CONTINGENCY OF PREDATION RISK-INDUCED TRAIT RESPONSES IN A MODEL FISH-ZOOPLANKTON COMMUNITY

By

Alexandra V. Rafalski

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife—Doctor of Philosophy
Ecology, Evolution, and Behavior—Dual Major

2020

ABSTRACT

CONTINGENCY OF PREDATION RISK-INDUCED TRAIT RESPONSES IN A MODEL FISH-ZOOPLANKTON COMMUNITY

By

Alexandra V. Rafalski

Predators affect prey populations both through consumption and by inducing anti-predator trait responses. In the mere presence of predators, many prey modify traits in order to reduce their risk of being consumed. Predation risk-induced trait responses (hereafter ‘trait responses’) are numerous and universal across ecosystems and across different taxa, from protists to large mammals. Increasing attention is being given to the proposition that trait responses can have large effects on prey fitness, with ensuing effects on prey population growth and interacting species. A thorough understanding of the role of such predation risk effects is important for the ecological theory of basic properties such as resilience and biodiversity, and for ecological models used in natural resources management.

While there are many studies that demonstrate a variety of trait responses in different taxa and examine the drivers of trait responses, it is still difficult to predict when trait responses will translate to population and community-level effects. The majority of theories and studies of trait responses have been conducted in simplified food webs such as predator-prey pairs. However, to examine the contribution of predation risk effects in addressing ecological questions, there is a need to understand how trait responses operate in larger food webs. To scale up from simplified systems, fundamental properties of populations and communities need to be considered including whether there is variation and contingency in trait responses among life history stages and similar species of prey. While there is a theoretical basis for expecting variation, empirical examples in a natural setting are lacking.

My dissertation research empirically examines the variation and contingency of behavioral trait responses induced by a fish predator within a diverse assemblage of zooplankton prey. Experiments were conducted in mesocosms with and without fish kairomone (produced by caged fish); the effect of kairomone on the position of zooplankton is used as a measure of behavioral response. Chapter 1 examines variation in behavioral responses among life history stages of copepods. The responses were highly stage-dependent, with nauplii shifting in the opposite direction than copepodites and adults. Chapters 2 and 3 examine variation in cladoceran behavioral responses and assess if the expression and magnitude of responses is contingent on differences in predation risk among taxa. In trying to understand the variation in trait responses among prey, it might be expected that more vulnerable prey would exhibit larger trait responses. Such positive relationships between trait responses and predation risk have been exhibited in some systems. We compared the relationship between behavioral responses and metrics of predation risk across cladocerans. Metrics included relative predation rate and net effect of the predator on density on each taxon (measured from a treatment with uncaged fish) as well as cladoceran body size and taxonomic identity (family). While cladocerans exhibited strong variation in behavioral responses, we did not find larger trait responses in more vulnerable prey.

Taken together, the chapters within this dissertation demonstrate there can be considerable variation in trait responses among prey and reinforces the complex nature of factors underlying trait responses. Explicit consideration of variation in trait responses and trade-offs that govern them can lead to better insight when scaling up the study of predation risk effects and their incorporation into models.

To my husband and my parents for
their love and support always.

ACKNOWLEDGMENTS

I thank my advisor, Scott Peacor, for his effort and mentorship in conducting this research, and most importantly to me, for his support and friendship as I navigated my graduate program and life. I also thank my committee member Ace Sarnelle for all the time and guidance he provided and my committee members Elena Litchman and Brian Roth for their guidance and support. I am very appreciative of the effort and guidance Kevin Pangle provided to me regarding data analysis. I thank Alex Wenner, Facilities Manager of the Edwin S. George reserve, for all his assistance.

This research was only possible due to the help, hard work, and dedication of numerous laboratory and field technicians. Whether our relationship lasted a summer, or we still Snapchat every day, I thank them all for the fun times we had and for their enthusiasm, curiosity, and life perspectives that enriched me. I also thank the former graduate students and postdocs of the LimnoLab who supported me in my research and teaching, and for the good times.

Lastly, I thank my mom, dad, husband Jeff, and brothers Nick and Mark for their love always.

PREFACE

This dissertation was prepared in manuscript format with each chapter to be submitted separately to peer-reviewed journals. Therefore, ‘we’ is used in place of ‘I’ throughout to reflect the contribution of co-authors of this work, including project conceptualization, data collection and analyses, and feedback on manuscript drafts. Coauthors are: Chapter 1, Scott Peacor, Kevin Pangle, and Clayton Cressler; Chapter 2, Scott Peacor; Chapter 3, Scott Peacor.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Trait responses and implications

Predators affect prey populations both through consumption and by inducing anti-predator trait responses. In the mere presence of predators, many prey modify traits in order to reduce their risk of being consumed (reviewed in Chivers and Smith 1998, Kats and Dill 1998, Lima and Dill 1990, Tollrian and Harvell 1999). Such predation risk-induced trait responses (hereafter, ‘trait responses’) can be expressed for different traits including behavior (Resetarits and Wilbur 1991), morphology (Dodson and Havel 1988), development (Peckarsky et al. 2002), growth (McPeek et al. 2001, Trussell et al. 2006), and physiology (Creel et al. 2007, Sheriff and Thaler 2014). Empirical examples of trait responses are numerous and universal across ecosystems and across different taxa, from protists to large mammals (reviewed in Lima and Dill 1990, Tollrian and Harvell 1999, Agrawal 2001). Trait responses can alter components of prey fitness including somatic growth rate, survival, and reproduction (McCauley et al. 2011, Peckarsky et al. 1993, Sheriff et al 2009, Trussell 2000, Turner and Montgomery 2003, Werner and Anholt, 1996, Zanette et al 2011), and affect prey population size. Trait responses and their population-level consequences can alter the interactions of the prey with other species and resources, translating to important implications of predation risk on community structure and ecosystem function (Bolker 2003, Werner and Peacor 2003, Schmitz et al. 2008, Peckarsky et al. 2008). While there are many studies that demonstrate a variety of trait responses in different taxa and examine the drivers of trait responses, it is still difficult to predict when trait responses will translate to population and community-level effects (Sheriff et al. 2020).

What leads to trait responses?

Inducible traits instead of fixed traits are expected when 1) predation pressure is variable, 2) there are costs and benefits to the trait change, and 3) there are reliable cues for detecting predators (Tollrian and Harvell 1999). Under these conditions, inducible traits instead likely evolve because fixed traits would have significant costs when predators are absent (Schultz 1988, Harvell 1990). Inducibility in some but not all traits arise because there can be high evolutionary costs and/limits (DeWitt et al. 1998, Agrawal 2001, Pigliucci 2005). Potential costs include maintenance, production, information acquisition, reproductive, and genetic costs (DeWitt et al. 1998, Agrawal 2001). Limits to inducibility include environmental mismatch or lag time between perception of risk and ability to produce a response and developmental constraints (DeWitt et al. 1998, Agrawal 2001). These costs and limits have ecological and evolutionary implications that contribute to the differences observed in trait responses across taxa (DeWitt et al. 1998, Pigliucci 2005).

The expression and magnitude of trait responses at any given time is dependent on the tradeoff between the consequent costs to fitness and the benefit from the reduction in predation rate due to the trait response (Abrams 1984, Werner and Anholt 1993, Lima and Dill 1990, Peacor et al. 2013). There are different theoretical approaches as to what aspects of fitness should be optimized as a function of trait response. Commonly, fitness may be expressed as the difference between growth rate and predation rate to maximize species' per capita growth rate (e.g., Abrams 1984, Ives and Dodson 1987, Krivan 2007). However, other approaches include minimizing the ratio of mortality to growth given specified relationships between the trait response and mortality and growth rates (Werner and Anholt 1983, Gilliam and Fraser 1987, Fraser and Gilliam 1992). In addition, there are theories that look explicitly at specific aspects of growth and predation. For example, the asset protection principle (Clark 1994) predicts that the expression or magnitude of trait responses should balance an individual's need to reduce predation rate while maintaining or increasing its reproductive fitness. Another theory is the risk allocation hypothesis (Lima and Bednekoff 1999) which predicts that trait responses should be based on variation in predation risk that may vary over time.

Fig. 1 provides a graphical framework to illustrate the costs and benefits of trait responses and how fitness influences the optimal trait response. Fig. 1 is modified from Peacor et al. (2013) to illustrate how different optimal trait responses are expected based on the shapes of a species' growth curve, predation curves, and resultant fitness curves. In Fig. 1, growth curves, predation curves, and resultant fitness curves are represented by solid, dashed, and dotted lines, respectively. Fitness is defined as the difference between growth rate and predation rate, with the fitness maximum corresponding to the optimal trait response which is indicated by arrows. The trait response reduces the predation rate of the predator, so the predation curve is depicted to decline as a function of trait response. The value of the trait response is represented by a relative scale of 0-1 for simplicity, though it represents traits that may increase (e.g., spine length) or decrease (e.g., swimming speed) in response to predation risk.

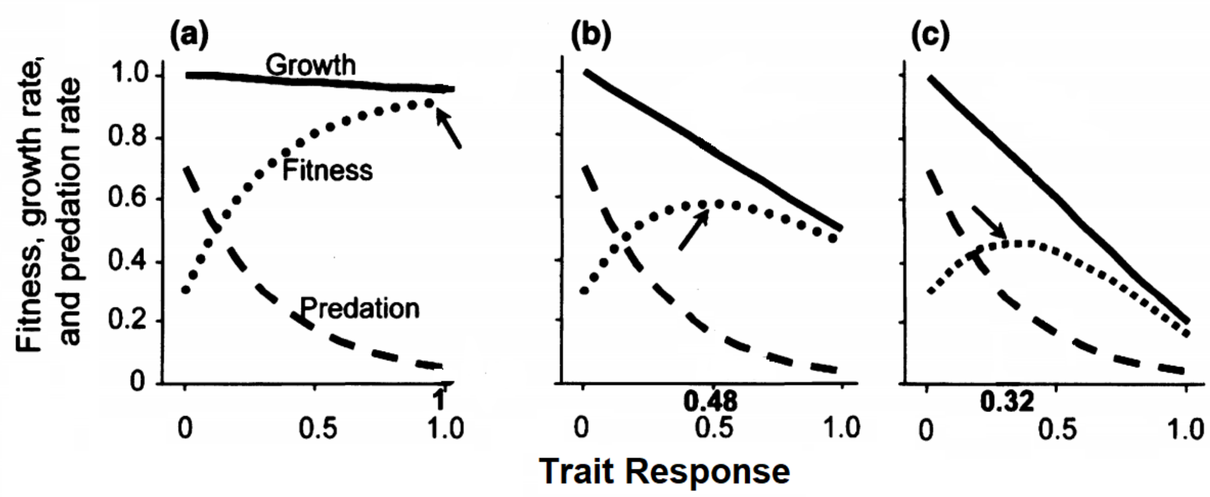

Figure 1. Modified from Peacor et al. (2013) to show how the value of the optimal trait response varies as a function of increased cost of the trait response. Panels a through c could represent a species in different environments where cost is represented as steeper growth curves moving from panel a to panel c. Growth curves, predation curves, and resultant fitness curves are represented by solid, dashed, and dotted lines, respectively. The value of the trait response is represented by a relative scale of 0-1 for simplicity. Fitness is defined as the difference between growth rate and predation rate, with the fitness maximum corresponding to the optimal trait change which is indicated by arrows and the predicted optimal trait value is labeled on the x-axis. An assumption is that the trait response reduces the predation rate of the predator, so the predation curve is depicted to decline as a function of trait response.

Different trait responses are adopted by a prey to balance costs and benefits of the trait response. This can be illustrated by changing the steepness of the growth curve in Fig. 1. Steeper growth curves represent a higher cost of a trait response. Moving from left to right in Fig. 1 (panel a to c), the growth curve becomes steeper while the predation curve is the same, resulting in a lower optimal trait response values from a to c. As an example of a trait response illustrated in Fig. 1, consider the behavioral response of refuge use, where higher trait response values correspond with more time spent in the refuge. A steeper growth curve could represent a change in the system that leads to reduced food levels in the refuge. Thus, compared to the growth curve in panel a, panel c represents more cost by spending time in the refuge, so the prey is predicted to spend less time in the refuge to optimize fitness. The example of different food levels in a refuge is just one example of factors that affect the costs of trait responses.

Any number of ecological and environmental factors can affect a species' costs and benefits of trait responses (reviewed by Lima and Dill 1990). These factors could be intrinsic or extrinsic and be variable over space and time. Examples of intrinsic factors include individual age, physiology, reproductive condition, experience, and ecology (reviewed in Lima and Dill 1990). Example extrinsic factors that affect these tradeoffs include predator type and density, habitat characteristics, resource availability, and social context (McNamara and Houston 1987, Bednekoff and Lima 2004, Higginson et al. 2012).

Contingency of trait responses among prey

The tradeoffs and factors that govern individual prey species' trait responses set an expectation for differences or contingency in the expression and magnitude of trait responses among similar prey species (Lima and Dill 1990, Randall et al. 1995, Peacor et al. 2013). Species coexistence in communities is promoted in part by variation in how species interact with and affect their environment (Chesson 2000, Leibold and McPeek 2006), and this variation leads to different costs and benefits for species and affects the expression and magnitude of their trait responses. Fig. 1 can be used to illustrate possible variation in trait responses among species based on having larger costs of responding. Using the example of time spent in a refuge, increased vulnerability of a species to a second predator as a result of responding or higher metabolic needs of a species that limit the maximum amount of time that can be spent in a refuge are costs that may be larger for one species compared to another. Costs also vary among life history stages of a species. For example, time spent in a refuge may have to balance with time spent searching for a mate. In this example, juvenile stages may have a shallower growth curve (Fig. 1 a) and spend more time in a refuge compared to mature stages that would have steeper growth curves (such as Fig. 1 c).

Current needs to understand contingency of trait responses

While there are many studies that demonstrate a variety of trait responses in different taxa and examine the drivers of trait responses, it is still difficult to predict when trait responses will translate to population and community-level effects (Sheriff et al. 2020). In order to predict how trait responses will translate to population and community-level effects, we need to evaluate population and community-level variation and contingency of trait responses of species (Bolker 2003). For example, in order to understand how trait responses influence populations and communities, we might consider: Do life history stages respond the same? Do prey species in a community respond the same? How much does context like abiotic factors matter? Can the magnitude of a species' response be predicted by its vulnerability to the predator? These questions are fundamental to understanding how predation risk affects populations and communities but there are very few studies that explore these properties in natural systems.

When trait responses among prey are different, consideration of this variation can lead to better insight of the effect of predation risk on interacting species. Models by Peacor and Werner (2000) demonstrate how different responses among competing prey can affect prey growth. Consider an example of three prey that compete for the same resource but exhibit different levels of foraging reduction as a trait response. By reducing the overall foraging activity of the assemblage of prey, the predator has an indirect positive effect on the amount of resources available to an individual prey. The contribution of the direct negative effect (from reduced foraging) and the indirect positive effects (mediated through resources) to growth are expected to be different for competitors that do not respond to the predator compared to those that respond. For a species that does not respond to the predator, the positive indirect effect dominates and there is a net positive effect of the predator on growth rate. However, for a species that responds by reducing foraging, there are negative direct and positive indirect effects of predation risk. It is thus necessary to explicitly consider if trait responses are different among prey in order to predict how predation risk will influence population growth.

Consideration of variation in trait responses among species can lead to better insight of the influence of predation risk on fundamental ecological questions. Ultimately, there is a need to scale up the understanding of predation risk effects and understand how they operate in large food webs (Boker 2003, Peacor and Cressler 2012, Peacor et al. 2012, Creel et al 2019). However, the majority of theories and studies of the importance of predation risk effects have been conducted in simplified food webs (Boker 2003, Peacor and Cressler 2012, Creel et al 2019). When there are different trait responses among prey in food webs, findings from simple food webs may not be appropriate for larger food webs. For example, simple food webs with single prey (e.g., Krivan and Schmitz 2004) demonstrate the potential for overriding trait-mediated effects on the prey compared to density-mediated effects. However, as shown by Peacor and Werner (2000, described above), the nature of the trait-mediated effect on any one prey species in an assemblage can vary depending on the trait responses of other prey. Thus, if trait responses differ in prey guilds or species groups, explicit consideration of variation in trait responses instead of assuming species have the same response should enhance the application of predation risk effects in larger food webs.

There is a need to explore underlying factors that affect trait responses to determine if there are generalizable principles that would help the understanding of predation risk effects in larger food webs. Trait-based approaches connecting species' traits to fitness in the environment have proven useful for addressing a number of ecological questions (Diaz and Cabido 2001). Studies that have investigated the relationship between traits and trait responses have shown that characteristics of the predator, prey, and environment influence the magnitude of trait responses (Preisser et al. 2007, Creel 2011, Creel et al. 2014). However, relatively little has been generalized across species that predicts the expression and magnitude of trait responses. Comparative studies across taxa that relate traits and characteristics of prey to trait responses could be useful in identifying underlying principles that can help predict how predation risk effects will influence population and communities.

Despite a theoretical basis for variation of trait responses and the implication for effects on population and communities, there are few studies in natural systems that explicitly examine the variation of trait responses at the population and community-levels of ecological organization such as through ontogeny and prey guilds. Such evidence could improve the consideration and application of scaling up the study of predation risk effects from simple food webs to larger food webs.

Dissertation Research

Differences in costs and benefits of trait responses set an expectation for different trait responses among prey stages or prey species in a community, but empirical examples that demonstrate variation in trait responses among prey that would be meaningful for scaling up the study of predation risk effects are lacking. Empirical studies from a wide array of taxa are needed to clarify the variation and contingency in the expression and magnitude of trait responses, and zooplankton-fish is a model system in which to explore trait responses.

It has long been shown that zooplanktivory by fish can have sweeping effects on the composition of freshwater communities, which can have cascading, top-down effects on aquatic ecosystem functioning (Gliwicz and Pijanowska 1999). Empirical work by Peacor et al. (2012) and others suggests that predation risk effects may be important in these large effects of fish on zooplankton. Peacor et al. (2012) demonstrated that predation risk alone can strongly affect zooplankton species' population growth and interactions over a multi-generational time scale. In these experiments by Peacor et al. (2012), zooplankton were exposed to predator chemical cues (kairomones) in mesocosms with caged fish to isolate potential non-consumptive effects from consumptive effect (CEs). Response to the mere presence of predator kairomones altered the community composition of zooplankton and the position of zooplankton in the water column. The experiments used in this dissertation use comparable mesocosm methodology to investigate variation and contingency in trait responses of zooplankton. A behavioral response is used as a measure of a trait response, which was calculated as the effect of kairomone on zooplankton position. This behavioral response reflects diel vertical and horizontal migration which are important anti-predator responses in a variety of zooplankton taxa in nature (DeWitt 1998, Tollrian and Harvell 1999, Lass and Spaak 2003).

Experimental Venue

The need to scale up the study of trait responses to investigate how they translate to population and community-level effects further motivates the work in this dissertation.

Arguments have been made that short-term (single generation of prey) and simple experiments may overestimate the importance of effects that result from trait responses (Boker 2003, Abrams 2010). An example of potential complexity not captured in studies of simple systems is the variation in trait responses among life history stages or similar prey species. In addition, experiments in simple systems may lack important species interactions (Holland 1995, Levin 1998, Grimm and Railsback 2005) that may affect fitness tradeoffs and the expression of trait responses. Whereas many studies of trait responses to predators are performed in highly controlled experiments, the experiments in this dissertation are performed in large 1100-liter mesocosms which capture much of the complexity of natural systems including a diversity of taxa, and variation in zooplankton densities and resource levels among mesocosm replicates. In this way, the trait responses are perhaps more robust to natural systems then compared to studies in which conditions are less variable such as in laboratory studies (as e.g., Tollrian, 1994, Pangle and Peacor 2006).

Chapter Outline

The chapter results come from three different mesocosm experiments conducted in three different years. The differences in the species that established in the experiments in different years permitted investigation of our questions. For example, the species of cladocerans that established in the experiment described in Chapter 3 were more diverse compared to Chapter 2 which allowed analysis based on taxonomic relatedness. In addition, mesocosm methodology was similar among years, but some methodological differences allowed for specific analyses. For example, an uncaged fish treatment was only performed in the experiment described in Chapter 2.

Chapter 1: Copepod stages respond to predation risk with spatial shifts in opposite directions

Chapter 1 examines variation in behavioral responses among copepod life history stages. We hypothesized that because fitness tradeoffs that underlie the expression of trait responses may change across life history stages, the nature of the trait responses could vary as a function of stage. We found that predation risk effects in copepods were strongly stage-dependent, with nauplii shifting in the opposite direction than copepodites and adults. These findings of differential trait responses of stages provide additional support for explicitly examining stage structure in order to understand systems.

Chapter 2: Evaluation of the expected relationship between trait responses and predation risk among prey

Chapter 2 provides evidence from the fish-zooplankton system that prey's vulnerability to the predator may not explain the differences in the expression and magnitude of the behavioral trait responses. A positive relationship between trait response and predation risk may be expected and has been exhibited in some systems. For example, in response to a gape or size-limited predator, only smaller stages or species demonstrate trait responses (Sih 1987, Kohler and McPeek 1989, Peckarsky 1996, Relyea 2001). However, for many predator-prey systems, a positive relationship between trait response and predation risk is not predicted based on ecological theory. We compared the behavioral responses of cladocerans to two metrics of predation risk measured from an uncaged fish treatment. Metrics included relative predation rate and net effect of the predator on density on each taxon. We found a negative relationship between trait responses and both metrics of predation risk. These findings indicate that the expression and magnitude of trait responses should be examined as the tradeoff in benefits and costs of the trait responses, rather than assuming that trait responses are directly related to a species' vulnerability.

Chapter 3: Investigation into the relationship between traits and variation in trait responses within group of similar prey

Chapter 3 examines the variation in behavioral responses of cladocerans and assesses the ability of fixed traits of body size and taxonomic identity (family), traits related to predation risk, to explain the variation. Trait-based approaches that connect species' traits to their fitness based on trade-offs of key functional activities have proven useful in classifying variation in species along meaningful trait axes to address ecological questions. While cladocerans demonstrated substantial variation in behavioral trait responses, we did not find that body size or taxonomic identity explained the variation. Results from our study and others suggest that there may be considerable variation in the relationship between body size and trait responses. However, the variation we found in trait responses among relatively similar prey taxa warrants further investigation into other traits that could be used to predict trait responses based on fitness trade-offs.

REFERENCES

REFERENCES

Abrams, P.A. 1984. Foraging time optimization and interactions in food webs. American Naturalist 124: 80–96.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326.

Bednekoff, P. A., and S. L. Lima. 2004. Risk allocation and competition in foraging groups: reserved effects of competition if group size varies under risk of predation. Proceedings of the Royal Society B: Biological Sciences 271: 1491–1496.

Bolker, B., M. Holyoak, V. Krivan, L. Rowe, and O. Schmitz. 2003. Connecting theoretical and empirical studies of trait-mediated interactions. Ecology 84: 1101–1114.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31: 343–366.

Chivers, D.P. and R.J. Smith. 1998. Chemical alarm signaling in aquatic predator–prey systems: A review and prospectus. Ecoscience 5: 338–352.

Clark, C.W. 1994. Antipredator behavior and the asset-protection principle. Behavioral Ecology 5: 159–170.

Creel, S., D. Christiansen, S. Liley, and J.A. Winnie. 2007. Predation risk affects reproductive physiology and demography of elk. Science 315: 960.

Creel, S. 2011. Toward a predictive theory of risk effects: hypotheses for prey attributes and compensatory mortality. Ecology 92: 2190–2195.

Creel, S., P. Schuette, and D. Christianson. 2014. Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. Behavioral Ecology 25: 773–784.

Creel, S, M. Becker, E. Dröge, J. M'soka, W. Matandiko, E. Rosenblatt, T. Mweetwa, H. Mwap, M. Vinks, B. Goodheart, J. Merkle, T. Mukula, D. Smith, C. Sanguinetti, C. Dart, D. Christianson, and P. Schuette. 2019. What explains variation in the strength of behavioral responses to predation risk? A standardized test with large carnivore and ungulate guilds in three Ecosystems. Biological Conservation 232: 164–172.

DeWitt, T.J., A. Sih, and D.S. Wilson. 1998. Costs and limits of phenotypic plasticity. Trends in Ecology and Evolution 13: 77–81.

Díaz, S. and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16: 646–655.

Dodson, S.I. and J.E. Havel.1988. Indirect prey effects: some morphological and life history responses of *Daphnia pulex* exposed to *Notonecta undulata*. Limnology and Oceanography 33: 1274–1285.

Fraser, G.F. and J.F. Gilliam. 1992. Nonlethal impacts of predator invasion: Facultative suppression of growth and reproduction. Ecology 73: 959–970.

Gilliam, J.F. and G.F. Fraser.1987. Habitat selection under predation hazard: Test of a model with foraging minnows. Ecology 68: 1856–1860.

Gliwicz, Z.M. and J. Pijanowska. 1999. The role of predation in zooplankton succession. Sommer, U. (ed.). Plankton Ecology. New York. pgs. 253–296.

Grimm, V. and S.F. Railsback. 2005. Individual Based Modeling and Ecology, Princeton, NJ: Princeton University Press.

Harvell, C.D. 1990. The ecology and evolution of inducible denfenses. The Quarterly Review of Biology 65: 323–340.

