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RACHEL NICOLE HARTNETT
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THEORETICAL AND EMPIRICAL STUDIES ON HIERARCHICAL EFFECTS OF SIZE DISTRIBUTIONS: LINKING THE INDIVIDUAL TO ITS COMMUNITY

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BY

Dr. Lawrence J. Weider, Chair

Dr. Michael E. Kaspari

Dr. Robert W. Nairn

Dr. Lara A. Souza

Dr. Caryn C. Vaughn

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Abstract

Population and community ecologists often view the world differently based on one assumption: community ecologists often assume that individuals within a population are functionally equivalent; population ecologists focus on these functional differences. The role that individual variation plays in population dynamics has been often overlooked in community and ecosystem ecology, although interest in this area continues to grow.

I focused primarily on how individual variation influences population dynamics and community properties, empirically testing ecological theory and using collected data to inform theory. I used the water flea, *Daphnia,* as a model organism because they are key primary consumers in aquatic food webs. A number of research groups have gathered valuable physiological data, and recent genomic resources have been developed as well. In addition, much work has been conducted on the important role that daphniids play at both the community and ecosystem level. Further, individual clonal lineages are easily maintained due to their mixed breeding cycles of asexual and sexual reproduction for experimental work. I use a combination of theoretical modeling and empirical testing to address questions at the interface of population and community ecology.

First, in Chapter One, I addressed how the individual phenotype was influenced by genotype and the environment. I wanted to know how sensitive body size was to food quality (i.e., high and low phosphorous content). I conducted a life-history table experiment using three distinct lineages of four species of *Daphnia* to compare variation in life-history traits among and between species, as well as across two different food quality levels. These data were used to explore the differences in using individual- and

biomass-based models that incorporate size-structure information in Chapter Two. I looked into the sensitivity of these types of models to changes in juvenile traits, adult traits, and density-dependence. In Chapter Three, I scaled up from the individual to the population-level, using simple microcosms to address how body size variation contributes to body size distributions. Finally in Chapter Four, I looked at how these species' variation in traits translated into a more complex environment and within a community context.

Combined, the work presented in my dissertation demonstrates that individual traits and population assemblages influence size-distributions, and trait variation and the standing community influence colonization success. With many communities and ecosystems undergoing rapid environmental changes, linking the role that individual trait variation plays in population dynamics will be key in helping to predict long-term persistence of community (e.g., diversity of heterospecifics) and ecosystem functions (e.g., alternative stable states).

Chapter One: Plasticity and sensitivity in life-history traits among *Daphnia* species under food stress

Rachel Hartnett

Abstract

Currently organisms are experiencing changes in their environment at an unprecedented rate. Therefore the study of life-history traits is crucial, as they are direct links between the environment and an organism's fitness. In addition, phenotypic plasticity is increasingly important to consider as a potential mechanism for population persistence given the fluctuations in environmental stressors we are currently experiencing. *Daphnia* is used as a model organism as the genus contains keystone primary consumers in aquatic food webs. A life-history table experiment (LHTE) using four species of *Daphnia* was conducted to compare variation in life-history traits among and between species, as well as across two different environmental conditions (i.e., high and low phosphorous availability). It was predicted that *Daphnia* would show a tradeoff between P-sensitivity and phenotypic plasticity because individuals with higher flexibility would show less change in phenotype between different phosphorous environments. Results indicate that clonal variation buffered the effects of nutrient availability at the species level. Plasticity was more evident in reproductive traits, while growth traits were found to be more constrained. Body size and clonal identity determined fitness in a food-stressed environment, indicating that both factors are important when considering zooplankton responses to environmental change.

Introduction

With increasing environmental stress, many suites of organismal traits are expected to experience strong selection, with life-history traits potentially being among the most impacted (Bradshaw and Holzapfel 2008, Reed *et al.* 2011). Organisms are facing ever-growing levels of stress due to environmental change (Walther *et al.* 2002). These stressors may come from processes such as eutrophication, land-use changes, and pollutants, or increased oscillations in seasonal patterns due to climate-change (Smith and Schindler 2009, IPCC 2014). Life-history traits have a direct link to fitness, as an organism's success is built upon an ability to grow to reproductive age, the timing of reproduction events, as well as cumulative reproductive output before death. Therefore, life-history theory has established direct associations between a population's environment and life-history trait evolution (Stearns 1992, Agrawal *et al.* 2013).

Food stress has been shown to create a variety of life-history trait effects in organisms, which include longer developmental time, decreases in body size, and lowered fecundity (Ellers & Van Alphen 1997; Nylin & Gotthard 1998). Food stress can be experimentally manipulated through lowering a limiting resource. In most freshwater lentic systems, phosphorus (P) is ultimately the most limiting nutrient (Wetzel 1983, Sterner 2008), with anthropogenic inputs of P in aquatic systems forcing rapid change in zooplankton populations (Frisch *et al.* 2014). Members of the genus *Daphnia* (Cladocera: Anomopoda) have one of the highest P contents amongst zooplankton, so they are predicted to be more responsive to P-limitation compared to other zooplankton taxa (Sterner and Schulz 1998). Daphniids can therefore be used as an indicator organism (Gannon & Stemberger 1978) in ecological risk assessment of stressed aquatic systems (Bettinetti *et al.* 2005). P-limitation (i.e., low food quality) has effects on *Daphnia* life-history traits such as growth, reproduction, and senescence (Dudycha 2003, Jeyasingh and Weider 2005).

Daphnia have a cyclically parthenogenic life-cycle, which includes bouts of asexual reproduction under good growing conditions, and sexual reproduction during times of food stress, changes in photoperiod, and crowding cues (Kleiven *et al.* 1992). Due to the hatching of sexually-produced offspring every year, genetic variation generally remains high in many natural *Daphnia* populations (Innes *et al.* 1986; Spitze *et al.* 1991; Weider *et al.* 1999). In addition, clones can be geographically widespread (Weider *et al.* 1999, Crease *et al.* 2012). Therefore, clonal variation can be considered equally (or nearly as) important as species identity in these systems. For *Daphnia*, clonal diversity is better maintained under P-limitation (Weider *et al.* 2008), therefore clonal variation may buffer species-specific tradeoffs seen in previous species-level work.

Another mechanism to mitigate environmental effects is an organism's capacity for phenotypic plasticity (Nunney 2015). Phenotypic plasticity is the ability of an organism to change its phenotype in response to environmental change. *Daphnia* have shown a great capacity for phenotypic plasticity in predator-avoidance (Spitze 1992; Weider & Pijanowska 1993), nutrient uptake/use efficiency (Lampert 1994), and other life-history traits (Lampert 1993). Here, where a changing environment would select for a more responsive organism, is fertile ground for the evolution of phenotypic plasticity when considering population persistence in these environments (Chevin *et al.* 2010).

Gathering information about the potential for phenotypically plastic traits via trait variation has been, and will continue to be, a goal toward predicting a species' ability to respond to continued environmental stress. However, there are costs and limits involved in maintaining plastic traits, including genetic and/or developmental constraints, competitive exclusion by a more optimal (and less plastic) trait during a stable period, or geographical limits (Whitlock 1996, Pigliucci 2005).

This present study aims to address the following: 1) Environmental variance is predicted to contribute a higher proportion of the total phenotypic variance (i.e., have a higher effect size) than the genetic (taxonomic) contribution (i.e., effect size of species/clonal identity). 2) Jeyasingh (2007) suggested that evolution should favor more plastic physiologies for smaller organisms in order to counter frequent shifts in nutrient limitation. And 3) what is the potential relationship between plasticity and trait variation? Clonal variation will buffer/reduce effects seen at the species level because clonal variation increases genetic and trait variation, and thereby flexibility in response to change. Species that are flexible in their use of phosphorus may compensate for P-limitation by being more plastic in life-history traits. As a result, I would predict a negative relationship between variation in traits and trait sensitivity to phosphorus.

Methods

Study organism

Daphnia are a cosmopolitan genus (Sarma *et al*. 2005, Lampert 2011). Three clonal lineages from four different *Daphnia* species (*D. magna*, *D. mendotae*, *D. obtusa*, and *D. pulex*) were collected from a variety of laboratory stocks (see

Table 1). These clonal lineages span the three subgenera of *Daphnia*, ranging across North America and Europe, and come from various aquatic habitats (Table 1). *D. magna* clones used in this study originated from South Dakota, Finland and Germany from a spectrum of habitats. The South Dakotan clone (MA3) came from a permanent lake, a shallow (< 2 m) prairie pot-hole (Weider *et al.* 2004). MA2 and MA1are both inbred lines from an original genetic cross between a Finnish clone and a German clone. MA2 was inbred for three generations and MA1 was inbred for one generation (Dieter Ebert, Switzerland, personal communication). The environment of the parental clones include a Finnish clone from a ephemeral with desiccation in spring/summer and freezing during autumn/winter and a clone from a German semi-permanent pond, with freezing in the winter (Roulin *et al.* 2013). In addition, *D. pulex* and *D. obtusa* clones came from temporary ponds in the U.S. Midwest, while *D. mendotae* came from permanent lakes in the U.S. Midwest. One *D. mendotae* clone (ME3) experienced high levels of mortality early on in the experiment, and was subsequently dropped from the analyses. These contrasting environments have created very different evolutionary trajectories for these species. However, one caveat that should be noted: a potential confounding issue with two of the three *D. magna* clones from their inbreeding (MA1 and MA2).

Experimental design

Clonal lineages were maintained as separate populations in 900 mL jars, with regular and plentiful feeding using the chemostatically-cultured green algae, *Scendesmus acutus*, at a constant 20°C in COMBO media (Kilham *et al.* 1998).

A small amount of cetyl alcohol (~10 mg) was added to act as a surfactant to prevent animals from being trapped in the air-water interface. Stock cultures received equal amounts of 24-hour incidental ambient lighting. Maternal lines for experimental animals were raised in individual 60 mL jars with 50 mL of COMBO and fed 1 mg C L^{-1} of *S. acutus* daily. Females were monitored every 24-hours, and first and second clutches were removed. Experimental animals (N = 20 per clone) were taken from third or later clutches within 24 hours to reduce maternal effects (Ebert 1991).

An initial body-length measurement (i.e., start length) was taken using a MOTICAM 2300 digital camera and software system (Motic®, S-05165) mounted to an Olympus BX51 compound dissecting microscope. Length measurements were taken from the top of the eyespot to the base of the core body, right above the top of the tail-spine. The tail-spine is known to be morphologically plastic depending on environmental conditions, and was not measured with core length due to potential confounding length measurements. Experimental animals were placed individually in 60 mL glass jars with 50 mL of COMBO at 20°C, and were divided into two environmental conditions, high and low phosphorus (N=10 per clonal line for each environmental treatment). Animals under a high phosphorus (HiP) feeding regime were fed daily with 1 mg C L^{-1} of *S. acutus* that was grown in nutrient-rich conditions (i.e., C:P, ~100:1). A low phosphorus (LoP) feeding regime consisted of daily 1mg C L^{-1} feeding of *S. acutus* grown in nutrient-poor conditions (i.e., C:P, ~750:1). Experimental animals were transferred every two days to fresh jars in order to avoid carbon (detrital) accumulation that could differentially affect resource availability based on inter-/intra-specific variation of filtering rates. Experimental animals were monitored daily and size was measured again at maturation, when first egg development was seen (i.e., age at maturation and length at maturation). Clutch size was recorded daily, as well as images for neonate body-lengths ($N \leq 5$ neonates per clutch in order to reduce small-clutch bias). Number of clutches, clutch size and mean neonate length (termed mean clutch length) were calculated from these daily recorded measurements. Dead experimental animals were measured with the day of death. The experiment ran for 28 days, and at the end of this period, experimental animals were measured (i.e., end length), as described above.

Statistical Analyses

Individuals (replicates) were dropped from analysis if they died within 5 days of the start of the experiment to prevent bias from missing data. Analyses were run using SPSS (Version 20, IBM). A Principal Component Analysis (PCA) was run for descriptive purposes in order to map out intra- and interspecific differences in multivariate space (Figure 1). This described which taxonomic (inter- vs intraspecific) level showed significant variation in life-history traits. Data collected for both treatments were run together to obtain principal components (PC), separate graphs were made for treatments for ease of viewing. Life-history traits were clustered into the two groups outlined by the PC axes, growth and reproduction. PC1 loadings correlated strongly (>0.8) with size variables (start length, size at maturation, end length, and mean clutch length). PC2 loadings correlated (>0.8) with reproductive variables (clutch size, number of clutches). A MANOVA was conducted to look at the significance of genetic (species, clonal) and environmental (phosphorous treatment) contributions for start length, length at maturation, end length, mean clutch length, and mean clutch size. Other variables (e.g., number of clutches, age at maturation) were too skewed to be used for parametric tests. Maternal effects are common among daphniid studies (Lampert 1993), so maternal line was also looked at as a potential confounding variable and was tested as a covariate. All collected data were screened for outliers using visual inspection of stem and leaf plots, and multivariate normality was checked for the dataset using post-hoc residuals from the MANOVA (Tabachnick & Fidell 2013).

Effect sizes were calculated using Cohen's d metric. Cohen's d is a common metric of effect size, in which the means from two groups (in this case, food treatments) are compared. A standard method was used instead of mean differences, so scale/unit-independent comparisons could be made between variables. The range of Cohen's d is infinite, so comparing absolute differences between studies can be challenging, without correction. However, since all of the animals were run simultaneously in the experiment, relative differences in Cohen's d are an appropriate metric for comparative purposes. Phenotypic plasticity was looked at in two ways: 1) the variance within life-history traits of clones and species and 2) the 'mobility' of clones and species across environments using PCA space. In order to look at variance, we looked at coefficients of variation (COVs) to estimate the spread of the traits. COVs were calculated from $COV = \frac{s}{\gamma}$, where s = standard deviation and γ = mean of the particular life-history trait. In addition, from the PCA, the movement of the center of data points per clone and per species (i.e., their centroid) can be used to approximate phenotypic plasticity potential, as it calculates responsiveness to low phosphorous conditions. Centroid calculations were done by calculating species and clonal centroids from principle components for HiP and LoP (i.e., high and low food quality). The change in centroid position and magnitude of the vector across environments were calculated on a finite set of points by $c_{(x,y)} = \frac{(x_1,y_1)+\cdots+(x_n,y_n)}{k}$, where each set of (x,y) coordinates is averaged by the k number of points.

Linear regressions were used to detect significant relationships between trait variation (COV) and P-sensitivity. P-sensitivity was calculated by using the differences in log-transformed values between phosphorous treatments (Seidendorf et al. 2010): P-sensitivity per trait = $\ln(\text{trait}_{\text{HIP}})-\ln(\text{trait}_{\text{LOP}})$.

Results

Under low-phosphorous (LoP) conditions, all clonal lines of all species showed smaller sizes both at first reproduction, and at the end of the experiment. Similarly, under LoP, clones exhibited delayed onset of reproduction and had smaller clutch sizes. The number of clutches varied per clone, as well as their mean clutch length (See Table 4).

At the interspecific level, *D. mendotae* showed no separation from the *D. pulex/D. obtusa* group along the growth axis (PC1), indicating that these three species were of similar size, while *D. magna* (as expected) was larger (Figure 1). Along the reproductive axis (PC2), the *D. pulex/D. obtusa* group showed some separation from *D. mendotae* and *D. magna*. These results indicated that *D. pulex/D. obtusa* expressed higher fecundities. While interspecific differences were apparent (Figure 1), it was clear that intraspecific (clonal) differences also were informative. Note that *D. magna* had a broad spread of traits, while the *D. pulex/D. obtusa* group showed overlap. Shifting from high to low phosphorus, the variation along the reproductive axis (PC2) was lost, however the size axis (PC1) still exhibited variation (Figure 1). This may have indicated a shift in resource allocation from reproduction to maintenance. In addition, the separation along the growth axis was maintained between low and high P. The MANOVA showed that both genetic factors (species, clone) as well as environmental factors (maternal effects, food treatment) significantly affected life-history traits (Table 2). The F statistic can be used as a proxy for the magnitude of effect size metric (Tabachnick & Fidell 2013). Using the F statistic as a relative effect size metric, the environment (i.e., food treatment) was found to have a relatively higher effect at the species level (F = 46.3) than the clonal level (F = 8.21). The environment (food treatment) had a stronger main effect than either genetic component (species or clone).

Effect sizes were calculated in an attempt to ascertain the relative contribution the environment had on each life-history trait. Effect sizes can be ranked from smallest to largest effect as follows: starting length of experimental animals, mean clutch length, number of clutches, length at maturation, end length, and clutch size were found to be the most affected by environment (Table

3). From a species perspective, the smaller species, *D. mendotae* and *D. obtusa*, were least affected by food quality (Cohen's d = 2.20 and 3.28, respectively), while the larger species, *D. pulex* and *D. magna*, were more affected (Cohen's d = 5.65 and 7.01, respectively; Table 3). However, smaller species did not show more plastic potential, as predicted. Instead, life-history traits of *D. magna* and *D. mendotae* were constrained (i.e., were unresponsive to food stress), while *D. pulex* and *D. obtusa* had more plastic traits (Figure 2).

Results from the phenotypic plasticity potential analyses, COVs and centroid calculations, indicated that reproductive traits were more responsive, and thus more plastic, than traits associated with size/growth. The first approach used COVs to compare variance of traits at the intra- and inter-specific levels (Figure 2). Growth-related traits were less variable (i.e. were more constrained) regardless of environment (median equals 4.88% in HiP and 5.52% in LoP), compared to reproductive traits (median equals 14.28% in HiP and 24.46% in LoP). In addition, low P conditions had higher COVs in general than nutrient-rich conditions (but see *D. pulex*-2). The movement of centroids along the reproductive axis further supported the notion that reproductive traits were less constrained than growth-related life-history traits (Figure 4). The magnitude of centroid change was consistent within clones for *Daphnia mendotae* and *D. obtusa*, while *D. magna* and *D. pulex* had their species centroids affected by single clonal lineages (Figure 4). When looking across traits of all clones and species, trait variation (COV) and P-sensitivity appear to be positively related, contrary to expectations ([All quadratic regressions fitted to the data were significant ($P < 0.05$).], Figure 3). Results indicated that reproductive traits were more responsive, and thus more sensitive, than traits associated with size/growth.

Discussion

Life-history traits cluster based on species identity, while clonal variation buffers the effects of poor food quality

Species identity clustered strongly in terms of composite life-history traits (i.e., PC axes) under high quality (high P) food conditions (Figure 1). However, there was no strong species-specific clustering under poor nutrient conditions, while clonal variation accounted for most of the data spread. The effects of low P were reduced at the clonal level rather than at the species level, indicating that clonal variation may play an important role in maintaining species persistence in different environments (Table 2). While these experimental clonal assemblages are somewhat of an artificial construct, for a single population it is clear that clonal (genotypic) variation plays a role in diversifying a population's portfolio.

