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Seasonal succession and variable Daphnia dominance in fishless ponds: ecological determinants and ecosystem consequences.

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## SEASONAL SUCCESSION AND VARIABLE DAPHNIA DOMINANCE IN FISHLESS PONDS: ECOLOGICAL DETERMINANTS AND ECOSYSTEM CONSEQUENCES

By

Christopher F. Steiner

## AN ABSTRACT OF A DISSERTATION

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

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Professor Gary G. Mittelbach

## ABSTRACT

## SEASONAL SUCCESSION AND VARIABLE DAPHNIA DOMINANCE IN FISHLESS PONDS: ECOLOGICAL DETERMINANTS AND ECOSYSTEM CONSEQUENCES

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Historically, variation in zooplankton size structure and dominance by large Daphnia species is thought to be driven by predatory fish which feed selectively on largebodied taxa permitting dominance by smaller forms. Yet, in small ponds that naturally lack fish, zooplankton communities exhibit a great deal of variation in dominance by large Daphnia pulex both spatially (among ponds) and temporally (seasonally within systems). This variation calls into question the reigning paradigm. In this study I explored two aspects of this ecological phenomenon: the ecological determinants of temporal and spatial variation in Daphnia dominance and the ecosystem consequences of this variation.

The interactive effects of consumer identity (*Daphnia pulex* or a small zooplankter, *Ceriodaphnia quadrangula*), algal heterogeneity and productivity on algal standing crop were explored experimentally using lab-based microcosms. Results showed that *Daphnia* exerted stronger top-down control on algal biomass but the effect was only manifest in high productivity treatments.

To determine the generality of this result, I repeated this experiment in a more natural setting using in situ mesocosms. *Daphnia* were compared with a diverse assemblage of small zooplankton in two ponds that differed naturally in productivity. I also manipulated productivity via nutrient additions to some enclosures. This experiment largely supported lab results; compared to small zooplankton, *Daphnia* more strongly controlled algae only in the high productivity pond and in nutrient enriched enclosures. These results were further confirmed by field surveys of natural ponds. I also utilized this mesocosm experiment to compare effects of zooplankton composition on nutrient limitation of algae. Though *Daphnia* appeared to enhance phosphorus limitation by the end of the experiment, on the whole, effects of zooplankton composition were weak.

I utilized in situ mesocosms to examine the effects of invertebrate predators (Notonectids) and nutrient enrichment on patterns of zooplankton dominance. Predator manipulations revealed strong effects of Notonectids on *Daphnia* populations. Thus, this predator can play the same role that fish do in lakes. However, *Daphnia* performed poorly even in the absence of predators, suggesting that resource competition or abiotic factors play a more important role in determining *Daphnia* dominance in these systems.

The final experiment assessed spatial and temporal variation in competitor and invertebrate predator (*Chaoborus*) effects on *Daphnia* population performance. Experimental manipulations of competitors and predators were deployed in five ponds that varied naturally in *Daphnia* relative abundance and twice in the growing season. In general, competition effects outweighed predator effects. Furthermore, competition intensity varied spatially and temporally. Effects were stronger in those ponds and times in which *Daphnia* relative abundance was low or non-existent. Effects also tended to intensify later in the growing season with the magnitude of intensification being the weakest in the single pond dominated by *Daphnia*. I dedicate this dissertation to the memory of my mom, Eiko Steiner.

#### Acknowledgements

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## Chapter 1

# INTRODUCTION: KING DAPHNIA? FURTHER EXPLORATIONS OF THE ZOOPLANKTONIC ARENA OF LIFE

The interplay of biotic interactions and abiotic factors is considered to be the primary determinant, on the proximal scale, of variation in community organization and dynamics. Much research has focused on biotic interactions set within a mosaic of spatial variation in abiotic conditions, often casting the abiotic as a species filter or environmental template upon which local communities assemble from larger species source pools (e.g. the resource-ratio competition models of Tilman 1982, in which spatial variation in nutrient supply determines, in part, differential community membership). In the upper latitudes of temperate regions environmental variation can take on an unquestionable temporal flavor as well; natural systems in these areas experience large and regular changes in climatic/abiotic conditions associated with seasonality. These fluctuations have the potential to affect community structure through direct effects on species membership (e.g. selecting species with differential, physiologically-based temperature tolerances) or through a more complex, indirect route. For example, seasonal variation in temperature or other abiotic factors may affect the strength of species interactions (predation and competition) and thus temporally alter the relative advantages and disadvantages of species within communities. Hence, variation in relative abundance of many species within a growing season may largely be a product of such seasonally associated modifications.

Effects of seasonality on the relative abundance of species within a growing season are most likely observed in organisms whose generation times are short and whose population dynamics play out entirely within the span of several months. Longer-

lived species, such as trees within a forest or large fish within lakes, may have total abundances set by dynamics integrated over several years of reproduction and recruitment. It is not surprising then that some of the most well studied examples of seasonal dynamics and succession come from planktonic communities of freshwater ecosystems whose primary and secondary producers are primarily algae and zooplankton (species that generally have very short generation times). Over several decades of research a healthy body of descriptive and experimental research centered on this phenomenon has accrued (contained in large part in the syntheses of Sommer et al. 1986 and Sommer 1989).

#### Background

The form that seasonal transitions in planktonic community structure can take is highly varied. Yet, a pattern of long-standing interest is variation in dominance by largebodied *Daphnia* species and the size-structure of zooplankton assemblages. Numerous investigations have shown that large-bodied *Daphnia* species tend to occur and sometimes dominate early in the growing season following ice-out and spring mixing. This early season peak is generally followed by dominance by small-bodied cladocera (e.g. *Ceriodaphnia* and *Diaphanosoma*) or small copepods (Sommer et al.1986, Gliwicz and Pijanowska 1989). The focus on variation in *Daphnia* goes beyond the purely academic, having a practical dimension as well. When compared to small-bodied taxa, these species are commonly thought to exert strong effects on aquatic ecosystem function, more strongly limiting algal standing crop (e.g. Leibold 1989) and driving systems towards greater phosphorus limitation via differential nutrient sequestration and excretion (Elser and Urabe 1999).

Historically, size selective predation by fish has been regarded as the primary determinant of variation in *Daphnia* dominance and the size structure of zooplankton communities -a notion dating back to the pioneering studies of Hrbacek et al. (1961) and Brooks and Dodson (1965). Both studies provided evidence that variation in the abundance of planktivorous fish can drive variation in dominance by large-bodied zooplankton; large zooplankton are selectively removed by predators permitting dominance by smaller taxa. Brooks and Dodson (1965) further hypothesized that large zooplankton are able to dominate in the absence of planktivory because they are superior resource competitors; predictions integrated into their "size-efficiency hypothesis" (hereafter referred to as SEH). While, Brooks and Dodson (1965) explored spatial (among lake) variation in size structure, their general concept has also been incorporated into successional models. Daphnia populations are believed to dominate early in the season through their quick use of abundant resources during spring turnover and tolerance for low temperatures. As the season progresses, rising temperatures are associated with an increase in top-down impacts on large taxa due to increasing fish predation, a result of hatching events, increases in activity levels and shifts in habitat usage to the open water. This in turn favors increases in the abundance of small-bodied taxa (Gliwicz and Pijanowska 1989).

An ample amount of evidence supports the contention that fish predation dramatically alters the size structure of zooplankton communities and patterns of *Daphnia* dominance both among water bodies and seasonally within systems (e.g. Hrbacek et al. 1961, Brooks and Dodson 1965, Hall et al. 1976, Carpenter and Kitchell 1993, Mittelbach et al. 1995, reviewed in Gliwicz and Pijanowska 1989). Yet, an

alternate body of research has shown that variable *Daphnia* dominance need not be invariably linked to fish predation. These studies question the second tenet of SEHcompetitive dominance by large-bodied taxa- and have instead purported that variation in size structure can be generated purely by exploitative resource competition, resource quality, abiotic factors (most notably temperature), or combinations thereof.

The idea that variable *Daphnia* dominance need not be driven by predation is further supported by data from shallow ponds in which fish are naturally excluded due to anoxia (i.e., winter fish kill). Figure 1 depicts survey data from 18 fishless ponds in southern Michigan (see figure legend and Chapter 3 for methodological details). As is readily apparent, these water bodies exhibit a tremendous amount of variation in zooplankton composition and dominance by Daphnia pulex (the primary large-bodied Daphnia inhabiting these systems). In addition to a clear temporal component (seasonal variation within ponds), these patterns vary greatly among ponds, with several showing late season declines in Daphnia abundance, some being dominated by Daphnia for much of the sample period, and some having little or no Daphnia. Though there is no shortage of studies in lakes, studies of seasonal succession in ponds have received limited attention -venturing little beyond studies of single systems (e.g. Lynch 1978, Smith and Cooper 1982). These data are unique in this respect and suggest that the factors determining Daphnia relative abundance in these systems may be complex, reaching beyond the simplistic SEH model framework.

In the following chapters I explore two very basic aspects of this ecological problem: the determinants of spatial and temporal variation in *Daphnia* relative abundance and the consequences of variable *Daphnia* dominance. I briefly outline, in

turn, the form that these investigations have taken and the processes and hypotheses explored in each.

#### Effects of Daphnia on Pond Ecosystem Function

Daphnia are commonly thought to occupy a central position in the functioning of aquatic ecosystems. Compared to small-bodied taxa, large Daphnia are believed to exert stronger top-down control of algae (Leibold 1989, Mazumder 1994, Cottingham and Schindler 2000), a result of higher per capita filtration rates (e.g. Knoechel and Holtby 1986, Mourelatos and Lacroix 1990) and the ability to consume a broader size spectrum of food particles (Burns 1968, Neill 1975a). Because of this, Daphnia presence is frequently cited as a chief ingredient of successful cascading trophic interactions and efficient bio-control of algal blooms. Despite a long history of Daphnia-centric aquatic ecological investigation very few direct experimental manipulations comparing the effects of large Daphnia and small-bodied taxa on trophic-level biomass of primary producers have been performed. Many studies have instead indirectly "manipulated" Daphnia presence via the presence or absence of planktivorous fish (introducing potential confounding effects).

Simple consumer-resource models provide a useful start point for predicting effects of grazer identity on the regulation and control of algal biomass. Empirical investigations suggest that larger algal taxa are favored by nutrient enrichment (Reynolds 1984, McCauley et al. 1988, Paerl 1988, Watson et al. 1992) and the presence of zooplankton grazers (McCauley and Briand 1979, Vanni 1987). Assuming algal-prey exhibit trade-offs in their ability to resist grazer consumption and competitive ability for limiting nutrients (e.g. small edible algae are better competitors while larger grazer

resistant algae are poorer resource competitors), keystone effects are feasible. Increasing supply of nutrients in the presence of grazers permits species turnover among algae; superior resource competitors are favored at low nutrients while large, grazer resistant algae (but poorer competitors) are favored at high levels of enrichment (the classic "keystone predator effect", sensu Paine 1966; see Leibold 1996 for details). A result of this dynamic is that both grazer and algal biomass at equilibrium should increase along gradients of enrichment (Leibold 1996, Leibold et al. 1997). Grazer identity however can alter the strength of algal biomass responses to enrichment. Efficient zooplankton (e.g. large Daphnia) with high rates of prey consumption and/or prey conversion are expected to more strongly control algal biomass responses. Furthermore, zooplankton that can only feed on a limited size range of algae (i.e., many small-bodied zooplankters) will result in some algal-prey being completely invulnerable to grazing pressure. This can result in weaker top-down control and stronger algal biomass responses to enrichment (Leibold 1996, Leibold et al. 1997). Thus, differences between large Daphnia and small-bodied zooplankton in their effects on algal biomass are predicted to be a function of enrichment (differences should be greater at higher levels of nutrient supply) and the degree of algal diversity present (i.e., a diverse prey assemblage exhibiting an array of edibilities will facilitate turnover and keystone responses).

Chapter 2 presents results of an experiment in which these hypotheses were tested using lab-based, experimental microcosms. In this experiment I assessed the interactive effects of grazer identity, prey heterogeneity (i.e., algal diversity) and nutrient enrichment on phytoplankton production. *Daphnia pulex* was compared with *Ceriodaphnia quadrangula* (a small-bodied cladoceran common in fishless ponds). This grazer

treatment was then crossed with two levels of enrichment in the form of nitrogen and phosphorus additions and two levels of algal-prey heterogeneity (a simple system initially composed of a single species of edible algae and a diverse system composed of numerous species of varying susceptibility to grazers). Algal response variables included total biomass, the biomass of edible and resistant algae (based on size), the relative biomass of inedible algae and zooplankton biomass.

In addition to direct effects on primary producers, large *Daphnia* are also thought to affect phytoplankton indirectly through excretion of limiting nutrients (i.e., recycling of phosphorus and nitrogen). Though exceptions are known, the tissues of large-bodied *Daphnia* generally have the lowest ratios of nitrogen to phosphorus among zooplankton taxa (Sterner et al. 1992, Sterner and Hessen 1994). Consequently, *Daphnia* may recycle more nitrogen relative to phosphorus, enhancing phosphorus limitation of primary producers. Several lab and field investigations have shown that large-bodied *Daphnia* can recycle lesser amounts of phosphorus relative to nitrogen when compared to many smallbodied taxa (reviewed in Elser and Urabe 1999). Yet, the biological relevance of such differential recycling awaits more thorough experimental evaluation; direct manipulations of zooplankton composition with the intent of testing above stated predictions are scarce.

Chapter 3 presents results from an in situ mesocosm experiment in which I investigated these hypotheses by comparing monocultures of *Daphnia pulex* with a diverse assemblage of small-bodied zooplankton extracted from ponds. Zooplankton manipulations were performed in two ponds that differed naturally in their relative abundances of *Daphnia*; one pond (P12; figure 1C) was dominated by *Daphnia* for the entire growing season, the other (P14; figure 1B) exhibited a seasonal loss of *Daphnia* 

and contained no *Daphnia* at the time of the experiment. I assessed zooplankton effects on nutrient content at the scale of the whole water column (i.e., total phosphorus and total nitrogen) and effects on algal nutrient limitation using measurements of C:P and N:P of algae. Comparisons between ponds allowed me to determine the effect of environmental context on these processes.

In addition to effects on carbon and nutrient responses, I also used this mesocosm experiment to further explore effects of zooplankton composition on algal biomass in a more natural setting than that afforded by the lab-based study. To determine the interactive effects of zooplankton composition and nutrient enrichment on algal standing crop, I crossed zooplankton manipulations (*Daphnia* versus small zooplankton) with two levels of enrichment (a "high nutrient" treatment consisting of nitrogen and phosphorus additions and a "low nutrient" treatment that received no additions). Nutrient manipulations were performed in P14 only. In addition to effects of nutrient enrichment, I also determined the interactive effects of pond identity and zooplankton composition on algal biomass.

Finally, Chapter 3 further presents results from a field survey of 18 fishless ponds performed during the year of the experimental manipulation. These ponds were all considered to be "semi-permanent," having water year round in the 1-2 years prior to the survey period. In addition to zooplankton data (presented in figure 1), I also made monthly measures of chlorophyll *a*, total nitrogen, total phosphorus, and C:N:P content of phytoplankton. This allowed me to explore the natural relationships between zooplankton composition, *Daphnia* dominance and measures of algal biomass and nutrient limitation (i.e., C:P and N:P ratios).

#### Determinants of Variable Daphnia Dominance

Chapters 4 and 5 both explore factors that may drive seasonal and among pond variation in the relative abundance of *Daphnia pulex*. As touched on above, there are numerous potential mechanisms that may drive variable dominance by *Daphnia* in fishless ponds that reach beyond overly simplistic assumptions of static hierarchies of competitive ability and vulnerability to predation. First, several investigations indicate that large-bodied *Daphnia* do not invariably dominate small-bodied taxa when competing for shared resources (e.g. Neill 1975b, Lynch 1978, Smith and Cooper 1982, Romanovsky and Feniova 1985, Bengtsson 1987). These studies have emphasized the importance of stage-based dynamics when considering exploitative resource competition between large and small taxa (Romanovsky and Feniova 1985). Juvenile Daphnia are thought be more prone to the negative effects of interspecific competition due to high overlap with competitors in prey preferences, lower filtration rates, and susceptibility to starvation. In contrast, Daphnia adults, when compared to small-bodied taxa, generally have higher filtration rates, feed on a larger size range of algae, are starvation resistant, and have higher biotic potential (i.e., the ability to convert resources consumed to reproduction; for reviews see Romanovsky and Feniova 1985, DeMott 1989). Because of these traits, the outcomes of zooplankton competitive interactions can be highly variable and dependent on resource concentration and system productivity (e.g. Tillmann and Lampert 1984, Romanovsky and Feniova 1985, Tessier and Goulden 1987). Low rates of algal-resource supply can favor small-bodied taxa by way of juvenile bottlenecks in Daphnia populations. High resource availability (or a pulse of food) can allow Daphnia populations to accrue a large number of adult stage individuals. These adults could in

turn depress resource levels (via high filtration rates) and essentially out-starve smallbodied competitors (see Romanovsky and Feniova 1985, Bengtsson 1987). Variation in nutrient availability, and consequently algal production, might be especially high in shallow water bodies such as ponds in which mixing events extend to nutrient-rich sediments. Thus, this model may account for variation in competition effects among and within ponds.

In addition to quantity, quality of resources may mediate the strength and outcome of zooplankton competition. Because *Daphnia* can consume large algal particles outside the range accessible by smaller species of zooplankton (Burns 1968), the sizestructure of algal assemblages could affect resource overlap and the outcome of competition among zooplankters. Recent attention has also focused on nutritional quality and algal stoichiometry as an important aspect of resource quality that can determine dominance by *Daphnia* or small-bodied zooplankton. As outlined above, *Daphnia* species have unusually high demands for phosphorus when compared to many taxa of small zooplankton. Consequently, *Daphnia* performance can be linked to the nutritional content of their food (i.e. relative concentrations of carbon, nitrogen, and phosphorus; Sterner 1993, Sterner and Hessen 1994, Urabe et al. 1997, Elser et al. 2001). Mineral limitation due to high C:P and N:P ratios of phytoplankton can theoretically enhance the negative effects of resource competition leading to competitive reversals between large *Daphnia* and small-bodied taxa (Sterner and Hessen 1994).

Though lacking fish, planktivorous invertebrates such as the phantom midge larvae (genus *Chaoborus*) and backswimming bugs (genus *Notonecta*) are plentiful in the pelagic zone of ponds. However, differential susceptibility of *Daphnia* life stages

complicates predictions of impacts; juveniles are more vulnerable to predation but adults may attain a size refuge (Swift and Fedorenko 1975, Vinyard and Menger 1980, Spitze 1985). Consequently, seasonal and spatial variation in productivity and/or resource quality may intensify or diminish top-down effects by affecting how long juveniles are exposed to predators and the ability of individuals to reach a size refuge (Chase 1999).

Chapter 4 presents results of an experiment in which I tested the effects of algal productivity on zooplankton dominance, examining patterns of *Daphnia* relative biomass in the presence of small-bodied competitors at three levels of nutrient enrichment (in the form of pulse additions). I also explored effects of invertebrate predators and the interaction of predation with enrichment by crossing two levels of nutrient addition with the presence or absence of *Notonecta undulata*, a predator known to have strong effects on *Daphnia* populations (Murdoch et al. 1984, Arner et al. 1998). Thus, I was able to address whether SEH operates in this pond. This experiment was conducted using mesocosms in a successional pond following the natural decline phase of *Daphnia* in the system.

Studies of single systems afford little power of extrapolation or generalization. Hence, my final field season (presented in Chapter 5) focused on a much broader objective: exploring the determinants of *Daphnia* relative abundance among ponds and seasonally within ponds. In this experiment, I assayed effects of competitor and invertebrates predators in five ponds and at two times in the growing season (once in late May/early June when *Daphnia pulex* were present in all systems and once in mid-August when a subset of ponds had experienced seasonal losses of *Daphnia*). I used a simple partial factorial design consisting of three treatments: (1) competitors and predators

excluded, (2) the natural competitor assemblage present at natural densities and predators excluded, and (3) the competitor assemblage and predators present at natural densities. Invertebrate predators were phantom midge larvae (genus Chaoborus). The experiment was run concurrently in all five ponds and all enclosures received the same initial density of target Daphnia pulex permitting across system quantification and comparison of competitor and predator effects on Daphnia population growth rates. This experiment allowed me to first determine the relative effects of top-down and bottom-up processes on Daphnia populations. It further permitted exploration of the relationship between spatiotemporal variation in competition and predation interaction strength and natural abundances of Daphnia in the ponds. Finally, I examined the potential determinants of variable competition and predation intensity, assessing the relationship between measures of interaction strength and several abiotic and biotic factors measured in the ponds. These factors included pH, temperature, oxygen concentration, aspects of the competitor assemblage (composition and relative biomass), and aspects of the resource base (including productivity measured as total phosphorus, total chlorophyll, algal size structure, and C:P and N:P content).

## **Literature Cited**

- Arner, M., S. Koivisto, J. Norberg, and N. Kautsky. 1998. Trophic interactions in rockpool food webs: regulation of zooplankton and phytoplankton by *Notonecta* and *Daphnia*. Freshwater Biology 39: 79-90.
- Bengtsson, J. 1987. Competitive dominance among Cladocera: are single-factor explanations enough? An examination of the experimental evidence. Hydrobiologia 145: 245-257.
- Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28-35.
- Chase, J. M. 1999. Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist 154: 559-570.
- Cottingham, K. L., and D. E. Schindler. 2000. Effects of grazer community structure on phytoplankton response to nutrient pulses. Ecology 81: 183-200.
- DeMott, W. R. 1989. The role of competition in zooplankton succession. Pages 195-252 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Elser, J. J., K. Hayakawa, and J. Urabe. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorous enrichment. Ecology 82: 898-903.
- Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology 80: 735-751.
- Gliwicz, Z. M., and J. Pijanowska. 1989. The role of predation in zooplankton succession. Pages 253-295 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- 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 Review of Ecology and Systematics 7: 177-208.
- Hrbacek, J., M. Dvorakova, V. Korinek, and L. Prochazkova. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 14: 192-195.
- Knoechel, R., and L. B. Holtby. 1986. Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. Limnology and Oceanography 31: 1-16.

- Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. American Naturalist 134: 922-949.
- Leibold, M. A. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence and diversity patterns in communities. American Naturalist 147: 784-812.
- Leibold, M. A., J. M. Chase, J. B. Shurin, and A. L. Downing. 1997. Species turnover and the regulation of trophic structure. Annual Review of Ecology and Systematics 28: 467-494.
- Lynch, M. 1978. Complex interactions between natural coexploiters-*Daphnia* and *Ceriodaphnia*. Ecology 59: 552-564.
- Mazumder, A. 1994. Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. Ecology 75: 1141-1149.
- McCauley, E., and F. Briand. 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. Limnology and Oceanography 24: 243-252.
- McCauley, E., W. W. Murdoch, and S. Watson. 1988. Simple models and variation in plankton densities among lakes. American Naturalist 132: 383-403.
- Mittelbach, G. G., A. M. Turner, D. J. Hall, J. E. Rettig, and C. W. Osenberg. 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. Ecology 76: 2347-2360.
- Mourelatos, S., and G. Lacroix. 1990. In situ filtering rates of Cladocera: effect of body length, temperature, and food concentration. Limnology and Oceanography 35: 1101-1111.
- Murdoch, W. W., M. A. Scott, and P. Ebsworth. 1984. Effects of the general predator Notonecta (Hemiptera) upon a freshwater community. Journal of Animal Ecology 53: 791-808.
- Neill, W. E. 1975a. Resource partitioning by competing microcrustaceans in stable laboratory microecosystems. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 19: 2885-2890.
- Neill, W. E. 1975b. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology 56: 809-826.
- Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist 100: 65-75.

- Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnology and Oceanography 33: 823-847.
- Reynolds, C. S. 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, U.K.
- Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among Cladocera: effect of different levels of food supply. Oikos 44: 243-252.
- Smith, D. W., and S. D. Cooper. 1982. Competition among cladocera. Ecology 63: 1004-1015.
- Sommer, U. 1989. Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Sommer, U., M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in freshwaters. Archiv fur Hydrobiologie 106: 433-471.
- Spitze, K. 1985. Functional response of an ambush predator: *Chaoborus americanus* predation on *Daphnia pulex*. Ecology 66: 938-949.
- Sterner, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology 74: 2350-2360.
- Sterner, R. W., J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17: 49-67.
- Sterner, R.W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1-29.
- Swift, M. C., and A. Y. Fedorenko. 1975. Some aspects of prey capture by *Chaoborus* larvae. Limnology and Oceanography 20: 418-425.
- Tessier, A. J. and C. E. Goulden. 1987. Cladoceran juvenile growth. Limnology and Oceanography 32: 680-686.
- Tillmann, U. and W. Lampert. 1984. Competitive ability of differently sized *Daphnia* species: an experimental test. Journal of Freshwater Ecology 2: 311-323.
- Tilman, D. 1982. Resource competition and community structure. Princeton University Press, Princeton, NJ.

- Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: is it real? Limnology and Oceanography 42: 1436-1443.
- Vinyard, G. L., and R. A. Menger. 1980. *Chaoborus americanus* predation on various zooplankters: functional response and behavioral observations. Oecologia 45: 90-93.
- Watson, S., E. McCauley, and J. A. Downing. 1992. Sigmoid relationships between phosphorus, algal biomass, and algal community structure. Canadian Journal of Fisheries and Aquatic Sciences 49: 2605-2610.

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Figure 1. Results of a survey of 18 fishless ponds (A-R) in southern Michigan conducted in 1998. Ponds are ordered from low to high productivity based on monthly total phosphorus measures averaged over the sampling period. Shown are biomass responses of *Daphnia pulex*, rotifers (all taxa combined), small-bodied cladocera (all taxa combined) and copepods (all taxa combined, excluding nauplii). Data points are the means of two replicate samples. All ponds were found within a 60 mile radius around the W. K. Kellogg Biological Station (latitudinal/longitudinal coordinates are shown for each pond) and were known to have water year-round in the 2 years prior to the survey (J. Chase, University of Pittsburgh, pers. comm.; personal observation). Samples were collected on 2-3 consecutive days in the last week of each month shown. Due to drought conditions and low water levels, samples could not be taken in six ponds during the August sample period.



