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9 Zooplankton grazing selectivity regulates herbivory and dominance of toxic
10 phytoplankton over multiple prey generations
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35 Abstract

36 Although biotic interactions have been recognized as a key factor shaping
37 communities, the role of indirect positive interactions is less understood. Although
38 top-down control of phytoplankton by zooplankton is a cornerstone of aquatic
39 ecology, cyanobacterial blooms often support high zooplankton biomass, indicating
40 that zooplankton might facilitate rather than control cyanobacteria. We tested the
41 hypothesis that a selective grazing copepod (*E. gracilis*) would facilitate the
42 dominance of a toxic cyanobacterium (*Microcystis*) by grazing on a competing
43 eukaryotic microalga (*Cryptomonas*) while a generalist cladoceran (*Daphnia magna*)
44 would have no effect on the dominance of cyanobacteria in a controlled laboratory
45 experiment where each grazer type was added to co-cultured phytoplankton.
46 Experiments started with a 9-fold initial dominance of *Cryptomonas* over *Microcystis*
47 by biomass. We found that generalist cladocerans had no effect on the relative
48 abundance of their prey while selective grazing copepods facilitated *Microcystis*
49 dominance. Moreover, the copepod had a higher overall mass-specific clearance rate
50 than the cladoceran. Our results suggest that selective grazing zooplankton can
51 promote the dominance of bloom-forming cyanobacteria, which may explain the co-
52 existence of these groups in nature. Moreover, the results emphasize that grazing
53 selectivity may be a key functional trait for zooplankton. We discuss implications for
54 the role of selective grazers in shaping the structure and function of pelagic
55 ecosystems.

Introduction

Positive interactions – those where at least one species benefits and none is harmed – are a key driver of ecosystem structure and function (Odum XX). Yet, their inclusion into theoretical and empirical research has been slower than negative interactions such as competition and predation (Polis and Strong, 1996, Bruno et al. 2003). Positive interactions (e.g., mutualism, commensalism) are often limited to non-consumer interactions (Bertness and Callaway 1994), likely because predation is typically considered as a negative antagonistic interaction. Yet, consumers are known to benefit their prey (Wilbur 1988) and herbivores may increase primary production via removal of intra- and inter-specific competitors (McNaughton 1985, Sterner 1990). Predators may also have a positive effect on the relative biomass of competing prey via non-uniform reductions in competition among prey species (Chase et al. 2002). Thus, selective predation can increase the relative biomass of less preferred prey. Facilitation is defined here as a positive effect on the relative abundance of a species mediated through the selective removal of a competitor by a shared predator. Examples include the facilitation of grazing resistant plants and the loss of high quality forage plants following grazing in rangelands (Dyksterhuis 1947, Rosenthal and Kotanen 1994, Cingolani et al. 2004). The facilitation of toxic phytoplankton blooms by zooplankton grazers, however, is less understood.

One of the most cherished paradigms in aquatic ecology is the trophic cascade via top-down control of phytoplankton by zooplankton (Sommer and Sommer 2006). Negative species interactions are implicit in this theory, as the top-down control of phytoplankton depends on a strong link between grazers and their phytoplankton prey (i.e., strong zooplankton herbivory). The tremendous potential for the management and restoration of eutrophied lakes and the prevention of harmful algal blooms (e.g., through biomanipulation) is one of the main reasons for

the recognition of this theory (Shapiro and Wright 1974, Jeppesen, Scheffer). Despite success stories, top-down control is limited to ecosystems with a strong link between phytoplankton and zooplankton (Sarnelle, Jeppesen papers). Aquatic ecosystems where blooms of inedible cyanobacteria dominate phytoplankton, however, are often beyond the scope of top-down control (Gliwicz 1990, Rondell et al. 2008, Lacerot et al. 2013 Elser and Goldman 1991). As cyanobacteria blooms generally support high zooplankton biomass (Bouvy et al. 2000, Sousa et al. 2008, Rangel et al. 2016), it has been suggested that zooplankton might actually, through selective removal of eukaryotic competitors, indirectly facilitate, rather than control cyanobacterial dominance (Porter 1977, Lynch 1980, Mitra and Flynn 2006).

The link between phytoplankton and zooplankton, and therefore, the potential for top-down control or facilitation of phytoplankton blooms by zooplankton likely depends on the traits of both grazer and prey (Ger et al. 2014). Ultimately, such traits regulate the edibility and nutritional quality of phytoplankton, rates of grazing and prey selection, stoichiometric constraints, as well as competition within grazers or their prey (Sterner 1989, Chen and Folt 1993, Legrand et al. 2003, Fulton and Paerl 1987). Key phytoplankton traits controlling the capacity of zooplankton to exert top-down control are morphological and chemical defenses (Kruk, Pohnert 2007, Ger et al. 2016 MC). Grazing pressure is also controlled by zooplankton traits such as body size, feeding mode, selectivity, and physiological tolerance to ingested toxins (DeMott 1989?, Kiorboe 2011, Litchman et al. 2013).

Overall, we expect that top-down control of bloom forming cyanobacteria is more likely with large, generalist grazers such as *Daphnia*, but only if they are also relatively tolerant to the ingestion of cyanobacterial toxins (Chislock et al. 2013) and if the cyanobacteria are in the edible size range (Gliwicz 1990). If they are not efficient at detoxifying toxins, we expect these large, generalist grazers to disappear during blooms, either because they cannot avoid ingesting toxic doses or because of reduced energy intake due to feeding inhibition (e.g., De Mott et al. 1991, Rohrlack et al. 1999, Lüring 2003). Tolerance to toxin ingestion is an adaptive trait that varies across *Daphnia* genotypes depending on evolutionary exposure history

(Hairston et al. 1999, Lemaire et al. 2012, Wilson and Sarnelle?) but can also be induced phenotypically via exposure during the lifetime of organisms (Gustaffsson et al. 2005, Jiang et al. 2013). Hence, one might expect an increase in the tolerance of large *Daphnia* parallel to increased warming and eutrophication (i.e., more cyanobacteria). Yet, large, generalist grazers are rare in warmer and more eutrophic waters due to a variety of reasons including high fish planktivory (Jeppesen et al. 2007, Moss et al. 2011, Havens et al. 2015), metabolic constraints linked to high temperatures (Atkinson, 1994), and their sensitivity to prolonged lack of abundant high quality food (Sommer PEG 2012, DeMott clogging filaments).

