

Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses

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With 4 figures and 2 tables

Abstract: We analyzed a large data set of laboratory experiments to examine the effects of cyanobacteria containing or lacking intracellular toxic metabolites and of different morphology on zooplankton population growth rates across multiple genera and species of cladocerans, rotifers and cyanobacteria. Twenty-one of the 29 zooplankton species maintained positive growth rates when fed a diet containing cyanobacteria even though cyanobacteria were a poor food source for half of the zooplankton species tested relative to a diet containing only green algae and/or flagellates. Differences among zooplankton species could not be explained by grazer species body lengths, even when experiments were restricted to those that used only filamentous cyanobacteria. Single-celled cyanobacteria were more detrimental to a larger number of zooplankton species compared to filamentous or chroococcoid colonial cyanobacteria. We also found no clear effect of putative cyanobacterial toxins on the growth of seven zooplankton species but we did detect a negative effect for the largest cladoceran species, *Daphnia magna*. Among the cyanobacterial genera, *Microcystis* had the largest negative effect on zooplankton population growth and there was no consistent difference between *M. aeruginosa* strains that produced microcystins and those that did not. Our results highlight the large variation in species-specific responses of zooplankton to cyanobacteria. Although cyanobacterial toxicity and mechanical interference may be important drivers in particular cyanobacteria-zooplankton interactions, we did not find general support for these mechanisms through the use of this meta-analysis.

Key words: growth rates, cyanobacterial diet, filamentous cyanobacteria, single-celled cyanobacteria, chroococcoid colonial cyanobacteria, cyanobacterial toxicity, cyanobacteria-zooplankton interactions.

Introduction

Cyanobacterial blooms in freshwater ecosystems are well-studied because some cyanobacteria are able to produce toxic secondary metabolites that contaminate water for use by humans and animals (Codd 2000). Multiple hypotheses have been proposed to explain the ability of cyanobacteria to dominate an ecosystem (e.g. Hyenstrand et al. 1998). One central hypothesis is that cyanobacteria are resistant to grazing by zooplankton (Porter & Orcutt 1980, Lampert 1987,

De Bernardi & Giussani 1990). Field observations of the absence of large-bodied cladocerans during cyanobacterial blooms (Edmondson & Litt 1982, Infante & Abella 1985) have provided support for this argument and have led to numerous laboratory studies investigating the mechanisms mediating interactions between herbivorous zooplankton and cyanobacteria. Almost three decades ago, Porter & Orcutt (1980) proposed three hypotheses to explain why cyanobacteria are inadequate food for zooplankton. First, metabolites produced by cyanobacteria may be toxic to zooplankton

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(toxicity hypothesis). Second, some cyanobacterial genera grow as chroococcoid or filamentous colonies which may interfere with grazer feeding appendages or be inedible to grazers due to their large size (morphology hypothesis). Finally, cyanobacteria may be deficient in macromolecules vital for zooplankton growth and reproduction (nutrition hypothesis).

A large number of laboratory and field studies have been conducted to directly or indirectly test the importance of these hypotheses, but conflicting results, compounded by different experimental conditions across studies, have made qualitative generalizations difficult (Lampert 1987). In situations such as these, quantitative comparison of published results using meta-analysis can uncover broad patterns across many disparate studies (Gurevitch & Hedges 1993, Osenberg et al. 1999). In a previous meta-analysis of laboratory studies we found, as expected, that cladocerans and rotifers grew significantly slower in general, when fed a diet containing cyanobacteria compared to a diet containing only chlorophytes and/or small flagellates (Wilson et al. 2006). In addition, zooplankton performed relatively better on diets containing filamentous cyanobacteria than diets containing single-celled or chroococcoid colonial cyanobacteria. Interestingly, presence/absence of putative cyanobacterial toxins, such as microcystin and anatoxin-*a*, had no clear effect on population growth rates across all studies. This previous meta-analysis was limited to comparisons across broad taxonomic groupings (e.g., cladocerans versus rotifers). In this paper, we examine patterns across genera and species of both zooplankton and cyanobacteria.

It is difficult to identify a predictor variable that can explain differences in zooplankton species responses to cyanobacteria because of conflicting results across studies, but one such variable with experimental support is the body length of cladocerans (Gliwicz & Lampert 1990). Past studies have shown that filamentous cyanobacteria can interfere with the feeding process, becoming tangled in the appendages of cladocerans (Webster & Peters 1978, Porter 1973, Hawkins & Lampert 1989) and that this negative effect increases with body size (Gliwicz & Siedlar 1980). Wilson et al. (2006) found a negative relationship between zooplankton body length and the magnitude of population growth inhibition for grazers fed filamentous cyanobacteria, providing general support for this hypothesis. Physiological tolerance to ingested cyanobacteria may also explain differences in zooplankton responses to cyanobacteria (DeMott et al. 1991). For example, *Ceriodaphnia* has been shown to be minimally affected

by a diet of cyanobacteria (Lampert 1982, Nandini 2000) and to have a higher tolerance to *Microcystis aeruginosa* compared to *Daphnia carinata* (Guo & Xie 2006). Tolerance towards cyanotoxins may be a function of increased production of the enzyme(s) affected by the toxin or of the enzyme(s) that detoxify the toxin present in zooplankton (DeMott & Dhawale 1995).