Figure 1



Figure 1 (continued)


Figure 1 (continued)



Figure 1 (continued)

#### **CHAPTER 2**

### THE EFFECTS OF PREY HETEROGENEITY AND CONSUMER IDENTITY ON THE LIMITATION OF TROPHIC-LEVEL BIOMASS

#### Abstract

The effects of prey heterogeneity and consumer identity on the strength of predator limitation of prey biomass were explored experimentally under controlled **laboratory** conditions. In this study, I utilized a model aquatic community composed of **zooplankton** as top predators, algae as prey, and nutrients as basal resources. To examine the effects of prey heterogeneity, I created a food chain initially composed of a single edible prey and a food web initially composed of a diverse assemblage of algae. These two prey treatments were then fully crossed with two predator treatments (a large-bodied Zooplankter, Daphnia pulex, and a small-bodied species, Ceriodaphnia quadrangula), and two levels of productivity. Prey heterogeneity had clear effects on the ability of predators to limit overall prey biomass. In food chains, predators had strong negative effects on algae and algal biomass exhibited a narrow response to enrichment. In contrast, **pred**ator limitation was weak in food webs with the consequence that predator and prey **biomass** both showed positive increases with productivity. The prey community in food webs also exhibited a striking increase in the relative abundance of large inedible algae with enrichment, in keeping with model predictions. These results indicate that prey heterogeneity can have substantial effects on predator-prey dynamics and trophic structure and can serve to shift systems from strong top-down control to ones in which **Prey** are co-limited by predators and resources. Comparisons between top predators showed that *Daphnia*, compared to *Ceriodaphnia*, more strongly limited the biomass of

large algae in food webs at high productivity and total algal biomass in all nutrient
enriched treatments (both chains and webs). Thus, consumer identity and ecological
context (productivity and heterogeneity of prey communities) may mediate the strength of
zooplankton-algae interactions and the efficacy of trophic cascades.

Keywords: algae, bottom-up, Ceriodaphnia, Daphnia, edibility, enrichment, food web dynamics, keystone predation, top-down, zooplankton.

#### Introduction

Ecologists have frequently focused on patterns of trophic-level biomass to infer ecological processes from community organization (Fretwell 1987, Power 1992, Leibold et al. 1997). The underlying rationale for much of this research has been predicated on the assumption that natural patterns of biomass partitioning, when examined along gradients of productivity, can provide insight into the factors controlling or limiting trophic levels (Power 1992, Leibold et al. 1997). The long-standing top-down, bottom-up debate is commonly approached in this manner and frequently centered on the predictions of Oksanen et al. (1981) (Fretwell 1987, Power 1992). Their mathematical treatment, hereafter referred to as EEH or the "exploitation ecosystems hypothesis" (Oksanen and Oksanen 2000), predicts that trophic-level biomass is exclusively top-down or bottom-up "Controlled" (sensu Osenberg and Mittelbach 1996). For example, in a two trophic-level system composed of plants and herbivores, EEH predicts that plants are top-down Controlled via the numerical response of herbivores, and thus their total biomass shows no response to increasing potential productivity at equilibrium. In contrast, herbivores, themselves having no predators, are bottom-up controlled and therefore respond positively. Hence, the EEH model predicts that consumer and resource control alternates as one moves up a food chain resulting in a concomitant decoupling of biomass in adjacent trophic levels along gradients of productivity (see Mittelbach et al. 1988, Oksanen and Oksanen 2000).

While the general EEH pattern has received some empirical support (e.g. Wootton and Power 1993, Mazumder 1994, Kaunzinger and Morin 1998, Oksanen and Oksanen **2000**), a growing body of evidence suggests that the biomasses of adjacent trophic levels often increase jointly as ecosystem productivity increases (e.g. McCauley and Kalff 1981, Hanson and Peters 1984, McNaughton et al. 1989, Ginzburg and Akcakaya 1992, Leibold et al. 1997). This pattern is particularly well documented in aquatic communities where **Zooplankton and phytoplankton biomasses are commonly positively correlated among** lakes and ponds of varying nutrient enrichment (McCauley and Kalff 1981, McCauley et al. 1988, Leibold et al. 1997). This chasm between model prediction and natural pattern has engendered an exploration of potential mechanisms that can generate positive correlations between the biomass of adjacent trophic levels and productivity (McCauley et al. 1988, Mittelbach et al. 1988, Arditi and Ginzburg 1989, Abrams 1996). Recent interest has been directed towards the effects of prey-heterogeneity and differential resource edibility. The abstraction of food chain interactions, as in EEH, implicitly assumes functional homogeneity of species within trophic levels. When models are extended to include within trophic level heterogeneity (i.e. food chains become food webs) the strength of predator-prey interactions weakens, EEH patterns dissolve, and

positive correlations between adjacent trophic levels can be generated (Abrams 1993, Leibold 1989, 1996).

To base predictions within a theoretical framework, consider a food web composed of a single top predator, a number of middle prey, and a single basal resource. As shown by Leibold (1996), if trade-offs exist among prey in predator susceptibility and competitive ability for resources, a serial replacement of prey species will occur as productivity increases (i.e. as the supply of basal resources increases). At low productivity only the most efficient consumers of resources can persist. As productivity increases the more predator-resistant but poorer resource competitors can invade (the well known "keystone predator" effect, sensu Paine 1966; see also Levin et al. 1977, Vance 1978, Holt et al. 1994). The keystone predator model predicts positive correlations between top predator and prey biomass along gradients of productivity, but weaker correlations if productivity is sufficient to allow the invasion of a completely inedible prey (see Leibold et al. 1997). The assumed trade-off between resource exploitation and predator resistance among prey is central to the model's predictions.

Predator identity can modify the response of heterogeneous prey assemblages by affecting variation in edibility. For example, in plankton communities, large-bodied grazers such as *Daphnia* have higher filtering rates (e.g. Knoechel and Holtby 1986, Mourelatos and Lacroix 1990) and consume a broader size spectrum of algae (Burns 1968, Neill 1975a) compared to small-bodied cladocera (e.g. species of *Ceriodaphnia*, *Diaphanosoma*, etc.). Consequently, *Daphnia* are thought to more effectively control **algal** communities (Pace 1984, McQueen et al. 1986, Leibold 1989, Mazumder 1994). Within the context of the keystone predator model, inefficient predators with lower per capita feeding and prey conversion rates (i.e. conversion of prey consumed to growth) will result in higher prey biomass at equilibrium. Additionally, if predators can only consume a limited subset of the prey community inedible prey will result, further weakening top-down effects along gradients of productivity.

Despite the growing body of theory, remarkably few experimental manipulations of both prey-heterogeneity and productivity have been attempted, the exceptions being the microbial study of Bohannan and Lenski (1999) and the mesocosm study of Leibold and Wilbur (1992). In the following experiment, I employed an aquatic system in which the top predator was composed of a single species of zooplankton (either Daphnia or *Ceriodaphnia*), algae were prey, and basal resources were nutrients (phosphorus and nitrogen). Two model communities were established: a simple food chain composed of a single top predator and a single species of algae, and a more complex food web composed of a single top predator and a heterogeneous (i.e. diverse) algal community. Productivity was then manipulated by adding nutrients. If within-trophic level heterogeneity is a key factor, communities with diverse algal-prey should display positive correlations between trophic level biomass and productivity, whereas food chains should exhibit the classic EEH response (increases in zooplankton biomass and no change in algae). Additionally, food webs should harbor a greater relative abundance of less edible species of prey under conditions of nutrient enrichment. Because algal productivity is channeled into less edible forms in this situation, we expect zooplankton to respond positively to enrichment but this increase should be lower in magnitude when compared to food chains with completely edible prey (Bohannan and Lenski 1999). Finally, predator identity should

further modify the degree of heterogeneity and hence the discrepancy between EEH and observed responses of food webs.

The aforementioned model predictions are based on the assumption that systems have reached a stable equilibrium. Experimentally, steady state conditions are difficult to obtain. An ideal experiment should last long enough to allow transient dynamics to diminish. Yet, in reality, the time frame required to obtain true steady state conditions is often sufficiently long to permit the intrusion of experimental artifacts (namely the invasion of new species or loss of nutrients from the system). The present experiment lasted six weeks, as a compromise between these issues, and was long enough to encompass several generations of predator and prey. Data analyses will rely largely upon exploration of dynamics to address model mechanisms. I discuss the validity and caveats of this approach in greater detail and in light of experimental results in the Discussion.

#### Methods

The experiment was conducted indoors, under controlled environmental conditions, at the Kellogg Biological Station, Experimental Pond Facility (Hickory Corners, MI). My experimental system consisted of white 20-liter polyethylene containers artificially illuminated with 80-watt overhead fluorescent fixtures equipped with full spectrum bulbs. Enclosures received continuous 24-hour light. Room temperature was regulated and water temperature remained within 20-21°C. All enclosures received 18 liters of well water (the same water used to culture all zooplankton). A 2x2x2 fully factorial design was then employed. Treatments included: two levels of productivity (low Versus high) crossed with two levels of algal-prey heterogeneity (a single species "food chain" treatment versus a diverse "food web" treatment) crossed with two zooplankton

treatments (*Daphnia pulex* versus *Ceriodaphnia quadrangula*). All treatments were replicated three times for a total of 24 enclosures.

Productivity was manipulated through addition of phosphorus (Na<sub>2</sub>HPO<sub>4</sub>) with high productivity treatments receiving a starting concentration of 180  $\mu$ g P per liter (well within the range considered hypereutrophic) and low productivity treatments receiving no additions for an initial concentration of 6  $\mu$ g P per liter. Nitrogen (NaNO<sub>3</sub>) was added with phosphorus in a 36:1 N to P molar ratio. This naturally high ratio was matched to that of the untreated well water to avoid changes in algal stoichiometry, and helped ensure that the system was limited by a single nutrient, phosphorus (Hecky and Kilham 1988). It also made it unlikely that nutrient retention/recycling by zooplankton could have shifted the system from phosphorus to nitrogen limitation (Sterner et al. 1992, Sterner and Hessen 1994). Nutrients were added once at the start of the experiment.

Single species algal-prey treatments were established with a lab-cultured inoculum of *Monoraphidium* unknown sp., a unicellular species of green-algae known to be both highly edible and nutritionally adequate for both species of zooplankton (personal observation). To minimize addition of algal culture medium, algae were first settled for 24 hours in a 1-liter bottle. Excess medium was then decanted leaving an algal concentrate that was then diluted to one liter in deionized water. Single prey treatments received a low-density inoculation of *Monoraphidium* resulting in a chlorophyll *a* startconcentration of 0.25 µg per liter. I established heterogeneous prey treatments by mixing *Monoraphidium* with an equal chlorophyll concentration of a diverse algal community extracted from ponds at the K.B.S. pond facility. A 10-liter sample of water was collected from the water column of each of three ponds using an integrated tube sampler and then filtered through 53-micron Nitex mesh to remove zooplankton. Samples were then pooled and thoroughly mixed before being added to enclosures. Total algal biomass after addition of both *Monoraphidium* and the diverse assemblage of algae equaled that of the single species enclosures (0.25 µg chlorophyll *a* per liter). Approximately 800 ml of pond water was added to each enclosure. This low volume was unlikely to cause substantial changes in nutrient concentrations or N:P ratios. However, as a precaution, an equal volume of pond water was filtered through Gelman A/E glass fiber filters (removing all particles larger than 1 micron) and added to the single prey treatments. Since most bacterioplankton are less than 1 micron in size (Lampert and Sommer 1997), the addition of filtered pond water made it likely that all treatments (chains and webs) received a similar bacterial assemblage at the start of the experiment.

Examination of samples of the algal community added to the food web treatments revealed an assemblage diverse in taxa and morphologies. Of the forms known to achieve sizes outside the range of edibility (see below for discussion of "edibility"), those present included pennate diatoms (Fragilariaceae, Naviculaceae), dinoflagellates (Peridiniales, Gonyaulacales), *Dinobryon* communities (Dinobryaceae), and several species of filamentous algae. Also present, were a number of taxa in the edible (<10 micron) size range of zooplankton. These were primarily unicellular algae and flagellates (Chlorophyceae).

Zooplankton populations were established with low initial densities to avoid immediate overgrazing and population crashes at the start of the experiment, especially at low levels of productivity. *Daphnia* and *Ceriodaphnia* treatments each received 9

haphazardly selected individuals concentrated from batch cultures. Cultures were established several months before the experiment by collecting *Daphnia pulex* and *Ceriodaphnia quadrangula* from several ponds in the area surrounding KBS. Species were isolated from samples and raised separately in untreated well water at high food density. Because *Daphnia* are larger than *Ceriodaphnia*, *Daphnia* treatments invariably received a larger biomass of zooplankton. However, given the low initial density relative to the potential population growth in these systems (see Results), the effects of differential biomass should have been minimal.

Algal growth on enclosure walls is largely inaccessible to zooplankton consumers and thus embodies a potential methodological artifact given the small size and high surface area to water volume ratio of the experimental enclosures. To minimize wall effects, I introduced snails (*Physa integra*) to both graze down algal growth and aid in recycling of nutrients to the planktonic community. Snails were obtained from ponds in the surrounding area, rinsed, and then lab-reared in large culture vats for several months. Prior to the experiment, snails were lightly scrubbed and rinsed in well water. Each experimental enclosure then received five size-matched individuals at the start of the experiment.

Algae were added to enclosures immediately following addition of water, with **ZOOP**lankton additions following two days later. A day after zooplankton inoculations, **nut**rients were added. All enclosures were gently mixed for one minute every 4 hours **With** low-pressure aeration from piston pumps. Mixing was intended to both resuspend **algae** and facilitate nutrient recycling within the water column. The experiment was **allowed** to run for 6 weeks and enclosures were sampled weekly, beginning in the first

week of the study for algae and the second week for zooplankton. Sampling was timed to occur midway between mixing events. In each period, zooplankton were sampled by taking a 1.2-liter sample of the water column using an integrated tube sampler. Zooplankton were then removed by filtering through 60-micron Nitex mesh and the water was returned to the source enclosure. Zooplankton were immediately preserved in acid Lugols solution and later enumerated in their entirety. Fifty randomly chosen individuals from each sample were measured to obtain biomass estimates from length-mass regressions. During each sample period, a 150-ml water sample was also extracted from each enclosure and filtered onto Gelman A/E glass fiber filters for subsequent analysis of chlorophyll a (sensu Welschmeyer 1994) as a correlate of algal biomass. Samples from single prev treatments were filtered in their entirety to measure total chlorophyll a. Water samples from heterogeneous prey treatments were divided into two size fractions. Half of the sample was filtered directly to obtain estimates of total chlorophyll a. The other half was first filtered through 35-micron Nitex mesh to remove large algae, and then filtered onto glass fiber filters to measure the edible size fraction of algae. Size was chosen as a general indicator of edibility because it is a common constraint on dietary preference of **Zooplankton** (Neill 1975a, Sommer 1988) and it is easily measured through size fractionation. Thirty-five microns was chosen as a proxy upper size limit for "highly edible" versus "predator-resistant" prey. The maximum upper size limit of consumable prey for Daphnia is approximately 60 microns (Burns 1968). Ceriodaphnia on the other hand have preferences that are much lower, below 10 microns (Neill 1975a). Thus, 35 microns represents a midpoint cutoff applicable to both predators (though in general, edibility may be overestimated for *Ceriodaphnia* and underestimated for *Daphnia*). It

should be noted that nutrients were added only at the start of the experiment. Hence, all nutrient resupply occurred via natural recycling within the enclosures. Other than minimal losses from zooplankton and algal sampling (an imposed mortality rate of approximately 7.5% per week), the enclosures represented closed systems.

Biomass responses of zooplankton and algae were analyzed using univariate repeated measures ANOVA (rm-ANOVA). Initial (day 0) densities were not included in analyses. When necessary, analyses of biomass from single sample dates were performed using ANOVA. Relative biomass values were arcsine square root transformed. All other data were log<sub>10</sub> transformed to conform to assumptions of homogeneity of variances. Because samples taken on adjacent dates from a given experimental unit are likely to show greater covariance than non-adjacent samples, assumptions of repeated measures ANOVA (sphericity) may be violated. To avoid type I errors when examining within subjects effects (i.e. response curves through time), both Greenhouse-Geiser (G-G) and Huynh-Feldt (H-F) adjusted probabilities are presented. All analyses were performed using Systat 8.0 (SPSS Inc., 1998).

#### Results

Focusing first on prey responses, algae in both food chains and food webs displayed a large initial increase in biomass in response to the initial nutrient pulse and delayed responses of the predators (figure 1A, 1B). Biomass by day 14 had decreased as <sup>20</sup>oplankton populations responded numerically but nutrient enriched food webs demonstrated a second marked increase in algal biomass nearing the end of the <sup>experiment</sup> (figure 1B). There was a significant interaction of time, nutrients and

heterogeneity (within subjects effect, G-G p<0.01, H-F p<0.001,  $F_{4,64}$ =6.79). However, a significant between subjects interaction between nutrients and heterogeneity was not detected (p=0.193,  $F_{1,6}$ =1.84). The lack of significance was likely a result of the large spike in algal biomass on day 7, present in both webs and chains. This early date represents prey responses under little or no predation pressure and is thus not representative of dynamics expected in the keystone model. When excluding day 7 and analyzing responses from days 14 (after predators had time to respond) to 42, a significant between subjects interaction between nutrients and prey heterogeneity was detected (table 1). Note that increases in algal biomass in food webs at the end of the experiment approached the maximal (day 7) levels, indicating that productivity treatments were still robust.

The degree of divergence in biomass in food webs relative to food chains was best seen when examining algal biomass on the final date of the experiment. Responses to productivity were subdued in single species enclosures, in clear contrast to the large increases in biomass of heterogeneous prey with enrichment (figure 2A, 2B) (a result borne out by a significant nutrient by heterogeneity interaction; p<0.00001,  $F_{1,16}=27.82$ ; table 2). In those communities with only a single species of algae, there were no significant effects of nutrient enrichment on biomass, regardless of predator identity (figure 2A, 2B; p>0.87, Tukey's HSD). In contrast, algal biomass in nutrient enriched food webs was significantly greater than all other treatments, independent of predator identity (figure 2A, 2B; p<0.05 for all comparisons, Tukey's HSD).

While edible algae (<35 microns) comprised the majority of algal biomass during the early spike in prey biomass (day 7), this size fraction was greatly reduced as predators increased in abundance (figure 3A, 3B). Focusing on days 14 to 42, there were no significant effects of nutrient enrichment on the edible size fraction using repeated measures (p>0.29, for all between and within subjects effects and interactions), nor was there a nutrient effect when examining just day 42 abundances (p>0.60, two-way ANOVA). In contrast, nutrient enrichment had a positive effect on resistant algae over time (p<0.0001, F<sub>1.8</sub>=54.96, between subjects effect, rm-ANOVA) and on day 42 (p<0.001,  $F_{1,8}$ =45.97, two-way ANOVA). Thus, the increases in total chlorophyll *a* on days 28 to 42 (figure 1B) were due purely to responses of resistant algae (figure 3A, 3B). These results were mirrored in analyses of relative biomass of resistant prey, calculated as the difference between total and <35 micron chlorophyll *a* divided by total chlorophyll *a*. Relative abundance of large algae was greatly enhanced by enrichment, showing a nearly four-fold increase by experiment's end (figure 4; between subjects effect of nutrients, p<0.001, F<sub>4.64</sub>=31.61, two-way rm-ANOVA). Though the species composition of the resistant algal fraction was not quantified, examination of net phytoplankton (species greater than 60 microns) revealed an assemblage dominated by filamentous green algae followed by the large dinoflagellate, Ceratium. Colonial forms of Monoraphidium were not observed, which would have complicated interpretations considerably.

There was evidence of an effect of predator identity on total algal biomass, but the effect was only manifest in high productivity treatments (figure 1A, 1B). There was a trend for a nutrient by predator interaction when examining all six sample dates (between

subjects effect, p=0.061, F<sub>4.64</sub>=4.06). The interaction was clearer when analysis was restricted to days 14 to 42 (between subjects effect, p=0.016,  $F_{1,6}$ =7.31). I performed separate analyses for low and high nutrient treatments for days 14 to 42, testing for effects of predator identity and prey heterogeneity. A between subjects predator effect was not detected in low productivity treatments regardless of prey composition (p=0.209,  $F_{1,8}=1.87$ ). In high productivity treatments, however, algal biomass was lower in the presence of Daphnia compared to Ceriodaphnia, (between subjects effect, p=0.043,  $F_{4,64}$ =5.81). When examining total algal biomass on the final date of the experiment, there was some indication of a three-way interaction between predator identity, prey heterogeneity and enrichment, but the effect was not significant at the 0.05 level (table 2). While predators did not differ in their effects on the edible size fraction (p>0.10, rm-ANOVA), a significant predator by nutrient interaction was detected when examining responses of predator-resistant algae (between subjects effect, p=0.048,  $F_{1,8}=5.45$ ). Daphnia more strongly limited resistant algae but only at high productivity levels (figure 3A, 3B). Predator identity had weak effects on the relative abundance of edible versus resistant algal-prey (p=0.067, two-way rm-ANOVA).

Zooplankton biomass responses depended on predator identity, nutrient enrichment, and algal heterogeneity. Both species responded positively to enrichment, regardless of the prey community presented, but *Daphnia* showed larger responses under conditions of enhanced productivity over time (figure 5). A significant interaction between nutrient enrichment and predator identity was detected when using rm-ANOVA (table 3). Differential responses of the two predators to the composition of the prey community and productivity were best perceived when inspecting each separately. As predicted by theory, both zooplankters exhibited trends towards lower biomass responses to enrichment in food webs compared to food chains (figure 5A, 5B) but a significant interaction between enrichment and prey heterogeneity was not detected for either (between and within subjects effects, p>0.15, two-way rm-ANOVA for each predator separately). Only positive main effects of enrichment were detected for both *Ceriodaphnia* (p<0.01,  $F_{1,8}$ =21.07) and *Daphnia* (p<0.0001,  $F_{1,8}$ =89.30) using repeated measures ANOVA.

#### Discussion

The top-down, bottom-up debate has routinely espoused two very different and opposing points of view: either systems exhibit donor-control dynamics in which trophic cascades are rare and resource limitation dictates trophic-level biomass (e.g. White 1978, Strong 1992, Polis and Strong 1996) or systems harbor the potential for strong consumerresource interactions in which top-down control alternates with bottom-up control at different trophic levels (e.g. Hairston et al. 1960, Oksanen et al. 1981, Oksanen and Oksanen 2000). However, recent treatments have emphasized a pluralistic approach in which both consumer and resource effects jointly determine trophic-level biomass (e.g. Leibold 1996, Osenberg and Mittelbach 1996, Leibold et al. 1997). In the keystone predator model, for example, top-down effects are dampened but both predators and resources determine total prey abundance. Moreover, the model accounts for shifts in community composition and diversity while explicitly considering underlying mechanisms, a significant departure from earlier treatments (e.g. Arditi and Ginzburg 1989). Thus, validation of the keystone model and the effects of prey heterogeneity are of potential import for understanding the myriad of community dynamics and patterns we see in natural systems.

The results of this experiment demonstrate that within trophic-level heterogeneity can have substantial effects on predator-prey dynamics and community structure. Simple food chains exhibited patterns of trophic biomass partitioning in keeping with predictions of EEH. Prey biomass showed little or no relationship with productivity, while predator biomass was positively affected, suggesting that predators exerted strong top-down effects. In contrast, when communities were composed of food webs, both predators and prey responded positively to enrichment. The increases in algal biomass in food webs were due entirely to increases in the large size fraction of algae, a pattern consistent with species turnover in the prey community and a shift in dominance towards predatorresistant prey (i.e. a keystone effect). Finally, because shifts in prey composition can allow system productivity to be channeled to species inaccessible to predators, theory predicts that predator responses to enrichment should be lower in food webs compared to food chains. In general, results from both predator treatments supported this prediction but trends were not significant at the 0.05 level.

These results are consistent with a hypothesized trade-off between competitive ability and predator-resistance among algal species in food webs. While I did not directly address the trade-off issue (a predator exclusion treatment was not included), a number of other studies have shown that smaller algal size generally leads to higher rates of nutrient uptake and population increase, suggesting that these species are superior resource competitors (reviewed in Reynolds 1984). Given that size is known to lead to decreased rates of zooplankton consumption, the existence of the trade-off appears to be a relatively sound assumption but one that clearly requires future experimental validation.

This study also examined the effect of consumer identity as a potential determinant of trophic structure. It is a widely-held truism that large-bodied Daphnia are strong interactors with algae and thus more effectively limit their prey communities compared to small-bodied species of zooplankton (Leibold 1989). Yet, a number of studies call into question the generality of this assertion (e.g. Leibold and Wilbur 1992, Turner and Mittelbach 1992, Leibold et al. 1997). My results suggest that ecological context plays an important role in mediating the effects of consumer size on the strength of zooplankton-algae interactions. Daphnia and Ceriodaphnia were comparable in their ability to control algal biomass at low productivity. However, Daphnia were able to effect larger reductions in algal-prey under enriched conditions (figure 1A, 1B). The two consumers also diverged in their effects on algae in food webs. While both affected edible algae to a similar degree, Daphnia more effectively controlled biomass of resistant algae in high nutrient treatments. This is not unexpected given Daphnia's known ability to feed on large algal particles, well outside the range of small-bodied species; the same algae favored under high nutrient conditions. Thus, my results support the argument that effective control of algal communities along gradients of enrichment is dependent not on prey heterogeneity alone but on the identity of their zooplankton consumers.

As with any experimental system, especially one that strives to address theory, a number of important caveats and concerns must be addressed. First, because my study system was not conducted under sterile conditions, food chain treatments were prone to invasion by predator-resistant algae. The relatively short duration of this experiment was

intended to minimize this problem. To monitor invasives, I size fractionated water samples from food chains at days 21 and 42 of the experiment. By mid-experiment, large algae had begun to appear in some food chain enclosures (in low nutrient treatments, average relative biomass of >35 micron algae=0.073, range=0.000-0.206; high nutrient treatments, average=0.101, range=0.050-0.146) and had reached greater proportions by the termination of the experiment (in low nutrient treatments, average relative biomass=0.169, range=0.000-0.392; high nutrient treatments, average=0.393. range=0.000-0.607). Thus, a longer experimental duration would likely have resulted in algal dynamics in food chain treatments similar to those of the food webs. Note, however, that invasions actually make detection of nutrient effects in food chains more likely (assuming they respond in the same manner as the food web treatments) and differences between chains and webs more difficult to detect. To explore the degree to which invading resistant algae affected my results, I reanalyzed data after eliminating replicates of food chain treatments with greater than 25% invasion by predator-resistant algae at the end of the experiment. Using t-tests to perform pairwise comparisons on algal biomass for the final date, when effects of invasives should have been most severe, results were similar to those using the full data set. There were no significant differences detected between low and high nutrient treatments in the food chains, though power is admittedly low (p=0.284 for Daphnia treatments, p=0.940 for Ceriodaphnia).

A second point to consider is that the Leibold (1996) keystone predation model and EEH food chain model both base predictions on the assumption that all community components have attained a stable equilibrium. Hence, a valid concern is whether the present experiment allows us to address these models. Qualitatively, algal biomass in

food chains as well as edible algae in food webs appeared relatively stable, but it was clear that resistant algae in food webs had not stabilized. Furthermore, *Daphnia* showed a pronounced drop in abundance in nutrient enriched food webs in the final two weeks of the experiment (figure 5A). This may have been due to the shift in dominance to large inedible algae combined with the genus' known propensity for starvation in juvenile stages (Neill 1975b, Romanovsky and Feniova 1985). Of concern is whether algal dynamics, specifically the increase in biomass of heterogeneous prey in *Daphnia* treatments, were a result of the aforementioned keystone processes or the drop in predator abundance. Two lines of reasoning support my conclusions. First, heterogeneous prey in the presence of *Daphnia* showed responses to productivity that paralleled those of *Ceriodaphnia* (figure 2B), suggesting that increases were not simply a result of decreases in predator abundance. That heterogeneous prey composition shifted towards larger, presumably less edible, forms with enrichment further supports the assertion that keystone processes were operating.