Warmer and more eutrophic waters select for smaller and more selective grazing zooplankton (Hansson et al. 2007, De Senerpont Domis et al. 2013), whose abundance is often positively related to cyanobacterial dominance in mesocosm experiments and monitoring studies (Bouvy 2001, Hong et al. 2015, Rangel et al. 2016). The advantage of selective grazers is the ability to grow in the presence of poor quality prey by grazing on beneficial food particles and avoiding harmful prey following the predictions of optimal foraging (DeMott 1989, Kirk 1990). Yet, complete avoidance of toxic prey is rare, and grazers must be able to grow and reproduce despite ingesting relatively small doses of toxic cells (DeMott and Moxter 1991, Colin and Dam 2003, Ger et al. 2016 review). Hence, selective grazers expected to facilitate cyanobacterial dominance are those that simultaneously select for edible phytoplankton but also able to detoxify any ingested toxins (e.g., Demott et al. 1991, Ger et al. 2016 JPR, Mitra and Flynn 2006, Hong et al. 2013).

The key difference among generalists vs. selective grazers in terms of facilitating toxic prey is that is that the former – assuming it is tolerant – is expected to uniformly reduce both toxic and non-toxic phytoplankton, while the latter is expected to reduce non-toxic prey more than toxic prey. Hence, while both grazing types can be expected to reduce the net growth and abundance of each prey type, selective grazing has the potential to change the relative growth and abundance of competing prey. Toxic phytoplankton subject to selective grazing may increase in biomass and growth relative to non-toxic competitors due to a combination of a grazer escape and competitive release. In contrast, the relative growth and

abundance of competing toxic and non-toxic phytoplankton is not expected to be affected by generalist grazers, which would uniformly reduce both prey types.

Although grazer regulation of phytoplankton communities has long been recognized (Porter 1977), the role of grazing selection is less understood. Decades of studies on copepod selectivity highlight strong avoidance of bloom forming cyanobacteria (Paerl and Fulton 1987, DeMott et al. 1991, Kirk and Gilbert, 1992, Ger et al. 2011, Hong et al. 2013, Ger et al. 2016 JPR), but these are based on short-term (<4 hours) grazing assays. Consequently, whether the avoidance of cyanobacteria persists across longer time scales is unknown. Copepods can switch prey when the preferred food is depleted (Kiørboe et al. 1996) and it is possible they ingest more cyanobacteria as nutritious prey declines over time. In a recent week-long study, a selective grazing tropical copepod (*Notodiaptomus iheringi*) promoted the dominance of cyanobacteria co-cultured with a competing eukaryotic phytoplankton, showing preliminary evidence for grazer facilitation of cyanobacterial dominance and no prey switching (Leitão et al. 2016). Yet, the effect of contrasting grazer selectivity on cyanobacterial dominance remains untested.

In the present study we compared the grazing impact of a selective grazer and two generalist grazers that potentially differed in their sensitivity to cyanobacteria toxins to test the predictions that (a) the selective grazer facilitates cyanobacterial dominance whereas the generalist grazer does not, (b) that the generalist grazer reduces cyanobacterial growth more than the selective grazer, and that (c) the top-down impact of the generalist grazer is reduced in the non-tolerant compared to the tolerant lineage. We evaluated the impact of the selective filter feeding calanoid copepod *Eudiaptomus gracilis* and two clones of the generalist filter feeder *Daphnia magna* with contrasting exposure history to cyanobacterial toxins on the biomass and relative abundances of the cyanobacterium *Microcystis aeruginosa* co-cultured with a nutritious eukaryotic competitor (*Cryptomonas pyrenoidifera*) in a 4-day laboratory experiment including no-grazer controls.

Methods

Organisms

Cultures of phytoplankton and zooplankton were combined to create laboratory food chains. The cyanobacterium *Microcystis aeruginosa* (NIVA-CYA 140) and the cryptophyte *Cryptomonas pyrenoidifera* (NIVA 2/81), both originating from the Norwegian Institute for Water Research (Norway), were grown in semi-continuous cultures exposed to a light intensity of 50 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ under a 16:8 h L:D cycle at $24 \pm 1^\circ\text{C}$, and used as prey species in the grazing experiments. The *Microcystis* strain used has previously been shown to produce three different variants of the hepatotoxin microcystin including MC-LR, -RR, and -YR (Ger et al. 2016). Phytoplankton cultures were maintained at the exponential growth phase by routine dilution with fresh media. *Microcystis* grew as single or double (roughly in equal proportion) spherical cells with a diameter of 4.5 μm , while *Cryptomonas* grew as single spheroid cells (length: 25 μm ; width = 12 μm). To establish mixtures with specific carbon ratios, the carbon biomass density of cultures was calculated from cell density and biovolume following the formula $\text{pgC cell}^{-1} = 0.1204 \times (\mu\text{m}^3)^{1.051}$ (Rocha and Duncan 1985) as previously detailed (Ger et al. 2016).

