Eutrophication mediates rapid clonal evolution in Daphnia pulicaria

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Eutrophication mediates rapid clonal evolution in *Daphnia pulicaria*

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Key Words: Nitrogen, phosphorus, ecological stoichiometry, phytoplankton, cyanobacteria, *Microcystis*

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Summary

1. Laboratory studies have revealed that *Daphnia* species can evolve to tolerate toxic cyanobacteria in the diet. Specifically, *Daphnia* from eutrophic lakes where cyanobacteria are common tend to have higher growth rates and survival when fed toxic cyanobacteria than populations from oligotrophic environments with low abundance of cyanobacteria.
2. We conducted an in-lake mesocosm (i.e., limnocorral) experiment during the fall of 2009 to assess the effects of nutrient enrichment on clonal evolution in *Daphnia pulicaria*. As nutrient enrichment often favors grazing-resistant cyanobacteria, we hypothesized that fertilization would influence the genotypic composition of *D. pulicaria* that vary in tolerance to cyanobacteria. Mesocosms were fertilized to manipulate phytoplankton and cyanobacterial abundance and concentrations of a cyanobacterial toxin (microcystin). Thus, half of the mesocosms were high-nutrient and half were low-nutrient. We then stocked half of the mesocosms with a mixture of six genetically-distinct *D. pulicaria* genotypes (three genotypes from oligotrophic lakes and three from eutrophic lakes) leaving half of the mesocosms *Daphnia*-free to assess grazing effects, using a fully factorial design.
3. When compared to the low nutrient treatment, high nutrient mesocosms had nearly five-fold higher chlorophyll *a* concentrations, eight-fold higher cyanobacterial dry biomass, and three-fold higher microcystin levels at the start of the experiment. In contrast, low nutrient mesocosms had phytoplankton concentrations typical of mesotrophic lakes.
4. Fertilization strongly affected *Daphnia* genetic diversity in the mesocosms. Final *Daphnia* genotype diversity in the mesocosms with low-cyanobacteria (richness = 5.83,

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Shannon-Weiner index = 1.55, evenness = 0.88) was similar to the initial stocked diversity (richness = 5.50, Shannon-Weiner index = 1.48, evenness = 0.87). In contrast, final diversity in fertilized mesocosms with high cyanobacteria was greatly reduced (richness = 2, Shannon-Weiner index = 0.17), with one *Daphnia* genotype that originated from the most-eutrophic lake being highly dominant (evenness = 0.25). Thus, eutrophication mediated strong clonal selection of a cyanobacteria-tolerant *Daphnia* genotype over just ten weeks.

5. By the end of the experiment, *Daphnia* significantly reduced phytoplankton biomass in the high-nutrient, but not in the low-nutrient treatment. This difference in effect size was largely driven by the five-fold higher initial phytoplankton biomass in the high-nutrient treatment. Thus, the ability of *Daphnia* to reduce phytoplankton biomass in eutrophic lakes may be driven more so by the abundance of planktivorous fishes, as opposed to the prevalence of cyanobacteria and associated toxins.

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Introduction

A growing body of evidence indicates that trait variation can have important consequences for ecological interactions (Thompson et al. 2001; Whitham et al. 2006; Post et al. 2008; Chislock et al. 2013a). Phenotypic expression, plasticity, and inducible defenses can all be important in mediating species interactions (Turner and Mittelbach 1990; Tollrian and Harvell 1999; Jeyasingh et al. 2009; Werner and Peacor 2003; Akbar et al. 2017; Des Roches et al. 2017). Although trait variation within keystone and foundation species is expected to have large effects on ecological interactions (Hughes and Stachowicz 2004; Whitham et al. 2006; Hughes et al. 2008; Post et al. 2008; Miner et al. 2012), there are relatively few field experiments that examine the role of intraspecific trait variation in freshwater environments (Palkovacs and Post 2009; Bassar et al. 2010). Environmental factors can strongly influence the presence and importance of species, including the evolution of trait frequencies (Darwin 1859), yet our knowledge is limited regarding the consequences of ecological-evolutionary interactions on ecosystem function (Alexander et al. 2016; Rudman et al. 2017).

Nutrient enrichment of freshwater ecosystems leads to a simultaneous increase in total phytoplankton biomass, the relative abundance of cyanobacteria, and cell toxin quota (Smith 1983; Watson et al. 1997; Paerl and Huisman 2008; Horst et al. 2014; Lürling et al. 2017). In freshwater lakes and ponds, cladocerans within the genus *Daphnia* can have large effects on phytoplankton abundance, transparency, and water quality (Leibold et al. 1989; Chislock et al. 2013a, 2013b; Ger et al. 2014, Ger et al. 2016; Sulcius et al. 2017). Strong trophic cascades are well documented in lakes with *Daphnia* (Leibold 1989). Furthermore, the magnitude of top-down control is thought to be affected by ecosystem productivity (Sarnelle 1992; Carpenter et al. 1995). A large body of literature has demonstrated that cyanobacteria and associated

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cyanotoxins, which are often concomitant with nutrient enrichment of lakes, can have strong negative effects on the survival and reproduction of several zooplankton species (DeMott et al. 1991; Wilson and Hay 2007). Furthermore, it is well established that predation rates on zooplankton by fishes can be greatly enhanced in eutrophic lakes, and that the success of biomanipulation in eutrophic lakes depends on the effectiveness of strategies aimed at reducing zooplanktivory (Carpenter et al. 1987; Sarnelle 1992).

Recent research has shown that populations of *Daphnia* in eutrophic lakes may evolve to tolerate toxic and grazing-resistant cyanobacteria, with tolerant *Daphnia* genotypes showing higher survival and growth rates when fed diets of toxic cyanobacteria (Hairston et al. 1999; Hairston et al. 2001; Sarnelle and Wilson 2005; Orsini et al. 2013; Frisch et al. 2017). It is also well known that many herbivores (including *Daphnia*) often trade-off the ability to exploit high resource levels with the ability to depress resources to low levels (Tessier et al. 2000; Tessier and Woodruff 2002; Jeyasingh et al. 2009). Numerous recent studies have documented substantial variation within *Daphnia* populations for traits associated with resource use, particularly with respect to nutrient enrichment and surface water management (Hairston et al. 1999; Sarnelle and Wilson 2005; Duffy 2010; Chislock et al. 2013a,b). Ecological trade-offs associated with food quantity and quality are hypothesized to cause clonal replacement in *Daphnia* populations that favor cyanobacteria-tolerant genotypes in eutrophic systems (Hairston et al. 1999; Hairston et al. 2001; Gustafsson and Hansson 2004; Sarnelle and Wilson 2005), but experiments to test this hypothesis are lacking and the time scale of clonal replacement is little documented.