Finally, inferring future dynamics from the present prey trajectories is potentially hampered if predators were to exhibit cyclic oscillations (e.g. stable limit cycles). This is especially true if unstable predator dynamics somehow deter dominance by inedible algae. *Ceriodaphnia* biomass appeared stable but *Daphnia* species are known to display an array of population behaviors from sustained, stable population levels over time to cyclic oscillations (Murdoch and McCauley 1985). Using simulations, Bohannan and Lenski (1999) show that keystone processes can be robust to such instabilities; oscillations in predator and edible prey abundances do not deter invasion and dominance by inedible prey under enriched conditions. This prediction was confirmed in their

experimental results. Abrams (1999) presents a general theoretical treatment of the ability of predator-resistant prey to invade an unstable system composed of a top predator and competitively superior edible prey. His results show that invasion can be hindered by predators that exhibit limit cycles, though invasibility is less restricted for those prey that are close to or completely inedible. Given these studies, consequences of cyclic Daphnia dynamics on prey responses in my experiment are uncertain. Accordingly, results from Daphnia treatments should be viewed with some caution. Ultimately, this question can only be resolved empirically and, given Abrams (1999) analyses, is a potentially important avenue of research to pursue. Yet, this would require an experimental duration much greater than the one I utilized, on the order of several months. Given the aforementioned caveats, I have attempted to enhance the robustness of my conclusions by combining analyses of dynamics (using repeated measures ANOVA) with examination of biomass at the end of the experiment. Even if we rely solely on *Ceriodaphnia* treatments, this combined analysis clearly indicates that prey heterogeneity can have important effects on patterns of biomass partitioning and the relative importance of top-down versus bottom-up processes. Algal biomass and size structure exhibited trajectories and divergences that were consistent with model predictions and hypothesized mechanisms.

In addition to prey heterogeneity, a number of alternative hypotheses have been proposed as potential explanations for why natural patterns of trophic-level biomass commonly deviate from EEH predictions. While some of the proposed mechanisms may have operated in my experiment, none appear to fully explain my results. These hypotheses include: 1) stage-structured interactions in which resources of juvenile and adult stages of predators are decoupled (Mittelbach et al. 1988, Persson et al. 1988, 1992), 2) adaptive responses by foraging prey (Abrams 1996), 3) unstable predator-prey dynamics (Abrams and Roth 1994), 4) spatial heterogeneity in the top predator (Arditi and Saiah 1992, Abrams 1994), 5) interference among predators (Sarnelle 1994, McCarthy et al. 1995, Oksanen et al. 1995), 6) increases in zooplankton death rates with productivity as a result of increasing planktivory (McCauley et al. 1988, Sarnelle 1992), and 7) ratio-dependence in predator functional responses (Arditi and Ginzburg 1988, Akcakaya et al. 1995). Hypotheses 1, 2 and 6 may be immediately dismissed as inapplicable to the present experiment. There was no evidence of predator-prey oscillations allowing rejection of hypothesis 3. Finally, hypotheses 4, 5, and 7 could have all occurred to some degree in my experiment. However, there is no obvious reason why these processes should have operated differentially in food chain and food web enclosures. Hence, they cannot explain observed differences between chains and webs.

This experiment represents one of only a handful in which the effects of prey heterogeneity were explored experimentally. Although a number of studies have experimentally documented keystone effects of predators on the composition of prey communities (e.g. Paine 1966, Levin et al. 1977, Lubchenco 1978, McCauley and Briand 1979), few have actually manipulated the prey assemblage or compared food chains to food webs with the intent of exploring trophic-level limitation. A notable exception is the microbial study of Bohannan and Lenski (1999). Their examination of food chain and food web responses to enrichment supported theoretical predictions and produced results similar to mine. Leibold and Wilbur (1996) also uncovered effects of within trophic level heterogeneity on patterns of trophic-biomass response to productivity in their aquatic mesocosms. However, in their study, food web architecture alone was manipulated; food

chains were not compared with food webs. The importance of these experimental results is bolstered by several lines of indirect evidence implicating prev heterogeneity as an important factor governing the organization of natural aquatic communities. Recent survey data and reviews of the literature indicate that the mechanisms of the keystone predator model may be operating among water bodies to generate patterns of trophic structure and community composition (Leibold et al. 1997, Leibold 1999), Furthermore, increases in grazer-resistant forms of phytoplankton are commonly observed in water bodies along gradients of nutrient enrichment (Reynolds 1984, McCauley et al. 1988, Paerl 1988, Watson et al. 1992), and the presence of zooplankton has been shown experimentally to favor the incidence of inedible forms of algae, in keeping with model expectations (McCauley and Briand 1979, Vanni 1987). There are a plethora of ecological mechanisms that can weaken predator effects and result in correlated biomass among adjacent trophic levels (e.g. Power 1992, Persson et al. 1996, Polis and Strong 1996, Abrams and Roth 1994), and certainly this study does not discount the alternatives. However, my results combined with previous investigations clearly show that along gradients of productivity, the presence of a diverse prey assemblage can have profound effects on predator-prey dynamics, trophic structure and the strength of predator control.

Though community ecology has long operated under the aegis of the mechanistic approach, tests of theory such as the keystone predator model are regrettably few. The focus on mechanism advances our understanding beyond the insights provided by phenomenological treatments by explicitly incorporating the biology underlying community dynamics and species interactions. This knowledge gained then offers the prospect of a greater capacity for predicting natural ecological patterns. While my study

addresses the effects of food web structure it does not explicitly test for the underlying autecological traits and trade-offs assumed to operate in the model. These traits will in general have physiological, morphological or behavioral bases to them and can be subject to selection. Thus, further examination of such mechanisms could offer an exciting linkage between evolutionary ecology and the ecological study of traits governing species coexistence.

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#### **Literature Cited**

- Abrams, P. A. 1993. Effect of increased productivity on the abundances of trophic levels. American Naturalist 141: 351-371.
- Abrams, P. A. 1994. The fallacies of "ratio-dependent" predation. Ecology 75: 1842-1850.
- Abrams, P. A. 1996. Dynamics and interactions in food webs with adaptive foragers. Pages 113-121 in G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics. Chapman and Hall, New York, USA.
- Abrams, P. A. 1999. Is predator-mediated coexistence possible in unstable systems? Ecology 80: 608-621.
- Abrams, P. A., and J. D. Roth. 1994. The effects of enrichment of three-species food chains with nonlinear functional responses. Ecology 75: 1118-1130.
- Akcakaya, H. R., R. Arditi, and L. R. Ginzburg. 1995. Ratio-dependent predation: an abstraction that works. Ecology 76: 995-1004.
- Arditi, R., and L. R. Ginzburg. 1989. Coupling in predator-prey dynamics: ratiodependence. Journal of Theoretical Biology 139: 311-326.
- Arditi, R., and H. Saiah. 1992. Empirical evidence and the theory of ratio-dependent consumption. Ecology 73: 1544-1551.
- Bohannan, B. J. M., and R. E. Lenski. 1999. Effect of prey heterogeneity on the response of a model food chain to resource-enrichment. American Naturalist 153: 73-82.
- Burns, R. J. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnology and Oceanography 13: 675-678.
- Fretwell, S. D. 1987. Food chain dynamics: the central theory of ecology? Oikos 50: 290-301.
- Ginzburg, L. R., and H. R. Akcakaya. 1992. Consequences of ratio-dependent predation for steady-state properties of ecosystems. Ecology 73: 1536-1543.
- Hairston, N. G., F. E. Smith, and L. B. Slobodkin. 1960. Community structure, population control, and competition. American Naturalist 44: 421-425.
- Hanson, J. M., and R. H. Peters. 1984. Empirical prediction of crustacean zooplankton biomass and profundal macrobenthos in lakes. Canadian Journal of Fisheries and Aquatic Sciences 41: 439-445.

- Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnology and Oceanography 33: 796-822.
- Holt, R. D., J. Grover, and D. Tilman. 1994. Simple rules for interspecific dominance in systems with exploitative and apparent competition. American Naturalist 144: 741-771.
- Kaunzinger, C. M. K., and P. J. Morin. 1998. Productivity controls food-chain properties in microbial communities. Nature 395: 495-497.
- Knoechel, R., and L. B. Holtby. 1986. Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. Limnology and Oceanography 31: 1-16.
- Lampert, W., and U. Sommer. 1997. Limnoecology: the ecology of lakes and streams. Oxford University Press, New York.
- Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. American Naturalist 134: 922-949.
- Leibold, M. A. 1996. A graphical model of keystone predators in food webs: trophic regulation of abundance, incidence and diversity patterns in communities. American Naturalist 147: 784-812.
- Leibold, M. A. 1999. Biodiversity and nutrient enrichment in pond plankton communities. Evolutionary Ecology Research 1: 73-95.
- Leibold, M. A., J. M. Chase, J. B. Shurin, and A. L. Downing. 1997. Species turnover and the regulation of trophic structure. Annual Review of Ecology and Systematics 28: 467-494.
- Leibold, M. A., and H. M. Wilbur. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature 360: 341-343.
- Levin, B. R., F. M. Stewart, and L. Chao. 1977. Resource-limited growth, competition, and predation: a model and experimental studies with bacteria and bacteriophage. American Naturalist 111: 3-24.
- Lubchenco, J. 1978. Plant species diversity in a marine intertidal community: importance of herbivore food preference and algal competitive abilities. American Naturalist 112: 23-39.

- McCarthy, M. A., L. R. Ginzburg, and H. R. Akcakaya. 1995. Predator interference across trophic chains. Ecology 76: 1310-1319.
- McCauley, E., and F. Briand. 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. Limnology and Oceanography 24: 243-252.
- McCauley, E., and J. Kalff. 1981. Empirical relationships between phytoplankton and zooplankton biomass in lakes. Canadian Journal of Fisheries and Aquatic Sciences 38: 458-463.
- McCauley, E., W. W. Murdoch, and S. Watson. 1988. Simple models and variation in plankton densities among lakes. American Naturalist 132: 383-403.
- McNaughton, S. J., M. Oesterheld, D. A. Frank, and K. J. Williams. 1989. Ecosystemlevel patterns of primary productivity and herbivory in terrestrial habitats. Nature 341: 142-144.
- McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Canadian Journal of Fisheries and Aquatic Sciences 43: 1571-1581.
- Mittelbach, G. G., C. W. Osenberg, and M. A. Leibold. 1988. Trophic relations and ontogenetic niche shifts in aquatic ecosystems. Pages 219-235 in B. Ebenman and L. Persson, editors. Size structured populations. Springer-Verlag, Berlin, Germany.
- Mourelatos, S., and G. Lacroix. 1990. In situ filtering rates of Cladocera: effect of body length, temperature, and food concentration. Limnology and Oceanography 35: 1101-1111.
- Murdoch, W. W., and E. McCauley. 1985. Three distinct types of dynamic behaviour shown by a single planktonic system. Nature 316: 628-630.
- Neill, W. E. 1975a. Resource partitioning by competing microcrustaceans in stable laboratory microecosystems. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 19: 2885-2890.
- Neill, W. E. 1975b. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology 56: 809-826.
- Oksanen, L., S. D. Fretwell, J. Arruda, and P. Niemela. 1981. Exploitation ecosystems in gradients of primary productivity. American Naturalist 118: 240-261.

- Oksanen, L., M. E. Power, and L. Oksanen. 1995. Ideal free habitat selection and consumer-resource dynamics. American Naturalist 146: 565-585.
- Oksanen, L., and T. Oksanen. 2000. The logic and realism of the hypothesis of exploitation ecosystems. American Naturalist 155: 703-723.
- Osenberg, C. W., and G. G. Mittelbach. 1996. The relative importance of resource limitation and predator limitation in food chains. Pages 134-148 in G. A. Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics. Chapman and Hall, New York, USA.
- Pace, M. L. 1984. Zooplankton community structure, but not biomass, influences the phosphorus-chlorophyll *a* relationship. Canadian Journal of Fisheries and Aquatic Sciences 41: 1089-1096.
- Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnology and Oceanography 33: 823-847.
- Paine, R. T. 1966. Food web complexity and species diversity. American Naturalist 100: 65-75.
- Persson, L., G. Andersson, S. F. Hamrin, and L. Johansson. 1988. Predator regulation and primary production along the productive gradient of temperate lake ecosystems. Pages 45-65 in S. R. Carpenter, ed. Complex interactions in lake communities. Springer, New York.
- Persson, L., J. Bengtsson, B. A. Menge, and M. E. Power. 1996. Productivity and consumer regulation-concepts, patterns, and mechanisms. Pages 396-434 in G. A.
  Polis and K. O. Winemiller, editors. Food webs: integration of patterns and dynamics. Chapman and Hall, New York, USA.
- Persson, L., S. Diehl, L. Johansson, G. Andersson, and S. F. Hamrin. 1992. Trophic interactions in lake ecosystems: a test of food chain theory. American Naturalist 140: 59-84.
- Polis, G. A., and D. R. Strong. 1996. Food web complexity and community dynamics. American Naturalist 147: 813-846.
- Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy? Ecology 73: 733-746.
- Reynolds, C. S. 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, U.K.

- Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among Cladocera: effect of different levels of food supply. Oikos 44: 243-252.
- Sarnelle, O. 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. Ecology 73: 551-560.
- Sarnelle, O. 1994. Inferring process from pattern: trophic level abundances and imbedded interactions. Ecology 75: 1835-1841.
- Sommer, U. 1988. Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnology and Oceanography 33: 1037-1054.
- Sterner, R. W., J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17: 49-67.
- Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25: 1-29.
- Strong, D. R. 1992. Are trophic cascades all wet? Differentiation and donor-control in speciose ecosystems. Ecology 73: 747-754.
- Turner, A. M., and G. G. Mittelbach. 1992. Effects of grazer community composition and fish on algal dynamics. Canadian Journal of Fisheries and Aquatic Sciences 49: 1908-1915.
- Vance, R. R. 1978. Predation and resource partitioning in one-predator-two prey model communities. American Naturalist 112: 797-813.
- Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology 68: 624-635.
- Watson, S., E. McCauley, and J. A. Downing. 1992. Sigmoid relationships between phosphorus, algal biomass, and algal community structure. Canadian Journal of Fisheries and Aquatic Sciences 49: 2605-2610.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnology and Oceanography 39: 1985-1992.
- White, T. C. R. 1978. The importance of a relative shortage of food in animal ecology. Oecologia 3: 71-86.

Table 1. Univariate repeated measures ANOVA testing for effects of predator identity, prey heterogeneity, nutrient enrichment, and time (i.e. sample date) on total chlorophyll a, for days 14 to 42.

Source of variation	df	MS	F	Р	
Univariate, between subjec	ts effects				
Predator identity	1	0.042	3.63	0.075	
Prey heterogeneity	1	0.240	20.82	<0.001	
Nutrients	1	0.575	49.85	<0.0001	
Predator X Prey het.	1	0.031	2.72	0.119	
Predator X Nutrients	1	0.084	7.31	0.016	
Prey het. X Nutrients	1	0.057	4.93	0.041	
Predator X Prey het.	1	0.031	2.71	0.119	
X Nutrients					
Error	16	0.012			

## Table 1. (continued)

Source of variation	df	MS	F	P (G-G)	P (H-F)		
Univariate, within subjects effects							
Time (T)	4	0.122	22.00	<0.0001	<0.0001		
T X Predator identity	4	0.003	0.62	0.584	0.649		
T X Prey Heterogeneity	4	0.227	40.97	<0.0001	<0.0001		
T X Nutrients	4	0.039	7.07	<0.001	<0.0001		
T X Pred. X Prey het.	4	0.007	1.20	0.317	0.317		
T X Pred. X Nutrients	4	0.005	0.87	0.451	0.486		
T X Prey het. X Nutrients	4	0.066	11.85	<0.0001	<0.0001		
T X Pred. X Prey het.	4	0.006	1.06	0.370	0.384		
X Nutrients							
Error	64	0.006					

Table 2. Results of three-way analysis of variance testing for effects of predator identity, prey heterogeneity, and nutrient enrichment on total chlorophyll *a* on the final sample date.

Source of variation	df	MS	F	Р
Predator identity	1	0.012	1.24	0.283
Prey heterogeneity	1	0.830	86.36	<0.0001
Nutrients	1	0.387	40.21	<0.0001
Predator X Prey het.	1	0.020	2.12	0.165
Predator X Nutrients	1	0.018	1.92	0.185
Prey Het. X Nutrients	1	0.268	27.82	<0.0001
Predator X Prey het.	1	0.043	4.43	0.051
X Nutrients				
Error	16	0.01		

Table 3. Univariate repeated measures ANOVA testing for effects of predator identity, prey heterogeneity, nutrient enrichment, and time (i.e. sample date) on predator biomass over the course of the experiment (days 14 to 42).

••••••••••••••••••••••••••••••••••••••				
Source of variation	df	MS	F	Р
Univariate, between subjec	ts effects			
Predator identity	1	0.126	46.44	<0.0001
Prey heterogeneity	1	0.001	0.29	0.595
Nutrients	1	0.296	109.03	<0.0001
Predator X Prey het.	1	0.002	0.68	0.422
Predator X Nutrients	1	0.084	30.77	<0.0001
Prey het. X Nutrients	1	0.005	1.82	0.197
Predator X Prey het.	1	0.004	1.48	0.241
X Nutrients				
Error	16	0.003		

# Table 3. (continued)

		· · · · · · · · · · · · · · · · · · ·					
Source of variation	df	MS	F	P (G-G)	P (H-F)		
Univariate, within subjects effects							
Time (T)	4	0.017	12.64	<0.0001	<0.0001		
T X Predator identity	4	0.008	5.99	0.004	0.0005		
T X Prey Heterogeneity	4	0.010	7.01	0.002	<0.001		
T X Nutrients	4	0.008	5.61	0.006	<0.001		
T X Pred. X Prey het.	4	0.002	1.35	0.274	0.263		
T X Pred. X Nutrients	4	0.006	4.58	0.014	0.003		
T X Prey het. X Nutrients	4	0.006	4.28	0.018	0.005		
T X Pred. X Prey het.	4	0.002	1.31	0.283	0.276		
X Nutrients							
Error	64	0.001					

Figure 1. Algal-prey response over the course of the experiment in the presence of each predator and at low or high productivity for (A) food chains (single algal-prey) and (B) food webs (heterogeneous algal-prey). Shown are means and standard errors.


Figure 1

Figure 2. Algal biomass on the final day of the experiment in the presence of (A) Daphnia predators or (B) Ceriodaphnia predators. Treatments shown are algal heterogeneity (chains versus webs) and productivity (low versus high). Shown are means and standard errors.



Figure 2

Figure 3. Responses of "edible" (<35 micron) and "resistant" (>35 micron) size fractions of algae over time at low or high productivity for (A) *Daphnia* treatments and (B) *Ceriodaphnia* treatments. Initial (day 0) algal samples were not size fractionated. Shown are mean and standard errors.







Figure 4. Dynamics of relative biomass of predator-resistant algae (in food webs) over the course of the experiment. Shown are mean responses to predator identity and productivity. Bars are standard errors.

Figure 5. Predator biomass response over the course of the experiment in food webs or food chains, and at low or high productivity levels, for (A) *Daphnia* and (B) *Ceriodaphnia*. Note differences in scale. Shown are means and standard errors.



Figure 5

### **CHAPTER 3**

# CONTEXT-DEPENDENT EFFECTS OF DAPHNIA PULEX ON POND ECOSYSTEM FUNCTION: OBSERVATIONAL AND EXPERIMENTAL EVIDENCE

### Abstract

Large-bodied zooplankton of the genus *Daphnia* are regarded as potential keystone species in freshwater pelagic habitats. Daphnia are thought to exert stronger top-down control and enhance or alter nitrogen-phosphorus limitation of algae, compared to other species of zooplankton. However, experimental exploration of these presumed properties via manipulations of *Daphnia* presence/absence is currently lacking. In the present study I examined in fishless ponds the degree to which Daphnia differ from small-bodied zooplankton in their effects on algal biomass, C:N:P content of the seston (as a measure of nutrient limitation), and total nitrogen and total phosphorus. This was done with both observational evidence from natural ponds and in situ experimental manipulations in which Daphnia pulex was compared with a diverse assemblage of small-bodied zooplankton. The role of environmental context was also explored experimentally by comparing zooplankton effects under low and high productivity conditions and in two different ponds. Experimental and observational results indicated that productivity can mediate Daphnia effects on algae. In natural ponds, Daphnia dominance resulted in a significantly weaker chlorophyll-total phosphorus relationship compared to small zooplankton dominated ponds, with the degree of divergence being greatest in high productivity systems. Experimental results showed that Daphnia exerted stronger top-down control in high productivity treatments only and exhibited a trend to graze algae to lower levels in the higher productivity pond. However, productivity by

zooplankton interactions were transient and algal biomass was similar in both *Daphnia* and small zooplankton treatments by the end of the experiment. Dynamics of C:N:P ratios over the course of the experiment did not reveal strong effects of zooplankton composition. However, ratios on the final date of the experiment provided evidence that *Daphnia* can enhance phosphorus limitation of algae; TP was lower and C:P higher in *Daphnia* treatments. N:P was also higher in *Daphnia* treatments, but only in one pond. Survey results revealed no effects of *Daphnia pulex* on seston C:N:P, suggesting that this species may not be an important factor governing the relative importance of nitrogen and phosphorus limitation of algae in natural ponds.

*Keywords*: algae, carbon, *Daphnia*, ecosystem function, keystone species, nitrogen, nutrient cycling, phosphorus, ponds, size structure, stoichiometry, zooplankton.

## Introduction

Recently the field of ecology has seen an upsurge of interest in the effects of species, species richness and functional groups on ecosystem function and stability (Schulze and Mooney 1993, Naeem et al. 1994, 1995, Tilman and Downing 1994, McGrady-Steed et al. 1997, Hulot et al. 2000). This research has been spurred in large part by practical needs, for gaining insight into such relationships undoubtedly holds important implications for how we manage natural resources as well as our ability to predict the outcome of environmental impacts and species losses. Traditionally, important or "keystone" species have been distinguished by their inordinately large effects on community properties relative to other species. Such key species may exact effects

through their ability to dominate community biomass (a numerical effect) or through extraordinarily high per capita effects. While conventionally measured at the level of communities (e.g. effects on species composition or richness), the presence of such species could have important repercussions on ecosystem-level properties, causing the relationship between species richness and ecosystem function to be disjunct. It is evident that our ability to comprehend and predict the consequences of variable species composition on ecosystem properties may depend vitally on identification and experimental validation of potential keystone species (Power et al. 1996).

In temperate freshwater systems, zooplankton body size is often considered to be an important determinant of planktonic community and ecosystem properties (e.g. Pace 1984, Vanni 1987, Elser et al. 1988, Quiros 1990, Mazumder 1994a, 1994b, Cottingham 1999). Commonly, large-bodied zooplankton of the genus *Daphnia* are thought to control total primary production more effectively than other zooplankton taxa, such as smallbodied cladocera or copepods (Leibold 1989, Mazumder 1994a, 1994b, 1994c, Cottingham and Schindler 2000), earmarking *Daphnia* as key components of trophic cascades and successful bio-control of algal blooms. Moreover, recent work has also focused on the carbon, phosphorus and nitrogen composition of zooplankton and the implications of among species variation in elemental stoichiometry on nutrient recycling. With few exceptions, *Daphnia* tissues appear to have the lowest carbon to phosphorus and nitrogen to phosphorus ratios among freshwater zooplankton (Sterner et al. 1992, Sterner and Hessen 1994, Elser and Urabe 1999). As a consequence of higher demands, *Daphnia* may sequester greater amounts of phosphorus relative to nitrogen, leading to

higher N to P excretion rates compared to less phosphorus demanding species of zooplankton (Sterner 1990, Sterner and Hessen 1994).

Taken together, these attributes single out *Daphnia* as potential keystone species whose presence can have significant impacts on the larger aquatic community and its ecosystem-level properties. While suspected, the reality of this assertion has rarely been tested via direct experimental manipulation of Daphnia in the field. For example, a number of lake studies have compared nitrogen and phosphorus excretion by Daphnia versus zooplankton species with higher N:P ratios (e.g. copepods). These studies have largely confirmed theorized predictions; Daphnia recycle nitrogen at greater rates than phosphorus (reviewed in Elser and Urabe 1999). However, experimental elucidation of the biological relevance at the ecosystem-level of such differential recycling (i.e. actual effects on algal assemblages in regards to elemental composition or nutrient limitation) is still in its formative stages (Elser and Urabe 1999). Furthermore, while indirect, observational evidence of strong top-down effects by Daphnia on primary producers is abundant (e.g. Mazumder 1994a, 1994c), there have been remarkably few investigations that have directly manipulated zooplankton composition with the intent of examining differences among taxa. Frequently, studies that have "manipulated" Daphnia presence or zooplankton size-structure have done so indirectly by manipulating the presence of planktivorous fish (e.g. Mazumder 1994b, and the studies analyzed in Sarnelle 1992 and Cottingham 1999), thus introducing potential confounding effects and experimental artifacts (e.g. nutrient recycling by fish; Vanni and Layne 1997). Those few investigations that have directly manipulated zooplankton composition have found highly variable effects of *Daphnia* presence on algal standing crop, with some experiments

finding small or insignificant differences (Turner and Mittelbach 1992, Brett et al. 1994), some revealing strong effects (Schoenberg and Carlson 1984, Rothaupt 1997), and some finding effects that were highly dependent on ecological context (Vanni 1984, Leibold and Wilbur 1992, Steiner in press). The specific circumstances that mediate the strength of *Daphnia* top-down control are poorly known. Yet, a number of studies, both experimental and observational, suggest that *Daphnia* may exert stronger effects under high productivity conditions (Vanni 1984, Sarnelle 1992, Mazumder 1994a, Steiner in press). Moreover, our understanding of *Daphnia* impacts has been largely limited to studies of lakes. The functional role that these species play in shallower water bodies such as ponds is generally unknown.

