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FRESHWATER ZOOPLANKTON: ECOLOGY, CRYPTIC SPECIES, AND GLOBAL CHANGE

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FRESHWATER ZOOPLANKTON: ECOLOGY, CRYPTIC SPECIES, AND GLOBAL CHANGE

A DISSERTATION APPROVED FOR THE
DEPARTMENT OF BIOLOGY

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Abstract

Modern threats to freshwater biota include anthropogenic eutrophication and invasive species, both of which may impact biodiversity. My four chapters focus on different aspects of these global changes. In Chapters 1 and 2, I focus on the effects of the harmful algal blooms caused by anthropogenic eutrophication on native freshwater grazers. In Chapter 3, I focus on a feature that complicates our understanding of diversity of zooplankton and other animals: cryptic species. In Chapter 4, I examine the environmental factors associated with abundance and morphology of an invasive zooplankter. Taken together, my work shows the myriad of effects and important considerations in studying the effects of global change on freshwater zooplankton.

In my first two chapters, I address the effects of harmful algal blooms that occur as a result of anthropogenic eutrophication. I tested how exposure to the toxigenic cyanobacteria *Microcystis aeruginosa* affects the common rotifer *Brachionus calyciflorus,* both within and across generations. In Chapter 1, using a laboratory experiment, I found evidence of carry over effects, meaning that individuals exposed to high amounts of good food (*Chlamydomonas* sp.) could later withstand exposure to *Microcystis* better than individuals that were fed low amounts of food during early development. Thus, as blooms of cyanobacteria become longer in duration and more frequent, rotifer populations may experience compounding negative effects. Further, I found that offspring produced by mothers fed *Microcystis* were of lower quality (measured as ovary:body ratio) and, if the mother had initially been fed a low amount of food, were also significantly smaller. However, offspring produced by mothers exposed

to *Microcystis* after having been reared on high amounts of good quality food produced significantly larger offspring. This led me to hypothesize that the larger size of these offspring could be adaptive under poor food conditions.

In Chapter 2, I tested this hypothesis by raising mothers in either *Microcystis* or *Chlamydomonas*, and then raising their offspring in either of the two treatments. Based on the maternal match hypothesis, I predicted that offspring would have higher population growth rates if reared on the same diet as their mother. However, the results of my experiment did not support this hypothesis. Instead, I found that offspring produced by mothers reared on *Chlamydomonas* had higher fecundity and survival than those produced by mothers reared on *Microcystis*, regardless of the offspring diet. The results of this chapter, taken together with those of Chapter 1, suggest that blooms of toxigenic cyanobacteria may have more extensive negative effects on grazer populations than have been previously suggested, based on single generation studies.

In Chapter 3, I consider patterns in the discovery of cryptic species across animal taxa. While earlier work had suggested that cryptic species are evenly distributed across taxa, I find significant variation in the number of cryptic species discoveries across both animal phyla and insect orders. In using a model comparison approach, I found that the best predictors of the frequency of cryptic species were those associated with research intensity, including human health association and taxonomic research effort. These results have consequences for our study of diversity in a changing world, as we can only

fully understand the effects of global change on diversity when the quantification of diversity includes cryptic species and is equivalent across taxa.

In Chapter 4, I investigated the drivers of abundance and morphology of an invasive zooplankter, *Daphnia lumholtzi*, twenty years after its initial introduction to lakes in the United States. I found that the single best predictor of *D. lumholtzi* abundances within Lake Texoma, OK-TX, was the abundance of cyanobacteria. Additionaly, patterns in the scaling relationships of head and tail spines led me to suggest that there is seasonal predation pressure by a gape-limited predator on *D. lumholtzi* within this subtropical reservoir. Both the abundance and morphology of *D. lumhotlzi* are associated with patterns in native biota.

Blooms of cyanobacteria will play a large role in structuring population dynamics of native zooplankton as climate change continues to favor increasing frequencies and durations of these blooms. Further, our understanding of the effects of these blooms on diversity and ecosystem function will be filtered through our understanding of this diversity. Molecular tests of cryptic speciation, particularly in less studied groups, will continue to be of the utmost importance.

Chapter 1 – Persistent and delayed effects of toxic cyanobacteria exposure on life history traits of a common zooplankter.

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Abstract

Anthropogenic eutrophication has resulted in shifts in phytoplankton community composition worldwide which represent dramatic changes in resource quality and availability for grazers such as rotifers. For these grazers, harmful algal blooms may have consequences that persist across several generations. We hypothesized that rotifers

exposed to a pulse of the toxigenic cyanobacterium *Microcystis aeruginosa,* would suffer demographic and physiological effects that decreased their ability to recover after cyanobacteria exposure. Additionally, we hypothesized that rotifer population recovery after harmful algal blooms is modulated by delayed effects of pre-bloom food availability. We used laboratory experiments to test the effects of switching from a high quality diet to toxigenic cyanobacteria on the physiological condition and associated life history changes of the common rotifer *Brachionus calyciflorus*. We found that *Microcystis aeruginosa* exposure decreased fecundity of rotifers by 51.5%, and early exposure to high levels of the high-quality food *Chlamydomonas* sp. did not ameliorate this negative effect. Rotifers exposed to *Microcystis* produced lower quality offspring (by 16.6%). However, we found that the effect of *Microcystis* on offspring body size was dependent on the density of food available in early life. Exposure to high-density food for the first three days of life tempered the negative effects of *Microcystis* exposure, whereas initial exposure to low-density food resulted in a 9.0% decrease in offspring length. We found that the negative effects of exposure to toxigenic cyanobacteria may accumulate across generations and limit the ability of rotifer populations to withstand the predicted increasing frequency and duration of harmful algal blooms.

Introduction

Increasing nutrient loading through agricultural runoff and wastewater discharge is changing resource availability globally in both marine and freshwater aquatic ecosystems (Keatley et al. 2011). This anthropogenic eutrophication has resulted in

phytoplankton community shifts due to altered species abundances and dominance by toxigenic, or otherwise harmful, algal species, including cyanobacteria (Taranu et al. 2015). Additionally, climate change and climate variability may lead to more frequent harmful algal blooms (HABs) through changes in surface water temperatures, vertical water column mixing, and precipitation and evaporation (Moore et al. 2008). These shifts in abundance, evenness, and composition of phytoplankton represent changes in resource quantity and quality for the organisms that feed on them, such as herbivorous zooplankton, including rotifers. Exposure to toxins and poor food quality associated with eutrophication can cause declines in population growth rates of herbivorous zooplankton (Tillmanns et al. 2008).

As herbivorous zooplankton play key roles in food web dynamics (Arndt 1993, Wallace and Snell 2010), as well as nutrient mineralization (Hambright et al. 2007), predicting the responses of these consumers to changes in phytoplankton assemblages is integral to understanding the ecosystem disruptive effects of algal blooms (Sunda et al. 2006). One important, yet little-studied, aspect of this trophic interaction is that herbivore responses to changes in resource availability may lag temporally behind resource changes. Moreover, algal blooms may have persistent demographic or physiological consequences for herbivorous zooplankton, which play out over several generations, with the most severe consequences occurring well after the initial shift in the algal community. While many researchers have investigated various effects of cyanobacteria on zooplankton (Tillmanns et al. 2008), time-lagged and persistent responses to

ecosystem disturbances have received little study in zooplankton-HAB interactions, particularly in rotifers.

Persistent effects of toxigenic cyanobacteria could occur across generations through two different mechanisms: phenotypic plasticity or natural selection. Over the short term, phenotypic plasticity, encompassing maternal effects and acclimation, could lead to changes in fitness independent of changes in genotype composition. For example, over the short time period of four to six generations, experimental exposure of *Daphnia magna* to toxin-producing *Microcystis aeruginosa* increased survivorship during a subsequent *Microcystis* feeding trial relative to a control population fed *Scenedesmus* (Gustafsson and Hansson 2004). The increased survivorship of offspring produced by *Daphnia* fed *Microcystis* was due to maternal effects, as demonstrated through switching experiments (Gustafsson et al. 2005).

Over a longer period, selection against susceptible genotypes could also produce an increase in grazer fitness. Selection by *Microcystis* against susceptible *Daphnia* genotypes may produce an increase in HAB tolerance. *Daphnia galeata* hatched from resting eggs isolated from different sediment ages showed a development of *Microcystis* toxin resistance over a period of 30 years, corresponding with an increase in cyanobacteria bloom frequency (Hairston et al. 1999). In rotifers, differences between species in susceptibility to the toxigenic algae *Karenia brevis*, suggest that natural selection may have led to an increase in fitness in a rotifer species with a shared evolutionary history with *K. brevis* when compared with a naïve species of rotifer

(Kubanek et al. 2007). Providing further support that natural selection may act on rotifer susceptibility to toxigenic algae, Snell (1980) demonstrated turnover in genotype composition of the rotifer *Asplanchna girodi* during a bloom of the toxigenic cyanobacteria *Anabaena flos-aquae*. Laboratory experiments demonstrated variation between these *Asplanchna girodi* clones in tolerance of *Anabaena flos-aquae.*

Within a single generation, cyanobacteria could have persistent effects on herbivorous zooplankton by changing the physiological status of an individual, possibly through effects on macronutrient storage, in a way that continues to have negative effects on survival or reproduction, even when the stressor is lifted. A stressor could therefore continue to affect population demographics by acting through changes in physiological status of individuals long after alleviation of that stressor. The importance of this phenomenon, known as carry-over effects, is increasingly recognized in the study of long-lived vertebrates, where effects from one season can affect reproductive success in the next (Harrison et al. 2011). In zooplankton, as in long-lived vertebrates, excess energy is stored as lipids, and can be used for somatic maintenance or reproduction. Thus, the environment during early life may affect late life survival and reproduction by increasing or decreasing the quantity of stored lipids. In the rotifer *Synchaeta pectinata*, surplus energy is stored as lipids within the ovary. Under low food conditions, a higher proportion of these energy stores are used for reproduction, increasing the chances of producing at least one offspring under limiting resources (Stelzer 2001). Thus the early storage of excess energy may mediate later exposure to stressors such as algae blooms. Although *Brachionus calyciflorus* is a well-studied model organism, the potential for

carry-over effects to modulate the response of this species to algae blooms, has not, to our knowledge been investigated. Yet cyanobacterial densities are regulated by meteorological and hydrological conditions, including sunlight, wind, and inflow, that may change quickly (Paerl 1996, Zhang et al. 2012). Given the spatial and temporal heterogeneity in cyanobacterial densities produced by these quickly changing factors as well as the temporal differences in the population dynamics of phytoplankton and zooplankton, it is quite likely that individual zooplankton experience temporal variation in resource availability within their lifespans.

We tested the hypothesis that the recovery of rotifer populations after algal blooms is modulated by delayed effects of these stressors within and across generations. Specifically, we predicted that rotifers exposed to a pulse of the toxigenic cyanobacterium *Microcystis aeruginosa* would suffer demographic and physiological effects that decreased their ability to recover, even across generations. We used laboratory experiments to test the effects of toxigenic cyanobacteria on the physiological condition and associated life history changes of a rotifer population. Rotifers were initially raised under high or low amounts of good quality food and then switched to toxigenic cyanobacteria. This experiment was designed to test whether internal food stores may buffer the life history responses to later stressors.

We hypothesized that because *Microcystis* is not nutritionally sufficient to sustain population growth of the rotifer *Brachionus calyciflorus* (Nandini and Rao 1997), individuals switched from nutritionally sufficient food (*Chlamydomonas* sp.) to diets of

only *Microcystis* would exhaust their internal lipid stores after several days. We predicted that this decrease in physiological condition would result in decreased maternal lifespans, decreased offspring size condition, and decreased total offspring number. Because internal lipid stores can be used for production of offspring in *Brachionus calyciflorus* (Gilbert 2004b), we predicted that rotifers switched from high *Chlamydomonas* to *Microcystis* would live longer and produce more offspring than those started in low *Chlamydomonas*. Overall, we predicted that carry-over effects would mediate the response of these consumers to changes in their algal diet.

Methods

The effects of unicellular *Microcystis aeruginosa* (UTEX LB 2385), a known toxigenic cyanobacterium (Hughes et al. 1958, Ouahid and Fernández del Campo 2009), were compared with *Chlamydomonas* sp. (Connecticut Valley Biological Supply), which produces high rotifer population growth rates under our culture conditions (see Supplemental Materials: Effect of food density on population growth rate). Both strains of algae used in this experiment were maintained through semi-continuous culture in COMBO medium (Kilham et al. 1998) at a 16:1 N:P (800 μM N and 50 μM P) in a 12-h-dark:12-h-light regime at 20°C. Before running this experiment, we confirmed toxicity of *Microcystis aeruginosa* cultures by measuring microcystin concentration using enzyme-linked immunosorbent assay (Abraxis LLC, Microcystins-ADDA ELISA kit).

For this experiment, we used a clonal strain of *Brachionus calyciflorus* originally hatched from a single resting egg isolated from Lake Texoma, OK-TX, which has frequent blooms of *Microcystis* spp. and other cyanobacteria. This rotifer strain reproduces via cyclical parthenogenesis, but this experiment included only asexually reproducing individuals. Individuals were maintained at low densities to reduce the likelihood of sexual reproduction, which can be triggered under high population density conditions (Gilbert 2004a). Additional experiments with this clone have shown that food level and quality do not significantly change the proportion of females reproducing sexually, and so exclusion of this aspect of the life cycle is not likely to affect our conclusions (see Appendix A: Effect of food quality and quantity on frequency of sexual reproduction).

To standardize backgrounds of individuals used in our experiment, we used third generation offspring, with the first and second generations kept in isolation under constant conditions. We maintained the first and second generations of rotifers in individual wells containing 2 mL of high concentration of *Chlamydomonas* (4×10^5 cells mL^{-1}) in COMBO medium at a 16:1 N:P (800 μM N and 50 μM P) in a 12-h-dark:12-h-light regime at 20°C. Algal cell densities were measured daily using microscopy (10 replicate haemocytometer counts; 200× magnification). Daily, we transferred individual rotifers to fresh food and checked for offspring. Starting on the second day, we checked second-generation females approximately every three hours and removed any neonates produced. We collected the first eight neonates produced by each second-generation female and randomly assigned them to one of eight treatment combinations consisting

of two levels of initial high-quality food (Treatment 1) and after three days, four levels of food quality (Treatment 2).

For the first three days of a third-generation neonate's life, it was randomly assigned to either low (1×10^4 cells mL^{-1}) or high *Chlamydomonas* (4×10^5 cells mL^{-1}) density treatments. Low and high *Chlamydomonas* treatments were chosen based on results of previous experiments, where an algae density which supports a population with a $\lambda\approx1$ (meaning that the population would sustain itself but not grow) was used for the low treatment and an algae density where the rotifer population reached maximum growth rates (λ_{max}) was used for the high treatment (see Appendix A). On the fourth day, the rotifer was transferred to one of four treatments: high *Chlamydomonas*, low (6.25×10^3 cells mL^{-1}) or high *Microcystis* (4×10^5 cells mL^{-1}), or starvation (autoclaved algal growth media without algae). Third-generation females were checked daily for neonate production and survival before being transferred to a well containing fresh treatment. After transferring females, all neonates produced in the past 24 hours by third-generation females were preserved in 95% ethanol, to ensure that all neonates were of similar age, which can affect body and ovary size. Then neonates were measured using an inverted microscope (100× magnification), digital camera, and ImageJ (http://imagej.nih.gov/ij/, accessed 27 Jul 2015). Preserved neonates were measured for total body area, ovary area, and body length. As an estimate of offspring condition, we calculated the ratio of the ovary area (including oocytes, developing egg, and vitellarium) to body area, which serves as a proxy for surplus energy of a rotifer (Stelzer 2001).

We performed all statistical analyses in R (version 3.2.1, R Core Team, http://www.R-project.org/, accessed 27 Jul 2015). Differences in survival among treatments were compared using Cox proportional hazards regression models (*survival* v2.37-4). Total lifetime fecundity was compared between treatments using two-way ANOVA. Offspring lengths were compared between treatments using a linear mixed-effects model to control for maternal identity and age (*nlme* v3.1-110). Differences in offspring condition among treatments were tested using a permutation based mixed effects model because the highly unequal samples sizes (as a consequence of treatments) and the distribution of the data did not fit the assumptions of a linear model (*lmPerm* v1.1.2)

Using the age-specific fecundity and mortality data, the projected population growth rate (λ) for each treatment was calculated as the dominant eigenvector of the corresponding population projection matrix. Confidence intervals (95%) for each λ were estimated with 2,000 bootstrap replicates (Caswell 2001). Briefly, this involved resampling, with replacement, the 4 to 6 rotifers within each treatment, and maintaining the original sample sizes. The growth rate was calculated for this bootstrap sample (λ^*). This process was repeated 2,000 times, generating 2,000 bootstrap estimates of λ^*. Then, the 95% confidence interval was estimated by the 2.5% and 97.5% percentiles of the distribution of bootstrap estimates (λ^*). All bootstrap distributions were examined for median bias by comparing the median of the bootstrap distribution to λ calculated from the original sample.

We implemented randomization tests in R to test for effects of Treatments 1 and 2 on rotifer population growth rates. Randomization tests are the best choice for testing for differences in population growth rates calculated from age-based survival and reproduction data because they take into account the structure of the data and are flexible in terms of distributional assumptions (Caswell 2001). In short, many random samples of rotifers were drawn and used to generate a distribution of a test statistic, assuming the null hypothesis of no effect was true. Then, the observed value of the test statistic was compared against this random distribution to calculate the probability of the experimental test statistic being observed, assuming the null hypothesis was true.

We used a randomization test to test the hypothesis that population growth rates differed between the two levels of Treatment 1 (low and high *Chlamydomonas*) regardless of which level of Treatment 2 was received. This was tested by randomly permuting individuals (including their entire reproductive and survival schedules) between the two levels of Treatment 1, maintaining the original sample sizes in both groups. Then the test statistic $\theta = \lambda^{\text{high}} - \lambda^{\text{low}}$, i.e., the difference in growth rate between the two groups, was calculated. This process of randomly permuting individuals then calculating the test statistic was repeated 2,000 times, giving 2,000 values for θ, which were then compared to the experimentally observed difference in growth rates of rotifers in high and low *Chlamydomonas* (θ_{obs}). Treatment 1 was considered to have a significant effect on growth rate if $\text{P}[\theta \geq \theta_{\text{obs}} | H_0] < 0.05$. That is, if the probability of the randomized test statistic being equal to or greater than the observed test statistic was less than 0.05, the null hypothesis was rejected.

To test the hypothesis that population growth rates differed between the four levels of Treatment 2, for each level of Treatment 1, individuals were randomly permuted between the four levels of Treatment 2, maintaining the original sample sizes in each group. The randomization testing process was very similar to the test of Treatment 1, but in this case, as more than two groups were being compared, the among-group standard deviation ($\theta = \mathrm{SD}(\lambda)$) was used as the test statistic.

To test for interaction between the two treatments, individuals were randomly permuted between the four levels of Treatment 2, again maintaining all original sample sizes and Treatment 1 groupings. If there were no interaction between the two treatments, the reaction norms would be parallel. To test for interaction between the two treatments, the standard deviation of the slopes of these lines was used as the test statistic (Caswell 2001).

Results

Treatments 1 (first three days of life) and 2 (day four until death) significantly affected survival (Fig. 1). Rotifers receiving high *Chlamydomonas* for Treatment 1 had significantly shorter lifespans than those receiving low *Chlamydomonas* (Cox proportional hazards regression model, $\chi^2 = 13.08$, df = 1, $p = 0.0003$). Rotifer lifespans were significantly shortened by starvation during Treatment 2 ($\chi^2 = 24.31$, df = 3, $p < 0.0001$), but the effects of *Microcystis* exposure could not be detected, likely due to

small sample size and high variability. There was no interaction between Treatments 1 and 2 ($\chi^2 = 2.19$, df = 3, p = 0.53).

Treatments 1 and 2 also significantly affected reproduction (Fig. 2). Females receiving high *Chlamydomonas* for Treatment 1 produced significantly more neonates over their lifespans than those receiving low *Chlamydomonas* (Two-way ANOVA, $F_{1,27} = 45.77$, p < 0.001). Treatment 2 significantly affected total offspring production ($F_{3,27} = 7.054$, p = 0.001). Rotifers receiving *Chlamydomonas* for Treatment 2 produced significantly more offspring than those receiving high *Microcystis,* low *Microcystis*, or no food (Tukey HSD, p < 0.02 for all pairwise comparisons). There were no differences in offspring production between either of the two *Microcystis* levels or starvation (Tukey HSD, p > 0.7 for all pairwise comparisons). There was no interaction between Treatments 1 and 2 ($F_{3,27} = 0.1949$, p = 0.899).

Only the first treatment had a significant effect on population growth rates. Rotifers that received high *Chlamydomonas* for Treatment 1 had significantly higher population growth rates than those that received low *Chlamydomonas* (Table 1, Randomization Test, p < 0.001). Within each level of Treatment 1, there was no significant effect of Treatment 2 on population growth rate (High *Chlamydomonas*, p = 0.4608; Low *Chlamydomonas*, p = 0.3448), and there was no significant interaction between Treatments 1 and 2 (p = 0.8436). The difference in population growth rates between high and low *Chlamydomonas* is attributable to changes in early life fecundity, and not

survival (see Appendix A: Relative contribution of survival and reproduction to population growth rate).

Rotifers fed high *Chlamydomonas* for Treatment 1 produced significantly larger offspring (Fig. 3, linear mixed-effects model, $F_{1,27} = 14.36$, $p < 0.001$). The effect of *Microcystis* on offspring body size was dependent on the initial *Chlamydomonas* density ($F_{3,27} = 3.300$, $p = 0.0354$). When switched to either low or high *Microcystis* treatment, there was a significant decline in offspring size, but only in rotifers receiving low *Chlamydomonas* during Treatment 1. Additionally, the condition of offspring produced, measured by ovary size relative to body size, was significantly affected by both the first and second maternal treatments (Fig. 4). Rotifers receiving low *Chlamydomonas* in Treatment 1 produced offspring with lower body condition (3% lower on average, Permutation-based mixed-effects model, $p = 0.0002$). Second treatments of *Microcystis* and starvation also decreased offspring condition. There was no interaction between Treatments 1 and 2 ($p = 0.1475$), although the small sample size in some treatment combinations could have reduced our power to detect this effect.

Discussion

We had hypothesized that early exposure of rotifers to high levels of food would alleviate the negative effects of later exposure to *Microcystis aeruginosa*. Contrary to our hypothesis, we found that *Microcystis* exposure decreased survival and fecundity of rotifers, regardless of the quantity of food they had received over the first three days of life. Therefore, survival and reproduction could not be rescued by early-life exposure to

high amounts of high quality food. However, when we examined the role of early-life food availability on the quality of offspring produced, we found that the effect of *Microcystis* on offspring body size was dependent on the initial *Chlamydomonas* density. When rotifers were switched to either the high or low *Microcystis* treatment, there was a significant decline in offspring size, but only in mothers that had received low *Chlamydomonas* for the first three days. This suggests that those individuals exposed to high concentrations of *Chlamydomonas* during growth and maturation had stored more energy that allowed them to continue to produce offspring of the same size and quality regardless of food level or quality offered after day three.

