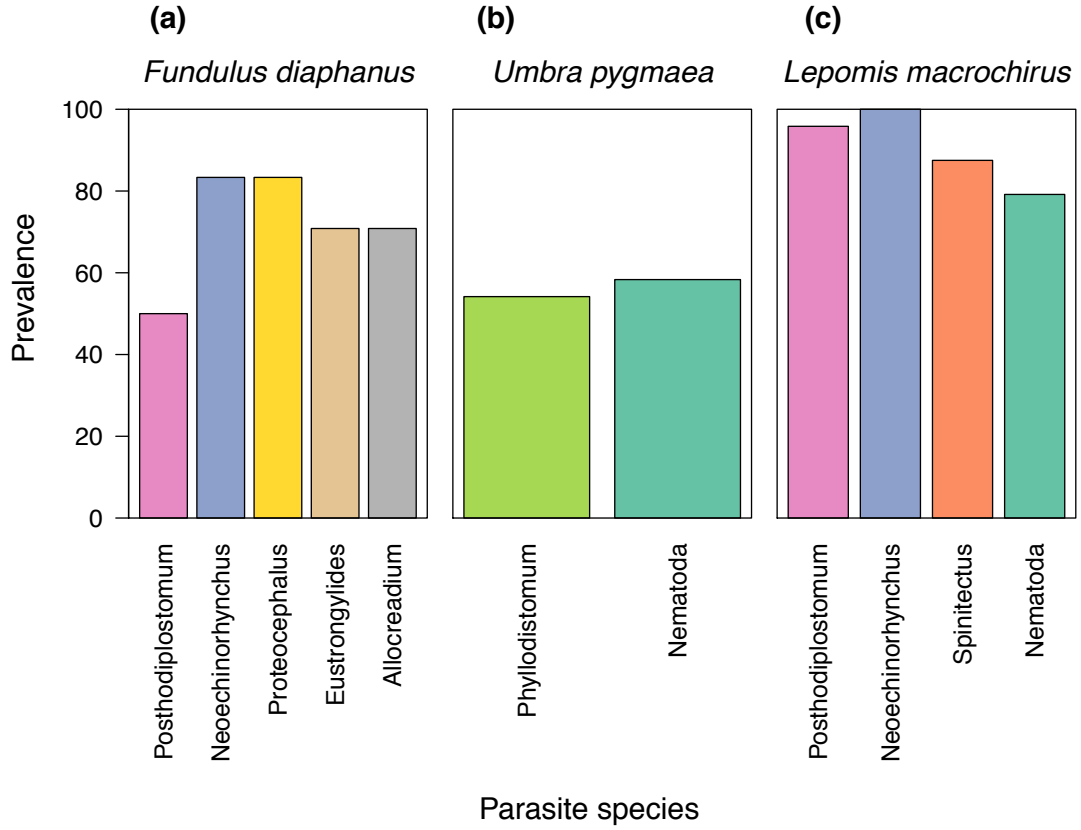


Figure 4.3: Variation in parasite load among host individuals in populations of *Fundulus diaphanus* (a), *Umbra pygmaea* (b), and *Lepomis macrochirus* (c).

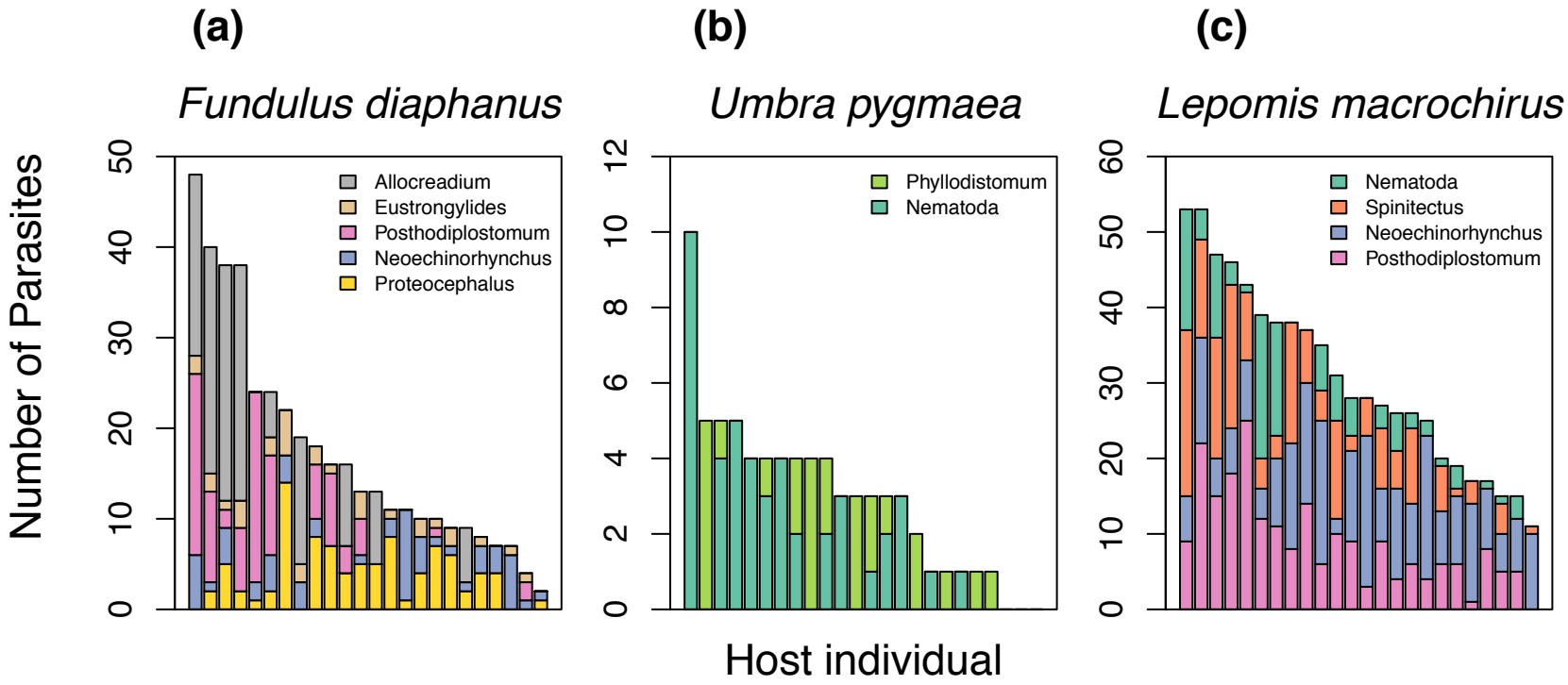


Figure 4.4. Variation in organismal stoichiometry among host individuals in populations of *Fundulus diaphanus* (a), *Umbra pygmaea* (b), and *Lepomis macrochirus* (c).