Higginson, A.D., T.W. Fawcett, P.C. Trimmer, J.M. McNamara, and A.I. Houston. 2012. Generalized optimal risk allocation: foraging and antipredator behavior in a fluctuating environment. American Naturalist 180: 589–603.

Hijazi, R. and R. Jernigan. 2009. Modeling compositional data using dirichlet regression models. Journal of Applied Probability and Statistics 4: 77–91.

Holland, J. 1995. Hidden order: how adaptation builds complexity. Reading, MA: Addison Wesley.

Ives, A.R. and A.P. Dobson. 1987. Antipredator behavior and the population dynamics of simple predator-prey systems. American Naturalist 130: 431–447.

Kohler, S.L. and M.A. McPeek. 1989. Predation risk and the foraging behavioral of competing stream insects. Ecology 70: 1811–1825.

Krivan, V. and O.J. Schmitz. 2004. Trait and density mediated indirect interactions in simple food webs. Oikos 107: 239–250.

Krivan, V. 2007, The Lotka-Volterra predator-prey model with foraging-predation risk trade offs. American Naturalist 170: 771–782.

Leibold, M.A. and M.A. McPeek. 2006. Coexistence of the niche and neutral perspectives in community ecology. Ecology 87: 1399–1410.

Levin, S.A. 1998. Ecosystems and the biosphere as complex adaptive systems. Ecosystems 1: 431–436.

Lima, S.L. and P.A. Bednekoff. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Naturalist 153: 649–659.

Lima, S.L. and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

McCauley, S. J., L. Rowe, and M.J. Fortin. 2011. The deadly effects of 'nonlethal' predators. Ecology 92: 2043–2048.

McNamara, J. M. 1996. Risk-prone behaviour under rules which have evolved in a changing environment. American Zoologist 36: 484–495.

McPeek, M.A., M. Grace, and J.M.L. Richardson. 2001. Physiological and behavioral responses to predators shape the growth/predation risk trade-off in damselflies. Ecology 82: 1535–1545.

Pangle, K.L. and S.D. Peacor. 2006. Non-lethal effect of the invasive predator *Bythotrephes longimanus* on*Daphnia mendotae*. Freshwater Biology 51: 1070–1078.

Peacor, S.D., and E.E. Werner. 2000. Predator effects on an assemblage of consumers through induced changes in consumer foraging behavior. Ecology 81: 1998–2010.

Peacor, S.D., K.L. Pangle, L. Schiesari, and E.E. Werner. 2012. Scaling-up anti-predator phenotypic responses of prey: impacts over multiple generations in a complex aquatic community. Proceedings of the Royal Society B 279: 122–128.

Peacor, S. D. and C.E. Cressler. 2012. The implications of adaptive prey behavior for ecological communities: A review of current theory. In T. Ohgushi, O. Schmitz, and R.D. Holt (Eds.), Trait-mediated indirect interactions: Ecological and evolutionary perspectives. Cambridge, UK: Cambridge University Press. pp. 131–160.

Peacor, S.D., B.L. Peckarsky, G.C. Trussell, and J.R. Vonesh. 2013. Costs of predator-induced phenotypic plasticity: A graphical model for predicting the contribution of nonconsumptive and consumptive effects of predators on prey. Oecologia 171: 1–10.

Peckarsky, B. 1996. Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. Ecology 77: 1888–1905.

Peckarsky, B.L., A.R. Mcintosh, B.R. Taylor, and J. Dahl. 2002. Predator chemicals induce changes in mayfly life history traits: a whole stream manipulation. Ecology 83: 612–618.

Peckarsky, B.L, B.L. Kerans, A.R. Mcintosh, and B.W. Taylor. 2008. Predator effects on prey population dynamics in open systems. Oecologia 156: 431–440.

Pigliucci, M. 2005. Evolution of phenotypic plasticity: Where are we going now? Trends in Ecology and Evolution 20: 481–486.

Preisser, E.L., J.L. Orrock, and O.J. Schmitz. 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. Ecology 88: 2744–2751.

Randall, J.A., S.M. Hatch, and E.R. Hekkala. 1995. Inter-specific variation in anti-predator behavior in sympatric species of kangaroo rat. Behavioral Ecology and Sociobiology 36: 243–250.

Relyea, R.A. 2001. The relationship between predation risk and antipredator responses in larval anurans. Ecology 82: 541–554.

Resetarits, W.J. and H.M. Wilbur. 1991. Choice of oviposition site by *Hyla chrysoscelis*: role of predators and competitors. Ecology 70: 220–228.

Schultz, J.C. 1988. Plant responses induced by herbivores. Trends in Ecology and Evolution 3: 45–49.

Schmitz, O.J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science 319: 952–954.

Sheriff, M.J., C.J. Krebs, and R. Boonstra. 2009. The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78: 1249–1258.

Sheriff, M.J. and J.S. Thaler. 2014. Ecophysiological effects of predation risk: an integration across disciplines. Oecologia 176: 607–611.

Sheriff, M.J., S.D. Peacor, D. Hawlena, and M. Thaker. 2020. Non-consumptive predator effects on prey population size: A dearth of evidence. Journal of Animal Ecology 89: 1302–1316.

Sih, A. 1987. Predators and Prey Lifestyles: An Evolutionary and Ecological Overview. In Predation: Direct and Indirect Impacts on Aquatic Communities. (eds Kerfoot, W.C., Sih, A.). University Press of New England, Hanover, New Hampshire, USA, pp. 203–224.

Tollrian, R. 1994. Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (SARS). Archiv für Hydrobiologie 130: 69–75.

Tollrian, R. and C.D. Harvell. 1999. The Ecology and Evolution of Inducible Defenses.Princeton University Press, Princeton, New Jersey.

Trussell, G.C., P.J. Ewanchuk, and C.M. Matassa. 2006. The fear of being eaten reduces energy transfer in a simple food chain. Ecology 87: 2979–2984.

Trussell, G.C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research 2: 803–822.

Turner, A.M. and S.L. Montgomery. 2003. Spatial and temporal scales of predator avoidance: Experiments with fish and snails. Ecology 84: 616–622.

Werner, E.E. and B.R. Anholt. 1993. Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. American Naturalist 142: 24–32.

Werner, E.E. and B.R. Anholt. 1996. Predation-induced behavioral indirect effects: Consequences to competitive interactions in anuran larvae. Ecology 77: 157–169.

Werner, E.E. and S.D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

Zanette, L.Y., A.F. White, M.C. Allen, and M. Clinchy. 2011. Perceived predation risk reduced the number of offspring songbirds produce per year. Science 334: 1398–1401.

CHAPTER 1

Copepod stages respond to predation risk with spatial shifts in opposite directions

Abstract

The mere presence of predators may induce anti-predator responses in prey, in which prey modify traits, including behavior, to reduce predation risk. Because the tradeoffs that underlie these trait responses may change across life history stages, the magnitude and even nature of the risk-induced trait responses could be a function of life history stage. Thus, differential responses of different stages to predator presence could affect the way in which stage structure influences predator-prey interactions and in turn prey fitness. We examined the behavioral response of freshwater copepods to fish predator cues (kairomones) in mesocosms with and without caged fish. Copepods responded strongly to fish kairomones by shifting position in the mesocosms. These predation-risk induced responses were strongly stage-dependent, with nauplii shifting in the opposite direction than copepodites and adults. These findings highlight stage-specific responses to predators, an understanding of which will enhance the ability to explain how predation risk affects prey fitness and population dynamics (i.e., predator non-consumptive effects).

Introduction

Predation can strongly influence prey species' dynamics and predation rates are often highly stage-dependent (de Roos and Persson, 2013, Nakazawa, 2015). Predators' abilities to capture prey, and similarly, prey vulnerability, may change with age or stage, due to intrinsic differences in niche, physiology, and/or behavior among species' stages. For example, prey size can be an important determinant of prey vulnerability (Claessen et al. 2002), shown in such examples as stage and size-specific differences in vulnerability of *Hyla pseudopuma* tadpoles to predatory insects (Crump 1984) and body size and shape determining predation on common bream and roach by pike (Nilsson and Bronmark 2000). Another stage-specific characteristic of prey is ontogenetic shifts in habitat preference (Werner and Gilliam 1984) as demonstrated in some species of butterflyfishes based on diet (Clark and Russ 2012) and nests and dens used as refuges until animals mature. Given the potentially large influence of stage-specific effects on predator-prey dynamics and community structure (Miller and Rudolf 2011, de Roos and Persson 2013), it is important to examine implications of stage-structure on predation.

Vulnerability to predation as a function of stage could further be influenced by differential responses of prey life history stages to predation risk. It is well known that predators can affect prey, not just through direct mortality, but also by inducing changes in prey traits such as behavior and morphology. These trait responses can in turn affect prey fitness and abundance, termed non-consumptive effects (NCEs), and have ensuing effects on other species in the community through trait-mediated indirect effects (TMIEs, Lima and Dill 1990, Werner and Peacor 2003, Peckarsky et al. 2008, Abrams 2010, Ohgushi et al. 2012). These trait changes will likely differ among stages due to differences in the fitness trade-offs associated with the trait. For example, if a species' vulnerability to predation decreases with size, the risk-induced trait response may vary with age or stage (Werner and Hall 1988, de Roos et al. 2002, Cressler et al. 2010). Perception of predation risk may also vary with stage; for example, Johnston et al. (2011) found that juvenile but not adult barnacles respond to dogwhelk kairomones. These examples of stage-dependent responses to predation risk are important because they underlie predator-prey interactions and may enhance the understanding of NCEs and TMIEs on community or ecosystem dynamics.

Stage-specific responses to predation risk may be particularly important in zooplankton. Zooplankton species can have a large size range and can be strongly susceptible to size-specific predation (Brooks and Dodson 1965). Moreover, zooplankton have been shown to respond to predation risk through changes in morphology, behavior, and life history (DeWitt 1998, Tollrian and Harvell 1999, Lass and Spaak 2003). These factors suggest that zooplankton are likely candidates to display stage-dependent trait-responses. Indeed, Weiss et al. (2016) found that *Daphnia* juveniles grow neckteeth when exposed to *Chaoborus* cues but neckteeth disappear as *Daphnia* grow larger, likely because they are less vulnerable to the gape-limited *Chaoborus*. Holliland et al. (2012) found stage-dependent magnitude of diel vertical migration in calanoid copepods *Eurytemora affinis* and *Acartia* spp. which they argued is due to predation risk changing as a function of size as individuals grew. Bourdeau et al. (2015) found *Bythotrephes* abundance in Lake Michigan affected the vertical position in the water column of adult stages of *Leptodiaptomus* spp. and juvenile and adult stages of *Diacyclops thomasii*, but there was no effect on juvenile diaptomids or copepod nauplii. It was hypothesized that these differences may be due to the energetic cost of the large vertical distances traveled, which different stages would be more or less able to pay.

Taken together, the evidence for stage-dependent anti-predator responses of other taxa and predation-risk induced effects on zooplankton highlight the need to understand stage-dependent responses of zooplankton to predators. We performed a mesocosm experiment to examine predator risk-induced behavioral responses of copepods to fish predators. This study has implications for understanding how stage-specific trait responses affect ecological interactions of copepods.

Methods

The results reported in this paper are from a mesocosm experiment designed to investigate the non-consumptive effects of predators on community composition of freshwater zooplankton by comparing community composition in predator-free mesocosms to the community composition of mesocosms with either fish kairomone (from fish in floating cages) or midge kairomone (from *Chaoborus* in floating cages). Using data from this broader study, we herein examine stage-specific risk-induced behavioral responses of copepods; in particular, we focus on three distinct stages of copepod ontogenetic development, including nauplii (the larval stage), copepodites (analogous to the juvenile or immature stage), and adults. We examine if and how predation risk affected habitat preference of different stages. For the purposes of this study, we combine the control and midge treatment, as midges had a negligible effect on zooplankton behavior, and because combining these two treatments increases power to examine the influence of kairomones. Experiments were conducted at the E.S. George Reserve (ESGR) of the University of Michigan near Pinkney, MI, USA. To guide presentation of the timing of different manipulations and events, days in the experiment are referenced relative to day 0 on 6 July when treatments were initiated by adding predators to the floating cages.

Mesocosms consisted of cylindrical cattle water tanks with a diameter of 168 cm and height of 60 cm. They were filled with 1100 L of treated well water, to a depth of approximately 45 cm. Twenty-five pounds of washed play sand were added to each mesocosm to cover the bottom. Mesocosms were inoculated with phytoplankton by adding 500 ml of 54 µm-filtered water from a pond in the ESGR on day -35. On day -27, zooplankton from a nearby lake were collected using 150 and 250 µm zooplankton nets, undesirable species such as amphipods and insects (e.g., *Chaoborus*) were removed, and the remaining animals were added to mesocosms to create a diverse zooplankton community. Thirty-five snails (*Planorbella* cf. *trivolvis*) of varying sizes but equal total biomass were added to each mesocosm to reduce periphyton growth. To increase zooplankton assemblage homogeneity among mesocosms, at day -13, zooplankton were collected from each mesocosm with a zooplankton net, mixed, and subsamples of this mixture were redelivered to each mesocosm. Inorganic nutrients were added to the mesocosms to support phytoplankton growth as a resource for zooplankton. An initial spike of 5.06 g of NH_4NO_3 and 0.37 g of KH_2PO_4 was added to each mesocosm on days -35 and -29. Starting at day -13, nutrients were added continuously via peristaltic pump at a rate of 0.29 g of NH_4NO_3 and 0.04 g of KH_2PO_4 per mesocosm per day.

The mesocosms were arranged in a 4 x 6 grid and were blocked (8 blocks) by three spatially adjacent mesocosms with eight replicates for each of the three treatments. Three floating mesh cages were used in each mesocosm to hold predators and allow kairomones to diffuse (no predators were added to the cages in the control treatment mesocosms). Cages were constructed from 41 X 27 X 26 cm plastic boxes with polystyrene foam glued to the sides to ensure flotation. The plastic on the sides and bottom of the boxes was cut out and replaced with mesh panels made of midge netting. The kairomone treatment consisted of one bluegill sunfish (*Lepomis macrochirus*, mean length ± SD of 4.7 ± 0.4 cm) in each cage. Fish originated from Patterson Lake, Livingston County, MI, USA. Each of the cages in the midge-kairomone treatment had 300 *Chaoborus* larvae, which were collected from a pond in the ESGR. Once a week, fish were rotated from the experimental mesocosms to culture mesocosms. Culture mesocosm fish were starved for 24 hours prior to being rotated back into the experiment.

Similarly, *Chaoborus* larvae were replaced with newly collected larvae once a week to ensure a relatively constant influx of *Chaoborus* kairomone. The mesh panels of all cages were sprayed with a hose each week to remove periphyton. 300 *Daphnia* of two species (*D. pulex* and *D. dentifera*; 700 – 1200 μm length) were added to each predator cage twice a week as a predator resource. Control cages received 300 *Daphnia* that had first been killed by microwaving to provide equal nutrient levels to each mesocosm.

On 15 September, day 71, zooplankton were sampled in all mesocosms at six distinct positions, including from three vertical strata: 'high' (just below the surface), 'middle' (at the midwater level) and 'low' (right above the bottom) crossed with two horizontal positions including ”side” (along the walls) and the “center” (within a 50 cm radius of the mesocosm center). A sample was collected in each of four quadrants for the “side” samples, and three samples were collected in the center at each stratum for the “center” samples. A 15 cm long 1.2 L cylindrical water sampler (Wildco) held parallel to the ground was used. The sampler was inserted into the water approximately 30 cm in front of, and then moved forward to, the collection position. At each of the six positions, the replicate samples were combined, passed through and collected on a 53 μm mesh sieve, and preserved in sugar formalin (e.g., the three samples from the high-center position were combined to make one sample for that one of six positions). Copepods were counted as nauplii, copepodites, or adults. The level of effort and training required to identify earlier stages precluded us from identifying copepodites beyond order (i.e., calanoid and cyclopoid), and nauplii were not identified as cyclopoid or calanoid. Adults of both taxa were identified to species.

Statistical Analyses

For all analyses, control and midge treatments were combined as no kairomone treatments and compared to the kairomone treatment. For each mesocosm, the percentage of a given taxa/stage found at a given position was calculated as the estimated abundance in that position divided by the total estimated abundance (e.g., the sum of the abundance at all six positions). We also calculated averaged position for vertical and horizontal directions. For vertical, we calculated the proportions in the high, middle, and low strata; for horizontal, we calculated the proportions in the center and side strata. For example, the proportion in the high strata was the abundance in the high side and high center positions divided by the sum abundance in all six positions. A mesocosm was only included in the positional analysis if there were enough individuals represented in the total number counted in the mesocosm to provide sufficient information for the positional estimates. We used 5 individuals counted in a mesocosm as the cut off based on a resampling exercise that showed an individual mesocosm with 5 or fewer individuals provided an estimate of spatial position inaccurate enough as to bias the estimate of the average position across mesocosms.

Compositional data, such as the proportion of individuals in different positional strata used in this study, are constrained in that the components sum to 1. As such, statistical methods designed for unconstrained data may lead to inappropriate inference (Hijazi and Jernigan 2009). Thus, to test for a behavioral response to kairomone, Dirchlet regression analysis was used. The Dirichlet distribution is the multivariate generalization of the beta distribution and performs wells for composite measurements, such as our response variables, that do not conform well to count-based or binomial-based analyses (reviewed in Douma and Weedon, 2019).

Separate Dirichlet regression analyses were run for the vertical position (as the proportion of individuals in high, middle, and low positions) and lateral position (as the proportion of individuals in center and side positions) because these were spatial axes along which shifts in zooplankton position are studied in natural systems (i.e., diel vertical and horizontal migration, e.g., Zaret and Suffern, Hays 2003, Burks et al 2012). To test for significant ($p \leq 0.05$) and marginally significant differences ($p \leq 0.10$) along each axis, we ran Dirichlet regression models with the proportions in the vertical or horizontal strata as the response with kairomone, removal, and the interaction of kairomone and removal as fixed effects. To identify if there were any significant fish x removal interactions, this full model was compared to a model without the interaction using a likelihood-ratio test approach. If no significant interaction was identified, the effect of kairomone was examined across no removal and removal mesocosms to have additional power to examine the kairomone effect. Specifically, Dirichlet regression models with the effects of kairomone and removal were compared to models with only kairomone or removal using a likelihood-ratio approach to test the significance of the effect of kairomone or removal. Analyses were performed in R version 3.5.2 (R Development Core Team, 2019) using the *DirichletReg* package (Maier, 2015).

Results

Community composition

Acanthocyclops vernalis, *Eucyclops* sp., *Mesocyclops edax*, and *Macrocyclops* spp. including *M. fuscus* and *M. albidus*, were the most common cyclopoid species in the mesocosms (Fig. 1a). Kairomone affected the community composition of cyclopoid species, with *M. edax* making up a larger proportion (42%) of the cyclopoid community in the kairomone treatment compared to the no kairomone treatment (17%), while the proportion of *A. vernalis* decreased with kairomone (6% in the kairomone compared to 28% in the no kairomone, Fig. 2a). *Skistodiaptomus oregenensis* was the most abundant calanoid in the mesocosms, and the community composition was similar between no kairomone and kairomone treatments (Fig. 2b). Whereas we discuss the potential influence of the changes in relative abundance of cyclopoid species on our results (see Discussion), the changes do not significantly influence our evaluation and thus we did not examine these trends in community composition statistically. Further, we do not examine potential effects of kairomone on zooplankton density (although density estimates are provided in Appendix A).

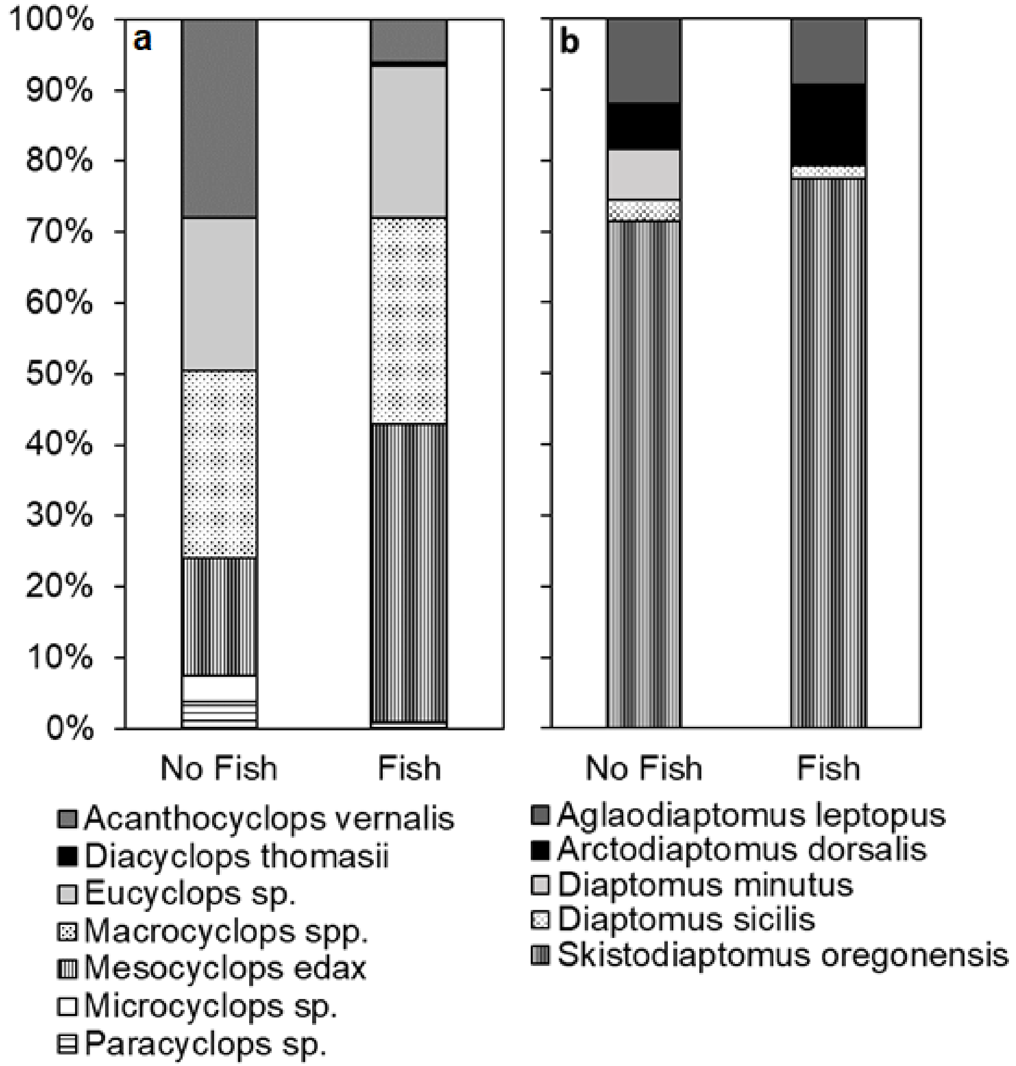

Figure 2. Species composition of adult copepods in no kairomone and kairomone treatments for a) cyclopoids and b) calanoids.

General location of copepod stages

The general trends of copepod position and the effect of kairomone can be seen in Fig. 3 which provides a graphical representation of the position of the different groups. This diagram does not capture the complexity of the distribution of copepods among all six positions, which is better captured in Fig. 4, but illustrates important trends. Also note (see Methods) that Fig. 3 uses the mean of the percentage of individuals found in each position, but statistics were performed on transformed data. Considering just the no kairomone treatments, there was spatial segregation among stages and among copepod groups (Fig. 3). Whereas calanoid copepodites and adults were generally located in the same place, cyclopoid stages appear more spatially segregated, with adult cyclopoids located lower and closer to the sides than cyclopoid copepodites. The calanoids were located farther from the mesocosm walls compared to the other groups (Fig. 3). The vertical and horizontal distribution of nauplii was closest to cyclopoid copepodites.

Effect of kairomone on position

Kairomone had a marginally or statistically significant effects on the vertical location of nauplii, cyclopoid adults, calanoid copepodites, and calanoid adults (Table 1). Different stages' locations shifted in opposite directions with nauplii being located higher and cyclopoid adults, calanoid copepodites, and calanoid adults being located lower (Fig. 3). There was a differential response of cyclopoid copepodites and adults in the vertical direction (no response and a strong response to move lower, respectively). In the horizontal direction, nauplii demonstrated a statistically significant shift towards the center of the tank while there was a trend for other stages to shift towards the side (Fig. 3). The shift was significant for cyclopoid copepodites and calanoid adults.

