COMMUNITY AND ECOSYSTEM LEVEL IMPLICATIONS OF HELMINTH PARASITISM

by

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Dedicated to the family, the friends, and the mentors who helped me along the way.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Pathogens and parasites are increasingly recognized as important components within host populations, communities, and ecosystems. Parasite contributions to ecosystem function most likely manifest as density-mediated impacts of parasites on their hosts, the direct contributions of parasite biomass to a system, and via parasite-induced changes in host behavior and physiology (trait-mediated impacts). Here, a framework was constructed that can be used to conceptualize parasite contributions to ecosystem function (Chapter 1). Then the influence of parasite attack on host movement was explored to further evince the mechanistic underpinnings of trait-mediated parasite impacts (Chapter 2). Additionally, mesocosms were created across a gradient of parasitism to examine how these mechanisms are likely to unfold at larger biological scales (Chapter 3). Lastly, a series of differential equations was created to model host-parasite-ecosystem interactions and generate theoretical predictions about how and when parasites are likely to influence ecosystem processes (Chapter 4). Parasites have many characteristics of ecosystem engineers, but their role has historically been ignored. These studies begin to explore the role that parasitism may have as one of the drivers of ecosystem processes

CHAPTER 1. PARASITES AND THEIR IMPACT ON ECOSYSTEM NUTRIENT CYCLING

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1.1 Abstract

Consumer species alter nutrient cycling through nutrient transformation, transfer, and bioturbation. Parasites have rarely been considered in this framework despite their ability to indirectly alter the cycling of nutrients via their hosts. A simple mathematical framework can be used to assess the relative importance of parasite-derived nutrients in an ecosystem.

1.2 Nutrient cycling by consumers: Where are the parasites?

Nutrient cycling, which describes the movement of elements through the biotic and abiotic environment, plays a central role in structuring ecosystems and food webs (Jones and Lawton 1995). Animals, from herbivores and detritivores to predators, are increasingly recognized as important contributors to nutrient cycling (Jones and Lawton 1995, Vanni 2002, Buck and Ripple 2017). However, the role of parasites in nutrient cycling has received little attention (Bernot 2013, Mischler et al. 2016, Chodkowski and Bernot 2017). This is surprising as parasitism is a common consumer strategy and helminth parasites contribute substantial biomass to ecological communities (Kuris et al. 2008).

A consumer's contribution to nutrient cycling involves three components: nutrient transformation, transfer, and bioturbation. Nutrient transformation refers to the intake of food and its assimilation into biomass or its release as waste (Vanni 2002). Nutrient transfer is the movement of nutrients throughout habitats, and bioturbation is the physical perturbation of sediments (Vanni 2002). Parasites can impact nutrient cycling directly through the transformation or transfer of nutrients within their own biomass. Parasites can also alter host behavior and physiology, which may indirectly contribute to nutrient cycling (Bernot and Lamberti 2008, Bernot 2013, Mischler et al. 2016, Buck and Ripple 2017). In this article, we explore the previously underemphasized role parasites can play in nutrient cycling by altering their hosts and outline a mathematical framework which addresses this outstanding question in parasitology.

1.3 Parasites alter host behavior and physiology

Parasites can alter host-consumer nutrient transformation through changes in host diet, egestion/excretion rates, waste stoichiometry, and/or nutrient storage (Vanni 2002). These aspects of nutrient transformation are not mutually exclusive and may offset or reinforce one another.

Parasitic infection can change a host-consumer's diet composition (Moore 2002, Bernot and Lamberti 2008). Snails infected with a trematode parasite selectively foraged on N-fixing, blue-green algae. In treatments with 50% compared to 0% trematode prevalence, infected snails reduced blue-green algal biovolume in mesocosms by ~70%; such effects may alter N fluxes within aquatic ecosystems (Bernot and Lamberti 2008). In addition to dietary changes, parasites can increase a host's egestion or excretion rate (Bernot 2013, Mischler et al. 2016), but hosts with parasite-induced reduced feeding rates may show decreases in these traits (Moore 2002). In one study, trematode infection in snails led to a ~30% increase in N excretion and a ~30% decrease in P excretion compared to uninfected snails (Bernot 2013). At the ecosystem-level, snails infected with high intensities of trematode metacercariae excreted N at a ~30% higher rate than snails with low intensity infections, a change which was great enough to rival other ecosystem-level N inputs (Mischler et al. 2016). Parasites can also impact the stoichiometry of host wastes (Bernot 2013, Mischler et al. 2016). In a snail-trematode system, infected snails had higher excretion N:P than uninfected snails (Bernot 2013), suggesting the rapid growth rate of some parasites may generate a P sink (Bernot 2013, Chodkowski and Bernot 2017). Parasites, by regulating population and individual host growth (e.g. mortality, stunted growth, gigantism), impact nutrient storage within host biomass (Sorensen and Minchella 2001, Vanni 2002, Buck and Ripple 2017).

Parasitic infection can also affect host movement and antipredator behavior, potentially changing both predation and nutrient transfer rates (Moore 2002, Sato et al. 2012, Buck and Ripple 2017). For example, trematode infection can reduce the cohesiveness of fish schools (Moore 2002) and nematomorph parasites, which manipulate infected crickets to jump into streams, have been considered as an energy and nutrient subsidy in some systems (Sato et al. 2012). Additionally, the risk of parasitism is now considered a factor in behaviorally mediated trophic cascades (Buck and Ripple 2017) and species migrations (Johns and Shaw 2016), which could drive nutrient transfers between ecosystems.

Despite the pervasiveness of parasitism demonstrated by these examples, the role of parasitism in nutrient cycling is just beginning to be understood (Sato et al. 2012, Bernot 2013,

Mischler et al. 2016, Chodkowski and Bernot 2017). Although multiple studies of snail-trematode interactions have suggested parasites may impact nutrient dynamics (Bernot 2003, 2013, Mischler et al. 2016), there are some conflicting results (Sato et al. 2012, Chodkowski and Bernot 2017). In the nematomorph study mentioned previously, there was no significant difference in the nutrient uptake rate between streams where cricket (nutrient) subsidies were excluded or added. However, more controlled studies may be required to best capture the role of parasitism in nutrient cycling.

1.4 A framework for parasites within nutrient cycling

Here, we develop a framework which allows researchers to set a threshold value at which the amount of parasite-derived nutrients become an important contributor to overall ecosystem nutrient inputs. In Figure 1.1, we present a simplified nutrient model that incorporates parasite contributions to nutrient cycling and examines hosts and parasites strictly as biomass and not individuals to simplify variability associated with infection intensity (Mischler et al. 2016). Although the model is not inherently dynamic, combining it with host-parasite population and SI models and iterating over multiple time steps will generate a more dynamic view of parasitic roles in nutrient cycling. Additionally, our framework assumes that only one limiting nutrient is of primary importance. However, nutrient cycles are often coupled with one another and the ratios in which nutrients are present is increasingly recognized as an important factor in structuring ecosystems (Bernot 2013, Mischler et al. 2016). Despite these limitations, the framework presented here provides a reasonable starting point for parasitologists new to ecosystem ecology and allows for improvements by integrating mass-balance approaches, individual-based modelling, and the addition of multiple nutrients.

The model involves a single nutrient which a producer, a host-consumer, and its parasite cycle back to the nutrient pool (see Figure 1.1). By quantifying the contributions of parasites to this cycle and comparing them to the total nutrient inputs and the nutrients that cycle through non-parasitic organisms, we can assess the relative importance of parasites to nutrient cycling in an ecosystem. If we define flux (F) as the rate of nutrient inputs (mg L-1 or ha-1 year-1) from any

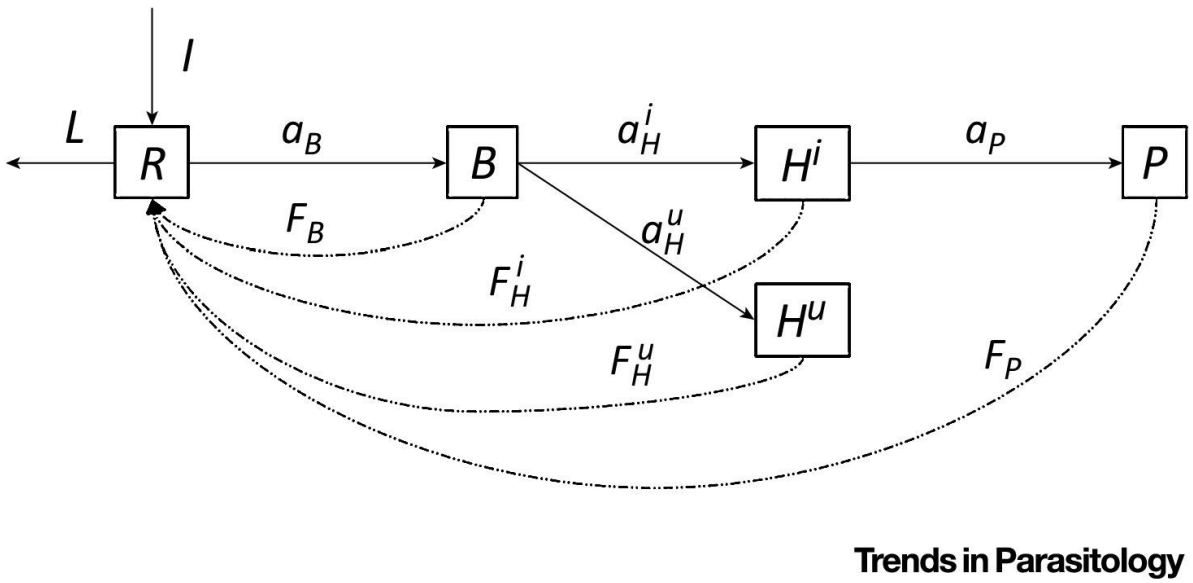

Figure 1.1. A framework considering parasitic roles in nutrient cycling. Dashed lines represent nutrients recycled back to the resource pool, R, by various organisms. In an ecosystem which receives a nutrient resource (*R*) at a rate of I (mg L-1 or ha-1 year-1), primary producers assimilate the nutrient into biomass (*B*; Figure 1.1 and Table 1.1). A host organism (*H*) ingests the producer and transfers material to a parasite (*P*). Some nutrients are lost from the nutrient pool (*L*), while nutrients are returned to the pool from producers, hosts, and parasites (F_X). The host flux (F_H) is the sum of the host's nutrient transformation, transfer, and bioturbation ($F_H = H_{tf} + H_{ts} + H_{bt}$). The parasite's direct contribution to nutrient cycling (F_P) includes the parasite biomass (within and outside the host). For the parasite's indirect contribution to nutrient cycling, we consider uninfected ($H^u$and infected (H^i) classes of hosts which have distinct fluxes (F_H^u and F_H^i, respectively) and distinct nutrient transformation, transfer, and bioturbation values (e.g. H_{tf}^u and H_{tf}^i). We assume that if the parasite is absent, all hosts would function similarly. Thus, we take the difference between infected and uninfected hosts and attribute the remainder to the parasite $\Delta F_H^i = F_H^i - F_H^u$). Therefore, the total parasite contribution to nutrient input can be summarized as $\Delta F_H^i + F_P$ which we compare to a threshold (T) defined as a proportion (p) of total nutrient inputs:$\Delta F_H^i + F_P = T = p(I - L + F_B + 2F_H^u)$. $2F_H^u$ in the preceding equation assumes equal biomass of infected and uninfected hosts.

Table 1.1. Parameter definitions within the model framework in figure 1.1.

Symbol	Definition
$\boldsymbol{R}$	Nutrient resource
$\boldsymbol{I}$	Rate of nutrient input to the ecosystem
$\boldsymbol{L}$	Rate of nutrient loss from the ecosystem
$\boldsymbol{B}$	Primary producer biomass
$\boldsymbol{H}$	Host-consumer biomass
$\boldsymbol{P}$	Parasite of host-consumer *H*'s biomass
$\boldsymbol{F_x}$	Flux of nutrients from various sources *B* (F_B), *H* (F_H), and *P* (F_P) back to *R*. These values are the sum of each organism's nutrient transformation, transfer, and bioturbation.
$\boldsymbol{a_X}$	Assimilation efficiency of organism X
$\boldsymbol{X_{tf}}$	Organism X's nutrient transformation
$\boldsymbol{X_{ts}}$	Organism X's nutrient transfer
$\boldsymbol{X_{bt}}$	Organism X's nutrient bioturbation
$\boldsymbol{H^x}$	The host-consumer's infection status, ^{u} = uninfected, ^{i} = infected
$\boldsymbol{\Delta F_H^i}$	The difference in nutrient flux between infected and uninfected host-consumers, $F_H^i - F_H^u$
$\boldsymbol{T}$	Threshold rate of nutrient input at which a parasite's $\Delta F_H^i + F_p$ is considered important
$\boldsymbol{p}$	A certain proportion of total nutrient fluxes which determines the value *T*

particular source, then the difference in fluxes between infected and uninfected individuals plus the nutrients stored with parasite biomass (direct contributions) that are returned to the nutrient pool ($F_H^i - F_H^u + F_P$) can be compared to a threshold proportion of the overall nutrient flux in the ecosystem (e.g. the sum of nutrients from precipitation, leaching, other consumers, etc.). This threshold is likely system dependent, however, existing data suggest consumer contributions of ≥ 5% of the total nutrient flux can be important (Vanni 2002).

1.5 Challenges and Opportunities

We also propose a set of general ecosystem characteristics which must be considered when studying how parasites impact nutrient cycling (Table 1.2). Studies of nutrient cycling may ignore parasites because the variability associated with parasitism is assumed to be accounted for when

measuring the host's role in nutrient cycling. If a complete sample of host organisms contains infected and uninfected individuals, then parasites are represented within the between-individual variability. However, this assumption misrepresents disease dynamics, as parasite prevalence and infection intensity are often spatially and temporally patchy. Thus, parasite-derived nutrients are potentially dynamic across time and space and may go unrecognized when averaging across entire study systems or over long periods of time.

A first step in assessing the role of parasitism in nutrient cycling is to examine how parasitism affects individuals hosts (Bernot 2013, Mischler et al. 2016, Chodkowski and Bernot 2017). Expanding our knowledge of individual host's contributions to nutrient cycling will best allow for the initial calculation of $F_H^i - F_H^u + F_P$. This should be done across various systems and parasite life stages to inform studies which encounter these variabilities. For example, trematode species have different larval stages which are functionally distinct in their interactions within their molluscan hosts. Do these larvae generate different responses in host contributions to nutrient cycling and are these differences consistent across taxa?

Mesocosms are well suited for studying parasitic roles in nutrient cycling as parasite presence, prevalence, and intensity can be manipulated. Using a consumer which is known to impact nutrient cycling, and which hosts a parasite that is suspected of altering relevant behaviors or physiology could reveal a role of parasites in nutrient cycling. A first step for field studies of nutrient cycling is to view parasitism as a covariate explaining variation in consumers. For example, researchers could sacrifice a subset of individuals for parasitological examination and relate infection status to a host's role in nutrient cycling. These studies would require interdisciplinary collaboration between ecosystem ecologists and disease ecologists which would greatly benefit both fields. Comparing nutrient dynamics in areas with variable parasitism may be possible in the future but separating how parasites affect co-occurring mechanisms of nutrient cycling can be challenging.

We suggest constructing a solid understanding of individual host-parasite relationships with regards to nutrient cycling before expanding conclusions to larger systems. Effect sizes for these host-parasite interactions are likely small for individual hosts. However, the cumulative impact of these parasite-induced changes in multiple individuals can be significant at the ecosystem level (Bernot 2013, Mischler et al. 2016). Nutrient cycling plays a pivotal role in structuring communities and ecosystems. The framework which we have outlined provides a first step in

integrating parasite ecology with this foundational aspect of ecology and emphasizes our need to understand the role of parasitism at the ecosystem level.

Table 1.2. Characteristics of the host-parasite-ecosystem unit that should be considered when studying the role of parasitism in ecosystem-level nutrient cycling.