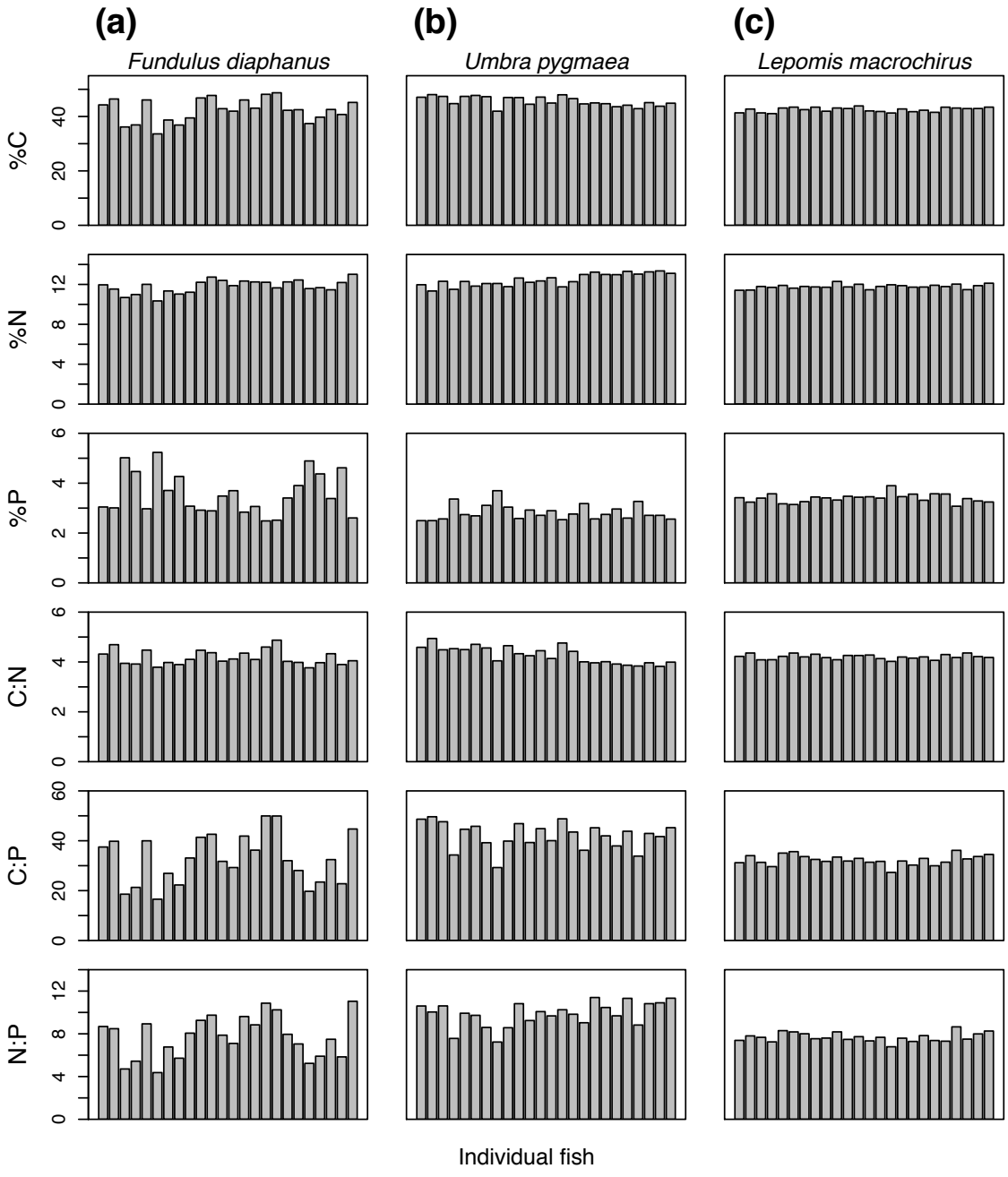


Figure 4.5. Variation in excretion chemistry within each host population sampled. Each bar represents a single observation for an individual fish.