Daphnia have a mixed asexual-sexual breeding system, which creates the unique advantage of establishing multiple clonal lineages in a population leading to the potential for maintaining high genetic diversity within a population. Researchers have found large clonal differences within a single species in response to predator cues (Spitze 1992, Weider and Pijanowska 1993), nutrient limitation (Lynch 1989, Weider *et al.* 2004), habitat selection (de Meester 1994) and toxins (Baird *et al.* 1990, Walls 1997). Intraspecific genetic variation has been shown to have population-wide effects on colonization (Crutsinger *et al.* 2008, Crawford and Whitney 2010), coexistence (Lankau *et al.* 2009), and predation (Post *et al.* 2008). In general, increased intraspecific variation allows for flexibility at the species level. Depending on intra- and inter-specific pressures, evolution will favor more or less specialized individuals within a generalist population (Araújo *et al.* 2011).

Trait variation in body size is constrained while there is flexibility in reproductive traits

The environment did not play a strong role in traits associated with body size (e.g., length at maturation and clutch lengths). Body-size traits in this study seemed to be conserved from both the PCA visualization and COV calculations (Figures 1 and 2). This indicates that *Daphnia* have size-based phenotypes that are somewhat genetically constrained. Allometric constraints may be one possible explanation for conserved morphological traits. It has been shown that regardless of body-size, daphniids all follow a similar pattern of resource allocation to growth and reproduction under different levels of food (carbon) quantity (Dudycha & Lynch 2005). The food quality levels from this study partly support the notion that larger-bodied *Daphnia* are more affected by food quality than smaller-bodied *Daphnia* (Table 3). Differently-sized species had differential responses to environmental changes. In particular, the two smaller species, *D. mendotae* and *D. obtusa*, had higher genetic contributions relative to *D. mag*na *and D. pulex* based upon their low environmental effect sizes (Table 3). Evidence points to some phylogenetic constraints (i.e., distinct evolutionary histories of these different taxa) that may explain differences between these two sets of "less plastic" and "more plastic" species. *D. magna* and *D. mendotae* are found in the subgenera Ctenodaphnia and Hyalodaphnia, respectively, while *D. pulex* and *D. obtusa* both stem from the subgenus *Daphnia*. However, evolution of traits such as ephippial spines and elongated setae have evolved in several subgenera due to environmental selection pressures (Colbourne *et al.* 1997). *Daphnia*'s physiology allow them to alter filtering rates under different food quality environments (Sahuquillo *et al.* 2007), although phylogenetic constraints are operating here via size.

Further, body size has been implicated in determining sensitivity to food quality, with larger individuals being affected by low food quality more so than smaller individuals (Peter and Lampert 1989). This study matches that prediction, P-sensitivity was highest in the largest species, *D. magna*, and decreased in rank order of species size (*D. pulex*, *D. obtusa*, and *D. mendotae*) (Figure 4). This is due to plasticity in reproductive output (Figure 1). Species consistently show a shift from high reproductive output in HiP to low output in LoP, while body size remains consistent between environments (Figure 2). Shifts toward lower reproduction has been seen for low levels of nitrogen and phosphorus (Sterner *et al.* 1992) and for low food concentrations (Lynch 1989); but under toxin-enriched environments, *Daphnia* have shown to maintain reproductive output (Forbes *et al.* 2016).

Reproductive trait variation and P-sensitivity

We hypothesized a tradeoff between an organism's sensitivity and trait variation. Influential life-history traits should have minimal trait variation under the hypothesis of environmental buffering, as fitness would be heavily dependent on minimal change within important vital rate constraints (Pfister 1998). However, in this present study, results were contradictory to expectations: organisms became more sensitive to changes in phosphorus with increasing trait variation. This is most likely due to reproductive traits being very P-intensive and very responsive to changes in food quality. In *Daphnia*, P and reproductive trait relationships have not been as well studied as somatic growth rate (SGR), a well-known proxy of fitness (Lampert & Trubetskova 1996). However depending on body size, *Daphnia* will be either more responsive to P-limitation through changes in clutch frequency (small-bodied species) or in clutch size (large-bodied species) (Hood and Sterner 2014). Plasticity in reproductive traits are generally considered less important in changing population growth rates based on previous modeling of growth and reproductive schedules (Pfister 1998). These results suggest that environmental buffering from P-limitation has canalized the highly vital growth traits over time, while leaving plasticity in reproductive rates sensitive to environmental change.

Conclusions

This present study provides evidence that species identity is important in determining body-size traits, but that may not translate into size-structured populations due to plasticity in reproductive traits across environments that vary in overall food quality (i.e. P-rich vs P-poor environments). Intraspecific trait variation, in particular, influenced responses to environmental change. Genetic differentiation of a population can reduce extinction risk in a multitude of organisms (Frankham 2005). In particular, it appears that the flexibility in reproductive traits may play an important role for population persistence in the face of environmental change. Intraspecific variation has been of interest to community ecologists in determining community composition (Macarthur and Levins 1967, Violle *et al.* 2012). Biomass alone is not sufficient to predict zooplankton structure across environments, but size and species identity, which incorporates size, trait variation, and P-sensitivity, are better indicators of zooplankton composition (Hessen *et al.* 1995). Determining how intra- and interspecific composition influences size-structure in zooplankton communities may better link populations to community-level processes.

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Tables

Table 1. Species list of *Daphnia* populations used in the Life-History Table Experiment (LHTE)

Species	Clone	Location	Habitat Type
D. magna	MA1	Munich, Germany	Semi-permanent lake (Roulin et al. 2013)
D. magna	MA2	Tvärminne, Finland	Ephemeral rockpool (Roulin et al. 2013)
D. magna	MA3	South Dakota, US	Shallow, permanent lake (Weider et al. 2004)
D. obtusa	OB1	Oklahoma, USA	Pond
D. obtusa	OB2	Illinois, USA	Pond
D. obtusa	OB3	Missouri, USA	Pond
D. pulex	PX1	Illinois, USA	Shallow pond (Lynch 1987)
D. pulex	PX2	Illinois, USA	Shallow pond (Lynch 1987)
D. pulex	PX3	Illinois, USA	Shallow pond (Lynch 1987)
D. mendotae	ME1	Minnesota, USA	Permanent lake
D. mendotae	ME2	Minnesota, USA	Permanent lake

Table 2. Factorial MANOVA scores. Main effects and two-way interactions from a Factorial MANOVA are shown here. “Food” indicates the main effect of the food treatment manipulation (high phosphorus - HiP/low phosphorus - LoP). “Species” indicates the main effect of species on the response variable. “Clone” indicates the level of effect at the clonal-level, nested within species, on the response variable. Body length (mm) at the start of the experiment, the mother of the experimental animals, and time blocks were used as covariates. Two-way interactions were also tested.

Source of variance	Wilk’s Lambda	df1	df2	Multivariate F
Start length (covariate)	0.937	5	166	2.220
Maternal effects (covariate)	0.969	5	166	1.051
Time (covariate)	0.942	5	166.000	2.063
Food	0.131	5	166.000	219.992***
Species	0.078	15	458	46.334***
Clone	0.235	35	700.729	8.219***
Species * Food	0.208	15	276.000	19.690***
Clone * Food	0.251	35	700.729	7.784***

*** $p < 0.0001$

Table 3. Calculated effect sizes (Cohen's d) between the high-phosphorous and low-phosphorous food treatments. Traits were classified into growth (start length, end length, and length at maturation) and reproduction (clutch length, number of clutches, and average clutch size). This classification system corresponds with the principal components (PCs). Note that on average, *D. pulex* and *D. magna* had higher effect sizes due to treatment when compared with either *D. mendotae* or *D. obtusa*.

	Start Length	Length at Maturation	End Length	Mean Clutch Length	Number of Clutches	Mean Clutch Size
D. mendotae (2.80)	-3.13	1.63	4.59	1.71	1.23	4.48
D. magna (7.01)	-0.76	12.42	12.72	1.11	4.25	10.82
D. pulex (5.65)	0.24	1.78	8.62	-0.16	2.71	18.64
D. obtusa (3.28)	-0.02	1.52	4.10	0.97	3.84	9.21
Mean effect	0.87	4.60	7.77	1.36	3.17	11.36

Table 4. Life-history traits for species and clones of *Daphnia*. Results of a life-history experiment that examined within and between species differences in traits. Mean trait values for seven life-history traits under two environmental conditions (high-phosphorus food – HiP; low phosphorus food –LoP) are given. Traits from left to right are as follows: core body length at the start of the experiment (mm), body length at maturation (mm), age of maturation (days), body length at the end of the experiment (mm), mean neonate body length per clutch (mm), the number of clutches, and the number of individuals in a clutch.

Species	Start Length (mm)		Length at Maturation (mm)		Age at Maturation (days)		End Length (mm)		Mean Clutch Length (mm)		Number of Clutches		Mean Clutch Size	
	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP
ME1	0.56	0.58	1.25	1.18	5.71	7.78	2.33	1.85	0.5	0.61	4.9	4.7	2.65	1.73
ME2	0.56	0.57	1.25	1.15	5.8	7.7	2.22	1.85	0.58	0.67	5.6	5.7	2.79	1.73
MA1	0.88	0.88	3.63	2.97	8	12.5	3.97	3.65	0.86	1.02	4.1	4.2	5.67	2.45
MA2	0.91	0.92	3.34	2.19	10.2	11	3.93	2.63	0.95	1.04	3.7	0.1	3.74	0.2
MA3	0.78	0.78	2.91	2.9	7.89	15.7	4.25	2.99	0.87	0.46	5.6	0.8	6.15	0.85
PX1	0.68	0.56	1.62	1.47	5.56	7	3.06	1.72	0.69	0.08	8.3	0.22	13.2	0.28
PX2	0.6	0.6	1.53	1.33	5.14	9.56	2.33	2.07	0.5	0.58	5.3	4.27	5.32	2.21
PX3	0.6	0.6	1.65	1.45	6.5	8	2.82	2.45	0.76	0.7	7.3	6.9	10.4	10.4
OB1	0.58	0.58	1.51	1.25	6	7.8	2.98	2.13	0.68	0.56	8.6	1.7	9.02	2.66
OB2	0.52	0.52	1.4	1.27	6.3	8.33	2.67	2.12	0.64	0.55	9.4	5.5	11	2.7
OB3	0.6	0.61	1.52	1.35	6	8.11	2.89	2.08	0.67	0.42	9.3	4.3	13.8	3.44

Figure Legends

Figure 1. Principal component (PC) analysis of seven life-history traits. Graphs were separated into a) high-P and b) low-P environments to distinguish environmental effects, with the first two principal components plotted. PC1 correlates with growth traits while PC2 correlates with reproductive traits. See Table 1 for species (letter) and clonal (number) abbreviations.

Figure 2. Coefficients of variation (COVs) for growth (1-4) and reproduction (5-6) life-history traits within and among species. Shaded symbols indicate HiP and open symbols indicate LoP food treatments. Because COVs are useful when comparing the spread of data across different groups, they were calculated for start length (1), length at maturation (2), end length (3), average length of neonates (4), number of clutches (5), and average clutch size (6).

Figure 3**.** Relationships between life-history trait variation (COV) and phosphorous sensitivity for a) high-P and b) low-P environments for all life-history traits (combined), grouped per species. All HiP regressions were significant ($P<0.05$).

Figure 4. The centroid positions of *Daphnia* species in PC space (A), and the magnitude of centroid change, |v|, between a high food quality (shaded-HiP) and poor food quality (open-LoP) environment (B). Average species position are represented by filled symbols. Centroid position changed more along the reproduction (y) axis than the growth (x) axis, except for clone MA2. The absolute value of centroid change was

consistent within clones for *Daphnia mendotae* and *D. obtusa*, while *D. magna* and *D. pulex* had their species centroid affected by single clones.

Figure 1. Principal component (PC) analysis of seven life-history traits.

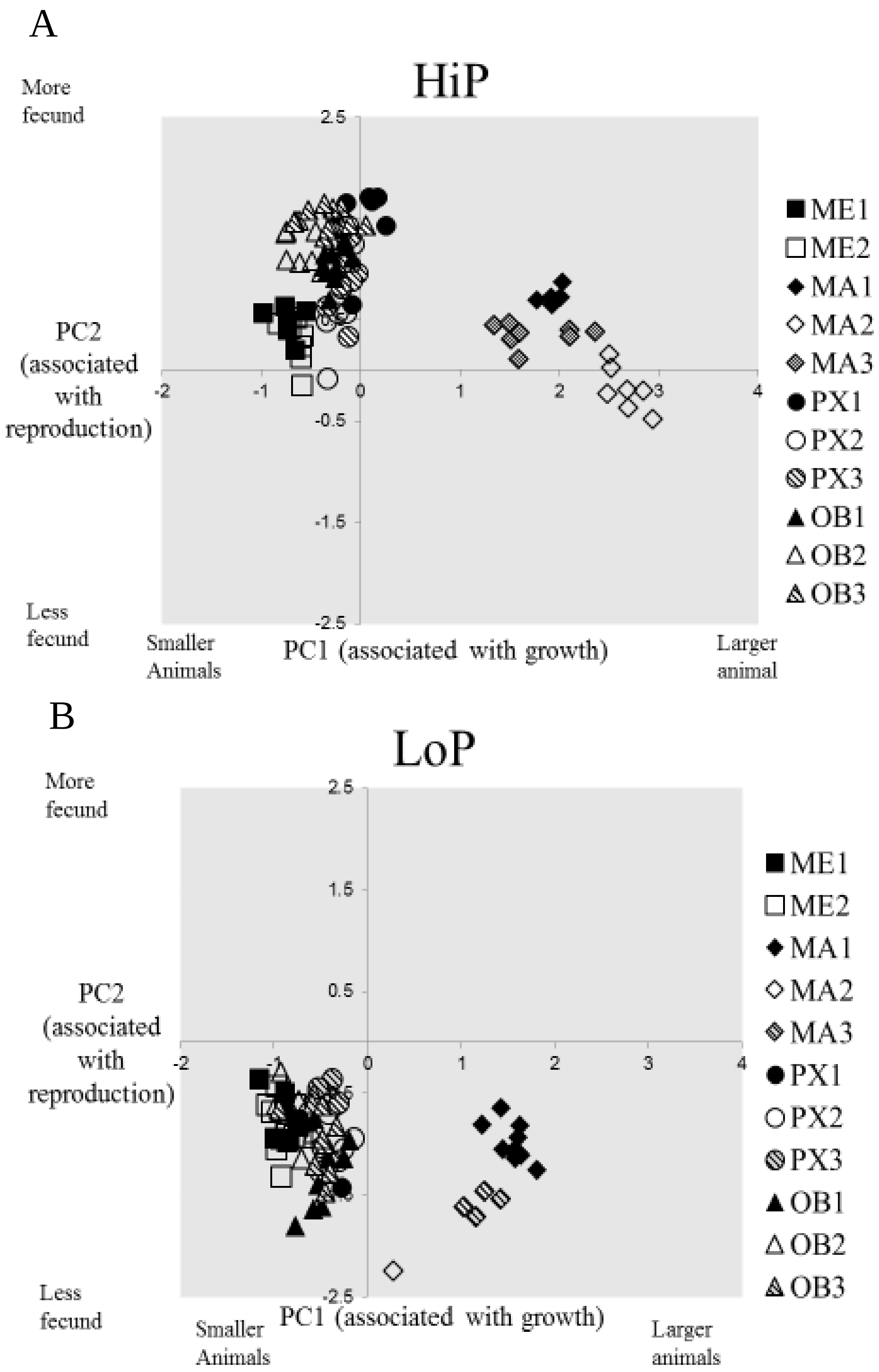


Figure 2. Coefficients of variation (COVs) for growth (1-4) and reproduction (5-6) life-history traits within and among species.

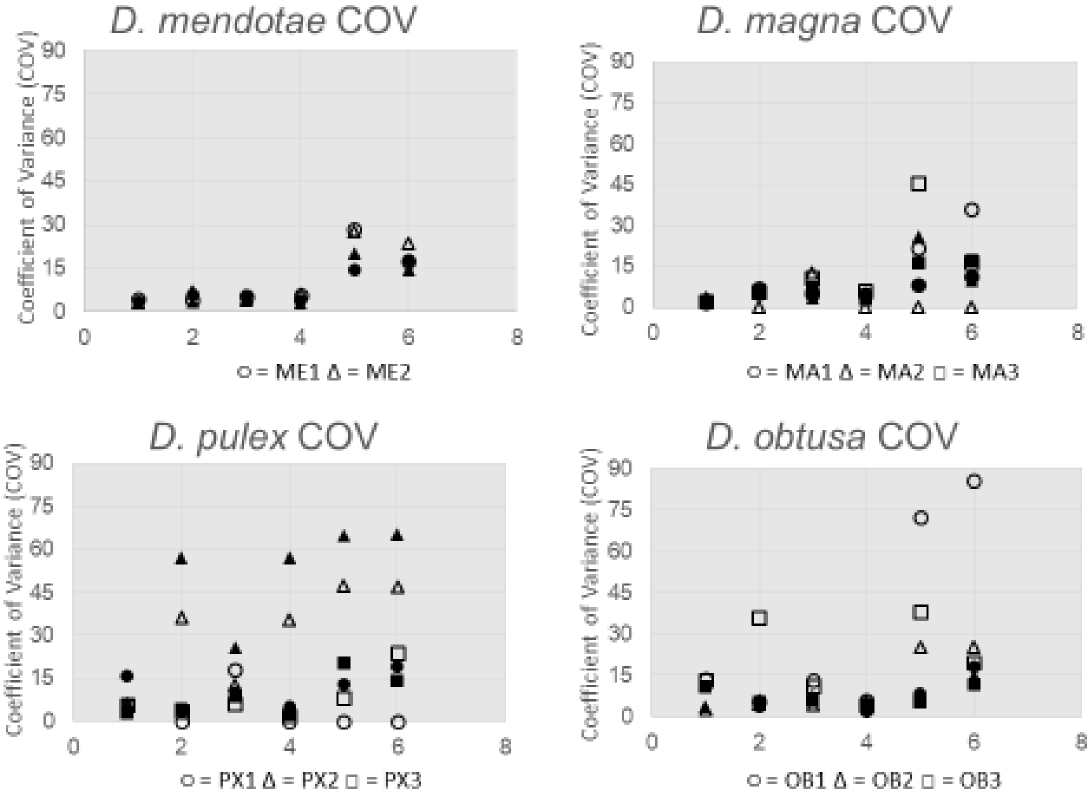


Figure 3. Relationships between life-history trait variation (COV) and phosphorous sensitivity for all life-history traits.