	Characteristics of the abiotic/biotic system	**Associated model parameter***
General considerations	Nutrient inputs to and losses from the system	I and L
	The amount of host and parasite biomass	H and P
	Host contributions to nutrient cycling	F_H
	Parasite prevalence	H^i
	Infected hosts, irrespective of infection intensity, show altered behavior/physiology compared to uninfected hosts	F_H^i
	The effect size of parasite-induced changes to host nutrient cycling	ΔF_H^i
	Mortality rate of infected hosts	H_{tf}^i
	Transmission success rate of parasite free-living stages	P_{tf}, P_{ts}, and F_H^i
Spatial considerations	Host aggregation in space	NA
	Parasite aggregation in space	NA
	How nutrient inputs from outside the system are mixed within the system	NA
Temporal considerations	Does parasitic infection peak seasonally	NA
	Does host population peak seasonally	NA
	Do nutrient inputs to the system peak seasonally (and does the nutrient peak coincide with a host/parasite biomass peak)	NA

**See Table1.1 for parameter definitions. NA refers to characteristics which are not present in the model framework.*

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CHAPTER 2. SHAKE IT OFF: BEHAVIOR OF THE SNAIL, *PHYSA ACUTA*, DURING AND AFTER PARASITE ATTACK

2.1 Abstract

Parasite avoidance has recently drawn attention from scientists due to its potential role in structuring ecological interactions. Interestingly, discussions of parasite avoidance have included very little information about snail hosts. Yet snails serve as important intermediate hosts for a number of ecologically important trematode species. Here, we use the common model species, *Physa acuta* host snails and *Echinostoma trivolvis* parasites, to explore how snails respond to parasite attack. Snails exposed to parasite cercariae displayed more shell shaking, more surfacing, and less time foraging than unexposed snails. However, these impacts did not persist beyond the initial exposure period. These results suggest that shell shaking, commonly assumed to be an antipredator response, also may be associated with parasite avoidance. The combination of these behaviors is likely to have ecological ramifications including interactions with predation, snail foraging, and bioturbation.

2.2 Introduction

Host manipulation by parasites can invoke sensational ideas about 'zombie' hosts and brain control (Doherty 2020). Certainly, hosts can show altered behavior following a parasitic infection (Moore 2002, Preston et al. 2014) but, more recently, research has begun to demonstrate that simply the presence of parasites can have substantial consequences for host behavior and potentially entire ecosystems (Behringer et al. 2018, Buck et al. 2018, Weinstein et al. 2018).

Parasite avoidance behaviors generate a 'landscape of disgust' (Weinstein et al. 2018) in which hosts alter their movement patterns, foraging decisions, mate selection, and energy allocation in order to balance the risk of parasitic infection with other components of fitness (Hart 1990, 1992, Hutchings et al. 2006, Koprivnikar and Penalva 2015, Buck et al. 2018). Parasite avoidance has likely influenced life history evolution of numerous organisms, as parasite avoidance is often seen as less costly than immunological, life history or behavioral responses following parasitic infection (Minchella 1985, Hart 1990, Curtis 2014, Behringer et al. 2018).

However, we know surprisingly little about how organisms avoid becoming parasitized (Hart 1990, Behringer et al. 2018).

One group of organisms of substantial ecological importance are the gastropods (snails and slugs; Dillon Jr. 2000, Johnson et al. 2013). Molluscs, including snails and slugs, are among the most imperiled organisms on the planet (Lydeard et al., 2004). However, snails can play substantial role in ecosystems as resources, consumers, and disease vectors (Dillon Jr., 2000; Graveland, 1996; Johnson et al., 2013; Reid et al., 2018). Snails play a central role in the transmission of numerous macroparasites (especially digenetic trematodes; Olsen 1974). Yet, literature reviews on parasite avoidance rarely discuss snail anti-parasite defense strategies (Hart 1990, Moore 2002, Curtis 2014, Behringer et al. 2018, Buck et al. 2018).

Trematodes are known to have influences on their hosts both after (Sorensen and Minchella 2001, Moore 2002) and before infection (Rohr et al. 2009, Koprivnikar and Penalva 2015). Before an infection is established, many trematodes use free-living larval stages which must identify and invade a potential host (Esch et al. 2001, Sukhdeo and Sukhdeo 2004, Vannatta et al. 2020). Some of these larvae are known to trigger defense responses in amphibian hosts (Rohr et al. 2009, Preston et al. 2014, Koprivnikar and Penalva 2015, Behringer et al. 2018), but we found few studies extensively documenting the behavior of snails in response to parasite attack (but see Davies and Knowles 2001, Wynne et al. 2016).

Physa acuta is one of the most widely distributed snails in the world (Burch and Tottenham J.L. 1980), is known to have elaborate anti-predator defenses (Frieswijk 1957, Wilken and Appleton 1991, Krupski et al. 2018), and can function as both a first and second host for trematodes (Olsen 1974). One species of trematode, *Echinostoma trivolvis*, is known to elicit anti-parasite behaviors in tadpoles when using these species as a host (Rohr et al. 2009, Preston et al. 2014). However, *E. trivolvis* can also use *Physa* snails as a second host (Olsen 1974). In this study, we use *P. acuta* and *E. trivolvis* as a model system to assess anti-parasite defense behaviors of snails. Additionally, we monitor snails for multiple days after infection to determine if parasitic infection may have longer term impacts on host movement in this system.

2.3 Methods

2.3.1 Parasite life cycle

Echinostoma trivolvis and *P. acuta* snails were used to study parasite avoidance and the influence of infection on host movement. *Echinostoma trivolvis* is a common trematode parasite of waterfowl and muskrats (Olsen, 1974). The parasite is discharged in the feces of waterfowl and muskrats, and then infects a snail first intermediate host. After maturation within the first intermediate host, swimming parasite larvae, called cercariae, are released and seek a second snail host in which they form metacercarial cysts. The life cycle is complete when second intermediate host snails (and sometimes tadpoles) are ingested by waterfowl and muskrats, and the adult parasite matures within the intestine.

2.3.2 Host and parasite collection

The second intermediate host snail, *P. acuta*, was raised in a trematode-free, laboratory colony at Purdue University. *Physa acuta* were originally collected from the Purdue Wildlife area in summer 2017. Snails were raised in a series of 31 fish tanks, established with pond sediment (also collected in 2017) and aquarium gravel. Within the colony tanks, snails were occasionally fed romaine lettuce, snail gelatin (Civitello et al. 2018), and consumed algae which grew on tank walls. This colony population was allowed to naturally grow for the next three years to ensure all infected snails and parasite propagules were gone. In fall of 2020, 15 first intermediate hosts, *Helisoma* spp. snails with active *E. trivolvis* infections were collected from the Purdue Wildlife area. Echinostome cercariae were isolated from *Helisoma* for use in our experiment by placing infected snails under bright artificial lights for a minimum of 90 minutes.

2.3.3 Experimental setup

Twenty-four hours prior to the beginning of each trial, adult *P. acuta* were randomly selected from the 31 colony tanks and placed into jars with ~180 mL of well water and a small amount of gelatin snail food (Civitello et al. 2018). After this 24-hour acclimation period, *P. acuta* snails were placed into 144 x 144 mm, square plastic arenas filled with 180 mL of well water (~8.5 mm water depth), a core of gelatin snail food, and allowed 3 – 5 minutes to acclimate to the arena. On day one of the

experiment, 30 *E. trivolvis* cercariae were added to the arenas and snails were filmed for ~1 hour (N= 26 exposed snails and N = 26 unexposed snails). Cameras were positioned directly above experimental arenas, facing straight down, between 21 and 28 cm from the arena. At the conclusion of the filming period, the snail and all contents of the arena (including parasite cercariae) were rinsed into the snail housing jar and left until the next day of observation.

In order to observe lasting impacts of parasitic infection, we filmed snails on days 3 and 5 of the experiment using the same procedure above but did not add parasites to the arenas on these days. Day 5 was selected as the end of the experiment as metacercariae are typically infective to the final host after this length of time and have settled in a fixed location within the host. After day 5 of the experiment or immediately following the death of a snail, snails were crushed and metacercarial cysts within the snail tissue were counted.

2.3.4 Data analysis

Snail defense strategies against parasites are not well documented. However, a number of studies have examined how snails respond to predation (Broenmark and Malmqvist 1986, Alexander and Covich 1991, McCarthy and Fisher 2000, Covich 2010, Wethington et al. 2018). Using these studies as guidance, we analyzed snail videos manually by enumerating: amount of time spent foraging/in proximity to gelatin food, number of trips to the water surface (defined as distorting the surface tension of the water; McCarthy and Fisher 2000), and number of shell shakes. Shell shaking is a common response to leech predation in some snails and involves rapid left and right movements of the shell (Townsend and McCarthy 1980). We defined a shake as a sudden movement of the shell in either direction at least 45 degrees from the center line of the animal (figure 2.1). Bouts of shell shaking were classified as separate bouts when separated by at least

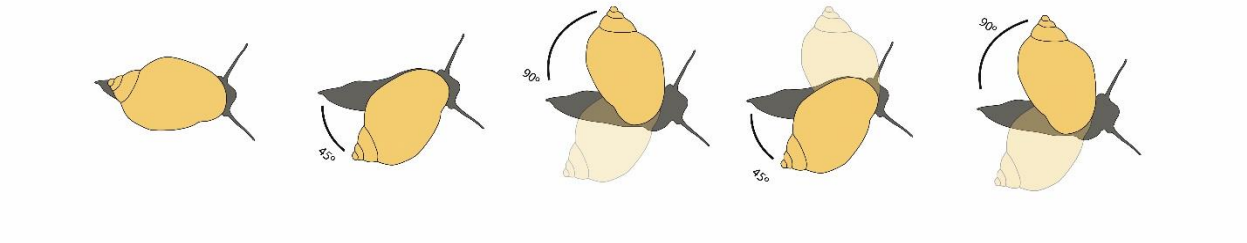

Figure 2.1. Dorsal view of a common shell shaking pattern. This figure shows four ‘shakes’ as defined in this study. Illustration by Gaby Sincich.

five seconds (Krupski et al. 2018). In addition, we analyzed videos in R version 3.6.3 (R Core Team 2019) using the manualPath() function in the pathtrackr package (Harmer and Thomas 2019). One frame of video was analyzed for every five seconds of video in order to determine mean velocity, variance in snail velocity, and variance in turn angle. Due to camera malfunctions, any video which had not recorded at least 55 minutes of video was excluded from analysis of shaking, foraging, and surfacing.

See table 2.1 for a summary of statistical models. Day 1 and days 3 and 5 were analyzed separately, as these times represent distinct biological differences (day 1 = during parasite attack, days 3 and 5 = after parasite attack). In day 1 models, we included the arena as a random factor as arenas were used more than once. We created zero-inflated, negative binomial GLMMs in glmmTMB (Brooks et al. 2017), gamma, gaussian, and negative binomial GLMMs in lme4 (Bates et al. 2015) and used ggplot2 for data visualization (Wickham 2016).

Table 2.1. Summary of statistical models and error distributions for analyzing snail behavior during and after parasite attack (day 1 and days 3 and 5, respectively). All models were formulated as GLMMs with Defense strategy ~ Exposure class + (1|arena). For models of after parasite attack behavior, days 3 and 5 were aggregated and an additional random factor (1|snail ID) was included.

Defense strategy	Error distribution (day 1)	Error distribution (days 3 and 5)
Number of shakes	Zero-inflated negative binomial	Zero-inflated negative binomial
Surfacing events	Negative binomial	Negative binomial
Time spent foraging	Negative binomial	Negative binomial
Mean velocity	Gaussian	Gamma
Velocity variance	Gaussian	Gaussian (with box-cox transform)

2.4 Results

Parasite exposure led to alterations in snail movement (figure 2.2). During parasite exposure, snails exhibited more shell shaking behavior (see table 2.2), had more surfacing events (Estimate = 0.5738 ± 0.2234, $z = 2.569$, $p = 0.0102$), and spent less time foraging (Estimate = -0.5218 ± 0.0127, $z = -40.98$, $p < 0.0001$). Exposed snails also tended to travel at higher velocities (Estimate = 0.2546

± 0.1358, t = 1.875, p = 0.0608) and have higher variance in velocity during parasite attack than unexposed snails (Estimate= 0.0831 ± 0.0439, t = 1.894, p = 0.0582) but these differences were not statistically significant.

Infected and uninfected snails (in days 3 and 5 of the experiment) showed no significant differences in any measure of movement. However, there was a non-significant trend where infected snails were more likely to shake their shells than uninfected snails at this point in the experiment (Zero-inflation model estimate = -1.633 ± 1.000, z = -1.652, p = 0.0985). No measure of snail movement was correlated with metacercarial infection intensity.

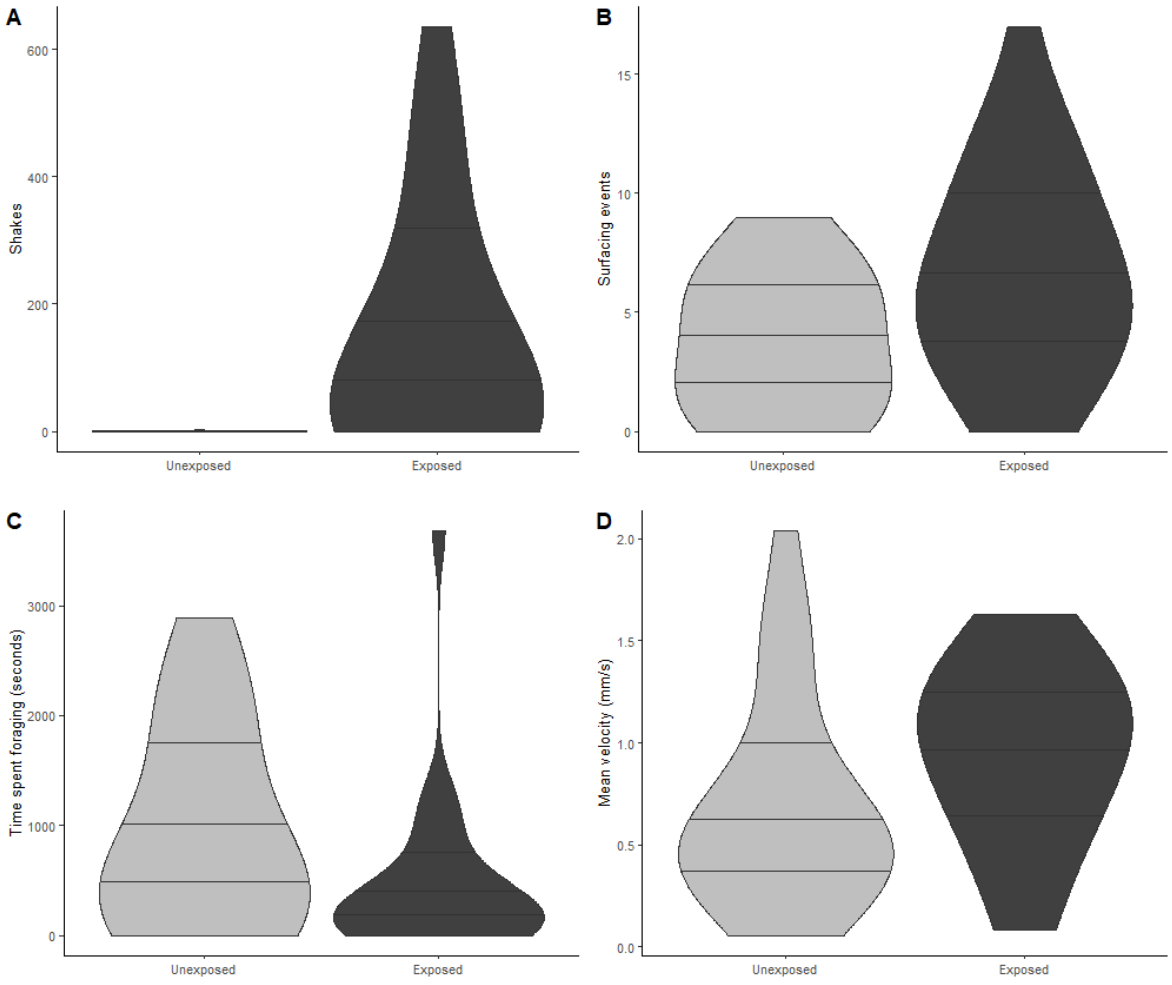

Figure 2.2. Snail movement in response to parasite attack. Exposed snails A) had a higher number of shell shakes, B) surfaced more often, C) foraged less, and D) travelled marginally faster than unexposed snails. Violin plots with data quantiles are shown due to data overdispersion.