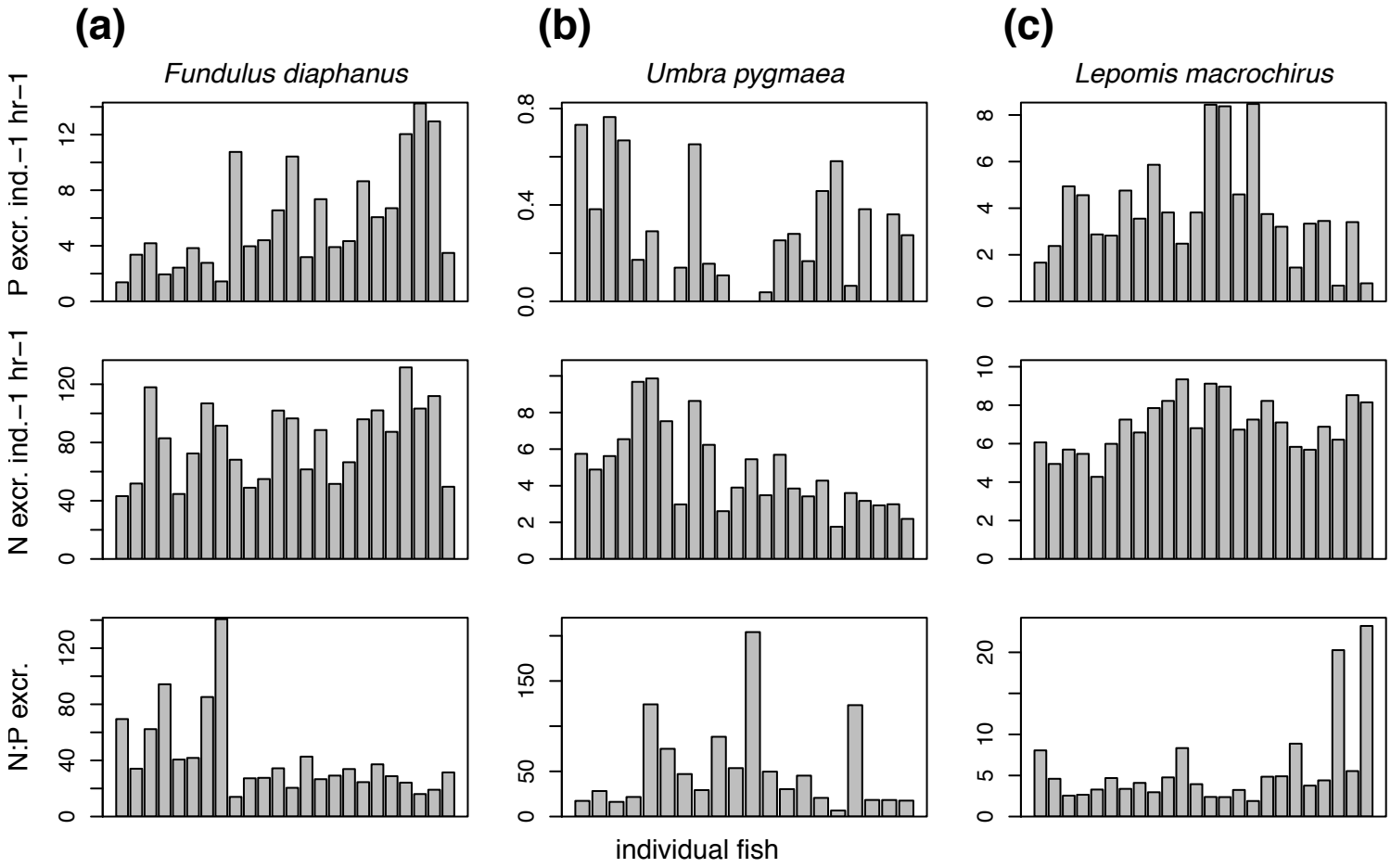


Figure 4.6. Structural equation models tested for each fish population in the absence of parasite data (Set A) and including individual parasite species (Set C). Black arrows represent significant relationships between pairs of variables, and faint arrows indicate non-significant relationships. In Set A, non-significant relationships (faint lines) were removed from the final model. In Set C, non-significant relationships (faint lines) were retained in the model. Red text inside boxes represents the R^2 values obtained for each response variable. Standardized path coefficients and *p*-values are given in Table 4.2, along with the results of an additional set of SEMs (Set B, not pictured) that used total parasite number instead of individual parasite species.