In the following paper I report on an experiment in which I assessed the effects of *Daphnia pulex* on pond ecosystem function- specifically algal standing crop, nitrogen and phosphorus content at the scale of the entire water column (TN and TP), and seston stoichiometry (C:N:P ratios) as an indicator of resource supply rates and nutrient limitation of algal growth. *Daphnia* alone were compared to a diverse assemblage of taxa composed of small-bodied cladocera, copepods, and rotifers. To explore the effect of environmental context, the experiment was performed in two fishless ponds that varied naturally in their ambient zooplankton communities. These included a pond that naturally lacked *Daphnia* and a pond dominated by *Daphnia*. Furthermore, the interactive effects of productivity and zooplankton composition on top-down control of algae were explored by comparing *Daphnia* and small-bodied zooplankton under nutrient enriched and unenriched conditions. To further investigate the effects of zooplankton composition and *Daphnia* dominance, I also surveyed natural ponds to examine the natural relationships

between *Daphnia* abundance and primary production and seston C:N:P. The main intent of this research was to evaluate the degree to which *Daphnia* compared to alternate taxa truly differ in their effects on these ecosystem functions and the ability for small-bodied species to compensate in the absence of this presumed strong interactor.

## Methods

# Field Experiment

The experiment was performed in August of 1998, in two fishless ponds (P12 and P14) at the W. K. Kellogg Biological Station, experimental pond facility (Hickory Corners, MI). Both ponds are 1.6 meters deep at their centers, with a surface area of approximately 700 m<sup>2</sup>. Though both ponds were fishless, zooplankton composition in the two differed greatly in the year of the study. In P12, *Daphnia pulex* were present and dominant for the majority of the summer, whereas the P14 zooplankton community exhibited a seasonal loss of *Daphnia* and was dominated by copepods and small-bodied cladocera (primarily *Diaphanosoma brachyurum*) during the entire growing season. Additional biotic and abiotic measures for P12 and P14 at the initiation of the experiment are presented in table 1.

The experiment was performed using 1200-liter polyethylene "bag" enclosures, sealed at their bottoms and suspended in the water column using floating frames. Bags were approximately 1m in diameter, extended to pond bottoms and were screened on their tops to exclude insects. To explore effects of zooplankton composition, two treatments were employed: *Daphnia pulex* alone and an assemblage of copepods, rotifers, and small-bodied cladocera alone (hereafter collectively referred to as "small" or "small-bodied" zooplankton for simplicity). *Daphnia pulex* was chosen since it is the dominant

Daphnia species found in permanent, fishless ponds in southwest Michigan (comprising, on a biomass basis, greater than 99% of *Daphnia* found in regional pond surveys; see below). The design was executed in both P12 and P14 concurrently. To explore the interaction of productivity and zooplankton composition on algal biomass, "Daphnia alone" and "small zooplankton" treatments were crossed with two productivity treatments ("low" and "high") in P14 only. Productivity manipulations consisted of additions of phosphorus (Na<sub>2</sub>HPO<sub>4</sub>) with high productivity treatments receiving an initial concentration of 75 µg P per liter and low productivity treatments receiving no additions for an initial concentration of 16 µg P per liter. Nitrogen (NaNO<sub>3</sub>) was added with phosphorus in a 70:1 N to P molar ratio, matched to that of the ambient pond water (based on TP:TN measurements made earlier in the growing season) to minimize changes in algal stoichiometry. Nutrients were added once, at the start of the experiment. All treatments were replicated three times for a total of 18 enclosures. All bags were filled by pumping water from their respective ponds through an 80-micron zooplankton net to remove the ambient zooplankton community and invertebrate predators. Small-bodied zooplankton were collected from a pond at the experimental facility that contained a population of pumpkinseed sunfish (Lepomis gibbosus). Thus, this pond contained no Daphnia and no invertebrate predators due to fish predation. Small zooplankton were collected using repeated vertical tows with a 150-micron plankton net. The isolated assemblage contained all the major small-bodied taxa found in the experimental ponds during the summer season, including calanoid and cyclopoid copepods, *Diaphanosoma* brachyurum, Ceriodaphnia quadrangula, Chydorus sphaericus, Bosmina longirostris, and numerous species of rotifers (dominated primarily by species of Keratella,

*Hexarthra*, *Lecane* and *Trichocerca*). To ensure their availability at the time of the experiment, *Daphnia pulex* were lab reared. *Daphnia* were isolated from several experimental ponds and cultures were initiated with numerous individuals to promote clonal diversity. *Daphnia* were maintained in batch cultures under high food conditions for two months prior to the experiment. One day after bags were filled with water, enclosures were inoculated with the same total biomass density of zooplankton (0.030 mg/liter). Nutrients were then added two days after zooplankton additions and the experiment was allowed to run for 40 days.

Zooplankton and phytoplankton were measured weekly beginning on the twelfth day of the experiment. Zooplankton were sampled using an integrated tube sampler and were immediately preserved in acid Lugol's solution for later enumeration. For each sample, fifty randomly chosen individuals of each species were also measured to obtain biomass estimates using published length-mass regressions. Water samples were collected using integrated tube samplers, immediately chilled on ice, and later filtered onto Gelman A/E glass fiber filters for subsequent fluorometric measurement of chlorophyll a (sensu Welschmeyer 1994) as a correlate of algal biomass. Carbon, nitrogen, and phosphorus of the seston were analyzed for the first, third and fifth sample periods only (corresponding to days 12, 26, and 40). Water samples were collected from enclosures using a tube sampler, chilled on ice, and later filtered through a 60-micron mesh to remove zooplankton. Filtered water was then divided in two and filtered through two separate, precombusted A/E glass fiber filters. One filter was immediately frozen for analysis of particulate phosphorus (see below). The other filter was first fumed with concentrated HCl to remove carbon contributions from CaCO<sub>3</sub> and then frozen for later

analysis of nitrogen and carbon content using a CHN analyzer. Note that Gelman A/E filters have an approximate pore size of 1.0 micron, minimizing bacterial contributions to C, N, and P estimates. Water samples (250 ml total) for total phosphorus (TP) and total nitrogen (TN) analyses were collected on days 12 and 40 using a tube sampler and then frozen for later analysis. TP and particulate phosphorus were analyzed using the ammonium molybdate method following persulfate digestion (Wetzel and Likens 1991). TN was analyzed using second-derivative, scanning spectroscopy (Crumpton et al. 1992, Bachmann and Canfield 1996). To eliminate cross contamination among enclosures, separate sampling gear were used for all treatment combinations. Only algal biomass (chlorophyll *a*) and zooplankton were sampled from high productivity treatments.

In the following analyses, pond identity is treated as a treatment factor or fixed effect. Thus, in order to examine zooplankton and pond effects, the experiment is treated as a 2 x 2 factorial design (i.e. zooplankton manipulations crossed with pond identity). To explore the interaction between productivity and zooplankton composition on algal biomass, P14 enclosures (zooplankton composition crossed with productivity manipulations) were analyzed as a separate 2 x 2 factorial experiment. Phytoplankton, zooplankton, TP, TN, and C:N:P responses through time were analyzed using univariate repeated measures ANOVA (rm-ANOVA), excluding initial (day 0) values. Due to potential violations of the assumption of sphericity, Greenhouse-Geiser (G-G) and Huynh-Feldt (H-F) adjusted probabilities are used for repeated measures analyses. Total phosphorus and total nitrogen were only sampled on two dates, therefore adjusted pvalues were not generated for these analyses. Examination of treatment effects for single sample dates was performed using ANOVA. All values were log<sub>10</sub> transformed to

conform to assumptions of homogeneity of variances and statistics were performed using Systat Version 8.0 (SPSS Inc., 1998).

## Field Survey

During the same year of the experimental study, a field survey of fifteen natural ponds plus three ponds from the K.B.S. experimental facility (including P12 and P14) was performed to determine the natural relationships among zooplankton composition, zooplankton biomass, algal standing crop and seston C:N:P. Only fishless ponds were included in the survey. All ponds were found within a 60 mile radius around K.B.S. and contained water year-round in the one to two years prior to the study (personal observation; J. Chase, University of Pittsburgh, pers. comm.).

Ponds were sampled monthly beginning in late April and ending in late August. Low water levels, due to drought conditions, made sampling unfeasible in six ponds during the August sample period. Zooplankton were collected using an integrated tube sampler, preserved in acid Lugol's, and later enumerated. Macrozooplankton were identified to the genus or species level, excepting copepods, which were classed as calanoid or cyclopoid. Rotifers were generally identified to the genus or species level with the exception of members of the Notommatidae and Proalidae, which were identified to the family level, and the Bdelloidea which were identified to the level of order. Fifty randomly chosen individuals of each taxon were also measured to obtain biomass estimates. During each sample period water was collected and chilled for later analysis of chlorophyll *a*, total phosphorus, total nitrogen, and seston C:N:P. Protocols matched those in the experiment except water for C:N:P analyses was filtered through a 35-micron mesh (instead of 60 microns) to remove zooplankton.

For descriptive analyses, measures from each sample period and pond were treated as separate data points. Though repeated measures in time within single ponds are not independent, treating the data in this manner permits inclusion of the considerable temporal variation in zooplankton composition and biotic/abiotic makeup that these systems exhibit over the growing season (variation that would be lost if a single grand mean was used for each pond). Macrozooplankton were first placed into four categories: copepods (including calanoids and cyclopoids), small-bodied cladocera (primarily Ceriodaphnia, Diaphanosoma, Chydorus, and Bosmina), rotifers, and Daphnia pulex. Excepting two sample dates in two ponds, in which Daphnia ambigua and Daphnia dubia were observed, Daphnia pulex was the only Daphnia species encountered in the survey. When present, these two alternate Daphnia species only comprised a minor fraction of zooplankton biomass (<5%) and were thus excluded from analyses. The relationships between pond zooplankton composition, TP, TN, total chlorophyll, and C:N:P data were explored using least squares, linear regressions. All analyses were performed using Systat Version 8.0 (SPSS Inc., 1998).

#### Results

## Field Experiment: Productivity and Zooplankton Effects

Predictably, nutrient enrichment had a significant positive effect on zooplankton biomass (figure 1A; p=0.001,  $F_{1,8}$ =25.16, between subjects effect, rm-ANOVA), as did zooplankton treatments- small-bodied zooplankton attained higher biomass levels regardless of productivity (figure 1A; p<0.0001,  $F_{1,8}$ =54.18, between subjects effect, rm-ANOVA). Enrichment also altered the composition of the small zooplankton assemblage. While *Diaphanosoma* remained a major component in both nutrient treatments, in high nutrient enclosures *Bosmina* biomass was more clearly sustained throughout the experiment, as was *Ceriodaphnia* (a species largely absent in low nutrient enclosures) (figure 2A, 2B).

In keeping with previous investigations, productivity and zooplankton composition had an interactive effect on algal biomass. Examining the levels of chlorophyll response curves through time, a significant zooplankton by nutrient interaction was detected using rm-ANOVA (figure 3A; p=0.003,  $F_{1,8}$ =17.82, between subjects effect). The nature of the zooplankton by productivity interaction was clear when analyzing low and high nutrient treatments separately as two-way rm-ANOVA's. *Daphnia* and small-bodied zooplankton did not differ in their effects on algal biomass at low nutrient levels (figure 3A; p=0.532,  $F_{1,4}$ =0.468, between subjects effect) but chlorophyll *a* was lower in the presence of *Daphnia* in high nutrient treatments (figure 3A; p=0.004,  $F_{1,4}$ =34.04, between subjects effect). However, effects of zooplankton composition and enrichment changed through time (G-G p=0.034, H-F p=0.011,  $F_{4,32}$ =3.86, time x nutrient x zooplankton interaction within subjects effect, rm-

ANOVA). When slicing the data (i.e. examining sample dates separately using two-way ANOVA's), the interactive effect of enrichment and *Daphnia* presence was evident only in the first three sample dates (p<0.03 for all contrasts). By days 33 and 40, zooplankton effects and zooplankton by nutrient interactions were no longer significant (p>0.30 for all contrasts).

### Field Experiment: Pond and Zooplankton Effects

Zooplankton responses were dependent on pond identity and zooplankton treatment. Biomass responses were lower in P14 compared to P12 (figure 1B), as indicated by a significant effect of pond identity (p<0.001,  $F_{1,8}$ =48.83, between subjects effect, rm-ANOVA). After day 12, biomass was generally higher in treatments with small-bodied zooplankton, compared to Daphnia treatments (figure 1B); a significant between subjects zooplankton effect was detected (p<0.0001, F<sub>1.8</sub>=71.00, rm-ANOVA). However, there was also a significant zooplankton by pond interaction (p=0.006,  $F_{1,8}$ =8.12, between subjects effect, rm-ANOVA), signifying that the magnitude of zooplankton effects were dependent on pond identity, with differences between small zooplankton and Daphnia being larger in P14 (figure 1B). By mid experiment, small zooplankton treatments were dominated primarily by Diaphanosoma, followed by copepods (calanoid, cyclopoid, and nauplii combined), and Bosmina (figure 2B, 2C). Not shown are rotifers and other small-bodied cladocera (e.g. Chydorus and Ceriodaphnia in P12), which, by day 19, comprised a small fraction of total biomass (<5%) and often fell to densities below the limits of detection.

Focusing on zooplankton and pond effects on algal biomass, there were no significant between subjects effects of pond identity on chlorophyll. Yet, the shape of the algal response curves differed between ponds (figure 3B); a significant within subjects effect was detected (G-G p=0.005, H-F p=0.001,  $F_{4,32}$ =6.41, rm-ANOVA). Examining responses through time, there were no significant main effects of zooplankton on chlorophyll (p>0.10 for all between subjects or within subjects effects). However, there

was some indication of an interaction between zooplankton composition and pond identity, but the effect was not significant at the 0.05 level (p=0.0639,  $F_{1,8}$ =4.62, between subjects effect). To further explore this potential interaction, I performed separate repeated measures ANOVA's for each pond separately. This analysis provided some evidence for an effect of zooplankton composition on algal responses. Examining P12 first, there appeared to be a trend for lower algal biomass in the presence of *Daphnia* (figure 3B), though the effect was only significant at the p=0.052 level ( $F_{1,4}$ =7.53, between subjects effect). In contrast, zooplankton composition had no effect on chlorophyll *a* in P14 (figure 3B; p=0.53,  $F_{1,4}$ =0.47, between subjects effect).

Zooplankton effects on algal stoichiometry were complex and highly dynamic. Looking first at seston C:P ratios, both pond identity and zooplankton composition had significant effects on the level of response curves through time (i.e. between subjects effects, p<0.01, rm-ANOVA). In general, ratios were higher in P14 when compared to P12 (figure 4A). However, interpretation of results was hampered by the fact that response curves crossed; C:P ratios in the presence of *Daphnia* were lower than small zooplankton treatments on day 12 but this trend had reversed by the final date of the experiment (figure 4A). Because chlorophyll levels and zooplankton composition and biomass required several weeks to stabilize, I also analyzed ratios on the final date of the experiment as a closer approximation to steady state responses. C:P ratios on day 40 were higher in P14, compared to P12 (figure 5A; p=0.003,  $F_{1,8}$ =18.73, two-way ANOVA). What's more, there was a significant effect of zooplankton composition on seston C:P; ratios were higher in the presence of *Daphnia* (figure 5A; p=0.016,  $F_{1,8}$ =9.25, two-way ANOVA).

Much like C:P results, analysis of seston C:N over the course of experiment was complicated by crossing response curves (fig 4B). Zooplankton composition and pond identity both had significant between subjects effects (p<0.005, rm-ANOVA). However, there was also a significant time by zooplankton by pond interaction (G-G p=0.008, H-F p=0.007,  $F_{2,16}$ =6.83) indicating that the shapes of response curves were dependent on both factors. By the end of experiment (figure 5B), zooplankton effects had dissipated (p=0.42,  $F_{1,8}$ =0.73, two-way ANOVA) but C:N ratios were lower in P12 compared to P14 (p=0.017,  $F_{1,8}$ =9.11, two-way ANOVA).

There were no significant between subjects effects of pond identity or zooplankton composition on seston N:P using repeated measures (p>0.10). Nevertheless, the shapes of responses through time were dependent on both treatment factors as indicated by a significant time by zooplankton by pond interaction (figure 4C; G-G p=0.004, H-F p=0.001,  $F_{2,16}=10.77$ , rm-ANOVA). When analyzing N:P ratios on the final date of the experiment (figure 5C), there was a significant main effect of zooplankton composition (p=0.025,  $F_{1,8}=7.54$ ) in addition to a significant interaction between pond identity and zooplankton treatment (p=0.030,  $F_{1,8}=6.91$ , two-way ANOVA). When compared to *Daphnia* treatments, N:P ratios were lower in the presence of small-bodied zooplankton but only in P12 (figure 5C; p=0.022, Tukey's HSD). Zooplankton composition had no effect on N:P in P14 (p=1.00, Tukey's HSD).

Total phosphorus decreased in all enclosures over the course of the experiment (figure 6A). There was a significant within subjects effect of time when using repeated measures ANOVA (p=0.0002, F<sub>1.8</sub>=45.05). Significant between subjects effects of pond identity (p=0.003,  $F_{1,8}$ =18.52) and zooplankton composition (p=0.001,  $F_{1,8}$ =22.39) were also detected. Total phosphorus appeared to be higher in P12 and in small zooplankton treatments (figure 6A). However, a significant time by zooplankton within subjects effect was also detected (p=0.021,  $F_{1.8}=8.22$ ), indicating that TP exhibited larger decreases in Daphnia treatments. To further explore this time effect, I performed separate ANOVA's for day 12 and day 40 TP data. These analyses demonstrated that near the start of the experiment (day 12) TP did not differ between zooplankton treatments (p=0.13,  $F_{1,8}$ =2.92), but total phosphorus was higher in P12 (p=0.0001,  $F_{1,8}$ =47.85). By the end of the experiment (day 40), pond effects had disappeared (p=0.21,  $F_{1,8}=1.82$ ) but TP was lower in Daphnia treatments compared to small-bodied zooplankton (figure 6A; p=0.004,  $F_{1.8}=16.61$ ).

There were no significant between subjects effects of pond or zooplankton treatment on total nitrogen (p>0.10, rm-ANOVA). However, responses over time differed between ponds. TN decreased in P12 but remained the same or showed a trend to increase in P14 (figure 6B; within subjects pond effect, p=0.018,  $F_{1,8}$ =8.75, rm-ANOVA). When examining day 12 and day 40 responses separately, there were no effects of zooplankton treatment or pond identity on total nitrogen concentrations (p>0.10, ANOVA). Examining day 12, TN:TP molar ratios, there were no treatment effects near the start of the experiment (figure 6C; p>0.10 for all effects, two-way ANOVA). By day 40, a significant pond by zooplankton interaction was detected (p=0.011,  $F_{1,8}$ =10.87, two-way ANOVA); this interaction was also detected using repeated measures (p=0.011,  $F_{1,8}$ =10.65, between subjects effect). At the end of the experiment, TN:TP was significantly higher in *Daphnia* treatments but only in P12 (figure 6C; p=0.008, Tukey's HSD).

# Field Survey

Field surveys revealed a striking amount of variation in zooplankton community structure and *Daphnia* dominance. Of the 18 ponds examined, half showed a complete loss of *Daphnia* by mid-season and only a minority (5 ponds) exhibited *Daphnia* relative abundances greater than 50%, when averaging biomass over the growing season. Hence, a large amount of temporal and spatial variation in *Daphnia* incidence and relative biomass appears to be the norm in fishless ponds, despite the absence of planktivorous fish.

Log total chlorophyll *a* was regressed against the log of the biomass of each zooplankton category (see Methods), total nitrogen, and total phosphorus using multiple linear regression. As expected, chlorophyll was strongly and positively correlated with log total phosphorus, a general indicator of productivity (table 2A). However, chlorophyll was also negatively related to *Daphnia* biomass (table 2A). When residuals from the log chlorophyll-log TP linear regression were regressed against the percent relative biomass of each zooplankton category separately, a negative relationship was only detected for *Daphnia* (log<sub>10</sub>y = -0.327log<sub>10</sub>x + 0.115; p=0.0110,  $r^2$ =0.0763, n=84). To further explore

effects of Daphnia on primary production, I first classed data as originating from a "Daphnia dominant" sample if Daphnia relative biomass equaled or exceeded 50 percent of total zooplankton biomass or "Daphnia subdominant" if less than 50 percent. Daphnia dominance clearly altered the chlorophyll-total phosphorus relationship (figure 7A). When using log-log linear regressions, relationships were significant at the p<0.01 level for both *Daphnia* dominant and subdominant samples;  $r^2$  values were 0.736 and 0.268, respectively. However, the scaling factor (slope) from *Daphnia* dominant samples was significantly lower than the relationship from Daphnia subdominant samples (p<0.05, ttest), though intercepts were not significantly different (p>0.10, t-test). The relationship between zooplankton biomass and chlorophyll a was also strongly affected by Daphnia dominance. When taken as a whole (Daphnia dominant plus subdominant samples), there was no relationship between log total zooplankton biomass and log chlorophyll (p=0.306, linear regression), nor was there a relationship between zooplankton abundance from Daphnia subdominant samples and algal biomass (figure 7B; p=0.609). In contrast, zooplankton biomass was significantly and negatively related to chlorophyll when Daphnia were a dominant component of the zooplankton community (figure 7B; p=0.002,  $r^2=0.284$ ). Multiple linear regressions revealed no effects of zooplankton composition on secton C:N:P. The log of C:N, N:P, and C:P showed no significant relationships with biomass of any of the four zooplankton categories (table 2B, 2C, 2D).

### Discussion

Interest in the size structure of zooplankton communities and variable dominance by *Daphnia* has a long and well-known history in aquatic ecology. Early research focused heavily on the determinants of this variation, elucidating the importance of size-selective predation (e.g. Brooks and Dodson 1965, Hall et al. 1976, Zaret 1980, Gliwicz and Pijanowska 1989), the effects of resource quantity and quality (Gliwicz 1977, Threlkeld 1986, Romanovsky and Feniova 1985, Elser and Urabe 1999), and interspecific competition (Neill 1975, Lynch 1978, Smith and Cooper 1982, Romanovsky and Feniova 1985). However, a separate body of work has also developed centered on the flipside of this question- the community and ecosystem-level consequences of variation in *Daphnia* incidence and dominance (e.g. Leibold 1989, Sarnelle 1992, Mazumder 1994a, 1994b, Rothaupt 1997, Elser and Urabe 1999, Steiner in press).

As outlined in the Introduction, much evidence indicates that *Daphnia* can have large impacts on primary production and the efficacy of trophic cascades in lake ecosystems. As suggested by the experimental evidence provided in the present study, the reality of these assertions may hinge on the specific context in which zooplankton (Daphnia versus small-bodied taxa) effects are measured. Though apparently not a longlived effect, productivity appears to mediate the strength of *Daphnia*-algal interactions and, consequently, the degree of divergence between this large-bodied taxon and its smaller cohabitants. The underlying mechanisms for this effect are not certain. It is possible that Daphnia were better able to graze the algal assemblage present in high productivity manipulations. Compared to most small-bodied taxa, large Daphnia can achieve higher per capita filtration rates on small edible algae (Knoechel and Holtby 1986, Mourelatos and Lacroix 1990) and are known to feed on a much broader size spectrum of resources (Burns 1968, Neill 1975b). Hence, Daphnia can access large algal particles outside the range of small taxa- the very same algae generally favored under the combined action of grazing pressure and enrichment (Leibold 1989, Cottingham 1999,

Steiner in press). Unfortunately, algal composition was not quantified in my study and therefore the validity of this mechanism cannot be assessed. It is also possible that *Daphnia* were able to exact effects through a more rapid numerical/biomass response under high productivity conditions; by the first sample period *Daphnia* biomass was higher than total small zooplankton biomass (figure 1A). This rapid response may have resulted from a greater capacity of *Daphnia* to consume and convert the large spike in algal production following the initial nutrient pulse (an  $r_{max}$  strategy). Furthermore, this initial algal assemblage was likely of greater nutritional quality (i.e. possessed a lower C:P ratio) compared to phytoplankton in the low nutrient treatment; an attribute that should have benefited a potentially phosphorus-limited species such as *Daphnia pulex*.

Pond environment seemed to mediate the ability of *Daphnia* to control algal standing crop as well. Though not significant at the p=0.05 level, there was a trend for stronger *Daphnia* effects in P12, while effects of zooplankton composition in P14 were largely absent. Disparate biomass responses may largely explain these results; in P14, *Daphnia* attained biomass levels far lower than small-bodied zooplankton by the end of the experiment (figure 1B). In contrast, *Daphnia* abundance was higher and more clearly sustained in P12. Note, however, that small zooplankton still attained higher biomass levels than *Daphnia* in P12; that *Daphnia* could graze algae to levels lower than or comparable to small zooplankton despite lower biomass responses is indicative of higher per unit biomass feeding rates and/or lower recycling rates of limiting nutrients (see below).

The mechanisms underlying pond effects on zooplankton numerical responses are difficult to discern. P12 and P14 clearly differed in their natural capacities to support

Daphnia populations- Daphnia pulex dominated the ambient P12 zooplankton community but were entirely absent from P14 at the time of the experiment. Furthermore, the two ponds differed in a number of other biotic and abiotic features (table 1). Most notably, P12 was the more productive of the two- exhibiting higher levels of ambient TP, chlorophyll *a*, and zooplankton biomass (table 1). In contrast, the ponds did not vary greatly in terms of abiotic factors (pH, temperature) or algal stoichiometry. Taken together, these data suggest that resources (productivity and/or quality) may have mediated differential Daphnia performance and grazing impacts in experimental enclosures in the two ponds.

Results from my field survey provided additional evidence that *Daphnia* dominance is an important factor governing trophic-level biomass of algae in natural ponds. In multiple linear regressions, algae tended to decrease with increasing *Daphnia* biomass. Furthermore, the chlorophyll-phosphorus relationship exhibited a weaker relationship when *Daphnia* comprised a major portion of the zooplankton community, with divergences between *Daphnia* dominant and subdominant regression lines being strongest at the highest levels of TP (figure 7A). Numerous investigations have also explored trophic structure and inferred ecological processes by examining the relationship between the biomass of adjacent trophic levels (e.g. McCauley and Kalff 1981, McCauley et al. 1988, Ginzburg and Ackakaya 1992, Power 1992, Leibold et al. 1997). A well documented pattern in aquatic systems is the positive relationship between zooplankton and phytoplankton abundance (Leibold et al. 1997). A notable finding from my study was the clear absence of such a pattern in fishless ponds; overall grazer biomass and algal biomass were not correlated. More striking was the clear effect of *Daphnia* dominance on the relationship (figure 7B), again indicative of stronger top-down control.