Table 2.2. Model results for shell shaking comparing parasite exposed snails relative to unexposed snails (intercept). Coefficients of the zero-inflation model show the probability of obtaining a zero value.

	Parameter	Estimate	SE	Z value	P value
Zero-inflation model					
	Exposed group	-2.148	1.212	-1.722	0.0764
Conditional model					
	Exposed group	5.5012	1.1393	4.828	< 0.0001

2.5 Discussion

In this study, we add a central piece to the growing literature on parasite avoidance. Here, we show that snails, important trematode intermediate hosts, display various behavioral responses to parasite stimuli. Snails exposed to parasites displayed shell shaking and surfacing behavior, spent less time foraging and may alter their velocity in response to parasite attack. However, these behavioral adaptations did not persist past initial exposure and there was no evidence that these behaviors altered parasite load.

Our experimental design is unable to separate the influence of direct stimulation by parasite cercariae and chemical cues from infected snail hosts. However, these cues would consistently occur together in natural environments. Additionally, in tadpoles, alterations in behavior were associated with parasites and not infected snail cures (Preston et al. 2014). It remains possible that in exposed versus unexposed trials *P. acuta* were responding partly to heterospecific snail cues. This is unlikely to be the case for most behaviors documented here as shell shaking is strongly linked with mechanosensory cues (Krupski et al. 2018), surfacing has largely been documented in relation to predation (McCarthy and Fisher 2000), and snail velocity shows little variation in response to other snails (Eliuk et al. 2020).

Evidence presented here suggests that snails display similar reactions as tadpoles to parasite attack. Tadpoles are known to increase their activity and display rapid swimming movement in response to attack by trematode cercariae (Koprivnikar et al. 2006, Rohr et al. 2009, Preston et al. 2014, Behringer et al. 2018). *Physa acuta* in the current study displayed similar behavior by altering surfacing behavior and engaging in shell shaking. These behaviors likely come at an energetic cost (Hart 1990, Hutchings et al. 2006, Koprivnikar and Penalva 2015),

although this cost is assumed to be less than the energetic investment in immune responses (Hutchings et al. 2006, Curtis 2014). Quantifying the cost of these defense mechanisms (via measuring metabolic rate of attacked snails) could present an effective method at quantifying the costs of parasitism as the amount of energy a host is willing to invest in parasite avoidance may provide one perspective on the potential energetic costs of a parasitic infection.

In terms of fitness costs, it is often assumed that predation represents a higher cost than parasitism and should thus illicit stronger avoidance responses (Rohr et al. 2009, Koprivnikar and Penalva 2015). For a century, shell shaking behavior has been assumed to be a general response to leech predation in *Physa* (Degner 1921, Wrede 1927, Frieswijk 1957). However, some authors have challenged this assumption citing shell shakings ineffectiveness at countering leech predation (Broenmark and Malmqvist 1986, Wilken and Appleton 1991) and somewhat ambiguous involvement in snail mating behavior (Dillon Jr. 2000, Wethington et al. 2018). Given the strong shell shaking response seen by *P. acuta* in response to parasite cercariae, we suggest that shell shaking may be an adaptive response to parasitism. Although we did not find any significant associations between shell shaking and infection intensity, our experimental conditions were aimed at facilitating parasite attack and not replicating the environment in which these species normally interact. The experimental conditions were designed to elicit and detect a host defensive response to parasite exposure. Under natural conditions it is unlikely that a snail host would experience such a high intensity exposure (30 cercariae) of extended duration in a small area (the parasites remained in contact with the host after the one-hour trial). Indeed, most *Physa* snails naturally infected with echinostome metacercariae have only accumulate 5 – 30 metacercariae within their lifetime (Zimmermann et al. 2017). Thus, it is not surprising that the shaking metrics did not correlate with infection intensity during the trial. This high intensity exposure may also contribute to the strength of response elicited compared to comparable experiments with predators. Experiments in more natural settings are needed to demonstrate the efficacy of these behaviors against parasites. It is also important to note that parasite avoidance behaviors can be countered by parasite manipulation (Gray et al. 2009, Eliuk et al. 2020) and exploited by other organisms (Hart 1992, Rohr et al. 2009, Kamiya and Poulin 2012, Koprivnikar and Penalva 2015, Behringer et al. 2018, Buck et al. 2018). For example, shell shaking behavior is known to increase snail mortality by allowing snails to be easily seen by visually guided predators (Ahlgren and Brönmark 2012).

Additionally, when snails engage in shell shaking and escape behaviors, resources are taken away from a snail's foraging opportunities.

Snails that engage in shell shaking use valuable metabolic resources and limit their ability to add resources via foraging. The high cost of this parasite avoidance runs contrary to the assumption that metacercarial infection with *E. trivolvis* in snails is of little consequence (Keeler and Huffman 2009), although some have challenged this assumption (Vannatta and Minchella *in review*). If indeed metacercarial infection is not costly to snails, we would expect selection to drive down the intensity of shell shaking responses to cercariae lest they become a burden on host energy use (Langerhans and DeWitt 2002). Behavioral defense mechanisms can also be dynamic with organismal physiological state. As our snails were fed ab libitum during the acclimation period and trials, it is possible that these behaviors are displayed when resources are abundant. Nevertheless, the current study presents an example of how parasite avoidance could alter community structure: by altering foraging, biomass accrual, and predation rates within snail communities.

We have shown that snails have their own suite of parasite avoidance behaviors including surfacing, shell shaking, and altering their velocity. Although these behaviors do not extend beyond the period of parasite attack, their ecological ramifications warrant further exploration. For example, shell shaking could cause bioturbation, introducing oxygen into sediment and making nutrients available to other organisms (Yang et al. 2020). Parasite avoidance behaviors of snails are likely to influence other biotic interactions, such as increasing snail vulnerability to predation. In fact, we suggest that shell shaking may be a response to parasite attack and that the assumption that this behavior functions primarily as an anti-predator strategy should be challenged. It is additionally worth noting that parasites themselves may counter host avoidance strategies such as by manipulating host aggregation patterns (Gray et al. 2009, Eliuk et al. 2020). Lastly, the apparent investment in parasite avoidance in snails suggests that parasitism may indeed have a previously underappreciated part in structuring aquatic ecosystems including a potential role in bioturbation in these systems.

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CHAPTER 3. THE INFLUENCE OF PARASITISM ON PRODUCERS AND NUTRIENTS IN MESOCOSM ECOSYSTEMS

3.1 Abstract

Pathogens and parasites are increasingly recognized as important components within host populations, communities, and ecosystems. Both density-mediated and trait-mediated impacts of parasites on ecosystems are known and likely operate together to influence ecosystem processes. Despite the assertion that impacts of parasites are pervasive, empirical evidence of these effects is lacking. Our aim is to fill this gap and test whether parasitism can have an impact on ecosystem processes within controlled mesocosm ecosystems. In the host-parasite (snail-trematode) system used, parasites form cysts in host tissue and cause minimal direct mortality (minimizing density mediated parasite impacts). We created mesocosms across a gradient of parasitism and measured water column nutrient concentrations, producer biomass, and invertebrate community composition. We demonstrate that trematode parasitism is correlated with an increase in periphyton biomass and ash-free dry mass. Additionally, water column carbon and phosphorus concentrations were influenced by producers but not parasites. We demonstrate that metacercarial parasites have limited impact as "ecosystem engineers", but some data suggest parasites may have a subtle influence on ecosystem processes.

3.2 Introduction

Pathogens and parasites play a significant role in the behavior and physiology of individuals, the stability and dynamics of host populations, and in community assembly and structure (Minchella and Scott 1991, Tompkins et al. 2011), but empirical evidence supporting the impact of disease at the ecosystem level has only recently been emphasized (Preston et al. 2016, Buck and Ripple 2017, Vannatta and Minchella 2018, Fischhoff et al. 2020, Paseka et al. 2020). Given the substantial influence of parasites on small scales, it seems plausible that parasitism could have cascading effects on higher levels of biological organization (Buck and Ripple 2017, Vannatta and Minchella 2018, Weinstein et al. 2018).

Ecosystem science deals primarily in two currencies: the flow of energy and the flow of materials (nutrient cycling; Preston, Mischler, Townsend, & Johnson, 2016). Most research to date

has focused on the energetic implications of parasitism in ecosystems (Kuris et al. 2008, Sato et al. 2011, Preston et al. 2013, 2016). However, recent reviews have suggested parasitism must also be considered within the context of ecosystem nutrient cycling (Preston et al. 2016, Bernot and Poulin 2018, Sanders and Taylor 2018, Vannatta and Minchella 2018, Fischhoff et al. 2020, Paseka et al. 2020). Studies have demonstrated links between parasitism and ecosystem nutrient cycling in producer communities (Eviner and Likens 2010, March and Watson 2010, Hatcher et al. 2012) as well as in consumer species (Holdo et al. 2009, Mischler et al. 2016, Brunner et al. 2017). Parasites influence nutrient cycling either directly via their own biomass or indirectly by altering nutrient transformation, nutrient transfer, and bioturbation by their hosts (Vannatta and Minchella 2018). Although ecosystem-level effects of parasites via regulation of host density have been documented (Holdo et al. 2009, Whiles et al. 2013, Buck and Ripple 2017), direct biomass effects and indirect parasite-induced changes in host behavior and physiology (trait-mediated effects of parasitism) are less understood (Mischler et al. 2016, Buck and Ripple 2017, Vannatta and Minchella 2018, Buck 2019). These trait-mediated impacts have potentially large impacts are ecosystems, but are often difficult to study independently of density-mediated effects for both predation and parasitism.

Parasites can contribute a substantial amount of biomass to some ecosystems (Kuris et al. 2008, Preston et al. 2013, but see Paseka 2017), but how this parasitic biomass influences ecosystem function is difficult to assess. In order to definitively show that parasitic influence can cascade to higher trophic levels, an entire system must have its parasite population manipulated while holding other variables constant. Some parasites produce a large amount of biomass (Kuris et al. 2008, Preston et al. 2013) which may directly contribute to nutrient cycling (Vanni 2002) and it is thought this biomass can operate in concert with density- and trait-mediated effects to influence ecosystem scale processes (Thomas et al. 1998, Sato et al. 2011, Buck and Ripple 2017, Vannatta and Minchella 2018, Weinstein et al. 2018, Buck 2019). In order to test whether parasitism is correlated with ecosystem processes, we selected a snail-trematode, host-parasite system where parasitism should have a limited impact on host population dynamics. We then experimentally manipulated the number of parasites in the system. Thus, if changes were detected in ecosystem dynamics across the gradient of parasitism, they were likely the result of parasite biomass or parasite-induced changes in host behavior and physiology. We assessed parasitism in two ways: infection prevalence (binary infected/uninfected responses to parasitism) and infection

intensity. We show that parasitism, despite previous assumptions, led to changes in host abundance which potentially operate alongside trait-mediated effects to alter producer biomass.

3.3 Methods

3.3.1 Summary

We created experimental mesocosms using a snail-trematode system in which one group of caged snails transmitted parasites to free-roaming snails. A gradient in parasitism was created by altering the number of caged snails transmitting parasites while keeping the total number of caged snails constant. We maintained our mesocosms for 12 weeks and examined dissolved nutrient concentration and stoichiometry, producer biomass, invertebrate community composition, and free-roaming snail abundance and infection intensities.

3.3.2 Parasite life cycle

We utilized the trematode parasite *Echinostoma trivolvis*. *Echinostoma trivolvis* begins its life cycle in the intestinal tracts of waterfowl or muskrats (Olsen, 1974; Figure 3.1). Adult parasites release eggs produced via sexual reproduction in host feces which mature in the environment. Once hatched, free-living miracidia penetrate *Helisoma* spp. snails and undergo asexual reproduction. After 4-6 weeks, free-living cercariae burst from *Helisoma* and encyst as metacercariae (parasitic cysts) in any available aquatic gastropod host (tadpoles may also be infected). These metacercariae are ingested by the vertebrate host when the snail is eaten, beginning the life cycle again. In our experiment, we replicated the snail-to-snail transmission pathway.

3.3.3 Experimental setup

Mesocosms were established at the Ross Biological Reserve in Tippecanoe County, Indiana, USA. Twenty-four mesocosms (150 L; 65 cm wide x 90 cm long x 30 cm deep) were randomly distributed on a concrete platform and established by rinsing 1 L of homogenized sediment from a pond at Purdue Wildlife Area through 1000 µm bolt cloth with well water. Bolt cloth removed large debris and unwanted gastropods from sediment. Sediment also served to establish

zooplankton and algal communities. This volume of sediment was chosen to cover the base of the mesocosm with a thin layer of sediment and provide a sufficient amount of algal inoculum (Johnson et al. 2007). One cinder block was placed in the center of each mesocosm to generate habitat structure and to add additional surface area for algal growth. Mesocosms were

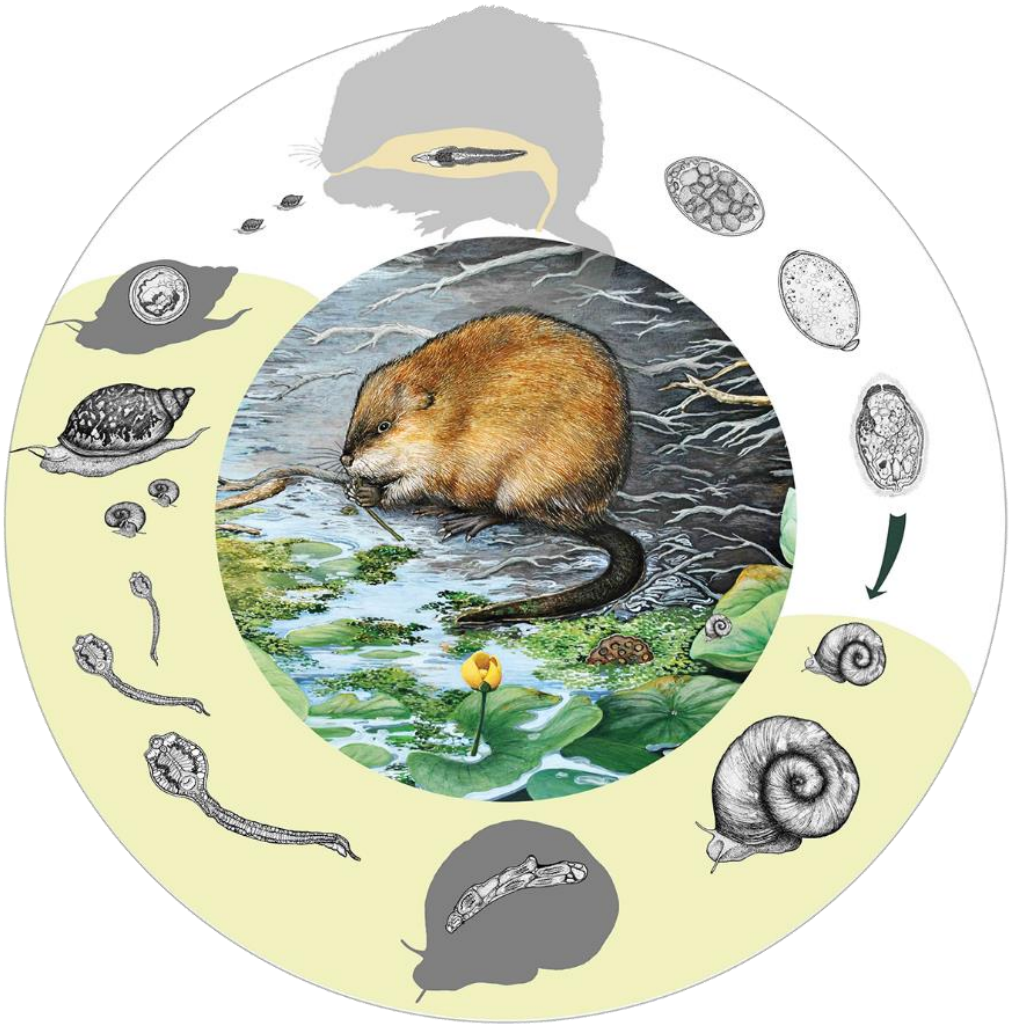


Figure 3.1. *Echinostoma trivolvis* life cycle. *Echinostoma trivolvis* adults reside in muskrat intestines. Eggs are released in feces, mature in aquatic environments, and hatch as miracidia. Miracidia penetrate *Helisoma* spp. snails and mature to rediae. Rediae release cercariae which actively seek out gastropod second intermediate hosts such as *Physa* and *Promenetus*. Cercariae enter the second intermediate host and form metacercariae, which develop into adult worms after ingestion by muskrats. The shaded region was replicated within our experimental mesocosms (illustrations by Gaby Sincich).

covered with 1 mm window screen lids to prevent colonization of additional organisms and left for two weeks (McCormick and Stevenson 1989) to allow algal and zooplankton communities to establish. Then, the snail-trematode parasite community components were added as well as ceramic tiles to assess periphyton growth (see below).

