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Effects of cyanobacteria on fitness components of the herbivore Daphnia

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**Effects of cyanobacteria on fitness components of the herbivore
*Daphnia***

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Key words: microcystin, Microcystis, *Daphnia*, toxin, cyanobacteria, limnology

28 *Abstract*

29 Cyanobacteria are known to negatively affect their herbivores either by being of low nutritional
30 value, by clogging the feeding apparatus, or by producing toxins; and specifically the role of
31 toxins has been debated. Hence, in order to assess to what extent cyanobacterial toxins affect a
32 major herbivore (*Daphnia magna*) that has previous experience of cyanobacterial toxins, we
33 conducted a life-table study using two otherwise-similar strains of *Microcystis aeruginosa*, one
34 producing and one not producing the toxin microcystin. In contrast to previous studies, we
35 found that *Daphnia* population growth was positive ($r > 0.1 \text{ d}^{-1}$) on a diet containing toxic
36 *Microcystis*. However, we also found that the presence of the toxin negatively affected early
37 survival and population growth of a microcystin-tolerant *D. magna* clone. Although there was
38 no effect of toxin presence on per capita fecundity of surviving adults, *Daphnia* produced
39 smaller neonates when fed toxin-containing *M. aeruginosa* than when fed the non-toxic
40 mutant. Hence, although *Daphnia* survival, population growth and neonate size were
41 negatively affected by microcystin presence, *Daphnia* populations that have prior experience
42 with toxic cyanobacteria may show positive population growth even at high concentrations of
43 cyanobacterial toxins. This conclusion may have considerable implications for interactions
44 between toxic cyanobacteria and herbivores in natural systems.

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46

47 *Introduction*

48 Negative relationships between bloom-forming cyanobacteria (for example species in the
49 genera *Anabaena*, *Aphanizomenon*, *Microcystis*, and *Oscillatoria/Planktothrix*, hereafter
50 referred to as "cyanobacteria") and the abundances of *Daphnia* have been observed in nature,
51 either across lakes that vary in trophic status (Hansson et al. 2007), or across the growth season
52 within eutrophic lakes (Threlkeld 1979). This may indicate negative effects of *Daphnia* grazing
53 on cyanobacterial abundance, negative effects of cyanobacteria on *Daphnia* population growth
54 rate, or both. These alternative explanations seem somewhat incompatible because
55 cyanobacterial inhibition of *Daphnia* growth and reproduction would be expected to interfere
56 with the ability of a *Daphnia* population to increase and so exert enough grazing pressure to
57 suppress cyanobacterial abundance. Despite this seeming incompatibility, evidence supportive
58 of both explanations has accumulated over the last several decades. However, the relative
59 importance of these mechanisms remains unresolved.

60 The negative effects of *Daphnia* grazing on cyanobacterial abundance have been
61 documented in field experiments in which the abundance of either zooplanktivorous fish (that
62 prey most intensively on large *Daphnia*), or in some cases the abundance of *Daphnia*, are
63 manipulated (Lynch and Shapiro 1981; Vanni 1984; Vanni and Findlay 1990; Sarnelle 1993;
64 Sarnelle 2007). The negative effects of cyanobacteria on *Daphnia* growth and reproduction
65 have been documented primarily in laboratory experiments in which *Daphnia* are fed various
66 species and strains of cyanobacteria and their performance (survival and/or fecundity) is
67 compared to that on a reference diet such as nutritious chlorophytes (Lampert 1987; Lürling
68 2003; Wilson et al. 2006).

