AN ABSTRACT OF THE DISSERTATION OF

Leah Mupas Segui for the degree of Doctor of Philosophy in Zoology presented on June 11, 2019.

Title: State-structured Species Interactions and Their Consequences Across Levels of Biological Organization

Abstract approved: _____
Mark Novak

A central challenge for ecology is to understand the dynamic nature of species interactions. A classic approach to community ecology assumes that individuals within a species are functionally identical and that consumer-resource dynamics can be predicted solely by using species abundances. However, one species can consist of multiple functional groups, as diet differences among life stages of a single species may be greater than the diet differences among species. Because of variation in diets over a lifetime, we must ask: under what circumstances can individuals be simplified into a collective species, and when does it matter to focus on intraspecific variation? I examined the role of stage structure in species interactions and its consequences across levels of biological organization.

In chapter 2, I conducted a functional response experiment in the laboratory to examine the role of body size, temperature, ontogenetic stage, and larval stonefly density on crayfish feeding rates. I found that the effect of temperature was stage-dependent as juveniles increased feeding with temperature, whereas adult feeding rate was similar across temperature treatments. Adult crayfish reduced their attack rate with increased stonefly density, which is either reflective of their biology or an artifact of experimental

design. Nonetheless, size- and temperature- dependent functional response models that included stage structure performed better than models without stage structure, highlighting the importance of incorporating stage structure into measures of species interaction strengths.

In chapter 3, I used metacommunity theory as a lens to explore how crayfish ontogenetic stage and sex alter the effect of diet on gut microbial communities. There was little overlap in the microbial community between crayfish in the lab and those in the wild, indicating that gut microbes are transient. The similarities in microbial community composition between food items and crayfish suggest that their gut microbes are mainly from their food and not from the surrounding environment. There were stage- and sex-dependent effects on gut microbial communities, likely due to differences in behavior and physiology between the stages and sexes. Though stage, sex, and diet influenced gut microbial communities, they had low explanatory power. Addressing connectivity between hosts or feedbacks between the host and the environment on gut microbial communities are potential avenues for future work.

Species introductions can alter the relationship between trophic interactions and ecosystem processes. Often, introduced species reduce the abundance and diversity of biota in recipient food webs. However, ontogenetic diet shifts in the introduced species can alter the presence, degree or direction of these impacts on native species, making it difficult for scientists and managers to predict the ecological consequences of species introductions. In chapter 4, I conducted a manipulative field experiment to assess the effects of crayfish species identity and ontogenetic stage on benthic invertebrate

composition and abundance as well as leaf litter breakdown by native signal crayfish (*Pacifastacus leniusculus*) and introduced ringed crayfish (*Faxonius neglectus neglectus*). Treatments with signal crayfish or adult crayfish had higher reductions in leaf litter relative to treatments with introduced crayfish and juvenile crayfish. Alpha and beta diversity of benthic invertebrates was similar among treatments, but there were fewer shredders in treatments with adult crayfish. Thus, I show that ontogenetic stage and native vs. non-native status both matter for understanding the impact of species introductions on local ecological communities and ecosystem processes.

In Chapter 5, I presented the challenges of uniting ecological theory and empirical data. I designed an experiment that investigated the roles of consumer body size and ontogenetic stage, environmental temperature, and resource quality on consumer-resource interactions. My goal was to bridge the Metabolic Theory of Ecology and Ecological Stoichiometry to describe how the balance leaf litter nutrient availability and crayfish elemental demand govern their feeding rates and nutrient cycling over ontogeny, but my models did not fit the data well. This developed into a philosophical dilemma: as scientists, do we choose to work in systems that will produce data that we know will fit our model, or do we test a model in various systems to see how generalizable our predictions can be? I explored the culture of science, focusing on the existence of paradigms in ecology that discourage "negative results" and the obstacles one might face in conducting functional response experiments. Specifically, I reviewed experimental design and statistical methods used in functional response literature and how they can be biased to maintain paradigms in ecology.

My dissertation contributes to our understanding on the role of intraspecific variation in ecology. I examined consumer-resource interactions (Ch. 2), community structure (Ch. 3), and ecosystem processes (Ch. 4 and 5) to assess the effect of stage structure on species interactions and their consequences across multiple scales of organization. I illustrated the challenges of bridging ecological theory and empirical data and the approaches that hinder or advance the field. Overall, my work has demonstrated the importance of incorporating stage structure in ecological studies and that this information advances both ecological theory and applied efforts.

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State-structured Species Interactions and Their Consequences Across Levels of Biological Organization

by

Leah Mupas Segui

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APPROVED:

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Major Professor, representing Integrative Biology

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Head of the Department of Integrative Biology

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Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

_____

Leah Mupas Segui, Author

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I would first like to thank my committee, starting with my advisor Mark Novak. My first goal in grad school was to not be afraid of equations/not have my eyes glaze over when reading an equation. My second goal was to craft a dissertation I can confidently call mine. Mark, thank you for helping me achieve these and numerous other goals. You have shown me such patience in helping me work through the ugliest code, unexpected data patterns, and poorly written drafts. You have definitely shaped the way I think about ecology. I've learned to become a more efficient coder, a better writer, and a more confident scientist. Thank you for the space grow, learn, and explore, to have broader conversations about science, and to shed a few tears. Most importantly, thank you for giving me the support to become the type of scientist I want to be. Dana Warren, thank you for the chats, the letters of recommendation, getting me to write my first chapter, the positivity in your self-deprecation, and for being there during the emotional rollercoaster that is grad school. Jason Dunham, for your perspectives on science outside of academia and for connecting me with Dave and Doug, which was a fantastic introduction to freshwater systems. Vrushali Bokil, for serving as my GCR and for your equity work for the university. Felipe Bareto, for joining the party during the last stretch. Though not officially part of my committee in the end, Dave Lytle for being part of my proposal meeting and Ben Dalziel for being part of my qualifying exam.

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CONTRIBUTIONS OF AUTHORS

Chapter 2: Mark Novak helped design the study, contributed to data analysis, and provided critical revisions.

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Chapter 5: Mark Novak helped design the study, contributed to data analysis, and provided critical revisions.

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DEDICATION

To my parents:

Mom,

for instilling a passion for food and event planning in me

&

Dad,

for encouraging me to talk to strangers and reminding me that

I can do anything.

PREFACE

"If we turned to the sea, or a fresh-water pond, or the inside of a horse, we should find similar communities of animals, and in every case we should notice that food is the factor which plays the biggest part in their lives, and that it forms the connecting link between members of communities." Charles Elton, 1927

State-structured Species Interactions and Their Consequences Across Levels of Biological Organization

CHAPTER 1 - Introduction

A central challenge for ecology is to understand the dynamic nature of species interactions. Such knowledge is considered key to develop models that can capture the influence of species interactions on ecosystem processes and to predict ecological responses to environmental change (Sutherland et al. 2013). At the core of food web theory are models that describe the reciprocal effects between consumers and their resources (reviewed in Wangersky 1978). A classic approach to community ecology assumes that individuals within a species are functionally identical and that consumer-resource dynamics can be predicted solely by using species abundances. However, one species can consist of multiple functional groups, as diet differences among life stages of a single species may be greater than the diet differences among species (Polis 1984, Woodward and Hildrew 2002, Rudolf and Lafferty 2011). Because of variation in diets over a lifetime, we must ask: under what circumstances can individuals be simplified into a collective species, and when does it matter to focus on intraspecific variation?

Stage-structured interactions

Individuals undergo ontogenetic development, starting as juveniles growing in body size and maturing into reproductive adults. Natural populations are structured, composed of different sizes, ages, and ontogenetic stages. Such populations undergo ontogenetic niche shifts in which energetic and nutrient requirements, resource use, competitive ability, and predator vulnerability change over an individual's lifetime (Elser et al. 1996, Werner and Giliiam 1984). Single species population models consider growth between ontogenetic stages and its effect on population dynamics (Caswell 2006). Ontogenetic niche shifts are prevalent in nature, occurring in 80% of animal taxa (Werner

1988), yet despite their ubiquity, stage structure is often left out of models depicting species interactions. Interaction types and strengths can vary over an individual's lifetime, making it difficult to predict the consequences of stage structure on community structure and ecosystem processes (Miller and Rudolf 2011,Figure 1). Individuals in larger, older stages are expected to have broader diets, consume more food, be more efficient in capturing resources, and be at less risk of predation than smaller, younger stages, meaning that an individual's ecological impact should increase with size (Osenberg and Mittelbach 1989, Woodward and Warren 2007, Vucic-Pestic et al. 2010). Alternatively, individuals in smaller stages have higher metabolic rates per unit mass, and can place a greater demand on resources than larger individuals (deRoos and Persson 2013). However, if certain interactions and processes correspond to an individual's ontogenetic stage regardless of size, then size-based approaches to measuring species will be inaccurate (Rudolf and Rasmussen 2013).

The consequences of ontogenetic development occurring at the individual level can be seen across levels of biological organization. Stage structure alters interaction types and strengths that govern population and community dynamics, which has implications for species coexistence and food web stability (Nakazawa 2014, Ramos-Jiliberto et al. 2016). At the ecosystem level, stages can vary in their functional role. Body size may not be functionally equivalent to ontogenetic stage; ecosystem processes such as net primary production, respiration, and nutrient cycling have been shown to vary as a function of an individual's ontogenetic stage regardless of body size (Gutiérrez-Yurrita and Montes 1999, Rudolf and Rasmussen 2013a,b).

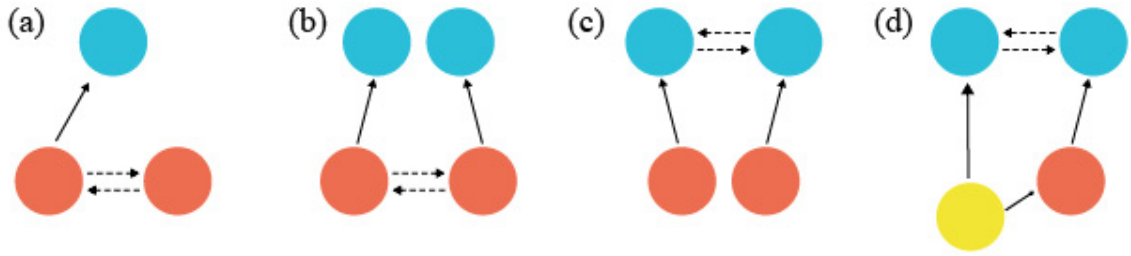

Figure 1 Examples of ontogenetic niche shifts in consumer-resource interactions. Blue circles represent consumers, red circles represent resources, and the yellow circle represents a basal resource. Multiple circles within a trophic level represent ontogenetic stages. Solid arrows represent trophic interactions and the direction of energy flow and dashed arrows represent transitions between juvenile and adult stages. A) The consumer feeds on one ontogenetic stage of the resource whereas the other stage has a refuge from predation. B) Each resource stage has a different consumer. C) Each consumer stage has a different resource. D) The consumer is both a predator of the intermediate consumer and competitor for the basal resource. Adapted from Miller and Rudolf 2011.

By ignoring such changes in life histories, we are left with incomplete depictions of food webs (Rudolf and Rasmussen 2013, Rudolf and Lafferty 2011, Polis 1984). This discrepancy can be problematic for natural resource managers as juveniles and adults may not respond similarly to biological control strategies (Buhle et al. 2005, Pardini et al. 2009, Zipkin et al. 2009). A major gap in food web theory is how the dynamic nature of such stage structure alters the forms and strengths of species interactions. While ecological theory on stage-structured interactions is increasingly well developed, they have vastly outpaced empirical tests (de Roos et al 2003).

Dissertation Overview

The primary focus of my dissertation work is to bring attention to the important but often ignored role of ontogenetic variation. I address the role of stage structure in population dynamics, community structure, and ecosystem processes in aquatic systems. Accurate characterizations of stage structure's effect across levels of biological organization both advance ecological theory and highlight the complex interactions that

need to be taken into account for natural resource management.

The functional response describes the effect of consumers on their resources and underlies measures of species interaction strengths. In Chapter 2, *Temperature-dependent effects on feeding rates across ontogeny*, I ask whether the strength of consumer-resource interactions is best predicted by consumer size, a reflection of metabolic demand, or by consumer ontogenetic stage regardless of body size. I use predictions from the Metabolic Theory of Ecology (Brown et al. 2004) in a functional response framework to test how crayfish (*Pacifastacus leniusculus*) ontogenetic stage, body size, and temperature affect their feeding rates on larval stoneflies (Perlidae) across varied stonefly densities. I conducted a laboratory experiment to measure crayfish feeding rates and used maximum likelihood model fitting techniques to estimate the effect of body size and temperature on their feeding behaviors.

The interactions between consumers and their resources not only affect the communities in which they reside, but also the microbial communities that reside within them. Microbial ecology is a growing field as scientists begin to understand their importance in human and animal health; yet despite their importance, microbial community assembly, dynamics, and persistence are not well understood. In Chapter 3, *host ontogenetic stage and sex modulate the effect of diet on gut microbial communitites*, I used metacommunity theory as a lens to explore how host traits affect gut microbial community structure (Leibold et al. 2004). Individual hosts can be viewed as patches of habitat that are colonized by microbes compatible with the host environment. Intraspecific variation in host traits, such as ontogenetic stage and sex, can affect gut microbial community structure through differences in behavior and physiology between

stages and sexes. I controlled the diets of juvenile and adult, male and female crayfish and used molecular techniques to examine how crayfish ontogenetic stage, sex, and diet affect gut microbial communities.

Species introductions pose considerable risks to ecosystem function. Introduced species can cause declines in local species richness, and mitigating their impact on ecosystem services can costs millions of dollars annually (Pyšek and Richardson 2010, Tobin 2018). In 2015, the ringed crayfish (*Faxonius neglectus*), native to the Ozark region of the United States, was discovered in the Willamette Valley river basin. Ringed crayfish have been found in southern Oregon since the 1970's and have displaced native signal crayfish in many areas (Bouchard 1977, Pearl et al. 2013). Despite their potential threat to the functioning of local freshwater systems, there are no ecological studies that measure their impacts on ecological freshwater communities in their introduced range. Previous studies have shown that invasive crayfish reduce invertebrate abundance more than native crayfish (Twardlocheb et al. 2013) but that their impacts on ecosystem processes are mixed (Usio et al. 2004, 2006). However, since most studies on introductions focus on the adult stage, the full impact of an introduced species across life stages is not well understood. In Chapter 4, *Diaspora and detritus: non-native crayfish influence leaf litter breakdown but not benthic invertebrate community composition,* I conducted a manipulative field experiment to elucidate the impacts of juvenile and adult crayfish on benthic invertebrate community structure and leaf litter breakdown, and to determine how these impacts vary between species.

In Chapter 5*, Approximating truth: challenges in bridging theory and data in a functional response framework*, I explored the challenges of uniting ecological theory and

empirical data. I designed an experiment that investigated the roles of consumer body size and ontogenetic stage, environmental temperature, and resource quality on consumer-resource interactions. This experiment incorporated ideas from Ecological Stoichiometry, a branch of ecology that explores how the balance of energy and elements governs ecological interactions and processes across biological scales of organization (Elser and Sterner 2002). Specifically, my goal was to bridge the Metabolic Theory of Ecology and Ecological Stoichiometry to describe how the balance leaf litter nutrient availability and crayfish elemental demand govern their feeding rates and nutrient cycling over ontogeny. However, my nutrient samples were mishandled and my models did not fit my data well. The latter developed into a philosophical dilemma: as scientists, do we choose to work in systems that will produce data that we know will fit our model, or do we test a model in various systems to see how generalizable our predictions can be? In this chapter, I explore the culture of science, focusing on the existence of paradigms in ecology that discourage "negative results" and the obstacles one might face in conducting functional response experiments. Specifically, I review experimental design and statistical methods used in functional response literature and how they can be biased to maintain paradigms in ecology. My hope for this chapter is that those who are learning how to work in the middle ground of ecological theory, data, and statistics gain insight into our limits of understanding and ways to move the field forward.

My dissertation contributes to our understanding on the role of intraspecific variation in ecology. I examined consumer-resource interactions (Ch. 2), community structure (Ch. 3), and ecosystem processes (Ch. 4 and 5) to assess the effect of stage structure on species interactions and their consequences across multiple scales of

organization. I used ecological theory to guide my questions and interpretations, and tested my hypotheses using manipulative laboratory and field experiments as well as molecular and statistical techniques. I illustrated the challenges of bridging ecological theory and empirical data and the approaches that hinder or advance the field. Overall, my work has demonstrated the importance of incorporating stage structure in ecological studies and that this information advances both ecological theory and applied efforts. If we want to manage our natural resources, we need to remember that every living thing needs to eat, but they do not all eat the same way.

CHAPTER 2 – TEMPERATURE-DEPENDENT EFFECTS ON FEEDING RATES ACROSS ONTOGENY

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Abstract

The functional response describes the effect of consumers on their resource and underlies measures of species interaction strengths. Metabolic theory provides predictions on how consumer body size and environmental temperature affect functional response parameters (attack rate and handling time), but how these affect feeding rates across consumer life stages in not well understood. We measured signal crayfish (*Pacifastacus leniusculus*) feeding rates to understand larval *Perlidae* stonefly density, crayfish body mass and ontogenetic stage, and water temperature affect functional response parameters. We found that stage-structured functional response models performed better than non-stage structured models in capturing crayfish feeding rates. Adult crayfish feeding rates were similar across temperature treatments and that juvenile crayfish feeding rates increased with temperature. Handling time had a positive relationship with temperature and negative relationship related to crayfish body mass. Attack rate was independent of temperature and had a negative relationship with body mass, though it is unclear whether the latter is reflective of adult crayfish behavior in the wild or a result of experimental arena size. These results emphasize the importance of incorporating stage structure in the functional response framework to increase accuracy in measuring the strength of species interactions.

Introduction

The functional response describes how consumer feeding rates respond to changing resource densities and are a key component to understanding the strength of consumer-resource interactions (Oaten and Murdoch 1975). In its most simplistic form, the functional response consists of two components: 1) the consumer's attack rate, the

rate at which a consumer encounters and captures its resource, and 2) handling time, the time needed for a consumer to kill, ingest, and digest its resource (Holling 1959). Classic functional response models assume that consumer feeding rate is solely influenced by resource density. However, ecologists now recognize that components of the functional response are also influenced by consumer traits, such as body size (Vucic-Pestic et al. 2010, Kalinkat et al. 2013). Individuals increase in size as they grow from juveniles to adults, and whether feeding rates vary predictably with consumer body mass or vary between life stages irrespective of body mass is not well understood.

Metabolic theory offers predictions on how functional response parameters, attack rate and handling time, are influenced by consumer body mass and environmental temperature. According to MTE, attack rate scales with a 3/4 power law relationship of body mass, and handling time scales with a negative 3/4 power law relationship of body mass, resulting in an increase in feeding rate with consumer body mass (Brown et al. 2004). For ectotherms, rising ambient temperature results in a higher metabolic rates and increased energetic demand (Clarke and Fraser 2004). Attack rate has been observed to increase with temperature (Vasseur and McCann 2005) and handling time is expected to decrease with temperature via increased digestion rate and/or decreased time capturing and ingesting a resource (Sentis et al. 2012). The ¾ power law is used to describe metabolic relationships across a wide range of taxa though other values have been proposed for metabolic scaling over ontogeny within a species (Glazier 2006). Metabolic theories in general, when applied to functional responses, predict patterns of higher attack rates, lower handling times, and overall greater feeding rates with increases in consumer body mass and temperature.