In this study, we used a mesocosm (i.e., limnocorral) experiment to explore (1) the role that fertilization-mediated shifts in phytoplankton communities have on clonal selection among six *Daphnia* genotypes that vary in their tolerance to cyanobacteria and their associated toxins

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(Sarnelle and Wilson 2005; Supplementary Figure 1) and (2) the ecosystem-level consequences of changes in genotypic and trait variation of a generalist aquatic consumer. We hypothesized that fertilization-mediated increases in grazing-resistant cyanobacteria would subsequently increase the prevalence of *D. pulicaria* genotypes that are tolerant of cyanobacteria.

Methods

Daphnia genotypes

The six *Daphnia* genotypes used in this experiment were descendants of single females isolated in 2004 from each of six small lakes (<0.3 km^2) in southern Michigan (Table 1). Three of the lakes (Lawrence, Sixteen, and Warner) are oligotrophic with few cyanobacteria, whereas the other three lakes (Baker, Wintergreen, MSU Lake 1) are eutrophic and have high cyanobacterial abundance during the summer months (Sarnelle and Wilson 2005). Notably, there was much greater variation in phosphorus level among the eutrophic lakes than the oligotrophic lakes (Table 1), which is typical (Watson et al. 1997; Downing et al. 2001).

All six *Daphnia* genotypes were grown under common-garden conditions to provide animals for stocking the enclosures. Prior to the field experiment, each *Daphnia* genotype was first maintained in the laboratory (25°C, 12h light: 12h dark) in 1-L glass beakers filled with autoclaved lake water and fed a nutritious green algae (*Chlorella vulgaris*) grown in a nutrient-rich medium (modified BG-11 medium; Wilson et al. 2005). Each *Daphnia* genotype was then transferred to separate outdoor 160-L tanks filled with 35-μm sieved lake water and supplemented with *Chlorella* as a food source. Clonal populations were grown in the tanks for several weeks before the start of the experiment.

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3 131 *Study site*
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6 132 The experiment was conducted at the E.W. Shell Fisheries Research Station at Auburn
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8 133 University, Alabama, in a small, eutrophic reservoir pond (S1). S1 is a shallow, polymictic pond
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10 134 with a surface area of approximately 8 ha, maximum depth of 3.5 m, and total nitrogen (TN) and
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12 135 total phosphorus (TP) concentrations in the mixed layer averaging about 1,000 μg L^{-1} and 150 μg
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14 136 L^{-1}, respectively in the fall (Boyd and Shelton 1984; A. E. Wilson and M. F. Chislock unpubl.).
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16 137 Consequently, S1 tends to be nitrogen limited (Chislock et al. 2014). Cyanobacteria begin to
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18 138 dominate phytoplankton communities in S1 during late spring (April), and cyanobacterial
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20 139 blooms typically persist into September (A. E. Wilson and M. F. Chislock unpubl.). Immediately
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22 140 prior to the initiation of the experiment (5 October 2009), chlorophyll *a* in S1 was approximately
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24 141 30 μg L^{-1}, and cyanobacteria comprised less than 10% of total phytoplankton biomass.
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28 143 *Mesocosm experiment*
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30 144 We manipulated nutrient concentrations in 2,500-L, clear polyethylene enclosures (i.e.,
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32 145 mesocosms) that were sealed at the bottom, open to the atmosphere at the top, and suspended
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34 146 from a floating platform (EZ-Dock) anchored in the middle of the pond. Twenty-four enclosures
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36 147 were filled on 5 October 2009 by pumping pond water through a 75-μm mesh net to exclude
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38 148 resident *Daphnia*. All enclosures were enriched at 400 μg L^{-1} phosphorus added as K_2HPO_4 at
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40 149 the beginning of the experiment. Low nitrogen enclosures received no addition of nitrogen
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42 150 (ambient), while high N enclosures were also enriched with 7,000 μg L^{-1} nitrogen added as
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44 151 NH_4Cl (n=12 enclosures per high N treatment) at the beginning of the experiment. Therefore, the
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46 152 ratio of total nitrogen to total phosphorus (TN:TP) in low N:P enclosures was ~2:1, by mass, and
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48 153 high N:P enclosures had a TN:TP ratio of ~16:1, by mass.
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3 154 We stocked approximately equal densities of six genetically-distinct (based on
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6 155 microsatellite analysis: see *Daphnia genotyping* section below) *Daphnia* genotypes from
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8 156 oligotrophic and eutrophic lakes (n=3 genotypes per lake type) into half (n=12) of the enclosures
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10 157 at a total density of 0.1 L^{-1} on 17 October 2009, and the remaining twelve enclosures served as
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13 158 no-*Daphnia* controls. Thus, the experimental design was completely factorial across both N and
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15 159 *Daphnia* treatments (six replicates per treatment). During *Daphnia* addition, we preserved two
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17 160 subsamples of the *Daphnia* inoculum in 95% ethanol for genetic analysis to assess the initial
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20 161 genetic composition of *Daphnia* stocked into each treatment. We sampled all enclosures
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22 162 biweekly-to-monthly beginning on 5 October 2009, and the experiment was concluded on 10
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24 163 December 2009 (10 weeks).
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27 164
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29 165 *Sample collection*
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31 166 Depth-integrated water samples for chlorophyll *a*, phytoplankton biomass and species
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34 167 composition, and microcystin were collected from each enclosure with a tube sampler (inside
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36 168 diameter = 51 mm) on each sampling date. We collected depth-integrated samples for *Daphnia*
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38 169 density, biomass, and genotypic composition at the end of the experiment only (10 December
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41 170 2009) to minimize opportunities for contamination. Zooplankton samples were preserved in 95%
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43 171 aqueous ethanol. Chlorophyll *a* concentrations were measured by extracting phytoplankton
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45 172 collected on Pall A/E filters in 90% ethanol for 24 h in the dark at 4°C followed by measurement
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48 173 with a fluorometer (Sartory and Grobbelaar 1984). Particulate microcystin concentrations were
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50 174 quantified using enzyme-linked immunosorbent assay (ELISA) (An and Carmichael 1994) after
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52 175 extraction from 75% aqueous methanol. Phytoplankton species abundance and composition were
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55 176 determined via the inverted microscope technique (Utermöhl 1958) using water samples
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preserved in 1% Lugol's solution. Biovolumes for each species were calculated using cell counts and estimates of cell volume based on measurements of cell dimensions. We then converted biovolumes ($mm^3 L^{-1}$) to dry biomass ($\mu g L^{-1}$) assuming a specific gravity of 1 g cm^{-3} and a dry biomass: wet biomass ratio of 0.40 (Riemann et al. 1989; Sarnelle and Wilson 2005; Knoll et al. 2008).

