Limnol. Oceanogr., 56(5), 2011, 1714–1724
© 2011, by the American Society of Limnology and Oceanography, Inc.
doi:10.4319/lo.2011.56.5.1714

Large variation in vulnerability to grazing within a population of the colonial phytoplankter, *Microcystis aeruginosa*

Jeffrey D. White,[a,*] RajReni B. Kaul,[b] Lesley B. Knoll,[c] Alan E. Wilson,[b] and Orlando Sarnelle[a]

[a] Department of Fisheries and Wildlife, Michigan State University, East Lansing, Michigan
[b] Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama
[c] Department of Zoology, Miami University, Oxford, Ohio

Abstract

We asked if intraspecific variation within a population of the toxic, bloom-forming phytoplankter, *Microcystis aeruginosa*, leads to differential vulnerability to grazing by the invasive, filter-feeding zebra mussel (*Dreissena polymorpha*). We performed two series of laboratory feeding experiments in which *D. polymorpha* were presented with several different sympatric *M. aeruginosa* clones in a two-species mixture with the high-quality, nutritious alga, *Ankistrodesmus falcatus*. Mean selectivity across *M. aeruginosa* clones ranged from near zero (i.e., no feeding on *M. aeruginosa*) to near one (equal feeding on the two algal species), evidence of maximal variation in grazing vulnerability across twenty clones of *M. aeruginosa*—a species assumed to be largely 'inedible' to grazers—from the same population. This range of vulnerability is essentially equal to that typically measured across all phytoplankton species. Large intraspecific variation in grazing vulnerability, and its ecological consequences, could influence the promotion or control of noxious blooms of toxigenic cyanobacteria.

Vulnerability to grazing is an ecological trait that critically influences the effect of consumers and trophic cascades on phytoplankton biomass, production, and species composition. Traditionally, phytoplankton species and even entire taxonomic groups have been classified as 'inedible' or 'resistant' to differentiate them from those that are 'edible' or otherwise more readily grazed (Agrawal 1998). These broad, often dichotomous, categories are typically based on algal size, morphology, digestibility, and production of compounds with putative herbivore-deterring properties (Porter 1973; Fulton and Paerl 1987; Jungmann and Benndorf 1994). A great deal of research has been directed toward elucidating interspecific differences in the vulnerability of phytoplankton taxa to grazing (Lehman and Sandgren 1985; Knisely and Geller 1986), although little is known about the range of susceptibility within any single species. Populations of some phytoplankton species are known to harbor large genetic and phenotypic variation (Rynearson and Armbrust 2000; Wilson et al. 2005; Logares et al. 2009), yet with the exceptions of grazer- and environmentally induced plasticity (van Gremberghe et al. 2009*a*; Van Donk et al. 2011), this intraspecific variation is generally ignored when classifying species with respect to grazing vulnerability. Recently, ecologists have begun to recognize that single species can exhibit a range in susceptibility to consumption by a single consumer, complicating the traditional edible vs. inedible categorizations (Long and Hay 2006; Vanormelingen et al. 2009).

Here, we explore the range in grazing vulnerability of the colonial cyanobacterium, *Microcystis aeruginosa. M. aeruginosa* is notorious for producing unsightly, toxic surface blooms (HABs, or harmful algal blooms) in eutrophic freshwaters, and so is of major importance with respect to water quality. *M. aeruginosa* is also routinely classified as inedible with respect to filter-feeding zooplankton, due to its colonial morphology and production of a suite of toxic compounds, including microcystins (Fulton and Paerl 1987; Agrawal 1998). In this paper, we examine the vulnerability of *M. aeruginosa* to a benthic filter-feeder, the invasive zebra mussel, *Dreissena polymorpha*. *D. polymorpha* continues to expand its range throughout North America with dramatic consequences for invaded ecosystems. In particular, the dreissenid invasion has yielded highly variable responses of *M. aeruginosa* biomass, with positive responses in some systems and negative responses in others (Smith et al. 1998; Vanderploeg et al. 2001; Raikow et al. 2004). Such opposing responses of *M. aeruginosa* to mussel invasion motivated our examination of intraspecific variation in the vulnerability of *M. aeruginosa* to dreissenid grazing.

Recent studies of *M. aeruginosa* have revealed marked genetic and phenotypic variability, both within (Kardinaal et al. 2007; Dyble et al. 2008) and across (Wilson et al. 2005; Martins et al. 2009; Tanabe et al. 2009) populations in traits such as morphology and toxicity. Intraspecific variation, in concert with grazing pressure, has been shown to drive both competition and facilitation among coexisting genotypes (van Gremberghe et al. 2009*b*). This could affect the composition and toxic properties of local *M. aeruginosa* populations and, therefore, HAB dynamics (Burkholder and Glibert 2009). Although variability in vulnerability to grazing might be expected across populations of *M. aeruginosa*, given documented variation in colony size and toxicity, we are aware of no studies that have quantified this variability within a single population of phytoplankton. Our experiments were motivated by two questions: do clones of *M. aeruginosa* isolated from a single lake vary in their vulnerability to grazing by *D. polymorpha*, and if so, how does the range of vulnerability within this one population of *M. aeruginosa* compare to the range of variation that corresponds to traditional edible–inedible classifications across phytoplankton species?