MTE's utility lies in its ability to broadly characterize species interactions based on body mass and temperature. However, MTE may not be appropriate in a functional response framework if individuals switch diets over course of their lifespan, thereby exhibiting ontogenetic niche shifts (Werner and Gilliam 1984). These shifts can occur in association with changes in morphology, habitat use, or physiological demand (Wilbur 1980). Intraspecific variation in body mass may result in multiple functional groups within a population, as individuals of the same species change their diet and trophic position as they grow(Woodward and Hildrew 2002, Rudolf and Lafferty 2011, Rudolf and Rasmussen 2013, deRoos and Persson 2013). Ontogenetic niche shifts are prevalent in nature, occurring across a broad spectrum of taxa and environments (Werner and Gilliam 1984). Despite this ubiquity, there are few studies that measure functional responses over ontogeny (Miller et al. 2008, McCoy et al. 2011, Long and Whitefleet-Smith 2013). Without stage structure, conventional and allometric functional response models may provide inaccurate information about a consumer's impact on resource populations, which is especially important when using consumer-resource dynamics for management decisions (Long and Whitefleet-Smith 2013). Shifts in interspecific interactions among consumer stages may deviate from MTE expectations on how body size and temperature influence how consumers respond to changing resource density, yet there is a lack of empirical studies that integrate metabolic theory and life history into a functional response framework.

To determine the degree to which metabolic theory captures variation in feeding rates across consumer ontogeny, we used signal crayfish (*Pacifastacus leniusculus*) to measure their feeding rates on Perlid stonefly larvae while experimentally varying

temperature and crayfish body size. Signal crayfish are omnivorous as a species; however, individuals are reported to undergo a diet shift, feeding primarily on aquatic invertebrates as juveniles and shifting to detritus as they grow in size (Mason 1963, but see Bondar et al. 2005). This shift has been attributed to juvenile crayfish requiring high amounts of protein for rapid growth (Momot 1995) and to adult crayfish being too large to capture fast moving invertebrates (Abrahamsson 1966). This contrasts with expectations from MTE, in which consumers are assumed to become more efficient at capturing resources as they grow larger. We hypothesized attack rate to be higher and handling time to be lower for juvenile crayfish compared to adult crayfish. We also hypothesized temperature to have a positive relationship with attack rate and a negative relationship with handling time regardless of crayfish stage. Within each stage, we hypothesized an increase in attack rate and decrease in handling time with juvenile crayfish body mass and the opposite pattern with adults.

Materials and Methods

Laboratory experiment

Crayfish (33-95 mm total length, measured from rostrum to tail) were collected from streams within Oregon State University's MacDonald-Dunn Research Forest northwest of Corvallis, OR, the traditional territory of the Chepenefa band of the Kalapuya. Based on preliminary crayfish diet data, larval stoneflies (*Perlidae* sp.) were the most common mobile aquatic invertebrate in crayfish stomachs. We captured Perlids stonefly larvae (8-12 mm) using kick nets to be used in feeding trials. Animals were held and experiments were conducted at the Aquatic Animal Health Laboratory in Corvallis,

OR. Crayfish and Perlids acclimated to laboratory conditions for 1 week and were fed algae pellets ad libitum daily.

Crayfish were starved for 48 hours prior to the start of the experiment at ambient stream temperature (14°C), followed by a 24 hour acclimation period to one of the treatment temperature. At the beginning of each trial, 1 crayfish was placed inside a circular plastic arena (26 cm diameter) and acclimated for 30 minutes. The arena was filled with filtered stream water of 10, 15, or 20°C to reflect the annual range of temperatures occurring in local streams. Water was shallow enough for crayfish to capture stoneflies at the surface of the water (20 cm), removing the possibility of a refuge for the stoneflies. Each experimental unit contained 1, 3, 5, 10, 20, 30, or 50 late instar larval stoneflies. Larval stonefly densities were based on previous benthic surveys from streams within the McDonald-Dunn forest (Preston et al. 2018). Treatment combinations were randomized per trial (n=142 trials). Consumed stoneflies were counted and immediately replaced with new individuals to maintain constant resource densities. After 30 minutes, crayfish were removed from the arena and measured for length (carapace length, CL and total length, TL, mm), sex, and placed in a drying oven for 24 hours to obtain dry weight (g).

Statistical methods

We used a hyperbolic Type II functional response model to describe the relationship between crayfish per-capita feeding rates and the larval stonefly density,

$$F = \frac{aN}{1 + ahN} \qquad (1),$$

(Holling 1959) where F is the individual consumption rate of a crayfish, N is number of stoneflies, a is the attack rate and h is the handling time. In accordance with metabolic theory, attack rate and handling time were assumed to follow a power-law relationship with consumer mass and an exponential relationship with temperature (Brown et al. 2004), with

$$a = a_0 m^{s_a} e^{\frac{E_a T - T_0}{kTT_0}} \quad (2)$$

and

$$h = h_0 m^{s_h} e^{\frac{E_h T - T_0}{kTT_0}} \quad , (3)$$

where h_0 and a_0 are normalization constants at temperature T_0 (15°C = 288.15 K), m is crayfish body mass (dry weight, g), s_a and s_h are allometric scaling exponents, E_a and E_h are activation energies, k is the Boltzmann constant (8.62 x 10-5 eV K^{-1}), and T is absolute temperature (K).

We used maximum likelihood estimation to fit several nested functional response models using the mle2 function in the "bbmle" package in R (Bolker 2017). As stoneflies were replaced after being consumed, we used a Poisson distribtuion to calculate the likelihood of our data based on the chosen model. The full model considered attack rate and handing time to be dependent on body size and temperature as described by equations 2 and 3. We then fit simplified models that removed all possible combinations of dependencies on body size or temperature (16 models total, supplementary material). The best performing model was selected on the basis of having the lowest AIC_C value (Burham and Anderson 2004). From this analysis, there were 2 top performing models

that were indistinguishable from each other ($\Delta AIC_c < 2$). We used these two models to repeat the analysis for only juveniles (total lengh < 60 mm) and only adults (total length ≥ 60 mm). We added the likelihoods of the juvenile-only and adult-only models and calcualted AICc to compare model performance without versus with the addition of stage structure, wand calculated AICc.

Results

Stonefly consumption by crayfish varied with crayfish body size and water temperature (Figure 2). Irrespective of size, crayfish feeding rates were 56% higher at 20°C than 10°C and 20°C (2.9 vs. 5.1 stoneflies per 30 min, respectively). Similarly, irrespective of temperature, adult crayfish consumed on average 45% more stoneflies than juvenile crayfish (22 vs. 10 stoneflies, respectively). Juvenile crayfish saturated at low densitites (~1 stonefly/m^2) at 10 and 15°C (Figure 2a,b).

Two non-staged structured models models outperformed all other non-stage structured models in describing the response of crayfish feeding rates to variation in prey density, crayfish size, and temperature and were indistinguishable from each other ($\Delta AIC_c < 2$, Figure 3, Appendix Table 1a). In the best performing model, attack rate (s_a = -0.979 ± 0.35 SE) and handling time (s_h = -0.846 ± -0.1 SE) decreased with crayfish body mass (Table 1). Attack rate was independent of temperature while handling time decreased with temperature (E_h = -0.615 ± 0.09 SE). In the second best performing model (ΔAIC_c = 1.32), both attack rate and handling time depended on crayfish body mass and water temperature (Table 1). The effects of body mass and water temperature on handing time and the effect of water temperature on attack rate were qualitatively similar to estimates in the best performing model (Table 1). Unlike the best performing

model, the second best performing model showed a positive relationship between the activation energy of attack rate and water temperature ($E_a = 0.44 \pm 0.44$ SE).

The stage structured model corresponding to the top-performing model out-performed the latter ($\Delta AIC_c = 5.15$). For this model, the normalization constant for attack rate (a_0) for juveniles was an order of magnitude higher than that for adults (Table 2). The mass scaling exponent of attack rate (s_a) was nine times greater for adults compared to juveniles, meaning that attack rate declined more rapidly with size for adults than for juveniles (Table 2, Figure 4b and a). Though there was no relationship between water temperature and attack rate, juveniles had a higher intercept for attack rate relative to adults (Figure 4c,d). Handling time decreased with body size for both juvenile and adult crayfish, with the the smallest juveniles exhibiting the longest handling times (Figure 4 e,f). Handling time decreased with temperature similarly between juveniles and adults (Figure 4g,h). Overall, attack rate and handling time estimates were more variable for adults than for juveniles (Figure 4).

Discussion

Metabolic theory provides predictions on how consumer body size and environmental temperature affect consumer-resource interactions. However, variation in feeding behaviors across ontogeny may not be captured by allometric scaling laws alone. Our study indicates that the effect of environmental temperature and consumer body size on feeding rates varies between consumer life stages and that accounting for stage structure is necessary to increase accuracy in measuring the strength of species interactions.

Plate 2.1. Physical model of an air horn used to celebrate a successful PCR run (Ch. 3), functioning R code (Ch. 2-5), or the completion of a dissertation (final page of this document). Verbal air horn preferred by the author of this dissertation, a hip hop enthusiast and costume connoisseur.

Incorporating ontogeny into the functional response framework provides additional biological information that is not captured in size-structured models. MTE predicts that attack rate increases with body mass and thus consumers are expected to be more efficient at searching an area and capturing resources as they grow (Brose 2010). However, crayfish are expected to become less efficient at capturing stoneflies as they grow as large crayfish are not efficient at capturing fast moving prey (Abrahamson 1966, but see Parkyn et al. 2001). Alternatively, juvenile crayfish experience the fastest growth rates and therefore must actively pursue invertebrates to meet their high energetic demands, whereas adult crayfish may not need invertebrates if their maintenance and reproductive costs are not as energetically demanding (Daborn 1975). We found that attack rate decreased with body mass, supporting our hypothesis on crayfish behavior over ontogeny. Juvenile crayfish encountered and captured stoneflies quickly, as shown by the higher attack rate, however they also had long handling times, which resulted in low feeding rates overall. When we conducted separate analyses for juveniles and adults, we found that these models performed better than models based on the full dataset. This shows that the effect of body size and temperature on functional response parameters is not the same between juveniles and adults.

Our results for the effect of temperature on functional response parameters are partially consistent with expectations from MTE. For the model including temperature dependence of the attack rate, we found the activation energy of attack rate and handling time to be within the range found in other studies on invertebrates (1.12 ± 0.51), meaning that crayfish feeding behavior is similarly affected by temperature as other ectotherms (Gillooly et al. 2001, Englund et al. 2011). Increased energetic costs associated with high

temperature are expected to increase feeding rate as individuals need more resources to meet metabolic demand (Gillooly et al. 2001). In order to meet this demand, consumers must either increase their attack rate or decrease their handling time, or both. Higher temperatures can lead to an increase in attack rate by affecting the behavior of both a consumer and its resource (Dell et al. 2011, Vucic-Pestic et al. 2011, Ohlund et al. 2014). For example, higher temperatures could have increased movement for both crayfish and stoneflies, increasing their encounter rates; higher temperatures could also negatively affect a stonefly's ability to escape a crayfish leading to an increase in capture success (Dell et al. 2011). However, since attack rate was independent of temperature in one of the top models, high feeding rates at the warmest temperature must be the result of a decrease in handling time with temperature. This is in line with predictions from MTE as handling time, the inverse of maximum feeding rate, negatively scales with warming (Rall et al. 2012).

Crayfish feeding rates varied across temperature treatments and depended body size. Our results showed that large adult crayfish fed similarly across temperature treatments while small juvenile crayfish increased feeding rates with temperature. We found that feeding rates of small crayfish saturated at the lowest stonefly density in the 10°C and 15°C treatments. In 20°C, small crayfish had the highest feeding rates and saturated at higher stonefly densities relative to the colder temperature treatments. This implies that temperature had more of an effect on the metabolic rate of small crayfish relative to large crayfish. Large adults in this study did not increase their feeding rates with temperature, which suggests that metabolic demand could outpace energy ingestion which would therefore lead to starvation (Vasseur and McCann 2005, Iles 2014). Small-

bodied individuals may have a higher capacity to adjust their metabolic rate with rising temperature and have a higher threshold for thermal stress relative to larger-bodied conspecifics (Lang et al. 2012, Messmer et al. 2017). The ability of small-bodied individuals to tolerate warm temperatures has been hypothesized to be due to their body's high surface area to volume ratio that allows them to more readily dissipate heat relative to large-bodied individuals. The pattern of declining body size with latitude has been attributed to an animal's capacity to regulate heat loss ('Bergmann's rule', Bergmann 1847). Thus, small-bodied animals may be better adapted to a warming world, and as a consequence, the shift towards smaller body sizes can change the strength of species interactions with implications for food web structure and function (Brose et al. 2012).

Experimental design has the potential to influence the behavior of consumers or their resource and may have influenced our measurement of attack rate. Uiterwaal and Delong (2018) found that ladybeetles are more efficient predators in larger arenas, as the constraints of smaller arenas may affect predator or prey behavior. In our study, small crayfish reached saturation at the lowest stonefly density (3 stoneflies arena^{-1}), meaning that they were not limited by attack rate, as they were able to locate and capture stoneflies throughout the arena. In contrast, large crayfish take up a larger portion of the arena and therefore have a more limited view of potential prey compared to small crayfish. Stoneflies were able hide behind large crayfish (personal observation); therefore it took longer for large crayfish to find the stoneflies, lowering their attack rate. To link theory with data, the use of short term, small scale manipulative experiments are used to parameterize models of consumer-resource interactions to understand dynamics at larger spatial and temporal scales (Sarnelle 2003, Berlow et al. 2004, Abrams 2001). However,

recent studies have highlighted issues with laboratory methods for estimating functional response parameters and have called for understanding the mechanisms by which experimental design influences results in order to make comparisons across studies possible (Li et al. 2018, Uiterwaal and Delong 2018).

Classic functional response models assume that consumer feeding rates are homogenous. The recent incorporation of metabolic theory into the functional response framework has led to predictions of the effect of temperature and body size on feeding rates and highlights the importance of intraspecific variation on species interactions. This study shows that body size alone may not capture important ecological processes that occur throughout an individual's lifetime and we suggest that future studies incorporate ontogenetic variation into the functional response framework.

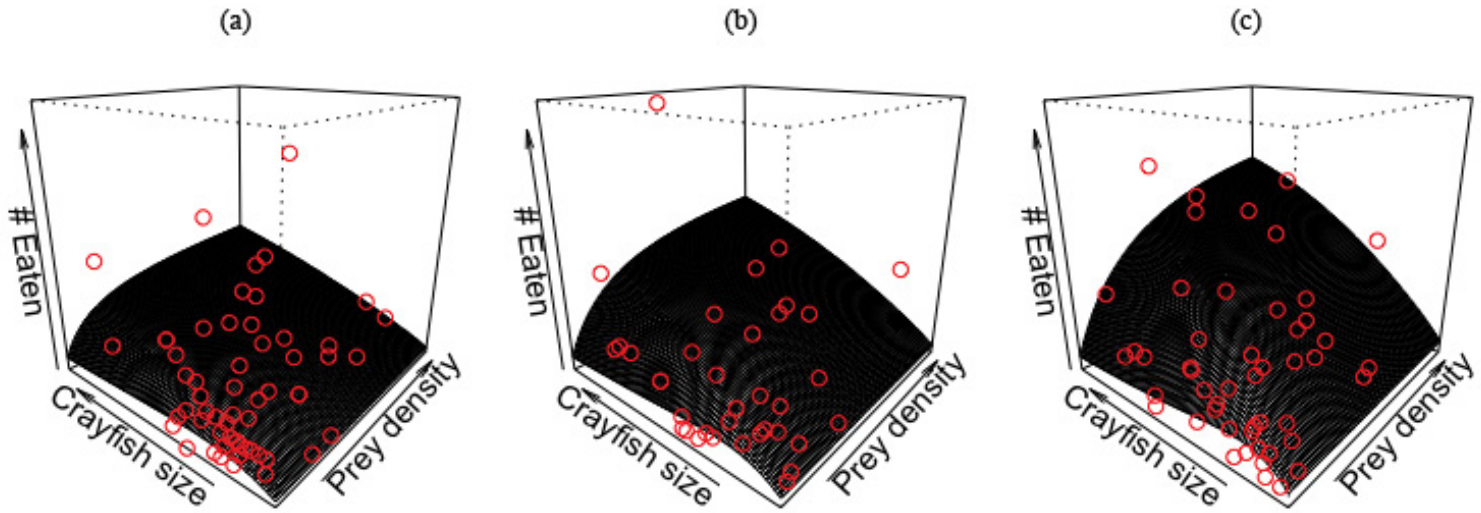

Figure 2.Three dimensional contour plot showing the number of stoneflies consumed as a function of crayfish body mass and stonefly density by temperature: 10°C (a), 15°C (b), and 20°C (c).

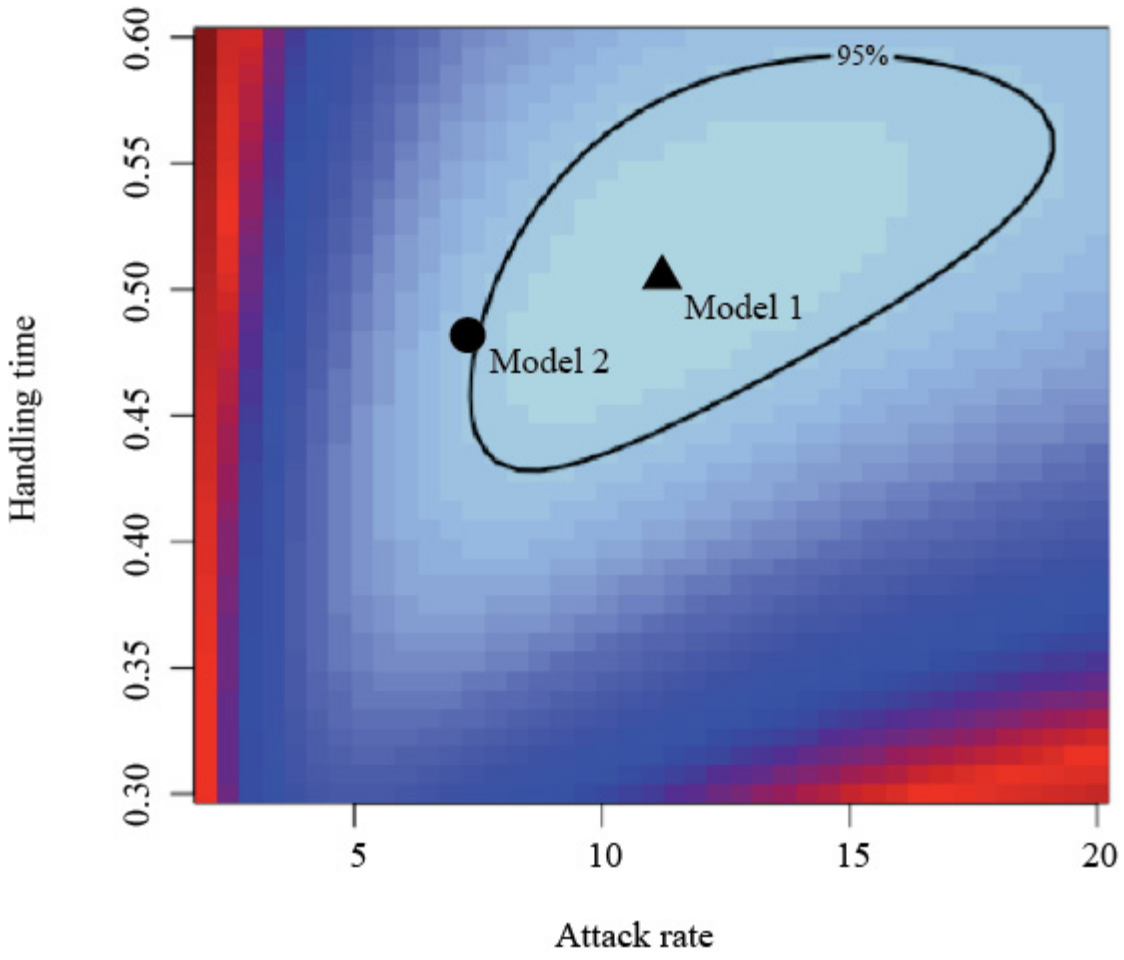

Figure 3: Likelihood surface of best performing model (Model 1) with attack rate dependent on crayfish body mass handling time dependent on both crayfish body mass and water temperature. The 95% CI is plotted for the maximum likelihood combination of attack rate and handling time. Points represent maximum likelihood estimates for two models: Model 1 (triangle) and Model 2 (circle), the second best performing model with attack rate and handling time both dependent on crayfish and body size and water temperature.