Daphnia genotyping

We genetically characterized 20-25 randomly selected *Daphnia* individuals from ethanol-preserved macrozooplankton samples for each inoculum subsample and for all *Daphnia* enclosures at the conclusion of the experiment. Prior to genotyping, *Daphnia* were measured and counted at 40X in a Sedgwick-Rafter cell, and *Daphnia* lengths were converted to biomass using a length-weight regression (O. Sarnelle unpubl.). *Daphnia* genotypes for each of the six clones used in the experiment were genetically discriminated using variation in two microsatellite loci (Dp3, Dp339; Colbourne et al. 2004) that have proven to be highly polymorphic for *Daphnia pulicaria* collected from several of our study lakes in Michigan (A. E. Wilson unpubl.). *Daphnia* from ethanol-preserved samples were rinsed thoroughly with distilled water to remove attached bacteria and phytoplankton. Genomic DNA was extracted by heating individual *Daphnia* to 95°C in 10 µL Lyse-N-Go PCR reagent (Pierce Chemical Co., Rockford, IL). Forward primers were modified with (-29)/IRDye labeled 19-mer M13 primer sequence in order to visualize polymerase chain reaction (PCR) products on a Li-Cor 4300 DNA Analyzer (Li-Cor Biosciences, Lincoln, NE). Amplification of microsatellite alleles was performed using polymerase chain reaction (PCR) in 12.5 µL volumes (~40 ng of DNA, 1X buffer [Promega Go Green Colorless Buffer], 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 pmol M13 labeled forward microsatellite primer, 2 pmol reverse microsatellite primer, 0.5 pmol IRDye labeled M13 primer,

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200 and 0.5 units of *Taq* DNA polymerase). Each locus was separately analyzed for each *Daphnia*
201 individual. PCR used a touchdown protocol under the following conditions: 95°C for 3 min
202 followed by 10 cycles of 94°C for 35 s, 65°C (-1°C/cycle) for 35 s, 72°C for 45 s, followed by an
203 additional 30 cycles with a constant annealing temperature of 55°C with a final extension at 72°C
204 for 10 min. Reactions were stopped with 6 µL stop buffer, diluted as necessary with deionized
205 water, denatured for 3 minutes at 90°C, and snapped cold before loading on a 6.5%
206 polyacrylamide gel.

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208 *Statistical analyses*

209 We used analysis of variance (ANOVA) to compare chlorophyll *a*, cyanobacterial dry
210 biomass, relative abundance of dominant phytoplankton taxa, and microcystin concentrations
211 between low- and high-nitrogen treatments immediately prior to the addition of *Daphnia* (16
212 October 2009). The effects of fertilization (primarily nitrogen) and *Daphnia* presence on
213 chlorophyll *a* and cyanobacterial dry biomass over time were tested using repeated measures
214 ANOVA (sampling date = repeated measures), and pairwise differences among treatments were
215 assessed with Tukey's test. The effect of fertilization on *Daphnia* density and biomass at the
216 conclusion of the experiment was tested using ANOVA. Standard diversity metrics, including
217 richness, Shannon-Weaver, and evenness indices, were calculated for the *Daphnia* inoculum
218 (two subsamples) and each fertilization treatment (six replicates per treatment) at the end of the
219 experiment to determine the effect of fertilization on *Daphnia* composition via ANOVA
220 followed by Tukey's multiple comparison tests. We also compared the relative abundance of
221 *Daphnia* genotypes using grouping based on their source lakes (i.e., 'eutrophic' versus
222 'oligotrophic) using ANOVA. To compare *Daphnia* genotypic composition at the beginning and

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223 conclusion of the experiment for low- and high-nitrogen treatments, we used principle
224 components analysis (PCA). We then used ANOVA of factor score #1 to compare *Daphnia*
225 genotypic composition at the beginning and conclusion of the experiment for low- and high-
226 nitrogen treatments. Relative abundance data for *Daphnia* genotypes were logit-transformed
227 prior to all statistical analyses. In some cases, specific genotypes were absent (e.g., 0%) or only
228 one genotype was present in an enclosure (e.g., 100%). To allow for logit-transformation in these
229 cases, we added or subtracted a small value (1%) where a genotype was absent or the only
230 genotype present, respectively. All other data were checked for normality and homogeneity prior
231 to being transformed, when necessary. All non-proportional data (e.g., chlorophyll *a*,
232 microcystin, cyanobacterial biomass, *Daphnia* biomass) were log_{10}-transformed while
233 proportional data were logit-transformed. Statistical analyses were conducted using SPSS and R.
234

235 **Results**

236 Elevated nitrogen concentration had a large and significant positive effect on the
237 concentrations of chlorophyll *a*, cyanobacterial dry biomass, and the toxin, microcystin (Figure
238 1), prior to adding *Daphnia* (i.e., the first eleven days of the experiment) and over the duration of
239 the experiment in the no-*Daphnia* control enclosures. Immediately prior to the stocking of
240 *Daphnia* genotypes (day 11: 16 Oct. 2009), mean chlorophyll *a* and cyanobacterial biomass for
241 high-nitrogen enclosures were nearly five- and eight-times higher, respectively, than for low
242 nitrogen enclosures (Figure 1A, chlorophyll *a*: $F_{1,22} = 19201.816$, $P < 0.0001$; Figure 1B,
243 cyanobacterial biomass: $F_{1,22} = 62.285$, $P < 0.0001$). Furthermore, mean concentrations of
244 microcystin were over three-times higher under high nitrogen than low nitrogen on day 11
245 (Figure 1C, $F_{1,22} = 8.002$, $P = 0.010$). By the fourth week of the experiment (~two weeks after