Two different clones of *Daphnia magna* with a contrasting *Microcystis* exposure history were used to test if previous toxin exposure (assumed to be related to tolerance) was a factor determining the relative abundance of the prey species. To represent the non-tolerant *Daphnia* (i.e., ND), we used a clone that previously suffered acute toxicity when exposed to several *Microcystis* strains, which was originally isolated from Lake Zwemlust in 1999 and has been maintained in the laboratory without exposure to cyanobacteria for > 17 years now in RT medium as described previously (Lürling 2003). For the tolerant *Daphnia* (TD), we used a clone (WV 32) previously shown to be tolerant to cyanobacteria toxins (Lemaire et al. 2012), which was isolated from a pond that shows recurrent intensive cyanobacteria blooms “Westveld Park” (Sint-Amandsberg, Belgium). Cultures of these two *Daphnia* (ND and TD) clones were fed with 0.5 $\text{mg C}\cdot\text{L}^{-1}\cdot\text{d}^{-1}$ equivalent of *Cryptomonas*, grown in 1L glass jars at $24 \pm 1^\circ\text{C}$ filled with synthetic zooplankton medium (Dutch Standard Water-REF) and maintained between 10-20 adults per liter for 3 weeks before the grazing experiment. The calanoid copepod

Eudiaptomus gracilis was obtained from Lake DeKuil, the Netherlands with a surface plankton tow. This lake underwent a restoration event in 2009 and cyanobacterial biomass was very low in the years before sampling the copepods in 2015 (Waajen et al., 2016). A new generation of copepods was grown from wild caught gravid females maintained in 4L glass beakers under identical conditions as the *Daphnia*. Only adult animals of similar age were used in subsequent experiments to minimize age related differences in grazing or death. Before the grazing experiment, *Daphnia* (either ND or TD) born on the same day were transferred to new cultures with 0.5 mgC.L⁻¹.d⁻¹ equivalent of *Cryptomonas*. Adults (i.e., 3.5 – 4.0 mm body length) from this new brood were used in the experiment. For copepods, either C5 stage copepodites or adults from the new laboratory raised brood were used in the experiment.

The length and dry weight of adult zooplankton was measured to create a normalized zooplankton biomass in subsequent experiments. Prior to experiments, the length of large adult *Daphnia* (3.74 ± 0.07 CI mm, n=15) and adult copepods (1.23 ± 0.02 CI mm, n=20) was individually measured under a dissecting microscope. Body length was measured as the distance from above the eye to the base of the tail spine for *Daphnia*, and the prosome for copepods. Dry weight was measured by filtering previously sized *Daphnia* (in batches of five animals each on three filters) on a pre-dried (24 h at 60° C) and weighed GF/C (Whatman, UK) filter. Copepod dry weight was measured the same way except that due to the higher number of copepods necessary for accurate measurement, we used 100 copepods (divided in batches of 25 animals each on four filters). This produced an estimate for the mean individual body weight and length for *Daphnia* (193 ± 45 CI $\mu\text{g.ind}^{-1}$) and the copepod *E. gracilis* (9 ± 2 CI $\mu\text{g.ind}^{-1}$). The relative individual dry weight of large adult *Daphnia* vs. adult copepods was used to normalize the zooplankton biomass in the grazing experiment. ND and TD were assumed to have the same size-weight relationship.

Grazing experiment

The effect of each zooplankton (i.e., ND, TD, copepod) on the competition between *Microcystis* (M) and *Cryptomonas* (C) was evaluated by comparing the abundance, growth, and grazing pressure on each prey, as well as the biomass ratio of M:C in no-grazer controls and treatments containing an equal biomass of one of the grazers (ND, TD, or copepod). Experiments took place in glass flasks, exposed to a light intensity of 50 $\mu\text{mol photons m}^{-2}\cdot\text{s}^{-1}$ under a 16:8 h L:D cycle at $24 \pm 1^\circ \text{C}$, sealed with silicone stoppers and gently bubbled via glass tubes sealed with a 0.22 μm vent (Millex, Millipore). Co-cultures of *Microcystis* and *Cryptomonas* were setup to start with a M:C biomass ratio of 1:9 by the dilution of exponentially growing single species cultures (explained above) in 1L of fresh WC medium. The *Daphnia* treatments (ND and TD) received two adult individuals each while copepod treatment received 25 individuals of adult *E. gracilis* each, which resulted in an equal dry zooplankton biomass per 1L flask (see results). Animals were concentrated on a 100 μm mesh, rinsed with distilled water, transferred to a petri dish, isolated in a drop of water using a wide-mouth plastic pipette under a dissecting microscope and transferred to the appropriate experimental flask. Controls and copepod treatments were replicated three times and the *Daphnia* (ND, TD) treatments were replicated four times. Phytoplankton resource limitation (i.e., light and nutrients) was assumed to be negligible under these conditions due to the nutrient rich WC medium and exponential growth in the absence of grazers (see results).

The abundance of phytoplankton in each flask was sub-sampled (10 mL) using a sterile pipette at the start and at every subsequent 24h for a total of four days (all zooplankton remained in the flask during sampling). In addition, the flasks were observed twice daily and any *Daphnia* neonates were removed to maintain the total grazer biomass. It was not possible to observe and remove copepod nauplii due to their small size. Flasks were also observed twice daily for any dead animals, which were replaced as soon as noticed. At the end of the experiment the contents of each flask were filtered on a 100 μm mesh to collect zooplankton in a petri dish and checked for mortality, which was indicated by either motility or heartbeat under a dissecting microscope. The live zooplankton of each flask were filtered on a

previously dried weighed GF/C filter, which was dried at 60° C for 24 hours and weighed again to calculate the total zooplankton dry mass per flask at the end of the experiment.