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4 69 Three properties of cyanobacteria may contribute to their relatively poor quality as a food
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6 70 source for *Daphnia*. Bloom-forming cyanobacteria typically exhibit a colonial or filamentous
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8 71 morphology in nature that can make them difficult for zooplankton to ingest in general
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10 72 (DeMott 1989) and may interfere with the food-collecting process in *Daphnia* in particular
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12 73 (Webster and Porter 1978; Gilbert 1990). In addition, cyanobacteria may lack certain
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14 74 biochemicals that are essential for *Daphnia* nutrition (Von Elert and Wolffrom 2001; Von Elert
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16 75 et al. 2002; Brett et al. 2009). Finally, some cyanobacteria produce compounds that may inhibit
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18 76 *Daphnia* survival, growth, reproduction and/or feeding (Rohrlack et al. 2001; Lürling 2003;
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20 77 Lürling and Van Der Grinten 2003; Rohrlack et al. 2003). A recent meta-analysis of laboratory
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22 78 life-table studies came to the unexpected conclusion that intracellular cyanobacterial toxins are
23
24 79 not a major factor underlying the negative effects of cyanobacteria on *Daphnia* population
25
26 80 growth (Wilson et al. 2006). This conclusion was based on a comparison of the effects of toxic
27
28 81 versus non-toxic strains across all studies. Further analyses suggested that any role of toxins
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30 82 may be limited to a small number of cyanobacterial genotypes (Wilson et al. 2006). A second
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32 83 meta-analysis of life-table studies that focused on variation across taxa suggested that
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34 84 *Microcystis* was the most inhibitory of the cyanobacterial genera, although the role of toxins in
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36 85 inhibiting *Daphnia* population growth was again uncertain (Tillmanns et al. 2008). The former
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38 86 result provides justification for the heavy emphasis on *Microcystis* among studies of the
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40 87 inhibitory effects of cyanobacteria on zooplankton (Wilson et al. 2006; Tillmanns et al. 2008).

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49 88 Several recent studies have shown that *Daphnia* individuals or populations can adapt to the
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51 89 presence of toxin-producing cyanobacteria in the diet (Hairston et al. 2001; Gustafsson and
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53 90 Hansson 2004; Sarnelle and Wilson 2005). This new information has led us to hypothesize that
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55 91 some of the conflicting nature of the evidence summarized above may be resolved if we can
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3 92 attribute variation in the negative effects of toxigenic cyanobacteria to variation in the toxin
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5 93 tolerance of the specific zooplankton genotypes used in life-table experiments, i.e. considering
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8 94 the co-evolutionary history of zooplankton-cyanobacteria interactions. As a first attempt to
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10 95 examine this hypothesis, we report the results of a laboratory experiment in which the effects
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12 96 of wild-type (produces the toxin, microcystin) and mutant (cannot produce microcystin)
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14 97 genotypes of *Microcystis aeruginosa* on the population growth of *Daphnia magna* were
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16 98 quantified.
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20 99 Several existing laboratory studies have demonstrated strong negative effects of one or both
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22 100 of these *M. aeruginosa* genotypes on the survival, growth and/or reproduction of *Daphnia*
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24 101 *magna* (Rohrlack et al. 1999; Rohrlack et al. 2001; Lürling 2003). The difference in our study
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26 102 is that we deliberately chose a *D. magna* genotype that we have found to be relatively tolerant
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28 103 of toxic cyanobacteria in the diet, which would better mirror the natural situation. Our
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30 104 objective was to determine how the effects of a highly toxic strain of *M. aeruginosa* are
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32 105 manifested on a relatively toxin-tolerant genotype of *D. magna*. *Daphnia* populations in
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34 106 habitats with high concentrations of toxic cyanobacteria should be dominated by genotypes that
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36 107 are toxin-tolerant (Sarnelle and Wilson 2005), making our experiment more like natural
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38 108 conditions. Knowledge about tolerance in herbivores may have considerable implications for
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40 109 our understanding of interactions between consumer and prey, and, in a broader perspective, on
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42 110 the dynamics of cyanobacterial blooms.
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50 112 *Method*