With respect to optimal offspring size, life history theory predicts a unimodal relationship between resource availability and offspring size, such that the largest offspring are produced at intermediate resource availability (Roff 2001). At high resource availability, many small offspring are predicted, and at low resource availability, few small offspring are predicted. Based on this hypothesis, if *Microcystis* acts as an intermediate level between starvation and *Chlamydomonas* in terms of resource availability, then we would predict that offspring size would be largest in offspring produced by rotifers fed *Microcystis*. For rotifers fed high *Chlamydomonas* for the first three days, we find support for this prediction. The largest offspring were produced by rotifers switched to *Microcystis,* compared to rotifers fed *Chlamydomonas* or starved. Rotifers fed *Chlamydomonas* after the third day produced many small offspring and starved rotifers produce few small offspring. However, when we consider the rotifers fed low *Chlamydomonas* for the first three days, we see no relationship

between the second treatment and offspring size. We suggest that this shows that the adaptive response of producing larger offspring at intermediate resource availability may be constrained by maternal resource background.

Our experiment provides evidence that under the resource limitation represented by *Microcystis* exposure, *Brachionus* is capable of using stored resources to maintain production of high quality offspring. However, under this resource limitation, the number of offspring produced declines, as does survival of the exposed rotifer, suggesting that the previously stored resources are used primarily for production of a few high-quality offspring, instead of production of many lower quality offspring or maintenance of somatic tissue. Similarly, in the rotifer, *Synchaeta pectinata*, stored resources are used to maintain a high reproductive effort even when rotifers are reared under low food availability (Stelzer 2001). This investment in the production of larger, high-quality offspring could be an adaptation to maximize fitness under limiting resources. Investment in larger offspring has been shown to increase offspring survival in young age classes (Walz 1995). If these larger, high-quality offspring were better at surviving and reproducing under poor conditions, then their investment would increase fitness relative to clones that do not produce larger offspring under such conditions.

In addition to this phenotypic plasticity in offspring size, some zooplankton have further maternal effects that increase offspring fitness under poor conditions. For example, in the cladoceran *Daphnia magna*, Gustafsson and Hansson (2004) found that over the course of four to six generations, the fitness of individuals exposed to a mixed diet of

Microcystis and *Scenedesmus* increased. With further experiments, Gustafsson and colleagues (2005) found that maternal effects were responsible for the increase in fitness. The likely mechanism underlying these observed maternal effects was the up-regulation of the production of detoxifying enzymes (Ortiz-Rodriguez et al. 2012). In contrast to these beneficial maternal effects observed in cladocerans, in *Brachionus calyciflorus*, we found that offspring born to mothers reared on *Microcystis* had significantly smaller body sizes and energy reserves. Based on this evidence, it seems unlikely that maternal effects are capable of lessening the negative effects of *Microcystis* on reproduction in this rotifer. It could be that while the effects of toxins can be ameliorated through maternal effects (e.g., up-regulation of enzyme production), nutritional deficiencies may not be so alleviated. Even non-toxic strains of *Microcystis* have strong negative effects on the population growth of the rotifer *Brachionus calyciflorus,* suggesting that nutrition plays a strong role in survival and reproduction (Zhao et al. 2014). In the experiments carried out with *Daphnia magna*, Gustafsson and Hansson (2004) used mixed diets of *Microcystis and Scenedesmus*, which may have alleviated any nutritional deficiencies of a pure *Microcystis* diet. That is, any negative effects attributable to the nutritional deficiencies of *Microcystis*, and not to its toxicity, may have been alleviated by supplementing with *Scenedesmus*, allowing the positive maternal effects to become apparent. Our own research provides evidence that nutritional deficiencies of pure cyanobacteria diets can be alleviated by including a mixed diet; in preliminary feeding experiments where the non-toxic cyanobacterium *Synechococcus leopoliensis* was supplemented with *Chlamydomonas* (see Appendix A:

Effect of food quality and quantity on frequency of sexual reproduction), rotifer population growth rates were higher than on a pure *Chlamydomonas* diet.

As we suggest above, the reduction in population growth rate in *Brachionus calyciflorus* exposed to unicellular *Microcystis aeruginosa* can be attributed to two mechanisms: toxicity and nutritional deficiency (Porter and Orcutt 1980, Lampert 1987). With the methods that we used, untangling the relative contributions of these two factors was not possible. In a comparative study of the effects of a toxic and non-toxic strain of *Microcystis aeruginosa*, Zhao and colleagues (2014) found that non-toxic strains of *Microcystis aeruginosa* could not sustain population growth of *Brachionus calyciflorus*. This suggests that although toxicity has a negative impact on *Brachionus calyciflorus*, it is not the sole reason for decreased population growth rates in rotifers exposed to *Microcystis aeruginosa* (Zhao et al. 2014). In another non-toxic cyanobacterium, *Synechococcus elongatus*, deficiencies in sterols and amino acids limited the population growth of *Brachionus calyciflorus* (Wacker and Martin-Creuzburg 2012). In a preliminary experiment, we found that *Synechococcus leopoliensis* supported population growth of *Brachionus calyciflorus* over two weeks, but population growth rates were lower than those produced by *Chlamydomonas* (see Appendix A: Effect of food quality and quantity on frequency of sexual reproduction). Combined, this evidence suggests that the observed decrease in offspring size and quality could be due to deficiencies in important macronutrients like fatty acids, sterols, or amino acids.

Although not directly tested in this study, cyanobacteria exposure could have a persistent effect on rotifers if cyanobacteria exposure altered the age structure of a zooplankton population. Biased survival of certain age or stage classes could have long-term demographic effects. For example, cyanobacteria exposure in rotifers may remove young individuals from the population at higher rates than older individuals (Barreiro Felpeto and Hairston 2013). As younger individuals have a higher reproductive contribution to the population growth rate (Roff 2001), this selective mortality could have a substantial effect on population growth rates of rotifers exposed to cyanobacteria. In the cladoceran zooplankter *Daphnia pulex*, exposure to *Microcystis aeruginosa* changed the age structure by increasing the proportion of adolescents in the population, as well as the production of resting eggs (Laurén-Määttä et al. 1997). Both of these responses would immediately decrease population growth rates, although an increased investment in resting egg production could allow for a reestablishment of the population after the threat of cyanobacteria dissipated. The decreased maternal investment of rotifers initially reared under low food conditions then transferred to *Microcystis* suggests a mechanism for increased susceptibility of juvenile offspring to cyanobacteria exposure. Neonates with lower lipid reserves have decreased energy reserves that could decrease their resilience in the face of toxin exposure and lower nutrient availability.

In our experiment, we isolated the roles of phenotypic plasticity and maternal effects by using only one genotype of *Brachionus calyciflorus*. We found in this population, negative effects of *Microcystis* exposure were transferred across generations, and

responses were mediated by prior food conditions. These results lead us to suggest that the effects of increasing frequency and duration of harmful algal blooms on rotifer populations may be even more deleterious than expected, as these negative effects carry across generations. However, in a lake ecosystem, coexistence of many genotypes (and many species) would present the possibility of selection among genotypes and competition among species. Further research incorporating multiple genotypes and species is needed to elucidate how the individual- and population-level effects we measured may interact with community interactions to structure the response of zooplankton and the wider lake community to persistent and frequent blooms of harmful algae.

As HABs are predicted to increase in frequency and duration due to climate change (Moore et al. 2008), grazers will experience changes in resources more regularly and for longer periods of time. This leads us to suggest that the presence of a negative feedback loop with more frequent and longer HABs which may reduce the resistance of rotifers to HABs over time. The poorly provisioned offspring produced by rotifers exposed to cyanobacteria could, over time, produce even fewer lower-quality offspring that have even lower tolerance to cyanobacteria. The observed declines in fitness could compound over generations, resulting in a negative feedback loop and smaller populations of primary consumers within lakes. Further experiments with rotifers are needed to elucidate the role of maternal exposure in offspring tolerance of cyanobacteria over many generations.

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Tables

Table 1: Projected population growth rate (λ) for each combination of Treatments 1 and 2 calculated from the corresponding population projection matrix. 95% confidence intervals for the population growth rate estimated through bootstrap resampling with 2,000 replicate samples.

Treatment 1	**Treatment 2**	**λ**	**CI (95%)**
High	C	2.422	(2.176, 2.654)
High	HM	2.263	(2.085, 2.407)
High	LM	2.043	(1.614, 2.451)
High	S	2.355	(2.140, 2.556)
Low	C	1.419	(1.183, 1.531)
Low	HM	1.332	(1.244, 1.428)
Low	LM	1.157	(1.061, 1.221)
Low	S	1.221	(1.080, 1.341)

Table 2: Results of linear mixed-effects model of offspring size. Asterisk represents significance at $\alpha = 0.05$.

	Numerator DF	Denominator DF	F	p-value
(Intercept)	1	201	11279.92	< 0.0001*
Treatment 1	1	27	14.36	0.0008*
Treatment 2	3	27	2.23	0.1079*
Maternal age	1	201	5.64	0.0185*
Treatments 1*2	3	27	3.300	0.0354*
Treatment 1* Maternal Age	1	201	4.919	0.0277*
Treatment 2* Maternal Age	3	201	0.607	0.6112
Treatments 1*2* Maternal Age	3	201	0.797	0.4968

Figures

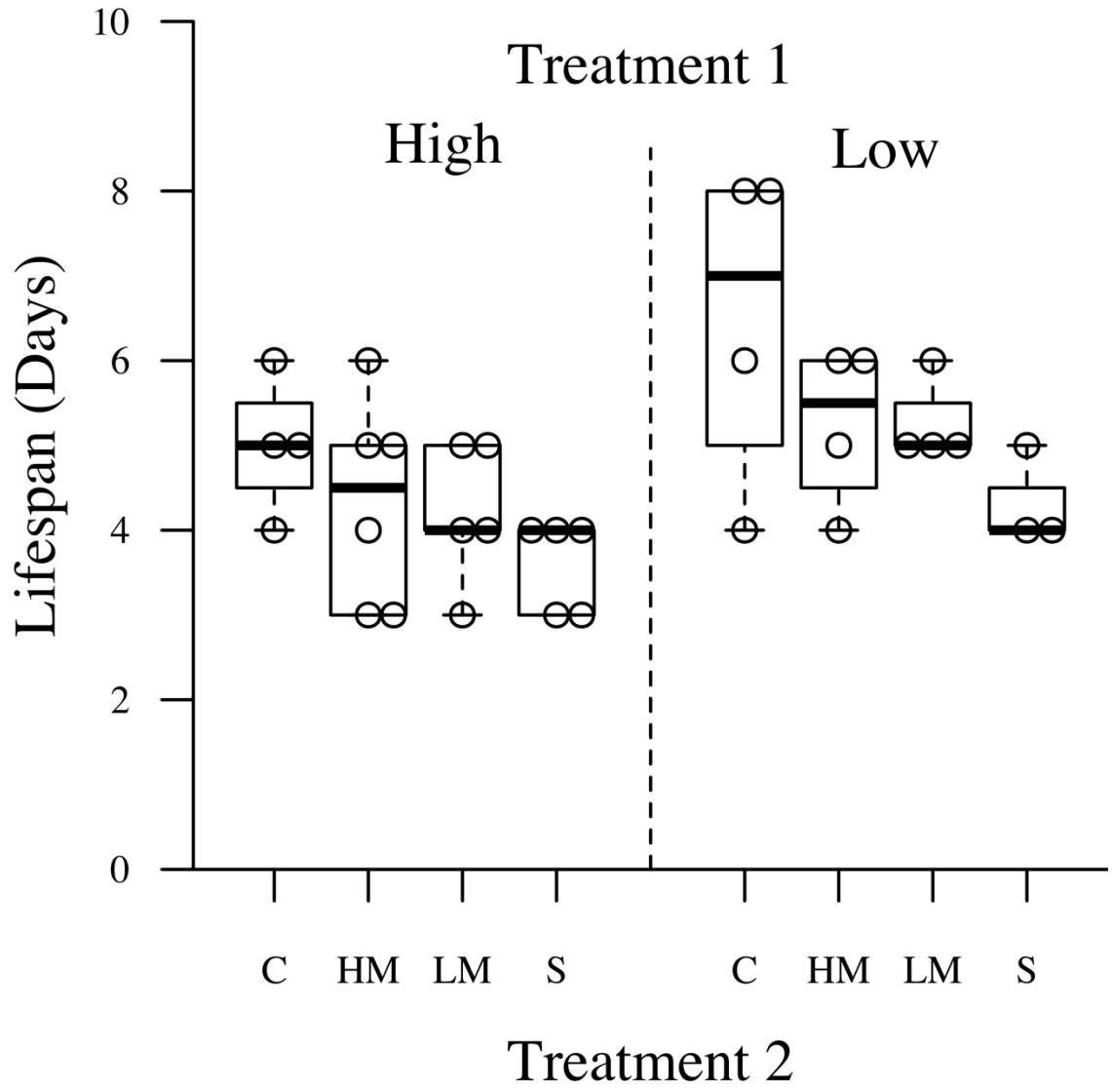

Figure 1: Lifespan of rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2 (C = *Chlamydomonas*, HM = High *Microcystis*, LM = Low *Microcystis*, and S = starvation). Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median. Individual data points are overlaid as small, open circles.

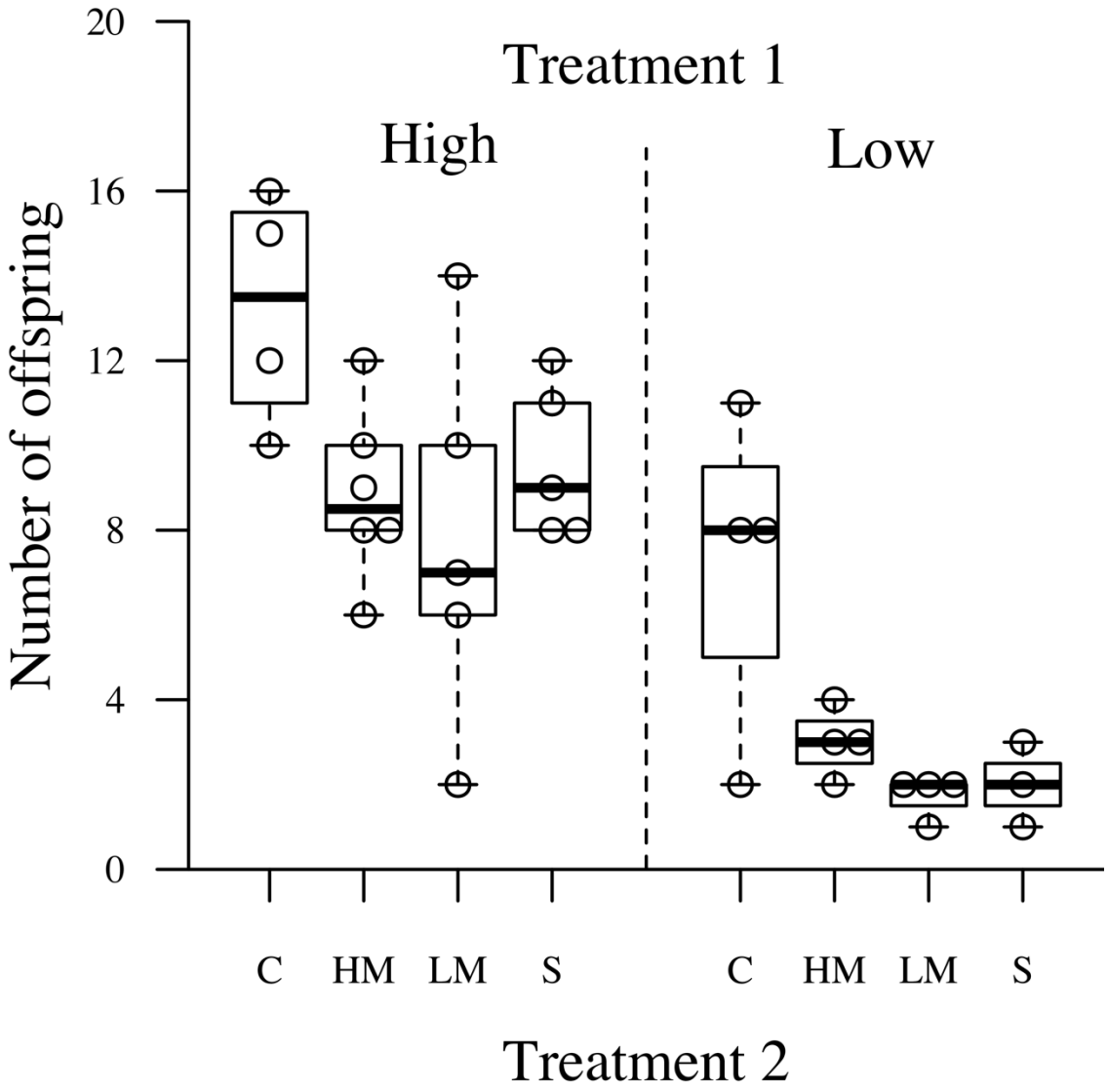

Figure 2: Number of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median. Individual data points are overlaid as small, open circles.

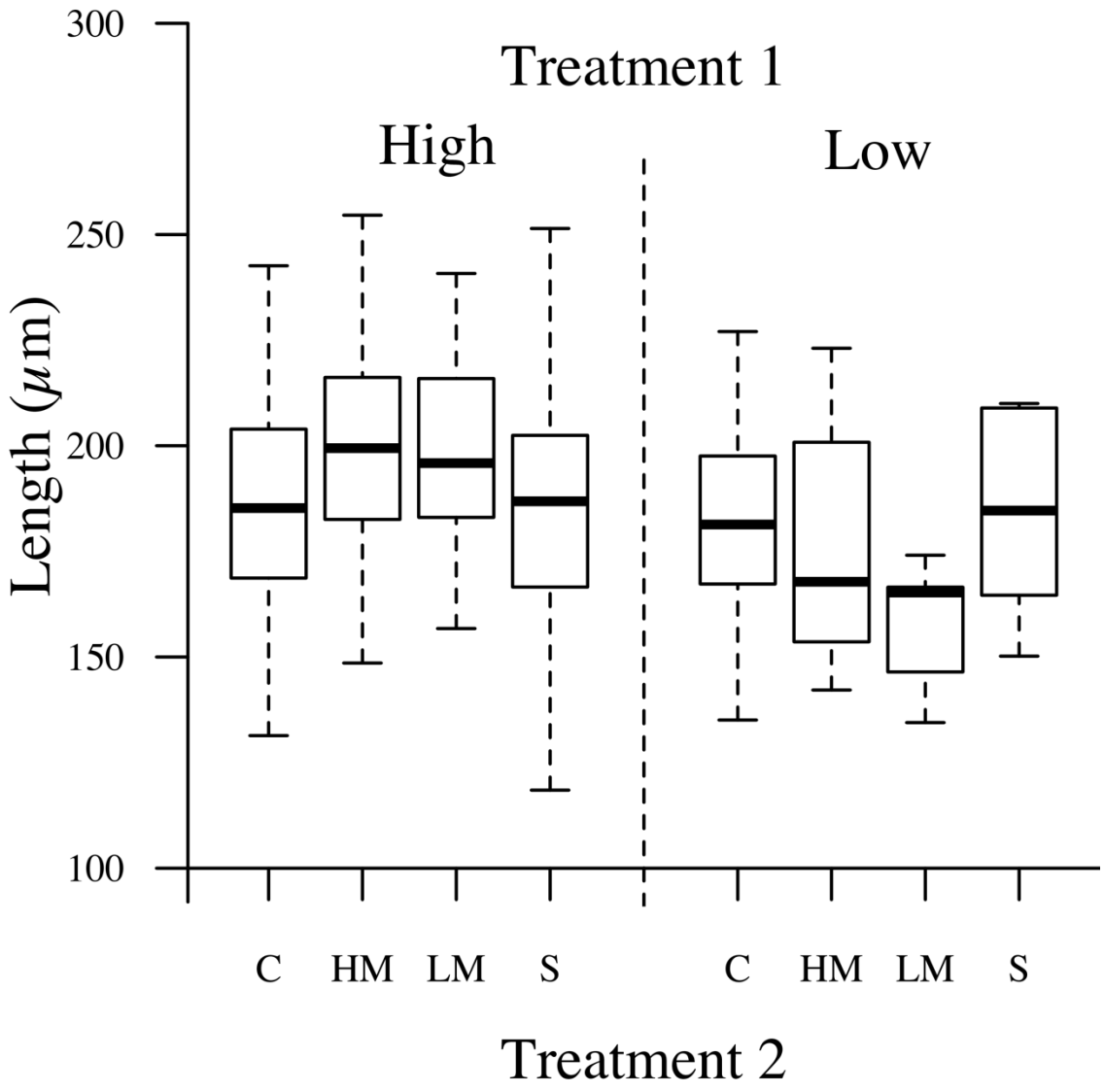

Figure 3: Length of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to the Treatment 2. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median.

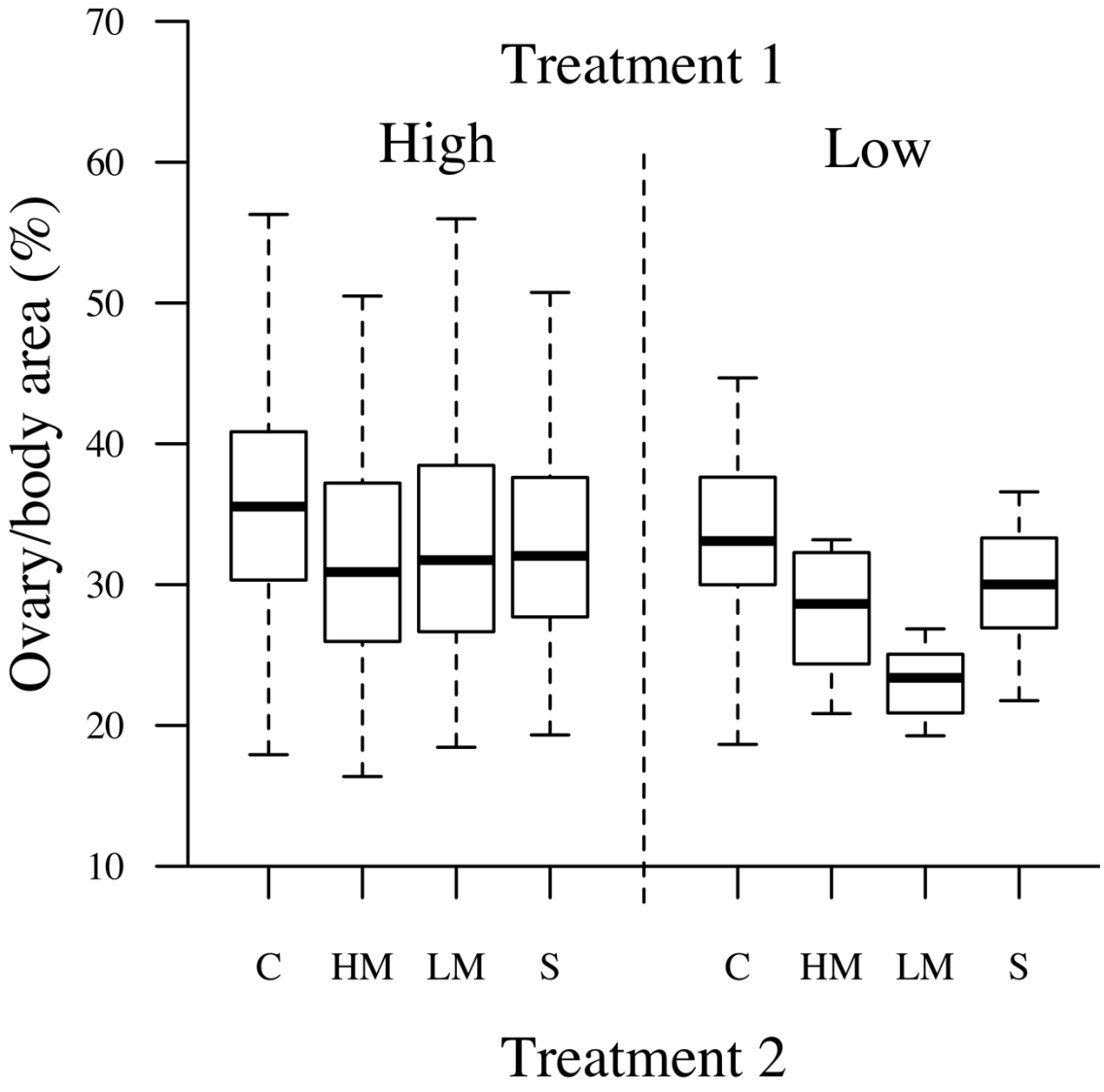

Figure 4: Condition of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2. Offspring condition was measured by dividing the ovary area by the total body area. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median.