To generate a gradient in parasitism, we placed 5 echinostome infected, caged *Helisoma* in 'high parasitism' treatments (100% prevalence), 2 infected and 3 uninfected, caged *Helisoma* in 'moderate infection' treatments (~ 40% field prevalence at Purdue Wildlife Area), and 5 uninfected, caged *Helisoma* in 'low parasitism/control' treatments (0% prevalence). These prevalence values were chosen based on estimates of snail densities and infection found during field sampling at Purdue Wildlife Area. In a few instances, the infection status of these snails was misidentified (false-positives and false-negatives) which introduced variability within the treatments.

Helisoma spp. snails were collected from Purdue Wildlife Area throughout the field season and screened for echinostome cercariae (identified according to Schell 1985). Both infected and uninfected "source" snails in the appropriate combination were placed in snail cages (2 L volume) constructed of 1000 µm bolt cloth and PVC to prevent caged snails from becoming lost or laying egg masses within mesocosms. Once a week, caged snails were screened to confirm survival. Additionally, snails were screened by putting snails in 6-well plates under artificial light for 90 minutes and checking for cercariae to ensure the appropriate number of snails were still shedding cercariae and that uninfected, caged snails had not developed active infections. During these weekly screenings, dead snails were replaced with live snails according to infection status and treatment type and surviving infected and uninfected snails were placed into separate containers. Snails in each container were mixed, and haphazardly redistributed across mesocosms. This was done to minimize differences related to infection intensity, snail size, and individual host responses to parasitism in caged snails.

Physa snails were visualized size-matched and distributed equally according to shell length in each mesocosm (20 snails per mesocosm) as free-roaming potential hosts for the parasitic cysts. Free-roaming *Physa* were raised in the laboratory for one year prior to mesocosm establishment to ensure that free-roaming snails would not initially harbor parasites. Additionally, gastropods smaller than 1000 µm were able to enter the mesocosms during establishment with pond sediment. This group primarily included *Promenetus* spp. snails.

3.3.4 Measurement of ecosystem function

A complete list of response variables is included in Table A.1. Invertebrate communities were assessed at the conclusion of the experiment. Free-roaming snails were stored in a refrigerator in order to slow their metabolism and prevent mortality. Due to personnel and time constraints, absolute size of snails was not recorded. However, *Physa* greater than 7 mm and those less than 7 mm were noted as being within distinct size classes. *Promenetus* snails had little variation in size based on visual inspection at the end of the experiment and were not separated into such classes. All snails were crushed and parasitic cysts (metacercariae) were counted by teasing apart host tissue. Prevalence was calculated within snail species as the number of snails containing cysts divided by the total number of snails retrieved from each mesocosm.

The remaining invertebrate community was assessed by collecting 1 L of mesocosm water from the surface, middle, and bottom of each tank (3 L total) and filtering the sample through 80 µm bolt cloth. Samples were refrigerated and processed within 1 week. Most invertebrates were keyed to family and, because of their distinctive characteristics, cladocerans were keyed to genus (Voshell Jr. 2002, Haney et al. 2013).

Primary production was measured using the *in situ diel* primary production method. This method uses the change in dissolved oxygen (DO) concentration between the DO maximum and minimum to approximate community respiration and photosynthesis (Howarth and Michaels 2000). *In situ diel* primary production was calculated by taking dissolved oxygen readings from 14:00 – 16:00 and then from 04:00 – 06:00 (the photosynthetic maximum and minimum, respectively) within a 24 hr period at weeks 0, 6, 9, and 12. Each mesocosm was recorded three times during these sessions and the average DO reading used as the value for that mesocosm. All remaining metrics of primary production were measured at the conclusion of the experiment. Periphyton ash-free dry mass was measured by scrubbing the 15 x 15 cm ceramic periphyton tiles into 380 mL of fresh, well water. Solutions were mixed thoroughly, and 50 mL of solution was vacuum filtered onto pre-ashed and weighed 0.7 µm glass fiber filters (GFF). These were then dried for at least 48 hr at 60 C, weighed, ashed at 550 C for 4 hr, cooled and re-weighed. For chlorophyll α, the second ceramic tile was scrubbed, and 50 mL of the solution filtered onto a 0.7 µm GFF. This filter was placed in a film canister and frozen at -80 C until processing. For extractions, filters were cut in half, placed in 10 mL of 90% ethanol for 24 hr and processed on a Turner Designs fluorometer using the Chl a-NA module. Surface vegetation was assessed by

collecting all floating vegetation with an aquarium net, drying the sample at 60 C for 48 hr and weighing. Before drying, 10 mL of packed vegetation was used as a subsample to determine the abundance of different floating vegetation species. This subsample was sorted by species, weighed, and included in the total vegetation biomass calculation.

Water nutrient concentration and stoichiometry were measured using a Shimadzu TOC/TNM-L analyzer and a SEAL AQ2 autoanalzyer. Water samples, conductivity, and pH were taken at week 0, 6, 9, and 12. Due to logistical constraints, samples were frozen before filtration. This is unlikely to have impacted our study as analyses were done relative to other treatments within this study. Carbon, nitrogen, and phosphorus were measured only for dissolved nutrient components (total dissolved organic carbon, total dissolved nitrogen, and total dissolved phosphorus) by filtering samples through 0.7 µm GFF filters.

3.3.5 Statistical analysis

We used linear regression with *Physa* mean infection intensity or prevalence of free-roaming snails (Number of *Physa* and *Promenetus* infected/ Number of *Physa* and *Promenetus* crushed) as a predictor. For time series nutrient data, linear models were produced with time as an additional predictor variable with an interaction term. Models examining nutrient concentration were conducted for weeks 6, 9, and 12 when parasitism could reasonably have had an effect on overall nutrient concentration. For analyses examining the change in nutrient concentration, week 0 was included to account for the starting point of each mesocosm. For stoichiometric analysis, C, N, or P values below the detection limit were set to one half the detection limit (Halvorson et al. 2017). Invertebrate community data were assessed using non-metric multidimensional scaling (NMDS) with the vegan package in R. Values were log transformed as needed to satisfy statistical assumptions. Nonparametric statistics were used on *Physa* prevalence data as this data continued to violate assumptions of linear models. All analyses were done in R version 3.6.3 (R Core Team 2019).

3.4 Results

3.4.1 Parasite infection metrics

Among all 24 mesocosms (8 experimental units per low, moderate, and high parasitism) over 1250 free-roaming snails were crushed and examined for parasitic infection. These yielded over 19,000 metacercariae. Prevalence and infection intensity in free-roaming snails was significantly, positively correlated with our treatment groups (Fig. 3.2).

3.4.2 Community metrics

Snail abundance was negatively correlated with *Physa* infection intensity (Figure 3.3). However, invertebrate community composition did not vary consistently by treatment group (PERMANOVA Adjusted r2 = 0.096, F2, 21 = 1.118, p = 0.346; Figure A.1). Additionally, the number of *Physa* greater than 7 mm was not significantly associated with infection. In some cases, *Promenetus* snails outnumbered *Physa* snails by the end of the experiment. However, due to their minute size, these snails likely have a smaller per capita influence on ecosystem processes.

3.4.3 Primary production

In situ diel primary production did not vary consistently in response to parasitism (p = 0.5361). Chlorophyll α also showed no significant relationship to parasitism (Figure 3.4b). However, the percent of periphyton ash-free dry mass increased significantly in relation to infection prevalence (Fig. 3.4a). Additionally, periphyton dry mass increased significantly with *Physa* infection intensity (Fig. 3.4c).

3.4.4 Nutrient concentration and stoichiometry

Dissolved organic carbon (DOC) and total dissolved phosphorus (TDP) concentrations in the water column were not influenced by parasitism. However, DOC and TDP were significantly related to surface vegetation biomass (Figure 3.5). Unfortunately, variable starting conditions for total dissolved nitrogen (TDN) obscure any influence of parasitism (Figure A.2). Absolute values of DOC and TDN match measurements and ranges from other systems (Hessen 1992, Johnson et al.

2006, Mischler et al. 2014). However, TDP in our experiment was low compared to others (Mischler et al. 2014).

Stoichiometry was highly variable within mesocosm water samples. Over time C:N, C:P, and N:P ratios all increased, but none of these changes were significantly related to parasitism (Figures A.3 – A.5). Molar DOC:TDN began near 15 and ended near 30, DOC:TDP ratios began near 400 and ended near 800, and TDN:TDP ratios remained near 30 throughout the experiments duration.

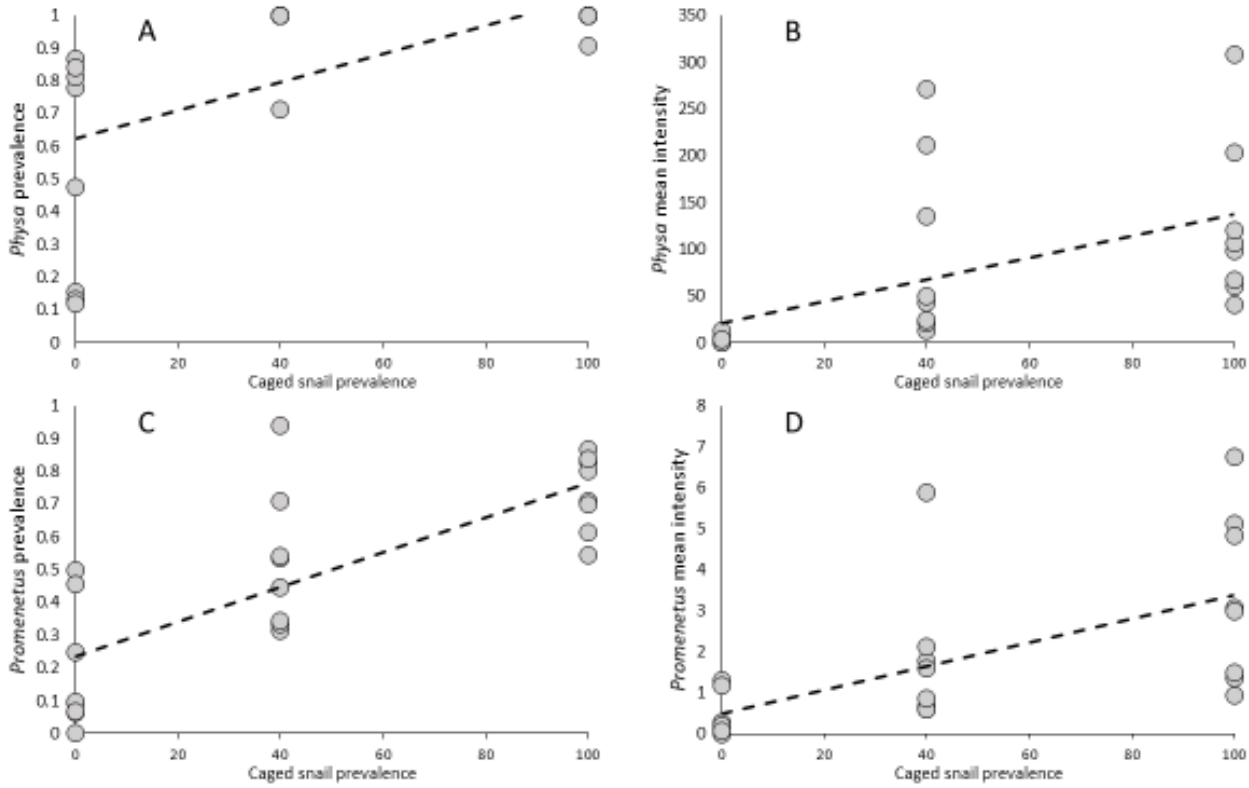


Figure 3.2. Caged snail treatments were a significant linear predictor of both A) *Physa* (Median-based linear model $p < 0.0001$) and C) *Promenetus* infection prevalence (Adjusted $r^2 = 0.5901$, $F_{1, 22} = 34.11$, $p < 0.0001$) and B) *Physa* (Adjusted $r^2 = 0.5461$, $F_{1, 22} = 28.68$, $p < 0.0001$; data shown untransformed) and D) *Promenetus* infection intensity (Adjusted $r^2 = 0.4777$, $F_{1, 22} = 22.03$, $p = 0.0001$; data shown untransformed).

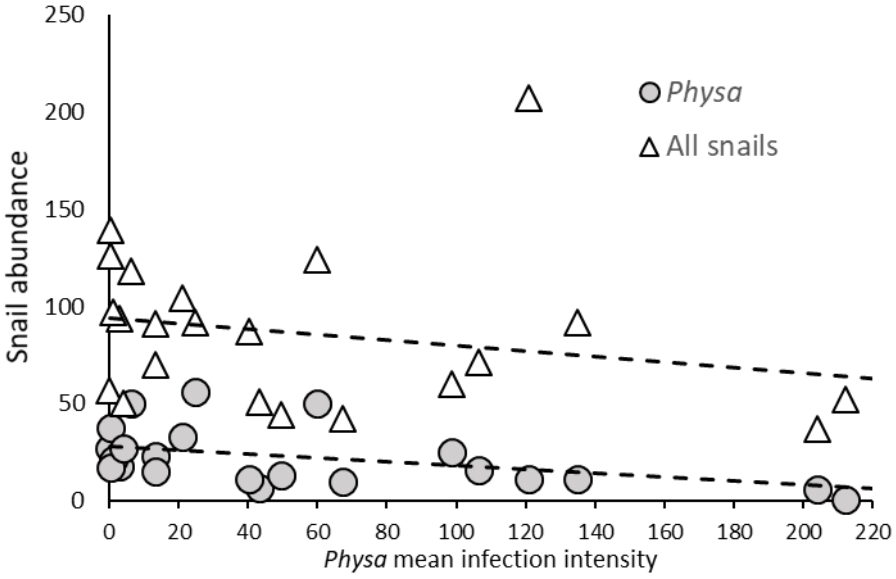
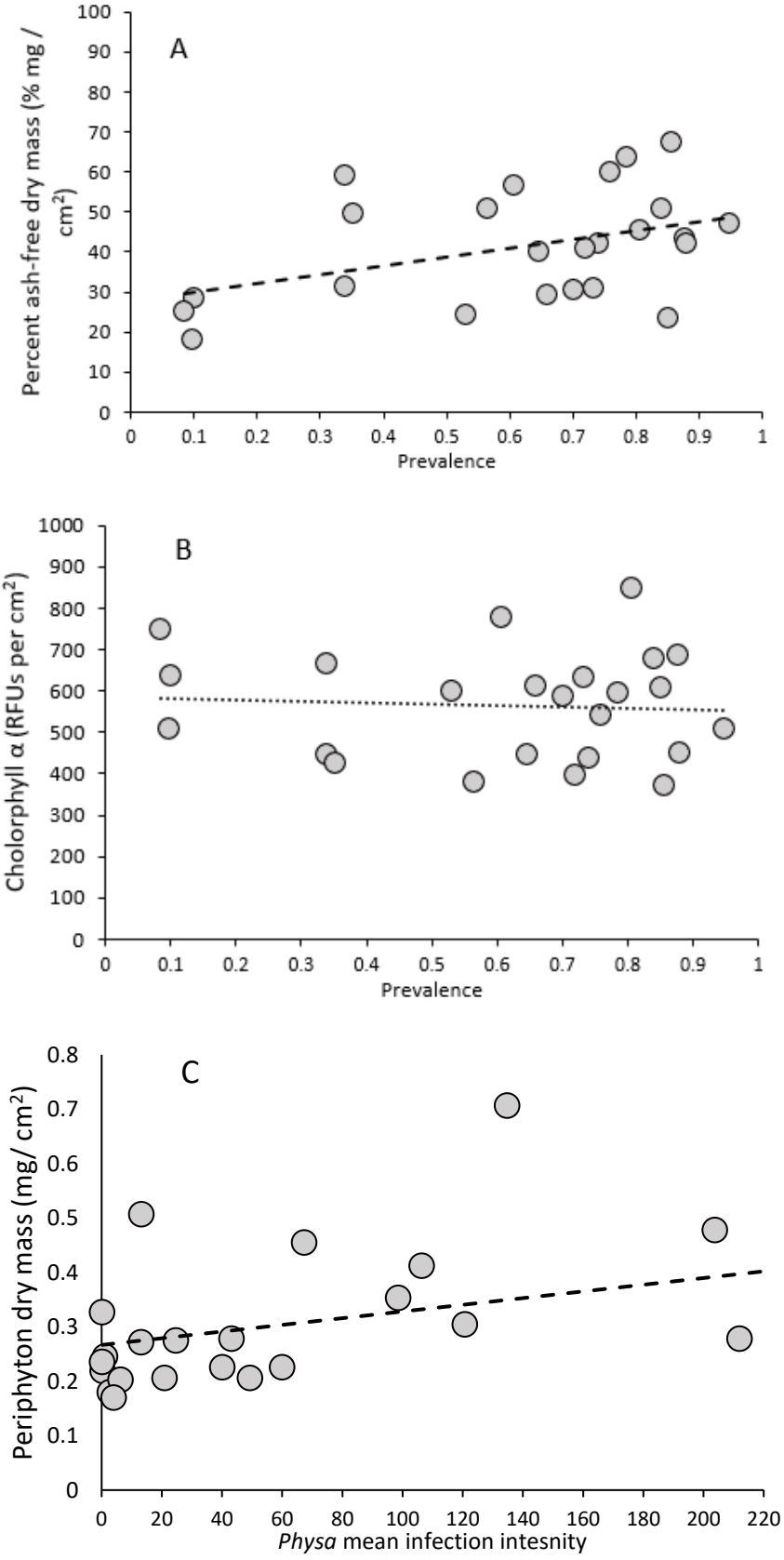