When examining these results as a whole they suggest that *Daphnia* dominance can play an important role in determining standing crop biomass of primary producers in high productivity environments. Though the mechanisms underlying productivity effects require further study, this insight complements previous investigations that have shown that productivity may temper the effects of variable size-structure and Daphnia dominance on algal production. For example, in a recent literature review of algal biomass patterns in lakes and enclosures, Mazumder (1994a) found that the chlorophylltotal phosphorus relationship was weaker in large Daphnia dominated systems compared to small zooplankton systems and that divergences were greatest in high productivity (high TP) systems. Using experimental microcosms, Steiner (in press) found that Daphnia pulex and the small zooplankter Ceriodaphnia quadrangula only differed in their effects on algal biomass under high productivity conditions. Similarly, in his comparative study of a hypereutrophic and a mesotrophic lake, Vanni (1984) found that Daphnia pulex only exerted stronger top-down control in the higher productivity system. Furthermore, when adding nutrients to enclosures in the mesotrophic system, Daphnia were able to significantly graze algae to lower levels when compared to small-bodied species. Productivity effects may also explain disparate findings in other experimental studies as well. For example, Turner and Mittelbach (1992) found negligible differences between Daphnia pulex and Ceriodaphnia. However, their experiment was performed under relatively low productivity conditions (comparable to my low nutrient treatments). Similarly, Brett et al. (1994) found small differences between Daphnia and calanoid

copepods in their study of an oligo-mesotrophic lake (when effects were corrected for zooplankton biomass differences among treatments that likely resulted from differences in initial conditions). In contrast, Schoenberg and Carlson (1984) found large differences between *Daphnia* and the small-bodied *Bosmina* in their study of a highly productive, hypereutrophic lake. Though other contextual variables may be equally important - e.g. food web architecture (Leibold and Wilbur 1992) or algal diversity (Steiner in press)productivity may be key to predicting keystone effects of *Daphnia* on algal production.

While these results are compelling they must be reconciled with the clear temporal component to Daphnia's ability to control algal standing crop in the enrichment experiment. The diminution of zooplankton effects on chlorophyll nearing the termination of the experiment suggests that small-bodied zooplankton can control algae as effectively as *Daphnia* if given enough time to respond numerically. Cottingham et al. (1997) drew similar conclusions. It is possible though that this was partly an artifact of the enclosure environment. Thick periphyton growth was observed on high nutrient enclosure walls mid-experiment (this algal fraction was not quantified). If the observed drop and apparent convergence in planktonic algal biomass was hastened by periphyton growth it is likely that the enclosure environment amplified this effect due to the unnaturally high surface area to volume ratio. Potential artifacts aside, the experimental results seem to contradict the clear patterns seen in the field survey. However, we must keep in mind that the survey data (as displayed in figures 7A and 7B) are separate samples in time. Daphnia abundance and dominance, and thus their effects on algal biomass, vary greatly over the growing season within a given pond. Thus, there likely is a temporal component to Daphnia effects in natural pond systems as well. Does this

temporal aspect negate *Daphnia*'s position as a potential keystone grazer? It is difficult to determine for there is no clear criterion for how long a species must exert stronger effects for it to be deemed a keystone species. At the very least, *Daphnia* presence may be a key factor determining peak biomass of algae in natural ponds.

This experiment also explored the effect of zooplankton identity on nutrient pools and seston stoichiometry. The role of zooplankton composition in pelagic nutrient cycling is at present a central area of research in aquatic ecosystem ecology (Anderson 1997, Elser and Urabe 1999). The potential for different species of zooplankton to differentially excrete nitrogen and phosphorus is largely accepted. Yet, the biological significance of this phenomenon- i.e. the ability of planktonic grazers to alter elemental limitation of primary producers- is an important aspect to this dynamic whose validity and widespread applicability await thorough experimental validation.

My experiment provided some support for theoretical predictions. When measuring phosphorus and nitrogen content at the level of the entire water column (TP and TN), total phosphorus was lower in *Daphnia* treatments regardless of pond identity. TP, as measured in this study, encompassed several different nutrient pools in the water column, including suspended algae, bacteria, heterotrophs (zooplankton and protists), detrital matter, and dissolved forms. A net loss is indicative of sedimentation and/or a shunting of nutrients to an organic component not included in a sample of the water column habitat (e.g. algal growth on enclosure walls). While surface bound algae were likely present during the experiment, wall growth would have to have been higher in *Daphnia* treatments in both ponds for this mechanism to fully account for differences in TP. *Daphnia* did depress phytoplankton in P12 to a greater extent than small

zooplankton, which could have resulted in enhanced wall growth due to competitive release (surface bound algae are largely immune from grazing by the zooplankton taxa in this study). However, phytoplankton levels in the two zooplankton treatments were similar in P14, making differential wall production in *Daphnia* versus small zooplankton treatments unlikely. It is also possible that *Daphnia* densities fell below the limits of detection. By the end of the experiment, densities in P12 were approximately 6 individuals per liter and 1 individual per liter in P14, compared to small zooplankton densities of 385 and 78 individuals per liter in P12 and P14, respectively. Hence, a 250 ml water sample may have been inadequate to sample this "pool" of nutrients (i.e. "lost" phosphorus may have been bound in living zooplankton biomass in *Daphnia* treatments). Using an estimate of 1.58 percent phosphorus per unit zooplankton mass (the maximal level for a Daphnia species from table 1 of Sterner et al. 1992), Daphnia biomass at the end of the experiment could only have comprised a mean of 1.085 µg P/liter in P12 and 0.206 µg P/liter in P14; levels far too low to fully account for differences in TP among zooplankton treatments. Therefore sedimentation seems a more probable explanatory mechanism. TP may have been lost as phosphorus-rich Daphnia senesced and sank to enclosure bottoms over the course of the experiment. The lack of extensive mixing in the enclosure environment may have made much of this nutrient pool inaccessible to algae in the water column.

A more pertinent issue is the extent to which nutrient sequestration by zooplankton alters or enhances nutrient limitation of primary producers. Carbon, nitrogen, and phosphorus ratios of seston are useful indicators of nutrient limitation, with C:P ratios greater than 130 and N:P ratios greater than 22 being indicative of phosphorus

limitation and C:N ratios greater than 9 indicating nitrogen limited growth (Healey and Hendzel 1980, Hecky et al. 1993). When examining seston carbon-nutrient ratios, experimental results were equivocal at best. Dynamics of C:P, C:N, and N:P over the course of the experiment provided no clear support for predicted zooplankton compositional effects. However, ratios of C:P and N:P on the final date of the experiment matched theoretical predictions somewhat; C:P was higher in the presence of Daphnia in both ponds, signifying enhanced phosphorus limitation. N:P was also higher in the presence of Daphnia, but only in P12; differences between Daphnia and small-bodied zooplankton were not significant in P14. Thus, Daphnia effects on TP (and the absence of effects on TN) translated into differences in elemental composition of the seston. However, basing conclusions on ratios from the final sample date alone is only justified if these data are representative of steady state conditions. Transient zooplankton dynamics (especially small zooplankton composition) and chlorophyll a levels appeared to stabilize by the end of the experiment. Unfortunately, C:N:P data do not allow us to infer whether nutrient ratios had stabilized or were still in flux at the termination of the experiment. Consequently, these results must be viewed with some caution.

One can only speculate on reasons for the apparent weak effects on algal stoichiometry in my study. Theory predicts that differential N and P excretion by zooplankton is only probable under a limited range of extant seston C:N:P ratios and nutrient loading ratios (Sterner 1990). This has been cited as a reason for the absence of strong differential effects of zooplankton on N and P limitation in previous studies (Moegenburg and Vanni 1991, Vanni and Layne 1997, Elser and Urabe 1999). When compared to taxa with higher tissue C:P and N:P, *Daphnia* are expected to excrete lesser

amounts of phosphorus when seston C:P molar ratios are less than 250 (Hessen and Andersen 1992). Furthermore, zooplankton-mediated transitions between nitrogen and phosphorus limitation are only predicted for N:P loading ratios between 14-40:1 (Andersen 1997, Elser and Urabe 1999). This may explain, in part, the apparent absence of zooplankton effects in the field survey. Using TN:TP as an estimate of nutrient loading ratios, only 43% of the pond survey samples fell within the requisite 14-40:1 range. What's more, seston C:P ratios fell below 250 for only 32% of samples (indicative of strong phosphorus limitation in these systems). I reanalyzed the survey results by first restricting analyses to data with seston C:P ratios below 250. This did not expose any heretofore unseen zooplankton effects on C:N:P. I also re-explored data by restricting samples to those with TN:TP ratios between 14-40:1. This too did not alter initial conclusions.

It is also important to consider the complex nature of nutrient recycling and inputs within a pond ecosystem. Nutrient supply rates and resultant ratios within natural water column habitats integrate numerous physical factors and potential inputs. In addition to excretion by zooplankton, important factors include allochthonous nutrient sources, input from microbial decomposition, excretion from higher level consumers (e.g. predaceous insects), exchange with adjacent aquatic habitats (e.g. transport from the littoral to pelagic habitat), light limitation (which can alter carbon:nutrient ratios; Sterner et al. 1997) and mixing events, to name a few. It is perhaps not surprising that zooplankton had no effects on algal stoichiometry in natural pond environments. These systems are generally shallow (less than 2m deep) and thus "planktonic systems" are commonly found within a matrix of rooted vegetation, itself home to a complex food web including

periphyton. More importantly, the photic zone and mixing events in these shallow systems extend to nutrient rich sediments. Hence, any effects of zooplankton on nutrient supply may easily be overwhelmed by inputs from this source.

In contrast to natural habitats, the physical conditions found within the experimental enclosures should have accentuated the effects of nutrient recycling by grazers within the water column; natural sediment inputs were completely eliminated and mixing events were likely minimized due to low water surface areas. However, much like the ponds, natural seston stoichiometry and nutrient loading conditions may have weakened potential zooplankton effects. At the initiation of the experiment, seston C:P levels in P12 and P14 were 256 and 290, while TN:TP molar ratios were 50 and 84, respectively. Hence, near the start of the experiment ambient ratios were likely within the range at which zooplankton effects were minimal or completely absent; at these high levels of phosphorus limitation all zooplankton are expected to excrete phosphorus at equally low rates. As the experiment progressed, seston C:P in enclosures began to fall to levels at which effects of zooplankton identity should have been manifest. Indeed, seston C:P by the end of the experiment was indicative of diminished phosphorus supply in Daphnia treatments. However, TN:TP ratios remained outside the range at which zooplankton composition should have shifted algal limitation between N and P limited growth. However, P12 small zooplankton treatments stood out from the rest, having markedly lower TN:TP ratios. This was largely due to their high TP levels. These levels, while still higher than the requisite 40:1 ratio, may have been low enough to allow differences between *Daphnia* and small zooplankton N and P excretion to be observable as differences in seston N:P.
Is *Daphnia pulex* a keystone species? A truly rigorous test of this question is only afforded by comparing effects of all zooplankton taxa either in isolation or by experimentally removing each species in turn and observing changes in community/ecosystem attributes, a daunting task and one that is truly intractable if one considers the copious species pool of planktonic grazers found within any given region. A more amenable course of inquiry will likely be comparisons of the sort used here, in which a suspected key species is experimentally contrasted with one or several alternate species. Such data can be complemented with observational evidence to enhance the robustness of conclusions. True and indisputable determination of the "keystoneness" of such focal species will lie in the synthesis of many such experiments and surveys. In itself, the present study imparts a rather indeterminate conclusion; the Daphnia hegemony is not entire. Grazer control of primary production may depend critically on Daphnia presence in pond ecosystems, but only under a subset of natural conditions (e.g. high productivity systems). Both survey and experimental evidence point to this, in addition to previously published work. Furthermore, keystone effects of Daphnia on algal standing crop may be transient, consigned to certain times of the year in high productivity ponds (e.g. early in the growing season when spring mixing events create spikes in primary production). The capacity for *Daphnia* to alter algal nutrient limitation, and the conditions that give rise to or hinder this function, remain far from certain. Within certain contexts (specifically artificial habitats that preclude mixing and sediment input), Daphnia may enhance phosphorus limitation beyond the effects imposed by small-bodied taxa. In more realistic settings (i.e. natural ponds), nutrient loading ratios alone may relegate the possibility of any Daphnia effect to a small subset of water-bodies. Alternate

nutrient supply sources (e.g. sediments and mixing events), may render strong zooplankton effects moot in even these ponds. Far-reaching generalities about *Daphnia's* central position in aquatic ecosystem function then must be tempered by considerations of the interplay of context and the function in question.

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## **Literature Cited**

- Andersen, T. 1997. Pelagic nutrient cycles: herbivores as sources and sinks. Springer-Verlag, New York.
- Bachmann, R. W., and D. E. Canfield, Jr. 1996. Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. Hydrobiologia 323: 1-8.
- Brett, M. T., K. Wiackowski, F. S. Lubnow, A. Mueller-Solger, and C. R. Goldman. 1994. Species-dependent effects of zooplankton on planktonic ecosystem processes in Castle Lake, California. Ecology 75: 2243-2254.
- Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28-35.
- Burns, R. J. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnology and Oceanography 13: 675-678.
- Cottingham, K. L. 1999. Nutrients and zooplankton as multiple stressors of phytoplankton communities: evidence from size structure. Limnology and Oceanography 44: 810-827.
- Cottingham, K. L., S. E. Knight, S. R. Carpenter, J. J. Cole, M. L. Pace, and A. E. Wagner. 1997. Responses of phytoplankton and bacteria to nutrients and zooplankton: a mesocosm experiment. Journal of Plankton Research 19: 995-1010.
- Cottingham, K. L., and D. E. Schindler. 2000. Effects of grazer community structure on phytoplankton response to nutrient pulses. Ecology 81: 183-200.
- Crumpton, W. G., T. M. Isenhart, and P. D. Mitchell. Nitrate and organic N analyses with second derivative spectroscopy. Limnology and Oceanography 37: 907-913.
- Elser, J. J., M. M. Elser, N. A. MacKay, and S. R. Carpenter. 1988. Zooplanktonmediated transitions between N- and P-limited algal growth. Limnology and Oceanography 33: 1-14.
- Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: theory, observations, and consequences. Ecology 80: 735-751.
- Ginzburg, L. R., and H. R. Akcakaya. 1992. Consequences of ratio-dependent predation for steady-state properties of ecosystems. Ecology 73: 1536-1543.
- Gliwicz, Z. M. 1977. Food size selection and seasonal succession of filter feeding zooplankton in a eutrophic lake. Ekologia Polska A 25: 179-225.

- Gliwicz, Z. M. and Pijanowska, J. 1989. The role of predation in zooplankton succession
   In: Sommer, U. (ed.), Plankton ecology: succession in plankton communities.
  Springer-Verlag, New York, pp. 253-295.
- 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 Review of Ecology and Systematics 7: 177-208.
- Healey, F. P., and L. L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Journal of the Fisheries Research Board of Canada 37: 442-453.
- Hecky, R. E., P. Campbell, and L. L. Hendzel. 1993. The stoichiometry of carbon, nitrogen and phosphorus in particulate matter of lakes and oceans. Limnology and Oceanography 38: 709-724.
- Hessen, D. O., and T. Andersen. 1992. The algae-grazer interface: feedback mechanisms linked to elemental ratios and nutrient recycling. Archiv fur Hydrobiologie Ergebnisse der Limnologie 35: 111-120.
- Hulot, F. D., G. Lacroix, M. F. Lescher, and M. Loreau. 2000. Functional diversity governs ecosystem response to nutrient enrichment. Nature 405: 340-344.
- Knoechel, R., and L. B. Holtby. 1986. Construction and validation of a body-length-based model for the prediction of cladoceran community filtering rates. Limnology and Oceanography 31: 1-16.
- Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the outcome of trophic interactions. American Naturalist 134: 922-949.
- Leibold, M. A., J. M. Chase, J. B. Shurin, and A. L. Downing. 1997. Species turnover and the regulation of trophic structure. Annual Review of Ecology and Systematics 28: 467-494.
- Leibold, M. A., and H. M. Wilbur. 1992. Interactions between food-web structure and nutrients on pond organisms. Nature 360: 341-343.
- Lynch, M. 1978. Complex interactions between natural coexploiters-*Daphnia* and *Ceriodaphnia*. Ecology 59: 552-564.
- Mazumder, A. 1994a. Patterns of algal biomass in dominant odd- vs. even-link lake ecosystems. Ecology 75: 1141-1149.
- Mazumder, A. 1994b. Phosphorus-chlorophyll relationships under contrasting zooplankton community structure: potential mechanisms. Canadian Journal of Fisheries and Aquatic Science 51: 401-407.

- Mazumder, A. 1994c. Phosphorus-chlorophyll relationships under contrasting herbivory and thermal stratification: predictions and patterns. Canadian Journal of Fisheries and Aquatic Science 51: 390-400.
- McCauley, E., and J. Kalff. 1981. Empirical relationships between phytoplankton and zooplankton biomass in lakes. Canadian Journal of Fisheries and Aquatic Sciences 38: 458-463.
- McCauley, E., W. W. Murdoch, and S. Watson. 1988. Simple models and variation in plankton densities among lakes. American Naturalist 132: 383-403.
- McGrady-Steed, J., P. M. Harris, and P. J. Morin. 1997. Biodiversity regulates ecosystem predictability. Nature 390: 162-165.
- Moegenburg, S. M., and M. J. Vanni. 1991. Nutrient regeneration by zooplankton: effects on nutrient limitation of phytoplankton in a eutrophic lake. Journal of Plankton Research 13: 573-588.
- Mourelatos, S., and G. Lacroix. 1990. In situ filtering rates of Cladocera: effect of body length, temperature, and food concentration. Limnology and Oceanography 35: 1101-1111.
- Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1994. Declining biodiversity can alter the performance of ecosystems. Nature 368: 734-737.
- Naeem, S., L. J. Thompson, S. P. Lawler, J. H. Lawton, and R. M. Woodfin. 1995. Empirical evidence that declining species diversity may alter the performance of terrestrial ecosystems. Philosophical Transactions of the Royal Society of London, B 347: 249-262.
- Neill, W. E. 1975a. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology 56: 809-826.
- Neill, W. E. 1975b. Resource partitioning by competing microcrustaceans in stable laboratory microecosystems. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 19: 2885-2890.
- Pace, M. L. 1984. Zooplankton community structure, but not biomass, influences the phosphorus-chlorophyll *a* relationship. Canadian Journal of Fisheries and Aquatic Sciences 41: 1089-1096.
- Power, M. E. 1992. Top-down and bottom-up forces in food webs: do plants have primacy? Ecology 73: 733-746.

- Power, M. E., D. Tilman, J. A. Estes, B. A. Menge, W. J. Bond, L. S. Mills, G. Daily, J. C. Castilla, J. Lubchenco, and R. T. Paine. 1996. Challenges in the quest for keystones. BioScience 46: 609-620.
- Quiros, R. 1990. Factors related to variance of residuals in chlorophyll-total phosphorus regressions in lakes and reservoirs of Argentina. Hydrobiologia 200/201: 343-355.
- Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among Cladocera: effect of different levels of food supply. Oikos 44: 243-252.
- Rothhaupt, K. O. 1997. Grazing and nutrient influences of *Daphnia* and *Eudiaptomus* on phytoplankton in laboratory microcosms. Journal of Plankton Research 19: 125-139.
- Sarnelle, O. 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. Ecology 73: 551-560.
- Schoenberg, S. A., and R. E. Carlson. 1984. Direct and indirect effects of zooplankton grazing on phytoplankton in a hypereutrophic lake. Oikos 42: 291-302.
- Schulze, E. D., and H. A. Mooney. 1993. Biodiversity and ecosystem function. Springer-Verlag, New York.
- Smith, D. W., and S. D. Cooper. 1982. Competition among cladocera. Ecology 63: 1004-1015.
- Steiner, C. F. The effects of prey heterogeneity and consumer identity on the limitation of trophic-level biomass. Ecology (*in press*).
- Sterner, R. W. 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: zooplankton and the algal competitive arena. American Naturalist 136: 209-229.
- Sterner, R. W., J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17: 49-67.
- Sterner, R. W., J. J. Elser, E. J. Fee, S. J. Guildford, and T. H. Chrzanowski. 1997. The light:nutrient ratio in lakes: The balance of energy and materials affects ecosystem structure and process. American Naturalist 150: 663-684.
- Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25: 1-29.
- Threlkeld, S. T. 1986. Resource-mediated demographic variation during the midsummer succession of a cladoceran community. Freshwater Biology 16: 673-683.

- Tilman, D., and J. A. Downing. 1994. Biodiversity and stability in grasslands. Nature 367: 363-365.
- Turner, A. M., and G. G. Mittelbach. 1992. Effects of grazer community composition and fish on algal dynamics. Canadian Journal of Fisheries and Aquatic Sciences 49: 1908-1915.
- Vanni, M. J. 1984. Biological control of nuisance algae by *Daphnia pulex*: experimental studies. –In: "Lake and reservoir management: Proceedings of the third annual conference, North American Lake Management Society." United States Environmental Protection Agency, Washington D. C. pp. 151-156.
- Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology 68: 624-635.
- Vanni, M. J., and C. D. Layne. 1997. Nutrient recycling and herbivory as mechanisms in the "top-down" effect of fish on algae in lakes. Ecology 78: 21-40.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnology and Oceanography 39: 1985-1992.
- Wetzel, R. G., and G. E. Likens. 1991. Limnological Analyses, 2<sup>nd</sup> Edition. Springer-Verlag, New York.
- Zaret, T. M. 1980. Predation and freshwater communities. Yale University Press, New Haven.

Table 1. Limnological characteristics of experimental ponds P12 and P14 at the initiation of the experiment.

Pond	рН	Oxy. (mg/l)	Temp.	Chl-a (µg/l)	Zoop. (mg/l)	Daphnia (mg/l)
P12	7.18	9.7	23	6.52	0.342	0.3
P14	7.9	9.24	24	2.62	0.048	0

Table 1. (cont'd)

Pond	TP (µg/l)	TN (mg/l)	TN:TP	C:N	C:P	N:P
P12	25.71	577.1298	49.645	13.11	284.16	21.68
P14	16.32	619.36	83.9178	11.3	256.41	22.7

Table 2. Results of multiple linear reg	gressions of pond ecosystem variables.
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	Effect	Coeff.	Std. Error	р	Overall p	r <sup>2</sup>
a. Dependent: Log <sub>10</sub> Chl-a					< 0.00001	0.741
Cor	nstant	-0.997	0.590	0.0965		
Log	g Daphnia	-0.0539	0.0184	0.00501		
Log	g Rotifers	0.0973	0.0319	0.00348		
Log	g Small Clad.	-0.0354	0.0294	0.233		
Log	g Copepods	-0.0941	0.0674	0.168		
Log	g TP	1.0381	0.106	< 0.00001		
Log	g TN	0.0451	0.197	0.820		
b. Dependent: Log <sub>10</sub> C:P (molar)				0.138	0.0834	
Co	nstant	2.405	0.0942	< 0.00001		
Log	g Daphnia	0.00734	0.00830	0.379		
Log	g Rotifers	-0.0203	0.0123	0.103		
Log	g Small Clad.	0.0151	0.0125	0.231		
Log	g Copepods	-0.0316	0.0292	0.282		
c. Dependent: Log <sub>10</sub> C:N (molar)			0.297	0.0595		
Co	nstant	0.897	0.0832	< 0.00001		
Log	g Daphnia	-0.00660	0.00733	0.374		
Log	g Rotifers	0.00862	0.0109	0.431		
Log	g Small Clad.	0.00360	0.0110	0.744		
Log	g Copepods	-0.0513	0.0258	0.050		
d. Dependent: Log <sub>10</sub> N:P (molar)			0.189	0.0739		
Co	nstant	1.508	0.117	< 0.00001		
Log	g Daphnia	0.0139	0.0103	0.181		
Log	g Rotifers	-0.0290	0.0153	0.0620		
Log	g Small Clad.	0.0114	0.0155	0.462		
Log	g Copepods	0.0197	0.0363	0.589		

Figure 1. Responses of *Daphnia pulex* and small-bodied zooplankton (all taxa combined) to (A) low nutrient (solid lines) and high nutrient treatments (dashed lines) and (B) pond identity. Productivity manipulations were only performed in P14. Shown are means and standard error bars. Initial (day 0) values are based on estimated biomass densities from enclosure inocula.



Figure 1

Figure 2. Responses of the major components of the small-bodied zooplankton assemblage in (A) high nutrient treatments, (B) low nutrient treatments and (C) P12 enclosures. Shown are means and standard errors.

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Figure 2



Figure 2 (cont'd)



Figure 2 (cont'd)

Figure 3. (A) Effects of zooplankton composition (*Daphnia pulex* versus small-bodied zooplankton) and nutrient enrichment (high, dashed lines, versus low, solid lines) on mean chlorophyll a (+/- S.E.). (B) Responses of mean chlorophyll a (+/- S.E.) to zooplankton composition and pond identity (P12 versus P14).



Figure 3

Figure 3. (A) Effects of zooplankton composition (*Daphnia pulex* versus small-bodied zooplankton) and nutrient enrichment (high, dashed lines, versus low, solid lines) on mean chlorophyll a (+/- S.E.). (B) Responses of mean chlorophyll a (+/- S.E.) to zooplankton composition and pond identity (P12 versus P14).

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Figure 3

Figure 4. Responses of seston C:N:P molar ratios to zooplankton composition (*Daphnia* versus small-bodied taxa) and pond identity (P12 versus P14). Shown are means (+/-S.E.) of (A) seston C:P, (B) seston C:N and (C) seston N:P. Day 0 values are based on pond samples taken at the initiation of the experiment.



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Figure 4



Figure 4 (cont'd)

Figure 5. Effects of zooplankton composition and pond identity on seston C:N:P on the final day of the experiment. Shown are means and standard error bars for (A) C:P, (B) C:N and (C) N:P.







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Figure 5 (cont'd)



Figure 5 (cont'd)

Figure 6. Effects of zooplankton composition and pond identity on (A) total phosphorus, (B) total nitrogen and (C) the molar ratio of total nitrogen to total phosphorus. Shown are means and standard errors for day 12 and day 40 of the experiment.

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Figure 6



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Figure 6 (cont'd)

Figure 7. Results from the field survey of permanent fishless ponds. (A) The relationship between chlorophyll *a* and total phosphorus in *Daphnia* dominant samples (>50% relative biomass; dashed line) and *Daphnia* subdominant samples (<50% relative biomass; solid line). Regression equations for *Daphnia* dominant samples:  $\log_{10}y =$ 0.655log<sub>10</sub>x - 0.117 (p=0.0024, r<sup>2</sup>=0.268, n=32); and *Daphnia* subdominant samples:  $\log_{10}y = 1.341\log_{10}x - 1.248$  (p=<0.0001, r<sup>2</sup>=0.736, n=52). (B) The relationship between zooplankton biomass and chlorophyll *a* in *Daphnia* dominant (dashed line) and subdominant (solid line) samples. Regression equations for *Daphnia* dominant samples:  $\log_{10}y = -0.753\log_{10}x - 0.0528$  (p=0.002, r<sup>2</sup>=0.284, n=32); and *Daphnia* subdominant subdominant (solid line) samples. Regression equations for *Daphnia* dominant samples:  $\log_{10}y = -0.753\log_{10}x - 0.0528$  (p=0.002, r<sup>2</sup>=0.284, n=32); and *Daphnia* subdominant subdominant samples. Regression equations for *Daphnia* dominant samples:  $\log_{10}y = -0.753\log_{10}x - 0.0528$  (p=0.002, r<sup>2</sup>=0.284, n=32); and *Daphnia* subdominant samples:  $\log_{10}y = 0.0642\log_{10}x - 1.104$  (p=0.609, r<sup>2</sup>=0.005, n=52). Shown are 95% confidence intervals.