Chapter 2 – Maternal effects are no match for stressful conditions: a test of the maternal match hypothesis in a common zooplankter

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Keywords: *Brachionus calyciflorus*, harmful algal blooms, life history, maternal match hypothesis, optimal allocation, rotifers

Summary

1. Maternal effects modulate population responses to environmental conditions and so are predicted to play a large role in the responses of organisms to global change.
2. In response to one such aspect of global change, the eutrophication of freshwaters and associated blooms of the toxin-producing cyanobacteria species *Microcystis aeruginosa*, the rotifer *Brachionus calyciflorus* produces larger offspring.

3. We hypothesized that rotifers exposed to *Microcystis* may be adaptively increasing offspring investment and offspring fitness (i.e. the maternal match hypothesis).
4. We explicitly tested the consequences of this differential investment by rearing offspring produced by rotifers reared under *Microcystis* and *Chlamydomonas* in a full factorial design, where offspring were raised under the maternal diet or the opposite food source.
5. We measured age-specific fecundity, survival, and population growth rates under these conditions and found that maternal exposure to *Microcystis* decreased offspring survival and fecundity, regardless of offspring diet. Population growth rates, tested using aster models, differed significantly among maternal and neonate diets, but there was no significant interaction between the two factors.
6. Our evidence thus leads us to reject the maternal match hypothesis in this case of rotifer-toxigenic algal bloom interactions and provides further support that toxigenic algal blooms may have extensive effects on grazer populations in ways that are not evaluated using traditional, single-generation experimental methods.

Introduction

Maternal effects on offspring phenotypic plasticity modulate population responses to environmental conditions (Schwarzenberger & Elert 2013; Moore, Landberg & Whiteman 2015) and so are predicted to play a large role in the responses of organisms

to global change (Meylan, Miles & Clobert 2012). One way in which the maternal environment may give rise to phenotypic plasticity in offspring is through anticipatory maternal effects taking the form of differential provisioning of offspring (Marshall and Uller 2007). For example, resource availability (here defined as food quality and quantity) during the maternal generation may affect the tradeoff in the number and size of offspring produced (Smith & Fretwell 1974). When a mother can predict the environment that her offspring will encounter, the tradeoff between the number and size of offspring may be modulated by the perceived survival risk of her offspring. According to optimal egg size theory (OEST), as resource availability declines, and thus offspring survival risk increases, the per capita investment by mothers should increase, leading to larger offspring, which comes at a cost of a decline in the number of offspring produced (Krist 2010). This theory has been tested in a broad variety of organisms, and support has been found in diverse taxa including collared flycatchers, *Ficedula albicollis* (Krist & Munclinger 2015); Atlantic salmon, *Salmo salar* (Rollinson & Hutchings 2013); and invertebrates like the crustacean zooplankter *Daphnia pulex* (Li & Jiang 2014) and the rotifer *Brachionus calyciflorus* (Beyer & Hambright 2016). However, support for the OEST is not universal (e.g., Brett 1993; Kirk 1997), leading to the questions of when and under which conditions the OEST operates.

Optimal provisioning of offspring will be particularly important under conditions of food scarcity or nutritional insufficiencies, when offspring fitness may be more dependent on resources acquired through maternal investment than resources acquired through feeding on those scarce resources. For example, young rotifers hatched from

larger eggs are more resistant to starvation than rotifers hatched from small eggs (Kirk 1997). The relative importance of maternal effects is context dependent because under poor conditions, offspring survival and reproduction will be more tightly linked to maternal investment than in good conditions. Thus, the external environment of the offspring will mediate the expression of maternal effects. We would thus predict that the realization of maternal effects as well as the affected life history traits depend on the environment. In a test of context-dependent maternal effects in soil mites, Plaistow et al. (2006) showed that effect of egg size differed between low-food and high-food conditions. Variation in egg size only affected tradeoffs in age and size at maturity under low-food conditions. One caveat of context-dependent maternal effects is that this maternal strategy of differential investment in offspring that will encounter good or poor environments assumes that mothers can identify their offspring's environment.

The maternal match hypothesis (MMH, described in Sheriff & Love 2013) was established as a framework to predict how maternally-derived stress would affect the phenotype of her offspring when imperfect information and changing environments lead to phenotypic mismatches between the offspring and the environment. Under the MMH, offspring born to mothers in stressful or poor environments are predicted to be more fit when raised in similarly stressful or poor environments compared with offspring born to mothers in good environments (Sheriff & Love 2013). The MMH suggests that, under stressful conditions, mothers will produce offspring that are more fit for the same stressful conditions. However, the phenotype of these offspring may be maladaptive under benign conditions. Thus, to fully test the MMH, offspring produced by mothers

reared in benign and stressful conditions must be raised under stressful conditions, and then evolutionarily meaningful response variables (e.g., lifetime fitness) must be monitored. Simultaneous empirical tests of the maternal match hypothesis and the optimal egg size theory are not well developed, yet herbivorous zooplankton provide an ideal system for these tests because they have short lifespans that can allow for the measurement of lifetime fitness, can be maintained as asexual (clonal) lineages, and are regularly exposed to natural variations in food availability.

Within aquatic systems, primary consumers like zooplankton are exposed to frequent variations in food quality and quantity. Rapid changes in phytoplankton composition, including blooms of harmful algae, represent abrupt changes in resource availability that may have dramatic effects on consumers (Sunda et al., 2006). Blooms of cyanobacteria act to change the nutritional quality (Lürling 2003) and toxicity (Tillmanns *et al.* 2008) of the resources available to freshwater grazers, and may have dramatic effects on the life histories of these grazers. Thus, we predicted that adaptive maternal effects in response to changes in resources should be well developed in freshwater grazers. A test of maternal effects in asexually reproducing organisms like some freshwater zooplankton effectively isolates the plastic effects of mothers from potential genetic differences between mother and offspring, allowing us to conveniently and directly test the role of context-dependent maternal effects. In one such asexually-reproducing freshwater zooplankton taxon, the rotifer *Synchaeta pectinata*, research has demonstrated that maternal diet affects investment in offspring (Stelzer 2001) and that investment has an effect on offspring fitness (Walz 1995). Even the life-extending

effects of caloric restriction can be passed to offspring in the rotifer *Brachionus plicatilis* (Kaneko *et al.* 2010).

Using the rotifer *Brachionus calyciflorus* (Fig. 1), we set out to test the consequences of differential offspring investment produced after maternal exposure to resources that differed in quality. The first generation of rotifers was raised with either toxigenic cyanobacteria (*Microcystis aeruginosa*) or good-quality food (*Chlamydomonas* sp.) and their offspring were either maintained on the same treatment as their mothers or switched to the other treatment. These two foods are known to produce differential resource investment, with fewer, larger offspring produced by rotifers exposed to *Microcystis* compared with those exposed to *Chlamydomonas,* if mothers were initially reared under high amounts of *Chlamydomonas* for the first three days of life (Beyer & Hambright 2016). We measured age-specific survival and fecundity of the four groups of second-generation rotifers and calculated the population growth rate and total offspring production, two measures of fitness which are predicted to change under the OEST and MMH. OEST would be supported if, under the poor food conditions of *Microcystis*, mothers produce fewer, larger offspring and these large offspring are more fit. However, MMH would be supported if offspring produced in *Microcystis* have higher fitness than those produced in *Chlamydomonas*, but only when reared in *Microcystis*.

Materials and methods

Study organisms and culture conditions

We used a clonal strain of the rotifer *Brachionus calyciflorus* (Pallas), that was started from a single resting egg isolated from a tributary of Lake Texoma, OK-TX, USA that has regular blooms of cyanobacteria. As is typical of rotifers, this strain reproduces by cyclic parthenogenesis, but we only considered females reproducing asexually in this experiment because earlier experiments have shown that the switch to sexual reproduction is mediated by rotifer population density (Gilbert 2004) and not by variation in resource quality or quantity (Beyer & Hambright 2016). Under our high-quality food conditions (see below for details), the median survival for this rotifer species is five days (Beyer & Hambright 2016). Typically, a newly-hatched female produces her first egg which hatches on the second day. She will continue to produce eggs, one at a time, throughout her life. Over days two through three, she may carry up to three eggs at a time, which will hatch into neonates, which will, in turn, produce their own offspring two days later. From these data, the average generation time is 3.1 days, and there is a very high degree of overlap between generations. Thus, mothers exist in nearly the exact same time and conditions as their daughters, and granddaughters.

All algae and rotifers were cultured in COMBO medium, which is optimized for both algal and zooplankton growth (Kilham *et al.* 1998). Cultures were maintained at 20°C on a 12-h-dark:12-h-light regime. We compared the effects of unicellular *Microcystis aeruginosa* (Kützing) (UTEX LB 2385, UTEX Culture Collection of Algae, Austin, TX, USA) with *Chlamydomonas* sp. (Ehrenberg) (Connecticut Valley Biological

Supply, Southampton, MA, USA). Under our conditions, high population growth rates of *Brachionus calyciflorus* are obtained on a diet of *Chlamydomonas* but not *Microcystis*. Additionally, this strain of *Microcystis* produces microcystins under our culture conditions (see Beyer & Hambright 2016 for further details).

Experimental test of maternal diet on offspring fitness

To test for the effects of maternal diet on offspring survival and reproduction, we raised populations of rotifers at densities below which sex is induced. These populations were fed either *Microcystis* (4×10^5 cells ml^{-1}) or *Chlamydomonas* (4×10^5 cells ml^{-1}) for three days. The densities of algae used for these treatments were selected to optimize population growth rates under each treatment (Beyer & Hambright, 2016). After three days, we collected neonates from the two treatments and transferred them to individual 2-mL wells of a 24-well plate (Grenier Bio-One, Monroe, NC, USA) with either the parental diet or the opposite food (six individuals raised in each factorial combination of maternal and offspring diet, random assignment of offspring to treatments using random number generator). Sample sizes were chosen based on sizes that were sufficient in to measure effects of diet on population growth rates in previous experiments (Beyer & Hambright, 2016). Each day, offspring were transferred to fresh food, and we measured survival and the number offspring produced.

Statistical analysis

All statistical tests were carried out in R (R Core Development Team, version 3.2.1, www.r-project.org). Differences in survival among treatments were compared using

Cox proportional hazards regression models (survival, Version 2.37-4). Assumptions of the Cox proportional hazards regression model were tested using the cox.zph function and examination of log-transformed survival curves, and we found that the proportional hazards assumption was met ($p > 0.05$ for all terms in model). We tested for differences in total number of offspring using a permutation-based ANOVA to account for the differences in dispersion observed between treatments (lmPerm, Version 1.1.2).

Fitness for each combination of maternal and offspring diets was quantified as the population growth rate (λ), which was calculated as the dominant eigenvalue of the corresponding Leslie matrix (Caswell, 2001). Confidence intervals were constructed using bootstrap resampling (2000 iterations). In assessing the relative contributions of maternal and offspring diet to fitness, we compared the utility of two approaches. For the first approach, we used randomization testing to test for significance of the contributions of maternal and offspring diet to the population growth rate of the offspring (Caswell 2001). Additionally, we modeled individual lifetime fitness using aster models (Shaw *et al.* 2008) (aster, version 0.8-31). Aster models are a parametric approach to modeling life history data that take into account the natural distributions of data (e.g., Poisson distributions for the number of offspring) and the interdependencies of the data (e.g., reproduction at age five is dependent on survival up until age five). Our model integrated both age specific survival and fecundity data. Survival to each age (S_x) was modeled using a Bernoulli distribution. As a result of the experimental treatments, there were too many zero values in the number of offspring produced at age x (B_x) to fit a Poisson distribution, so instead, reproduction was modeled as a two-step

process. For each age, we modeled the success of reproduction as a variable that took two values (Bernoulli distribution, 0 or 1) with 1 indicating that the rotifer produced offspring at that age. Then, for those rotifers that produced at least one offspring, the number of offspring produced at that age was modeled using a zero-truncated Poisson distribution (B_x). Using these aster models, we tested for the effect of maternal and offspring diet on the population growth rate, and calculated standard errors for those estimates. We then compared the conclusions drawn from aster modeling with those drawn from randomization testing to test the effectiveness of these two approaches that are commonly used in individual-based life history testing.

Results

Survival and Reproduction

We found that maternal diet had a strong influence on survival and reproduction of offspring (Figs 1, 2). The offspring of rotifers fed *Microcystis* had lower survival than those produced by rotifers fed *Chlamydomonas* (Cox proportional hazards model, Wald $z = 2.50$, $p = 0.012$). There was no detectable effect of offspring diet on survival (Wald $z = 1.02$, $p = 0.310$). Exposure to *Microcystis*, in both first and second-generation diets, severely reduced reproduction in the second-generation rotifers (Permutation-based ANOVA; $p = 0.007$, $p = 0.004$). None of the rotifers raised in *Microcystis* with maternal diets of *Microcystis* produced any offspring (Fig. 2).

Fitness

Using randomization testing, we found that lifetime fitness was significantly affected by maternal diet ($p = 0.017$, Table 1). Within those rotifers with maternal diet of *Chlamydomonas*, there was no significant effect of offspring diet on fitness ($p = 0.116$). Within those rotifers with maternal diet of *Microcystis*, there was no effect of offspring diet on fitness ($p = 0.423$). There was no interactive effect between maternal and offspring diet on fitness ($p = 0.351$). Using aster modeling, we found that lifetime fitness was significantly affected by maternal diet and offspring diet, but the interaction between the two had no effect (Table 1).

When we made a direct comparison of parametric (aster model) and nonparametric methods (randomization testing) in estimating population growth rates and effects of treatments on population growth rates, we found some similarities and some differences in drawn inferences (Table 1). Estimates of population growth rates were nearly equivalent for three of the four treatments (Tables 2, 3), but the population growth rate of rotifers where both the neonate and mother were raised in *Microcystis* was over-estimated by the aster model. This is an unusual case where absolutely no offspring were produced by any of the second-generation individuals raised in *Microcystis,* and the variation in number of offspring produced was zero (Fig. 2). Both parametric and nonparametric methods detected a significant effect of maternal diet on offspring fitness (Table 1). Neither approach supported an interactive effect of maternal and offspring diets on offspring fitness. However, using the parametric approach (aster model), we

found support for a significant effect of offspring diet on offspring fitness (Table 1), which was not detected using the nonparametric approach (randomization testing).

Discussion

OEST makes the prediction that under lower resource availability (*Microcystis*) the produced eggs and offspring will be larger than under high resource availability (*Chlamydomonas*). We found support for this in earlier work (Beyer & Hambright 2016). The maternal match hypothesis makes the further prediction that offspring produced by *Microcystis*-reared mothers will be more fit than those produced by *Chlamydomonas*-reared mothers when raised with *Microcystis*. That is, the stressed mothers will produce offspring that are more fit under stressful conditions. We did not find support for the MMH within this experiment. In comparing offspring raised with low quality food (*Microcystis*), we found that offspring produced by mothers fed *Microcystis* had lower fitness when fed *Microcystis* (measured as estimated population growth rate and as total offspring produced) than those produced by mothers fed *Chlamydomonas*. That is, the offspring produced by stressed mothers fared worse in stressful situations than the offspring produced by unstressed mothers, even though they were larger. It is clear, then, that offspring size cannot be used as a proxy for fitness in *Brachionus calyciflorus*, and instead total lifetime fitness must be directly measured in order to understand the fitness consequences of environmental stressors and to validly test the OEST and MMH.

In describing the MMH, Sheriff and Love (2013) lay out the conditions under which the MMH is expected to operate. Any life history or ecological traits that increase the likelihood of a match between the maternal and offspring environments will increase the adaptive potential of maternally derived stress responses. For example, r-selected organisms (those that are fast maturing and short lived) should have greater responses to maternally derived stress than k-selected organisms, because offspring are more likely to exist within the same ecological conditions that were stressful to the maternal generation. Additionally, organisms with stronger philopatry and low dispersal should have stronger adaptive responses to maternally derived stress. Based on these criteria, we would predict that *Brachionus calyciflorus* would have an adaptive response to maternally derived stress, because these rotifers have short lifetimes, reproduce early in life, and do not actively disperse, leading to overlapping generations.

In considering the relationship between offspring size and fitness, our results, that larger offspring had lower fitness, are counterintuitive when considered within the traditional framework of the OEST. That these offspring were less fit, even though they have presumably larger energy reserves for somatic maintenance and reproduction, warrants speculation. While the majority of studies show that larger offspring are more fit (Walz 1995; Kirk 1997), not all research supports this expected relationship between offspring size and fitness. Multiple mechanisms (including lower boundary on egg volume, Guinnee et al., 2007; physiological constraints, Stelzer, 2002; and feeding behavior, Garbutt and Little, 2014) have been invoked to explain this paradoxical empirical result in freshwater zooplankton. In a test of the maternal match hypothesis in *Daphnia*

magna, Guinnee et al. (2007) took larger offspring produced by individuals fed low amounts of food and reared them in either low or high food environments. These larger offspring produced by resource-limited mothers had lower fitness in both environments, compared with offspring born to mothers fed high amounts of food, because they took longer to reproduce and did not have a survival advantage. In this case, larger eggs were not adaptive (as OEST would predict), but instead the explanation for this pattern was that there was a lower boundary on egg volume, or that there is a minimum viable size for eggs, and mothers do not produce eggs smaller than this (Guinnee *et al.* 2007). In the rotifer, *Macrotrachela quadricornifera*, Santo et al. (2001) found that larger egg sizes decreased developmental time and time to reproduction, but did not confer benefits under stressful conditions. In the rotifer *Synchaeta pectinata*, Stelzer (2002) found that although low temperatures produce larger eggs and larger offspring, these offspring were not more fit than those smaller offspring produced at warmer temperatures, when tested under colder temperatures. When tested under warmer temperatures, the smaller offspring were found to have higher fitness. In *Daphnia magna*, smaller offspring produced at higher food concentrations have paradoxically higher fitness when compared with larger offspring produced at low food concentrations (Garbutt & Little 2014). This difference may be due to the feeding behavior of the offspring, because the maternal environment was found to affect feeding rates of offspring, independent of the environment in which the offspring live. These four examples highlight the many factors governing the relationship between egg size and fitness, and illustrate mechanisms that could produce the counterintuitive pattern of larger eggs having lower fitness. The design of our experiment does not allow us to

disentangle the factors leading to the cumulative decreased fitness under the *Microcystis* feeding regime, but many of the causes described above could be playing a role. As both nutrition and toxicity contribute to the negative affects of *Microcystis aeruginosa* on *Brachionus calyciflorus* (Zhao et al. 2014), we suggest that this offspring size-fitness relationship could be produced by constraints on the ability of rotifers to upregulate detoxifying enzymes, by inability to compensate for the nutritional inadequacy of *Microcystis aeruginosa*, or by interaction of these two mechanisms.

Although maternal effects have been tested in rotifers relative to resting egg production (Gilbert & Schröder 2007), morphology (Gilbert & McPeek 2013), lifespan (Gribble & Mark Welch 2013), and many other conditions, the contribution of maternal effects to responses to harmful algal blooms including cyanobacteria has not been measured. However, in *Daphnia*, maternal effects play a strong role in population responses to cyanobacteria exposure. Depending on the species and clonal identity, and likely depending on experimental design, exposure of mothers to cyanobacteria may increase (Gustafsson, Rengefors & Hansson 2005; Ortiz-Rodríguez, Dao & Wiegand 2012) or decrease (Dao, Do-Hong & Wiegand 2010) offspring fitness under similar conditions of cyanobacteria exposure. Maternal exposure of *Daphnia magna* to toxins from *Microcystis* sp. was found to decrease offspring survival, even when offspring were switched to control media (Dao *et al.* 2010). When comparing neonates fed *Microcystis*, those neonates produced by mothers fed a non-toxic algae (*Rhodomonas*) had higher fitness than those produced by mothers fed *Microcystis* (Brett 1993). That is, maternal exposure to *Microcystis* was harmful to offspring raised in *Microcystis*, rather than

beneficial, as was later shown by (Gustafsson *et al.* 2005). The results of Brett (1993), where offspring from mothers fed *Microcystis* were less fit, contrary to the predictions of the maternal match hypothesis, square with our results quite well, possibly because in both studies, pure cultures were used rather than the mixed diets employed by Gustafsson and colleagues (2005). It could be that the mixed diets employed by Gustafsson et al. (2005) allow rotifers to compensate for nutritional inadequacies of the poor food making apparent only effects of toxins, as opposed to a pure diet approach, where rotifers are subjected to simultaneous differences in nutrition and toxicity, with both factors influencing offspring fitness and transgenerational effects.

Bet hedging, which addresses the question of how mothers should invest resources in a fluctuating environment, may play a role in the response of rotifers to environmental changes. Although we did not directly test for the presence of bet-hedging strategies, in other experiments, researchers have found that responses to mixis (sexual reproduction) cues (Gilbert & Schröder 2007) and hatching cues (García-Roger, Serra & Carmona 2014) vary within clones, supporting a role of diversified bet-hedging in response to a changing environment. With imperfect information about the environmental quality that an offspring will encounter, it may increase a mother's fitness if she produces a range of offspring within or across clutches (diversified bet-hedging). Although we didn't directly test for the presence of diversified bet-hedging in response to food quality, this strategy also could account for some of the variation observed within treatments.

We set out to test the maternal match hypothesis in an asexually reproducing invertebrate subject to natural and frequent changes in resource quality and availability. In an earlier experiment, we found that mothers exposed to poor food quality produced fewer and larger offspring, contingent upon early exposure to high amounts of high quality food, thus supporting the OEST. Based on the maternal match hypothesis, we predicted that the larger size of these offspring produced under poor food quality would have higher fitness if they were raised in the same food quality. Our evidence leads us to reject the maternal match hypothesis under these conditions and provides further support that toxigenic algal blooms may have more extensive effects on grazer populations than can be evaluated using traditional experimental methods, which use only single generations of rotifers, because the effects of exposure to toxigenic algae propagate and intensify across generations.

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Tables

Table 1: Summary of results from aster model comparison testing the effects of maternal diet, offspring diet, and their interaction on lifetime individual fitness. The interaction was tested relative to the full model, and the individual effects of maternal diet and offspring diet were tested relative to a model including only the main effects and not interactions. Results of randomization testing are shown, for comparison, in the far-right column.

Term	**Model degrees of freedom**	**Test degrees of freedom**	**Deviance**	***p* (aster model)**	***p* (randomization testing)**
Full Model	13				
Maternal × offspring diet	12	1	0.7496	0.387	0.351
Main effects only	12				
Maternal diet	11	1	12.89	< 0.001	0.017
Offspring diet	11	1	5.845	0.012	*Chlamydomonas* 0.116 *Microcystis* 0.423

Table 2: Population growth rates and standard errors estimated from aster models for each combination of maternal and offspring diet.