* Corresponding author: whitej82@msu.edu

Methods

We conducted two series of feeding experiments with *D. polymorpha* and *M. aeruginosa*. The first set, run in 2001, was aimed at both screening a large number of co-occurring *M. aeruginosa* genotypes for variation in vulnerability to *D. polymorpha* and comparing the vulnerabilities of these recently isolated, colonial clones against single-celled clones purchased from a culture collection. A second set of feeding experiments, conducted in 2010, more thoroughly quantified the magnitude of the range in vulnerability across recently isolated, colonial clones, while also exploring potential explanatory correlates of vulnerability.

Collection and maintenance of organisms—Zebra mussels were collected from the littoral zone of Gull Lake, Michigan (Barry and Kalamazoo counties; summer total phosphorus ~ 10 μg L^{-1}). Gull Lake was invaded by zebra mussels in 1994 and, thereafter, experienced an increase in *M. aeruginosa* abundance (Sarnelle et al. 2005). Mussels were removed from rocks by cutting the byssal threads with a razor, and gently scrubbed clean of periphyton and marl. Mussels with valve lengths of 17–19 mm were retained for use in experiments. Mussels were acclimated to room temperature (~ 20°C) and fed a diet of *Ankistrodesmus falcatus* (~ 4 μg L^{-1} chlorophyll *a* [Chl *a*]), a high-quality alga on which mussels filter at rates generally comparable to published maximal filtration rates (Kryger and Riisgård 1988).

Colonies of *M. aeruginosa* were collected from the mixed layer of Gull Lake with horizontal tows of a 100-μm-mesh zooplankton net (2001 experiments) or with a depth-integrating tube sampler (2010 experiments). Establishment of new clones for the 2010 experiments was necessitated by the loss of clones used in 2001 due to the tendency of *M. aeruginosa* to lose its natural colonial morphology after an extended period of time in culture. Clones were isolated by pipetting individual colonies sequentially through a series of well plates containing sterile deionized water (2001 experiments) or sterile growth medium (2010 experiments), prior to being transferred into individual test tubes of BG-11 medium (2001 experiments) or 0.5× WC-S growth medium (2010 experiments). A typical success rate for Gull Lake colonies isolated into the latter medium is about 80% (O. Sarnelle pers. obs.), much higher than for BG-11 (Wilson et al. 2005). Gull Lake clones were designated according to the year of isolation followed by an arbitrary letter (e.g., 2000K).

Successful clones were maintained in batch cultures of 0.5× WC-S, with an inoculum of culture transferred to fresh, sterile media every 4–8 weeks. *A. falcatus* was grown in semi-continuous culture in full-strength WC medium. Phytoplankton were cultured at 20°C under fluorescent lights at 70 μmol m^{-2} s^{-1} on a 12 : 12 h light : dark cycle. In all of our experiments, mussels were fed colonial Gull Lake clones that were in culture for $<$ 4 yr (Table 1).

Feeding experiments—The basic design of the feeding experiments was similar in 2001 and 2010, with methodological differences as noted below. Two mussels were allowed to feed for 0.5 h on a 0.5-liter suspension (2001) or for 1 h on a 0.9-liter suspension (2010). Mussel shell length did not statistically differ across treatments within any experiment (ANOVA, $p > 0.20$; 2001 range = 16.7–17.6 mm, 2010 range = 17.9–19.1 mm). All experiments were conducted in 1-liter glass beakers. Immediately prior to a feeding experiment, mussels were allowed to clear their guts for 30 min (2001) or 3 h (2010), by holding them in Gull Lake water that had been filtered through a cartridge filter with a nominal particle retention of 1 μm (hereafter, referred to as 'filtered lake water'; Chl *a* reduced by ~ 98%). Feeding suspensions were created by adding targeted amounts of *M. aeruginosa* and *A. falcatus* from exponentially growing cultures to filtered lake water. Targeted total biomass in the feeding suspensions was 5 μg Chl *a* L^{-1}, which is within the natural range encountered by mussels in Gull Lake (J. White unpubl.).

Table 1. Sampling and isolation information for all Gull Lake *Microcystis aeruginosa* clones employed in the 2001 and 2010 experiments.