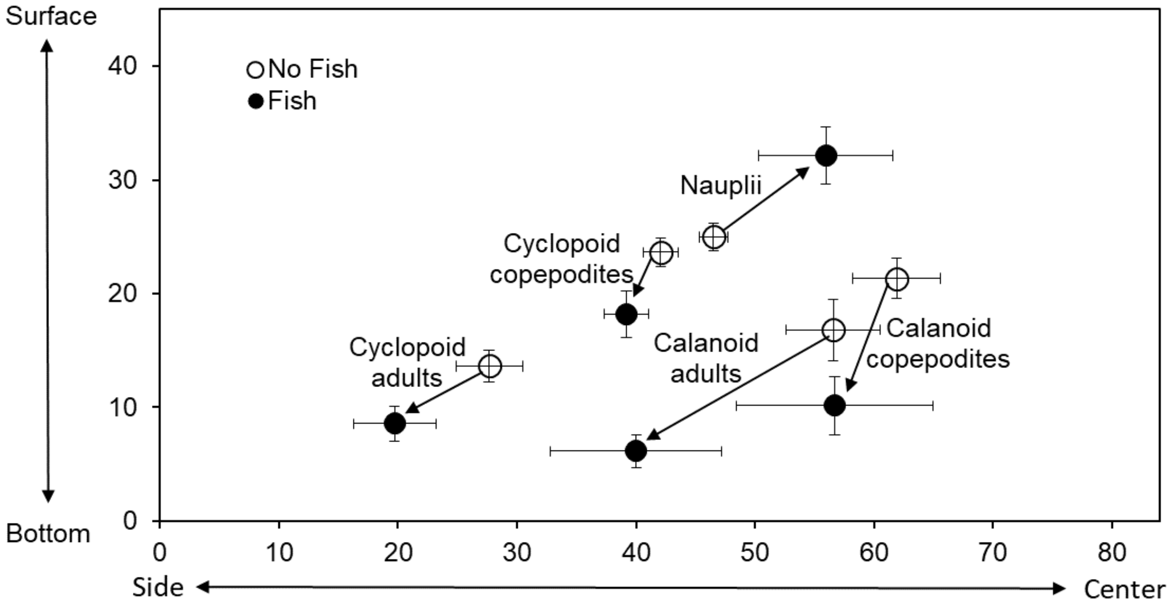

Figure 3. Average location (± SE) of stages in the no kairomone and kairomone treatments where the x-axis represents distance from the wall towards the center of the mesocosm in cm and where the y-axis represents the depth from the water's surface to the bottom of the mesocosm where the bottom is 0 cm and the water's surface is the depth of the water (45 cm). The horizontal position value (x-axis) was calculated as the average percentage of individuals in the center strata multiplied by the radius of the mesocosms (84 cm). The vertical position value (y-axis) was calculated by summing the product of the percentage of individuals in the high, middle and low strata by 1, 0.5, and 0, respectively, and then multiplying the sum (range 0 to 1) by the mesocosm water depth.

Table 1. Statistical results of the Dirichlet regression of the effects of fish kairomone on each stage including total N for all treatments, X (Chi) statistic, and significance values. Significance of p-values are denoted for $p \leq 0.05$ with * and $p \leq 0.1$ with · . To indicate the direction of significant effects, grey-shaded cells in the vertical direction indicate taxa individuals were located lower with the treatment effect while dark-outlined values indicate individuals were located higher. For the horizontal position, grey-shaded values indicate more toward the center with the treatment effect.

	Vertical Position			**Horizontal Position**		
Stage	**X**	***p*-value**		**X**	***p*-value**	
Nauplii	7.03	0.071	.	15.9	0.000	*
Cyclopoid copepodites	3.13	0.370		9.4	0.009	*
Cyclopoid adults	8.22	0.042	*	3.7	0.158	
Calanoid copepodites	11.2	0.011	*	1.46	0.480	
Calanoid adults	10.9	0.012	*	8.65	0.013	*

In contrast to other stages, nauplii were located higher with kairomone, going from near uniformity among the six positions in the no kairomone treatment to a large shift with more (38%) of individuals located in the high center position with kairomone, and fewer individuals in the low and middle side positions (Fig. 4a). The most dramatic change in position was the near disappearance of calanoids away from the surface of the mesocosm (Fig. 4b-c). Although the percentage of cyclopoids in the high strata was larger than it was for calanoids, the cyclopoids' locations similarly shifted lower in the mesocosms. With kairomone, cyclopoid copepodites' location shifted toward the low center and low side from the high center of the mesocosms, whereas cyclopoid adults shifted mostly from the high side (but also from all center positions) towards the low side (Fig. 4d-e).

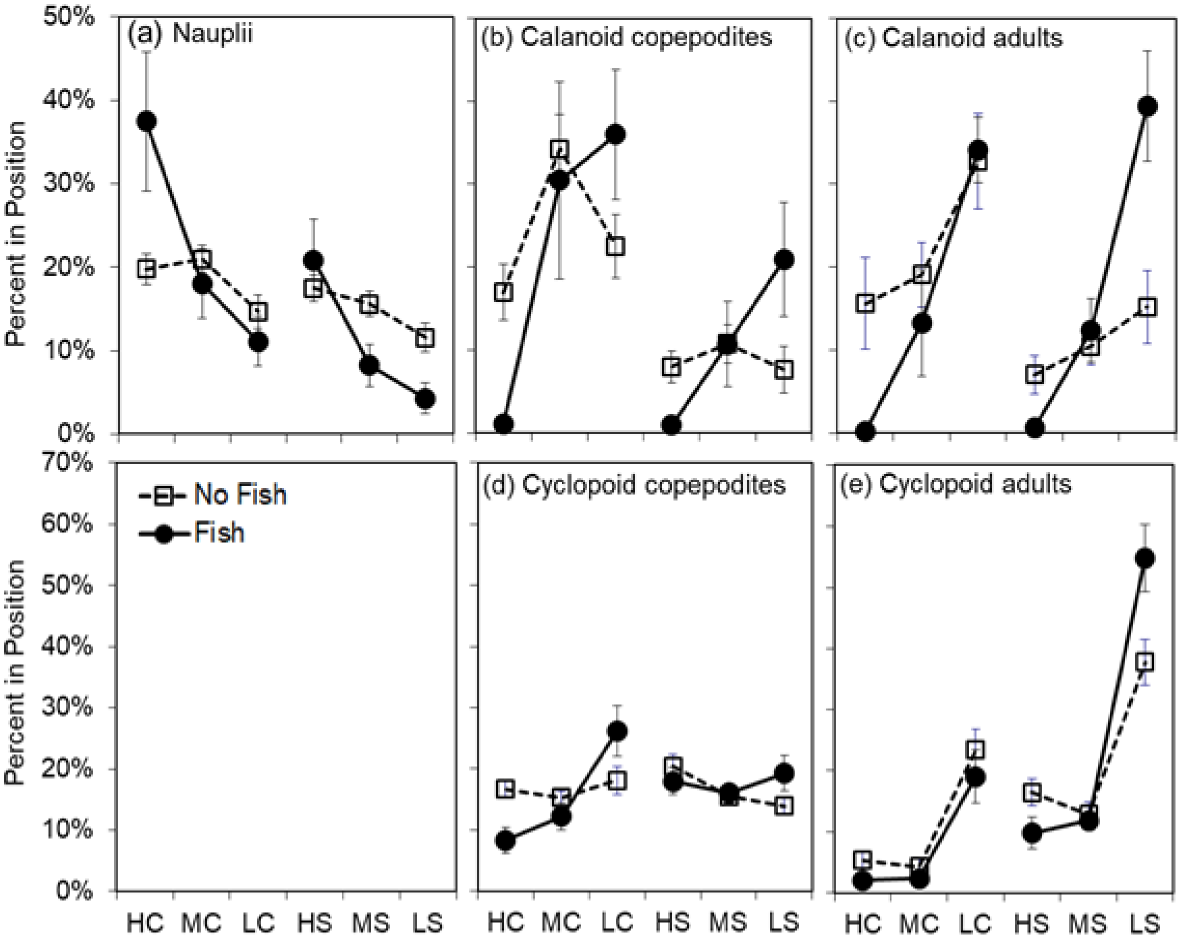

Figure 4. Percent (± 1 SE) of copepod stages found in the six different positions in the no kairomone (open squares) and kairomone (filled circles) mesocosms (e.g., high, middle, low vertical strata at center or sides of mesocosms) for (a) nauplii, (b) cyclopoid copepodites, (c) cyclopoid adults, (d) calanoid copepodites, and (e) calanoid adults. Positions: HC = high center, MC = middle center, LC = low center, HS = high side, MS = middle side, LS = low side.

Discussion

Predation risk by fish strongly affected the location of all copepod stages, with differential effects on nauplii compared to other stages. Nauplii were the only stage to be located higher and towards the center with fish kairomone. All other stages demonstrated a significant shift in the vertical or horizontal positions in the opposite direction from nauplii, being located lower and more towards the side with fish kairomone. The shift of other stages to be located lower with fish kairomone would be expected to reduce predation risk (deeper with fish predators). Predation risk has been demonstrated to influence zooplankton position in nature (Zaret and Suffern 1976, White 1998, Tollrian and Harvell 1999, Lass and Spaak 2003) and these predation-risk effects may be important drivers of community and ecosystem dynamics (Pangle et al. 2007, Boeing and Ramcharan 2010, Peacor et al. 2012). Our finding of a stage-specific response to fish predator is important because it suggests that consideration of differential responses of stages could enhance the ability to explain the importance of predator risk-induced effects (NCEs and TMIEs) on community and ecosystem dynamics.

We evaluated two potentially confounding issues related to our findings of stage-specific responses to predation risk. First, nauplii were not identified to taxon so we cannot determine if it was the cyclopoid, calanoid, or nauplii from both groups that responded. It is possible that only one of the group's nauplii responded. However, the significant effects of kairomone on the copepodites and adults of cyclopoids and calanoids were in the opposite direction of the nauplii. Therefore, the results provide evidence that kairomone had a different effect on the stage of a least one, if not both, orders. Second, changes in relative density of cyclopoid species due to kairomone and not trait responses could potentially explain the effect of kairomone on the location of cyclopoids. In particular, the proportion of *A. vernalis* was smaller and *M. edax* was larger in the kairomone treatment (Fig. 2). A kairomone effect on position of the cyclopoids could be due to the changes in proportion of these species and not a behavioral response to kairomone if these species prefer different locations. We examined the kairomone effect on the position of the cyclopoids by excluding *A. vernalis* or *M. edax* data. The position results remained significant when *A. vernalis* was removed but was not significant when *M. edax* was removed (p =0.15, Appendix B). It is possible that predation risk did influence stages through indirect effects on density and not only through trait responses. However, the findings still demonstrate important consideration of stage differences and structure when examining the importance of predation risk effects. This potentially confounding effect does not apply to calanoids and thus even if it did apply to cyclopoids does not confound our overall finding of stage-specific responses.

There are a number of mechanisms that could underlie the difference in the response of the copepod stages. One possibility is that susceptibility of zooplankton to predation by bluegill may be size dependent, with bluegill feeding preferentially on the larger zooplankton (Mittelbach 1981, Bremigan and Stein 1994), and hence more vulnerable stages are expected to respond more strongly. Prey size could also affect zooplankton metabolism and hence the tradeoff of predation risk and energy gain (per McNamara and Houston 1987), i.e., small stages with higher energy requirements per unit size would respond less to predation risk. Lastly, there is the interesting possibility that nauplii may seek refuge near fish predators as it could confer safety from predation by cyclopoids which shifted lower with kairomone. Such refuge near predators of a prey’s predator has been seen in other prey species (Berger 2007).

The effects of kairomone on copepod habitat preference could be due directly to predator avoidance behavior or due to indirect effects caused by the kairomone effects on other habitat parameters. That is, given the duration and complex community of zooplankton in the experiment, it is possible that kairomone had NCEs on community characteristics such as species densities and resource abundance which may affect interactions among competing zooplankton. There is increased understanding that the effect of NCEs can influence species interactions, and in turn affect species abundances and dynamics (Werner and Peacor 2003, Peckarsky et al. 2008). We cannot rule out that indirect effects of fish may have altered habitat characteristics including species' densities and resource abundance in such a way to drive copepod habitat preference. Thus, the kairomones could be causing the copepod habitat shifts indirectly, directly due copepod behavioral responses to the fish, or a combination of both.

The stage-specific responses to predation risk that we found demonstrate that the study of NCEs needs to be scaled up beyond investigating NCEs in species pairs or pared-down communities to truly understand their importance. Even just considering the copepods in our experiment, the habitat shifts due to kairomone may not only affect the interactions between fish and copepods but also within and among copepod stages through changes in competitive and predation interactions. For example, with kairomone, nauplii were located higher while cyclopoid adults were located lower and therefore possibly separated from each other (Fig. 3). This could lead to positive trait-mediated indirect effects by fish on calanoids through cyclopoids because cyclopoid adults may feed on other zooplankton including nauplii and copepodites (Williamson, 1980), and so the spatial separation may reduce predation on calanoid nauplii. This example demonstrates how predation risk-induced trait responses and their effects may be based not only on an individual's species but also on its stage. This indicates that consideration of the multitude of potential species and stage-dependent interactions and responses could enhance the understanding of predation risk on communities.

Whereas many studies of phenotypic responses to predators are performed in highly controlled experiments, the implications of our study to a natural setting deserve attention. The present experiment was performed in large 1100-liter mesocosms which capture much of the complexity of natural systems, with large variation in e.g., zooplankton densities and resource levels. In this way, the significant responses are perhaps more robust then compared to studies in which conditions are less variable such as in laboratory studies (as e.g., Tollrian 1994, Pangle and Peacor 2006). Nevertheless, our findings (Fig. 4) likely only offer qualitative, rather than quantitative, insights into the effects of fish on copepods in natural systems. Thus, we believe that the vertical shifts in response to the presence of kairomones, which was to be located deeper for copepodites and adults, but nearer the surface for nauplii (Fig. 3), are likely robust to natural systems. Although only significant for calanoid adults (Table 1), we interpret the trend for all copepodites and adults to shift closer to the sides as evidence for lateral movement in a natural setting to move more toward the littoral zone where there are macrophytes, as found by Burks et al. (2001) for *Daphnia*.

Our results and others' show that stages of species may respond differently to predation risk and this has implications for understanding how species will function in communities and ecosystems. We hypothesized that intraspecific variation would extend to prey's behavioral responses to predation risk and found differential response between nauplii and other copepod stages. Ecological niches, for example habitat use and diet, can differ among a species' stages and influence ecosystem processes and these niche differences highlight a need to not assume a species' function is the same across its stages (Rasmussen and Rudolf 2013). We extend their work to include stage-specific responses to predation risk. If the same response is assumed for all stages, population-level NCEs may be exaggerated or missed. For example, in the copepods that we studied, the response of copepodites and adults to be deeper with kairomone may confer a cost to growth in colder and/or less productive habitat and the expected population-level NCE would be negative. However, when nauplii of the same taxa are located higher with kairomone and may escape from resource limitation or predation by other animals, it is hard to predict the net NCE on the species. Thus, an understanding of stage-specific responses to predators will enhance the ability to explain NCEs in communities, similar to the methods used to model consumptive effects.

Acknowledgements

We thank Chris Davies, Scott Meihls, and Glen Carter for zooplankton enumeration. We thank the Ecology and Evolutionary Biology department of the University of Michigan for use of the E. S. George Reserve. Funding for this study was provided by the National Science Foundation DEB-0089809 and DEB-1145808.

APPENDICES

APPENDIX A. Copepod densities

Table 2. Mean densities ± standard deviation (individuals L^{-1}) for each stage in each treatment.

Copepod	No Kairomone	Kairomone
Nauplii	199 ± 259	66 ± 119
Calanoid copepodites	2.2 ± 4.2	9.1 ± 18
Calanoid adults	1.0 ± 1.6	9.5 ± 18
Cyclopoid copepodites	20 ± 39	13 ± 23
Cyclopoid adults	2.2 ± 3.5	9.9 ± 20

APPENDIX B. Cyclopoid position analysis with the exclusion of *Acanthocyclops vernalis* or *Mesocyclops edax*

Examination of the kairomone effect on the vertical and horizontal position of the cyclopoid adults using Dirichlet regression and excluding *Acanthocyclops vernalis* and *Mesocyclops edax.*

Table 3. Dirichlet p-values for effect of kairomone on the percentage high and side of cyclopoid adults without *Acanthocyclops vernalis* or *Mesocyclops edax* using Dirichlet regression test. An asterisk denotes p-value < 0.05.

Species	Vertical	Horizontal
Acanthocyclops vernalis excluded	0.014*	0.120
Mesocyclops edax excluded	0.155	0.200

REFERENCES

REFERENCES

Abrams, P.A. 2010. Implications of flexible foraging for interspecific interactions: lessons from simple models. Functional Ecology 24: 7–17.

Berger, J., 2007. Fear, human shields and the redistribution of prey and predators in protected areas. Biology Letters 3: 620–623.

Boeing, W.J. and C.W. Ramcharan. 2010. Inducible defences are a stabilizing factor for predator and prey populations: a field experiment. Freshwater Biology 55: 2332–2338.

Bourdeau, P.E., K.L. Pangle, and S.D. Peacor. 2015. Factors affecting the vertical distribution of the zooplankton assemblage in Lake Michigan: The role of the invasive predator *Bythotrephes longimanus*. Journal of Great Lakes Research 41: 115–124.

Bremigan, M.T. and R.A. Stein. 1994. Gape-dependent larval foraging and zooplankton size implications for fish recruitment across systems. Canadian Journal of Fisheries and Aquatic Sciences 51: 913–922.

Brooks, J.L. and S.I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28–35.

Burks, R.L., E. Jeppesen, and D.M. Lodge. 2001. Littoral zone structures as *Daphnia* refugia against fish predators. Limnology and Oceanography 46: 230–237.

Claessen, D., C. van Oss, A.M. de Roos, and L. Persson. 2002. The impact of size-dependent predation on population dynamics and individual life history. Ecology 83: 1660–1675.

Clark, N.J. and G.R. Russ. 2012. Ontogenetic shifts in the habitat associations of butterflyfishes (F. Chaetodontidae). Environmental Biology of Fishes 94: 579–590.

Cressler, C.E., A.A. King, and E.E. Werner. 2010. Interactions between behavioral and life history trade-offs in the evolution of integrated predator-defense plasticity. American Naturalist 176: 276–288.

Crump, M.L. 1984. Ontogenetic changes in vulnerability to predation in tadpoles of *Hyla psudopuma*. Herpetologica 40: 265–271.

De Roos, A.M., K. Leonardsson, L. Persson, and G.G. Mittelbach. 2002. Ontogenetic niche shifts and flexible behavior in size-structured populations. Ecological Monographs 72: 271–292.

De Roos, A.M. and L. Persson. 2013. Population and Community Ecology of Ontogenetic Development. Princeton University Press, Princeton, New Jersey.

DeWitt, T.J. 1998. Costs and limits of phenotypic plasticity: tests with predator-induced morphology and life history in a freshwater snail. Journal of Evolutionary Biology 11: 465–480.

Douma, J.C. and J.T. Weedon. 2019. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. Methods in Ecology and Evolution 10: 1412–1430.

Ferrari, S.L.P. and F. Cribari-Neto. 2004. Beta regression for modelling rates and proportions. Journal of Applied Statistics 31: 799–815.

Hays, G.C. 2003. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. Hydrobiologia 503: 163–170.

Hijazi, R. and R. Jernigan. 2009. Modeling compositional data using dirichlet regression models. Journal of Applied Probability and Statistics 4: 77–91.

Holliland, P.B., I. Ahlbeck, E. Westlund, and S. Hansson. 2012. Ontogenetic and seasonal changes in dielvertical migration amplitude of the calanoid copepods *Eurytemora affinis* and *Acartia* spp. in a coastal area of the northern Baltic proper. Journal of Plankton Research 34: 298–307.

Johnston, B.R., M. Molis, and R.A. Scrosati. 2012. Predator chemical cues affect prey feeding activity differently in juveniles and adults. Canadian Journal of Fisheries and Aquatic Sciences 90: 128–132.

Lass, S. and P. Spaak. 2003. Chemically induced anti-predator defences in plankton: a review. Hydrobiologia 491: 221–239.

Lima, S.L. and L. Dill. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

Maier, M.J. 2015. DirichletReg: Dirichlet regression in R. R package version 0.6-3.1. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://cran.r project.org/web/packages/DirichletR eg/vignettes/DirichletReg-vig.pdf.

McNamara, J.M. and A.I. Houston. 1987. Starvation and predation as factors limiting population size. Ecology 68: 1515–1519.

Miller, T.E.X. and V.H.W. Rudolf. 2011. Thinking inside the box: Community-level consequences of stage structured populations. Trends in Ecology and Evolution 26: 457–466.

Mittelbach, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62: 1370–1386.

Nakazawa, T. 2015. Ontogenetic niche shifts matter in community ecology: a review and future perspectives. Population Ecology 57: 347–354.

Nilsson, P.A. and C. Bronmark. 2000. Prey vulnerability to a gape-size limited predator: behavioural and morphological impacts on northern pike piscivory. Oikos 88: 539–546.

Ohgushi, T., O.J. Schmitz, and R.D. Holt. 2012. Trait-Mediated Indirect Interactions: Ecological and Evolutionary Perspectives. Cambridge University Press, Cambridge, New York.

Pangle, K.L. and S.D. Peacor. 2006. Non-lethal effect of the invasive predator *Bythotrephes longimanus* on*Daphnia mendotae*. Freshwater Biology 51: 1070–1078.

Pangle, K.L., S.D. Peacor, and O.E. Johansson. 2007. Large non-lethal effects of an invasive invertebrate predator on zooplankton population growth rate. Ecology 88: 402–412.

Peacor, S.D., K.L. Pangle, L. Schiesari, and E.E. Werner. 2012. Scaling-up anti-predator phenotypic responses of prey: impacts over multiple generations in a complex aquatic community. Proceedings of the Royal Society B 279: 122–128.

Peckarsky, B.L., P.A. Abrams, D.I. Bolnick, L.M. Dill, J.H. Grabowski, B. Luttbeg, J.L. Orrock, S.D. Peacor, E.L. Preisser, O.J. Schmitz, and G.C. Trussell. 2008. Revisiting the classics: considering nonconsumptive effects in textbook examples of predator-prey interactions. Ecology 89: 2416–2425.

R Core Team. 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing Computing, Vienna, Austria. http://www.r-project.org.

Smithson, M. and J. Verkuilen. 2006. A better lemon squeezer? Maximum-likelihood regression with beta distributed dependent variables. Psychological Methods 11: 54–71.

Tollrian, R. 1994. Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (SARS). Archiv für Hydrobiologie 130: 69–75.

Tollrian, R. and C.D. Harvell. 1999. The Ecology and Evolution of Inducible Defenses.Princeton University Press, Princeton, New Jersey.

Weiss, L.C., E. Heilgenberg, L. Deussen, S.M. Becker, S. Kruppert, and R. Tollrian. 2016. Onset of kairomone sensitivity and the development of inducible morphological defenses in *Daphnia pulex*.Hydrobiologia 779: 135–145.

Werner, E.E. and J.F. Gilliam. 1984. The ontogenetic niche and species interactions in size structured populations. Annual Reviews of Ecological Systems 15: 393–425.

Werner, E.E. and D.J. Hall. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate predation risk trade-off. Ecology 69: 1352–1366.

Werner, E.E. and S.D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

White, M.D. 1998. Horizontal distribution of pelagic zooplankton in relation to predation gradients. Ecography 21: 44–62.

Williamson, C.E. 1980. The predatory behavior of *Mesocyclops edax*: Predator preferences, prey defenses, and starvation-induced changes. Limnology and Oceanography 25: 903–909.

Zaret, T.M and J.S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnology and Oceanography 21: 804–813.

CHAPTER 2

Evaluation of the expected relationship between trait responses and predation risk among prey

Abstract

Predators affect prey populations both through consumption and by inducing anti-predator responses, in which prey modify traits, including behavior, morphology, and life history, to reduce predation risk. Trait responses have been demonstrated in a variety of taxa and can affect population and community dynamics of prey. In trying to understand the variation in trait responses among prey, it might be expected that more vulnerable prey would exhibit larger trait responses. Such positive relationships between trait response and predation risk have been exhibited in some systems. For example, when prey size limits the ability of the predator to consume the prey and larger species do not respond. However, for many predator-prey systems, positive, negative, or neutral relationships between trait response and predation risk are possible based on ecological theory. Using a fish-cladoceran system, we provide empirical evidence of unexpected relationships between trait responses and two metrics of predation risk. Experiments were conducted in mesocosms with and without fish kairomone (produced by caged fish). A difference in zooplankton position in kairomone relative to no kairomone treatments was used as measure of a behavioral response. Metrics of predation risk included relative predation rate and net effect of the predator on density on each taxon measured from a treatment with uncaged fish. We found that taxa that exhibited the largest responses were the least vulnerable to the predator, and that highly vulnerable taxa did not respond. These findings demonstrate the complex nature of factors underlying trait responses. Examining the expression of trait responses as the tradeoff

between reduction in predation and decreased fitness rather than assuming that responses are directly related to the predation risk can enhance the understanding of how predation risk shapes communities and ecosystems.

Introduction

Predators affect prey populations through both direct consumption and through antipredator risk-induced trait responses (Sih 1987, Werner and Peacor 2003, Bolker et al. 2003). Empirical examples of trait responses which include changes in behavior, morphology, physiology, or life history, are numerous and observed across ecosystems and across different taxa, from protists to large mammals (reviewed in Lima and Dill 1990, Tollrian and Harvell 1999, Agrawal 2001). These trait responses come with costs that can alter prey fitness including reduced individual somatic growth rate, survival, and reproduction (Werner and Anholt, 1996, Trussell 2000, Turner and Montgomery 2003, Sheriff et al 2009, McCauley et al. 2011, Zanette et al 2011), and can have with corresponding population-level consequences (non-consumptive effects or NCEs). Through direct and indirect interactions with other community members including competitors, resources, and predators, trait responses can influence population and community dynamics (Bolker 2003, Werner and Peacor 2003). Thus, understanding trait responses has important implications for predicting how predation risk affects community structure, ecosystem processes, and species evolution (Agrawal 2001, Peckarsky et al. 2008, Schmitz et al. 2008).