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3 246 *Daphnia* addition: 3 Nov. 2009), *Daphnia* had suppressed cyanobacterial biomass by ~96%
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6 247 relative to no-*Daphnia* controls at high nitrogen (Tukey's test: $P = 0.009$) but had no effect on
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8 248 chlorophyll *a* (Figure 1A, Tukey's test: $P = 1.000$). At low nitrogen, *Daphnia* had no effect on
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10 249 either chlorophyll *a* (Figure 1A, Tukey's test: $P = 0.972$) or cyanobacterial biomass (Figure 1B,
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12 250 Tukey's test: $P = 1.000$) by day 29 (3 November 2009). Repeated measures ANOVA over the
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14 251 10-week experiment revealed significant effects of nitrogen addition ($F_{1,20} = 519.876$, $P <$
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16 252 0.0001), *Daphnia* presence ($F_{1,20} = 9.931$, $P = 0.005$), and the interaction of nitrogen and
17
18 253 *Daphnia* presence ($F_{1,20} = 6.221$, $P = 0.022$) on chlorophyll *a*. Furthermore, nitrogen ($F_{1,20} =$
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20 254 31.837, $P < 0.0001$), *Daphnia* presence ($F_{1,20} = 8.635$, $P = 0.008$), and the interaction of nitrogen
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22 255 addition and *Daphnia* presence ($F_{1,20} = 8.241$, $P = 0.0009$) all had significant effects on
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24 256 cyanobacterial biomass over time. At the conclusion of the experiment (day 66), *Daphnia* had
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26 257 reduced chlorophyll *a* by approximately 46%, relative to the no-*Daphnia* control at high nitrogen
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28 258 (Tukey's test: $P = 0.005$). In contrast, *Daphnia* had no effect on chlorophyll *a* at low nitrogen
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30 259 (Tukey's test: $P = 0.963$).
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32 260 Although *Daphnia* biomass was similar for low- and high-nitrogen enclosures ($P = 0.78$) at
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34 261 the conclusion of the experiment, *Daphnia* genetic diversity was significantly different between
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36 262 nitrogen treatments (Figure 2; Table 1). The relative abundance of eutrophic-lake *Daphnia* was
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38 263 nearly 100% for high-nitrogen enclosures where cyanobacteria dominated, while *Daphnia*
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40 264 genotypes from oligotrophic and eutrophic lakes were equally represented at low nitrogen
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42 265 (Figure 2). In fact, relative abundances of the six *Daphnia* genotypes were extremely similar (all
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44 266 Tukey pairwise comparisons $P \geq 0.83$) at the start (richness = 5.5, Shannon-Weiner index = 1.48,
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46 267 evenness = 0.87; Table 2) and end (richness = 5.83, Shannon-Weiner index = 1.55, evenness =
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48 268 0.88) of the experiment in the low nitrogen treatment (Figure 2) where phytoplankton abundance
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or species composition changed little over time (Figure 1). In contrast, the high-nitrogen treatment differed significantly from the inoculum (i.e., initial) and low nitrogen treatment (all Tukey pairwise comparisons $P \leq 0.001$; Table 1). For example, in the high-nitrogen treatment, three genotypes were undetectable or near the detection limits, and a single genotype (MSU Lake 1) dominated at the end of the experiment (Table 2; richness = 2, Shannon-Weiner index = 0.17, evenness = 0.25). PCA of relative abundances of *Daphnia* genotypes revealed that 87% of the total variance could be explained by a single factor (factor 1) that could be attributed to the relative abundance of the MSU Lake 1 genotype. ANOVA using PCA factor 1 scores indicated that the relative abundance (based on PCA factor 1 scores) of the MSU Lake 1 genotype at the end of the experiment was significantly higher at high nitrogen versus low nitrogen ($P < 0.0001$; Tukey's test: $P < 0.0001$) or when compared to the beginning of the experiment ($P < 0.0001$). In contrast, the relative abundance (based on PCA factor 1 scores) of the MSU Lake 1 genotype was similar at the beginning versus end of the experiment for low nitrogen enclosures (Figure 2; $P = 0.216$). Similar analyses using only the MSU Lake 1 genotype logit-transformed relative abundance data showed the same patterns (initial and low nitrogen treatment were statistically similar (Tukey's test: $P = 0.362$); however, both relative abundances of initial and for the low nitrogen treatment were different from the high-nitrogen treatment at the end of the experiment (both Tukey's tests: $P < 0.001$)).

Discussion

Nutrient enrichment of freshwater ecosystems frequently leads to increased phytoplankton biomass, especially cyanobacteria (Paerl and Huisman 2008). As grazing-resistant cyanobacteria can inhibit *Daphnia* feeding, growth, survival, and reproduction (Gliwicz and Lampert 1990;

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DeMott et al. 1991; Lurling and van Der Grinten 2003; Wilson et al. 2006, Tillmanns et al. 2008), eutrophication could influence the genotypic composition of *Daphnia* populations in which tolerance to cyanobacteria varies (Hairston et al. 1999; Sarnelle and Wilson 2005). We created environments with high and low concentrations of cyanobacteria via two fertilization regimes and exposed a mixture of *Daphnia* genotypes to these two environments. The *Daphnia* genotype from the most eutrophic lake (MSU Lake 1) dominated the *Daphnia* population at high nitrogen, accounting for 95% of the *Daphnia* population at the conclusion of the experiment. Despite the increased abundance of cyanobacteria at high nitrogen by week four, *Daphnia* addition resulted in a large reduction in cyanobacterial biomass after only two weeks and the effect was maintained until the end of the experiment. In contrast, *Daphnia* had no effect on cyanobacterial biomass at low nitrogen. Limited sampling precluded our ability to better assess *Daphnia* grazing effects and potential grazer selectivity over time. However, initial grazing by *Daphnia* on cyanobacteria may have indirectly facilitated small, rapidly growing chlorophytes (e.g., *Scenedesmus* species) by reducing competition with cyanobacteria (e.g., increased light availability) in the high-nutrient treatment.

A single *Daphnia* genotype (MSU Lake 1) dominated the *Daphnia* population in the high-nitrogen treatment, which was characterized by higher phytoplankton biomass, cyanobacterial abundance, and concentrations of the cyanotoxin, microcystin. The MSU Lake 1 genotype was collected from a eutrophic lake and identified to be tolerant to toxic *Microcystis* in a laboratory experiment. Interestingly, the other two genotypes from eutrophic lakes nearly disappeared in these fertilized enclosures. These data suggest that being from a eutrophic lake may not fully explain tolerance to cyanobacteria or their toxins or alternatively that tolerance is a continuous, rather than dichotomous, phenomenon (Sarnelle and Wilson 2005). In the case of this study, it

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appears that clonal replacement may have been driven by the rapid growth rate of the MSU Lake 1 genotype on nutritious green algae (i.e., *Ankistrodesmus*), in addition to surviving on the toxic diet (i.e., *Microcystis*). While we estimated tolerance by comparing somatic growth rates of *Daphnia* genotypes on toxic versus nutritious phytoplankton, shifts in *Daphnia* populations will also be affected by differences among genotypes in genotype-specific birth and death rates. Alternatively, different *Daphnia* genotypes from eutrophic lakes may also differ in diel vertical migration behavior, allowing access to additional food sources (e.g., bacteria) and affecting vulnerability to zooplanktivorous fishes. All three genotypes from oligotrophic lakes that were identified to be sensitive to toxic *Microcystis* were quickly driven to (Warner) or near (Lawrence and Sixteen) extinction in the high-nitrogen environment. These patterns follow observed phenotypic bottlenecks for a *Daphnia* population in Lake Constance as it underwent eutrophication over a decade (Hairston et al. 1999). In our study, near exclusion of half of the stocked genotypes occurred in just two months.