Figure 3.3. *Physa* and total snail abundances within mesocosms. Abundance was negatively correlated with *Physa* mean infection intensity, but coefficients are small (*Physa*: Estimate = -0.008096 ± 0.0014, $F_{1, 22} = 31.61$, $p < 0.0001$; All snails: Estimate = -0.0025 ± 0.0011, $F_{1, 22} = 0.0299$).

Figure 3.4. Influence of parasitism on A) the percent of periphyton ash-free dry mass, B) Chlorphyll α RFUs, and C) Periphyton dry mass (mg/cm^2). Ash-free dry mass has a linear relationship with prevalence (Adjusted $r^2 = 0.1377$, $F_{1, 22} = 4.673$, $p = 0.042$), chlorophyll α had no relationship with prevalence (Adjusted $r^2 = -0.040$, $F_{1, 22} = 0.113$, $p = 0.740$), and periphyton dry mass was positively correlated with infection intensity (Adjusted $r^2 = 0.1972$, $F_{1, 22} = 6.651$, $p = 0.0171$).


A
Percent ash-free dry mass (% mg / cm²)
Prevalence
B
Cholorphyll α (RFUs per cm²)
Prevalence
C
Periphyton dry mass (mg/ cm²)
Physa mean infection intesnity

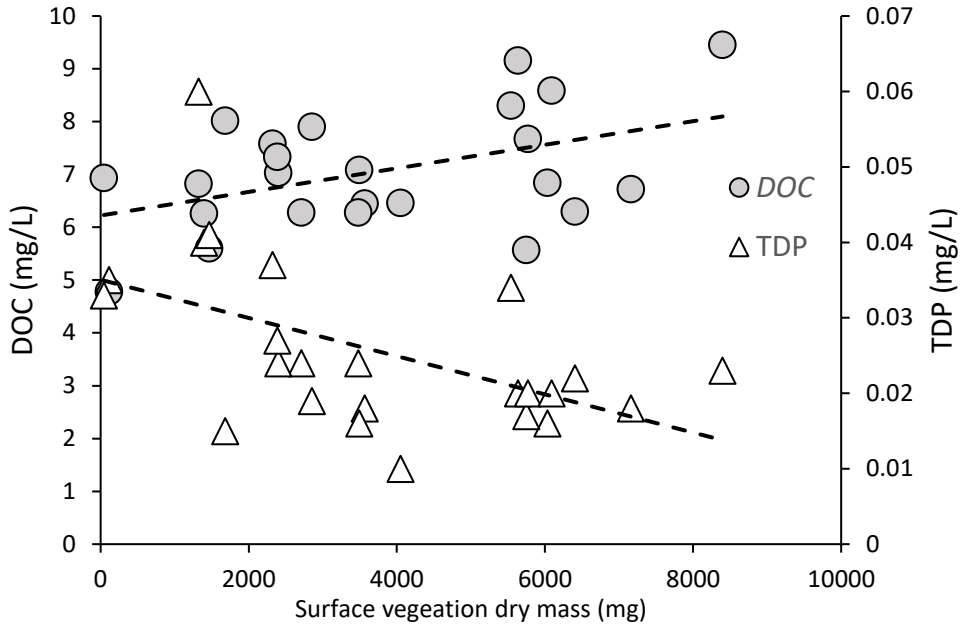


Figure 3.5. Influence of surface vegetation on water column A) carbon and B) phosphorus in the final week of the experiment (Carbon: Adjusted $r^2 = 0.1706$, $F_{1,22} = 5.731$, $p = 0.0256$; Phosphorus: Adjusted $r^2 = 0.2385$, $F_{1, 22} = 8.203$, $p = 0.0090$).

3.5 Discussion

In order to test whether parasitism was correlated with an impact on an ecosystem, we selected a system where parasitism would have a limited impact on host population dynamics. That is, the effect of metacercarial parasites on host abundance is small. Thus, if changes were detected in ecosystem dynamics when parasitism was present, they were likely the result of parasite biomass or the indirect result of parasite-induced changes in host behavior and physiology (trait-mediated effects). Unfortunately, snail abundance was significantly correlated with infection intensity. This limits our ability to distinguish between the role of density and trait-mediated impacts of parasitism. Despite this parasitism was associated with a significant impact on the dry mass of periphyton, while surface vegetation was significantly associated with water column carbon and phosphorus concentrations.

Intensity and prevalence data show that our experiment successfully created a gradient in parasitism, but the specific mechanisms by which parasite biomass influences nutrient concentrations and producer biomass remains unclear. Four non-mutually exclusive mechanisms

could explain the results: 1) the decrease in host density had a cascading influence on producers (Holdo et al., 2009), 2) cercariae which are unsuccessful at finding a snail host may contribute energy and nutrients to these systems by their death (Kuris et al., 2008; Lambden and Johnson, 2013; Preston et al., 2013), 3) infection with trematode metacercariae and/or rediae (in caged snails) may influence the C, N, and P excretion rate in hosts (Bernot, 2013; Mischler et al., 2016), and/or 4) metacercarial infection may alter snail foraging patterns and movement (Keeler and Huffman, 2009; Mouritsen and Poulin, 2005; Webber et al., 1987). Although these variables were not directly measured here, we explore how these factors may have influenced our results below.

Disease is known to have various top-down effects on producers (Buck and Ripple, 2017; Holdo et al., 2009). The significant association between host abundance and infection intensity, although small, could influence producer biomass. As total periphyton dry biomass increased with infection intensity and not prevalence, it is most likely that reductions in host density or a reduction in foraging (a host trait) due to high infection intensities drive this association. However, the correlation between infection intensity and host abundance make further conclusions tenuous. Additionally, trematode cercariae can produce a substantial amount of biomass within certain systems (Kuris et al., 2008; Preston et al., 2013). It remains possible that decomposing cercariae supply a fraction of periphyton nutrient requirements, but the lack of correlation between parasitism and nutrients would suggest this is not the case.

Although many species of trematode metacercariae are assumed to have little impact on their hosts (Keeler and Huffman, 2009; Kuris and Warren, 1980), a number of studies have documented that metacercariae can remain metabolically active, even after encystment (Keeler and Huffman, 2009; Lowenberger et al., 1994; Siddiqui and Nizami, 1981; Thomas and Gallicchio, 1967). Lambden and Johnson (2013) showed that in the transition from free-living cercariae to encysted metacercariae, *Echinostoma trivolvis* (the parasite used in this study) will increase its dry mass by 80%. This mass increase suggests metabolic activity as *E. trivolvis* transitions from cercariae to metacercariae, which could generate physiological impacts on its hosts. One potential physiological impact of parasitism is altered nutrient excretion. Mischler et al. (2016) showed that infection with trematode metacercariae led to an intensity dependent increase in N excretion from infected snails. A similar relationship may be present in free-roaming *Physa* and *Promenetus* or caged *Helisoma* infected with metacercariae in our experiment. Additionally, infections in caged snails could also have altered nutrient inputs into the system. Sporocysts (a parasitic life stage

comparable to rediae) are known to influence host nutrient excretion rates (Bernot, 2013). In the *Helisoma-Echinostoma* system used here, both mass-corrected foraging rate and egestion rate are significantly higher in infected than uninfected snails (Vannatta and Minchella, in prep). Although it is possible that *E. trivolvis* metacercarial or redial infection generates a similar increase in N excretion that could contribute to changes in periphyton, the chemical analyses do not support this explanation. Almost all N in our mesocosms was in the dissolved organic instead of dissolved inorganic form (see mesocosm.master.csv in the data repository) indicating that available N is not coming from excretion.

Although parasitism was not likely associated with nutrient concentrations, surface vegetation was correlated with both dissolved organic carbon and total dissolved phosphorus. The relationship between aquatic vegetation and release of DOC is well known (Wetzel, 2001). Loss of organic carbon in the form of amino acids and other compounds is common in aquatic environments. It also follows that in a phosphorus limited ecosystem (as was created here) aquatic vegetation will rapidly uptake and assimilate phosphorus leading to the negative correlation between vegetation biomass and P concentrations.

In addition, the percent of periphyton represented as ash-free dry mass (% AFDM) increased with parasite prevalence. This pattern is likely related to host movement under parasite attack. For example, Webber et al. (1987) found that metacercarial infection can alter the activity of infected individuals. In our system, *Physa* show vigorous shell shaking responses, surface from the water, and spend less time foraging while under parasite attack (Vannatta unpublished data) which may increase bioturbation and limit foraging. During the experiment, snails were mostly observed near the water surface. As periphyton near the surface is sloughed during shell shaking, this material can accumulate as particulate organic matter at the tank bottom where the periphyton tiles were located (Evans-White and Lamberti, 2005; Grimm, 1988; Halvorson et al., 2017; Halvorson and Atkinson, 2019; Morales and Ward, 2000) Particulate organic matter can trap other particles and create a substrate for bacterial growth (Mulholland et al., 1991). Thus, increased bioturbation may lead to an increasing proportion AFDM in periphyton, but not increasing photosynthetic activity. This assertion is supported as the additional AFDM was not coupled with any change in chlorophyll concentration in our system.

In summary, we show that parasitism is associated with altered producer biomass which may have cascading impacts on ecosystems. Mechanistically, parasitism may alter the

accumulation of particulate organic matter through parasite-induced changes in host foraging and bioturbation. Metacercariae, the parasitic stage used in this experiment, are commonly considered to have little ecological significance. Trematodes in general, and metacercariae in particular, are common within ecosystems, can reach high densities, are distributed widely within habitats, persist for long periods of time, and may impact resources (as suggested in this study). All of these characteristics can be important for ecosystem function. We have demonstrated that parasitism can be an important factor structuring mesocosm ecosystems. However, studies at larger (field manipulations) and smaller scales (examining specific mechanistic pathways) are needed in this snail-trematode system in order to better understand the magnitude of the impact. Additionally, alterations in the nutrient status of an ecosystem could alter the importance of parasitism. As such, examining parasitism across a gradient of nutrient inputs must also be considered. This study documents the importance of considering parasites not only at the individual, population, and community levels, but also as integral components of ecosystems.

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CHAPTER 4. ASSESSING THE RELATIVE ROLES OF DENSITY-MEDIATED, TRAIT-MEDIATED, AND DIRECT IMPACTS OF PARASITISM ON ECOSYSTEMS

4.1 Abstract

Rapid environmental change is having dramatic impacts on species interactions and ecosystem functioning. Despite the ubiquity of parasitism, little is known about the influence of parasites on ecosystems processes. Of particular interest are the density-mediate, trait-mediated, and direct effects of parasitism. The relative influence of these factors is difficult to assess in field settings. Here, a model of host-parasite-ecosystem interactions was constructed. Parameter values are varied in order to assess: 1) how and when parasitism can influence nutrient cycling, 2) the relative impact of parasite biomass, host density-mediated, and host trait-mediated effects on nutrient cycling, and 3) whether parasitic impacts on nutrient cycling feedback on host-parasite dynamics. The model results indicate that parasitism can increase or decrease nutrient recycling under certain conditions and possibly have counterbalancing influences on ecosystem function depending upon host mortality, consumption, and assimilation rates. Also, density-mediated impacts of parasitism tend to impact host biomass compartments whereas trait-mediated impacts have larger systemic impacts, and for parasites with density-independent transmission, changes in parasite virulence have the largest impact on parasite transmission by altering biomass of uninfected hosts.

4.2 Introduction

Anthropogenic climate change and habitat loss have profound impacts on both biotic and abiotic processes (Hoegh-Guldberg et al. 2008, Stein et al. 2013). Climate change creates new opportunities, but the disruption of ecosystems also presents challenges (Brooks et al. 2019). As climate changes, species must disperse to remain within their climate envelope or adapt to new environments (Pickles et al. 2013). Parasites which rely on multiple hosts face significant extinction risk in association with their hosts (Dunn et al. 2009, Pickles et al. 2013, Carlson et al. 2017). Parasitism is of particular interest, because over 40% of species are parasitic at some point in their life cycles (Dobson et al. 2008) and these parasites contribute to biomass and energy flow in many ecosystems (Kuris et al. 2008, Sato et al. 2011).The loss of parasitic species is likely to

alter species interactions and disease transmission with consequences at the ecosystem scale (Poulin 2006, Estes et al. 2011, Paull and Johnson 2011, Pickles et al. 2013, Buck and Ripple 2017, Silliman et al. 2018, Weinstein et al. 2018, Brooks et al. 2019). To date, few studies have explored the theoretical impacts of parasitism at the ecosystem scale. However, as these environments change, understanding how parasitism and other biotic interactions impact ecosystem processes will be necessary.

Parasitism has only recently been incorporated in ecosystem studies (Minchella and Scott 1991, Hudson et al. 2006, Preston et al. 2016). However, scientists are beginning to realize the importance of parasites in structuring communities and ecosystems (Lafferty et al. 2008, Kuris et al. 2008, Koprivnikar and Johnson 2016, Preston et al. 2016). Many researchers are exploring the role of parasites in energy flow through ecosystems (Lafferty et al. 2008, Kuris et al. 2008, Lettini and Sukhdeo 2010, Sato et al. 2011), but energy flow is only part of what dictates the distribution and abundance of species. Material flow (referred to here as nutrient cycling) is also inextricably linked to the structuring of communities and ecosystems (Lindeman 1942, Colinvaux 1993, Sterner and Elser 2002). Our knowledge of how parasites may impact these processes is sparse (Bernot 2013, Mischler et al. 2016, Preston et al. 2016, Vannatta and Minchella 2018, Fischhoff et al. 2020). It is possible that parasites play an unrecognized role in nutrient cycling as they release large amounts of free-living biomass (Preston et al. 2013) and also profoundly influence host behaviors and physiology (Vannatta and Minchella 2018). As such, parasitic influences on nutrient cycling are often categorized as density-mediated (controlled by host death), trait-mediated (controlled by alterations to host traits, such as foraging rate), or directly mediated (i.e. free-living parasite biomass; Preston et al. 2013, Vannatta and Minchella 2018, Fischhoff et al. 2020). While density-mediated influences of parasitism on ecosystems are known (Holdo et al. 2009) and likely have similar impacts as predation (Buck and Ripple 2017), both trait-mediated and direct effects of parasitism are less understood at the ecosystem scale. Changes in animal traits (foraging, space use, assimilation, etc.) can have cascading impacts on entire ecosystems and parasites are well documented to alter relevant host traits (Buck and Ripple 2017). Lastly, some parasites (i.e. trematodes) can represent a large amount of biomass with certain ecosystems (Kuris et al. 2008, Preston et al. 2013) and direct contributions of animal biomass is a central mechanism of consumer-driven nutrient recycling by which parasites may play an underappreciated role (Vannatta and Minchella 2018).