Chlorophyll *a* (µg / liter)

#### CHAPTER 4

# PLANKTONIC SEASONAL SUCCESSION IN FISHLESS PONDS: EFFECTS OF ENRICHMENT AND INVERTEBRATE PREDATORS

with Allison H. Roy

## Abstract

We investigated the potential determinants of Daphnia pulex abundance in fishless ponds. Historically, the size-efficiency hypothesis (SEH) has been evoked to explain variation in Daphnia relative abundance. This hypothesis assumes that largebodied zooplankton, such as Daphnia, are superior competitors compared to small-bodied zooplankton. Size-selective predation can favor small zooplankton by removing large Daphnia. An alternate body of evidence suggests that large-bodied Daphnia are not invariably superior resource competitors. Rather, small-bodied taxa may be favored by low resource/productivity levels while large *Daphnia* may be favored under high resource conditions or following a resource/productivity pulse. We tested first whether SEH operates in a fishless pond that exhibits a seasonal decline in *Daphnia* abundance and if variation in initial system productivity (via nutrient manipulations) can affect the outcome of competitive interactions and *Daphnia*'s susceptibility to invertebrate predation by the backswimming bug, Notonecta undulata. We found little support for SEH; though predators had detectable negative effects, *Daphnia* performed poorly regardless of predator presence or absence. This indicates that resource-based effects and competition are of greater import for understanding mid-summer declines and the late season absence of Daphnia in ponds. Moreover, we found no supporting evidence for the resource levels hypothesis; productivity had no effect on Daphnia dominance or predator

susceptibility (likely due to a shift in algal quality to large inedible forms in high productivity treatments).

Keywords: competition, Daphnia, enrichment, interaction strength, Notonecta, ponds, predation, seasonal succession, size-efficiency hypothesis, zooplankton.

### Introduction

A commonly observed successional pattern in planktonic communities is a seasonal shift from early season dominance by large-bodied species of Daphnia to late season dominance by small-bodied cladocera (such as *Diaphanosoma* and *Ceriodaphnia*) and copepods (Sommer et al. 1986, Gliwicz and Pijanowska 1989). To date, this seasonal dynamic has been investigated primarily in lake communities with an associated emphasis on size-selective predation by fish as a principal determinant of zooplankton size-structure (e.g. Sommer et al. 1986, Gliwicz and Pijanowska 1989, Tessier and Welser 1991), a view rooted in the size-efficiency hypothesis (SEH) of Brooks and Dodson (1965). SEH assumes that large-bodied zooplankton, compared to small-bodied taxa, are superior resource competitors and consequently dominate in the absence of predators. Size-selective predation by fish on larger individuals can reduce the abundance of large Daphnia, permitting dominance by small-bodied zooplankton. Given this topdown-centric framework, it is interesting to note that in small permanent ponds where fish are absent, zooplankton communities frequently exhibit early season peaks followed by declines in Daphnia abundance (e.g. figure 1; see also Lynch 1978). Furthermore, seasonal patterns of Daphnia relative biomass can vary greatly among these water bodies,

with some systems being dominated by *Daphnia* for the entire growing season, some showing successional patterns (as in figure 1), and some having little or no *Daphnia* (Steiner, unpublished data). The determinants of this variation remain a mystery and hint at a biological complexity not encompassed within the traditional top-down view.

There are numerous potential mechanisms that may drive variable dominance by Daphnia in fishless ponds. First, several investigations indicate that large-bodied Daphnia do not invariably dominate small-bodied taxa when competing for shared resources (e.g. Neill 1975a, Lynch 1978, Smith and Cooper 1982). Many of these studies have emphasized the importance of stage-based dynamics when considering exploitative resource competition between large and small taxa (Romanovsky and Feniova 1985). Within the context of interspecific competition for algal resources, *Daphnia* adults, when compared to small-bodied taxa, generally have higher filtration rates, feed on a larger size range of algae, are more resistant to periods of low resources (i.e., are starvation resistant), and have higher biotic potential (i.e., the ability to convert resources consumed to reproduction). In contrast, juveniles greatly overlap competitors in prey preferences, have lower filtration rates compared to many small-bodied species, and are more prone to starvation (for reviews see Romanovsky and Feniova 1985, DeMott 1989). Because of this, Daphnia juveniles are generally more susceptible to the negative effects of interspecific competition and may be prone to demographic bottlenecks (Neill 1975a, Lynch 1978, Smith and Cooper 1982, Romanovsky and Feniova 1985).

Given this dynamic, the outcomes of zooplankton competitive interactions are potentially variable and dependent on several factors. A number of studies have emphasized the importance of resource concentration and system productivity on

differential performance of large versus small-bodied cladocera (e.g. Goulden et al. 1978, Tillmann and Lampert 1984, Romanovsky and Feniova 1985, Tessier and Goulden 1987). Low rates of algal-resource supply can favor small-bodied taxa by way of juvenile bottlenecks in Daphnia populations (Neill 1975a, Romanovsky and Feniova 1985). High resource availability (or a pulse of food) can allow *Daphnia* populations to accrue a large number of adult stage individuals. These adults could in turn depress resource levels (via high filtration rates) and essentially out-starve small-bodied competitors. This novel mechanism makes alternative stable states possible in zooplankton competitive arenas and could explain variation in competitive outcomes among ponds as well as seasonally within systems, especially if ponds are prone to mixing events. To date, a number of studies have suggested that competitive outcomes between large and small zooplankton could be dependent on initial resource levels or productivity/nutrient pulses during community development (Tillmann and Lampert 1984, Romanovsky and Feniova 1985, Bengtsson 1987). However, these studies, in addition to the general model (sensu Romanovsky and Feniova 1987), do not account for changes in resource quality that can occur concomitantly with enrichment events and/or grazing pressure (McCauley and Briand 1979, Reynolds 1984, McCauley et al. 1988, Paerl 1988, Vanni 1987, Leibold 1996). There is evidence that larger sizes of algae are favored by enrichment (e.g. Reynolds 1984, McCauley et al. 1988, Paerl 1988). This dynamic could favor Daphnia if algal size shifts outside the range of edibility for small zooplankton. However, one can just as easily foresee a shift towards extremely large forms (e.g. filamentous green or blue-green algae) of low edibility for Daphnia. These complications are discussed in greater detail in light of results in the Discussion.

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Resource effects notwithstanding, top-down effects could also generate midsummer declines of *Daphnia* in fishless ponds. Planktivorous invertebrates are plentiful in pond systems; yet how effective these predators are in shifting the size-structure of zooplankton communities remains equivocal. A number of studies have documented a general preference by invertebrate predators for small or intermediate size classes of zooplankton (Lynch 1979, Pastorak 1981, Spitze 1985), though some species (such as the backswimming bugs, Notonectidae) can feed on larger individuals (Scott and Murdoch 1983, Murdoch et al. 1984, Arner et al. 1998). The potential impact of invertebrate predators on community size structure is complicated by differential susceptibility of Daphnia life stages; juveniles are more vulnerable to predation but adults may attain a relative size refuge (Swift and Fedorenko 1975, Vinyard and Menger 1980, Spitze 1985). Consequently, seasonal variation in productivity and/or resource quality may enhance top-down effects by affecting how long juveniles are exposed to predators and the ability of individuals to reach a size refuge (Chase 1999). Just as a pulse of resource production may affect competitive outcomes, high levels of resources may weaken predator interactions with Daphnia.

In the present paper we report on an in situ mesocosm experiment in which we explored a number of these hypotheses and processes in a semi-natural pond known to exhibit a seasonal loss of large-bodied *Daphnia pulex*. We set out to address the following questions: (1) does the size efficiency hypothesis operate, i.e., can large-bodied *Daphnia* dominate zooplankton communities under late season conditions and in the absence of predation, (2) does increasing algal productivity alter competitive outcomes

and favor dominance by large *Daphnia* and (3) what effects do invertebrate predators have on size-structure and do effects on *Daphnia* vary with enrichment.

## Methods

All experiments were performed in a single pond (P14) at the W. K. Kellogg Biological Station, experimental pond facility (Hickory Corners, MI). This pond is fishless and permanent, with a taxonomically diverse planktonic community. It is approximately 1.6 meters deep, at its deepest point, with a surface area of approximately 700 m<sup>2</sup>. Productivity, as measured by total phosphorus, is in the mesotrophic range (mean, +/- 1 S.E., over three years, 15.3 ug P/liter, +/- 6.1). The past four years (1997-2000), P14's *Daphnia pulex* population has undergone the abovementioned pattern of seasonal succession with the late season zooplankton assemblage dominated primarily by *Diaphanosoma* and calanoid copepods (e.g. figure 1). The pond supports an invertebrate predator assemblage, resident year-round, dominated by the phantom midge larvae (genus *Chaoborus*), plus backswimming bugs (*Notonecta*) and dytiscid beetle larvae (*Dytiscus*).

The experiment took place in the summer of 1997. Our experimental system consisted of impermeable 1200-liter polyethylene "bag" enclosures, open at their tops, sealed at their bottoms and suspended in the water column by floating frames. Tops of the frames were covered with fiberglass window screening to prevent invasion by insects. Enclosures extended to the pond bottom and were deployed in late June, following the natural decline phase of *Daphnia* in this pond (*Daphnia* were completely absent by midexperiment). Water was pumped from the pond into bags through a 150-micron mesh net to remove the majority of the zooplankton population. We then inoculated all enclosures

with an equal biomass of *Daphnia pulex* and a diverse assemblage of small-bodied zooplankton. Small zooplankton were obtained from a neighboring pond at the pond facility. This source pond contained a population of pumpkinseed sunfish (*Lepomis gibbosus*) and thus contained no *Daphnia* and no invertebrate predators due to fish predation. The zooplankton assemblage extracted from this pond included all the major late-season taxa found in P14, including species of *Diaphanosoma*, *Ceriodaphnia*, *Chydorus*, *Bosmina*, *Scapholeberis*, calanoid and cyclopoid copepods, and several species of rotifers (dominated primarily by *Keratella*, *Asplanchna*, *Lecane*, and *Trichocerca*). We lab reared all *Daphnia* to ensure their availability at the time of the experiment. Early in the spring, we isolated *Daphnia pulex* individuals from several experimental ponds and reared these in large batch cultures for approximately 2 months. Cultures were initiated with numerous individuals to promote a clonally diverse population and were periodically thinned to maintain high food conditions.

A day after enclosures were filled with water we inoculated bags with an equal biomass concentration of *Daphnia pulex* and the small-bodied zooplankton community. Total zooplankton biomass at the experiment's initiation was 0.0245 mg/liter. To explore effects of a productivity pulse on zooplankton dynamics we subjected enclosures to three nutrient treatments (high, medium and low) consisting of additions of phosphorus (Na<sub>2</sub>HPO<sub>4</sub>) and nitrogen (NaNO<sub>3</sub>) with a N:P molar ratio of 57.3:1, matched to that of the ambient pond water to avoid changes in algal stoichiometry. High nutrient treatments received a start concentration of 150 ug P/liter (well within the range considered hypereutrophic), medium treatments received 80 ug P/liter, and low nutrient treatments received no additions for a start concentration of 10.7 ug P/liter. Nutrients were added

once, one day after zooplankton additions. To explore effects of invertebrate predators and their interaction with nutrient addition we crossed presence/absence of the predator *Notonecta undulata* with low and high nutrients. Notonectids were collected from ponds at the experimental pond facility using horizontal tows with a plankton net. Each predator treatment received 7 adult *Notonecta*, on the high end of densities found in ponds at the experimental facility but low when considering the range of variation encountered in fishless ponds (J. Chase, University of Pittsburgh, pers. comm.). Because Notonectids are known to feed preferentially on *Daphnia* (Scott and Murdoch 1983), we biased our experiment towards seeing predator effects on the large zooplankter. Predator additions occurred four days following zooplankton inoculations.

All treatments were replicated three times for a total of 15 enclosures and the experiment was allowed to run for 32 days. Every 8 days, beginning on the eighth day, we sampled the zooplankton community using an integrated tube sampler. Zooplankton were immediately preserved in chilled sucrose formalin and stored for later enumeration. Fifty randomly chosen individuals of each species were also measured to obtain biomass estimates using length-mass regressions. During each sample period, 500 ml of water was collected from the water column of each enclosure using an integrated tube sampler. Water samples were chilled and later filtered for subsequent analysis of chlorophyll *a* as a correlate of algal biomass (sensu Welschmeyer 1994). Samples were fractionated into two size classes. Half of the water sample was directly filtered onto glass fiber filters to measure total chlorophyll *a*, the other half was first filtered through a 35-micron Nitex mesh to measure the "edible" size fraction of algae. A number of algal characteristics can impart resistance to grazers (e.g. toxicity, gelatinous sheathing, spines). Size was chosen

as a general indicator of edibility in this study because it is a common constraint on dietary preference of zooplankton (Neill 1975b, Sommer 1988) and it is easily measured through size fractionation. Thirty-five microns was chosen as a proxy upper size limit for "highly edible" versus "grazer-resistant" prey based on known size preferences of Daphnia and small-bodied cladocera. The maximum upper size limit of consumable prey for Daphnia is approximately 60 microns (Burns 1968). Small-bodied zooplankton (excluding copepods that can feed selectively on a large size range) have preferences that are much lower, below 15 microns (Neill 1975b). Thus, 35 microns represents a midpoint cutoff applicable to both size fractions of the zooplankton community (though in general, edibility may be poorly estimated for copepods, overestimated for the remaining smallbodied zooplankton assemblage, and underestimated for *Daphnia*). Midway through the experiment a mat of filamentous algae (dominated by *Oedogonium* and *Spyrogyra*) appeared on the water surface of the medium and high nutrient enclosures. Surface algae was collected in totality on the final day of the experiment using d-nets, subsampled and filtered to measure chlorophyll a. In all subsequent analyses, we treat surface algae as grazer resistant.

Biomass responses of zooplankton and algae were analyzed using univariate repeated measures ANOVA (rm-ANOVA). Initial (day 0) densities were not included in analyses. When necessary, analyses of biomass from single sample dates were performed using ANOVA. Chlorophyll a measures were  $\log_{10}$  transformed to conform to assumptions of homogeneity of variances. Due to zero values, all zooplankton biomass measures were  $\log_{10} (x + 1)$  transformed. All proportional data (percent relative biomass) were arc-sine square root transformed. Because samples taken on adjacent dates from a given experimental unit are likely to show greater covariance than non-adjacent samples, assumptions of repeated measures ANOVA (sphericity) may be violated. To avoid type I errors when examining within subjects effects (i.e., response curves through time), both Greenhouse-Geiser (G-G) and Huynh-Feldt (H-F) adjusted probabilities are presented. All analyses were performed using Systat 8.0 (SPSS Inc., 1998).

## **Results**

Focusing on zooplankton responses in the absence of predation, nutrient enrichment had weak effects on Daphnia biomass (figure 2; p=0.090, F<sub>2.6</sub>=3.695, between subjects effects, rm-ANOVA). Mid-experiment Daphnia biomass began to drop in all treatments and by day 32 there was no significant difference detected (p=0.248,  $F_{2,6}$ =1.772, one-way ANOVA). Enrichment had positive and sustained effects on total small-bodied zooplankton biomass (figure 2; p=0.009, F<sub>2,6</sub>=11.539, between subjects effect). The small zooplankton assemblage was dominated primarily by cladocera (of which Diaphanosoma, Bosmina, and Chydorus were the dominant components). Rotifers, after day 16, comprised less than 5% of total zooplankton biomass. The majority of the small-bodied zooplankton response was due to the significant positive responses of cladocera to enrichment (p=0.007,  $F_{2,6}$ =12.994, between subjects effect). Copepod biomass dynamics may have been dependent on enrichment, as indicated by time by nutrient within subjects effect (G-G p=0.072, H-F p=0.053,  $F_{6,18}$ =0.053). However, there was no overall effect of nutrients on the levels of copepod responses through time (p=0.642,  $F_{2,6}$ =0.478, between subjects effect, repeated measures ANOVA).

Previous work has suggested that nutrient enrichment can alter competitive outcomes between *Daphnia* and small-bodied zooplankton (see Introduction). Examining percent relative biomass, *Daphnia* did not dominate the zooplankton community at any time during the experiment, regardless of nutrient enrichment (figure 3). Enrichment had no effect on the relative biomass of *Daphnia* through time (p=0.248,  $F_{2,6}$ =1.775, between subjects effect; p=0.246,  $F_{6,18}$ =1.462, within subjects effect, rm-ANOVA). In contrast, small-bodied zooplankton comprised, on average, greater that 70% of the biomass in all non-predator enclosures.

Notonectid predators had no effect on the biomass of total small-bodied zooplankton (figure 4A) or its major components- small-bodied cladocera, rotifers, or copepods (p>0.17 for all within and between subjects predator effects and predator by nutrient interactions, rm-ANOVA). Notonectids did have negative effects on *Daphnia pulex* (figure 4B), independent of nutrient level (p=0.012,  $F_{1,8}$ =10.603, between subjects effect). However, there were no significant predator by time interactions (within subjects effects), only a significant time by nutrient interaction (G-G p=0.055, H-F p=0.039,  $F_{3,24}$ =3.258), suggesting that declines in *Daphnia* abundance were not a function of predator presence or absence.

Algae appeared to respond positively to nutrient enrichment, as indicated by chlorophyll *a* concentrations through time (fig 5; p=0.057,  $F_{2,6}$ =4.795, between subjects effect). Relative biomass of grazer resistant (>35 micron) algae was calculated as the difference between total and <35 micron fractions divided by total chlorophyll *a*. Repeated measures analysis of the relative abundance of this size fraction revealed weak positive effects of enrichment (figure 6; p=0.075,  $F_{2,6}$ =4.118, between subjects effect). However, these measures do not take into account the sizable fraction in the form of filamentous surface algae. Its inclusion reveals that the majority of primary production by the end of the experiment was resistant (figure 7; p<0.001,  $F_{2,6}$ =36.168, one way ANOVA of nutrient effects on relative abundance of resistant algae for the final sample date).

### Discussion

As a driver of temporal and spatial variation in zooplankton community size structure, size-selective predation by fish has a formidable body of supporting evidence (e.g. Hrbacek et al. 1961, Brooks and Dodson 1965, Hall et al. 1976, Gliwicz and Pijanowska 1989, Carpenter and Kitchell 1993, Mittelbach et al. 1995). However, the importance of top-down effects in fishless ponds is uncertain. Our experiment provided strong evidence that invertebrate predators can have negative effects on Daphnia populations. Notonectids inflicted significant reductions in *Daphnia* populations, a result consistent with previous investigations (e.g. Murdoch et al 1984, Arner et al. 1998). Thus, this invertebrate predator has the capacity to play the role that planktivorous fish do in the original formulation of SEH. Nonetheless, while biomass was depressed in the presence of Notonecta, Daphnia at no point dominated the zooplankton community and by mid-experiment had begun to decline in all enclosures, independent of predator presence or absence. This was true regardless of productivity levels; enrichment had no effect on the magnitude or strength of predator effects on Daphnia. Hence, the heart of the size efficiency hypothesis -competitive dominance by large bodied zooplankton in the absence of predators- was not supported by our experiment.

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As previously outlined, absolute quantity of resources has been linked to variable Daphnia performance, with several papers suggesting that small-bodied species perform better at low resource levels and large-bodied species at high resource concentrations (Goulden et al. 1982, Tillmann and Lampert 1984, Romanovsky and Feniova 1985, Bengtsson 1986, 1987, Tessier and Goulden 1987). Using simulations, Romanovsky and Feniova (1985) have shown that resource pulses may facilitate competitive reversals by diminishing effects of small-bodied species on *Daphnia* and strengthening the negative effects of *Daphnia* on competitors. Variation in nutrient availability, and consequently algal production, might be especially high in shallow water bodies such as ponds in which mixing events extend through the entire water column to nutrient-rich sediments. Thus, this model may account for variation in competition effects among ponds (along gradients of productivity and/or mixing regime) as well as variation in competitor interaction strength within ponds (due to mixing events and seasonal changes in productivity). Though we did not measure the effects of competition directly in our experiment, the small-bodied assemblage was composed largely of cladocera, species known to compete with Daphnia for shared resources (reviewed in DeMott 1989). Using relative biomass as an indicator of competitive ability and performance, Daphnia at no point exhibited clear competitive dominance, comprising a minor fraction of the zooplankton community at all times during the experiment. This was true despite a nutrient pulse an order of magnitude higher than ambient levels. While Daphnia biomass did increase in response to enrichment, the effect was short-term and in clear contrast to the more sustained responses of the small-bodied zooplankton fraction. Furthermore, the

lack of dominance does not appear to be an artifact of experimental duration since *Daphnia* responses peaked mid-experiment and then declined in all treatments.

In order for productivity pulses to generate shifts in competitive outcomes, Daphnia must exhibit a substantial population increase and dominate the zooplankton community during the early onset of community development. During this early community growth phase, adult *Daphnia*, through their high per capita filtration rates, can drive resources to low levels and out-starve competitors (Romanovsky and Feniova 1985). This dynamic response was clearly not attained in the present investigation suggesting that abiotic conditions or some aspect of resource quality may have suppressed Daphnia population responses. Though the majority of algal production was in the edible (less than 35 micron) range early in the experiment (surface filamentous forms appeared later), this course level of resolution can miss important aspects of resource quality and algal size structure. Zooplankton taxa can vary greatly in their preferences for different particle sizes and taxa (e.g., Neill 1975b). Moreover, digestibility and nutritional adequacy (i.e., carbon, phosphorus and nitrogen content), may differentially favor zooplankton taxa. Finally, resource effects could have interacted with high pond temperatures.

Poor *Daphnia* performance and declines later in the experiment may have been due to our use of a dynamic resource community. Prior investigations that have uncovered effects of resource concentration on competitive outcomes have used static algal assemblages. Clearly phytoplankton community composition can change in response to both enrichment and grazing pressure (McCauley and Briand 1979, Reynolds 1984, McCauley et al. 1988, Paerl 1988, Vanni 1987). In our study, algae increased in

response to enrichment but also exhibited a striking increase in the grazer-resistant size fraction (>35 microns). By the termination of the experiment, much of this was in the form of surface-bound filamentous algae that comprised close to 60% of algal biomass in the highest nutrient treatments. If primary productivity is channeled to species of algae inaccessible to *Daphnia*, the non-equilibrial dynamics envisioned in Romanovsky and Feniova (1985) may not be approximated. Other studies have shown that allocation of resource production towards less edible forms can attenuate consumer biomass responses to enhanced productivity (e.g. Bohannan and Lenski 1999). Indeed, *Daphnia* relative biomass was low even during its highest population peak. These results do not negate the idea that temporally fluctuating resources may facilitate coexistence among competing zooplankton or that pulses can prolong *Daphnia* persistence in otherwise unfavorable environments. It does however call into question the capacity for a nutrient pulse during community development to generate alternative competitive outcomes and *Daphnia* dominated systems in natural pond environments.

These results, when combined with data from Chapters 2 and 3, suggest that the ability of *Daphnia pulex* to control algal resources and competitively dominate zooplankton communities is highly variable. Those chapters showed that *Daphnia*, when compared to small-bodied taxa, reduce algal biomass to lower levels only under a limited subset of environmental conditions. Using algal standing crop as an indicator of a species' R\* (and consequently its competitive ability), competitive outcomes between small-bodied zooplankton and *Daphnia pulex* should be highly variable in space and time. This conclusion is consistent with previous studies that have uncovered variable

competition effects between large *Daphnia* and small taxa (e.g. Lynch 1978, Smith and Cooper 1982).

A major challenge in ecology is understanding the limits and acceptable application of our existing body of theory. The size-efficiency hypothesis has attained a paradigmatic stature in aquatic ecology and is commonly found incorporated in trophic cascade models as well as general models of zooplankton succession. Our results clearly showed that top-down effects, while present, are not needed to explain the poor performance of *Daphnia* following mid-summer declines in ponds; abiotic conditions and/ or resource-based effects, likely competition with small-bodied taxa, appear to be more essential. The inability of *Daphnia* to dominate small-bodied taxa in the absence of predators is further evidence that SEH may have limited applicability in shallow pond systems. Finally, the effects of varying resource levels and productivity on competitive outcomes between large and small-bodied taxa may be insubstantial outside of a laboratory setting (i.e., when applied to systems in which algal composition is dynamic and itself a function of enrichment and grazing events).

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## Literature Cited

- Arner, M., S. Koivisto, J. Norberg, and N. Kautsky. 1998. Trophic interactions in rockpool food webs: regulation of zooplankton and phytoplankton by *Notonecta* and *Daphnia*. Freshwater Biology 39: 79-90.
- Bengtsson, J. 1986. Life histories and interspecific competition between three *Daphnia* species in rockpools. Journal of Animal Ecology 55: 641-655.
- Bengtsson, J. 1987. Competitive dominance among Cladocera: are single-factor explanations enough? An examination of the experimental evidence. Hydrobiologia 145: 245-257.
- Bohannan, B. J. M., and R. E. Lenski. 1999. Effect of prey heterogeneity on the response of a model food chain to resource-enrichment. American Naturalist 153: 73-82.
- Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28-35.
- Burns, R. J. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnology and Oceanography 13: 675-678.
- Carpenter, S. R., and J. F. Kitchell. 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge, U. K.
- Chase, J. M. 1999. Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist 154: 559-570.
- DeMott, W. R. 1989. The role of competition in zooplankton succession. Pages 195-252 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Gliwicz, Z. M., and J. Pijanowska. 1989. The role of predation in zooplankton succession. Pages 253-295 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Goulden, C. E., L. L. Hornig, and C. Wilson. 1978. Why do large zooplankton species dominate? Internationale Vereinigung fur Theoretische und Angewandte Limnologie, Verhandlungen 20: 2457-2460.
- Goulden, C. E., L. L. Henry, and A. J. Tessier. 1982. Body size, energy reserves, and competitive ability in three species of cladocera. Ecology 63: 1780-1789.
- Gurevitch, J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist 140: 539-572.

- Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. American Naturalist 155: 435-453.
- 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 Review of Ecology and Systematics 7: 177-208.
- Hrbacek, J., M. Dvorakova, V. Korinek, and L. Prochazkova. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 14: 192-195.
- Lynch, M. 1978. Complex interactions between natural coexploiters-*Daphnia* and *Ceriodaphnia*. Ecology 59: 552-564.
- Lynch, M. 1979. Predation, competition, and zooplankton community structure: an experimental study. Limnology and Oceanography 24: 253-272.
- McCauley, E., and F. Briand. 1979. Zooplankton grazing and phytoplankton species richness: field tests of the predation hypothesis. Limnology and Oceanography 24: 243-252.
- McCauley, E., W. W. Murdoch, and S. Watson. 1988. Simple models and variation in plankton densities among lakes. American Naturalist 132: 383-403.
- Mittelbach, G. G., A. M. Turner, D. J. Hall, J. E. Rettig, and C. W. Osenberg. 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. Ecology 76: 2347-2360.
- Murdoch, W. W., M. A. Scott, and P. Ebsworth. 1984. Effects of the general predator *Notonecta* (Hemiptera) upon a freshwater community. Journal of Animal Ecology 53: 791-808.
- Neill, W. E. 1975a. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology 56: 809-826.
- Neill, W. E. 1975b. Resource partitioning by competing microcrustaceans in stable laboratory microecosystems. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 19: 2885-2890.
- Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine, and inland waters. Limnology and Oceanography 33: 823-847.
- Pastorak, R. A. 1981. Prey vulnerability and size selection by *Chaoborus* larvae. Ecology 62: 1311-1324.

