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Ph.D. degree in Zoology

Major Professor's Signature

2/23/04

Date

SPECIES INTERACTIONS AND THE FUNCTIONING OF POND ECOSYSTEMS.

By

Jeremy M. Wojdak

A DISSERTATION

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Department of Zoology

2004

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ABSTRACT

SPECIES INTERACTIONS AND THE FUNCTIONING OF POND ECOSYSTEMS.

By

Jeremy M. Wojdak

Ecologists have become interested in the functional roles of biodiversity at the ecosystem level. Positive effects of diversity on the rates of ecosystem processes have been observed, but mechanisms are rarely understood. Moreover, variation in the strength of species richness effects across abiotic or biotic gradients remains largely unexplored. This dissertation addresses gaps in our understanding of the functional roles of biodiversity by studying herbivorous freshwater snail communities as a model system.

I examined the consequences of aquatic snail species richness on the biomass of several functional groups and ecosystem processes in laboratory microcosms. Higher snail species richness led to higher herbivore biomass, and less algal biomass and total organic matter. Snail species used five habitat types differently, and groups of species with dissimilar habitat use had stronger diversity effects for several response variables. These results suggest that niche complementarity is the mechanism responsible for snail species richness effects on pond ecosystem properties and community structure.

However, niche complementarity among species is likely to vary as species' niches change in response to their abiotic and biotic environment. I

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manipulated the physiological state of pond snails, the abundance of algal resources, and predation cues in a full factorial design to assess their effects on snail habitat use, an important aspect of the niche of snails. In general, snails foraged more and spent less time in refuge when resources were abundant, when predators were absent, and when previously exposed to physiological stress (i.e. starvation).

Since the traits of species vary along ecological gradients, the complementarity among species' niches should be context dependent. To assess this possibility, and the consequences for the functioning of pond ecosystems, I varied snail species richness, predation intensity, and resource availability in a mesocosm field experiment. Greater snail species led to greater secondary production, consumer biomass, and macrophyte stem growth, and lower epiphyton and periphyton biomass. However, predators reduced the effect of snail species richness on the biomass of attached algae. Snail species richness effects on many functional groups were stronger than those of a substantial nutrient enrichment or a voracious top-predator.

DEDICATION

To my parents, Lark and Michael Wojdak.

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ACKNOWLEDGEMENTS

I thank N. Dorn, H. Wojdak, G. Mittelbach, M. Dorn, S. Whitmire, T. Darcy, E. Thobaben, B. Luttbeg, and E. Garcia for assistance in the field and laboratory. G. Mittelbach, E. Thobaben, S. Hamilton, N. Dubois, N. Dorn, C. Steiner, J. Conner, T. Getty, and M. Leibold provided helpful advice regarding conceptual issues, experimental design, statistics, and sampling techniques. S. Hamilton, M. Leibold (support from NSF-DEB 9815799), and M. Klug supplied critical equipment. Comments from K. Gross, O. Sarnelle, S. Hamilton, G. Mittelbach, R. Smith, N. Dorn, B. Luttbeg, E. Thobaben, and H. Wojdak greatly improved the text. I received financial support from a NSF-funded Research Training Grant (NSF DIR-09113598 and DBI-9602252), a Dissertation Completion Fellowship (College of Natural Science, MSU), the Department of Zoology and the Ecology, Evolutionary Biology and Behavior Program at MSU, and G. H. Lauff Graduate Research Awards. N. Consolatti, A. Gillespie, S. Shaw, C. Adams, and J. Gorentz provided fantastic “behind the scenes” support.

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CHAPTER ONE

SPECIES INTERACTIONS AND THE FUNCTIONING OF POND ECOSYSTEMS.

The past ten years have seen an explosion of interest among ecologists in the functional roles of biodiversity at the ecosystem level (Loreau et al. 2001). This interest is largely motivated by the high rates of extinction for many taxa and the dependence of human society on the functioning of ecosystems. Despite much activity, many fundamental questions remained unanswered or unexplored. For instance, many empirical examples of positive effects of species diversity on the rates of ecosystem processes exist (e.g., Tilman et al. 2001, Downing and Leibold 2002), but very rarely has a mechanism been established to explain those patterns (but see Cardinale et al. 2001). Moreover, whether the strength of species diversity effects varies across abiotic or biotic gradients remains relatively unknown. The work presented here hopes to address some of these gaps in our understanding of the functional roles of biodiversity. This chapter provides an overview of the remainder of the dissertation.

Chapter Two examines the consequences of snail species richness on the biomass of various functional groups (e.g., snails, algae, plants) and on several ecosystem processes (e.g., primary productivity, accrual of organic matter) in a microcosm experiment, and evaluates whether these effects can be explained by the niche complementarity hypothesis. The niche

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complementarity hypothesis suggests that diverse assemblages of species will be able to utilize a greater proportion of the total resource pool because species have different niches (e.g., resource use, habitat use, phenology). I found that herbivore (snail) species richness influenced the biomass of herbivores and algae, and the accrual of organic matter in microcosms. Moreover, there were significant differences among the six snails species in their use of five distinct habitat types. Among snail species that used similar habitats, I saw little change in the community and ecosystem level response variables with increasing diversity, but among those with divergent habitat use I saw greater changes. This work is novel in that it demonstrates that the effects of increased species richness are quantitatively predictable using measures of niche overlap (e.g., similarity in habitat use) among species.

Complementarity between species' niches is not a static property, however. The resource use, habitat use, and behavior of species can be plastic, responding to both biotic and abiotic contexts. For instance, the habitat use of prey is often influenced strongly by the risk of predation (Werner et al. 1983, Turner and Mittelbach 1990). In Chapter Three I investigated the plasticity of a snail's (*Physa gyrina*) behavior and interactions with its resource, as a function of predation risk, resource abundance, and the snails' energetic state. *Physa* were found to hide more/forage less when predators were present, resources were scarce, and when in good energetic state. Although these results seem intuitive, they seem to contradict a body of well-reasoned theory (e.g., Abrams 1991, Werner and Anholt 1993) that suggests that foraging

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effort should decrease with increasing resource abundance. The strength of the interaction between the prey (snails) and the resource (periphytic algae) was also observed to depend on the presence of predation risk, suggesting that predators can have community-wide effects mediated by changes in the traits of their prey.

The ability of a predator to influence the abundance of a basal resource (i.e. two trophic levels below) by inducing changes in the traits of its prey has only recently been appreciated by ecologists (Abrams 1984, Lampert 1987, Turner and Mittelbach 1990, Huang and Sih 1991, Turner 1997). Peacor and Werner (2000, 2001) suggest that at least in some systems the effects of predators mediated through prey traits can be as large or larger than those mediated by changes in the density of prey. However, as described in Chapter Three, the abundance of a resource can influence the response of prey to predation risk. For instance, if resources are very abundant, prey facing predation risk may continue to forage despite that risk (e.g., no/small change in prey traits), while at low resources prey may decide to seek refuge (e.g., large change in prey traits). Chapter Four (a collaborative effort with Bernard Luttbeg) explores the strength of trait-mediated and density-mediated indirect interactions between a predator and the basal resource in a three-trophic level experimental food chain, across a gradient of resource abundance. Trait-mediated effects exceeded those mediated by changing density at low resource abundance, while the reverse was true at high initial resource levels. These results suggest that trait-mediated indirect effects may represent a large

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proportion of the total effect of predators under some conditions, and thus deserve greater attention from community ecologists.

The divergent traits of species are the fuel for the niche complementarity mechanism, and Chapters Three and Four demonstrate that the traits of at least one aquatic snail species are quite plastic, as has been shown for other snail species (e.g., Chase 1999). Chapter Five explores the consequences of trait variability among snails (induced by resource and predation gradients) for the strength of species richness effects on ecosystems and food webs. In general, snail species richness had strong effects on snail biomass, algal biomass, and the growth of aquatic vascular plants. However, the appearance of some effects of species richness depended on the ecological context (e.g., the presence of predators and the initial resource levels). Species richness effects could not, for the most part, be explained by sampling effects (i.e. the higher probability of having a particularly dominant species in a diverse assemblage - Huston 1997). These strong food web effects did not translate into strong ecosystem level effects probably because of compensatory responses between the two main producer groups – algae and plants. Overall, species richness effects were similar in magnitude to strong predator and resource manipulations, suggesting a prominent role for herbivore diversity in structuring aquatic communities.

The appendices contain several experiments and a field survey that were used to collect ancillary information relevant to understanding the functional roles of the snail species used in previous experiments. The reproductive rates

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of six snail species were determined in a set of semi-natural field observations (Appendix A). Patterns of movement (e.g., speed, probability of changing direction) across low and high resource environments were determined for six snail species, as was the ability to find high resource patches in a spatially heterogeneous foraging arena (Appendix B). A field survey of 16 ponds near the Kellogg Biological Station was conducted to understand the patterns of occurrence of snail species across natural gradients of species richness (Appendix C). Experiments that evaluate the effects of species richness on ecosystem processes often used random community compositions. Natural communities are not thought to be randomly assembled, so such experiments may not accurately describe natural ecosystem's response to variation in species richness. The field survey data presented in Appendix C were used in Chapter Five to evaluate the implications of differences between compositions of experimental and natural communities for interpreting species richness effects on ecosystems. Appendix D evaluates whether the predator *Belostoma flumineum* has preferences among the snail species used in the experiment reported in Chapter Five.

Together, the research presented here suggests that the diversity of herbivorous animals can have meaningful effects on the structure of food webs and possibly on ecosystem processes, and that the strength of these effects is likely to vary in predictable ways across common ecological gradients. Moreover, these results demonstrate that it is necessary to understand the behavior of

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behavior of individuals to fully understand the dynamics of ecological communities.

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CHAPTER TWO

CONSEQUENCES OF HABITAT PARTITIONING AMONG SNAILS FOR POND ECOSYSTEM FUNCTION.

Abstract:

While the number of studies investigating the effects of species diversity on ecosystem properties continues to grow rapidly, few have examined how ecosystem functioning depends on the degree of niche similarity among species (i.e. the niche complementarity hypothesis). The results of a microcosm experiment are reported where similarity in habitat use among a guild of aquatic snails successfully predicts changes in ecosystem properties with increasing species richness.

Replicate microcosms with all possible one and two species combinations of a guild of six snail species were started with identical initial snail biomass. By the end of the experiment, microcosms with two species of snails had greater snail biomass, less total organic matter, and less periphyton biomass than monocultures. The identity of species present in the microcosms had strong effects on total organic matter, snail biomass, primary productivity, periphyton biomass, and sedimentation rate. Snail species differed in their use of five distinct habitat types in the microcosms. Similarity in habitat use between a species pair was negatively related to the magnitude of diversity effects (for dissolved oxygen, periphyton biomass, and accrual of organic matter). Sampling effects were ruled out as the cause of diversity effects in some cases, but not others. These results suggest that niche complementarity

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Introduction:

Theory and experiments demonstrate that the number of species in a local area can influence the rates of ecosystem processes (reviewed in Schalpfer and Schmid 1999, Loreau et al. 2001), but few studies have examined the mechanisms behind these effects. Understanding the mechanistic basis for positive effects of diversity on ecosystem functioning will be important if ecologists hope to predict the consequences of species loss in natural communities.

Some have suggested (Aarssen 1997, Huston 1997, Wardle 1999, Tilman et al. 1997) that “sampling” or “selection probability” effects may explain positive changes in ecosystem function with increasing species richness. In experiments with random subsets of species at each of several levels of diversity, for example, dominant species will occur more frequently in diverse communities and could by themselves lead to increasing ecosystem function with increasing diversity. Sampling effects have been described as experimental artifacts by some (Huston 1997, Wardle 1999), and defended as a viable mechanism in nature by others (Tilman et al. 1997). Indirect statistical approaches have been developed to separate sampling effects from other diversity mechanisms (Garnier et al. 1997, Hector 1998, Loreau 1998, Emmerson and Rafaelli 2000, Loreau and Hector 2001, Adler and Bradford 2002, Petchey 2003), but there has been debate about the application and interpretation of these statistics (Loreau and Hector 2001, Hector et al. 2002, Petchey 2003). Moreover, some techniques require knowledge of the

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contribution of individual species to ecosystem function in mixture, data that are difficult or impossible to obtain for many responses.

Only two other mechanisms, facilitation and niche complementarity, have received much consideration. Cardinale et al. (2002) propose that diverse assemblages of stream insects achieve greater total resource capture than less diverse assemblages because of a physical facilitation mechanism. Complementary resource use has been suggested to explain greater plant productivity in diverse plant communities (e.g., Tilman et al. 2001), and is the most commonly discussed and modeled diversity mechanism (Tilman et al. 1997, Hooper 1998, Petchey 2000, Loreau et al. 2001). However, few if any studies have attempted to predict the consequences of increases in species richness using a quantitative measure of niche overlap (e.g., resource partitioning, similarity in habitat use).

If niche complementarity is a mechanism operating to enhance ecosystem function, sets of species that are very similar in niche should show little if any positive effects of diversity on ecosystem functioning (Figure 1A, species X and Y), whereas those with pronounced niche differentiation should show strong diversity effects (Figure 1A, species Y and Z). Thus, strong evidence for the niche complementarity mechanism would require that: 1) species richness has a positive effect on ecosystem function, and 2) a negative relationship exists between the similarity of species' niches and the magnitude of richness effects. The first requirement can be evaluated by comparing observed polyculture performance with that expected from the performance of the constitu

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the constituent species in monoculture. The measure δEF (defined in Petchey 2003 and elsewhere) describes the effects of diversity on an ecosystem function in this way:

> δEF = observed polyculture yield – expected polyculture yield,
>
> where expected polyculture yield = $\Sigma(p_i * EF_i\text{monoculture})$ for all species *i*,
>
> p*i* = proportion of species *i* in mixture, and EF*i*monoculture = the yield of species *i* in monoculture.

The second requirement can be examined with a simple regression between an index of niche overlap and δEF (Figure 1B).

The above approach circumvents the need to rely on post-hoc statistical partitioning of diversity effects into that due to sampling effects and that due to complementarity among species (*sensu* Loreau and Hector 2001), which is especially important when it is impossible to measure the contribution of individual species in a mixture to ecosystem functioning. If sampling effects are the sole mechanism operating in an experiment the second criterion above will fail (e.g., non-significant regression in Figure 1B). Here I present the results of a laboratory microcosm experiment where snail species richness effects on ecosystem functioning were accurately predicted by an index of habitat use similarity.

Figure 1. The niche complementarity hypothesis suggests that ecosystem functioning should increase with increasing species richness because of niche differences among species (e.g., resource use, habitat use, phenology). A hypothetical example is presented in A) where species X, Y, and Z are maintained in monoculture and polyculture. Species X and Y are very similar in niche (represented by strongly overlapping circles), and so δEF (defined in text) should be small. Species Y and Z are very different in niche, and so δEF should be large. With many such pair-wise comparisons the relationship between niche overlap and δEF could be examined, as in B). If niche complementarity is responsible for species richness effects on ecosystems, the slope of the regression line in B) should be negative.

Figure 1

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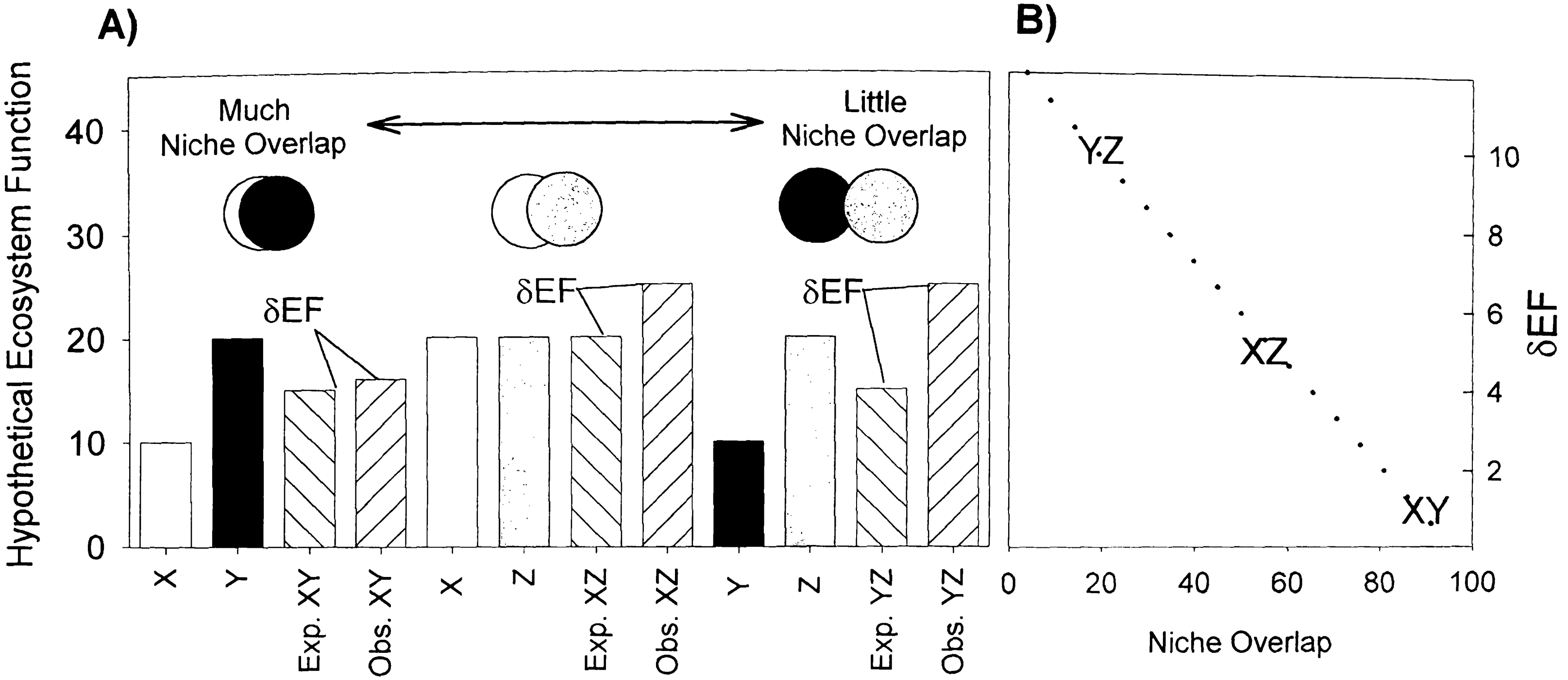
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Methods:

The experiment was conducted in 100 18 L plastic microcosms, filled on 6 Jun 2001 with 16 L of water (filtered through an 80 μm sieve) from a reservoir at the W. K. Kellogg Biological Station's Experimental Pond Facility. The microcosms were set up in a greenhouse (under semi-natural light and temperature regimes). Air conditioning kept the room and water temperatures moderated on exceedingly warm days (maximum temperature recorded = 29° C). Algae were collected from six local ponds, homogenized by vigorous mixing, and added as an inoculum to each microcosm. Nutrients were added (75 μg/L P and 1875 μg/L N, as KH_2PO_4 and NH_4NO_3) to raise the microcosms' productivity to eutrophic status. The aquatic plant *Ceratophyllum demersum* was added to each microcosm (5 g wet mass), as was an equal amount of dead *Ceratophyllum* to serve as detritus. Sand was added as a benthic substrate. Microcosms were covered with window screen lids to prevent entry/exit of organisms.

The simplest possible diversity effect is that resulting from having two species instead of one, and a pair of species similarly represents the simplest case for understanding niche complementarity. To facilitate the planned analyses I setup one and two species combinations. This low level of snail species richness is realistic; a survey of 16 ponds near the W. K. Kellogg Biological Station revealed an average snail species richness of 2.25 species per pond (Appendix C). Individuals of six snail species common to SW Michigan (*Amnicola limosa*, *Bithynia tentaculata*, *Fossaria obrussa*, *Gyraulus*

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parvus, *Helisoma trivolvis*, and *Physa gyrina* - hereafter referred to by generic names) were added to the microcosms on 23 Jun in all possible one and two species combinations (i.e. six monoculture and fifteen polyculture treatments). I used a replacement design (similar to many competition experiments) such that initial snail biomass was 20 mg dry weight (without shells) in each microcosm; monocultures received 20 mg of one species and polycultures received 10 mg each of two species. Each monoculture was replicated six times, while each polyculture was replicated four times. This difference in sample sizes was designed to partially alleviate the difference in total numbers of replicates contributing to the species richness level means. In addition, four control microcosms were established that contained no snails. Treatments were assigned to experimental units randomly.

Snail habitat use was observed five times during the experiment (3 Jul, 14 Jul, 26 Jul, 6 Aug, 21 Aug). Snails were recorded as being on the sides of the container, on the plant, on sand, or on detritus, or at the air-water interface. The area of the five habitat types was estimated to provide a null expectation of random distribution throughout the microcosms (i.e. if habitat A was 5% of total, 5% of snails should be observed in habitat A). The area of three dimensional habitats (plants and detritus) was estimated by calculating the surface area of cylinders of the same dimensions as the plant branches. Snail biomass and abundance were quantified at the end of the experiment (10 Oct) by counting and measuring all snails of each species in each treatment using a dissecting microscope and digitizing tablet, and using length-weight regressions (C.

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Osenberg, *unpublished data*) to convert linear measurements of the shells to dry mass of animals. A few snails and odonate larvae invaded the microcosms, most likely as eggs attached to the planted macrophytes. Effects of invasion on response variables were not detected.

Plants were weighed at the end of the experiment. Periphyton biomass was measured on 3 Jul, 18 Jul, 30 Jul, 18 Aug, and 19 Sep by removing plastic strips that had been adhered to the bucket sides before the experiment began. These strips were placed into 95% ethanol to extract pigments, and the resulting chlorophyll *a* concentration (a surrogate for periphyton biomass) was determined using narrow band flourometry (Welschmeyer 1994). Phytoplankton biomass was quantified on 20 Jul and 13 Aug by removing 100 mL of water from the center of each microcosm, filtering on a glass fiber filter, and determining chlorophyll *a* concentration as above for periphyton.

The diel peak concentration of dissolved oxygen can indicate the relative balance of productivity and respiration in aquatic systems. In this experiment I measured dissolved oxygen at dusk on 3 Jul, 13 Jul, 24 Jul, 7 Aug, and 10 Sep, using a YSI Model 57 meter. On 8 Aug I also measured dissolved oxygen in the microcosms at dawn, which allowed calculation of total system respiration rates over the previous ~12 h period of darkness. Respiration rates were not different among treatments (ANOVA - species richness df = 1, 75, F= 0.12, P=0.72, composition df = 19, 75, F = 1.57, P=0.09), so I consider any differences in dissolved oxygen to reflect differences in primary productivity.

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The accumulation of organic sediment was quantified on 2 Sep in the microcosms by placing a syringe just above the sand substrate and withdrawing 50 mL of water and substrate. Sand grains were heavy enough to fall out of the syringe tip, leaving the organic component of the sediment, which was subsequently filtered onto glass fiber filters, dried (60° C for 24 h), weighed, combusted in a muffle furnace (550° C for 1 h), and reweighed to calculate the ash-free dry weight of the sediment. Accrued sediment was dominated by snail feces in all treatments. At the end of the experiment the total organic matter in the microcosms was quantified by brushing all surfaces, filtering the entire microcosms' contents (minus the plant and snails) onto a glass fiber filter, drying, and weighing.

Statistical Methods

Snail habitat use measures (number of snails observed in a given habitat) were summed across the five observation dates, and arcsin-square root transformed to meet normality assumptions of the analyses. MANOVA was used to test for overall differences between species' habitat use in monoculture, and was followed by ANOVAs for individual habitats (e.g., do species differ in their use of sand habitat). Repeated measurements of dissolved oxygen, periphyton biomass, and phytoplankton were averaged. The effects of species richness and species composition (nested within richness) on the suite of ecosystem/food web response variables were examined using ANOVA. Total organic matter, sedimentation rate, periphyton biomass, and phytoplankton biomass data were all log-transformed prior to analyses to meet assumptions of

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ANOVA. Two statistics were calculated to describe the response of ecosystem functions to changes in species richness, δEF (defined above) and D_{max} (as in Loreau 1998) defined as:

$$D_{\max} = \frac{O_T - MAX(M_i)}{MAX(M_i)},$$

where O_T is the observed yield of a mixture and MAX(M_i) is the maximum observed yield of any species in monoculture.

D_{max} can be thought of as the proportional deviation of the total polyculture yield from the highest yielding of the constituent species in monoculture. Complementarity mechanisms could result in the polyculture having greater ecosystem function than the average of the monocultures ($\delta EF>0$), and would definitively be acting if the yield was greater than the highest monoculture (Figure 2, $D_{max}>0$). If only sampling effects are operating, the level of ecosystem functioning in polyculture could match the level of the highest monoculture, but not exceed it (Figure 2, $D_{max}\leq 0$). Thus, a range of values of these two statistics exists (e.g., $\delta EF>0$, $D_{max}\leq 0$) where either sampling effects or niche complementarity may account for diversity effects.

The above statistics, developed for studies of plant diversity and production, can easily accommodate diversity effects that cause reductions in some ecosystem process or in the biomass of a functional group. For example, increasing snail richness is expected to increase snail biomass and consequently decrease algal biomass, so the snail species that reduces

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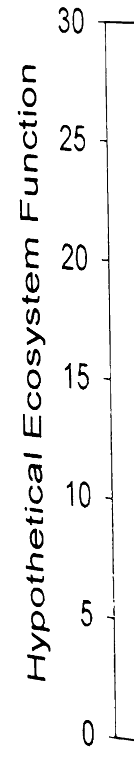
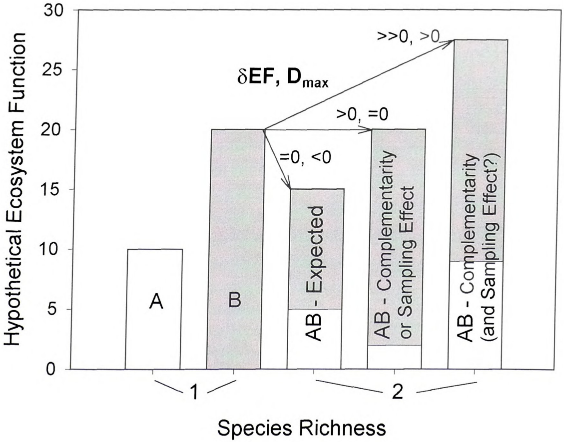


Figure 2. Hypothetical results of combining two species, A and B, in a polyculture. The polyculture could yield the average of what the two monocultures did, the expectation if there are no effects of species richness. Alternatively, the polyculture could yield more than that average (either because of sampling effects or niche complementarity), or even more than the "dominant" species in monoculture (species B in this case). This last possibility implies the action of some form of complementarity or facilitation. The values of δEF and D_{max} are displayed for each possible outcome.



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periphyton the most would be the "dominant", and $D_{max} > 0$ would mean that the polyculture had less periphyton than the "dominant" species had in monoculture. Total system organic matter, periphyton, and dissolved oxygen were expected *a priori* to respond negatively to increasing biomass/activity of snails, and thus D_{max} and δEF were calculated to reflect those expectations (e.g., $D_{\text{"max"}} = (\text{MIN}(M_i)-O_T)/\text{MIN}(M_i)$).

To evaluate the hypothesis that niche overlap between a pair of species should be related to the magnitude of species richness effects on various response variables, I performed regression analyses between an index of similarity in habitat use and the effect of diversity (δEF) for each response variable. Similarity in habitat use was calculated as percent similarity (Krebs 1989).

Results:

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Results:

All species of snails reproduced during the experiment, so changes in snail biomass reflect reproduction, growth, and death. The final biomass of snails was strongly influenced by both the number and identity of snail species present (Table 1, Figures 3A, 4A). Microcosms with two snail species had greater final biomass than those with one species (Figure 3A). *Bithynia* was particularly productive, exceeding the next most productive species by a factor of three. The biomass of *Gyraulus*, *Physa*, and *Amnicola* all decreased dramatically from initial stocking levels (Figure 4A).

Periphyton, the primary food resource of these snails, was affected by both snail species richness and composition (Table 1, Figure 3C, 4C). Polycultures had less periphyton than monocultures (Figure 3C), corresponding to the greater snail biomass with greater snail species richness. *Bithynia* did not reduce periphyton as much as one might expect based on its very high biomass (Figure 4C). *Fossaria* and *Helisoma* were the next most abundant snail species in monoculture and reduced periphyton biomass strongly, but *Physa* had the strongest per-unit biomass effects on periphyton (Figure 4C). Polycultures with *Helisoma* present had noticeably less periphyton than did those without, suggesting that *Helisoma* is a particularly strong interactant with the attached algae community. Phytoplankton and macrophyte biomass did not respond to snail richness or composition (Table 1, Figure 3B, D and Figure 4B, D).

Table 1. ANOVA results for snail species richness and composition effects on A) snail, periphyton, phytoplankton, and

and B) sedimentation rate, dissolved oxygen concentration, and total system organic matter.

Table 1. ANOVA results for snail species richness and composition effects on A) snail, periphyton, phytoplankton, and macrophyte biomass, and B) sedimentation rate, dissolved oxygen concentration, and total system organic matter. Periphyton, phytoplankton, organic matter and sedimentation data were log transformed prior to analyses. Dissolved oxygen, periphyton, and phytoplankton data were means of several repeated measures. Response variables with error degrees of freedom not equal to 75 had missing data for one or two microcosms.