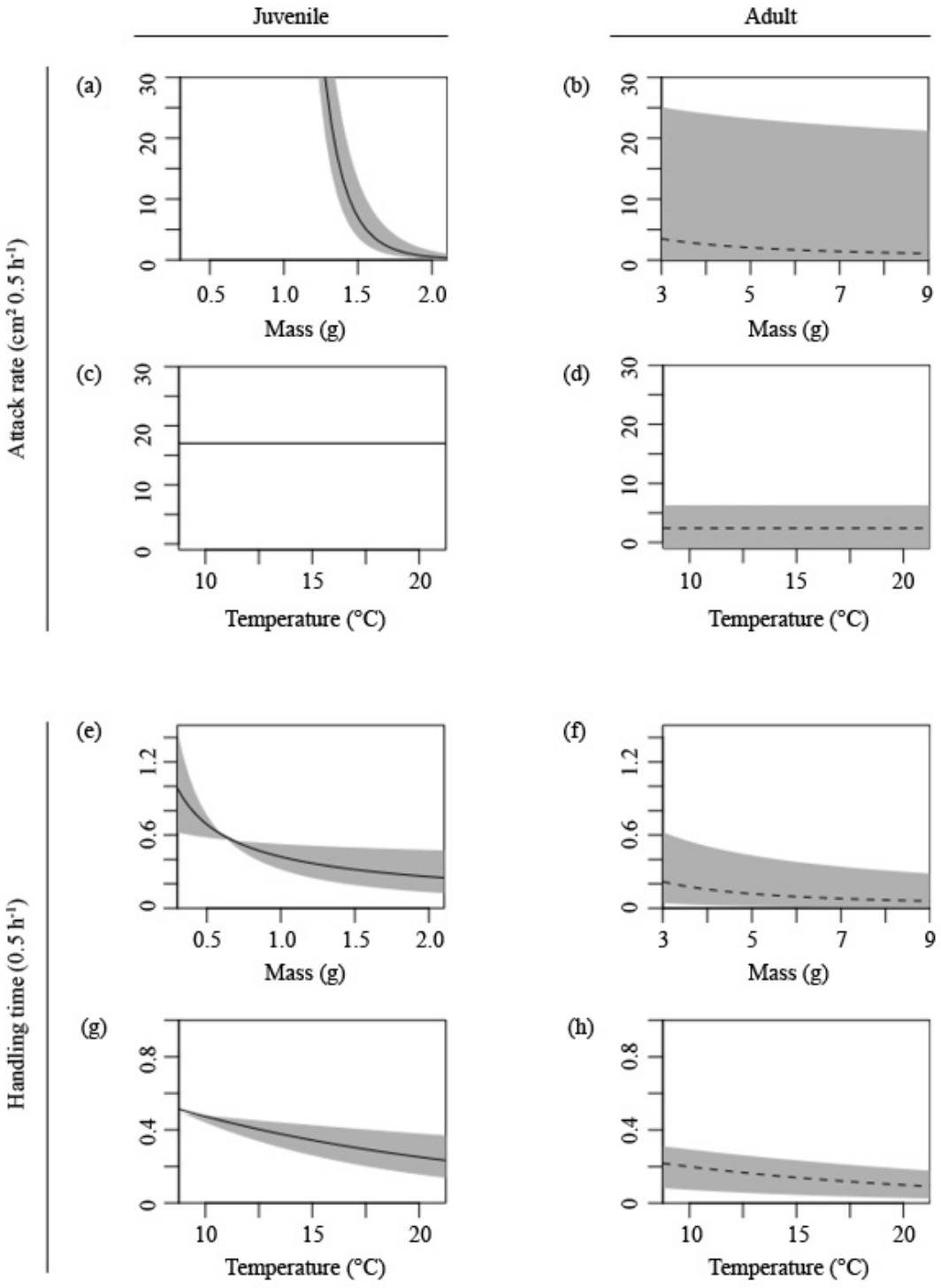

Figure 4. Juvenile (solid lines, a-d) and adult (dashed lines, e-h) attack rates and handling times as a function of crayfish mass and water temperature. Gray bands represent 95% CI**.**

Table 1: Parameter estimates of the top two performing functional response models (Δ AICc = 1.32). Estimates for Model 1 are from the best performing model with the lowest AIC_c value that includes activation energy of attack rate and handling time and body mass scaling of handling time. Estimates for Model 2 are from the second best performing model that includes activation energy and body mass scaling of attack rate and handling time.

	Estimate		S.E.	
Parameter	Model 1	Model 2	Model 1	Model 2
Normalization constant for attack rate (a_0)	11.21	7.37	6.96	4.85
Mass-scaling exponent of attack rate (s_a)	-0.98	-0.83	0.35	0.35
Activation energy of attack rate (E_a)	---	0.44	---	0.44
Normalization constant for handling time (h_0)	0.51	0.48	0.06	0.07
Mass-scaling exponent of handling time (s_h)	-0.85	-0.84	0.10	0.11
Activation energy of handling time (E_h)	-0.62	-0.53	0.09	0.14

Table 2: Parameter estimates of the top two performing functional response models for juveniles (TL < 60 mm , a) and adults (TL ≥ 60 mm, b). Estimates for Model 1 are from the best performing model with the lowest AIC_c value that includes activation energy of attack rate and handling time and body mass scaling of handling time. Estimates for Model 2 are from the second best performing model that includes activation energy and body mass scaling of attack rate and handling time.

a)

	Estimate		S.E.	
Juvenile crayfish (total length ≤ 60 mm)	Model 1	Model 2	Model 1	Model 2
Normalization constant for attack rate (a_0)	2.82×10^2	1.74×10^2	0.004	2.48×10^2
Mass-scaling exponent of attack rate (s_a)	-9.12	-8.54	0.80	2.18
Activation energy of attack rate (E_a)	---	0.51	---	0.68
Normalization constant for handling time (h_0)	0.42	0.41	0.05	0.06
Mass-scaling exponent of handling time (s_h)	-0.70	-0.74	0.29	0.30
Activation energy of handling time (E_h)	-0.57	-0.50	0.18	0.21

b)

Adult crayfish (total length > 60 mm)	Model 1	Model 2	Model 1	Model 2
Normalization constant for attack rate (a_0)	11.33	1.69	9.36	2.58
Mass-scaling exponent of attack rate (s_a)	-1.06	-0.03	0.47	1.09
Activation energy of attack rate (E_a)	---	1.10	---	0.80
Normalization constant for handling time (h_0)	.791	0.33	0.28	0.33
Mass-scaling exponent of handling time (s_h)	-1.20	-0.67	0.25	0.67
Activation energy of handling time (E_h)	-0.63	-0.17	0.11	0.42

CHAPTER 3 – HOST ONTOGENETIC STAGE AND SEX MODULATE THE EFFECT OF DIET ON GUT MICROBIAL COMMUNITIES

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Abstract

Microbiota influence host fitness, yet despite their importance, microbial community assembly, dynamics, and persistence is not well understood. By using meta-community theory as a lens to understand variation in microbial communities, individual hosts can be viewed as patches of habitats that are colonized by microbes compatible with the host environment. The host environment is dynamic, and it is unclear how variation of the host traits alters microbial communities. We conducted a manipulative experiment in the laboratory to understand how host diet (natural, algae-only, mussels-only), ontogenetic stage, and sex alter gut microbial communities in signal crayfish (*Pacifastacus leniusculus*). We found one core taxa among all crayfish and microbial composition similar to their food items, suggesting that gut microbes are transient and largely driven by diet. We also found interactions between life stage (juvenile and adult) and sex, as well as between sex and diet in shaping the gut microbiota of crayfish. Differences between juveniles and adults are likely due to physiological changes that accompany reproductive organ growth and hormonal changes. Behavioral differences between male and female crayfish as well as hormonal differences between the sexes may be responsible for sex-dependent differences in gut microbial communities for crayfish in the wild. Though diet, ontogenetic stage, and sex were important factors for crayfish gut microbial communities, their low explanatory power suggests that other factors, such as dynamics occurring at different scales or feedbacks between host and microbes, are driving variation in gut microbial communities.

Introduction

Microbial communities are integral to the ecology of their hosts by influencing their growth, reproduction, and nutrition (Turnbaugh et al. 2006, Himler et al. 2011, Shin et al. 2011). Community composition within an individual host relies on colonization of microbes from the surrounding environment, interactions between the host and microbes, and interactions among microbes within the host (Douglas and Lindsey 2016, Miller et al. 2018). Microbes from the environment can internally colonize hosts by being consumed along with food items, however it is unclear to which degree the gut microbe community are from the resources consumed or how much host traits influence the resulting community (Smith et al. 2015).

By integrating with community ecology, microbiology can leverage established ecological theories to characterize the patterns of microbe distributions, abundances, and interactions. Metacommunity theory examines how the dispersal of organisms between communities alters local and regional community dynamics (Leibold et al. 2004). One of the major paradigms in metacommunity theory is “species sorting”, which focuses on the effect of environmental gradients on community structure. Under this view, the world consists of heterogeneous patches, each with local environmental conditions that affect the abundance and diversity of species that colonize them (Leibold et al. 2004). By delineating individual hosts as patches, metacommunity theory can be used to understand how host traits affect their microbial communities and how these communities change over space and time (Miller et al. 2018).

Host life history characteristics drive intraspecific variation in gut microbial communities that further influence host development and reproduction (McFall-Ngai et

al. 2013). Animals that molt or undergo metamorphosis restructure their digestive tracts (Kohl et al. 2013); this turnover in microbial habitat results in either a random community dominated by early colonizers or there could be selection to maintain a consistent community composition. Furthermore, stage-specific interactions between hosts and their resources can lead to diet differences among individuals within a species (Werner and Gilliam 1984, Miller and Rudolf 2011, de Roos and Persson 2013). Such ontogenetic diet shifts can arise if hosts forage in different habitats or are able to consume different prey items as they grow (Werner and Gilliam 1984). Thus, variation in diet between ontogenetic stages results in distinct gut flora for juveniles and adults (Hongoh et al. 2006, Givens et al. 2015).

Physiological differences between sexes also influence intraspecific variation in gut microbial communities. Sex-specific hormones may be responsible for differences in the gastrointestinal environment and digestion rate of certain diet types between males and females (Freire et al. 2011, Bolnick et al. 2014, Klein and Flanagan 2016). Aggressive behaviors between males cause stress and the resulting microbial community, and ultimately metabolic pathways, is affected by the change in physiology (Zha et al. 2018). For adult females, gut microbes contribute additional energy to meet elevated energetic demands for reproduction (Amato 2013). Therefore, the interaction between host diet, ontogenetic stage, and sex has implications for gut microbial community structure and ultimately host health.

We conducted a laboratory experiment using signal crayfish (*Pacifastacus leniusculus*) to test how diet, ontogenetic stage, and sex influence gut microbial community structure and the degree to which the surrounding environment and prey

items influence gut microbial communities. Like most crayfish, signal crayfish are an omnivorous species but have been noted to undergo an ontogenetic diet shift where juveniles feed primarily on protein-rich sources (e.g. other invertebrates) to aid in rapid growth before switching to plant-based sources as adults (Mason 1963 but see Bondar et al. 2005). Though crayfish do not display sexual dimorphism or differences in feeding strategies, males have been found to be more aggressive than females (Mathews et al. 2009). This suggests that physiological differences between the sexes may play a role in altering gut microbial communities. With these behaviors, we hypothesized that diet would have stage- and sex-dependent effects on gut microbial community structure.

Materials and Methods

Study system

Lake Erken is a meso-eutrophic lake in southeastern Sweden (59°85′18″N, 18°83′59″E) with a surface area of 24 km^2 and mean depth of 9 m (Naddafi and Pettersson 2007). Adult crayfish (Total length = 64 - 123 mm) occupy deeper regions of the lake and juveniles (total length = 47 - 85 mm) are commonly found along the shore. Previous research in this system shows that signal crayfish have a preference for the algae *Chara vulgaris* compared to other macrophytes (Nyström and Strand 1996) and are predators of zebra mussels (Schreiber et al 1998, Naddafi et al. 2007).

Experimental design

Crayfish were captured using minnow traps and by hand netting individuals while snorkeling. A subset of 80 individuals were frozen at -80°C immediately after capture to characterize gut microbial communities in the field (hereafter referred to as "field

crayfish"). The rest (n=100) were transported live to Uppsala University to be used for the laboratory experiment. We recorded crayfish length (carapace length and total length, mm), sex, and reproductive status (visually assessed by searching for the presence of dark eggs in mature females and white sperm masses in mature males; (Yazicioglu and Kozák 2016). We collected three resource types: algae (*Chara* sp., average wet mass per sample = 0.059 g), zebra mussels (muscle mass only, average wet mass per individual = 0.165 g), and Chironomidae larvae (average wet mass per individual = 0.001 g) (n=3 samples per resource type). We also sampled water from Lake Erken and laboratory tap water and filtered 500 ml and on Whatman glass microfiber filter (n=3 each water type). All samples were frozen at -80°C until further processing for microbial community characterization.

Laboratory crayfish (n = 100) were housed individually in 5L aquaria filled with tap water at the Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden. Room temperature was 14°C and the light:dark cycle was set to 14:10 to reflect daylight length late summer. Each tank contained either an adult or juvenile male or female crayfish which were fed either algae or mussels daily for 4 weeks ad libitum between 23 August and 26 September 2017. Individuals were immediately frozen at -80°C at the end of the experiment. The hindgut of partially defrosted field and laboratory crayfish were dissected using sterile dissection techniques, placed into microcentrifuge tubes, and frozen until further processing. Crayfish that molted during the experiment (n = 5) were not processed since crayfish do not feed after molting until after their carapace has hardened.

DNA extraction, PCR amplification and sequencing

DNA was extracted from crayfish hindguts, lake water, tap water, and food items using DNeasy Powersoil (Qiagen, No./ID: 12888-10) following the manufacture's protocol with an additional incubation at 65°C for 10 min after adding the C1 solution. The hypervariable V4 region of the 16S ribosomal RNA gene was amplified in a two-step PCR using primer pair 515F and 805R. For the first step, PCRs were performed in triplicate using Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, No./ID: F-530XL). 25 cycles were performed following Phusion polymerase protocol. Negative controls were run during DNA extraction and 16S PCR amplification to check for contamination. Triplicate PCR products of each sample were pooled and subsequently purified using AMPure XP magnetic beads for Purification (Beckman Coulter, No./ID: A63882). For the second step, Illumina adaptor sequences and barcodes were attached to the PCR primers to provide each sample a unique identifier. Samples were then purified again using magnetic beads. An equal concentration of DNA from each sample was pooled and run through agarose gel. 400-500 bp band was then excised and purified using the QIAquick gel extraction kit (Qiagen, No./ID: 28104). PCR products were sequenced on IlluminaMiSeq to obtain 250 bp paired-end reads at Science for Life Laboratory (SciLifeLab), Uppsala, Sweden.

Sequence preprocessing

Only samples with 3 successful PCR runs were included in the analysis. Sequences were processed using DADA2 (Callahan et al. 2016), an R package for data quality filtering, paired-end merging, and chimera removal that produces amplicon sequence variants (ASV). ASVs, which are exact amplicon sequences as opposed to

clustered sequences based on a threshold, were classified to family using the Ribosomal Database Project 16S rRNA database (release 11.5, Wang et al. 2007) and rarefied to 4,000 reads per sample.

Core OTUs

We visualized community composition using relative abundance of OTUs and identified core OTUs among all crayfish. We agglomerated samples to the order level as higher taxonomic resolution is dependent on the classifier or database. Since there is no standard threshold for what constitutes as a "core" (Shade and Handelsman 2012), we created three core datasets for 50%, 75%, and 95% prevalence.

Alpha diversity metrics

Bacterial alpha diversity was estimated using Chao1 (richness) and Shannon diversity index in the phyloseq package (McMurdie and Holmes 2013) and Faith's phylogenetic diversity (branch length based richness, Faith 1992) in the picante package (Kembel et al. 2010). We conducted a one-way ANOVA to test the difference in alpha diversity metrics among the food items and Tukeys post-hoc test to reveal differences between food items. For the following alpha and the beta diversity measures for crayfish, "all diets" refers to all three diet groups: laboratory crayfish fed algae, laboratory crayfish fed mussels, or field crayfish with diets that reflect their feeding habits in the wild. We conducted a permutation test on an two-way ANOVA to test the effect of crayfish traits and all two-way interactions on alpha diversity metrics (all diets + life stage + reproductive status + all diets:sex + all diets:reproductive status + sex:reproductive status).

Beta diversity

We tested the effect of diet, crayfish traits, and all interactions on bacterial beta diversity metrics (all diets + life stage + reproductive status + all diets x sex + all diets x reproductive status + sex x reproductive status + all diets x reproductive status x sex) using permutational ANOVA (PERMANOVA) in the vegan package (Oksanen 2013). We measured beta diversity using the following metrics: Jaccard (based on the presence or absence), Bray-Curtis (based on richness), unweighted UniFrac (measure of richness that incorporates phylogenetic relationships across the taxa), and weighted UniFrac (phylogenetic relationships weighted by relative abundances, (Lozupone and Knight 2005). We repeated the analysis using a subset of the data for crayfish only in the lab in order to determine if differences in community composition were due to diet or laboratory artifacts (hereafter "lab diets"). To visualize beta diversity of the full dataset (both laboratory and field crayfish), we used Constrained Analysis of Principle Coordinates (CAP) which is a Principal Coordinates Analysis with axes that explain variance by the groups of interest.

Indicator taxa

To identify taxa associated with crayfish diet types, ontogenetic stage, or sex, we conducted an indicator analysis on the top 100 OTUs agglomerated to the order level using the indicspecies package (De Cáceres and Legendre 2009). The analysis produces an indicator value, which measures the relationship between taxa presence-absence or abundance in a set of similarly classified groups (Dufrêne and Legendre 1997, De Cáceres et al. 2010).