- Reynolds, C. S. 1984. The Ecology of Freshwater Phytoplankton. Cambridge University Press, Cambridge, U.K.
- Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among Cladocera: effect of different levels of food supply. Oikos 44: 243-252.
- Scott, M. A. and W. M. Murdoch. 1983. Selective predation by the backswimmer, *Notonecta*. Limnology and Oceanography 28: 352-366.
- Smith, D. W., and S. D. Cooper. 1982. Competition among cladocera. Ecology 63: 1004-1015.
- Sommer, U. 1988. Phytoplankton succession in microcosm experiments under simultaneous grazing pressure and resource limitation. Limnology and Oceanography 33: 1037-1054.
- Sommer, U., M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. Archiv fur Hydrobiologie 106: 433-471.
- Spitze, K. 1985. Functional response of an ambush predator: *Chaoborus americanus* predation on *Daphnia pulex*. Ecology 66: 938-949.
- Swift, M. C., and A. Y. Fedorenko. 1975. Some aspects of prey capture by *Chaoborus* larvae. Limnology and Oceanography 20: 418-425.
- Tessier, A. J. and C. E. Goulden. 1987. Cladoceran juvenile growth. Limnology and Oceanography 32: 680-686.
- Tessier, A. J. and J. Welser. 1991. Cladoceran assemblages, seasonal succession and the importance of a hypolimnetic refuge. Freshwater Biology 25: 85-93.
- Tillmann, U. and W. Lampert. 1984. Competitive ability of differently sized *Daphnia* species: an experimental test. Journal of Freshwater Ecology 2: 311-323.
- Vanni, M. J. 1987. Effects of nutrients and zooplankton size on the structure of a phytoplankton community. Ecology 68: 624-635.
- Vinyard, G. L., and R. A. Menger. 1980. *Chaoborus americanus* predation on various zooplankters: functional response and behavioral observations. Oecologia 45: 90-93.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnology and Oceanography 39: 1985-1992.



Figure1. Seasonal dynamics in P14 during the 1997 growing season. Shown is mean biomass (mg/liter) from two vertical tows (+/- 1 S.E.) for the dominant species of zooplankton.



Figure 2. Responses of small-bodied zooplankton (solid lines; cladocera, copepods, and rotifers combined) and *Daphnia pulex* (dashed lines) in low, medium and high nutrient treatments, in the absence of Notonectid predators. Shown are means and standard errors.



Figure 3. Percent relative biomass of *Daphnia* in low, medium and high nutrient treatments, in the absence of predators. Shown are means and standard errors.



Figure 4. Effects of the presence or absence of *Notonecta undulata* at low and high nutrients on (A) responses of small-bodied zooplankton and (B) *Daphnia pulex*. Shown are means and standard errors.



Figure 4. (cont'd).



Figure 5. Algal responses (measured as total chlorophyll *a*) to low, medium and high nutrient treatments, in the absence of predators. Shown are means and standard errors.



Figure 6. Percent relative biomass of "grazer-resistant" algae (>35 micron chlorophyll *a* fraction) over the course of the experiment in low, medium and high nutrients treatments, in the absence of predators. Means are shown with standard error bars.



Figure 7. Percent relative biomass of "grazer-resistant" algae (>35 micron chlorophyll *a* fraction plus filamentous algae chlorophyll *a*) for the final date of the experiment in low, medium, and high nutrient treatments, in the absence of predators. Means and standard errors are shown.

#### CHAPTER 5

# SEASONAL SUCCESSION AND VARIABLE DOMINANCE IN POND PLANKTONIC COMMUNITIES: ASSESSING SPATIAL AND TEMPORAL VARIATION IN PREDATOR AND COMPETITOR EFFECTS

## Abstract

Aquatic ecologists have long recognized the importance of variable competitor and predator interaction strength as drivers of seasonal succession of planktonic communities. A common successional pattern is early season occurrence of large-bodied Daphnia followed by mid-season declines and shifts to dominance by small-bodied taxa. Most studies of this successional pattern have focused largely on lake systems with an associated emphasis on variable fish predation as a primary determinant of zooplankton seasonal dynamics. The overriding influence of fish predation is called into question by pond systems in which patterns of dominance by large Daphnia is independent of fish presence/absence, being highly variable both seasonally and spatially (among ponds). The mechanisms generating these patterns are unknown. In this paper I present results of a multi-pond, in situ experiment in which I assessed the effects of competition and invertebrate predation on *Daphnia* populations in fishless ponds. The experiment was conducted in five ponds that varied naturally in *Daphnia* relative abundance and patterns of seasonal succession. Daphnia pulex population growth rates were measured in three treatments: (1) a predator/competitor exclusion, (2) the natural competitor assemblage present and predators excluded and (3) competitors present and the predator *Chaoborus* present at natural pond densities. To assess temporal variation in interaction strength, the experiment was conducted twice in the growing season, once early when Daphnia were present in all ponds and once late when *Daphnia* had gone extinct in a subset. Results

showed that effects of resource competition greatly outweighed effects of predation. However, competition intensity was variable among ponds (being strongest in those ponds/times in which *Daphnia* relative abundance was low, and weak or non-existent when *Daphnia* were dominant). Ponds also showed a significant temporal intensification of competition effects, increasing late in the season; this temporal trend also appeared to vary among ponds (showing little or no increase in the single pond dominated by *Daphnia* during both experimental runs). Several potential determinants (biotic and abiotic) of variable competitor interaction strength were explored using regressions. Only the C:P ratio of edible algae showed a significant relationship. Negative effects of competition increased as algal resources became more phosphorus limited, in keeping with recent stoichiometric models.

*Keywords*: algae, carbon, *Chaoborus*, competition, *Daphnia pulex*, phosphorus, ponds, predation, seasonal succession, size-efficiency hypothesis, size structure, stoichiometry, zooplankton.

#### Introduction

The field of community ecology has amassed an impressive body of evidence supporting the importance of competition and predation in structuring natural communities (Sih et al. 1985, Gurevitch et al. 1992, 2000). Having acquired the knowledge that such density-dependent processes indeed operate in nature, one could argue that a more daunting task now facing ecologists is attaining the capacity to predict when, where, and with what intensity these organizational forces operate. Understanding

the factors underlying temporal and spatial variation in competitor and predator effects can enhance our understanding of community organization and our ability to predict the dynamics of populations and food webs. The phenomenon of seasonal succession has long provided fertile ground for experimental and theoretical exploration of the ecological mechanisms forcing community dynamics. This has been no more apparent than in aquatic ecosystems where seasonal turnover in plankton species composition and dominance is commonplace in temperate regions (Sommer 1989). Such striking temporal transformations in species advantages and disadvantages present a novel challenge to familiar equilibrial approaches (models that assume a static hierarchy of competitive ability and/or susceptibility to predation), requiring instead that top-down and bottom-up processes be approached as dynamic phenomena. Since patterns of succession can also vary pronouncedly among water bodies, seasonally dynamic systems, by their very nature, are ideal model systems for examining spatiotemporal variation in competitor and predator interaction strength and the factors, be they biotic or abiotic, underlying this variation.

Zooplanktonic systems commonly exhibit seasonal turnover in community sizestructure, with large-bodied *Daphnia* species dominating early in the growing season followed by dominance by small-bodied cladocera (e.g. *Ceriodaphnia* and *Diaphanosoma*) or small copepods (Sommer et al. 1986, Gliwicz and Pijanowska 1989, Tessier and Welser 1991). Both resource competition and predation are thought to drive variation in zooplankton size-structure and the size-efficiency hypothesis (SEH) has frequently provided the theoretical framework for understanding the action and relative importance of these forces (Brooks and Dodson 1965, Gliwicz and Pijanowska 1989).

SEH postulates that large species are competitively superior to small-bodied species of zooplankton. Size-selective predation on large zooplankton by efficient planktivores (i.e. fish) can shift dominance to smaller forms. Despite the prevalence of this model, a sizable body of evidence suggests that variable dominance by large versus small-bodied zooplankton may be dependent on interspecific competition and its interaction with environmental context, independent of fish predator presence or absence (see DeMott 1989). As examples: resource quality, temperature, productivity, and variability in resource levels have all been cited as factors that may influence zooplankton performance as well as the strength and outcome of competitive interactions between large and small-bodied taxa (e.g. Lynch 1978, 1979, Romanovsky and Feniova 1985, Tillmann and Lampert 1984, Threlkeld 1986, Bengtsson 1987).

The notion that variation in *Daphnia* relative abundance may not be invariably linked to size-selective predation by fish is further bolstered by field surveys of permanent ponds that naturally lack fish. Figure 1 shows percent relative biomass data for *Daphnia pulex* obtained from a survey of 18 permanent, fishless ponds in southwest Michigan. These systems clearly exhibit considerable spatial (among pond) and temporal (within pond) variation in the incidence and relative abundance of *Daphnia pulex* (the primary large-bodied zooplankter in these systems). Though lacking fish, zooplankton predators are present in these pond systems (e.g. predaceous invertebrates such as *Chaoborus*). At present, however, evidence that these predators can control large-bodied *Daphnia* populations is equivocal (Gliwicz and Pijanowska. 1989); some studies have provided evidence for strong top-down control (Murdoch et al. 1984, Arner et al. 1998) while others have revealed weak or even positive effects (Lynch 1979, Neill 1981).

Numerous factors could control spatiotemporal variation in predator effects and consequently the variation in figure 1. For example, large *Daphnia* can attain a relative size refuge from invertebrate predators (Swift and Fedorenko 1975, Vinyard and Menger 1980, Spitze 1985). Hence, variation in algal-resources (quantity, productivity, and quality) could mediate the strength of predator effects via effects on *Daphnia* growth rates and its ability to attain invulnerable sizes (Chase 1999). Furthermore, predator phenology and timing of emergence events (as in the case of Chaoborids) could further alter the strength of top-down control.

The role that resource competition plays in generating the variation in figure 1 is equally ambiguous. Lab studies have shown that small zooplankton can be competitively dominant to large Daphnia (Neill 1975a), but others have shown that competitive outcomes may be highly variable (e.g. Lynch 1978) and dependent on levels of resource supply coupled with temperature (Romanovsky and Feniova 1985, Bengtsson 1985). Additionally, the strength and outcome of zooplankton competition may be mediated by resource quality. While large-bodied Daphnia can consume large algal particles, well outside the range accessible by smaller species of zooplankton (Burns 1968), some smallbodied cladocera are more efficient at filtering small seston particles (e.g. picoplankton and bacteria; Neill 1975b, Geller and Muller 1981, Brendelberger 1991). Thus, the sizestructure of algal assemblages could affect resource overlap and the outcome of competition among zooplankters within and among ponds. Furthermore, attention has recently focused on algal stoichiometry as an important aspect of resource quality. Compared to most small-bodied cladocera and copepods, Daphnia tissues have some of the lowest carbon to phosphorus and nitrogen to phosphorus ratios among zooplankton

taxa (Sterner et al. 1992, Sterner and Hessen 1994). Due to their high demands for phosphorus, *Daphnia* performance can be linked to the nutritional content of their food (i.e. relative concentrations of carbon, nitrogen, and phosphorus; Sterner 1993, Sterner and Hessen 1994, Urabe et al. 1997, Elser et al. 2001). It has been hypothesized that mineral limitation due to high C:P and N:P ratios of algae could enhance the negative effects of resource competition leading to competitive reversals between large *Daphnia* and small-bodied taxa (Sterner and Hessen 1994). This has yet to be shown empirically. Finally, the abundance and composition of the competitor assemblage can greatly affect the strength of resource competition through differential taxon-specific filtration rates, assimilation/growth efficiencies, and resource overlap. For example, *Daphnia* are known to compete more intensely with small-bodied cladocera than with copepods (reviewed in DeMott 1989).

To date, studies of plankton seasonal succession have been largely relegated to studies of lakes, explaining to a large degree the emphasis on consumer effects by planktivorous fish. The survey data presented in figure 1 are unique in this respect and though potential mechanisms are clearly in abundance, how these forces actually shape these patterns is currently unknown. The magnitude of variation exhibited by these systems and its incorporation of both temporal and spatial components makes them ideal models with which to explore variation in the strength of species interactions and the factors influencing this variation. That a single species, *Daphnia pulex*, is the dominant large-bodied zooplankter in these systems further facilitates across system quantification and comparison of variation in species interaction strength. In this paper I present results from a series of experiments in which I explored the relative effects of competition and

predation on *Daphnia pulex* abundance in these systems, focusing on both among pond variation and seasonal, within pond variation. I then examine several factors that could be affecting variation in competitor and predator interaction strength.

## Methods

Experiments were performed in five fishless ponds found within a 60-mile radius around the W. K. Kellogg Biological Station (Hickory Corners, MI). These included three ponds at the K.B.S. experimental pond facility (P12, P14, and P15) and two natural systems (Shaw4 and Lux16). All ponds were known to have water year-round in the two to three years prior to the experiments (personal observation; J. Chase, University of Pittsburgh, pers. comm.). *Daphnia pulex* was chosen as the focal species since it is the dominant *Daphnia* species encountered in field surveys of permanent, fishless ponds (comprising greater than 99% of *Daphnia* species observed, on a biomass basis; Steiner unpublished data). Ponds were chosen to represent a natural gradient of *Daphnia pulex* relative abundance, based on field survey data collected the previous year. In the year previous, all five ponds were known to have *Daphnia pulex* early in the growing season, but two of the five (P14 and P15) exhibited a complete loss of *Daphnia* midseason.

I used a partial factorial design consisting of three treatments (3 replicates each): a competitor/predator exclusion (-C –P), a treatment with competitors present at natural density and predators excluded (+C -P), and a treatment in which both predators and competitors were present at natural densities (+C +P). The design was implemented concurrently in all ponds. To assess temporal variation in treatment effects I executed the experiment twice in the growing season, once in late May/early June when *Daphnia* were still present in all ponds but were declining in some (hereafter referred to as the June or

"early season" run), and once in mid August (hereafter referred to as the August or "late season" run) after seasonal succession had occurred and *Daphnia* were absent from a subset of the ponds (table 1).

Predator/competitor exclusions consisted of impermeable 27-liter polyethylene bags, sealed at their bottoms and suspended from frames in the water column. These enclosures were filled with water from their respective ponds filtered through 80-micron mesh to remove zooplankton. Care was taken to gently filter the water to minimize inadvertent nutrient enrichment through physical destruction of zooplankton and algae. These treatments allowed me to evaluate the potential productivity of the pond for Daphnia as well as their ability to cope with ambient temperature conditions. Competitor and predator enclosures consisted of permeable 27-liter polyester cloth bags with an average mesh size of 250 microns. Cursory examination of bag contents revealed that this mesh is large enough to permit the natural algal assemblage (excepting the largest net phytoplankton), as well as the majority of the small-bodied zooplankton community, to move into the enclosure when initially expanded in the pond. Yet, it is small enough to keep Daphnia in the enclosures and predators in or out. Competitor treatments allowed me to measure Daphnia response to the natural pond environment, in terms of ambient resource and abiotic conditions, in the absence of direct negative effects of predators. Predator enclosures allowed me to measure this response but with the added direct, topdown effect. Mesh bags were expanded in the water column allowing them to be inoculated with the natural competitor and algal community. Bottoms of all bags were anchored to keep bags expanded in the water column and tops were covered with fiberglass screening to prevent insect invasion.

*Chaoborus* was chosen as the focal predator because it is the biomass dominant zooplankton predator in these systems and its high natural abundance allowed me to stock bags at natural densities. Prior to each experimental run I collected *Chaoborus* from the water column of each pond using repeated vertical tows (or horizontal tows through mid-water column in Lux16 and Shaw4) with a 150-micron plankton net. I sampled a volume of water equal to that of the enclosures and then hand picked *Chaoborus* individuals from the samples. Predator treatments thus received an initial predator density equal to the natural density of the pond at the time of the run (table 1).

To ensure their availability at the time of the experiment, all *Daphnia* were labreared. *Daphnia pulex* were collected from several ponds in the area, isolated and then raised in batch cultures under high food conditions. All enclosures, for both runs, received a low-density inoculum of *Daphnia* resulting in an initial density of approximately 4 individuals per liter (or approximately 0.012 mg/liter). To minimize the effects of high food, culture conditions on initial population growth rates, I prevented the addition of egg-carrying adults to enclosures. All *Daphnia* were filtered through a 425micron screen and any large adults and/or individuals carrying eggs that made it through this initial filtration were removed by hand. This method was successful in removing all egg-carrying individuals and resulted in an inoculum dominated by juveniles (over 80% <900microns in length).

After 12 days, all enclosures were exhaustively sampled by repeated vertical tows with a 150-micron plankton net (each enclosure volume was over-sampled 5 times). *Daphnia* individuals were then immediately preserved in acid Lugol's solution for later enumeration. The short experimental duration minimized enclosure artifacts due to their

high surface area to volume ratio and ensured that mesh bags remained largely free of periphyton growth that could have clogged mesh enclosures. For my primary response variable I calculated a per capita, instantaneous population growth rate as  $r = [ln (N_2 / N_1)] (1 / t)$ , where t was the duration of the experiment (12 days), N<sub>1</sub> was the initial stocking density, and N<sub>2</sub> was the density after time duration t.

In the following analyses, pond identity is treated as a treatment or fixed effect. Treatment effects on r for each run were explored using ANOVA. Comparisons between -C -P and +C -P treatments were used as a measure of competitor effects, while comparisons of +C -P and +C +P treatments assessed predator effects, over and above competitor effects. Temporal changes in treatment effects were explored using univariate repeated measures ANOVA.

In the time between the initiation and termination of each experimental run (both early and late season), I also measured a number of limnological variables in each pond (Table 1). Temperature, oxygen and pH were recorded in the field at mid-depth at two central locations using a portable probe. Ambient macrozooplankton communities were sampled using an integrated tube sampler, preserved, later enumerated and grouped as copepods or small-bodied cladocera. I also took 900ml water samples for subsequent measurement of chlorophyll *a* (sensu Welschmeyer 1994). To determine algal size structure, chlorophyll samples were divided into three size fractions (less than 15 microns, less than 60 microns, and total) using Nitex mesh screens and filtered onto Gelman A/E glass fiber filters. I chose 60 microns as the upper size limit of edible algae for *Daphnia*, and 15 microns as the upper size limit for small-bodied zooplankton

(though this may not hold true for copepods). To determine seston C:P and N:P ratios, a 500ml water sample was collected and later filtered through a 35-micron mesh to remove zooplankton. The water was then divided in two and filtered onto two separate, precombusted A/E filters. One filter was first fumed with concentrated HCl to remove carbon contributions from CaCO<sub>3</sub> and then frozen for later analysis of carbon and nitrogen content using a CHN analyzer. The other was frozen for later measurement of particulate phosphorus using the ammonium molybdate method following persulfate digestion (Wetzel and Likens 1991).

I used least squares, linear regressions to explore the relationships between six measures of competitor/predator effects and pond environmental variables. I first calculated "interaction strength" in a given pond as  $\Delta r$  (analogous to an effect size), equal to the difference between the mean r from the experimental and the mean r from its control (Osenberg et al. 1997). Hence, to measure competitor interaction strength, the mean from its control (-C –P treatments) was subtracted from the mean of the competition treatment (+C –P). Predator interaction strength was measured as the difference between the mean r from the predator treatment (+C +P) and the mean of its control (+C –P). "Total interaction strength" (competitor plus predator effects) was calculated as the difference between the predator treatment (+C +P) and the no predator/no competitor control (-C –P). "Relative interaction strength" was calculated for competitors, predators, and competitors plus predators (total effects) by dividing the interaction strength ( $\Delta r$ ) by the absolute value of the mean r of its control.

All statistical analyses were performed using Systat Version 8.0 (1998, SPSS Inc.).
#### Results

Pond dynamics during the year of the investigation differed from the year previous. Though *Daphnia pulex* was present in all ponds at the start of the first experimental period, P14, P15 and Shaw4 all exhibited a complete loss of *Daphnia* by the second run. In contrast, *Daphnia* dominated the Lux16 community and was present, but at low biomass densities, in P12 during both early and late season assays (table 1).

Daphnia populations exhibited positive growth responses in all controls during both experimental periods, regardless of ambient temperatures (figure 2A, 2B, solid black bars). Examining competition effects first, competitors had significant negative effects on population growth rates during both runs (figure 2A, 2B). However, the magnitude of the effect was dependent on pond identity, as indicated by a significant pond by competition interaction during both early and late season assays (table 2A, 2B). Competition effects were only significant in P12, P14, P15 and Shaw4 during both runs (p < 0.05, Tukey's HSD, for all comparisons). Competitors had no effects on Daphnia in Lux16 (p>0.30, Tukey's HSD, for both early and late season). Significant effects of competition and a pond by competition interaction were also detected when using repeated measures ANOVA (table 3). However, there was also a significant temporal component to the effects of competitors; a significant time by competition interaction was detected, indicative of a seasonal intensification (table 3). This was clear when examining mean differences between competition treatments and controls (i.e.  $\Delta r$ ) through time (figure 3A). There was also some indication that the magnitude of this seasonal intensification differed among ponds (i.e. slopes in figure 3A appear to be heterogeneous); a time by

pond by competitor interaction was detected but only at the p=0.0596 level of significance (table 3). Lux16, the pond populated by *Daphnia* during both experimental periods, exhibited only a small increase in competition effects relative to the other ponds (figure 3A). Patterns in relative competition intensity largely mirrored raw interaction strength (figure 3B).

Predators had significant negative effects on *Daphnia* population growth rates during both early and late season assays (figure 2A, 2B, table 2B, 2C). However, a significant pond by predator interaction was detected during the second run (table 2C); late in the growing season, predators had negative effects in Lux16 only (p<0.01, Tukey's HSD; p>0.90, Tukey's HSD, for all other ponds). When examining both dates together using repeated measures, predators had an overall significant effect on *Daphnia* r (table 4). Moreover, the magnitude of these effects appeared to shift as the season progressed, decreasing in most ponds while increasing in Lux16 (figure 4A). However, significant time by predator and time by pond by predator interactions were not detected (table 4). Relative effects of predators through time were rather erratic, increasing in P14 and Lux16 while decreasing in all other ponds (figure 4B). Since the predator component was generally outweighed by competition effects, total interaction strength (predators plus competitors) and relative total interaction strength mimicked trends in competitor interaction strength (figure 5A, 5B).

To explore the relative effects of competition and predation, I performed partial factorial ANOVA's for each pond separately, using the GLM feature in Systat 8.0. I modeled competition and predation as main effects and performed the analysis for both early and late season experiments. Percent variation explained by competition or

predation was calculated as the sum-of-squares for each factor divided by the total sumof-squares (i.e. the sum of competition, predation, and the residual error components) multiplied by 100 (Welden and Slauson 1986, Underwood and Petraitis 1993). Focusing first on the June experiment, the percent of experimental variation explained by competition ranged from 37.1% to 97.9% and generally outweighed predation effects (figure 6A). The single exception was P14, in which predation accounted for 55.5% and competition 37.1% of the variation. Later in the growing season, the relative effects of competition increased greatly in four of the five ponds accounting for the majority of experimental variation and overwhelming the predator component (figure 6B). Lux16 was anomalous and variation explained by predator effects (51.6%) was greater than that of competition (17.4%).

These results do not reveal the actual relevance of experimentally derived measures of competition and predation to natural *Daphnia* abundances in the ponds. To examine this I performed linear regressions of percent relative biomass of *Daphnia* measured in the ponds at the time of the experiments against the six measures of competitor and predator effects. Only relative total interaction strength (y=0.368x + 0.622, p=0.032,  $r^2$ =0.458, n=10) and relative competitor interaction strengths (y=0.317x + 0.500, p=0.039,  $r^2$ =0.433, n=10) showed significant relationships with *Daphnia* relative biomass (p>0.40, for all regressions with measures of predator effects). Figure 7 depicts relative total effects; data from Lux16 in August appear to be strongly influencing the regression. However, the relationship was still significant when removing this outlier (p=0.022,  $r^2$ =0.553, n=9). Thus, ponds in which competitor plus predator effects were strongest (most negative) were ponds with the lowest natural relative biomasses of *Daphnia*. This relationship was largely due to the competition component, with the addition of predator effects adding little to the explanatory power of the model.

I used multiple linear regressions to examine potential determinants of variation in competitor and predator effects. The large number of limnological variables measured in the ponds precluded inclusion of all factors in a regression model. Instead, I determined the key environmental measures that varied among the ponds using principal components analysis (PCA). The variables in table 1 were included in the PCA (biomass and chlorophyll a measures were  $\log_{10}$  transformed). This analysis generated 5 principal components with the first PCA axis explaining 43.9% of the total variance and the second axis accounting for an additional 18.6%. I treated the first PCA axis as the primary environmental gradient among the ponds and then determined which limnological variables correlated most strongly with its factor scores. Temperature, log total chlorophyll, seston C:P ratio, and the relative biomass of the <15 micron fraction of chlorophyll were positively correlated with the first axis (all p < 0.05, Bonferroni corrected). Only the relative biomass of the 15 to 60 micron fraction of chlorophyll was correlated (positively) with the second PCA axis (p=0.063, Bonferroni corrected). Given the small amount of additional variation explained by this axis, this measure was not included in the following analyses. However, including it does not qualitatively alter my conclusions.

I performed stepwise regressions to identify which of the four pond variables best explained variation in competitor and predator interaction strengths. Examining competitor effects first, in both forward and backward stepwise regressions, interaction

strength was significantly and negatively related only to seston C:P (p=0.0223,  $r^2$ =0.499, n=10), as was relative interaction strength (p=0.021,  $r^2$ =0.506, n=10). Thus, competitor interaction strength was greater in ponds with higher carbon to phosphorus ratios of edible resources (figure 8). Predator interaction strength and relative interaction strength were not significantly related to any of the four pond variables.

Though the PCA permitted identification of the limnological variables varying most strongly among ponds, this does not mean other factors could not have influenced competition and predation intensity. As a precaution, I fully explored the data set by performing separate linear regressions with all measures of interaction strength against all pond variables not included in the stepwise multiple regression models (I also included measures of the percent relative biomass of small-bodied cladocera and copepods). This analysis revealed only one heretofore unseen pattern. Relative competitor interaction strength was weakly related to the percent relative biomass of small-bodied cladocera (y=-0.351-1.327x, p=0.069,  $r^2$ =0.356, n=10). Given the large number of comparisons this analysis entailed, the reported p-value and confidence in these parameter estimates must be viewed with caution.