Maternal diet	**Offspring diet**	**Aster λ**	**Standard Error**
Chlamydomonas	*Chlamydomonas*	1.29	0.10
Chlamydomonas	*Microcystis*	0.97	0.27
Microcystis	*Chlamydomonas*	0.79	0.60
Microcystis	*Microcystis*	0.58	2.31

Table 3: Population growth rates and standard errors estimated with bootstrap resampling for each combination of maternal and offspring diet.

Maternal diet	**Offspring diet**	**Leslie matrix λ**	**Standard Error**
Chlamydomonas	*Chlamydomonas*	1.53	0.41
Chlamydomonas	*Microcystis*	0.62	0.18
Microcystis	*Chlamydomonas*	0.36	0.18
Microcystis	*Microcystis*	0.00	0

Figures

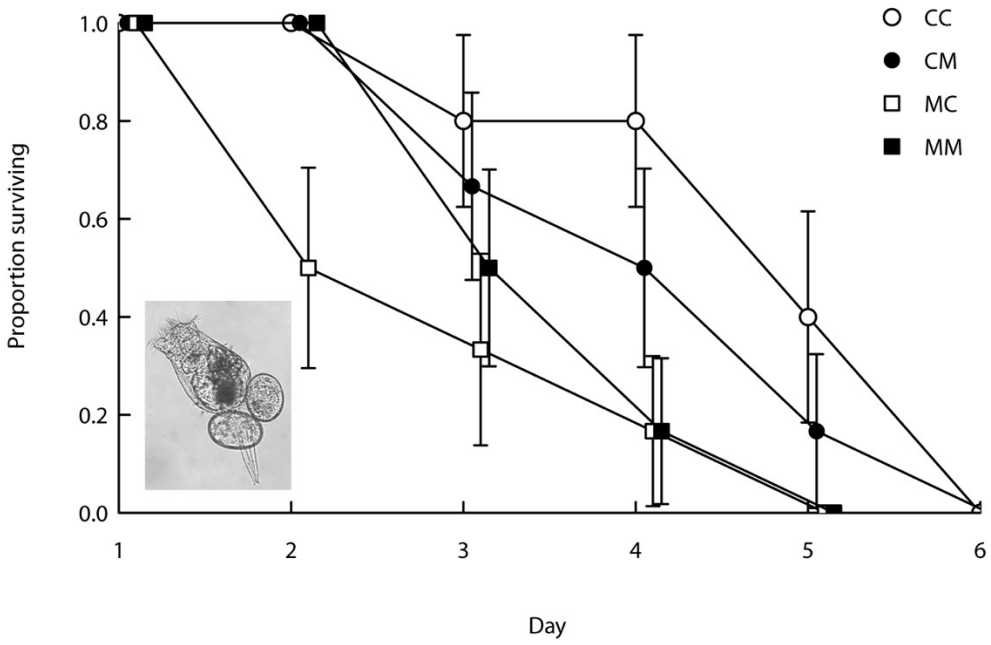

Figure 1: Comparison of survival of the second generation of rotifers subjected to four treatments. Points represent mean proportion surviving ± 1 s.d. (calculated from 2000 bootstrap replicates). Inset photo shows female *Brachionus calyciflorus* with two asexually produced eggs.

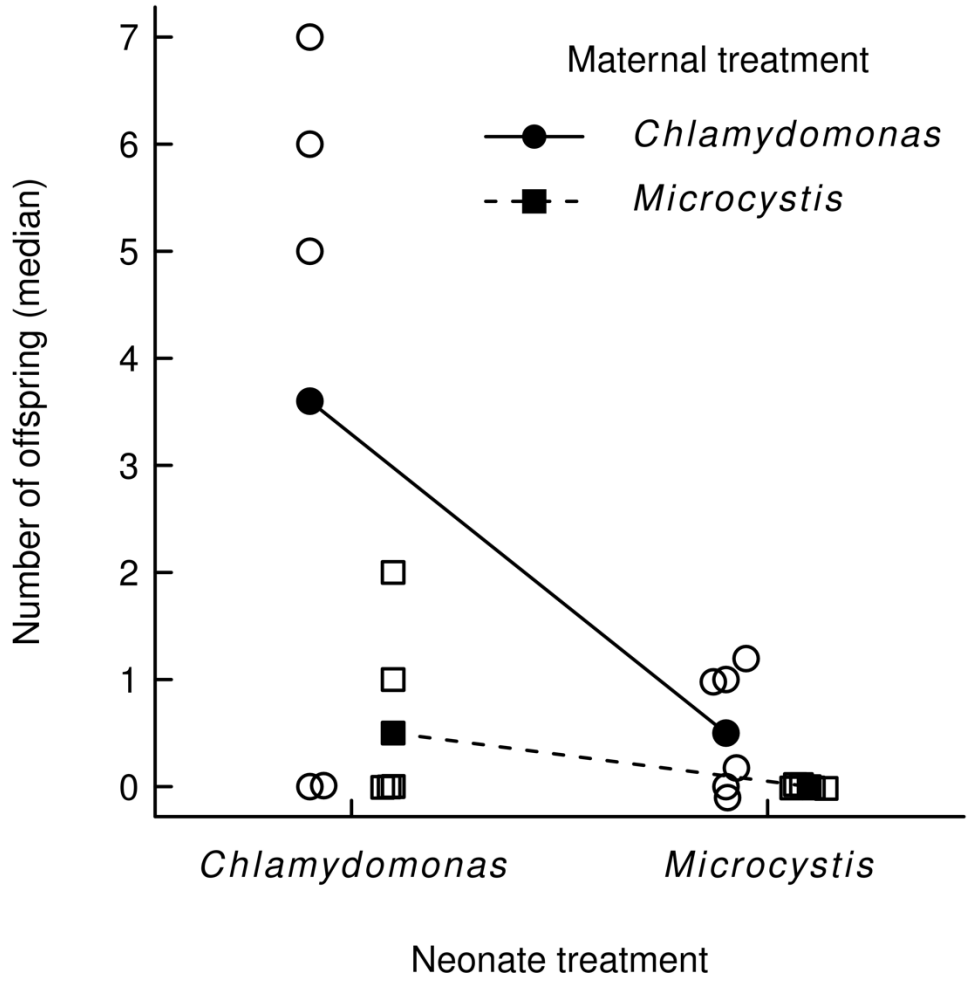

Figure 2: Comparison of fecundity among the four treatments. Individual data points are overlaid as small, open shapes. Median values for each treatment are represented by solid shapes, and lines indicate the norm of reaction.

Chapter 3 – Research intensity predicts cryptic species discovery rates

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Keywords: cryptic species, taxonomy, systematics, diversity, meta-analysis

Abstract

To fully describe the diversity of life on earth, we need estimates of how many cryptic species there are. Individual species within cryptic species groups are indistinguishable based on traditional morphological techniques, but have been identified as distinct species based on molecular or other methods. The number of cryptic species discoveries is increasing at an exponential rate, yet there is no consensus on frequencies of cryptic species or whether cryptic species are more common in some taxa. Using a meta-analysis, I found that cryptic species frequencies are not homogeneous across animal phyla or insect orders, but by using metrics of research intensity, the number of cryptic species can be predicted with great accuracy. As research intensity shapes our view of the frequency of cryptic species among animal taxa, conclusions about the true frequency of cryptic species or about the biological traits giving rise to cryptic species cannot be drawn until efforts to standardize for research intensity are carried out.

Introduction

The discovery of cryptic species is increasing in frequency across all taxa (Bickford et al. 2007). These species, while indistinguishable from each other using traditional morphological techniques, can be recognized as distinct species using a combination of molecular, behavioral, ecological, or morphometric analyses. While the question of how many species exist has been historically well developed (Costello 2015), it's unclear how many cryptic species exist, a question which has strong implications for estimates of biodiversity (Bickford et al. 2007, Adams et al. 2014).

In a meta-analysis of cryptic species publications, Pfenninger and Schwenk (2007), looking only at groups within major metazoan phyla (including Chordata, Arthropoda, and Mollusca), found that for these groups, the $\log_{10}$-transformed number of estimated species was well correlated with the $\log_{10}$-transformed number of cryptic species reports. From this, they concluded that cryptic species are distributed evenly across major metazoan taxa (Pfenninger and Schwenk 2007). However, Trontelj and Fišer (2009) pointed out flaws in this approach, including the use of non-monophyletic groups and exclusion of minor metazoan taxa. When tested using a different analytical approach (ratios of cryptic to total species), the distribution of cryptic species across taxa was not even—the frequency of cryptic species within metazoan phyla varied by up to two orders of magnitude (Trontelj and Fišer 2009).

A further quantification of cryptic species was made by using expert opinions to estimate the number of remaining molecular cryptic species within 58 marine taxa,

ranging from birds to isopods (Appeltans et al. 2012). Based on these estimates, description of these cryptic species would increase the total number of species by 11-43%. Additionally, nine taxa (Brachyura, Siphonophorae, Aves, Sirenia, Euphausiacea, Amphionidacea, Branchiopoda, Mictacea, and Isopoda) were predicted by these experts to have no cryptic species. However, since its publication, there have been cryptic species discoveries within many of these taxa (e.g. Brachyura (van der Meij 2015), Aves (Grosser et al. 2015), Siphonophorae (Pontin and Cruickshank 2012)), highlighting the problem in using only expert opinion and providing motivation for a quantitative, predictive framework for describing cryptic species discoveries.

If cryptic species are evenly distributed across taxa, as suggested initially by Pfenninger and Schwenk (2007) and perpetuated by Scheffers et al. (2012), the number of cryptic species should scale linearly with the total number of described species. That is, a certain percentage of described species can be expected to contain yet unrecognized cryptic species, but this percentage should be constant across phyla or orders. However, it seems likely that taxon-level traits including ecology, morphology, biogeography, and evolutionary rates could influence the likelihood of finding cryptic species within a given taxon. For example, smaller taxa may have more unrecognized cryptic species, as diagnostic characters may be more difficult to discern (Bickford et al. 2007). With respect to ecology, Manter (1955) described his first rule, which postulated that morphological evolution rates are slower in parasites than in hosts, which would predict that cryptic species rates should be higher among taxa with higher parasite prevalence rates. Likewise, molecular evolution rates may be faster in parasites relative to non-

parasitic taxa (Bromham et al. 2013), which would, again, lead to higher rates of cryptic speciation among taxa with high parasite prevalence rates. However, the effects of species-level traits on cryptic species frequencies are not addressable if research intensity varies systematically across taxa. Mayr (1948) and Knowlton (1993), concluded that cryptic species are unlikely to be evenly distributed among animal taxa. Instead, both researchers identified research intensity as a likely correlate of cryptic species diversity.

To truly understand diversity, we need to explain and predict the variation in cryptic diversity among taxa beyond expert opinion, broad patterns, or speculation (Adams et al. 2014). Within parasitic helminthes, the variation in the number of cryptic species described within a taxon using molecular markers was well correlated with the number of individuals sequenced (Poulin 2011). That is, within a small subset of invertebrates, increased research effort was associated with increased cryptic species recognition. However, the research effort expended on the paraphyletic group of parasitic helminthes varied only one order of magnitude across its constituent taxa (which do vary in taxonomic rank: Phylum Acanthocephala, Class Trematoda, Phylum Nematoda, Class Cestoda) and so it is unknown whether this pattern will hold across larger scales.

There has been growing disagreement about the nature of trends in taxonomy, which clearly play a role in cryptic species discovery rates. Surveys and interviews of taxonomists tend to produce pervasively negative views of the future of taxonomy (Coleman 2015). However, the data tell another story. The number of taxonomists

worldwide is increasing (Costello et al. 2014) (although more taxonomists are still needed to describe all of earth's taxa (Wägele et al. 2011, Drew 2011, Bacher 2012, Costello et al. 2013)). Concurrently, the number of species described per taxonomist is declining and may represent a declining number of species yet to be described (Joppa et al. 2011, Costello et al. 2012). An alternative hypothesis for this phenomenon of declining return on taxonomic investment was put forth by Sangster and Luksenburg (2015), who found that, over this same time period where the number of species described per taxonomist declined, the detail and thoroughness of species descriptions had increased. Sangster and Luksenburg (2015) thus concluded that the declining rates of catch-per-unit-effort are not due to a decline in the number of species yet to be described, but due to an increase in the difficulty of describing species once they are found.

I identified three pivotal questions related to cryptic species discoveries in animals. First, does the variation in cryptic species frequencies across taxonomic groups change when taxonomically valid groupings (phyla or orders) are used? Second, does research intensity predict the discovery of cryptic species within a taxon? And third, do observed patterns hold at different taxonomic scales (i.e., insect orders and animal phyla)? I took a meta-analytical approach, examining the frequency of published cryptic species discoveries between 1988 and 2011. I compared cryptic species discoveries relative to many predictor variables that have been postulated to govern the discovery rate of cryptic species or, alternatively, the cryptic speciation rate. For both animal phyla and

insect taxa, I compared the number of cryptic species discoveries within this time period to the total number of species described within that taxon.

Methods

To quantify cryptic species publication rates, I searched for papers (9 Nov 2012) including 'cryptic' or 'sibling' and 'species' in the title, abstract or keywords in Web of Knowledge (Thomson Scientific) published between 1988 (commercialization of PCR) and 2011 (7,529 records). The phrase 'sibling species' can be used interchangeably with cryptic species (Knowlton 1993), although sibling species may additionally include the connotation of more recent divergence between species pairs. I evaluated each record by reading the title and abstract, and included only records that tested for the presence of cryptic species (2,831). I excluded papers were if they were irrelevant to the definition of cryptic species (species indistinguishable from each other based on traditional morphological techniques, that are identified as distinct species using a combination of molecular, behavioral, or morphometric analyses). To prevent double-counting any species, duplicate records were removed, keeping the oldest record of each cryptic species (1,904 records included). Each record was categorized by phylum, and for insects (636 records), by order.

The total number of described species was calculated from the Catalogue of Life 2015 (CoL2015) annual checklist (Roskov et al. 2015). The database was downloaded, run locally with MAMP (Meyer et al. 2015), and queried from within R using the RMySQL package (Ooms et al. 2016). All further analyses were carried out in R (version 3.2.4).

In counting the total number of described species, I only included accepted names, excluding provisionally accepted names and synonyms.

For phylum Nematoda, 749 out of 3469 records in CoL (21.6%) were missing author and date information, which could lead to biases in estimates of taxonomic research activity and species accumulation rates. To resolve some of these records, I used the R package, taxize (Chamberlain and Szöcs 2013) to look up author and date information from an additional seventeen databases. This resolved a further 211 cases, leaving 15.5% of cases without author or date of description, and the remaining authorless records were removed from the further analyses where publication date or author name was required.

Several measurements of research intensity were calculated and used in models of cryptic species frequencies. Molecular research effort (MRE) was quantified using the total number of genomes sequenced per animal phylum or insect order (*Genome information by organism* n.d.). Each taxon included in the genome list was identified to phylum, and for insects to order (Chamberlain and Szöcs 2013). I quantified potential human health associations (HHA) by performing queries in PubMed for each phylum or order name, and 'health'. HHA was quantified by the total number of records returned. PubMed was accessed using PubMedXML.r (Magnusson n.d.). To quantify taxonomic research effort (TRE), I used CoL2015 to identify the unique number of first author taxonomists in each year, as well as the number of species described per first author

taxonomist per year. Earlier efforts have shown that using only the first author is a valid approach to quantifying the numbers of practicing taxonomists (Joppa et al. 2011).

I included two biological predictors of cryptic species frequencies. For each cryptic species record, I identified whether the organism was a parasite or a host to parasites, using the R package helminthR (Dallas 2016), which accesses the London Natural History Museum's Host-Parasite database. As with all host-parasite checklists (Poulin et al. 2015), the Host-Parasite database is undoubtedly incomplete. However, even in its incompleteness, it can still serve as a measurement of research intensity. For animal phyla, I included the average body size of each animal phylum, measured as biovolume (Orme et al. 2002), as a predictor of total number of cryptic species. For phylum Chordata, the average size was not provided for the phylum, but only for subphyla. In this case, the average size was calculated as a weighted average, taking the total number of species number reported by Orme et al. (2002) into account. For phylum Platyhelminthes, the average size was calculated as the average of the three reported multiple biovolumes.

I estimated the total number of species remaining to be described for each taxon using a non-linear model of logistic growth. The fitted model was used to identify characteristics of the species discovery curve, including the point of inflection of the model, which represents the point in time when half of the total estimated species have been described, and the asymptote, which represents the estimate of total number of species. This model choice fit the species accumulation curves well (Figs. 1, 2), and so

these parameter estimates (asymptote and inflection point) were used in further analyses. However, this approach also assumes that if there is a decline in the rate of species descriptions, it is because the number of described species is approaching the total number of species, which may or may not be the case (Joppa et al. 2011, Sangster and Luksenburg 2015). While there are many approaches to estimations of species richness (Costello 2015), non-linear regression was optimal in this case as it can be readily and consistently applied across taxa. Other approaches, including expert opinions (Fisher et al. 2015), species traits like body size (Stork et al. 2015) or geographic location (Costello et al. 2015) work well for well-characterized taxa or subsets of taxa, but may not be as easily applied to less-studied or larger collections of taxa. Earlier species richness estimates using logistic growth equations (Costello et al. 2012) found that species records post 1980 had to be excluded from analyses because there is a delay in entering these records into databases; thus an artificial asymptote (decline in numbers of species described) was observed, and caused not by declines in species description rates, but a lag in database entry of these descriptions. However, in preliminary analyses, by excluding data from 1980-2005, the total numbers of species described was underestimated. For this reason, logistic growth models were fitted to all species records from 1750-2005 (Fig. 3, 4).

I used a model comparison approach to find the best combination of predictors (Table 1) to model the number of cryptic species. I fit generalized linear models with Poisson response distribution between the predictors and response variable (number of cryptic species records 1988-2011). First, to test the hypothesis that the collected predictors

better modeled the number of cryptic species described than the approach of Pfenninger and Schwenk (2007), I compared the goodness of fit of the full model (all predictors) to a highly reduced model with only the total number of described species as predictor. If the full model was significantly better than the reduced model, I then used the corrected Akaike information criterion (AICc) to rank all candidate models, considering models with ΔAICc between 0-2 to have substantial support (Burnham and Anderson 2002). I also examined plots of the predicted versus the actual numbers of cryptic species, for both the full and reduced models.

Results

Relationships between cryptic and total species are linear when $\log_{10}$-transformed, for both animal phyla and insect orders (Figs. 3, 4). However, there are some notable outliers, including Chaetognatha, Sipuncula, Gastrotricha, Brachiopoda, Acanthocephala, and Bryozoa, all of which have fewer cryptic species than predicted from the total number of species described per taxon. Chordata, Platyhelminthes, Nematoda, and Onychophora have more cryptic species than predicted based on the number of species described in each taxon. Even after accounting for the total number of described species, there is still substantial variation in the numbers of cryptic species.

In comparing the full model (all predictors from Table 1) to a reduced model with only total described species as the predictor, the full model performed significantly better than the model with only total described species for both animal phyla (Chi-square test, df = 10, deviance = 2036.5, $p < 0.0001$) and insect orders (df = 8, deviance = 282.43, p

< 0.0001). The full model predicted cryptic species numbers with high accuracy, as can be seen by comparing the number of predicted and actual cryptic species numbers for animal phyla and insect orders (Fig. 5).

For both animal phyla and insect orders, total species (measured as either number described or estimated total number), human health association, recent taxonomic productivity, and taxonomic research effort (average number of species described per author) were important predictors (Tables 2 and 3). For animal phyla, body size was not included in either of the two best models (Table 2).

Discussion

I found that there was a roughly linear relationship between $\log_{10}$-transformed cryptic species records and total species records for animal phyla and insect orders. However, even after log-transforming both of these variables, and thus masking a very large amount of inherent variation, there were many groups that had more or fewer cryptic species than predicted. Thus standardizing to uniform taxonomic rank was insufficient to remove the variation in cryptic species frequencies. By including measures of research intensity, I was able to better predict the number of cryptic species in both animal phyla and insect orders. Variables associated with higher cryptic species numbers were consistent between these two scales. I found that human health associations were positively related to the number of cryptic species discoveries. Additionally, there were more cryptic species found in taxa with higher numbers of

species described per taxonomist. For animal phyla, the number of genomes published per phylum was also positively related to cryptic species numbers.

In this study, cryptic species were unevenly distributed among animal taxa, directly contradicting Pfenninger and Schwenk's (2007) results and supporting the results of Trontelj and Fišer (2009). In a focused examination of parasitic helminthes, Poulin (2011) found that research intensity (as quantified by sequencing effort) was a strong predictor of cryptic species discoveries. In this survey across animal phyla and insect orders, I found a strong predictive relationship between multiple quantifications of research intensity and cryptic species discoveries.

Pfenninger and Schwenk (2007) showed that there is a linear relationship between the $\log_{10}$-transformed number of total species and number of cryptic species within a taxon. That a rough correlation would exist between the number of total species and the incidence of cryptic species is unsurprising. Arthropods, with 100,000 times as many species as brachiopods, are clearly likely to have a higher number of cryptic species. However, there was high variation around this linear relationship, and I found that the research effort expended on a taxon explains the differences in cryptic species frequencies. Since our understanding of the frequency of cryptic species, and species in general, is shaped by the research intensity on each taxon, it is not yet possible to draw conclusions as to how biological features, like body size, ecology, or biogeography, affect the cryptic speciation.

Using the new understanding that research effort shapes cryptic species discovery rates, we can better direct our investment of resources. For example, if poorly studied taxa are comparatively rich in cryptic species, then efforts to increase research in these taxa may provide more return on investment than efforts to increase research in already well-studied taxa. For practical cases of human and animal health, the recognition and description of cryptic species is vital (de León and Nadler 2010); this is supported by a positive relationship between human health associations and cryptic species descriptions in models of both animal phyla and insect orders. Many of the recently described mammals are cryptic species, which has major implications for conservation (Ceballos and Erlich 2009); the taxonomic ranking (species vs. subspecies vs. population) may directly influence decisions about conservation of an organism. If the cryptic species differ in ecological niche, distribution, or susceptibility to human disturbance, it could be necessary to implement different conservation strategies for the newly recognized cryptic species (e.g., Sattler et al. 2007, Feckler et al. 2014). Discoveries of cryptic species have implications for tests of fundamental ecological theory, as well. The biodiversity ecosystem function (BEF) theory postulates relationships between ecosystem diversity and function (Hooper et al. 2005). Inadequate descriptions of diversity through the omission of cryptic species identification can only hinder testing of this pivotal theory (Peay et al. 2008). As we move forward in studying the crucial interactions between biodiversity, ecosystem function, and global change, a thorough quantification of animal diversity necessitates consideration of cryptic species and their roles in these interactions.

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Tables

Table 1: Descriptions of predictors included in model of cryptic species numbers ([a] indicates predictor omitted from insect orders model).