A) Functional group biomass

	Snail Biomass				*Periphyton Biomass*				*Plant Biomass*				*Phytoplankton Biomass*			
Source	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**
Species Richness	1	14842.8	29.328	**<0.00001**	1	0.807	4.162	**0.0449**	1	0.482	1.527	0.2204	1	0.024	0.122	0.7281
Composition(SR)	19	4118.8	8.138	**<0.00001**	19	3.748	19.340	**<0.00001**	19	0.402	1.273	0.2270	19	0.159	0.821	0.6764
Error	75	506.1			74	0.194			75	0.316			75	0.193		

B) System level properties

	Dissolved oxygen				*Sedimentation Rate*				*Total Organic Matter*			
Source	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**	**df**	**MS**	**F**	**P**
Species Richness	1	0.292	2.663	0.1069	1	0.486	1.452	0.2320	1	1.030	4.124	**0.0459**
Composition(SR)	19	0.532	4.853	**<0.00001**	19	0.692	2.065	**0.0142**	19	0.714	2.859	**0.0007**
Error	75	0.110			75	0.335			73	0.250		

Figure 3. Snail spe

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Figure 3. Snail species richness effects on A) snail biomass, B) plant biomass, C) periphyton biomass, D) phytoplankton biomass, E) dissolved oxygen, F) sedimentation rate, and G) total system organic matter. Data (untransformed) are means + 1 SE. Asterisks indicate statistically significant effects of species richness ($P<0.05$). The dashed line in panel A represent the initial snail biomass (20 mg dry mass).

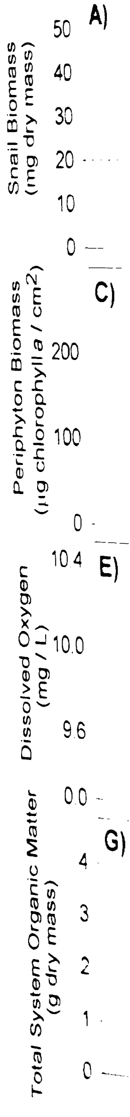
A)
Snail Biomass (mg dry mass)
50
40
30
20
10
0
C)
Periphyton Biomass (µg chlorophyll a / cm2)
200
100
0
E)
Dissolved Oxygen (mg / L)
10.4
10.0
9.6
0.0
G)
Total System Organic Matter (g dry mass)
4
3
2
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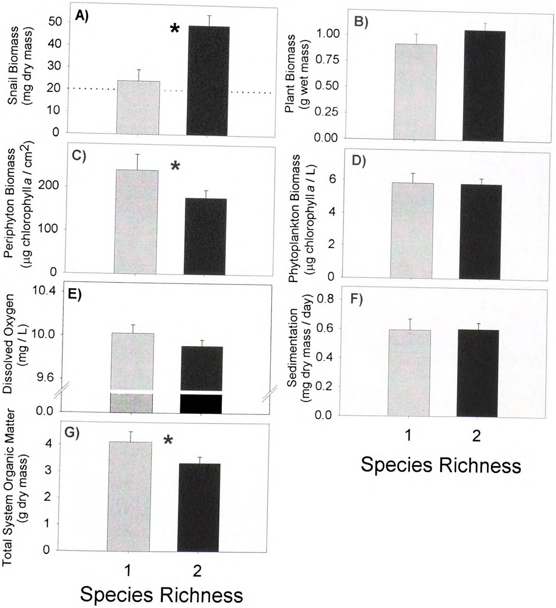
Figure 3


A)
Snail Biomass
(mg dry mass)
0
10
20
30
40
50
*
B)
Plant Biomass
(g wet mass)
0.00
0.25
0.50
0.75
1.00
C)
Periphyton Biomass
(μg chlorophyll a / cm2)
0
100
200
*
D)
Phytoplankton Biomass
(μg chlorophyll a / L)
0
2
4
6
E)
Dissolved Oxygen
(mg / L)
0.0
9.6
10.0
10.4
F)
Sedimentation
(mg dry mass / day)
0.0
0.2
0.4
0.6
0.8
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Total System Organic Matter
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0
1
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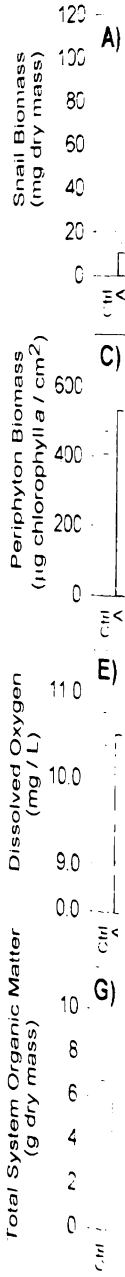
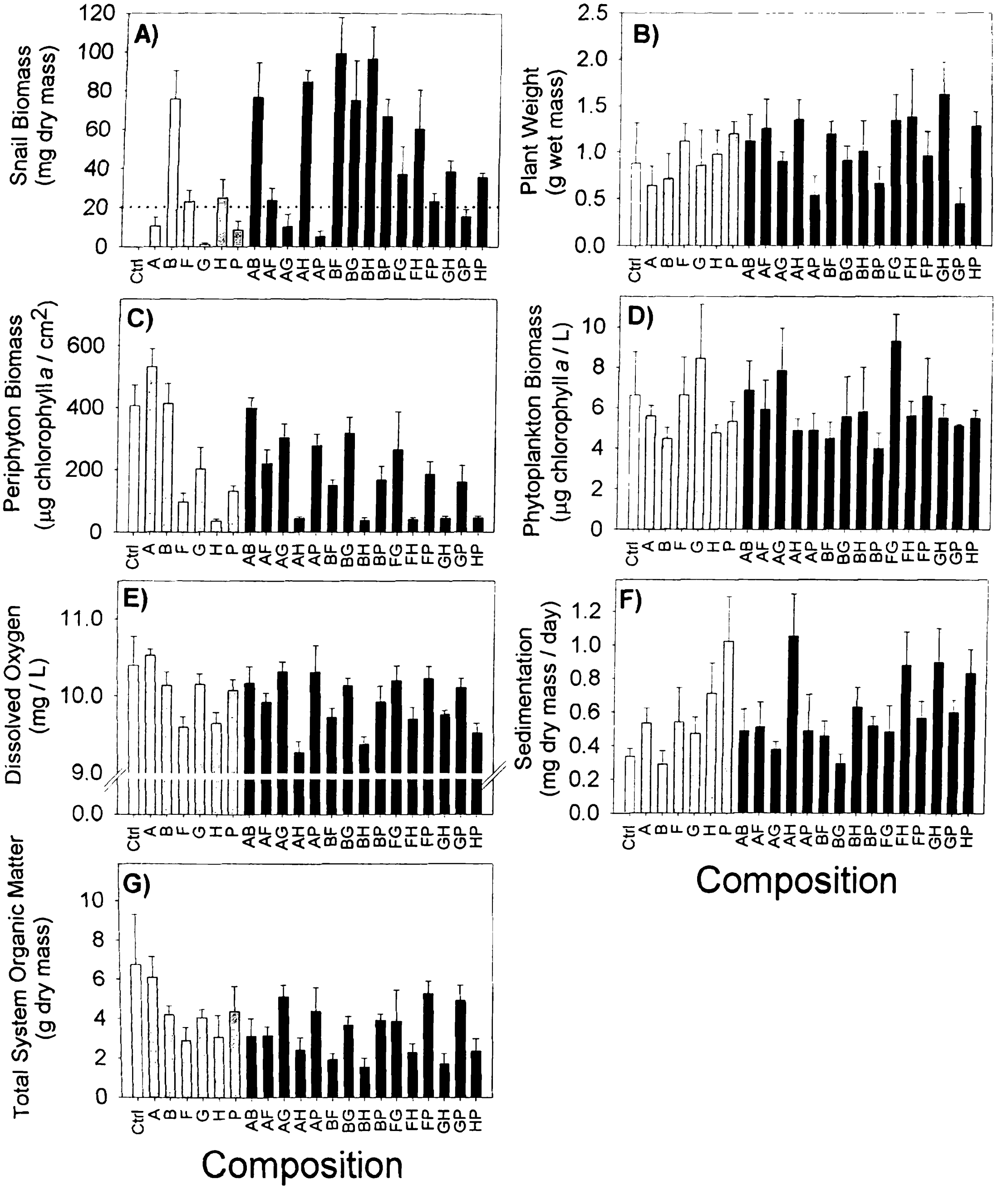


Figure 4. Snail species composition effects on A) snail biomass, B) plant biomass, C) periphyton biomass, D) phytoplankton biomass, E) dissolved oxygen, F) sedimentation rate, and G) total system organic matter. White bars represent data from no snail control microcosms, grey bars represent monocultures, and black bars represent polycultures. Snail composition treatments are labeled with the initials of the genera present. Data (untransformed) are means + 1 SE. The dashed line in panel A represent the initial snail biomass (20 mg dry mass).

Figure 4


A)
Snail Biomass (mg dry mass)
0
20
40
60
80
100
120
B)
Plant Weight (g wet mass)
0.0
0.5
1.0
1.5
2.0
2.5
C)
Periphyton Biomass (µg chlorophyll a / cm²)
0
200
400
600
D)
Phytoplankton Biomass (µg chlorophyll a / L)
0
2
4
6
8
10
E)
Dissolved Oxygen (mg / L)
0.0
9.0
10.0
11.0
F)
Sedimentation (mg dry mass / day)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
G)
Total System Organic Matter (g dry mass)
0
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Dissolved oxygen measured at its diel peak provided a good surrogate for primary productivity in this experiment (because respiration did not differ between treatments). Snail species richness did not significantly affect dissolved oxygen in the microcosms (Table 1, Figure 3E), but snail species composition did (Table 1, Figure 4E). Macrophytes were larger (but not significantly so) in microcosms with more snail species, thus the lack of species richness effects on dissolved oxygen may be due to opposite (i.e. compensatory) changes in the production of algae and plants. But in general, where periphyton biomass was low so was dissolved oxygen, which suggests that periphyton was dominating primary production in the microcosms (Figure 4E).

Snail species richness did not affect sedimentation rates, but snail species composition did (Table 1, Figures 3F, 4F). Most notably, *Bithynia* monocultures were the only single species treatment where sedimentation rate was lower than in the no snail controls (Figure 4F). Total system organic matter was lower in species-rich microcosms than in monocultures (Table 1, Figure 3G). The response of total organic matter to snail composition was largely similar to the response of periphyton biomass (Figure 4F). For instance, *Fossaria* and *Helisoma* monocultures had the lowest organic matter of any single species treatments, and polycultures containing *Helisoma* had particularly low levels of total organic matter (Figure 4G).

D_{max} was calculated for each polyculture and for each response variable where snail species richness had significant effects. The average D_{max} (across

Table 2. Summary statistics for D_{max} calculations, for the three response variables that were sensitive to the number of snail species present: snail biomass, total system organic matter, and periphyton biomass. Average D_{max} is the mean of D_{max} for all 15 polycultures. δEF is expressed in the units of measurement for that response variable.

	Snail Biomass	*Total Organic Matter*	*Periphyton Biomass*
Average D_{max}	0.407	0.042	-0.537
# of polycultures where $D_{max} > 0$	10/15	9/15	1/15
Average δEF	25.684	0.856	58.506
# of polycultures where $\delta EF > 0$	14/15	11/15	12/15

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the 15 polycultures) was greater than zero for final snail biomass and total organic matter (Table 2), suggesting that sampling effects are an insufficient explanation for those species richness effects. Moreover, most of the individual polycultures had positive D_{max} values. The average D_{max} was far below zero for periphyton biomass (as chlorophyll *a*), however, and only one polyculture had a positive D_{max} (Table 2). This suggests that either sampling effects or complementarity could explain changes in periphyton biomass with increasing snail species richness, but the uniformly low periphyton biomass in treatments containing *Helisoma* supports the action of sampling effects.

Snail species used habitats to different degrees (MANOVA Wilk's λ=0.0182, df=25.98, F=7.62, P<0.0001, Table 3, Figure 5). *Amnicola* used the sides of the bucket to a greater extent than did the other snails (Figure 5). *Bithynia* was particularly abundant on sand habitat, while *Amnicola* was found on sand less than the other species. *Fossaria* and *Physa* seemed to be randomly distributed throughout the microcosms (Figure 5, i.e. similar habitat use to null expectations based on relative areas of habitat types). Snails did not change their habitat use in response to the presence/absence of other snail species (MANOVAs, Wilk's λ >0.12), except for *Fossaria* which was never found on plants in the presence of *Helisoma* or *Physa*, but was found on plants when alone or with the other snail species (MANOVA, Wilk's λ = 0.01, ANOVA for plant habitat, P=0.003).

Similarity in habitat use was a significant predictor of the magnitude of diversity effects (δEF) for total organic matter, periphyton biomass, and

Table 3. ANOVA results for differences among six snail species in their use of five habitat types – water surface, bucket sides, plants, sand, and detritus.

		Surface			*Sides*			*Plant*			*Sand*			*Detritus*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species	5	0.168	17.954	**<0.00001**	0.333	18.332	**<0.00001**	0.062	4.744	**0.0026**	0.261	10.244	**<0.00001**	0.085	2.829	**0.03292**
Error	30	0.009			0.018			0.013			0.025			0.030		

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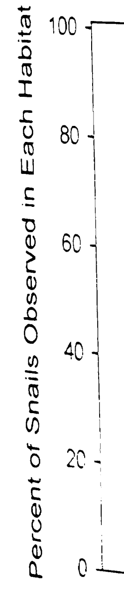


Figure 5. Habitat use (surface, bucket sides, plants, sand, and detritus habitats) for six snail species. Data are the mean percent of the total number of snails of a given species observed in an experimental unit that were in a particular habitat during five observation periods. Species treatments are labeled with the initial of the genera name. A null expectation is also presented, which represents the percent area of the different habitats in the microcosm environment.

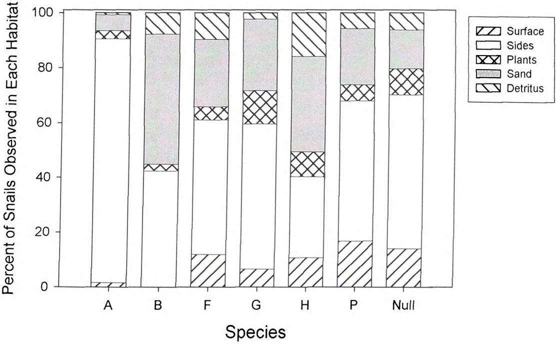


Figure 6. Regression of percent similarity in habitat use between a pair of species against the magnitude of diversity effects (δEF), for A) snail biomass, B) plant biomass, C) periphyton biomass, D) phytoplankton biomass, E) dissolved oxygen, F) sedimentation rate, and G) total system organic matter. Points in the coordinate plane are coded by the species pair being considered (initials of genera names).

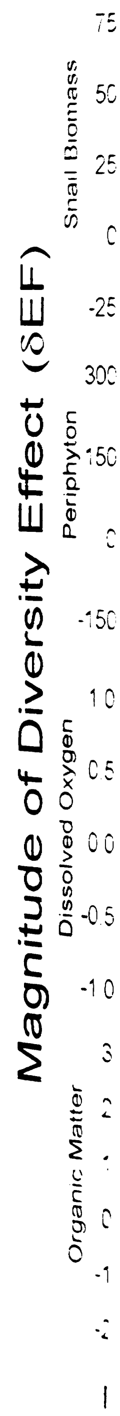
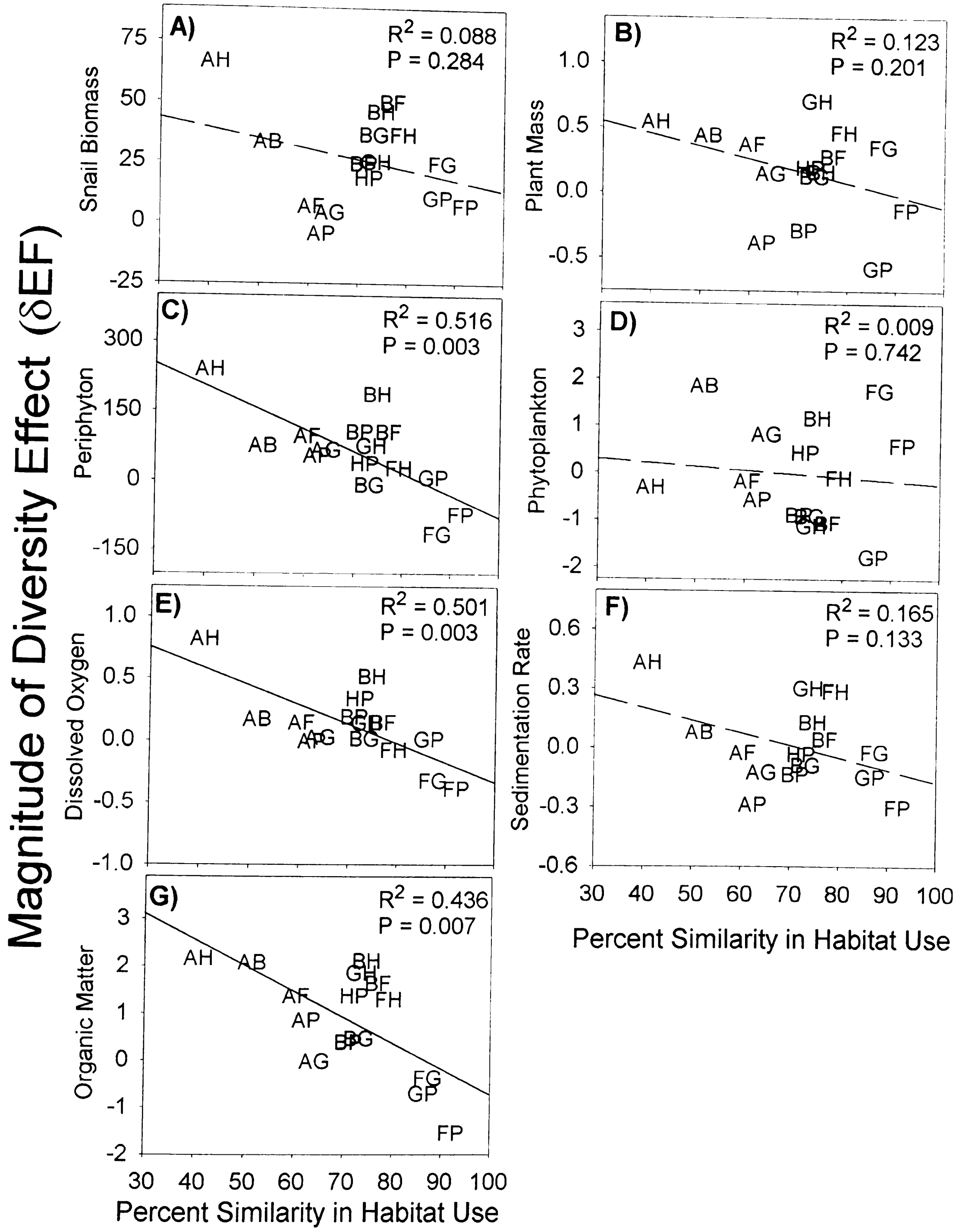


Figure 6



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dissolved oxygen (Figure 6 G, C, E), indicating the action of a niche complementarity mechanism. Notably, this significant negative relationship was observed for a response variable where the average species richness effects were not statistically significant (dissolved oxygen, Figure 6 E).

Interestingly, the slopes of the regressions for all response variables were negative (whether the regressions were significant or not), which means that diversity effects always tended to be larger when combining species that were divergent in habitat use. The probability that seven regression analyses would all have negative slopes by chance alone is 1/128, sufficiently unlikely to warrant interpretation of the slopes.

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Discussion:

Accurately predicting the consequences of species loss in ecosystems will require an understanding of the mechanisms by which species affect ecosystem functioning. The niche complementarity mechanism has received much consideration, but rarely have niche differences among species been concretely linked to the consequences of variation in species richness. This would require one to first establish that species vary in some aspect of their niche relevant to ecosystem functioning. Second, one would need to demonstrate that systems are more sensitive to the removal or addition of unique (in terms of niche) species.

In this experiment significant differences in habitat use were observed among the snail species (Table 3, Figure 5), and it seems clear that systems containing species that occupy different habitats should have greater rates of resource utilization than those with some habitats left unexploited (all else being equal). Correspondingly, in this experiment microcosms containing species with strongly divergent habitat use were generally more sensitive to changes in snail species richness (Figure 6). Thus, the results reported here strongly support the action of a niche complementarity mechanism. Moreover, species richness effects were larger when combining species that use different habitats even when average species richness effects were not statistically significant (e.g., dissolved oxygen, Figure 6E). There was also evidence for niche complementarity effects on periphyton biomass (Figure 6C), even though this response variable was also influenced by sampling effects (Table 2).

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Interestingly, increases in snail biomass with increasing snail richness were not the result of differentiation among the snails in habitat use (Figure 6A). However, I only measured one aspect of the niches of these species, and it is likely they differ in other important ways. For example, Chase et al. (2001) suggest an interesting kind of resource specialization that may occur among some of the same snail taxa used here, and has been described for other taxa (Schmitt 1996). That is, some species may forage over a large area, only removing the loosely attached algal cells (typical of *Physa*) while other species may specialize in removing the tightly adherent algal cells (typical of *Helisoma*). I have performed other experiments which confirm *Physa*'s and *Helisoma*'s opposing foraging strategies, and which suggest that prosobranch snail species (e.g., *Bithynia tentaculata, Amnicola limosa*) are, like *Helisoma*, slow to find resource patches (Appendix B). So, although one could always argue some unmeasured aspect of species niches could exist, here it seems that real and meaningful niche differences among these species existed but were not quantified, and potentially could explain positive effects of snail richness on snail biomass. This seems particularly reasonable considering the inability of sampling effects to explain the snail biomass response to snail species richness, and the frequently positive values for D_{max} (Table 2).

On the other hand, sampling effects were apparent for some response variables. For instance, significant species richness effects on periphyton were due to the functional dominance of *Helisoma* and an experimental design that resulted in *Helisoma* being twice as likely to occur in polycultures (1/3) as monocultures

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monocultures (1/6). Sampling effects have been vigorously attacked as insidious and misleading design artifacts (e.g., Huston 1997), but also defended as viable diversity mechanisms that could act in nature if dominant species occur more frequently in more diverse systems (e.g., Tilman et al. 1997). However, most ecologists do not view community assembly to be the result of a random draw from the regional species pool, and might expect functionally dominant species to occur in most systems regardless of species richness. Yet, a survey of 16 ponds in southwest Michigan (Appendix C) found *Helisoma* was present much more frequently in higher diversity ponds than in low (0% of 1 species ponds, 50% of 2 species ponds, 88% of 3 species ponds, and 100% of ponds with four species). So in this particular case the "sampling effect" mimics a meaningful pattern of occurrence of a functionally dominant species in nature. Generally, though, the occurrence of functionally dominant species across natural ecosystems of varying diversity is unknown and deserves further empirical consideration.

While the action of a niche complementarity mechanism is apparent in this study, no mechanism can be established unequivocally without manipulating the hypothesized causal factor. Here I hypothesized that similarity in niche (e.g., habitat use) among sets of species would influence the total ecosystem function realized by those sets of species. Manipulating the number and types of habitat types would be the best way to concretely establish this mechanism. For instance, if snails were put into microcosms with varying numbers of habitat types and ecosystem functioning increased with diversity

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equally in all cases, then we would have reason to doubt the hypothesized mechanism. In contrast, if function increased with richness only where numerous habitat types were available, then we could confidently ascribe positive effects of diversity to niche differentiation. This approach would require an enormous experimental design (to perform the present study at three levels of habitat diversity would require around 300 microcosms).

Short of manipulating the number of available habitat types, quantifying the differences among species' niches and predicting changes in ecosystem function with increasing diversity is the next best alternative. With this approach we can compare empirical results with our expectations assuming that a niche complementarity mechanism was operating.

The simple approach applied here has some potential problems, however. For instance, species may expand their use of habitats when in monoculture, and restrict their use of habitats when in the presence of other species (i.e. fundamental niche may differ from realized niche). We could imagine a set of five hypothetical species each capable of exploiting the entirety of a theoretical niche space when alone, but occupying only 20% of that space when in the presence of the other species. If we only measured their niches in polyculture we would conclude species are all fairly different in niche. We would expect that species loss will leave some niche space (e.g., resources) unutilized and consequently losses of species would have strong effects on ecosystem functioning. However, after species loss the niches of the remaining species would likely expand to include the "free" niche space, and ecosystem

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functioning would be maintained. Thus, if we hope to use similarity in niche to predict the effects of gaining or losing species on ecosystem functioning, it may be more appropriate to measure the niches of species in monoculture (e.g., the fundamental niches). As it turns out, the habitat use of the snails in this experiment was largely insensitive to the presence or absence of any other species. However, this may not be generally true.

The correspondence between the predicted effects of niche complementarity on ecosystem functioning and previous considerations of interspecific competition has not received due attention (but see Jonsson and Malmqvist 2002). Each of the possibilities in Figure 2 corresponds to different relationships between the strength of inter- versus intra-specific competition. For instance, if inter- and intra- specific competition are equal in strength, one could expect the "additive" result in Figure 2. If interspecific competition was asymmetric one might expect the "sampling effect" result (i.e. where a dominant species is essentially unaffected by the presence of a second species), and if interspecific competition was weaker than intraspecific one would expect overyielding, or the "complementarity" result. The wealth of empirical studies of competition remains an untapped resource for understanding diversity effects on ecosystems, presumably because those studies stopped short of measuring an "ecosystem function", or didn't call their responses such. However, population growth rates of competing species and the effects on the abundance of resources have been measured often, and have clear implications for the functioning of ecosystems.

Conclusions

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Conclusions

The consequences of species loss for natural ecosystems are unclear, in large part because a mechanistic understanding of diversity's role in ecosystems has been elusive. This study provides a novel method for evaluating the action of one diversity mechanism, niche complementarity, and demonstrates its action in a laboratory experiment.

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CHAPTER THREE

FORAGING AND REFUGE USE BY THE POND SNAIL *PHYSA GYRINA*: EFFECTS OF PHYSIOLOGICAL STATE, PREDATORS, AND RESOURCE LEVELS.

Abstract:

The costs and benefits of anti-predator behavioral responses should be functions of the actual risk of predation, the availability of the prey's resources, and the physiological state of the prey. Empirical studies of state-dependent behavior are only beginning, however, and few studies have investigated interactions between all three factors. Here I present the results of a laboratory experiment where I manipulated the physiological state of pond snails (*Physa gyrina*), the abundance of algal resources, and predation cues (*Belostoma flumineum* consuming conspecific snails) in a full factorial design to assess their direct effects on snail behavior and indirect effects on algal biomass.

On average, snails foraged more when resources were abundant, and when predators were absent. Snails also foraged more when previously exposed to physiological stress (i.e. starvation). Snails spent more time at the water's surface (a refuge) in the presence of predation cues, but predation, resource levels, and prey state had interactive effects on refuge use. There was a consistent positive trait-mediated indirect effect of predators on algal biomass, across all resource levels and prey states.

Introduction

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Prey can respond to predation by reducing their activity (Lima and Dill 1990, Kolar and Rahel 1993), investing in defensive structures (Hanazato and Yasuno 1989, Brønmark and Miner 1992) or chemicals (Coley 1983, Bryant et al. 1983, 1985), modifying their life-history (Crowl and Covich 1990, DeWitt 1998, Chase 1999) or restricting their use of habitat (Turner and Mittelbach 1990, Turner et al. 1999). However, prey often sacrifice some ability to acquire resources by employing anti-predator defenses (Sih 1980, Gilliam 1982, Werner and Gilliam 1984, Lampert 1987), and the costs of predator avoidance behavior may outweigh the benefits if the actual risk of mortality is low or resources are plentiful.

Recently there has been a realization that the physiological state of an organism should influence its behavior (Kohler and McPeek 1989, Pettersson and Bronmark 1993, Werner and Anholt 1993, Lima 1998, Luttbeg et al. 2003) because individuals with varying physiological conditions may realize different costs and benefits from the same behavior (Godin and Crossman 1994). For example, a weak individual risks death from starvation if it is too vigilant in its attempts to avoid predation, while a well-fed organism is better able to hide or remain inactive when a predator is near. Similarly, the benefit of resource acquisition is probably highest for the prey in the poorest state, and for many species there may be diminishing returns for prey nearing satiation. Along these lines, energetically stressed fish are found to exhibit "predator inspection" behaviors more often than unstressed fish, presumably because stressed fish need to feed

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need to feed and benefit the most from accurately assessing predation risk (Godin and Crossman 1994, McLeod and Huntingford 1994).

While studies of anti-predator responses of prey continue to accrue (reviewed in Lima and Dill 1990, Lima 1998), few studies have addressed the potential interactions between all three of the main factors that should influence prey decision making: predation risk, resource availability, and physiological state of the prey (but see Kohler and McPeek 1989). Here I experimentally manipulate all three factors to test these simple predictions: 1) Increased predation risk should cause prey to spend more time in a refuge and less time foraging. 2) Prey will choose riskier behavior (i.e. greater foraging effort) when resources are more abundant. 3) Prey in a poor physiological state will take more risk than those in a good state. There also may be interactions between these factors, which necessitates their study in concert – for instance, prey in poor condition may be willing to take more risk in general, but if resources are very low there is no benefit to that risk-taking, and thus prey behaving optimally will continue to remain inactive or in refuge.