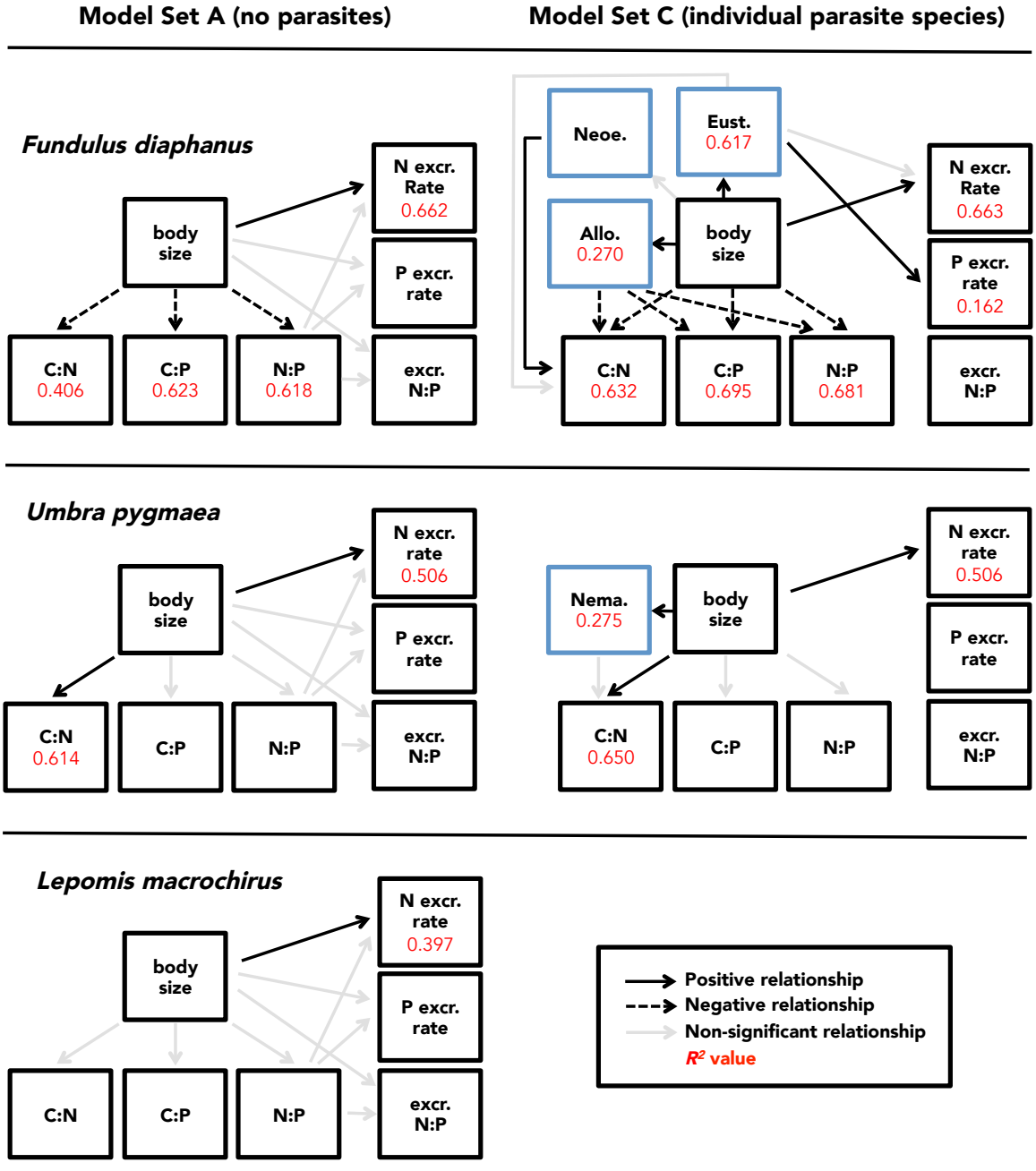


Table 4.1: Parasite species infecting three populations of fish. Bracketed letters following parasite species names indicate the phyla Acanthocephala, Nematoda, and Platyhelminthes.

Host species	Parasite species	Stage	Infection site	Trophic strategy	Infection prev. (%)
Fundulus diaphanus	*Posthodiplostomum minimum* [P]	metacercaria	liver	encysted	50.0
	Neoechinorhynchus cylindratus [A]	cystacanth	liver	encysted	83.3
	Proteocephalus ambloplitis [P]	plerocercoid	liver	absorber	83.3
	Eustrongylides sp. [N]	larval	body cavity	encysted	70.8
	Allocreadium commune [P]	adult	intestine	tissue grazer	70.8
Lepomis macrochirus	*Posthodiplostomum minimum* [P]	metacercaria	liver	encysted	95.8
	Neoechinorhynchus cylindratus [A]	cystacanth	liver	encysted	100.0
	Spinitectus sp. [N]	adult	intestine	tissue grazer	87.5
	Nematoda [N]	larval	body cavity	encysted	79.2
Umbra pygmaea	*Phyllodistomum pearsei* [P]	adult	urinary bladder	tissue grazer	54.2
	Nematoda [N]	larval	body cavity	encysted	58.3

Table 4.2. (following page)

Path coefficients from three sets of structural equation models constructed for each fish population. Model Set A describes observed relationships between body size, tissue stoichiometry, and excretion chemistry in the absence of infection data. Sets B and C are extensions of the models in Set A that include total parasite infection and infection with individual parasite species, respectively. Regression coefficients and *p*-values are provided for each path in the models, and path coefficients have been standardized to facilitate comparisons of paths within and among models. R^2 values are given for each response variable. Criteria for the choice of parameters included in each model are explained in the text

Fundulus diaphanus												
Response	**Model Set A (no parasites)**				**Model Set B (total infection)**				**Model Set C (individual parasite species)**			
	R^2	**Predictor**	**Std. Coeff.**	***p*-value**	R^2	**Predictor**	**Std. Coeff.**	***p*-value**	R^2	**Predictor**	**Std. Coeff.**	***p*-value**
C:N	**0.406**	size	-0.637	<0.001	**0.468**	size	-0.540	0.005	**0.632**	size	-0.505	0.012
						infection	-0.267	0.134		Allo.	-0.339	0.030
										Eust.	-0.004	0.981
										Neoe.	0.341	0.025
C:P	**0.623**	size	-0.789	<0.001	**0.682**	size	-0.694	<0.001	**0.695**	size	-0.719	<0.001
						infection	-0.261	0.062		Allo.	-0.279	0.042
										Eust.	0.009	0.956
N:P	**0.618**	size	-0.786	<0.001	**0.671**	size	-0.695	<0.001	**0.681**	size	-0.728	<0.001
						infection	-0.249	0.078		Allo.	-0.260	0.062
										Eust.	0.019	0.905
log-N excr. rate	**0.662**	size	0.814	<0.001	-	-	-	-	**0.663**	size	0.786	<0.001
										Eust.	0.044	0.787
log-P excr. rate	-	-	-	-	-	-	-	-	**0.162**	Eust.	0.403	0.051
Umbra pygmaea												
Response	**Model Set A (no parasites)**				**Model Set B (total infection)**				**Model Set C (individual parasites)**			
	R^2	**Predictor**	**Std. Coeff.**	***p*-value**	R^2	**Predictor**	**Std. Coeff.**	***p*-value**	R^2	**Predictor**	**Std. Coeff.**	***p*-value**
C:N	**0.614**	size	0.784	<0.001	**0.618**	size	0.759	<0.001	**0.650**	size	0.730	<0.001
						infection	0.061	0.686		Nema.	0.195	0.162
log-N excr. rate	**0.506**	size	0.712	<0.001	**0.515**	size	0.670	<0.001	-	-	-	-
						infection	0.100	0.558				
Lepomis macrochirus												
Response	**Model Set A (no parasites)**				**Model Set B (total infection)**				**Model Set C (individual parasites)**			
	R^2	**Predictor**	**Std. Coeff.**	***p*-value**	-				-			
log-N excr. rate	**0.397**	size	0.6304	0.001	-				-			

Table 4.3. Fit statistics for component models in the SEM. For each response variable that was predicted by multiple SEMs, the R^2 value, *p*-value and AIC scores are given to allow comparison among competing models. Fit statistics for component models in piecewise SEMs are the results of simple or multiple regressions including the predictor variables used in each model.