Predictor	**Description**
Body size[a]	Biovolume estimates for each animal phylum
Described species	Total number of valid species names
Early taxonomic productivity	Proportion of species described before 1850
Estimated total number of species	Estimated asymptote from logistic growth model (1750-2005)
Host ratio	For each taxon, the proportion of cryptic species that are hosts
Human health associations	Number of records returned from PubMed for 'health' and 'taxon name'
Inflection point	Estimated inflection point from logistic growth model (1750-2005)
Molecular research intensity	Total number of genomes sequenced per animal phylum or insect order
Parasite ratio[a]	For each taxon, the proportion of cryptic species that are parasites
Recent taxonomic productivity	Proportion of species described after 1949
Taxonomic research effort	Median number of species described per author (1750-2005)

Table 2: Results of AICc model comparison for animal phyla (only models with $\Delta AIC_c < 2$), including parameter estimates. Blank cells indicate that the variable was not included in that model.

Value	**Model 1**	**Model 2**
Intercept	-53.6	-50.0
Body size		
Described species		-8.83×10^{-6}
Estimated total number of species	-8.42×10^{-6}	
Early taxonomic productivity		
Human health association	0.00200	0.00198
Host ratio		
Inflection point	0.0294	0.0275
Molecular research intensity	0.00641	0.00658
Parasite ratio	1.02	1.03
Recent taxonomic productivity	-6.91	-6.57
Taxonomic research effort	0.952	0.949
df	8	8
AICc	144.8	145.4
ΔAICc	0.00	0.56
weight	0.411	0.310

Table 3: Results of AICc model comparison for insect orders (only models with ΔAIC_c < 2). Blank cells indicate that the variable was not included in that model.

Value	**Model 1**	**Model 2**	**Model 3**	**Model 4**	**Model 5**
Intercept	1.59	0.919	-26.97	1.57	1.05
Described species	-1.42×10^{-5}			-1.25×10^{-5}	
Estimated total number of species		-7.72×10^{-6}	-1.80×10^{-5}		
Early taxonomic productivity		-8.34		-5.25	-8.51
Human health association	0.000958	0.000919	0.00130	0.000920	
Host ratio					6.21
Inflection point			0.0150		
Molecular research intensity					
Recent taxonomic productivity	-2.31		-4.55	-1.88	
Taxonomic research effort	0.565	0.517	0.647	0.563	0.381
df	5	5	6	6	4
AICc	108.0	108.7	108.9	109.0	109.5
ΔAICc	0	0.68	0.93	0.98	1.50
weight	0.215	0.153	0.135	0.132	0.102

Figures

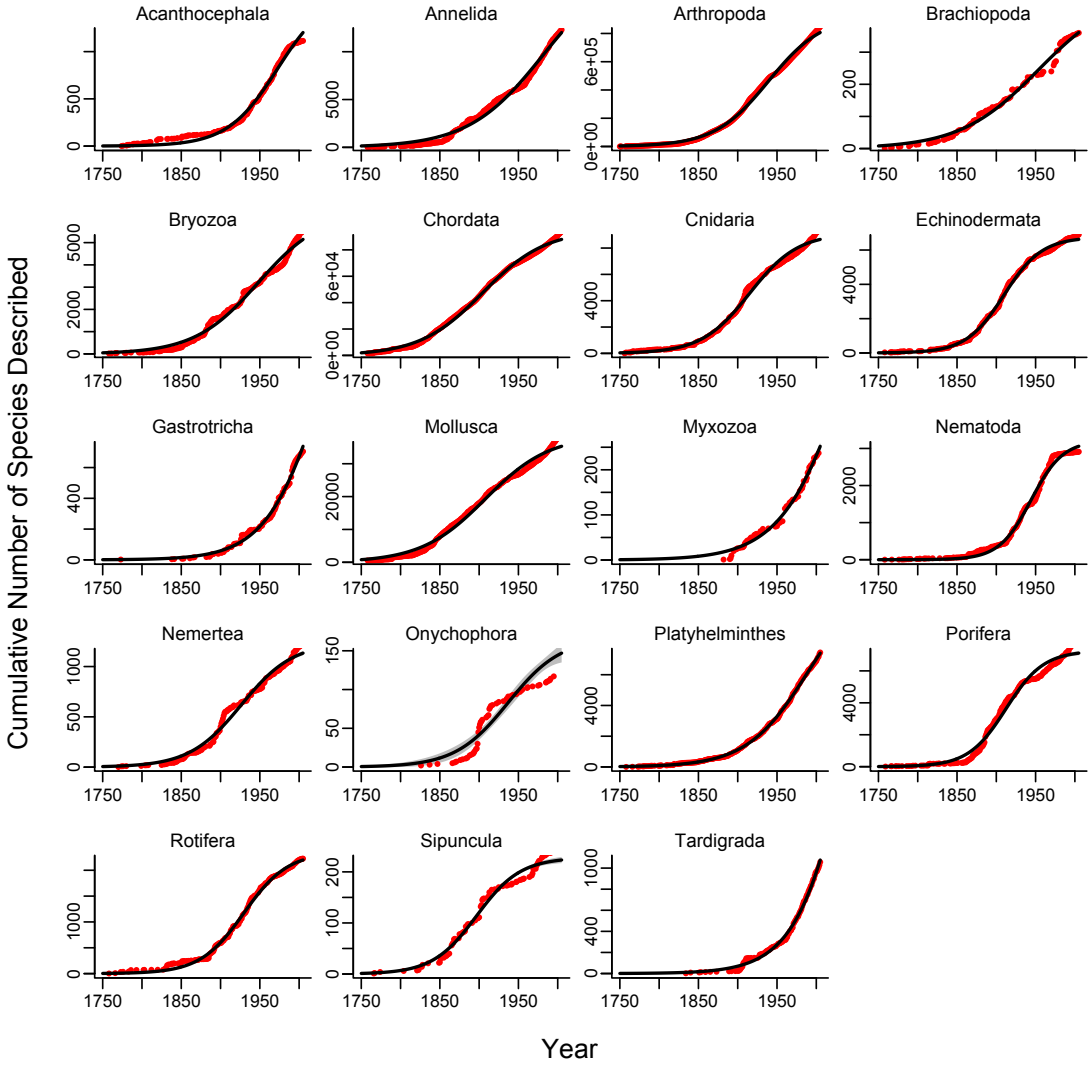

Figure 1: Plot of cumulative number of species described for each animal phylum. Red dots indicate observed counts of cumulative species for each year in which species were described. Black lines represent the nonlinear regression fit of the logistic growth model. Grey bands around black line represent 95% confidence intervals around line, but are too narrow to be seen behind black line for most taxa.

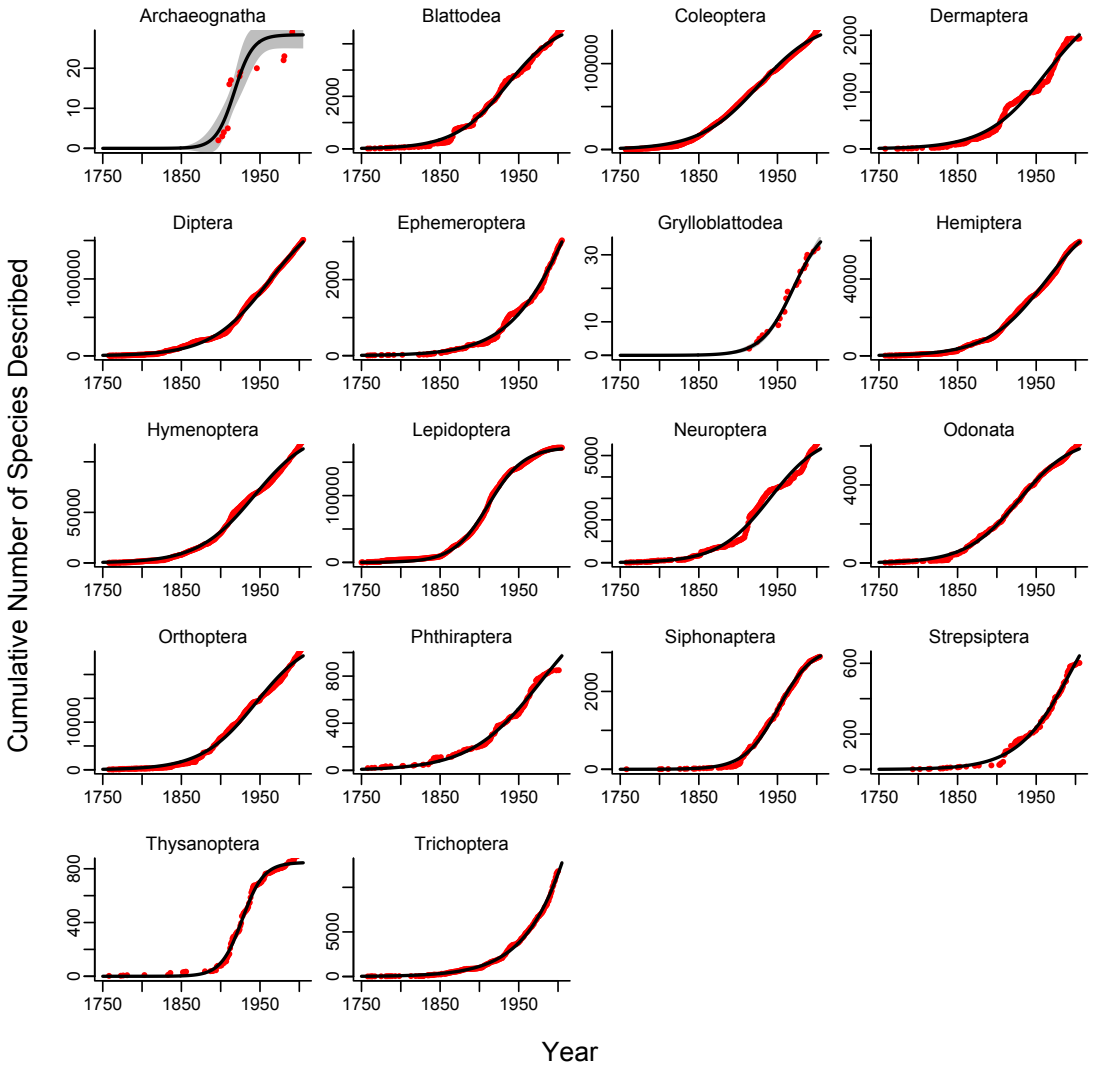

Figure 2: Plot of cumulative number of species described for each insect order. Red dots indicate observed counts of cumulative species for each year in which species were described. Black lines represent the nonlinear regression fit of the logistic growth model. Grey bands around black line represent 95% confidence intervals around line, but are too narrow to be seen behind black line for most taxa.

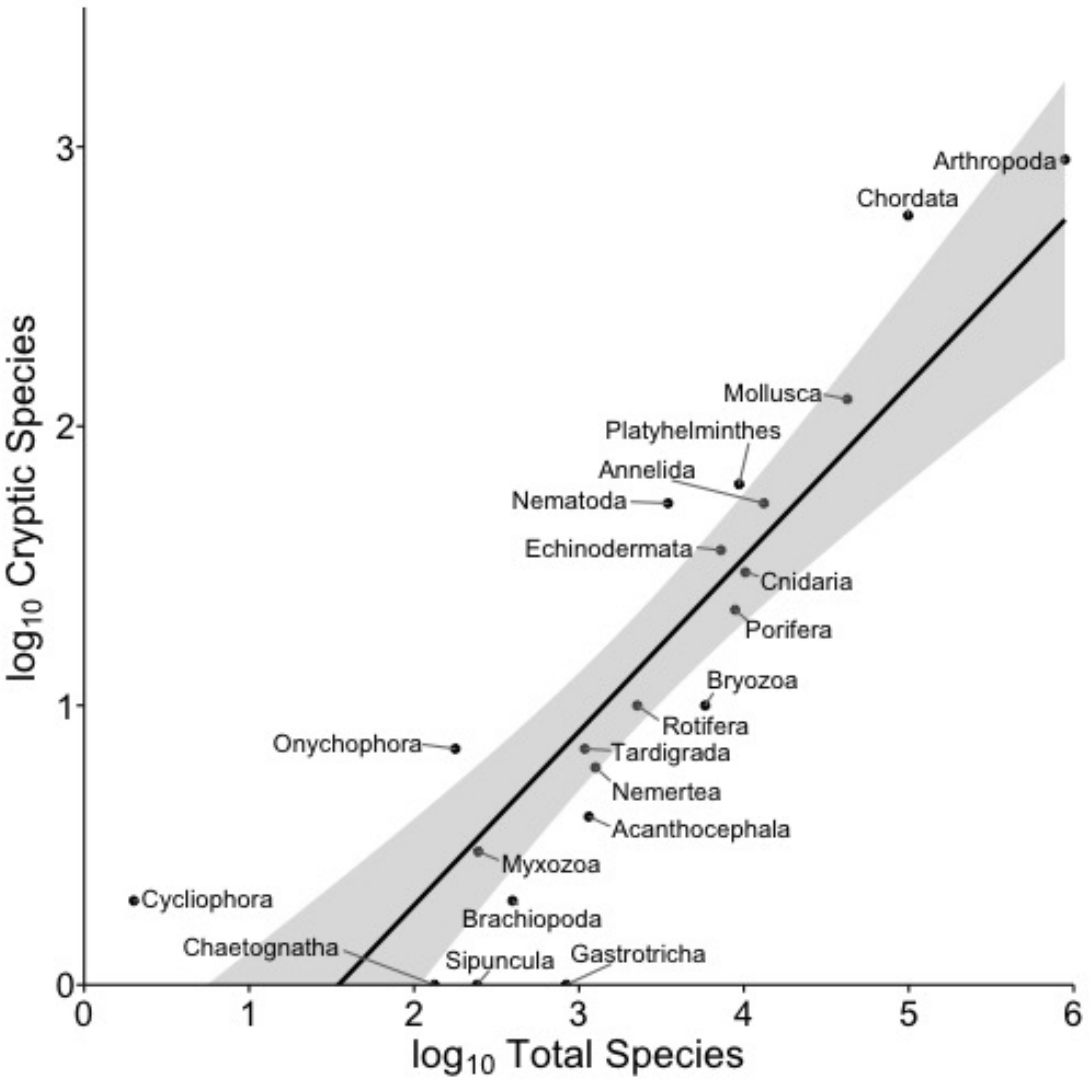

Figure 3: Relationship between $\log_{10}$-transformed total species and cryptic species records for animal phyla. Only animal phyla with at least one cryptic species record were included in the plot. The black line represents the linear regression of $\log_{10}$-transformed total and cryptic species ($y = -0.96 + 0.62x$, $F_{1,19} = 55.74$, $R^2 = 0.73$, $p < 0.001$), and the shaded region represents the 95% confidence interval.

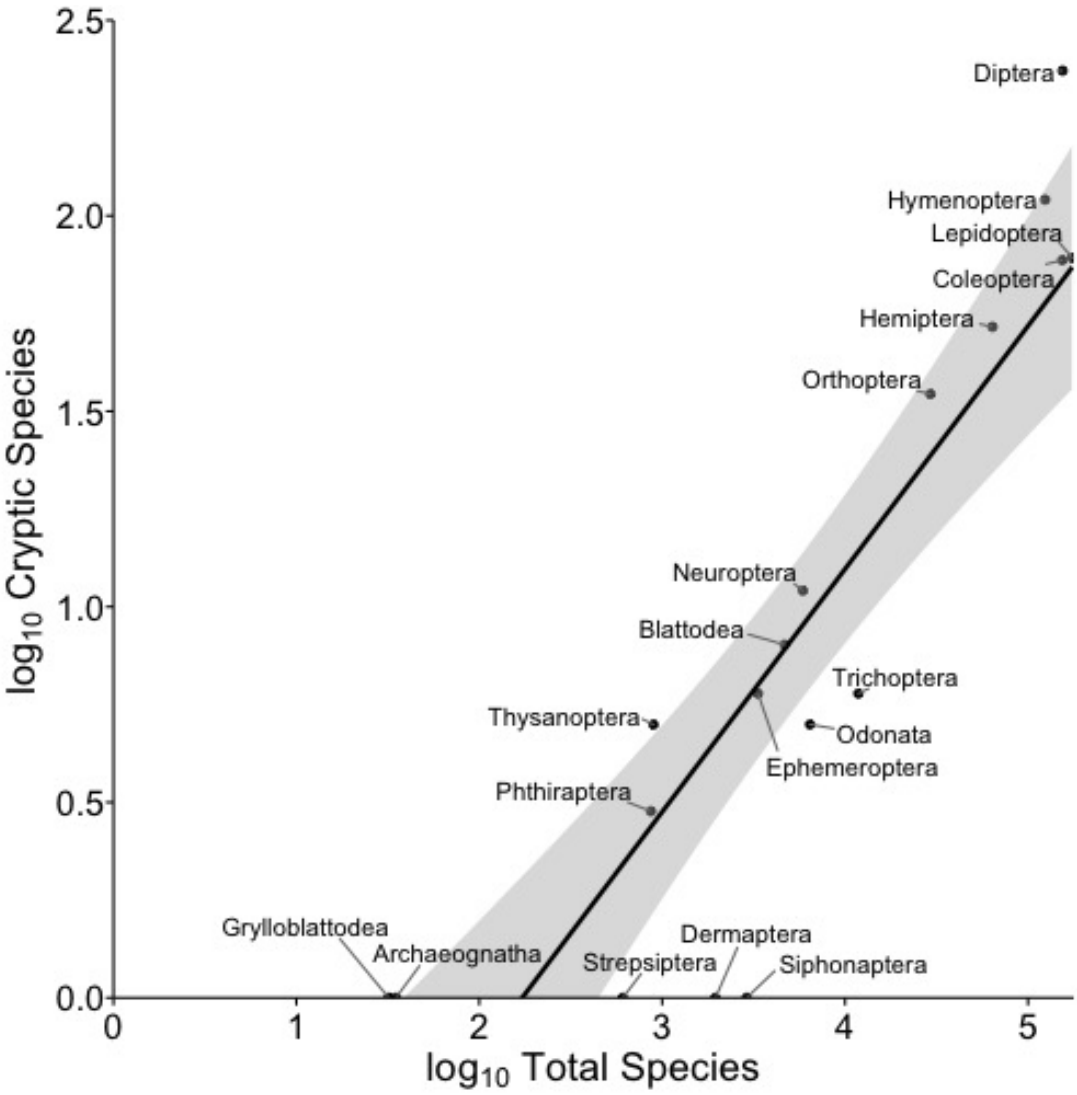

Figure 4: Relationship between $\log_{10}$-transformed total species and cryptic species records for insect orders. Only insect orders with at least one cryptic species record were included in the plot. The black line represents the linear regression of $\log_{10}$-transformed total and cryptic species ($y = -1.39 + 0.62*x$, $F_{1,16} = 62.82$, $R^2 = 0.78$, $p < 0.001$), and the shaded region represents the 95% confidence interval.

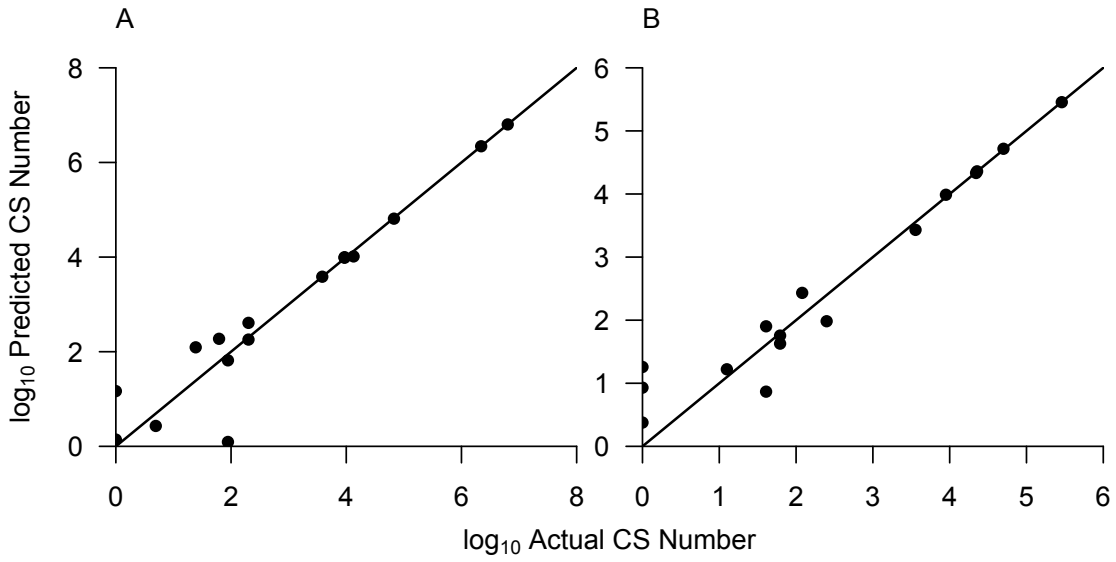

Figure 5: Comparison of actual and predicted numbers of cryptic species for full models for (A) animal phyla and (B) insect orders. Black line represents equal numbers of actual and predicted cryptic species.

Chapter 4 – The niche and morphology of the invasive *Daphnia lumholtzi* in a subtropical reservoir, twenty years after invasion

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Keywords: *Daphnia lumholtzi*, invasive species, allometry, cyanobacteria

Abstract

Invasive species can have long-lasting effects on invaded systems, yet most research focuses on immediate effects of invasions. Twenty years after the invasion of Lake Texoma, OK-TX by *Daphnia lumholtzi*, we investigated the factors associated with abundances and morphology of this species. We uncovered seasonality in abundances and defensive morphology of *D. lumholtzi* within Lake Texoma. Tail spine growth was hyperallometric (faster than core body growth) in summer and fall, but allometric (growing at equivalent rate as body) in winter. Helmet growth rate declined with increasing body size, and was curvilinear in summer and fall, but linear in winter. This leads us to suggest that the selective predation pressure acting upon these traits is strongest in summer and fall. Additionally, we found that abundances of *D. lumholtzi* in

Lake Texoma were best predicted by cyanobacteria concentration. Other factors that have been previously associated with *D. lumholtzi* abundance, including temperature, salinity, nutrients, were either weakly or not at all associated with *D. lumholtzi* abundance in Lake Texoma. The niche of this invasive species within Lake Texoma appears to be primarily limited by predation pressure and cyanobacterial abundance. Thus, interactions with native predators and primary producers appear to be structuring the niche of *D. lumholtzi* in this subtropical reservoir, and further studies may illuminate whether these limiting factors are responsible for range limits and unsuccessful introductions within the southern United States.

Introduction

Invasive species are often treated as static once they arrive in a new system, but global change means that these species may be part of shifting ecosystems. Thus, ongoing characterization of the realized niche of these invasive species will allow more comprehensive understanding of their impacts. Further, by studying changes in these invaded systems, as well, we can make predictions about the evolution of the realized niche of these invasive species relative to global change.

One such invasive species is *Daphnia lumholtzi,* a crustacean zooplankter native to Australia, Africa, and Asia, which has invaded the United States over the past twenty-five years (Sorensen and Sterner 1992), with the source of these introductions likely being from Africa or Asia, but not Australia (Havel and Shurin 2004). The primary introduction of *Daphnia lumholtzi* occurred in the southern US (Frisch et al. 2012), with

the first records occurring in Missouri in 1990 (Havel et al. 1995), Texas in 1991 (Sorensen and Sterner 1992), and in Lake Texoma, OK-TX in 1991 (Work and Gophen 1995). Following the initial introduction, molecular data show that there were possible later introductions across US, coupled with spread from established populations (Frisch et al. 2012). The current distribution of *Daphnia lumholtzi* stretches west to California (Havel and Shurin 2004), north to Lake Erie (Muzinic 2000) and Lake St. Clair (Tudorancea et al. 2009), east to North Carolina (Finn et al. 2012), and south to Florida (Havel and Hebert 1993). Throughout this period, researchers have focused in particular on understanding which biotic and abiotic factors drive *D. lumholzi* invasion success and abundances, as well as their interactions with native competitors and predators.