When predators influence the traits of prey species they can have indirect effects on the prey's resource (Peacor and Werner 1997, 2001, Bernot and Turner 2001). Peacor and Werner (2001) demonstrated that trait-mediated indirect interactions of this kind can exceed the strength of density-mediated indirect interactions under some conditions, and suggest that in some systems trait changes among prey may be responsible for a large part of the community-wide structuring influence of predators. Here I also examine the consequences of behavioral

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of behavioral changes among the consumers (elicited from resource, predation, and prey state treatments) on the abundance of the basal resource.

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Methods:

I used a simple three trophic-level system consisting of the insect predator *Belostoma flumineum* (Hemiptera), the common freshwater pond snail *Physa gyrina*, and periphytic algae, to test the predictions described above. The behavioral and morphological responses of freshwater snails to predation risk are well described (Osenberg 1988, Turner 1996, Turner et al. 1999, Bernot and Turner 2001), which makes them a particularly good taxonomic group for this study. *Physa* are known to assess predation risk chemically (Turner 1996), and can distinguish between predator types; *Physa* climb out of the water when crayfish are present and seek covered shelter when fish are present (Turner et al. 1999, Bernot and Turner 2001). Less is known about the behavioral responses of snails to *Belostoma*, which are voracious snail predators and may consume up to six snails per day in laboratory settings (J. Wojdak *unpublished data*, Crowl and Alexander 1990).

I experimentally manipulated prey state, resource level, and predation risk in a 2 X 2 X 2 design, replicated seven times. Two separate trials were run, with three replicates of each treatment in the first trial (23-25 Sep 2002) and four replicates of each treatment in the second run (26-28 Sep). There was no effect of trial number on any response variable, and so trials were pooled for statistical analysis. Treatments were randomly assigned to experimental units within a trial. One replicate of each the "good" state-low resource-predator present and "poor" state-low resource-predator present treatments were lost due to accident.

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Each experimental unit (18 L plastic bucket) contained 6 L of low nutrient well water, and a suspended predator enclosure. The predator enclosure was a clear plastic tube (10 cm diameter) with fine mesh (250 μm) on each end that allowed water, but not snails or predators, to pass through. Organisms were collected from ponds in the Lux Arbor Reserve (W. K. Kellogg Biological Station, MI, USA) where snails and *Belostoma* naturally co-occur. Snails were either fed spinach to satiation or starved for six days prior to the experimental trials, creating snails in "good" and "poor" energetic state, respectively. Six *Physa* were placed into each bucket and were allowed to acclimate for 12 h. At the beginning of each trial I placed three *Physa* into each predator enclosure, and one *Belostoma* into half of the enclosures. Three resource tiles (23 cm^2 each) were placed into each experimental unit. "High" resource treatments received tiles that had been incubated with a diverse inoculum of local algae species in a high nutrient, high light environment for three weeks (mean algal biomass (s.e.) = 2.08 (0.22) mg / cm^2 ash free dry weight - AFDW). The low resource treatments received similar tiles, but after removing some periphyton by physical agitation (resulting mean algal biomass (s.e.) = 0.54 (0.18) mg / cm^2 AFDW). The AFDW of the algae on "high" and "low" resource tiles was determined as the difference in mass of a dried sample (60° C for 24 h) and that sample after combustion (550° C for 1 h). Experimental units were maintained indoors in a controlled temperature and light environment (24 h light from 55 W full spectrum florescent bulbs).

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Snail habitat use was recorded at ten irregular intervals (0.25, 0.5, 1, 1.5, 2.5, 4.5, 19, 26, 43, and 48 h). Snails within 2.5 cm of the surface or above the water were considered to be evading predators by "crawling out" (*sensu* Turner 1996). Crawling out clearly protects snails from crayfish, but it is less certain that crawling out of the water represents a true refuge in nature from an amphibious insect like *Belostoma*. However, I have observed *Physa* crawling out in response to *Belostoma* in laboratory settings. Snails on resource tiles were considered to be foraging. Surface habitat and resource patches represented 8% and 7% of the total habitat area available for snails respectively, so an average of >0.50 snails in either area represents over-representation relative to the null expectation of random spatial distribution of prey. At the end of the second trial one resource tile from each experimental unit was selected at random (n=4 per treatment) to determine the AFDW of the algae remaining on the tiles.

Snail behavior data were analyzed with repeated measure ANOVA. No treatment effects were evident in the first hours of the experimental trials, so analyses were restricted to the final four measurements (19, 26, 43, and 48 h). Algal biomass (AFDW) data were log-transformed, after which all data met normality and homogeneity of variance assumptions (verified with modified Levene's tests and probability plots of residuals). All analyses were performed in SYSTAT v8.0.

Results:

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Results*:*

Behavior

Nineteen hours after the start of the experimental trials, the presence of predators had reduced the number of snails found on resource tiles by 60% (Table 4, predator P<0.001, Figure 7). The difference declined to only 38% by the end of the experiment (after 48h - Table 4, predator*time, P<0.001), mostly because of a reduction in foraging through time in the absence of predators. On average, nearly 70% more snails were found near the surface of the water when predators were present than in their absence (Table 4, predator P<0.001, Figure 2).

The abundance of algal resources significantly affected snail behavior; on average 70% more snails were found foraging in high resource treatments than in low (Table 4, resource P<0.001, Figure 7) and snails spent less time near the surface in high resource treatments, relative to low (Table 4, resource P=0.001, Figure 8).

The physiological state of the snails, manipulated by the antecedent food supply, had significant main effects on snail foraging effort but not refuge use. Snails in poor condition were found 34% more often on resource tiles than snails in good condition (Table 4, state P=0.013, Figure 7a vs. 7b). There was, however, a significant time*resource*state*predation interaction for snail refuge use (Table 4, P=0.018, Figure 8). Four way interactions can be difficult to interpret, but it appears this effect was driven by the dynamics in the "good" state treatments. After 19 h, snails in low resource environments with predation

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Table 4. Repeated-measures ANOVA results describing the foraging effort and refuge use of *Physa gyrina* through time in response to prey energetic state, predation risk, and resource manipulations. Significant effects ($P < 0.05$) are bolded.

		Foraging Effort			*Refuge Use*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
Between Subject							
State	1	25.63	6.66	**0.013**	11.07	2.18	0.147
Predator	1	128.76	33.46	**<0.001**	182.16	35.80	**<0.001**
Resource	1	55.05	14.30	**<0.001**	63.96	12.57	**0.001**
Predator*State	1	9.33	2.43	0.126	3.16	0.62	0.434
Resource*Predator	1	0.19	0.05	0.825	0.60	0.12	0.733
Resource*State	1	0.37	0.10	0.757	2.64	0.52	0.475
State*Resource*Predator	1	0.03	0.01	0.929	0.03	0.01	0.935
Error	46	3.85			5.09		
Within Subject							
Time	3	9.23	15.31	**<0.001**	7.19	10.35	**<0.001**
Time*State	3	1.35	2.24	0.086	1.42	2.05	0.110
Time*Predator	3	6.91	11.45	**<0.001**	0.95	1.37	0.254
Time*Resource	3	0.22	0.36	0.779	1.04	1.49	0.219
Time*Predator*State	3	0.81	1.35	0.262	1.10	1.58	0.197
Time*Resource*Predator	3	0.74	1.22	0.304	1.71	2.46	0.065
Time*Resource*State	3	0.21	0.35	0.791	0.76	1.09	0.354
Time*Resource*State*Predator	3	0.90	1.49	0.221	2.42	3.48	**0.018**
Error	138	0.60			0.70		

Figure 7. The response of foraging effort (defined as the number of snails on resource tiles) for snails in A) “good” state, and B) “poor” state, to predation risk and resource manipulations. Triangles represent the presence of *Belostoma* predators, circles represent the absence of predators. Dark symbols represent high initial resource levels, open symbols represent low initial resource levels. Data are means ± 1 SE. The analyses in Table 4 only include the last four observations.

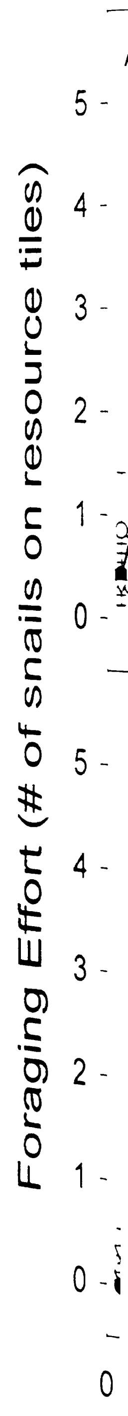

Figure 7

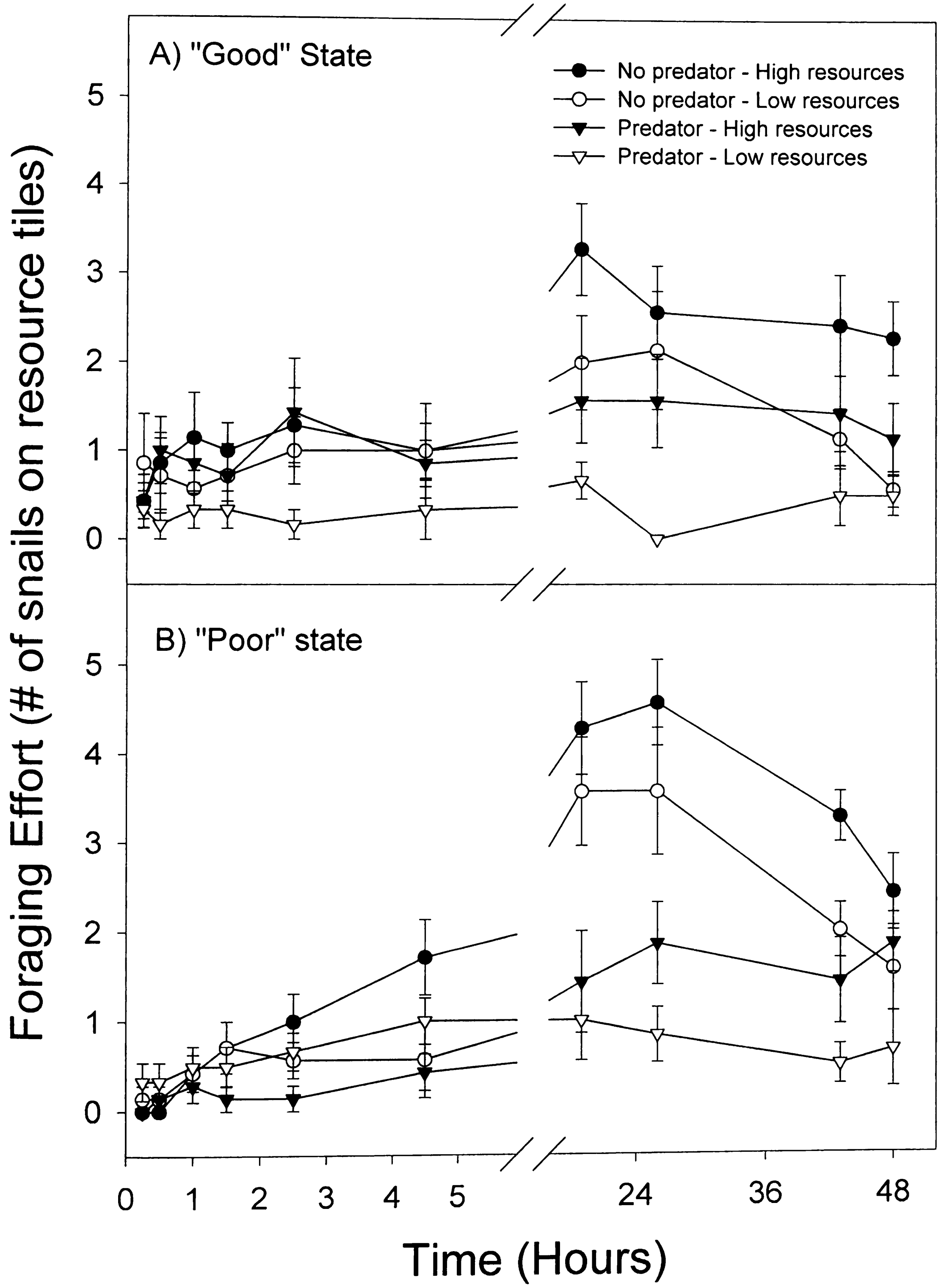

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Figure 8. The response of refuge use (defined as the number of snails near or above the water surface) for snails in A) "good" state, and B) "poor" state, to predation risk and resource manipulations. Triangles represent the presence of Belostoma predators, circles represent the absence of predators. Dark symbols represent high initial resource levels, open symbols represent low initial resource levels. Data are means ± 1 SE. The analyses in Table 4 only include the last four observations.

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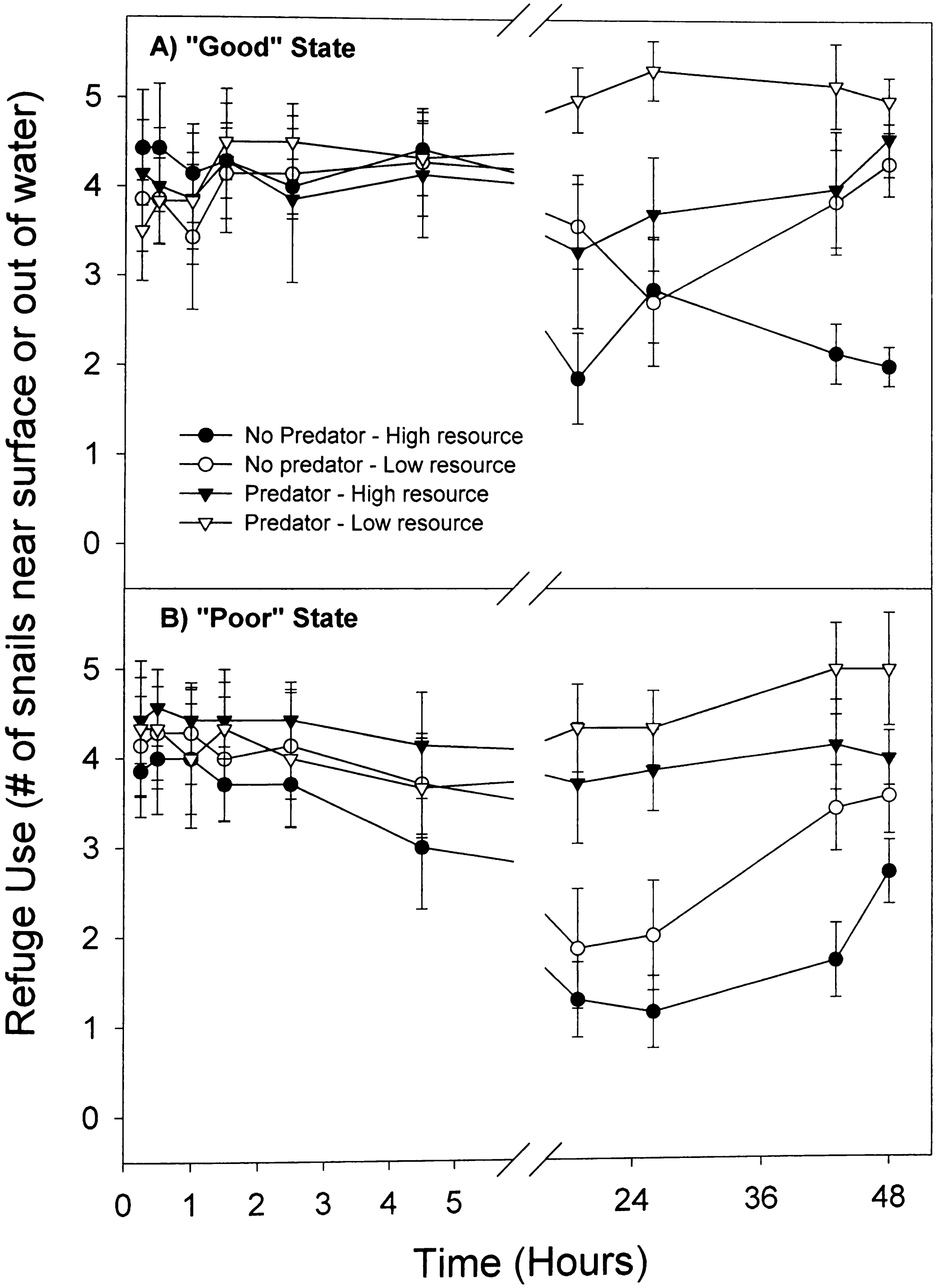

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cues were using the water surface refuge more than other treatments, but by the end of the experiment snails in every resource and predator treatment, except high resource-no predator environments, were using the surface habitat to a great extent. In comparison, the relative ordering of predation and resource level combinations was more constant through time when the snails had experienced prior physiological stress ("poor" state).

Trait-Mediated Indirect Interactions

Predators can induce behavioral changes among prey, which can indirectly affect organisms at lower trophic levels (called trait-mediated indirect interactions). Here predation cues had strong, positive indirect effects on algae; final algal biomass was on average 45% higher in the presence of predators than in their absence (Table 5, predator P=0.049, Figure 9). The indirect effects of predators on algal resources did not depend on the initial physiological state of the snails or on the original resource level (Table 5, no significant interactions).

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Table 5. ANOVA results for response of ash-free dry weight (AFDW) of algae on resource tiles to prey state, predation risk, and resource manipulations. AFDW data were log-transformed to meet assumptions of the ANOVA procedure. Significant effects (P < 0.05) are bolded.

Source	**df**	**MS**	**F**	**P**
State	1	0.01	0.04	0.846
Predator	1	1.32	4.32	**0.049**
Resource	1	23.90	78.21	**0.000**
Predator*State	1	0.20	0.66	0.425
Resource*Predator	1	0.16	0.51	0.481
Resource*State	1	0.08	0.27	0.611
State*Resource*Predator	1	0.01	0.02	0.884
Error	22	0.31		

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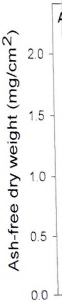
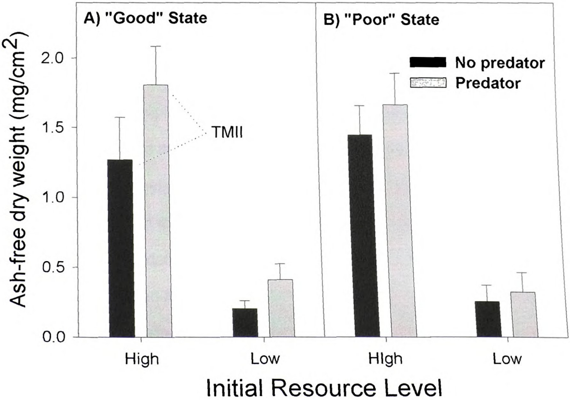


Figure 9. Ash-free dry weight (AFDW) of algae on resource tiles at the end of the experiment, for snails in A) "good" state and B) "poor" state. Black bars represent the absence of *Belostoma* predators, while gray bars represent the presence of predators. Trait-mediated indirect interactions (TMII) can be visualized by comparing the AFDW in the presence and absence of predators (at the same level of resources and prey energetic state). Data (untransformed) are means + 1 SE.



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Discussion:

In general, snails reduced their time spent foraging and increased their time in refuge when predators were present, when resources were scarce, and when they were in good energetic state. However, refuge use by *Physa* was influenced by all three factors in an interactive fashion, while foraging effort was influenced by each factor independently. The anti-predator behavioral response of snails had significant positive effects on the biomass of the basal resource, algae. Interestingly, the magnitude of trait-mediated indirect interactions did not vary with resource availability or physiological state, despite differences in prey behavior across those treatments. These results are discussed below relative to past theoretical and empirical work.

Formal models (e.g., Werner and Anholt 1993) and intuition suggest that resource levels, prey state, and predation risk should have interactive effects on prey behavior, but empirical results are conflicting. For instance, Kohler and McPeek (1989) found that predation risk and resource levels acted independently on mayfly behavior. However, well-fed mayfly larvae reduced their foraging effort in the presence of predators, but starved mayflies did not (i.e. prey state X predation interaction). Dill and Fraser (1984) observed that Coho salmon reduced their attack distance (a component of foraging effort) as they neared satiation in the presence of predators, but not in the absence (i.e. state X predation interaction). Cerri and Fraser (1983) found resources and predators acted independently on stream minnow patch choice, while in a similar system Gilliam and Fraser (1987) found interactive effects. Holbrook and Schmitt (

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and Schmitt (1988) used a particularly comprehensive set of experiments to examine the effects of predation risk and food reward on the patch selection of juvenile surfperch. They found that when prey were presented with all combinations of predation risk and resource levels simultaneously, the factors acted interactively on surfperch patch use, but when fish were confined to one condition and their behavior compared between the conditions (a design similar to this study) predation and resource levels effects were additive. Horat and Semlitsch (1994) found hunger and predation risk acted independently on several aspects of tadpole foraging behavior. Abramsky et al. (2002) observed gerbils responded to the threat of owl predation by congregating in a safe habitat where they paid an energetic cost of increased intraspecific competition, but that increases in seed resources in the risky habitat induced the gerbils to shift back to the risky habitat (resource X predation interaction).

Many subsequent studies have addressed the trade-off between foraging success and mortality from predators, but the results remain at least superficially conflicting. Indeed, even within this experiment refuge use by *Physa* was influenced by all three factors in an interactive fashion, while foraging effort was influenced by each factor independently. Several possible explanations exist for the seemingly contradictory empirical results. First, different prey taxa may have fundamentally different responses (i.e. interactive or additive responses) to predation risk, hunger, and the abundance of resources. Also, different aspects of prey behavior (e.g., foraging, refuge use) may respond in different ways to these factors. There may not be generality in

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prey responses. Second, the statistical power of individual experiments (and individual comparisons within experiments) can vary by orders of magnitude, so even if the magnitude of the interactive effects were identical across all studies one might expect to find both significant and non-significant interaction terms among analyses of variance. Factorial meta-analysis, a tool for quantitatively synthesizing the results of factorial experiments, was recently developed and applied to a multifactor ecological question (do competition and predation interact? – Gurevitch et al. 2000), and represents a way of evaluating both of the suggested explanations above.

Another possible explanation for the seemingly contradictory empirical evidence among published studies may be that the particular levels of the predation risk, resource, and state manipulations in an experiment are critical for detecting interactions. For example, prey very near starvation may be less concerned about predators than prey facing mild energetic stress, and so a very strong state manipulation may interact with a predation risk manipulation while a smaller state manipulation may not. Behavioral changes induced by deteriorating state, resource level, or predation risk may happen at thresholds (hypothetical examples in Figure 10), rather than being linear changes across gradients of those factors. If manipulations are large, one might assume thresholds will be exceeded and interactive effects detected, but it is often hard to judge whether a particular manipulation was large or small. Future work should employ designs with multiple levels of each factor, and should ensure that

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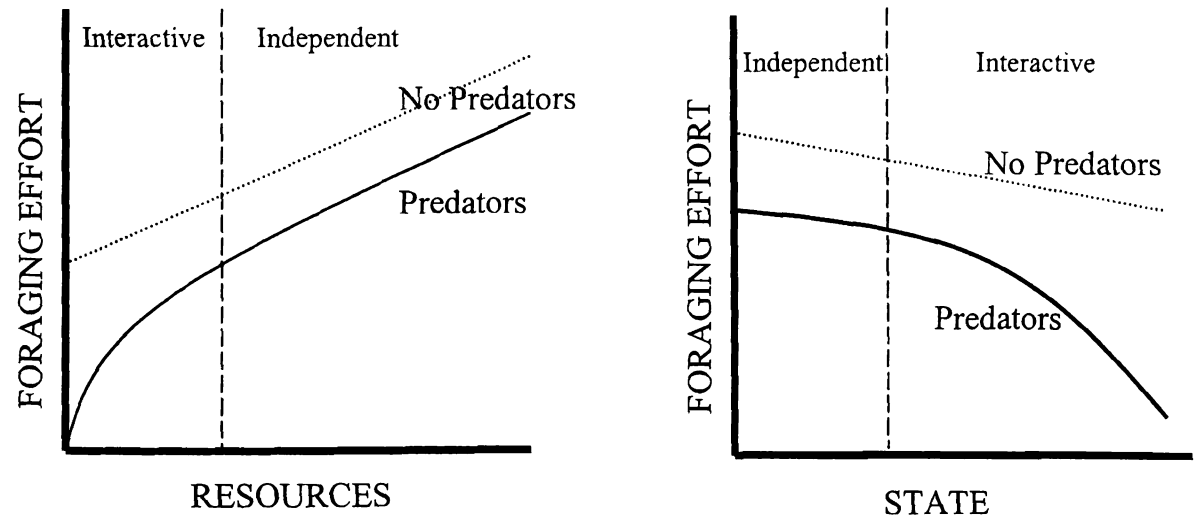
Figure 10. A) Hypothetical relationships between foraging effort, resource levels, and predation risk. The dotted line represents no/low predation risk, and the solid line represents high predation risk. Predation risk and resource levels interact to determine foraging effort at low resource levels, but they act independently at higher resource levels. B) Hypothetical relationships between foraging effort, prey state, and predation risk. The dotted line represents no/low predation risk, the solid line represents high risk. State and predation risk act independently when prey are in poor state, but interact as prey state improves.



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manipulations fully cover the natural range of each factor. Especially helpful would be more studies that use explicit models to predict thresholds in behavior and test for those behavioral shifts with treatment levels defined by the model (e.g., Gilliam and Fraser 1987).

Some theoretical models (Abrams 1991, Werner and Anholt 1993) predict that in the presence of predation risk the time prey spend foraging should decrease with increasing resource levels, assuming growth rate is a decelerating function of activity or time spent foraging. Some experiments support those predictions (Anholt et al. 2000) but numerous examples exist where in the presence of predators, the time prey spent foraging increases with resource levels (e.g., this study, Kohler and McPeek 1989, Gilliam and Fraser 1987).

Several explanations are possible. First, a particular empirical system may have a linear relationship between foraging effort and growth rate – if this relationship is assumed in models like Anholt and Werner (1993), foraging effort increases with increasing resources. Second, resource manipulations in empirical studies may be at levels that are still on the linear part of a saturating relationship between foraging effort and growth rate, and thus the response is like that predicted from models assuming a linear relationship.

Predictions from such models may also depend critically on the functional response of the consumer. For instance, if we assume a Type II functional response we expect foraging effort to decrease with increasing resources, because consumers are spending a greater proportion of their time

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handling prey (not foraging for prey). However, if we assume a Type III functional response, foraging effort will first increase with increasing resource levels, then decrease. Thus, to generate predictions for a specific system (or to rectify disparate empirical results) we may need to know the type of functional response that typifies the predator-prey interaction, and we may need to know the position along the resource gradient.

Lastly, as resource levels increase prey may need to move less to find resource patches (Formanowicz 1982, Formanowicz and Bradley 1987, Formanowicz et al. 1989), or may move less because it takes longer to exhaust resource patches. An increase in the time spent foraging may be coupled with a decrease in movement speed (the case in Kohler and McPeek 1989), with no net change in predation risk (because encounter rate with predators does not increase). Studies of periphyton grazing invertebrates are probably well described by this hypothetical scenario (e.g., this study, Kohler and McPeek 1989). This last possibility demonstrates the necessity of considering different aspects of foraging behavior (time spent foraging, rate of activity) separately (Werner and Anholt 1993), as they may be simultaneously adjusted to maximize growth and minimize mortality.

Empirical demonstrations that predators can have strong impacts on non-prey organisms by changing traits of prey are accumulating (Lampert 1987, Turner and Mittelbach 1990, Huang and Sih 1991, McIntosh and Townsend 1996, Beckerman et al. 1997, Turner 1997, Peckarsky and McIntosh 1998, Peacor and Werner 2001, 2002), and were forecasted by earlier mathematical

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models (Abrams 1984). Less well understood, though, is how the strength of trait-mediated indirect interactions (TMIIs) vary across important ecological gradients. Again, because the costs and benefits of predator-avoidance behaviors should depend on prey state and resource levels, we have reason to expect that TMIIs will vary in strength. For instance, at low resource levels there is little energetic cost for prey hiding in a refuge, so it may be adaptive for prey to respond strongly to even a mild threat of predation (and therefore TMIIs should be large). In contrast, prey hiding while in high resource environments pay a large opportunity cost, and therefore may choose riskier behaviors (consequently TMIIs may be small or non-existent). In this experiment the effect of predators on refuge use and foraging effort were generally larger (Figures 7, 8) when prey were in good physiological state, compared to prey that had experienced energetic stress. Despite the prey's stronger behavioral reaction to predation cues, I did not detect any difference in the magnitude of TMIIs between the two resource levels, or between prey of different initial physiological states. It is possible that no true differences exist. However, algal biomass was only measured in four of the seven replicates for each treatment, and indirect effects involving multiple trophic levels may involve greater intrinsic experimental error. Thus, it is possible that greater statistical power would reveal differences not seen here.