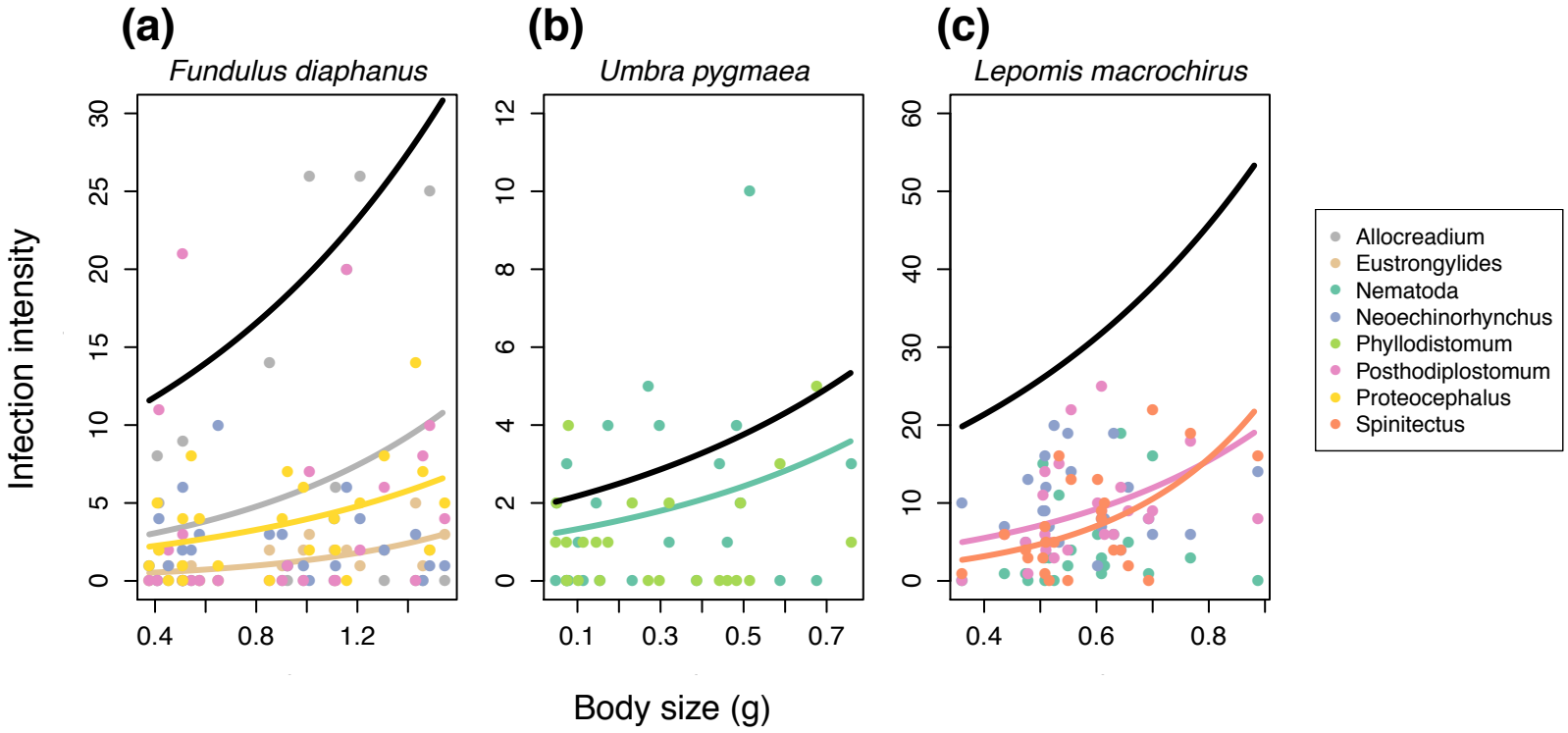
Population	Response	Predictors	Model Set	R^2	*p*-value	AIC	AICc	ΔAICc
F. diaphanus	C:N	size	A	0.406	0.001	2.08	3.28	1.71
		size, infection	B	0.468	0.001	1.46	3.57	1.99
		size, Allo., Eust., Neoe.	C	0.632	0.001	-3.37	1.57	0
	C:P	size	A	0.623	< 0.001	159.73	160.93	1.19
		size, infection	B	0.682	< 0.001	157.63	159.74	0
		size, Allo., Eust.	C	0.695	< 0.001	158.62	161.96	2.22
	N:P	size	A	0.618	< 0.001	81.69	82.89	0.72
		size, infection	B	0.671	< 0.001	80.07	82.17	0
		size, Allo., Eust.	C	0.681	< 0.001	81.39	84.72	2.55
	log N-excr rate	size	A	0.662	< 0.001	-44.54	-43.34	2.819
		size, Eust.	C	0.663	< 0.001	-42.63	-40.52	0
U. pygmaea	C:N	size	A	0.614	< 0.001	-2.32	-1.12	0
		size, infection	B	0.618	< 0.001	-0.51	1.60	2.71
		size, Nema.	C	0.65	< 0.001	-2.61	-0.51	0.613
	log N-excr rate	size	A	0.506	< 0.001	-20.94	-19.74	0
		size, infection	B	0.515	0.001	-19.34	-17.24	2.503

Table 4.4. Overall fit statistics for each SEM. Fisher's *C* statistics were derived from Shipley's test of directed separation and used to conduct χ^2 tests to assess the overall fit of each SEM. *p*-values resulting from these tests are given for each model, with values > 0.05 indicating that the SEM fits the data and that no important paths among variables have been omitted. *K* indicates the number of free parameters in the model. AIC and AICc scores were calculated from Fisher's *C* statistic, with the later being corrected for small sample size.