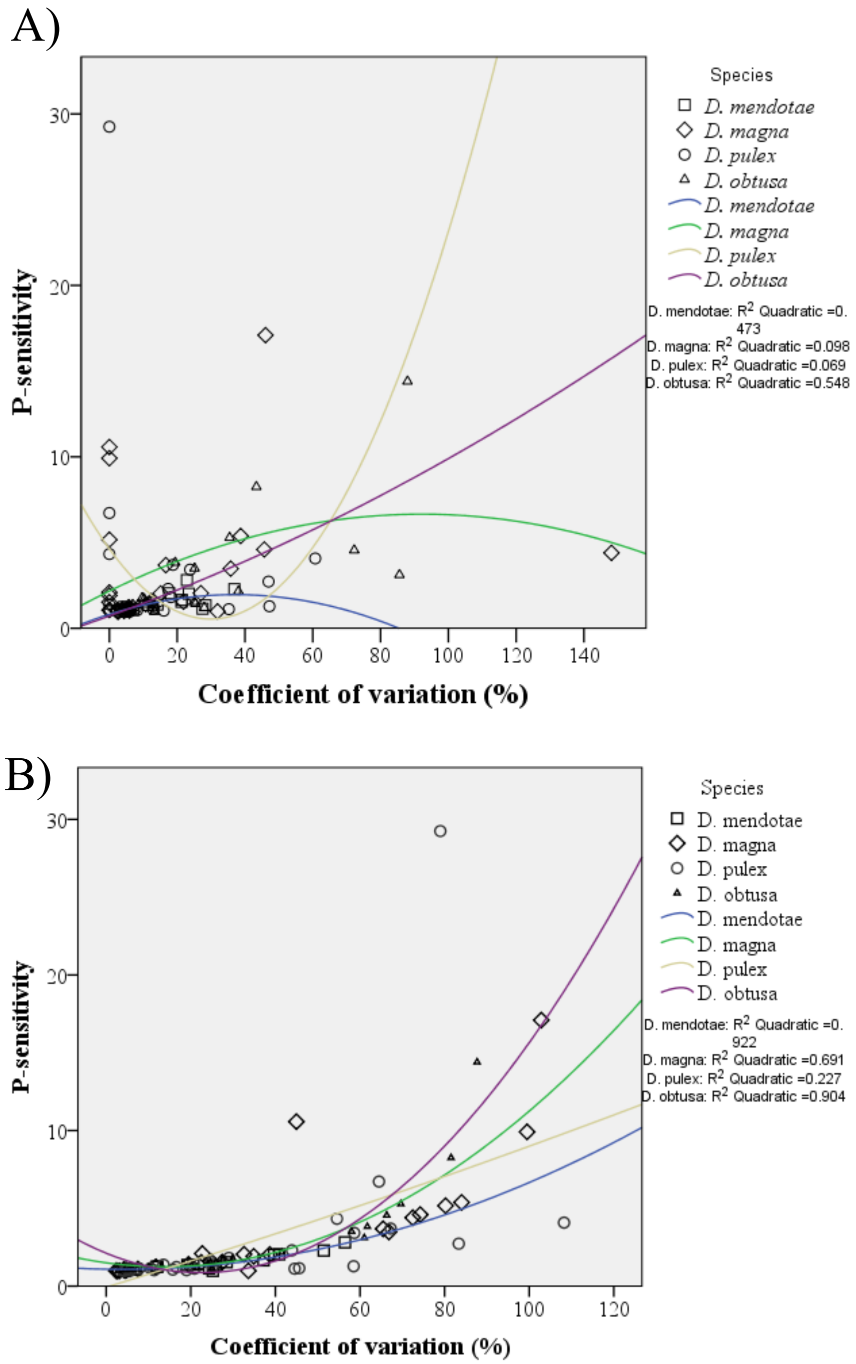
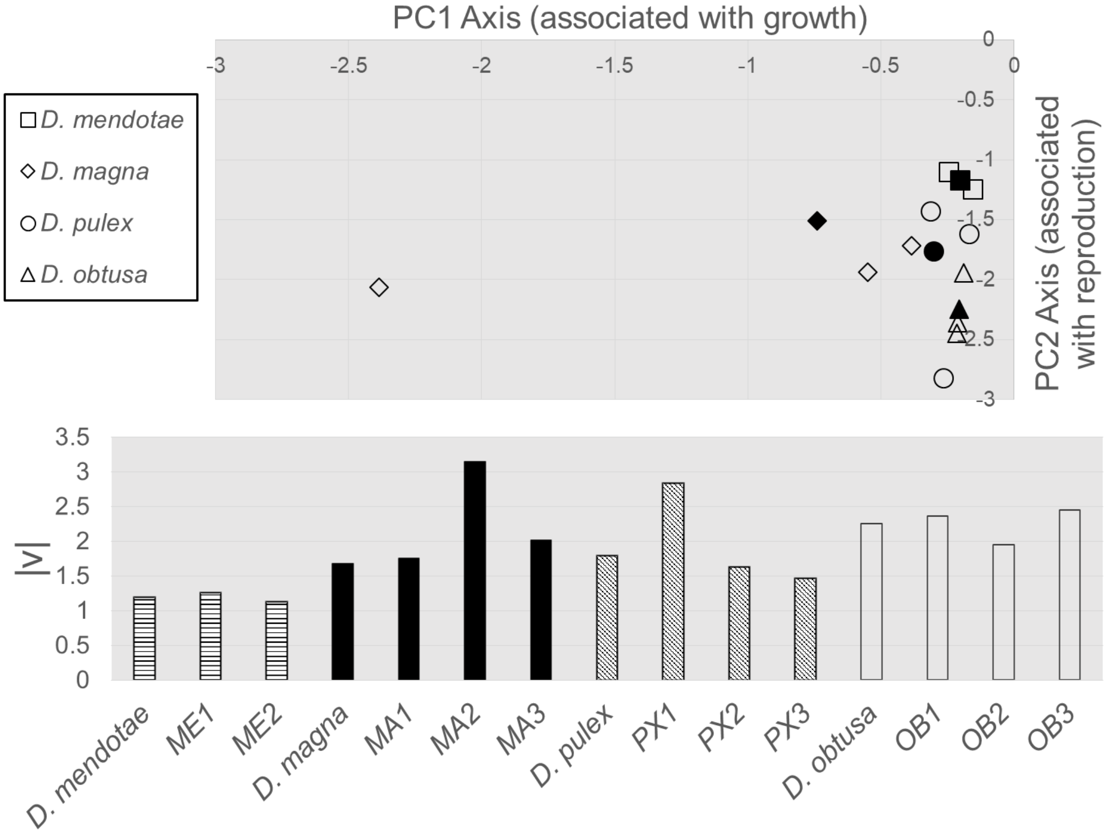


Figure 4. The centroid positions of *Daphnia* species in PCA space, and the magnitude of centroid change, |v|, between a nutrient rich (shaded-HiP) to nutrient poor (open-LoP) environment.



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Chapter Two: Is the individual more than the sum of its parts? Parameterization of biomass- and individual-based models under differing food quality environments

Rachel Hartnett

Abstract

Metabolic theory, which ties body size to population and community energetics, has been one approach in unifying an individual trait to ecosystem function. Models and predictions that have stemmed from this work use biomass as their common currency. However, population biology typically utilizes individual-based models to predict population size, extinction risk, and productivity. Direct comparison of these different types of models is needed to judge robustness across many taxa and environments. A life-history table experiment using four different species of an aquatic invertebrate, *Daphnia,* that were fed either high or poor quality food, was conducted to prime two models based upon life-history strategy: 1) using an individual-based and 2) using a biomass-based approach. I predicted that the biomass model will be less accurate across food quality systems, but more generalizable across *Daphnia* taxa. I further predicted that biomass models would be insensitive to changes in reproductive output. Results indicate that the biomass-based model is generalizable among *Daphnia* species, and insensitive to changes in food quality due to its emphasis on biomass scaling. Individual-based models continue to better incorporate system-specific properties, but are not generalizable across taxa or systems. Model development needs to continue toward a testable and generalizable model that can be effectively applied to better predict ecological processes.

Introduction

Attempts have been made in the past few decades to unify ecological theory using neutral models (Hubbell 2011), stoichiometry (Sterner and Elser 2002), metabolism (Brown et al. 2004), fundamental principles (Scheiner and Willig 2011), and energy flows (Loreau 2010). The theoretical underpinnings of these unifying principles predominately use continuous, rather than discrete, measurements and projections: mainly through biomass of organisms. Continuous functions use the biomass of species, rather than discrete individuals, in their prediction of community and ecosystem properties or function. Through the lens of conservation, can these functions be applied to predict real populations, communities, and ecosystems? Neutral models in particular have been debated; opponents of such models say conservation efforts benefit from full knowledge of how and when species succeed (Clark 2012), while proponents suggest full knowledge is not a reality and conservation can benefit from broader patterns (Rosindell et al. 2012).

Modeling approaches in ecology strive toward two key characteristics, testability and generality, which are often incompatible (DeAngelis and Gross 1992). Simple models that aim to grasp abstract properties of ecosystems (e.g., (May 2001)) are often not conducive to direct testing, but hope to be generally applicable across most, if not all, systems. Simple models do have predicted outcomes, but may not be able to predict the complexity of real systems (Evans et al. 2013). Unifying theoretical models are, by necessity, simple models due to their aim of being generalizable. On the other end of the spectrum, models that aim to describe a particular system’s mechanics are highly testable, but are species and population specific within a system. Ecologists have developed individual-based models (IBMs) to study the mechanisms behind a single population's demographic patterns. IBMs combine individual life-history data with discrete time intervals to project population growth and dynamics (Bacaer 2010). While these models are effective in applied conservation and management, they are normally too complex to be generalized across systems (Forbes et al. 2016).

In contrast biomass-based models (BBMs), use the amount of carbon, or food quantity, to simulate food availability (Nisbet et al. 2000). However, food quality can play a role in shaping life-history traits across taxa (Riddington et al. 1997, Ball et al. 2000). For the keystone freshwater herbivore, *Daphnia*, food quantity thresholds play an important role in growth, maintenance, and reproduction (Lampert 2011). Large-bodied *Daphnia* have lower food quantity thresholds due to higher filtering efficiencies (Burns 1969) and greater size range for food particles, and therefore are less likely to be food limited than small-bodied *Daphnia* (Gliwicz 1990). However, food quality, quantified by elemental ratios in the growing field of ecological stoichiometry (Sterner and Elser 2002), influences life-history traits (and population outcomes) as well. In many aquatic systems, phosphorus (P) has been found to be the most limiting nutrient (Wetzel 1983). P-limitation has been shown to have effects on *Daphnia* life-history traits such as growth, reproduction, and senescence (Dudycha 2003, Jeyasingh and Weider 2005). Effects can be dependent on population structure; for example in *Daphnia galeata*, juveniles are more impacted by food stress, which results in a decrease in growth rate and delayed maturity (Vanni and Lampert 1992).

This study compares the testability and generality of an IBM and a BBM, by using trait data from a life-history table experiment of four species of *Daphnia*, across differing food quality, to parameterize the models. de Roos and Persson (2013) have taken an interesting approach in developing a simple BBM and incorporating some intraspecific processes between adult and juvenile biomass. When comparing the mechanisms of a BBM to an IBM, the structured BBM model of de Roos and Persson is the best direct comparison to a stage-structured IBM due to their incorporation of population structure into their model.

Within this study, the goals of model comparison will be to: 1) check for generality; and 2) compare models for sensitivity among taxa and between environmental systems. Because the IBM uses discrete functions and can account for more variability in trait data, I predict that the IBM will be less generalizable across *Daphnia* species, but will be more accurate in predicting the amount of biomass populations generate under different levels of food quality. In contrast, I predict that the BBM will be generalizable across *Daphnia* species, but not between environmental systems due to its emphasis on biomass (via body length) and insensitivity to changes in reproductive output.

Methods

Study organism

In order to make parameter ranges biologically accurate, field data were mined from the freshwater zooplankter, *Daphnia*. These organisms are a well-established ecological model system with extensive studies on their life-history, resource use, and predator-prey interactions (Lampert 2011). Their populations have distinctive stage-structuring classified by instars. *Daphnia* are a cosmopolitan genus (Sarma et al. 2005, Lampert 2011). Three clonal lineages from four different *Daphnia* species (*D. magna*, *D. mendotae*, *D. obtusa*, and *D. pulex*) were collected from a variety of laboratory stocks. These clonal lineages span the three subgenera of *Daphnia*, and range across North America and Europe.

Life-history table experiment

A life-history table experiment was conducted on three clonal lineages for four different species of *Daphnia*. Measurements were taken for an initial body-length measurement (i.e., start length), body-length after first egg development was seen (i.e., length at maturation), number of clutches, clutch size, and body-length at the end of the experiment (i.e., end length). Juvenile growth rate was calculated from the difference between length at maturation and start length, as well as adult growth rate from the difference between length at the end of the experiment and length at maturation. See chapter one methods for more details.

Biomass-based model (BBM)

To accomplish this objective, the tested model is adapted from Yodzis and Innes (1992)population equations, as highlighted in de Roos and Persson (2013). This model incorporates a structured population of juveniles and adults with the population's resource, but uses biomass to track the population dynamics in a continuous function. This model was chosen for its incorporation of population structure, which lends itself better to direct comparison with a stage-structured IBM compared to other BBMs. The model is driven by the following equations for resource growth, juvenile biomass growth, and adult biomass growth.

The growth of the resource is determined by $G(R) = \rho(R_{max} - R)$ where ρ is the intrinsic turnover rate and R_{max} is the maximum growth potential of the resource. The consumer is modeled by ingestion rates, assimilation rates, and a maturation rate of the juveniles to adults. Juvenile ingestion is given by $\omega_J(R) = M_c R/(H_c + R)$, where M_c is the mass-specific maximum ingestion rate and H_c is the half-saturation density of the resource. Ingestion rates are differentiated between adults and juveniles by a factor of q. Thus, adult ingestion rate is the same, except modified by q as follows: $\omega_A(R) = qM_c R/(H_c + R)$. Assimilation is calculated by $v(R) = \sigma_c \omega(R) - T_c$ for both juveniles and adults, where σ_c is the conversion efficiency, $\omega(R)$ is the resource intake rate and adjusted for juvenile or adult ingestion rates, and T_c is the mass-specific maintenance rate. Juveniles mature at a rate of $\gamma\left(v_J, \mu_J\right) = (v_J(R) - \mu_J)/(1 - z^{\left(1 - \frac{\mu_J}{v_J(R)}\right)})$, where $v_J(R)$ is the net energy production of juveniles, μ_J is the background mortality of juveniles, and z is the newborn to adult consumer size ratio. This model is assuming that all assimilated resource is going to growth for the juvenile and reproduction for the adult (see discussion).

The dynamic equations are as follows for changes in resource, juvenile consumers, and adult consumers, respectively:

$$\frac{dR}{dt} = G(R) - \omega_J(R)J - \omega_A(R)A$$

$$\frac{dJ}{dt} = v_A(R)A + v_J(R)J - \gamma\left(v_J(R), \mu_J\right)J - \mu_J J$$

$$\frac{dA}{dt} = \gamma\left(v_J(R), \mu_j\right)J - \mu_A A$$

Individual-based model (IBM)

The model, adapted from Gotelli (2008), runs iterative, discrete steps to calculate population size. The Leslie matrix (Gotelli 2008) in this study has individuals moving uni-directionally toward increasingly-larger size-classes (Table 2). After reaching a size at maturation, individuals begin to reproduce, with fecundity increasing with size. For the purpose of this project, I will assume that *Daphnia* in the field spend approximately one fourth of their life as a juvenile (Schwartz 1984), which led me to have six adult size-classes in addition to two juvenile size-classes. Each offspring that is produced starts in the first size-class and has a probability of surviving and growing to a new size-class: $s_i*(1-g_i)$ where s_i is the class-specific survival and g_i is the class-specific growth rate. When an individual reaches maturity (i.e. size-class three and above), reproductive success is calculated by f_i*s_1 where f_i is the class-specific fecundity and s_1 is the survival rate of neonates.

Parameterization and model runs

Modeling simulations were run in MATLAB (Mathworks 2016a). BBM parameters changed by the user include: W_a, the estimated average body length (mm) of the species being modeled. W_a is used to calculate the other size-specific parameters (e.g., assimilation constant). Therefore, even this single parameter could have large differences in projected population outcomes. Biomass is calculated iteratively for resource, juvenile consumer, and adult consumer using the dynamic equations listed above. Biomass projections are carried out over 35 time steps, and each projection had 100 iterations. Vectors of total biomass and the proportion of juveniles (juvenile biomass: total biomass) were calculated for later analysis.

IBM parameters changed by the user include: juvenile size, juvenile growth, adult size, adult growth, and the average clutch size (Table 3). These parameters are used in determining the survivorship, stage-specific growth, and reproductive success in the Leslie matrix component of the model. Each iteration of the model generates a Leslie matrix using randomly drawn life-history traits, constrained by ranges from the life-history table experiment. Random traits were calculated by using stretchbetaval and betaval functions in MATLAB (Mathworks 2016a), which draw a random number from the user's mean and standard deviation of a trait to create survivorship, growth, and fecundity parameters. The stretched beta distribution was used when the minimum and maximum points did not range between 0 and 1 (Morris and Doak 2002).

As life-history data were collected as body lengths (mm), body length was converted into biomass using the conversion equation $W = \alpha L^{\beta} *10^{-6}$, where W is the organism's weight in milligrams, α and β are the intercept and slope of the length-weight regression, respectively, and L is the measured length in millimeters (McCauley 1984). A general length-weight regression for the wet weight of *Daphnia* was used with $\alpha = 4.3405$ and $\beta = 2.829$ (Watkins et al. 2011). Projections of the model were carried out over 35 time steps, and bootstrapped over 100 iterations. Biomass was then calculated from the summed lengths within juvenile and adult stages, and converted using the length-weight conversion equation. Vectors of total biomass and the proportion of juveniles (juvenile biomass: total biomass) were calculated for later analysis. Subsequently the change in biomass was calculated as Δ biomass = total biomass HiP – total biomass LoP/ (total biomass LoP) x 100.

Results

Species showed differences in both growth and reproductive life history traits in the life history experiment. Under low phosphorus conditions, all species showed smaller sizes at first reproduction and at the end of the experiment, with a 75% (±2.5 S.E.) reduction in size traits in low phosphorus conditions. They exhibited delayed onset of reproduction and had smaller clutch sizes (Table 2).

After parameterizing both models with the life-history data collected, the IBM was sensitive to system differences with an average change in biomass across systems of 236900% ± 16150 S.E., while the BBM had an average change in biomass of 10% ± 50 S.E. (Table 1). The projected amount of total biomass after 35 time steps was relatively consistent within the BBM, among *Daphnia* species and across high and low quality systems (35 mg ± 2.5 S.E., Figure 1). The IBM showed less consistency in projected total biomass across *Daphnia* species in the high phosphorus system (Figure 1a), but consistently projected low total biomass across *Daphnia* species in the low phosphorus system (Figure 1b).

The magnitude of change in biomass between high and low phosphorus environments was consistently small within the BBM, except in the case of *D. magna*. *D. magna*'s difference in projected biomass in the BBM compared to the other species is due to the proportional change in juvenile biomass. The percent of juvenile biomass decreases with low food quality (Figure 2b), resulting in a higher projected total biomass (Figure 1). The IBM model was sensitive to changes in food quality (Figure 2a); as reproductive success was diminished under poor food quality, more of the biomass was contained in the juvenile stage.