Prey in natural populations likely exist at varying physiological or energetic states, reflecting environmental conditions and the behavior of the prey in previous time intervals. For instance, an individual may be in poor

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condition now because it recently sensed predation risk, and chose to hide in a refuge. Now, even given the same conditions (e.g., predation risk) the prey may make different choices because of its new state. Short-term experiments that manipulate prey state may be useful to generate parameters for dynamic models that simulate prey behavior across a growing season or a cohort lifetime. In other words, knowing how prey make short-term behavioral choices given a certain state, predation risk, and resource levels will be helpful in predicting what dynamic strategies prey may use over longer time scales. Evaluating the adaptive significance of prey behavior is only appropriate over longer time scales, as some strategies may have negative effects on short-term fitness but positive effects on lifetime fitness.

Another factor not considered here, but that may influence prey behavioral decisions, is ontogeny. Prey that are nearing reproductive maturity, or the reproductive season, may make different choices than those whose reproduction is less imminent. For instance, an organism that is in good condition and that will have some positive fitness if it survives to reproduce might benefit more on average from hiding, and foregoing further energetic gain from foraging and the consequent predation risk (Werner and Anholt 1993, Luttbeg et al. 2003). Ontogeny and prey state seem especially likely to have interactive effects on prey foraging behavior.

Conclusions

Foraging often exposes prey to greater risk of predation, and thus prey face a trade-off between safety and energetic gain. Prey behavior should also

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reflect the physiological state of the individuals because the relative costs and benefits of certain behaviors may differ for energetically stressed and satiated animals. In this experiment, I found that prey state, resource abundance, and predators all affected snail behavior.

Because predator-induced changes in prey traits can have meaningful effects on other functional groups, understanding the behavioral decisions of prey will be a necessary step towards understand the functioning of ecological communities. Especially useful will be experiments that examine prey behavior and trait-mediated indirect interactions along gradients of predation risk and resource availability.

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CHAPTER FOUR

RELATIVE STRENGTHS OF TRAIT-MEDIATED AND DENSITY-MEDIATED INDIRECT EFFECTS OF A SNAIL PREDATOR (*BELOSTOMA FLUMINEUM*) VARY WITH RESOURCE LEVELS.

with Bernard Luttbeg

Abstract:

Predators can affect the density of their prey, and can change prey traits (e.g., morphology, behavior). Both changes in prey density and prey traits may influence how the prey interacts with its resources. Thus, predators can interact indirectly with resource species (i.e. two trophic levels below) mediated by two separate mechanisms. Moreover, prey balance the conflicting demands of maximizing energy return from foraging and avoiding mortality by predators, and therefore the availability of the prey's resource should influence the strength of anti-predator behavioral responses (e.g., reductions in activity, seeking refuge). I investigated the relative strength of trait- and density-mediated indirect effects of an insect predator *Belostoma flumineum* on algal communities through a pond snail, *Physa gyrina*, across a gradient of basal resource abundance. I found that at low initial resource levels, trait-mediated indirect effects exceed density-mediated indirect effects, while at high initial resources the reverse is true. The total effect of predators remained constant across the basal resource gradient. These results support the predictions of

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simple optimization models, and contradict the predictions of a dynamic state-dependent model.

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Introduction:

Predators can affect both the density and traits (e.g., physiology, morphology, behavior) of their prey, but until recently most ecologists have assumed the strong indirect effects of predation in food webs (e.g., trophic cascades) are primarily the result of changes in prey density. However, the magnitude of trait-mediated indirect interactions (TMIIs) can rival and even exceed density-mediated indirect interactions (DMIIs) (Peacor and Werner 2000, 2001). While this result is somewhat surprising, Peacor and Werner (2001) set forth a plausible explanation: the reduction in foraging rate induced by the presence of a predator is immediate, affects all prey individuals, and may occur over the entire lifespan of the prey (but see Turner 1997). Despite the potential importance of TMIIs, there are very few empirical comparisons of the relative strength of TMIIs and DMIIs.

Variation in the relative strength of TMIIs and DMIIs across common ecological gradients is virtually unexplored. However, how strongly prey react to changes in predation risk should depend on the level of resources available for the prey, but models differ in the predicted direction of this effect. As resource levels are reduced, the rate of energetic gain for the consumer should be reduced. Optimality theory predicts that when resources are scarce prey should respond strongly to the risk of predation (e.g., cease foraging, seek refuge, remain inactive) because the ratio of the risk of mortality to energetic gain is high (Gilliam 1983, Werner and Gilliam 1984). Thus, optimality theory predicts that as resource levels decrease, 1) TMIIs should increase, because prey are resp

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prey are responding more to predation risk, and 2) DMIIs should decrease, because prey will be less willing to expose themselves to predation. However, theory based on state dependent behavior (i.e. an animal's behavior depends on its physiological or energetic state) predicts opposite responses; when resources are scarce, prey should respond weakly to the risk of predation because they must forage either to avoid starvation or because foraging success greatly increases their fitness (Luttbeg et al. 2003). Thus, as resource levels decrease these models predict that 1) TMIIs will decrease, because prey are responding less to predation risk, and 2) DMIIs will increase, because prey expose themselves to more predation. I test which of these theories best describes the community dynamics of a simple three trophic level community consisting of an insect predator (*Belostoma flumineum*), the pond snail *Physa gyrina*, and periphytic algae.

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Methods:

Trait-mediated indirect interactions (TMIIs) can be estimated by comparing the abundance of the basal resource in the absence of predators and in the presence of predators that cannot kill prey. Typically, predators are rendered non-lethal by caging them, and are fed conspecific prey so that the focal prey may sense the predators visually and chemically (Peacor 1997, Bernot and Turner 2001). Density-mediated indirect interactions (DMIIs) can be estimated by comparing the level of resources found when predators are present and able to kill prey versus when predators are caged and can only "scare" prey. Here I define TMII, DMII, and the total indirect interaction (TII) as:

TMII = (resources with caged predator / average resources with no predator) – 1,
DMII = (resources with deadly predator /average resources with caged predator) – 1,
TII = (resources with deadly predators / average resources with no predators) - 1.

Thus, indirect effects are defined as the proportional increase in the resources present at the end of the experiment due to trait changes among prey (TMII), density changes among the prey (DMII), or both (TII). These definitions avoid the problem of indirect effects increasing with resource abundance as a statistical consequence of comparing larger means.

In order to examine the effects of initial resource availability on the strength of trait- and density-mediated indirect effects, I assembled and studied a simple three trophic level community consisting of an insect predator (*Belostoma flumineum*), a pond snail (*Physa gyrina*), and the snail's resource, periphytic alg

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periphytic algae. I varied the algal resources initially available to snails, and crossed that manipulation with the three predator treatments necessary to evaluate TMIIs and DMIIs.

Initial algal biomass was manipulated by adding ceramic tiles (23 cm^2; 12 tiles/tank) with different amounts of algae to the tanks. I therefore varied the resource available per unit area of resource substrate, rather than varying the area of resource substrate itself. The amount of algae per tile was manipulated by growing algae in tubs with different levels of inorganic nutrients (nitrogen as NH_4NO_3 and phosphorus as KH_2PO_4), under 24 h light from 55 W full spectrum florescent bulbs. Initial algal biomass was estimated by placing tiles (4 per resource level) in 95% ethanol for 24 h, and then determining the concentration of chlorophyll *a* using narrow-band flourometry (Welschmeyer 1994).

Two separate trials were conducted, the first (13-21 Jul 2003) with six levels of resource availability (0.12, 0.22, 0.80, 1.09, 3.27, and 3.28 μg chlorophyll *a* / cm^2) crossed with the three predator treatments, and the second (27 Jul – 4 Aug) with two levels of available resource (1.78 and 4.63 μg chlorophyll *a* / cm^2), again crossed with the predator treatments. The levels of algal resource in the second trial overlap with those from the first trial, and the results presented were largely insensitive to including the second trial. I included the second trial because together the two trials provide a larger and more complete resource gradient. Each combination of resource level and predator treatment was replicated twice, and treatments were assigned to experimental units randomly, within trials. Algal biomass remaining after the 9 d trials was est

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Animals were collected from a pond in the Lux Arbor Reserve (W. K. Kellogg Biological Station, SW Michigan, USA) where they naturally co-occur. I added 19 snails (average shell length (SE): 6.97 (0.18) mm, shell length (SE): 2.71 (0.19) mg dry weight) to each experimental unit, which were 300 L outdoor "cattle tanks". The tanks were filled with low-nutrient well water, had a sand substrate, and were subject to natural levels of light, temperature, and rainfall. One third of the tanks received one free swimming (and thus deadly) predator each, another third received one caged predator each, while the remaining third received no predators. The predator cages were clear plastic tubes (10 cm diameter) with fine mesh (250μm) on each end that allowed water, but not snails or predators, to pass through. Cages were suspended at the water's surface in the middle of the tanks. Enclosed predators were fed two snails every 48 h, a rate close to field estimates of snail consumption rates by *Belostoma* (0.5 snails per day, Kesler and Munns 1989). Organisms were only used for one trial. A few tiles of each initial resource level were placed in several control tanks (containing no snails or predators). Four of these tiles per resource level were harvested after 9 d, and algal biomass was determined as above.

Snail habitat use was observed during the afternoon hours four times per trial. During each observation I recorded the number of snails on resource tiles, on the sand near the resource tiles, on the sand away from tiles, within 10 cm of

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the surface, and on the sides of the tank. I also noted the number of dead snails. I expected actively foraging snails to be on or near resource tiles. In other experiments *Physa* have responded to *Belostoma* predation risk by crawling out of the water and staying near the water-air interface (Chapter Three), so I expected snails to increase their use of surface habitat in response to predation risk.

Simple linear regressions of effect sizes for TMIIs and DMIIs versus initial algal abundance were used to evaluate the sensitivity of predator induced indirect effects to initial algal biomass. The interaction term in an ANOVA with predator treatment and initial algal biomass as factors was used to determine if slopes of the relationships between initial and final algal biomass for different predator treatments were similar. Regression analysis was used to characterize the relationships between initial and final algal biomass for each treatment, and to examine the dependency of snail habitat use and snail mortality on initial algal biomass. Chi-square goodness of fit tests were used to compare snail habitat use for the three predator treatments. Analyses were performed in SYSTAT v8.0.

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Results and Discussion:

Predators (*Belostoma*) had meaningful indirect effects on the abundance of the basal resource, periphytic algae, and the relative strength of TMIIs and DMIIs varied with initial algal abundance. As initial algal abundance increased, the magnitude of TMIIs decreased (Figure 11, Table 6) and the magnitudes of DMIIs increased (Figure 11, Table 6). There was a data point at low resources that seemed to have a strong influence on the relationship between TMIIs and initial resource abundance. However, a significant ($P<0.01$) negative relationship remains after removing that point. The resulting relationship has a larger R^2 (0.44) but a shallower slope (-0.2175).

Regardless of treatment, systems with greater initial algal biomass had higher final algal biomass (Figure 12). However, the slopes of those relationships were marginally different between predator treatments (ANOVA, initial algal biomass * predator treatment, $P=0.088$). Changes in the strength of TMIIs and DMIIs across the initial algal resource gradient result from tanks with non-lethal predators having higher than average (e.g., the grand mean) final algal biomass at very low initial resources and lower than average final biomass at high initial resources (i.e. shallow slope in Figure 12). The data reported in Figure 12 suggest there may have been some systematic differences between trials 1 and 2 (see Methods for what data come from what trial); final algal biomass appears to be higher in trial 2 in all tanks except non-lethal predator tanks, compared to trial 1. However, the inclusion of trial 2 doesn't qualitatively change any of the conclusions.

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Table 6. Simple linear regression results for the relationships between several dependent variables and initial algal abundance.

Dependent Variable	slope	intercept	R^2	P
TMIIs	-0.3954	1.10	0.290	**0.0313**
DMIIs	0.4297	-0.17	0.448	**0.0046**
TIIs	0.0579	0.53	0.022	0.5829
Snail mortality				
no predators	-0.1360	2.63	0.009	0.7321
non-lethal predators	-0.2918	2.99	0.048	0.4156
lethal predators	-0.5157	19.29	0.214	0.0712

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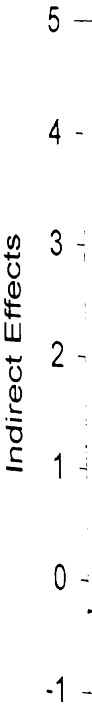
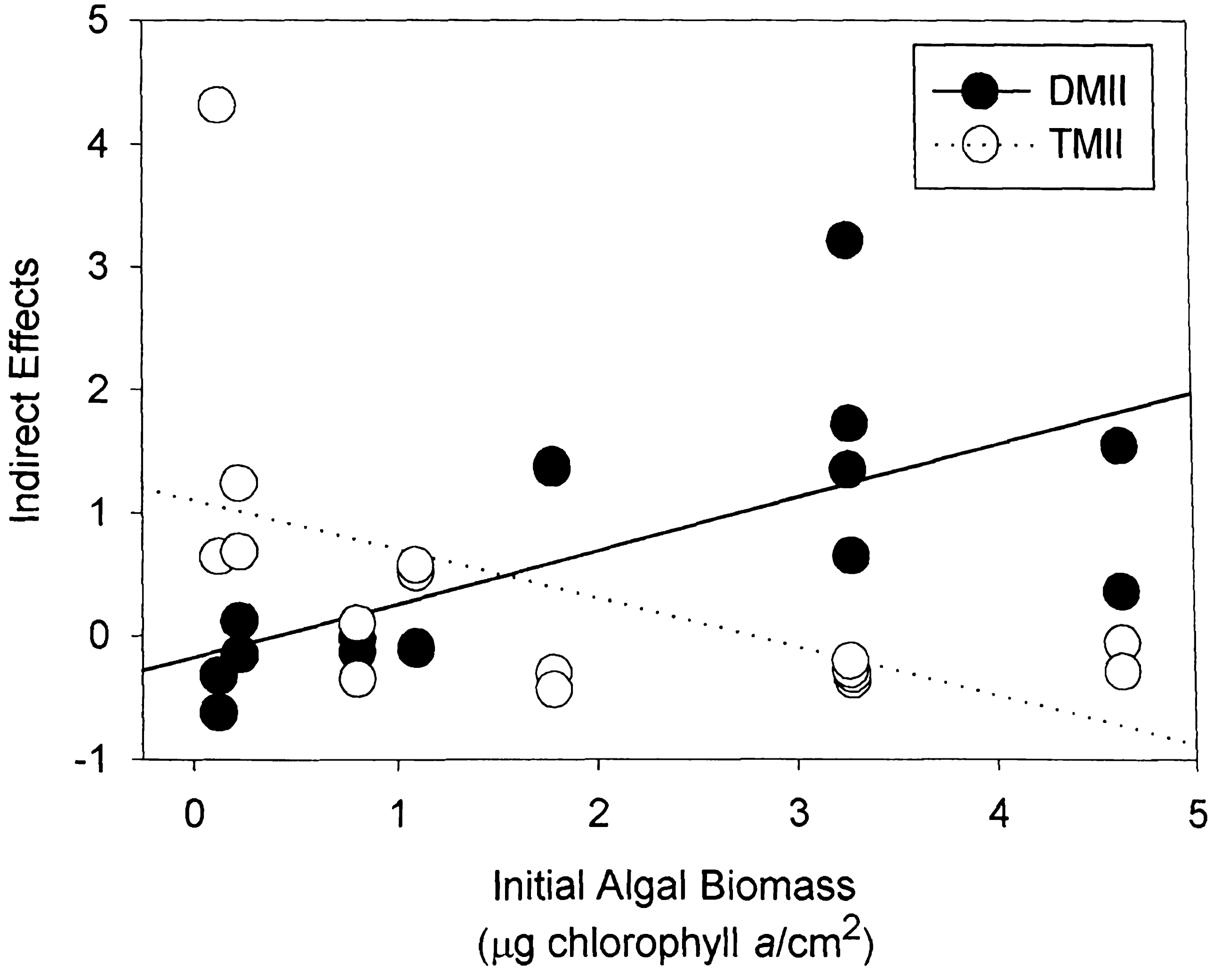


Figure 11. The strength of indirect effects of the predator *Belostoma flumineum* on algal abundance, as a function of initial basal resource availability. Density-mediated indirect interactions (DMIIs) are represented by solid circles, while trait-mediated indirect interactions (TMIIs) are represented by open circles. Lines (DMIIs as a solid line, TMIIs as a dashed line) represent simple linear regressions described in Table 6.



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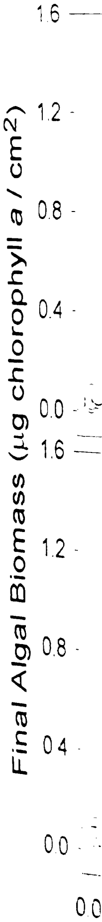
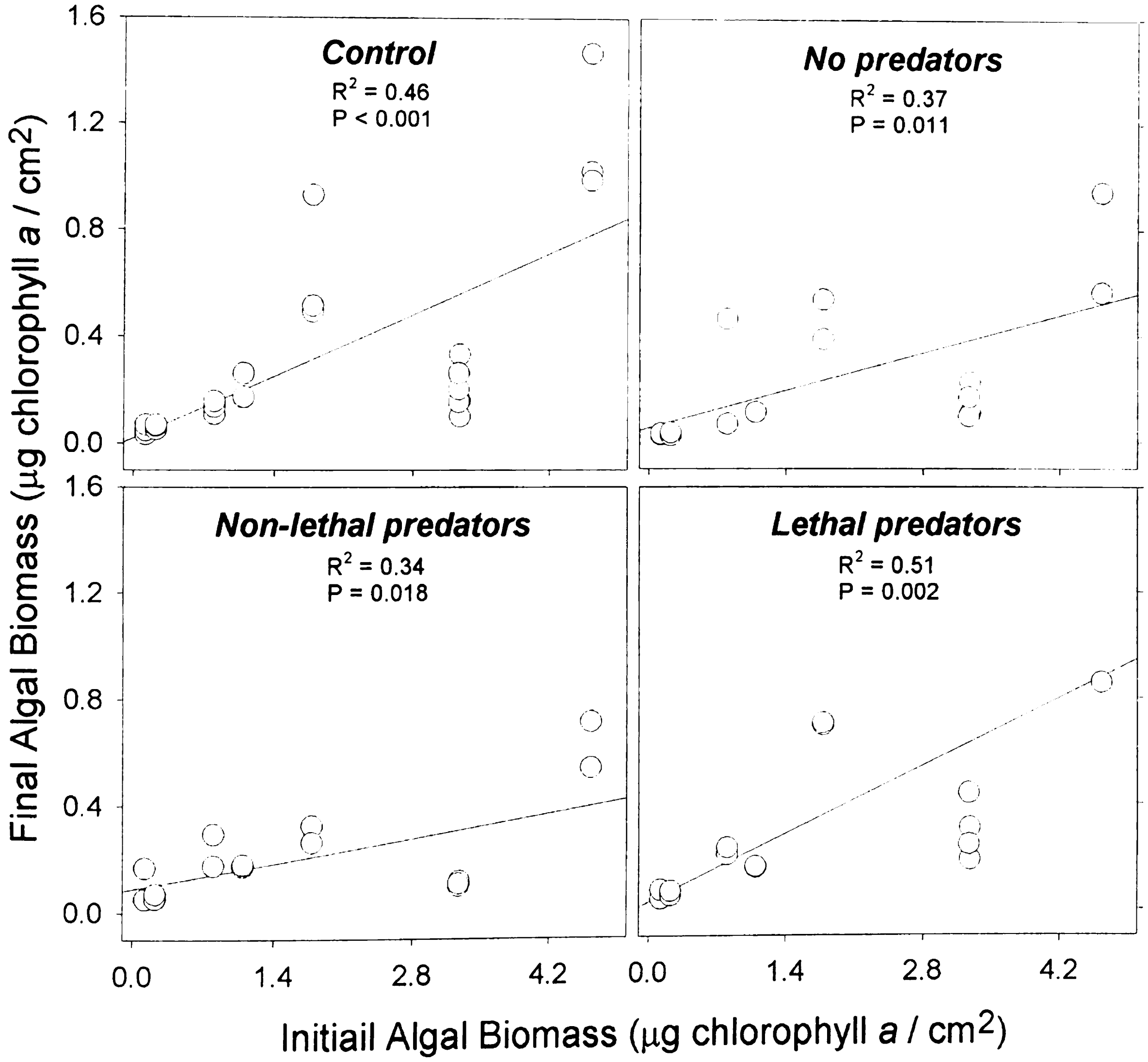


Figure 12. Linear regressions of final algal biomass on initial algal biomass for controls (no snails), no predator, non-lethal predator, and lethal predator treatments.



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Snails exhibited changes in habitat use in response to the predator treatments, but not in the ways I expected. Snail behavior differed between the no predator and non-lethal predator treatments; snails in the no predator treatment were found more often on the sand away from the resource tiles while snails in the non-lethal predator treatment were found on the sand near the resource tiles more often (Figure 13, Chi-square goodness of fit test, $\chi_4^2=10.88$, $P<0.05$). When the two "sand" categories were combined, prey behavior did not differ between the no predator and non-lethal predator treatments (Chi-square goodness of fit test, $\chi_3^2=1.03$, $P>0.5$).

Snail behavior was also significantly different between the non-lethal and lethal predator treatments (Chi-square goodness of fit test, $\chi_4^2=24.88$, $P<0.05$). This difference was primarily driven by snails in the lethal predator treatment being found on the sides of the tanks less often than in the non-lethal predator treatment (Figure 13). When deadly predators were present, shells of dead snails were often found on the bottom of tanks near the sides, indicating that the snails were being captured and consumed while on the sides of the tank. Thus mortality may explain why fewer snails were seen on the sides of the tanks in the lethal predator treatment. With the side of tank category eliminated from the behavioral data, there were no significant differences in prey behavior between the non-lethal and lethal predator treatments (Chi-square goodness of fit test, $\chi_4^2=6.51$, $P>0.05$).

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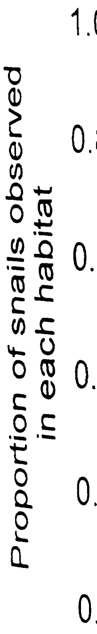
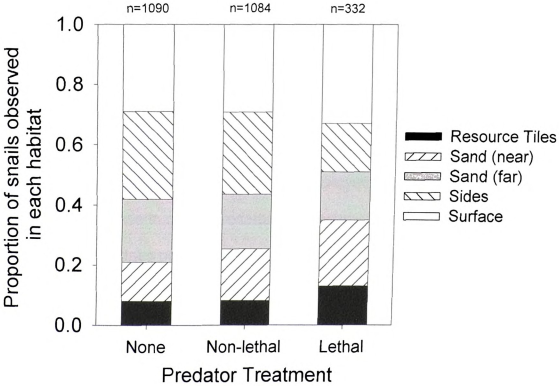


Figure 13. The use of habitat by *Physa gyrina* in the absence of predators, in the presence of non-lethal predators, and in the presence of lethal predators. Data are mean proportions (across four observations periods) of snails still alive found in a given habitat. Number of total snails observed in each treatment (n) is reported in the figure.



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Thus, I don't feel the behavioral observations captured the trait changes of the snail prey responsible for the trait-mediated indirect effects. In previous experiments aquatic snails have climbed to the water surface as a behavioral response to crayfish and *Belostoma* predators (Turner 1996, Alexander and Covich 1991, Covich et al. 1994, Chapter Three). I did not see an increase in surface habitat use in response to either non-lethal or lethal predators (Figure 13). However, the frequency of the behavioral observations may have been insufficient to detect the behavioral changes responsible for the measured TMIIs. In a laboratory experiment (Chapter Three) where behavior was observed more frequently (10 times over 48 h), snails showed little immediate response to predation cues, showed marked reductions in foraging effort and increases in refuge use 19 h after predators killed prey, and started to resume "pre-threat" behavior by 48 h. In the present experiment caged predators were fed every 48 h, and most of the observations were conducted before feedings (i.e. 47 h after previous feeding). If I had observed behavior more frequently, or closer to the predator feedings, I may have observed the expected anti-predator behavioral response to caged predators (i.e. increased use of surface habitat). This explanation does not, however, explain the lack of behavioral response to free-swimming predators, who were consuming prey continually throughout the experiment. Prey may have also changed their rate of movement in response to predation risk, thereby reducing their encounter rate with the predator. This aspect of prey behavior was not measured.

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Predators had strong direct effects on prey densities. In the presence of free-swimming predators, 39% of snails were dead after 24 h and 97% of snails were dead by the end of each trial, on average. In contrast, snail mortality in the non-lethal and no predator treatments was low - 13% on average. The initial abundance of algal resources did not significantly affect snail mortality in any predator treatment (Table 6). There was a trend towards greater total mortality at low resources in the lethal predator treatment (P>0.07, Table 6), but that trend was generated by one high resource tank with very low mortality. There was also no effect of initial resource abundance on the rate (i.e. deaths pre unit time) of mortality (regressions, all P>0.15). Thus, even if prey were seeking refuge more (or remaining inactive/foraging less) at some resource levels than others, mortality from predation remained relatively constant across the resource gradient. I observed few differences in snail behavior across the resource gradient; snails in the presence of non-lethal predators were less frequently observed on the tank sides as resources increased in abundance (regression, P=0.03).

One might expect that DMIIs should be uniformly strong across the resource gradient, because mortality was uniformly high. However, the TMIIs at low resource levels were so strong that even a 97% final mortality rate didn't increase the total effect of predators. In effect, increases in algal abundance due to changes in the traits of the prey preempted those due to density changes. The total indirect effect of predators (TIIs - mean(SE) 0.64 (0.16)) didn't vary with initial resource levels (Table 6).

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If real predators are used in an experiment, it will be impossible to separate trait and density changes completely; it is difficult to imagine an experiment where the actual density effects of a predator could be imposed without also inducing the behavioral effects of that predator. With the treatment structure used here one can observe the effect of predator induced trait changes among the prey (non-lethal predator treatment), and the combined effect of prey trait and density changes (lethal predator treatment), on the abundance of the basal resource. Then, one can subtract out the effect of trait changes from the effect of both trait and density changes to estimate the effect of density changes. This approach is reasonable, but does ignore the potential for density and trait changes to have interactive effects, as observed in Peacor and Werner (2001). Using this approach, interactive effects will be perceived as density effects, with the potential for overestimating the importance of density changes, relative to the effect of trait changes.

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Conclusions:

Both the results here and those of Peacor and Werner (2001) suggest that predator induced changes in the traits of prey can have large effects on the abundance of basal resources even when mortality rates of prey (due to predation) are high, and that the magnitude of trait mediated effects can surpass the magnitude of density-mediated effects. These results emphasize that the usual conception of ecological communities in terms of population sizes and/or biomass in different compartments, ignoring the dynamic nature of the traits of species, is insufficient to understand interactions in the community studied here. Empirical examples of strong behaviorally-mediated effects of a top predator on primary producers exist (e.g., Turner and Mittelbach 1990, Schmitz et al. 1997, Beckerman et al. 1997), but rarely have the strength of behavior- and density-mediated indirect interactions been compared. The comparisons to date suggest predator-induced changes in the traits of prey may constitute a powerful mechanism structuring some communities.

These results also show that the relative importance of prey behavior in community dynamics is a function of resource densities. Thus, community dynamics may be shaped by how resource levels affect prey's behavioral response to predation risk and the feedback of how prey behavior affects resource densities. This adds another level of complexity for studying communities, but it may be possible to use natural variation in resources over space and time as opportunities to study the relative importance of prey behavior in community dynamics.

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I found that the magnitudes of TMIIs decreased and of DMIIs increased as initial resource levels were increased. This matches the prediction of simple optimization models where prey respond less to predation risk when resource levels are higher because of the increased foraging success (Gilliam 1983, Werner and Gilliam 1984). A dynamic state-dependent model proposes that high resource levels will lead to improved prey state (i.e. less hungry, larger mass), and subsequently prey will respond strongly to predation risk because the fitness benefits of foraging are small when prey state is high (Luttbeg et al. 2003). These results do not match the predictions of this state dependent model. However, if the fitness benefits of foraging for *Physa gyrina* are not reduced when their physiological state is improved or the states of *Physa gyrina* individuals did not change greatly during this experiment, these results would be consistent with a state-dependent model.

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CHAPTER FIVE

TOP-DOWN, BOTTOM-UP, AND CONSUMER SPECIES RICHNESS EFFECTS ON ECOSYSTEMS: CONTEXT DEPENDENCY AND RELATIVE EFFECT STRENGTHS.

Abstract:

Theory and experiments demonstrate that the number of species in a local area can determine rates of ecosystem processes, but we know little about how the strength of that control compares with other influences or how it might vary across ecological gradients. Here I report results of a mesocosm experiment with aquatic gastropod grazers where consumer species richness, predation intensity, and resource availability were crossed in a full-factorial design. This design allowed a direct comparison of the strength of the different factors on food web properties and ecosystem functioning, and an evaluation of how the factors may interact.

Systems with higher snail species richness had greater secondary production, consumer biomass, and macrophyte stem growth, and lower epiphyton and periphyton biomass. However, snail species richness effects on periphyton and epiphyton were context-dependent; predators reduced the effect of increasing snail richness on the biomass of attached algae. Species richness effects were statistically determined to be the result of a biological mechanism (e.g., differential resource use) rather than being solely artifacts of the experimental design (e.g., sampling effects). The effects of nutrient

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enrichment and predation were mostly predictable from simple food chain models; increases in nutrient availability led to increased algal biomass, snail biomass, and primary production, while predators decreased snail biomass and indirectly augmented algae.