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53 113 *Experimental organisms:* The *Daphnia magna* clone used in this study was hatched from
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55 114 ephippia collected in 2001 from the water's edge, in the highly eutrophic Lake Bysjön (Total
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3 115 phosphorus and nitrogen concentrations: 1200 and 2400 $\mu\text{g/L}$, respectively) situated in
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6 116 southern Skåne, Sweden. Lake Bysjön has blooms of cyanobacteria every summer, generally
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8 117 dominated by *Aphanizomenon*, but different species of *Microcystis* also occur and occasionally
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10 118 these blooms produce microcystins (unpublished data). Ehippia were hatched in 2006,
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12 119 resulting in the establishment of 13 clonal lines of *D. magna*. These 13 clones were exposed to
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14 120 increasing concentrations of microcystin-producing *M. aeruginosa* (wild-type, PCC 7806) to
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16 121 identify clones that were most tolerant of this strain (Gustafsson 2007). The clone used in this
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18 122 study was the most tolerant of the 13 based on fastest time to maturity on a diet containing
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20 123 PCC 7806. Animals were cultivated in an aquarium (17°C, 16:8 light:dark cycle) and fed only
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22 124 *Scenedesmus obliquus* for 8 months prior to the start of the experiment.
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27 125 *Scenedesmus obliquus* and two strains of *Microcystis aeruginosa*, the microcystin-
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29 126 producing PCC 7806 (wild-type) and a genetically-engineered mutant, PCC 7806 mcy A
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31 127 (Dittmann et al. 1997) that cannot produce microcystin, were cultivated in Z8 medium at 20°C
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33 128 with a light dark cycle of 12:12 and a light intensity of 15-20 $\mu\text{mol quanta}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The cells
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35 129 were maintained in exponential growth phase. Total algal concentrations used in the
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37 130 experiment correspond to 1 mg Carbon/L, which is equivalent to 60,000 cells mL^{-1} (0.5 mg
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39 131 C/L) of *Scenedesmus obliquus* plus 140,000 cells mL^{-1} (0.5 mg C/L) for *Microcystis*
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41 132 *aeruginosa*. Both phytoplankton species were within the size range of particles that are
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43 133 efficiently consumed by *Daphnia* (*Scenedesmus*: 7.5 μm x 5 μm , *Microcystis*: 3 - 4 μm ,
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45 134 DeMott 1989). There were no differences in size among *Microcystis* clones and neither
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47 135 *Microcystis* nor *Scenedesmus* produced colonies or gelatinous sheets of any substantial size.
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49 136 Algal cells were enumerated in a Palmer- Maloney counting chamber (Wildlife Supply,
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51 137 Buffalo, New York, USA) using an Nikon inverted microscope at 400 X magnification. The
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3 138 animals were fed twice this concentration every second day. Samples of approximately 1 mL
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6 139 from both the toxic and non-toxic treatment were stored at -20°C for later analysis of
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8 140 microcystin content.

10 141 *Experimental design:* *Daphnia* neonates were exposed to three dietary treatments: a
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12 142 control consisting of 100% *Scenedesmus*, a "non-toxic" treatment with 50% *Scenedesmus* and
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14 143 50% mutant *Microcystis* PCC 7608 and a "toxic" treatment with 50% wild-type *Microcystis*
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16 144 and 50% *Scenedesmus* (all percentages based on carbon content). Neonates born within 24 h
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18 145 from ten parthenogenetically reproducing females were randomly transferred to 250 mL
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20 146 beakers to start the experiment. Four replicate beakers were assigned to each treatment and
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22 147 each beaker contained 5 or 6 animals. Every second day animals were pipetted to new algal
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24 148 solution in clean beakers. The animals were checked for survival, maturity and offspring every
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26 149 day and the water was stirred with a pipette to re-suspend the phytoplankton. Offspring were
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28 150 removed every day, counted and measured from the eye to the base of the tail spine. Length
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30 151 measurements were used to calculate biomass based on length-weight regressions (Bottrell et
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32 152 al. 1976). The experiment was continued for 28 days, which allowed for approximately three
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34 153 adult instars (Lürling 2003).