In this study, we examine the effects of cyanobacterial toxicity and morphology as they pertain to species-specific variation in cyanobacterial effects on zooplankton. The response of zooplankton population growth rates to treatment diets with varying proportions of cyanobacteria versus control diets containing only chlorophytes and/or flagellates was used as a measure of effect size. Experimental diets containing cyanobacteria producing known toxins (e. g., microcystin, anatoxin, etc.) were expected to decrease grazer population growth rates compared with cyanobacteria that tested negative for known toxins (Wilson & Hay 2007). Filamentous and chroococcoid colonial cyanobacteria were expected to have a greater inhibitory effect on population growth rates than single-celled cyanobacteria, and this inhibitory effect was expected to be greater on larger zooplankton species relative to smaller species. The effect of nutritional deficiency could not be separated from inhibitory compounds produced by cyanobacteria in this meta-analysis and so could not be directly tested. However, indirectly we expected a negative relationship between grazer population growth rates and percentage of cyanobacteria in treatment diets (Wilson et al. 2006). Examination of these meta-data allowed us to assess the generality of previous findings (Wilson et al. 2006) for specific zooplankton and cyanobacterial taxa.

Methods

The methodology for data assemblage and analysis was described in detail previously (Wilson et al. 2006) and is only briefly described here. Data were collected by searching the Web of Science (from 1945 to 2007) and Aquatic Sciences and Fisheries Abstract (from 1971 to 2007) databases and the references cited therein, for studies that examined the influence of cyanobacteria on zooplankton population growth rates under laboratory conditions. Selected studies consisted of paired experiments with test populations fed a control diet of nutritious food such as chlorophytes and/or small flagellates, and a treatment diet consisting of either 100% cyanobacteria or cyanobacteria together with chlorophytes and/or small flagellates (1–99% cyanobacteria, by carbon content). Zooplankton species were grouped as reported by individual studies and hybrids were not included in the analysis. Only studies that provided

population growth rates (r) or studies where these rates could be calculated were included. This excluded multiple studies that used somatic growth rates (g) as experimental endpoints. Although g and r have been shown to be closely correlated in *Daphnia* when fed a nutritious diet (Lampert & Trubetskova 1996), the inclusion of cyanobacteria in treatment diets may decouple the relationship between g and r (Hietala et al. 1995, Reinikainen et al. 1999, Sarnelle & Wilson 2005). Cyanobacteria were labeled toxic if the strain tested positive via a chemical analysis, such as HPLC or ELISA, for a previously described metabolite with a demonstrated toxic effect on any organism and non-toxic if the strain tested negative for toxins common to its genus (Wilson et al. 2006). In total, 47 research papers were retrieved with data that fit these criteria giving a sum of 376 treatment-control experimental pairs (a partial list of studies is given in Appendix 1 of Wilson et al. 2006; newer studies included in this analysis but not used in Wilson et al. 2006 were Martin-Creuzburg et al. 2005, Geng et al. 2006, and Wilson & Hay 2007).

In meta-analysis, differences in precision among studies can be incorporated into the analysis by weighting the effect size by the reciprocal of the within-study error variance (Gurevitch & Hedges 1999). However in this data set, within-study error variance ($\sigma_{\text{treatment error}} = 0.003$; $\sigma_{\text{control error}} = 0.001$) was about two orders of magnitude less than among-study variance ($\sigma_{\text{treatment } r} = 0.205$; $\sigma_{\text{control } r} = 0.206$) indicating that weighted and unweighted results should not differ substantially. Few differences between weighted and unweighted results were found in the previous meta-analysis (Wilson et al. 2006). Therefore, in this study, weighted results were not calculated, so as to maximize the number of observations and avoid rejecting relevant data for purely statistical reasons, which can bias the results (Englund et al. 1999).

Each treatment within each study was paired with the experimental control to calculate an effect size using the equation: $(r_{\text{treatment}} - r_{\text{control}})/r_{\text{control}}$ to give a total of 376 effect sizes used in subsequent analyses. The effect size is proportional to the percent decrease in population growth rate fed a diet containing cyanobacteria relative to a control diet so grazers with larger effect sizes did better on diets containing cyanobacteria than grazers with smaller effect sizes. Mean control and treatment r values and mean effect sizes for all treatments (all with 95% confidence intervals, CI) were reported for each zooplankton species and genera, and mean effect sizes were reported for cyanobacterial strains, species and genera. The sample size for each zooplankton or cyanobacterial species or genus used in this analysis was the total number of effect sizes, or when r was reported, the total number of population growth experiments for all treatments that used the corresponding organism. Rather than reporting multiple P -values for each taxon to determine if there was a significant effect of the treatment diet (i.e. the effect size was below zero), the 95% confidence limits were presented graphically. If the upper confidence interval of a negative effect size does not overlap with zero then growth rates were significantly lower on diets containing cyanobacteria relative to control diets (Steidl & Thomas 2001).

Two-tailed, two-sample t -tests were used to test for differences in mean effect sizes between toxic and non-toxic treatments diets, single and colonial *M. aeruginosa* and microcystin producing and non-producing *M. aeruginosa*. One-way ANOVAs were used to test for differences among more than two groups and Tukey's post hoc test was used to compare multiple means. The non-parametric Kruskal-Wallis test was used when

heteroscedasticity could not be corrected through transformations. Pearson's correlations were used to test for relationships between mean species body length and mean species effect size within each group: cladocerans, rotifers and *Daphnia*. Linear regressions were used to test for a negative relationship between effect size and percent cyanobacteria in treatment diets for taxa that had a range of at least fifty percent cyanobacteria in grazer diets. A general linear model was used to test if grazer genera responded differently to an increasing percent of cyanobacteria in treatment diets (percent cyanobacteria \times genus interaction). P values for significance tests were set *a priori* at 0.05.

Results

Mean treatment r values for 6 out of 8 rotifer and 15 out of 21 cladoceran species and as well as for three commonly-used genera, *Ceriodaphnia*, *Daphnia* and *Keratella* were significantly greater than zero, indicating positive population growth rates in the presence of cyanobacteria (Fig. 1A). Although most cladoceran and rotifer species were able to maintain a positive growth rate, 6 rotifer and 13 cladoceran species had lower population growth rates when fed a treatment diet containing varying proportions of cyanobacteria (1–100% by carbon) relative to growth rates observed on diets containing only chlorophytes and/or small flagellates (mean effect size < 0 ; Fig. 1B). Among the cladoceran genera that were negatively affected, *Chydorus* and *Moina* had the largest average decrease in r when fed treatment diets containing cyanobacteria (–143% and –82%, respectively) and amongst the rotifers, *Hexartha* (–194%) and *Brachionus* (–85%) were most negatively affected (Fig. 1B).

