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

Jessica E. Beyer* and K. David Hambright

Program in Ecology and Evolutionary Biology and Plankton Ecology and Limnology Laboratory, Department of Biology, University of Oklahoma, Norman, OK, USA

*Corresponding author. Tel.: +1 405-595-6521; e-mail address: beyer@ou.edu

Keywords: rotifers, harmful algal blooms, life history, *Brachionus calyciflorus*, cyanobacteria-zooplankton interactions, offspring size, allocation

Running head: HAB effects on rotifer life history

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 Supplemental Materials: 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

Supplemental Materials). 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 ($\theta = \text{SD}(\lambda)$) 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 Supplemental Materials: 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 Supplemental Materials: 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 Supplemental Materials: 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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Acknowledgements

We would like to thank the members of the Plankton Ecology and Limnology Laboratory for helpful discussions and, in particular, Dani Glidewell for lab assistance. Two anonymous reviewers also provided constructive suggestions. Funding was provided by the Oklahoma Department of Wildlife Conservation (through the Sport Fish Restoration Program, Grant F-61-R to KDH), the University of Oklahoma Department of Biology, and OU Biological Station (through a Graduate Research Fellowship to JEB). This work was submitted by JEB as partial fulfillment of the requirements for a Ph.D. degree from the University of Oklahoma.

Figure legends

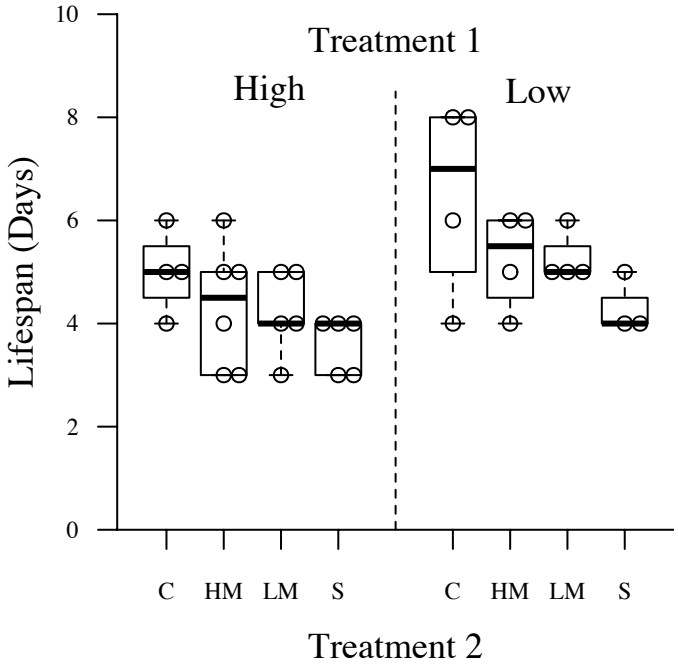

Figure 1: Lifespan of rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2 (C = *Chlamydomonas*, HM = High *Microcystis*, LM = Low *Microcystis*, and S = starvation). Boxes indicate the first and third quartiles, and the darker, horizontal line represents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