Discussion

The BBM was insensitive across high and low phosphorus conditions, showing little change in biomass across systems (Table 1), and was generalizable across taxa (Figure 1). The BBM proved to be insensitive to changes in food quality, as food quality mostly affected reproductive traits rather than traits associated with body length (Table 3). Food quality has particular effects on reproductive success across taxa (Bomford and Redhead 1987, Wacker and Elert 2003), which may explain the BBM's lack of response to high and low phosphorus parameters. Most of the BBM's variables are scaled with body size, using a mass-specific metabolic approach, and focuses on carbon availability in the system. However, traits associated with body length (i.e., biomass) in the life-history experiment were more constrained than reproductive traits (Table 3). The amount of carbon given in the life-history experiment (1 mgC/L/day) is considered to be a high level of food quantity (Lampert 2011). It has been shown that regardless of body-size, daphniids all follow a similar pattern of resource allocation to growth and reproduction under different levels of food (carbon) quantity (Dudycha and Lynch 2005). Therefore, the generality of the BBM among taxa does reflect a natural system of high food quantity.

The IBM was successful in modeling changes in biomass due to differing food quality. As reproductive output (i.e., number of clutches produced) decreased considerably in the low phosphorus environment, the IBM predicted substantial decreases in biomass across all *Daphnia* species. In addition, P-sensitivity differs depending on the *Daphnia* species. A recent study by Hood and Sterner (2014) showed that *D. mendotae* was strictly insensitive, or completely inflexible, in terms of body phosphorus content. *D. magna*, *D. pulex*, and *D. obtusa* exhibited similar sensitivities in their ability to change their body phosphorus content. This may account for the lack of change in the IBM total biomass in *D. mendotae* (Table 1). However, parameters that might predict sensitivity to phosphorous remain elusive, especially given that sensitivity to phosphorus is independent of body size (Tessier and Woodruff 2002b), habitat (Tessier and Woodruff 2002a, Seidendorf et al. 2009), and phylogenetic history (Seidendorf et al. 2009).

After model development, sensitivity analysis on the model is important to determine where data collection is most valuable to accurately parameterize the model and predict the system's parameters like the population's biomass. Sensitivity analysis yields the absolute change in a modeled outcome with an incremental change in a parameter (Caswell 2006). From such an analysis, the most sensitive parameters should be the focus of data collection, as their accuracy most strongly affects the modeled outcome. Theory from IBMs predicts juvenile survivorship has the strongest effect on population growth rate (Gotelli 2008), while empirical studies across a variety of taxa (i.e., insects to large mammals - (Miller et al. 1973, Hunter et al. 2010)) show that adult fecundity can also have strong effects in population growth rates. Juvenile stages have also been shown to have strong effects on population growth and competitive ability in BBMs (de Roos and Persson 2013).

Juvenile survivorship was high in the life history experiment, which may not reflect what is happening in the field if invertebrate predators are in the community (Schwartz 1984). Nevertheless, the parameterization of both models used high juvenile survivorship, yet the IBM had significantly lower proportion of juveniles, while the

BBM consistently had about a third of its biomass comprised of juveniles. The IBM showed increases in proportional juvenile biomass under low phosphorus conditions, while the BBM remained insensitive to juvenile biomass, except in the case of *D. magna*, which showed a sharp decrease in the proportion of juvenile biomass and an overall decrease in total biomass under low phosphorus (Figure 1, Figure 2b). This may be due to *D. magna*'s relatively large size in comparison with the other species; however, its proportional change in size is similar to the other species (Table 3). Regardless, the BBM was not able to accurately predict differences in population dynamics due to changes in food quality, leading back to the question of whether simple models can be adequately adjusted to remain generalizable, yet respond to changes in system properties.

Populations of many species are declining at an alarming rate due to environmental change (i.e., climate change, landscape-use change (Parmesan 2006, Turner et al. 2007)). Monitoring population declines through extensive demographic surveys are constrained by limited resources and time (Simberloff 1988), so modeling efforts are often implemented in order to give policy-makers informed recommendations. IBMs have been used in conservation biology efforts to protect particular life-stages that are critical to positive population growth rates (Olsen et al. 2004). For example, fisheries have a long history of over-exploitation. Modeling has been employed to predict available fish biomass and acceptable fishing limits, which has been effective in restoring global fisheries (NOAA 2016). Results presented here indicate that biomass alone is not sufficient to create a model that will be sensitive to changes in the system and generalizable across taxa. Future modeling efforts must integrate the detailed lines of individual behavior into the broad strokes of energy flows or biochemical processes (Grimm et al. 2017). Several authors have proposed that individual-based models (IBMs) showed the most promise in developing models that were both testable and generalizable (DeAngelis and Gross 1992, Grimm et al. 2017). And indeed, individual-based models that integrate stoichiometric principles show promise in balancing these desired characteristics (Smith et al. 2014, Kaiser et al. 2014). There is a great need to develop and test these integrated models for accuracy using demographic data under shifting conditions of food quantity and quality. Such advancement in modeling should increase the predictive value for use by a variety of stakeholders (e.g. conservation, management) in better understanding the dynamics of both natural and human-altered ecological systems.

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Tables

Table 1. Proportional change in biomass across systems among different species of *Daphnia*. Model comparison of the computed change in biomass (%), across parameter data collected from high and low food quality. The biomass based model (BBM) had a change in initial adult weight, while multiple parameters associated with growth and reproduction were changed in the individual-based model (IBM).

Species	BBM Δ biomass	IBM Δ biomass
D. magna	28	14299
D. mendotae	4	982
D. obtusa	5	71449
D. pulex	5	8020

Table 2. Leslie matrix generated by the individual-based model (IBM). Vital rates: survivorship (s_i), stage-specific growth (g_i), and reproductive success (f_i) were calculated using randomly drawn life-history traits, constrained by ranges from the life-history table experiment (Table 3).

0	0	f3*s1	f4*s1	f5*s1	f6*s1	f7*s1
s2	s3*(1-g1)	0	0	0	0	0
0	s3*g1	s4*(1-g1)	0	0	0	0
0	0	s4*g2	s5*(1-g2)	0	0	0
0	0	0	s5*g2	s6*(1-g2)	0	0
0	0	0	0	s6*g2	s7*(1-g2)	0
0	0	0	0	0	s7*g2	s7*(1-g2)

Table 3. Life-history traits for the four species of *Daphnia* under two environmental conditions (high-phosphorus food – HiP; low phosphorus food –LoP). Mean trait values for seven life-history traits are given. Traits from left to right are as follows: core body length at the start of the experiment (mm), body length at maturation (mm), juvenile growth rate (mm/day), body length at the end of the experiment (mm), adult growth rate (mm/day), the number of clutches, and the number of individuals in a clutch.

Species	Start Length (mm)		Length at Maturation (mm)		Juvenile growth rate (mm/day)		End Length (mm)		Adult growth rate (mm/day)		Number of Clutches		Mean Clutch Size	
	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP	HiP	LoP
D. magna	0.86	0.86	3.29	2.69	0.73	0.08	4.05	3.09	0.89	0.84	4.47	1.7	5.19	1.17
D. mendotae	0.56	0.58	1.25	1.17	0.76	0.74	2.28	1.85	0.54	0.64	5.25	5.2	2.72	1.73
D. obtusa	0.57	0.57	1.48	1.29	0.1	0.08	2.85	2.11	0.66	0.51	9.1	3.83	11.28	2.93
D. pulex	0.63	0.59	1.6	1.42	0.73	0.19	2.74	2.08	0.65	0.45	6.97	3.8	9.67	4.31

Figure Legends

Figure 1. Comparison of biomass among the four species of *Daphnia* used in this study. Total biomass (mg) was computed by parameterizing an individual-based model (IBM) and biomass-based model (BBM) with data collected from four *Daphnia* species and across: a) a high (HiP) and b) a low (LoP) phosphorus environment. Models were compared within systems to test outcome generality in high and low food quality systems, and among *Daphnia* species. Bars indicate ± 1 S.E., but may be difficult to see in b) as they are very small.

Figure 2. Change in the proportion of juvenile biomass across systems. The proportion of juvenile biomass (%) was computed for high (HiP) and low (LoP) phosphorus environments using collected life-history data to parameterize: a) an individual-based model (IBM) and b) biomass-based model (BBM). Reaction norms were plotted using a linear regression to test model sensitivity to food quality.

Figure 1. Comparison of biomass among the four species of *Daphnia*

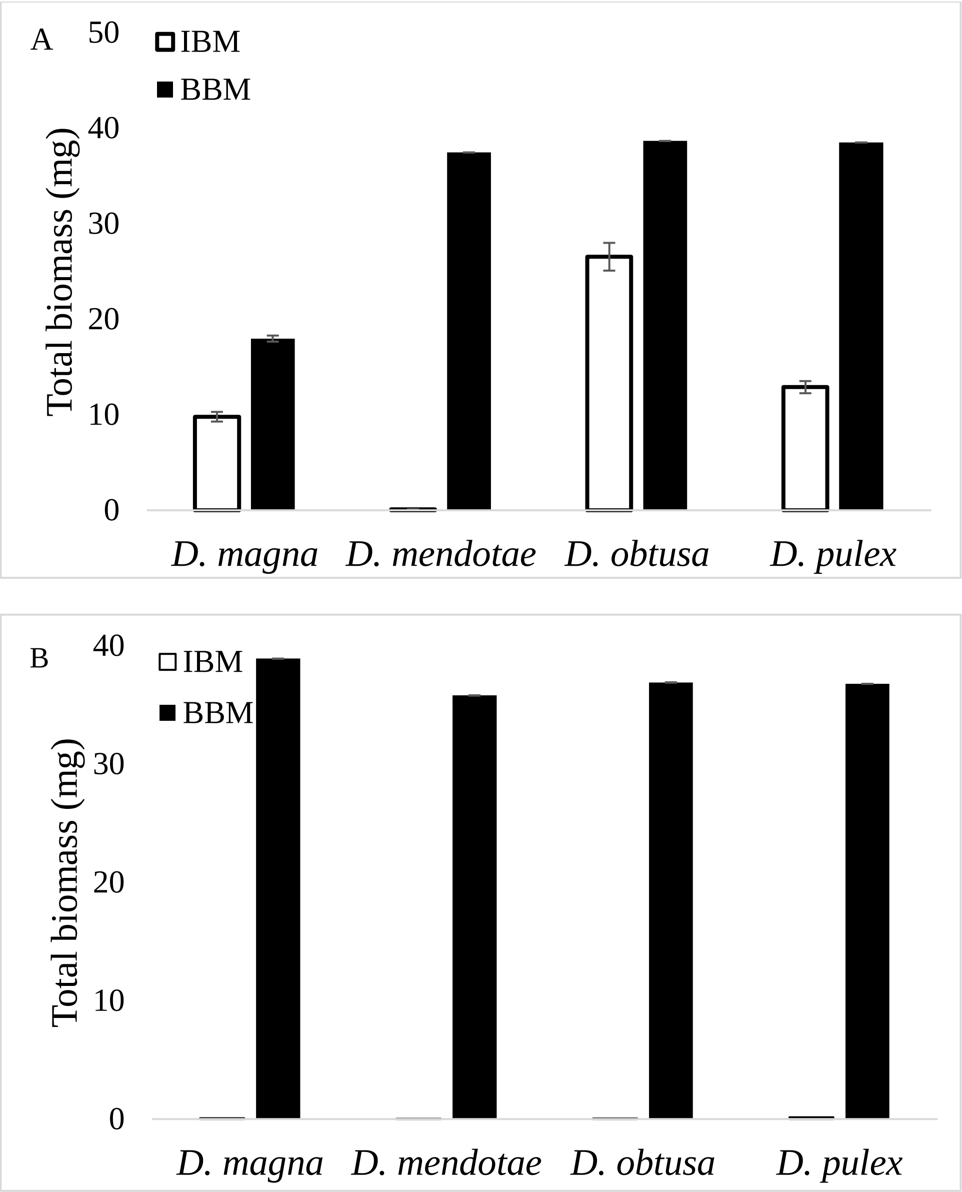
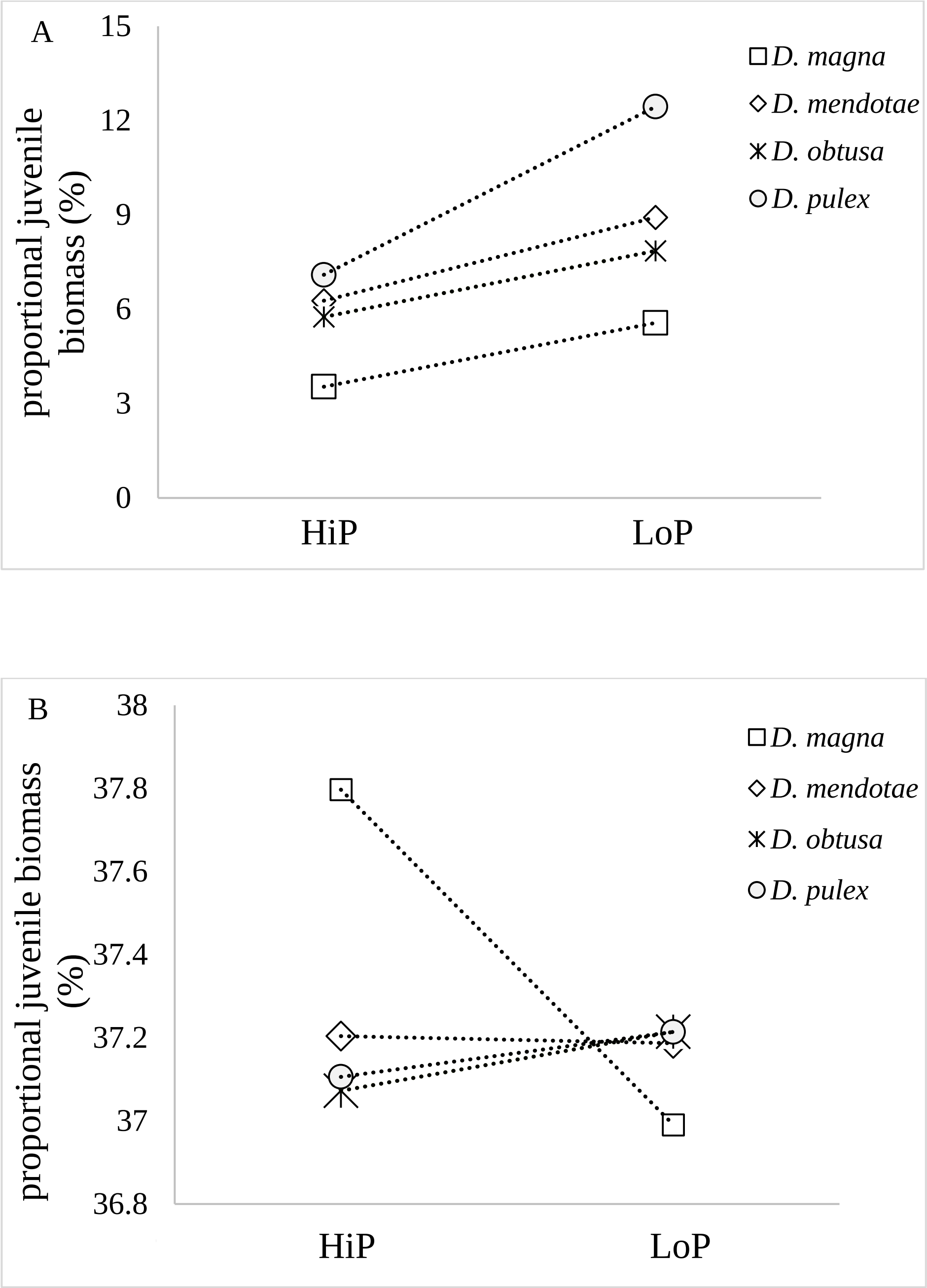


Figure 2. Change in the proportion of juvenile biomass across systems



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Chapter Three: Impacts of intraspecific and interspecific composition on body size-distributions and competitive interactions among *Daphnia*

Rachel Hartnett

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Abstract

As scientists become more certain that evolutionary processes can occur at the same rate as ecological processes, the role of intraspecific trait variation has been increasingly important in community ecology. Variation in body size has been used as a proxy for a wide array of functional traits. While extrinsic factors, like competition and predation, have been well-studied, intrinsic mechanisms shaping size distributions are less well known. This study aimed to look at the effects of intrinsic mechanisms, specifically population composition, intraspecific competition, and density-dependence, on shaping size distributions, in experimental assemblages of the keystone aquatic herbivore, *Daphnia*. I predicted that distributions would reflect dominance by individual clones, both within and among species assemblages, and small individuals would be favored under high densities. To test the effect of these mechanisms on size-distributions, single clone populations, mixed clones, and mixed species assemblages were set-up in microcosms. Size-distribution shifts as well as clonal composition changes were monitored. Body size distributions remained consistent throughout the experiment, with species differentiating by median size and interquartile range (IQR). I predicted that the largest species tested, *Daphnia magna,* would have complete competitive dominance among mixed species assemblages based on traditional niche and allometric theory; however this was not the case. It appears intraspecific competition may be inhibiting dominance at the species level. Intraspecific competition may explain weak trends between niche overlap and coexistence among clones. In light of these findings, intrinsic factors may play a larger role in shaping zooplankton composition than previously recognized.

Introductions

Intraspecific variation has experienced a resurgence of interest in community ecology (Agrawal *et al.*, 2007; Bolnick *et al.*, 2011; Violle *et al.*, 2012), especially in cases of dominant species (Whitham *et al.*, 2006). Intraspecific variation is modulated directly by evolutionary processes, which allows community ecology to account for evolutionary processes as well as ecological consequences operating on a population from observed patterns. Indeed, researchers are becoming increasingly aware of the interplay between evolution and ecology (Hairston *et al.*, 2005; Carroll *et al.*, 2007; Stoks *et al.*, 2016) and the positive ecosystem-wide effects such as faster ecosystem recovery, increases in primary production, and increases in species richness from increased intraspecific variation (Reusch *et al.*, 2005; Crutsinger *et al.*, 2006; Hughes *et al.*, 2008; Gibert *et al.*, 2015).

Intraspecific variation is thought to exert a positive effect on populations by buffering effects from biotic and abiotic factors (Oney *et al.*, 2013), increasing niche diversity (Van Valen, 1965), and increasing individual specialization (Bolnick *et al.*, 2003). Intraspecific variation can also promote coexistence between species in cases where intraspecific variation results in stronger negative control on conspecifics rather than heterospecifics (May, 2001; McPeek, 2012); although intraspecific variation could decrease the chances of species coexistence by increasing niche overlap of competing species (Hart, Schreiber & Levine, 2016). The resulting diversity due to both intraspecific diversity and increased richness from species coexistence can impact community structure and ecosystem processes (Crutsinger *et al.*, 2006).

In particular, variation in body size has been extensively studied because of its strong linkages to life-history traits (Kerr 1974), body energetics (Brown *et al.*, 2004), and demography (Brooks & Dodson 1965). Research into the role of body size has been shown, in a variety of taxa, to influence critical life-history traits like recruitment (Cargnelli & Gross, 1996; Rode, Amstrup & Regehr, 2010), reproduction (Levitan, 1991; Wiklund & Kaitala, 1995; Dickerson *et al.*, 2005), and survivorship (Cargnelli & Gross, 1996; Congdon *et al.*, 1999). Body size is considered to be a trait that accounts for other critical growth and reproductive traits that would determine fitness due to allometric scaling patterns found across geographic scales and taxa (Gould, 1966; West, Brown & Enquist, 1997; Elser *et al.*, 2010). Because of the importance associated with body size, ecologists have commonly used body-size distribution (BSD) data to tie individuals and populations to community-level functions (Jennings *et al.*, 2001; Cohen, Jonsson & Carpenter, 2003; Downing *et al.*, 2014).