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36 154 Population growth rates were calculated from daily survival and fecundity schedules using
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38 155 Euler's equation (Stearns 1992). Treatment effects on survival were analyzed via repeated-
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40 156 measures MANOVA on arcsin square root-transformed proportions in each beaker
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42 157 (experimental unit) on days 2-28. Per capita fecundity (offspring produced per surviving
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44 158 female summed over 28 days), and instantaneous population growth (r, d^{-1}) were analyzed via
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46 159 One-Way Analysis of Variance (ANOVA), followed by an *a priori* Tukey's HSD test for
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48 160 differences among individual means. Fecundities and population growth rates were log-
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3 161 transformed as needed to homogenize variances. All statistical tests were performed using
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5 162 SPSS for Macintosh.
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8 163 *Microcystin analysis:* Microcystin was analyzed by means of ELISA (enzyme-linked
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10 164 immunosorbent assay) using the Abraxis Microcystins ELISA Kit (Environmental Assurance
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12 165 Monitoring, LLC.). Before the analysis, algal cells were lysed by freezing and thawing three
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14 166 times and then sonicated for two minutes in an ice bath (Ultrasonic Liquid Processor, Heat
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16 167 Systems Inc.). Samples were centrifuged for 20 min at 6500 rpm and the supernatant was
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18 168 analyzed. A negative control, five standards (0.15, 0.40, 1.0, 2.0 and 5.0 $\mu\text{g L}^{-1}$, respectively),
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20 169 a control at 0.75 $\mu\text{g L}^{-1}$ and samples were all analyzed in duplicate. Absorbance was measured
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22 170 at 450 nm on a Hyperion Microreader 3, (Hyperion, Miami FL, USA).
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30 172 *Results*

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32 173 Total microcystin concentrations averaged 10 $\mu\text{g L}^{-1}$ (range 9 - 11 $\mu\text{g L}^{-1}$) in the toxic treatment
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34 174 and were undetectable in the non-toxic treatment. We found no significant overall effect of
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36 175 treatment on *Daphnia* survival over 28 days (ANOVA F-test on arcsine-transformed data, $p >$
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38 176 0.15), although mean survivorship in the toxic treatment (63%, SE = 0.22) was considerably
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40 177 lower and more variable than in either of the other treatments (control: 87%, SE = 0.16, non-
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42 178 toxic: 83%, SE = 0.14, Fig. 1). Further, the repeated measures analysis was ambiguous with
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44 179 respect to a time x treatment interaction: by the Greenhouse-Geisser criterion, the interaction
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46 180 was not significant at the 5% level ($p = 0.09$) whereas by the less conservative Huynh-Feldt
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48 181 criterion, the interaction was significant ($p = 0.04$). Given these results and the pattern of mean
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50 182 responses across the three treatments (Fig. 1), we re-ran the analysis to test the hypothesis that
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54 183 *Daphnia* survival was different in toxic *Microcystis* (N = 4) versus pooled non-toxic
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3 184 (*Microcystis* or *Scenedesmus*, N = 8) treatments. In this case, we found overall lower survival in
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5 185 the toxic treatment (ANOVA F-test on arcsine-transformed data, $p < 0.04$). Mortality in the
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8 186 toxic treatment was limited to the first two weeks of the experiment (Fig. 1).
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11 187 With respect to *Daphnia* fecundity, we found an overall effect of treatment (ANOVA F-
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13 188 test, $p < 0.01$), but the means for the non-toxic *Microcystis* and toxic *Microcystis* treatments
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15 189 were essentially identical (Fig. 2), so the treatment effect was purely a function of diet species
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17 190 (*Scenedesmus* versus *Microcystis*). As seen for survival, error variation was higher in the toxic
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19 191 treatment (SE = 4.6 versus 1.9 for non-toxic and 2.6 in the control). *Daphnia* population
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21 192 growth was also affected by treatment (ANOVA F-test, $p < 0.0001$), and in this case all three
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23 193 treatment means were different from each other (Fig. 2), indicating a further negative effect of
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25 194 the toxic strain of *Microcystis*, along with an effect of diet species. Finally, there was an overall
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27 195 effect of treatment on mean neonate length over the course of the experiment (ANOVA F-test,
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29 196 $p < 0.0001$), with mean length highest in the non-toxic *Microcystis* treatment and lowest in the
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31 197 *Scenedesmus* control (Fig. 3). The neonate biomass produced during the experiment (28 days)
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33 198 was 152, 93 and 82 $\mu\text{g L}^{-1}$ in the control, non-toxic and toxic treatments, respectively
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35 199 (ANOVA, F-test, $p < 0.001$; Fig. 4).
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44 201 *Discussion*