In the low nitrogen treatment, we found no significant changes in genotypic composition in the *Daphnia* populations suggesting that the mesocosm environment (that lacked planktivorous fish) was suitable habitat for all six *Daphnia* genotypes. Moreover, considering that the low nitrogen treatments maintained chlorophyll *a* at elevated levels (>20 $\mu g\ L^{-1}$), competition for resources may have been low, thus minimizing the potential for one or a few genotypes to out-compete the others. The low abundance of cyanobacteria and microcystin in the low nitrogen treatment likely also limited the potential for eutrophic lake *Daphnia* (especially MSU Lake 1) to dominate; however, as we only sampled *Daphnia* populations at the end of the experiment to minimize contamination of *Daphnia* genotypes, the dynamics of relative abundances for *Daphnia* genotypes over time are unknown.

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338 Ecological genetics is a rapidly growing field that merges aspects of population genetics and
339 community ecology to understand the development and consequences of intraspecific variation
340 for communities and ecosystems (Whitham et al. 2006; Wade 2007). In freshwater ecosystems,
341 rapid adaptive evolution by major consumers may play an important role in the response of lake
342 ecosystems to cultural eutrophication and food web manipulations (Hairston et al. 2001; Sarnelle
343 and Wilson 2005; Jiang et al. 2013; Orsini et al. 2013; Lyu et al. 2015; Frisch et al. 2017).
344 Hairston et al. (1999) made the seminal observation that a generalist herbivore in freshwater
345 lakes (*Daphnia*) can evolve to tolerate toxic cyanobacteria in the diet in response to nutrient
346 enrichment. *Daphnia* tolerance to toxic cyanobacteria has now been observed for a *D. galeata*
347 population following eutrophication of Lake Constance in Europe (Hairston et al. 2001) and for
348 several *D. pulicaria* populations in North America (Sarnelle and Wilson 2005; Frisch et al.
349 2017). Despite several recent studies documenting tolerance of zooplankton to toxic
350 cyanobacteria, understanding the specific mechanisms allowing for grazer tolerance has
351 remained elusive (but see Macke et al. 2017). In this study, we highlight a significant,
352 unexplored threat of eutrophication, namely that it mediated, through the promotion of toxic
353 cyanobacteria, the significant loss of *Daphnia* genetic diversity within a single growing season.

354 Understanding the causes and consequences of intraspecific variation within *Daphnia* may aid
355 in the future management of eutrophic lakes (Lyu et al. 2016; Ger et al. 2016; Lyu et al. 2017).
356 Given the considerable variation in ecologically important traits observed for *Daphnia*
357 populations (Tessier et al. 2000; Duffy et al. 2010), an emphasis on intraspecific trait variation
358 provides an interesting conceptual framework for linking diversity to ecosystem function, and
359 the results of our study and several previous studies suggest that this approach may be profitable,
360 particularly for plankton food webs (Hairston et al. 1999; Tessier et al. 2000; Sarnelle and

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361 Wilson 2005; Post et al. 2008; Duffy et al. 2010; Orsini et al. 2013).
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Conflicts of interest

The authors declare no conflicts of interests.

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3 375 **References**
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5
6 376 Akbar, S., J. J. Du, H. Lin, X.S. Kong, S.C. Sun, and X.J. Tian. 2017. Understanding interactive
7
8 377 inducible defenses of *Daphnia* and its phytoplankton prey. Harmful Algae **66**:47-56.
9
10 378 Alexander, T. J., P. Vonlanthen, and O. Seehausen. 2016. Does eutrophication-driven evolution
11
12 379 change aquatic systems? Philosophical Transactions of the Royal Society B 372: 20160041.
13
14 380 An, J. S., and W. W. Carmichael. 1994. Use of a colorimetric protein phosphatase inhibition
15
16 381 assay and enzyme-linked-immunosorbent-assay for the study of microcystins and nodularins.
17
18 382 Toxicon **32**: 1495-1507.
19
20 383 Bassar, R. D., M. C. Marshall, A. Lopez-Sepulcre, E. Zandona, S. K. Auer, J. Travis, C. M.
21
22 384 Pringle, A. S. Flecker, S. A. Thomas, D. F. Fraser, and D. N. Reznick. 2010. Local adaptation
23
24 385 in Trinidadian guppies alters ecosystem processes. Proceedings of the National Academy of
25
26 386 Sciences of the United States of America 107:3616-3621.
27
28 387 Boyd, C. E., and J. L. Shelton. 1984. Observations on the hydrology and morphometry of ponds
29
30 388 on the Auburn University Fisheries Research Unit. Alabama Agricultural Experiment Station
31
32 389 Bulletin**:** 1-64.
33
34 390 Carpenter, S. R., J. F. Kitchell, J. R. Hodgson, P. A. Cochran, J. J. Elser, M. M. Elser, D. M.
35
36 391 Lodge, D. Kretchmer, X. He, and C. N. von Ende. 1987. Regulation of lake primary
37
38 392 productivity by food web structure. Ecology **68**:1863-1876.
39
40 393 Carpenter, S. R., D. L. Christensen, J. J. Cole, K. L. Cottingham, X. He, J. R. Hodgson, J. F.
41
42 394 Kitchell, S. E. Knight, and M. L. Pace. 1995. Biological control of eutrophication in lakes.
43
44 395 Environmental Science and Technology **29**:784-786.
45
46 396 Chislock, M.F., K.L. Sharp, and A.E. Wilson. 2014. *Cylindrospermopsis raciborskii* dominates
47
48 397 under very low and high nitrogen-to-phosphorus ratios. Water Research **49**: 207-214.
49
50
51
52
53
54
55
56
57
58
59
60

Copy for Review

398 Chislock, M.F., O. Sarnelle, B.K. Olsen, E. Doster, and A.E. Wilson. 2013a. Large effects of
399 consumer offense on ecosystem structure and function. Ecology **94**: 2375-2380.

400 Chislock, M.F., O. Sarnelle, L.M. Jernigan, and A.E. Wilson. 2013b. Do high concentrations of
401 microcystin prevent *Daphnia* control of phytoplankton? Water Research **47**:1961-1970.

402 Colbourne, J. K., B. Robison, K. Bogart, and M. Lynch. 2004. Five hundred and twenty-eight
403 microsatellite markers for ecological genomic investigations using *Daphnia*. Molecular
404 Ecology Notes **4**: 485-490.

405 Darwin, C. 1859. On the origin of species by means of natural selection. John Murray, London.

406 DeMott, W. R., Q.- X. Zhang, and W. W. Carmichael. 1991. Effects of toxic cyanobacteria and
407 purified toxins on the survival and feeding of a copepod and three species of *Daphnia*.
408 Limnology and Oceanography **36**: 1346-1357.

409 Des Roches, S., D.M. Post, N.E. Turley, J.K. Bailey, A.P. Hendry, M.T. Kinnison, J.A.
410 Schweitzer, and E.P. Palkovacs. 2017. The ecological importance of intraspecific variation.
411 Nature Ecology & Evolution DOI:10.1038/s41559-017-0402-5

412 Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in
413 lakes. Canadian Journal of Fisheries and Aquatic Sciences **58**: 1905-1908.