Previous research into abiotic drivers of *D. lumholtzi* invasion success has identified temperature, turbidity, and phosphorous concentration as key environmental characteristics. Temperature is positively associated with *D. lumholtzi* abundance and population growth rates in field-based observational studies (Havel et al. 1995, Work and Gophen 1995, 1999b, Yurista et al. 2000, Lennon et al. 2001, Havens et al. 2012), and laboratory-based experimental studies (Work and Gophen 1999a, Lennon et al. 2001, Yurista 2004). Turbidity negatively affects *D. lumholtzi* population growth rates in the laboratory (Work and Gophen 1999a) and abundances in lakes (Havens et al. 2012), however, *D. lumholtzi* may tolerate intermediate suspended sediment concentrations better than native *Daphnia* (Soeken-Gittinger et al. 2009). Additionally, phosphorous concentrations and the associated increase in primary productivity have been associated with both increased *D. lumholtzi* invasion success (Havel et al. 2005)

and abundance (Havens et al. 2012) as well as decreased invasion success (Dzialowski and O'Brien 2000). Composition of the primary producers may account for these contradictory findings, as differences in the tolerance of cyanobacteria have been found between *D. lumholtzi* and native *Daphnia* spp. (Pattinson et al. 2003, Fey and Cottingham 2011).

Daphnia lumholtzi interact with native predators and competitors within their invasive range. Competition with native *Daphnia* spp. may affect the abundance of *Daphnia lumholtzi*, although the results from several studies are contradictory. Fey and Cottingham (2011) found that the outcome of competition experiments was mediated by temperature and algal composition, with warmer temperatures promoting *D. lumholtzi* and higher cyanobacterial abundances promoting the native *D. pulex* (Fey and Cottingham 2011). On the other hand, in field experiments, Johnson and Havel (2001) found that the presence of *D. lumholtzi* has a negative affect on native *D. parvula* population growth rates during the late summer and fall, when *D. lumholtzi* occurs at high abundances. However, this competitive interaction was asymmetrical, with the presence of *D. parvula* not affecting *D. lumholtzi* population growth rates (Johnson and Havel 2001). Others have suggested that the coexistence of native *Daphnia* and *D. lumholtzi* may be mediated by the presence of invertebrate predators (Celik et al. 2002), with *Chaoborus* presence increasing *D. lumholtzi* abundances relative to native *Daphnia*.

D. lumholtzi was first discovered in Lake Texoma, OK-TX in 1991 (Work and Gophen 1995). Shortly after this invasion, *D. lumholtzi* abundances in 1994 and 1995 were driven primarily by temperature (Work and Gophen 1995), with peak abundances appearing in June and July. During this midsummer abundance peak, *Daphnia lumholtzi* constituted a large fraction of the diet of inland silversides (*Menidia beryllina*), itself also a likely invader (Hubbs 1982), suggesting that they had been incorporated into the carbon flow in Lake Texoma (Lienesch and Gophen 2001). Further, predation by inland silversides on *D. lumholtzi* is size-selective, with smaller silversides preying less readily on large *D. lumholtzi* (Lienesch and Gophen 2005). Small changes in *D. lumholtzi* morphology may thus have large effects on survival and population growth if they shift individuals into the 'inedible' category of silversides. If silverside predation is driving *D. lumholtzi* morphology, we can make some predictions about relative strength of this selective pressure during the course of a year based on the life history and ontogeny of silversides in Lake Texoma (Hubbs 1982).

Interactions between predators and *D. lumholtzi* are mediated by the large, defensive head and tail spines of *D. lumholtzi*. In *D. lumholtzi*, growth of head and tail spines is plastic, and mothers produce offspring with larger head and tail spines in response to the presence of vertebrate and invertebrate predator kairomones (Dzialowski et al. 2003), as well as increased temperature (Yurista 2000) and increased cyanobacteria concentration (Whittington and Walsh 2015). Temperature, in this case, is likely acting as a proxy cue for predation risk, as has been shown in other cladocerans (Miehls et al. 2013). Longer spines decrease the risk of predation from both vertebrates and

invertebrates (Engel et al. 2014). However, as in other *Daphnia* species, predator identity beyond 'vertebrate' vs. 'invertebrate' matters (Herzog and Laforsch 2013), and growth of head and tail spines does not protect against all invertebrate predators (e.g., *Leptodora kindtii*) (Effert and Pederson 2006). In the presence of vertebrate predators, the inducible defenses of *Daphnia lumholtzi* allow it to outcompete native *Daphnia*, which do not occur when fish are absent (Engel and Tollrian 2009). Similarly, the competitive interactions of *D. lumholtzi* and native *Daphnia* may be affected by the presence of the invertebrate predator *Chaoborus* sp., with the 'winner' being native *Daphnia* in the absence of *Chaoborus*, while *D. lumholtzi* wins in the presence of *Chaoborus* (Celik et al. 2002).

One way of investigating growth of head and tail spines is to take an allometric approach. In this framework, the growth of a trait (e.g., tail spine) is measured relative to body size growth by log-transforming both variables, and performing a linear regression on the log-transformed trait size against the log-transformed body size. If the slope of the relationship is equal to one, the trait is considered allometric, and the trait grows at the same rate as the body. If the slope of the relationship is significantly greater than one, the trait is considered hyperallometric, and the trait grows at a faster rate than the body. If the slope is less than one, the trait is considered hypoallometric, and the trait grows at a slower rate than the body.

Two studies tested the allometry of head and tail spines in field-collected *D. lumholtzi*. Sorensen and Sterner (1992) measured head spine, tail spine, and body size in *D.*

lumholtzi individuals collected from Fairfield Reservoir, TX from January to March 1991. There were differences between months in spine investment; increases in temperature were associated with increased growth of tail spines relative to body size. Yurista (2000) collected *D. lumholtzi* individuals from Kentucky Lake, KY from July to September. Within both populations, growth of head and tail spines were hyperallometric (Sorensen and Sterner 1992, Yurista 2000). However, these two studies found differential investment in the two spines. Yurista (2000) found that tail spines were both absolutely larger than head spines, and the relative growth rate of tail spines was higher than that of head spines. Sorensen and Sterner (1992) found that tail spines were absolutely larger, but that the growth rate of head spines was greater than that of tail spines.

Two other studies took alternative approaches that make the results difficult to directly compare to other published work. Work and Gophen (1995) measured head spine + head, tail spine, and body size in *D. lumholtzi* from February-July 1993 in Lake Texoma, OK-TX. They found positive relationships between head spine + head and body size, as well as between tail spine and body size. They found nonlinear relationships between some of these variables, although without reporting regression equations, these claims are difficult to substantiate. For both head and tail spines, the proportion of total length increased throughout the season, suggesting a greater investment in spines from February to July within this subtropical reservoir (Work and Gophen 1995). Schnake (2002) measured *D. lumholtzi* collected from Lake Taylorville, IL from May to December in 1993, 1994, 1999, and 2000. Measurements were

transformed to proportion of total length before being arcsine transformed. These transformed proportional values varied across months and by site, and variation in head and tail spine investment was associated with many water quality variables, including dissolved solids, Secchi depth, temperature, dissolved oxygen, chlorophyll a, conductivity, and phosphate (Schnake 2002).

The analytical methods employed in these four studies effectively assume linear scaling between traits and body size, and ignore the potential for nonlinear relationships. However, tests of non-linear allometry in *Daphnia lumholtzi* are important because a curvilinear pattern could suggest that growth of spines is sustained only until the cost of producing the trait exceeds the benefits the trait confers. There is a cost of helmet and tail spine production in *Daphnia* (Spaak and Boersma 1997, Boeing et al. 2005), and so individuals should only invest in this trait if the cost of growth is outweighed by the benefit. Thus, if there is a size refuge from predation, we would predict a curvilinear relationship between body size and size of defensive traits. For example, if large *D. lumholtzi* escape gape-limited predators, there would be no selective benefit to sustaining further spine growth. Thus, the rate of head spine growth may decline with increasing body size. There is evidence of nonlinear allometry in the defensive traits of other cladocerans, including helmet and tail spine length of *Daphnia cucullata* (Lagergren et al. 2007), however no one has tested for nonlinearity in these scaling relationships in *D. lumholtzi.*

The goal of this study was to characterize the niche of *D. lumholtzi* within Lake Texoma, with comparisons to earlier work in Lake Texoma and other invaded lakes. We tested the hypothesis that environmental factors associated with *D. lumholtzi* abundance in other lakes and from earlier work in Lake Texoma are associated with current patterns of *D. lumholtzi* abundance in Lake Texoma. Our second hypothesis was that the morphology of *D. lumholtzi* reflects seasonal predation pressure by gape limited predators. Given that the predominant predators of *D. lumholtzi* in Lake Texoma are likely gape-limited with seasonal abundances, we hypothesized that growth of defensive traits (helmet and tail spine) would vary seasonally.

Methods

Over the course of a year (June 2010—July 2011), vertical, depth-integrated zooplankton tows with a Wisconsin-type net (350-μm mesh) were taken throughout the entire water column monthly at five pelagic sites on Lake Texoma, OK-TX (Fig. 1). These sites span two watersheds, with the Red River entering the western edge of the lake and the Washita River entering the eastern portion. Environmental measurements, including temperature, salinity, cyanobacterial abundance, pH, and dissolved oxygen were taken concurrently with zooplankton sampling (for details, see (Hambright et al. 2015)).

From each zooplankton sample, we measured abundances of *Daphnia lumholtzi* (females L^{-1}) as well as helmet, body, and tail spine length in mm of each female (Fig. 2). We measured 571 individuals collected June 2010—July 2011, although in some

individuals, the tail spine had broken, and so the sample size was larger for helmet analyses (n = 571) than tail spines (n = 558). We divided these samples into three seasons: summer (June-August), fall (September-November), and winter (December-February). Only one individual was collected in the spring (March-May), so that season was omitted from morphological analyses.

To test for seasonal differences in the allometry of the helmet and tail spine, we used nonlinear regression, following the approach of Lagergren et al. (2007). For each season, we fitted the logarithmic form of the complex allometry function (Jolicoeur 1989) in R (Version 3.2.4, (R Core Team 2016)) as shown in Eq. 1.

$$\ln(y) = \ln(A) - C(\ln(x_{max}) - \ln(x))^D$$

To test whether *D*, the curvature parameter, differed significantly from one (which would indicate a curvilinear relationship and deviation from simple allometry), we constructed 95% confidence intervals for *D* using the confint function in the MASS package (Version 7.3-45 (Venables and Ripley 2002)) and determined whether each interval overlapped one. If *D* was not significantly different from one, I carried out a linear allometric analysis on the natural log-transformed trait and body lengths, and reported the results of both analyses.

We used regression trees to test contributions of environmental variables to the abundance of *Daphnia lumholtzi* during this time period. Regression trees are well suited to this data set because they allow modeling of complicated interactions among predictors and nonlinear relationships between predictor and response variables (De'ath

and Fabricius 2000), both of which are common in aquatic ecology. For predictor variables, I used water temperature (˚C), salinity (practical salinity units, psu), dissolved oxygen (mg L^{-1}), chlorophyll (μg L^{-1}), phycocyanin (proportional to μg L^{-1}), total nitrogen (mg L^{-1}), total phosphorous (mg L^{-1}), and Secchi depth (m). I constructed a regression tree using recursive partitioning with the ctree function in the party package (Version 1.0-25 (Hothorn et al. 2006)).

Results

As body size of *D. lumholtzi* increased, so did head spine length (Fig. 3). The relationship between head spine and body length was significantly curvilinear (D > 1) for *D. lumholtzi* collected during fall and summer (Table 1). The relative growth of head spines declined with increasing body sizes. For *D. lumholtzi* collected in the winter, however, the relationship between head spine and body length was linear and allometric (head spines grew at the same rate as body size). There was substantially more variation in helmet size in *D. lumholtzi* collected during the winter (Fig. 3). Overall, for a given body size, head spines were smaller in the winter than in the summer or fall, as shown by the lower intercept value fitted to winter individuals (Table 1).

In all seasons, we found a linear relationship between tail spine and body length (Table 2), with tail spine length increasing with body length (Fig. 4). In the summer and fall, tail spine growth was hyperallometric (growing at a faster rate than the body), whereas in winter, tail spine growth was allometric (Table 2). As with head spines, we found that tail spines were, for a given body size, smaller in the winter than in the summer or fall

(Fig. 4). Additionally, for *D. lumholtzi* of a given size, the tail spine was longer than the head spine.

Phycocyanin was the only predictor variable included in the regression tree for *D. lumholtzi* abundances (Fig. 5). *D. lumholtzi* abundances were positively associated with cyanobacteria concentrations (Fig. 6). The highest *D. lumholtzi* abundances (mean ± st dev: 0.274 ± 0.355) were associated with phycocyanin above 20.9 μg L^{-1}. At low phycocyanin concentrations (<16.5), *D. lumholtzi* abundances were very low (0.022 ± 0.037). Intermediate phycocyanin concentrations were associated with intermediate *D. lumholtzi* abundances (0.092 ± 0.09). Although these factors weren't retained by the regression tree model, *D. lumholtzi* abundance appeared unimodally related to salinity, positively related to pH, and negatively related to Secchi depth (Fig. 6). We found no relationship between temperature and *D. lumholtzi* abundance (Fig. 6).

Discussion

We set out to characterize factors associated with abundances and morphology of *Daphnia lumholtzi* within Lake Texoma. We found that the single best predictor of *D. lumholtzi* abundances was a positive association with cyanobacteria abundances. Although temperature, salinity, Secchi, and other factors found to be important in previous studies were included in the model, none of them were included in the final regression tree. Additionally, we found no relationship between temperature and abundance of *D. lumholtzi* within Lake Texoma, contrary to earlier work in this lake (Work and Gophen 1995). The positive association between cyanobacteria and *D.*

lumholtzi within Lake Texoma suggests that *D. lumholtzi* may be able to persist during blooms of cyanobacteria that typically occur during the summer with warmer temperatures, but may also occur year-round in Lake Texoma. These are the same conditions where native *Daphnia* are usually found at low abundances (Work and Gophen 1995). Thus *D. lumholtzi* may be colonizing a niche that native *Daphnia* species cannot.

The defensive morphology of *D. lumholtzi* further allows their persistence during the summer and fall when predation pressure by young-of-year fishes is likely higher in Lake Texoma (Hubbs 1982, Lienesch and Gophen 2001). We found that growth of head spines in *D. lumholtzi* followed a curvilinear relationship, with decreasing investment in head spines as body sized increased. This pattern is consistent with the selection pressure on *D. lumholtzi* spine size being a small, gape-limited predator. Tail spine growth was linear and hyperallometric in the summer and fall, but allometric in the winter. Earlier research has found tail spine growth to be hyperallometric (Sorensen and Sterner 1992, Yurista 2000). Sorensen and Sterner measured individuals in the winter and spring (January-March), and Yurista collected primarily in the summer (July—early September, P. Yurista, personal communication). The seasonal differences in allometry of both head and tail spines suggest that the selective agent is itself seasonal.

Within Lake Texoma, several species fit the profile as small, gape-limited predators exerting seasonal pressure. Lake Texoma has two large-bodied invertebrate predators that can feed on *D. lumholtzi:* the cladoceran, *Leptodora kindti* (Holt et al. 1978), and

the dipteran, *Chaoborus punctipennis* (Sublette 1957). In laboratory experiments, *Leptodora kindti* prefers *D. lumholtzi* over the native *Daphnia pulex*, and is not discouraged by its defensive spines (Effert and Pederson 2006), and thus is unlikely to be the predator shaping the nonlinear and seasonal allometry of head spines of *D. lumholtzi* within Lake Texoma. *Chaoborus punctipennis*, on the other hand, is a size-selective predator of *D. lumholtzi* and laboratory experiments have shown that it is unable to consume individuals with total length greater than 1.84 mm and helmets greater than 0.51 mm (Engel et al. 2014). Further, the daily migration behavior of the two species results in microhabitat overlap, which puts *D. lumholtzi* at risk of predation by *Chaoborus*. Within other invaded lakes, *D. lumholtzi* performs diel vertical migration (Sorensen and Sterner 1992, Williams and Pederson 2004). *Chaoborus punctipennis* migrates in the same way within Lake Texoma (Sublette 1957), moving into the hypolimnion during the day to avoid vertebrate predators, and moving into the epilimnion during the night to feed. Thus, assuming *D. lumholtzi* is migrating as it does in other invaded lakes, it likely overlaps in depth preferences with *Chaoborus punctipennis* within Lake Texoma. Additionally, if *Chaoborus* abundances in Lake Texoma peak in late summer to fall, as they do in North Carolina (Celik et al. 2002), then the trend in head spine growth may reflect population densities of and predation pressure by *Chaoborus*. Within Lake Texoma, inland silversides consume *D. lumholtzi* as well (Lienesch and Gophen 2001), and cannot consume larger-spined *D. lumholtzi* (Lienesch and Gophen 2001). Predation pressure by young-of-year inland silversides would be seasonal, with highest abundances observed May through July (Hubbs 1982). Evidence of non-linear helmet growth and seasonal patterns in allometry lead us to

suggest that size-selective predation on *D. lumholtzi* is carried out by either *Chaoborus punctipennis* or inland silversides. With the likely overlap in vertical migration profiles of *D. lumholtzi* and *Chaoborus punctipennis*, *Chaoborus* is the more likely culprit of the two.

Daphnia lumholtzi reach high abundances in eutrophic lakes during the summer, which makes them unique relative to native *Daphnia* that tend to peak in abundances in late spring. The higher temperature tolerance of *D. lumholtzi*, paired with their unique defensive morphology, allows them to exploit late summer resources in subtropical reservoirs. The relatively high abundances of *D. lumholtzi* observed during summers in the southern US (Havel et al. 1995, Work and Gophen 1995, Yurista et al. 2000, Havens et al. 2012) co-occur with native non-cladoceran grazers, including copepods, rotifers, and ciliates. Although tests of competition have focused on native *Daphnia*, the two do not temporally overlap in most invaded systems. Further work should be done to characterize the interactions between *D. lumholtzi* and the native grazers that are abundant during the summer peaks of *D. lumholtzi*, including copepods and rotifers (Hambright et al. 2010). Copepods are considered to be more tolerant of cyanobacteria than native *Daphnia* due to their selective feeding mechanisms. So there may be either direct or indirect competition between *D. lumholtzi* and copepods during the blooms of cyanobacteria that are associated with high abundances of *D. lumholtzi*. As global change is predicted to lead to increases in both the frequency and duration of blooms of cyanobacteria (Paerl and Paul 2012), we need further research to characterize the

proximate causes of positive relationships between *D. lumholtzi* and cyanobacteria, as well as potential competitive interactions with native grazers.

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Tables

Table 1: Parameter estimates ± standard error, and confidence intervals from nonlinear regression of ln-transformed helmet length against body length. Where the curvature parameter, *D*, is not significantly different from 1, results of linear regression of the same variables are also provided.

	Parameter	Summer (n = 163)	Fall (n = 331)	Winter (n = 77)
Nonlinear regression	*A*	0.671 ± 0.0374	0.665 ± 0.0411	0.567 ± 0.135
	C	0.776 ± 0.0694	0.951 ± 0.0703	1.01 ± 0.290
	D	1.82 ± 0.262	1.69 ± 0.200	1.16 ± 0.432
	95% CI for *D*	(1.37, 2.33)	(1.32, 2.09)	(0.587, 2.06)
	Residual standard error	0.245	0.293	0.497
Linear regression	Slope			1.12 ± 0.122
	Intercept			-2.19 ± 0.845
	95% CI for Slope			(0.876, 1.36)

Table 2: Parameter estimates ± standard error, and confidence intervals from nonlinear regression of ln-transformed tail spine length against body length. Where the curvature parameter, *D*, is not significantly different from 1, results of linear regression of the same variables are also provided.

	Parameter	Summer (n = 162)	Fall (n = 323)	Winter (n=73)
Nonlinear regression	*A*	2.05 ± 0.158	1.95 ± 0.144	1.54 ± 0.290
	C	1.26 ± 0.0804	1.29 ± 0.0755	0.994 ± 0.220
	D	0.946 ± 0.0985	0.851 ± 0.0776	1.02 ± 0.296
	95% CI for *D*	(0.765, 1.14)	(0.702, 1.01)	(0.584, 1.65)
	Residual standard error	0.145	0.149	0.328
Linear regression	Slope	1.22 ± 0.0325	1.17 ± 0.0260	1.00 ± 0.0833
	Intercept	-1.43 ± 0.217	-1.17 ± 0.172	-0.408 ± 0.575
	95% CI for Slope	(1.16, 1.29)	(1.12, 1.22)	(0.842, 1.17)

Figures

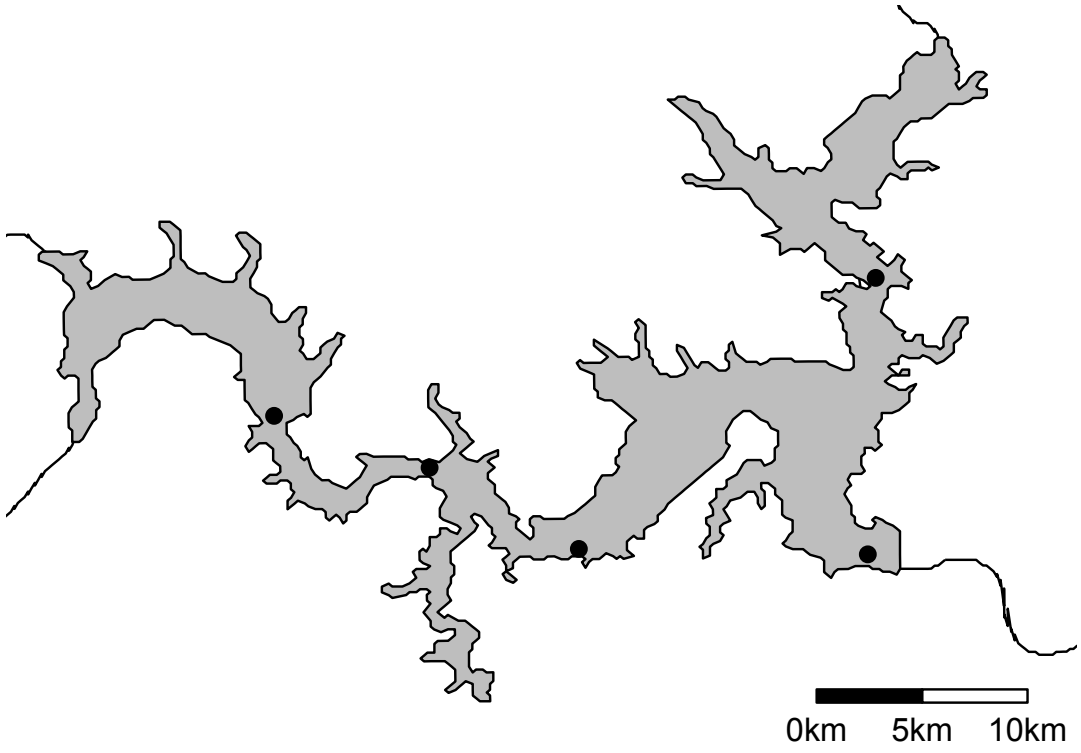

Figure 1: Map of Lake Texoma, OK-TX, with five pelagic sampling sites indicated by black circles.