Differences among species could not be explained by zooplankton mean species body length or when grouping by genera (Fig. 1). There was no relationship between mean species body length and mean species effect size among cladocerans (Pearson's $r = 0.087$, $P = 0.716$, $n = 20$), rotifers ($r = -0.652$, $P = 0.161$, $n = 8$) or only *Daphnia* species ($r = -0.310$, $P = 0.354$, $n = 11$). There was also no correlation when the data were restricted to experiments using filamentous cyanobacteria (all species, $r = -0.465$, $P = 0.070$, $n = 16$).

Zooplankton taxa showed varied responses to different cyanobacterial morphologies. In general, single-celled cyanobacteria were the most detrimental to zooplankton and caused a negative effect on the majority (18 out of 22) of zooplankton species (Fig. 2A) relative to diets containing only control foods. Filamentous cyanobacteria had a negative effect on fewer species with half (8 out of 17) of the zooplankton species negatively affected (Fig. 2B); chroococoid colonial cyanobacteria had a negative effect on

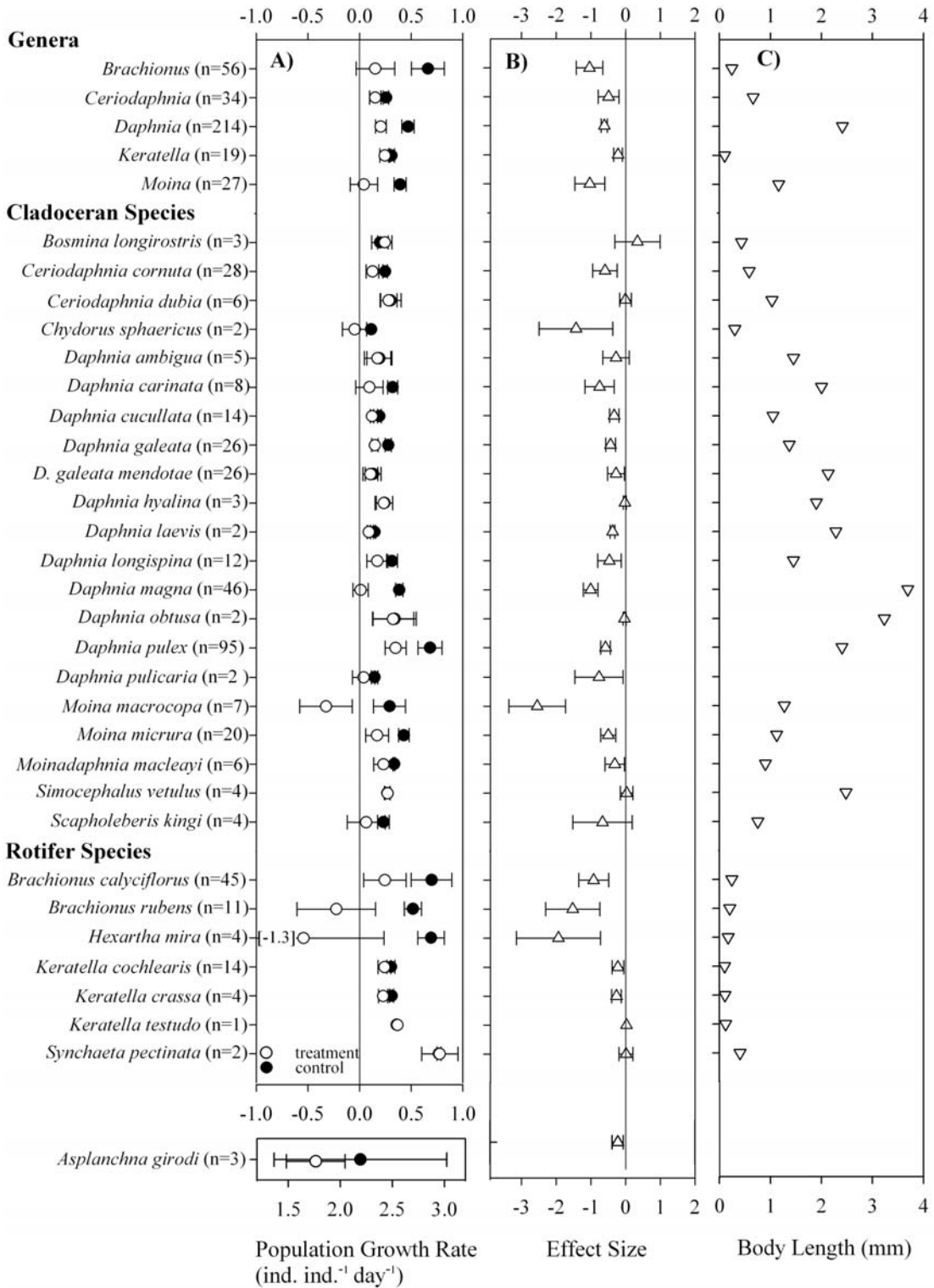


Fig. 1. Panel A: Mean population growth rates (\pm 95% CI) of herbivorous zooplankton fed a control diet of small chlorophytes and/or flagellates or a treatment diet containing varying proportions of cyanobacteria (1–100%) combined with green algae and/or flagellates. Panel B: Mean effect sizes (\pm 95% CI) for zooplankton taxa calculated from control and treatment population growth rates ($[r_{\text{treatment}} - r_{\text{control}}] / r_{\text{control}}$). Grazers with larger effect sizes did better on diets containing cyanobacteria than grazers with smaller effect sizes. Effect sizes with confidence intervals below zero indicate a significant negative effect of cyanobacteria on zooplankton population growth rate relative to diets lacking cyanobacteria. Panel C: Mean adult body length of zooplankton taxa taken from each study or estimated from the literature. The number of treatment – control experimental pairs used to calculate means for each zooplankton is given in parenthesis. Genus entries give mean values for all species in the specified genus.

