EFFECTS OF VARYING ENVIRONMENTS ON THE ECOLOGY AND EVOLUTION OF THE NEW ZEALAND MUD

SNAIL AND ITS INTERACTORS

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of SARAH M. REDD find it satisfactory and recommend that it be accepted.

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EFFECTS OF VARYING ENVIRONMENTS ON THE ECOLOGY AND EVOLUTION OF THE NEW ZEALAND MUD

SNAIL AND ITS INTERACTORS

Abstract

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Changes in environmental variables have long been known to influence biological interactions, and neglecting to account for environmental differences can lead to disastrous environmental scenarios. Here, I present the results of two studies looking at environmental effects on interactions involving the New Zealand Mud Snail (Potamopyrgus antipodarum), a worldwide invasive species. Chapter 1 describes an infection experiment designed to test for environmental effects on the interaction between the mud snail and its trematode parasite, Microphallus sp. I used parasites from different environments (lake versus stream) and snails raised under different food regimes in a laboratory infection experiment. Both parasite origin and food level impacted snail growth, and snails raised under high food levels also exhibited higher infection rates, indicating that environment is important to the snail host. Results failed to find evidence of environmental influence on the genotypic specificity of the snail-parasite interaction, indicating an absence of geographic selection mosaics for the tested variables.

In chapter 2, I investigated environmental impacts on ecological interactions between invasive populations of the New Zealand Mud Snail, the narrowly endemic Jackson Lake Spring Snail (Pyrgulopsis robusta), and Rainbow Trout (Oncorhynchus mykiss). Presence of the mud snail negatively impacts the spring snail, currently known in only a single stream. Previous work indicates the existence of predator

avoidance behavior in the mud snail, and I was interested in the effect this might have on resource use and competition. In a laboratory experiment, I tested for indirect effects of Rainbow Trout on the consumer-resource interactions of both snails at different competitive densities. Snails of both species were more likely to exhibit predator avoidance behaviors when competition was low, indicating that strong competition may pose a greater threat to snails than weak predation. I found strong effects of snail density on chlorophyll density, but consumer-resource interaction strengths indicated stronger per biomass interactions at low snail density. Combined results indicate the importance of quantifying environmental effects to prediction and management of the New Zealand Mud Snail.

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CHAPTER ONE

INTRODUCTION

Environmental variation has long been known to affect both the strength and outcome of

species interactions. Accounting for site-specific characteristics is necessary to ensure success in

management situations involving sensitive species (Griffith et al. 1989), biological control (Howarth

1991; Simberloff and Stiling 1996), nursery stocks (Puttonen 1989; Jobidon et al. 2003), and disease

control (Vale et al. 2008). In controlled studies, environmental differences have been the cause of

reversals in the outcome of competition (Morin 1981; Levine et al. 1998; Relyea 2000), in the spectrum

between parasitism and mutualism (Johnson et al. 1997; Jones and Smith 2004; Thompson and

Fernandez 2006), and in the prevalence of interactions itself (Benkman et al. 2001; Pauw et al. 2009).

Increasingly, environmental effects are being recognized as possible mediators of coevolutionary

interactions. By altering the genetic specificity of species interactions, environmental variation might

lead to variation in the prevalence and outcome of coevolution across species ranges (Thompson 2005).

Here, I discuss experimental findings looking at environmental effects on the New Zealand Mud Snail

(Potamopyrgus antipodarum) and its coevolved trematode parasite (Microphallus sp.). I addressed this

subject in a laboratory experiment by varying two environmental variables and measuring their effects

on host growth and on the host parasite interaction, measured as the rate of parasite infection on

specific snail host genotypes.

Environmental influences on species interactions might be particularly important in explaining

the outcome of coevolution under the Geographic Mosaic Theory of Coevolution (Thompson 2005). The

geographic mosaic theory ('GMTC') proposes that coevolution is a geographically determined process,

where the existence and characteristics of coevolutionary interactions depend on environmental

factors. The GMTC states that many interactions exist in geographic selection mosaics, where

environmental context affects interspecific genotype by genotype interactions. In other words, there are geographic differences in the fitness effects of one species on another (GxGxE effects). Theory predicts that these geographic differences should lead to broad-scale coevolutionary dynamics such as coevolutionary hot spots and cold spots that occur due to perpetual shifting of the geographic boundaries between populations that occurs due to evolutionary processes such as drift and migration (Thompson 2005).

A number of studies have investigated apparent environmental differences in coevolved interactions. Of those that explicitly test the first assumption of the theory – that the environment should change GxG interactions– there have been mixed results, and strong GxGxE effects (Tetard-Jones et al. 2007; Piculell et al. 2008) appear less common than weak or non-significant ones (Heath and Tiffin 2007; Laine 2007; Vale and Little 2009). However, the number of studies that test for the existence of geographic selection mosaics is still relatively small, composed mostly of laboratory experiments and lacking broad-scale investigations of how natural systems behave. Explicit tests of the geographic mosaic theory are needed, both to illuminate coevolutionary dynamics and to ensure accurate prediction of broad-scale interactions.

At its most basic level, coevolution is reciprocal selection between species, where the distribution of genotypes of one species affects fitness in another species and vice versa (Thompson 2005). Proof of the existence of a selection mosaic requires positive identification of reciprocal selection in a species grouping across multiple sites, and evidence that the frequency-dependent fitness functions for these species vary across sites. While corroboration of the ecological predictions of the GMTC indicates potential for selection mosaics, the former criteria are necessary to conclusively demonstrate their existence (Gomulkiewicz et al. 2007). In practice, this has meant testing multiple genotypes of each interacting species against each other while varying some environmental factor. While positive identification of selection mosaics in this manner is conclusive, negative results indicate an absence of

GxGxE effects solely for the specific environments and genotypes tested. The danger of making conclusions based on limited tests is evidenced by a study performed by Mitchell et al. (2005), who found a significant genotype by environment effect in the same water flea system where Little et al. (2007) found no significance. This is likely due to the increased number of genotypes tested by Mitchell et al. (8 genotypes as opposed to 4 tested by Little et al.), which increased their statistical power. Where possible, researchers testing the GMTC should utilize realistic ranges of genotypes.

Host-parasite models are a natural choice for the study of environmental effects on the evolution of interactions, firstly because host-parasite relationships are likely to be coevolved (Connell 1980). Infection by parasites on hosts often has strong fitness consequences for both species. Parasites, by definition, are not free living and must infect a host or hosts before reproduction can occur. While the effects of parasitism on hosts are always negative, the importance of this interaction to host fitness varies between systems and can be strongly context-dependent within systems. Where host fitness is strongly affected by parasitism, reciprocal selection is likely.   Secondly, environmental conditions often mediate the rate of interaction between hosts and parasites. Environmental stressors have long been known to influence host susceptibility to parasitism (reviewed by Lafferty and Kuris 1999), but the direction and size of these effects vary between study systems. Indeed, a recent review found evidence of significant environmental effects on traits mediating host-parasite interactions (e.g. infectivity, infection severity, host mortality) in 43 of the 69 papers the authors evaluated (Wolinska and King 2009).