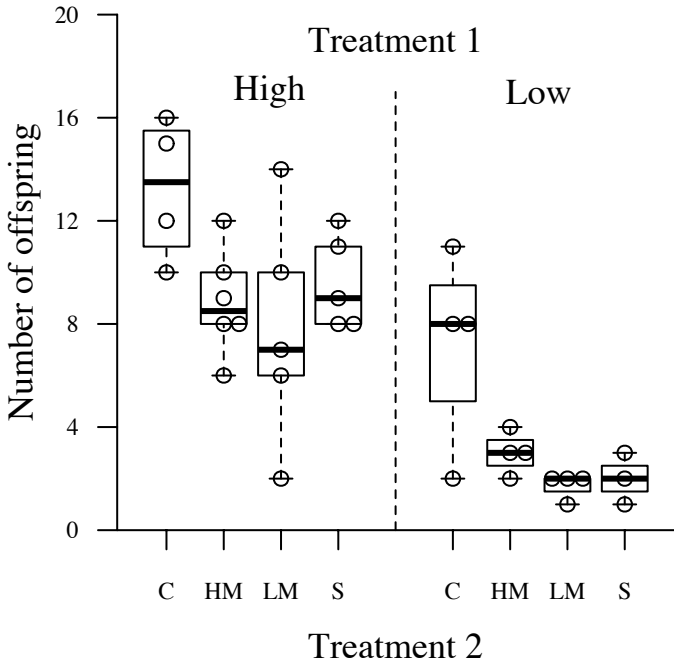

Figure 2: Number of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median. Individual data points are overlaid as small, open circles.

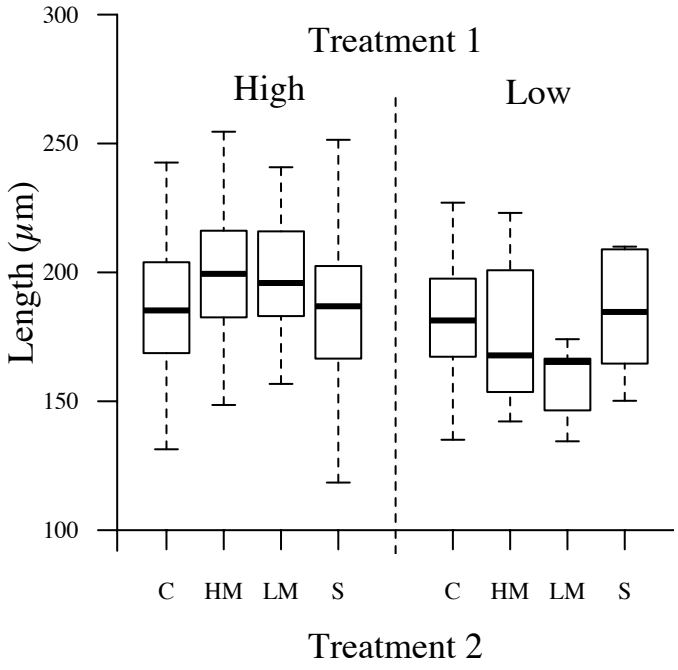

Figure 3: Length of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to the Treatment 2. Boxes indicate the first and third quartiles, and the darker, horizontal line represents the median.

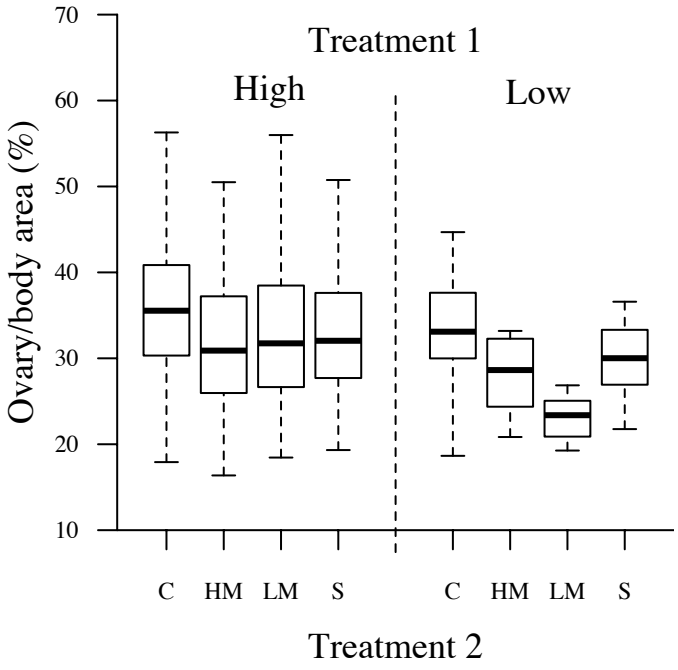

Figure 4: Condition of offspring produced by rotifers exposed to Treatment 1 for the first three days of life and then switched to Treatment 2. Offspring condition was measured by dividing the ovary area by the total body area. Boxes indicate the first and third quartiles, and the darker, horizontal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

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.

	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