Experiments and theory indicate that the expression and magnitude of trait responses of a species is dependent on the tradeoff between the consequent costs to fitness and the benefit from the reduction in predation rate (Abrams 1984, Werner and Anholt 1993, Lima and Dill 1990). Different trait responses are expected at different levels of risk dependent on costs. Fig. 5

illustrates different possible relationships between predation risk and trait responses for a species. If a trait response is accompanied by a large cost, but is effective at reducing predation risk, then smaller trait changes are expected at low predation risk and larger trait responses are expected at high risk (Line A, Fig. 5). Intermediate trait responses would be expected depending on the curvature of the relationship between the trait response and the cost and benefit (Peacor et al. 2013). If there is a small cost associated with a highly effective trait response, then a steep increasing leading to a large trait response is expected at both low and high risk levels (Line B, Fig. 5). In contrast, if there is a large cost associated with a trait response that is not effective against the predator, then no trait response is expected (Line C, Fig. 5). Costs and benefits of trait responses will be influenced by multiple intrinsic and extrinsic factors (reviewed by Lima, 1998, Lima and Dill, 1990, Peacor et al. 2013). Examples of intrinsic factors include individual age, physiology, reproductive condition, experience, and ecology (reviewed in Lima and Dill 1990). Examples of extrinsic factors include predator type and density, habitat characteristics, resource availability, and social context (McNamara and Houston 1987, Bednekoff and Lima 2004, Higginson et al. 2012).

When considering how the magnitude of trait responses may vary within a group of prey species, it might be expected that there would be a positive relationship between species' trait responses and their vulnerability (predation risk). This might be expected based on a simple logical argument that predation risk would induce large responses to decrease that risk. Certainly, if a species is not consumed by the predator, no trait response would be expected. Such monotonically increasing relationships of the trait response and predation risk within species of prey have been observed. For example, a positive relationship between predation risk and trait responses has been observed when prey size limits the ability of the predator to consume the prey and larger species do not respond (Sih 1987, Kohler and McPeek 1989, Peckarsky 1996, Relyea 2001). However, there can be positive, negative, or no relationship between the trait response and predation risk of different prey.

Fig. 5 can be used to examine the different possibilities for how trait responses may be related to predation risk of different prey by considering different possible trait-response-predation risk relationships simultaneously. Lines A, B, and C could represent three different prey species in a community where each species has a different trait response-predation risk relationship due to different costs and benefits. Comparing the three lines simultaneously can be used to examine the possibilities of the relationship between trait responses and predation risk of different prey. Points 1, 2, and 3 represent these three prey species in a system with the same number of predators. The magnitude of their trait response under this number of predators is dictated by their own costs of responding, but they have different vulnerability to the predator such that 1, 2, and 3 have increasing vulnerability, as indicated by higher predation risk values. When species represented by points 1, 2, and 3 are present in a community, a negative relationship between trait responses and predation risk would be expected (grey arrow).

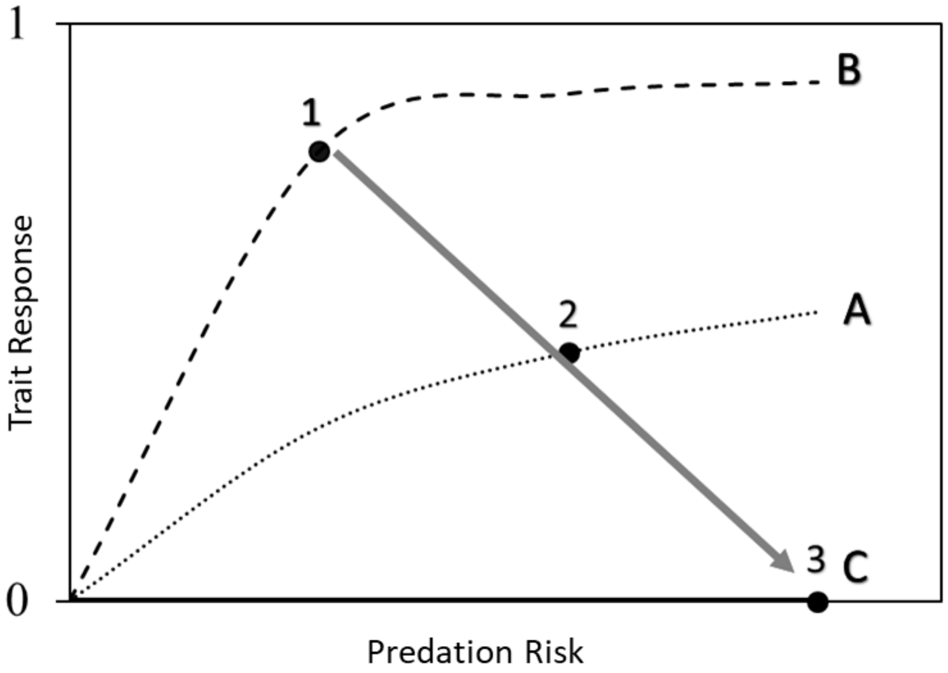

Figure 5. Conceptual diagram demonstrating how the predicted trait response can vary as function of predation risk experienced among prey. Lines A, B, and C represent three species with different costs of responding where B has the lowest cost and C has the highest cost. Points 1, 2, and 3 represent these three prey species in a system with the same number of predators. When species represented by points 1, 2, and 3 are present in a community, a negative relationship between trait responses and predation risk would be expected (grey arrow).

Despite a theoretical basis for a positive, negative, or no relationship between trait responses and predation risk, many studies of predation risk effects assume that the magnitude of trait responses is positively related to the magnitude of risk posed by the predator. This may be assumed based on empirical examples that demonstrate that the magnitude of behavioral responses is higher for species that evolved under higher average predation rates (Sih 1987, Lima and Dill 1990, Relyea 2001, Martin and Briskie 2009, Ghalambor et al. 2013). This may also be assumed based on empirical evidence of the threat sensitivity hypothesis that demonstrate larger trait responses with greater perception of predation risk (Sih, 1986, Chivers et al. 2001). Even if not explicitly stated, when studies investigate trait responses only to voracious predators or in the most susceptible prey, there is an implicit assumption that the magnitude of predator-induced responses are dependent on predation risk. However, as described by Fig. 5, a positive relationship is not the only possible relationship between trait responses and predation risk among different prey.

Very few studies have examined the relationship between trait responses and predation risk in a community of prey that represent natural variation in vulnerability to predators. This study examines the relationship between behavioral trait responses induced by fish and predation risk in a community of freshwater cladocerans. Fish-cladocerans represent a model system to examine this relationship because cladocerans have been shown to demonstrate behavioral responses to fish predators (Tollrian and Harvell 1999, Lass and Spaak 2003) and have different vulnerability to fish predation, based primarily on size (Brooks and Dodson 1965). This study was performed with two metrics of predation risk as a robustness examination of the relationship between trait responses and predation risk.

Methods

Cladoceran behavioral trait responses, metrics of predation risk, and zooplankton size were measured from a mesocosm experiment that was designed to simulate two components of a predator, the non-consumptive effects and consumptive effects. The experimental design included five treatments: presence/absence of fish kairomone (from fish in floating cages) crossed with presence/absence of removing zooplankton with a net (to simulate the density effects of fish predation), and lastly, a treatment with uncaged fish. A difference in zooplankton position in kairomone relative to no kairomone treatments was used as measure of a behavioral response to predation risk. We examine the effect of kairomones across the presence and absence of removal treatments, and accounted for any influence of the removal effect, because including both the no removal and removal treatments increased the number of replicates and there is no a priori reason to use one treatment over the other. We describe the removal treatment methodology for completeness, although the effect of removal is not a focus of this study.

Data from the uncaged treatment were used to calculate two metrics of predation risk of each taxon including a relative predation rate and net density effect. These metrics represent measures of interaction strength of predators on prey as indicators of predation risk. Our metric of relative predation rate could represent attack rate or per capita interaction strength in basic theoretical models (Lotka-Volterra, Laska and Wooton 1998). The other metric of net density effect is intended to capture the overall net effect (non-consumptive and consumptive effect) of a predator on prey density in a community (sensu Paine 1992).

Mesocosm experiment

Experiments were conducted at the E.S. George Reserve (ESGR) of the University of Michigan near Pinkney, MI, USA. To guide presentation of the experimental schedule, we reference manipulations and events relative to day 0 when treatments were initiated on 8 July 2013. The experiment was terminated on 23 August 2013 (day 46). Experimental mesocosms were started well before treatment initiation in order to create established, homogenous zooplankton communities in the mesocosms. On days -62 through -60, black round plastic tanks with a diameter of 168 cm and height of 60 cm were filled with 1100 L of treated well water, to a depth of approximately 50 cm. 11 kg of washed play sand were added to each to cover the plastic bottom to produce a more natural substrate. On day -56, mesocosms were inoculated with phytoplankton by adding 500 mL of 35 µm-filtered lake water from nearby Sayles Lake in Pinckney, Michigan to each mesocosm. Sayles Lake is a 10-hectare lake with a maximum depth of approximately 3 m and an average depth of less than 2m with a diverse fish community including bluegill. On days -49 and -38, a diverse community of zooplankton was added to mesocosms. The zooplankton were collected from Sayles Lake using 150 µm zooplankton nets towed at night through areas of macrophytes and open water. Undesirable species such as amphipods, *Hydra*, and insects (e.g., *Chaoborus*) were removed. To increase zooplankton assemblage homogeneity among mesocosms, on days -24 and -6, a portion of zooplankton were collected from each mesocosm with a 64 µm zooplankton net, mixed, and subsamples of this mixture were redelivered to each mesocosm. Dominant Crustacean zooplankton taxa that established in the mesocosms included cladocerans *Daphnia pulicaria*, *Ceriodaphnia* sp., *Scapholeberis* sp., *Diaphanosoma* sp., *Bosmina* sp., *Alona* sp., *Chydorus* sp., calanoid, cyclopoid copepods, and ostracods.

Inorganic nutrients were added at a N:P ratio of 20:1 to the mesocosms to support phytoplankton growth as a resource for zooplankton. An initial spike of 4.35 g of NH_4NO_3 and 0.37 g of KH_2PO_4 was added to each mesocosm on day -56. From days -38 to day -2, nutrients were added three days per week with 1.65 g NH_4NO_3 and 0.14 g of KH_2PO_4 added on these days. Starting on day 0, nutrients were added five days per week with 0.57 g NH_4NO_3 and 0.049 g of KH_2PO_4 added on these days. On days 15 and 38, these amounts were reduced by 50% and 50% again, respectively, in order to reduce an observed increase in phytoplankton and filamentous algae growth.

Treatment manipulations

Mesocosms were randomly assigned to the five treatments in nine experimental blocks. Treatments were initiated on Day 0 by adding fish to the corresponding mesocosms. All mesocosms had two floating cages that were constructed from 41 X 27 X 26 cm plastic boxes with polystyrene foam glued to the sides. The cages had windows on all sides and the bottom with mesh panels made of midge netting. Cages were clipped to opposite sides of the mesocosms. The cages' mesh panels were sprayed with a hose each week to reduce potential periphyton growth that would decrease diffusion of kairomones from the cages. Bluegill sunfish (*Lepomis macrochirus*, mean length ± SD of 5.5 ±0. 4 cm) were collected from nearby Patterson Lake. One bluegill per cage was used in the kairomone treatments. Once a week, fish from cages were exchanged with fish from culture tanks. Culture mesocosms consisted of 1100 L plastic cattle tanks that housed approximately 25-50 fish of a similar size and fed a mixed assemblage of zooplankton three times per week. For the uncaged predator treatment, two fish were added to the uncaged treatment mesocosms and allowed to swim freely. Uncaged fish were removed (and replaced with fish from the culture mesocosms) with a net from the mesocosms every 2-4 days through day 14, and then once per week through the end of the experiment. Other treatments received the same net activity used to capture the fish to control for disturbance. The removed fish were immediately euthanized using an overdose of aqueous Tricaine Methanesulfonate (MS-222) and frozen for stomach content analysis.

Zooplankton removal in the removal treatments was conducted two times per week. It consisted of three tows around the mesocosms with a 363 µm Nitex conical plankton net with a diameter of 50 cm. This sized mesh was used to remove larger zooplankton to simulate the size-selectivity of fish predation. The removal net was also towed in the center of the mesocosms and we avoided towing too close to the walls so as to remove the larger, pelagic species such as Daphnia and to leave the small, littoral species such as *Alona* sp. and *Chydorus* sp. While not significant, the removal did affect the zooplankton community by creating a community where smaller-bodied cladoceran were proportionally more abundant than Daphnia with removal (Rafalski and Peacor unpublished). This resulting zooplankton community is similar to a community that would be expected from zooplanktivory (Brooks and Dodson 1965, Hall et al. 1976). The removal was performed at night because zooplankton were more evenly distributed throughout the mesocosms. To control for potential nutrient loss that might result from removal, the zooplankton that were removed were killed by microwaving and added back to the mesocosm they were removed from over the next three days in equal daily amounts to control for potential nutrient loss that would accompany zooplankton removal. In order to control for towing disturbance, the same towing procedure was performed in the no removal treatments; zooplankton were immediately released by removing the net's cod end while still in the mesocosm's water.

A natural concern in an experiment with a predator and no-predator treatment is that nutrients excreted from the fish could influence phytoplankton growth and hence zooplankton. Our calculations indicate that nutrient recycling from zooplankton in the mesocosms and nutrients added to the mesocosms far exceed any nutrient excretion by fish (Peacor et al. 2012, Appendix A). Further, on days 8-10, we performed a three-day in-situ study comparing the growth of *Ankistrodesmus* (from culture) in 35 µm Nitex chambers in seven replicates each of no fish, caged fish, and uncaged fish mesocosms. Chambers were hung on the northwest sides of the mesocosms, at a depth of 20 cm and 30 cm from the mesocosm wall just beyond where the floating fish cage was tethered. We found no significant difference in the phytoplankton growth rate (µg chlorophyll-*a* L^{-1} day^{-1}) among treatments (Rafalski and Peacor unpublished), suggesting no effect of fish excretion on phytoplankton growth. We nevertheless were conservative by taking several measures to reduce any potential differences in nutrient addition between treatments. First, culture fish were starved the day before they were rotated into the experiment to minimize their nutrient excretion. Second, each caged fish was fed a metabolically minimal diet of 200 *D. pulicaria (*700 – 1200 µm length) twice a week, and to provide equal nutrients, cages in all other treatments received 200 *D. pulicaria* that had first been killed by microwaving. Lastly, inorganic nutrients were added at levels that far exceeded the predicted excretion of fish and were added near continuously (i.e., 5 days per week) to overwhelm any pulses in nutrient supply by fish or other treatment manipulations.

Sampling and metrics of predator density effects and zooplankton position

In order to calculate metrics of predator density effect, zooplankton density was sampled at the beginning (day 4) and the end (day 44) of the experiment. Samples were collected with a 5 cm diameter vertical tube sampler to collect water samples of the entire water column (i.e., from the surface to the bottom of the mesocosm). Zooplankton were sampled after sunset because we observed less spatial segregation that could improve the sampling estimate. Twenty tube casts were taken at points along a grid placed over the tanks and combined to yield one 18 L sample (~2% of mesocosm volume). Sampling was stratified such that all areas from the sides to the center of the mesocosms were proportionally represented. This method allowed accurate assessment of total mesocosm density even if zooplankton density varied as a function of radial position. Collected water was filtered through a 64 µm mesh sieve and preserved with 90% ethanol. Crustacean zooplankton were enumerated via compound microscope and identified to species or genus. Zooplankton lengths were measured from twenty random individuals of each taxa in each of the samples from day 44 using a calibrated microscope digitizing tube and board (Roff and Hopcroft 1986). Digitizing points were based on methods described in Rosen (1981).

As a measure of relative predation rate on each taxon, Chesson's α was calculated from the counts of zooplankton in the stomachs of uncaged fish relative to the zooplankton density in the mesocosms. Specifically, stomach contents of the preserved uncaged fish from days 2 and 4 were compared to the zooplankton density in the same mesocosms the fish were removed from on day 4. The stomachs were removed from each bluegill, and the stomach contents were rinsed out and zooplankton were enumerated. Chesson's α (Chesson 1978) was calculated for each cladoceran in each mesocosm per the equation:

$$\alpha_i = \frac{r_i/n_i}{\sum_{j=1}^{m} r_j/n_j} \quad , \; i = 1, \ldots, m$$

Where α_i is equal to is the proportion (r_i/n_i) of individuals of taxon type *i* in the stomach counts for all taxa *m* of the sacrificed fish, divided by the sum proportions (r_i/n_i) of the density of each taxon in the mesocosm for all *m* taxa. All crustacean zooplankton taxa included copepods and ostracods were used to calculate Chesson's α, though only cladocerans were used in this study of behavioral responses. The counts of the two fishes' stomach contents were combined to get one Chesson's α for each cladoceran per date per mesocosm, and the calculated Chesson's α for the two dates were averaged together to get one Chesson's α per cladoceran per mesocosm. The calculated Chesson's α were averaged across the mesocosms for each cladoceran using only mesocosms which had sample counts greater than 5 in accordance with sampling accuracy as described below. Two additional mesocosms were excluded because they had many chironomid midges, which were preferentially consumed by fish compared to zooplankton.

As the measure of net density effect on each taxon, we calculated the effect of uncaged fish on density of each taxon at the end of the experiment (day 44). The effect was calculated as the ratio of the average density in the uncaged fish treatment to the average density in the control (no kairomone - no removal) treatment.

To measure zooplankton spatial position, zooplankton were sampled in all mesocosms at six distinct positions at the end of the experiment (day 44). Sampling was done at three vertical strata: ‘high’ (just below the surface), ‘middle’ (at the midwater level) and ‘low’ (right above the bottom) crossed with two horizontal positions including ”side” (along the walls) and the “center” (within 25 cm of the mesocosm center). Four sub-samples were collected in each of four quadrants and combined for the “side” samples, and three sub-samples were collected in the center at each stratum and combined for the “center” samples. A 15 cm long 1.2 L cylindrical water sampler (Wildco) held parallel to the ground was used. The sampler was inserted into the water approximately 30 cm behind, and then moved forward to, the collection position. The order of the samples was chosen as to not disturb subsequent samples. Each of the six positional samples were collected on a 64 µm mesh sieve and preserved in 90% ethanol. Crustacean zooplankton were enumerated via compound microscope and identified to species or genus.

For each mesocosm, the proportion of each cladoceran at each of the six position was calculated as the abundance in that position divided by the sum of the abundance at all six positions. We also calculated averaged position for vertical and horizontal directions. For vertical, we calculated the proportions in the high, middle, and low strata; for horizontal, we calculated the proportions in the center and side strata. For example, the proportion in the high strata was the sum of the abundance in the high side and high center positions divided by the sum abundance in all six positions. Proportions were only included for mesocosms if there were 5 or more individuals represented in the total number counted in the mesocosm. We used 5 individuals as the cut off based on a resampling exercise that showed that 5 is the threshold number of individuals counted in a mesocosm below which the estimates of spatial position in that mesocosm negatively affects the accuracy of the estimates of the average position across mesocosms.

Calculation of behavioral response

To produce an approximation of the magnitude of each cladoceran's behavioral response, we calculated the sum of the absolute values of the differences in the treatment means of the proportions in all six positions. This sum difference was used in order to produce a single metric that would capture differences in both the vertical and horizontal directions. Standard error was propagated from the treatment standard errors.

Statistical Analyses

Regression plots were used to examine the relationship between the behavioral responses and the relative predation rate, net density effect, and size (length in µm) using the average data of each cladoceran taxon. The relationships were qualitatively compared to expected relationships between trait response and predation risk.

Both significant and not-significant behavioral responses were used in the analyses of the relationship between behavioral trait response and predation risk because any magnitude of behavioral response (including no response) could be expected. However, we tested for and examined any significant kairomone x removal interactions that may affect our detection or inference of kairomone effects (described below).

Compositional data, such as the proportion of individuals in different positional strata used in this study, are constrained in that the components sum to 1. As such, statistical methods designed for unconstrained data may lead to inappropriate inference (Hijazi and Jernigan 2009). Dirichlet regression was used to analyze the significance of the behavioral responses. The Dirichlet distribution is the multivariate generalization of the beta distribution and performs wells for composite measurements, such as our response variables, that do not conform well to count-based or binomial-based analyses (reviewed in Douma and Weedon, 2019).

Separate Dirichlet regression analyses were run for the vertical position (as the proportion of individuals in high, middle, and low positions) and horizontal position (as the proportion of individuals in center and side positions) because these are spatial axes along which shifts in zooplankton position are studied in natural systems (i.e., diel vertical and horizontal migration, e.g., Zaret and Suffern 1976, Hays 2003, Burks et al. 2012). To test for significant ($p \leq 0.05$) differences along each axis, we ran Dirichlet regression models with the proportions in the vertical or horizontal strata as the response with kairomone, removal, and the interaction of kairomone and removal as fixed effects. To identify if there were any significant fish x removal interactions, this full model was compared to a model without the interaction using a likelihood-ratio test approach. If no significant interaction was identified, the effect of kairomone was examined across no removal and removal mesocosms to have additional power to examine the kairomone effect. Specifically, Dirichlet regression models with the effects of kairomone and removal were compared to models with only kairomone or removal using a likelihood-ratio approach to test if the positional response differed with kairomone or removal. Analyses were performed in R version 3.5.2 (R Development Core Team, 2019) using the *DirichletReg* package (Maier, 2015).

We tested for the interaction to be conservative in our analysis of the relationship between trait response and predation risk to ensure there were no behavioral responses that were missed by combining removal and no removal treatments. For example, a significant interaction could mean that that the kairomone had opposite effects on position in the treatments e.g., located higher in no removal and lower in removal. By combining no removal and removal treatments, the kairomone effect would be lost. For taxa that had significant fish x removal interactions, boxplots of data in each treatment were examined to identify if kairomone had opposite effects in no removal and removal treatments, and cladocerans were included in the analysis with predator density effect so long as the position effects were not in opposite directions.

Results

Effect of fish kairomone on position

Fish kairomone had a statistically significant effect on the vertical position of *Alona* sp., *Chydorus* sp., and *Bosmina* sp., and the horizontal position *Chydorus* sp. (Table 4). Whereas *Alona* sp. and *Chydorus* sp. were located lower with kairomone, *Bosmina* sp. was located higher (Table 4). In the horizontal direction, *Chydorus* sp. was located more towards the center. The positions *Ceriodaphnia* sp., *D. pulicaria*, and *Diaphanosoma* sp. were not significantly affected by fish kairomone (Table 4). Simulated predation (removal) significantly affected the position of *D. pulicaria* to be lower with removal, while there was a trend for *Diaphanosoma* sp. to be higher with removal. One marginally significant kairomone x removal interaction was identified which was for *Scapholeberis* sp. in the horizontal direction. Inspection of the kairomone effect on Scapholeberis sp. in no removal only and removal only treatments revealed that the trend of the kairomone effect was in the same direction (more center with kairomone) in both the no removal and removal treatments but apparently to a different enough degree to yield a significant interaction (Appendix B).

Table 4. Statistical results of the Dirichlet regression of the effects of kairomone, removal, and their interaction on the vertical and horizontal position of each taxa, including total N for all treatments, Chi (X) statistic, and significance values. Significance of p-values are denoted for p ≤ 0.05 with * and p ≤ 0.1 with · . To indicate the direction of significant effects, grey-shaded cells in the vertical direction indicate taxa individuals were located lower with the treatment effect while dark-outlined values indicate individuals were located higher. For the horizontal position, grey-shaded values indicate more toward the center with the treatment effect and dark-outlined values indicate more toward the side.

			Vertical Position		**Horizontal Position**	
Cladoceran	**Effect**	**N**	**X**	***p*-value**	**X**	***p*-value**
Alona sp.	Kairomone	35	8.22	0.041 *	3.97	0.137
	Removal		0.252	0.969	3.69	0.158
	Kairomone:Removal		0.801	0.849	1.60	0.450
Chydorus sp.	Kairomone	32	10.6	0.014 *	8.06	0.018 *
	Removal		1.30	0.729	1.79	0.410
	Kairomone:Removal		1.61	0.658	1.08	0.584
Bosmina sp.	Kairomone	28	9.08	0.028 *	0.367	0.832
	Removal		2.63	0.452	0.170	0.919
	Kairomone:Removal		0.342	0.952	2.82	0.244
Ceriodaphnia sp.	Kairomone	35	0.119	0.990	0.955	0.620
	Removal		0.266	0.966	0.347	0.840
	Kairomone:Removal		5.69	0.128	3.83	0.147
Daphnia pulicaria	Kairomone	30	0.612	0.894	2.22	0.330
	Removal		8.37	0.039 *	0.178	0.915
	Kairomone:Removal		4.39	0.222	2.72	0.256
Scapholeberis sp.	Kairomone	22	2.01	0.570	0.379	0.150
	Removal		3.91	0.272	0.012	0.994
	Kairomone:Removal		2.55	0.314	5.57	0.062 ·
Diaphanosoma sp.	Kairomone	17	1.98	0.577	1.28	0.529
	Removal		6.49	0.090 ·	1.00	0.607
	Kairomone:Removal		4.07	0.254	0.382	0.826

Relationship between trait responses and predator density effects

A negative trend was found between the behavioral responses and the relative predation rate across taxa. Two cladocerans which were most consumed (Chesson's α different from equal preference) did not respond to predator kairomone, while some of the least preferred taxa responded significantly (Fig. 6).