Phytoplankton cell density was estimated via two methods: flow cytometry and the Phyto-Pam fluorometer. This was done to test the validity of the latter during longer term grazing experiments where cellular pigment concentrations may vary. For cytometry, 4 mL of the sub-sample from each flask was preserved with paraformaldehyde (1% final vol.) and kept in the dark at 4° C until analysis. The cellular abundance of both species was determined with a flow cytometer [MoFlo Legacy Cell Sorter; Beckman Coulter, Miami, FL, USA], which were separated based on their relative size (time of flight) and fluorescent emission signal in the 630-790nm range while excited by a 488nm and 633nm laser. Reference particles (Fluoresbrite® YG Carboxylate Microspheres 1.00µm, Polysciences Inc.) were added to calculate the analyzed volume. Cells were sorted and identified by microscopy to verify the gating strategy. Blank samples (WC media) and cultures of each phytoplankton strain were also included in triplicates. As the second estimate of relative phytoplankton biomass, the phytoplankton specific chlorophyll concentration in the same 10 mL sub-sample was measured with a Phyto-Pam fluorometer (Heinz Walz GmbH Effeltrich, Germany) following the method of Lürling and Panosso (2010). This method detects and separates the in-vivo fluorescence of *Microcystis* ('Blue' chlorophyll) from *Cryptomonas* ('Brown' chlorophyll). We created a specific reference spectrum program for each phytoplankton strain used during the experiments using uni-algal cultures. A previous test verified that dissolved *Microcystis* pigments had no effect on the fluorescence based chlorophyll (chl-a) and PSII efficiency of *Cryptomonas*. Hence, there is no reason to expect that *Microcystis* allelopathy interfered with *Cryptomonas* fluorescence-based biomass estimates. Estimates of growth and the relative biomass of each phytoplankton were then compared among flow cytometry vs. Phyto-Pam (as described below) to identify potential biases among the methods used to estimate cell density.

Phytoplankton growth rate was calculated by the net change in the concentration of cells (for cytometry estimates) or pigment specific chl-a (for Phyto-Pam estimates) of each species during the 4-day grazing experiment using the formula $\ln(C_f/C_i)/4$, where C_f and C_i is the final and initial cell concentration, respectively. Phytoplankton carbon biomass was estimated from the biovolume of each phytoplankton species as explained above, based on cell density (i.e., flow cytometry estimate). In addition, the relative abundance in the biomass of *Microcystis:Cryptomonas* (i.e., M:C biomass ratio) was calculated for estimates based on flow cytometry as well as Phyto-Pam (i.e., Blue:Brown chl-a ratio).

Change in the prey cell concentration between controls and treatments during the experiment were used to calculate rates of individual and weight-specific clearance (CR) according to Frost (1972), as shown below (Ger et al. 2011).

$$CR = (b - a) \times V/N$$

$$b = \ln(C_f / C_i) / t$$

$$a = \ln(T_f / T_i) / t$$

Where V is the volume of the flask, N is the number of zooplankton per flask, C_f and C_i are the final and initial algal concentration ($\mu\text{g C.L}^{-1}$) in controls, T_f and T_i are the final and initial algal concentration in the treatments, and t is the grazing period.

The ingestion rate on each prey was calculated by multiplying the CR with the mean cell concentration (C) of each prey, according to the following formula (Frost, 1972):

$$C = (T_f - T_0) / \ln(T_f / T_0)$$

Finally, mass specific CR and IR for each prey was calculated by dividing individual CR and IR by the previously measured dry weight of each zooplankton grazer (see above). We calculated grazing rates (CR and IR) across four different time periods (i.e., day 0-1; 0-2; 0-3; 0-4) to evaluate changes in grazing pressure due to varied prey concentrations during the 4-day experiment. Prey specific rates of grazing are abbreviated as CR_C and IR_C (*Cryptomonas*) and CR_M and IR_M (*Microcystis*).

The effect of treatments on responses was evaluated with post-hoc one-way ANOVAs, pairwise HSD Tukey tests, and generalized linear models. The normality of distribution and homogeneity of variance was tested with a Shapiro-Wilk test and Levene's test, respectively, as well as standard diagnostic plots. The effect of

treatments on responses with non-normally distributed data or with non-homogenous variance was log-transformed to meet these criteria. The relationship between the phytoplankton growth estimates or the M:C biomass ratio obtained from flow cytometry vs. Phyto-Pam was evaluated with linear regression. All statistical analysis was performed using R (REF).

Results

Cell density of both *Cryptomonas* and *Microcystis* increased exponentially in the no-grazer controls (Figure 1). All three zooplankton treatments (i.e., ND, TD, copepod) reduced the abundance of both *Cryptomonas* and *Microcystis* relative to controls with no grazers (Figure 1). Hence, by the end of the 4-day experiment, zooplankton significantly reduced the biomass of both phytoplankton prey compared to controls, but the effect varied with treatment and prey type. The reduction in *Microcystis* biomass was highest with tolerant *Daphnia*, intermediate with copepods, and weakest with the non-tolerant *Daphnia* (Figure 2). Yet, the difference was not statistically significant and all three zooplankton treatments reduced *Microcystis* biomass relative to controls similarly by a factor between 2 - 3× (Figure 2). In contrast, the reduction in *Cryptomonas* biomass by zooplankton relative to controls was much stronger with copepods (~6×) compared to the *Daphnia* treatments (~2×) and was statistically significant (Figure 2). Thus, the greatest reduction in total phytoplankton biomass occurred with copepods, followed by tolerant *Daphnia* and non-tolerant *Daphnia* (Figure 2). A post-hoc ANOVA confirmed the significant negative effect of treatments on *Microcystis* ($F_{3,10}=8.217$, $p=0.005$), *Cryptomonas* ($F_{3,10}=19.43$, $p<0.001$), and total phytoplankton biomass ($F_{3,10}=18.34$, $p<0.001$) using estimates based on flow cytometry. Significant differences among treatments based on the pairwise HSD Tukey test results are shown in Figure 2.