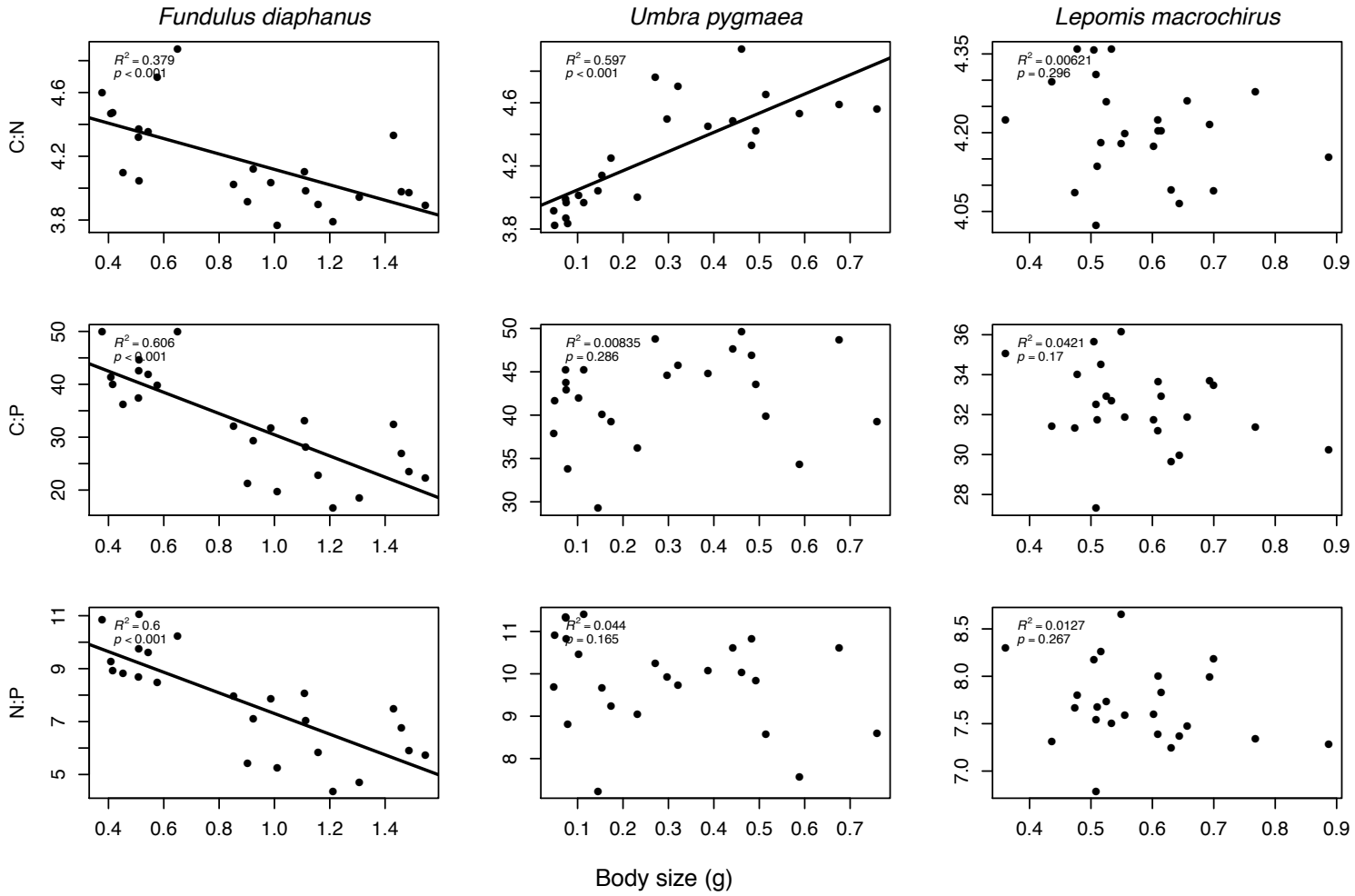
Population	Model Set	Fisher's *C*	*p*-value	*K*	AIC	AICc
F. diaphanus	A	6.44	0.375	12	30.44	66.42
	B	9.43	0.307	17	43.43	173.72
	C	22.75	0.647	29	80.75	-323.00
U. pygmaea	A	3.10	0.212	6	15.10	21.32
	B	2.87	0.239	10	22.87	42.21
	C	4.71	0.319	9	22.71	38.93

Chapter 4 Appendix

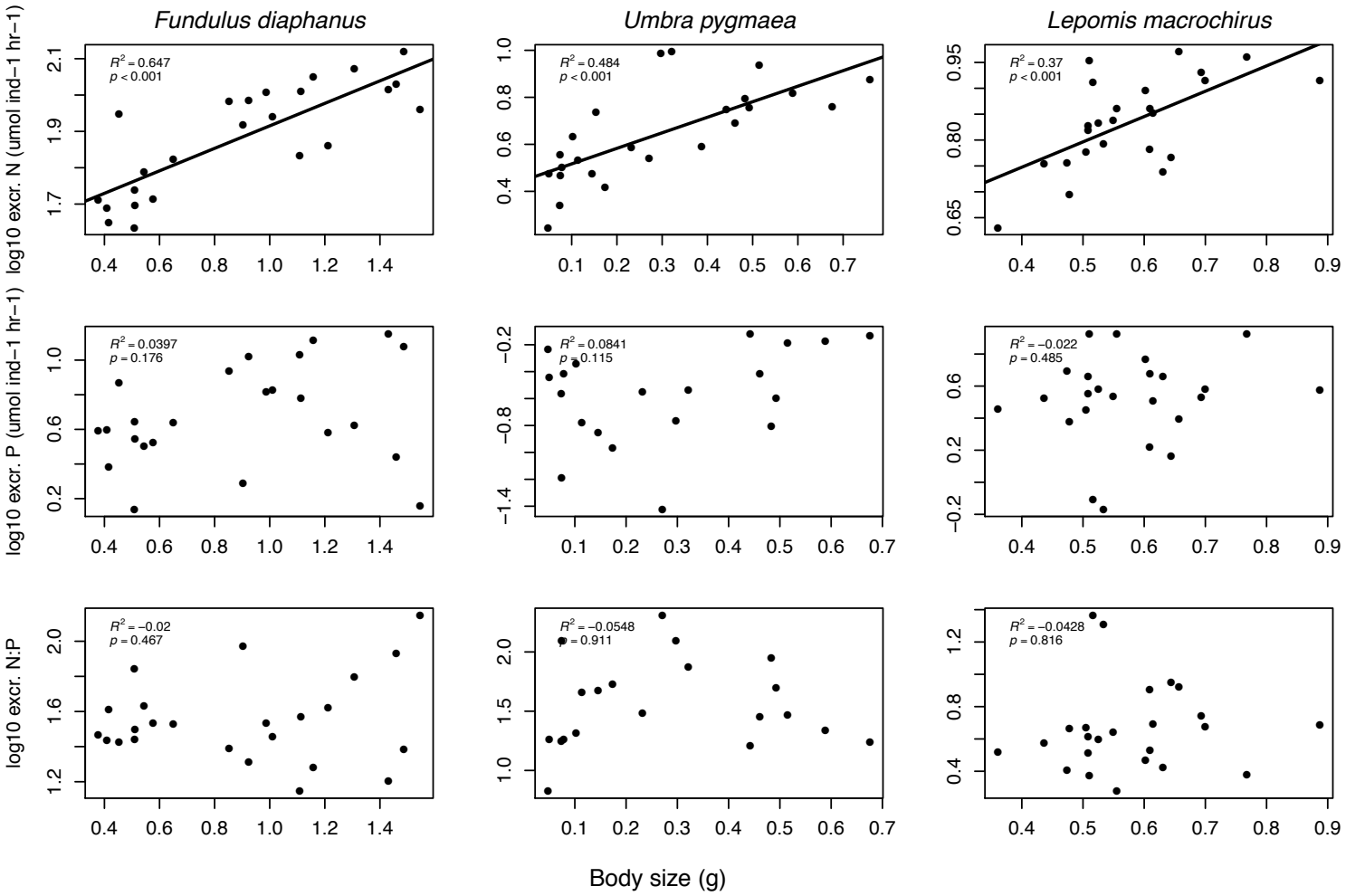
Sup. Fig. S4.1. Relationships between infection intensity (number of parasites of one species in a single host) and host body size for each parasite species (colored points and lines) and the total parasite burden of each host (black lines). For individual species with no trend line plotted, there was no relationship between infection intensity and body size.