In general, ecological communities of both plants and animals have abundant small species and few larger species, following a reciprocal function. Predominant theory and empirical work has focused on how body-sizes are shaped by different extrinsic forces such as prey composition and availability, predator pressure, habitat heterogeneity and species composition (reviewed in Peters, 1983; Kozłowski & Gawelczyk, 2002; Purvis, Orme & Dolphin, 2003). In animals, it is proposed that selective forces favor large-bodied individuals that are better able to escape predation and consume more resources, more efficiently (Brooks & Dodson, 1965; Hall & Threlkeld, 1976); however energetic costs, predation pressure, and nutrient limitation restrict the abundance of larger individuals and results in many more small-bodied species (Brown, Marquet & Taper, 1993; Wahlström *et al.*, 2000; Cottenie *et al.*, 2001). In plants, it has been proposed that adaptations to the niche occupied by large plants would have been uncommon until recent evolutionary time, that small species would be more differentiated, and/or smaller plants would have higher fecundities (Aarssen, Schamp & Pither, 2006).

The zooplankter, *Daphnia*, hold a key functional niche, as important grazers in lakes and ponds, and are often dominant when present in an aquatic system. *Daphnia* size is known to determine grazing rates (Burns, 1969), competitive ability (Lynch, 1977; DeMott, 1989), and predation risk (Brooks & Dodson, 1965; Stibor, 1992; Jeyasingh & Weider, 2005) within and among *Daphnia* species. *Daphnia* generally follow the expectation of the size-efficiency hypothesis (Brooks & Dodson, 1965; Hall *et al.*, 1976) in which large-bodied *Daphnia* species are expected to dominate under high food quantity and quality, and low predation risk, while small-bodied *Daphnia* dominate in communities with high predation risk or poor food quality (DeMott, Gulati & Van Donk, 2001; Iglesias *et al.*, 2011). The BSD and taxonomic composition of zooplankton communities will shape phytoplankton community structure, thereby influencing overall aquatic function (Cyr & Curtis, 1999). It is known that extrinsic factors, predation rate and resource abundance, help shape overall zooplankton composition and BSD, but the role of intrinsic factors is less studied.

One intrinsic factor, density dependence, is thought to influence small-bodied individuals more than large-bodied ones due to the overlapping prey availability of small and large organisms, in which large organisms have the advantage (Werner & Gilliam, 1984; Cottenie *et al.*, 2001). Therefore, increases in population density, going from exponential to log growth phases, results in trends favoring large-bodied individuals (Lynch, 1977; Kozłowski & Gawelczyk, 2002). However the effects of other intrinsic factors such as intraspecific variation and intraspecific competition on body-size distributions has received less attention (but see, Weiner, 1985).

The questions that I set out to address were: (1) at the population level, to what extent will shifts in body-size distributions (BSDs) be due to changes in population composition? (2) how does intraspecific variation in body size affect competitive ability and eventual coexistence of clones and species?

I conducted a competition experiment that consisted of single and mixed-species assemblages to test these predictions in the model organism, *Daphnia*. For this study, I have used a *Daphnia* assemblage consisting of three clones of four different species spanning the genus, including representatives from each of the three subgenera that have been shown to exhibit different growth and reproductive schedules within and between species (Hartnett, unpublished). I varied clonal and species composition within microcosms being fed a single food source to look at the effects of intra- and interspecific variation in body size on shifts in size distributions and species coexistence.

Methods

2.1 *Daphnia* collection and maintenance

This work was conducted using four species of *Daphnia* (*D. magna*, *D. mendotae*, *D. obtusa*, and *D. pulex*). Three clonal lines from each of these *Daphnia* species were kept in stock cultures of 8 L buckets containing an artificial pond-water medium, COMBO

(Kilham *et al.*, 1998). I mass-cultured these clonal lines, by feeding the buckets *ad libitum* with a single species of the green algae, *Scendesmus acutus*.

2.2 Microcosm set-up and sampling

To synchronize the age/size-structure of experimental animals, gravid females (N=20-60 per clone depending on clutch size) were raised in 700ml of COMBO and fed daily with *Scenedesmus acutus* (1mgC/L). Neonates were removed daily. When possible, <24hour neonates (*Daphnia mendotae*) were used for experimental animals, if not, it was acceptable to pool <48hour (*D. pulex, D. obtusa*) or even <72 hour animals (*D. magna*). 900ml glass jars filled with 700ml COMBO were inoculated with individuals (N=12). Single clone treatments had 12 animals from a single clone, multi-clonal treatments had four individuals each from three clones, and multi-species treatments had two species, each with two individuals from each of six clones (three clones from each species). Jars were then fed *Scenedesmus acutus* (1mgC/L) every day for eight weeks. Initial samples were taken (t= day 0), after a period of time to grow to a substantial density (t= day 28), and subsequently at two-week intervals (t= day 42, day 56). The experiment was maintained at ambient (20-22^{o}C) room temperatures under naturally fluctuating light conditions.

On each sampling day, ephippia, resting eggs produced sexually, were removed and enumerated. Subsampling was conducted by first sealing the jars with water-tight lids, gently inverting the jars three times (to mix the contents), and then decanting off a 100ml aliquot per experimental jar. Jars were then replenished with fresh COMBO up to the original 700 ml. Aliquots were filtered through 540 um Nitex ® mesh into a 125ml plastic bottle. The mesh samples containing the daphniids were placed into

60mm x 15mm x 1.5 mm petri dishes with a Kimwipe ® (to absorb excess media), covered, and stored at -20° C until sample processing. The coarsely-filtered media was stored overnight at 4° C. The next day, *in vitro* chlorophyll-a (chl-a) was extracted and measured by filtering the media through 25mm GF/F filters (#1825-025), and grinding this filter in 90% acetone. After incubating for 3-4 hours, the filter/acetone was spun down using a Eppendorf centrifuge (Model 5804) at 1500 rpm for five minutes. Then chl-a was measured (after calibration from a random subsample) using the chl NA module in a Turner model TD 700 bench top fluorometer (Turner Designs, Sunnyvale, California, USA).

2.3 Sample processing

Samples were processed for: 1) body size distribution and 2) clonal identification using allozyme markers. Size distributions were recorded for each sample using a MOTICAM 2300 digital camera and software system (Motic®, S-05165) mounted to an Olympus BX51 compound dissecting microscope. Length measurements were calculated from the top of the eyespot to the base of the core body, right above the top of the tail-spine. Tail-spine is known to be morphologically plastic depending on environmental conditions, and was not measured with core length due to potential confounding length measurements. Next, I haphazardly sampled animals (~20 per sample) from the frozen sub-sample by taking a random quadrat (using a random number generator) and selecting large individuals within the quadrat. For the multi-species treatments, individuals were identified to species before conducting the allozyme screening. Allozymes were run using standard methods (Hebert & Beaton, 1993) for two loci per species. Allozymes included: *phosphoglucose isomerase* (PGI, EC 5.3.1.9), *phosphoglucomutase* (PGM, EC 5.4.2.2), *glutamate-oxalacetate-transaminase* (GOT, EC 2.6.1.1), and *mannose-6-phosphate* isomerase (MPI, EC 5.3.1.8). *D. mendotae* were identified using PGI, *D. magna* were identified using PGM and GOT, *D. obtusa* were identified using MPI, and *D. pulex* were identified using a combination of PGI, PGM, GOT and/or MPI depending on the species treatment.

2.4 Statistical analyses

Analyses for body size distributions were calculated using two metrics: 1) median size as a proxy of central tendency and 2) interquartile range (IQR) as a metric of variation. As variation and central tendency are used to describe distributions, median size and IQR were used to determine significant effects of species and clonal composition on body-size distributions (BSDs) over time using a profile analysis (Greenhouse & Geisser, 1959). A profile analysis takes a multivariate approach to the repeated measures ANOVA and determines main effects of clonal and species composition, main effects of time, and interaction effects between time and main effects. Significance testing of the profile analysis uses Hotelling's Trace, a modified F-statistic, similar to that used in a repeated- measures ANOVA. The significance threshold value was Bonferroni-corrected for running multiple tests to $\alpha=0.025$. In addition, size distributions were compared using a distribution overlap index, DOI, where distributions were standardized to sum to 1, and then indexed by: $DOI = \sum_{k=1}^{p} \left|\left(y_{ak} - y_{bk}\right)\right|$, where DOI compares size distributions between sites a and b for each size bin, k (Ernest, 2005). Because of this standardization, values of DOI are expected to range from 0 (complete overlap) to 2 (completely non-overlapping).

Significance testing was done using the Kolmogorov-Smirnov test for goodness-of-fit (Sokal & Rohlf, 1995).

Clonal dominance was determined to be stochastic (null expectation) or deterministic, using a G-test (Sokal & Rohlf, 1995). Pooled G (G_p), G for heterogeneity (G_H), and total G (G_T) were calculated to account for variation within treatments (i.e., repeated measures). Multiple G tests were accounted for by correcting the significance threshold value to $\alpha = 0.005$. Total ephippial counts were analyzed using a repeated measures ANOVA, with species identity and treatment type (i.e., single clones, pooled clones of a single species, or mixed clones of paired species). All statistical tests were run using SPSS (Version 20, IBM).

Results

3.1 Changes in body-size distributions

Median body size for individual species did not show an overall trend from small to larger-bodied individuals, indicating that populations were in log-phase population growth (Figure 1). In addition, variation in size distributions was conserved throughout the experiment (Figure 1). A profile analysis confirmed that median size and IQR did not change over time, nor was there a significant time by species interaction (Table 1). However, species composition did have a significant effect on median body-size (profile analysis, F = 35.639, P < 0.001) and body size variation (profile analysis, F = 3.815, P = 0.013) (Table 1). Post-hoc pairwise comparisons of the profile analysis indicated that *D. magna* IQR differed significantly from *D. mendotae* (Tukey HSD, P<0.001), *D. obtusa* (Tukey HSD, P=0.006), and *D. pulex* (Tukey HSD, P = 0.007).

Clonal composition did not play a significant role in influencing body size variation. See figures 4 and 5 for size information within each species.

Analysis of DOIs revealed that pairwise comparisons showed marginal amounts of overlap between distributions (range 0.11 to 1.11), with the most distinct distributions (i.e., greatest degree of non-overlap) being found, 1) between two *D. magna* clones, and 2) pairwise comparisons between species distributions within mixed species treatments. However, DOIs did not correlate significantly with coexistence among clones (Figure 2, r^2=0.04). Nor did DOIs differ significantly among species. Density of the species in jars did differ among single, pooled, and mixed diversity treatments (repeated measures ANOVA for clonal type, F= 8.31, P=0.002), but did not differ significantly over time or within species, with the exception of *D. obtusa* (Figure 1). IQR did show a weak correlation with abundance in samples (Pearson's correlation between IQR and abundance r^2=0.195, P<0.01) (Figure 3).

3.2 Clonal dominance

Clonal composition remained mixed throughout the experiment for multi-clonal treatments. Clonal composition consisted of 2.06 (mean) ± 0.61 (SD) clones per microcosm (700 ml) by the end of the experiment. Within the multi-species treatments, clonal composition slightly increased with an average of 2.89 (mean) ± 1.00 (SD) clones per microcosm (700 ml). From the G-test analysis, clonal competition appears to be *stochastically* determined in the pooled clonal cultures of *D. mendotae*, and deterministically in all other treatments (see Table 2 and Table 3 for more details). Total ephippia production was high in one clone of *D. mendotae* (mean 77 ±50 (SD) ephippia/700 ml), and all three clones of *D. obtusa* (mean 57 ± 50 (SD) ephippia/ 700 ml). Pooled clones of single species had relatively low ephippial production across species (mean 18 ± 23 (SD) ephippia/700 ml), while multi-species ephippial production was moderate (mean 37 ± 27 (SD) ephippia/ 700 ml). Within the multi-species samples, *D. obtusa* had consistently higher production (mean 70 ± 2 (SD) ephippia/700 ml) than their paired species. *D. magna* had lower relative ephippial abundances except when paired with *D. pulex*. See figure 6 for species composition ranked by ephippia totals. A repeated measures ANOVA shows significant effects of sampling time (repeated measures ANOVA, df = 2, F = 35.31, P <0.0001), and of species identity over sampling events (repeated measures ANOVA, df = 12, F = 14.44, P < 0.0001). But there was not a significant effect on treatment type (repeated measures ANOVA, df = 2, F = 0.42, P = 0.659) nor their interaction.

Discussion

Based on theory (Peters, 1983), changes from small to larger-bodied individuals are expected as a population reaches carrying-capacity. In the present study, however, neither median body size, interquartile range (IQR), nor density differed among sampling times, indicating that sampling was done after populations' had reached the log-phase of growth (Figure 1, density not shown). This is important to note, as therefore, I am unable to say anything about shifts in population-level body size distributions (BSD) or composition during colonization, but instead report on patterns in population composition and BSD after establishment.

4.1 Evidence of minimal niche partitioning within and between Daphnia species

Niche partitioning between large and small-bodied *Daphnia* species has been well-studied, with primary focus on the competitive advantage of large-bodied *Daphnia*

(Brooks & Dodson, 1965; DeMott, 1989), with a strong cost in cases of high predation and poor food quality (DeMott *et al.*, 2001; Iglesias *et al.*, 2011). The capacity of *Daphnia magna* to span the body size range of other congeners (Figure 1) may give the species a competitive advantage in terms of niche width. Intraspecific variation was weakly positive, but significantly, associated with density (Figure 3, Pearson's correlation between IQR and density $r^2=0.195$, $P<0.01$), which may indicate that an increase in niche width of a *Daphnia* population can increase carrying capacity of a population. This gives further evidence that BSDs may have important implications in population success. This finding, along with the above implications of *D. magna's* niche capacity, suggests that there is no potential cost for species dominance in these microcosms. However, allometric constraints on density were still apparent in this study. In addition, in nature, *D. magna* would be most susceptible to visual predation; often a tradeoff can be seen between zooplankton body size and predation rates (Brooks & Dodson, 1965; Iglesias *et al.*, 2011).

BSDs have a long history as a proxy for niche width (Wilson, 1975; Werner & Gilliam, 1984; Scharf, Juanes & Rountree, 2000). Due to allometric relationships, BSDs should encompass demography and productivity of a community (Enquist, West & Brown, 2009), although there is evidence that size distributions of mammals may not follow these trends (Ernest, 2005). While relationships between mean body size and abundance are well-characterized (reviewed in White *et al.*, 2007), the connection between niche width and body size is less well-known (Heino, 2005); although there seems to be a positive correlation between body size and niche width in marine systems (Costa, 2009). Few studies have looked into directly testing the relationship between

BSDs, niche width and overlap, and coexistence. One such study in birds found that competition strength increased when bird body mass was more similar (Leyequién, Boer & Cleef, 2007). Here, differentiation in niches is minimal via BSDs (e.g. low DOIs). Hart et al. (2016) indicated that individual variation within niches may ultimately weaken coexistence between species by reducing the ratio between intraspecific and interspecific competition. My results support this theoretical prediction, although the mechanism cannot be directly tested in this current study.

4.2 Species coexistence occurs despite overlapping niches, due to strong intraspecific competition

Competition within and among *Daphnia* clones has been shown to reduce coexistence of clonal assemblages. For example, previous work (Weider *et al.*, 2005; Weider, Jeyasingh & Looper, 2008) using a *D. pulex* x *D. pulicaria* hybrid clonal assemblage found rapid erosion of genetic diversity (as measured by the effective number of clones) along a manipulated gradient of food quality and quantity. These authors noted that loss of diversity was slowed under poor food quantity and quality conditions. In the present study, I tested coexistence on a single resource, which should produce higher levels of coexistence when conspecific competition is greater than interspecific competition (May, 2001; McPeek, 2012). Clonal diversity was winnowed away slightly at the intraspecific level, while it was winnowed away more significantly at the interspecific level (with an average loss of one clone within species and an average loss of 3 clones between species). Further, clonal diversity was significantly different from a uniform distribution. Stochastic processes such as priority effects and environmental stochasticity were reduced, if not completely removed, by the experimental design.

Genetic drift due to low starting densities and diversity bottlenecks (as a potential mechanism for the observed reduction of clonal diversity) is possible, even though most clones of each species were represented at the end of the experiment. In particular, *D. mendotae* clones were prone to extinction in some paired species treatments, and may have been more susceptible to the impact of drift. However, pairwise coexistence of all species was maintained on a single resource, and the mechanism(s) must be intrinsic (barring bacterial contributions), indicating that mechanisms driving intraspecific competition to interspecific competition will be important in predicting the presence of co-occurring species.

Daphnia are able to switch their reproductive mode from asexual reproduction of genetically-identical daughters to sexual reproduction of males and ephippia during times of food stress (Epp, 1996). The increased ephippial production from single species to multiple species seen in this study (Figure 6) indicates some additional level of stress with the addition of conspecifics. Previous work (Burns, 2000) has shown that crowding conditions induce conspecific cues, independent of food depletion, among *Daphnia* that slow the growth of smaller individuals, while larger individuals appear primarily unaffected, potentially giving an additional advantage to larger individuals. This could explain the greater abundance of *D. magna* found in my mixed species assemblages as they are the largest species, although there is other evidence of allelopathy from previous work that shows negative effects on life-history traits in this species (Matveev, 1993; Goser & Ratte, 1994). These signaling mechanisms should lead to dominance of larger-bodied species, which we see in this present study; however it would not explain the prominence of coexistence with smaller species.

As seen here, increasing species and clonal composition proved to have minimal effects on BSDs. This has also been seen in a previous study that examined *Daphnia* resource competition when measuring grazing rates under increasing clonal and species richness (Hargrave, Hambright & Weider, 2011). These authors found that monocultures of *Daphnia* were just as efficient at grazing, as multi-clonal and multi-species assemblages. They concluded that this was likely due to a combination of chemical and mechanical interference competition, perhaps due to the homogeneous nature of the environment or due to high functional overlap among *Daphnia* assemblages.

Beyond allelopathic signaling and intraspecific control, the coexistence of smaller-bodied organisms could be due to a switch in feeding behavior. DeMott & Kerfoot (1982) showed previously that the small-bodied cladoceran, *Bosmina*, was able to coexist with *Daphnia* by more efficiently removing high-quality food items. *Daphnia* species have also been shown to feed on bacteria, especially under competitive conditions by selectively feeding using the setae found within their filtering appendages (Burns, 1969; Peterson, Hobbie & Haney, 1978; Geller & Müller, 1981; Hessen, 1985). Bacterial variance in composition and productivity was not included in the scope of this current study, so I cannot say whether coexistence is due to selectivity of smaller species on bacteria or other small food particles. A third alternative would be that resource competition was not strong enough of a force to drive exclusion of lineages. However, chl-a analysis indicated that chl-a remained at mesotrophic levels (mean = 21.2 ± mg/L), which is fair ground for competition to occur (Carney & Elser, 1990). Chl-a levels increased significantly over time (i.e., accumulated) only in samples of *D.*

mendotae or samples that went extinct over the course of the experiment. This may explain why *D. mendotae* did not show clonal abundances significantly different from a uniform distribution, and it also provides evidence that *D. mendotae* may be a poor competitor due to an inability to graze a single resource as efficiently as the other three species.