Zooplankton reduced the growth rate of both phytoplankton when compared to controls without grazers, but growth reduction depended on the specific treatment and phytoplankton species (Figure 3). Overall, zooplankton only marginally reduced *Microcystis* growth ($F_{3,10}=3.068$, $p=0.078$), and differences

among zooplankton treatments were not statistically significant for *Microcystis* growth (Figure 3). Yet, compared to controls, the greatest growth reduction in *Microcystis* occurred with tolerant *Daphnia* (HSD Tukey test $p = 0.058$), which was followed by copepods and naïve *Daphnia*. In contrast, all zooplankton treatments significantly reduced *Cryptomonas* growth ($F_{3,10}=19.24$, $p<0.001$), and differences among zooplankton treatments were statistically significant, as indicated by the pairwise HSD Tukey test (Figure 3). Copepods reduced growth in *Cryptomonas* much more than either *Daphnia*. Specifically, the copepods reduced *Cryptomonas* growth to negative values, while the two *Daphnia* reduced growth by a factor of two.

The mean biomass ratio of *Microcystis*:*Cryptomonas* (M:C), as estimated from relative cellular abundance (via flow cytometry) increased during the experiment in all flasks including in the no-grazer controls (Figure 4). Yet, while the M:C ratio increased similarly among the controls and the two *Daphnia* treatments, the copepod increased the M:C ratio to considerably higher levels by the end of the experiment (Figure 4). The mean M:C with the copepod increased to a peak of about 0.7 on the third day and then declined. At the end of the 4-day experiment the treatments had a significant effect on log transformed M:C ratio ($F_{3,10}=7.37$, $p=0.006$). Specifically, when compared to controls the M:C ratio was significantly higher in the copepod treatment, marginally lower in the tolerant *Daphnia*, and similar in the non-tolerant *Daphnia* (Table 1).

Comparing among zooplankton treatments, the mean mass-specific clearance rate on *Cryptomonas* (CR_C) and total food (CR_T) were highest with the copepod, intermediate with the tolerant *Daphnia*, and lowest with the non-tolerant *Daphnia* across all periods of measurement (Figure 5). This difference in clearance rates among grazers was most pronounced on the second day of measurement when the mean CR_C or CR_T of the copepod was about $3\times$ that of the non-tolerant *Daphnia* and $2\times$ the tolerant *Daphnia* (HSD Tukey test $p<0.05$) (Figure 5). Mean CR_C was significantly higher with the copepod compared to one or both *Daphnia* clones on all days of measurement except for the first. Mean mass-specific clearance rate on *Microcystis* (CR_M) was similar between all zooplankton treatments. With longer measurement time, mean CR_C and CR_T among the zooplankton treatments were

relatively constant while CR_M increased (Figure 5). Comparing among prey specific grazing rates, CR_M was generally less than CR_C for all zooplankton treatments, except when measured at the end of the experiment (day 4), when $CR_M \approx CR_C$ for both *Daphnia* clones (but not the copepod). Differences in mass-specific ingestion rates ($IR_{M,C,T}$) among zooplankton treatments was similar to the overall trends observed for clearance rates above (Appendix Figure 1). Individual prey specific rates of clearance ($mL\ ind^{-1}\ h^{-1}$) and ingestion ($ngC\ ind^{-1}\ h^{-1}$) for each zooplankton treatment are provided in the appendix table 2.

Total zooplankton dry weight was similar across all treatments at the end of the experiment ($F_{2,8}=0.616$, $p=0.564$), with a mean of $468 \pm 19\ \mu g\ CI$ ($n=11$) per 1L flask. The mean dry weight of individual *Daphnia* was $237 \pm 9\ \mu g$ ($n=8$), which was based on the average of two individuals per 8 replicate flasks of either tolerant or non-tolerant *Daphnia* treatments collected at the end of the experiment. The dry weight of individual *E. gracilis* (copepod) was $9\ \mu g \pm 1\ CI\ \mu g$ ($n=3$), based on the average of 49 individuals per three replicate flasks collected from the copepod treatments at the end of the experiment. Zooplankton mortality at the end of the experiment was low, with two (out of 8 total) naïve *Daphnia*, and four copepods (out of 150 total) on the final day. A total of eight and three neonates were removed from the non-tolerant and tolerant *Daphnia* treatments, respectively.

The relationship between phytoplankton growth estimates via flow cytometry vs. Phyto-Pam were positive and linear (Appendix Figure 2). While growth estimates for *Cryptomonas* were similar to Phyto-Pam when compared to flow cytometry and the slope was similar to unity (Appendix Figure 2) and a slightly negative intercept ($-0.043 \pm 0.036\ CI$). In contrast, growth estimates for *Microcystis* were underestimated by Phyto-Pam when compared to flow cytometry by a third (Appendix Figure 2) with an intercept similar to zero ($0.057 \pm 0.062\ CI$). The relationship between the M:C biomass ratio estimates via flow cytometry vs. Phyto-Pam was also linear between the two methods with a slope similar to unity (Appendix Figure 3). Overall, the growth and relative biomass of each

phytoplankton was estimated similarly between the two methods, though Phyto-Pam underestimated *Microcystis* growth compared to flow cytometry.

Discussion

We compared how zooplankton with contrasting grazing traits regulated the dominance of a common toxic bloom-forming cyanobacterium (i.e., *Microcystis*) in a four-day laboratory experiment with artificial food webs. In the absence of grazers (i.e., controls), both *Microcystis* and its competitor *Cryptomonas* grew exponentially although the relative abundance of *Microcystis* increased slightly after four days due to higher growth rates. As predicted, the addition of the non-tolerant or tolerant clone of generalist grazing *Daphnia* to the co-cultured phytoplankton had no effect on the relative abundance of *Microcystis* compared to controls. Adding the selectively grazing copepod *E. gracilis*, however, increased the relative abundance of *Microcystis* compared to controls. Results support our hypothesis that the selective grazing copepod would facilitate cyanobacteria dominance (i.e., increase the M:C biomass ratio) while the generalist *Daphnia* would not. We believe these are unique results experimentally demonstrating the importance of zooplankton grazing traits as potential drivers of cyanobacterial dominance.