Study System

The New Zealand Mud Snail (Potamopyrgus antipodarum; NZMS) is native to New Zealand but introduced populations occur worldwide. NZMS populations consist of either diploid sexuals or triploid parthenogic females. Snails in Australia and New Zealand are infected by >15 parasites, most commonly
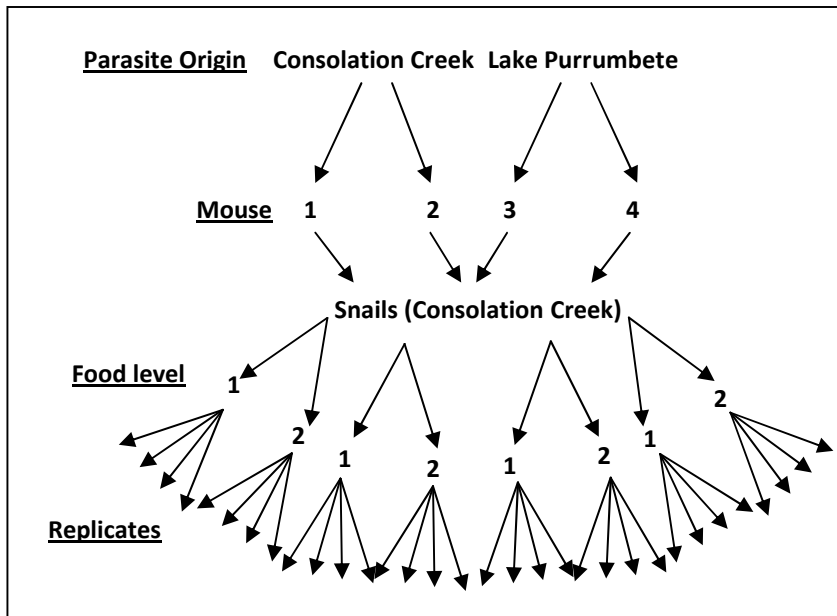
Microphallus sp., an undescribed digenetic trematode (Jokela and Lively 1995). Genetic data indicates

that Microphallus is a single species (Dybdahl and Lively 1996) that infects waterfowl as its definitive

host. Parasites infect snails that ingest eggs found in avian feces and infect a new final host when snails

are consumed by waterfowl. Infection by Microphallus eliminates the snail gonad and prevents its

reproduction, essentially eliminating that snail's fitness (Dybdahl and Lively 1996; Lively et al. 2004).

In invertebrates, infection is generally thought of as a matching alleles model, where parasite

alleles at any infection loci must match the host allele at that site, or no infection will result. A separate

model, termed 'gene for gene' was developed in plant systems and results in a broader range of host

genotypes that can be infected by any one parasite genotype. For a review of both these models, see

Agrawal and Lively (2002). Matching alleles models predict cycles of local adaptation and maladaptation

in host-parasite systems. Selection on the parasite should promote matches between parasite and host

alleles, whereas selection on the host to outrun the parasite should promote mismatches. This should

lead to negative frequency dependence, where hosts with rare alleles are at an advantage as less

prominent targets for parasite selection.

Data for the New Zealand Mud Snail is compatible with the expectations of a matching alleles

model. Asexual populations of NZMS tend to consist of a few common and many rare snail genotypes

(e.g. Dybdahl and Krist 2004). It has been hypothesized that Microphallus specializes on the most

common snail clone in an area (after Bell 1982; first hypothesized by Haldane 1949), and most data

supports the prediction that Microphallus should have higher infection success on snails from common

local clones (Dybdahl and Lively 1998; reviewed by Lively et al. 2004). This should lead to frequency-

dependent boom and bust cycles, where low reproduction in highly infected common clones allows

other genotypes to rise to prominence and promotes host switching in the parasite, which has been

hypothesized as a mechanism for the maintenance of sex in hosts (Glesener and Tilman 1978; Bell 1982;

Hamilton 1982; Antonovics and Ellstrand 1984; Lively 1987; Howard and Lively 1994).

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In New Zealand, mud snail clonal diversity is so high that no clone is locally common at multiple sites (Mark Dybdahl, unpublished data; see also Dybdahl and Lively 1995; Jokela et al. 2003). However, invaded sites are much less diverse and many Australian sites are home to high proportions of the same common snail genotypes. The US1 and AUS1 snail clones are the most frequently occurring genotypes in 7 of 14 sampled sites in Victoria, Australia, and one of these clones is common in 4 additional sites (Mark Dybdahl, unpublished data). Using parasite populations from Australia allowed us to choose sites with the same common snail genotypes and control for the selective effect of snails on parasites across locations. This enabled us to investigate whether selection by the external environment impacted infection rates on specific host genotypes.

Snails and parasites used in this experiment were collected from two sites in Victoria, Australia. The volcanic Lake Purrumbete is promoted by the local government as a vacation site for tourists and is regionally known for its lucrative trout fishery. Also protected as a wildlife sanctuary, tourism at Lake Purrumbete helps generate $3 million Australian dollars ($2.28 million USD) annually (Lynch 2009). By contrast, the second collection site, Consolation Creek, is a stream outside of Leongatha, a town of 4,550 with a prominent dairy industry. Also located in Victoria, Consolation Creek is located 314 km (195 mi.) east of Lake Purrumbete.

Figure 1: Diagram showing experimental treatment combinations

METHODS

To test for the existence of geographic selection mosaics, I varied two environmental factors, parasite population of origin and snail food level. By measuring the rate of parasite infection on specific snail genotypes, I was able to statistically test for GxGxE interaction by looking for snail genotype x environment interactions. The validity of this test stems from the assumption that the distribution of parasite genotypes was constant across both tested parasite populations of origin. Maternal effects are not known in Microphallus sp., and parasites used in this experiment were offspring of field-collected parasites and subject to identical conditions in the laboratory. Prior studies indicate a strong genetic basis for parasite infectivity. Thus, any meaningful difference in the distribution of parasite genotypes across sites should be detected as a difference in infection rates between the two parasite populations of origin. Critics may contend that a difference in snail genotype-specific infection across environments represents a GxE and not a GxGxE interaction. For this contention to hold water, one would have to assume that either a) parasite genotype was not a significant factor in patterns of infection across snail genotypes or b) multiple parasite genotypes were not present in our collection. All previous evidence from this system strongly contradicts such assertions.

We collected snails from a single population at Consolation Creek, New South Wales, Australia. Parasites (Microphallus sp.) were collected from Lake Purrumbete and Consolation Creek. We exposed juvenile snails to Microphallus using the following method: encysted parasites collected from infected NZMS were fed to laboratory mice, whose feces were collected approximately every 4 hours over the following week. Because snail age is highly correlated with shell length (Baudoin 1975; Hughes and Answer 1982; Sousa 1983), experimental snails were chosen based on size (< 1.5 mm). Juvenile snails were used because laboratory infection rates have been highest in juveniles (Dybdahl and Krist 2004), despite adults having higher field infection rates (Jokela and Lively 1995).   In addition, juveniles have

not yet reached asymptotic size, enabling us to measure growth. Following collection, mouse feces

were soaked in water and rinsed regularly. Snails were placed, 35 to a replicate, in square plastic

containers for two weeks, during which they received no food except for feces from infected mice.

Following the two week exposure period, snails were separated into two treatments based on

the amount of food provided, with 4 replicates of each treatment combination (see Figure 1). High food

treatments received 0.004g of Spirulina daily per 35 snails, while low food treatments received 1/10th

this amount. A study by Dybdahl and Krist (2004) found that altering food levels caused significant

changes in infection rates of P. antipodarum by Microphallus, indicating that food level might be an

important mediator of host-parasite interactions in this system.   Food treatments were continued for 4

months, after which parasites were expected to have developed to the stage of metacercariae and were

easily detected. All snails were measured lengthwise and dissected to determine snail brooding

condition and parasite presence. Dissected snails were stored individually, frozen in 20 µl of crushing

buffer. Allozyme electrophoresis was performed on all 472 snails, and snails collected from Willow

Creek and Russian River, CA (US1 clone) were used as controls for evaluating banding patterns.

Variation was recorded at six allozyme loci (for methods see Dybdahl and Lively 1995).

Analysis

Statistical analysis was performed using SAS software version 9.2 (SAS Foundation). Snail length,

mortality and presence/absence of infection were used as the response variables, with fixed effects of

parasite population, food environment, and snail genotype. Mouse and replicate were included as

random effects. Snail length and mortality analyses were performed using SAS Proc Mixed, and

infection was analyzed as a binomial logit model using SAS Proc GenMod. Length data failed to meet

the normality assumption despite attempts at transformation. I corrected for this problem by rank

transforming the data, essentially a non-parametric procedure, and then reanalyzing the transformed

data using Proc Mixed. Proc GenMod does not have mixed model capability, thus all variables were included as fixed effects in the infection analysis.