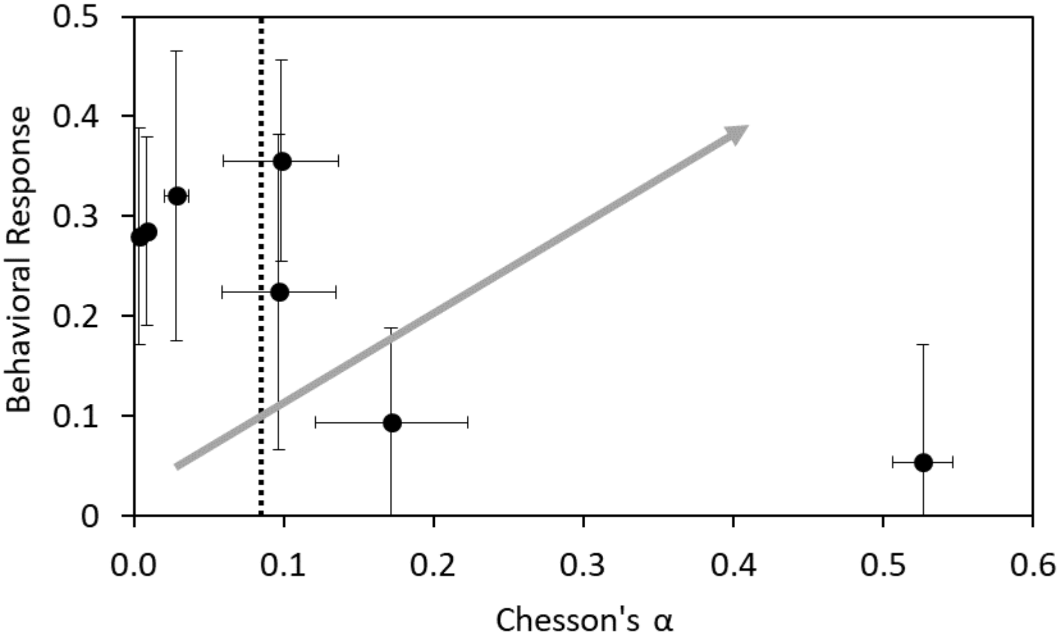

Figure 6. Behavioral response (± SE) versus average Chesson's α (± SE) (metric of relative predation rate) of fish across all cladoceran taxa. The behavioral response error bars are truncated at 0 because the sum difference cannot be negative. The grey arrow is used to illustrate the positive relationship that might be commonly expected between trait responses and predation risk to show how our results deviate from this expectation. Taxa can be identified by their Chesson's α (listed lowest to highest): *Alona* sp., *Chydorus* sp., *Bosmina* sp., *Diaphanosoma* sp., *Scapholeberis* sp., *Ceriodaphnia* sp., *D. pulicaria*. The dotted line at x = 0.08 represents equal dietary preference of taxa used to calculate Chesson's α (all taxa consumed at same relative rate).

A positive trend was found between the behavioral response and the net density effect of fish on zooplankton density. The cladocerans whose density was positively affected by uncaged predators demonstrated the largest trait responses while taxa whose density was negatively affected demonstrated some of the smallest trait responses (Fig. 7). Fish had positive effects on the density of *Bosmina* sp., *Alona* sp., and *Chydorus* sp., and negative effects on *D. pulicaria* and *Diaphanosoma* sp. (Fig. 7; density estimates are presented in Appendix B).

Relationship between trait responses and zooplankton size

A negative trend was found between the behavioral response and the length of the cladocerans (Fig. 8).

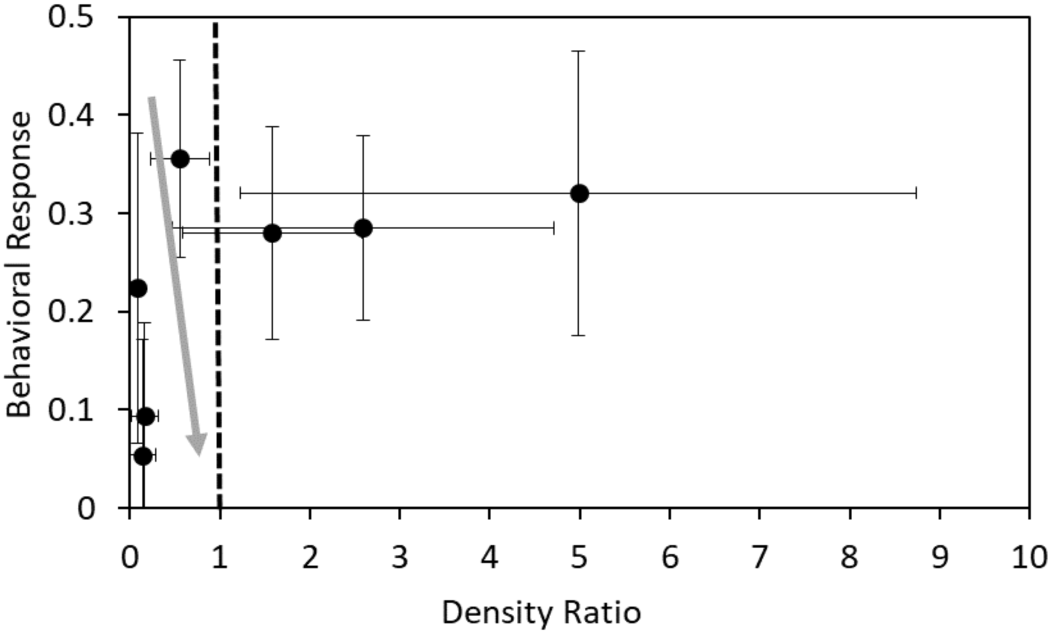

Figure 7. Behavioral response (± SE) versus the ratio (± SE) of average density in the uncaged fish treatment to the control treatment (metric of net density effect) across all cladoceran taxa. The behavioral response error bars are truncated at 0 because the sum difference cannot be negative. The grey arrow is used to illustrate the relationship that might be commonly expected between trait response and predation risk to show how our results deviate from this expectation. In this figure, a negative trend between trait responses and net density effect would reflect the common expectation of a positive relationship between trait response and predation risk. The expectation line stops at density ratio = 1 because it is expected that a predator would only have negative effects on prey density. Taxa can be identified by their net density effect (listed lowest to highest): *Diaphanosoma* sp., *D. pulicaria*, *Ceriodaphnia* sp., *Scapholeberis* sp., *Alona* sp., *Chydorus* sp., and *Bosmina* sp. The dashed line at x = 1 represents no effect of the uncaged fish on zooplankton density.

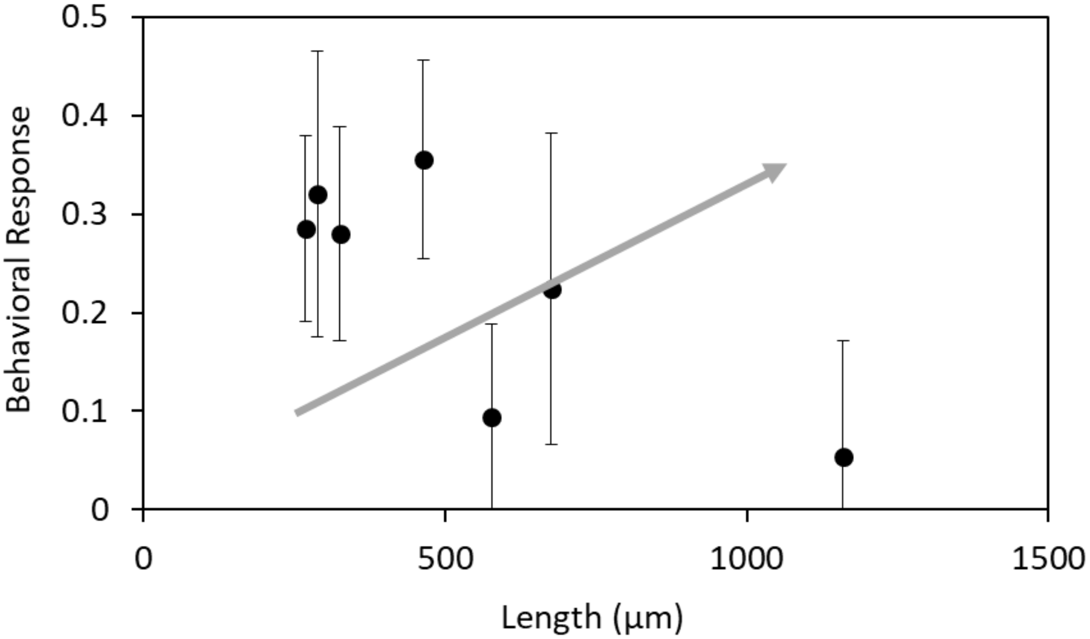

Figure 8. Behavioral response versus the average size (measured as length in µm) across all taxa. The behavioral response error bars are truncated at 0 because the sum difference cannot be negative. The grey arrow is used to illustrate the positive relationship that might be commonly expected between trait responses and length to show how our results deviate from this expectation. Taxa can be identified by their lengths (listed smallest to largest): *Bosmina* sp., *Alona* sp., *Chydorus* sp., *Ceriodaphnia* sp., *Scapholeberis* sp., *Diaphanosoma* sp., and *D. pulicaria*.

Discussion

We did not find the positive relationship that might be commonly expected between trait responses and predation risk for either of our two metrics. Based on the predator density effects we measured, our prey exhibited a large range of vulnerability to the predator, which allowed us to examine this relationship. While a positive relationship may be the common expectation between trait responses and relative predation rate (see Intro), we found a negative trend (Fig. 6).

The taxa that had one of the largest trait responses was the least vulnerable to the predator. A negative trend between trait responses and net density effect would reflect the common expectation of a positive relationship between trait responses and predation risk. It would also be expected that the predator would have a negative effect on prey density such that no trait responses would be expected at a density ratio > 1. However, not only did we find an unexpected positive trend, we also found that densities of taxa were positively affected by the predator and that these taxa demonstrated the largest trait responses (Fig. 7). Our findings support the theory that any relationship between trait responses and predation risk is possible. The finding is strengthened by the fact that this was true for two different metrics of predation risk. This highlights that a larger trait response is not necessarily expected with more predation risk and has implications for predicting the magnitude of trait responses among prey.

These two metrics of predator density effect were used as indicators of predation risk and represent well known measures of interaction strength of predators on prey and the range of metrics of predator effect used by the scientific community (Laska and Wootton 1998, Wootton and Emmerson 2005). The measurement of relative predation rate could represent attack rate or per capita interaction strength in basic theoretical models (Lotka-Volterra, Laska and Wooton 1998). The second metric represents the overall net effect of a predator on prey density in a community (Paine 1992). This net density effect metric includes both the consumptive and non-consumptive effects of the predator on density.

We did not find a potentially expected result that larger cladocerans would demonstrate a larger trait response (Fig. 8). This may be expected because vulnerability of cladocerans to consumption by fish increases with size (Mittelbach 1981, Bremigan and Stein 1994). In other taxa, some studies have found a relationship between trait responses and size including mayflies, snails, and sunfish (e.g., Peckarsky 1996, Werner and Hall 1988, Crowl and Covich 1990). In these taxa, higher trait responses may be expected at lower sizes, because body size can affect the susceptibility of prey to attack and the costs of predator avoidance (Pekarsky 1996, Preisser and Orrock 2012). The findings of our study and others' findings suggest that it is likely that the relationship between trait responses and size is not a generalizable relationship across taxa.

Factors that may explain how negative relationships arise between trait response and predation risk can be explored by comparing the responses of two species that are on opposite ends of the spectrum of their vulnerability to consumption by fish. For example, while the more vulnerable *Ceriodaphnia* sp. demonstrated no significant position effect, the less vulnerable *Chydorus* sp. was located lower with kairomone. However, there are other characteristics of these two species that may affect the cost associated with a response to fish that may underlie the different expression of their behavioral response. For example, these two taxa are both present in littoral habitats of shallow lakes where they may encounter predation risk by other predators such as *Chaoborous sp.*, to which *Ceriodaphnia* is highly susceptible (Riessen et al. 1988). Thus, *Ceriodaphnia* sp. may have limited response to fish kairomone because movement may incur costs of consumption by the alternative predator. This is just one possible scenario of the multitude of species traits and environmental parameters that may affect the costs and benefits of trait responses to predation risk that could lead more vulnerable prey to respond less.

The findings in this study provide additional evidence that a larger trait response is not expected with more predation risk. We are only aware of three other studies from two systems that address the relationship between trait responses and predation risk (i.e., Relyea 2001, Creel et al. 2014, Creel et al. 2019). Using 30 predator-prey combinations of tadpoles and predators, Relyea (2001) examined the relationship between predation rate and trait responses and found that predation risk had no significant effect on how a given prey responds to the predator. Similarly, recent studies conducted with large carnivores and ungulate prey in African grasslands revealed no relationship between predation risk and antipredator responses of the ungulate prey (Creel et al. 2014, Creel et al. 2019). The evidence from studies of trait responses from a wide array of taxa are useful to consider if vulnerability does not predict the magnitude of trait responses, are there other attributes of predators, prey, or the environment that better relate to the magnitude of trait responses (Liley and Creel 2008).

It might be considered that the lack of responses in some taxa is due to lack of infochemicals that would elicit their response. However, there were significant responses of several taxa, indicating the presence of a cue to elicit responses across a diversity of taxa. Studies show that prey respond to a variety of infochemicals including those produced directly by the predators and chemicals produced by injured prey (Laforsch et al. 2006). Zooplankton have demonstrated trait responses to chemicals produced by predators and injured heterospecifics from a wide variety of taxa (Laforsch et al. 2006, Pecor et al. 2016). Daphnia from fishless lakes have been shown in the laboratory experiments to not respond to fish kairomones (Boeing et al. 2005), but the zooplankton in our experiment were collected from a lake with a diverse fish assemblage. Thus, it is likely that kairomones were present to serve as a cue of fish presence and that our experiment contained zooplankton that would respond to predator cues.

We found that the vertical position of *D. pulicaria* and *Diaphanosoma* sp. were marginally or significantly different in the no removal and removal treatments; however, the causes underlying this behavioral response as a result of removal remain unknown. Examination of the potential mechanistic causes of how removal may affect position was beyond the scope of this study, so we can only speculate as to how removal may have influenced the position of

Daphnia sp. and *Diaphanosoma* sp. It is possible that removal would influence zooplankton density and community structure and affect the competition of resources (i.e., phytoplankton) through differences in density. While not significant, the removal did affect the zooplankton community by creating a community where smaller-bodied cladoceran were proportionally more abundant than Daphnia with removal (Rafalski and Peacor unpublished). This finding is consistent with the effect that planktivorous fish such as bluegill may have on a zooplankton community (Brooks and Dodson 1965, Hall et al. 1976). Regardless, because we found no significant fish x removal interactions, removal effects do not influence the inferences made about the relationship between behavioral responses and predator density effects.

Our findings when compared to findings of others demonstrate how variation in trait responses can be expected (Fig. 5) contingent on environmental factors. We found that *D. pulicaria* and *Ceriodaphnia* sp. did not respond to fish kairomone while other studies have found behavioral trait responses of both taxa. For example, studies show *D. pulicaria* demonstrate diel vertical migration (Dodson 1989, Nihongi et al., 2016). Other studies have shown responses of Ceriodaphnia sp. For example, in a similar mesocosm to the one performed in this study, Peacor et al. (2012) found *Ceriodaphnia* sp. did respond to *L. macrochris* by being located higher in the mesocosms with kairomones. Lauridsen and Lodge (1996) demonstrated diel horizontal migration of these taxa. A factor that could explain our finding, compared to studies that showed diel horizontal migration by these taxa include the presence of macrophyte refuge in other studies (Lauridsen and Lodge 1996, Burks et al. 2002). Regardless, context-dependency of trait responses supports our premise that any relationship between trait-responses and predation risk is possible since the magnitude of the trait responses may change with environmental factors and not predation risk.

A particular strength of the experimental approach used in this study is that our experimental venue captures the variation that is present in natural systems. The fact that significant responses were observed with this variation suggests a robustness of this measurement of trait responses. One parameter that varied among the mesocosms is the diversity of the zooplankton community. For example, the coefficient of variation of taxa's densities averaged in the control and fish kairomone treatments ranged from 110% - 330% across the different the taxa (densities and SE presented in Appendix A). Similarly, the coefficient of variation of chlorophyll-*a* concentrations averaged in the control and fish kairomone treatments were 115% and 214%, respectively.

This study demonstrates the complex nature of factors underlying trait responses. This study and others show that response cannot always be predicted by vulnerability to predators, so it is worth further investigation to identify attributes of species and their environment to understand the contingency of these responses. A better understanding of these attributes will aid in the understanding of the importance of trait-mediated effects in communities and ecosystems. In this experiment, the fact that many prey species exhibited trait responses, including those that are less vulnerable, does highlight a possibility that both trait-mediated and density-mediated effects of planktivorous fish may be important in structuring zooplankton communities. Moreover, examining predator-induced responses as the tradeoff between reduction in predation and decreased fitness rather than assuming that responses are directly related to the amount of predation risk can improve the understanding of trait responses and how predation risk shapes communities and ecosystems.

Acknowledgements

We thank Katie Heffernan, Rob Emelander, Mitch Nisbet, Kiley Brothers, Keenan Scriber, Adam Haynes, Hope Healey, Ellary Marano, Casey Geisland, and Julie Markiewicz for field assistance and/or zooplankton enumeration. We thank the Ecology and Evolutionary Biology department of the University of Michigan for use of the E. S. George Reserve. Funding for this study was provided by the National Science Foundation DEB-0089809 and DEB-1145808.

APPENDICES

APPENDIX A. *Scapholeberis* sp. position

This appendix includes boxplots of proportions of *Scapholeberis* sp. individuals in the high, middle, low, and center strata. This was used to examine the direction of the kairomone effect in the no removal and removal treatments because there was a marginally significant fish x removal interaction on the horizontal position (main text Table 4). Inspection reveals that the trend of the kairomone effect was in the same direction (Fig. 9, more center with kairomone) in both the no removal and removal treatments but apparently to a different enough degree to yield a marginally significant interaction. Because the kairomone effect was in the same direction, *Scapholeberis* sp. was included in the analysis between trait response and predation risk.

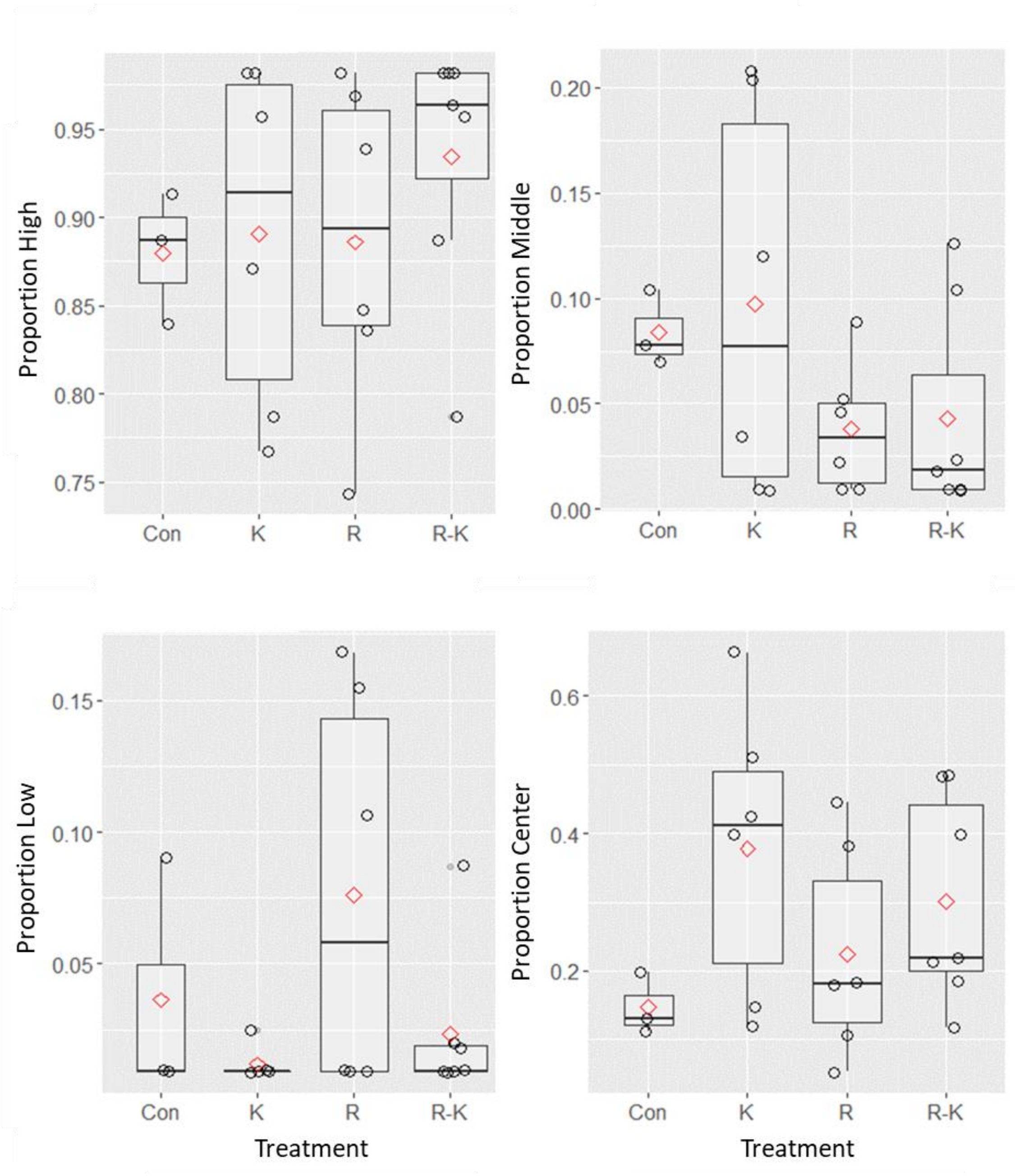

Figure 9. Proportion of *Scapholeberis* sp. individuals in the high, middle, low, and center positions in each mesocosm in Control, Kairomone (K), Removal (R), and Removal-Kairomone (R-K) treatments. The red diamond is the treatment average. The box shows the 25th percentile, median, and 75th percentile. Whiskers extend to the non-outlier minimum and maximum, outliers are plotted as points.

APPENDIX B. Uncaged and control treatment densities

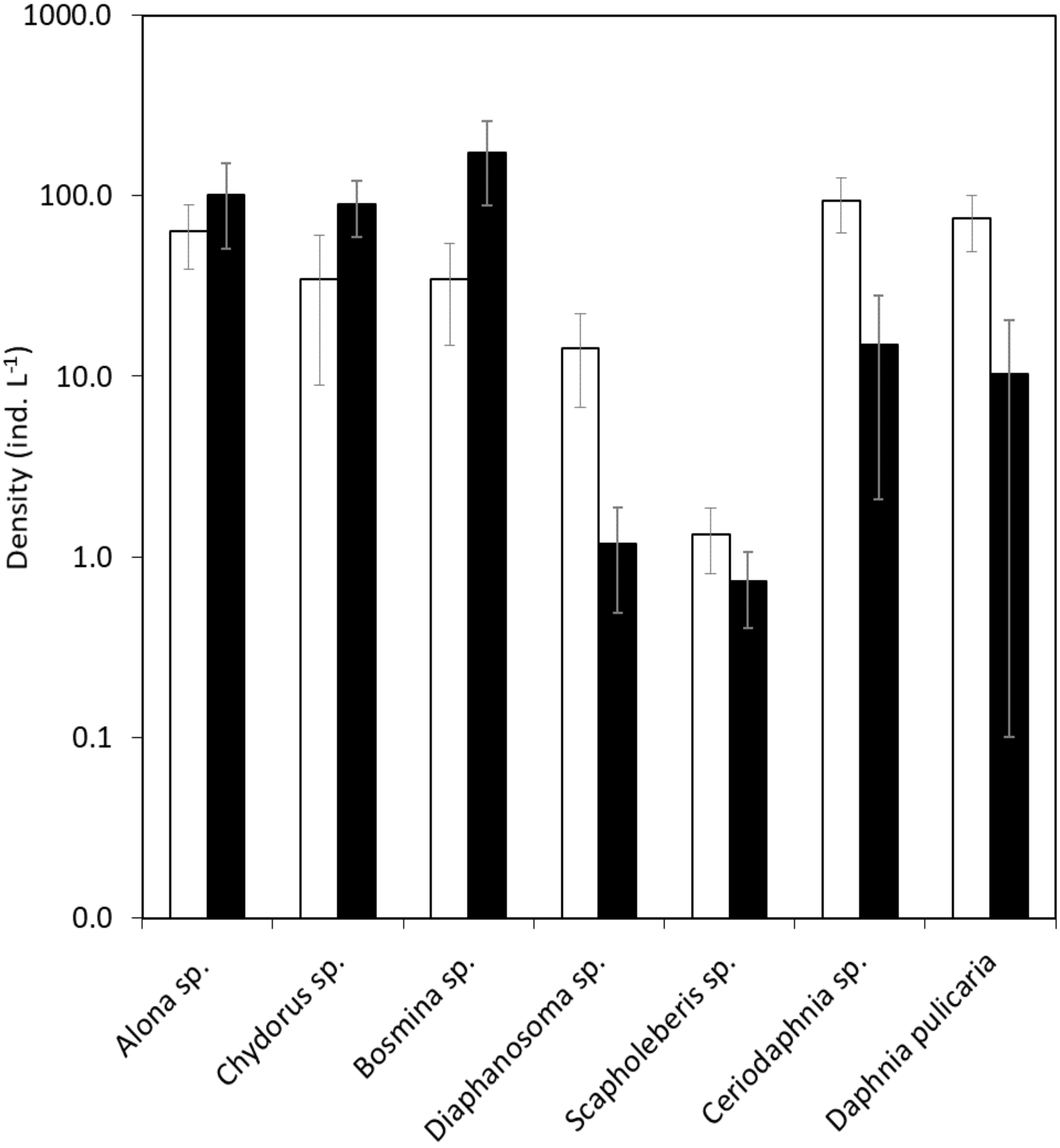

Figure 10. Average (± 1 SE) densities (individual L-1, logarithmic scale) of cladocerans in control (unfilled bars) and uncaged (filled bars) treatments.