Here, a mathematical model is developed to explore: 1) how and when parasitism can influence nutrient cycling, 2) the relative impact of parasite biomass, host density-mediated, and host trait-mediated effects on nutrient cycling, and 3) whether parasitic impacts on nutrient cycling feedback on host-parasite dynamics. We expand on a general consumer-resource model originally developed to examine nutrient recycling and predation (Leroux and Loreau 2010) and apply this conceptualization to a trematode parasitism system. Previous ecosystem models of parasitism often include producers as a resource for a host (Smith and Grenfell 1994, Long et al. 2012, Koltz et al. *in review*) or have focused on disease within producers (Borer et al. 2020). The model presented here is, to our knowledge, the first to examine explicit nutrient and producer compartments, nutrient recycling, and parasitism, simultaneously. Our model formulation yields a more wholistic picture of the role of trematode parasitism within an ecosystem. Three key variables are manipulated (host death, consumption, and assimilation rate) in order to explore these objectives. These results will help identify specific systems in which trematode parasitism may have a significant role in ecosystem processes and help integrate trematodes into a larger ecological context using nutrients as a common currency (Jones and Lawton 1995).

4.3 Methods

A general consumer-resource model with predation, explicit nutrient compartment, and nutrient recycling was adapted and applied to a host-parasite system (Figure 4.1; Leroux and Loreau 2010). Our model conceptualizes a multi-host parasite with an intermediate host that lives and reproduces within the ecosystem of interest. A second, definitive host (not modelled explicitly) introduces parasite propagules into the ecosystem but otherwise has no influence on the system. To begin, consider a simple nutrient pool (N) governed by nutrient inputs, outputs, and uptake by a primary producer (V):

$$\frac{dN}{dt} = i - oN - \rho_V NV \qquad \text{(eq. 1a)}$$

where i represents a nutrient input rate (i.e. atmospheric deposition, allochthonous materials etc.; see Table 4.1 for variable descriptions) and o represents a concentration dependent output rate (i.e. leaching, material translocation etc.). Additionally, nutrients are removed from the nutrient pool via uptake by producers. Uptake is dependent on the uptake rate (ρ_V), nutrient concentration (N),

and producer biomass (V). Change in producer biomass is in turn dictated by the following equation:

$$\frac{dV}{dt} = \rho_V NV - (\rho_{Hu} H_u + \rho_{Hi} H_i)V - d_V V$$

(eq. 2)

where the first term shows density dependent producer growth. It is assumed here that all nutrients that are taken up by producers are subsequently assimilated. The next term states that biomass is removed from the producer compartment in a density dependent fashion by foraging uninfected (H_u) and infected hosts (H_i). The final term represents a density dependent background death rate for producers, d_V.

The change in uninfected host biomass is represented by the equation:

$$\frac{dH_u}{dt} = \chi_{Hu} \rho_{Hu} VH_u - d_{Hu} H_u - \beta H_u$$

(eq. 3)

where biomass is added to uninfected hosts based on a consumption rate (ρ_{Hu}) and assimilation efficiency (χ_{Hu}). Additionally, uninfected hosts have a background death rate, d_{Hu}. The final term, βH_u, represents the movement of uninfected host biomass into the infected host compartment at a transmission rate, β. Our model assumes that transmission is not influenced by the density of infected hosts. This assumption would apply in cases where parasites with complex life cycles are transmitted to long-lived definitive hosts which have a limited influence on the intermediate host's ecosystem (i.e., human hosts in schistosomiasis). Infected host biomass changes according to the expression:

$$\frac{dH_i}{dt} = \beta H_u - (d_{Hu} + v)H_i$$

(eq. 4)

where infected hosts have an enhanced death rate from virulence, v (Civitello et al., 2018). Parasite biomass is dictated by a similar expression:

$$\frac{dP}{dt} = \chi_{Hi} \rho_{Hi} VH_i - d_p P$$

(eq. 5)

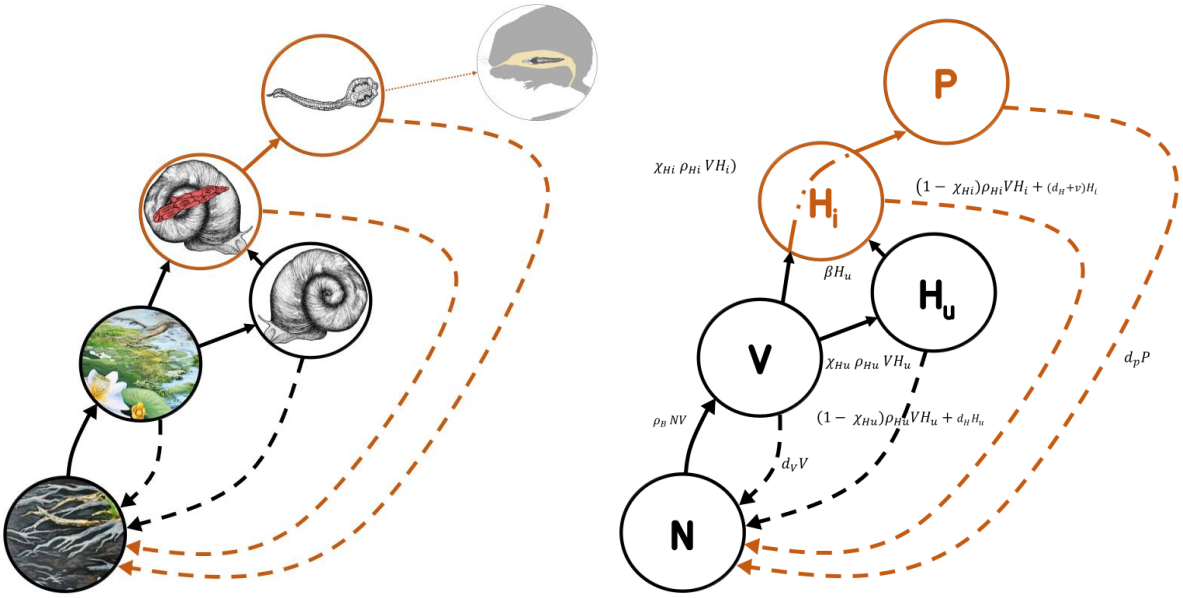

Figure 4.1. A hypothetical ecosystem with host (H), parasite (P), producer (V), and nutrient resource (N). Transmission occurs via a host outside the ecosystem with constant population size (top center of figure). Equations governing nutrient assimilation, infection dynamics, and nutrient recycling are displayed at right (illustrations by Gaby Sincich).

This set of equations assumes that infected hosts are unable to reproduce once they become infected. Additionally, parasite biomass in the model ecosystem is represented in the form of parasite propagules released from infected hosts.

In order to represent nutrient recycling, we return to eq. 1 and add recycling of waste and dead organisms giving this compartment the final form:

$$\frac{dN}{dt} = i - oN - \rho_V NV + d_V V + (1-\chi_{Hu})\,\rho_{Hu}\,VH_u + (1-\chi_{Hi})\,\rho_{Hi}\,VH_i + d_{Hu}H_u + (d_{Hu}+v)H_i + d_pP$$

(eq. 1b)

In this equation, materials that are consumed but not assimilated (e.g. $(1-\chi_{Hu})\,\rho_{Hu}\,VH_u$) are recycled back to the nutrient pool as well as the biomass lost via death of organisms (Leroux and Loreau, 2010). These additions generate a final series of differential equations:

Table 4.1. Variables, descriptions, and initial conditions for simulations.

Variable	Description	Initial conditions
State variables		
N	Nutrients	200
V	Producers	50
H_u	Uninfected hosts	50
H_i	Infected hosts	100
P	Parasites	20
Rates		
i	Nutrient input	41
o	Nutrient loss	0.1
$\boldsymbol{\rho}$	Consumption/uptake rate of producers ($_V$), uninfected ($_{Hu}$), and infected hosts ($_{Hi}$)	V = 0.1, Hu = 0.1, Hi = *varied*
$\boldsymbol{\chi}$	Assimilation efficiency of uninfected ($_{Hu}$) and infected hosts ($_{Hi}$)	Hu = 0.2, Hi = *varied*
$\boldsymbol{d}$	Death rate of producers ($_V$), uninfected hosts ($_{Hu}$), and parasites ($_P$)	V = 0.05, Hu = 0.1, P = 0.95
v	Virulence factor	*Varied*
$\boldsymbol{\beta}$	Transmission rate	0.3

$$\frac{dN}{dt} = i - oN - \rho_V NV + d_V V + (1 - \chi_{Hu})\,\rho_{Hu}\,VH_u + (1 - \chi_{Hi})\,\rho_{Hi}\,VH_i + d_{Hu}H_u + (d_{Hu} + v)H_i + d_p P$$

(eq. 1b)

$$\frac{dV}{dt} = \rho_V\,NV - (\rho_{Hu}H_u + \rho_{Hi}\,H_i)V - d_V V$$

(eq. 2)

$$\frac{dH_u}{dt} = \chi_{Hu}\,\rho_{Hu}\,VH_u - d_{Hu}H_u - \beta H_u$$

(eq. 3)

$$\frac{dH_i}{dt} = \beta H_u - (d_{Hu} + v)H_i$$

(eq. 4)

$$\frac{dP}{dt} = \chi_{Hi}\,\rho_{Hi}\,VH_i - d_p P$$

(eq. 5)

To address the specific objectives, the total contributions of parasites to nutrient cycling were quantified as defined by Vannatta and Minchella (2018). These authors suggested that all nutrients recycled by an infected host cannot be defined as parasite derived nutrients as these hosts would still contribute to nutrient recycling in the absence of parasites. Instead, parasitic contributions must be defined as the change in nutrient recycling between infected hosts and uninfected hosts. As such, we define parasite derived nutrient recycling (PNR) as:

$$PNR = \{(1 - \chi_{Hi})\, \rho_{Hi}\, VH_i - \ (1 - \chi_{Hu})\, \rho_{Hu}\, VH_i\} + \{(d_{Hu} + \ v)\, H_i - \ d_{Hu} H_i\} + \ \ d_p P \quad \text{(eq. 6)}$$

This expression is created by calculating how much nutrient would be recycled via parasitic processes and subtracts the amount that would be recycled if infected host biomass had the parameter values of uninfected hosts. Additionally, the model was run with parasite density-mediated, trait-mediated, and direct impacts quantified or omitted. In order to determine parasite density-mediated impacts, simulations were conducted at different levels of virulence, v, while holding host traits constant (such that $\chi_{Hu}\rho_{Hu}$ and $\chi_{Hi}\rho_{Hi}$ are equivalent). In a similar fashion, there were simulations with virulence set to 0 (equivalent death rate for infected and uninfected hosts). Simulations were also compared with and without direct parasite contributions, $d_p P$. Lastly, we examined interactive effects by simultaneously changing two parameters of interest and calculating parasite-derived nutrient recycling within this parameter space. The parameterizations of others were followed in that the analyses were constrained to parameter values that yielded numerically tractable solutions (Leroux and Loreau, 2010; Long et al., 2012). As such, parameter values are relative and do not fully replicate a specific host-parasite system. However, many parameter values are within the bounds of documented host-parasite systems. All biomass and other estimates were extracted from model simulations after biomass values had stabilized (100-time steps). All analyses were conducted in R (R Core Team, 2019) using the deSolve package (Soetaert et al., 2010).

4.4 Results

4.4.1 Parasitism and nutrient recycling's influence on biomass

Parasitism and nutrient recycling have interactive and marked impacts on the amount of biomass within the model ecosystem. In the absence of system-wide nutrient recycling, (referred to as

nutrient cycling below) parasites decrease the amount of biomass within the ecosystem (figure 4.2). However, when nutrient recycling is included, parasites contribute to a substantial increase in biomass. Nutrient recycling consistently leads to an increase in biomass.

4.4.2 Density-mediated impacts

Density-mediated impacts of parasitism (alterations to the death rate/virulence of infected hosts, $d_{Hu} + v$, while removing trait mediated impacts of parasitism) had the largest impact on uninfected host biomass (figure 4.3B). Increases in density-mediated impacts additionally led to decreases in infected host biomass but had little influence on other compartments (figure 4.3). Lastly, alterations to density-mediated parasite impacts had minimal influence on parasite derived nutrient recycling (PNR; figure 4.4).

4.4.3 Trait-mediated impacts

Trait-mediated impacts of parasitism were shown by altering consumption rate (ρ_{Hi}) and assimilation rate (χ_{Hi}) of infected hosts while holding density-mediated impacts constant. Increasing infected host consumption rates led to a decrease in biomass within the entire ecosystem (figure 4.3E). Parasite and nutrient mass increased with increasing host consumption rates (figure 4.3 A, D) but changes in the nutrient compartment were minimal. Consumption rate had a marked impact on uninfected and infected host biomass (figure 4.3 B, C) with increasing consumption generating large decreases in these compartments. Consumption rate had the largest influence on parasite derived nutrient recycling compared to the other parameters of interest (figure 4.4).

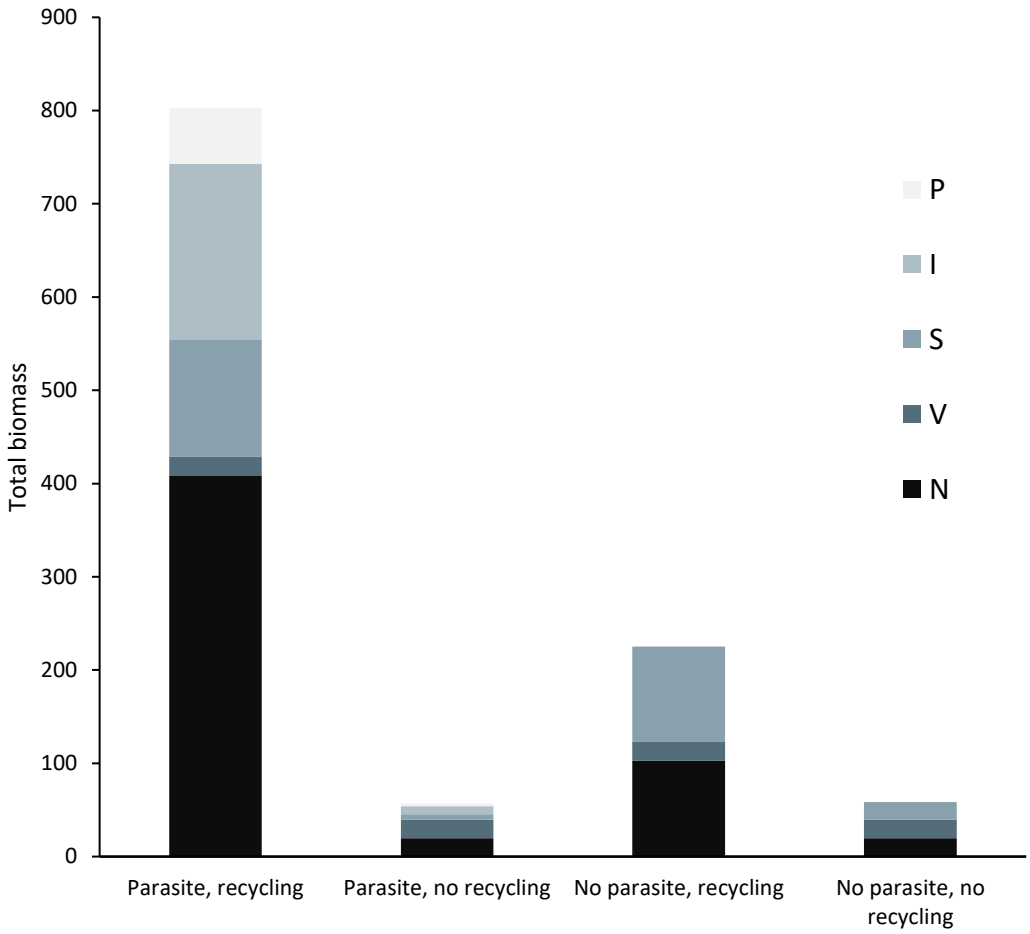

Figure 4.2. Biomass distribution in model ecosystems with and without parasitism and with and without nutrient recycling. State variables are as defined in Table 4.1: N = nutrient, V = producers, S = susceptible, uninfected hosts, I = infected hosts, and P = parasites. The influence of parasitism and nutrient recycling are synergistic generating an increase in biomass greater than the either individually. In these simulations χ_{Hi}= 0.1, ρ_{Hi}= 0.15, and ν = 0.1. All other parameters were set as shown in Table 4.1.