RESULTS

A total of 472 snails survived and were used in the length analysis. However, due to ambiguous banding patterns in the allozyme data, 74 snails were eliminated from the infection analysis, resulting in a sample size of 398. Total snail mortality was 51%, and ranged from 3 – 96% depending on the individual replicate container. A factorial ANOVA indicated that mortality was not significantly different across treatment combinations. The most common snail genotype across treatments was US1 (149 of 398 snails), followed by AUS1 (32 of 398 snails). Because we could not know the genotypes of live snails, replication of genotypes across treatment combinations was

Figure 2: Snail length by food treatment and parasite population of origin, both of which were significant predictors of snail length. Error bars: ± 1 SE. *p=<.0001   **p=0.0013

unequal. To make statistical analysis possible, I grouped snail genotypes into 3 categories: US1, AUS1, and Rare. Genotypes representing less than 6% of the snail sample were categorized as rare. The rare genotype grouping represented 217 snails with 77 unique genotypes.   All individual rare genotypes were represented by 22 or fewer snails, with 65 rare genotypes represented by fewer than 5 snails. Analyzing more genotypes and classifying a smaller number of clones as rare did not change statistical significance, though including more than five distinct genotypes in the dataset resulted in a lack of

model convergence and an inability to calculate statistics. Similarly, the presence of replicate in the model did not change significance.

Length Analysis

Both parasite origin and snail food level were significant predictors of final snail length (See Figure 2). Snails raised with high food were longer than snails raised with low food by 0.21mm on
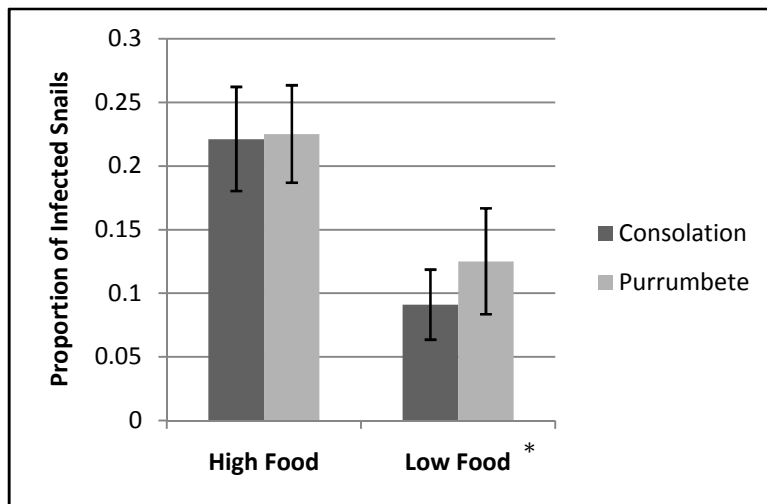
Figure 3: Infection rate in snails by parasite origin and food level. Error bars: ± 1 SE.   *p = 0.0198

average, indicating that differences in food levels were significant to snails. Snails exposed to allopatric parasites (Purrembete) were also longer by 0.36mm on average (figure 2). In addition, snails from the US1 genotype were significantly longer than other genotypes [US1: 3.68 ± 0.038, AUS1: 3.46 ± 0.108, Rare: 3.44 ± 0.035 mm (mean ± SE)]. The relatively longer length of the US1 clone might be caused by inherent genetic differences in growth rates or by intraspecific competition. The US1 clone was by far the most common in the sample (149 of 398 genotyped snails were US1). Members of the US1 clone were more likely to be competing against snails of the same genotype than other snails were. In a field situation, one would expect intragenotype competition to be more intense than intergenotype competition because of greater resource overlap within genotypes.   However, in the lab situation only one food source was provided, so all snails effectively had completely overlapping niches. Any genotype that was faster or better able to exploit that niche might have an advantage.
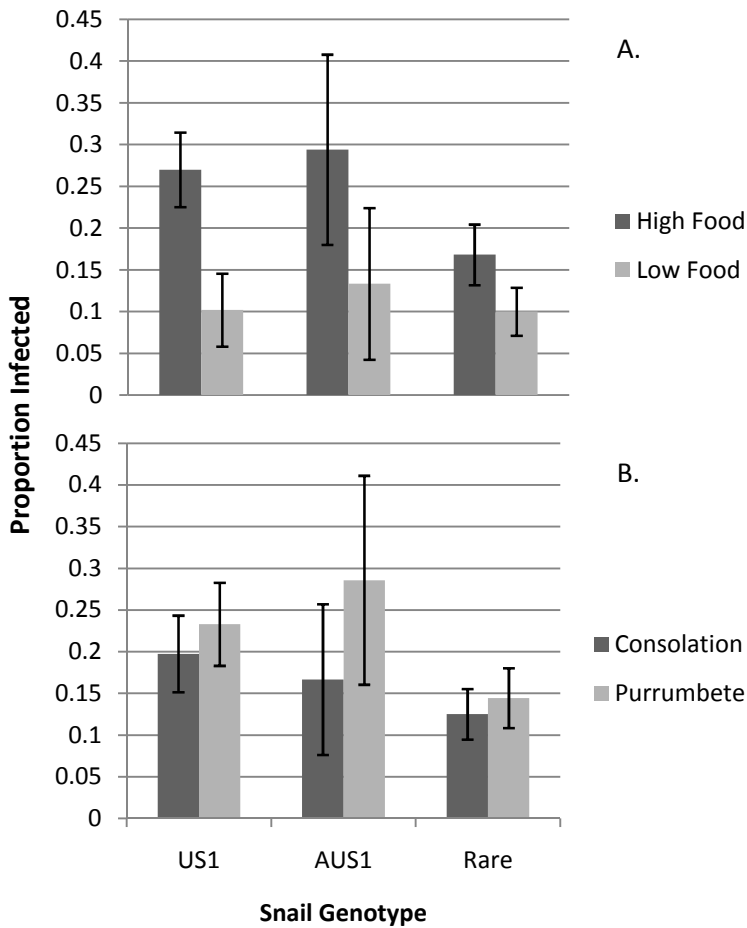
Figure 4: Infection rate in snails by snail genotype and food level (A) and snail genotype and parasite origin (B). Error bars: ± 1 SE.

Unexpectedly, infection predicted snail length, with infected snails longer than their uninfected counterparts [Infected: 3.90 ± 0.041, Uninfected: 3.46 ± 0.028 mm (mean ± SE)]. Field samples of New Zealand Mud Snails have found that larger snails are more likely to be parasitized (Jokela and Lively 1995). Because of the correlation between snail age and size, it has been hypothesized that increased infection in large P. antipodarum could be due to the greater length of time that older snails had been exposed to parasitism. However, the results of my experiment suggest that infection might be a cause of greater snail length. P. antipodarum reaches asymptotic size around the time of first reproduction, but infected snails do not reproduce. Perhaps non-reproductive snails could continue to allocate energy to growth longer than their uninfected conspecifics. No interaction effects were statistically significant predictors of snail length.

Infection Analysis

Snails raised under high food levels were infected at significantly higher rates than snails raised with low food (Figure 3). While it seems intuitive that hosts in poor condition would have fewer

resources to allocate toward resisting infection, my results indicated that the opposite occurred: snails in the high food environment were more frequently infected. Krist et al. (2004) obtained similar results and attributed them to high mortality in low food treatments. In my experiment, however, there was no difference in mortality between the two food treatments, indicating that parasites were not inducing mortality at higher rates for snails in poor condition. This result, if corroborated in the field, may indicate lowered fitness for snails in good condition in areas where parasite infection rates are high.

The mouse effect was also significant, perhaps due to differential parasite survival and reproduction in the mouse GI tract. No interaction effects were statistically significant, indicating that the environmental conditions I tested do not cause variation in the genotype dependence of these populations on each other (figure 4).

DISCUSSION

The purpose of this experiment was to assess the possibility of geographic selection mosaics caused by variation in the fitness dependence of Microphallus sp. on the genotypic distribution of its host, the New Zealand Mud Snail. I varied two environmental factors: snail food level and parasite environment of origin. While results suggested that both factors altered snail length and food level changed the risk of infection, neither factor interacted with snail genotype. This indicates that, while geographic selection mosaics may be possible in this species, food level and parasite origin can influence infection rates without changing the genotypic dependence of the interaction.