REFERENCES

REFERENCES

Abrams, P.A. 2010. Implications of flexible foraging for interspecific interactions: lessons from simple models. Functional Ecology 24: 7–17.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326.

Bednekoff, P.A. and S.L. Lima. 2004. Risk allocation and competition in foraging groups: Reversed effects of competition if group size varies under risk of predation. Biological Sciences: 271: 11491–1496.

Boeing, W.J. and C.W. Ramcharan. 2010. Inducible defences are a stabilizing factor for predator and prey populations: a field experiment. Freshwater Biology 55: 2332–2338.

Bolker, B., M. Holyoak, V. Krivan, L. Rowe, and O. Schmitz. 2003. Connecting theoretical and empirical studies of trait-mediated interactions. Ecology 84: 1101–1114.

Bremigan, M.T. and R.A. Stein. 1994. Gape-dependent larval foraging and zooplankton size implications for fish recruitment across systems. Canadian Journal of Fisheries and Aquatic Sciences 51: 913–922.

Brooks, J.L. and S.I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28–35.

Burks, R.L., D.M. Lodge, E. Jeppesen, and T.L. Lauridsen. 2002. Diel horizontal migration of zooplankton: costs and benefits of inhabiting the littoral. Freshwater Biology 47: 343–365.

Chesson, J. 1978. Measuring preference in selective predation. Ecology 59: 211–215.

Chivers, D.P., J.M. Kiesecker, A. Marco, J. DeVito, M.T. Anderson, and A.R. Blaustein. 2001. Predator-induced life history changes in amphibians: egg predation induces hatching. Oikos 92: 135–142.

Creel, S., P. Schuette, and D. Christianson. 2014. Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. Behavioral Ecology 25: 773–784.

Creel, S, M. Becker, E. Dröge, J. M'soka, W. Matandiko, E. Rosenblatt, T. Mweetwa, H. Mwap, M. Vinks, B. Goodheart, J. Merkle, T. Mukula, D. Smitb, C. Sanguinetti, C. Dart, D. Christianson, and P. Schuette. 2019. What explains variation in the strength of behavioral responses to predation risk? A standardized test with large carnivore and ungulate guilds in three Ecosystems. Biological Conservation 232: 164–172.

Crowl, T. and A. Covich. 1990. Predator-induced life history shifts in a freshwater snail. Science 247: 949–951.

Dodson, S.I. 1989. The ecological role of chemical stimuli for the zooplankton: predator-induced morphology in Daphnia. Oecologia 78: 361–367.

Douma, J.C. and J.T. Weedon. 2019. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. Methods in Ecology and Evolution 10: 1412–1430.

Ghalambor, C.K., S.I. Peluc, and T.E. Martin. 2013. Plasticity of parental care under the risk of predation: how much should parents reduce care? Biology Letters 9: 20130154.

Hall, D.J., S.T. Threlkeld, C.W. Burns, and P.H. Crowley. 1976. The size-efficiency hypothesis and the size structure of zooplankton communities. Annual Reviews of Ecology and Systematics 7: 177–208.

Hays, G.C. 2003. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. Hydrobiologia 503: 163–170.

Higginson, A.D., T.W. Fawcett, P.C. Trimmer, J.M. McNamara, and A.I. Houston. 2012. Generalized optimal risk allocation: foraging and antipredator behavior in a fluctuating environment. American Naturalist 180: 589–603.

Kohler, S.L. and M.A. McPeek. 1989. Predation risk and the foraging behavioral of competing stream insects. Ecology 70: 1811–1825.

Laforsch, C., L. Beccara, L. and R. Tollrian. 2006 Inducible defenses: the relevance of chemical alarm cues in Daphnia. Limnology and Oceanography 51: 1466 –1472.

Laska, M.S. and J.T. Wootton. 1998. Theoretical concepts and empirical approaches to measuring interaction strength. Ecology 79: 461–476.

Lass, S. and P. Spaak. 2003. Chemically induced anti-predator defences in plankton: a review. Hydrobiologia 491: 221–239.

Lauridsen, T. and D. M. Lodge. 1996. Avoidance by Daphnia magna of fish and macrophytes: chemical cues and predator-mediated use of macrophyte habitat. Limnology and Oceanography 4: 794–798.

Lima, S. L. 1998. Nonlethal effects in the ecology of predator prey interactions. Bioscience 48: 25–34.

Lima, S.L and P.A. Bednekoff. 1999. Temporal variation in danger drives antipredator behavior: the predation risk allocation hypothesis. American Naturalist 153: 649–659.

Lima, S.L and L.M. Dill. 1990. Behavioral decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

Liley, S. and S. Creel. 2008. What best explains vigilance in elk: characteristics of prey, predators, or the environment? Behavioral Ecology 19: 245–254.

Maier, M. J. 2015. DirichletReg: Dirichlet regression in R. R package version 0.6-3.1. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://cran.r project.org/web/packages/DirichletR eg/vignettes/DirichletReg-vig.pdf.

Martin, T.E. and J.V. Briskie. 2009. Predation on dependent offspring. Annual Proceedings New York Academy of Sciences 1168: 201–217.

McCauley, S. J., L. Rowe, and M.J. Fortin. 2011. The deadly effects of ‘nonlethal’ predators. Ecology 92: 2043–2048.

McNamara, J.M. and A.I. Houston. 1987. Starvation and predation as factors limiting population size. Ecology 68: 1515–1519.

Mittelbach, G.G. 1981. Foraging efficiency and body size: a study of optimal diet and habitat use by bluegills. Ecology 62: 1370–1386.

Nihongi, A., J.J. Ziarek, M. Uttieri, R. Sandulli, E. Zambianchi, and J.R. Strickler. 2016. Behavioural interseasonal adaptations in *Daphnia pulicaria* (Crustacea: Cladocera) as induced by predation infochemicals. Aquatic Ecology 50: 667–684.

Paine, R.T. 1992. Food-web analysis through field measurement of per capita interaction strength. Nature 355: 73–75.

Peacor, S. D. and E.E. Werner. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. Proceedings of the National Academy of Sciences USA 98: 3904–3908.

Peacor, S.D., K.L. Pangle, L. Schiesari, and E.E. Werner. 2012. Scaling-up anti-predator phenotypic responses of prey: impacts over multiple generations in a complex aquatic community. Proceedings of the Royal Society B 279: 122–128.

Peacor, S.D., B. L. Peckarsky, G.C. Trussell, and J.R. Vonesh. 2013. Costs of predator-induced phenotypic plasticity: A graphical model for predicting the contribution of nonconsumptive and consumptive effects of predators on prey. Oecologia 171: 1–10.

Peckarsky, B. 1996. Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. Ecology 77: 1888–1905.

Peckarsky, B.L, B.L. Kerans, A.R. Mcintosh, and B.W. Taylor. 2008. Predator effects on prey population dynamics in open systems. Oecologia 156: 431–440.

Pecor, K., T. Sarris, J. Shaffer, and E. Thom. 2016. Responses to injured conspecifics and heterospecifics by water fleas, *Chydorus sphaericus.* Marine and Freshwater Behaviour and Physiology 49: 415–419.

Preisser, E.L. and J.L. Orrock. 2012. The allometry of fear: interspecific relationships between body size and response to predation risk. Ecosphere 3: 77.

R Core Team. 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing Computing, Vienna, Austria. http://www.r-project.org.

Relyea, R. A. 2001. The relationship between predation risk and antipredator responses in larval anurans. Ecology 82: 541–554.

Riessen, H.P., J.W. Sommerville, C. Chiappari, and D. Gustafson. 1988. *Chaoborus* predation, prey vulnerability, and their effect in zooplankton communities. Canadian Journal of Fisheries and Aquatic Sciences 45: 1912–1920.

Roff, J. C. and R.R. Hopcroft. 1986 High precision microcomputer based measuring system for ecological research. Canadian Journal of Fisheries and Aquatic Sciences 43: 2044–2048.

Rosen, R.A. 1986. Length-dry weight relationships of some freshwater zooplankton. Journal of Freshwater Ecology 1: 225–229.

Schmitz, O.J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science 319: 952–954.

Sheriff, M.J., C.J. Krebs, and R. Boonstra. 2009. The sensitive hare: Sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78: 1249–1258.

Sih, A. 1986. Antipredator responses and the perception of danger by mosquito larvae Ecology 67: 434–441

Sih, A. 1987. Predators and Prey Lifestyles: an Evolutionary and Ecological Overview. In Predation: Direct and Indirect Impacts on Aquatic Communities. (eds Kerfoot, W.C., Sih, A.). University Press of New England, Hanover, New Hampshire, USA, pp. 203–224.

Tollrian, R. and C.D. Harvell. 1999. The Ecology and Evolution of Inducible Defenses. Princeton University Press, Princeton, New Jersey.

Trussell, G.C. 2000. Predator-induced plasticity and morphological trade-offs in latitudinally separated populations of *Littorina obtusata*. Evolutionary Ecology Research 2: 803–822.

Turner, A.M. and S.L. Montgomery. 2003. Spatial and temporal scales of predator avoidance: Experiments with fish and snails. Ecology 84: 616–622.

Werner, E.E. and B.R. Anholt. 1993. Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. American Naturalist 142: 24.

Werner, E.E. and B.R. Anholt. 1996. Predation-induced behavioral indirect effects: Consequences to competitive interactions in anuran larvae. Ecology 77: 157–169.

Werner, E.E. and D.J. Hall. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate predation risk trade-off. Ecology 69: 1352–1366.

Werner, E.E. and S.D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

Wootton, J.T. and M. Emmerson. 2005. Measurement of interaction strength in nature. Annual Review of Ecology, Evolution, and Systematics 36: 419–444.

Zanette, L.Y., A.F. White, M.C. Allen, and M. Clinchy. 2011. Perceived predation risk reduced the number of offspring songbirds produce per year. Science 334: 1398–1401.

Zaret, T.M and J.S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnology and Oceanography 21: 804–813.

CHAPTER 3

Characterizing variation in trait responses within group of similar prey

Abstract

Predators affect prey populations both through consumption and by inducing anti-predator responses, in which prey modify traits, including behavior, to reduce predation risk. Despite trait responses being well-studied in a variety of taxa, it is still difficult to predict when trait responses will translate to population and community-level effects. One reason for this is that assumptions may be made about the variation and contingency of trait responses of species within groups of prey. Trait-based approaches that connect species' traits to their fitness based on trade-offs of key functional activities have proven useful in classifying variation in species along meaningful trait axes to address ecological questions. To explore how trait responses may be related to traits associated with predation risk, we compared the behavioral trait responses induced by fish kairomones in cladocerans to their body size and taxonomic identity. We found considerable variation in the responses in relation to taxonomy with the four Daphniidae taxa demonstrating a range of responses from no response to the largest responses, comparable to a taxon from family Sididae. We found no clear relationship between the behavioral responses and size. Results from our study and others suggest that there may be considerable variation in the relationship between body size and trait responses among taxa. The substantial variation we found among relatively similar prey taxa warrants further investigation of trait axes that explain the expression of trait responses and can be related to predator-prey interactions.

Introduction

A thorough understanding of the variation among species is important for being able to make predictions about community structure and ecosystem level processes (Chesson 2000, Hooper et al. 2005). There are different ways to classify species and their variation including based on phylogeny, morphology, or ecological role or function (Grime 1977, Tilman et al. 1997, Walker et al. 1999, Reynolds et al. 2002). Of these different ways to classify variation, frameworks that describe how species interact and affect their environment have particular application to addressing ecological questions (Diaz and Cabido 2001, Petchey and Gaston 2002, Norberg 2004, Mason et al. 2005). In particular, frameworks that use a trait-based approach have been gaining interest (reviewed in Zakharova et al. 2019). Trait-based approaches connect organisms' traits to their fitness based on trade-offs of key functional activities including feeding, growth, survival, and reproduction (Litchman et al. 2013). Examples of such traits include body size, life history, morphology, physiology, and behavior (Barnett et al. 2007, Litchman and Klausmeier 2008, Litchman et al. 2013).

Trait-based approaches have been applied to explain ecological processes such as community assembly, invasive species, ecosystem services (reviewed in Zakharova et al. 2019), and the application of trait-based approaches to understand the foraging ecology of predators (Spitz et al. 2014, Green and Côté 2014). It is recognized that traits of both predators and prey may affect the encounter rate, attack rate, and capture rate and ultimately consumption of prey by a predator (Ohman 1988, Green and Côté 2014). Studies have investigated how traits such as behavior, morphology, habitat, and physiology affect predator-prey encounters (Beukers and Jones 1997, Pressier et al. 2007, Andriskiw et al. 2008, Gorini et al. 2012, Clements et al. 2016, Juanes 2016).

In order to create trait-based classifications and apply them to ecological questions, there is a need to identify the functionally relevant traits, tradeoffs that govern the trait, and the variation in those traits (Barnett et al. 2007, Litchman et al. 2013, Sodré and Bozelli 2019). In exploring the relationship between traits and predator-prey interactions, fixed traits have received most of the attention (Spitz et al. 2014, Green and Côté 2014). However, many taxa demonstrate plastic trait responses in morphological, life history, and behavioral traits in response to predation risk (reviewed in Tollrian and Harvell 1999, Lima and Dill 1990, Agrawal 2001). The expression and magnitude of trait responses is expected to optimize fitness dependent on the tradeoff of reducing loss to predation and costs to growth of the trait response. Thus, the expression and magnitude of trait responses may be related to aspects of fitness that are linked to fixed traits.

Studies that have investigated the relationship between traits and trait responses have shown that characteristics of the predator, prey, and the environment influence the magnitude of trait responses (Preisser et al. 2007, Creel 2011, Creel et al. 2014). However, relatively little has been generalized across species that predicts the expression and magnitude of trait responses. For example, hunting mode of predator has been shown to influence trait responses of prey in multiple systems (Schmitz 2008, Thaker et al. 2011). However, it is expected that prey traits may be a better predictor of trait responses than predator traits because prey traits reflect the benefits and costs of responding to predation risk (Creel 2011, Spitz et al. 2014). Some limited examples exist from studies in African savannas in which foraging strategy and body size of prey have been shown to be related to trait responses (Creel et al. 2014).

In this study, we assess the ability of fixed traits related to predation risk to predict the variation in trait responses in cladocerans induced by fish predators. Specifically, we examine how the behavioral response to fish kairomones of compares to the fixed traits of body size and the taxonomic identity. Cladoceran body size is an important determinant of predation risk with larger taxa being more vulnerable (Brooks and Dodson 1965, Litchman et al. 2013, Sodré and Bozelli 2019), so it may be expected that larger taxa would demonstrate larger behavioral responses. We investigate the relationship between taxonomic identify (family) and behavioral responses because taxonomic groups of zooplankton such as cladocerans or Daphnids are often grouped together in applied food web models (NOAA Great Lakes) and assumed to be similarly affected by a fish predator.

Methods

Cladoceran behavioral trait responses were measured from a mesocosm experiment that was designed to simulate the non-consumptive effects and consumptive effects of a fish predator. The experimental design included four treatments including the presence/absence of fish kairomone (from fish in floating cages) crossed with presence/absence of removing zooplankton with a net (to simulate the density effects of fish predation). A difference in zooplankton position in kairomone relative to no kairomone treatments was used as measure of a behavioral response to predation risk. We examine the effect of kairomones across the presence and absence of removal treatments, and account for any effect of removal, because including both the no removal and removal treatments increased the number of replicates and there is no a priori reason to use one treatment over the other. To be complete, we describe the removal treatment methodology, but the effect of removal on trait responses is not a focus of this analysis.

The mesocosm experiments were conducted at the E.S. George Reserve (ESGR) of the University of Michigan near Pinkney, MI, USA. We reference experimental manipulations and measurements relative to day 0 when treatments were initiated on 8 July 2013. The experiment was terminated on 23 August 2013 (day 46). Experimental mesocosms were started well before treatment initiation in order to create established, homogenous zooplankton communities in the mesocosms. On days -36 and -35, black round plastic tanks with a diameter of 168 cm and height of 60 cm were filled with 1100 L of treated well water, to a depth of approximately 50 cm. 11 kg of washed play sand were added to each to cover the plastic bottom to produce a more natural substrate.

On day -33, mesocosms were inoculated with phytoplankton by adding 500 mL of 35 µm-filtered lake water from nearby Sayles Lake in Pinckney, Michigan to each mesocosm. Sayles Lake is a 10-hectare lake with a maximum depth of less than 3 m and an average depth of less than 2m with a diverse fish community including bluegill. On days -27, a diverse community of zooplankton was added to mesocosms. The zooplankton were collected from Sayles Lake using 150 µm zooplankton nets towed at night through areas of macrophytes and open water. Undesirable species such as amphipods, *Hydra*, and insects (e.g., *Chaoborus*) were removed. To increase zooplankton assemblage homogeneity among mesocosms, on days -8, a portion of zooplankton were collected from each mesocosm with a 64 µm zooplankton net, mixed, and subsamples of this mixture were redelivered to each mesocosm. Dominant crustacean zooplankton taxa that established in the mesocosms include cladocerans *Daphnia pulicaria*, *D. dentifera*, *Ceriodaphnia* sp., *Scapholeberis* sp., *Diaphanosoma* sp., *Simocephalus* sp., *Bosmina* sp., *Alona* sp., *Chydorus* sp., calanoid copepods, cyclopoid copepods, and ostracods.

Inorganic nutrients were added at a N:P ratio of 20:1 to the mesocosms to support phytoplankton growth as a resource for zooplankton. An initial spike of 4.35 g of NH_4NO_3 and 0.37 g of KH_2PO_4 was added to each mesocosm on day -33. From days -33 to -1, nutrients were added one day per week with 1.65 g NH_4NO_3 and 0.14 g of KH_2PO_4 added on these days.

Starting on day 0, nutrients were added five days per week with 0.29 g NH_4NO_3 and 0.024 g of KH_2PO_4 added on these days.

Treatment manipulations

Mesocosms were randomly assigned to the four treatments in eight experimental blocks. Treatments were initiated on day 0 by adding one bluegill sunfish (*Lepomis macrochirus*, mean length ± SD of 5.5 ± 0. 4 cm) to each of two cages in the kairomone treatment mesocosms. Cages were constructed from 41 X 27 X 26 cm plastic boxes with polystyrene foam glued to the sides, with mesh windows made from midge netting on all sides and the bottom. No kairomone treatments also received two (empty) cages. Cages in all treatments were clipped to opposite sides of the mesocosms. Once a week, caged fish were exchanged with fish from culture tanks. Culture tanks consisted of 1100 L plastic cattle tanks that housed approximately 25-50 fish of a similar size and fed a mixed assemblage of zooplankton three times per week. The cages' mesh panels were sprayed with a hose each week to reduce potential periphyton growth that would decrease diffusion of kairomones from the cages.

A 363 µm Nitex conical plankton net with a diameter of 50 cm was used for the physical removal of zooplankton in the removal treatments and was conducted two times per week. Removal consisted of three tows of the net around the perimeter of the mesocosms and was performed at night because zooplankton were more evenly distributed throughout the mesocosms. To control for potential nutrient loss that might result from removal, the zooplankton that were removed were killed by microwaving and added back to the mesocosm they were removed from over the next three days in equal daily amounts. To control for the towing disturbance, the same towing procedure was performed in the no removal treatments. As soon as the towing was complete, zooplankton were released by removing the net's cod end while it was in the mesocosm's water.

A natural concern in an experiment with a predator and no-predator treatment is that nutrients excreted from the fish could influence phytoplankton growth and hence zooplankton. Our calculations indicate that nutrient recycling from zooplankton in the mesocosms and nutrients added to the mesocosms far exceeded any nutrient excretion by fish (Peacor et al. 2012). We nevertheless were conservative by taking several measures to reduce any potential differences in nutrient addition between treatments. Fish were fed metabolically minimal diets while in the experiment cages or before being exchange into the cages. Specifically, each caged fish was fed 200 *D. pulicaria (*700 – 1200 µm length) twice a week, cages in all other treatments received 200 *D. pulicaria* that had first been killed by microwaving to provide equal nutrients. In addition, NH_4NO_3 and KH_2PO_4 were added at levels that were calculated to far exceed the amount of nutrients from excretion of fish, and were added near continuously (i.e., 5 days per week) to overwhelm any pulses in nutrients generated by fish or other treatment manipulations.

Qualitative assessments of mesocosm clarity and other characteristics were performed at least weekly as a matter of mesocosm experiment protocol. Such assessments served to keep us apprised of any developments in the tank and can serve as a check to add information to more quantitative measurements (e.g., a qualitative measurement of clarity can serve to augment chlorophyll measurements). These assessments led to the observation of corixids in several mesocosms on day 7. Corixids consume zooplankton, so their presence in mesocosms could make the zooplankton community too different among treatment replicates. Subsequently, we conducted qualitative assessments of the abundance of corixids in each mesocosm each week. We corroborated this assessment data with counts of corixids present in samples that were collected for zooplankton. From these counts, we determined that five control and two kairomone only mesocosms were invaded with corixids. We suspect that corixids invaded mesocosms of only the no removal treatments due to the timing of the treatment manipulations because we controlled for the disturbance in all the no removal treatment mesocosms at dusk, when corixids are most actively foraging, but performed the manipulation in all the removal treatment mesocosms after sunset.

Sampling of zooplankton position

To evaluate how kairomone affected zooplankton position, zooplankton were sampled in all mesocosms at six distinct positions at the end of the experiment (day 63). Sampling was done at three vertical strata: ‘high’ (just below the surface), ‘middle’ (at the midwater level) and ‘low’ (right above the bottom) crossed with two horizontal positions including ”side” (along the walls) and the “center” (within 25 cm of the mesocosm center). Four sub-samples were collected in each of four quadrants and combined for the “side” samples, and three sub-samples were collected in the center at each stratum and combined for the “center” samples. A 15 cm long 1.2 L cylindrical water sampler (Wildco) held parallel to the ground was used. The sampler was inserted into the water approximately 30 cm behind, and then moved forward to, the collection position. The order of the samples was chosen as to not disturb subsequent samples. Each of the six positional samples were collected on a 64 µm mesh sieve and preserved in 90% ethanol. Crustacean zooplankton were enumerated via compound microscope and identified to species or genus.

For each mesocosm, the proportion of each cladoceran at a given position was calculated as the abundance in that position divided by the sum of the abundance at all six positions. We also calculated averaged position for vertical and horizontal directions. For vertical, we calculated the proportions in the high, middle, and low strata; for horizontal, we calculated the proportions in the center and side strata. For example, the proportion in the high strata was the abundance in the high side and high center positions divided by the sum abundance in all six positions. A mesocosm was only included in the positional analysis for a given cladoceran if there were 5 or more individuals represented in the total number counted in the mesocosm. We used 5 individuals as the cut off based on a resampling exercise that showed that 5 is the threshold number of individuals counted in a mesocosm below which the estimates of spatial position in that mesocosm negatively affects the accuracy of the estimates of the average position across mesocosms. In addition, the five control and two kairomone only mesocosms that contained corixids were excluded from the position analysis because corixids could have effects on zooplankton position.

Calculation of behavioral response

To produce an approximation of the magnitude of each cladoceran's behavioral response, we calculated the sum of the absolute values of the differences in the treatment means of the proportions in all six positions. This sum difference was used in order to produce a single metric that would capture differences in both the vertical and horizontal directions. Standard error was propagated from the treatment standard errors.

Statistical Analyses

Regression plots were used to examine the relationship between the behavioral responses and size (length in µm) using the average data of each cladoceran taxon. Comparison of responses among and within cladoceran families was qualitative.

Both significant and not-significant behavioral responses were used in the analyses of the relationship between behavioral trait responses and size and taxonomy because any magnitude of behavioral response (including no response) could be expected. However, we tested for and examined any significant kairomone x removal interactions that may affect our detection or inference of kairomone effects (see below).

The proportion of individuals in different positional strata used in this study do not conform to parametric assumptions (Hijazi and Jernigan 2009). We utilized Dirichlet regression to analyze the treatment effects on positional response. The Dirichlet distribution is the multivariate generalization of the beta distribution and performs wells for composite measurements, such as our response variables, that also do not conform well to count-based or binomial-based analyses (reviewed in Douma and Weedon, 2019).