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46 202 Our study shows that exposure to *Microcystis aeruginosa* had a negative impact on population
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48 203 growth rate, survival and fecundity of a microcystin-tolerant clone of *Daphnia* and that the
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50 204 presence of microcystin in the wild-type of *Microcystis* significantly enhanced the negative
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52 205 effect on population growth rate and reduced the biomass of neonates produced. Our
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54 206 experimental treatment with 50% cyanobacteria is a common feature in natural eutrophic lakes
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3 207 (e.g. Bergman et al. 1999; Cronberg et al. 1999). Moreover, the amount of toxic compounds,
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5 208 such a microcystin, may vary temporally from zero to 35 $\mu\text{g L}^{-1}$ within the same lake (Hansson
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8 209 et al. 2007). Hence, with respect to the amount of cyanobacteria and toxins, our study may well
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10 210 mirror the situation in natural systems.

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13 211 Of the three potential mechanisms that may underlie the reduction in population growth
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15 212 (morphology, nutrition, toxic metabolites), we can in our study rule out morphology as an
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17 213 explanation because the *Microcystis* strains we used were similar in size to the *Scenedesmus*
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19 214 control and both species were well within the highly-edible size range for *Daphnia* (DeMott
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21 215 1989).

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24 216 With respect to the effect of food quality (i.e. difference between *Scenedesmus* and non-
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26 217 toxic *Microcystis*), it appears that the negative effect on population growth was driven
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28 218 primarily by reduced fecundity because survival was similar between the *Scenedesmus* diet and
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30 219 the non-toxic *Microcystis* diet (Fig. 1). In contrast, the negative effect of the toxic metabolite
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32 220 on *Daphnia* population growth (difference between non-toxic and toxic *Microcystis*) was
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34 221 clearly not driven by reduced fecundity (Fig. 2). Fecundity was essentially identical between
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36 222 the non-toxic *Microcystis* and toxic *Microcystis* diets, although the size of neonates was larger
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38 223 in the non-toxic *Microcystis* treatment than in the other two treatments (Fig. 3). An increase in
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40 224 *Daphnia* neonate size is often observed when food supply is scarce (Boersma 1997) and has
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42 225 been interpreted as a way to enhance survival capability of the neonates. At ample food
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44 226 conditions, such as in the *Scenedesmus* treatment, *Daphnia* generally produce small, but
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46 227 numerous neonates, which are able to survive with less investment from the mother (Boersma
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48 228 1997; Hammers-Wirtz and Ratte 2000; Pieters and Liess 2006). On a diet of toxic *Microcystis*,
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50 229 however, neonates were smaller suggesting that mothers were not able to invest as much in
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3 230 individual neonates. These differences in size, maternal mortality and fecundity, mean that the
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5 231 total neonate biomass produced during the investigation period was highest in the *Scenedesmus*
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8 232 treatment and lowest when mothers were fed toxic *Microcystis* (Fig. 4).
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10 233 We suggest that the presence of microcystin affected *Daphnia* population growth by
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12 234 reducing survival, as indicated by our re-analysis of the survival data. Moreover, the negative
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15 235 effect of microcystin on survival was manifested solely during the first 2 weeks of life, with no
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17 236 additional treatment-related mortality thereafter (Fig. 1). Our results contrast with those of
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19
20 237 previous studies in terms of the strength of the negative effect of toxic *Microcystis aeruginosa*.
21
22 238 Most notably, we found that *D. magna* was able to maintain a positive population growth (~
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24 239 0.12 d^{-1}) on a diet containing 50% toxic *M. aeruginosa*. Previous studies have found that this
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27 240 strain of *Microcystis* (PCC 7806) did not support positive population growth of *Daphnia* when
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29 241 presented alone or in a 50:50 mixture with *Scenedesmus* (Rohrlack et al. 1999, 2001; Lüring
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31 242 2003; Semyalo et al. 2009). One potential explanation for this contrast in the effect of *M.*
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33 243 *aeruginosa* PCC 7806 could have been that microcystin levels differed among experiments.
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36 244 However, microcystin levels in our toxic treatment ($10 \mu\text{g L}^{-1}$) were comparable to the level of
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38 245 $\sim 9 \mu\text{g L}^{-1}$ employed by Rohrlack et al. (2001) who found 90-100% mortality of 6 *Daphnia*
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40 246 clones within 10 days of exposure (compare to our Fig. 1). However, Rohrlack et al. (2001) did
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42
43 247 not provide any nutritious algae to the animals in their toxic treatment. Lüring (2003) did not
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46 248 measure microcystin levels directly, but we can estimate the levels he employed in his 50%
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48 249 toxic PCC 7806/50% *Scenedesmus* treatment as $\sim 4.5 \mu\text{g L}^{-1}$, based on the biovolume
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50 250 concentration of *Microcystis* used ($5 \text{ mm}^3 \text{ L}^{-1}$) and a microcystin quota of $0.9 \mu\text{g mm}^{-1}$ for this
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53 251 strain, as reported by Rohrlack et al. (2001). Thus, we did not use lower microcystin levels than
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3 252 previous studies that reported negative population growth, making it unlikely that this can
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6 253 explain the difference among studies.