Sup. Fig. S4.2. Relationships between tissue stoichiometry and body size for each host population sampled.



Sup. Fig. S4.3. Relationships between excretion chemistry and body size for each host population sampled.



Sup. Table S4.1. Multivariate analysis of variance results for the effects of fish sex on (1) fish body variables (size, C:N, C:P, N:P), (2) excretion variables (N excretion rate, P excretion rate, excretion N:P), and (3) infection variables (individual infection intensities for each parasite species present).

Fundulus diaphanus			Sex ratio in sample: 11F / 13M	
Response variable set	***Wilk's statistic***	***F***	***df***	***p-value***
(1) Fish body	0.70	1.99	1, 22	0.14
(2) Excretion	0.98	0.17	1, 22	0.92
(3) Infection	0.81	0.86	1, 22	0.53
Lepomis macrochirus			Sex ratio in sample: 15F / 9M	
Response variable set	***Wilk's statistic***	***F***	***df***	***p-value***
(1) Fish body	0.86	0.80	1, 22	0.54
(2) Excretion	0.81	1.54	1, 22	0.23
(3) Infection	0.85	0.81	1, 22	0.53
Umbra pygmaea			Sex ratio in sample: 10F / 14M	
Response variable set	***Wilk's statistic***	***F***	***df***	***p-value***
(1) Fish body	0.66	2.44	1, 22	0.08
(2) Excretion	0.98	0.12	1, 18	0.95
(3) Infection	0.91	1.05	1, 22	0.37

Sup. Table S4.2. Multivariate analysis of variance results for differences in fish tissue stoichiometry (%C, %N, %P, C:N, C:P, and N:P) with and without accounting for the distinct elemental content of parasites.

Population	***Wilk's statistic***	***F***	***df***	***p-value***
Fundulus diaphanus	0.98	0.14	1, 46	0.99
Umbra pygmaea	1.00	<0.01	1, 46	1.00
Lepomis macrochirus	1.00	0.01	1, 46	1.00

Sup. Table S4.3. Results of simple linear regressions for the effects of *Fundulus diaphanus* body size and infection variables on tissue stoichiometry (C:N, C:P, and N:P) and excretion chemistry (N excretion rate, P excretion rate, and excretion N:P). Bold text indicates relationships that were significant or marginally significant ($p \leq 0.07$) and included in SEM Model Sets B and C.

Dep. var.	Statistic	Body size (g)	Total parasite #	Allo.	Eust.	Neoe.	Post.	Prot.
C:N	**slope**	-0.48	**-0.01**	**-0.02**	**-0.09**	**0.05**	-0.01	-0.01
	intercept	4.60	**4.32**	**4.26**	**4.29**	**4.04**	4.21	4.19
	R^2	0.41	**0.22**	**0.24**	**0.15**	**0.17**	0.05	0.01
	***p*-value**	<0.01	**0.02**	**0.01**	**0.06**	**0.04**	0.31	0.74
C:P	**slope**	-20.05	**-0.30**	**-0.50**	**-3.87**	0.93	-0.41	-0.61
	intercept	50.51	**38.24**	**35.51**	**37.75**	30.31	34.21	34.92
	R^2	0.62	**0.27**	**0.22**	**0.23**	0.05	0.06	0.04
	***p*-value**	<0.01	**0.01**	**0.02**	**0.02**	0.29	0.23	0.33
N:P	**slope**	-3.90	**-0.06**	**-0.09**	**-0.74**	0.10	-0.08	-0.13
	intercept	11.2	**8.80**	**8.27**	**8.71**	7.46	8.03	8.20
	R^2	0.62	**0.25**	**0.21**	**0.23**	0.02	0.06	0.05
	***p*-value**	<0.01	**0.01**	**0.03**	**0.02**	0.55	0.24	0.30
log-N excretion rate	**slope**	0.31	<0.01	<0.01	**0.06**	-0.01	<0.01	0.01
	intercept	0.46	0.70	0.71	**0.65**	0.76	0.73	0.69
	R^2	0.66	0.06	0.06	**0.28**	0.02	<0.01	0.06
	***p*-value**	<0.01	0.25	0.23	**0.01**	0.52	0.89	0.24
log-P excretion rate	**slope**	0.21	<0.01	0.01	**0.09**	0.01	-0.01	0.01
	intercept	-1.00	-0.86	-0.87	**-0.94**	-0.84	-0.79	-0.84
	R^2	0.08	0.02	0.12	**0.16**	0.01	0.02	0.01
	***p*-value**	0.18	0.52	0.10	**0.05**	0.67	0.57	0.74
log-excretion N:P	**slope**	0.10	<0.01	-0.01	-0.03	-0.02	0.01	<0.01
	intercept	1.46	1.55	1.59	1.59	1.60	1.52	1.53
	R^2	0.02	<0.01	0.06	0.02	0.04	0.03	<0.01
	***p*-value**	0.47	0.94	0.24	0.47	0.37	0.44	0.76

Sup. Table S4.4. Results of simple linear regressions for the effects of *Umbra pygmaea* body size and infection variables on tissue stoichiometry (C:N, C:P, and N:P) and excretion chemistry (N excretion rate, P excretion rate, and excretion N:P). Bold text indicates relationships that were significant or marginally significant ($p \leq 0.07$) and included in SEM Model Sets B and C.