The results suggest that disproportionately higher grazing pressure on *Cryptomonas* by the copepod *E. gracilis* was the main driver for increasing the relative biomass of *Microcystis*. Compared to the two *Daphnia* clones, an equal biomass of *E. gracilis* cleared up to 3× more nutritious prey (*Cryptomonas*). Mass specific clearance on the toxic prey (*Microcystis*), however, was similar across all zooplankton treatments. Thus, as expected, the two *Daphnia* clones uniformly reduced the growth of both prey, indicating generalist grazing (REF), while the copepod reduced the nutritious prey more than the toxic one, indicating selective grazing (REF). That the copepod maintained a higher clearance rate on *Cryptomonas* vs. *Microcystis* even as the relative abundance of the former was declining further indicates selective grazing.

Positive selection is commonly observed in *E. gracilis* and calanoid copepods (DeMott 1986, DeMott and Moxter 1991, Ger et al. 2016) and is based on the remote

detection of chemical or mechanical cues before ingestion combined with individual handling of prey items (Svensen and Kiorboe 2000, Kiorboe 2011). Major cues for calanoid copepods that generate feeding currents, including *E. gracilis*, are prey size and chemistry (DeMott 1986, Rangel et al. 2016b). Copepods generally select larger sized prey within an optimal prey size range that scales with their body size (Demott 1986, Hanson et al. 1994, Sommer and Sommer 2006). Hence, the positive selection of *Cryptomonas* cells by *E. gracilis*, which are an order of magnitude larger than those of *Microcystis*, is likely due, at least partially, to size selection. Yet, prey chemistry, especially toxin content, may be more important than prey size for selective grazing calanoid copepods (DeMott et al. 1991). Indeed, the ingestion of identical sized *Microcystis* cells by *E. gracilis* was shown to be inversely proportional to the content of microcystin toxins (Ger et al. 2016). Moreover, ingestion of a smaller (3 μm) non-microcystin producing *Microcystis* strain was 3-fold higher than a larger (5 μm) microcystin producing strain by another calanoid copepod (*Notodiaptomus iheringi*) similar in body size to *E. gracilis* (Ger et al. 2016).

Considering this, it is likely that the avoidance and subsequent facilitation of *Microcystis* by *E. gracilis* was mainly driven by the toxicity of this cyanobacterium.

Prey toxicity, which reduces food quality, likely caused the difference in the mass-specific grazing rates among the copepod and the two *Daphnia* clones. Large generalist *Daphnia* like the ones used here are often reported to have higher mass specific grazing rates than copepods (Peters and Downing 1984, Miner et al. 2013, but see also Hansen et al. 1997). Therefore, the *Daphnia* treatments in our study might be expected to have higher herbivory rates than *E. gracilis*. In contrast, an equal biomass of *E. gracilis* ingested about 2 to 3 $\times$ more phytoplankton than the tolerant and non-tolerant *Daphnia* clones, respectively. Our results corroborate with the inhibitory effect of non-filamentous toxic cyanobacteria on cladocerans and suggest that *Daphnia* ingestion rate depends on the overall quality of available prey (Rohrlack et al. 1999, Lüring 2003). This is because the only option for *Daphnia* in the presence of toxic cyanobacteria is to reject ingestion of all prey items, including nutritious particles (Demott 1986). Grazing inhibition may also explain why our second hypothesis – that *Daphnia* would reduce cyanobacteria biomass more than

E. gracilis – was rejected. Moreover, despite the slightly higher clearance rates of the tolerant *Daphnia* on both prey species, the top-down effect of either *Daphnia* clone on total phytoplankton was similar. Although tolerance here was not quantified, any potential difference in tolerance among these clones had little effect on total herbivory. Consequently, inhibition also might explain why our third hypothesis (i.e., stronger top-down control with the tolerant *Daphnia*) was also rejected. In contrast, the higher total clearance rate of *E. gracilis* in this study corroborates previous reports of uninhibited grazing by this copepod in the presence of toxic *Microcystis* (Ger et al. 2011, 2016 HA). Taken together, our results suggest that 1) mass-specific clearance rates for selective grazing copepods can be higher than that of large *Daphnia* in the presence of toxic prey, and 2) selective grazing zooplankton may dominate overall herbivory in eutrophic aquatic ecosystems.

Our results highlight how zooplankton grazing traits can regulate phytoplankton community structure. Although top-down effects on phytoplankton communities have long been recognized (Porter 1977), most studies consider zooplankton body size – not grazing behavior – as the key variable (Gianuca et al. 2016). Indeed, body size is a master functional trait, controlling individual ingestion rate, prey size spectrum, and the potential to graze down cyanobacteria blooms, among others (Litchman et al. 2013, Hebert et al. 2016). In mesocosm experiments, increased cladoceran body size promoted the dominance of ‘inedible’ phytoplankton species that were larger than the prey size range of their generalist grazers due to passive size-based selection (Lynch 1980, Vanni 1987). In contrast, active cue-based selection by copepods operates within the grazer prey size range and is more efficient because it allows individual handling and rejection of prey items with little metabolic cost (DeMott 1986). Copepod prey selection has been linked to a weaker top-down effect compared to cladocerans (Sommer and Sommer, 2006) but its effect on the dominance of toxic phytoplankton has only been evaluated in modeling studies (Mittra and Flynn 2006, Scotti et al. 2015). The current results therefore build on the earlier predictions regarding the role of grazers promoting inedible phytoplankton (e.g., Porter 1977, Lynch 1980) by emphasizing active grazing selectivity – in addition to body size – as a major

zooplankton functional trait. The evaluation of zooplankton selectivity on the structure and function of plankton ecosystems merits further attention (Litchman et al. 2013, Hebert et al. 2016). This point is further underlined given future scenarios predicting warmer and more eutrophic conditions for freshwaters (Kosten et al. 2013), which are expected to increase the dominance of selective grazing zooplankton including copepods and rotifers (Jeppesen, Senepont Domis et al. 2013).