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8 254 An alternative explanation for the difference between our results and those of Rohrlack et
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10 255 al. (1999, 2001) and Lürling (2003) is related to our use of a strain of *D. magna* that had
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12 256 previous experience from *Microcystis* in the diet (Gustafsson and Hansson 2004). Several
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14 257 studies have now demonstrated that *Daphnia* populations can either adapt genetically or
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16 258 acclimate phenotypically, to better tolerate the presence of toxic cyanobacteria in the diet
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18 259 (Hairston et al. 2001; Gustafsson et al. 2005; Sarnelle and Wilson 2005). Previous studies
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20 260 using toxic *Microcystis* may have used *Daphnia* strains that had no previous experience of
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22 261 cyanobacteria. We therefore suggest that the ecological experience of consumers be taken into
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24 262 consideration when selecting strains for study and when interpreting results with respect to
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26 263 natural conditions (Gustafsson et al. 2005; Sarnelle and Wilson 2005).

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29 264 We found a significant difference in *Daphnia* population growth rate between diets
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31 265 containing toxic wild-type *Microcystis* versus the non-toxic mutant, the same basic result
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33 266 reported by Rohrlack et al. (1999, 2001). Our conclusion that the added effects of microcystin
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35 267 only affected early-life survival (given that there was no effect of microcystin on per capita
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37 268 fecundity, Fig. 2) is also consistent with the conclusions of Rohrlack et al. (1999, 2001). That
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39 269 toxins primarily affect survival can also be implied from the meta-analysis of Wilson et al.
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41 270 (2006). However, negative effects of microcystin on the fecundity of *Daphnia* have also been
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43 271 reported, manifested as later maturation in individuals exposed to the wild-type (Semyalo et al
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45 272 2009). There may exist a threshold level for microcystin where concentration above the
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47 273 threshold level has an instant effect on survival while concentrations below mainly affect
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3 274 fecundity and growth rate. This threshold level could differ for different species and clones of
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6 275 *Daphnia* but also be related to the daphnids earlier experience of microcystin.

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8 276 Our results contrast with those of Lürling (2003) who was not able to detect a difference in
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10 277 survival between toxic and non-toxic strains. The latter result led Lürling (2003) to conclude
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12 278 that other toxic compounds produced by *Microcystis* were driving poor *Daphnia* performance
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15 279 (Jungmann 1992; Rohrlack et al. 2003). Given that the *Daphnia* in our experiment performed
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17 280 much better on both strains, we conclude that our animals are also tolerant of other cyanotoxins
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19
20 281 possibly present during the experiment. We also found significant effects of treatment on
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22 282 neonate length, where mean length was greater in the non-toxic *Microcystis* treatments relative
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24 283 to the high-quality *Scenedesmus* diet (Fig. 3) indicating greater maternal investment in each
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27 284 offspring under deteriorating environmental conditions (Boersma 1997; Hammers-Wirtz and
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29 285 Ratte 2000; Pieters and Liess 2006).