#### Discussion

The size efficiency hypothesis makes two very clear predictions regarding the operation of top-down forces and exploitative resource competition in the organization of zooplankton assemblages: large-bodied taxa are superior resource competitors and size-selective predation by fish on large-bodied zooplankton can shift dominance to smaller species. The central role that planktivorous fish can play in determining the size structure

of zooplankton communities is largely undisputed; clear examples of shifts in zooplankton size structure due to variation in selective predation on large-bodied taxa are numerous (e.g. Hrbacek et al. 1961, Brooks and Dodson 1965, Hall et al. 1976, Carpenter and Kitchell 1993, Mittelbach et al. 1995, reviewed in Gliwicz and Pijanowska 1989). However, the ability of large-bodied Daphnia to invariably dominate small-bodied taxa in competition is not as certain (Bengtsson 1987). A number of lab-based studies have shown that competitive dominance by small-bodied taxa is both empirically and theoretically feasible (e.g. Neill 1975a, Goulden et al. 1982, Romanovsky and Feniova 1985, Bengtsson 1987, Tessier and Goulden 1987). Yet, a framework that embraces resource competition between large *Daphnia* and small-bodied taxa as a primary determinant of Daphnia seasonal dynamics in fishless systems must also allow for variability in the strength and outcome of competitive interactions; evidence for this in field settings has been less forthcoming. The studies of Lynch (1978) and Smith and Cooper (1982), both performed in fishless ponds, are perhaps the best examples of field experiments that exposed variable effects of competition and competitive reversals between large *Daphnia* and small zooplankters. My study presents two notable findings: dominance by *Daphnia* can be highly variable (both temporally and spatially) in fishless systems, and the effects of resource competition (and variation in these effects), as opposed to predation, may have an overriding influence on these patterns.

In general, effects of competition outweighed predator effects in my experiment, explaining a greater proportion of experimental variation in both the June and August experimental periods. Spatiotemporal variation in competitor effects (pond by competitor and time by competitor interactions) was considerable and qualitatively consistent with

natural patterns of Daphnia abundance in the ponds. For example, effects of competitors were completely absent in Lux16, the pond with the highest levels of *Daphnia* relative biomass. Temporal trends in competitor effects were also consistent with natural patterns of Daphnia seasonal dynamics. With the exception of Lux16, all the experimental ponds experienced seasonal declines in *Daphnia pulex* abundance. Concomitant with this pattern was an increase in the negative effects of competition later in the season; Daphnia exhibited negative population growth rates in all +C-P enclosures during the August run, with the exception of Lux16. In fact, *Daphnia* were completely eliminated from all competitor enclosures in P12 and Shaw4. This temporal trend was confirmed by a significant time by competitor interaction using rm-ANOVA. No such temporal trend was evident in predator effect sizes; indeed predator effects had waned in most ponds by August. Furthermore, it appeared that the degree of intensification of competitive effects varied among ponds, increasing in all systems with the exception of Lux16 (the only experimental pond dominated by *Daphnia* in August). This pattern was detected as a time by pond by competition interaction, but was only significant at the p < 0.06 level.

When considering experimental results as a whole, *Daphnia* relative biomass was related to the magnitude of relative competitor interaction strength, rather than predator effects. Though relative total effects were related to *Daphnia* relative biomass, the addition of predator effects added little to the model fit. Though obviously not a direct causal link to dynamics of *Daphnia* in the ponds, this pattern is at least consistent with competition as a central forcing factor. This result also suggests that relative interaction strength (i.e. effect size standardized by controls) is a more informative and biologically relevant measure of "competition intensity;" a conclusion congruent with previous

assertions (e.g. Grace 1995). The reasoning for this is clear if we imagine two ponds in which competitors have equal interaction strength (i.e. equal mean differences in r between controls and competition treatments). However, in one pond this effect size may be minute relative to the potential growth response of the target species (i.e. r is large in controls and in treatments with competitors). In the other, the effect size may be large relative to the controls (i.e. the target species has a very small growth potential even in the absence of competitors). Relative interaction strength can capture such among system differences (Grace 1995).

As briefly outlined in the Introduction, factors that can influence variation in competition effects on Daphnia fall under three broad classes: variation in the structure of the co-exploiter (i.e. small-bodied zooplankton) assemblage, variation in the quantity/quality of resources, and variation in abiotic conditions (most notably temperature). Of course, these factors need not operate independently and can do so synergistically. Aspects of all three varied among the ponds. However, regressions revealed a strong relationship between competitor interaction strength (as well as relative interaction strength) and a single measure of resource quality: the carbon to phosphorus ratio of the less than 35-micron fraction of seston. Algal stoichiometry is currently an area of central focus in aquatic ecology, drawing linkages between population, community and ecosystem levels of biotic organization (Elser et al. 1996, Elser and Urabe 1999). Decreasing nutritional quality of resources (i.e. increasing C:P) can affect consumer resource-use efficiency by increasing the minimal resource quantity (i.e. R\*) needed to maintain zero net population growth rates (Sterner and Hessen 1994). This will most severely impact the most phosphorus-demanding consumers (e.g. Daphnia),

potentially altering competitive outcomes (Sterner and Hessen 1994). My experimental results were qualitatively consistent with this scenario; competition effects increased as seston C:P increased. Furthermore, competition intensity tended to be most severe for those points in space/time at which C:P was greater than 300 (figure 8), the threshold food ratio above which *Daphnia* production is estimated to be limited by phosphorus (Urabe and Watanabe 1992). Several studies have documented effects of algal C:P and N:P on *Daphnia* population performance (e.g. Sterner 1993, Urabe et al. 1997, Elser et al. 2001), yet this is the first study to reveal covariation between algal stoichiometry and experimentally quantified effects of competition.

While intriguing, some caution is warranted when interpreting this pattern. First, seston stoichiometry did not vary independently among the ponds and hidden colinearities could have accounted for variation in competition effects. For example, high C:P is indicative of algal nutrient limitation, which in turn could be associated with changes in algal composition. The fact that several variables, in addition to seston C:P, were significantly correlated with the first PCA axis is evidence that numerous factors covaried among the ponds. This is a potential drawback of any across system experiment of this type and only further experimental manipulation of potential causal factors themselves can reveal true causal relationships.

While their impacts relative to competition were small, invertebrate predators did have measurable effects over and above competitors, most notably in two ponds on two dates. During August in Lux16, predator effect size (interaction strength) was nearly two times higher than competitor effects and predation accounted for 3 times the percent variation explained by competition. However, this pond was dominated by *Daphnia* 

during the August run and overall effects of predators and competitors (total interaction strength) were the lowest among the five ponds, suggesting that neither force was an effective controlling agent. Predators also had large effects on *Daphnia* r in P14 during June; predator interaction strength was nearly 2.5 times higher than competitor effects and explained 1.5 times more experimental variation. Unlike Lux16, total interaction strength was high in P14 during July and predator effects comprised a large fraction of this negative effect. Thus predators likely had the capacity to control and regulate *Daphnia* populations in this pond at the time of the experiment.

Despite this, competition effects on the whole outweighed predator effects at those times and in those ponds that harbored low or non-existent Daphnia densities. The solidity of this result, though, must be considered in light of some potential caveats. First, my experiment only measured predator effects over and above the effects of competition. Even though predator presence decreased Daphnia r by a small degree in most of the assays, this does not mean predators are incapable of imposing strong negative effects on Daphnia population growth. Assessing this requires a treatment in which competitors were excluded and predators were present. Furthermore, my design precluded detection of competitor by predator interactions; this could have occurred if predators shifted feeding preference in the presence of alternate prey taxa. Finally, in addition to Chaoborids, natural ponds are populated by several taxa of planktivorous invertebrates (e.g. Notonectidae and Dytiscidae), some of which may have direct negative impacts on large-bodied Daphnia. Because these predators have relatively low pond densities they could not be stocked in my enclosures at natural densities. Thus, my experiment does not allow me to say with absolute certainty that invertebrate predator assemblages have little

or no effect on *Daphnia* relative to competition. On the other hand, I am able to infer that predators (all taxa included) would have had insubstantial effects in 3 of the 5 ponds during the August experimental period. P12, P15 and Shaw4 exhibited total or near complete losses of *Daphnia* in +competitor –predator enclosures. In these systems, predator presence is not needed to explain the absence of *Daphnia* in the ponds themselves, reinforcing the general argument that top-down factors are not paramount in explaining late season declines and extinction of *Daphnia* in fishless ponds.

In addition to biotic factors, zooplankton face a changing abiotic milieu as the growing season progresses. Abiotic stressors (such as changes in pH, oxygen, temperature etc.) may exacerbate poor conditions by interacting with ambient resource quantity/quality and predation pressure. High temperature and its interaction with resources have commonly been cited as potential drivers of *Daphnia* performance (Lynch 1977, Orcutt and Porter 1984), competitive outcomes between large and small-bodied zooplankton (e.g. Lynch 1978, Bengtsson 1987), and temporal shifts in competitor interaction strength (Lynch 1978). However, *Daphnia* exhibited positive responses in all control enclosures during both runs and regressions failed to reveal a relationship between temperature and competitor/predator interaction intensity. Similarly, oxygen concentration and pH were not significantly related to measures of competition and predation interaction strength. Thus, these abiotic measures alone do not appear to be major determinants of *Daphnia* success in these ponds nor does spatiotemporal variation in biotic interactions appear to covary with these variables.

Many models of species interactions base predictions on steady-state conditions and assume static hierarchies of competitive ability and/or predator-vulnerability (e.g.

Brooks and Dodson 1965, Holt et al. 1994, Leibold 1996). Seasonally dynamic systems present a unique challenge to this form of thinking by incorporating the potential for shifts in the magnitude and direction of competitor and predator effects. This study provides clear evidence that such changes are present and the likely cause of seasonal and spatial variation in the abundance of what is considered to be a major component of planktonic communities. Furthermore, the effects of competition appear to be central in determining Daphnia incidence and abundance in fishless ponds. Though resource competition has long been recognized as an important determinant of zooplanktonic community structure, aquatic ecology has long held to the view that dominance by large Daphnia populations is controlled principally by predation pressure. This presumption is commonly found incorporated in trophic cascade models as well as general models of zooplankton succession. My results question the applicability of this model in pond systems by emphasizing bottom-up factors over top-down forces. Furthermore, resourcebased effects were not static phenomena but highly dynamic, varying both spatially and temporally in relation to the nutritional quality of resources. The impacts of algal stoichiometry on competitive outcomes between Daphnia and alternate taxa have long been suspected but actual quantification of effects on competition intensity have been deficient. The present study provides an intriguing intimation that such processes are occurring and of central importance in pond communities. Future work will need to further explore and verify whether the causal pathway underlying this pattern is a direct or indirect one.

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## **Literature Cited**

- Arner, M., S. Koivisto, J. Norberg, and N. Kautsky. 1998. Trophic interactions in rockpool food webs: regulation of zooplankton and phytoplankton by *Notonecta* and *Daphnia*. Freshwater Biology 39: 79-90.
- Bengtsson, J. 1987. Competitive dominance among Cladocera: are single-factor explanations enough? An examination of the experimental evidence. Hydrobiologia 145: 245-257.
- Brendelberger, H. 1991. Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnology and Oceanography 36: 884-894.
- Brooks, J. L., and S. I. Dodson. 1965. Predation, body size, and composition of plankton. Science 150: 28-35.
- Burns, R. J. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnology and Oceanography 13: 675-678.
- Carpenter, S. R., and J. F. Kitchell. 1993. The trophic cascade in lakes. Cambridge University Press, Cambridge, U. K.
- Chase, J. M. 1999. Food web effects of prey size refugia: Variable interactions and alternative stable equilibria. American Naturalist 154: 559-570.
- DeMott, W. R. 1989. The role of competition in zooplankton succession. Pages 195-252 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Elser, J. J., D. R. Dobberfuhl, N. A. Mackay, and J. H. Schampel. 1996. Organism size, life history, and N:P stoichiometry. Bioscience 46: 674-684.
- Elser, J. J., K. Hayakawa, and J. Urabe. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology 82: 898-903.
- Geller, W. and H. Muller. 1981. The filtration apparatus of cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia 49: 316-321.
- Gliwicz, Z. M., and J. Pijanowska. 1989. The role of predation in zooplankton succession. Pages 253-295 in U. Z. Sommer (editor). Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Goulden, C. E., L. L. Henry, and A. J. Tessier. 1982. Body size, energy reserves, and competitive ability in three species of cladocera. Ecology 63: 1780-1789.

- Grace, J. B. 1995. On the measurement of plant competition intensity. Ecology 76: 305-308.
- Gurevitch, J., L. L. Morrow, A. Wallace, and J. S. Walsh. 1992. A meta-analysis of competition in field experiments. American Naturalist 140: 539-572.
- Gurevitch, J., J. A. Morrison, and L. V. Hedges. 2000. The interaction between competition and predation: a meta-analysis of field experiments. American Naturalist 155: 435-453.
- 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 Review of Ecology and Systematics 7: 177-208.
- Hrbacek, J., M. Dvorakova, V. Korinek, and L. Prochazkova. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 14: 192-195.
- Lynch, M. 1977. Fitness and optimal size in zooplankton population. Ecology 58: 763-774.
- Lynch, M. 1978. Complex interactions between natural coexploiters-*Daphnia* and *Ceriodaphnia*. Ecology 59: 552-564.
- Lynch, M. 1979. Predation, competition, and zooplankton community structure: an experimental study. Limnology and Oceanography 24: 253-272.
- Mittelbach, G. G., A. M. Turner, D. J. Hall, J. E. Rettig, and C. W. Osenberg. 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. Ecology 76: 2347-2360.
- Murdoch, W. W., M. A. Scott, and P. Ebsworth. 1984. Effects of the general predator *Notonecta* (Hemiptera) upon a freshwater community. Journal of Animal Ecology 53: 791-808.
- Neill, W. E. 1975a. Experimental studies of microcrustacean competition, community composition, and efficiency of resource utilization. Ecology 56: 809-826.
- Neill, W. E. 1975b. Resource partitioning by competing microcrustaceans in stable laboratory microecosystems. Internationale Vereinigung fur Theoretische und Angewandte Limnologie 19: 2885-2890.
- Neill, W. E. 1981. Impact of *Chaoborus* predation upon the structure and dynamics of a crustacean zooplankton community. Oecologia 48: 164-177.

- Orcutt, J. D., and K. G. Porter. 1984. The synergistic effects of temperature and food concentration on life history parameters of *Daphnia*. Oecologia 63: 300-306.
- Osenberg, C. W., O. Sarnelle, and S. D. Cooper. 1997. Effect size in ecological experiments: the application of biological models in meta-analysis. American Naturalist 150: 798-812.
- Romanovsky, Y. E., and I. Y. Feniova. 1985. Competition among Cladocera: effect of different levels of food supply. Oikos 44: 243-252.
- Scott, M. A. and W. M. Murdoch. 1983. Selective predation by the backswimmer, *Notonecta*. Limnology and Oceanography 28: 352-366.
- Sih, A., P. Crowley, M. McPeek, J. Petranka, and K. Strohmeier. 1985. Predation, competition, and prey communities: a review of field experiments. Annual Review of Ecology and Systematics 16: 269-311.
- Smith, D. W., and S. D. Cooper. 1982. Competition among cladocera. Ecology 63: 1004-1015.
- Sommer, U. 1989. Plankton ecology: succession in plankton communities. Springer-Verlag, New York, New York.
- Sommer, U., M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG-model of seasonal succession of planktonic events in freshwaters. Archiv fur Hydrobiologie 106: 433-471.
- Spitze, K. 1985. Functional response of an ambush predator: *Chaoborus americanus* predation on *Daphnia pulex*. Ecology 66: 938-949.
- Sterner, R. W. 1993. *Daphnia* growth on varying quality of *Scenedesmus*: mineral limitation of zooplankton. Ecology 74: 2350-2360.
- Sterner, R. W., J. J. Elser, and D. O. Hessen. 1992. Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. Biogeochemistry 17: 49-67.
- Sterner, R.W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1-29.
- Swift, M. C., and A. Y. Fedorenko. 1975. Some aspects of prey capture by *Chaoborus* larvae. Limnology and Oceanography 20: 418-425.
- Tessier, A. J. and C. E. Goulden. 1987. Cladoceran juvenile growth. Limnology and Oceanography 32: 680-686.

- Tessier, A. J. and J. Welser. 1991. Cladoceran assemblages, seasonal succession and the importance of a hypolimnetic refuge. Freshwater Biology 25: 85-93.
- Threlkeld, S. T. 1986. Resource-mediated demographic variation during the midsummer succession of a cladoceran community. Freshwater Biology 16: 673-683.
- Tillmann, U. and W. Lampert. 1984. Competitive ability of differently sized *Daphnia* species: an experimental test. Journal of Freshwater Ecology 2: 311-323.
- Underwood, A. J., and P. S. Petraitis. 1993. Structure of intertidal assemblages in different locations: how can local processes be compared? Pages 39-51 in R. E. Ricklefs and D. Schluter (editors). Species diversity in ecological communities. University of Chicago Press, Chicago, Illinois.
- Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: is it real? Limnology and Oceanography 42: 1436-1443.
- Urabe, J., and Y. Watanabe. 1992. Possibility of N or P limitation for planktonic cladocerans: an experimental test. Limnology and Oceanography 37: 244-251.
- Vinyard, G. L., and R. A. Menger. 1980. *Chaoborus americanus* predation on various zooplankters: functional response and behavioral observations. Oecologia 45: 90-93.
- Welden, C. W., and W. L. Slauson. 1986. The intensity of competition versus its importance: an overlooked distinction and some implications. The Quarterly Review of Biology 61: 23-44.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. Limnology and Oceanography 39: 1985-1992.
- Wetzel, R. G., and G. E. Likens. 1991. Limnological Analyses, 2<sup>nd</sup> Edition. Springer-Verlag, New York.

Pond	pН	Oxygen (mg/l)	Temp.	Total Chl-a (µg/l)	Relative <15µ Chl-a	Relative <60µ Chl-a	Relative 15 to 60µ Chl-a
Early Seas	son (Ju	ine)				- <u>1</u> 2	
P12	6.90	6.01	25.3	5.27	0.91	0.96	0.05
P14	6.92	6.00	25.5	8.65	0.52	0.74	0.22
P15	7.05	11.30	25.8	6.47	0.31	0.48	0.17
Shaw4	4.60	9.00	23.5	18.44	0.15	0.54	0.38
Lux16	6.05	8.91	24.5	188.16	0.20	0.82	0.62
Late Seaso	on (Au	igust)					
P12	8.09	2.64	26.0	4.93	0.39	0.72	0.33
P14	8.22	5.52	26.0	7.25	0.60	0.65	0.05
P15	8.18	6.19	26.5	3.48	0.54	0.79	0.25
Shaw4	5.30	0.28	25.0	72.38	0.23	0.70	0.47
Lux16	6.60	0.75	22.0	49.16	0.20	0.31	0.12

Table 1. Limnological variables measured during the early season (June) and. late season (August) experimental periods.

Total Zoop. Biomass (mg/l)	Daphnia Biomass (mg/l)	Copepod Biomass (mg/l)	Small Cladocera Biomass (mg/l)	Chaoborus (no./l)	C:P (molar)	N:P (molar)
n (June)						
0.145	0.005	0.102	0.038	0.70	416.73	105.76
0.115	0.005	0.033	0.073	1.66	488.66	142.97
0.155	0.000	0.056	0.091	2.15	437.12	53.32
0.083	0.018	0.019	0.039	0.52	238.12	24.33
0.120	0.078	0.040	0.000	4.11	121.02	14.16
Late Season (August)						
0.124	0.001	0.050	0.073	1.60	548.70	36.59
0.022	0.000	0.012	0.010	0.13	439.63	34.94
0.012	0.000	0.005	0.008	0.72	497.17	36.56
0.011	0.000	0.000	0.011	0.69	498.76	36.64
0.180	0.165	0.007	0.000	6.33	155.59	11.33
	Total Zoop. Biomass (mg/l) n (June) 0.145 0.115 0.155 0.083 0.120 n (August) 0.124 0.022 0.012 0.012 0.011 0.180	Total Zoop. Biomass (mg/l) Daphnia Biomass (mg/l)   n (June) 0.145 0.005   0.145 0.005 0.115   0.155 0.000 0.083 0.018   0.120 0.078 0.001   0.022 0.000 0.012 0.000   0.011 0.000 0.011 0.000   0.180 0.165 0.165	Total Zoop, Biomass (mg/l)Daphnia Biomass (mg/l)Copepod Biomass (mg/l)n (June)0.1450.0050.1020.1450.0050.0330.1150.0050.0330.1550.0000.0560.0830.0180.0190.1200.0780.040(August)0.0220.0000.0120.0000.0120.0120.0000.0050.0110.0000.0000.1800.1650.007	Total Zoop. Biomass (mg/l) Daphnia Biomass (mg/l) Copepod Biomass (mg/l) Small Cladocera Biomass (mg/l)   n (June) 0.145 0.005 0.102 0.038   0.145 0.005 0.102 0.038   0.115 0.005 0.033 0.073   0.155 0.000 0.056 0.091   0.083 0.018 0.019 0.039   0.120 0.078 0.040 0.000   0.124 0.001 0.050 0.073   0.124 0.001 0.050 0.073   0.012 0.000 0.012 0.008   0.011 0.000 0.005 0.008   0.011 0.000 0.000 0.011	Total Zoop. Daphnia Biomass (mg/l) Copepod Biomass (mg/l) Small Cladocera Biomass (mg/l) Chaoborus (no./l)   n (June) 0.145 0.005 0.102 0.038 0.70   0.145 0.005 0.102 0.038 0.70   0.115 0.005 0.033 0.073 1.66   0.155 0.000 0.056 0.091 2.15   0.083 0.018 0.019 0.039 0.52   0.120 0.078 0.040 0.000 4.11   1 (August) 0.12 0.010 0.13   0.012 0.000 0.005 0.008 0.72   0.011 0.000 0.000 0.011 0.69   0.180 0.165 0.007 0.000 6.33	Total Zoop. Biomass (mg/l) Daphnia Biomass (mg/l) Copepod Biomass (mg/l) Small Cladocera Biomass (mg/l) Chaoborus (no./l) C:P (molar)   n (June) 0.145 0.005 0.102 0.038 0.70 416.73   0.145 0.005 0.102 0.038 0.70 416.73   0.115 0.005 0.033 0.073 1.66 488.66   0.155 0.000 0.056 0.091 2.15 437.12   0.083 0.018 0.019 0.039 0.52 238.12   0.120 0.078 0.040 0.000 4.11 121.02   n(August) 0.122 0.000 0.012 0.010 0.13 439.63   0.012 0.000 0.005 0.008 0.72 497.17   0.011 0.000 0.000 0.011 0.69 498.76   0.180 0.165 0.007 0.000 6.33 155.59

Table 2. Results of two-way analyses of variance testing for effects of competition and pond identity (A, B) or predation and pond identity (C, D) on *Daphnia* per capita population growth rate (r) at two times in the growing season (early versus late season).

Source of variation	df	SS	F	Р
A) Early Season				
Competition	1	0.075	243.866	<0.0001
Pond	4	0.210	171.254	<0.0001
Pond x Competition	4	0.023	18.906	<0.0001
Error	20	0.006		
B) Late Season				
Competition	1	0.249	466.573	<0.0001
Pond	4	0.117	54.509	<0.0001
Pond x Competition	4	0.058	27.112	<0.0001
Error	20	0.011		

# Table 2. cont'd.

Source of variation	df	SS	F	Р	
C) Early Season					
Predation	1	0.006	7.036	0.015	
Pond	4	0.319	96.481	<0.0001	
Pond x Predation	4	0.005	1.491	0.243	
Error	20	0.017			
D) Late Season					
Predation	1	0.004	6.255	0.021	
Pond	4	0.205	91.497	<0.0001	
Pond x Predation	4	0.007	3.262	0.033	
Error	20	0.011			

Table 3. Univariate repeated measures ANOVA testing for effects of competition, pond identity and time (i.e. early versus late season) on *Daphnia* per capita population growth rate (r).

Source of variation	df	SS	F	Р		
Univariate, between subjects effects						
Competition	1	0.298	981.913	<0.0001		
Pond	4	0.311	255.482	<0.0001		
Pond x Competition	4	0.075	61.940	<0.0001		
Error	20	0.006				
Univariate, within subjects e	ffects					
Time	1	0.077	143.000	<0.0001		
Time x Competition	1	0.026	47.707	<0.0001		
Time x Pond	4	0.016	7.241	<0.001		
Time x Pond	4	0.006	2.707	0.0596		
x Competition						
Error	20	0.011				

Table 4. Univariate repeated measures ANOVA testing for effects of predation, pond an	ıd
time (i.e. early versus late season) on Daphnia per capita population growth rate (r).	

Source of variation	df	SS	F	Р	
Univariate, between subjec	cts effects				
Predation	1	0.009	12.778	0.002	
Pond	4	0.511	177.717	<0.0001	
Pond x Predation	4	0.007	2.401	0.084	
Error	20	0.014			
Univariate, within subjects	effects				
Time	1	0.181	270.000	<0.0001	
Time x Predation	1	0.0001	0.217	0.646	
Time x Pond	4	0.014	5.081	0.005	
Time x Pond	4	0.005	1.998	0.134	
x Predation					
Error	20	0.013			

Figure 1. Results of a survey of 18 permanent, fishless pond in southern Michigan. Shown is relative biomass of *Daphnia pulex* (mean of two replicate samples, +/- 1 S. E.) for A) late April, 1998 and B) late July, 1998. Ponds are ordered from low to high productivity based on mean total phosphorus measures. Ponds 1, 2, 3, 10 and 16 correspond to the experimental ponds P15, P14, P12, Shaw4 and Lux16, respectively.



Figure 1

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Figure 2. Population growth rates of *Daphnia* in each pond and in the three experimental treatments for A) the early season (June) run and B) the late season (August) run. Shown are means, +/- 1 S. E.



Figure 2

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Figure 3. Temporal trends and among pond differences in A) competitor interaction strength ( $\Delta r$ ) and B) relative competitor interaction strength.



Figure 3

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Figure 4. Temporal trends and among pond differences in A) predator interaction strength  $(\Delta r)$  and B) relative predator interaction strength.





اند موجد. سۇرىيارىڭ Figure 5. Temporal trends and among pond differences in A) total interaction strength (competitor plus predator effects) and B) relative total interaction strength.



Figure 5

Figure 6. Percent of total variation explained by competition and predation effects in partial factorial ANOVA's performed separately for each pond. Shown are results for A) June experiments and B) August experiments.



Figure 6

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Figure 7. The relationship between *Daphnia* relative biomass (measured in the ponds during the experimental runs) and relative total interaction strength, for all ponds and both experimental periods.


Figure 8. The relationship between relative competitor interaction strength and seston C:P.