Whether copepods and other selective grazers can facilitate the dominance of toxic phytoplankton in nature is an important question highlighted by our results. Future studies, however, are necessary to verify the prevalence, persistence, and magnitude of grazer facilitated dominance of cyanobacteria and other harmful algae. For example, prey switching is a commonly observed behavior in copepods (Gasparini and Kastelt 1997, Kiørboe et al. 1997). If prey switching occurs, the selective avoidance and facilitation of cyanobacteria may be relaxed or even reversed. The stable ingestion rate of *E. gracilis* on *Cryptomonas* despite the decline in the relative abundance of this prey indicates no prey switching during our experiment. A previous and longer duration (i.e., 7-day) study also found no evidence of prey switching by copepods grazing on *Microcystis* (Leitão et al. 2018). Yet, the traits of copepods (e.g., tolerance, selectivity) and cyanobacteria (toxicity, morphology) are extremely diverse and we therefore expect a large variation in the interaction of these organisms (Ger et al. 2016).

Although our results provide novel evidence for the role of contrasting grazing traits (i.e., generalist vs. selective) on cyanobacterial dominance, further research is required before more specific generalizations can be made. Including independent replicates for each grazer trait – multiple species of selective vs. generalist grazing zooplankton – would enable a more rigorous test for the role of grazer selectivity suggested by the results. Likewise, future experiments involving cyanobacteria with contrasting defensive traits would help unravel the role of chemical vs. morphological defenses. Indeed, cyanobacteria have various morphological defenses that may limit both generalist and selective grazers (Gliwicz 1990, Rangel et al. 2016), which were not addressed here. For instance, not all

cyanobacteria are toxic and copepods are known to ingest large amounts of filamentous cyanobacteria (James & Forsyth, 1990, Engström et al. 2015, Hogfors et al. 2014, Motwani et al. 2017).

There are other factors that were not within the scope of this study but could affect grazer facilitation of toxic phytoplankton in nature. For example zooplankton can change the competitive interactions of primary producers via nutrient recycling (Lehman 1980, Sterner 1986) and also grazer specific stoichiometry, i.e., by altering elemental nutrient ratios available to phytoplankton (Sterner 1990, Elser and Hassett 1994). Given that *E. gracilis* selectively avoided *Microcystis* in our study, it is possible that this cyanobacterium also benefited from increased nutrient availability or altered nutrient ratio, in addition to the reduced abundance of its competitor. Yet, the nutrient rich medium used here can be expected to minimize any limitation of this resource for either phytoplankton species during the experiment. Indeed, both *Microcystis* and *Cryptomonas* grew at relatively high rates in the absence of grazers, providing further evidence that nutrient limitation was unlikely in the current setup. Copepod selection for edible phytoplankton, however, highlights the potential for selective grazing zooplankton to further benefit cyanobacteria by regenerating nutrients, especially when nutrients are limiting (Mitra and Flynn 2006). Thus, how nutrient limitation affects top-down grazer facilitation of cyanobacterial dominance is an important line for future work.

The use of artificial foodwebs to test the effect of grazers on the facilitation of cyanobacterial dominance provides a useful method that can be extended to compare the interactions across multiple species, clones, and functional traits. Such experiments with controlled conditions and a simplified complexity of interacting species are necessary for isolating the role of specific traits of grazers and prey on phytoplankton community structure. Natural plankton communities, however, are complex and include multiple co-existing competitors and heterotrophic protozoans that graze on phytoplankton or phytoplankton parasites, either of which can be grazed upon by meta-zooplankton (Engström et al. 2002, Bec et al. 2006, Agha et al. 2016). Thus, testing the hypothesis that grazer selectivity can facilitate cyanobacterial dominance would also benefit from mesocosm studies with natural

plankton communities. Indeed, increased concentrations of natural zooplankton communities dominated by copepods have been shown to promote the dominance of bloom forming cyanobacteria (Wang et al. 2010, Hong et al. 2015). Yet, the role of specific grazing traits is obscured in such studies due to multiple interacting zooplankton with contrasting traits. Controlling the abundance of specific species with known grazing traits would enhance mechanism-based understanding in future mesocosms. Taken together, both laboratory and field experiments are needed to further evaluate how grazer traits may facilitate blooms of cyanobacteria.

The current results also have implications for the methods used to quantify phytoplankton abundance in the current setup. The slope of unity and tight correlation among flow cytometry vs. Phyto-Pam for quantifying the relative abundance (i.e., biomass ratio) of *Microcystis* vs. *Cryptomonas* suggests that both methods worked equally well in our experiment. Though overall patterns in phytoplankton growth was also similarly estimated by either method, Phyto-Pam underestimated *Microcystis* growth by about 30% compared to flow cytometry, suggesting that growth estimates may be improved by complementing measurements made via specific pigment concentrations with electronic particle counting.

In conclusion, results confirmed the hypothesis that the selective grazing copepod *E. gracilis* would facilitate the dominance of toxic cyanobacteria, while the generalist grazing *Daphnia magna* would not. Hence, the facilitation of inedible or toxic primary producers by selective herbivores appears to be likely in eutrophic pelagic ecosystems. Moreover, mass specific total herbivory was up to 3-fold higher for copepods when compared to *Daphnia*, which contrasts predictions that the latter dominates specific herbivory rates (Peters and Downing 1984, Miner et al. 2013), and instead supports predictions that copepods may have higher mass-specific ingestion rates than large cladocerans (Hansen et al. 1997), at least in the presence of toxic phytoplankton. Overall, our results emphasize grazing selection as a potentially key trait regulating herbivory and the phytoplankton community composition. Thus, in addition to the master trait of organism size (Kruk et al, Litchman et al. 2013, Colina et al. Hebert et al.), grazing selectivity emerges as an

important zooplankton functional trait. Although more work is necessary to generalize these conclusions (as detailed above), the results have implications for the structure and function of eutrophic waters where selective grazers are expected to dominate zooplankton communities.