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32 286 Our results suggest three general conclusions. Firstly, that the presence of microcystin
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34 287 further enhances the negative impact of cyanobacteria on *Daphnia* population growth.
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36 288 Secondly, intraspecific variation in microcystin tolerance within *Daphnia* species can be large,
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38 289 given that some genotypes rapidly go extinct (Rohrlack et al. 1999, 2001), while others may
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41 290 show considerable population growth (Fig. 2). Thus, although our data suggest that co-occurring
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43 291 *Daphnia* populations can increase in the presence of high levels of microcystin, population
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46 292 growth should be impaired relative to when microcystin is absent because of reduced early
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48 293 survival, suggesting that microcystin matters even for tolerant *D. magna*. This reduction in
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50 294 population growth rate could be enough to affect a *Daphnia* decline in habitats with other
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53 295 significant sources of mortality, such as predation and food shortage.

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Figure Legends

Figure 1. Effects of dietary treatment on survival of *Daphnia magna* over 28 days of exposure.

Squares- 100% *Scenedesmus*, circles- non-toxic *Microcystis* (50% microcystin-lacking *Microcystis* + 50% *Scenedesmus*), triangles- toxic *Microcystis* (50% microcystin-containing *Microcystis* + 50% *Scenedesmus*). Error bars represent standard errors of the mean.

Figure 2. Effects of dietary treatment on fecundity (upper panel) and per capita population growth rate (day^{-1} ; lower panel) of *Daphnia magna* over 28 days of exposure. Probability (p)

values are for significance tests (ANOVA) of overall differences among treatments. Columns with different letters are significantly different from the adjacent column at $p < 0.05$ (multiple comparison tests). Error bars represent standard errors of the mean. Treatments are:

Scenedesmus (100%), Non-toxic *Microcystis* (50%) + *Scenedesmus* 50% and Toxic *Microcystis* (50%) + *Scenedesmus* 50%.

Figure 3. Effects of dietary treatment on carapace length of *Daphnia magna* neonates over 28

days of exposure. Probability (p) values are for significance tests (ANOVA) of overall differences among treatments. Columns with different letters are significantly different from the adjacent column at $p < 0.05$ (multiple comparison tests). Error bars represent standard

errors of the mean. Treatments are: *Scenedesmus* (100%), Non-toxic *Microcystis* (50%) + *Scenedesmus* 50% and Toxic *Microcystis* (50%) + *Scenedesmus* 50%.

Figure 4. Effects of dietary treatment on biomass ($\mu\text{g L}^{-1}$, $\pm 1\text{SE}$) of *Daphnia magna* neonates

over 28 days of exposure. P values are for significance tests (ANOVA) of overall differences among treatments. Columns with different letters are significantly different from the adjacent column at $p < 0.05$ (multiple comparison tests). Treatments are: *Scenedesmus* (100%), Non-

toxic *Microcystis* (50%) + *Scenedesmus* 50% and Toxic *Microcystis* (50%) + *Scenedesmus* 50%.