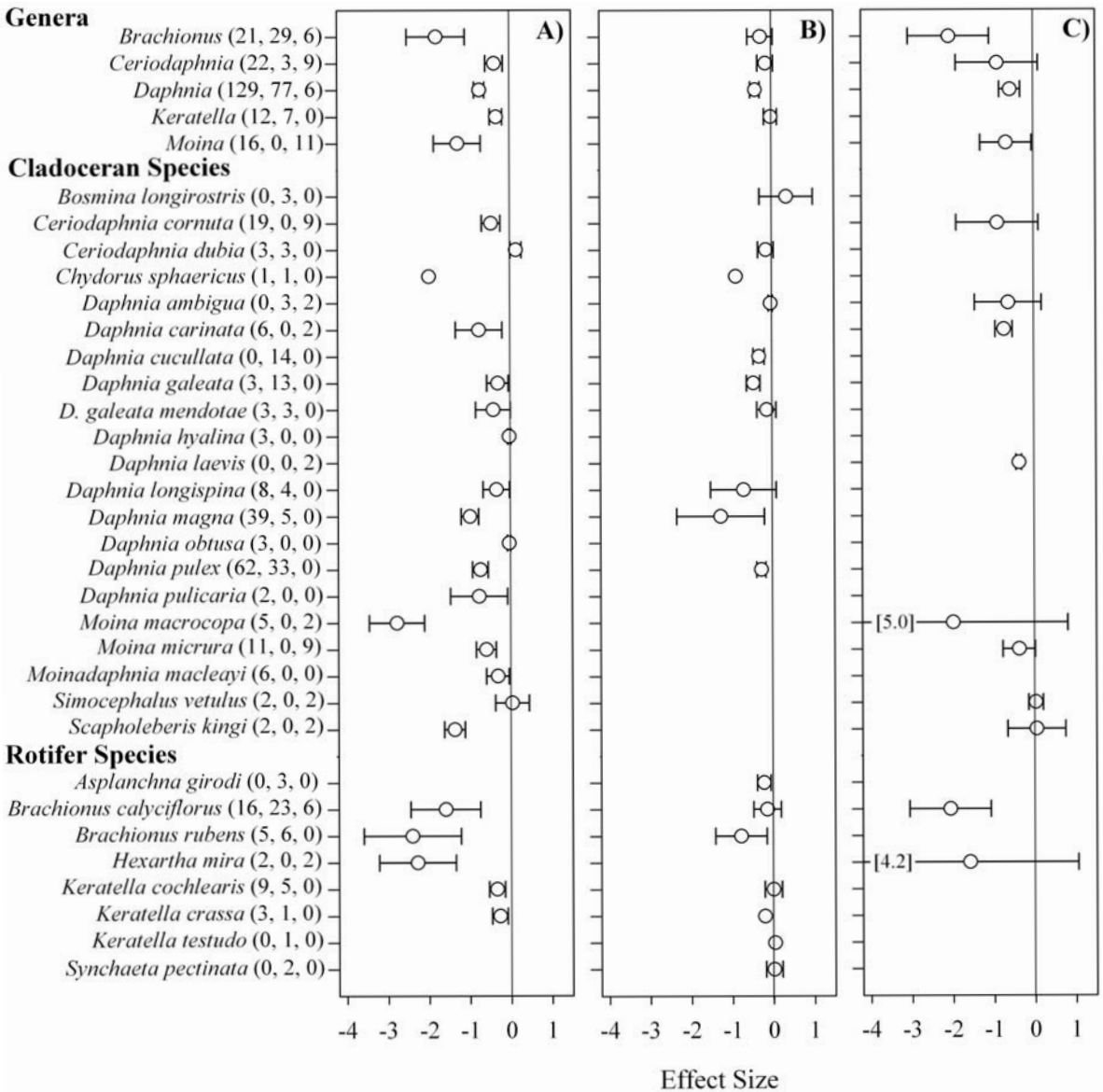


Fig. 2. A comparison of mean effect sizes and 95 % confidence intervals for taxa fed a treatment diet containing: **A)** single-celled, **B)** filamentous, and **C)** chroococcoid colonial cyanobacteria. Sample sizes for each morphology are given in order after each species or genus (single, filamentous, chroococcoid colonial). Effect size was calculated from r for paired experiments of grazers fed a control diet of chlorophytes and/or flagellates and a treatment diet containing cyanobacteria. Genus entries give mean values for all species in the specified genus.

the fewest species tested (3 out of 10; Fig. 2C). There were differences amongst the cladoceran species when treatment diets contained single-celled ($F_{16, 161} = 5.46$, $P < 0.001$, $n = 178$; Fig. 2A) or filamentous ($F_{9, 72} = 4.10$, $P < 0.001$, $n = 82$; Fig. 2B) cyanobacteria but not chroococcoid colonial cyanobacteria ($F_{7, 22} = 0.803$, $P = 0.593$, $n = 30$; Fig. 2C). Differences amongst rotifer species could only be detected for treatment diets containing single-celled cyanobacteria (single-celled: $F_{4, 30} = 2.92$, $P = 0.037$, $n = 35$; filamentous: $F_{6, 34} =$