Snail species richness effects on the biomass of many functional groups were as strong or stronger than those of a substantial nutrient enrichment or of the addition of a voracious top-predator (*Belostoma flumineum*). Nutrient enrichment had the most pronounced effects on whole system processes (e.g., respiration, primary production, sedimentation). Species richness had very small effects on ecosystem properties, probably because of compensatory responses among different producer functional groups. This experiment suggests that the number of consumer species in a system can have large and meaningful effects on the distribution of biomass in a food web, that these effects can depend on ecological context, and that nutrient availability may be more important for ecosystem processes than is species richness.

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Introduction:

The effect of species richness on the functioning of ecosystems has been the focus of much ecological research (Loreau et al. 2001). Empirical evidence to date is equivocal (Schalpfer and Schmid 1999, Tilman 1999), and has sparked an intense debate over the interpretation of diversity-ecosystem function experiments (Huston 1997, Wardle 1999, Emmerson and Raffaelli 2000, Adler and Bradford 2002, Petchey 2003). While some ecosystem processes do appear sensitive to the number of species present (e.g., Tilman et al. 2001, Downing and Leibold 2002), many important questions have only begun to be addressed empirically. For instance, how do species richness effects on ecosystem function compare in strength to other effects (e.g., keystone species, trophic effects) and what system properties (e.g., food web structure, underlying productivity) mediate richness effects on ecosystems?

The first question goes to the heart of many ecologists' concerns about the importance of species richness-ecosystem function experiments. The magnitude of a statistically significant effect of richness on ecosystem function in a tightly controlled and well-replicated experiment is hard to interpret in isolation of other important factors. In contrast, if the magnitude of richness effects could be compared directly to that of factors known to frequently regulate community structure, then meaningful inferences could be drawn about how important richness effects *could be* in natural systems. Only the magnitudes of compositional effects (e.g., Hooper and Vitousek 1997, Hooper 1998, Tilman et al. 1997b, Downing and Leibold 2002) and resource availability

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(terrestrial plants - Fridley 2002, stream fungi - Bärlocher and Corkum 2003) have been compared to those of diversity, and in most cases diversity effects were relatively weak. A wider set of comparisons (more factors and more systems) is needed to evaluate the importance of richness effects relative to other factors ecologists routinely consider.

How other biotic and abiotic factors strengthen or weaken diversity effects on ecosystem function is of theoretical interest for ecologists, but also is important to appropriately interpret diversity-ecosystem function experiments performed to date. We do not yet know, for instance, whether the particular experimental conditions used in a given study provide a liberal or conservative test of diversity effects on ecosystem function (Fridley 2001). By examining diversity-function relationships in a number of ecological contexts we may begin to learn what factors mediate those relationships. The first diversity-function studies to examine context-dependency have explored resource availability (Jonsson et al. 2001, Fridley 2002), the presence of mutualists (Klironomos et al. 2000), and disturbance (Cardinale et al. 2000, 2002), and in each case there was a particular condition that enhanced the strength of diversity effects. All species face threats from predators, pathogens, and/or parasites, and all species must acquire resources to grow and reproduce. Therefore, predation intensity and resource availability may provide general gradients on which to focus study of context-dependent species richness effects on ecosystem function.

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Prey respond in many ways to predation (see Lima and Dill 1990, Lima 1998 for comprehensive reviews), but very often reduce their activity (Crowl and Covich 1994, Kolar and Rahel 1993), invest in defensive structures (DeWitt 1998, Brønmark and Miner 1992) or chemicals (Coley 1983, Bryant et al. 1983, 1985), modify their life-history (Crowl and Covich 1990, DeWitt 1998, Chase 1999) or restrict their use of habitat (Werner et al. 1983, Turner and Mittelbach 1990, Turner 1996, 1997, Turner et al. 1999). By employing anti-predator defenses, prey often sacrifice some ability to acquire resources (Sih 1980, Gilliam 1982, Werner and Gilliam 1984, Lampert 1987). High predation environments will generally be dominated by predator-resistant taxa and these taxa are expected to be less effective in their resource use (Leibold 1989). Predation is therefore predicted to reduce functional diversity among prey species, and species diversity effects on ecosystems that depend on functional uniqueness (e.g., differential resource use) may be less likely or less intense when predation is strong.

The availability of resources (Jeffries 1990, Nonacs 1990, Chapter Three), an organism's energetic state (Kohler and McPeek 1989, Sih 1992, Chapter Three), or the intensity of competition can interact with predation to determine prey response to the risk of predation (Pettersson and Brønmark 1993). Thus, species effects on ecosystems should be influenced by both predation and resource availability. For instance, prey are often willing to accept more risk when resources are scarce (Anholt and Werner 1995), and one might predict that functional diversity should not be as sensitive to

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predation under low-resource conditions, because prey may not respond to predation risk as strongly. Resource abundance could also mediate species effects on ecosystems if resource heterogeneity increases with resource abundance. For most parameters variance does increase with the mean, and patchy distributions of resources can provide an axis for niche differentiation (Chase et al. 2001).

Here I investigate consumer species richness and composition effects on ecosystem functioning and food web properties in a replicated mesocosm experiment with pond snails, invertebrate predators, macrophytes, and algae. The experiment was performed in four distinct ecological contexts to examine how underlying system productivity and predators influence the effects of consumer species richness on system properties. This design allows both a consideration of the context-dependency of richness effects on whole systems, and a comparison of effect strength between diversity effects and two factors that frequently structure aquatic communities, top-down (predator) and bottom-up (resource availability) forces.

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Methods:

The experimental design consisted of a snail species richness and composition manipulation replicated in four ecological contexts, defined by the four possibilities of a 2x2 factorial crossing nutrient enrichment and predator presence/absence. Each combination of snail richness/composition, nutrient status, and predator presence was replicated four times, with the exception of the most species rich treatments which were replicated six times. The purpose of this unbalanced design was to partially alleviate the difference in sample size for species richness main effects (which average over several levels of composition at low species richness and only one level of composition at the highest richness level).

Setup

The experiment was established in 120 aquatic mesocosms (outdoor "cattle tanks") at the W. K. Kellogg Biological Station Experimental Pond Facility, each filled with 275 L of filtered well water (conductivity ~ 300 μS, pH ~ 9.6, total nitrogen (TN) ~ 96 μg/L, total phosphorus (TP) ~ 17 μg/L) on 20 May 2002. Phosphorus and nitrogen were added to all tanks in the form of KH_2PO_4 and NH_4NO_3; "low nutrient" tanks were raised to two times ambient nutrient concentrations, while "high nutrient" tanks were raised to eight times ambient concentrations. These nutrient levels are nominally high, but are reasonable because all of the production in the tanks would stem from the dissolved nutrients (there were no nutrients stored in organic sediments as in a natural pond). Moreover, small forested ponds in Michigan routinely have water column conc

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column concentrations of TN >1750 μg/L and TP >250 μg/L (P. Geddes, *personal communication*). Sand was added to each tank as substrate. Fiberglass screen lids covered each tank to limit entry/exit of organisms.

Organisms

A diverse algal inoculum collected from ten local ponds was introduced into each tank soon after they were filled. Vascular macrophytes (*Potamogeton illinoensis*) were added to each tank on 9 Jun at 4.09 g/m^2 dry mass. Zooplankton, fungi, bacteria, and some insects (mainly odonates and notonectids) colonized the tanks through the introduction of algae and macrophytes.

Three snail species, *Physa gyrina, Fossaria obrussa*, and *Helisoma trivolvis* (hereafter referred to by generic names), comprised the grazer species assemblage. All are common snails that co-occur in ditches, shallow ponds, and lakes in Michigan, and were selected for use because of their ubiquity (these species account for >82% of snail biomass in small Michigan ponds on average (n=16 ponds); Appendix C). Chase et al. (2001) have described the different foraging modes employed by these snail taxa; *Helisoma* finds resources patches slowly but efficiently removes most of the available resource ("digger"), while *Physa* finds new patches quickly but removes less of the algae in a patch before moving on ("grazer"). *Fossaria* was not studied by Chase et al. (2001), but is likely intermediate in both traits as were two members of its taxonomic family (*Pseudosuccinea columella* and *Lymnaea elodes* - Family Lymnaeidae).

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Snail species richness and composition were manipulated by stocking tanks with every possible combination of one, two, and three species, resulting in seven snail treatments. In a field survey of 16 southern Michigan ponds, snail species richness averaged 2.25 (mode = 3), and ranged from 0-4 (Appendix C). Thus, a manipulation of snail species richness including three species treatments covers a majority of the natural range in richness, and is ideal for understanding the consequences of species loss from communities of this type. Snails were stocked on 19-20 June at 275 mg dry mass per tank, with that mass divided equally by the number of species present (i.e. monoculture tanks had 275 mg of snail biomass, two species tanks had 137.5 mg of each species, etc.). Because of differences in average body size between species, starting with equal biomass across treatments necessitated differences in the number of individuals of each species used (i.e. monocultures contained 64 *Fossaria*, 64 *Physa*, or 16 *Helisoma*). The snail densities (on a per area basis) used in this experiment are well within the natural range of densities and appropriate for the mesocosms given their inherent productivity (evidenced by the average final live biomass of snails per tank, 271.4 mg dry mass).

One adult waterbug (Hemiptera: *Belostoma flumineum*) was added to each of sixty randomly chosen predator tanks. *Belostoma* are efficient snail predators, able to consume up to six adult snails per day in lab settings (Crowl and Alexander 1990), and 0.5 snails/day in the field (Kesler and Munns 1989). All snails were susceptible to *Belostoma* except for the largest *Helisoma*, which can grow to

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can grow to a size refuge (>10mm) where they are essentially invulnerable (Chase 1999). In laboratory experiments *Belostoma* displayed a hierarchy of prey preference; *Physa* was the most preferred prey species used here, followed by *Helisoma*, while *Fossaria* was least preferred (Appendix D). Several *Belostoma* died during the experiment and were replaced within 24 h.

Response Variables

Some variables were measured more than once during the experiment to ensure adequate estimation, not because temporal dynamics were of primary interest. Therefore, multiple measurements for a single response were averaged over time for all subsequent analyses.

Snail Biomass

At the end of the experiment snail biomass was estimated by sieving each tank's contents (1mm mesh), preserving snails in 70% ethanol, counting all snails and measuring shell length of the first ~100 live and ~100 dead individuals encountered for each species from each tank. Length measurements were converted to dry mass using species-specific length-weight regressions (C. Osenberg, *unpublished data*), and an average snail mass was calculated. Snail production was calculated as the biomass of all live and dead snails (number times average mass) minus the initial biomass of snails, while standing snail biomass reflects only snails alive at the end of the experiment.

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Primary Producers

Periphyton biomass was estimated on 8 Jul, 2 Aug, and 29 Aug. A section of plastic tape (3.63 cm^2), which had been adhered to the tank wall at the start of the experiment, was removed on each sampling date. These pieces were placed into 95% ethanol to extract chlorophyll *a*, and the chlorophyll concentrations were determined using narrow-band flourometry (Welschmeyer 1994).

Epiphyton, metaphytic algae, and macrophytes were censused during the first week of August and again at the end of the experiment (31 Aug - 2 Sep). I assessed epiphyton biomass visually using a six-point scale (i.e. 0=no visible epiphyton, 1, 2, 3=most stems are covered, 4, 5=macrophyte completely covered/ significant damage). On 18 Aug I calibrated the qualitative scale to actual chlorophyll *a* concentrations by haphazardly removing one 10cm long stem of macrophyte from each of 24 random tanks, brushing the epiphyton onto a glass fiber filter, and then determining the chlorophyll *a* concentration as above. The qualitative epiphyton score was strongly and linearly related ($P<0.0001$, $R^2=0.764$) to chlorophyll *a* of the epiphyton, therefore I converted epiphyton scores to chlorophyll *a* concentrations using that regression equation. I estimated metaphyton algal abundance visually as percent cover of the surface. Macrophyte growth was estimated on 2 Aug by counting the number of new stems emerging from the sediment. Macrophyte biomass was measured at the end of the experiment by weighing the plants after wringing away excess water. Macrophyte biomass was converted to dry mass using a wet to dry we

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wet to dry weight regression derived for *Potamogeton illinoensis* (g dry mass = 0.324 * (g wet mass) - 2.39, R^2=0.891). Phytoplankton biomass was measured on 9 Aug by filtering 100 mL of water from each tank onto a glass fiber filter, then determining chlorophyll *a* concentration as above. Phytoplankton chlorophyll a concentrations were very low (averaged 3.6 μg/L), and did not respond to the treatments (ANOVA not shown), therefore phytoplankton is ignored in the remaining analyses. There was a marginally significant effect (P=0.065) of nutrient enrichment on phytoplankton, however.

Whole-System Properties

Whole system primary productivity and respiration rates were measured by determining the diel flux of dissolved oxygen (Howarth and Michaels 2000). I measured oxygen concentrations in each tank with a YSI Model 600XL-100-m at sunset and sunrise at the beginning of the experiment (13-14 Jul), and at sunset, sunrise, and the next sunset during the middle (6-7 Aug) and end of the experiment (28-29 Aug). The difference in dissolved oxygen from dusk to dawn, divided by the number of hours of darkness, gives the respiration rate per hour for the entire mesocosm community. The difference between dissolved oxygen concentrations at dawn and dusk, divided by the number of hours of sunlight, gives the net primary productivity (i.e. primary productivity minus respiration) per hour. Gross primary productivity was calculated by accounting for daytime respiration. Both respiration and gross primary productivity rates were scaled to 24 h (e.g., total respiration per day).

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I measured the accumulation of sediments during the experiment on 16-18 Aug by randomly placing a small circular quadrat (314 cm^2) on the bottom of each tank. A large syringe was used to withdraw the sediment within the quadrat, which was then filtered onto a glass fiber filter for ash free dry weight analysis. Ash free dry weight was calculated as the difference in mass between a dried (48 h at 50° C) sample and that sample after combustion (1 h at 550° C), and represents the dry mass of organic material in the sample. It is possible that attached algae could be inadvertently included in the sediment samples, but visually the sediment was dominated by snail feces in all tanks.

Statistical Methods

Sampling effects (Huston 1997, Aarssen 1997, Tilman et al. 1997a) are positive changes in an ecosystem function with increasing diversity because of the greater chance of including a particularly influential species in a diverse system compared to a less diverse system. Various methodologies (Garnier et al. 1997, Wardle 1999, Hector 1998, Loreau 1998, Emmerson and Rafaelli 2000, Loreau and Hector 2001, Adler and Bradford 2002, Petchey 2003) have been proposed to separate sampling effects from "real" diversity mechanisms like differential resource use and facilitation. Here I use the statistics suggested by Loreau (1998). Two important parameters, D_i and D_{max}, are defined:

$$D_i = \frac{O_i - E_i}{E_i},$$

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$$D_{\max} = \frac{O_T - MAX(M_i)}{MAX(M_i)},$$

where O_T is the observed yield of all species in a mixture and MAX(M_i) is the maximum yield in monoculture of any species.

Thus, D_i is the proportional deviation of species *i*'s yield in polyculture from the yield predicted from its monoculture performance (positive D_i means a species had net positive interactions with the other species, relative to when by itself), and D_{max} is the proportional deviation of the total polyculture yield from the highest yielding monoculture. Loreau (1998) argues that $D_{max} > 0$, or $D_i > 0$ for all species, indicates a positive effect of diversity (e.g., niche complementarity or facilitation) beyond any potential sampling effect, and I use those criteria here.

The above definitions, developed for studies of plant diversity and production, can also be used to reveal diversity effects that cause reductions in some process or in the biomass of a functional group. For example, increasing snail richness is expected to increase snail biomass and consequently decrease attached algal biomass (e.g., periphyton, epiphyton), so the snail species that reduced algae the most was the "dominant", and $D_{max} > 0$ for algae would mean that the three species treatment had less algae than the "dominant" species treatment had in monoculture.

ANOVA (GLM procedure) was used to identify treatment effects and interactions for each response variable. Species composition was modeled as a nested factor within species richness because a given composition (e.g.,

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Helisoma + *Physa*) was only possible at a single level of richness. Interactions between species composition and nutrient enrichment and predation were not included in the ANOVA models; they were never significant and did not qualitatively affect the significance of other effects if included. Significant species richness by predation or by nutrient enrichment interactions demonstrate context dependency of species richness effects. Periphyton biomass and sedimentation data were log transformed, and metaphyton cover data were arcsine-square root transformed, to meet the assumptions of normality and homogenous error variance. Subsequently all response variables met ANOVA assumptions (examined with Levene's tests and probability plots of residuals). Tukey's HSD multiple comparison tests were used to dissect significant species composition effects. All statistics were performed using SYSTAT version 8.

Calculating effect sizes in factorial experiments is complicated by the potential for significant interactions to make interpreting main effects problematic (i.e. main effects may be small or zero if a factor has strong positive effects at one level of another factor and strong negative effects at the other level of the other factor, and thus may not accurately describe the response). Although methods have been developed to calculate effect sizes for main and interactive effects in simple factorial experiments (2X2 factorial - Gurevitch et al. 2000), it remains unclear how to expand those methods to tackle more complex experimental designs like the one presented here (e.g., 3–way factorial with a nested factor). In experiments with three manipulated factors and their many

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possible interactions it is difficult to visually examine the data and reach conclusions regarding the relative importance of the factors for a particular response variable.

Thus, in order to assess the relative importance of the three factors for each response variable, I calculated the percent change in the mean induced by manipulating the focal factor, separately at each level of the other factors. I then averaged the absolute values of those percent changes, which results in a statistic that describes the average percent change (ignoring direction) in a response because of changes in the focal factor. For instance, to use this methodology in a simple 2 by 2 factorial one would determine the change in the mean of a response variable induced by manipulating factor A, separately at levels 1 and 2 of factor B (e.g., +30% at B1, -20% at B2). Then, one would calculate the average of the absolute values of those means (e.g., 25%). This statistic would be interpreted as the average percent change in the response resulting from changes in factor A, across factor B. One would likewise calculate this statistic for factor B, and then could compare their relative importance for the response of interest. For example, if manipulating factor B meant on average a change of 8% in the response, one would conclude that factor A is more important than factor B.

This methodology does result in the loss of some potentially important information (e.g., direction of responses), but is useful here to summarize the effect of a large number of factors and interactions on a long list of responses, and captures information that is difficult to extract from figures (e.g., relative magnitude c

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Results:

Anax dragonflies invaded many tanks (44/120), most likely entering as eggs attached to macrophytes. *Anax* are effective snail predators (J. Wojdak, *personal observation*), and had direct effects on snails and indirect effects on other response variables. Invasion was random with respect to treatment (χ^2 goodness of fit tests for each factor, with the expectation of equal invasion of each treatment, all P > 0.19). *Anax* presence/absence (observed during routine attempts to remove them) was used as a concomitant variable in all ANOVAs to reduce error variance, and appears in tables with the treatment factors.

Snail Biomass

Total snail production was 87% higher on average in tanks with three species than in monocultures (Figure 14A; Table 7 – species richness P=0.0019) and the effect of species richness on total snail production was essentially the same in all four ecological contexts (Table 7, no significant interactions with species richness). “High” nutrient tanks had 18% greater total snail production (Figure 14A, Table 7, nutrients P=0.0004) than “low” nutrient tanks. Tanks with *Physa* present had less snail production than those without (Figure 15A, Table 7, species composition P=0.0053).

The total production of snails did not depend on *Belostoma* presence (Figure 14A, Table 7, *Belostoma* P=0.48), but the standing biomass of live snails at the end of the experiment did (Figure 14B, Table 7, *Belostoma* P=0.0377). In contrast, both the total production of snail biomass and standing

Table 7. A

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SR*P
N*P
SR*N*P
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Table 7. ANOVA results for snail production and standing biomass. Significant (P<0.05) treatment factors and interactions are bolded.

		Total Snail Production			*Snail Standing Biomass*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species Richness	2	287300	6.67	**0.0019**	138381	5.94	**0.0036**
Nutrient Enrichment	1	579032	13.44	**0.0004**	166786	7.16	**0.0087**
Predation	1	21268	0.05	0.4839	103265	4.43	**0.0377**
SR*N	2	29683	0.69	0.5044	934	0.04	0.9607
SR*P	2	30714	0.71	0.4927	10920	0.47	0.6270
N*P	1	15047	0.35	0.5559	25790	1.11	0.2951
SR*N*P	2	13628	0.32	0.7296	957	0.04	0.9598
Species Composition (SR)	4	168885	3.92	**0.0053**	94621	4.06	**0.0042**
Anax	1	1578200	36.63	**<0.0001**	732296	31.44	**<0.0001**
Error	103	43089	-	-	23288	-	-

Figure 14.

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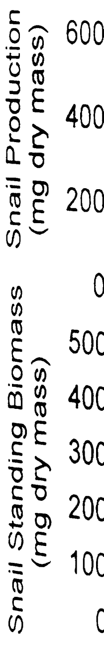

Figure 14. The response of A) snail production and B) snail standing biomass to species richness, nutrient, and predation manipulations. The left column of figures is from “low” nutrient tanks and the right column is from “high” nutrient tanks. Solid symbols represent the absence of *Belostoma* predators, open circles represent the presence of *Belostoma*. Means ± 1 SE are reported. Refer to ANOVA table (Table 7) for statistics.

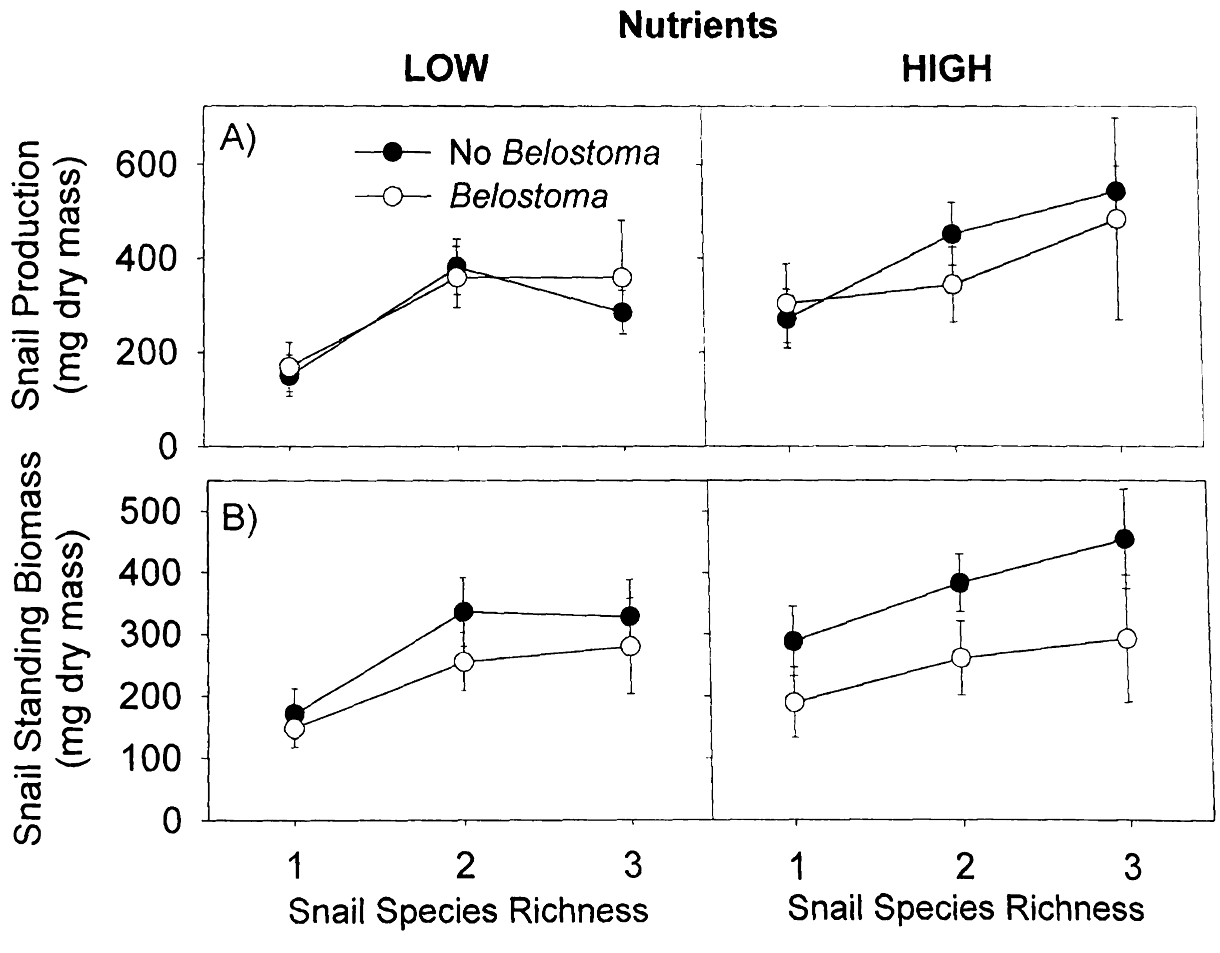

Figure 15.

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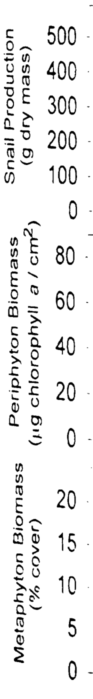
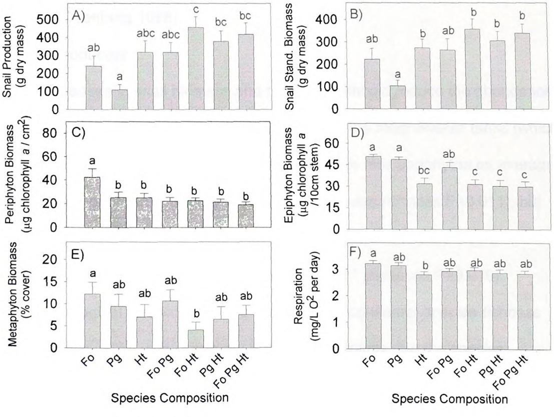


Figure 15. Species composition effects on A) snail production, B) snail standing biomass, C) periphyton, D) epiphyton, E) metaphyton, and F) ecosystem respiration. Means + 1 SE are reported. Fo = *Fossaria obrussa*, Pg = *Physa gyrina*, Ht = *Helisoma trivolvis*, Fo Pg = *Fossaria obrussa* + *Physa gyrina*, etc. Statistically significant differences (Tukey's HSD multiple comparisons) are noted with different letters.



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snail biomass were lower when *Anax* invaded (Table 7, *Anax*, both P<0.0001). Snail species richness, composition, and nutrient enrichment had qualitatively similar effects on standing snail biomass as they did on production.

The number of snails increased from the original 16-64 individuals added per tank to a mean of 1037 (SE = 75.3) per tank, and as mentioned above total snail biomass at the end of the experiment was nearly identical to that at the beginning. Thus, snail age and size distributions changed during the experiment from highly skewed towards older, larger adult snails to being dominated by younger, smaller animals, much as it does in natural lakes from spring to summer (Osenberg 1988).