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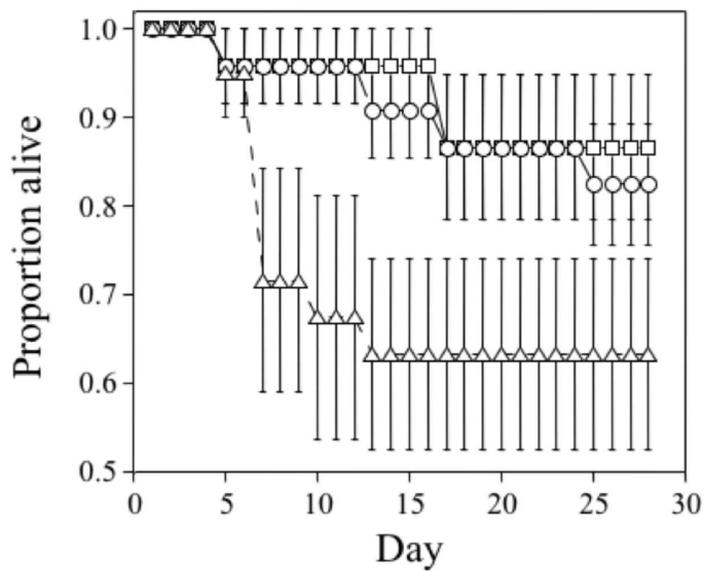
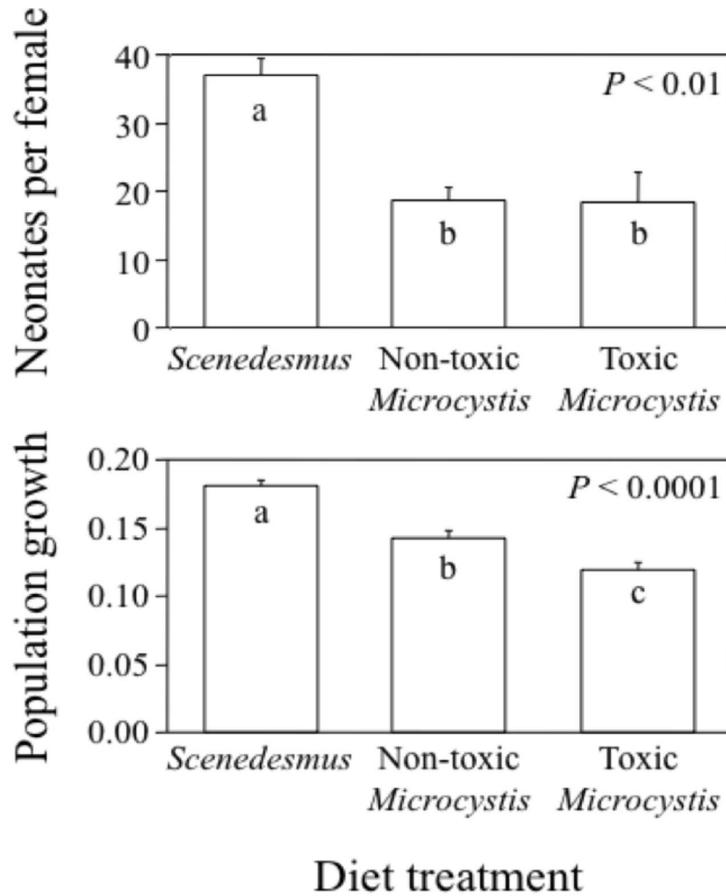
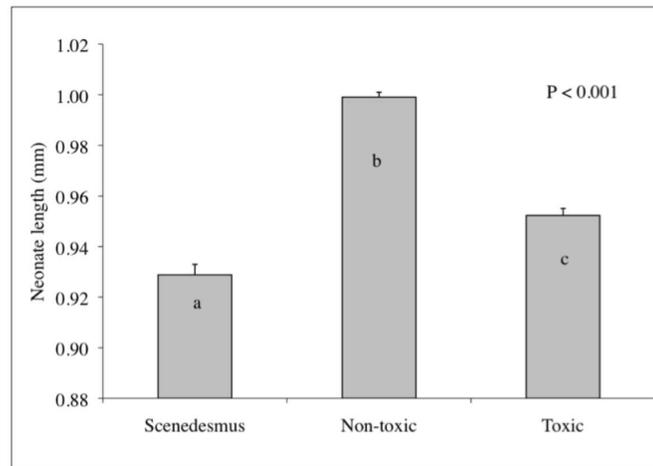


Fig 1

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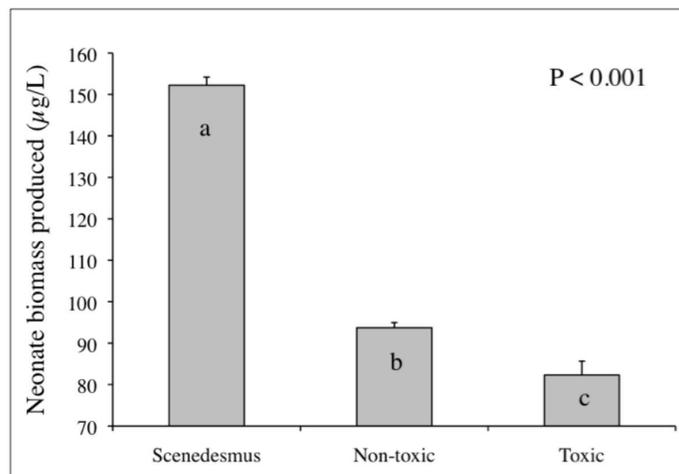


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