0.076 , $P = 0.603$, $n = 41$; chroococcoid colonial: $F_{1, 6} = 0.181$, $P = 0.385$, $n = 8$). Filamentous cyanobacteria had less of a negative impact than single-celled cyanobacteria for *Keratella* (Student's t -test; $t_{17} = 2.51$, $P = 0.023$, $n = 19$), *Daphnia* (One-way ANOVA; $F_{2, 209} = 6.27$, $P = 0.002$, $n = 214$; Tukey's Post Hoc Test $P = 0.001$), and *Brachionus* ($F_{2, 53} = 11.21$, $P < 0.001$, $n = 56$; Tukey's $P < 0.001$). There was no difference between single-celled and chroococcoid colonial cyanobacteria for *Daphnia* ($P = 0.801$) or *Moina*

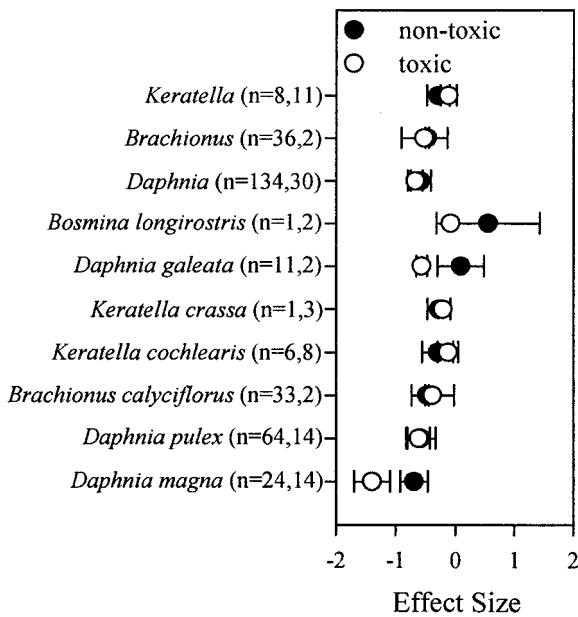


Fig. 3. Mean effect sizes and 95 % confidence intervals for zooplankton taxa fed treatment diets of cyanobacteria which produce putative toxins (*n* is given as first number in parenthesis) and those that do not produce toxins (*n* is second number in parenthesis). Entries at the top give mean values for all species in a specific genus.

($t_{25} = 2.51$, $P = 0.185$, $n = 27$) but there was a larger negative effect of chroococcoid colonial over filamentous cyanobacteria for *Brachionus* ($P = 0.007$).

Three genera and seven species of zooplankton were used in both experiments using toxic cyanobacteria as well as experiments using non-toxic cyanobacteria (Fig. 3). In most cases there was little difference between the toxic/non-toxic effect sizes; only *D. galeata* and *D. magna* had significantly lower growth on toxic diets (*D. galeata*, $t_{11} = 4.97$, $P < 0.001$; *D. magna*, $t_{36} = 3.2$, $P = 0.003$; Fig. 4). The data for *D. galeata*, however, included only two observations of growth on non-toxic diets.

There was a negative relationship between effect size and percent of cyanobacteria in the treatment diets in only four of the ten zooplankton species tested and four out of five grazer genera (*Brachionus*, *Daphnia*, *Keratella*, and *Moina*; Table 1). The response of these four genera differed in slope (percent cyanobacteria \times genus interaction; $F_{3,311} = 14.7$, $P < 0.001$). When treatment diets consisting of 100 % cyanobacteria were considered, five *Daphnia* species and *Simonephalus vetulus* were able to maintain significantly positive growth rates (Table 2). Additionally, the lower confidence intervals of the genera *Daphnia* and *Ceriodaphnia* and the species *D. longispina* and *D. pulex*

Table 1. Results of linear regressions which test for the dependence of effect size on percent cyanobacteria in treatment foods for zooplankton genera and species. Ranges for percent cyanobacteria in treatment diets are also given. Only taxa with a range of at least fifty percent cyanobacteria across treatment diets were considered suitable for a linear regression model. Genus entries give mean values for all species in the specified genus.

Zooplankton taxa	n	range	slope	P value
<i>Brachionus</i>	56	1 – 100	-0.018	<0.001
<i>B. calyciflorus</i>	45	1 – 100	-0.020	<0.001
<i>B. rubens</i>	11	1 – 100	-0.019	0.138
<i>Ceriodaphnia</i>	34	2 – 100	-0.002	0.606
<i>C. cornuta</i>	28	5 – 100	-0.004	0.400
<i>Daphnia</i>	214	1 – 100	-0.004	0.001
<i>D. ambigua</i>	5	2 – 90	-0.011	0.018
<i>D. carinata</i>	8	6 – 100	-0.010	0.075
<i>D. galeata</i>	16	20 – 100	-0.008	0.001
<i>D. longispina</i>	12	23 – 100	-0.008	0.191
<i>D. magna</i>	46	5 – 100	-0.012	<0.001
<i>D. pulex</i>	95	1 – 100	-0.003	0.159
<i>Keratella</i>	19	2 – 60	-0.011	0.003
<i>Moina</i>	27	5 – 100	-0.017	0.001
<i>M. micrura</i>	20	5 – 100	-0.006	0.050

only minimally overlapped with zero indicating that the actual mean was most likely above zero.

In all there were 26 unique culture collection strains of cyanobacteria from 10 genera represented in the combined dataset but 229 of the 378 treatment-control experimental pairs used *Microcystis aeruginosa* (Fig. 4). The strain with the largest negative effect was an anatoxin producing *Anabaena flos-aquae* NRC-44-1 (mean \pm 95 % CI; -1.68 ± 0.92) followed by microcystin producing *M. aeruginosa* PCC 7806 (-1.58 ± 0.06). The cyanobacterial taxa that were the best foods for zooplankton, relative to population growth rates on control diets lacking cyanobacteria, were *Aphanizomenon flexuosum* (0.33 ± 0.47) and the microcystin producing strain, *M. aeruginosa* NPLJ42 (-0.13 ± 0.20). The most commonly used strain was *M. aeruginosa* PCC 7820 ($n = 52$) which produces microcystins and was used predominantly in tests with *D. pulex* ($n = 31$). *D. pulex* fed strain PCC 7820 as part of the treatment diet was more negatively impacted than *D. pulex* fed all other strains of *M. aeruginosa* (PCC 7820: -0.93 ± 0.35 , $n = 31$; all other *M. aeruginosa* strains: -0.32 ± 0.17 , $n = 17$; $t_{46} = 2.44$, $P = 0.019$).