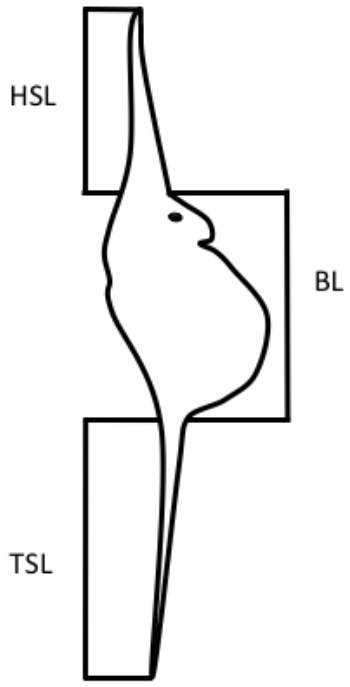

Figure 2: Diagram of measurements of *Daphnia lumholtzi* including head spine length (HSL), body length (BL), and tail spine length (TSL).

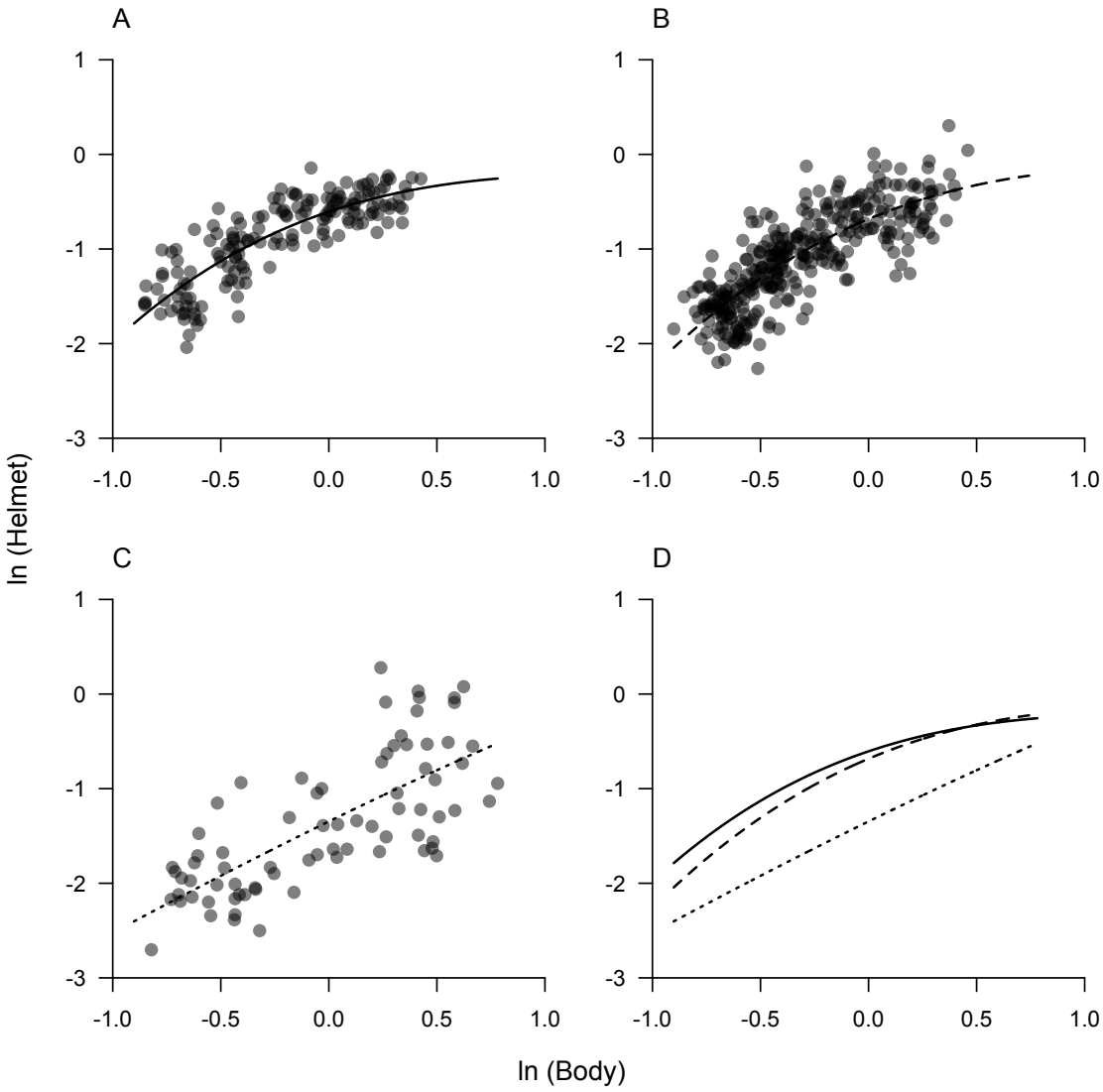

Figure 3: Relationship between log-transformed body and helmet size by season: (A) Summer, (B) Fall, (C) Winter, (D) nonlinear regression fits from 3 seasons. Lines represent nonlinear regression fit.

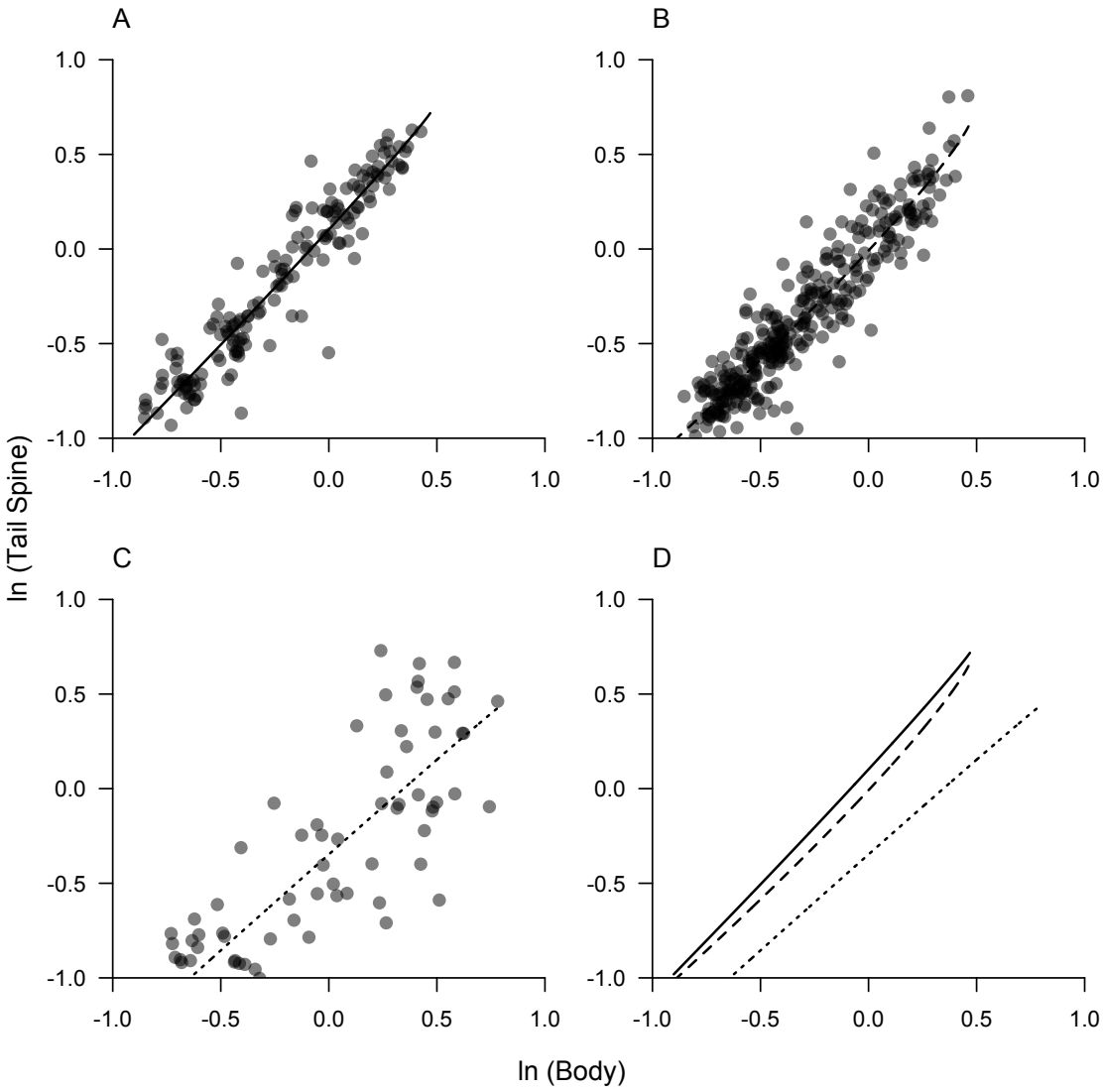

Figure 4: Relationship between log-transformed body and tail spine size by season: (A) Summer, (B) Fall, (C) Winter, (D) nonlinear regression fits from 3 seasons. Lines represent nonlinear regression fit.

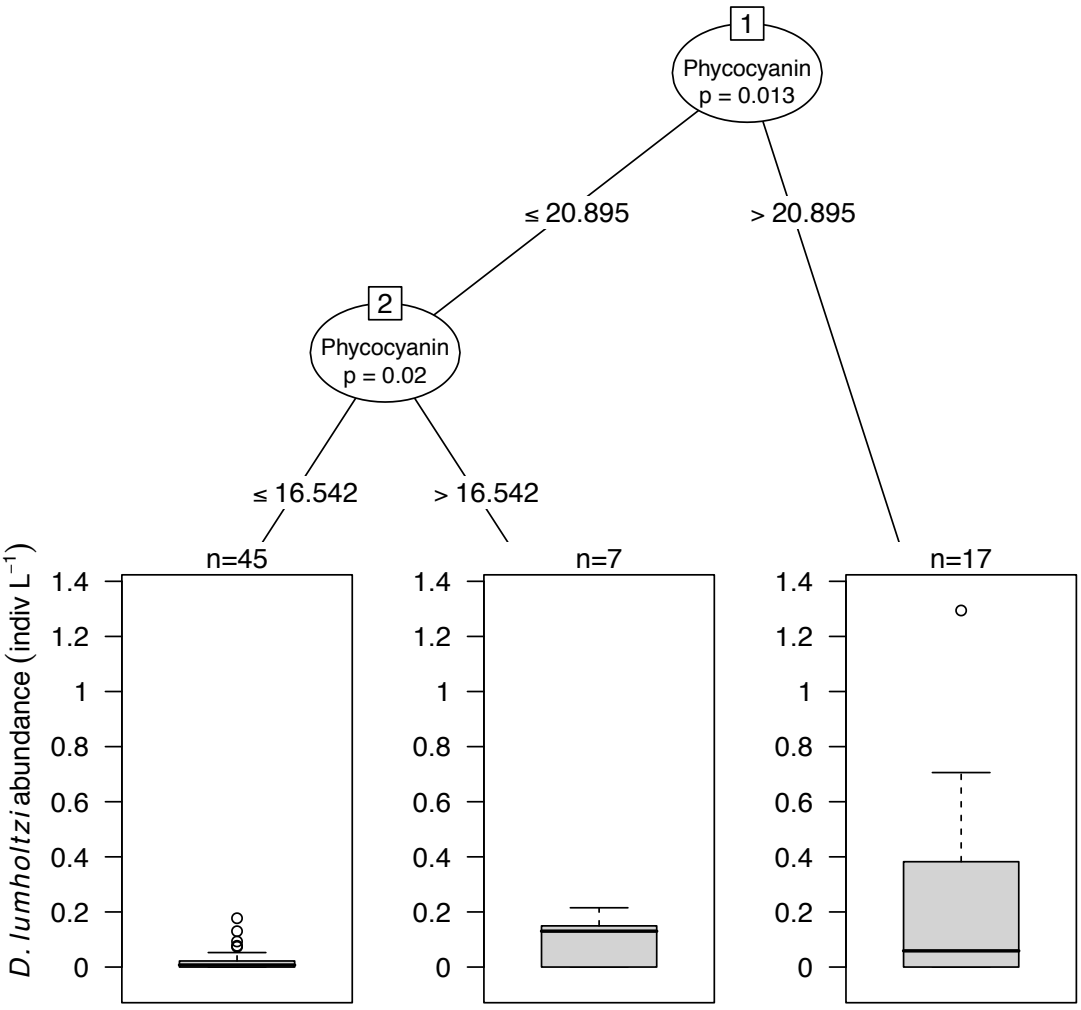

Figure 5: Regression tree for *Daphnia lumholtzi* abundance. Box and whisker plots show the abundance of *D. lumholtzi* in the samples at each node.

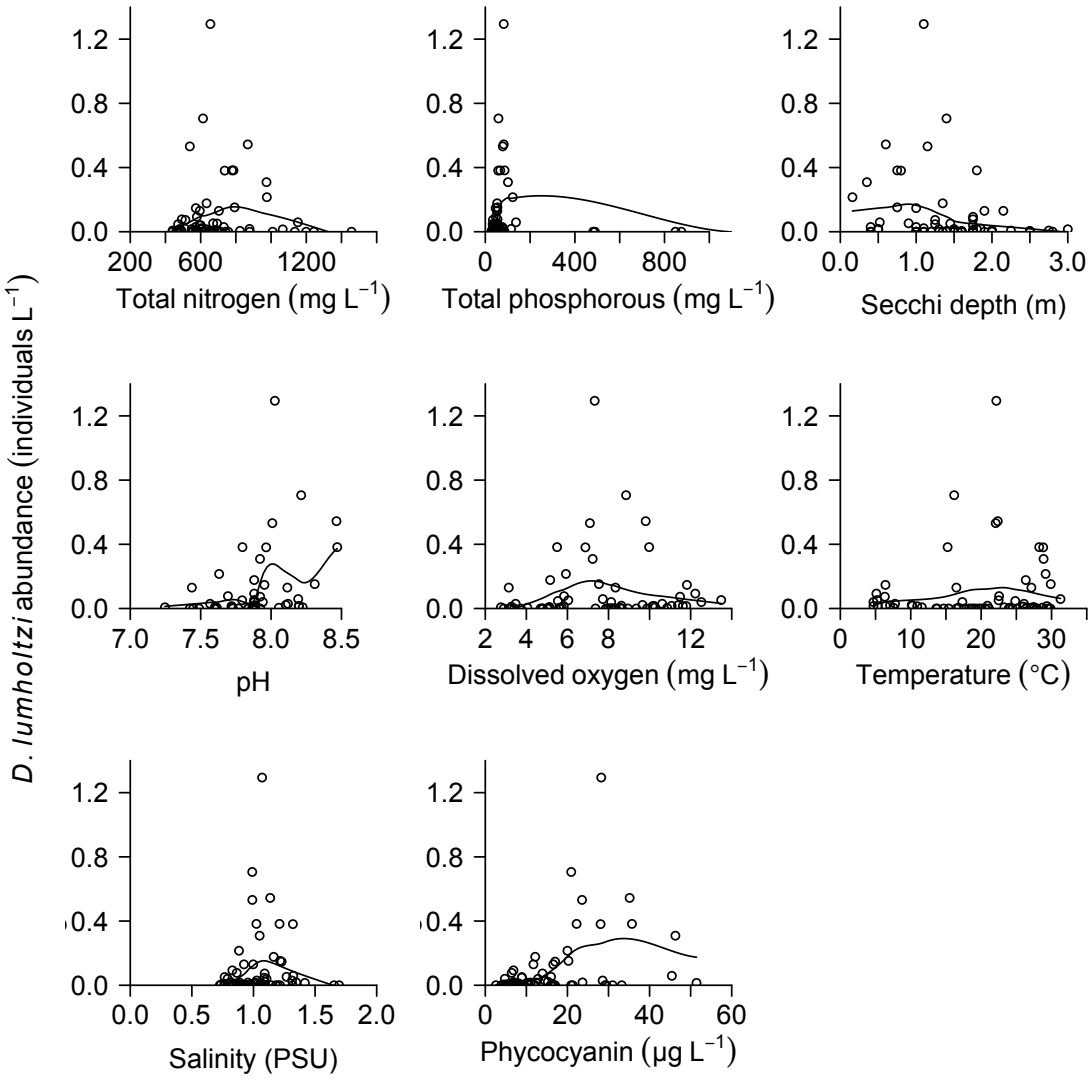

Figure 6: Scatterplots of predictors included in regression tree analysis and *Daphnia lumholtzi* abundance. Lines represent loess curves.

Appendix A: Supplemental material to Chapter 1

Contents

1. Effect of food density on population growth rate

2. Effect of food quality and quantity on frequency of sexual reproduction

3. Relative contribution of survival and reproduction to population growth rate

1. Effect of food density on population growth rate

Methods

We ran an experiment to test the effect of *Chlamydomonas* sp. density on the population growth rate of *Brachionus calyciflorus*. These results were used to select the density of algae used in the switching experiments described in the main text. We tested the effects of five densities of *Chlamydomonas* sp. on the population growth of *Brachionus calyciflorus*. Each replicate consisted of ten *Brachionus calyciflorus* females in one well of a six-well tissue culture plate in 8 mL of COMBO, fed at one of five *Chlamydomonas* densities (0, 1×10^4, 2.5×10^4, 1×10^5, and 4×10^5 cells mL^{-1}). These densities were selected based on the range of values previously used to calculate the population growth rate of *Brachionus calyciflorus* (Barreiro Felpeto and Hairston 2013). Daily, we counted the number of rotifers and eggs in each well. Then, we transferred 10 individuals to a new well with fresh media and algae. This daily transfer allowed us to control the algal density and prevent the accumulation of mixis inducing protein to prevent the production of males and resting eggs. We repeated this process for six days, at which point there was no survival in the lowest density treatment (0 cells mL^{-1}). All well plates were stored at 20°C on 12:12 light:dark cycle. At the end of the

experiment, we calculated the population growth rate (λ) for each replicate as the multiplicative rate of increase in the rotifer population in one day ($N_t N_{t-1}^{-1}$), averaged (within replicate) across days four to six of the experiment. We fit numerical response curves (Holling Type II and Type IV models) to population growth (Barreiro Felpeto and Hairston 2013). We used a combination of likelihood ratio tests and Akaike Information Criterion corrected for small samples (AIC_c) to determine which of the two models best fit the data (AICcmodavg package, version 2.0-3, Mazerolle 2015).

Results

The population growth rate increased with increasing resource density (Fig. A1). There was no significant difference in the fit of the Type II and Type IV models ($F_{12,11}$ = 1.0274; p = 0.3325), but the estimate of parameter i in the Type IV model was very close to zero (Table A1, suggesting very little decay in growth rate after reaching λ_{max}). Using the Akaike Information Criterion corrected for small samples (AIC_c), the Type II model was preferred because it had fewer parameters than the Type IV model but the same fit. Using Type II model, the maximal population growth parameter (λ_{max}) was estimated at 2.18 rotifers female^{-1} day^{-1}.

2. Effect of food quality and quantity on frequency of sexual reproduction

Methods

Two experiments were run to test whether food quality and quantity affected the frequency of sexual reproduction in the rotifer clone used in the switching experiments. *Brachionus calyciflorus*, like most monogonont rotifers, is a cyclic parthenogen, with

reproductive mode responsive to environmental cues. Typically, females reproduce asexually, producing genetically identical, asexually-reproducing daughters until a cue (crowding, in the case of *Brachionus calyciflorus*) leads to production of sexually-reproducing daughters that produce haploid eggs. If unfertilized, these haploid eggs develop into males, but if fertilized, eggs enter diapause and develop into females following the return of favorable conditions (Gilbert 2003). As resting eggs are energetically more costly for females to produce (Gilbert 2010), we hypothesized that food quantity and quality could negatively affect the frequency of sexual reproduction.

The first of the two experiments exploring effect of diet on the frequency of sexual reproduction was designed to test the hypothesis that the probability of producing mictic (sexual) daughters varies based on food density. To test this, two generations of *Brachionus calyciflorus* were raised without crowding with high concentrations of *Chlamydomonas* sp. (4×10^5 cells mL^{-1}). Individual rotifers (first generation) collected from uncrowded cultures were raised in wells of 24-well plate containing 2 mL of algae culture. We collected ten second-generation individuals on the day they were born and transferred them to high concentrations of *Chlamydomonas* (4×10^5 cells mL^{-1}). These second-generation rotifers were checked daily for neonate production and transferred to fresh media. All produced neonates (third generation) were transferred to individual wells with either high or low concentrations of *Chlamydomonas* (4×10^5 cells mL^{-1} and 1×10^4 cells mL^{-1}, respectively). They were monitored until production of their first offspring (fourth generation). Production of a male neonate identified the third-generation rotifer as mictic, whereas production of a female neonate identified the third-

generation rotifer as amictic. To test the hypothesis that food density affected the probability of rotifers producing sexual offspring, we used a generalized linear mixed model with binomial response (mictic or amictic response) with maternal identity specified as random effect and birth order and food quantity specified as fixed effects (lme4 package, version 1.1-8, Bates et al. 2015). Significance of fixed effects was tested using likelihood ratio tests.

The second experiment was designed to test whether food quality influenced the reproductive mode of *Brachionus calyciflorus*. In this experiment, the effect of *Chlamydomonas* sp. (CVBS) on sexual reproduction frequency was compared with that of *Synechococcus leopoliensis* (UTEX B625), a non-toxigenic cyanobacterium. We used three treatments of equivalent carbon concentration, but varying quality. For our high quality food, we used *Chlamydomonas* sp., at the concentration that produced the maximal population growth (4×10^5 cells mL^{-1}). For our low quality food, we used *Synechococcus*, which is deficient in several components necessary for reproduction and survival in *Brachionus calyciflorus* (Wacker and Martin-Creuzburg 2012). The concentration of *Synechococcus* used (1.4×10^7 cells mL^{-1}) was equivalent in carbon content to the pure *Chlamydomonas* treatment. We compared the effects of these pure diets with the effect of a mixed diet, closer to the conditions a rotifer would experience in a lake (2×10^5 cells mL^{-1} *Chlamydomonas* and 7×10^6 cells mL^{-1} *Synechococcus*). To isolate the effect of diet and remove any potential confounding effects of crowding, we mixed pre-cultured media with our algae cultures to produce the final treatment that the rotifers experienced. This resulted in final treatments containing cues equivalent to 20

rotifers mL^{-1}. Populations of ten rotifers were maintained in wells of 6-well tissue culture plate containing 8 mL of treatment. Every day, we counted the number of rotifers carrying amictic and mictic eggs (including both male eggs and resting eggs), and then randomly transferred ten rotifers to another well containing freshly prepared treatment. To test the effect of food quality on the probability of rotifers producing sexual offspring, we used a generalized linear mixed model with binomial response (number of mictic and amictic rotifers) with replicate ID specified as random effect (to account for repeated measurements) and day and food quality specified as fixed effects (lme4 package, version 1.1-8, Bates et al. 2015). We used likelihood ratio tests to test the significance of the fixed effects. We calculated population growth rates (λ) as the multiplicative rate of increase in the rotifer population in one day ($N_t N_{t-1}^{-1}$). We used RM ANOVA to test for differences in population growth rates between treatments.