414 Duffy, M. A. 2010. Ecological consequences of intraspecific variation in lake *Daphnia*.
415 Freshwater Biology **55**: 995-1004.

416 Frisch, D., P. K. Morton, B. W. Culver, M. B. Edlund, P. D. Jeyasingh, and L. J. Weider. 2017.
417 Paleogenetic records of *Daphnia pulicaria* in two North American lakes reveal the impact of
418 cultural eutrophication. Global Change Biology 23:708-718.

419 Ger, K.A., L.A. Hansson, and M. Lurling. 2014. Understanding cyanobacteria-zooplankton
420 interactions in a more eutrophic world. Freshwater Biology **59**:1783-1798.

Copy for Review

421 Ger, K.A., P. Urrutia-Cordero, P.C. Frost, L.A. Hansson, O. Sarnelle, A.E. Wilson, and M.
422 Lürling. 2016. The interaction between cyanobacteria and zooplankton in a more eutrophic
423 world. Harmful Algae **54**:128-144.
424 Ger, K. A., R. Panosso, and M. Lurling. 2011. Consequences of acclimation to *Microcystis* on
425 the selective feeding behavior of the calanoid copepod *Eudiaptomus gracilis*. Limnology and
426 Oceanography **56**: 2103-2114.
427 Gliwicz, Z. M., and W. Lampert. 1990. Food thresholds in *Daphnia* species in the absence and
428 presence of blue-green filaments. Ecology **71**: 691-702.
429 Gustafsson, S., and L.-A. Hansson. 2004. Development of tolerance against toxic cyanobacteria
430 in *Daphnia*. Aquatic Ecology **38**: 37-44.
431 Hairston, N. G., W. Lampert, C. E. Caceres, C. L. Holtmeier, L. J. Weider, U. Gaedke, *et al.*
432 1999. Lake ecosystems - Rapid evolution revealed by dormant eggs. Nature **401**: 446-446.
433 Hairston, N. G., C. L. Holtmeier, W. Lampert, L. J. Weider, D. M. Post, J. M. Fischer, *et al.*
434 2001. Natural selection for grazer resistance to toxic cyanobacteria: Evolution of phenotypic
435 plasticity? Evolution **55**: 2203-2214.
436 Horst, G.P., O. Sarnelle, J.D. White, S.K. Hamilton, R.B. Kaul, and J.D. Bressie. 2014. Nitrogen
437 availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*.
438 Water Research **54**:188-198.
439 Hughes, A. R. and J. J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass
440 ecosystem to disturbance. Proceedings of the National Academy of Sciences **101**: 8998-9002.
441 Hughes, A. R., B. D. Inouye, M. T. J. Johnson, N. Underwood, and M. Vellend. 2008. Ecological
442 consequences of genetic diversity. Ecology Letters **11**: 609-623.

Copy for Review

443 Jeyasingh, P. D., L. J. Weider, and R. W. Sterner. 2009. Genetically-based trade-offs in response
444 to stoichiometric food quality influence competition in a keystone aquatic herbivore. Ecology
445 Letters 12:1229-1237.
446 Jiang, X.D., L.H. Zhang, H.S. Liang, Q.M. Li, Y.L. Zhao, L.Q. Chen, W. Yang, and S.Y. Zhao.
447 2013. Resistance variation within a *Daphnia pulex* population against toxic cyanobacteria.
448 Journal of Plankton Research **35**:1177-1181.
449 Knoll, L. B. S., O.; Hamilton, S. K.; Kissman, C. E. H., Wilson, A. E., Rose, J. B., and Morgan,
450 M. R. 2008. Corrigendum: Invasive zebra mussels (*Dreissena polymorpha*) increase
451 cyanobacterial toxin concentrations in low-nutrient lakes. Canadian Journal of Fisheries and
452 Aquatic Sciences **65**: 2552-2552.
453 Leibold, M. A. 1989. Resource edibility and the effects of predators and productivity on the
454 outcome of trophic interactions. American Naturalist **134**: 922-949.
455 Lürling, M., and E. van Der Grinten. 2003. Life-history characteristics of *Daphnia* exposed to
456 dissolved microcystin-LR and to the cyanobacterium *Microcystis aeruginosa* with and
457 without microcystins. Environmental Toxicology and Chemistry **22**: 1281-1287.
458 Lürling, M., F. Van Oosterhout, and E. Faassen. 2017. Eutrophication and warming boost
459 cyanobacterial biomass and microcystins. Toxins **9**.
460 Lyu, K., H.Y. Guan, C.C. Wu, X.Y. Wang, A.E. Wilson, and Z. Yang. 2016. Maternal
461 consumption of non-toxic *Microcystis* by *Daphnia magna* induces tolerance to toxic
462 *Microcystis* in offspring. Freshwater Biology **61**:219-228.
463 Lyu, K., L. Zhang, L. Gu, X.X. Zhu, A.E. Wilson, and Z. Yang. 2017. Cladoceran offspring
464 tolerance to toxic *Microcystis* is promoted by maternal warming. Environmental Pollution
465 **227**:451-459.

Copy for Review

466 Lyu, K., L. Zhang, X.X. Zhu, G.L. Cui, A.E. Wilson, and Z. Yang. 2015. Arginine kinase in the
467 cladoceran *Daphnia magna*: cDNA sequencing and expression is associated with resistance
468 to toxic *Microcystis*. Aquatic Toxicology **160**:13-21.

469 Macke, E., M. Callens, L. De Meester, and E. Decaestecker. 2017. Host-genotype dependent gut
470 microbiota drives zooplankton tolerance to toxic cyanobacteria. Nature Communications **8**:
471 1608.

472 Miner, B. E., L. De Meester, M. E. Pfrender, W. Lampert, N. G. Hairston. 2012. Linking genes
473 to communities and ecosystems: *Daphnia* as an ecogenomic model. Proceedings of the Royal
474 Society of London B **279**: 1873-1882.

475 Orsini, L., K. Schwenk, L. De Meester, J. K. Colbourne, M. E. Pfrender, and L. J. Weider. 2013.
476 The evolutionary time machine: using dormant propagules to forecast how populations can
477 adapt to changing environments. Trends in Ecology & Evolution 28:274-282.

478 Paerl, H. W., and J. Huisman. 2008. Climate - Blooms like it hot. Science **320**:57-58.

479 Palkovacs, E. P., and D. M. Post. 2009. Experimental evidence that phenotypic divergence in
480 predators drives community divergence in prey. Ecology 90:300-305.

481 Post, D. M., E. P. Palkovacs, E. G. Schielke, and S. I. Dodson. 2008. Intraspecific variation in a
482 predator affects community structure and cascading trophic interactions. Ecology **89**: 2019-
483 2032.