Primary Producers

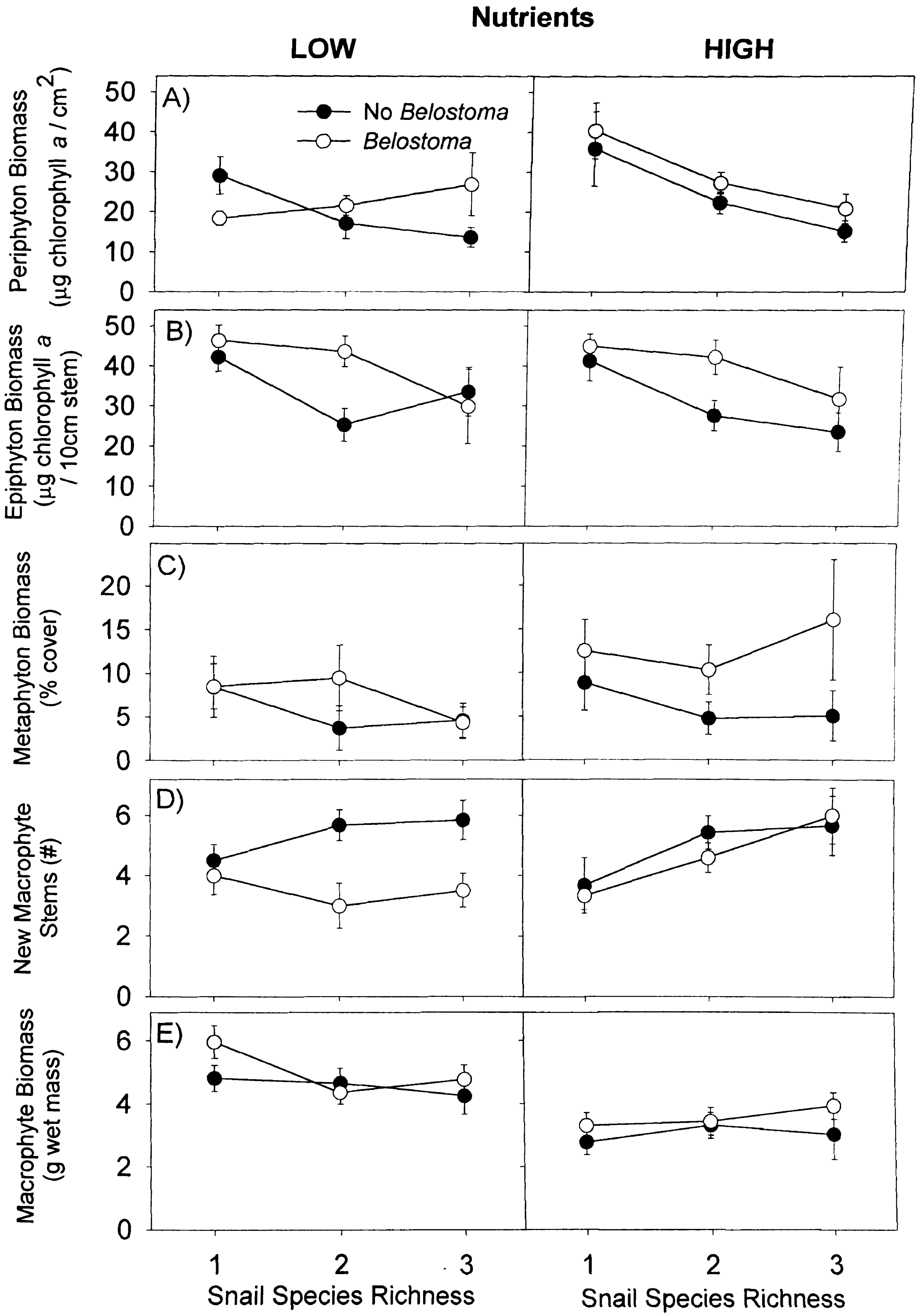
Increases in snail biomass and production should reduce the abundance of snails' primary forage, attached algae. Indeed, the most diverse tanks (which had more snail biomass and production) had 38.3% less periphyton on average than species poor tanks (Figure 16A, Table 8, species richness P=0.011), but the effects of snail richness on periphyton depended on the presence of *Belostoma* (Figure 16A, Table 8, species richness * predation P=0.049). Hence, ecological context mediated the effects of consumer species richness on periphyton. This was especially true at low nutrients, but the species richness*predation*nutrients interaction was not statistically significant. Nutrient enrichment enhanced periphyton biomass overall (Figure 16A, Table 8, P=0.048), as expected. The strength of the interactions between snails and the

Table 8. ANOVA results for algal biomass (periphyton, epiphyton, and metaphyton) and macrophyte (stem growth and biomass) responses. Significant (P<0.05) treatment factors and interactions are bolded. Error degrees of freedom are parenthetically noted if different from the first column. “n/a” means the term was not included in the model.

		Periphyton Biomass			*Epiphyton Biomass*			*Macrophyte Stems*			*Macrophyte Mass*			*Metaphyton*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species Richness	2	1.18	4.71	**0.0110**	1310.29	9.14	**0.0002**	16.73	3.68	**0.0287**	0.98	0.45	0.6380	0.04	1.47	0.2357
Nutrient Enrichment	1	1.00	4.00	**0.0481**	242.48	1.69	0.1963	3.52	0.77	0.3810	61.60	28.23	**<0.0001**	0.06	1.91	0.1702
Predation	1	0.54	2.15	0.1458	888.23	6.20	**0.0144**	30.08	6.61	**0.0115**	6.49	2.98	0.0875	0.16	5.33	**0.0229**
SR*N	2	0.28	1.14	0.3243	24.29	0.17	0.8444	9.53	2.09	0.1284	5.58	2.56	0.0825	0.00	0.15	0.8645
SR*P	2	0.77	3.09	**0.0497**	619.05	4.32	**0.0158**	5.35	1.18	0.3125	2.86	1.31	0.2742	0.02	0.75	0.4799
N*P	1	0.02	0.08	0.7811	18.21	0.13	0.7222	16.33	3.59	0.0609	0.02	0.01	0.9184	0.03	1.09	0.2984
SR*N*P	2	0.49	1.96	0.1462	97.91	0.68	0.5073	3.75	0.82	0.4414	0.98	0.45	0.6396	0.02	0.73	0.4850
Species Composition (SR)	4	0.76	3.05	**0.0204**	1526.88	10.65	**<0.0001**	2.85	0.63	0.6440	3.15	1.44	0.2251	0.07	2.51	**0.0460**
Anax	1	2.53	10.13	**0.0019**	3137.23	21.89	**<0.0001**	n/a	-	-	n/a	-	-	n/a	-	-
Error	103	0.25	-	-	143.32	-	-	4.55	(104)	-	2.18	(104)	-	0.03	(104)	-

Figure 16. The response of A) periphyton, B) epiphyton, C) metaphyton, D) emergence of new macrophyte stems, and E) macrophyte biomass to species richness, nutrient and predation manipulations. The left column of figures is from “low” nutrient tanks and the right column is from “high” nutrient tanks. Solid symbols represent the absence of *Belostoma* predators, open circles represent the presence of *Belostoma*. Means ± 1 SE are reported. Refer to ANOVA table (Table 8) for statistics.

Figure 16



Nutrients
LOW
HIGH
Periphyton Biomass (µg chlorophyll *a* / cm^2)
A)
No *Belostoma*
Belostoma
Epiphyton Biomass (µg chlorophyll *a* / 10cm stem)
B)
Metaphyton Biomass (% cover)
C)
New Macrophyte Stems (#)
D)
Macrophyte Biomass (g wet mass)
E)
0
10
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periphyton community depended on which snail species were present (Table 8, species composition P=0.020), with *Fossaria* monocultures interacting particularly weakly and thus having abundant periphyton (Figure 15C).

Like periphyton, epiphyton biomass was lower in more species-rich mesocosms (Figure 16B, Table 8, species richness P=0.0002); three species tanks had 32.2% less epiphyton than monocultures on average. Epiphyton always decreased with increasing snail species richness, but the shape of that relationship depended on the presence or absence of *Belostoma* (Figure 16B, Table 8, species richness * predation P=0.016). When *Belostoma* were present, three-species treatments had significantly less epiphyton than one- and two-species treatments, but in the absence of *Belostoma,* treatments with two and three species had less epiphyton than monocultures. Epiphyton biomass was less abundant in treatments with *Helisoma* relative to those without (Figure 15D, Table 8, species composition P<0.0001). Both *Belostoma* and *Anax* predators had indirect positive effects on epiphyton biomass (Figure 16B, Table 8, *Belostoma* P=0.014, *Anax* P<0.0001). *Belostoma* also had indirect positive effects on the abundance of metaphyton (Figure 16C, Table 8, predation P=0.023). Treatments with *Helisoma* had less metaphyton than did treatments without (Figure 15E, Table 8, species composition P=0.046).

Growth of new macrophyte stems was 36% greater in three snail species systems relative to systems with only one species (Figure 16D, Table 8, species richness P=0.029). *Belostoma* predators had indirect negative effects on macrophyte stem growth (Figure 16D, Table 8, predation P=0.012), and there was a

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there was a marginally significant predation * nutrient enrichment interaction (Table 8, P=0.061) where the effects of predators were more pronounced at LOW nutrient status. However, macrophytes reached a higher final biomass in LOW nutrient tanks than in HIGH tanks (Figure 16E, Table 8, nutrient enrichment P<0.0001).

Whole-system properties

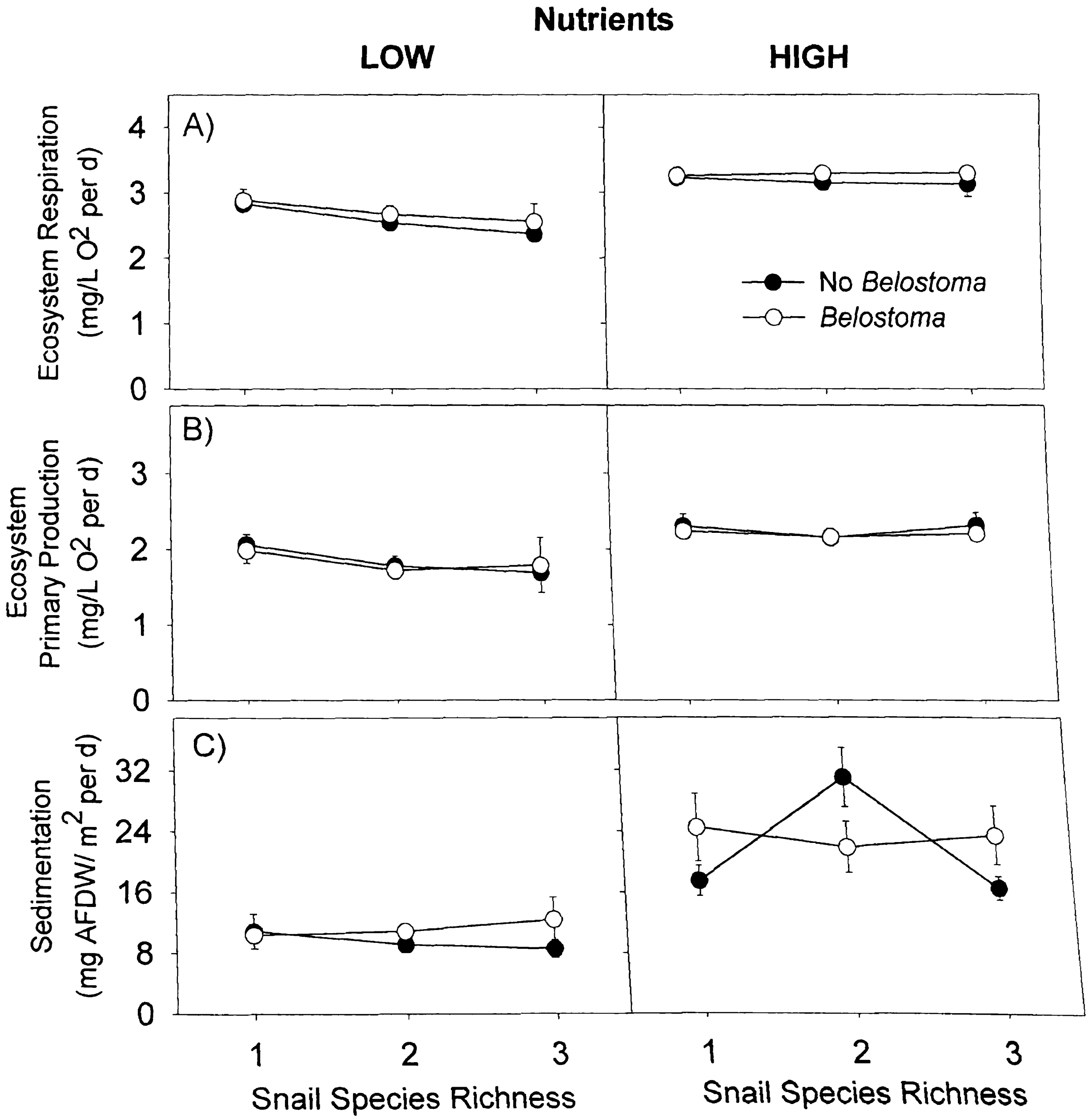
Primary producer functional groups responded in a variety of ways to the treatments, but we can also ask what effects the treatments had on whole system production and respiration. Nutrient enrichment led to higher rates of whole-ecosystem respiration (Figure 17A, Table 9, nutrient enrichment P<0.0001) and primary production (Figure 17B, Table 9, nutrient enrichment P<0.0001). Nutrient concentrations decreased greatly during the course of the experiment in all tanks (average soluble reactive phosphorus <3μg/L on 30 Jul), and likewise rates of production decreased through time (analysis not shown). Overall respiration and primary productivity were highly correlated (P<0.0001, r^2=0.71), and were both probably dominated by the metabolism of algae. The composition of snails influenced respiration rates (Figure 15F, Table 9, species composition P=0.033); *Fossaria* monocultures had greater respiration rates than did *Helisoma* monocultures, most likely because *Fossaria* treatments had greater epiphyton, periphyton, and metaphyton biomass (Figure 15C-E). Accumulation of organic sediments (the result of snail consumption and defecation) was more than two times greater in HIGH nutrient treatments

Table 9. ANOVA results for ecosystem respiration, primary production, and sedimentation. Significant ($P<0.05$) treatment factors and interactions are bolded. Error degrees of freedom are parenthetically noted if different than the first column. “n/a” means the term was not included in the model.

		Ecosystem Respiration			*Primary Production*			*Sedimentation*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species Richness	2	0.30	1.96	0.1464	0.47	2.27	0.1080	0.25	1.31	0.2742
Nutrient Enrichment	1	8.49	54.82	**<0.0001**	4.17	20.21	**<0.0001**	16.33	85.08	**<0.0001**
Predation	1	0.23	1.47	0.2277	0.03	0.16	0.6919	0.71	3.69	0.0575
SR*N	2	0.26	1.69	0.1888	0.17	0.81	0.4497	0.32	1.66	0.1943
SR*P	2	0.06	0.39	0.6809	0.01	0.05	0.9468	0.33	1.70	0.1874
N*P	1	0.00	0.03	0.8635	0.01	0.07	0.7947	0.10	0.52	0.4719
SR*N*P	2	0.00	0.02	0.9816	0.04	0.19	0.8243	0.54	2.82	0.0642
Species Composition (SR)	4	0.42	2.73	**0.0331**	0.20	0.98	0.4210	0.34	1.77	0.1415
Anax	1	0.78	5.01	**0.0274**	n/a	-	-	1.11	5.80	**0.0178**
Error	103	0.15	-	-	0.21	(104)	-	0.19	-	-

Figure 17. The response of A) ecosystem respiration, B) ecosystem primary production, and C) sedimentation to species richness, nutrient, and predation manipulations. The left column of figures is from "low" nutrient tanks and the right column is from "high" nutrient tanks. Solid symbols represent the absence of *Belostoma* predators, open circles represent the presence of *Belostoma*. Means ± 1 SE are reported. Refer to ANOVA tables (Table 9) for statistics.

Figure 17


Nutrients
LOW
HIGH
A)
Ecosystem Respiration (mg/L O2 per d)
4
3
2
1
0
No *Belostoma*
Belostoma
B)
Ecosystem Primary Production (mg/L O2 per d)
3
2
1
0
C)
Sedimentation (mg AFDW/ m2 per d)
32
24
16
8
0
1
2
3
Snail Species Richness
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Snail Species Richness

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(Figure 17C, Table 9, nutrient enrichment $P<0.0001$) than in LOW. The presence of *Anax* reduced the rate of sedimentation (Table 9, *Anax* $P=0.018$).

Mechanisms

I tested for species richness effects that could not be explained by sampling effects using the D statistics suggested by Wardle (1999) and Loreau (1998) (see *Methods* for definitions), and found convincing support for the presence of a biologically based diversity mechanism. D_i was nearly always positive, for all species in all contexts, for both snail production and live standing biomass (Table 10), which suggests that for these snails interspecific interactions are either positive (e.g., facilitation) or less negative (e.g., niche differentiation) than intraspecific interactions. D_i cannot be calculated for the remaining response variables because the aggregate community response cannot be partitioned into species-specific responses (Petchey 2003). The only D statistic that could be calculated for these response variables was D_{max} (equivalent to overyielding - Hector 1998), which again represents the proportional increase in performance in polyculture relative to the "best" monoculture (which was usually *Helisoma*). In this experiment D_{max} was almost always positive (Table 10), indicating significant overyielding. There was significant overyielding for every response variable when averaged across all contexts, but most response variables saw at least one context without overyielding.

Table 10.

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Table 10. D_i and D_{max} statistics calculated for A) snail production, B) standing snail biomass, C) other response variables sensitive to snail species richness (only D_{max} could be calculated for these variables because responses could not be decomposed into species-specific effects). The "dominant" species (i.e. highest value in monoculture) are noted for each response in each context. Species names are abbreviated with the initials of the genera and species names. *'s indicate a response variable where the effect of snails is expected to be negative, so a positive D_{max} means the polyculture reduced that response more than the most dominant species in monoculture did.

		D_{Fo}	D_{Pg}	D_{Ht}	D_{max}	"dominant"
A) Snail Production						
LOW	**No predator**	0.180	0.343	0.449	0.060	Ht
LOW	**Predator present**	0.436	-0.071	1.033	0.339	Pg
HIGH	**No predator**	0.376	0.764	0.477	0.204	Ht
HIGH	**Predator present**	-0.062	0.318	0.660	0.018	Ht
-	-	0.212	0.320	0.627	0.167	Ht
B) Snail Standing Biomass						
LOW	**No predator**	0.715	1.190	0.953	0.236	Ht
LOW	**Predator present**	1.103	-0.130	2.117	0.653	Pg
HIGH	**No predator**	0.131	2.635	0.482	0.098	Ht
HIGH	**Predator present**	0.276	1.954	0.591	-0.005	Ht
-	-	0.440	1.079	0.803	0.241	Ht

C) D_{max} *for other response variables*

		Epiphyton*		**Periphyton***		**Macrophyte Stems**	
LOW	**No predator**	0.050	Ht	0.320	Pg	*0.061*	Pg
LOW	**Predator present**	0.190	Ht	-0.890	Fo	*-0.440*	Fo
HIGH	**No predator**	-0.158	Ht	0.263	Pg	0.079	Ht
HIGH	**Predator present**	0.067	Ht	0.097	Ht	0.500	Pg
-	-	0.062	Ht	0.240	Ht	0.292	Ht/Fo

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Variation in D_{max} across the contexts could have two sources: variation in the strength of diversity effects, and variation in the relative contribution of sampling effects to the overall diversity effect. For instance, the D_{max} for periphyton in low nutrients/no predator treatments was 0.32, while it was -0.89 in low nutrients/predator treatments (Table 10C), because the overall diversity effect was negative in the first case and positive in the second (e.g., variation in the overall effect of diversity – Figure 16A). In contrast, the D_{max} for periphyton in high nutrient/no predator treatments was 0.263, while it was 0.097 for high nutrient/predator present treatments (Table 10C). In both of these cases the effect of diversity on periphyton was negative and fairly strong (Figure 16A), but in the presence of predators sampling effects were stronger (i.e. the dominant monoculture was closer to the three-species mean in the presence of predators than in the absence, data not shown).

Comparison of species richness effects in natural and artificial communities

Although sampling effects alone cannot explain the effects of diversity in this experiment, it is possible that some combinations of species present in the experiment never occur in nature, and therefore the conclusions reached here would be less directly applicable to natural systems. A survey of 16 ponds in SW Michigan (Appendix C) revealed that most compositions used in this experiment are in fact represented in nature (although *Gyraulus parvus*, a biomass subordinate, was often present as well). However, a comparison of the degree of nestedness (using NestCalc - Atmar and Patterson 1995) in the experimental and natural communities revealed important differences in the frequency

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frequency of occurrence of various snail community compositions. Nestedness measures the degree to which less diverse communities are nested subsets of more diverse communities, and to what degree the order of species' "extinction" is constant, and is expressed on a temperature scale (T – 0° equals complete nestedness, 100° equals complete anti-nesting). Examples of every possible order of "extinction" are present in the experimental communities – the result is significant "anti-nesting" (T=54.78°, P=0.029, where t = random temperature calculated using 500 Monte Carlo simulations). In contrast, the natural communities surveyed were significantly more nested than one would expect at random (T=27.34°, P=0.055), and had a regular order of "extinction". For instance, *Fossaria* was most often the first species "lost" as pond snail diversity went from three to two species, and *Physa* was never observed alone in a natural pond.

I explored the implications of these differences in the distribution of community types in the natural and experimental settings by examining the effects of species richness on various response variables after weighting the data by the frequency with which the particular snail composition was seen in the survey of ponds. For instance, if *Fossaria* and *Helisoma* were equally represented in natural ponds with only one snail species, the new mean for 'species richness = 1' would be calculated as: 0.5 * mean {*Fossaria* monocultures} + 0.5 * mean {*Helisoma* monocultures}. In order to do this analysis I had to ignore the frequent presence in the natural ponds of *Gyraulus parvus* (almost always <5% of snail community biomass), and the infrequent

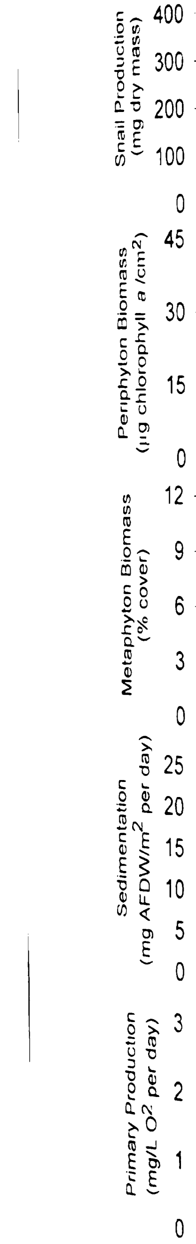
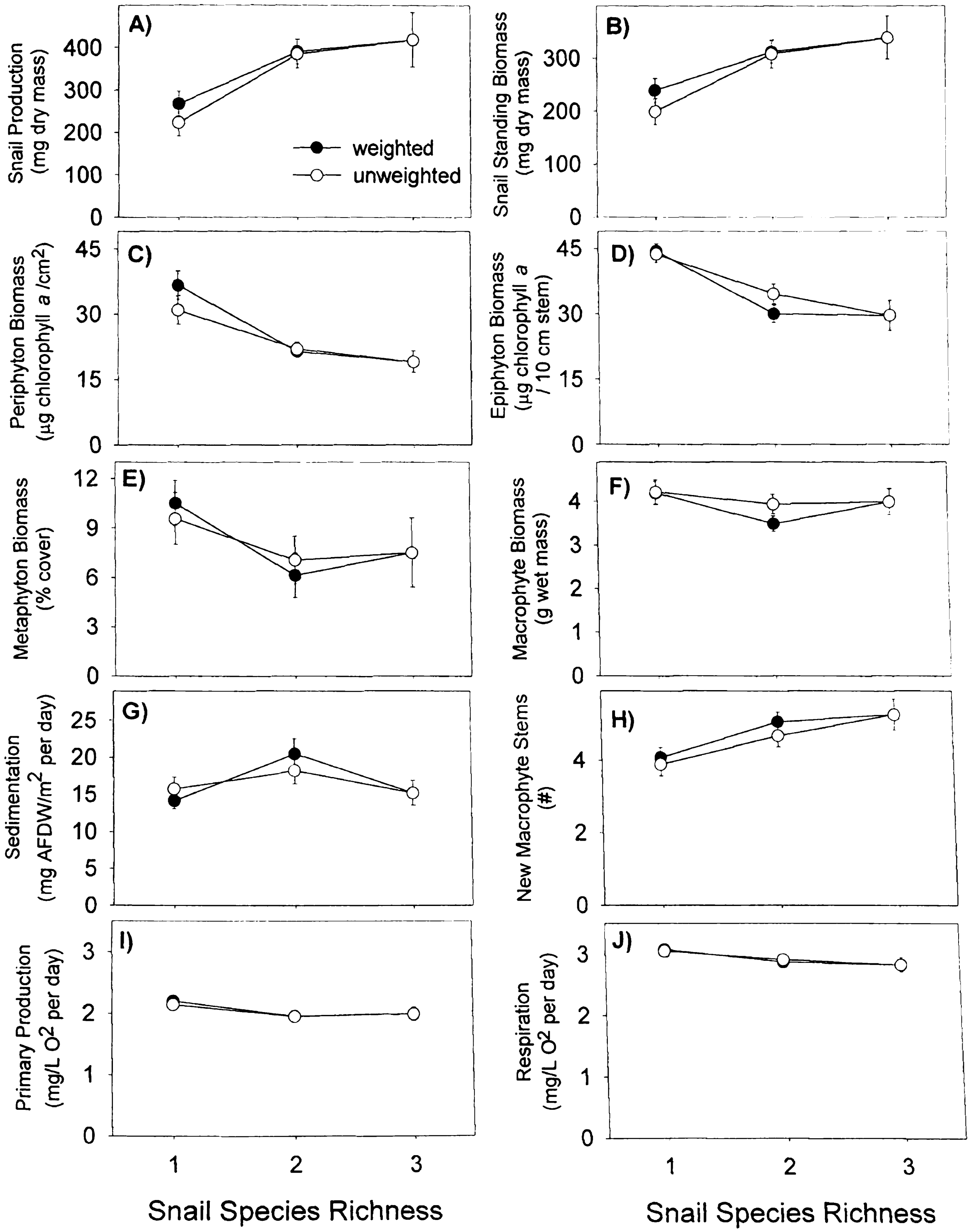


Figure 18. Effects of species richness in the original data and data weighted by the frequency of specific snail compositions in a survey of 16 natural ponds, for A) snail production, B) snail standing biomass, C) periphyton, D) epiphyton, E) metaphyton, F) new macrophyte stems, G) sedimentation, H) macrophyte biomass, I) ecosystem primary productivity, and J) ecosystem respiration. Open circles represent original, unweighted data. Dark circles represented weighted data. Data are means ± 1 SE.

Figure 18


A)
Snail Production (mg dry mass)
0
100
200
300
400
weighted
unweighted
B)
Snail Standing Biomass (mg dry mass)
0
100
200
300
C)
Periphyton Biomass (µg chlorophyll a /cm2)
0
15
30
45
D)
Epiphyton Biomass (µg chlorophyll a / 10 cm stem)
0
15
30
45
E)
Metaphyton Biomass (% cover)
0
3
6
9
12
F)
Macrophyte Biomass (g wet mass)
0
1
2
3
4
G)
Sedimentation (mg AFDW/m2 per day)
0
5
10
15
20
25
H)
New Macrophyte Stems (#)
0
2
4
I)
Primary Production (mg/L O2 per day)
0
1
2
3
J)
Respiration (mg/L O2 per day)
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Snail Species Richness
1
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a 37%

presence (2 ponds) of *Pseudosuccinea columella*, which were not used in the experiment.

In general, weighting the experimental data by the frequency of occurrence of various compositions in natural ponds did not change responses to species richness qualitatively (Figure 18). Species richness effects on snail biomass, snail production, and macrophyte stem emergence got slightly weaker, while richness effects were slightly stronger for periphyton and epiphyton biomass, ecosystem respiration, sedimentation, and macrophyte cover (Figure 18). Statistically, the effects of species richness in the weighted data are rarely significant, even where they had been with the unweighted data. However, because weighting the data to represent only the compositions seen in nature rarefies the dataset from 120 observations to 88, the power to detect effects and the precision of estimates decreases substantially.

Effect Sizes

Because methods for calculating main and interactive effect sizes in factorial experiments have not been established (except in the simplest case: Gurevitch et al. 2000), here I developed a simple statistic that describes the average magnitude of change in a response variable that results from manipulating a focal factor. For instance, in this experiment manipulating species richness from one species to three species resulted in a 47% increase in snail production in low nutrient-no predator tanks, a 53% increase in low nutrient-predator tanks, a 50% increase in high nutrient-no predator tanks, and a 37% increase in high nutrient-predator tanks, for an average of a 46.9%

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change across the other factors. In contrast, adding predators to the mesocosms had +6% to -37% effects on snail production in the various nutrient and species richness treatments, an average 19.6% change in mean snail production. From these calculations I would conclude that on average, changes in species richness resulted in bigger changes in snail production than did changes in predation.

Figure 18 reports the average percent change resulting from predation, nutrient, and species richness manipulations for each response variable. Snail species richness had the largest effects on snail production and standing biomass, epiphyton, periphyton, metaphyton, and macrophyte stem growth (Figure 19A-E). Nutrient enrichment had stronger effects on macrophyte biomass and on whole-system properties like primary production, respiration, and sedimentation (Figure 19F, G, I-J) than did either predation or consumer species richness. *Belostoma* predators had the strongest effects only on the emergence of macrophyte stems (Figure 19H). The exact details of these calculations were not critical; qualitatively similar results were observed when looking at the size of main effects (because of the infrequence of significant interactions), or by looking at absolute changes in responses rather than percent changes.

Figure 19. Average percent change in A) snail production, B) snail standing biomass, C) periphyton, D) epiphyton, E) metaphyton, F) new macrophyte stems, G) sedimentation, H) macrophyte biomass, I) ecosystem primary productivity, and J) ecosystem respiration, induced by snail species richness, nutrient, and predation manipulations. See text for details of calculations. All effect sizes are calculated with untransformed data. Error bars (1 SE) describe the variability in the strength of a focal factors' effects on the response variable, across the levels of the other factors. The number of observations for these means are the number of unique levels of the other factors; n=4 for species richness effects, n=6 for nutrient and predator effects. * Notice different scale of y-axis for periphyton biomass. Black bars represent nutrient effects, white bars represent effects of predators, and gray bars represent the effects of snail species richness.