Results

Pre-processing

After removing chloroplasts, we obtained 761,265 OTUs total and 5,639 unique OTUs from 135 samples, 120 crayfish and n = 3 for each food item and water source. We further filtered the data by removing OTUs that did not occur more than 5 times in the whole dataset to reduce noise resulting in 5406 unique OTUs. After rarefying to 4,000 reads per sample, we obtained 4721 unique OTUs from 117 samples: 31 crayfish fed algae, 38 crayfish fed mussels, 33 field crayfish, and all food items and water sources.

Crayfish gut microbial community composition reflects microbes found in food items but not water samples

Algal microbial communities were primarily composed of Proteobacteria (91%) (Figure 6a). Mussels were composed of Proteobacteria (74%) and Actinobacteria (21%) (Figure 6a). Chironomids had Proteobacteria (36%), Bacteroidetes (33%), Firmicutes (25%) (Figure 6a). Actinobacteria was dominant in Erken water (83%) and tap water (42%) (Figure 6b).

Proteobacteria dominated lab crayfish microbial composition regardless of diet type, with approximately 90% for laboratory crayfish fed mussels or algae and 55% for field crayfish (Figure 5, Figure 6c). Bacteroidetes (4%) and Firmicutes (31%) were the second most common phyla for laboratory and field crayfish, respectively (Figure 5).

The core microbiome of crayfish consists of orders associated with gastrointestinal processes

Crayfish shared 8% of the total OTUs across diet treatments (266 OTUs; Figure 8). *Enterobacteriales, Vibrionales, Rhodobacterales, Burkholderiales, Alteromonadales,*

Xanthomonadales, and *Actinomycetales* were present in 50% of crayfish. *Enterobacteriales, Vibrionales,* and *Rhodobacterales* were present in 75% of crayfish. Enterobacteriales were present in 95% of crayfish.

Differences in alpha diversity were more apparent among food items than among crayfish

There was a detectable difference in richness (Chao1) and phylogenetic diversity (Faith's PD) between the microbiomes of algae and the microbiomes of both mussels and chironomids and a detectable difference in diversity (Shannon index) among all three food items (Table 3). There was not a detectable difference in richness or phylogenetic diversity among all crayfish (Table 4). However, there was an interaction between sex and reproductive status using Shannon diversity index (Figure 7).

Diet and host traits affect gut microbial beta diversity

The effect of diet on gut microbial communities was mostly modulated by host traits (Table 3). Community composition was affected by an interaction between diet and reproductive status (Jaccard, Figure 9) whereas community structure was influenced by both diet and reproductive status individually (Bray-Curtis). Phylogenetically informed community composition was affected by reproductive status and an interaction between diet and sex (Unweighted UniFrac). Phylogenetically informed community structure was influenced by an interaction among diet, reproductive status, and sex (Weighted UniFrac).

For laboratory crayfish, both phylogenetically uninformed and informed community composition were influenced by diet and reproductive status (Jaccard and Unweighted UniFrac, respectively, Table 3). Community composition (Bray-Curtis) and

both measures of richness (Jaccard, Unweighted UniFrac) were affected by diet (Table 3). Phylogenetically informed community structure was influenced by an interaction between reproductive status and sex (Table 3).

Indicator taxa

Indicator taxa were found for the microbiomes of field crayfish and laboratory crayfish fed mussels but were not found for laboratory crayfish fed algae, between reproductive statuses, or between sexes. Two Proteobacteria orders (Xanthomonadales and Desulvibrionales, FDR q-value = 0.025) and a Firmicutes order (Clostridiales, FDR q-value < 0.001) were associated with the microbiomes of field crayfish. For lab crayfish, a Proteobacteria order (Burkholderiales, FDR q-value = 0.001, FDR q-value = 0.002) and an Actinobacteria order (Actinomycetales) were associated with the microbiomes of crayfish on a mussel diet.

Discussion

In this study, we used metacommunity theory to motivate the role of diet and host traits in determining gut microbial community composition. By treating individual hosts as habitat patches, we explored how host characteristics influence microbial communities. We found that microbial diversity varied among host diet treatments and that these differences were modulated by host sex and life stage in signal crayfish. This is the first study to describe the crayfish gut microbiome using next generation sequencing and supports Skelton et al. (2017) in that processes regulated by host biology influence internal microbial communities.

We found prominent taxa in crayfish guts known for providing nutrition and pathogen defense in other animals. Enterobacteriales, which was the most prevalent bacterial order among laboratory and field crayfish in this study, is commonly found in invertebrate hindguts (Behar et al. 2009, Colman et al. 2012, Hernández et al. 2015). It has been shown to be involved in nitrogen fixation, providing an important source of nitrogen for amino acid synthesis or assimilation of plant compounds, thereby allowing invertebrates to live on plant based diets (Hernandez et al 2015). This would allow crayfish to benefit from consuming algae even during the juvenile phase where protein is necessary for rapid growth. Firmicutes is one of the most common phyla found in animal guts (Ley et al. 2008), was the second most common phylum in field crayfish but was absent in laboratory crayfish. Taxa within Firmicutes are associated with a high protein diet and protein breakdown (Costello et al. 2010, Li et al. 2017). Since it was found in field crayfish and in chironomids but not in either lake or laboratory water samples, this suggests that Firmicutes might be from other animal sources than those used in the laboratory experiment. As crayfish are also known as scavengers, the presence of Firmicutes in field crayfish could also indicate the importance of dead animals in their diet. Proteobacteria was the most abundant phylum in both laboratory and field crayfish and their food items and has been found to be the most abundant phylum in crustaceans (Cheung et al. 2015) and marine and freshwater fishes (Sullam et al. 2009, Romero et al. 2014, Tarnecki et al. 2017). The dominance of Proteobacteria in crayfish samples was reflective of the abundance of Proteobacteria in their invertebrate and algal food items but not in lab or lake water samples, suggesting that selectivity for Proteobacteria in aquatic organisms occurs in areas other than digestive tract.

Gut microbial community structure and composition in crayfish was largely influenced by diet. Wang et al. (2011) found similar gut microbial diversity between wild and lab adult mosquitos, providing evidence that adults maintain a constant internal environment regardless of external conditions. In contrast, we found only one core taxa and 266 OTUs shared among crayfish treatments, which suggests that crayfish gut microbes are highly transient and are affected by diet. All beta diversity metrics provide support that diet is important for the presence and phylogenetic relatedness of OTUs present in crayfish guts. Crayfish gut microbial composition was similar to the microbial composition of their prey items, which may mean that diet rather than the gut environment alters gut microbial diversity (David et al. 2014). The ordination plot (Fig. 4) demonstrated that field crayfish were clustered along axis 2, whereas crayfish fed either mussels or algae were tightly clustered along axis 1. These clusters provide evidence for differences between specialist and generalist diets; between the specialist diets, there was more variation in microbial communities of crayfish with herbivorous diets relative to carnivorous diets, a pattern that has been seen in mammals (Ley et al. 2008). Generalists have diverse gut environments that can sustain a few dominant microbes that may not persist in specialists (Bolnick et al. 2014). Alternatively, generalists are more likely to encounter food with compounds that may inhibit the presence of certain taxa (Bolnick et al. 2014). Lab crayfish microbial communities diverged from that of field crayfish within a month and this quick turnover in gut microbiota may help crayfish maintain and benefit from an omnivorous, opportunistic diet (David et al. 2014).

Physiological differences between life stages may be responsible for the variation

of gut microbial communities over ontogeny. Juvenile crayfish have high molting rates as growth occurs most rapidly during this stage. Since molting removes the gut lining in crayfish (Mente et al. 2016), juvenile crayfish may have higher turnover in microbial composition compared to adult crayfish. Crayfish used in analyses did not molt during the study, but juvenile crayfish are more likely to have recently molted relative to adults. Microbial differences between life stages may also be a result of tradeoffs between reproductive organ development for adults and immune function for juveniles (Cheung et al. 2015). During our study period, adults were about a month away from mating; crayfish may harbor microbes that increase energy availability for gamete production or the change in physiology affects which microbes colonize (Amato 2013). Spatial distribution of crayfish may also affect gut microbial communities in the wild. In Lake Erken, we found juveniles close to shore and adults in deeper water, which may result in differences in resource availability and changes in gut microbial diversity. Since we saw differences between stages in the lab, this suggests gut microbial communities are influenced by different physiology between stages and not behavior.

Behavioral differences between male and females could explain the differences in microbial communities between sexes. We found an effect of sex on alpha diversity (Shannon) and gut microbial community structure in 3 out of the 4 beta diversity metrics in the full dataset that included field crayfish; in contrast, we found the effect of sex for laboratory crayfish in only one beta diversity metric (Weighted UniFrac). Sexual dimorphism, different feeding strategies between males and females, or stress- and sex-hormones has been hypothesized to describe the sex-dependent effects on microbial diversity in vertebrates (Bolnick et al. 2014, Zha et al. 2018). Crayfish do not display

sexual dimorphism or differences in feeding strategies between sexes but male crayfish have been found to be more aggressive than females (Gherardi and Cioni 2004). When crayfish are isolated from one another in the lab and from the threat of predation, they do not have the stressors that would elicit hormonal changes that effect their gut microbial communities. Sex-by-diet interactions have been shown to affect gut microbiomes in vertebrates (Bolnick et al. 2014), but to our knowledge, this has never been shown in an invertebrate. This suggest that sex-by-diet interactions are more common and general that previously known.

Other factors other than diet, sex, life stage, or the interaction among these three factors explain differences in microbial community structure among crayfish. We found that these factors have low explanatory power as our models only capture about 20% of the variation in microbial community structure. Though the majority of the variation is left unexplained, it is within the range of other microbial studies on humans (Falony et al. 2016). We hypothesized that host traits would be the prominent driver of microbial community structure but there are other processes occurring either simultaneously or alternatively. Burns et al. (2016) found that neutral processes were important in structuring juvenile zebrafish microbial communities. Future studies that expand on metacommunity theory by incorporating feedback between the host and environment as well as feedback between microbes and the host may better capture host-microbiome dynamics (Miller et al. 2018). We did not assess the functions of bacteria found in this study, but is possible that the functions that the bacteria serve may be conserved even with changes in microbial community structure. We cannot assess microbe-microbe interactions in this study, however the varied results among the different beta diversity

measures provide hypotheses about drivers of community structure to be further explored.

Our study highlights how host sex and life stage modulate the effect of diet on gut microbial community structure. Since behavior is a possible contributing factor to our results, future studies should explicitly incorporate a metacommunity framework to understand how connectivity among hosts influences gut microbial communities. Plasticity in microbial diversity may be host taxa-specific, and the integration of microbiology and ecological theory is needed to understand how hosts maintain physiological functions with stable or dynamic microbial communities.

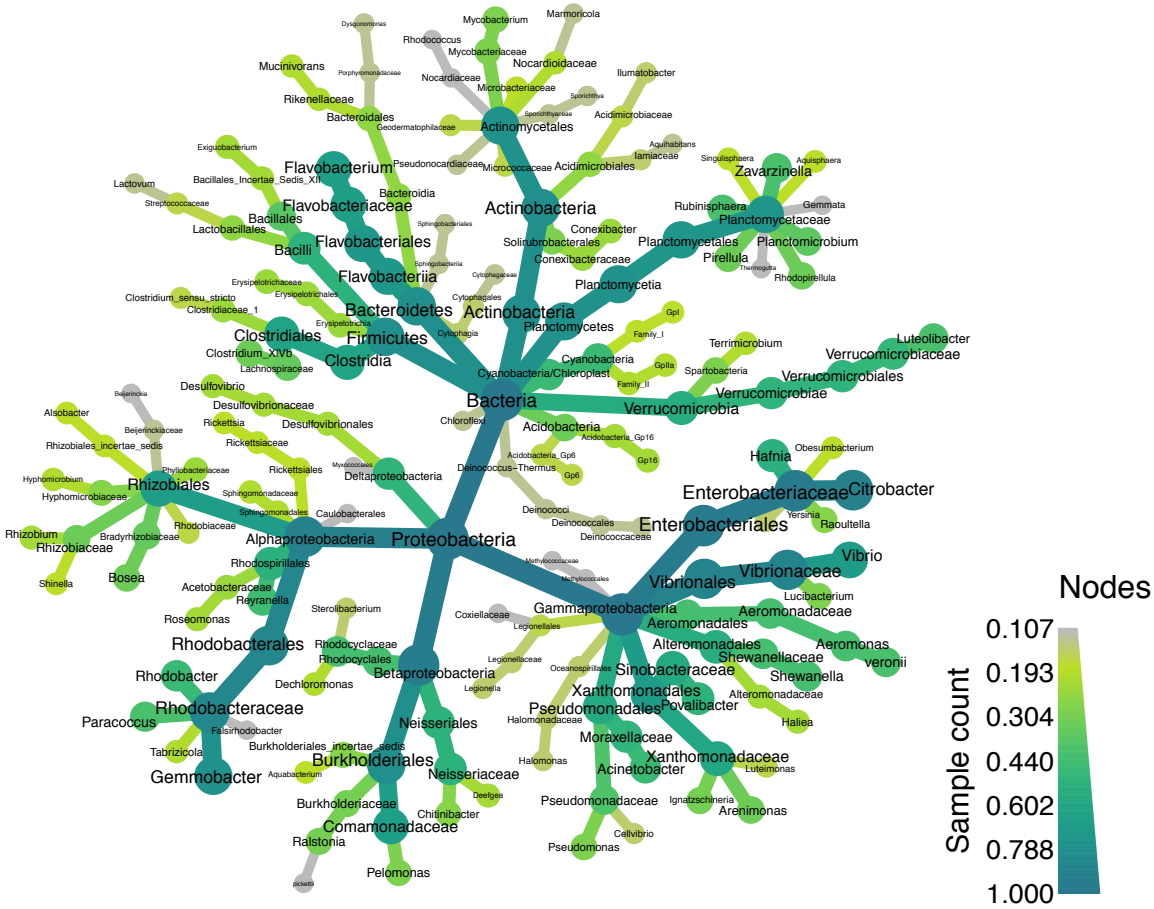
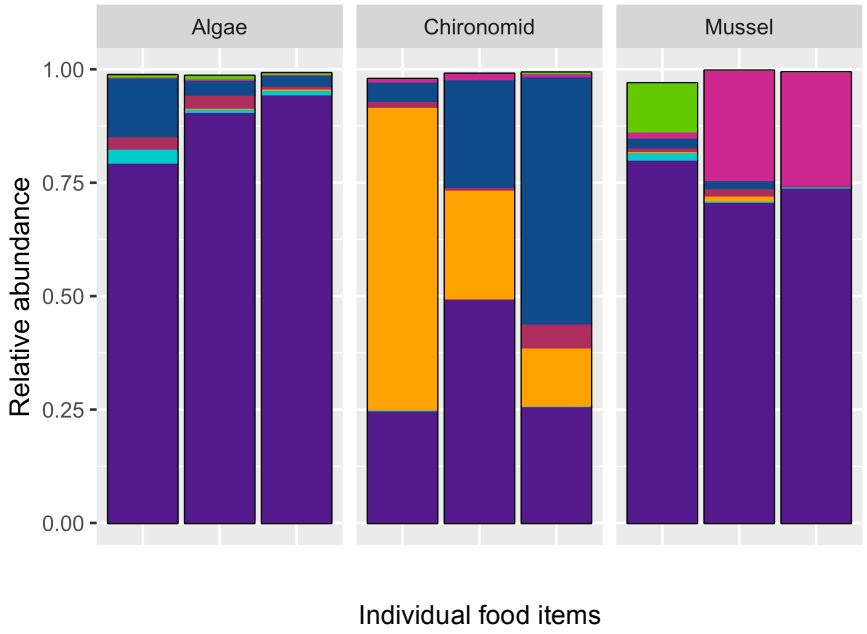


Figure 5. Phylogenetic heat tree of bacterial taxa represented in crayfish guts. Colors represent taxa prevalence- dark blue represents taxa found in 100% of crayfish guts, gray represents taxa found in 10% or fewer crayfish guts.

A.



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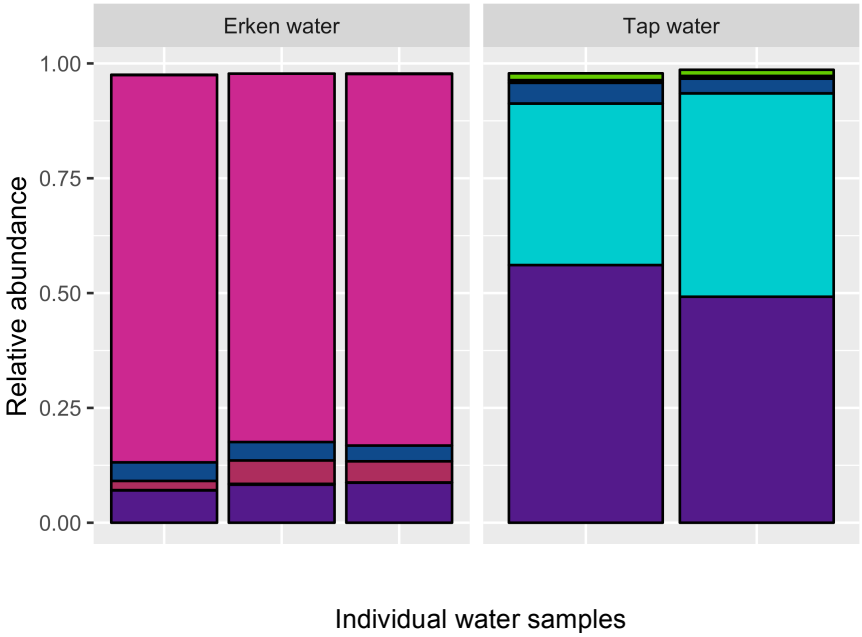

Figure 6. Relative abundance of bacteria phyla by A) diet treatment, B) prey items, and C) habitat where each bar represents an individual sample.

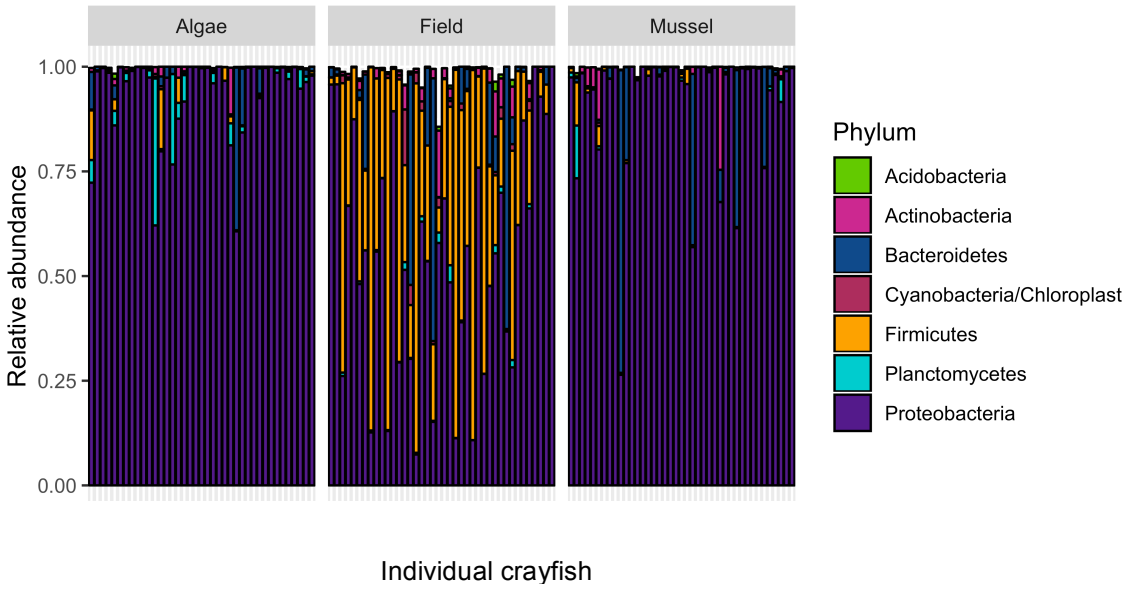

Figure 5 cont: Relative abundance of bacteria phyla by C) habitat where each bar represents an individual sample.