Whereas consumption rate changes had a large impact on uninfected host, infected host, and total biomass, assimilation rate changes had a large impact on parasite and total biomass. For total biomass, consumption rate and assimilation rate had opposite impacts with increases in assimilation rate leading to increases in total biomass (4.3E). Increasing assimilation efficiency in infected hosts had little impact on parasite derived nutrient recycling (figure 4.4).

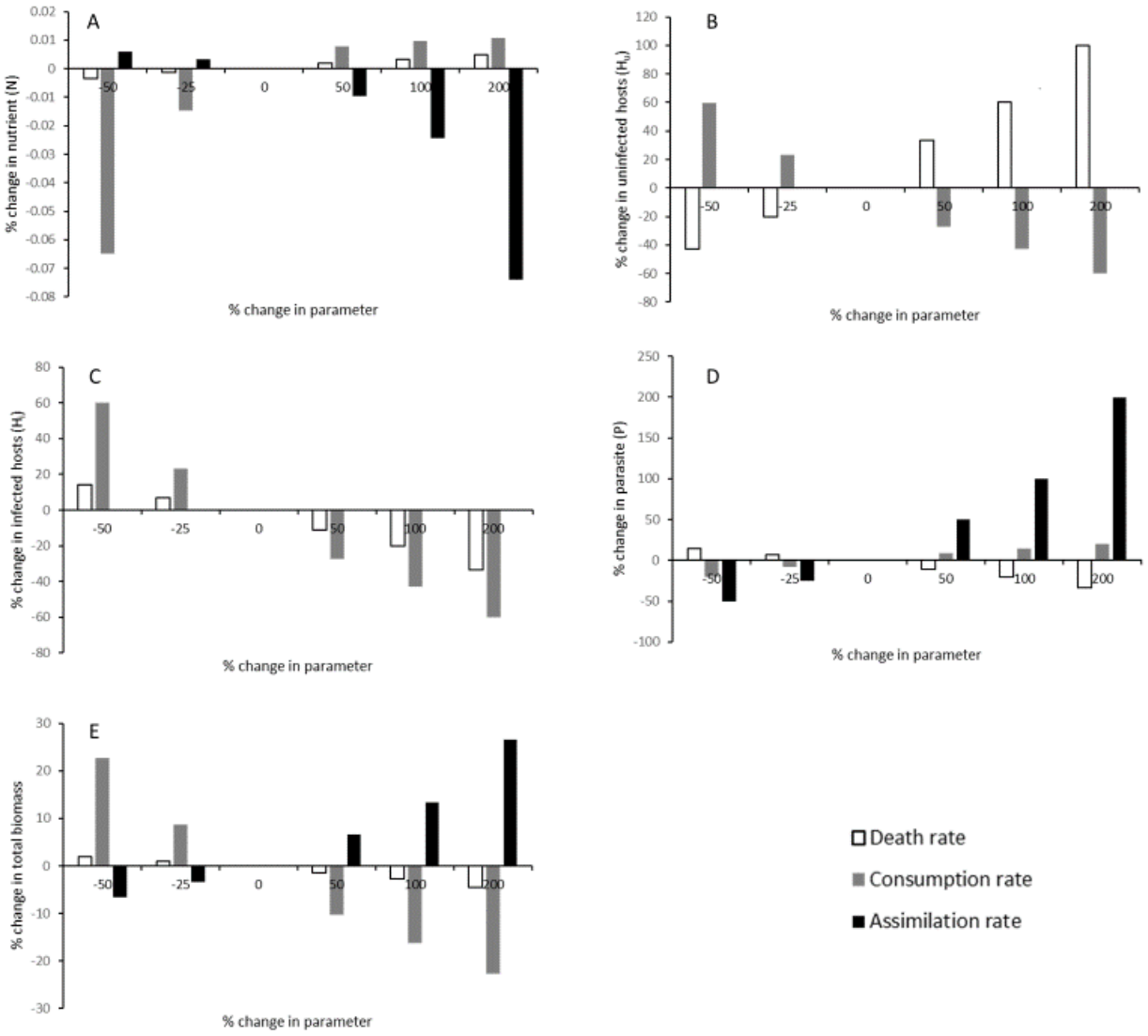

Figure 4.3. Percent change in infected host parameters compared to uninfected host parameters and corresponding change in model compartments. Results show changes as rates were varied independently. Parameters: death rate ($d_{Hu} + v$), consumption rate (ρ_{Hi}), and assimilation rate (χ_{Hi}).

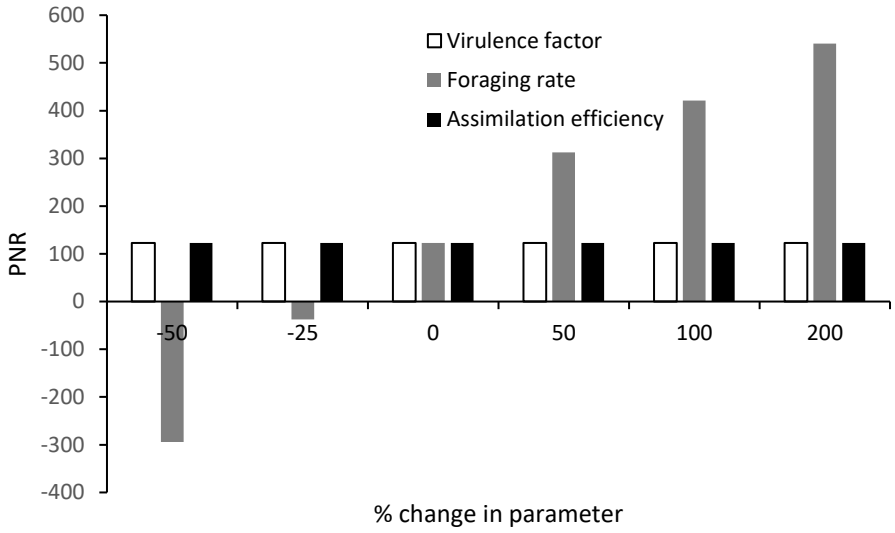

Figure 4.4. Influence of parameter changes on parasite derived nutrient recycling (PNR). In simulations only parameters of interest were varied.

4.4.4 Interactive effects

Examining simultaneous parameter changes suggests that consumption rate is the largest driving factor of parasite-derived nutrient recycling (PNR; Figure 4.5). While changes in assimilation and death rate lead to minimal changes in PNR (figure 4.5 B, C), alterations to consumption rate have a clear influence on the amount of nutrient derived from parasitism. Consumption rate does interact with death rate such that reductions in infected host foraging and death reduce parasite-derived nutrient recycling, whereas increasing consumption rate and reducing death rate maximize parasite-derived nutrient recycling.

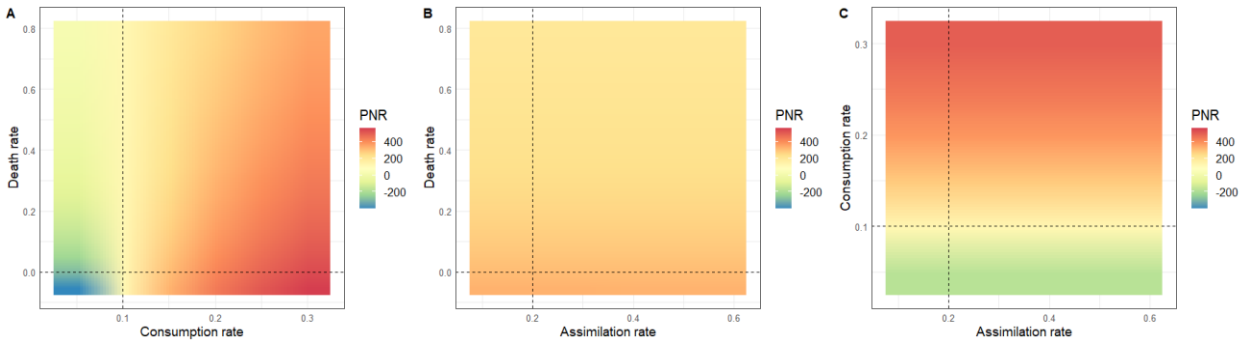

Figure 4.5. Influence of simultaneous, infected host parameter changes on parasite-derived nutrient recycling (PNR). Dashed lines show uninfected host rates.

4.5 Discussion

A mathematical model of host-parasite-ecosystem interactions was used to explore the role that parasitism might play in ecosystem processes and address three objectives: 1) how and when parasitism can influence nutrient cycling, 2) the relative impact of parasite biomass, host density-mediated, and host trait-mediated effects on nutrient cycling, and 3) whether parasitic impacts on nutrient cycling feedback on host-parasite dynamics. By varying the presence of parasites and nutrient recycling, as well as density-mediated (death rate) and trait-mediated (host consumption and assimilation rates) impacts of parasitism independently, the results suggest that parasitism will 1) have variable and possibly counterbalancing influences on ecosystem function depending upon the interaction between host death and foraging, 2) density-mediated impacts of parasitism tend to impact host biomass compartments, whereas trait-mediated impacts have more systemic impacts, and 3) for parasites with density-independent transmission, changes in host death rate have the largest impact on parasite transmission by altering biomass of uninfected hosts. Interestingly, no parameter changes had a substantial impact on producer biomass.

4.5.1 Parasitism and nutrient recycling's influence on biomass

Nutrient recycling uniformly led to an increase in biomass within the model ecosystem. This is not surprising as the addition of nutrient inputs into a system will allow for more nutrient assimilation. Parasitism, however, had a variable impact on total biomass. When recycling was included, parasitism generated a large increase in biomass. When recycling was not included, parasitism led to a small decrease in biomass. In this way parasitism behaves much like predation (Leroux and Loreau, 2010). It is known that parasites account for a large amount of biomass within some ecosystems such as estuaries and small ponds (Kuris et al., 2008; Preston et al., 2013). These systems tend to have high overall productivity which is thought to support parasite biomass. Our results suggest that parasite biomass and nutrient recycling could additionally support productivity in these systems.

Oligotrophic stream systems have low parasite biomass (Paseka, 2017). Due to the directional flow of nutrients in lotic systems, streams will also have low levels of localized nutrient recycling. In concert, our model suggests the presence of parasitism and minimal nutrient recycling may reinforce oligotrophy (and thus low biomass) in these systems. The comparison here between

fairly eutrophic and oligotrophic systems highlights the context dependency of parasitism: parasitism in eutrophic and oligotrophic systems may reinforce these trophic states. As such, we expect parasitism to have a stronger influence on a system when nutrient recycling is promoted as this allows the density and trait-mediated impacts of parasitism to fully feedback on the local system.

4.5.2 Density-mediated and trait-mediated impacts on biomass

Density-mediated impacts of parasites had the largest influence on uninfected and infected host biomass. This is a consequence of host density-independent disease transmission. By driving down the infected host population and thus the amount of parasite biomass, density-mediated parasite impacts allowed uninfected hosts to assimilate and store biomass. This result contrasts with the classic example of rinderpest virus in Africa. The mass die-off of livestock (a density-mediated impact) systemically altered ecosystem function and carbon storage (Holdo et al., 2009). However, rinderpest is a directly transmitted pathogen which stores little biomass in pathogen tissue. With direct transmission, uninfected hosts are unlikely to consistently replace infected hosts as this would generate disease outbreaks driving the total population back down unlike the dynamics of the current model. As such, it is important to consider the ecology of the pathogen in question when considering how density and trait-mediated impacts of parasitism effect ecosystems. Density-mediated impacts are likely minimized when parasites show density-independent transmission (i.e. schistosomiasis infection in aquatic snails).

Alterations in infected host consumption and assimilation rates (hosts traits) generated contrasting directional effects on total biomass, however, consumption rate had larger systemic impacts. Increases in consumption rate led to decreases in uninfected host biomass, which subsequently decreased infected host biomass due to density-independent transmission and competition for forage. While decreasing biomass in these compartments, alterations in consumption rate interestingly led to an increase in parasite derived nutrient recycling (figure 4.4). This occurs partly because increases in infected host foraging moves more biomass into the short-lived parasite propagules, but more so because infected host biomass is having a larger mass specific impact on vegetation. Alterations in host foraging in response to parasites is very common (de Roode et al., 2013; Ezenwa et al., 2020; Hart, 1990; Hite et al., 2020; Hutchings et al., 2006; Vannatta and Minchella, 2018) suggesting that trait-mediated impacts of parasites via consumption

may be pervasive. The coupling of altered consumption rates without a corresponding change in assimilation efficiency by necessity alters the amount of waste produced by infected hosts. Few studies have examined how parasites simultaneously alter these parameters in natural settings (but see Lettini and Sukhdeo 2010), but the interaction between assimilation rate and consumption rate likely has an influence on how parasites drive nutrient recycling in ecosystems.

In contrast to consumption rate, assimilation efficiency alters nutrient recycling by altering biomass within the parasite compartment. In our model, all infected host assimilation is co-opted by the parasite for its own reproduction, an impact seen in many snail-trematode systems (Civitello et al., 2018; Sorensen and Minchella, 2001). This characteristic drives up the amount of parasite biomass. When nutrient recycling via parasite death is removed from simulations, these nutrients leave the system via parasite death, lowering total biomass within the system and acting as an additional nutrient output term. In some systems, such as oligotrophic streams mentioned above (Paseka, 2017), the removal of parasite biomass upon parasite death is a reasonable assumption. However, this model structure underscores the idea that density, trait, and directly-mediated impacts of parasites rarely ever occur in isolation.

4.5.3 Interactive effects

When considered simultaneously, it is clear that consumption rate of infected hosts is a strong determinant of parasite-derived nutrient recycling. While consumption rate does interact with death rate, death rate had little influence in combination with assimilation or independently. As mentioned above, alterations in host consumption are very common with parasitic infections. Given the stark influence of consumption rate on parasite-derived nutrient dynamics, we predict that systems with low parasite virulence and large alterations in host consumption should be most likely to generate substantial parasitic influences on nutrient recycling.

4.5.4 Influence of density and trait-mediated impacts on disease dynamics

In our model, density-mediated effects (infected host death rates) were the only parameter to influence disease dynamics. Increased death of infected hosts shifted biomass into the uninfected host compartment leading to decreased prevalence (figure 4.5). This same interaction minimizes

parasite derived nutrient recycling (PNR) as reduced infected host and parasite biomass by definition reduces this quantity (figure 4.4).