Food level was a significant predictor of infection, indicating that the environmental conditions I chose are important to this host-parasite interaction. Significantly higher infection rates in the high food environment indicate that primary productivity may mediate the frequency with which this interaction occurs. Changes in the frequency of infection might influence the speed of evolution for this system, and could shape the relative strengths of drift and selection.

Neither of the interaction effects that would indicate potential for geographic selection mosaics (genotype x food level and genotype x parasite origin) were significant at α=0.05. That my experiment tested for the existence of genotype by genotype by environment effects rests on the assumptions that parasite genotypes are tightly coupled to specific snail genotypes they infect and that the distribution of parasite genotypes is similar across the environments we chose. As infection rates did not differ across parasite origins, we can safely assume that the distribution of genotypes was similar across parasite environments, assuming infection is highly dependent on host and parasite genotypes. My results indicate a lack of geographic selection mosaics for the genotypes and environmental factors I tested. These results arguably indicate a lack of geographic selection mosaics, even if my assumptions are not met. For selection mosaics to occur, infection rates would have to differ across snail genotypes and environments, a signal that was not detected in my data.

One alternative explanation of the results I obtained is that the frequencies of different parasite genotypes in two environments were exactly reversed. Results from this scenario would indicate equal infection rates across environments when in fact the genotype-dependent infection rates were changing due to host genotype switching by parasites under changing environmental conditions. While this explanation seems unlikely, it cannot be ruled out barring knowledge of parasite genotypes.

An absence of geographic selection mosaics under the study conditions suggests that hosts can drive parasite selection to the exclusion of other factors, even when those factors impact parasite fitness (e.g. by changing the rate of host-parasite interaction). This confirms the view that hosts are the most relevant environments for their parasites. While the results I obtained may not hold true for all possible environmental variations in this system, they emphasize the importance of scale to the GMTC. To understand the broader implications of the GMTC for coevolution, studies need to identify environmental factors that lead to geographic selection mosaics and determine what scale they operate at across a landscape. Selection mosaics between P. antipodarum and Microphallus sp. were not

detected in Victoria, Australia, but might operate at a broader, perhaps interisland, level. Future studies

should focus on broad-scale environmental effects where possible, refocusing at finer resolutions where

evidence of selection mosaics is found. Knowing the scale at which geography changes the genotype-

dependence of coevolutionary interactions will show us precisely where our ability to predict

coevolutionary dynamics using traditional genetic models breaks down.

CHAPTER TWO

INTRODUCTION

Environmental effects can mediate ecological interactions both directly, by causing a response in a monitored species, or indirectly, by impacting a chain of interacting species or modifying interactions themselves (Wootton 1993). Trophic cascades and exploitative competition are two examples of interaction chains, whereby a species exerts influence over the density of another species by its direct interaction with a third species. Interaction modifications occur when a third species changes the rate of interaction between two or more other species (such as when predator presence reduces activity of a consumer, creating an indirect positive effect on that consumer's resource). In this chapter, I present the results of an experiment looking at resource competition (an interaction chain) between the invasive New Zealand Mud Snail (*Potamopyrgus antipodarum*; NZMS) and the narrowly endemic Jackson Lake Spring Snail (*Pyrgulopsis robusta*) and how a third species, Rainbow Trout (*Oncorhynchus mykiss*) modifies this interaction (interaction modification).

Though direct biotic and abiotic effects have traditionally been viewed as the main forces that structure communities, it is becoming apparent that indirect effects can also influence the frequency and type of interactions (Werner and Peacor 2003). Two major types of indirect effects have been identified: density-mediated effects, and trait-mediated effects. Interactions such as competition are deemed 'density-mediated', because the outcome often depends on the initial densities of the competing species and their modification of the density of a resource. The effects of keystone species and trophic cascades are also traditionally seen as density-mediated (Abrams 1995).

In contrast, trait-mediated indirect effects (TMIEs) refer to phenotypic changes in a species of interest that change the rate of its interactions with other species (Relyea 2000). These phenotypic changes are often caused by the reaction of one species to another (e.g. behavioral changes in response

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to predator presence), and can provide insights into complex interactions that affect multiple trophic levels. Many interactions consist of both density- and trait-mediated effects. For instance, the presence of predators might alter the density of a resource both by reducing the density of an intermediate consumer and altering its feeding activity. For an extensive review of TMIEs, see Werner and Peacor (2003).

Many studies of TMIEs have been conducted in aquatic systems (Werner and Peacor 2003). It is relatively easy to measure and control nutrient availability in closed aquatic systems, and the three-dimensionality imposed by water allows a wide variety of phenotypic expression related to habitat preference (Braithwaite 1998). Since many aquatic animals can sense chemical signals released by predators (e.g. Holomuzki and Short 1988; Kats et al. 1994; Ferrari and Chivers 2006) these systems also enable researchers to study trait-mediated indirect effects induced by predators while avoiding actual predation, isolating the trait-mediated effect (Agrawal 2001).

Phenotypic Plasticity Requirement

A necessary requirement for the occurrence of TMIEs is trait plasticity at the individual level. Phenotypic plasticity is the evolved ability of a species to alter its phenotype in response to environmental conditions (Via et al. 1995, Agrawal 2001). Plastic traits can be altered over the course of an individual's lifetime, while multiple generations are required for evolutionary change. Therefore, while evolution occurs at the population level, phenotypic change due to plasticity occurs at the scale of the individual (Agrawal 2001).

Despite costs, phenotypic plasticity has been demonstrated for a variety of organisms, and some degree seems ubiquitous (Agrawal 2001). Trait-mediated indirect effects have been documented in a variety of systems (reviewed by Werner and Peacor 2003). Most of these indirect effects have impacts that resonate through multiple interactions in the community, though ecologists have by neccessity

studied specific interactions in isolation (e.g. Huang and Sih 1991, Wissinger and McGrady 1993, Relyea 2001).

Indirect effects can play important roles in ecosystem services and should be incorporated into theory underlying management plans and restoration efforts. Trait-mediated indirect effects are especially relevant to species invasions, where high levels of behavioral and trait-level plasticity in some invasive species may allow them to escape predation and parasitism by native species. Some studies support this view: Carlsson et al. (2004) demonstrated that the invasive golden apple snail exhibits a behavioral response to native predators in the new range, and Trussell et al. (2003) showed that trait plasticity in native species can increase resistance to invasive predators.

Phenotypic plasticity in native species may also influence the invasibility of biotic communities. For instance, Peacor and Werner (1997) found that the presence of larval odonate predators in aquaria containing tadpoles facilitated the invasion of midges, while predator-free tanks were not invaded. Peacor et al. (2006) modeled behavior in response to predators, and suggested that phenotypic plasticity in native communities alters the steepness of fitness surfaces, making it difficult for novel organisms to adapt to the new community quickly enough to persist. The idea of biotic resistance to invaders is generally accepted, but vastly understudied. Facon et al. (2006) suggest that the success of any invasion is caused by a match between the invaded area and the invasive species, rather than by specific traits that promote invasiveness. Plasticity in both invasive and native organisms may be one component of that match.

Study System

While islands in general and New Zealand in particular are famous as sinks for deleterious introduced species from other countries, a number of New Zealand's own species have recently surfaced as invasive (Yeates and Williams 2006). Potamopyrgus antipodarum is probably the island

country's most invasive export. This New Zealand Mud Snail is a worldwide invader that has spread throughout the Snake and Columbia River watersheds in the Western United States. Recent work indicates that the snail exerts strong negative effects on the narrowly endemic Jackson Lake Spring Snail (Riley et al. 2008). Studies of NZMS in its native range indicate that fish modify the interaction between the mud snail and its resources. NZMS responds behaviorally to fish predators by reducing activity on the tops of rocks where it is most accessible to predators, and environmental factors such as snail size, parasite infection and temporal variation in predator activity may mediate this response (Levri and Lively 1996; Levri 1998). While behavioral plasticity has not been studied for invasive populations of NZMS, plasticity in growth (Dybdahl and Kane 2005) and shell shape (Erica Kistner, unpublished data) are present in Western United States populations. JLSS has not been studied in the context of phenotypic plasticity, but its growth rates slow under intra- and interspecific competition (Riley et al. 2008). I was curious whether the native snail would exhibit a similar, and perhaps stronger, behavioral response than NZMS due to its shared evolutionary history with trout. Such a response would likely benefit the invasive snail in competition where an experimental setup disallowed predation. I also wanted to know how the addition of trout would impact resource use in both snail species.