Conclusions

Results from this study, indicate that intraspecific interactions may play an under-appreciated role in interspecific interactions. Taken together, these different levels of interaction impact body-size distributions (BSDs) of organisms in communities, as exemplified in the present *Daphnia* case. It is clear that research into the maintenance of BSDs needs further exploration, particularly in testing the relative effects between intraspecific and interspecific competitive interactions. In a rapidly changing environment, understanding mechanisms and drivers is critical in moving toward predictive ecology and applied efforts. Understanding changes in BSDs is especially important in aquatic systems, given that metabolic and trophic ecology studies have used zooplankton size distributions as proxies for community functions such as grazing rates and prey availability, as well as overall ecosystem productivity and top-down versus bottom-up control (Pace, 1986; Carpenter & Kitchell, 1996). Size shifts due to intrinsic mechanisms via intra- and interspecific variation will inform ecological processes at all scales, from individuals to ecosystems.

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Tables

Table 1. Significance testing of body-size distributions (BSDs) was examined via profile analyses for central tendency (median) and variance (interquartile range). A profile analysis gives information on significant differences between groups (main effect), over time (flatness), or if there was an interaction between groups and time (parallelism). We looked at species for group membership. The significance threshold value (α) was corrected for running multiple simultaneous tests using the Bonferroni correction, resulting in α = 0.025.

	Median				**Interquartile Range**		
	F	**df**	**P-level**		**F**	**df**	**P-level**
Main effect	35.64	3.00	>**0.001**	Main effect	3.82	3.00	**0.01**
Flatness	2.09	2.00	0.13	Flatness	1.39	2.00	0.25
Parallelism	2.87	6.00	**0.01**	Parallelism	2.03	6.00	0.06

Table 2. Goodness of fit tests of allozyme data using the G-statistic, under the null expectation of a uniform distribution across clones. G-tests were partitioned into information about individual replicates matching null expectation, homogeneity between replicates (G for heterogeneity or G_H), information from pooling the replicates (pooled G or G_p), and whether the data as a whole fit null expectations (total G or G_T). The significance threshold value (α) was corrected for running multiple simultaneous tests using the Bonferroni correction (resulting in α = 0.005). Significant values of G are in bold.

	Tests	df	G	Subject	df	G		Tests	df	G	Subject	df	G
ME	Gp	1	-	1,1	1	1.02	OB	Gp	2	**101.6**	1,1	2	**26.99**
	G_H	17	20.47	1,2	1	5.55		G_H	30	**178.3**	1,2	2	**14.82**
	G_T	18	0.00	1,3	1	1.39		G_T	32	**279.9**	1,3	2	**19.85**
				2,1	1	0.40					2,1	2	9.75
				2,2	1	0.11					2,2	2	**18.05**
				2,3	1	2.77					3,1	2	**13.86**
				3,1	1	0.33					3,2	2	5.55
				3,2	1	1.39					3,3	2	**20.48**
				3,3	1	0.20					4,1	2	10.24
				4,1	1	4.16					4,2	2	**18.80**
				4,2	1	0.40					5,1	2	**10.41**
				4,3	1	0.40					5,2	2	**15.47**
				5,1	1	0.40					5,3	2	**28.96**
				5,2	1	1.65					6,1	2	**22.19**
				5,3	1	1.05					6,2	2	**28.96**
				6,1	1	0.40					6,3	2	**15.56**
				6,2	1	0.14					Total	32	279.9
				6,3	1	0.05							
				Total	18	21.82							

	Tests	df	G	Subject	df	G		Tests	df	G	Subject	df	G
MA	Gp	2	3.18	1,1	2	5.45	PX	Gp	2	-	1,1	2	7.00
	G_H	30	**84.03**	1,3	2	7.41		G_H	26	**84.91**	2,1	2	3.14
	G_T	32	**87.21**	2,1	2	1.77		G_T	28	**0.00**	2,2	2	4.26
				2,3	2	8.58					2,3	2	**15.47**
				3,1	2	**16.76**					3,1	2	0.19
				3,2	2	6.59					3,2	2	0.26
				3,3	2	7.78					3,3	2	**11.87**
				4,1	2	4.50					4,1	2	1.48
				4,2	2	2.77					4,2	2	8.79
				4,3	2	2.98					4,3	2	**27.04**
				5,1	2	0.00					5,1	2	4.26
				5,2	2	**8.79**					6,1	2	8.32
				5,3	2	0.66					6,2	2	3.17
				6,1	2	4.39					6,3	2	10.28
				6,2	2	0.00					Total	28	105.5
				6,3	2	**8.79**							
				Total	32	87.21							

	Tests	df	G	Subject	df	G		Tests	df	G	Subject	df	G
MEx	Gp	4	**-**	1,1	4	12.11	MEx	Gp	4	**-**	1,1	4	**16.66**
	G_H	64	**229.3**	1,2	4	7.33		G_H	44	**113.5**	1,2	4	11.09
	G_T	68	**0.00**	1,3	4	0.00		G_T	48	**0.00**	1,3	4	**28.71**
				2,1	4	9.87					2,1	4	**17.51**
				2,2	4	0.00					3,1	4	**20.25**
				3,1	4	**25.17**					3,3	4	**71.53**
				3,2	4	11.09					4,1	4	5.38
				3,3	4	13.32					4,2	4	13.18
				4,1	4	4.65					5,1	4	**19.41**
				4,2	4	13.91					5,2	4	**17.49**
				4,3	4	**44.36**					6,1	4	6.34
				5,1	4	8.29					6,2	4	9.36
				5,2	4	**16.75**					6,3	4	**23.19**
				5,3	4	**36.65**					Total	52	260.1
				6,1	4	**19.52**							
				6,2	4	**15.79**							
				6,3	4	8.29							
				Total	68	247.0							

	Tests	**df**	**G**	**Subject**	**df**	**G**		**Tests**	**df**	**G**	**Subject**	**df**	**G**
MEx	Gp	4	**-**	1,1	4	**48.03**	MAx	Gp	5	**97.19**	1,1	5	4.39
	G_H	52	**155.3**	1,2	4	**16.79**		G_H	75	**141.8**	1,3	5	12.91
	G_T	0	**0.00**	2,1	4	**19.72**		G_T	80	**239.0**	2,1	5	7.41
				3,1	4	**19.41**					2,2	5	11.19
				3,2	4	**45.06**					2,3	5	**16.20**
				3,3	4	1.18					3,1	5	15.39
				4,1	4	5.84					3,2	5	**26.03**
				4,2	4	10.16					3,3	5	**37.12**
				5,1	4	**27.81**					4,1	5	**18.08**
				5,2	4	11.35					4,2	5	8.32
				5,3	4	**31.74**					4,3	5	12.37
				6,1	4	12.75					5,1	5	12.64
				6,2	4	**16.15**					5,2	5	**16.83**
				6,3	4	12.78					5,3	5	11.21
				Total	56	278.7					6,1	5	**17.88**
											6,2	5	11.09
											Total	80	239.0

	Tests	df	G	Subject	df	G		Tests	df	G	Subject	df	G
MAx	Gp	5	**-**	1,1	5	13.08	PXx	Gp	5	**75.61**	1,1	5	11.70
	G_H	80	**223.4**	1,2	5	7.17		G_H	60	**150.5**	1,2	5	**25.08**
	G_T	0	**0.00**	1,3	5	**19.80**		G_T	65	**226.1**	2,1	5	12.12
				2,1	5	**32.99**					2,2	5	13.08
				2,3	5	**39.42**					2,3	5	**71.67**
				3,1	5	**16.97**					3,2	5	11.42
				3,2	5	**16.83**					3,3	5	14.34
				3,3	5	10.24					4,2	5	10.24
				4,1	5	13.94					4,3	5	**16.83**
				4,2	5	1.01					5,1	5	4.69
				4,3	5	**63.73**					5,2	5	13.85
				5,1	5	2.77					6,1	5	5.88
				5,2	5	14.33					6,2	5	15.24
				5,3	5	**71.67**					Total	65	226.1
				6,1	5	**16.09**							
				6,2	5	7.37							
				6,3	5	**47.16**							
				Total	85	394.5							

Figure Legends

Figure 1. Species-specific median sizes and interquartile ranges (IQR), given in millimeters, for *D. magna* (○), *D. mendotae* (□), *D. obtusa* (x), *D. pulex* (Δ). Median size and IQRs were not found to change significantly over time (flatness test, Table 1); however, there was a significant contribution of species on median size and IQR (main effects, Table 1).

Figure 2. Distribution Overlap Indices (DOIs), which are standardized from 0 to 2, indicate either complete overlap of distributions or complete non-overlap, respectively, and their relationship to the mean number of clones found coexisting within mixed species assemblages. There is not a significant correlation between the two variables ($r^2 = 0.04$).

Figure 3. The relationship between trait variation and density was examined via interquartile range (IQR) as a proxy for trait variation. A weak but significant association between trait variation and density was found ($r^2 = 0.195$, $P<0.01$).

Figure 4. Species-specific mean interquartile ranges (IQR) for single clone, pooled clones, and pairwise species treatments with 95% confidence intervals.

Figure 5. Species-specific mean median sizes (mm) for single clone, pooled clones, and pairwise species treatments with 95% confidence intervals.

Figure 6. Total ephippial counts in the microcosms. Total ephippia counts in microcosms with 95% confidence intervals. Data are provided for each single species (ME = *Daphnia mendotae*, OB = *D. obtusa*, MA = *D. magna*, PX = *D. pulex*) as well as

species pairs of pooled clones. Species composition is ranked by ephippial production along the x-axis.

Figure 1. Species-specific median sizes and interquartile ranges (IQR), given in millimeters, for *D. magna* (○), *D. mendotae* (□), *D. obtusa* (x), *D. pulex* (Δ).

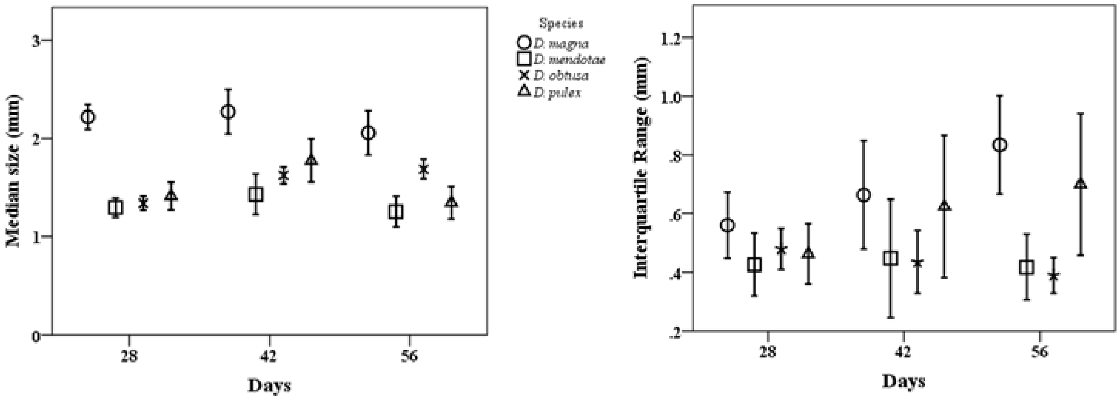


Figure 2. Distribution Overlap Indices (DOIs), which are standardized from 0 to 2, indicate either complete overlap of distributions or complete non-overlap, respectively, and their relationship to the mean number of clones found coexisting within mixed species assemblages.

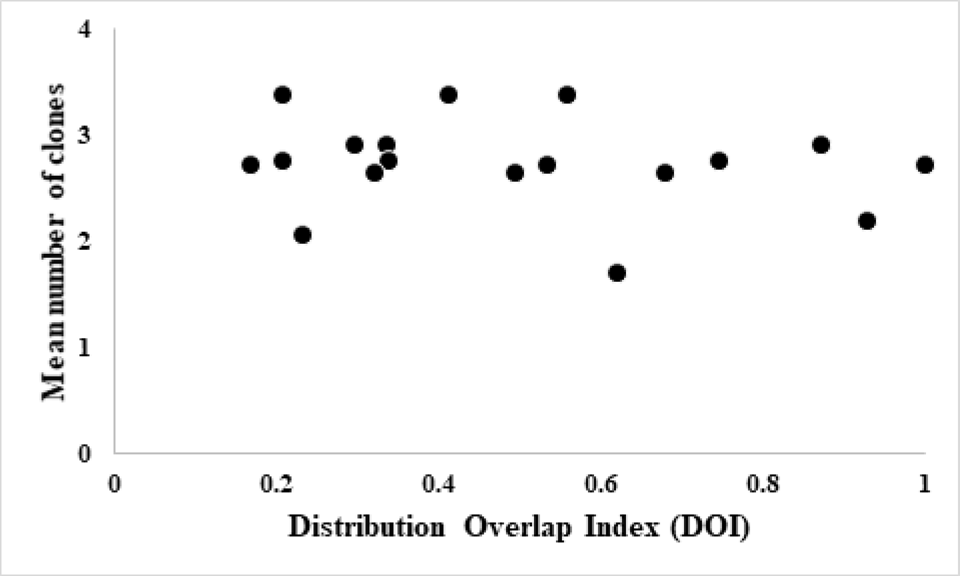


Figure 3. The relationship between trait variation and density was examined via interquartile range (IQR) as a proxy for trait variation.

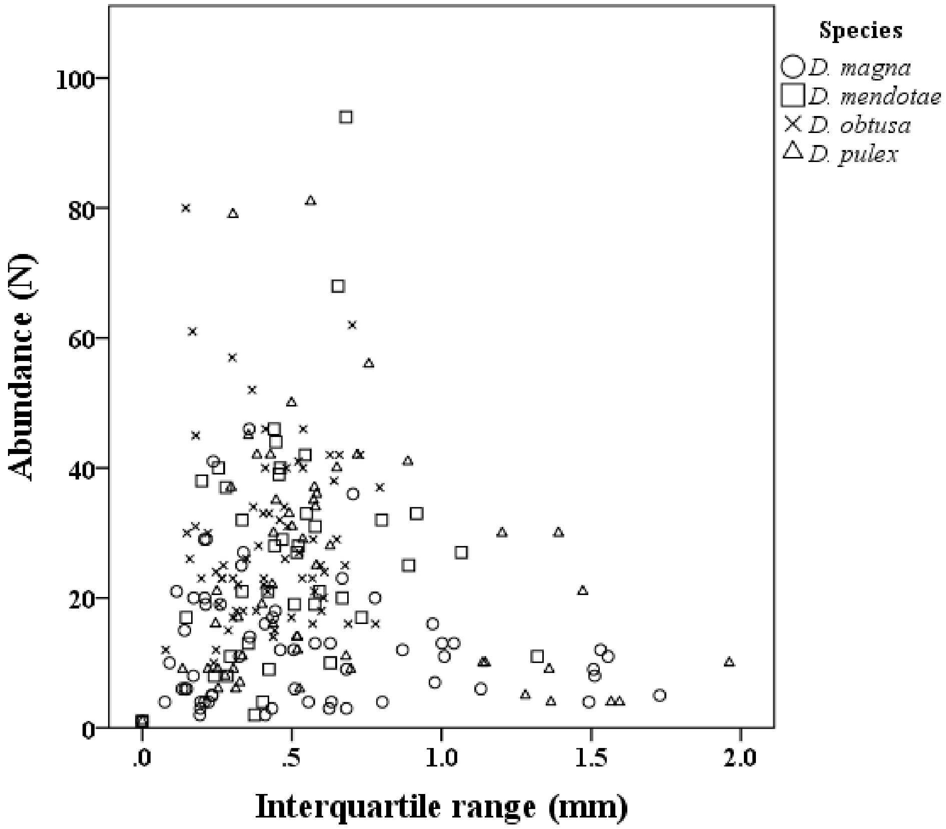


Figure 4. Species-specific mean interquartile ranges (IQR) for single clone, pooled clones, and pairwise species treatments with 95% confidence intervals.

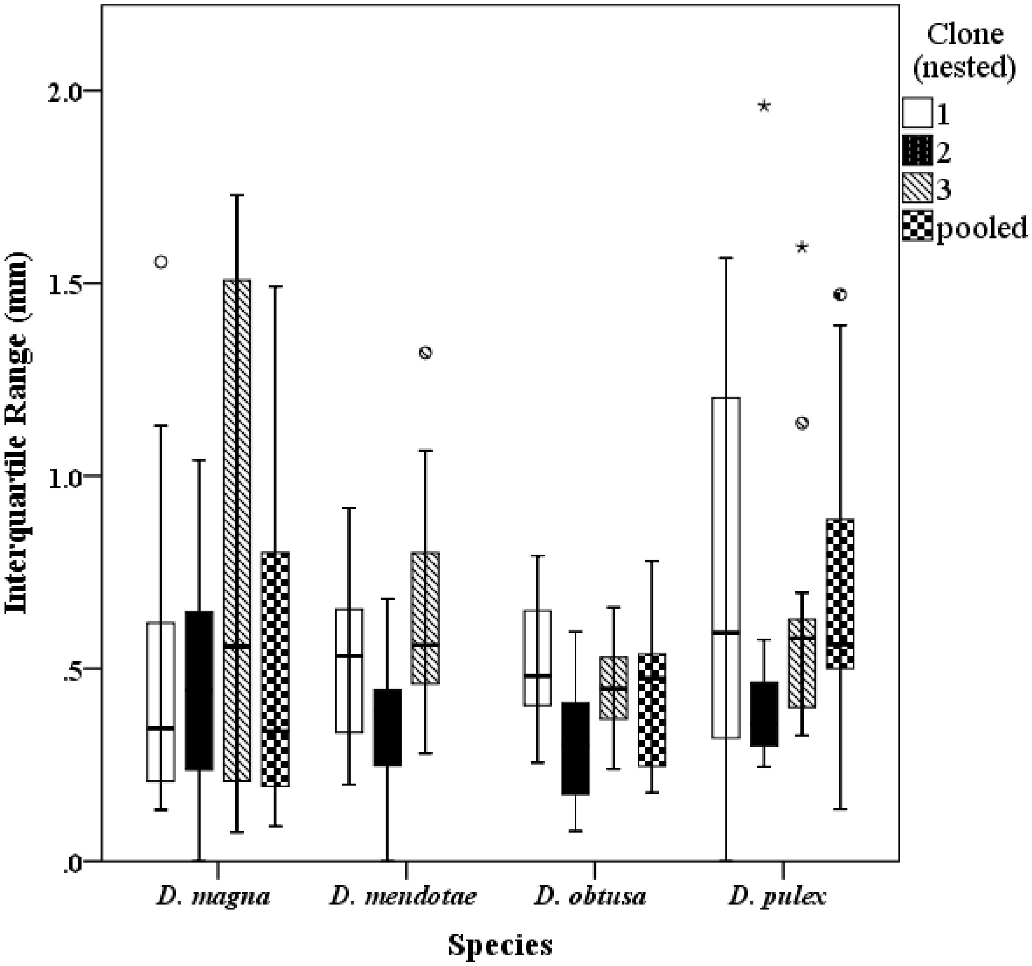


Figure 5. Species-specific mean median sizes (mm) for single clone, pooled clones, and pairwise species treatments with 95% confidence intervals.