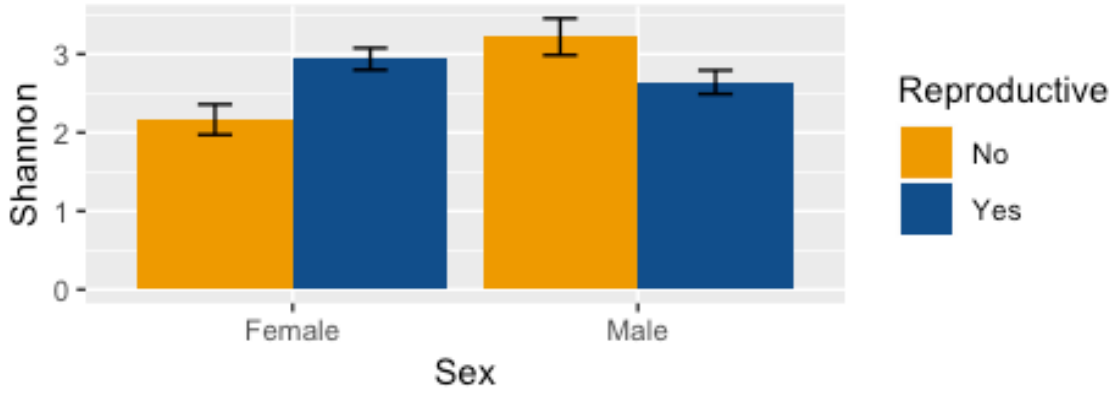

Figure 7: Shannon diversity of crayfish gut microbial communities by crayfish sex and reproductive stat

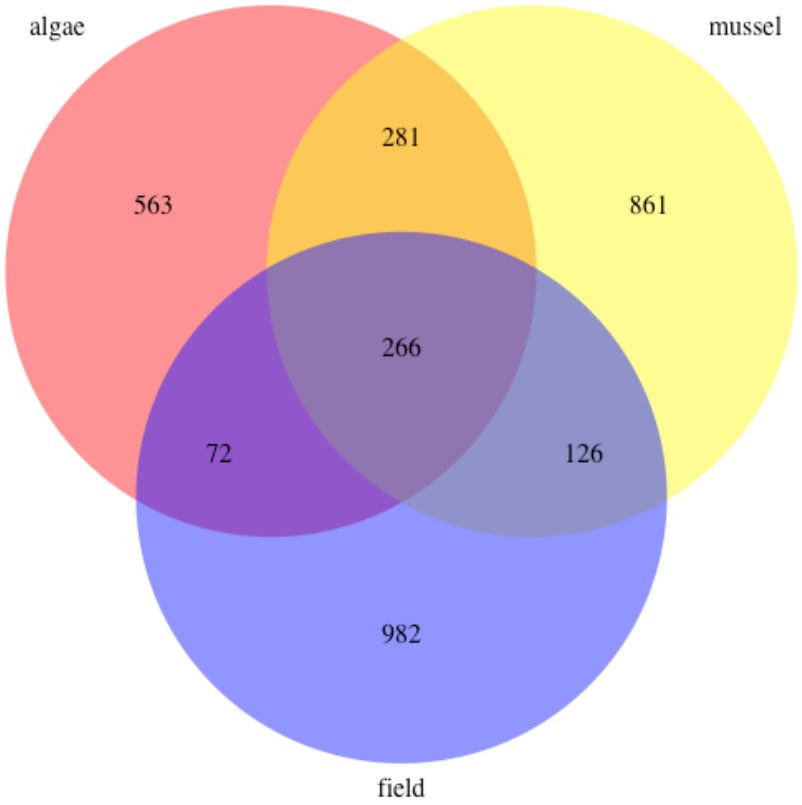

Figure 8. Venn diagram showing the number of OTUs in crayfish fed algae (red), mussels (yellow) or crayfish in the field (blue).

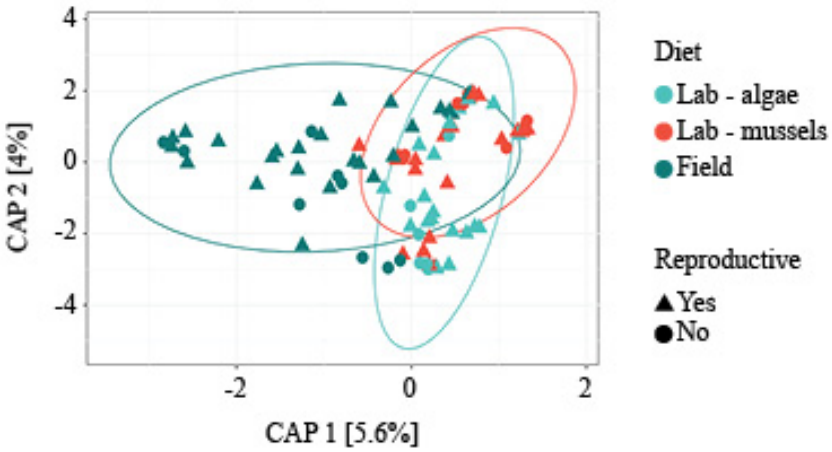

Figure 9. Constrained Analysis of Principle Coordinates ordination plot of microbial community composition (Jaccard) among crayfish diet treatments and between crayfish ontogenetic stages. Color indicates diet and shape indicates reproductive status.

Table 3. Effect of crayfish diet, sex, and reproductive status on gut microbial alpha diversity using Chao1, Shannon diversity index, and Faith's phylogenetic diversity metrics.

Factor	F-value	p-value	p-value from permutation
Chao 1			
Diet	2.43	0.09	0.09
Sex	0.04	0.85	0.97
Reproductive status	0.72	0.09	0.49
Diet:Sex	2.42	2.42	0.09
Diet:Reproductive status	0.72	0.72	0.49
Sex:Reproductive status	0.71	0.71	0.50
Shannon			
Diet	1.78	0.17	0.17
Sex	<0.01	1	1
Reproductive status	0.44	0.64	0.64
Diet:Sex	1.24	0.29	0.29
Diet:Reproductive status	0.52	0.67	0.59
Sex:Reproductive status	10.62	<0.01	<0.01
Phylogenetic Diversity			
Diet	1.04	0.36	0.36
Sex	0.12	0.73	0.89
Reproductive status	1.10	0.34	0.34
Diet:Sex	2.07	0.13	0.13
Diet:Reproductive status	0.18	0.91	0.83
Sex:Reproductive status	0.28	0.60	0.76

Table 4. Post-hoc tests of pairwise comparisons of alpha diversity metrics between food items.

Factor	Stat	p-value	p-value from permutation
Chao 1			
Algae - Chironomid	1.99	0.05	0.07
Algae - Mussel	2.05	0.04	0.07
Chironomid - Mussel	1.92	0.23	0.23
Shannon			
Algae - Chironomid	2.15	0.03	0.05
Algae - Mussel	2.15	0.03	0.05
Chironomid - Mussel	1.85	0.06	0.06
Phylogenetic Diversity			
Algae - Chironomid	2.02	0.04	0.08
Algae - Mussel	1.93	0.05	0.08
Chironomid - Mussel	0.42	0.68	0.68

Table 5. Effect of crayfish diet, reproductive status, and sex on gut microbial beta diversity using Jaccard, Bray-Curtis, Unweighted UniFrac, and Weighted UniFrac.

All crayfish

		Jaccard			Bray-Curtis		
	df	F	R^2	p	F	R^2	p
Diet	2	3.01	0.06	**<0.01**	5.31	0.10	**<0.01**
Reproductive status	1	1.48	0.01	**<0.01**	1.16	0.01	0.28
Sex	1	0.94	0.01	0.60	0.72	<0.01	0.68
Diet x Reproductive status	2	1.23	0.02	**0.04**	1.38	0.02	0.14
Diet x Sex	2	1.06	0.02	0.24	1.04	0.02	0.36
Reproductive status x Sex	1	1.12	0.01	0.18	1.85	0.02	**0.08**
Diet x Reproductive status x Sex	2	1.10	0.02	0.18	1.35	0.02	0.14

		Unweighted UniFrac			Weighted UniFrac		
Diet	2	4.50	0.08	**<0.01**	9.43	0.15	**<0.01**
Reproductive status	1	1.93	0.02	**<0.01**	1.60	0.01	0.12
Sex	1	0.88	0.01	0.68	0.87	0.01	0.54
Diet x Reproductive status	2	1.22	0.02	0.13	1.38	0.02	0.14
Diet x Sex	2	1.45	0.03	**0.02**	1.17	0.02	0.28
Reproductive status x Sex	1	1.08	0.01	0.32	2.82	0.02	**0.01**
Diet x Reproductive status x Sex	2	1.19	0.02	0.13	1.99	0.03	**0.03**

Lab crayfish

		Jaccard			Bray-Curtis		
Diet	1	1.87	0.03	**<0.01**	3.04	0.04	**0.01**
Reproductive status	1	1.26	0.02	**0.08**	0.44	0.01	0.88
Sex	1	1.01	0.01	0.42	1.14	0.02	0.31
Diet x Reproductive status	1	1.09	0.01	0.27	1.61	0.02	0.13
Diet x Sex	1	1.01	0.01	0.38	0.72	0.01	0.63
Reproductive status x Sex	1	1.11	0.02	0.22	1.45	0.02	0.16
Diet x Reproductive status x Sex	1	1.11	0.02	0.25	0.82	0.01	0.52

		Unweighted UniFrac			Weighted UniFrac		
Diet	1	1.75	0.03	**0.02**	1.80	0.03	0.11
Reproductive status	1	1.41	0.02	**0.08**	0.42	0.01	0.83
Sex	1	0.87	0.01	0.68	0.80	0.01	0.49
Diet x Reproductive status	1	1.04	0.02	0.36	0.71	0.01	0.60
Diet x Sex	1	1.51	0.02	0.04	0.72	0.01	0.58
Reproductive status x Sex	1	1.06	0.02	0.32	4.27	0.06	**0.01**
Diet x Reproductive status x Sex	1	1.31	0.02	0.11	0.90	0.01	0.43

CHAPTER 4 – DIASPORA AND DETRITUS: NON-NATIVE CRAYFISH IMPACT LEAF LITTER BREAKDOWN BUT NOT BENTHIC INVERTEBRATE COMMUNITY STRUCTURE

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Abstract

Species introductions can alter the relationship between trophic interactions and ecosystem processes. Often, introduced species reduce the abundance and diversity of biota in recipient food webs. However, ontogenetic diet shifts in the introduced species can alter the presence, degree or direction of these impacts on native species, making it difficult for scientists and managers to predict the ecological consequences of species introductions. I conducted a manipulative field experiment to assess the effects of crayfish species identity and ontogenetic stage on benthic invertebrate composition and abundance as well as leaf litter breakdown by native signal crayfish (*Pacifastacus leniusculus*) and introduced ringed crayfish (*Faxonius neglectus neglectus*). Treatments with signal crayfish and adult crayfish had higher reductions in leaf litter relative to treatments with introduced crayfish and juvenile crayfish. Alpha and beta diversity of benthic invertebrates was similar among treatments, but there were fewer shredders in treatments with adult crayfish. Thus, I show that ontogenetic stage and native vs. non-native status both matter for understanding the impact of species introductions on local ecological communities and ecosystem processes.

Introduction

Species interactions and ecosystem function are inextricably linked as the trophic connections among individuals affect decomposition, nutrient cycling, and energy flows in a system. These processes may be altered in the presence of an introduced species, which can directly or indirectly alter the abundance, behavior, and distribution of species in the recipient community and the functions they provide (D'Antonio 1992, Baxter et al. 2004). Studies that assess the impact of introduced species are biased towards terrestrial

systems (Lowry et al. 2013) and are often incomplete because many do not consider their stage structure (Persson 2016). Life stages within a species can be functionally different from one another, and this intraspecific ontogenetic difference can be greater than that between species (Rudolf and Lafferty 2011). As a result, the strength, and potentially direction, of their interactions with other members of the food web changes with age (Miller and Rudolf 2011). To predict how ecosystems will be altered by species introductions, it is necessary to understand 1) how different life history stages of focal species can differentially affect benthic invertebrate community structure and ecosystem functioning, and 2) if these differences are consistent between native and introduced species.

Crayfish are one of the most widespread aquatic invaders in the world. As both predators and detritivores, crayfish occupy multiple trophic positions and their ecological impacts can propagate throughout recipient systems (Momot 1995). Invasive crayfish often display aggressive behaviors, which confer a competitive advantage over native crayfish for resources (Gherardi and Cioni 2004, Pintor and Sih 2009, Lodge et al. 2000). Non-native crayfish typically cause greater reductions on the abundances of invertebrates and basal resources compared to native crayfish (Twardochleb et al. 2013). Their effects on ecosystem processes, such as leaf litter breakdown, can vary. For example, by directly processing detritus they may increase its availability for other consumers in streams, or they may indirectly reduce leaf litter breakdown by removing other detritivore species (Creed et al. 2004, Jackson et al. 2014). If the balance of these processes differ between native and non-native crayfish, species replacement may not provide the equivalent ecosystem functions (Usio et al. 2006).

A recent crayfish introduction in Oregon streams has presented an opportunity to examine the relative impacts of native and non-native crayfish on local ecological communities and ecosystem processes, and whether these impacts are consistent between juvenile and adult stages. The native range of the signal crayfish (*Pacifastacus leniusculus*) extends from northern California to southern British Columbia (Larson and Olden 2011). Ringed crayfish (*Faxonius neglectus neglectus*), originally from the Ozark region, were discovered in western Oregon in 2015 (Jeff Ziller, personal communication). The functional role of both crayfish species varies over ontogeny, as juvenile crayfish are known to feed predominantly on aquatic invertebrates whereas adults feed on detritus (Mason 1963, France 1996, Whitledge and Rabeni 2011, but see Bondar et al. 2005). Ringed crayfish have displaced signal crayfish in southern Oregon where they have been introduced (Bouchard 1977, Pearl et al. 2013). While it is assumed that ringed crayfish will be more aggressive and competitive relative to signal crayfish, no studies have documented the interactions between ringed and signal crayfish where the species co-occur.

I conducted a field caging experiment to test five hypotheses about how crayfish species identity and ontogenetic stage impact aquatic invertebrate community structure and leaf litter breakdown. Introduced crayfish are expected to cause greater reductions in native biota and disrupt ecosystem processes relative to native crayfish, and crayfish ecological roles vary between life stages. Therefore, I hypothesized that invertebrate diversity and abundance would be reduced by a greater extend in 1) ringed crayfish relative to signal crayfish and 2) juvenile crayfish relative to adults. I also hypothesized that leaf litter reductions would be higher for 3) ringed crayfish relative to signal crayfish

and 4) adults relative to juveniles. Lastly, I hypothesized that 5) ringed crayfish and juvenile crayfish would reduce leaf litter processing by other detritivores

Methods

Field experiment

I conducted the experiment in Smith Creek (43° 45', -122° 54'), a 3rd order stream that empties into Dorena Lake within the Willamette River drainage in western Oregon. The experiment involved treatments with each combination of crayfish species (ringed or signal) and life stage (adult or juvenile, Table 1). Each treatment was replicated 9 times (36 total cages).

I constructed enclosures (500 cm x 500 cm x 150 cm) with 1.25 cm PVC pipe and plastic hardware cloth (mesh size = 1 cm^2). I placed four 5 g leaf packs in each enclosure. Two leaf packs were covered by plastic hardware cloth (mesh size = 0.5 cm^2) to prevent crayfish access but allow access by other invertebrates (hereafter "mesh") and two leaf packs were exposed (hereafter "open"). Leaf packs were composed of senescent red alder (*Alnus rubrus*) leaves that were collected in the previous autumn and air-dried in the lab. Enclosures were lined with cobble and left in the stream for two weeks prior to the start of the experiment to allow for colonization of aquatic invertebrates. I placed three crayfish in each enclosure after the two-week conditioning period. The experiment ran for 6 weeks, from August to mid-September 2016.

The main response variables were leaf litter loss and invertebrate abundance. Leaf packs were rinsed and subsequently air-dried for 72 h. To quantify leaf litter breakdown,

I recorded the leaf litter loss as the initial – final leaf litter weight (g). I removed the enclosures from the stream, washed the cobble from each enclosure in a bucket, and drained the debris through a micron sieve. To quantify invertebrate abundance, invertebrates from the cobble were preserved in 70% ethanol, identified to the lowest taxonomic rank possible (no lower than family) and enumerated. Each family was assigned one of the following feeding groups to indicate their trophic role in streams: "shredder", "collector", "scraper", or "predator" (Merritt and Cummings 1996, Appendix Table 1).

Statistical analyses

Five of the 36 cages were dropped from analyses as they were damaged over the 6-week study and had fewer crayfish than were present at the start of the experiment; however, this adjustment was relatively equal across treatments (Table 1). I estimated the variation in aquatic invertebrate community across replicates within a crayfish species or ontogenetic stage treatment using the Shannon diversity index, henceforth referred to as alpha diversity (McMurdie and Holmes 2013). I used general linearized models (GLM) with a negative binomial distribution to determine the effect of crayfish species, stage, and the species-by-stage interaction (without controls) on alpha diversity and on the total invertebrate abundance and abundance of each functional feeding group. Specimens that were not identified to family were not included in the functional feeding group analysis because a single order can encompass multiple feeding groups (Merrit and Cummins 1996). I estimated the effect of crayfish species, stage, and their interactive effect on the variation in aquatic invertebrate community structure between treatments using

permutational ANOVA (PERMANOVA) using Bray-Curtis dissimilarity of invertebrate presence and abundance, henceforth referred to as beta diversity. I conducted a two-way ANOVA to assess the effect of crayfish species, stage, and species-by-stage interaction on leaf litter loss. I visually examined residual plots that confirmed that the assumptions of normality and homogeneity of variance were met. I then followed the ANOVA with a Tukey post-hoc test to compare treatments. All analyses were done using R (version 1.1.463) using the packages vegan (Oksanen et al. 2018) and *emmeans* package (Length 2018).

Results

Aquatic invertebrate composition

There were a total of 15 orders of aquatic invertebrates represented by 48 families in the enclosures (Supplementary material S1). Predominant taxa in the cobble samples include Heptageniid mayflies (30%), Chironomid larvae (18%), Juga snails (10%), and Leptophlebiid mayflies (7%, Supplementary material C1).