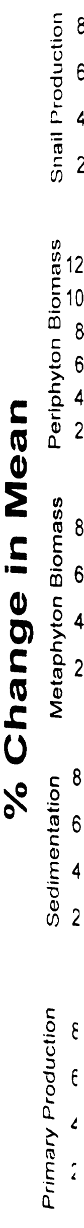
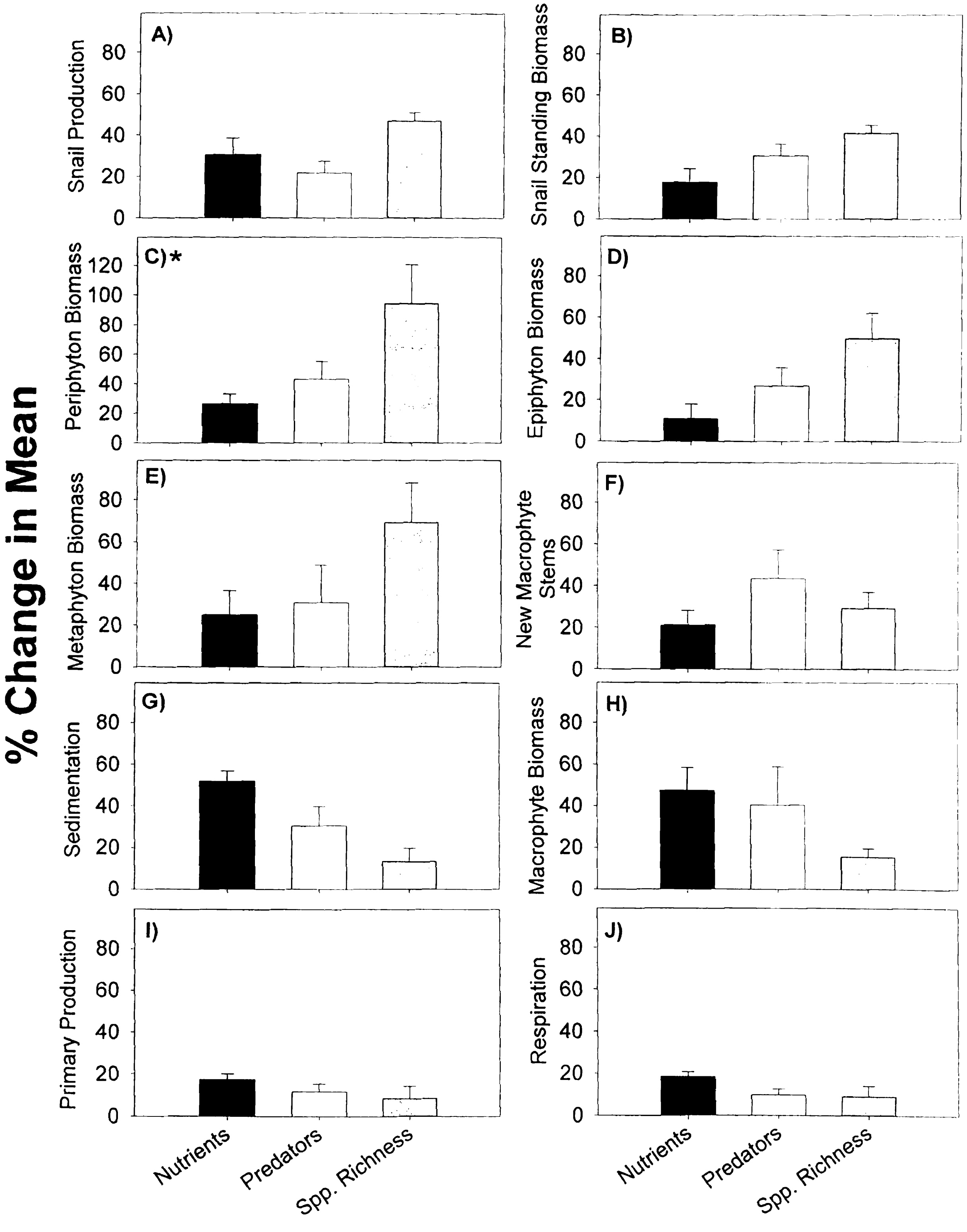


Figure 19


% Change in Mean
A)
Snail Production
0
20
40
60
80
B)
Snail Standing Biomass
0
20
40
60
80
C)*
Periphyton Biomass
0
20
40
60
80
100
120
D)
Epiphyton Biomass
0
20
40
60
80
E)
Metaphyton Biomass
0
20
40
60
80
F)
New Macrophyte Stems
0
20
40
60
80
G)
Sedimentation
0
20
40
60
80
H)
Macrophyte Biomass
0
20
40
60
80
I)
Primary Production
0
20
40
60
80
J)
Respiration
0
20
40
60
80
Nutrients
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Discussion:

The goals of this experiment were to determine how species richness effects would depend on ecological context, to identify possible mechanisms operating to generate diversity effects, and to compare the strength of species richness effects with other factors (e.g., predation and resource availability) that often shape community structure and ecosystem processes.

Context Dependency

Species richness had meaningful effects on secondary production and standing biomass of snails, periphyton and epiphyton biomass, and growth of new macrophyte stems. Logically any effect of snail species richness would have to originate as an effect on snail production, standing biomass, or behavior, and only then cascade to affect other food web or system properties. In this experiment greater secondary production in diverse mesocosms in all contexts meant that the mechanism of species richness effects (e.g., sampling effect, niche complementarity, and/or facilitation) was not sensitive to food web structure or system productivity.

While there were more snails in species rich mesocosms, the ability of these snails to affect other functional groups was contingent on the biotic and abiotic conditions of the system. For instance, periphyton biomass decreased with increasing snail richness in all contexts except when *Belostoma* was present in low nutrient tanks (Figure 16A). This was the context with the lowest overall standing biomass of snails (Figure 14B), suggesting that snails need to

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be abundant in order for snail richness effects to manifest in other functional groups.

Herbivore guilds often exhibit foraging trade-offs where some species move quickly from patch to patch grazing lightly (e.g., grazers) while other species are more sedentary, exhausting resources before moving on (e.g., diggers) (Schmitt 1996, Chase et al. 2001). Snails often respond to predation by reducing their activity and by seeking refuge (Bernot and Turner 2001, Turner 1996, 1997) and indeed in previous experiments I have observed that *Physa gyrina* moves less, feeds less, and spends more time near the water's surface in the presence of *Belostoma* (Chapter Three). Thus under the threat of predation species' foraging strategies may converge on a slow-moving, intense grazing, "digger" mode (e.g., *Helisoma*'s default strategy) to maximize energy intake while minimizing encounters with predators, and this would result in a reduction of functional diversity across the guild of snails. The effects of increasing snail species richness on epiphyton biomass were not apparent until high levels of richness (e.g., three species), in the presence of *Belostoma* (Figure 16B). In contrast, in the absence of predators snail species richness had effects on epiphyton biomass at lower richness levels (e.g., two species - Figure 16B). These results are consistent with a reduction of functional diversity among the snails because of anti-predator behavioral responses. However, snail biomass would be expected to be less sensitive to species richness if predators induced a reduction in functional diversity among the snails. This was not the case here (i.e. species richness effects on snail biomass were equally large in the presence and absence of predators- Figure 14).

Epiphyton physically covers its macrophyte substrate, and if abundant can shade the plant causing reductions in growth (Brønmark 1985). Macrophyte stem growth was higher in diverse tanks, where snail biomass was higher and epiphyton was lower (Figures 14 and 16). This was most likely the result of direct consumption of epiphyton by snails (Underwood et al 1992, Brønmark 1985), but through consumption and defecation snails move organic material from other substrates to the bottom of the tank, which could have increased the availability of nutrients to the plants. The predator *Belostoma* mediated the strength of the snail-epiphyton-macrophyte interactions; when predators were present epiphyton was more abundant, and macrophytes performed more poorly (Figure 16B, D). The context dependency of the effects of snail richness on epiphyton was not statistically evident in measurements of macrophytes, however.

Mechanisms

Any study of the effects of species richness should identify, to the degree possible, the mechanism responsible for those effects. The sampling effect (Tilman et al. 1997, Wardle 1999, Huston 1997) can produce richness effects merely by the inclusion of dominant (i.e. disproportionately influential) species in a greater fraction of species rich units than in species poor units. Facilitation can allow species to utilize resources or avoid predators that they could not in isolation, and thus polycultures can have higher process rates (e.g., grazing, sedimentation) than monocultures. Differential resource use, where species using unique resources or the same resource in unique ways (e.g., phenological differences), can similarly lead to positive effects of diversity on process rates.

D statistics (Table 10) indicate a biologically based mechanism is largely responsible for effects of diversity on system properties in this experiment. Differential resource use is possible, but I did not quantify specialization on different resource types here. Appendix B demonstrates the existence of differences in foraging mode between species of aquatic snails. Also, Chase et al. (2001) describe how pond snails can partition patchy periphyton resources and suggest that a trade-off in foraging traits (e.g., digger vs. grazer strategies) can act as a mechanism of coexistence, reducing competition intensity between species. Thus, niche complementarity could explain the effects of diversity seen with these pond snails either through differential use of the same resource or through use of different resources. In a previous experiment, I observed significant differences in the use of habitat among a larger set of snails (Chapter Two). Moreover, the relative magnitude of diversity effects was predictable using an index of niche overlap between species, strongly suggesting the operation of a niche complementarity mechanism. It may also be true that species with one grazing strategy may facilitate growth of the resources of a second species with another strategy (e.g., low-lying or tightly adhered algae may increase in abundance after an inefficient grazer removes the "overstory" – Underwood et al. 1992, Lowe and Hunter 1988).

D_{max}, which describes the degree of overyielding in mixtures, varied across the four ecological contexts studied here because of variation in both the strength of diversity effects and of sampling effects. Thus, it appears that even with a constant pool of species the degree to which sampling effects influence the results of a diversity experiment will depend on the conditions in that experiment (as suggested in Fridley 2001). This result is not surprising; sampling effects depend on the dominance of individual taxa, and the relative dominance of species depends on the biotic and abiotic conditions in a community.

There has been debate about whether sampling effects represent a mechanism that could operate in natural systems or are simply experimental artifacts (Huston 1997, Tilman et al. 1997a). In general, if species presence across a set of communities is related to the functional attributes of the species (e.g., the functional dominant species is present in all systems and species with little functional effect are found only in diverse systems), experiments that create random communities could misgauge the true functional effect of species richness in nature. It may be true that the relationship between presence in a community and the functional attributes of species is strong in communities primarily structured by competition (where community membership is determined by traits related to resource acquisition), and weaker in communities structured by disturbance or immigration processes (where community membership is determined by dispersal traits and resistance to harsh conditions). The applicability of species richness-ecosystem function studies to natural systems will be more apparent as this relationship is measured for a variety of taxa, and as the response of ecosystems to species richness is evaluated for sets of random and non-random communities as is done here and elsewhere (Petchey et al. 1999, Jonsson et al. 2002, Smith and Knapp 2003).

Effect Sizes

By manipulating species richness, predation, and resource availability in the same experiment, comparison of the magnitude of these different effects is possible. However, the absolute magnitude of the different effects depends on the strength of the treatments imposed, which here were reasonably but arbitrarily chosen. Both nutrient and predator manipulations were strong; there was a four-fold difference in starting nutrient concentrations, and predators could potentially consume up to six snails per day (9.4 - 37.5% of initial snail abundance per day, depending on snail species composition). Despite the predator and nutrient manipulations being quite strong, species richness effects on the biomass of various functional groups were often as large or larger than those of predators and nutrients. It appears, then, that the effects of consumer species richness can rival the strength of factors that historically have been considered the strongest regulators of aquatic community structure: top-down and bottom-up forces.

In contrast, other studies have reported that diversity effects are weaker than those of nutrient enrichment (Bärlocher and Corkum 2003, Fridley 2002). However, in Bärlocher and Corkum's study of stream fungi where nutrient effects on leaf mass loss were three times as large as the effects of diversity, nitrogen and phosphorous were increased 100-fold, while the species richness manipulation was only a five-fold increase (1 to 5 species). If effect sizes are scaled to the magnitude of the manipulations (percent change in response over the percent change in the experimental factor) scaled diversity effects would be nearly seven times as strong as scaled nutrient effects. Similarly, Fridley (2002) used a very strong nutrient manipulation (added 90 g N, 30 g P, 60 g K per m^2 to an old-field plant community), but because ambient nutrient conditions were not described I am unable to rescale the effect sizes by the strength of the manipulations as above. However, Fridley's nutrient addition was nearly an order of magnitude higher than the recommended yearly application rates for cornfields in North Carolina (5X, 15X, and 7X times the rates for nitrogen, phosphorus, and potassium, respectively – Hardy et al. 2003), where that study was performed. This suggests the dominating effects of nutrients in Fridley's experiment were probably due to an exceedingly large nutrient manipulation, compared with a more modest species richness manipulation. Studies that hope to compare the strengths of various factors need to incorporate, if possible, the strength of the manipulations into calculations of effect size (as when calculating sensitivity analyses). This may be impossible, however, if an experimental manipulation is of a qualitative nature (such as predator presence/absence in this study) and cannot be compared directly to the manipulation of another factor. In this case, careful consideration of the system (i.e. what variation is seen in the manipulated factors across natural systems) and the manipulations should guide interpretation. Alternatively, experiments could be designed to examine ecosystem functioning across gradients of important ecological factors (e.g., multiple treatments of different magnitudes).

Predator control of prey trophic level biomass is predicted to be weak when some prey species are at least partially invulnerable (Leibold 1989). When abundant, snails were able to reduce algal abundance, but macrophytes were inedible or not preferred. Macrophytes had increased stem emergence rates (but not increased biomass) in treatments where snails reduced algal biomass strongly (e.g., at higher snail richness). New stems may have greater production: biomass ratios, so increases in macrophyte stem growth could represent increased primary productivity. Compensatory interactions between algae and macrophytes may explain why despite strong "local" food web effects of consumer species richness (i.e. on consumer biomass and the biomass of the functional groups those consumers interact with most directly), ecosystem functioning (e.g., primary production, respiration, sedimentation) did not respond strongly to consumer richness. The generality of this result is unknown because so few studies have manipulated species richness in complex enough systems (multiple functional groups within a trophic level) to allow for such a compensatory response. Appreciation for the interconnectedness between sub-compartments of food webs and ecosystems is growing (Persson 1999, Persson et al. 2001, Vadeboncoeur et al. 2002, Schindler and Scheurell 2002), but has been largely ignored in diversity-ecosystem function studies. Such interconnectedness may lend stability to ecosystem processes.

Conclusions

The number of studies explicitly considering how the richness of consumer species can affect ecosystem properties is small, but growing (see Duffy et al. 2001, Downing and Leibold 2002, Cardinale et al. 2000, 2002, Jonsson and Malmqvist 2000) and is comprised solely of studies in aquatic systems. Interestingly, most studies of the effects of primary producer species richness on ecosystems have been conducted in terrestrial systems. Individual consumer and predator species can have remarkably strong effects on aquatic ecosystems (Mittelbach et al. 1995), and it appears that in some cases the number of species of aquatic consumers can also have dramatic effects on food webs and ecosystems (this study, Cardinale et al. 2000, 2002, Jonsson and Malmqvist 2000, Downing and Leibold 2002). This study begins to put the strength and generality of species richness effects on ecosystem function into perspective, relative to well-studied factors like predation and resource availability. Future diversity-ecosystem function studies should attempt to include as much of the natural food web as possible, to allow for complex and compensatory responses of different functional groups and increase the applicability of the results to natural systems. Moreover, applicability could be facilitated by more explicitly considering the differences in the distribution of species compositions across sets of experimental and natural communities.

APPENDICES

APPENDIX A

ESTIMATES OF THE REPRODUCTIVE RATES OF SIX AQUATIC SNAIL SPECIES UNDER SEMI-NATURAL FIELD CONDITIONS.

Introduction:

The reproductive rate of an animal is a fundamental characteristic of its life-history that can influence the types of habitats and communities it can inhabit. Moreover, reproductive rates often are related to other important niche dimensions such as minimum resource requirements and competitive ability. Thus, understanding the potential reproductive rates of a guild of species may lend insight into mechanisms of coexistence, patterns of distribution and abundance of species, and the functional complementarity among species. Here I used a semi-natural field experiment to estimate the reproductive rates of six common aquatic snail species.

Methods:

Six snail species common to lakes and ponds in southwest Michigan were used in these experiment: *Amnicola limosa*, *Bithynia tentaculata*, *Physa gyrina*, *Promenetus exacuous*, *Pseudosuccinea columella*, and *Valvata tricarinata*, referred to hereafter by their generic names. Animals were collected from Gull Lake, Kalamazoo County, MI.

Sixty glass jars (0.47 L) were suspended on floats just under the water surface in Pond 1 of the W. K. Kellogg Biological Station's Experimental Pond Facility on 30 May 2000, ten for each species listed above. Six snails of a given species were added to each jar, as was 10 g of the macroalga *Chara* spp. that had been collected from Three Lakes, Kalamazoo County, MI and cleaned free of macroinvertebrates and their eggs. Each jar had a window screen lid that allowed water to flow through, but prevented the movement of macroinvertebrate species in or out of the jars. On days 10, 26, and 43 of the experiment all jars were collected from the pond, snails measured and recorded as alive or dead, and the number of eggs was counted. After sampling on days 10 and 26 fresh *Chara* was added to the jars and they were redeployed (with the same snails). Data were analyzed with ANOVA.

Results and Discussion:

Snail species differed strongly in initial length and weight (Table 11, Figure 20A), reflecting differences in the natural size distributions of the source populations. Larger snails can generally produce more eggs (Osenberg 1988), as is the case for most invertebrate animals. Interestingly, while reproduction is generally thought to be energetically expensive for invertebrates, snails in this experiment were able to grow while reproducing (Table 11, Figure 20B). Snail species differed in their total production of eggs during the 43 d observation period (Table 11, Figure 21).

Bithynia were large (Figure 20A), grew well during the experiment (Figure 20B) and produced the most eggs (Figure 21). *Bithynia* are typically found in deeper lakes, not near the surface of small ponds as was the case in this study, and thus their high rates of growth and reproduction is surprising. *Pseudosuccinea* did not do well, in contrast. Most *Pseudosuccinea* died before the end of the experiment (8/10 experimental units went "extinct"), and those remaining produced very few eggs considering their size. *Pseudosuccinea* has an amphibious habit, and may not have performed well because they were held completely under the surface of the water in the jars. *Amnicola* produced more eggs than other snail species of a similar size. These results suggest that there are strong differences in the rate at which snail species can produce eggs.

Table 11. ANOVA results for differences among species in length, weight, growth, and egg production.

Response	**df**	**F**	**P**
Snail Length	5, 54	21.55	<0.0001
Snail Mass	5, 54	8.35	<0.0001
Mass Gain	5, 45	7.02	<0.0001
Total Eggs Produced	5, 46	3.08	0.018

Figure 20. A) Average body length and mass of six snail species used in reproductive rate observations. Average length in mm = black bars, average weight in mg = gray bars. Data are mean + 1 SE. B) Average mass gain per day for six snails species during the 43 days. Data are means + 1 SE.

Figure 20

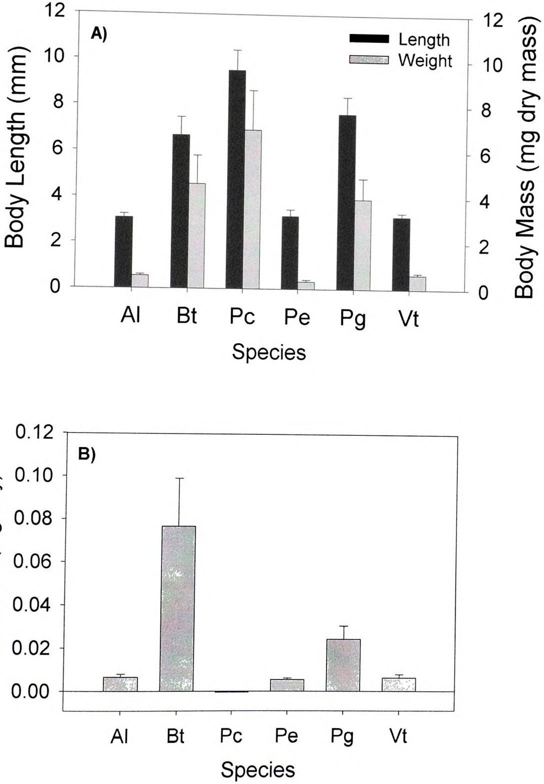
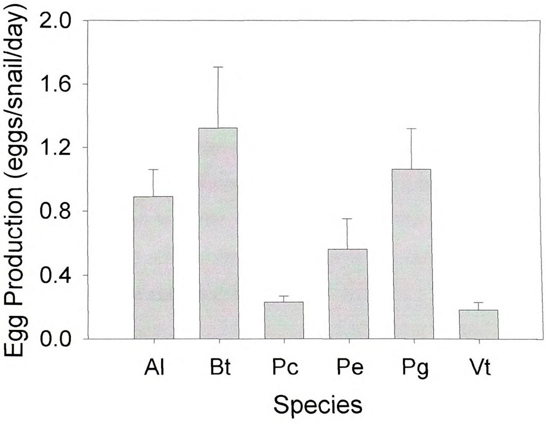

A)
Body Length (mm)
0
2
4
6
8
10
12
Body Mass (mg dry mass)
0
2
4
6
8
10
12
Length
Weight
Al
Bt
Pc
Pe
Pg
Vt
Species
B)
Mass Gain (mg/day)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
Al
Bt
Pc
Pe
Pg
Vt
Species

Figure 21. Egg production per snail per day over a 43 d period for six aquatic snail species. Data are means + 1 SE. Species are coded by the first letters of the genus and species names: *Amnicola limosa* = Al, *Bithynia tentaculata* = Bt, *Physa gyrina* =Pg, *Promenetus exacuous* = Pe, *Pseudosuccinea columella* = Pc, and *Valvata tricarinata* = Vt.



APPENDIX B

MOVEMENT AND FORAGING BEHAVIOR OF SIX AQUATIC SNAIL SPECIES.

Introduction:

Many factors can influence the foraging success of an animal. The speed at which an individual moves through a habitat, for instance, can influence the total amount of resource available to the forager (Werner and Anholt 1993). Moreover, movement speed should influence the frequency of finding new resource patches in a spatially heterogeneous habitat. The ability of species to fine-tune their movement patterns (e.g., speed, turning frequency) to optimize foraging success given a set of environmental conditions could be an important component of foraging behavior. I quantified the movement speed of six species of aquatic snails in high and low resource environments in laboratory experiments to identify potential differences in foraging mode or ability (Schmitt 1996). Finally, I measured the speed with which snails found resource patches in a spatially heterogeneous environment.

Methods:

Six snail species common to lakes and ponds in southwest Michigan were used in these experiment: *Amnicola limosa*, *Bithynia tentaculata*, *Promenetus exacuous*, *Helisoma trivolvis, Physa gyrina*, and *Valvata tricarinata*, referred to hereafter by their generic names. Animals were collected from Gull Lake, Kalamazoo County, MI.

Speed Trials

Each speed trial took place in a 38 L aquarium, filled with 10L of room-temperature (21° C) reservoir water (KBS Experimental Pond Laboratory reservoir), under artificial light from fluorescent lamps. "Low" resource aquaria had no visible periphyton present, while "high" resource aquaria had moderate amounts of periphyton growing on the walls and bottom of the tanks. Unfortunately, the quantity of periphyton was not measured. One snail was placed into the center of each aquaria. Snail location was recorded every minute for 20 consecutive minutes on 1 cm^2 grids placed under the aquaria. Rates of movement and the probability of turning during a 2 minute interval (turning was defined as changing direction more than 45 degrees) were calculated from the position data. Twenty separate trials were run for each species in low resource environments, and fourteen trials per species in high resource environments between 9-17 Oct, 2000. Results were analyzed with simple linear regression and ANOVA.

Resource matrix trials

Four snails of one species were placed into the center of each aquarium (19 L). The bottoms of the aquaria were covered with a matrix of 22 clean ceramic tiles and 3 "high" resource tiles (incubated in a high-nutrient, high-light environment with a diverse assemblage of algal species for 10 d). The physical position of the "high" resource tiles was determined at random for a given set of trials, but was the same for every trial performed at the same time. The proportion of snails on resource tiles was recorded at planned but irregular intervals (every two minutes for the first 20 minutes, then at 30, 60, 240, and 1440 minutes). Five trials were performed for each species (one trial each on 18 Oct, 29 Oct, 14 Nov, and two trials each on 30 Nov 2000). Tanks were illuminated with fluorescent lights and were held at room temperature (21° C). Differences in initial body size between species were analyzed with ANOVA.

Results and Discussion:

Speed Trials

Snail species differed greatly in initial size in the speed trials (Figure 22A), and large snails moved greater distances on average than did small snails (Figure 22B). As expected, species differed in their average movement rates. However, different species responded to changes in resource levels in different ways (i.e. species * resource interaction, Figure 23A, Table 12); every species increased their rate of movement in low resource environments relative to high resource ones, except for *Physa*. *Physa* moved much faster in high resource environments, and was the fastest snail in general. *Physa*'s high movement rate coincides with the results of Chase et al. (2001), who describe *Physa* as an area-extensive forager (*sensu* Schmitt 1996). *Helisoma* moved much slower than expected given its large size, which again coincides with Chase et al. (2001) where *Helisoma* was characterized as an area-intensive, or digger, species. The prosobranch species (*Amnicola*, *Bithynia*, and *Valvata*), which respire through gills, typically have heavier shells, and inhabit deeper lakes than the pulmonates (*Physa*, *Helisoma*, and *Promenetus*), were all relatively slow. Species did not differ in the probability that they would turn during a given time interval, but all snails turned more in low resource environments than in high, on average (Table 12). However, the magnitude of the changes in turning probability may not be biologically meaningful (Figure 23B).

Table 12. ANOVA results for species and resource main effects and their interaction on snail movement speed and the probability of turning. Bold type indicates P<0.05.

		Speed			*Probability of Turning*		
Source	**df**	**MS**	**F**	**P**	**MS**	**F**	**P**
Species	5	21.587	43.628	**<0.0001**	0.025	0.599	0.7008
Resources	1	0.001	0.001	0.9744	0.190	4.623	**0.0330**
Species*Resources	5	3.051	6.166	**<0.0001**	0.068	1.658	0.1473
Error	168	0.495			0.041		

Figure 22. A) The average body size (shell length in mm) of each species used in the speed trials. Species are coded by the first letter of the species and genus names (*Amnicola limosa* =Al, *Bithynia tentaculata* = Bt, *Promenetus exacuous* = Pe, *Helisoma trivolvis* = Ht, *Physa gyrina* = Pg, and *Valvata tricarinata* = Vt). Data are means + 1 SE. B) The relationship between body size and movement speed. Line represents simple linear regression, points are coded by species (as above). ANOVA results for a test of differences in size among the species are reported in the figure.

Figure 22

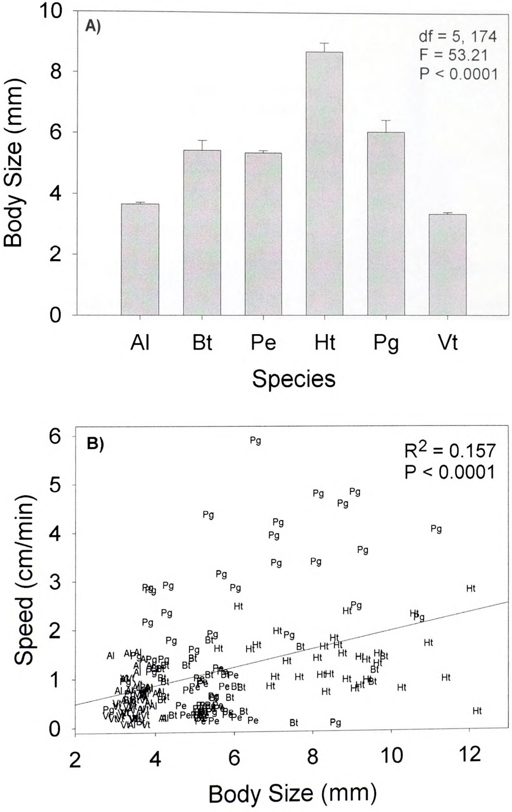
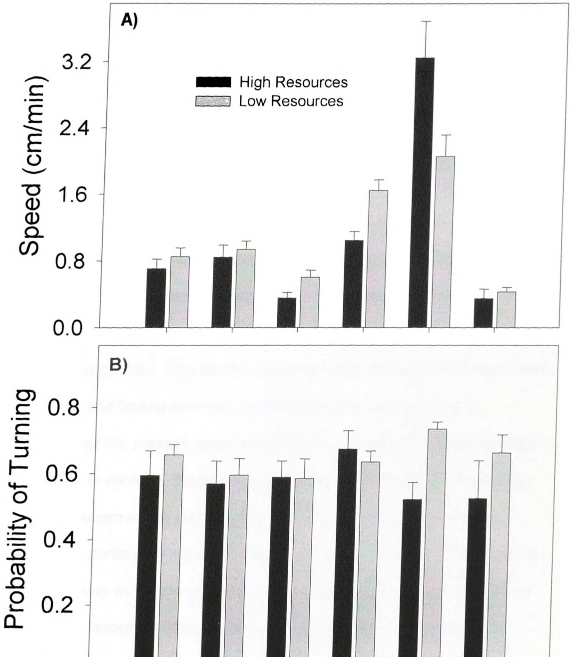

A)
Body Size (mm)
0
2
4
6
8
10
Al
Bt
Pe
Ht
Pg
Vt
Species
df = 5, 174
F = 53.21
P < 0.0001
B)
Speed (cm/min)
0
1
2
3
4
5
6
Body Size (mm)
2
4
6
8
10
12
R^2 = 0.157
P < 0.0001
Al
Bt
Pe
Ht
Pg
Vt

Figure 23. A) Average speed (cm/min) of six snail species in low and high resource environments. Data from high resource environments are represented in black, data from low resource environments are represented in gray. Species are coded by the first letter of the species and genus names (*Amnicola limosa* =Al, *Bithynia tentaculata* = Bt, *Promenetus exacuous* = Pe, *Helisoma trivolvis* = Ht, *Physa gyrina* = Pg, and *Valvata tricarinata* = Vt). Data are means + 1 SE. B) Average probability of turning during a 2 minute interval for six snail species in low and high resource environments. Resource environments are species are coded as above. Data are means + 1 SE.