Separate Dirichlet regression analyses were run for the vertical position (as the proportion of individuals in high, middle, and low positions) and horizontal position (as the proportion of individuals in center and side positions). This allowed us to compare the direction of the behavioral responses among taxa on spatial axes along which shifts in zooplankton position are studied in natural systems (i.e., diel vertical and horizontal migration, e.g., Zaret and Suffern 1976, Hays 2003, Burks et al. 2012). To test for significant ($p \leq 0.05$) or marginally significant ($p \leq 0.10$) differences along each axis, we ran Dirichlet regression models with the proportions in the vertical or horizontal strata as the response with kairomone, removal, and the interaction of kairomone and removal as fixed effects. To identify if there were any significant fish x removal interactions, this full model was compared to a model without the interaction using a likelihood-ratio test approach. If no significant interaction was identified, the interaction term was dropped, and the effect of kairomone was examined across no removal and removal mesocosms to have sufficient power to examine the kairomone effect. Specifically, we ran Dirichlet regression models with the effects of kairomone and removal and comparing this model to models with only kairomone or removal using a likelihood-ratio approach to test if the positional response to test the significance of the kairomone or removal effects.

For cladocerans with marginally significant or significant fish x removal interactions, we considered that the interaction could mean two things. First, it could mean that the position differed with kairomone in either the no removal or removal treatments but there was no significant difference in the other treatment. Second, it could mean that that the kairomone had opposite effects on position in the treatments e.g., located higher in no removal and lower in removal. In both cases, if the kairomone had marginally significant or significant opposite effects on position in the no removal and removal treatments, there would be more than one position effect to compare to other taxa and we have no a priori reason to use one treatment over the other. Thus, we used boxplots of the proportion data in each treatment to examine if kairomone had opposite effects in no removal and removal treatments, and cladocerans were included in our examination of variation in trait responses so long as the position effects were not in opposite directions. Analyses were done in R version 3.5.2 (R Development Core Team, 2019) using the *DirichletReg* package (Maier, 2015).

Results

We first looked at the statistical results from the Dirichlet regression to determine which cladocerans not to continue in the analysis with size and taxonomy. This was based on whether taxa had marginally significant or significant fish x removal interactions. The vertical position and/horizontal positions of *Chydorus* sp., *Alona* sp., and *Scapholeberis* sp. had a marginally significant or significant kairomone x removal interaction (Table 5). Inspection of the proportion data for each mesocosm using boxplots (Appendix A), revealed that the trend of the kairomone effect was in the opposite direction in the no removal and removal treatments. Due to the opposite effects of kairomone in the no removal and removal treatments, the effects of kairomone on the position of *Chydorus* sp., *Alona* sp., and *Scapholeberis* sp. are not included in the analyses between trait responses and size or taxonomy because there would be more than one position effect to compare to other taxa and we have no a priori reason to use one treatment over the other. Inspection of the kairomone effect on *Simocephalus* sp. in no removal only mesocosms and removal only mesocosms revealed that the trend of the kairomone effect was in the same direction (more center with kairomone) in both the no removal and removal treatments but apparently to different enough degree to yield a significant interaction (Appendix B). *Simoceaphalus* sp. was included in our analysis.

Table 5. Statistical results of the Dirichlet regression of the effects of kairomone, removal, and their interaction on the vertical and horizontal position of each species or taxa, including total N for all treatments, X (Chi) statistic, and significance values. Significance of p-values are denoted for p ≤ 0.05 with * and p ≤ 0.1 with · . To indicate the direction of significant effects, grey-shaded cells in the vertical direction indicate taxa individuals were located lower with the treatment effect while dark-outlined values indicate individuals were located higher. For the horizontal position, grey-shaded values indicate more toward the center with the treatment effect.

			Vertical Position			Horizontal Position		
Taxon	**Effect**	**N**	**X**	**p-value**		**X**	**p-value**	
Alona sp.	Kairomone	24	3.49	0.320		0.768	0.681	
	Removal		4.14	0.247		0.130	0.937	
	Kairomone:Removal		8.12	0.044	*	5.47	0.065	·
Chydorus sp.	Kairomone	24	9.20	0.027	*	2.11	0.348	
	Removal		4.60	0.204		0.157	0.925	
	Kairomone:Removal		6.58	0.086	·	1.43	0.49	
Bosmina sp.	Kairomone	24	2.44	0.486		2.43	0.297	
	Removal		8.89	0.031	*	0.895	0.639	
	Kairomone:Removal		2.95	0.399		2.72	0.257	
Ceriodaphnia sp.	Kairomone	24	1.32	0.724		1.33	0.513	
	Removal		3.24	0.356		2.80	0.247	
	Kairomone:Removal		1.44	0.696		2.42	0.298	
Daphnia pulicaria	Kairomone	24	3.50	0.320		3.82	0.148	
	Removal		1.24	0.744		2.93	0.231	
	Kairomone:Removal		2.37	0.500		2.31	0.315	
Daphnia dentifera	Kairomone	24	19.0	0.0003	*	8.23	0.016	*
	Removal		4.76	0.190		9.97	0.009	*
	Kairomone:Removal		0.314	0.958		3.63	0.163	
Scapholeberis sp.	Kairomone	24	2.14	0.545		10.2	0.006	*
	Removal		2.52	0.471		6.13	0.047	*
	Kairomone:Removal		2.19	0.534		10.6	0.005	*
Simocephalus sp.	Kairomone	23	11.3	0.010	*	1.08	0.582	
	Removal		0.788	0.852		0.075	0.963	
	Kairomone:Removal		0.963	0.810		5.04	0.080	·
Diaphanosoma sp.	Kairomone	24	17.3	0.0006	*	0.102	0.950	
	Removal		8.10	0.044	*	1.290	0.526	
	Kairomone:Removal		6.05	0.109		0.660	0.719	

For the remaining taxa (*Bosmina* sp., *Ceriodaphnia* sp., *D. dentifera*, *D. pulicaria*, *Simocephalus* sp., and *Diaphanosoma* sp.), the average proportion of each cladoceran taxa at the six distinct sampling positions in the no kairomone and kairomone treatments is shown in Fig. 11 which provides information on the distribution and the direction of the kairomone effect on position. Note that Fig. 11 uses the mean of the proportion of individuals found in each position, but statistics (Table 5) were performed on a multivariate response that represented the vertical or horizontal direction (e.g., proportion high, middle, and low strata for the vertical position).

General location of cladocerans

Considering just the no kairomone treatments, the majority of individuals of *D. dentifera* and *Bosmina sp*. were located in the middle strata (Fig. 11a and 11c, average ± SE of 68% ± 5% and 75% ± 5%, respectively), whereas the majority of individuals of *Ceriodaphnia* sp., *D. pulex, and Simocephalus* sp. and *Diaphanosoma* sp. were located in the low strata (59% ± 3%, 66% ± 5% and 60% ± 3%, and 49% ± 3%, respectively). Relatively few individuals of any taxa were located in the high strata (the taxa with the highest amount was Simocephalus sp. with an average of 18% ± 2%). Moreso than other taxa, *Bosmina* sp. and *Ceriodaphnia* sp. tended to be located nearer to the side of the mesocosm relative to the center.

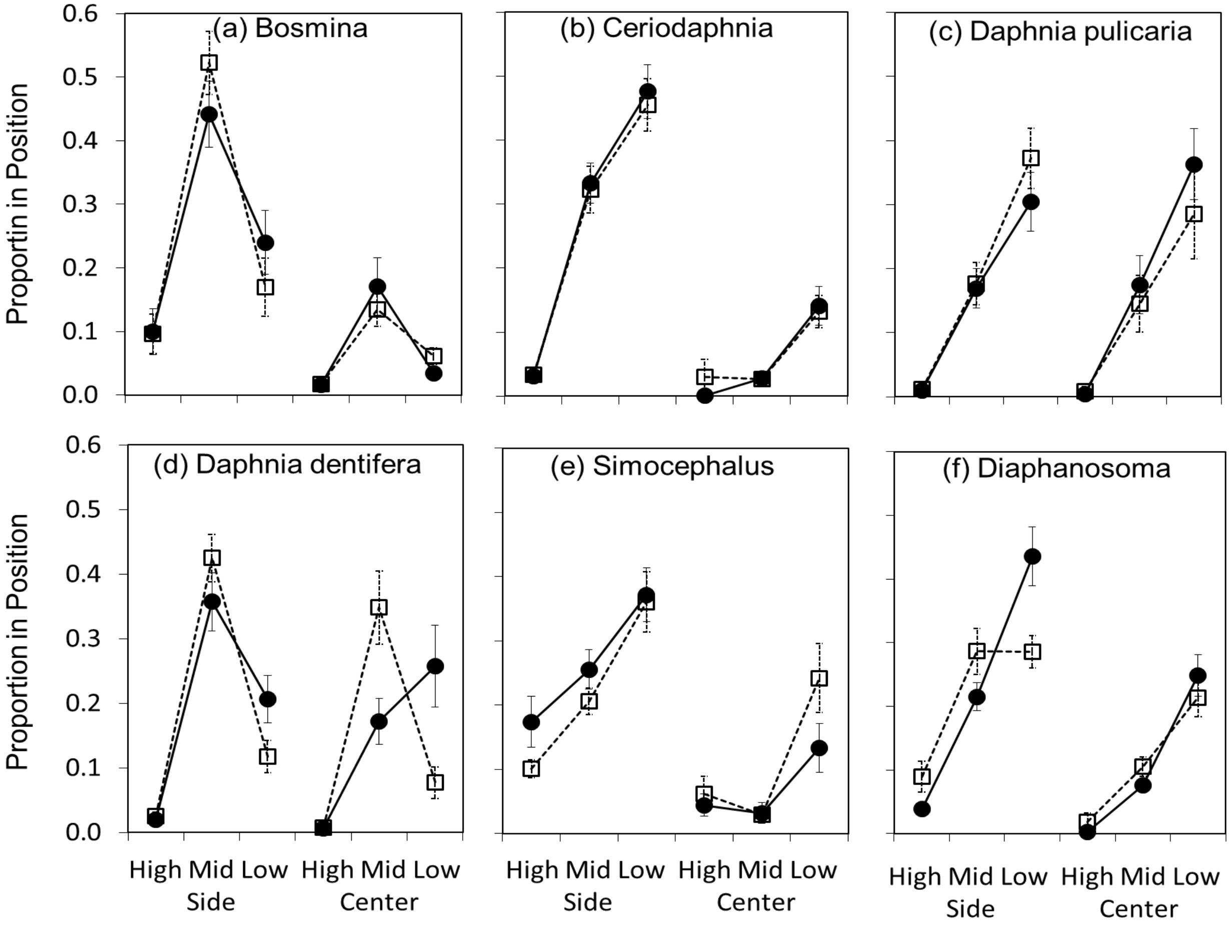

Figure 11. Average proportion (± 1 SE, averaged across removal treatments) of cladocerans found in the six different positions in the no kairomone (open squares) and kairomone (filled circles) mesocosms for (a) *Bosmina* sp., (b) *Ceriodaphnia* sp., (c) *Daphnia dentifera*, (d) *Daphnia pulicaria*, (e) *Simocephalus* sp., and (f) *Diaphansomsoma* sp. Positions: high side, middle side, low side, high center, middle center, and low center.

Effect of kairomone on position

The six cladoceran taxa demonstrated differential behavioral responses to fish kairomone. Kairomone had a statistically significant effect on the vertical position of *D. dentifera,* Diaphanosoma sp., and Simocephalus sp. (Table 5) and on the horizontal position of *D.*

dentifera. Both *D. dentifera* and *Diaphanosoma* were located lower with fish kairomone (Fig. 11 d and f), but *D. dentifera* shifted from the middle center to the low center and side positions (Fig. 11d), whereas *Diaphanosoma* shifted from the high and middle side to the low side position (Fig. 11f). In contrast to *D. dentifera* and *Diaphanosoma*, *Simocephalus* was located higher with fish kairomone, with shifts from the low center to the high and middle side positions (Fig 11e). Kairomone had no effect on the behavioral responses of *Bosmina* sp., *Ceriodaphnia* sp., and *D. pulicaria* (Table 5, Fig. 11a-c).

Relationship between behavioral response and size and taxonomy

No relationship was found between the behavioral response and the length of the cladocerans (Fig. 12). The largest cladoceran (*D. pulicaria*) did not respond to fish kairomone. Cladocerans of intermediate size had the largest behavioral responses. We also found no relationship between the behavioral responses and family (Fig. 12). The four Daphniidae taxa demonstrated a range of responses from no significant response (*Ceriodaphnia* sp.) to the largest responses (*Simocephalus* sp. and *D. dentifera*). Moreover, taxa from Daphniidae demonstrated opposite responses of moving higher and lower (Fig. 11, *Simocephalus* sp. and *D. dentifera*, respectively), while taxa from different families (Sididae and Daphniidae) demonstrated a response in the same direction (Fig. 11).

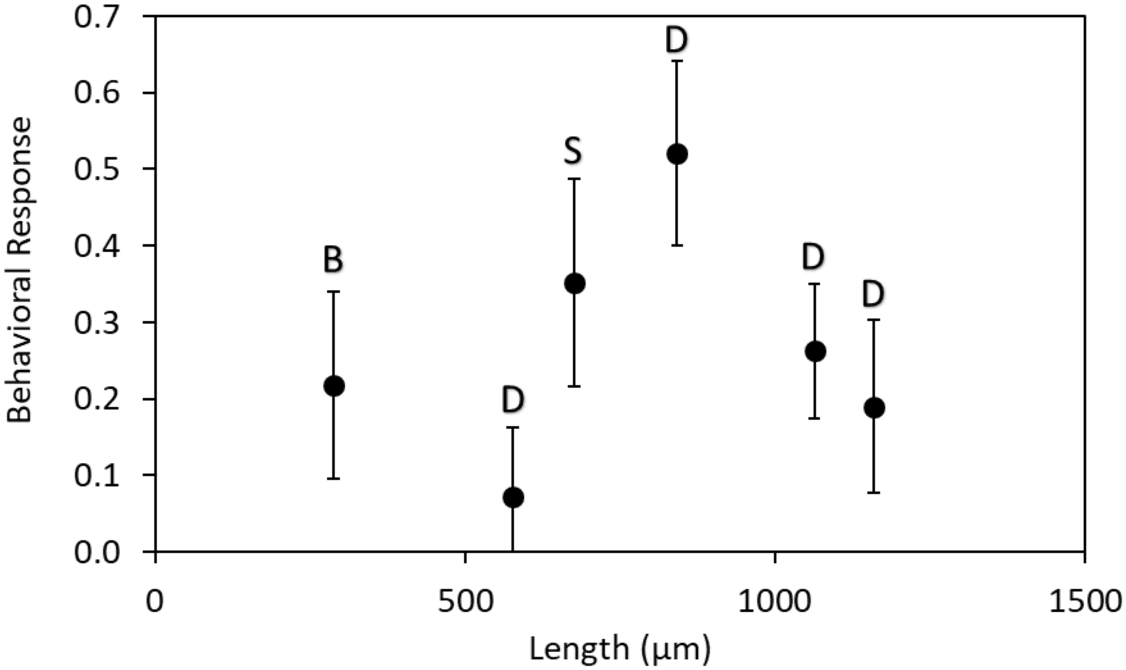

Figure 12. Behavioral response (± SE) versus the average length (μm) across all taxa. Taxa can be identified by their lengths (listed smallest to largest): *Bosmina* sp., *Ceriodaphnia* sp. *Diaphanosoma* sp., *D. dentifera*, *Simocephalus* sp., and *D. pulicaria*. The behavioral responses of *Diaphanosoma* sp., *D. dentifera*., and *Simocephalus* sp. were significant in the vertical and/or horizontal directions. Letters denote taxon families: B = Bosminidae, D = Daphniidae, S = Sididae.

Discussion

Freshwater cladocerans demonstrated substantial variation in a behavioral trait response to fish kairomone. We found no relationship between the behavioral response and body size or family, which are fixed traits associated with predation risk. Body size is often considered an important trait influencing predator-prey interactions because body size can affect the susceptibility of prey to attack and the costs of predator avoidance (Pekarsky 1996, Preisser and Orrock 2012). Our findings of a lack of relationship between trait responses and these traits related to predation risk suggest that consideration of multiple traits may complement the information obtained from body size and taxonomy when examining what prey traits may predict the expression or magnitude of trait responses.

While we did not find a relationship between body size and trait responses in this study, body size has been a good determinant of trait responses in other taxa including mayflies, snails, and sunfish (e.g., Peckarsky 1996, Werner and Hall 1988, Crowl and Covich 1990). However, in an examination of the relationship between body size and behavioral responses across a wide variety of taxa, Preisser and Orrock (2012) found the potential for substantial variation among similar species when compared with variation within broader taxonomic groups, which is consistent with our findings. This suggests that there may be considerable variation in the relationship between body size and trait responses among taxa and depending on taxonomic level. This highlights that consideration of additional traits and/or how traits interact with body size may enhance the understanding of the expression of trait responses.

We considered that taxonomic relatedness may explain variation in trait responses but found no clear pattern relating taxonomy to trait responses. A lack of pattern is consistent with findings from other studies that have also found substantial variation in functional traits in a variety of taxa (reviewed in Weiss and Ray 2019). Specifically, for zooplankton, Barnett et al. (2007) found that taxonomic differences between species did not fully capture differences in functional traits of body length, habitat, trophic group, and feeding type.

The variation found in trait responses among cladocerans may be based on variation in their other traits that affect their fitness tradeoffs. For example, when examined closely, cladocerans demonstrate substantial differences in traits such as clearance rate and selectivity, maximum growth rate, habitat, and stoichiometric requirements (Barnett et al. 2007, Litchman et al. 2013). These differences in how cladoceran interact with their environment can lead to different tradeoffs among fitness components such as survival, growth rate, and reproduction. The expression and magnitude of trait responses at any given time is dependent on the tradeoff between the consequent costs to fitness and the benefit from the trait response, of which predation risk is just one factor affecting the tradeoff (Abrams 1984, Werner and Anholt 1993, Lima and Dill 1990, Peacor et al. 2013). It is likely that consideration of multiple traits may enhance our ability to predict variation in trait responses.

Consideration of tradeoffs can be used to explore the differences in trait responses among cladocerans in our experiment. *D. pulicaria* and *D. dentifera* are the most closely related pair of taxa in our experiment and only *D. dentifera* responded to the predator. This difference is consistent with findings in some Michigan Lakes that *D. pulicaria* consistently occupy the hypolimnion whereas *D. dentifera* may exhibit diel vertical migration from the hypolimnion to the epilimnion at night (Duffy 2010). D. pulicaria may be located at deep depths to occupy a hypolimnetic refuge from fish predation to which it is highly vulnerable (Tessier and Welser 1991). While counterintuitive that the species that is more vulnerable to fish predation did not respond to fish kairomones, trait responses cannot be predicted in isolate of other factors that influence fitness. For example, the presence of a refuge influences the expression of trait responses (Dewitt et al. 1999); however, consideration of species interactions in a refuge is also important. Between these two Daphnia species, *D. pulicaria* is the superior competitor over *D. dentifera* (VIijverbery and Vos 2006), so migration out of refuge by *D. dentifera* may result from the tradeoff between foraging and predation risk (Tessier and Welser, 1991, Duffy 2010).

We found that simulated predation (removal) and the interaction of kairomone and removal significantly affected the positions of some taxa. However, this does not affect our findings related to variation in traits and trait responses. We did not include *Alona* sp., *Chydorus* sp., and *Scapholeberis* sp. in the analysis because based on inspection of boxplots of the data, there was a trend for fish kairomone to affect their position in opposite directions in the no removal and removal treatments. For this reason, there was not a single behavioral response to compare directly to the responses of the other cladocerans. However, these taxa did demonstrate behavioral responses and would be among the smallest taxa in our study and belong to an additional family (Chydoridae). Thus, if these taxa had been included, we would have found that some but not all small taxa exhibited behavioral responses, and that small taxa exhibit similar responses as intermediate-sized taxa. Such variation is consistent with our results from the limited group that we did include in our analysis.

The effects of kairomone on cladoceran position could be due directly to predator avoidance behavior or due to indirect effects caused by the kairomone effects on other habitat parameters. There is increased understanding that non-consumptive effects can influence species' population growth and in turn affect the abundances and dynamics of other species (Werner and Peacor 2003, Peckarsky et al. 2008). We cannot rule out that non-consumptive effects may have altered habitat characteristics in such a way to influence habitat preference. Thus, the kairomones could be causing changes in cladoceran position indirectly, directly due to predator avoidance, or a combination of both. However, at least some taxa would need to exhibit trait responses for non-consumptive effects to occur, and this is consistent with the variation we found and would expect among prey.

Whereas most studies of trait responses are performed in highly controlled systems, the experimental venue in this study captures much of the ecological complexity that is present in natural systems. This design may produce variation in trait responses that may be robust to natural settings. The mesocosms had multi-species assemblages of species that are interaction rich. Interactions among prey is expected to influence the fitness tradeoffs that affect the expression and magnitude of trait responses (Teplitsky and Laurila 2007). As such, the trait responses we found may be less artificial than highly controlled in laboratory studies (as e.g., Tollrian, 1994, Pangle and Peacor 2006).

The trait responses we found demonstrate that the study of predation risk effects needs to be scaled up beyond investigating predation risk effects in species pairs to understand their effects on populations and communities. Even just considering the cladocerans in our experiment, the different behavioral responses due to fish kairomone may not only affect the interactions between fish and cladocerans but also within and among the cladocerans who may compete for resources. For example, *Diaphanosoma* sp. was found to shift to the low side position with fish kairomone, which is where *Ceriodaphnia* sp. were abundant. The habitat overlap could influence growth rates of *Diaphanosoma* sp. and *Ceriodpahnia* sp. based on competition for resources (Mateev 1987, Mateev and Gabriel 1994). On the other hand, *D. dentifera* and *Diaphanosoma* sp. moved in opposite directions with kairomone so competition may be relaxed due to spatial separation. This suggests that consideration of the multitude of potential species interactions and trait responses could enhance the understanding of the importance of how predation risk effects operate in communities.

Our results and others' show that a complex relationship may exist between traits that influence predation risk and the expression and magnitude of trait responses. However, the variation we found in trait responses among relatively similar prey taxa warrants further investigation into factors that influence trait responses. Exploring contingency of trait responses among prey based on underlying tradeoffs should enhance the understanding of how trait responses influence population and community-level dynamics. While some studies have revealed characteristics of predator and prey that explain the variation in trait responses in particular systems, few generalities exist.

Acknowledgements

We thank Keenan Scriber, Adam Haynes, Rachael Donabedian, Hope Healey, Katie Heffernan, Amanda Rice, and Julie Markiewicz for field assistance and/or zooplankton enumeration. We thank the Ecology and Evolutionary Biology department of the University of Michigan for use of the E. S. George Reserve. Funding for this study was provided by the National Science Foundation DEB-0089809 and DEB-1145808.

APPENDICES

APPENDIX A. Kairomone x removal interactions

The vertical position of *Chydorus* sp. and *Alona* sp. had a marginally significant or significant kairomone x removal interaction, respectively, while the horizontal position of *Alona* sp. and *Scapholeberis* sp. had a marginally significant or significant kairomone x removal interaction, respectively (Table 5). Inspection of the proportion data for each mesocosm using boxplots (Fig. 13-15), revealed that the trend of the kairomone effect was in the opposite direction in the no removal and removal treatments. In the vertical position, there was a trend for *Alona* sp. and *Chydorus* sp. to be located higher with kairomone in the no removal and lower with kairomone in the removal treatment; trends were weak but apparently to a different enough degree to yield marginally or significant interactions (Fig. 13-14). In the horizontal position, there was a trend for *Alona* sp. to be located more toward the center with kairomone in the no removal and a weak trend to be more toward the side in the removal treatment; the trends were weak but apparently to a different enough degree to yield marginally significant interactions. There were significant opposite effects for *Scapholeberis* sp. to be located more toward the center with kairomone in no removal and more toward the side in removal (Fig. 15).