Enclosures with adult crayfish had fewer aquatic invertebrates relative to juveniles

Crayfish stage had an effect on overall aquatic invertebrate abundance (GLM $p = 0.004$, Fig. 1a), such that enclosures with juvenile crayfish had on average 30% higher invertebrate abundance compared adult crayfish. Shredder abundance was on average 45% higher in enclosures with juveniles compared to adults ($p = 0.06$, Fig. 1b). The effect of crayfish species and stage on collector, scraper, and predator abundance was statistically unclear ($p > 0.1$, Fig. 1c, d, e).

Neither species identity nor stage had a clear impact on aquatic invertebrate diversity

Overall, the diversity of native benthic invertebrates was not affected by species identity or stage. The difference in alpha diversity of benthic invertebrates was not statistically clear between stages (GLM $p = 0.67$) or between species (GLM $p = 0.98$), and there was not a statistically clear interaction between species and stage (GLM $p = 0.97$). The difference in beta diversity between species (PERMANOVA, $F = 0.62$, $p = 0.21$) and ontogenetic stages (PERMANOVA, $F = 1.27$, $p = 0.82$) was not statistically clear, and was not a statistically clear interaction between the species and stage (PERMANOVA, $F = 0.8$, $p = 0.59$).

Species identity and stage influenced leaf litter loss

Both crayfish species identity and ontogenetic stage interacted with leaf treatment (open or mesh) to determine leaf litter loss (Table 2). There was lower leaf litter loss from open leaf packs in treatments with ringed crayfish compared to signal crayfish (Tukey's post-hoc test, $p < 0.001$, Figure 1). The treatments with adult crayfish, regardless of species, had higher leaf litter loss from open leaf packs compared to juveniles (Tukey's post-hoc test, $p < 0.001$, Figure 1). The effect of species identity on leaf litter loss did not clearly depend on ontogenetic stage (ANOVA species x stage interaction $p = 0.3$) or on the combination of stage and leaf treatment (ANOVA species x stage x leaf treatment interaction $p = 0.72$). There was no detectable difference in leaf litter loss from mesh covered leaf packs regardless of crayfish species or stage (Tukey's post-hoc test, $p > .1$).

Discussion

Generalizing the impacts of introduced species is challenging when their trophic roles vary over ontogeny. However, these nuances matter; here, I show that ontogenetic stage can modify the effects of an introduction on aquatic invertebrate community structure and leaf litter decay. Despite evidence from previous studies that non-native crayfish greatly reduce the abundance of native biota and alter ecosystem processes (Twardlocheb et al. 2013), in this study ringed crayfish did not have strong effects on invertebrate abundance and did not reduce leaf litter as much as signal crayfish. Further, their effects on stream invertebrate communities and leaf litter processing differed between adult and juvenile stages, suggesting that species non-native status alone is not enough to predict its impact on local ecological communities.

If ringed crayfish continue to displace signal crayfish, as they have in southern Oregon, this study suggests that leaf litter breakdown rate may slow and hence coarse and fine particulate organic matter may be less available for other detritivores. Signal crayfish can be effective detritus processors in areas where they have been introduced (Usio and Townsend 2004), and they have similar leaf litter processing rates between adult and juvenile crayfish in their native range (Bondar and Richardson 2009). Ringed crayfish had lower detritus processing than signal crayfish, especially at juvenile stages. Surprisingly, leaf litter breakdown in open leaf packs by juvenile ringed crayfish was not different than leaf packs in mesh (inaccessible by crayfish). Reductions in leaf processing by ringed crayfish as a whole may have long-term impacts on benthic invertebrates and their predators if they displace signal crayfish.

Biomass can be an important predictor of the impact of a species on ecological functioning. For example, Bondar and Richardson (2009) found that higher crayfish biomass resulted in higher leaf litter breakdown regardless of stage. In this study, we had higher juvenile biomass of signal crayfish than ringed crayfish. Therefore, the effect of juveniles from each species in this study may not be directly comparable if crayfish biomass is a better predictor of detritus breakdown than ontogenetic stage. Nevertheless, adult crayfish biomass was comparable across species, allowing differences on leaf litter breakdown to be related to species identity.

Crayfish had mixed, sometimes surprising effects on stream invertebrate communities. In this study, diversity measures indicated that invertebrate community composition in enclosures were similar regardless of crayfish species identity or ontogenetic stage. However, crayfish stage did affect stream invertebrate abundance in a surprising way. Because juveniles are known to consume invertebrates whereas adults primarily consume detritus, I hypothesized that invertebrate abundance would be lower in enclosures with juveniles relative to adults. In contrast to my expectations, I found fewer invertebrates in enclosures with adult crayfish compared to cages with juveniles, regardless of species. I did not track individual diets in this study. However, if adult crayfish did consume invertebrates, then adults would be likely to have a greater negative effect on invertebrate abundance than juveniles as they are larger and need to consume more prey to meet metabolic demands. The effect of introduced crayfish may not be immediately apparent in the stream where they co-occur, as reductions in leaf litter breakdown by ringed crayfish can have delayed effects on other detritivores in the community.

Beyond the effect of trophic interactions, non-trophic interactions can also shape community patterns (Kefi et al 2012). As the largest invertebrates in small streams,

crayfish movement disturbs the sediment, which displaces other benthic invertebrates (Usio and Townsend et al. 2004, Parkyn et al. 1997). Reduced total invertebrate abundance in treatments with adult crayfish may be due to greater bioturbidity by adult crayfish. Other indirect effects such as chemical cues can cause invertebrates to alter their behavior to avoid predation (Richmond and Lasenby 2006, McIntosh et al. 2002, Turner et al. 2000). However, there may be a threshold for chemical cues to take effect. I found more invertebrates in enclosures with juvenile compared to adult crayfish, meaning that juveniles are either less efficient at capturing invertebrates or do not produce enough chemical cues or have recognizable cues to be detected by potential prey compared to adults (Bondar and Richardson 2009). The combination of trophic and non-trophic interactions may swamp species- or stage-effects on invertebrate community structure, resulting in similar communities between species and stages seen here (Helms and Reed 2005).

Changes to detritivore abundance within functional feeding groups can affect energy pathways throughout stream food webs. Shredders may have been more abundant in treatments with juveniles because juveniles had the lowest impact on leaf litter breakdown, meaning that more leaf litter was available for detritivores other than crayfish. Alternatively, since leaf litter in mesh packs (inaccessible to crayfish) was not reduced in treatments where shredders are most abundant, this suggests that shredders did not exert strong effects on leaf litter in the presence of crayfish. It has been proposed that shredders facilitate collectors by breaking down coarse particulate organic matter into fine particulate organic matter that is usable by collectors (Heard and Richardson 1995, Short and Maslin 1977). However, I did not find more collectors in enclosures with juvenile crayfish where shredder abundance was the highest, in accordance to Bondar and Richardson (2009). I also did not find differences in abundance of scrapers or predators

between crayfish stage or species. Each functional feeding group may have responded similarly to the presence of crayfish regardless of stage or species, but this level of organization may mask differences in the response of individual benthic invertebrate taxa (Usio and Townsend, Bondar and Richardson, Alcolro et al., Lodge et al. 1994).

Non-native crayfish are commonly thought to disrupt bottom-up energy pathways to higher trophic levels by reducing the abundance of other invertebrates. However, I did not find a clear effect of crayfish species on total invertebrate abundance. The effect of non-native crayfish on benthic invertebrates may be more prominent in systems where they are the dominant benthic consumer (Helms and Reed 2005, Lagrue et al. 2014). The presence of other consumers, like fishes and salamanders, may have caused high benthic invertebrate immigration and emigration among cages, overwhelming the effect of crayfish species or stage (Lagrue et al. 2014). I did not find evidence for short-term impacts on invertebrate communities, and it is not currently known whether ringed crayfish provide a functionally equivalent role or if they have altered invertebrate communities where signal crayfish have been displaced.

Demographic differences between native and non-native species can determine whether a non-native species will displace or co-occur with native species (Larson and Magoulick 2008). Saepharn et al. (unpublished data) found that in the laboratory, signal crayfish displayed more aggressive postures and captured food more frequently compared to ringed crayfish of comparable size, suggesting that signal crayfish should resist ringed crayfish invasion. However, if ringed crayfish have higher fecundity or growth rates relative to signal crayfish, their high abundance can lead to long term shifts in community structure and ecosystem processes not seen in this laboratory study (Mathers et al. 2016).

Ecologists have been tasked to measure and predict the effect of non-native species in order to help prioritize management efforts. Non-native species are expected to have detrimental effects on recipient food webs, but ontogenetic diet shifts make it difficult to predict their impact on community structure and ecosystem processes. In this study, I did not see major differences in benthic invertebrate communities between native signal crayfish and non-native ringed crayfish. However, life history stage modified the abundance of invertebrate functional feeding groups, even in this relatively short-term study. In addition, ringed crayfish altered patterns of leaf litter breakdown relative to native signal crayfish, an ecosystem function that can have delayed but important effects on the stream community. Signal crayfish had stronger effects on leaf processing compared to ringed crayfish and their displacement may result in changes to species interactions that alter energy flows. Whether a species is native or non-native is not enough to predict their impact on local ecological communities, and this study shows that ontogenetic stage is an important factor to consider when assessing the effect of introduced species on native community structure and ecosystem processes.

Figure 10. Mean invertebrate abundance (± 1 SEM) by crayfish species and ontogenetic stage: a) overall, b) shredder, c) collector, d) scraper, and e) predator.

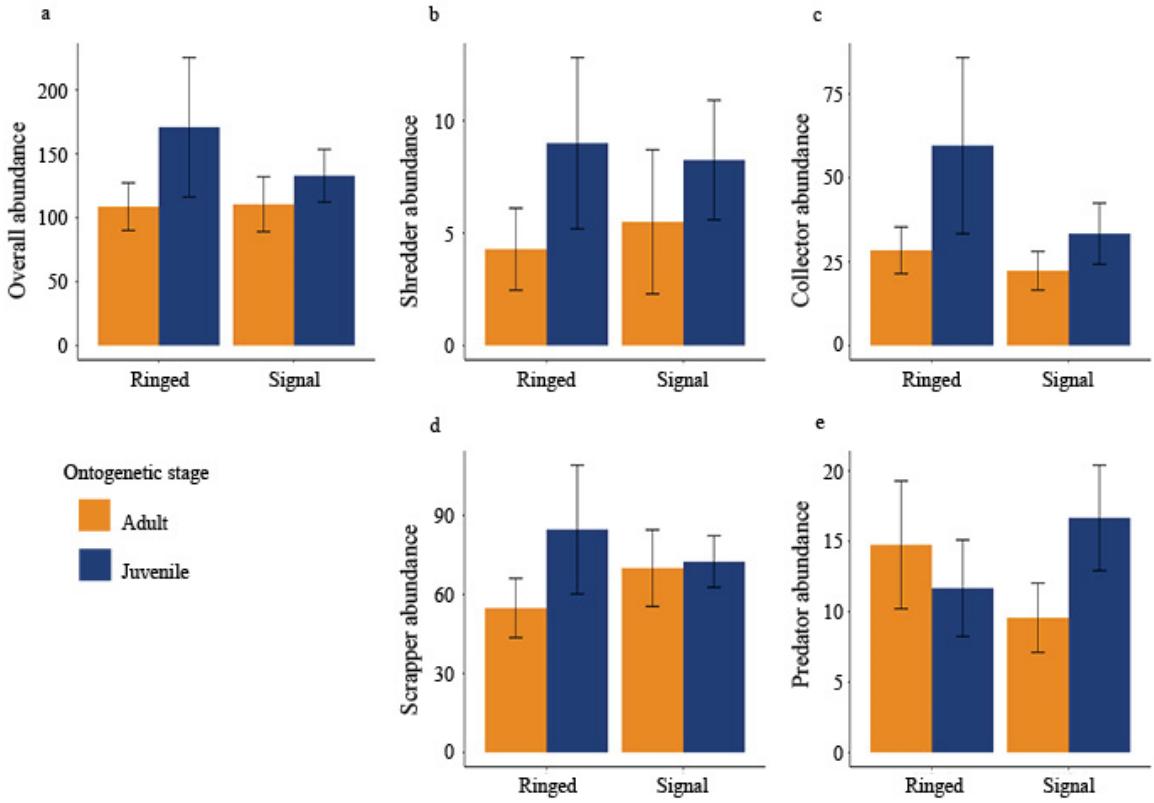

Figure 11. Mean (± 1 SEM) leaf litter remaining (g) after 6 weeks by crayfish treatment. Blue bars represent leaf packs covered in mesh and inaccessible to crayfish. Green bars represent open leaf packs accessible to crayfish.

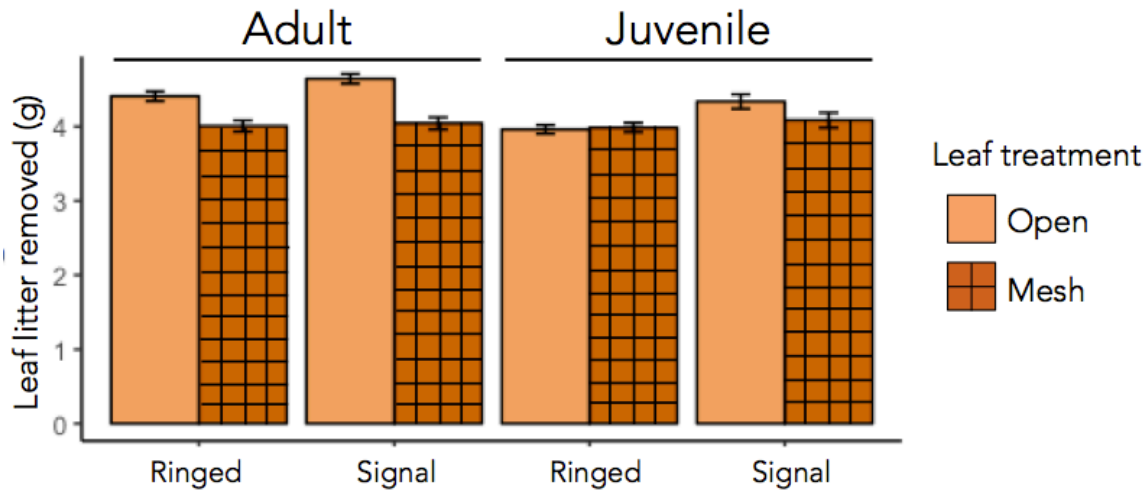

Table 6. Mean crayfish total length (measured from rostrum to tail, mm) and mean mass (blotted wet weight, g) (± 1 SEM) by species and ontogenetic stage.

Species	Stage	Mean total length (mm)	Mean mass (g)	# Replicates
Ringed	Adult	74.29 ± 0.55	21.03 ± 0.69	7
	Juvenile	43.11 ± 1.23	2.77 ± 0.24	8
Signal	Adult	74.57 ± 1.32	17.47 ± 1.01	7
	Juvenile	52.62 ± 1.46	6.19 ± 0.63	7

Table 7. Results of two-way ANOVA testing the effect of crayfish species identity, ontogenetic stage, and leaf treatment on leaf litter loss.

Treatment	df	F value	p-value
Crayfish species (ringed, signal)	2	8.348	<0.001
Crayfish stage (juvenile, adult)	1	10.632	0.001
Leaf treatment (open, mesh)	1	21.865	<0.001
Species x stage	1	1.172	0.281
Species x leaf treatment	2	5.717	0.004
Stage x leaf treatment	1	13.730	<0.001
Species x stage x leaf treatment	1	0.138	0.711

CHAPTER 5 - APPROXIMATING TRUTH: CHALLENGES IN BRIDGING THEORY AND DATA IN A FUNCTIONAL RESPONSE FRAMEWORK

Abstract

The functional response captures the relationship between resource density and consumer feeding rates. It also serves as a model connecting empirical data on feeding rates to ecological theory to gain insight into consumer-resource dynamics. Statistics quantifies the confidence of our understanding of the link between models and data to determine the robustness of existing theory. These three components, theory, data, and statistics, are used in conjunction to create new knowledge but are also subject to biases from the scientific framework in which they are used. Failing to recognize these biases can hinder progress in science. Using one of my functional response experiments as a case study, I review the challenges of designing and analyzing functional response experiments and suggest ways to move forward.

Part 1: Paradigms in science

The central goal in ecology is to understand the mechanisms that regulate the abundance and distribution of species. Since its inception, various theories have been developed to serve as a conceptual framework to explain patterns in natural history observations and predict how ecological communities will respond to environmental change. As ecologists, we bring together incomplete pieces to understand how the world works: we test theories by constructing models with simplifying assumptions and collect data that are imperfect samples of the whole we are trying to measure. Theories are explanations of phenomena that have been supported through a vast accumulation of evidence and are general enough to be applied to different systems. Models are

simplifications of a system that allow ecologists to evaluate hypotheses about how the world works and can be used as a bridge connecting empirical data and theory. Statistics allows us to quantify the confidence of our understanding of the link between models and data to determine the robustness of existing theory. However, the models we create and the data we collect are always conducted within a given scientific framework. These frameworks can create biases in the interpretation of data and statistical inferences. Failing to recognize these biases can hinder progress in science.

"What a man sees depends both upon what he looks at and also upon what his previous visual-conceptual experience has taught him to see" – Thomas Kuhn

In his book "The Structure of Scientific Revolutions", philosopher Thomas Kuhn describes the process of how science moves forward. The way we conduct science is guided by paradigms, which are methodological, philosophical, or societal constructs that capture the current state of scientific understanding. A scientific paradigm dictates what types of experiments to perform, what data to collect, and how to interpret the data. As scientists test the paradigm within their systems, data that do not fit within the paradigm begin to emerge. This brings about a "state of crisis" until a new paradigm is developed that address these anomalies and the cycle continues.

Though paradigms allow us to organize our endeavors, they can also limit us from gaining new knowledge. Early studies in a field are likely to be confirmatory to the paradigm in which they were developed. Kuhn has noted that conducting confirmatory studies are preferred over studies that test paradigm assumptions (Kuhn 1970, Austin 1999, Kamath and Losos 2017). The demand for positive results in high impact journals has led to a bias towards large effect sizes and a citation bias as these high profile

publications are more likely to be cited than those from lower impact journals (Jennions and Møller 2002, Murtaugh 2002). Studies in languages other than English may not be index into databases or incorporated into other analyses (Egger and Smith 1998). There are also biases in study system location, as it is easier to conduct research where financial resources are abundant and study sites are easily accessible (Martin et al. 2012). Societal context also influences the language that is used within a paradigm. For example, during World War II, Charles Elton was tasked to find ways to control introduced pests in an effort to protect England's food supply (Davis et al. 2011). His writings on species invasions included war metaphors as the fear of invasion by Germany was at the forefront of England's concern. The dominance of men in western science has similarly influenced the language used in foundational work to describe behavioral differences between sexes in animal mating systems as it reflected society's view of women in the era these studies were conducted (Kamath and Losos 2017). Words such as "passive" or "less eager" were used to describe female Anolis lizard social behavior, and any evidence to the contrary was omitted. Because researchers continued to cite these works, assumptions turned into "quasi-facts" and work on Anolis lizards is currently undergoing a paradigm shift as more scientists are embracing results counter to the prevailing paradigm. More generally, biases are also subject to external factors, such as funding sources, which can benefit certain types of questions more than others (Gravem et al. 2017). All together, the search for broad patterns in nature is limited by what information scientists chose to include, which can ultimately hinder scientific progress (Parker et al. 2016).