A comparison of the most common cyanobacterial genera showed that *Microcystis* (comprised of only *M. aeruginosa*) had a larger negative effect on zooplank-

Table 2. Population growth rates (*r*) of zooplankton taxa fed treatment diets consisting solely of 100 % cyanobacteria. *r* values with lower confidence intervals (CI) that are above zero maintained positive growth even when fed diets of 100 % cyanobacteria. Genus entries give mean values for all species in the specified genus.

Zooplankton taxa	n	Mean	Lower CI	Upper CI
<i>Brachionus</i>	20	-0.32	-0.57	-0.07
<i>B. calyciflorus</i>	18	-0.27	-0.52	-0.02
<i>B. rubens</i>	2	-0.78	-1.81	0.25
<i>Ceriodaphnia</i>	12	0.12	0	0.24
<i>C. cornuta</i>	9	0.09	-0.07	0.25
<i>C. dubia</i>	3	0.21	0.19	0.24
<i>Chydorus sphaericus</i>	2	-0.05	-0.17	0.07
<i>Daphnia</i>	75	0.04	-0.01	0.09
<i>D. carinata</i>	4	-0.05	-0.2	0.1
<i>D. cucullata</i>	2	0.14	0.11	0.18
<i>D. galeata</i>	11	0.12	0.1	0.14
<i>D. hyalina</i>	3	0.24	0.15	0.33
<i>D. laevis</i>	2	0.09	0.07	0.11
<i>D. longispina</i>	8	0.15	-0.01	0.3
<i>D. magna</i>	18	-0.13	-0.25	0
<i>D. obtusa</i>	3	0.33	0.13	0.53
<i>D. pulex</i>	22	0.05	-0.04	0.13
<i>Hexarthra mira</i>	4	-0.54	-1.33	0.24
<i>Moina</i>	10	-0.30	-0.52	-0.08
<i>Moina macrocopa</i>	7	-0.33	-0.58	-0.07
<i>Moina micrura</i>	3	-0.25	-0.74	0.24
<i>Simocephalus vetulus</i>	4	0.27	0.25	0.3
<i>Scapholeberis kingi</i>	4	0.07	-0.12	0.25

ton than *Anabaena* and *Planktothrix* (One-way ANOVA, $F_{4,361} = 6.20, P < 0.001$; Fig. 4). Although there were significant differences in mean effect size among strains of *M. aeruginosa* (Kruskal-Wallis, $H_{14} = 28.2, P = 0.011$; Fig. 4) no difference could be detected between *M. aeruginosa* strains that produce microcystins (mean effect size $\pm 95\%$ CI; $-0.72 \pm 0.14, n = 125$) and those that do not ($-0.53 \pm 0.15, n = 27; t_{150} = 1.19, P = 0.237$). There was also no detectable difference in mean effect size between single-celled ($-0.83 \pm 0.14, n = 191$) and colonial ($-0.91 \pm 0.39, n = 38$) strains of *M. aeruginosa* ($t_{227} = -0.440, P = 0.661$).

Discussion

In this study, we examined variation among zooplankton and cyanobacterial taxa, with respect to cyanobacterial effects on zooplankton population growth and found some major differences among zooplankton taxa. Treatment diets containing cyanobacteria caused a significant decrease in zooplankton population growth