The New Zealand Mud Snail is a worldwide invasive species, with populations in the United States, Australia, Tasmania, Japan, Europe, and Britain. The first mud snails in the Western United States were recorded in the Snake River watershed near Hagerman, Idaho in 1987 and were thought to have been transported through ballast water (National Management and Control Plan 2006). The snail is now common throughout the Snake and Columbia River watersheds and is thought to spread to new streams on fishing and recreational equipment.

The mud snail reaches its highest densities in Polecat Creek, Wyoming, just south of the border between Yellowstone and Grand Teton National Parks (lat 44°6'33.025"N, long 110°41'28.020"W). In parts of Polecat creek, the mud snail reaches densities of >500,000 per m^2 and consumes 75% of gross

primary production (Hall et al. 2003; Hall et al. 2006). While populations are estimated to be much

smaller in the winter months, geothermal input keeps water temperature in the area relatively stable.

Polecat Creek is fed by two smaller streams. One of these unnamed streams, referred to hereafter as

Marmot Spring (lat 44°8'6.553"N, long 110°42'50.431"W), is home to the narrowly endemic Jackson

Lake Spring Snail. While historical data indicates that the spring snail was once common in Jackson Lake

and many of its associated streams, recent searches have failed to locate it in these areas, and Marmot

Spring is the only remaining location where this snail is known (Riley and Dybdahl 2006). Both the native

and invasive snails are abundant at the interface between Marmot Spring and Polecat Creek, and the

mud snail becomes rare as you move upstream. Leslie Riley's work indicates that the invasive P.

antipodarum exerts strong negative effects on the growth of its native counterpart, while the presence

of P. robusta facilitates growth in the invasive snail (Riley et al. 2008).

The spread of the mud snail may also be taking its toll on trout. A study by Vinson and Baker

(2008) indicates that New Zealand Mud Snails are nutritionally poor, and wild-caught Brown (Salmo

trutta) and Rainbow trout whose stomach contents included P. antipodarum rated lower on a condition

index. In addition, lab-raised brown trout put on a diet that consisted solely of New Zealand Mud Snails

lost weight, and 53.8% of ingested mud snails remained alive after passing through the trout's gut

(Vinson and Baker 2008).

Preliminary trials in the laboratory indicated that both snail species avoided the tops of rocks

more often in the presence of Rainbow Trout. The New Zealand Mud snail also exhibited a behavioral

response to small Northern Pikeminnow (P. robusta was not tested against this species) but lack of

replication necessitates conservative interpretation of these data (Redd, unpublished data).

METHODS

My goal was to measure the strength of the interactions of the two snails with their common

algal resource in the presence and absence of trout predators. Mud Snails and Spring Snails were

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collected from Polecat Creek and Marmot Spring, respectively, on May 31, 2009. I used a factorial

response surface design, which incorporates elements of both density and replacement series, to

measure the response of each species to its competitor (Inouye 2001; Riley et al. 2008). This type of

design involves holding the biomass of a target species constant while varying the biomass of a

competitor species in separate treatments (Forrester et al. 2006). Snail densities were determined on a

per biomass basis, using a length to mass regression (P. robusta: Riley et al. 2008; P. antipodarum: Hall

et al. 2006). Target snails measured 2.5-3 mm on average. Sizes were chosen because they were below

the asymptotic size for each species, indicating continued growth potential. I varied 3 factors: species

composition (intraspecific vs. interspecific), total snail biomass (two levels, high and low), and trout

presence/absence. Low biomass was 0.66 g/m^2 AFDM, which corresponds to approximately 1/7th of

ambient biomass of both species as measured by Leslie Riley in Marmot Spring in 2002 (Riley et al.

2008). Using very low biomasses minimizes the potential for intraspecific competition. High density was

4.98g/m^2 AFDM, slightly greater than ambient density (measured as 4.47g/m^2 AFDM by Leslie Riley). In

total, there were seven competition treatments, four intraspecific and three interspecific. In addition, I

used a snail-free control treatment to estimate algal densities on ungrazed rocks. Each competition

treatment was tested under trout presence and absence, yielding a total of 16 treatment combinations.

Available space and equipment precluded testing more than 32 treatment combinations at one time. By

running the same experiment three times, each with different snails and rocks, I replicated each

treatment combination 4-6 times.

Snails were placed in 5-gallon glass fish tanks on a grouping of rocks collected from the Snake

River at Boyer Park Marina, WA. Rocks were collected 0-3 days prior to beginning the experiment, and

care was taken to place the rocks so that the side with the greatest density of algae faced upward, as

would be the case under natural conditions. I expected this setup to create a tradeoff between resource

acquisition and predator avoidance. A plastic sandwich container with walls cut out and replaced with

fiberglass window screening (mesh size 1mm) was placed over the snails and rock grouping in each tank and weighted down with 2-inch square ceramic tiles. In fish presence treatments, trout were allowed to swim freely around the container.

Tanks were fed by a recirculating water system originating in separate reservoirs for fish and non-fish treatments to avoid exposing control tanks to water contaminated by fish scent and snail alarm cues. The reservoir that fed tanks containing fish was drained and filled with dechlorinated (using sodium thiosulfate) tap water daily, due to a fish die-off early in the first round of the experiment caused by high ammonium levels. Because of space and hardware constraints in the room, one reservoir fed tanks on the top bench while the other fed tanks on the bottom bench. Because the experiment was performed in three different 11-day time periods, trout could be rotated between the two benches at the beginning of each time period, but any interaction between bench and time period would be confounded. Trout were fed alternating diets of crushed P. antipodarum and P. robusta and trout chow, with crushed snails fed every 3 days.

Snails in each treatment were weighed as a group prior to and following the experiment. Excess water was removed using a 10μl syringe before weighing. Living and dead snails were counted following the experiment to determine mortality and biomass per snail. Snail behavior was observed daily by removing fish and containers and counting the number of visible snails of each species that were on and around the rocks and container. Escaped snails were noted, and containers were replaced if mesh became loose and allowed many snails to escape during the course of the experiment. Behavioral observations were made by the author and Amy Klein, an undergraduate student at Washington State University. The total percentage of snails on the rocks was estimated to be the number of escaped snails plus the number of visible snails divided by the initial number of snails in each tank. This method tends to underestimate the percentage of living snails on tops of rocks toward the end of each experiment in tanks when mortality occurs during the course of the study.

Chlorophyll α density on each rock grouping was determined by the spectrophotometric

method (APHA 1995; Hauer and Lamberti 2007) for the second and third runs of the experiment. Rocks

were scrubbed with toothbrushes to create slurries, and 10-ml samples were passed through glass fiber

filters. Filtered chlorophyll was extracted in 90% buffered acetone for 24 h, and density was measured

using a spectrophotometer set at 664, 665, and 750 nm. Tracings of rocks were used to estimate

surface area for determining chlorophyll density.

Calculating Interaction Strengths

Interaction strengths, which measure the per capita or per biomass impact of one species on

another, are a valuable tool for estimating species impacts. By multiplying interaction strength by

population size, one can estimate population level effects of a species. This makes interaction strengths

a valuable addition to an ecologist's or manager's toolkit, especially in the context of determining

invasive species impacts (Riley et al. 2008). Indeed, Parker et al. (1999) argued that interaction strengths

should be one of the key components for measuring the impact of invasive species. Traditionally

applied to predator-prey systems, Riley et al. (2008) recently adapted equations for dynamic index

interaction strengths from Wooton (1997) and applied them to competitive interactions between the

New Zealand Mud Snail and the Jackson Lake Spring Snail.   I applied the same equations to measure the

strength of the biotic interactions between these two snail species and a resource, and calculated the

change in interaction strength when Rainbow Trout were present.