A challenge in using models to test predictions from theory is choosing a system to work in. Study systems can be chosen by the reliability of data to fit the model of interest

or to test the generality of a model. When data do not conform to theoretical predictions, their omission from publication further perpetuates existing paradigms. Ecological data is messy necessitating the use of statistics to see patterns in the noise, but this can still overwhelm signals needed to make inferences on biological processes.

I struggled with these issues for one of my experiments. In this chapter, I will use my experiment as a case study to explore Kuhn's ideas of paradigms under the functional response framework and the challenges of using statistics to bridge ecological theory and empirical data. Part 2 will describe this experiment and its motivation. In part 3, I will review the challenges in conducting and analyzing my type of experiment.

Part 2: Uniting ecological theories in a functional response framework to describe detritus breakdown by crayfish

Background

Functional responses measure the effect of resource density on a consumer's feeding rate and can provide important information on the processes that regulate population dynamics, community structure, and food web stability. In its most simplistic form, this relationship is governed by the consumer's attack rate, or the rate at which a consumer encounters and captures its resource, as well as its handling time, which is the time needed to kill, ingest, and digest its resource. Classic functional response models assume that consumer feeding rate is solely influenced by resource density, but recent studies have recognized the influence of consumer traits on consumer-resource interactions (Vucic-Pestic et al. 2010, Kalinkat et al. 2013). Two unifying theories in ecology, Metabolic Theory of Ecology (MTE) and Theory of Ecological Stoichiometry

(ES), provide mechanistic predictions of the factors that influence the functional response, but how these theories together contribute to our understanding of consumer feeding rates is not well understood.

The Metabolic Theory of Ecology posits that metabolic rate governs biological processes and can explain patterns seen in nature across scales (Brown et al. 2004). Central to metabolic theory is the importance of body size and an organism's temperature on its metabolic rate. According to MTE, attack rate scales with a 3/4 power law relationship of body mass, and handling time scales with a negative 3/4 power law relationship of body mass, resulting in an increase in feeding rate with consumer body mass (Brown et al. 2004,Figure 12a, d, g). For ectotherms, rising ambient temperature results in a higher metabolic rates and increased energetic demand (Clarke and Fraser 2004). Attack rate has been observed to increase with temperature (Vasseur and McCann 2005, Figure 12b) and handling time is expected to decrease with temperature via increased digestion rate and/or decreased time capturing and ingesting a resource (Sentis et al. 2012, Figure 1e), resulting in an increase in per-capita feeding rate with temperature (Figure 12). Therefore, MTE provides predictions by which body size and temperature govern consumer attack rate and handling time and ultimately overall feeding rates.

Ecological Stoichiometry focuses on the balance of energy and elements between consumers and their resources (Elser and Sterner 2002). The elemental makeup of an organism, for most organisms the ratio of carbon, nitrogen, and phosphorus (C:N:P), dictates its nutrient demands (Stern and Elser 2002). The stoichiometric mismatch between a consumer's elemental make up relative to its resource influences a consumer's feeding rate. Consumer per-capita feeding rate is predicted to increase with grater

stoichiometric mismatch, via increased attack rate and decreased handling time, to compensate for the low nutritive quality of their resource (Figure 12c,f). Conversely, if a consumer cannot undergo compensatory feeding or avoids low quality resources, per-capita feeding rates are predicted to stay the same or even decline (Hillebrand et al. 2009), Figure 12i). Therefore, energetic demand driven by body size and temperature as well as the relationship between consumer nutrient demand and resource quality drive consumer-resource dynamics.

Signal crayfish as a study system to test MTE and ES over ontogeny

Like most crayfish, signal crayfish are omnivorous as a species. However, individuals are reported to undergo a diet shift, feeding primarily on aquatic invertebrates as juveniles and switching to detritus as adults (Mason 1963). This differs from most consumers that become carnivorous as adults, particularly in aquatic ecosystems. This shift has been attributed to juveniles requiring high amounts of protein for rapid growth (Momot 1995) and to adults being too large to capture fast moving invertebrates (Abrahamsson 1966, but see Parkyn et al. 2001). Therefore, stoichiometric demands may vary over ontogeny. Stoichiometric mismatches may be particularly important for detritus-detritivore interactions as detritus is low in N and P, which are elements that are necessary for growth and body maintenance (Evans-White et al. 2005). Research on stoichiometric imbalance has focused primarily on herbivores, though 80% of global net primary production becomes part of detrital food webs (Cebrian 2004). Thus, using crayfish as a study system addresses this gap of knowledge in energy pathways of primary producers.

The goal of this study was to estimate how body size, temperature, and resource quality affect detritus consumption by crayfish. I addressed the role of body size and temperature in Chapter 2. In this experiment, I assessed how the effects of body size and temperature on feeding rates are modulated by resource quality. Specifically, I compared feeding strategies when crayfish are faced with high (alder; *Alnus rubra*) or low (ash; *Fraxinus latifolia*) detritus quality. Alder leaves have high N:P and low C:N due to its symbiotic relationship with nitrogen-fixing bacteria. Since lignin can inhibit the accessibility of these elements to detritivores, I used alder and ash leaves as they are similar in lignin composition to alder but differ in C:N (Frainer et al. 2015, Table 1).

Methods

C:N measurements

Fallen Alder (*Alnus rubra*) ash (*Fraxinus latifolia*), Oregon white oak (*Quergus garryana*), big leaf maple (*Acer macophyllum*), and vine maple (*Acer circinatum*) leaves were collected in autumn 2014 along stream banks within Oregon State University's MacDonald-Dunn Research Forest, the traditional territory of the Chepenefa band of the Kalapuya, in northwest Corvallis, Oregon. Leaves were dried in an oven at 50°C for 48 hrs and subsequently ground with a mortar and pestle. Leaf matter (1 g per replicate, 3 replicates per tree species) was placed into plastic vials and sent to the Wildlife Habitat and Nutrition Lab at Washington State University to perform Van Soest sequential fiber analysis. The amount of lignin in leaves influences a consumer's ability to consume and digest the leaf, therefore it was important for us to find leaves of similar lignin composition for the feeding experiment. The analysis measured C, N, cellulose, and lignin in leaves. C represents total carbon, cellulose represents the portion of labile

carbon that is accessible to consumers, and lignin represents the portion of recalcitrant carbon that is resistant to decomposition.

Functional response experiment

Crayfish (28 - 92 mm total length) were collected from the Siletz River, Oregon (44°43'N 123°55'W). Crayfish were acclimated to laboratory conditions for 1 week and were fed algae pellets daily ad libitum. Crayfish were starved for 48 hours prior to the start of the laboratory experiment at ambient stream temperature (14°C), followed by a 24 hr acclimation period to one of the three temperature treatments. At the beginning of each trial, 1 crayfish was placed inside a rectangular aquarium (35x20x13 cm) and acclimated for 30 minutes. The aquarium was filled with filtered stream water at 10, 15, or 20°C to reflect the range of temperatures occurring in local streams throughout the year. Leaves were soaked in stream water for 1 week prior to the experiment to allow for conditioning. I used a 15 mm diameter cork borer to cut evenly sized disks out of leaves. Crayfish were fed leaf disks of either alder or ash at the following total wet weights: 0.3, 0.5, 1, 1.5, or 2 g (0.02-0.06 g per leaf disk). The experiment ran for 24 hrs and I subsequently recorded wet weight (g) of detritus after the experiment, as well as crayfish length (total length and carapace length, mm) and sex. Crayfish were then dried in an oven overnight to obtain dry weight.

I used a hyperbolic Type II functional response model to describe the relationship between crayfish per-capita feeding rates and detritus biomass,

$$F = \frac{aN}{1 + ahN} \qquad (1)$$

(Holling 1959), where F is the per-capita consumption rate, N is resource abundance, a is the attack rate and h is the crayfish handling time of detritus. In accordance with metabolic theory, attack rate and handling time follow a power-law relationship with consumer mass, and an exponential relationship with temperature, respectively,

$$a = a_0 m^{s_a} e^{\frac{E_a T - T_0}{kTT_0}} \quad (2),$$

$$h = h_0 m^{s_h} e^{\frac{E_h T - T_0}{kTT_0}} \quad (3),$$

where h_0 and a_0 are normalization constants at temperature T_0 (15°C = 288.15 K), m is consumer body mass (dry weight, g), s_a and s_h are the allometric scaling exponents for attack rate and handling time, respectively, E_a and E_h are the activation energies for attack rate and handling time, respectively, k is the Boltzmann constant (8.62 x 10-5 eV K^{-1}), and T is the absolute temperature (K).

Leaf matter was not replaced during the experimental period, therefore we used the Rogers random consumer equation with the Lambert W function to estimate attack rate and handling time while accounting for prey depletion (Rogers 1972, Bolker 2008),

$$N_e = \frac{\omega(ahN_0 e^{Pt - hN_0})}{ah} \quad (4),$$

where ω is the Lambert W function, N_e is the biomass of detritus eaten, N_0 is the initial detritus biomass, P is crayfish density, and t is the experimental duration (1 day).

We used maximum likelihood estimation to fit several nested functional response models using the *spblx* function in the "nloptr" package (Rowan 1990, Johnson 2019) and mle2 function in the "bblme" package in R (Bolker 2017). As leaf disks were not replaced after being consumed and represents a continuous variable, we used a gamma and log-normal distributions to calculate the likelihood of our data based on the chosen model. The full model considered attack rate and handing time to be dependent on crayfish body mass, water temperature, and detritus quality as described by equations 2, 3, and 4. We then fit simplified models that removed all possible combinations of dependencies on body mass, temperature, or detritus quality (30 models total). The best performing model was selected on the basis of having the lowest AIC_C value (Burnham and Anderson 2004).

Results

Alder (*Alnus rubra*) and ash (*Fraxinus latifolia*) had similar lignin percentages (Table 8). Ash had higher percent cellulose compared to alder, and higher C:N. Leaf consumption by crayfish varied with leaf biomass irrespective of crayfish body mass or water temperature (Figure 13). The best performing model for models using a gamma distribution had the normalization constants of attack rate and handling time dependent on leaf quality (Table 8a). Attack rate had a positive relationship with crayfish body mass and temperature (Table 8a). Handling time had a positive relationship with body mass and a negative relationship with temperature (Table 8a). For models using a log-normal distribution, attack rate increased with body size and temperature (Table 8b). Handling time increased with body mass. The relationship between handling time and temperature was dependent on resource quality: handling time increased on ash leaves and handling

time decreased on alder leaves (Table 8b). The top gamma model better than the top log-normal model (ΔAICc > 5). The best performing model overall overestimated feeding rates at low initial leaf biomass and overestimated feeding rates as initial leaf biomass increased and had a low fit to the data (pseudo-R^2 = 0.17, Figure 14).

Part 3: Challenges encountered in using models and statistics to bridge theory and data

Over the past 60 years, theoreticians and empiricists have developed many functional response models to understand consumer-resource interactions (Jeschke et al. 2002). Ecologists have modified the original Hollings disc equation by accounting for prey depletion (Rogers 1972), the ratio between consumer and resource density (Abrams and Ginzburg 2000) habitat complexity (Barrios-O'Neill et al. 2016) and dimensionality (Pawar et al. 2012), to name a few. My contribution to this rich literature was to further the unification of Metabolic Theory of Ecology and Ecological Stoichiometry through explaining feeding rates over consumer life stages, and to bring attention to the important but often ignored role of ontogenetic variation.

Though my methods were straightforward, the results deviated from my expectations. I had expected the data to follow a typical Type II pattern with crayfish feeding rates saturating at some leaf density. However, the best performing model had a pseudo-R^2 of 0.17, meaning that it only captured 17% of variation in crayfish feeding rates. I was able to obtain estimates of the functional response parameters, but with such an overall poor fit I was not confident that these estimates would provide biological insight into my system. In the following sections, I therefore review the process and challenges in designing and analyzing functional response experiments.

Experimental design

The challenge of using short temporal and small spatial scale experiments is extrapolating the results to dynamics occurring at longer and larger scales (Levin 1966, Witman et al. 2015). For a functional response experiment, choosing the experimental

duration, mesocosm size, and resource densities can be difficult and can have an effect on our inferences of biological processes. For example, in a recent meta-analysis of functional response experiments, Li et al. (2018) found that attack rate estimates decreased with increasing experimental duration because short-term experiments miss important time constraints on an individual predator's foraging behavior. Longer experiments capture non-feeding activities, and the handling times of starved predators are consistently shorter than those of satiated predators (Li et al. 2018). Mesocosm size can similarly effect consumer and resource behavior, leading to a bias in functional response parameter estimates (Uiterwaal et al. 2017, Uiterwaal and Delong 2018). Additionally, there has been conversation in the literature as to whether resource densities used in functional response experiments should reflect natural densities (Sarnelle 2003, Sarnelle and Wilson 2008) or use unnaturally high densities so that consumers are saturated, thereby making the data fit a type 2 or type 3 functional response (Kalinkat et al. 2013).

My own experiments came across issues with resource densities. Crayfish feeding rates did not saturate in my experiment (Figure 1), which suggests that higher leaf densities should have been used. Holling's original experiment had densities that spanned two orders of magnitude and found saturation (Holling 1959). Similar to my results, Maselou et al. (2015) experiment with a predatory bug on aphid prey had one order of magnitude difference between the lowest and the highest aphid density yet predator feeding rate did not reach saturation. Filter feeders are known to be the only taxa that exhibit a type 1 functional response (Jeschke et al. 2004), but in other taxa it has been suggested that consumer-resource interactions are linear across a range of naturally

occurring resource densities (Wootton and Emmerson 2005, Novak 2010). By using unnaturally high resource densities to achieve saturation, those who conduct functional response experiments may maintain the paradigm of the ubiquity of type 2 functional responses even though it is rarely realized, which has consequences for predictions of consumer-resource interactions.

The art of model fitting

Faster computing power and the increasing complexity of statistical techniques has enabled the analysis of functional response experiments to evolve considerably over time. An early statistical method for model fitting was nonlinear least squares but it is acknowledged that overdispersion has a positive relationship with resource density that is not accounted for using this method (Trexler et al. 1988, Fenlon and Faddy 2006). Logistic regression has been used to distinguish between Type I, II, and III (Trexler et al. 1988) but has been criticized (Okuyama 2013).

Current maximum likelihood methods are used for nonlinear model fitting with the flexibility to choose the appropriate error distribution to reflect experimental design (Bolker et al. 2013). This method finds the parameter values that make the observed data most likely to have happened. Next, one must choose initial starting values for each parameter to be used in a specified algorithm used to search for the maximum likelihood estimates, which can be challenging with complex models that have multiple parameters. This is particularly challenging when colinearity is present, as is often the case (Dormann et al. 2013). Once estimates are obtained, it is important to plot a likelihood surface to see how the likelihood changes as a function of parameter values. Parameter estimates for multiple functional response models can be calculated and Akaike Information Criteria

(AIC) can be used help determine which model "best" represents the data. To use AIC, an AIC value is calculated using the likelihood and number of parameters in a model and the preferred model is the one with the lowest AIC value.

Model selection is subject to which models the researcher deems worth testing, which are subject to the prevailing scientific paradigm that guides the types questions are asked in the first place. The "best performing model" is the preferred model relative to the other models tested, but may not be true or the most accurate model for describing the focal system (in terms of R^2 or Root Mean Square Error). For example, the best performing model may have a poor fit to the data, therefore inferences made on these parameter estimates would be misleading. A review of the literature is needed to know how often studies report goodness-of-fit measures to define the limits of their inferences, similar to how effect sizes can be reported alongside p-values to improve the readers comprehension of the statistical clarity of the effect of a parameter on a measured outcome. Without this additional information, adhering to established models regardless of model fit reinforces paradigms and prevents new knowledge.

I faced my own challenges using maximum likelihood to understand how crayfish body size, temperature, and resource quality affect crayfish feeding rates. To obtain functional response parameter estimate, I chose log-normal and gamma distributions as they were both are appropriate for my data. The use of each error distribution in the likelihoods resulted in different top performing models: log-normal highlighted the importance of resource quality on handling time, whereas gamma highlighted the importance of resource quality on attack rate and handling time intercepts. I moved forward with the analysis using the gamma distribution as these models performed better

based on AIC than models with a log-normal distribution (ΔAICc > 5). I had 12 parameters to estimate for the full model that included body size, water temperature, and resource quality and found that the R package I was using (*bbmle*, Bolker 2017) was sensitive to initial conditions. I used the default algorithm (Nelder-Mead) to search for a global minimum, which is another way to say that the algorithm searched for the single best estimate to maximize the likelihood (or minimize the negative log likelihood). One can picture the best estimate at the bottom of a valley of a likelihood surface. Instead, my likelihood surface was relatively flat and the algorithm could not find a global minimum, which explains why the parameter estimates were sensitive to initial conditions. The best performing model had a pseudo-R^2 of 0.14. In an attempt to improve model fit, I switched to *nloptr*, a nonlinear optimizer in R, to obtain parameter estimates and used these as starting values in mle2 in order to get error estimates. This method resulted in a pseudo-R^2 of 0.17. Ultimately, I tried various statistical methods with our "best performing model" and obtained parameter estimates but was not confident in my ability to make inferences on crayfish feeding rates based on my model.

"A theory has only the alternative of being right or wrong. A model has a third possibility: it may be right, but irrelevant." – Manfred Eigen

My challenges with these data brings up questions about how studies are conducted in the first place: 1) was the experimental design appropriate for the question? 2) Do we choose systems that will produce data that will fit our models? 3) Are our models appropriate to test our hypotheses? 4) Do our models test the correct theory? These questions highlight the different ways to bring empirical data and theoretical

ecology together. Theories can't predict specific outcomes in any one system, but testing theory-derived models in different systems allows us to understand how generalizable theories can be. Some researchers explicitly test theory; for example, Wang et al. (2018) tests whether activation energy of attack rate follows the ¾ power scaling law that is canonical to Metabolic Theory of Ecology. My research was motivated by theory as I searched for qualitative patterns in functional response parameters as predicted by MTE and ES. I was expecting crayfish to exhibit a Type II functional response and hence fit that model to the data, but my ability to hypothesize the ways in which the model did not capture the biology of the system might have limited because of this expectation. The type II functional response fit well when crayfish feed on stoneflies (Chapter 2) but not when they feed on leaf litter (this chapter). The high amount of variation in crayfish feeding rates on leaf litter (Figure 2) suggests that the interaction of some consumer-resource pairs may be more difficult to describe than others. Because all models are wrong, the challenge is to understand what is "importantly wrong" (Box 1976).

Approximating truth

Ecology is progressing towards more complex statistical methods and it is important to know the limits of inference with each method. A long-standing paradigm in the use of statistics in ecology is the use of p-values to determine the "significance" of a result based on the probability of the data given that the null hypothesis is true. Bayesian statistics is an alternative approach that focuses on the probability of a hypothesis given the data and is gaining more popularity in ecology. The newest frontier of statistics in ecology is equation free modeling and machine learning. These are phenomenological models, which focus strictly on the relationship between variables and not the causes for

the relationship, as opposed to mechanistic models, which attempt to explicitly include biological processes of the focal system (Hilborn and Mangel 1997). There is debate on whether models need to be mechanistic to be useful (Hartig and Dormann 2013, Perretti et al. 2013, White and Marshall 2019). Phenomenological models may have predictive power, but recognizing patterns does not equate to understanding them. Mechanistic models may be grounded in biology and generalizable to different systems, but some processes can be difficult to derive measure. The use of each model type will depend on the goal of the study. Both model types can be used to uphold or challenge existing paradigms.