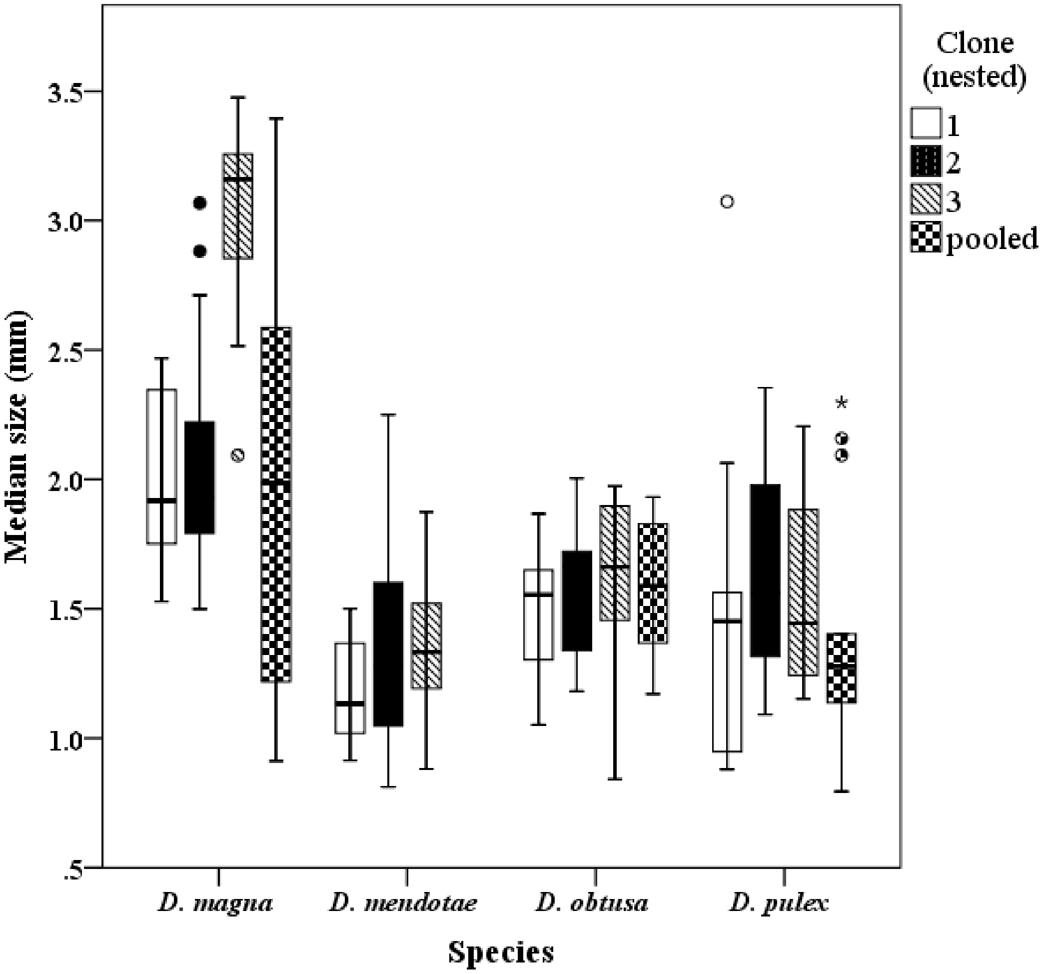
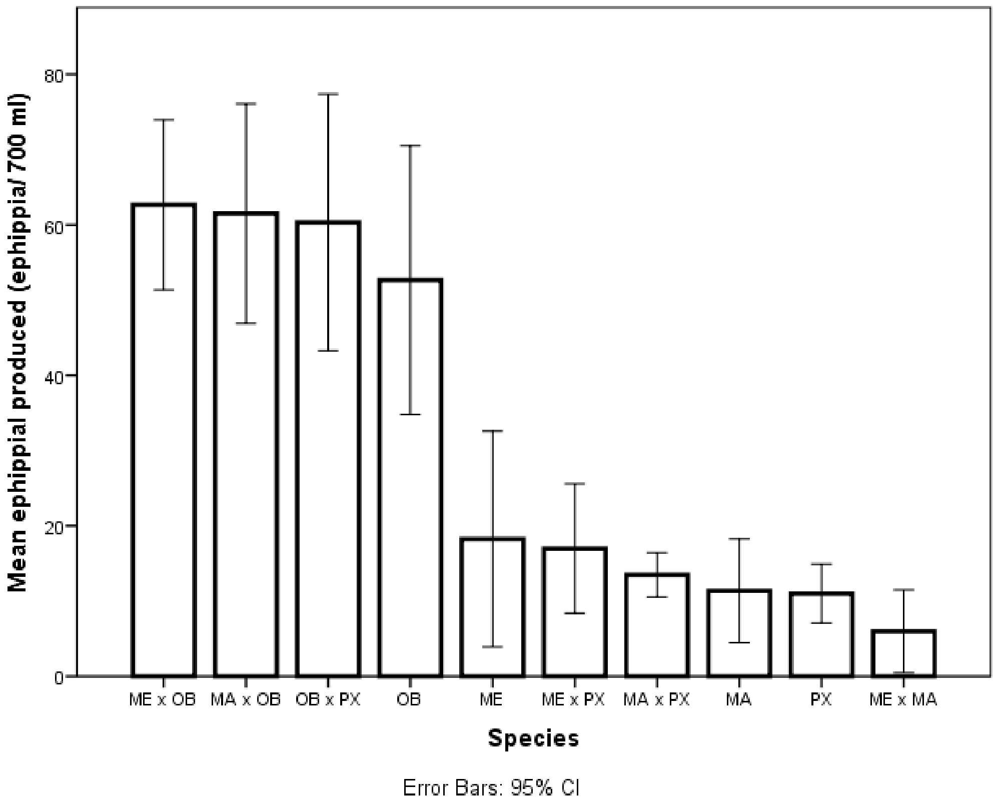


Figure 6. Total ephippia counts in the microcosms.



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Chapter Four: Intraspecific trait variation and colonization success in natural assemblages of zooplankton

Rachel Hartnett

Abstract

Intraspecific variation in organismal traits has been of increasing value in linking population-level processes to community properties. Increased intraspecific variation should enable populations to colonize new communities more readily, as an increase in variation will lead to a wider niche breadth. Colonization success should also be a function of biodiversity of the standing community, rather than abundance if niche processes are driving the community. To test these mechanisms, I added three different *Daphnia* species to natural assemblages of zooplankton in order to determine how colonizer traits and the properties of the standing community affected colonization success in greenhouse mesocosms. There was differential success among *Daphnia* species, potentially due to differences in variation in body size. I found that colonization success was associated with a low species richness, high productivity, and high abundance. I also found a correlation between species evenness and colonization success of *D. magna* (the largest bodied daphniid species), where communities with less even communities were more likely to be colonized. These findings suggest that both colonizer traits and properties of the standing community are important in determining colonizer success. Moving toward a more predictive field of ecology, monitoring of the standing community and intraspecific trait variation is crucial for long-term community and ecosystem persistence.

Introduction

Individuals of a population differ in a variety of traits like body size, sex, behavior, or physiology. This trait variation within populations, or intraspecific variation, has recently received increased interest from community ecologists (Bolnick et al. 2011). Empirical evidence has shown that intraspecific variation can play a positive role in coexistence (Stoll and Prati 2001), stability of community assemblage (Post et al. 2008), and juvenile recruitment (Gamfeldt et al. 2005). Niche theory predicts that increased intraspecific diversification should increase the colonization ability of that species, as it has more ecological opportunities to exploit the available habitat. In addition, colonization success should be a function of species richness rather than abundance of the invaded community. Species traits as well as the standing community's environment and composition play a role in determining colonization success.

When it comes to the traits of invasive colonizers, certain life-history traits, like that of the freshwater zooplankter, *D. lumholtzi*'s ability to produce dormant eggs under poor food conditions (Smith et al. 2009), have been implicated in increasing this species' ability to colonize and persist in novel environments. In *Daphnia* (like many other organisms) body size is a key trait known to affect other life-history traits (Ebert 1991) and to affect an individual's ability to survive and persist in the community (Brooks and Dodson 1965, Cyr and Curtis 1999). Aquatic systems are highly size-structured; the body size of an individual often determines the trophic position and participation in community network interactions, which has implications for the success of a colonizing population (Schröder et al. 2009). However, individual traits can be weak indicators of colonization success, although it has been shown that increased genetic diversity of the colonizing species increases colonization success (Kolbe et al. 2004, Crawford and Whitney 2010). So even if a colonization event involved individuals of the same size, intraspecific variation in size and other traits can still have consequences on colonization success.

The standing community is also important in determining colonizer success. Niche theory posits that species richness will increase community resistance to colonization, as in the classic experiments in grasslands (Tilman 1997) and in marine sessile invertebrates (Stachowicz and Byrnes 2006). Niche theory also predicts that communities with populations that exhibit high functional diversity should support fewer species, as they can fill just as many niches with fewer species. For example, arbuscular mycorrhizal fungi, which have a mutualistic relationship with the majority of vascular plants, are depauperate in species (with only 154 described species); yet they have high functional diversity within species (Munkvold et al. 2004). However, this diversity in functional traits may not translate across species richness; functional trait diversity may not increase strongly with increased species richness (Stuart-Smith et al. 2013).

Another metric of biodiversity that may play a role is the relative abundance of species in a community, or species evenness. Grime (1998) published a ‘mass ratio’ hypothesis in which there is a positive correlation between species richness and species evenness, essentially stating there is more evenness when there are more species. However, Mulder et al. (2004) observed that increased species richness resulted in lowered evenness. There is a lack of strong experimental evidence on the relationships between species richness and species evenness in ecosystems. In a meta-analysis of aquatic ecosystems, Soininen et al. (2012) found evidence to suggest that species evenness and richness are independent axes of biodiversity. These authors found a significant correlation in one third of the studies, of which one third were positively correlated and two thirds were negatively correlated. In terms of colonization success, a study by Mattingly et al. (2007) found no correlation with species evenness and colonization success, but did find a positive correlation between evenness and primary productivity.I set out to test which traits of the keystone aquatic herbivore, *Daphnia*, promote colonization success (e.g., body size, flexibility in reproductive output) and to determine how niche theory may be governing colonization ability in aquatic mesocosms. The hypotheses that I tested were: 1) if individual specialization occurs within a species due to large intra-populational variation in a trait and increases the niche breadth for that population in a community network, then colonization success will be higher in *Daphnia* species with greater variation in important colonizing traits including life-history traits (e.g., rapid growth rates and fast egg maturation) and high physiological rates (e.g., decomposition rates) (Wheat et al. 2011, Cline and Zak 2015); 2) if niche processes are governing colonization ability within the standing community, then colonization success will be a function of species richness and not the total abundance of organisms in the mesocosms (Loreau 2000). To investigate these hypotheses, I used simple mesocosm communities, and colonized natural assemblages of zooplankton with non-native species of *Daphnia (D. magna*, *D. pulex, D. obtusa)*, with known life-history characteristics.

Methods

Initial set up

In mid-June of 2015, I filled thirty 378 L Agrimaster poly stock tanks (mesocosms; Behlen Country) with a 1:1 ratio of well water and pond water filtered through a 153 µm Nitex ® mesh plankton net from a shallow experimental pond in the Aquatic Research Facility on the University of Oklahoma campus in Norman, Oklahoma (35.183737° N, -97.448117°W). I randomly assigned twelve HOBO Pendant Temperature/Light Data Loggers (UA-002-08) to mesocosms and zip-tied them to the left or right side, again randomly, at about half the total depth (i.e., 24 cm) of each mesocosm. These data loggers recorded temperature every two hours for the duration of the experiment. Mesocosms were inoculated with a natural assemblage of zooplankton from the same experimental pond; I inoculated each mesocosm with zooplankton > 153 µm from about ~25 L of pond water.

Addition of algae and Daphnia

After an equilibration period of four weeks, I determined that chlorophyll-a (chl-a) levels were below carbon thresholds suitable for supporting *Daphnia* reproduction, thus algal supplements were subsequently added. I cultured algae in 100 L plexiglass cylinders with a 1:1 ratio of well water and filtered pond water up to 90 L and 5 L of a filtered algal culture. These cultures have a turnover of ~3-4 days. The algal assemblage varied as the season progressed, but the primary alga was a *Scendesmus* species. I added 1 L of cultured algae to each tank every two weeks. Further, I randomly assigned three *Daphnia* species, *D. magna*, *D. obtusa*, and *D. pulex*, to each mesocosm (n = 10 tanks per species); each tank was inoculated with 30 individuals of a mixed assemblage of three genotypes per species on July 23rd, 2015.

Sampling

I took abiotic measurements every two weeks from the initial set-up of the mesocosms in June 2015 until the end of sampling in mid-September 2015. These measurements included pH, conductivity ($\mu_{siemens}$), total dissolved solutes (ppm), salinity (ppm), temperature (°C), dissolved oxygen concentration (mg/L), and percent dissolved oxygen (%) using a Hach meter (Hach, HQ36d: dissolved oxygen and temperature) and a PCSTestr Multi-Parameter (Oakton Instruments, PCSTestr 35 model WD-35425-10; pH, conductivity, TDS, salinity). I also took water samples for chl-a and nutrient processing. I used a PVC pipe (6.35 cm inner diameter) with a stringed half-tennis ball as a make-shift vertical sampler of ~1 L, and made six vertical draws in a star pattern in the mesocosm. I collected these draws into a bucket, and sampled 50 ml for chl-a, 30 ml for soluble reactive phosphorus (SRP), and 30 ml for ammonia (NH3) analyses. I filtered the water samples for chl-a onto a 25mm GF/F filters (#1825-025), wrapped the filter in tin foil, and stored the samples at -20°C until samples were processed. Likewise, the SRP and NH3 samples were stored at -20°C until processed. After water samples were taken for chl-a and nutrients, I stirred the tanks to make sure the whole zooplankton community was represented in the zooplankton sample. I again collected six draws using my sampler into a bucket and filtered the 6 L through 153 μm Nitex ® mesh. Zooplankton were rinsed into 30 ml bottles and stored in 70% ethanol.

Sample processing

Chlorophyll-a: I extracted and measured *in vitro* chl-a by filtering the media through the GF/F filter and grinding each filter in 90% acetone. After incubating for 3-4 hours, the filter/acetone was spun down using an Eppendorf centrifuge (Model 5804) at 1500 rpm for five minutes. Then chl-a was measured (after calibration from a random subsample) using the chl NA module in a Turner model TD 700 bench top fluorometer (Turner Designs, Sunnyvale, California, USA).

SRP and NH3: I used the spectrophometric methods outlined in the *Standard methods for the examination of water and wastewater* (Clesceri et al. 1995) and ran all samples on a Beckman spectrophotometer (Model DU520). Zooplankton samples were subsampled in triplicate using a 1 ml Hensen-Stempel pipette and Sedgewick-Rafter cell, and identified and enumerated specimens using an Olympus BX51 compound dissecting microscope.

Statistical analysis

Analyses were run using SPSS (Version 20, IBM). A correlation matrix of the environmental variables was used to reduce the environmental variables to independent variables (temperature, pH, salinity, dissolved oxygen concentration, SRP, and NH3) (Tabachnick & Fidell 2013). A redundancy analysis (RDA) was run with correlation scaling in order to determine which environmental variables (temperature, pH, salinity, dissolved oxygen concentration, SRP, and NH3) influenced the species composition (abundance, richness, and evenness) of the mesocosms. A discriminant analysis was run in order to determine if environmental variables and species composition were sufficient in predicting colonization success of *Daphnia* species (presence or absence of

Daphnia species added to the mesocosm). General linear models (GLMs) were subsequently run to evaluate which predictor variables in the discriminant analysis were most important in predicting colonization success (Tabachnick & Fidell 2013).

Results

The natural variation of initial abundance (N_o), ranged considerably (312 +/- 158 individuals/L), but initial richness (S_o) was less variable (6.6 +/- 2.5 species). A discriminant analysis indicated that there were no differences between mesocosms assigned to each of the three colonizing species, as an analysis was unable to assign each species to their mesocosms over random chance (i.e., > 50% of the time) (Figure 2). High species richness in the mesocosms was associated with lower mesocosm primary productivity (lower pH, lower temperature environments with less soluble reactive phosphate [SRP]), while high abundance was associated with higher mesocosm primary productivity (higher pH, higher temperature environments with more SRP) (Figure 1). The environmental predictors explained 31.9% of variation in community composition, with 62.77% of this variation accounted for in axis 1 and 20.29% in axis 2.

Daphnia species exhibited differential colonization/establishment success between species and along mesocosm environmental axes. *D. magna* was most successful in colonizing and establishing populations in 7 out of 10 mesocosms, compared to 4 out of 10 mesocosms for *D. obtusa*, and 2 out of 10 mesocosms for *D. pulex*. Colonization success was not predicated on the abundance of potential predators (cyclopoid copepods [Gliwicz and Stibor 1993], and *Chaoborus* [(Spitze 1992]). *Daphnia* abundance was actually marginally positive with both predators (r = 0.373, 0.577 respectively; $P < 0.05$). Colonization success could be predicted by environmental variables 90% of the time with the discriminant function. The discriminant function revealed a significant association between colonization success and all predictors, accounting for 72.8% of between group variability, although closer analysis of the structure matrix revealed only three significant predictors, temperature (GLM, $F = 19.92$, $p < 0.001$, $df = 1$), dissolved oxygen concentration (GLM, $F = 9.6$, $p = 0.003$, $df = 1$), and richness (GLM, $F = 6.413$, $p = 0.014$, $df = 1$), with pH, salinity, SRP, NH_3, abundance, and evenness as non-significant predictors.

Discussion

Niche processes appear to be governing the colonization of aquatic mesocosms by *Daphnia* species. Colonization success was predicted by the community's species richness and other environmental factors. *Daphnia* were better able to colonize mesocosms with higher productivity, high abundance of other zooplankton, and a less even community composition, as these variables were not associated with species richness (Figure 1). This happened despite a high prevalence of taxa with similar functional roles that were already established for two weeks and stayed at high abundances throughout the experiment (Table 1). *Daphnia magna*, in particular, was positively correlated with total mesocosm abundance (Pearson, $r = 0.653$, $p = 0.041$) and a less even community (Pearson, $r = 0.206$, $p = 0.025$). While this study supports previous work on the relationship between colonization success and species richness, it also shows that species evenness has an independent relationship with colonization ability that is counter-intuitive. As a community is dominated by one or a few taxa (becoming less even), one would expect these few taxa to encompass a niche previously occupied by several taxa (Hillebrand et al. 2008).