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Table 1: Summary statistics for the effect of each zooplankton treatment on the log-transformed biomass ratio of *Microcystis:Cryptomonas* on the final day of the experiment relative to the no grazer controls as evaluated by a generalized linear model.

Factor	Estimate	<i>t</i> value	<i>p</i>
Intercept	-1.556	0.246	<0.001
Non-tolerant <i>Daphnia</i>	-0.065	0.325	0.845
Tolerant <i>Daphnia</i>	-0.613	0.325	0.089
Copepod	0.912	0.348	0.026

Figure 1: The abundance ($1000 \times \text{cell mL}^{-1}$) of a) *Cryptomonas* and b) *Microcystis* co-cultured in no-grazer controls (C) and treatments with zooplankton representing contrasting functional traits including non-tolerant *Daphnia* (NT), tolerant *Daphnia* (TD), and the copepod *E. gracilis* (Cop) during the experiment. Lines link the daily mean value for each treatment and shaded areas show the 95% confidence intervals.

Figure 2: The mean final biomass ($\mu\text{gC L}^{-1}$) of co-cultured *Microcystis* (a), *Cryptomonas* (b), and their sum (c) at the end of 4-day grazing experiment compared across the zooplankton treatments (ND: naïve *Daphnia*, TD: tolerant *Daphnia*, Cop: *E. gracilis*) and the no-grazer control. Error bars show 95% confidence intervals letters represent significant differences based on an HSD Tukey test ($p < 0.05$).

Figure 3: Mean net growth of *Microcystis* and *Cryptomonas* co-cultured with the zooplankton treatments (ND: naïve *Daphnia*, TD: tolerant *Daphnia*, Cop: *E. gracilis*) and in no-grazer controls during the 4-day experiment. Error bars show 95% confidence intervals and letters represent significant differences based on an HSD Tukey test ($p < 0.05$).

Figure 4: The biomass ratio of *Microcystis*:*Cryptomonas* during the 4-day experiment in no-grazer controls and the zooplankton treatments (ND: non-tolerant *Daphnia*, TD: tolerant *Daphnia*, Cop: the copepod *E. gracilis*). Lines link the daily mean value for each treatment and shaded areas show the 95% confidence intervals.

Figure 5: Mean dry weight specific clearance rate ($\text{mL mg}^{-1} \text{h}^{-1}$) on *Microcystis* (CR_M), *Cryptomonas* (CR_C), and the total of these two prey (CR_T), measured from the start to a given day of the experiment for each zooplankton treatment (ND: non-tolerant *Daphnia*, TD: tolerant *Daphnia*, Cop: the copepod *E. gracilis*) Bars show the 95% confidence intervals.

Appendix Table 1: Mean individual rates of clearance (CR: mL ind⁻¹ hour⁻¹) or ingestion (IR: ngC ind⁻¹ hour⁻¹) compared between *E. gracilis* and the two clones of *D. magna* (ND: non-tolerant *Daphnia*, TD: tolerant *Daphnia*, Cop: *E. gracilis*) as measured over each time period (days). Grazing rates are for *Microcystis* (CR_M or IR_M) or *Cryptomonas* (CR_C or IR_C). Values in parenthesis show the standard deviation for each measurement.

Zooplankton	CR _M	CR _C	IR _M	IR _C	Time
ND	1.55 (1.26)	5.65 (8.22)	47.09 (39.8)	1644 (2180)	0-1
	1.52 (1.33)	4.51 (2.79)	57.41 (48.8)	1157 (432)	0-2
	3.45 (3.12)	3.43 (2.47)	198.7 (166)	1313 (770)	0-3
	3.72 (2.32)	4.64 (2.55)	357.2 (144)	2808 (721)	0-4
TD	3.37 (3.48)	10.28 (3.41)	95.46 (99.4)	2586 (349)	0-1
	3.13 (2.95)	8.84 (3.14)	106.9 (94.1)	2541 (810)	0-2
	4.16 (2.52)	8.19 (2.73)	183.5 (96.6)	2510 (541)	0-3
	6.89 (4.55)	4.22 (1.16)	306.1 (90.5)	2405 (1110)	0-4
Cop	0.04 (0.07)	0.56 (0.27)	1.38 (2.40)	142.6 (70.4)	0-1
	0.13 (0.11)	0.78 (0.17)	4.96 (4.45)	151.6 (46.3)	0-2
	0.18 (0.11)	0.54 (0.11)	8.48 (4.52)	124.7 (30.2)	0-3
	0.20 (0.08)	0.41 (0.02)	13.16 (4.08)	127.2 (31.7)	0-4

Appendix Figure 1: Mean dry weight specific ingestion rate (ngC mg⁻¹ h⁻¹) on *Microcystis* (IR_M), *Cryptomonas* (IR_C), and the total of these two prey (IR_T), measured from the start to a given day of the experiment for each zooplankton treatment (ND: non-tolerant *Daphnia*, TD: tolerant *Daphnia*, Cop: the copepod *E. gracilis*) Bars show the 95% confidence intervals.

Appendix Figure 2: Relationship between phytoplankton growth rate estimated via flow cytometry versus the Phyto-Pam fluorometer for (a) *Cryptomonas* (slope = 0.921 ± 0.165 CI, $p < 0.001$, Adjusted $R^2 = 0.901$) and (b) *Microcystis* (slope = 0.673 ± 0.148 CI, $p < 0.001$, Adjusted $R^2 = 0.836$). The grey shaded area indicates the 95% confidence interval fitted with a linear regression.

Appendix Figure 3: Relationship between the biomass ratio of *Microcystis:Cryptomonas* estimated by flow cytometry versus the Phyto-Pam fluorometer. The grey shaded area indicates the 95% confidence interval fitted with a linear regression (slope = 1.169 ± 0.121 CI, $p < 0.001$, Adjusted $R^2 = 0.847$).