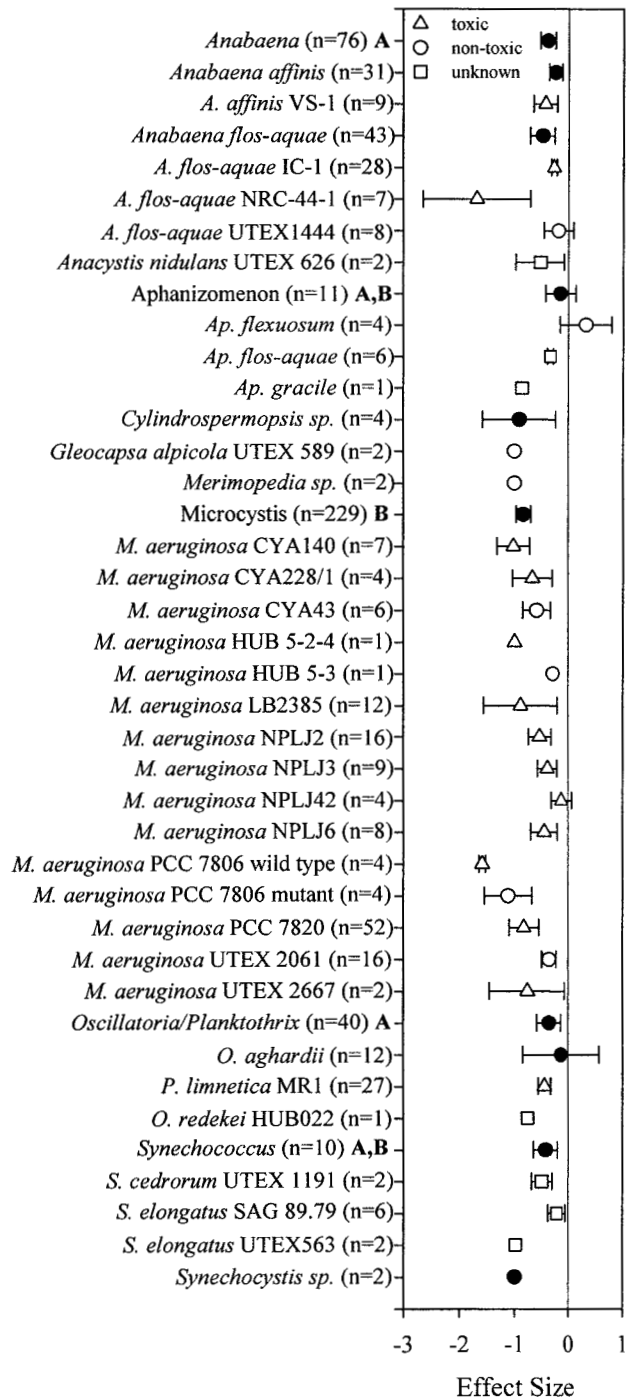


Fig. 4. A comparison of mean effect sizes ($\pm 95\%$ CI) for cyanobacterial taxa used in treatment diets of herbivorous zooplankton. Black symbols denote mean values for all species in a specified genus. Black symbols also give means of a species when strains were not identified. Differences between cyanobacterial genera were tested by one-way ANOVA. There is no difference in means between genera sharing the same letter (Tukey's Post Hoc Test, $p < 0.05$). Error bars are presented for all species with $n > 1$ but may be smaller than the symbol.

relative to control diets of small chlorophytes/flagellates for the majority of zooplankton species tested (Fig. 1B) but surprisingly, 6 out of 8 rotifer species and 14 out of 20 cladoceran species maintained positive growth rates when fed a diet containing cyanobacteria (Fig. 1A). The latter results indicate that populations of some zooplankton genotypes may be able to increase even though their population growth rate is negatively impacted by cyanobacteria (Sarnelle 2007).

Differences in mean effect size among both rotifer and cladoceran species were evident in the experiments using single-celled cyanobacteria (Fig. 2). Since mechanical interference is not pertinent in this case, differences among grazers must be due to either physiological tolerance to cyanobacteria or a behavioral response (DeMott & Dhawale 1995, Sarnelle & Wilson 2005, Wilson & Hay 2007). Differences in grazer performance on a diet containing single-celled cyanobacteria have been reported previously across grazer genera, species within a genus and genotypes within species (Nandini & Rao 1998, Lundstedt & Brett 1991, Ferrão-Filho et al. 2000, DeMott 1999, Hietala et al. 1995, Sarnelle & Wilson 2005).

Field observations of the lack of large *Daphnia* during cyanobacterial blooms (Edmondson & Litt 1982, Infante & Abella 1985, DeMott et al. 2001) as well as results from some laboratory experiments (Webster & Peters 1978, Porter & Orcutt 1980, Gliwicz & Lampert 1990) have emphasized mechanical interference as a major mechanism limiting the growth of large bodied zooplankton species during cyanobacterial blooms. However, in the present study we could detect no relationship between mean species effect sizes and mean species body lengths for 20 cladoceran species (body length [BL] range: 0.3 to 3.9 mm; effect size [ES] range: -5.1 to 1), 11 *Daphnia* species (BL: 1.0 to 3.9 mm; ES: -5.1 to 0.3) or eight rotifer species (BL: 0.1 to 0.4 mm; ES: -4.3 to 2.1); nor could we detect a relationship when experiments were limited to those that used filamentous cyanobacteria in treatment diets (12 species, BL: 0.1 to 3.6 mm; ES: -1.2 to 0.3). Also, no difference in mean effect size could be detected between colonial and single celled *M. aeruginosa*. Both of these results suggest that, in general, mechanical interference may not be a major inhibitor of zooplankton growth (Ferrão-Filho et al. 2000). Other factors may be responsible such as susceptibility of zooplankton to unidentified secondary metabolites produced by cyanobacteria (Kurmayer 2001, Rohrlack et al. 2004, Wilson & Hay 2007) or differences in susceptibility to cyanobacteria due to grazer life history characteristics (Lynch 1980, Ferrão-Filho et al. 2000).

Comparisons of experiments with treatment diets containing toxic cyanobacteria and experiments using non-toxic cyanobacteria failed to reveal any clear effect of toxicity which is consistent with findings from our previous meta-analyses (Wilson et al. 2006). Of the seven zooplankton species and three genera that were used in both experiments, only *D. magna* and possibly *D. galeata* were more negatively affected by the presence of cyanobacteria containing known intracellular toxins (Fig 3). For both species, the most frequently identified cyanobacterial secondary metabolites (about 80 %) were microcystins. Also, no difference in mean effect size could be detected between *M. aeruginosa* that produced microcystins and those that did not. Non-toxic cyanobacteria may, however, contain yet unidentified toxins (Rohrlack et al. 2004, Wilson & Hay 2007) or may not have been tested for all known toxins, only those most common to the genus.

Though there appears to be no overall effect of known cyanobacterial toxins, except in the case of *D. magna*, these metabolites may nevertheless have an effect on some genotypes. For example, *D. pulicaria* taken from a lake with documented cyanobacterial blooms was less susceptible to toxic cyanobacteria than *D. pulicaria* taken from a less eutrophic lake (Sarnelle & Wilson 2005). In a direct test of toxicity, Wilson & Hay (2007) found microcystin-LR added to freeze dried *Chlorella* and fed to zooplankton significantly reduced the population growth rate of one clone of *D. pulicaria* while having no effect on a second *D. pulicaria* clone. Also, Rohrlack et al. (2001) found a mutant strain of *M. aeruginosa* not capable of producing microcystins to be less toxic to five clones of common *Daphnia* species than the microcystin-producing wildtype. However, the effects of toxins may be fundamentally altered when the toxin is separated from its normal biochemical setting within a cyanobacterium (Wilson & Hay 2007), and knocking-out the genetic sequence necessary to produce microcystins may alter the strain in other undocumented ways.