Figure 23


A)
Speed (cm/min)
3.2
2.4
1.6
0.8
0.0
High Resources
Low Resources
B)
Probability of Turning
0.8
0.6
0.4
0.2
0.0
Al
Bt
Pe
Ht
Pg
Vt
Species

Resource matrix trials

Snail species differed strongly in body size again in the resource matrix trials (Figure 24). As demonstrated in the speed trials, larger snails move more quickly in general. Thus, we might expect larger snails to find resource patches more quickly if snail foraging consists of random movement across a habitat. *Physa* were found to be particularly fast movers, and considering their larger mean size in the resource matrix trials than the speed trials, *Physa* should have traveled much faster than the other species in these trials.

Physa found resource patches very quickly. After four minutes nearly 40% of *Physa*, on average, were on resource patches (Figure 25). Interestingly, the proportion of *Physa* on resource patches did not continue to increase through time. Examination of the raw data indicated that *Physa* were both leaving and finding resource patches during the remainder of the experiment, as the average proportion of *Physa* on resources varied between ~ 0.2 and 0.4. In contrast, the proportion of all other snail species on resource patches increased rather steadily through time (Figure 25). *Helisoma* and *Valvata* were particularly slow to find resource patches. Interestingly, *Valvata* and *Promenetus* were slow to find resource patches, but reached the highest proportion on resources of any species. It appears, then, that these snail species may use different foraging strategies: some move quickly and give up on patches quickly (e.g., *Physa*), while other species are slow to find patches and even slower to leave them (e.g., *Valvata* and *Promenetus*). Differences in foraging behavior such as these suggest that diverse snail communities may be

Figure 24. Average body size of snails used in the resource matrix trials. Species are coded by the first letter of the species and genus names (*Amnicola limosa* =Al, *Bithynia tentaculata* = Bt, *Promenetus exacuous* = Pe, *Helisoma trivolvis* = Ht, *Physa gyrina* = Pg, and *Valvata tricarinata* = Vt). Data are means + 1 SE. ANOVA results for a test of differences in size among the species are reported in the figure.

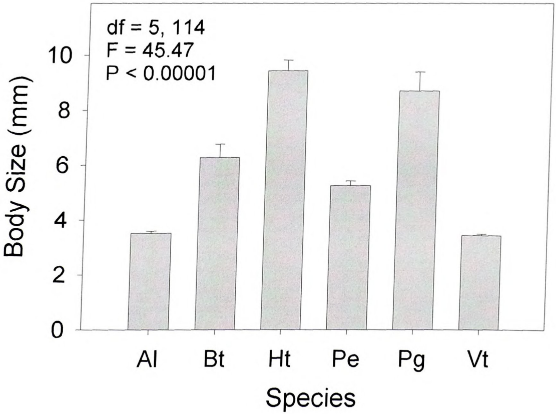
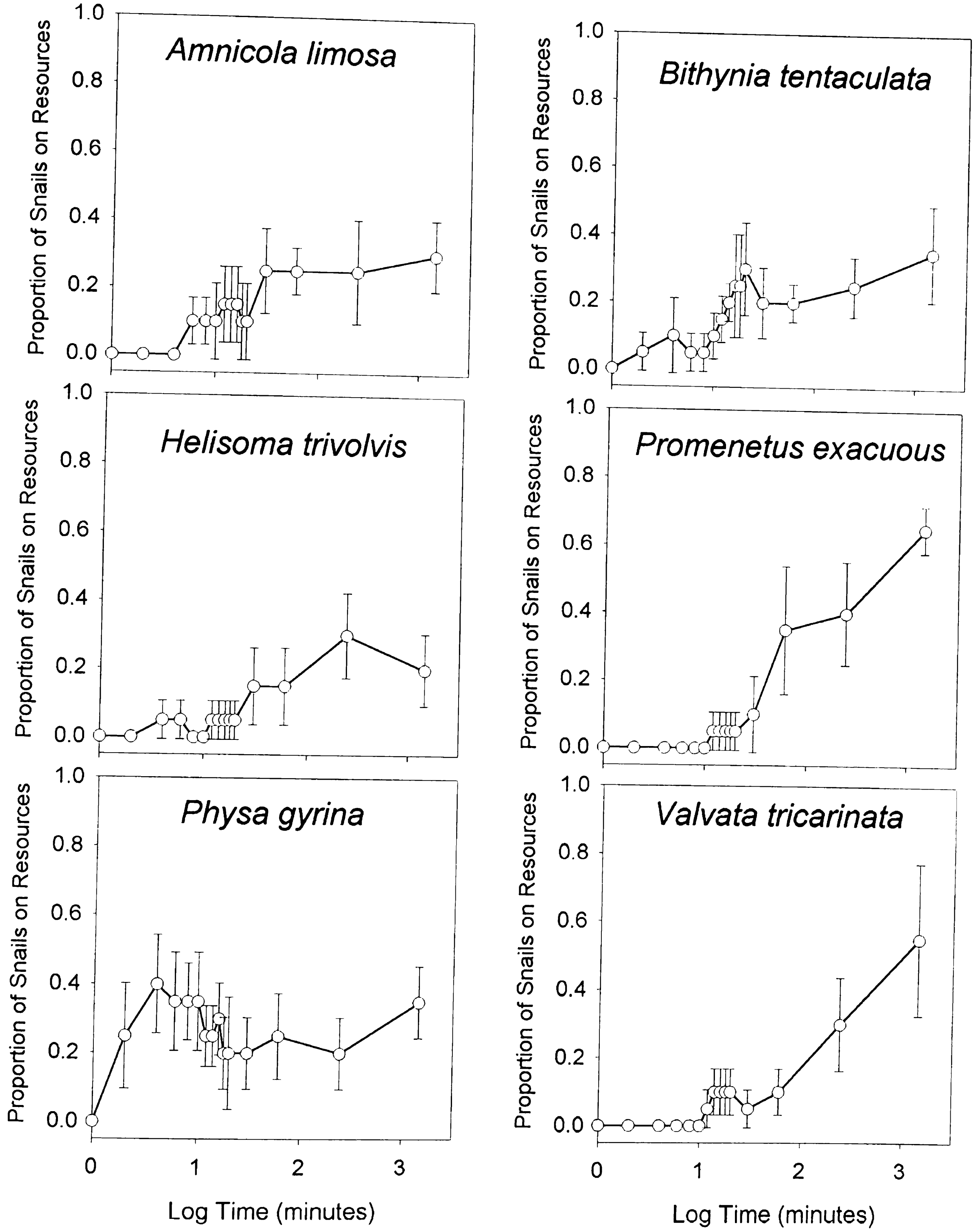


Figure 25. Average proportion of snails observed on resource tiles over the course of the 24h trials. Each panel reports the results for a given snail species. Data are means ± 1 SE. The abscissa is log transformed in order to more clearly show data.

Figure 25


Amnicola limosa
Bithynia tentaculata
Helisoma trivolvis
Promenetus exacuous
Physa gyrina
Valvata tricarinata
Proportion of Snails on Resources
1.0
0.8
0.6
0.4
0.2
0.0
0
1
2
3
Log Time (minutes)

able to achieve greater total resource utilization than species poor snail communities. In other words, the contrasting foraging behavior of these snail species could fuel a niche complementarity mechanism, leading to greater ecosystem process rates in diverse systems.

APPENDIX C

DISTRIBUTION AND ABUNDANCE OF AQUATIC SNAILS IN SOUTHWEST MICHIGAN PONDS.

Introduction:

Theory and experiments demonstrate that the number of species in local habitats can influence the rates of some ecosystem processes (Loreau et al. 2001). However, the distribution of species across natural gradients of species diversity may not match that used in experiments (Wardle 1999). For instance, most experiments create diversity gradients by drawing different numbers of species randomly from a larger pool of species (e.g., Tilman et al. 1997). Sometimes this process is replicated so that at each level of diversity there are multiple (or all possible) combinations of species. Natural communities are not generally thought to be random assemblages; rather they are thought to be the product of interspecific interactions, dispersal, stochastic demographic processes, physiological tolerances, and other factors. Thus, experiments may not accurately predict the response of ecosystem function to changes in species diversity. I conducted a field survey of ponds in southwest Michigan to examine the patterns of species occurrence across natural variation in species diversity. I use this information in Chapter Five to examine the differences between experimental results based on random assemblages and results based on only those assemblages actually observed in nature.

Methods:

I sampled snail communities in 16 ponds near the W. K. Kellogg Biological Station on 5 Sep 2003. Nine ponds were located at the Lux Arbor Reserve, while seven ponds were located at the Kellogg Biological Station's Experimental Pond Facility. Figure 26 shows the physical location of the Lux Arbor ponds. The experimental ponds varied in pond age (time since construction), composition and extent of vegetation, and the presence or absence of sunfish (e.g., *Lepomis macrochirus*). Natural ponds varied in depth, size, composition and extent of macrophytes, and in many other factors. I took repeated sweep net samples (between five and nine) from benthic sediments and submerged macrophytes in each pond, until five subsequent sweep nets produced no new snail species (evaluated with macroscopic inspection of samples in the field). I attempted to sample across any visible heterogeneity in each pond. Multiple samples for a given pond were pooled, and then preserved in 95% ethanol. Snails were identified, counted, and measured in the laboratory (with dissecting microscopes and digitizing tablets). Snail shell lengths were converted to mg of dry animal biomass using length-weight regressions (C. Osenberg, *unpublished data*). Experimental ponds are numbered using the scheme established by D. Hall and used there since, and the Lux Arbor ponds are numbered using the numbering scheme routinely used there.

Results and Discussion:

Average snail species richness among the 16 ponds surveyed was 2.25, and both the mode and median species richness was 3.0 (Table 13). The total snail species pool consisted of only five species. Interestingly, the most species rich pond (PL4) was at the Experimental Pond Facility. Because this sampling was performed only once, it is possible other species of snails that peak in abundance during other parts of the year could inhabit these ponds. Also, sampling was thorough but obviously not complete, so all species present may not have been detected. Thus, I consider the estimates of species richness reported here to be conservative.

The total biomass of snails found in natural (Lux Arbor) and semi-natural (Experimental Pond Facility) ponds was significantly related to snail species richness. Ponds with more snail species tended to have greater snail biomass (Figure 27). The relationship between snail richness and biomass does not appear to be an artifact of combining disparate data sets. In other words, the relationship appears when looking at either the semi-natural or the natural ponds separately.

Helisoma commonly dominated the biomass of snail communities when present (Table 13, Figure 28). *Physa* was not found in ponds with low snail species richness (e.g., below three species - Table 13, Figure 28). *Gyraulus* was present in many ponds (Table 13, Figure 28), but rarely accounted for much of the total snail biomass (Figure 28).

Table 13. The occurrence of five snail species in a survey of 16 ponds in southwest Michigan (1= present, 0=absent). Snail species are labeled with the first letter of each the species and genus names: *Fossaria obrussa* = Fo, *Gyraulus parvus* = Gp, *Helisoma trivolvis* = Ht, *Pseudosuccinea columella* = Pc, *Physa gyrina* = Pg.

Pond	**Snail Species**					**Snail Species Richness**
	Fo	*Gp*	*Ht*	*Pc*	*Pg*	
PL2	0	0	0	0	0	0
LA16	0	0	0	0	0	0
PL11	0	1	0	0	0	1
PL17	1	0	0	0	0	1
LA26a	0	1	0	1	0	2
LA26b	0	1	1	0	0	2
PL8	1	1	0	0	0	2
LA8	0	1	1	0	1	3
PL16	1	1	1	0	0	3
LA5	0	1	1	0	1	3
LA7	0	1	1	0	1	3
LA18	0	1	1	0	1	3
LA30	0	1	1	0	1	3
LA9	0	0	1	1	1	3
PL10	1	0	1	0	1	3
PL4	1	1	1	0	1	4

Figure 26. Aerial photograph of Lux Arbor Reserve, MI showing the location of ponds sampled during the survey. Photo was taken in 1993 and is archived on the KBS LTER web site (www.lter.kbs.msu.edu).

Figure 27. Relationship between snail species richness and snail biomass. Snail biomass data was log-transformed after adding a constant (1). Points are labeled with their site codes (PLx =Pond Lab pond x, LAx = Lux Arbor pond x). The dotted line is a simple linear regression model. Symbols for sites LA16 and LA7 were moved slightly higher on the y-axis for visual clarity.

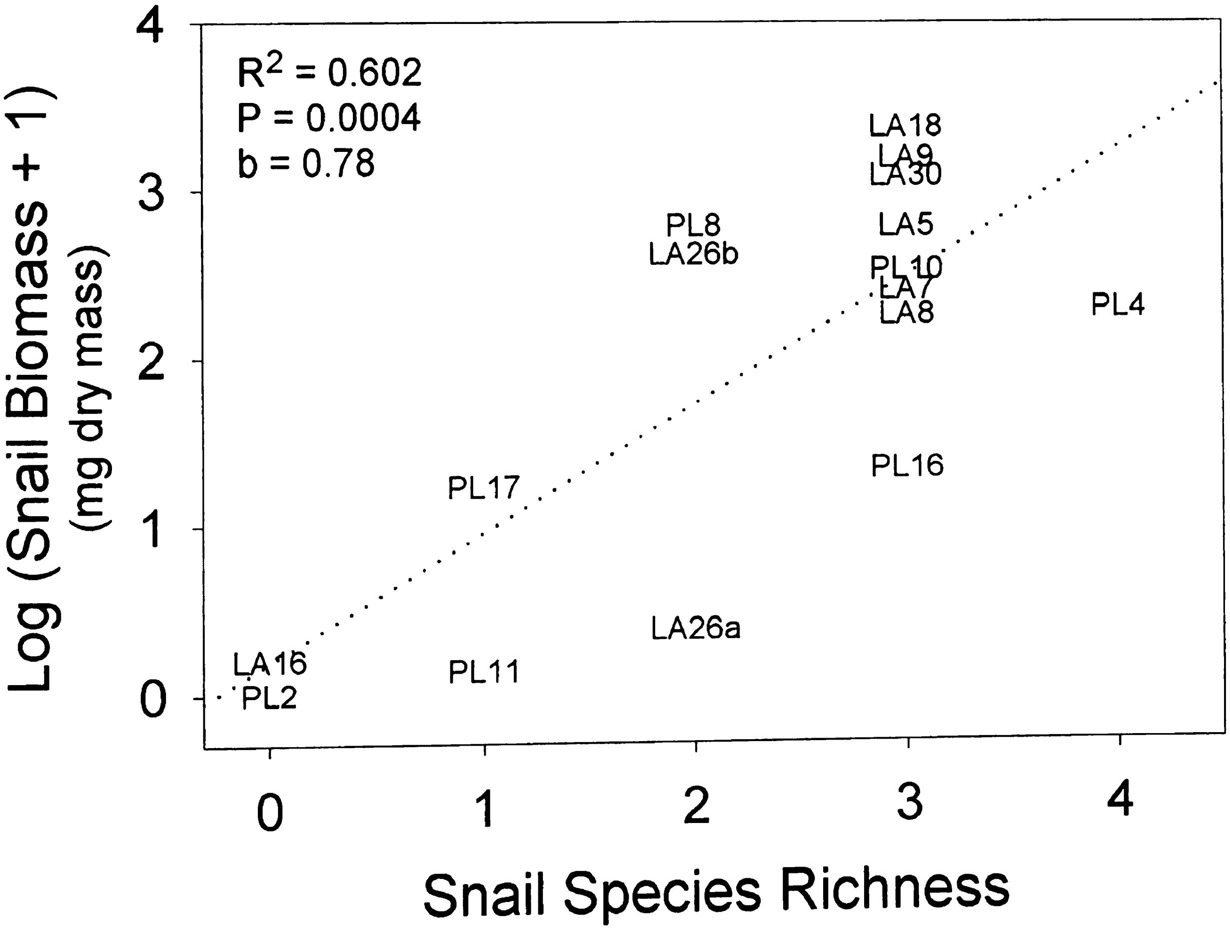
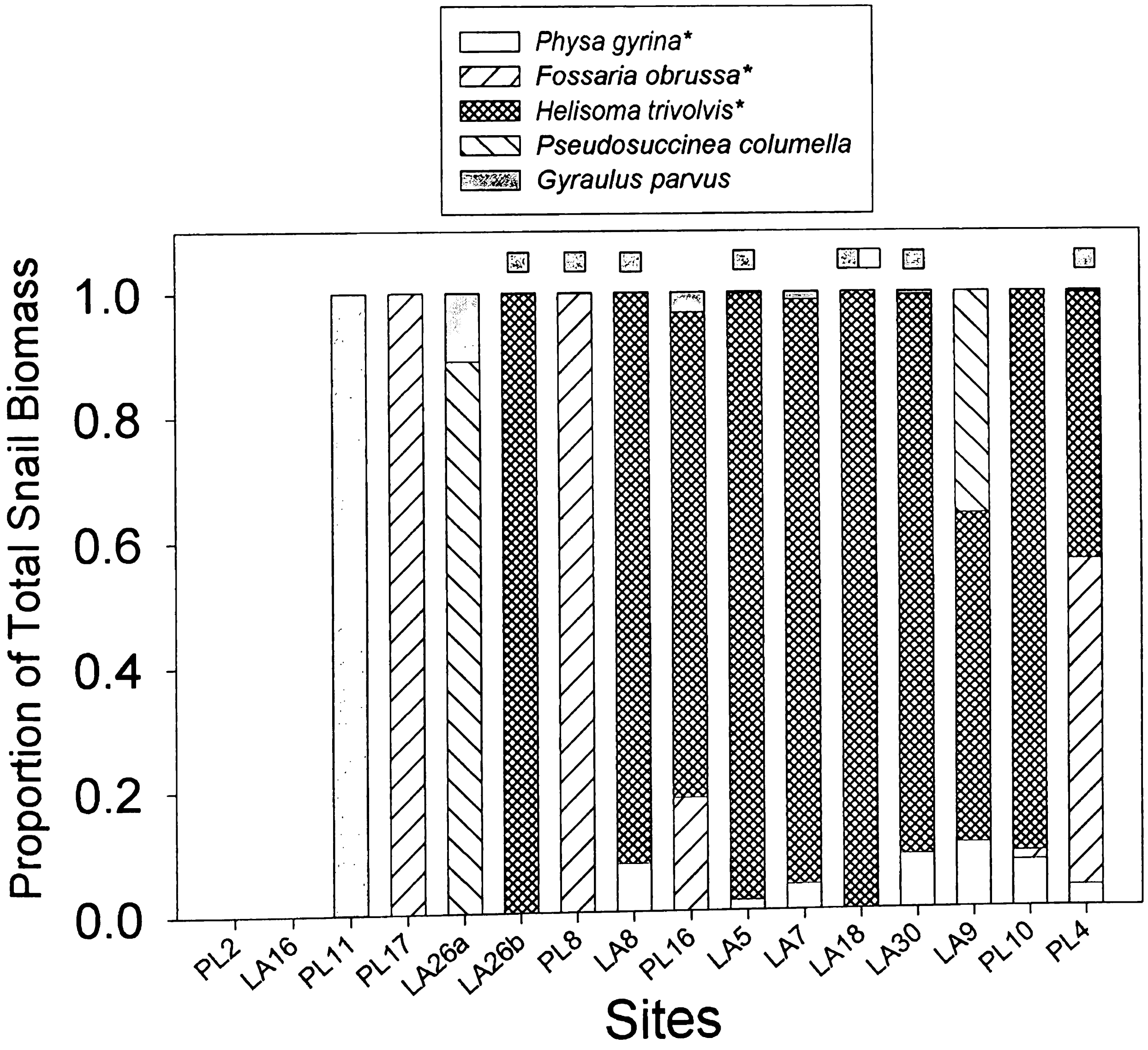


Figure 28. The proportion of total snail biomass of five snail species in each of 16 ponds in southwest Michigan. The sites are ordered by their snail species richness (refer to Figure 26 for snail richness and biomass values for each site). Species comprising less than 1% of the total snail biomass in a pond are coded with a symbol above the data in the figure to indicate their presence. Asterisks in the legend indicate species used in the experiment reported in Chapter Five.



The pattern of species occurrence across the ponds examine here appears non-random. This suggests that experiments that use random assemblages of species may not accurately characterize natural ecosystems' response to changes in species richness. See Chapter Five for a comparison of the patterns of species occurrence in species diversity-ecosystem function studies and this survey.

APPENDIX D

RELATIVE PREFERENCES OF THE PREDATOR *BELOSTOMA FLUMINEUM* FOR SEVERAL SNAIL PREY.

Introduction:

Predators are not indiscriminate in what prey they choose to ingest. Instead, they often have strong preferences for some prey items. These preferences can reflect differential energetic gain realized from different prey items, differences in the effort needed to capture and handle prey, or differences in the abundance of prey items. Understanding predators' preference for certain prey species is a key step towards understanding what effects predators will have at the community level. Preference is often calculated by comparing the frequency of a prey item in the predators' diet with the frequency of that prey in the environment. A common approach to assess preference is to expose predator individuals to equal densities of a variety of prey in the laboratory, and to record which prey the predator consumes.

Methods:

In order to better understand the potential effects of a voracious snail predator, *Belostoma flumineum*, on aquatic snail communities I performed feeding preference trials in the laboratory (10-12 Jul 2002). Three common prey species were considered: *Helisoma trivolvis*, *Fossaria obrussa*, and *Physa gyrina* (referred to by the initial of the genera names). Preference trials were performed in a pair-wise design (i.e. H vs. F, H vs. P, F vs. P). Three snails each of two different species were added to 650 mL plastic cups containing 500 mL of pond water, a plastic perch, and a *Belostoma* predator. Snails were obtained from ponds at the W. K. Kellogg Biological Station's Experimental Pond Facility, and thus reflect natural size distributions. *Belostoma* were collected from Pond 7 in the Lux Arbor Reserve (see Appendix C for map). Unfortunately, sizes of the snails used in this experiment were not recorded, but in general *Helisoma* was the largest, *Physa* the next largest, and *Fossaria* the smallest. All snails were vulnerable to predation by *Belostoma*; *Helisoma* larger than ~10mm in shell length are invulnerable to *Belostoma* (Chase 1999, and in previous feeding trials) and so were not used. Preference was evaluated in two ways; the identity of the first snail eaten (within 10 minutes of initiation) and the final number of each species killed after 12 h were recorded. The number of each species killed was used to compute Ivlev's electivity index (Ivlev 1961, as described in Krebs 1989), a common measure of dietary preference. Ivlev's electivity index is calculated as the difference of the percentages of prey item *i* in the diet and environment, over the sum of the percentages of prey item *i* in the diet and environment.

Results and Discussion:

Preference for different snail prey species by the predator *Belostoma* was evaluated by recording what prey species was selected first when multiple species were present, and by comparing the frequency of prey species in the diet versus their frequency in the environment (Ivlev's index). The results from both approaches provide similar results; *Physa* was chosen over either *Helisoma* or *Fossaria*, and *Helisoma* was chosen over *Fossaria* (Figures 29, 30). Belostoma attack snail prey by grasping them, rotating them so the snail's aperture faces the predator, and then injecting their long piercing/sucking mouthpart. *Belostoma* may prefer *Physa* because they have a relatively large aperture, and are incapable of pulling their bodies far into the shell (as do large, invulnerable *Helisoma*). *Fossaria* may have been chosen less often because they have a relatively small aperture, and a smaller body (less energetic return for same handling time) to shell size ratio.

Values of Ivlev's index greater than zero indicate preference for a prey item. However, the results reported here are mean index values over many trials, and sometimes average below zero despite the predator generally preferring that prey item (Figure 30). For example, if in an individual trial no prey of a certain type were consumed, Ivlev's index would have a value of –1.0, which can bring a grand mean of multiple trials below zero even when the predator prefers that prey item generally. Thus, for the purposes of this study the absolute value of Ivlev's index are less important than the relative values compared to the other prey species in the trial.

Figure 29. The proportion of feeding trials where a focal prey species was chosen first by the predator *Belostoma flumineum*. Two species of prey were present in each trial (labeled “HF” for *Helisoma* and *Fossaria*, etc.). The number of trials (n) with different combinations of prey are reported in the figure.

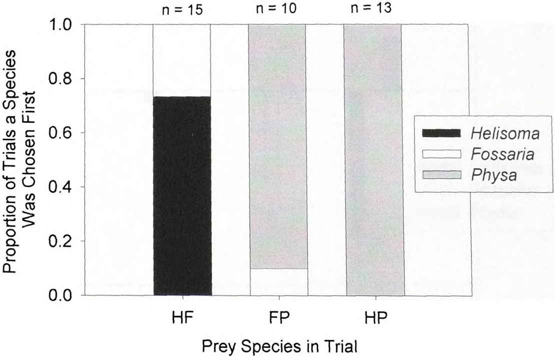
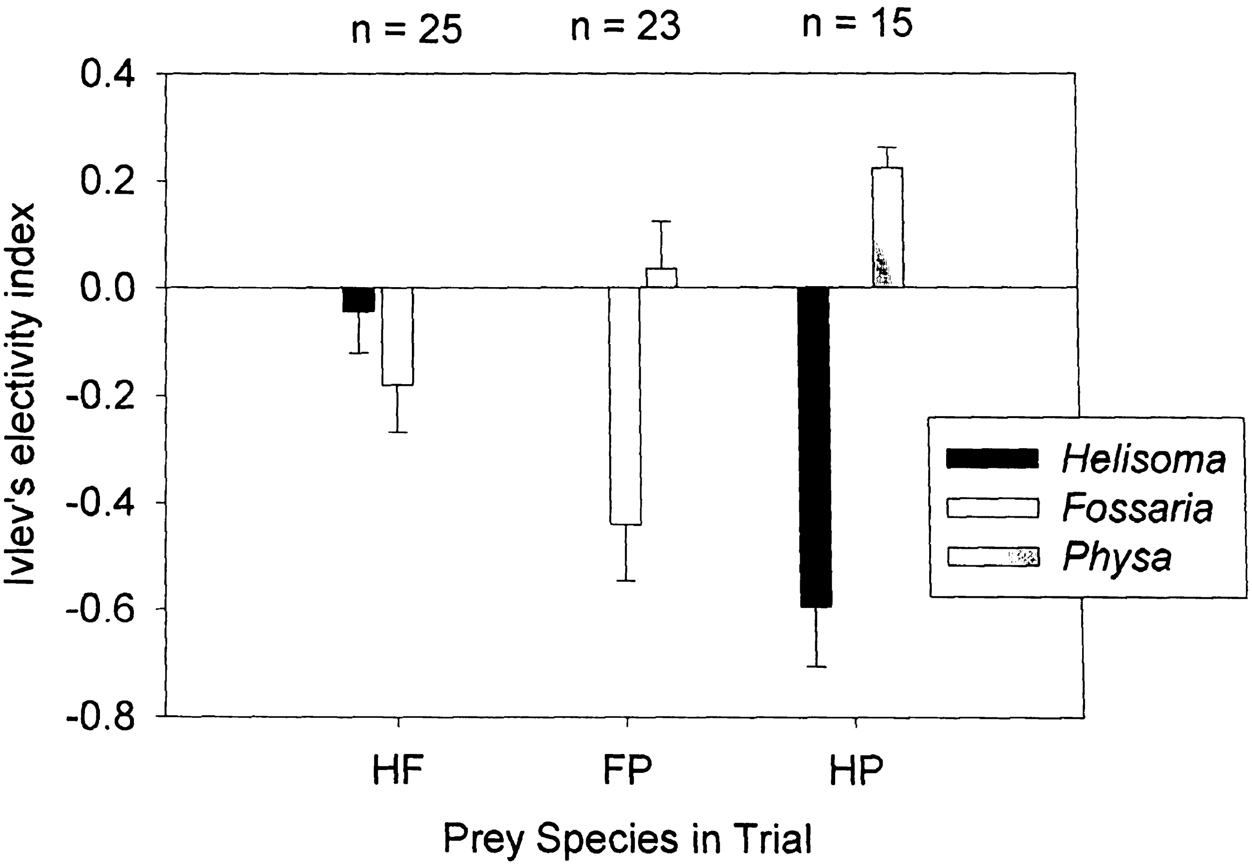


Figure 30. Ivlev's electivity index describing the relative preferences of the predator *Belostoma flumineum* for snail prey species. Two species of prey were present in each trial (labeled "HF" for *Helisoma* and *Fossaria*, etc.). The number of trials (n) with different combinations of prey are reported in the figure.



The demonstrable preferences among snail prey of the predator *Belostoma flumineum* suggest that predation could cause shifts in the relative abundance of these snail species. Many of the ponds in Lux Arbor (e.g., Ponds 5, 7) and in the Barry State Game Area seem to be densely populated with *Physa* early in the summer, but by late summer large *Helisoma* dominate the snail communities (J. Wojdak, *personal observations*). These changes coincide in time with increases in *Belostoma* density (or at least the ease of collecting *Belostoma*).

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