To calculate the consumer-resource interaction strength, I followed the equation:

$$-\quad = \frac{\ln -}{-}$$

[1]

From Riley et al. (2008), where -c denotes the interaction strength, N_s is chlorophyll α concentration

with snails, N_0 is chlorophyll α concentration with snails absent, M is the biomass of snails in N_s, and t is

time in days. This measures the gram for gram impact of consumers on resources, where more negative

values indicate more deleterious effects of the interaction on the resource.

Analysis

All analyses were performed using SAS software version 9.2 Proc Mixed (SAS Institute). Growth

and behavioral responses were analyzed separately for each snail species. I used a repeated measures

ANOVA in SAS Proc Mixed to look at behavior of each snail species in response to trout. Behavior data

for P. robusta failed to meet normality assumptions, and a rank transformation was used to correct for
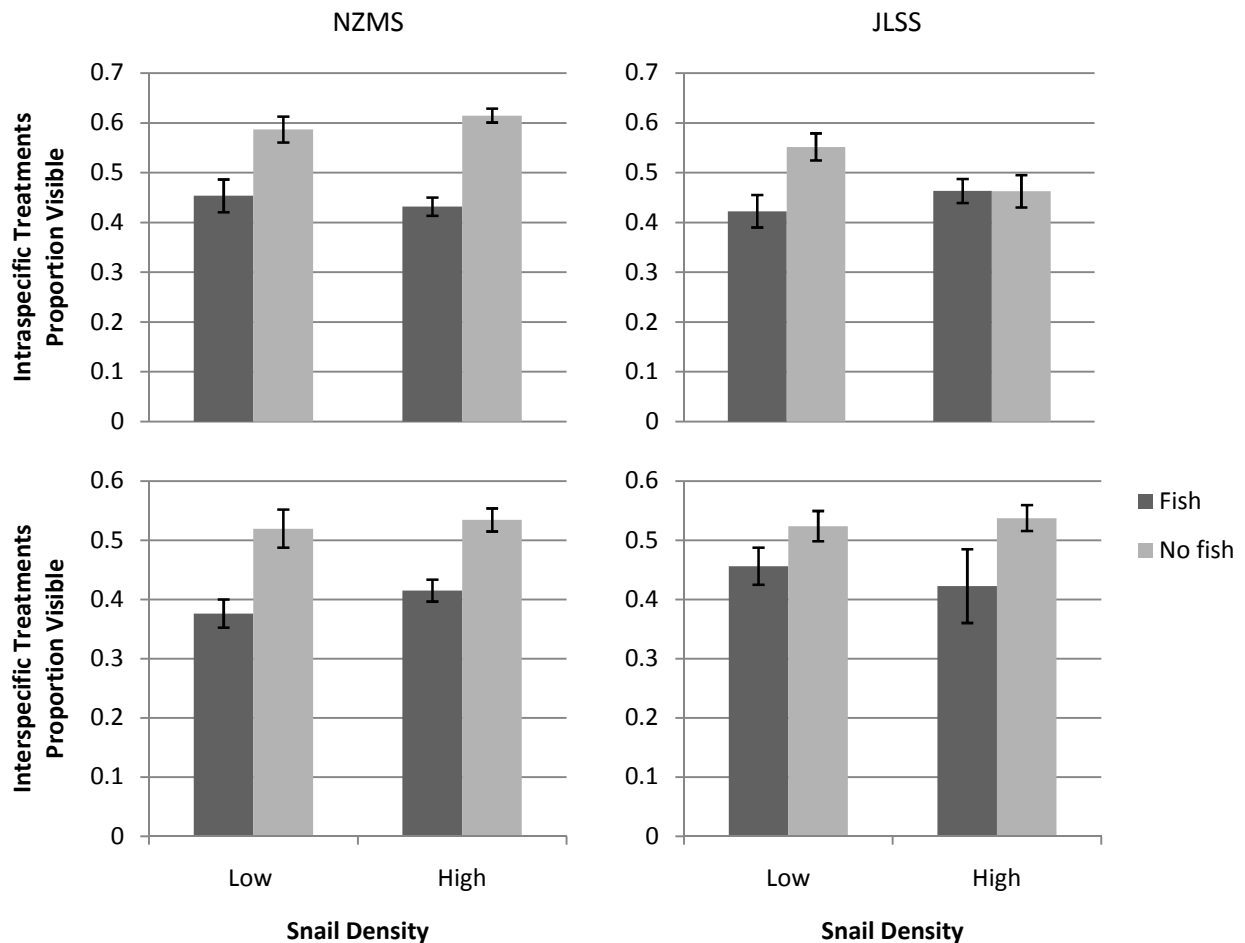
Figure 5: Snail behavior as measured by the proportion of snails visible from the top of experimental units. The two graphs on the left side indicate responses of the New Zealand Mud Snail, while graphs on the right show behavior of the Jackson Lake Spring Snail. The two upper graphs represent intraspecific treatments and lower show interspecific treatments. For all graphs, low density treatments are on the left side and dark bars indicate the presence of fish. Error bars: ± 1 SE.

this after other attempts at transformation failed to improve normality. I performed a factorial ANOVA in SAS Proc Mixed to look at snail growth for each species individually. Chlorophyll density was analyzed as a factorial ANOVA in Proc Mixed with fixed effects of competition type (intraspecific vs. interspecific), snail density, and fish presence/absence, and was analyzed twice to include different combinations of experimental runs.

RESULTS

Snail Behavior

More invasive snails were visible from the tops of tanks when fish were absent than in their presence [Fish present: 54.98% ± 5.87%; Fish absent: 70.13% ± 5.99% visible snails (mean ± SE); see figure 5]. Competition type also impacted invasive snail activity, and snails in intraspecific treatments were more frequently visible [Intraspecific: 65.74% ± 5.92%; Interspecific: 59.36% ± 5.93% visible snails (mean ± SE)]. In the native snail, trout presence but not competition type influenced behavior [Fish present: 44.56% ± 2.82%; Fish absent: 51.64% ± 2.91% visible snails (mean ± SE)]. These results indicate that the New Zealand Mud Snail is able to respond behaviorally to predators in its invasive range, despite having only been exposed to said predators for a few decades. The Jackson Lake Spring Snail exhibited a similar predator avoidance response, though not quite as dramatically as its invasive competitor.

Snail density did not affect behavior in either species. Interestingly, invasive but not native snails behaved differently depending on the day, and the day x fish effect was also significant in the invasive snail. This may be related to the feeding schedule for trout, which were fed crushed snails every 3rd day. Previous studies of NZMS behavior indicated stronger behavioral responses to fish when fish were fed crushed snails (Levri 1998b), and predator avoidance theory in aquatic organisms suggests

that a pairing of predator scent and alarm cues from damaged prey aids learning in conspecifics (Ferrari and Chivers 2006).

I also found a significant difference in measurements between the two observers for native snail behavior (p < 0.0001), an effect that was narrowly non-significant for the invasive snail (p=0.0530). This is probably due to human error, as snails often blended in with algae and rocks and could be difficult to see. As care was taken to ensure that observers did not consistently measure the same tanks, the model should account for this effect.

Snail Growth and Mortality

In neither snail species did snail growth differ from zero, and none of the measured variables influenced growth. While previous field studies have shown evidence for growth in the studied species on similar time frames to my experiment (e.g. Riley et al. 2008), snails in the laboratory often grow slower than their free-living counterparts (L.A. Riley, pers. observation).