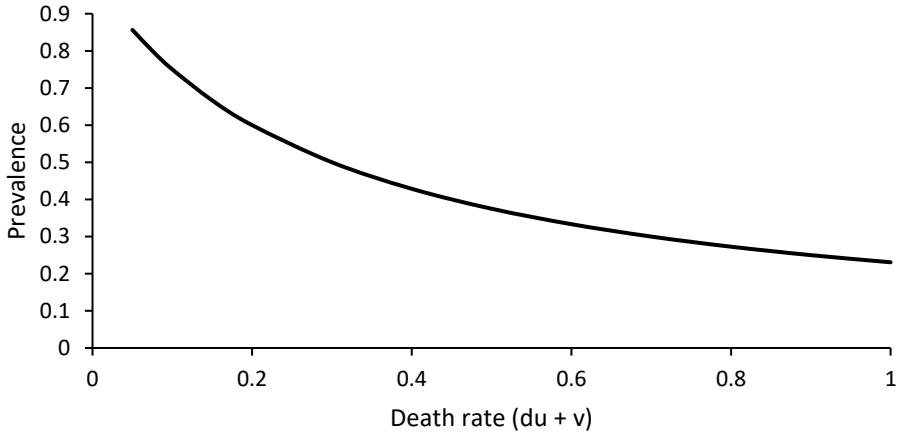

Figure 4.6. Prevalence decreases with increasing infected host death rate.

4.5.5 Conclusion

Explicit consideration of density, trait, and directly-mediated influences of parasitism can be useful for characterizing the behavior of model ecosystems. Unfortunately, empirical evidence to support our theoretical results is limited (Fischhoff et al., 2020; Paseka et al., 2020; Preston et al., 2016; Vannatta and Minchella, 2018). As such, a number of studies are needed both to parameterize the model and empirically test how ecosystems vary under differing parasite regimes. For future studies, it is important to consider that density, trait, and directly-mediated parasitic impacts often occur simultaneously. Additionally, all forms of nutrient recycling are not equal. Creating separate nutrient compartments for dead organisms, sloppy feeding, feces/egestion, and host urine/excretion with different rates of nutrient transfer to the bioavailable nutrient compartments could generate a more accurate representation of nutrient dynamics. If certain nutrient types generate fast and slow nutrient releases, the duration of these feedbacks is likely to alter host-parasite-ecosystem dynamics. Combining theoretical, empirical, laboratory, and field methods in order to explore these relationships will aid in parameterizing biologically relevant models and in predicting how ecosystems will respond to changes in parasite abundance. In this time of unprecedented environmental change and species extinction, gathering an inclusive picture of ecosystem function is pivotal. A robust understanding of relevant functional groups, such as parasites, will be integral in maintaining healthy ecosystems.

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APPENDIX A. CHAPTER 3 SUPPLEMENTAL MATERIALS

Table A.1. Summary of measurements taken from each mesocosm, the method used, and the process measured.

Measurement	Method
Dissolved Organic Carbon-C (DOC)	Shimadzu TC via NPOC
Total Dissolved Nitrogen-N (TDN)	Shimadzu TN via ASTM D8083
Total Dissolved Phosphorus-P (TDP	SEAL AQ2 analyzer via APHA method 4500 P
pH	Oakton PCTS Testr 50
Conductivity	Oakton PCTS Testr 50
***In situ diel* Primary Production**	Measurements at DO maximum and minimum; YSI probe
Periphyton Ash-Free Dry Mass (AFDM)	Combustion oven
Periphyton Chl α	Turner Designs Chl a-NA module; in RFU
Surface Vegetation Biomass	Dry mass
Snail abundance	Counting
Infection Prevalence/ Intensity	Snail crushing
Invertebrate Community Composition	

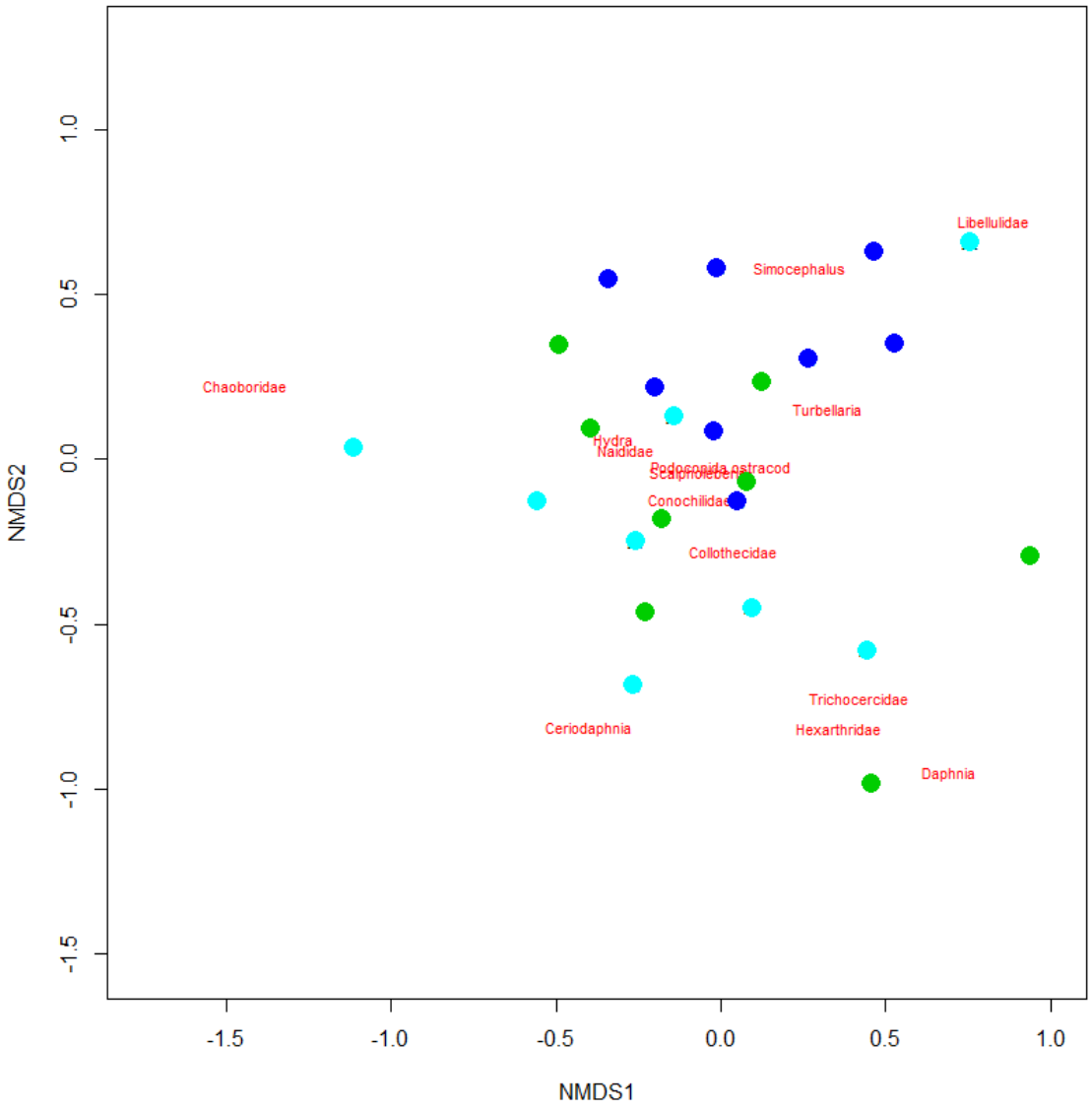

Figure A.1. Non-metric multidimensional scaling biplot of non-gastropod community members using presence/absence data. Species occurring in only one mesocosm were removed to prevent bias. PERMANOVA analysis showed no significant differences based on treatment group (green = low, dark blue = moderate, and light blue = high parasitism; Adjusted r2 = 0.096, F2, 21 = 1.118, p = 0.346). See data file for raw data: community.csv

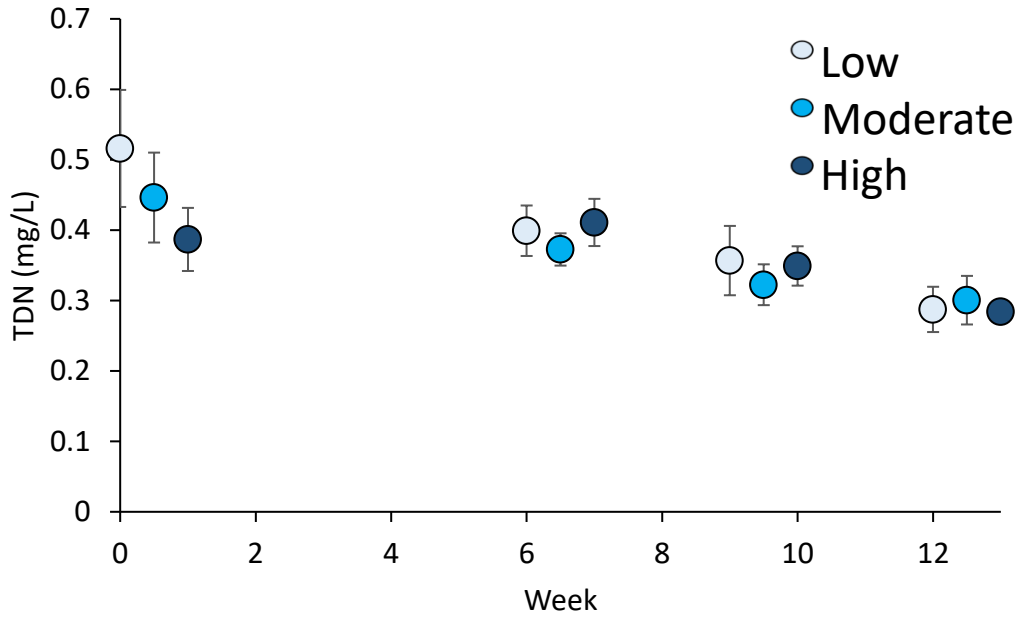

Figure A.2. Change in total dissolved N shows a shallower slope with increasing parasitism. For TDN, untransformed data is shown. Data is shown by treatment group for ease of visualization. Error bars are 95% confidence intervals.

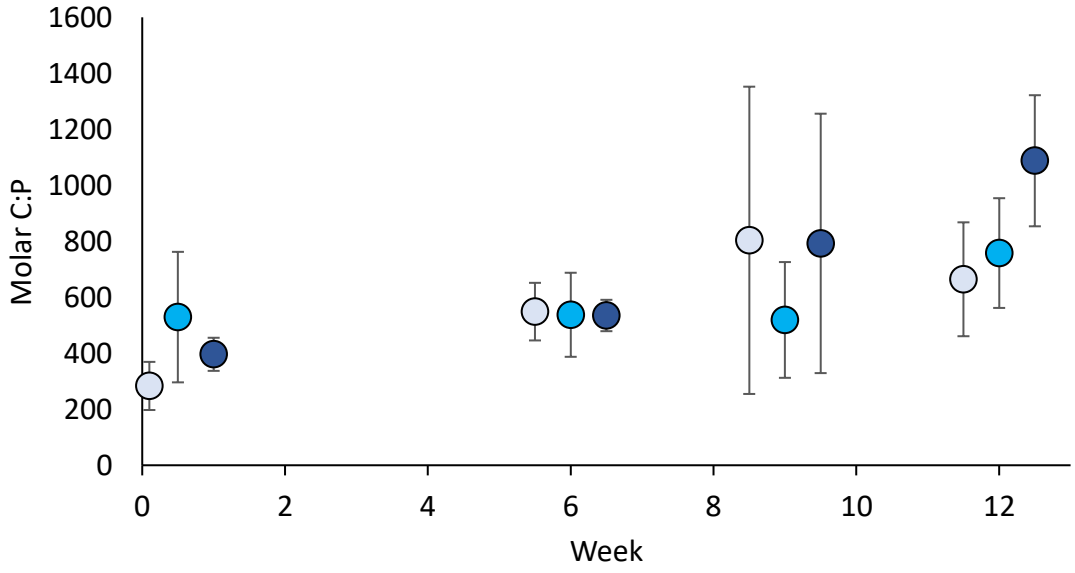

Figure A.3. Molar C:P increased over time, but the change was not significantly different between treatments. Error bars show 95% confidence intervals.

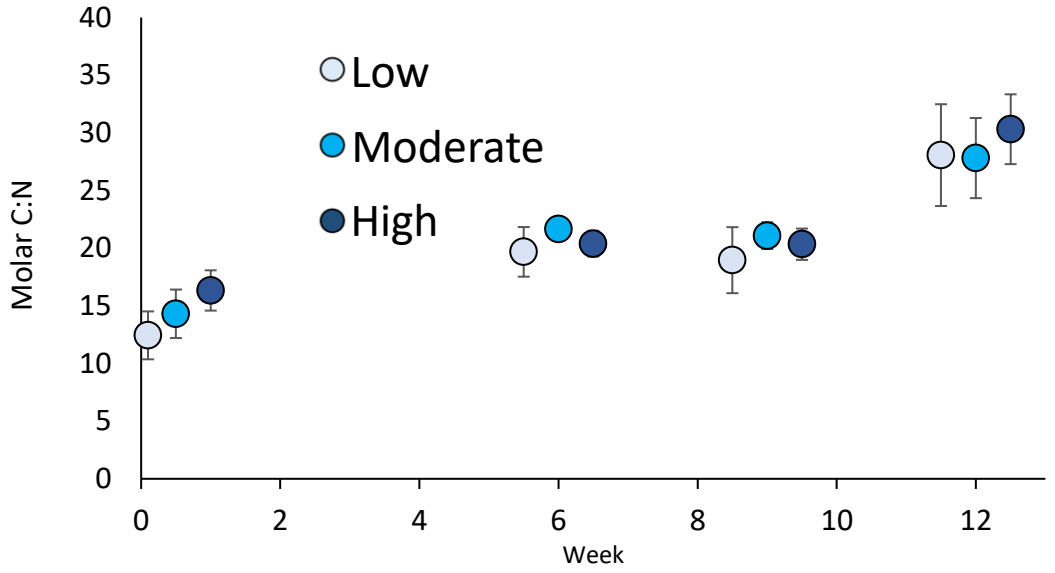

Figure A.4. Molar C:N increased over time, but the change was not significantly different between treatments. Error bars show 95% confidence intervals.

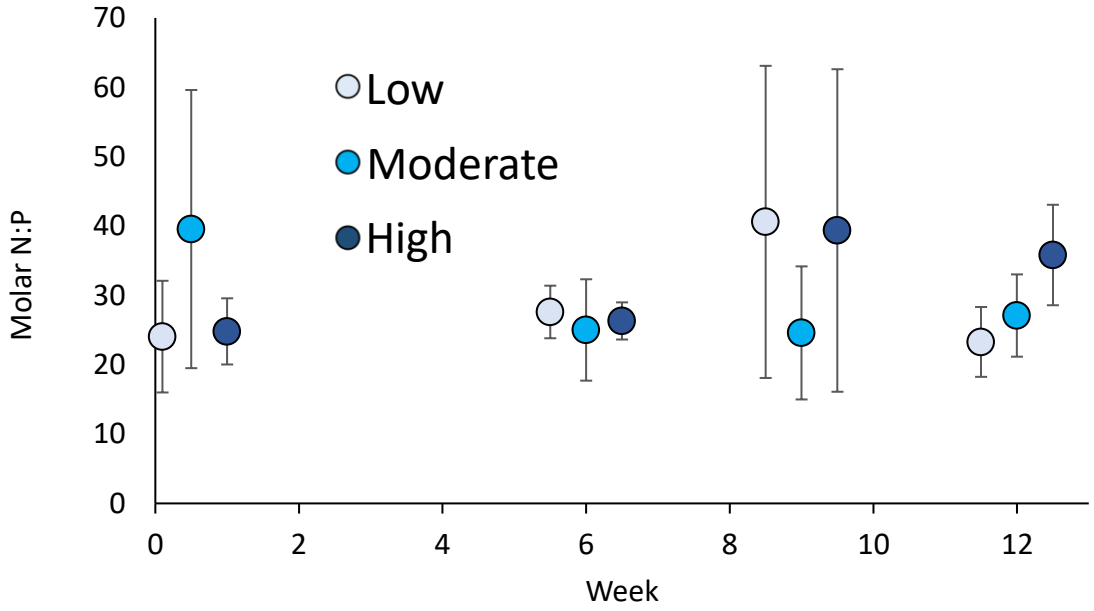

Figure A.5. Molar N:P remained mostly constant and the change was not significantly different between treatments. Error bars show 95% confidence intervals.