Colonization success is mediated by environmental fluctuations, including productivity of the system (Davis et al. 2000). Communities that are highly productive may have higher rates of colonization success, according to the *"more individuals hypothesis"* (Wright 1983). There may be a possible role for mesocosm productivity in colonization success here, but previous studies have noted that highly productive habitats had stronger trends with extinction rates (where more energy lowers extinction rates of abundant taxa) and weaker trends with colonization rates (Evans et al. 2005). Habitat quality and habitat size can both increase the ability of populations of *Daphnia* to persist through time by having an effect on both their carrying capacity and intrinsic growth rate; the latter would be particularly important in establishment of a population (Griffen and Drake 2008).

The number of individuals of *Daphnia* added to the mesocosms was standardized across mesocosms and should have minimal effects on colonization success compared to overall propagule pressure, which was not manipulated in this study (Drake et al. 2005). However, the role of the number and frequency of addition, or propagule pressure, would also play a large role in natural systems. The immigration rate of *Daphnia* had profound effects on extinction rates, and therefore, influenced the ability to persist in microcosms without competing zooplankton (Drake et al. 2005).

One caveat to note is that communities were measured for an initial numerical abundance, N_o, and initial species richness S_o, which occurred naturally in each mesocosm. I expected the natural variation of both abundance and richness to range considerably, but richness may have been less variable due to community-stabilizing effects (May 2001) through competitive tradeoffs (Leibold and McPeek 2006) or overyielding by complementary species that increase productivity over temporal variation (Hector et al. 2010). Another complicating factor that may have limited species richness was the amount of precipitation the shallow experimental pond received before the experiment began. May of 2015 had historic levels of rainfall, potentially resulting in lower levels of nutrients and species richness during the initial set-up to the experiment (Kloesel et al. 2015).

However, the variables of the standing community accounted for 72.8% of the variation between successfully-colonized and non-colonized mesocosms. Intraspecific differences in the three colonizing species of *Daphnia* may account for the differential success in colonization ability. These *Daphnia* species are known to have differing competitive abilities based upon body size and differences in filtering appendages (Burns 1969, Hessen 1985, Peter and Lampert 1989). *D. magna*'s body size range differs from *D. pulex* and *D. obtusa*, with *D. magna* having greater variation in body size, and an overall larger body size; other key life-history traits do not exhibit variation to the same degree among species (Hartnett, unpublished life-history table experiment in chapter one). The extended range in available sizes for *D. magna* may have increased their colonization success, as well as the size ranges of the standing community. Colonization success was moderately positively (but not significantly) correlated with potential predators of *Daphnia* (i.e., cyclopoid copepods, *Chaoborus*). The predatory zooplankton may have consumed competing size-classes in those mesocosms, allowing for more available habitat. However, body size was not monitored during this experiment, so no definitive conclusions can be made about the possible impact of predation in this study.

Colonization is a fundamental process in ecology. The properties of the standing community and the intraspecific variation in the colonizing population have been shown to be important in this study. Body size is a trait that has been implicated in a variety of functions in aquatic ecosystems, and it is used as the sole measure of conservation management in some cases (Petchey and Belgrano 2010). In addition, colonization of non-native organisms is a major concern in conservation management (Lockwood et al. 2013). While it is clear that the traits of the colonizer are important to determine colonization success, it is rare to be able to track an invasion event in its early stages of colonization.

Often managers do not have all the information they need about the colonizing population to effectively predict colonization rates at all stages of invasion (Ricciardi and MacIsaac 2011). Scientists should continue efforts to determine common traits among successful colonizers. However, conservation managers might focus efforts into monitoring the standing community. Biodiversity, particularly species richness, of the standing community is known to be important in resisting invasion (Tilman 1997), although here, the relative abundance of these species also has effects on the colonization ability of organisms. Resistance to colonization (i.e., invasion) is an important community-level trait, as theory suggests that increasing colonization events can de-stabilize a system through a buildup of introduced feedback cycles (Mooney 2005). This has been seen in urban systems (Rebele 1994) and desert plant communities (Báez and Collins 2008). As communities and ecosystems are bombarded with both non-native colonizers and other rapid anthropogenic changes, the results from this present study indicate that addressing long-term stability, resistance, and resilience of these systems will be crucial in the coming years.

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Tables

Table 1. Common taxa found in the mesocosms (Summer 2015). Potential predators of *Daphnia*, % occurrence of a given taxon in the mesocosms, mean abundance, body size information (from Carpenter and Kitchell 1996), and Pearson's correlation coefficient are given for each taxon commonly found in the mesocosms. * indicates that the correlation coefficient is significantly different from 0.

Taxa	Potential predator	% occurrence	Mean abundance (ind./L)	Mean size (mm)	Corr. Coeff. with *Daphnia* abdundance
Ceriodaphnia		100	22467	0.6-1.4	-0.182*
Chydorus		97	11968	0.3-0.5	-0.076
Calanoid adults		92	6075	1.8-2.0	0.563*
Cyclopoid adults	X	88	3283	1.0-1.5	0.373*
Bosmina		62	14414	0.4-0.6	0.226*
Ostrocoda		42	2893	0.1-1.0	0.033
Chaoborus	X	32	754	N.A.	0.577*

Figure Legends

Figure 1. Redundancy analysis (RDA) biplot of environmental variables (X – solid circles) and community composition (Y – open squares) of the mesocosms. A RDA shows an association between the environment and the community composition of the mesocosms ($p<0.001$ with 100 permutations). The environmental predictors explained 31.9% of variation in community composition, with 62.77% of this variation accounted for in axis 1 and 20.29% in axis 2.

Figure 2. Discriminant Analysis (DA) of mesocosms, with added *Daphnia* species as the predictor. The DA used the variables of temperature, dissolved oxygen concentration, richness, pH, salinity, soluble reactive phosphorus (SRP), ammonia (NH3), abundance, and evenness in the discriminant functions, of which the top two functions are plotted here. The mesocosms assigned to each species of *Daphnia* do not separate out among this environmental space, as seen by the close centroids of these groups.

Figure 1. Redundancy analysis (RDA) biplot of environmental variables (X – solid circles) and community composition (Y – open squares) of the mesocosms.

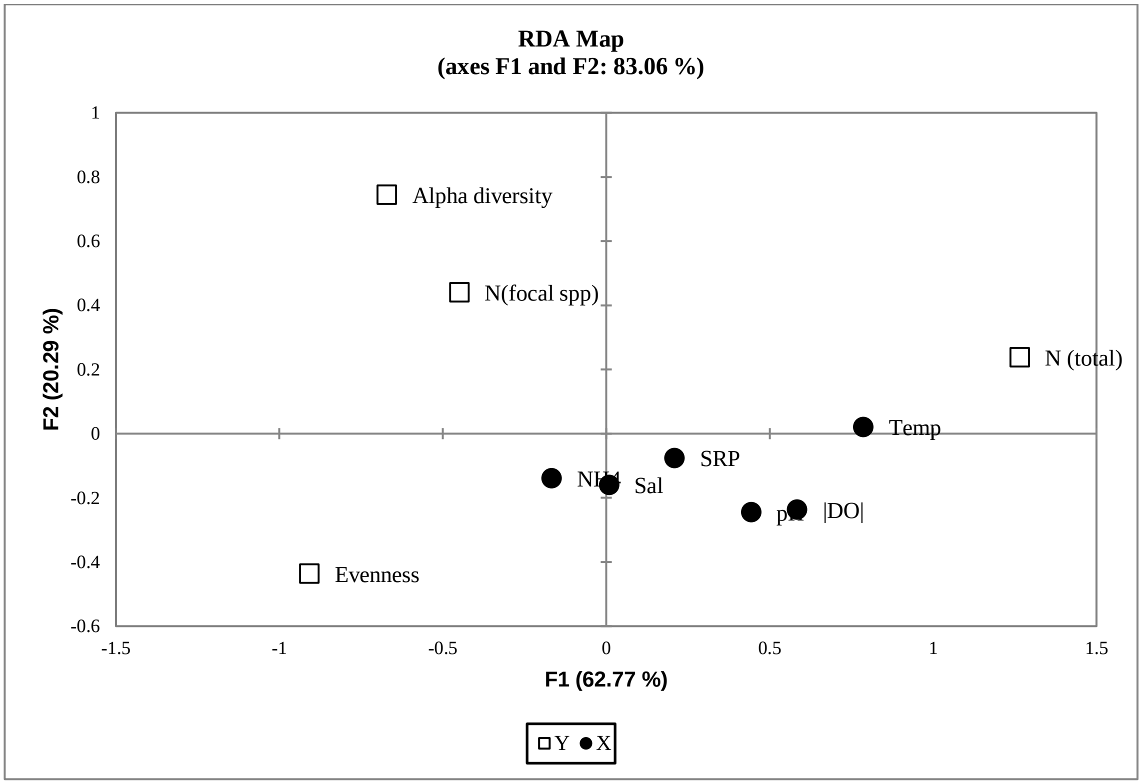
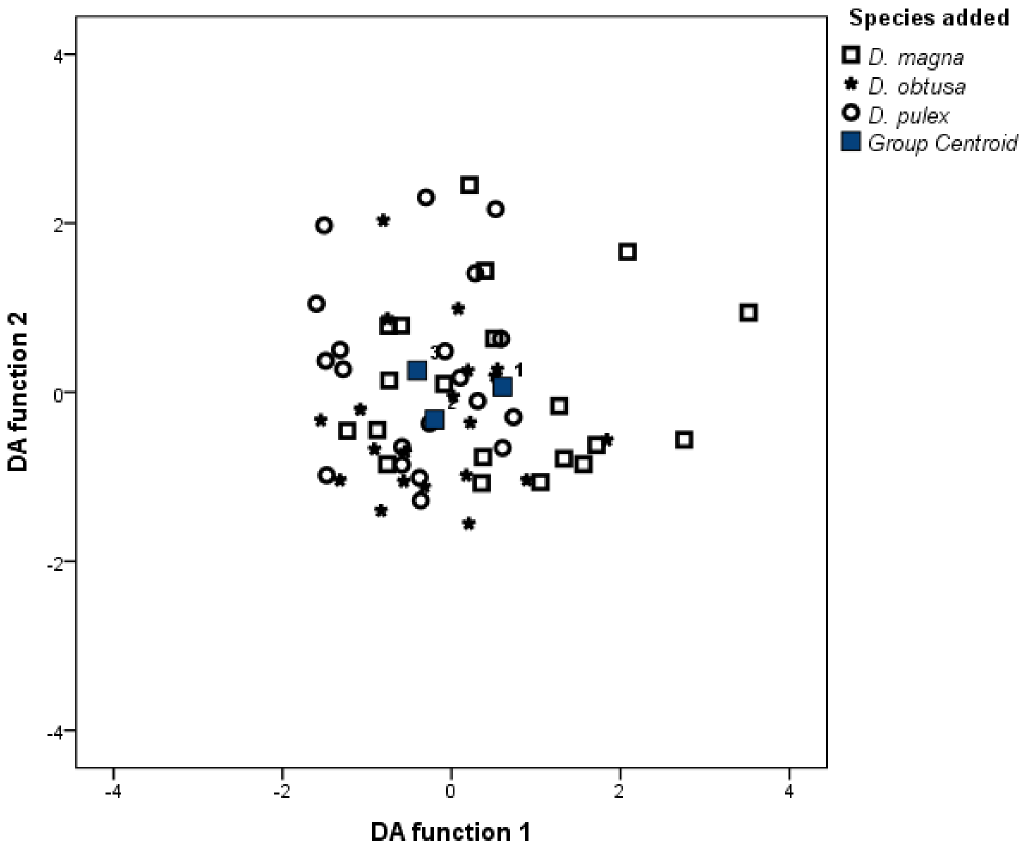


Figure 2. Discriminant Analysis (DA) of mesocosms, with added *Daphnia* species as the predictor.



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Synthesis

Monitoring every individual in a community is a daunting task for any ecologist. However, population biologists have been documenting the importance of intraspecific variation (i.e., variation between individuals in a population) and its effects on individual fitness in a population (Reznick et al. 1997, Bolnick and Smith 2004). Intraspecific variation can also influence higher-tiered processes; plant ecologists have shown that the genetic variation of a dominant tree species and the resulting phenotypic variation of defensive traits have strong impacts on the surrounding arthropod community (Schweitzer et al. 2004). Intraspecific variation feeds into the role of niche and neutral processes at the community level. Neutral theory has been formed on the assumption of functional redundancies among species within a group (Hubbell 2011), and intraspecific variation could increase the overlap between species (Polis 1984). On the other hand, niche theory has strong foundations in both empirical and theoretical work (MacArthur 1955, Hutchinson 1961). Intraspecific variation could also influence the size of a species niche, increasing niche diversity (Van Valen 1965) and individual specialization (Bolnick et al. 2003). Svanbäck and Bolnick (2007) provide evidence among aquatic communities of generalists that are composed of specialized individuals, providing wider niche breadth. This can happen due to temporal and ontogenic shifts in niche utilization patterns (Polis et al. 1989, McCann 2011). Community ecology could benefit greatly from studying intraspecific variation, but we should prioritize the traits that can be easily measured, and will have the strongest and farthest-reaching effects.

A universally important trait exhibiting intraspecific variation across taxa

Variation in body size has been extensively studied because of its strong linkages to other life-history traits (Kerr 1974), body energetics (Brown et al. 2004), and demography (Brooks & Dodson 1965). Research into the role of body size has been shown, in a variety of taxa, to influence a number of critical life-history traits (Cargnelli & Gross, 1996; Dickerson *et al.*, 2005; Rode, Amstrup & Regehr, 2010). Thus, it is considered to be a trait that influences other critical growth and reproductive traits, and thereby could determine fitness of an organism. Due to allometric scaling patterns, the effects of body size can be found across geographic scales and among various taxa (Gould 1966, West et al. 1997, Elser et al. 2010). Because of the importance associated with body size, ecologists have commonly used size distribution data to tie individuals and populations to community-level functions (Jennings et al. 2001, Cohen et al. 2003, Downing et al. 2014). Size distributions have been used as a proxy for species interaction strength, trophic position, and ecosystem productivity (Sprules and Munawar 1986, Woodward et al. 2005b, 2005a). Therefore, I set out to study the patterns of intraspecific variation in body size.

Empirical evidence for linking individuals to their community through body size

Body size is particularly important in aquatic systems as (1) it affects competition, larger-sized zooplankton in general are better competitors (Vanni 1986, Achenbach and Lampert 1997); (2) and it affects predation, because fish prefer larger, compared to smaller, zooplankton (Persson et al. 1996). Within *Daphnia*, size classes will influence the phytoplankton community (Vanni 1987, Cyr and Curtis 1999), competing species (Pace and Vaqué 1994), and their partitioning in the water column (Dini et al. 1987). As body size is so important in population dynamics and community structure, it would be valuable to know how variation in body size is generated. I looked at (i) the variation of body size within species, between species, and across food quality treatments, and (ii) how this variation influences intra- and inter-specific competition and (iii) colonization ability. In addition, (iv) I used some empirical evidence to test the ability of a model more reliant on size differences (biomass-based) to perform against a more traditional model (individual-based) in predicting population dynamics.

(i) There is still ongoing discussion about what the relative effects species identity and body size may play in community structure (Woodward et al. 2010). Does species identity constrain size distributions through physiological limits (Nylin and Gotthard 1998)? Or would physiological processes like food quantity, food quality, and maternal condition shape the size of offspring in *Daphnia* through plasticity (Gliwicz 1990, Vanni and Lampert 1992, Lampert 1993)? I found that both body size and clonal identity were important factors in determining overall fitness in a low food quality environment. Within species variation buffered the effects of food quality differences at the interspecific level. In addition, body size was less sensitive to food quality than traits associated with reproduction, indicating again that species identity cannot be ignored.

(ii) Extrinsic factors like predation and food availability are known to shape body-size distributions (Brooks and Dodson 1965, Sprules and Munawar 1986). Larger individuals have a competitive advantage over smaller individuals, but face higher predation (Brooks and Dodson 1965, Lynch 1977). However, the effects of intrinsic factors like intraspecific competition and population composition are less well-known.

In a competition experiment, I found that size distributions of a single lineage do not differ from mixed lineage or species assemblages, with the exception of the large-bodied *Daphnia magna*. *D. magna*'s size distribution has a broader range than the other *Daphnia* studied, which may play a role in their competitive success. However, I also found that there was a higher number of coexisting clones within mixed lineages and species assemblages. This maintenance of clonal and species diversity was unexpected, but could be due to intraspecific competition. At the population rather than the individual level, it seems that species identity plays a role in shaping size distributions in a limited capacity.

(iii) As colonizer traits and the standing community richness is known to influence colonization ability (Tilman 1997, Kolbe et al. 2004, Crawford and Whitney 2010), I investigated the extent of these two factors, particularly in regards to: 1) potential intraspecific variation of traits in the colonizing species; and 2) the abundance and richness of the standing community. My results indicated that *D. magna* (i.e., the largest-bodied species tested) was the best colonizer and that establishment success was associated with low species richness of the colonized community, high mesocosm productivity, and high abundance of resident species. The variation in body size of *D. magna* was advantageous as both a competitor (iv) and a colonizer (iii).

(iv) Finally, body size has been tied to theory through allometric scaling laws and the flow of energy via carbon (West et al. 1997, Brown et al. 2004). Theoretical models in these veins have been based on the biomass of organisms. In order to see the role of species identity (i.e., individual-based) versus body size (i.e., biomass-based), I compared the ability of two stage-structured models to predict changes in population growth under two different food qualities, using data from individual data (i). Results indicate that the biomass-based model is sensitive to juvenile traits, generalizable among *Daphnia* species, and insensitive to changes in food quality due to its emphasis on biomass scaling. Individual-based models continue to better incorporate system-specific properties. Specifically, these models showed sensitivity to changes in adult reproduction with differing food quality, but are not generalizable across taxa or systems.

Scope: Currently organisms are experiencing environmental change at an unprecedented rate (Parmesan 2006, Fabry et al. 2008). Ecologists are therefore seeking out what traits or functions enhance a community's ability to persist through time, resist environmental change, and be resilient post-environmental events. The study of functional traits has emerged in community ecology to make better conservation and management decisions (Cadotte et al. 2011). Intraspecific variation of important functional traits can have strong effects on the community (Whitham et al. 2006), causing a renewed interest in intraspecific variation (Cadotte et al. 2011). In addition, the mechanisms that maintain diversity are open questions in biology (Loreau 2004, McCann 2011). Individual specialization due to intraspecific variation would result in less intraspecific competition, and therefore reduce coexistence of species (May 2001). The scope of my work has shown that key traits like body size can be (i) physiologically conserved within species, (ii) conserved across closely-related taxa at the population level, and 3) important in determining population-level success in competition and colonization when variation in the trait is high (iii, iv). However, species identity still played a role in the maintenance of diversity (iii). Intraspecific variation therefore has a role in unifying principles between population and community ecology.

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