The most dramatic factor influencing mortality was experimental run (NZMS: p=0.0026; JLSS: p<0.0001), with a significantly higher percentage of snails dying in the third run of the experiment. Average mortality across runs was 38.27% ± 3.16% for NZMS and 25.05% ± 3.91% for JLSS. Trout presence also increased mortality in the invasive snail [Trout present: 46.29% ± 5.03%; Trout absent: 32.67% ± 4.09% (mean ± SE)], probably due to the greater tendency of the smaller and faster invasive snails to escape from mesh containers. Competitive density was narrowly nonsignificant (p = 0.0591) for the invasive snail, and mean mortality was higher where snail density was high. For the native snail, I found a snail density x fish presence interaction. Where fish were present, mortality increased with snail density. In the absence of trout, mortality decreased with increasing snail density. This may indicate that high competitive density combined with predation pressure could have negative effects greater than what would be expected simply by combining the isolated effects of fish and density.
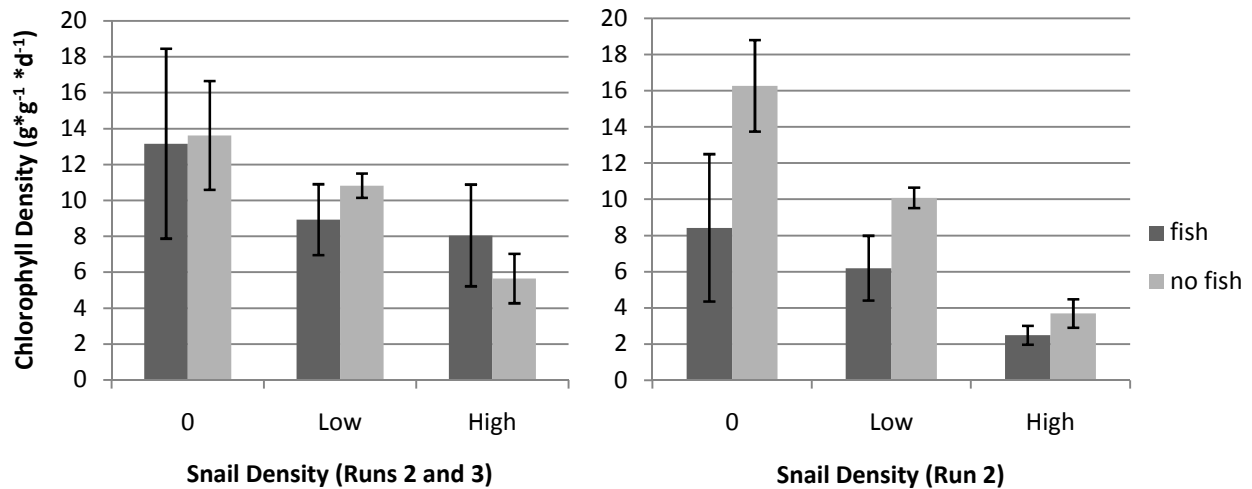
Figure 6: Chlorophyll α densities grouped by snail density and trout presence/absence. The graph on the left includes data from both experimental runs, while the graph on the right represents run 2 alone. Snail density and trout treatment were significant predictors of chlorophyll density within round 2 (p<0.0001 and p=0.0011 respectively). Error bars: ± 1 SE.

Chlorophyll Analysis

Each run of the experiment had vastly different values for chlorophyll density, with values from Run 3 sometimes reaching 10 times the values from the same treatment in Run 2. Analyzing the total data resulted in no significant effects besides experimental run (p<0.0001). In analyzing run 2 of the experiment alone, both trout presence (p=0.0011) and competitive density (p < 0.0001) were significant predictors of chlorophyll density (see figure 6). The interaction effect was non-significant (p=0.5269). Run 3 only contained one replicate and therefore could not be analyzed alone.

Interaction Strengths

Consumer-resource (C-R) interaction strengths were calculated for each treatment and measured as the daily per biomass effect of snails on chlorophyll. While snail density did not predict snail behavior, it appeared to be the most prominent influence on interaction strength (figure 7). Per biomass effects of snails on chlorophyll were more negative when snail density was low, indicating that snail-snail competition may have limited food intake when snail density was high. In intraspecific
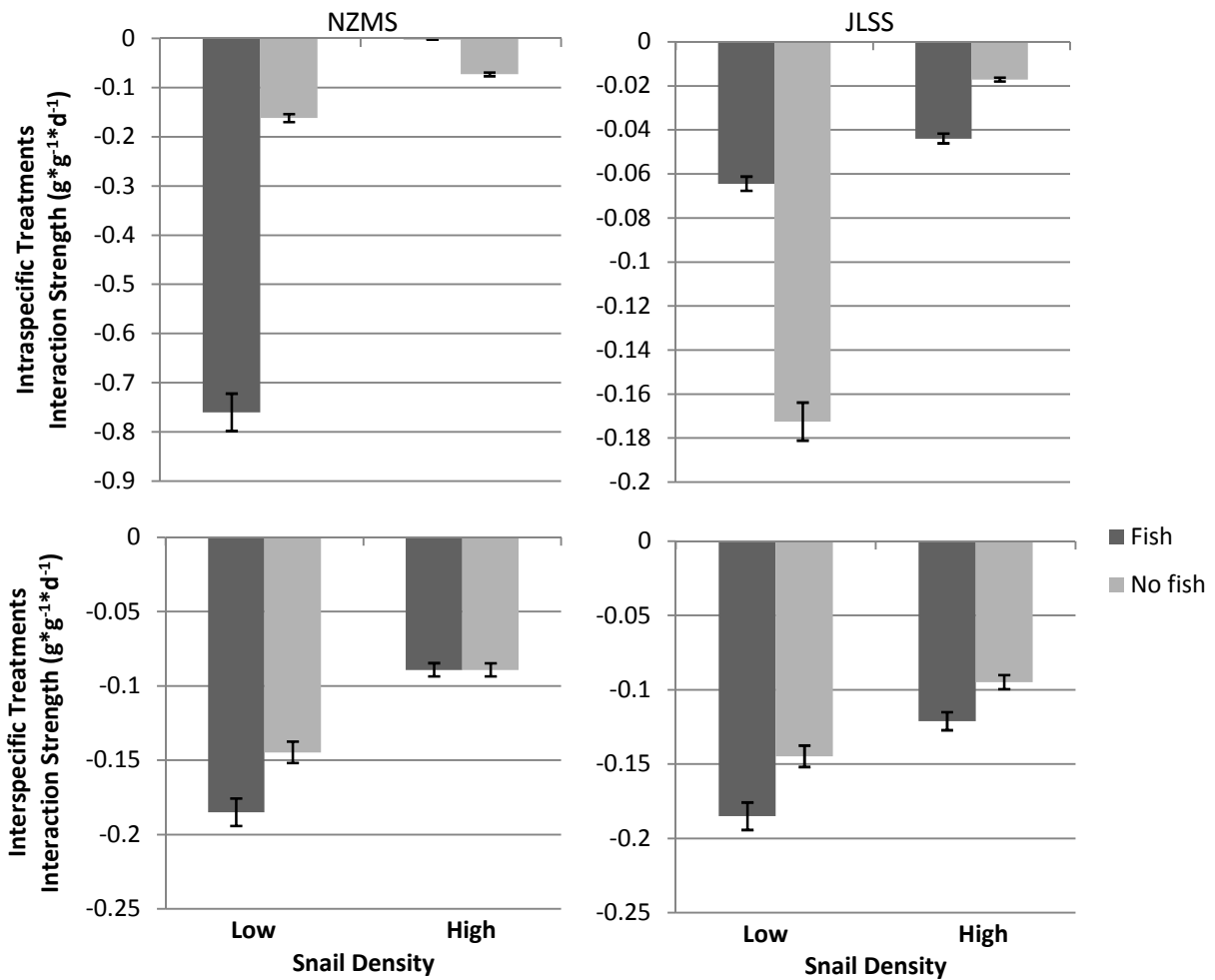
Figure 7: Consumer-resource interaction strengths based on chlorophyll densities from run 2. The two graphs on the left reference the density of NZMS in intraspecific (top) and interspecific (bottom) treatments. The graphs on the right present results of the C-R interaction with JLSS as consumer. Error bars: ± 5%.

treatments, variation between interaction strengths in fish presence and absence treatments was much

more extreme in NZMS than in JLSS. Interestingly, under high intraspecific densities, the negative effect

of NZMS on chlorophyll biomass was much higher when fish were present than in their absence, which

contrasts with expectations derived from snail behavior. P. robusta depressed chlorophyll in

approximately equal amounts in fish and no fish treatments under high intraspecific competition.

Consumer-resource interactions were stronger in interspecific than intraspecific competition treatments

when snail density was comparable, indicating more efficient resource use when both species were

present.