Dep. var.	Statistic	Body size (g)	Total parasite #	Nema.	Phyl.
C:N	**slope**	1.21	**0.06**	**0.06**	-0.02
	intercept	3.93	**4.11**	**4.18**	4.30
	R^2	0.61	**0.14**	**0.16**	0.01
	***p*-value**	<0.01	**0.07**	**0.06**	0.73
C:P	**slope**	5.46	-0.16	0.20	-0.99
	intercept	40.54	42.59	41.77	43.2
	R^2	0.05	<0.01	0.01	0.07
	***p*-value**	0.29	0.76	0.68	0.22
N:P	**slope**	-1.52	-0.17	-0.08	-0.20
	intercept	10.3	10.35	10.01	10.07
	R^2	0.09	0.11	0.03	0.06
	***p*-value**	0.17	0.11	0.43	0.25
log-N excretion rate	**slope**	0.66	**0.03**	0.03	<0.01
	intercept	-0.70	**-0.60**	-0.56	-0.50
	R^2	0.51	**0.14**	0.12	<0.01
	***p*-value**	<0.01	**0.07**	0.09	0.99
log-P excretion rate	**slope**	0.64	0.02	-0.02	0.10
	intercept	-2.25	-2.12	-2.03	-2.19
	R^2	0.13	0.01	0.02	0.15
	***p*-value**	0.11	0.64	0.60	0.09
log-excretion N:P	**slope**	0.10	0.02	0.05	-0.10
	intercept	1.53	1.49	1.46	1.68
	R^2	<0.01	0.02	0.12	0.15
	***p*-value**	0.81	0.57	0.14	0.09

Sup. Table S4.5. Results of simple linear regressions for the effects of *Lepomis macrochirus* body size and infection variables on tissue stoichiometry (C:N, C:P, and N:P) and excretion chemistry (N excretion rate, P excretion rate, and excretion N:P). No relationships between host nutrients and infection were statistically significant, so they were omitted from SEMs.

Dep. var.	Statistic	Body size (g)	Total parasite #	Nema.	Neoe.	Post.	Spin.
C:N	**slope**	-0.19	<0.01	<0.01	<0.01	<0.01	<0.01
	intercept	4.31	4.24	4.21	4.22	4.21	4.21
	R^2	0.05	0.02	0.01	0.01	<0.01	0.01
	***p*-value**	0.30	0.53	0.67	0.65	0.87	0.72
C:P	**slope**	-5.17	-0.04	0.01	-0.02	-0.08	-0.09
	intercept	35.36	33.73	32.34	32.56	33.09	32.99
	R^2	0.08	0.07	<0.01	<0.01	0.06	0.07
	***p*-value**	0.17	0.20	0.91	0.84	0.25	0.20
N:P	**slope**	-0.88	-0.01	0.01	<0.01	-0.02	-0.02
	intercept	8.20	7.96	7.67	7.71	7.86	7.83
	R^2	0.06	0.06	<0.01	<0.01	0.07	0.07
	***p*-value**	0.27	0.23	0.76	0.93	0.21	0.20
log-N excretion rate	**slope**	0.49	<0.01	<0.01	<0.01	<0.01	<0.01
	intercept	-0.59	-0.38	-0.31	-0.30	-0.35	-0.35
	R^2	0.40	0.10	<0.01	0.01	0.11	0.12
	***p*-value**	<0.01	0.13	0.92	0.74	0.12	0.10
log-P excretion rate	**slope**	0.37	<0.01	-0.01	0.01	0.01	0.01
	intercept	-1.19	-1.11	-0.91	-1.12	-1.05	-1.04
	R^2	0.02	0.04	0.08	0.07	0.04	0.05
	***p*-value**	0.48	0.37	0.18	0.23	0.37	0.30
log-excretion N:P	**slope**	0.12	<0.01	0.02	-0.02	<0.01	-0.01
	intercept	0.59	0.72	0.60	0.82	0.70	0.70
	R^2	<0.01	0.01	0.09	0.08	0.01	0.01
	***p*-value**	0.82	0.67	0.15	0.17	0.67	0.58

CONCLUSION

I started thinking about using ecological stoichiometry to study host-parasite interactions and to link these interactions with ecosystem processes just over six years ago, and I have been both surprised and encouraged by the number of papers published on this theme since then. Despite this growing interest, we still have more questions than answers on the topic of parasite stoichiometry, and there remains a major conceptual gap between parasite ecology and ecosystem ecology.

Moving forward, additional empirical and theoretical work on the ecological stoichiometry of parasitism will shed greater light on the broad questions that I posed in this dissertation. My hope is that future work will extend far beyond my initial goals and lead to a stoichiometric framework to address an array of questions on the ecology and evolution of parasitism and disease. Some of the topics that seem especially promising as research frontiers are assessing the elemental costs of host-parasite coevolution, merging stoichiometric theory with concepts from metabolic ecology to better quantify the energetic and nutrient dynamics of infection, and using stoichiometric theory to predict the effects of environmental nutrients on diseases relevant to human health and conservation.

This dissertation's completion is concurrent with an expansion of stoichiometric theory out of the zooplankton-phytoplankton systems in which it originated and into broader use in biology. Evaluating the applicability of stoichiometry to a broad array of taxa and interaction types, including parasites and their interactions with hosts and the environment, is essential to the continued development of ecological stoichiometry as a unifying framework for biology.