484 Riemann, B., P. Simonsen, and L. Stensgaard. 1989. The carbon and chlorophyll content of
485 phytoplankton from various nutrient regimes. Journal of Plankton Research **11**: 1037-1045.

486 Rudman, S. M., M. Kreitzman, K. M. A. Chan, and D. Schluter. 2017. Evosystem services: rapid
487 evolution and the provision of ecosystem services. Trends in Ecology & Evolution **52**: 403-
488 415.

Copy for Review

489 Sarnelle, O. 1992. Nutrient enrichment and grazer effects on phytoplankton in lakes. Ecology
490 **73**:551-560.
491 Sarnelle, O., and A. E. Wilson. 2005. Local adaptation of *Daphnia pulicaria* to toxic
492 cyanobacteria. Limnology and Oceanography **50**: 1565-1570.
493 Sartory, D. P., and J. U. Grobbelaar. 1984. Extraction of chlorophyll-a from fresh-water
494 phytoplankton for spectrophotometric analysis. Hydrobiologia **114**: 177-187.
495 Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green-algae in
496 lake phytoplankton. Science **221**: 669-671.
497 Sulcius, S., D. Montvydiene, H. Mazur-Marzec, J. Kasperoviciene, R. Rulevicius, and Z.
498 Cibulskaite. 2017. The profound effect of harmful cyanobacterial blooms: From food-web
499 and management perspectives. Science of the Total Environment **609**:1443-1450.
500 Tessier, A. J., and P. Woodruff. 2002. Trading off the ability to exploit rich versus poor food
501 quality. Ecology Letters **5**: 685-692.
502 Tessier, A. J., M. A. Leibold, and J. Tsao. 2000. A fundamental trade-off in resource exploitation
503 by *Daphnia* and consequences to plankton communities. Ecology **81**: 826-841.
504 Thompson, J. N., O. J. Reichman, P. J. Morin, G. A. Polis, M. E. Power, R. W. Sterner, *et al.*
505 2001. Frontiers of ecology. BioScience **51**: 15-24.
506 Tillmanns, A. R., A. E. Wilson, F. R. Pick, and O. Sarnelle. 2008. Meta-analysis of
507 cyanobacterial effects on zooplankton population growth rate: species-specific responses.
508 Fundamental and Applied Limnology **171**: 285-295.
509 Tollrian, R., and C. D. Harvell. 1999. The ecology and evolution of inducible defenses. Princeton
510 Univ. Press.
511 Turner, A. M., and G. G. Mittelbach. 1990. Predator avoidance and community structure:

Copy for Review

interactions among piscivores, planktivores, and plankton. Ecology **71**: 2241-2254.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Mitt. int. Ver. theor. angew. Limnol. **9**: 1-38.

Wade, M. J. 2007. The co-evolutionary genetics of ecological communities. Nature Reviews Genetics **8**: 185-195.

Watson, S. B., E. McCauley, and J. A. Downing. 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. Limnology and Oceanography **42**: 487-495.

Werner, E. E., and S. D. Peacor. 2003. A review of trait-mediated indirect interactions in ecological communities. Ecology **84**: 1083-1100.

Whitham, T. G., J. K. Bailey, J. A. Schweitzer, S. M. Shuster, R. K. Bangert, C. J. Leroy, *et al.* 2006. A framework for community and ecosystem genetics: from genes to ecosystems. Nature Reviews Genetics **7**: 510-523.

Wilson, A. E., O. Sarnelle, and A. R. Tillmanns. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. Limnology and Oceanography **51**: 1915-1924.

Wilson, A.E., O. Sarnelle, B.A. Neilan, T.P. Salmon, M.M. Gehringer, and M.E. Hay. 2005. Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa*: implications for harmful algal blooms. Applied Environmental Microbiology **71**: 6126-6133.

Wilson, A. E., and M. E. Hay. 2007. A direct test of cyanobacterial chemical defense: variable effects of microcystin-treated food on two *Daphnia pulicaria* clones. Limnology and Oceanography **52**:1467-1479.

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Tables

Table 1. Source lakes and genetic characterizations of the six *Daphnia* genotypes. Three *Daphnia* genotypes were collected from oligotrophic lakes where cyanobacteria were absent (Lawrence, Sixteen, and Warner), and three genotypes were collected from eutrophic lakes with abundant cyanobacteria (Baker, MSU Lake 1, and Wintergreen). Each *Daphnia* genotype was characterized using two microsatellite markers (Dp3 and Dp339; Colbourne et al. 2004), and microsatellite nucleotide lengths were determined for both loci.

Lake	Location Lat. N, Long. W	TP (µg l^{-1})	Chl (µg l^{-1})	Dp3 length (bp)	Dp339 length (bp)
Lawrence	42°26’27”, 85°21’03”	8-10	4	282, 291	175, 180
Sixteen	42°33’90”, 85°36’80”	9-12	5	282, 286	175, 180
Warner	42°28’16”, 85°31’30”	12-14	3	282, 282	175, 180
Baker	42°26’27”, 85°21’03”	21-40	9-74	291, 291	175, 180
Wintergreen	42°23’50”, 85°23’07”	50-70	13-25	282, 282	175, 175
MSU Lake 1	42°40’53”, 84°28’57”	170-300	60-250	282, 286	175, 190

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Table 2. Diversity estimates of *Daphnia* populations at the start (inoculum; 2 subsample replicates) and end the mesocosm experiment for the two treatments (Low N and High N; 6 replicates each). Twenty to twenty-five individuals were sampled from each inoculum subsample and for all enclosures at the end. Note that two High N enclosures only had the MSU Lake 1 *D. pulicaria* genotype so evenness could not be calculated for these enclosures. Means (one standard error). Letters denote statistically similar treatment means ($\alpha < 0.05$; ANOVA followed by Tukey's multiple comparison tests).

Diversity metric	Inoculum	Low N end	High N end
Genotypic richness	5.50 (0.5)[a]	5.83 (0.17)[a]	2.00 (0.37)[b]
Shannon-Weiner index	1.48 (0.16)[a]	1.55 (0.04)[a]	0.17 (0.10)[b]
Evenness	0.87 (0.05)[a]	0.88 (0.02)[a]	0.25 (0.11)[b]

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Figure captions

Figure 1. Dynamics of (A) chlorophyll *a* (μg L^{-1}), (B) cyanobacterial dry biomass (μg L^{-1}), and microcystin (ng L^{-1}) over the ten-week enclosure experiment across four treatments, including no *Daphnia* under high nitrogen (black square), no *Daphnia* under low nitrogen (white square), *Daphnia* under high nitrogen (black circle), and *Daphnia* under low nitrogen (white circle). The experiment was initiated on 5 October 2009 ('day 0'), *Daphnia* were added on 17 October 2009 (day 12) after sampling, and the experimented ended on 10 December 2009 (day 80). Data represent means ± one standard error for each treatment. Note: the figure legend for all three panels is provided in (C).