<div align="center">**DISCUSSION**</div>

This study aimed to determine whether predators could indirectly mediate interactions between

the New Zealand Mud Snail, the Jackson Lake Spring Snail, and their food resources. A plastic response

is required for the expression of trait-mediated indirect effects (Peacor and Werner 2003). By

measuring snail behavior in response to a change in the biotic environment, I determined that both

NZMS and JLSS avoid visibility more often when predators are present. I also found that the responses

of the two snail species to other environmental factors, such as competition, differed. Species

differences in response to external conditions can mediate their interactions with other community

members, changing the rate or direction of interactions. In this study, behavioral plasticity mediated the

strength of interactions between two snail species and their food resources.

As expected, the percentage of visible *P. antipodarum* decreased when fish were present. In

virtually all treatment combinations, the proportion of visible snails was higher in the absence of fish.

This behavioral response was especially striking in the invasive snail, which may indicate a higher degree

of plasticity in NZMS than in JLSS. However, other possibilities exist; predation may be less of a risk to *P.*

robusta, which tends to have a greenish tint to its shell and blends in more easily with algae. To test this

prediction, one could determine the optimal foraging rate for each species when predation is possible,

and estimate how well that species conforms to the optimal strategy.   In either case, both species

responded to the presence of trout, indicating the presence of plasticity and the potential for trout to

modify interactions between snails and their food resources.

Behavioral modification of this kind might drive changes in the rate of interaction

between consumers and their resources, which could in turn mediate exploitative competition between

consumers. I measured the reduction in a common resource for both snail species and found that trout

presence was a significant predictor of chlorophyll density, which, in contrast to my expectations, was highest when trout were absent. Behavioral data suggested that snails are more visible when trout are absent, indicating that interactions between snails and their resources are limited by trout. Chlorophyll density was inversely related to snail density, but the two species did not differ in their ability to reduce chlorophyll. Since chlorophyll was actually higher when trout were absent, we may not be able to assume that visible snails are more efficient foragers. Interactions between fish treatment, snail density, and competition type (intraspecific vs. interspecific) were narrowly non-significant, which suggests the possibility of interference competition.

The strength of interactions between both snail species and their resource was more negative when snail density was low. This indicates that individual snails were more successful foragers when competition was low. The strength of the consumer-resource interaction also increased when multiple species were present, indicating that intraspecific competition may reduce foraging efficiency to a greater extent than interspecific competition, possibly due to greater niche overlap.

Overall, the indirect effects of trout on snail behavior and consumer-resource interactions were clearly significant, indicating the presence of TMIEs for this system. However, despite a plausible behavioral mechanism for reducing snail foraging, chlorophyll content was lower where trout were present. This raises many questions for future research and underscores the necessity of studying these indirect effects, which may prove surprising. Future studies may indicate that trout are mediating interference competition, rather than exploitation, in this system.

These results indicate that predator presence may decrease the competitive pressures that the Jackson Lake Spring Snail faces from the New Zealand Mud Snail by reframing consumer foraging priorities and suppressing competition. Such a result indicates that competition between the snails may already be modified by species that coexist with P. antipodarum and P. robusta, a possibility which could help to explain why the invasive snail was able to quickly spread over Polecat Creek but not Marmot

Spring. If possible, field studies should test this prediction in the natural habitat of these snails. My

work conclusively demonstrates the importance of environmental effects to the New Zealand Mud Snail

and its interactors and underlines the importance of field experiments for determining the variables that

drive species interactions and invasions.

APPENDIX A: Summary of Statistics for Chapter I: Host-Parasite Coevolution

Table 1: Summary statistics for coevolution experiment, including F-statistics for the length analysis and likelihood ratio chi-square values for the infection analysis

	Model Effects	df	F-value	p-value
Length analysis				
	Parasite origin	1	10.48	0.0013
	Food level	1	44.65	<0.0001
	Origin x Food level	1	0.80	0.3728
Infection analysis				
	Parasite origin	1	0.34	0.5599
	Mouse	2	27.35	<.0001
	Food level	1	5.43	0.0198
	Replicate	3	4.48	0.2139
	Genotype	1	4.33	0.2277
	Origin x Food level	1	0.66	0.4166
	Origin x Genotype	2	0.19	0.9093
	Food level x Genotype	2	1.30	0.5232

Table 2: Summary statistics for snail behavior and resource use, including F statistics for snail behavior under a repeated measures ANOVA as well as factorial ANOVAs for snail growth, mortality, and chlorophyll α density.

Model Effects	df	F-value	p-value
Snail behavior			
P. antipodarum			
Fish	1	31.46	<.0001
Snail Density	1	0.18	0.6771
Competition Type	1	5.74	0.0213
Day	10	5.21	<.0001
Run	1	0.02	0.8951
Bench	1	2.47	0.1236
Observer	1	3.12	0.0530
Fish*Density	1	0.00	0.9583
Fish*Comp Type	1	0.11	0.7384
Density*Comp Type	1	0.00	0.9900
Day*Fish	10	2.96	0.0013
Day*Comp Type	10	0.71	0.7117
Day*Density	10	0.77	0.6537
P. robusta			
Fish	1	7.69	0.0066
Snail Density	1	0.92	0.3387
Competition Type	1	0.18	0.6696
Day	10	1.43	0.1659
Run	1	7.96	0.0057
Bench	1	3.60	0.0606
Observer	1	16.18	<.0001
Fish*Density	1	0.05	0.8282
Fish*Comp Type	1	0.10	0.7505
Density*Comp Type	1	0.02	0.8813
Day*Fish	10	2.25	0.0146
Day*Comp Type	10	0.94	0.4970
Day*Density	10	1.42	0.1705

Table 2 cont.

Model Effects	df	F-value	p-value
Snail growth			
P. antipodarum			
Run	2	1.41	0.2572
Bench	1	0.13	0.7166
Fish	1	0.32	0.5741
Snail Density	1	2.81	0.1017
Competition Type	1	0.20	0.6601
Fish*Density	1	1.24	0.2716
Fish*Comp Type	1	1.00	0.3230
Density*Comp Type	1	0.53	0.4694
Fish*Density*Comp Type	1	0.15	0.6961
P. robusta			
Run	2	0.77	0.4689
Bench	1	1.61	0.2116
Fish	1	0.08	0.7847
Snail Density	1	2.71	0.1077
Competition Type	1	1.18	0.2850
Fish*Density	1	1.18	0.2835
Fish*Comp Type	1	2.43	0.1275
Density*Comp Type	1	0.09	0.7641
Fish*Density*Comp Type	1	1.70	0.6961
Mortality			
P. antipodarum			
Run	2	6.98	0.0026
Bench	1	0.00	0.9536
Fish	1	5.83	0.0205
Snail Density	1	3.78	0.0591
Competition Type	1	0.98	0.3285
Fish*Density	1	0.69	0.4104
Fish*Comp Type	1	0.23	0.6366
Density*Comp Type	1	0.11	0.7472
Fish*Density*Comp Type	1	0.90	0.3488
P. robusta			
Run	2	31.06	<.0001
Bench	1	1.51	0.2272
Fish	1	1.20	0.2796
Snail Density	1	1.46	0.2346
Competition Type	1	0.00	0.9552
Fish*Density	1	5.07	0.0302
Fish*Comp Type	1	0.56	0.4587
Density*Comp Type	1	0.53	0.4724
Fish*Density*Comp Type	1	0.09	0.7668

Table 2 cont.

Model Effects	df	F-value	p-value
Chlorophyll density			
Experimental runs 2 and 3			
Run	1	20.92	<.0001
Snail Density	1	2.79	0.1037
Competition Type	1	1.85	0.1817
Fish	1	0.20	0.6584
Density*Comp Type	1	0.59	0.4487
Density*Fish	1	1.47	0.2338
Comp Type*Fish	1	0.16	0.6932
Density*Comp Type*Fish	1	2.13	0.1530
Run 2			
Snail Density	1	27.37	<.0001
Competition Type	1	1.77	0.1967
Fish	1	14.00	0.0011
Density*Comp Type	1	0.02	0.8864
Density*Fish	1	0.41	0.5269
Comp Type*Fish	1	3.00	0.0970
Density*Comp Type*Fish	1	3.56	0.0725

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