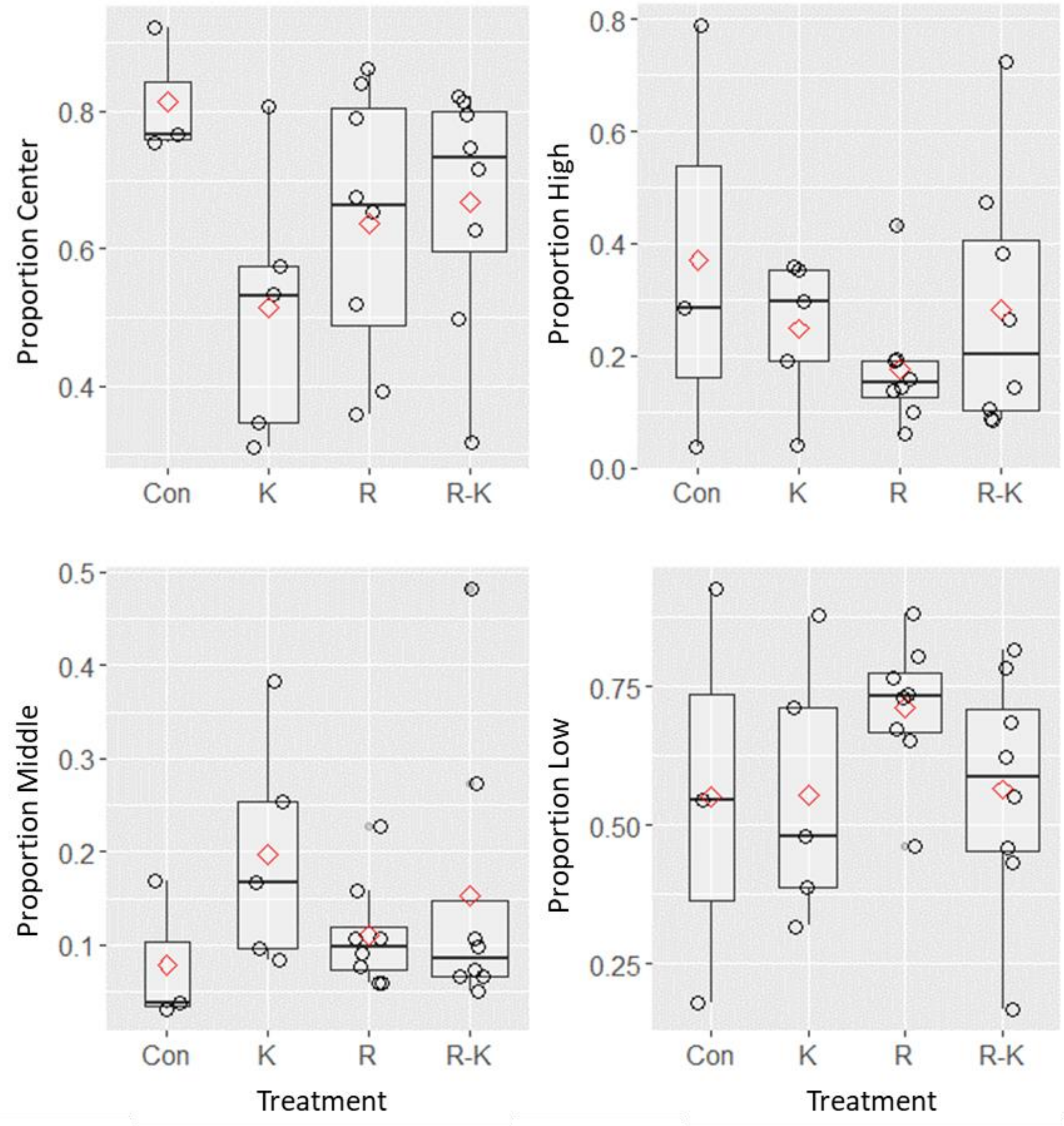

Figure 13. Proportion of *Alona* sp. individuals in center, high, middle, and low positions in each mesocosm in Control, Kairomone (K), Removal (R), and Removal-Kairomone (R-K) treatments. The red diamond is the treatment average. The box shows the 25th percentile, median, and 75th percentile. Whiskers extend to the non-outlier minimum and maximum, outliers are plotted as points.

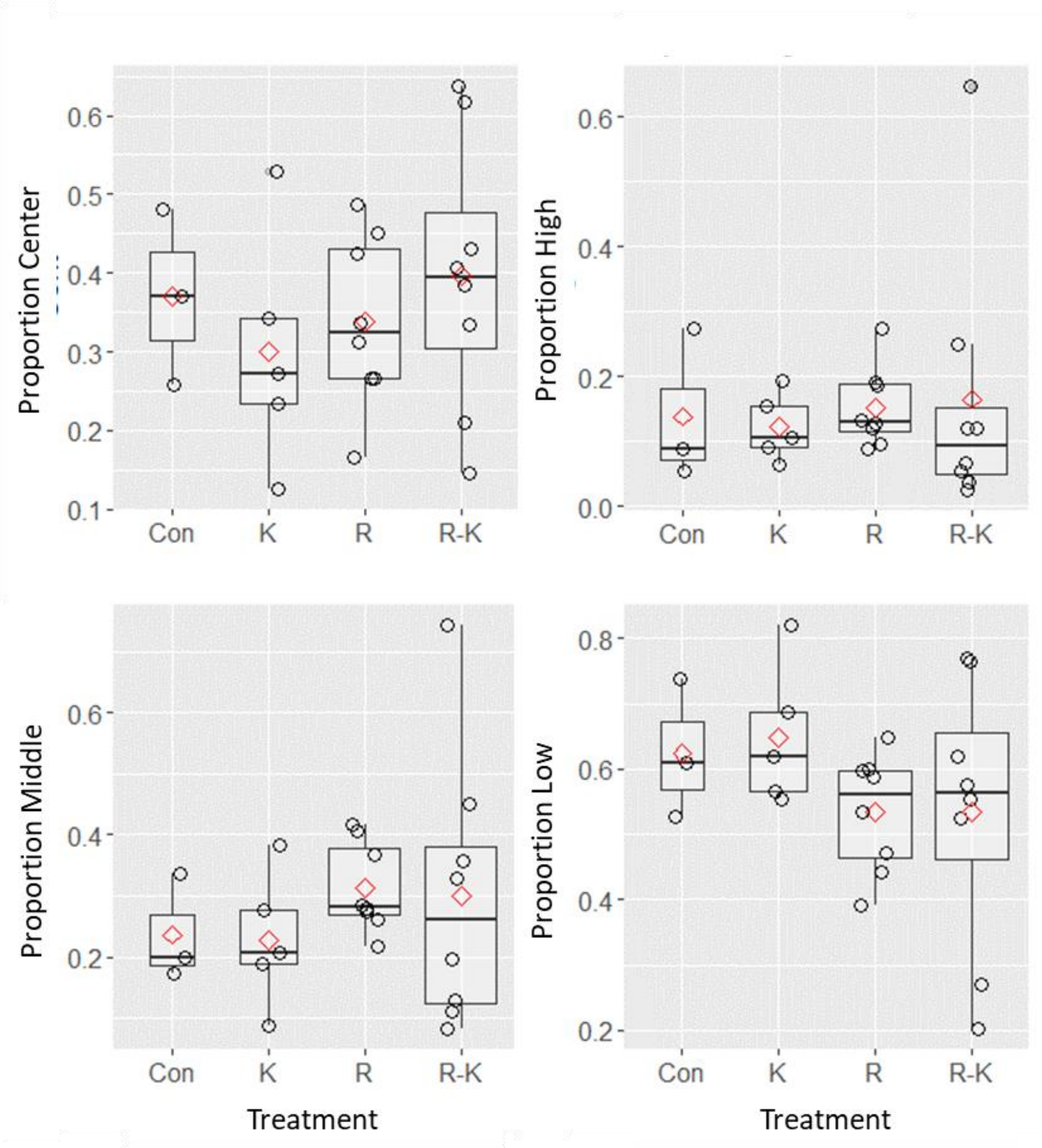

Figure 14. Proportion of *Chydorus* sp. individuals in center, high, middle, and low positions in each mesocosm in Control, Kairomone (K), Removal (R), and Removal-Kairomone (R-K) treatments. The red diamond is the treatment average. The box shows the 25th percentile, median, and 75th percentile. Whiskers extend to the non-outlier minimum and maximum, outliers are plotted as points.

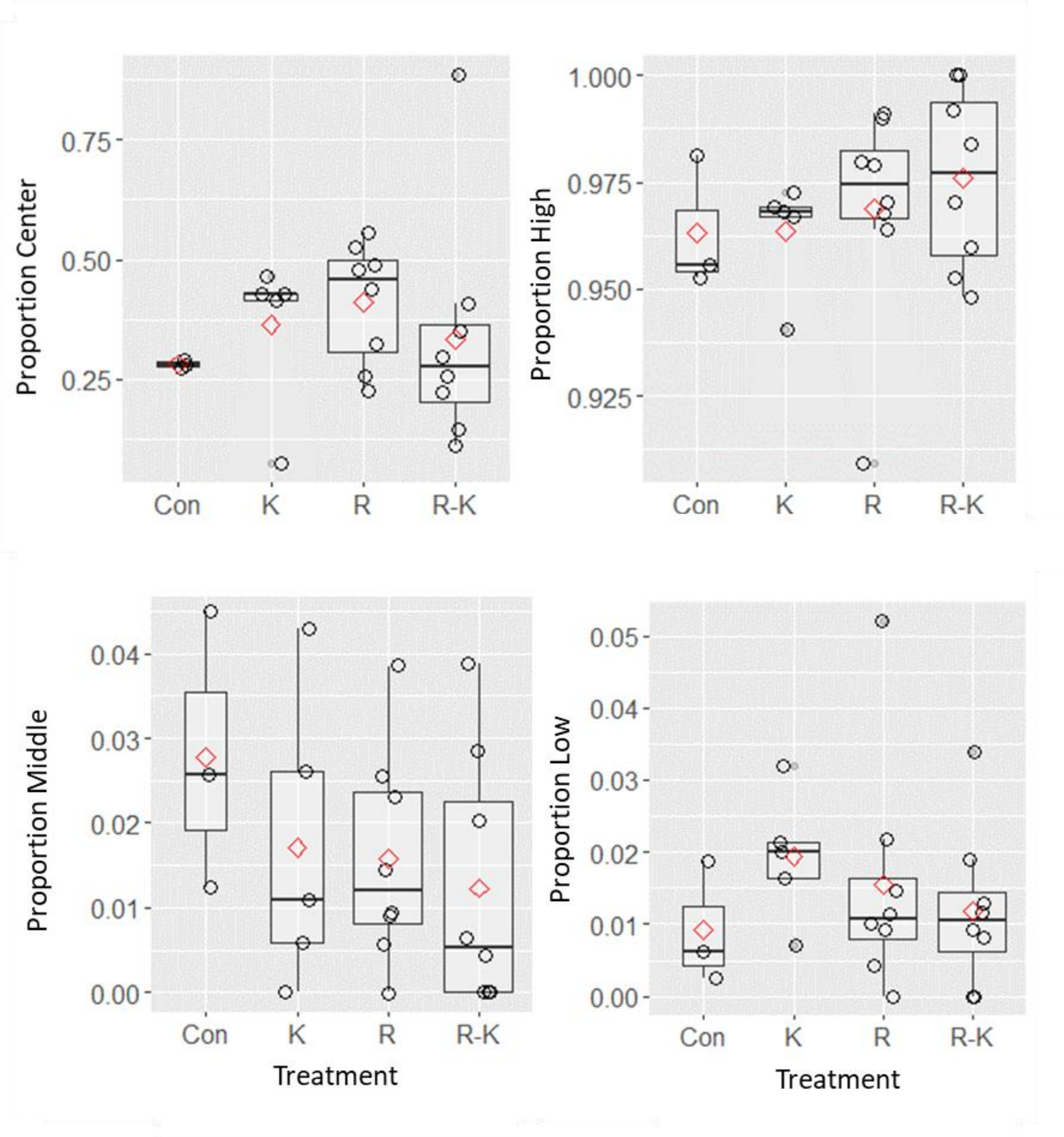

Figure 15. Proportion of *Scapholeberis* sp. individuals in center, high, middle, and low positions in each mesocosm in Control, Kairomone (K), Removal (R), and Removal-Kairomone (R-K) treatments. The red diamond is the treatment average. The box shows the 25th percentile, median, and 75th percentile. Whiskers extend to the non-outlier minimum and maximum, outliers are plotted as points.

APPENDIX B. *Simocephalus* sp. position

Inspection of the kairomone effect on *Simocephalus* sp. in no removal only mesocosms and removal only mesocosms revealed that the trend of the kairomone effect was in the same direction (more center with kairomone) in both the no removal and removal treatments but apparently to different enough degree to yield a significant interaction (Fig. 16). Boxplots for proportion in vertical position strata are included for completeness, there was a significant effect of kairomone on *Simocephalus* sp. position (Table 5, $X(3, 24) = 11.3$, $P = 0.010$).

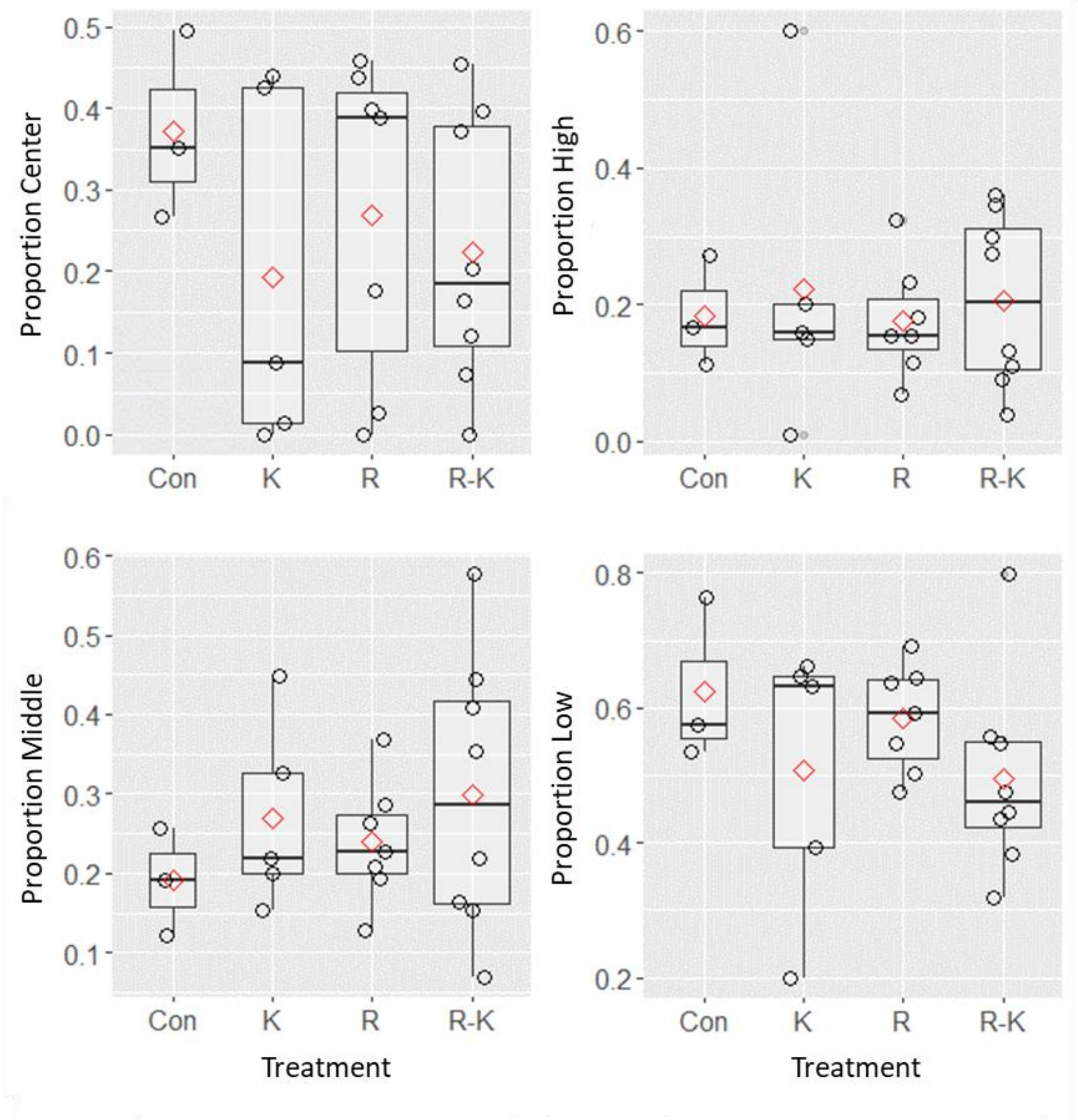

Figure 16. Proportion of *Simocephalus* sp. individuals in center, high, middle, and low positions in each mesocosm in Control, Kairomone (K), Removal (R), and Removal-Kairomone (R-K) treatments. The red diamond is the treatment average. The box shows the 25th percentile, median, and 75th percentile. Whiskers extend to the non-outlier minimum and maximum, outliers are plotted as points.

REFERENCES

REFERENCES

Abrams, P.A. 1984. Foraging time optimization and interactions in food webs. American Naturalist 124: 80–96.

Agrawal, A.A. 2001. Phenotypic plasticity in the interactions and evolution of species. Science 294: 321–326.

Andriskiw, M., J.M. Fryxell, I.D. Thompson, and J.A. Baker. 2008. Habitat-mediated variation in predation risk by the American marten. Ecology 89: 2273–2280.

Barnett, A,J., K. Finlay, B.B. Beisner. 2007. Functional diversity of crustacean zooplankton communities: Towards a trait-based classification. Freshwater Biology 52: 796–813.

Beukers, J.S., and G.P. Jones. 1997. Habitat complexity modifies the impact of piscivores on a coral reef population. Oecologia 114: 50–59.

Burks, R.L., E. Jeppesen, and D.M. Lodge. 2001. Littoral zone structures as *Daphnia* refugia against fish predators. Limnology and Oceanography 46: 230–237.

Chesson, P. 2000. Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics 31: 343–366.

Clements, H.S., C.J. Tambling, and G.I.H. Kerley. 2016. Prey morphology and predator sociality drive predator prey preferences. Journal of Mammalogy 97: 919–927.

Creel, S. 2011. Toward a predictive theory of risk effects: hypotheses for prey attributes and compensatory mortality. Ecology 92: 2190–2195.

Creel, S., P. Schuette, and D. Christianson. 2014. Effects of predation risk on group size, vigilance, and foraging behavior in an African ungulate community. Behavioral Ecology 25: 773–784.

Creel, S, M. Becker, E. Dröge, J. M'soka, W. Matandiko, E. Rosenblatt, T. Mweetwa, H. Mwap, M. Vinks, B. Goodheart, J. Merkle, T. Mukula, D. Smitb, C. Sanguinetti, C. Dart, D. Christianson, and P. Schuette. 2019. What explains variation in the strength of behavioral responses to predation risk? A standardized test with large carnivore and ungulate guilds in three Ecosystems. Biological Conservation 232: 164–172.

Crowl, T. and A. Covich. 1990. Predator-induced life history shifts in a freshwater snail. Science 247: 949–951.

Dewitt, T.J., A. Sih, and J.A. Hucko. 1999. Trait compensation and cospecialization in a freshwater snail: size, shape and antipredator behavior. Animal Behaviour 58: 397–407.

Díaz, S. and M. Cabido. 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16: 646–655.

Douma, J.C. and J.T. Weedon. 2019. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. Methods in Ecology and Evolution 10: 1412–1430.

Duffy, M.A. 2010. Ecological consequences of intraspecific variation in lake *Daphnia*. Freshwater Biology 55: 995–1004.

Gorini, L., J.D.C. Linnell, R. May, M. Panzancchi, L. Boitani, M. Odden, and E.B. Nilsen. 2012. Habitat heterogeneity and mammalian predator-prey interactions. Mammal Review 42: 55–77.

Green, S.J., and I.M. Côté. 2014. Trait-based diet selection: Prey behavior and morphology predict vulnerability to predation in reef fish communities. Journal of Animal Ecology 83: 1451–1460.

Grime, J.P. 1977. Evidence for the existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. American Naturalist 111: 1169–1194.

Hays, G.C. 2003. A review of the adaptive significance and ecosystem consequences of zooplankton diel vertical migrations. Hydrobiologia 503: 163–170.

Hijazi, R. and R. Jernigan. 2009. Modeling compositional data using dirichlet regression models. Journal of Applied Probability and Statistics 4: 77–91.

Hooper, D.U. and P.M Vitousek. 1997. Effects of plant composition and diversity on ecosystem processes. Science 277: 1302–1305.

Juanes, F. 2016. A length-based approach to predator-prey relationships in marine predators. Canadian Journal of Fisheries and Aquatic Sciences 73: 677– 684.

Lima, S.L. and L. Dill. 1990. Behavioural decisions made under the risk of predation: a review and prospectus. Canadian Journal of Zoology 68: 619–640.

Litchman, E. and C.A. Klausmeier. 2008. Trait-based community ecology of phytoplankton. Annual Reviews of Ecology and Systematics 39: 615–639.

Litchman, E., M.D. Ohman, and T. Kiørboe. 2013. Trait-based approaches to zooplankton communities. Journal of Plankton Research 35: 473–484.

Maier, M. J. 2015. DirichletReg: Dirichlet regression in R. R package version 0.6-3.1. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://cran.r project.org/web/packages/DirichletR eg/vignettes/DirichletReg-vig.pdf.

Mason, N.W.H., D. Mouillot, W.G. Lee, and J.B. Wilson. 2005. Functional richness, functional evenness and functional divergence: the primary components of functional diversity. Oikos 111: 112–118.

Mateev, V.F. 1987. Self-maintaining plankton: pelagic Cladocera in small microcosms with lake water. Hydrobiologia 225: 301–307.

Mateev, V.F. and W. Gabriel. 1994. Competitive exclusion in Cladocera through elevated mortality of adults. Journal of Plankton Research 16: 1083–1094.

Norberg, J. 2004. Biodiversity and ecosystem functioning: a complex adaptive systems approach. Limnology and Oceanography 49: 1269–1277.

Ohman, M.D. 1988. Behavioral responses of zooplankton to predation. Bulletin of Marine Sciences 43: 530 –550.

Pangle, K.L. and S.D. Peacor. 2006. Non-lethal effect of the invasive predator *Bythotrephes longimanus* on*Daphnia mendotae*. Freshwater Biology 51: 1070–1078.

Peacor, S.D., B. L. Peckarsky, G.C. Trussell, and J.R. Vonesh. 2013. Costs of predator-induced phenotypic plasticity: A graphical model for predicting the contribution of nonconsumptive and consumptive effects of predators on prey. Oecologia 171: 1–10.

Peckarsky, B. 1996. Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. Ecology 77: 1888–1905.

Peckarsky, B.L, B.L. Kerans, A.R. Mcintosh, and B.W. Taylor. 2008. Predator effects on prey population dynamics in open systems. Oecologia 156: 431–440.

Petchey, O.L. and K.J. Gaston. 2002. Functional diversity (FD), species richness and community composition. Ecology Letters 5: 402–411.

Preisser, E.L., J.L. Orrock, and O.J. Schmitz. 2007. Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. Ecology 88: 2744–2751.

Preisser, E.L. and J.L. Orrock. 2012. The allometry of fear: interspecific relationships between body size and response to predation risk. Ecosphere 3: 77.

R Core Team. 2019. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing Computing, Vienna, Austria. http://www.r-project.org.

Reynolds C.S., V. Huszar, C. Kruk, L. Naselli-Flores, and S. Melo. 2002. Towards a functional classification of the freshwater phytoplankton. Journal of Plankton Research 24: 417–428.

Schmitz, O.J. 2008. Effects of predator hunting mode on grassland ecosystem function. Science 319: 952–954.

Spitz, J.,V. Ridoux, and A. Brind'Amour. 2014. Let's go beyond taxonomy in diet description: testing a trait-based approach to prey–predator relationships. Journal of Animal Ecology 83: 1137–1148.

Sodré, E.O. and R.L. Bozelli. 2019. How planktonic microcrustaceans respond to environment and affect ecosystem: a functional trait perspective. International Aquatic Research 11: 207–223.

Teplitsky, C. and A. Laurila. Flexible defense strategies: Competition modifies investment in behavioral vs. morphological defenses. Ecology 88: 1641–1646.

Tessier A.J. and J. Welser.1991. Cladoceran assemblages, seasonal succession and the importance of a hypolimnetic refuge. Freshwater Biology 25: 85– 93.

Thaker, M., A.T. Vanak, C.R. Owen, M.B. Ogden, S.M. Niemann, and R. Slotow. 2011. Minimizing predation risk in a landscape of multiple predators: effects on the spatial distribution of African ungulates. Ecology. 92: 398–407.

Tilman. 1997. Biotic control over the functioning of ecosystems. Science 277: 500–504.

Tilman, D., J. Knops, D. Wedin, P. Reich, P., Ritchie, and E. Siemann. 1997. The influence of functional diversity and composition on ecosystem processes. Science 277: 1300–1302.

Tollrian, R. 1994. Fish-kairomone induced morphological changes in *Daphnia lumholtzi* (SARS). Archiv für Hydrobiologie 130: 69–75.

Tollrian, R. and C.D. Harvell. 1999. The Ecology and Evolution of Inducible Defenses.Princeton University Press, Princeton, New Jersey.

Vijverberg, J. and M. Vos. 2006. Predator-released compounds, ambient temperature and competitive exclusion among differently sized *Daphnia* species. Freshwater Biology 51: 756–767.

Walker B., A. Kinzig, and J. Langridge. 1999. Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. Ecosystems 2: 95–113.

Walker, B.H. and J.L. Langridge. 2002. Measuring functional diversity in plant communities with mixed life forms: a problem of hard and soft attributes. Ecosystems 5: 529–538.

Weiss, K.C.B. and C.A. Ray. 2019. Unifying functional trait approaches to understand the assemblage of ecological communities: synthesizing taxonomic divides. Ecography 42: 2012–2020.

Werner, E.E. and D.J. Hall. 1988. Ontogenetic habitat shifts in bluegill: the foraging rate predation risk trade-off. Ecology 69: 1352–1366.

Werner, E.E. and B.R. Anholt. 1993. Ecological consequences of the tradeoff between growth and mortality rates mediated by foraging activity. American Naturalist 142: 24–32.

Werner, E.E. and S.D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology 84: 1083–1100.

Zakharova, L., K.M. Meyer, and M. Seifan. 2019. Trait-based modelling in ecology: A review of two decades of research. Ecological Modelling 407: 108703.

Zaret, T.M and J.S. Suffern. 1976. Vertical migration in zooplankton as a predator avoidance mechanism. Limnology and Oceanography 21: 804–813.