Results

Overall, we found no effect of food quality or quantity on the probability of sexual reproduction in the rotifer, *Brachionus calyciflorus*. The quantity of food available to a female did not affect the probability of producing mictic offspring (Fig. A2, $\chi^2 = 0.0015$, df = 1, $p = 0.969$). However, with increasing birth order, offspring were less likely to be mictic ($\chi^2 = 11.09$, df = 1, $p < 0.001$). That is, offspring born earlier within a mother's lifespan were more likely to reproduce sexually (Fig. A3). There was no significant interaction between food quantity and birth order ($\chi^2 = 2.72$, df = 1, $p = 0.10$).

Food quality, manipulated using carbon-equivalent densities of *Chlamydomonas*, *Synechococcus*, and a mixture of the two, did not affect the frequency of sexual reproduction when rotifers received constant density cues ($\chi^2 = 2.15$, df = 2, $p = 0.341$). Food quality affected population growth rates ($F_{2,9} = 28.71$, $p < 0.001$), with the mixed diet producing the highest growth rate (Table A2). The differences in average frequency of sexual reproduction between the two experiments can be attributed to the presence or absence of density cues. In the first experiment, rotifers were maintained individually and were transferred to fresh culture media every day. In the second experiment, however, rotifers were maintained in populations of at least ten individuals, and were maintained in conditioned media equivalent to rotifer density of 20 individuals mL^{-1}.

3. Relative contribution of survival and reproduction to population growth rate

Methods

We performed a life table response experiment analysis to identify the relative contributions of changes in survival and fecundity to the changes in population growth rate (Caswell 2001). For this analysis, we used the life table data generated from the switching experiment (see main text for details). Briefly, we calculated the overall mean projection matrix ($\mathbf{A}^{(\cdot\cdot)}$) as the reference matrix. Then, for each of the eight combinations of Treatments 1 and 2, we calculated the contributions of changes in the age specific fecundity and survival rates to changes in the population growth rates (popbio package, version 2.4, Stubben and Milligan 2007). This process allowed us to determine which of these two vital rates was most important, and at which ages the contribution was the strongest

Results

The differences in population growth rates observed in our treatments (see main text for details) were primarily due to changes in reproductive output (Fig. A4) and not survival (Fig. A5). The contribution of differences in fertility to observed differences in the population growth rate was nearly 100 times greater than the contribution of differences in survival. Thus, although we detected significant differences in survival, the differences in population growth rates are attributable to differences in the reproductive schedules of rotifers in the switching experiments.

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Tables

Table A1: Model parameters from *Chlamydomonas* density experiment (presented as estimate ± standard error).

Model Type	df	AIC_c	Maximum growth rate (λ_{max})	Half-saturation constant (K_f)	Food concentration where $\lambda = 0$ (f_0)	Rate of decay (i)
II	12	12.52	2.18 ± 0.15	16594.53 ± 6298.38	-2006.42 ± 1661.80	NA
IV	11	15.85	1.86 ± 0.29	10210 ± 6340	-1326 ± 1.285	-4.231×10^{-7} ± 3.537×10^{-7}

Table A2: Population growth rates of *Brachionus calyciflorus* fed three diets of different food quality for 14 days (presented as mean ± standard deviation).

Treatment	Population growth rate (λ)
Chlamydomonas	1.92 ± 0.86
Synechococcus	1.3 ± 0.61
Mixed	2.48 ± 0.89

Figure Legends

Figure A1: Population growth rate of rotifers as a function of *Chlamydomonas density*. Growth rate, λ, represents the multiplicative rate of increase in the rotifer population in 1 day ($N_t N_{t-1}^{-1}$), averaged (within replicate) across days 4 to 6 of the experiment. Results are shown as the means of the three replicates ± 1 SE. Line represents non-linear regression fit of Type II functional response curve.

Figure A2: Frequency of sexual reproduction under different food quality and quantity treatments. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median. Individual data points are overlaid as small, open circles.

Figure A3: Conditional probability of mictic and amictic offspring based on birth order. The black line represents the estimated changes in the conditional probability of producing mictic offspring with increasing birth order.

Figure A4: The contributions to λ of effects of treatment on age specific fertility. For each combination of Treatment 1 and Treatment 2, the bars represent the contribution of age specific fertility to differences observed in the population growth rate, λ. Positive bars represent a positive effect on the growth rate, relative to the average, and negative bars represent a negative effect on the population growth rate, relative to the average.

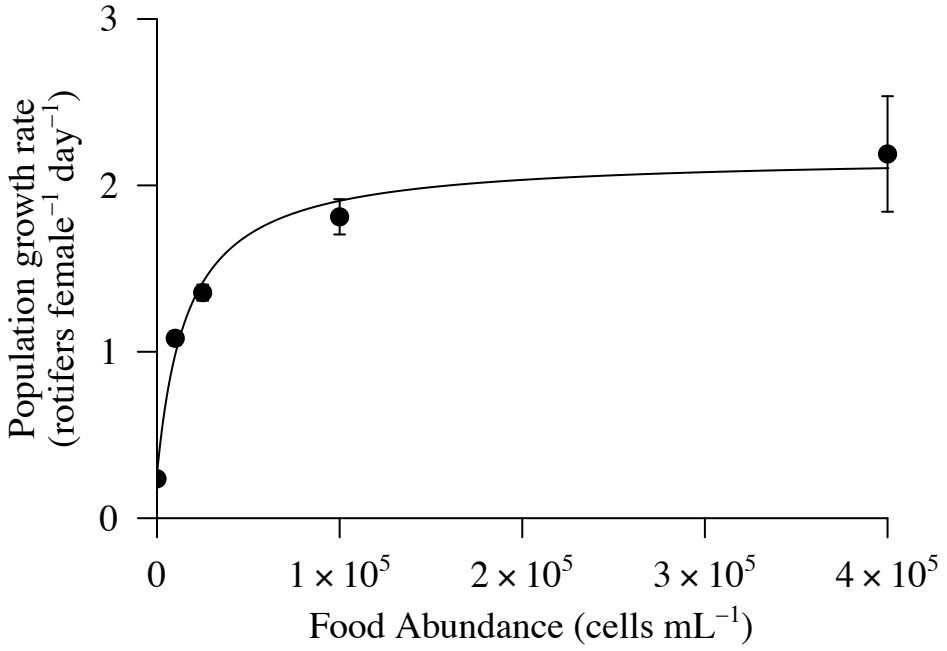
Figure A5: The contributions to λ of effects of treatment on age specific survival (note change in y-axis scale from Fig. 4). For each combination of Treatment 1 and Treatment 2, the bars represent the contribution of age specific survival to differences observed in the population growth rate, λ.

Figure A6: Age-specific survival curves from switching experiment described in main text. Top panel shows rotifers fed high concentration of *Chlamydomonas* for Treatment

1, and lower panel shows survival of rotifers fed low concentration of *Chlamydomonas* for Treatment 1. Error bars represent ± 1 standard deviation (calculated from 1000 bootstrap replicates).

Figure A7: Effect of maternal age and Treatment 1 on offspring length as described in main text. Circles represent mean length and error bars represent ± 1 standard error.

Figure A1


Population growth rate
(rotifers female^{-1} day^{-1})
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2
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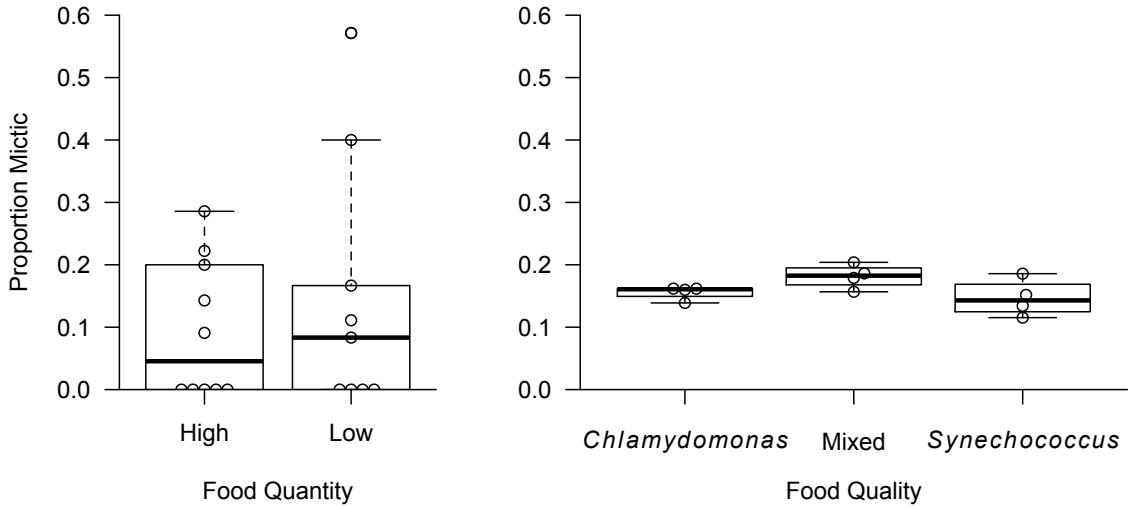
0
1×10^5
2×10^5
3×10^5
4×10^5
Food Abundance (cells mL^{-1})

Figure A2


Proportion Mictic
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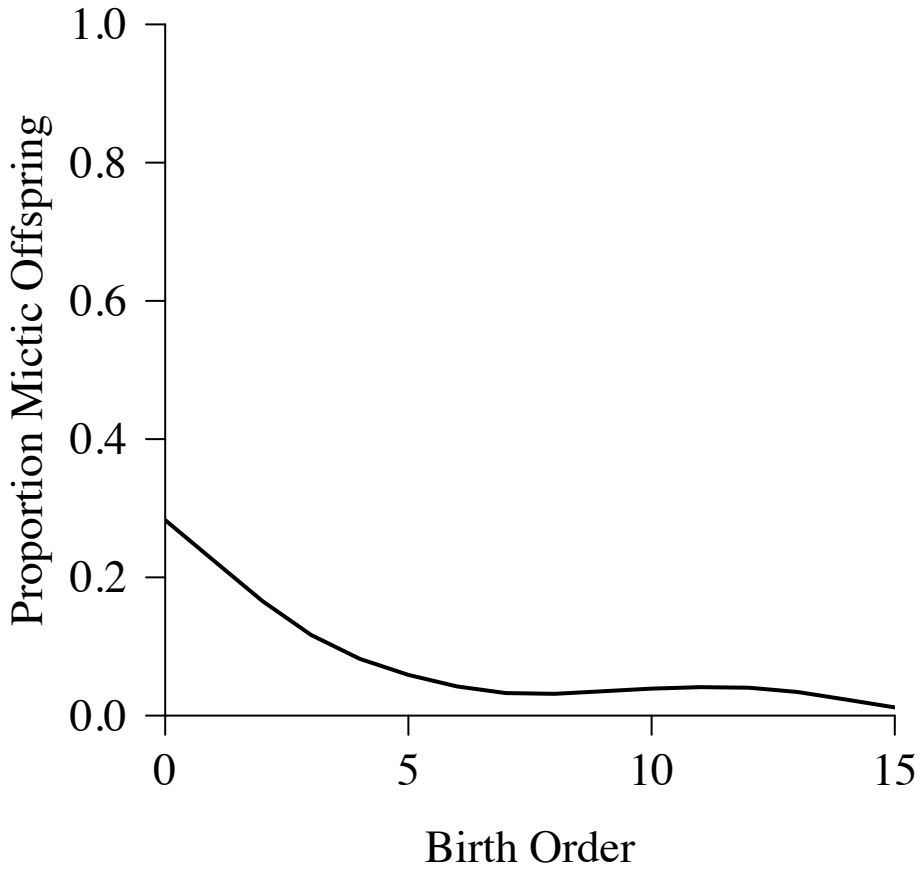
High
Low
Food Quantity
Chlamydomonas
Mixed
Synechococcus
Food Quality

Figure A3


1.0
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0.2
0.0
Proportion Mictic Offspring
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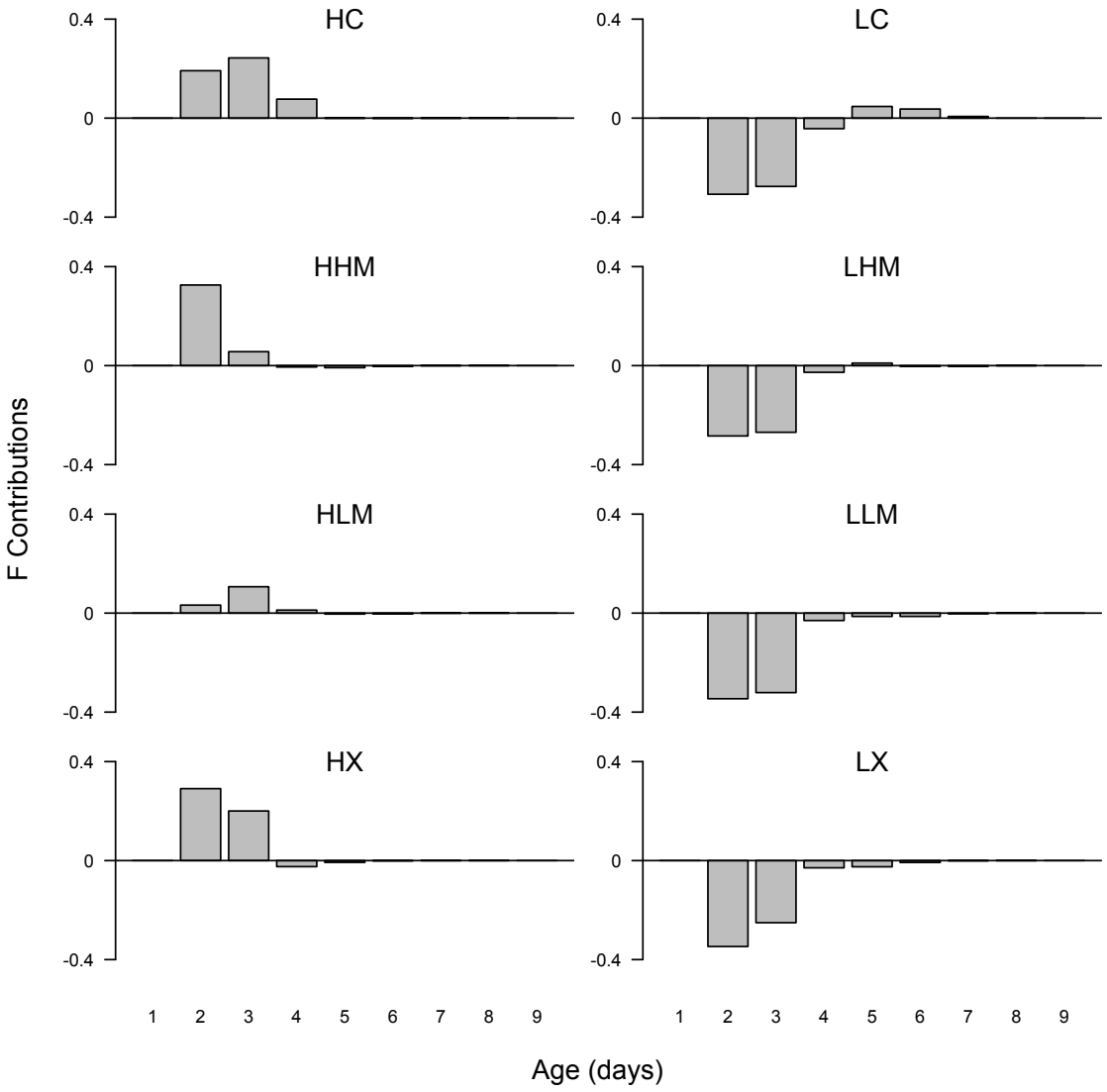
5
10
15
Birth Order

Figure A4


HC
LC
HHM
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-0.4
F Contributions
1
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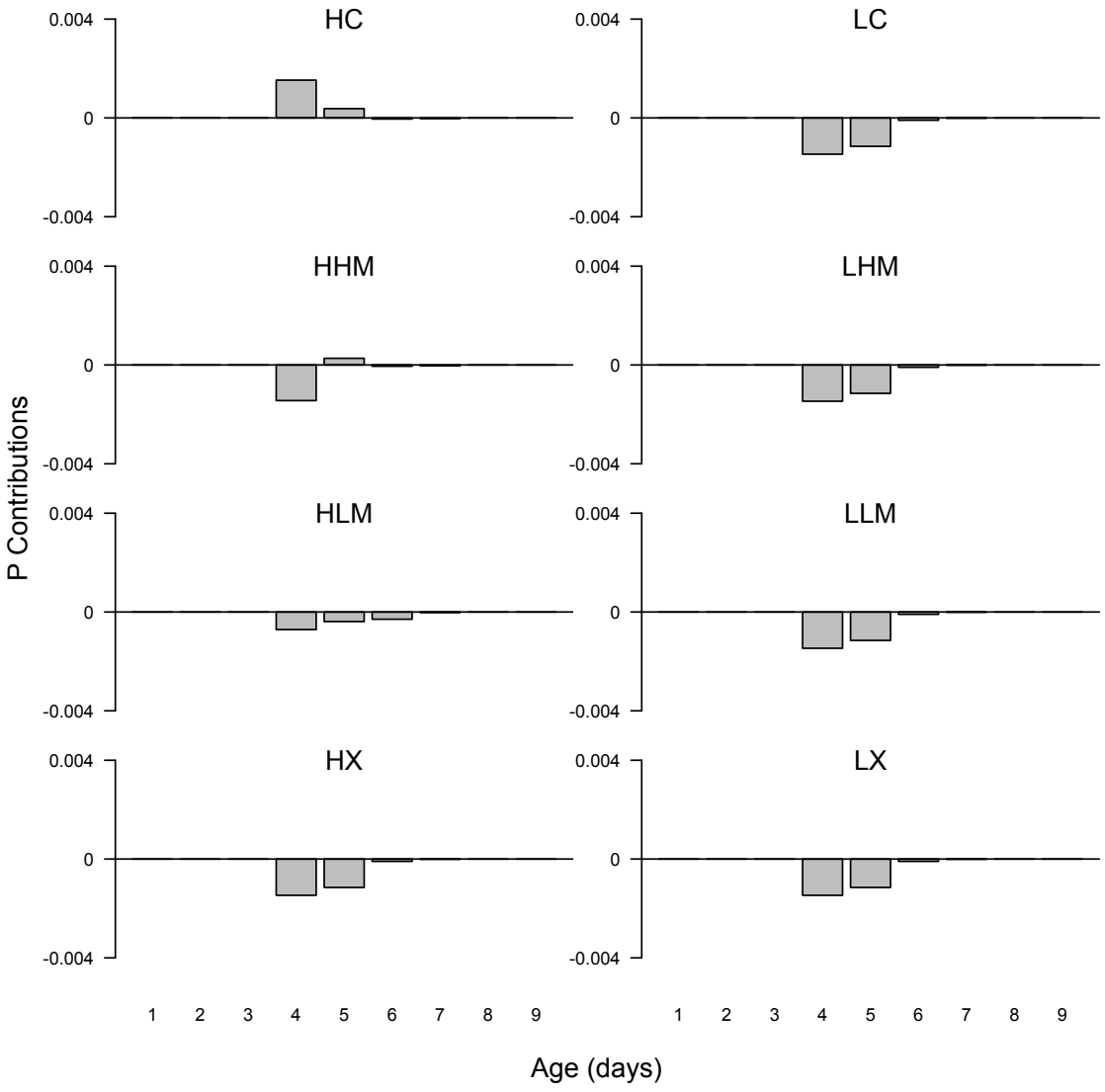
7
8
9
Age (days)

Figure A5


HC
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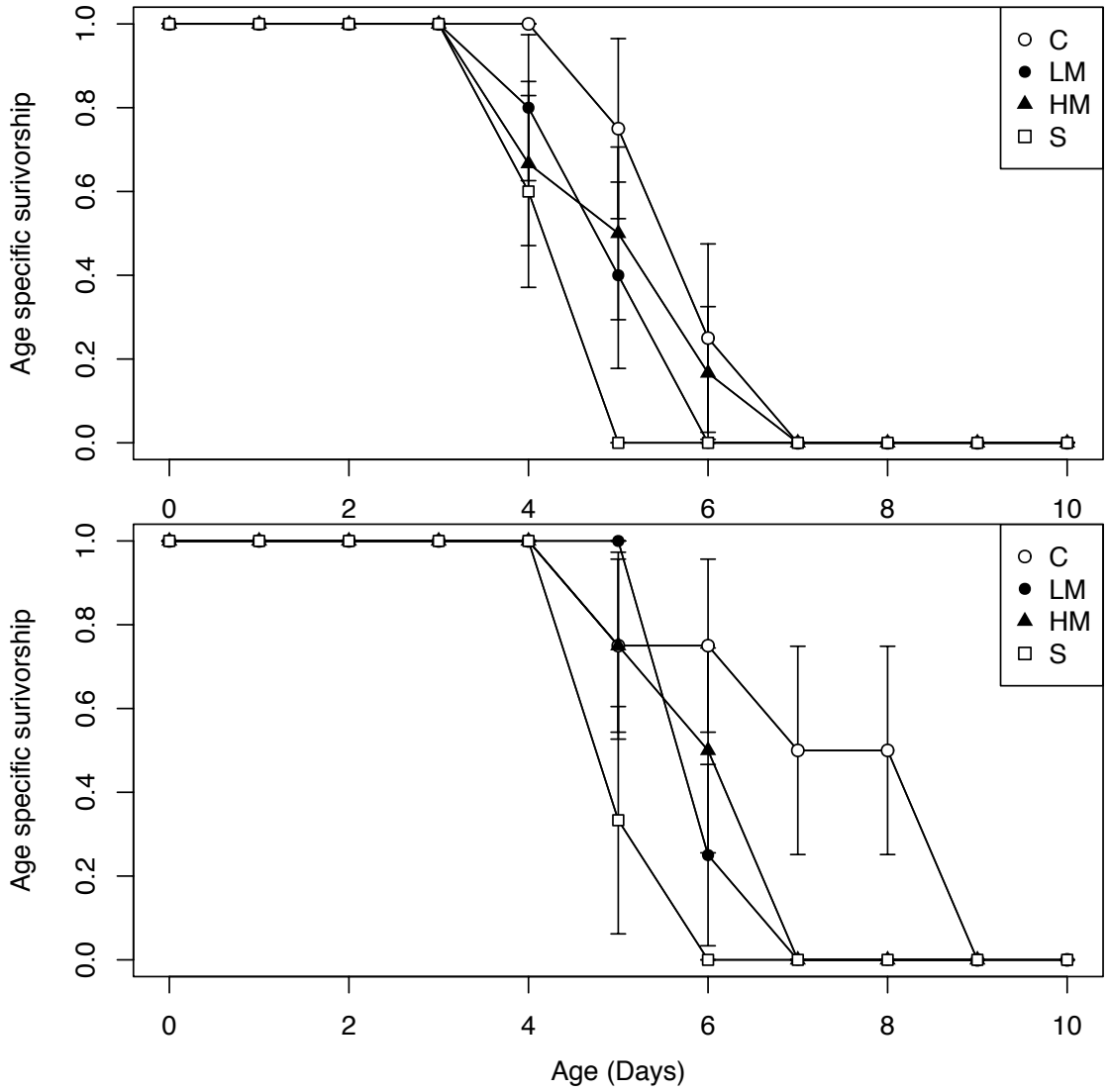
P Contributions
1 2 3 4 5 6 7 8 9
Age (days)

Figure A6


Age specific surivorship
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0.2
0.4
0.6
0.8
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2
4
6
8
10
C
LM
HM
S
Age specific surivorship
0.0
0.2
0.4
0.6
0.8
1.0
0
2
4
6
8
10
C
LM
HM
S
Age (Days)

Figure A7