Nutritional inadequacy of cyanobacteria may be responsible for limiting zooplankton growth (DeMott & Müller-Navarra 1997). Cyanobacteria have reduced amounts of highly unsaturated fatty acids compared with green algae (Ahlgren et al. 1992, DeMott & Müller-Navarra 1997), and the addition of a second algal species with sufficient fatty acids to treatment diets has been shown to decrease the negative effect of cyanobacteria (Hietala et al. 1997, Reinikainen et al. 1994), though this result may be due to the addition of sterols rather than fatty acids (von Elert et al. 2003). Here we found a negative relationship between

effect size and percent of cyanobacteria in treatment diets for only four of the ten species that were fed a range in percent cyanobacteria (Table 1). Though other mechanisms such as the presence of harmful bioactive compounds could cause this negative relationship, the absence of a relationship in six of the ten species suggests a supplementary food source was not necessary to maintain population growth. Additionally, six species and to some extent the genera *Daphnia* and *Ceriodaphnia*, were capable of maintaining a positive growth rate even when fed 100% cyanobacteria (Table 2). Thus earlier findings that showed a negative relationship between effect size and percent cyanobacteria in the treatment diet (Wilson et al. 2006) may not hold for all zooplankton – cyanobacteria interactions. Other studies (Brett 1993, De Bernardi et al. 1981, Repka 1996) have also reported positive growth rates of zooplankton species on diets of solely cyanobacteria.

In general, the genus *Microcystis* was a poorer food source for zooplankton than *Anabaena* or *Planktothrix*, but the effect size was strain specific and did not depend on the strain's capacity to produce microcystins (Fig. 4; Nizan et al. 1986, Ferrão-Filho et al. 2000, Lürling 2003). Microcystin-free extracts of *M. aeruginosa* can still be detrimental to herbivorous zooplankton (Kurmayer 2001, Rohrlack et al. 2004). The cyanobacterial strain with the largest negative effect on zooplankton was *Anabaena flos-aquae* NRC-44-1 which produces anatoxin-*a* (Porter & Orcutt 1980, Starkweather & Kellar 1983). Although there was insufficient data to statistically compare toxic and non-toxic *Anabaena* strains, it is interesting to note that the one non-toxic *Anabaena* strain, *A. flos-aquae* UTEX 1444, was also the only strain that did not cause a clear negative effect on zooplankton grazers. Although our results and those previously reported (Wilson et al. 2006) suggest no clear effect of putative cyanobacterial toxins in general, further research on the effects of anatoxin-*a* on grazer growth rates is needed.

Daphnia pulex (n = 95) and *Daphnia magna* (n = 46) were the most commonly used species in this meta-analysis, and because of the large sample sizes for these two species it is particularly interesting to note the differences. Both *D. pulex* and *D. magna* were significantly affected by treatment diets containing cyanobacteria but only *D. pulex* could maintain a positive growth rate when fed diets containing cyanobacteria. *D. magna* was affected by treatment diets containing putative toxins while *D. pulex* was not (Fig. 3) which is congruent with the fact that there was a negative effect of percent cyanobacteria on effect size for *D. magna* but not *D. pulex* (Table 2).

This meta-analysis and the previous study (Wilson et al. 2006) have provided quantitative generalizations across laboratory studies of zooplankton population growth rates when fed treatment diets containing cyanobacteria. Neither toxins nor colonial morphology appear to have specific negative effects in general across zooplankton species. The nutritional hypothesis could not be addressed directly, as it is difficult to separate the possible effects of unidentified bioactive cyanobacterial metabolites from nutritional inadequacies. However, the finding that numerous zooplankton species could maintain a positive growth rate when fed diets of only cyanobacteria implies that some cyanobacterial genotypes contain adequate nutritional value to maintain population growth of some zooplankton species, at least for the duration typical of laboratory studies.

The majority of studies summarized by this meta-analysis used single clones, typically from laboratory cultures, making it difficult to extrapolate these results to natural systems. This meta-analysis generalizes the results of these studies which used a range of treatments (cyanobacteria species, concentrations, morphology – including filament length and concentration), and genetic strains of both zooplankton and algae. These findings do not negate the results of individual studies but suggest that many of the hypotheses put forward may not adequately explain why cyanobacteria are of poor food quality to zooplankton. Furthermore, as this meta-analysis was conducted at the scale of species, it does not consider the importance of intra-specific variation which, given the large amount of variation in *r* among studies compared to within studies as well as demonstrated differences in responses between zooplankton clones (Repka 1996, Sarnelle & Wilson 2005), may be quite high. A qualitative review of the literature would find many examples and exceptions to each of the generalizations made in this meta-analysis. However, the strength of a meta-analysis is to draw quantitative generalizations across multiple studies, which used different conditions and different strains of organisms to assess if the hypotheses found prevalently in the literature are supported by the available data.

In general, our results support the conclusion that cyanobacteria are a poorer food resource for zooplankton than small chlorophytes and/or flagellates, though some species were able to maintain positive growth rates on diets containing cyanobacteria. More importantly, our results highlight the high degree of variation in species-specific responses of zooplankton to cyanobacteria. For example, some strains of cyanobacteria were particularly detrimental to some spe-

cies of zooplankton, while other zooplankton species were capable of reproducing even on diets composed entirely of cyanobacteria. The effects of cyanobacterial morphology and known toxins were not general or strong enough to stand out over variation among strains and species. Although a meta-analysis can provide a summary of available data, experiments using multiple clones isolated from multiple water bodies with documented limnological history are necessary to document the range of traits within each species. Only once this range is established, can we begin the process of understanding variation in the interaction between any two species (Holt 2005).

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Submitted: 28 August 2007; accepted: 27 February 2008.