The challenge in using models and statistics to bridge ecological theory and empirical data is that models, data, and theory incomplete representation of nature. Models can't simultaneously be precise, realistic, and general (Levins 1966), and it is important to understand the boundaries of our inferential abilities. In this chapter, I reviewed the theoretical, empirical, and statistical difficulties in conducting a functional response experiment. Those learning how to be in the middle of these 3 worlds need the language competency to translate between math, biology, and statistics. People who specialize in any of these fields are more adept at doing each of these things, but specialization also prevents scientific progress (Haller 2014, Graham and Dayton 2002). Advances in computing power have allowed us to conduct analyses that were previously impossible, which has led us to use more technical methods that are only fully understood by a few. Technology is enabling us to bridge theory and data, but lack of communication and universal understanding impede their unity. Additionally, specialization can prevent scientists from being in touch with the history of their field, making it likely that they will

revisit old ideas instead of synthesizing past information and challenging existing paradigms (Graham and Dayton 2002).

To move the field of ecology forward, what is needed is clear communication among ecologists, statisticians, and mathematicians so that ecologists understand how to rigorously test theory and the strengths, assumptions, and limitations of available methods. Our role as ecologists should be to "approximate truth" (Petkov 2018), to resolve theoretical contradictions by understanding the contexts in which certain theoretical predictions are supported instead of dismissing studies that do not fit the prevailing paradigm. We need more evidence of anomalies, a cultural shift towards publishing "negative" results to fully understand how robust our theories are, to develop new theories, and find ways to unify our observations. By recognizing the limits of our methods for understanding, we can integrate our incomplete pieces of information and avoid being trapped existing paradigms to move the field forward.

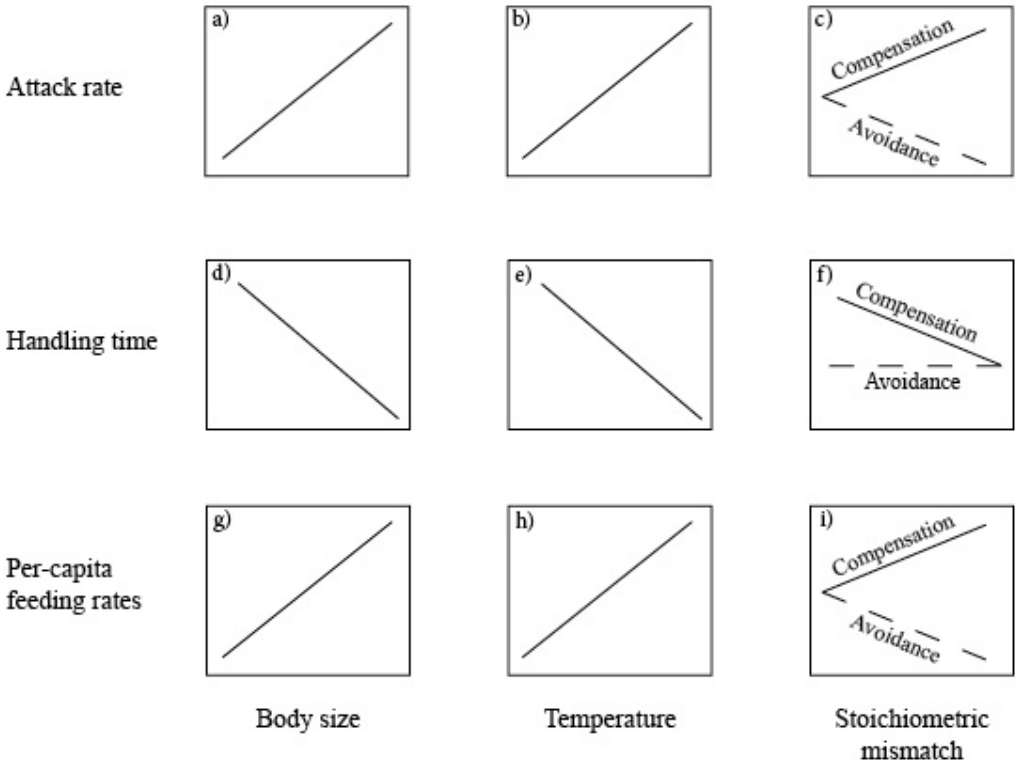

Figure 12. Patterns of attack rate, handling time, and per-capita feeding rates as a function of consumer body size, environmental temperature (for ectotherms), and stoichiometric mismatch. Figure adapted from Hillebrand et al. 2009.

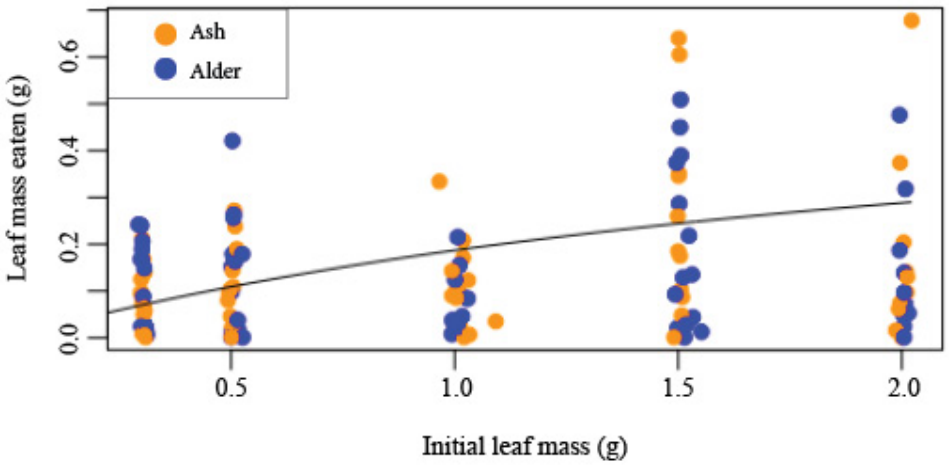

Figure 13. Leaf litter eaten as a function of initial leaf litter mass by leaf type. Orange circles represent ash leaves and blue circles represent alder leaves.

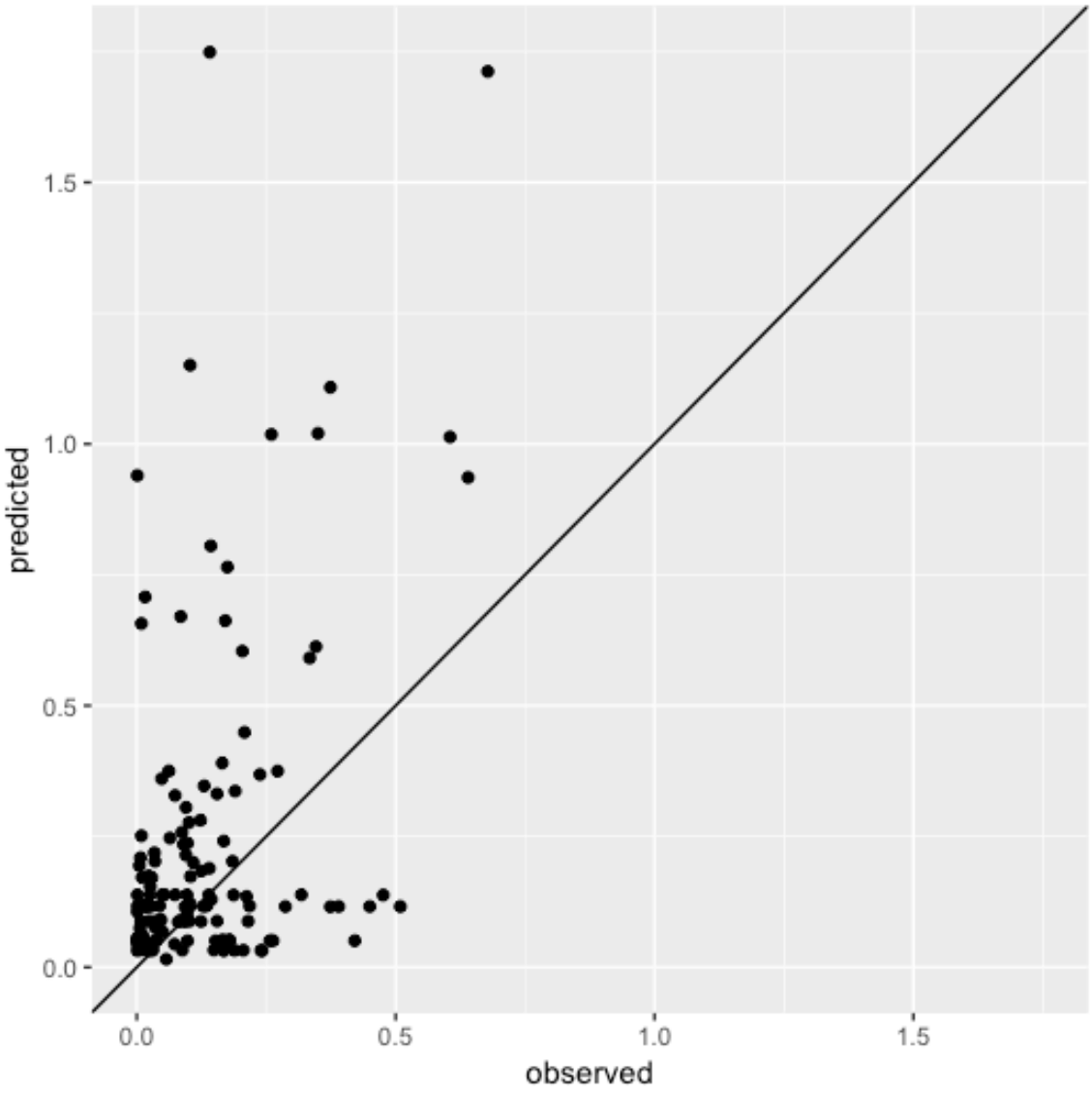

Figure 14. Observed vs. predicted for the best performing model that included the effect of resource quality on the normalization constant of attack rate and handling time. Line represents 1:1.

Table 8. Chemical composition of leaves from the five most common trees in the MacDonald-Dunn forest. C:N represents the ratio between carbon and nitrogen. Percent cellulose and lignin (mean ± SD) are based on 1 g dry mass.

Leaf type	C:N	% cellulose	% lignin
Alder (*Alnus rubra*)	34.28	24.93 ± 1.01	20.21 ± 0.44
Ash (*Fraxinus latifolia*)	52.28	52.42 ± 1.55	22.47 ± 1.28
Oak (*Quercus garryana*)	44.42	50.53 ± 1.32	37.9 ± 1.11
Big leaf maple (*Acer macophyllum*)	46.75	31.64 ± 4.60	40.21 ± 6.99
Vine maple (*Acer circinatum*)	95.22	23.81 ± 3.20	15.21 ± 2.30

Table 9. Functional response parameters and estimates from the best performing model using gamma (a) and log-normal (b) distributions using the *mle* function in the *bbmle* package. For the gamma-distributed model, all parameters were independent of resource quality except the normalization constants of attack rate (a0) and handling time (h0). For the log-normal distributed model, all parameters were independent of resource quality except the activation energy of handling time.

a)

Leaf type	Parameter	Est.	S.E.
	Mass scaling exponent of attack rate (sa)	1.095	0.321
	Activation energy of attack rate (Ea)	1.174	0.442
	Mass scaling exponent of handling time (sh)	1.023	0.781
	Activation energy of handling time (Eh)	-0.207	0.381
Ash (*Fraxinus latifolia*)	Normalization constant of attack rate (a0)	0.119	0.345
Alder (*Alnus rubra*)	Normalization constant of attack rate (a0)	0.126	0.135
Ash (*Fraxinus latifolia*)	Normalization constant of handling time (h0)	0.906	1.319
Alder (*Alnus rubra*)	Normalization constant of handling time (h0)	22.85	NA*

*could not be estimated using profiling methods of mle2

b)

Leaf type	Parameter	Est.	S.E.
	Mass scaling exponent of attack rate (sa)	0.92	0.28
	Activation energy of attack rate (Ea)	0.90	0.31
	Mass scaling exponent of handling time (sh)	0.01	1.08
Ash (*Fraxinus latifolia*)	Activation energy of handling time (Eh1)	1.57	4.65
Alder (*Alnus rubra*)	Activation energy of handling time (Eh2)	-2.09	5.77
	Normalization constant of attack rate (a0)	0.03	0.01
	Normalization constant of handling time (h0)	0.19	0.40

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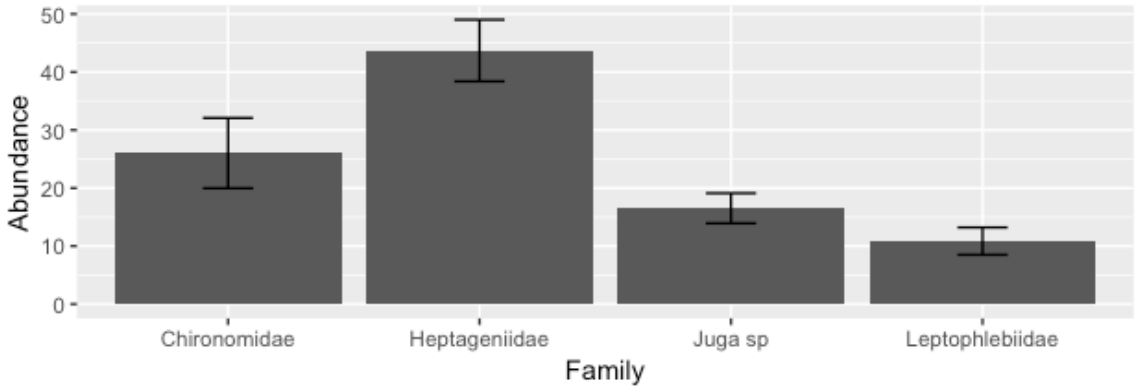
APPENDICES

Appendix A: Supplementary materials for Chapter 2

Appendix Table A1: All non-stage structured models tested for AICc model selection in order from lowest to highest AICc value. Dashes represent parameters that were removed from analysis. Estimates and standard errors for the normalization constant for attack rate (a_0), mass-scaling exponent of attack rate (s_a), activation energy of attack rate (E_a), normalization constant for handling time (h_0), mass-scaling exponent of handling time (s_h), activation energy of handling time (E_h) from fitting the functional response model (equation 1) with body size and temperature scaling of attack rate and handling time (equations 2 and 3).

Model	a_0	s_a	E_a	h_0	s_h	E_h	L	df	AICc	Δ AICc	Model weight
1	11.21 ± 0.10	-0.98 ± 0.35		0.60 ± 0.06	-0.85 ± 0.85	-0.62 ± 0.62	796.51	5	796.943	0	.62
2	7.37 ± 4.89	-0.83 ± 0.35	0.44 ± 0.44	0.48 ± 0.07	-0.84 ± 0.11	-0.53 ± 0.14	797.65	6	798.27	1.33	.32
3	2.24 ± 0.53	---	0.88 ± 0.40	0.39 ± 0.05	-0.67 ± 0.10	-0.38 ± 0.15	802.33	5	802.771	5.83	0.03
4	2.92 ± 0.73	---	---	0.43 ± 0.05	-0.69 ± 0.09	-0.59 ± 0.09	804.40	4	804.694	7.75	0.01
5	2.14 ± 0.54	---	1.59 ± 0.30	0.35 ± 0.04	-0.63 ± 0.09	---	806.65	4	806.942	10.00	<0.01
6	3.12 ± 1.25	-0.36 ± 0.28	1.47 ± 0.27	0.37 ± 0.05	-0.73 ± 0.12	---	807.15	5	807.586	10.64	<0.01
7	0.73 ± 0.18	0.87 ± 0.20	1.63 ± 0.25	0.16 ± 0.01	---	---	840.58	4	840.327	43.38	< 0.01
8	0.74 ± 0.18	0.88 ± 0.20	1.52 ± 0.31	0.16 ± 0.01	---	-0.09 ± 0.14	841.58	5	842.018	45.07	< 0.01
9	7.98 ± 4.91	-0.72 ± 0.35	---	0.45 ± 0.05	-0.73 ± 0.10	---	843.97	4	844.256	47.31	< 0.01
10	3.22 ± 0.96	---	---	0.41 ± 0.05	-0.63 ± 0.09	---	846.59	3	859.691	49.83	< 0.01
11	2.03 ± 0.53	---	1.615 ± 0.3	0.17 ± 0.02	---		859.52	3	859.691	62.78	< 0.01
12	2.05 ± 0.51	---	1.328 ± 0.38	0.18 ± 0.02	---	-0.17 ± 0.15	860.16	4	860.446	63.50	< 0.01
13	1.31 ± 0.36	0.70 ± 0.23	---	0.19 ± 0.02	---	-0.54 ± 0.09	860.57	4	860.856	63.91	< 0.01
14	2.78 ± 0.72	---	---	0.19 ± 0.02	---	-0.54 ± 0.09	869.27	3	869.442	72.50	< 0.01
15	1.13 ± 0.32	0.77 ± 0.22	---	0.18 ± 0.02	---	---	894.72	3	894.892	97.95	< 0.01
16	2.76 ± 0.77	---	---	0.20 ± 0.02	---	---	904.60	2	904.686	107.74	< 0.01

Appendix B: Supplementary materials for Chapter 4



Appendix Figure B1: Mean (± S.E) abundance of the common benthic aquatic families found in all crayfish enclosures

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Appendix Table B1. Aquatic invertebrate taxa found in enclosures by Order and Family.

Order	Family
Basommatophora	Lymnaeidae
Coleoptera	Certopogonidae
Coleoptera	Chrysomelidae
Coleoptera	Coleoptera sp.
Coleoptera	Elmidae
Coleoptera	Hydropsychidae
Coleoptera	Lepidostomatidae
Coleoptera	Psephenidae
Collembola	Collembola sp.
Diptera	Athericidae
Diptera	Ceratopogonidae
Diptera	Chironomide
Diptera	Diptera sp.
Diptera	Dixidae
Diptera	Empididae
Diptera	Pelecorhynchidae
Diptera	Simuliidae
Diptera	Tipulidae
Ephemeroptera	Ameletidae/Baetidae complex
Ephemeroptera	Ephemereliidae
Ephemeroptera	Heptageniidae
Ephemeroptera	Hydropsychidae
Ephemeroptera	Leptageniidae
Ephemeroptera	Leptophlebiidae
Ephemeroptera	Potamanthidae
Hydracarina	Hydracarina sp.

Order	Family
Juga	Juga sp.
Neuroptera	Neuroptera sp.
Odonata	Gomphidae
Ostracoda	Ostracoda sp.
Plecoptera	Capniidae
Plecoptera	Chloroperlidae
Plecoptera	Hydropsychidae
Plecoptera	Leptophlebiidae
Plecoptera	Leutridae/Capniidae complex
Plecoptera	Perlidae
Plecoptera	Perlodidae
Plecoptera	Plecoptera
Plecoptera	Polycentropodidae
Plecoptera	Taeniopterygidae
Gastropoda	Gastropoda sp.
Thysanoptera	Thysanoptera sp.
Trichoptera	Glossosomatidae
Trichoptera	Hydrophychidae
Trichoptera	Lepidostomatidae
Trichoptera	Philopomatidae
Trichoptera	Phryganeidae
Trichoptera	Polycentropodidae
Trichoptera	Psychomyiidae
Trichoptera	Rhyacophilidae
Trichoptera	Trichoptera sp.