Figure 2. Relative abundance of each of the six *Daphnia* genotypes at stocking ('Initial' = 16 October 2009) and the conclusion of the experiment (10 December 2009). Three gray bars represent *Daphnia* from oligotrophic sites (Lawrence, Sixteen, Warner) and three white bars represent *Daphnia* from eutrophic sites (Baker, MSU Lake 1, Wintergreen). Data represent means ± one standard error for each nitrogen treatment.

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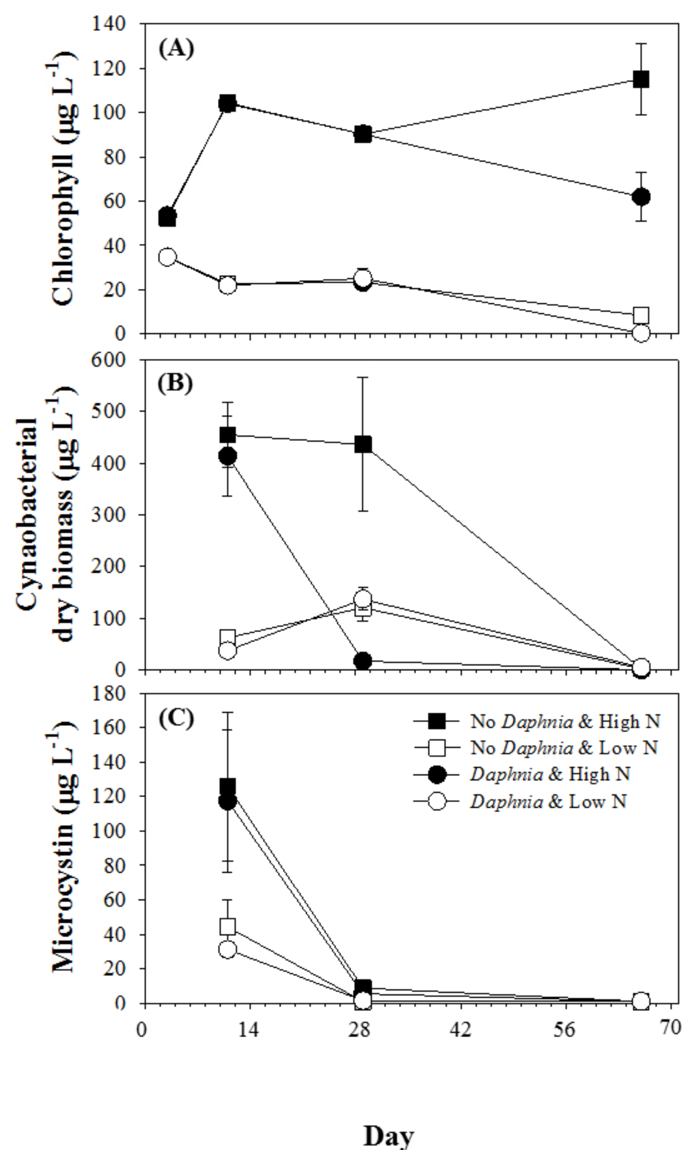

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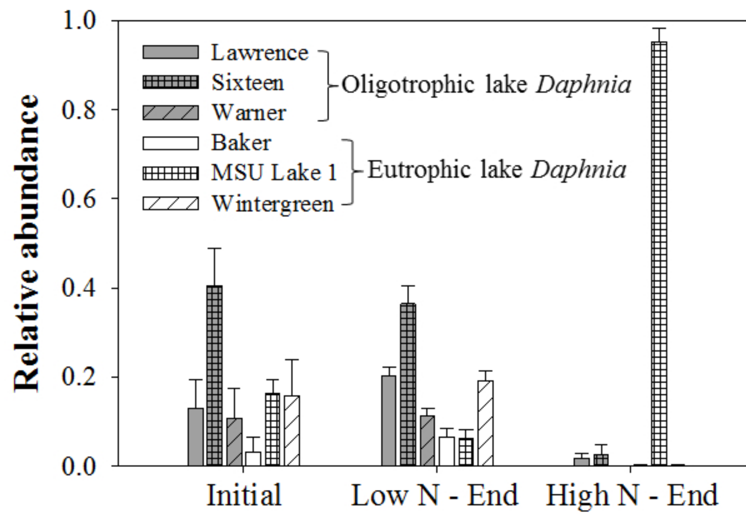

Figure 2

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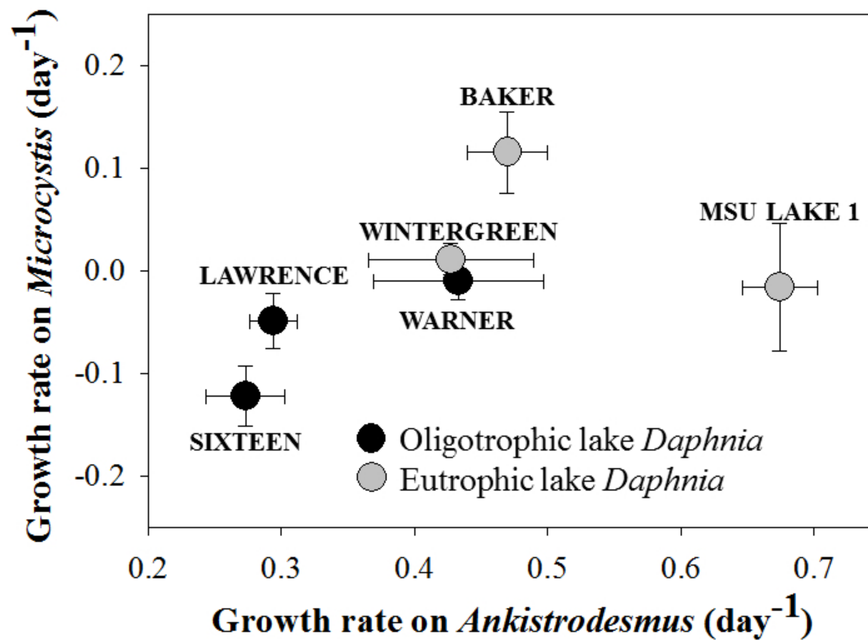

Supplementary Figure 1. Mean juvenile somatic growth rates for the six *Daphnia* genotypes on two diets: a nutritious green alga (100% *Ankistrodesmus*) and a toxic cyanobacterium (100% *Microcystis aeruginosa*). Data represent means ± one standard error for each genotype (Sarnelle and Wilson 2005).

Supplementary Figure 1

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