Clone	Sample date	Isolation medium	Experiment
2000JN19	19 Jun 2000	BG-11	2001
2000B–2000S	29 Jun 2000	BG-11	2001
2000AG23	23 Aug 2000	BG-11	2001
2006A, 2006B	05 Sep 2006	0.5× WC-S	2010
2008C	25 Jul 2008	0.5× WC-S	2010
2009A, 2009C	23 Jul 2009	0.5× WC-S	2010

Beakers were sampled for algal cell counts (preserved in 1% Lugol's iodine), and in some cases also for Chl *a* (Pall A/E glass fiber filters), immediately before mussels were added (initial) and immediately after mussels were removed (final). Beakers were stirred before taking final samples to resuspend any pseudofeces that could contain undigested cells, which might remain viable (Vanderploeg et al. 2001). Thus, measured filtering rates represent mortality rates inflicted by mussels on the phytoplankton in the beakers. Mussels were observed for the initiation of feeding (siphons fully extended), which marked the start of the feeding period (time 0). Mussels typically began filtering within 5 min of placement into beakers. Beakers were gently aerated during all experiments, which succeeded at keeping phytoplankton in suspension and deprived buoyant *Microcystis* of any spatial refuge from mussels. In 2001, we usually employed four replicate beakers with mussels (in two cases, only two and three beakers were employed) and two control beakers lacking mussels for each feeding suspension. Given that we observed no significant changes in cell densities within control beakers in 2001 (paired *t*-test, $t = -0.41$, df = 17, $p > 0.80$), we employed five replicates with mussels and one control in 2010.

In 2001, mussels were not acclimated to specific feeding suspensions, although of course, all experimental mussels were exposed to the *M. aeruginosa* population in Gull Lake prior to being collected. In 2010, mussels were acclimated to the specific feeding suspensions they would experience in the experiment for 24 h before each experiment (Dionisio Pires and Van Donk 2002). We conducted a test of the effect of acclimation time on mussel filtering rates in 2010

using a colonial Gull Lake clone of *M. aeruginosa* that was shown to be consumed by mussels in preliminary experiments. We observed no significant change in filtering rates (ANOVA, $p > 0.90$, $n = 20$) with increasing acclimation time (0 h, 12 h, 24 h, 48 h).

Most of our experiments were conducted as 'particle-choice' assays, with an individual *M. aeruginosa* clone supplied in a two-species mixture with *A. falcatus*, with a targeted biomass composition of 80% *M. aeruginosa*. Particle-choice experiments, although more time-consuming to analyze, more closely resemble nature, where grazers can choose between more than one type of particle. In addition, particle-choice experiments facilitate more precise comparisons across treatments and experiments, because filtering rates on the particles of interest (the *M. aeruginosa* clones) are scaled to filtering rates on a standardized particle (*A. falcatus*), via calculation of a selectivity index (*see* below).

In 2001, we assayed selectivity for 12 colonial, Gull Lake *M. aeruginosa* clones and 3 single-celled clones from the Pasteur Culture Collection (designated with PCC followed by a number; also grown in 0.5× WC-S medium) via a series of particle-choice experiments. We were primarily interested in examining variation in grazing vulnerability that was not a function of colony size, so each colonial *M. aeruginosa* culture was sieved through 100-μm mesh to increase the likelihood that all colonies were within the edible size range for mussels (Horgan and Mills 1997).

In 2010, we conducted two preliminary 'no-choice' assays with eight newly isolated, colonial Gull Lake *M. aeruginosa* clones to identify a subset that seemed to vary greatly in vulnerability to mussel consumption. These assays consisted of feeding mussels unialgal suspensions of each clone, and monitoring Chl *a* depletion over 1 h. We also included beakers with mussels and 100% *A. falcatus* for comparison. On the basis of these preliminary assays, we selected five clones for inclusion in the 2010 particle-choice experiment.

Sample and data analysis—For each initial and final sample from the experimental beakers, a subsample was settled in a 10-mL settling chamber and *A. falcatus* cells counted at 200× on an inverted microscope (Nikon Eclipse TE2000-S). The subsample was then treated with 1 mol L^{-1} sodium hydroxide to dissolve the colony matrix of *M. aeruginosa*, resettled, and individual *M. aeruginosa* cells counted at 400×. Species-specific filtering rates (L individual^{-1} d^{-1}) were calculated for each beaker as: $F = [(\ln C_i - \ln C_f)/t] \times V/N$, where C_i and C_f are initial and final cell densities (cells L^{-1}) respectively, t is the length of the feeding period (d), V is the volume of the suspension (L) and N is the number of mussels in the beaker. This calculation assumes that there are no changes in cell densities in control beakers lacking mussels. In both the 2001 (paired *t*-test, $t = -0.41$, df = 17, $p > 0.80$) and 2010 (paired *t*-test, $t = 0.84$, df = 4, $p > 0.40$) experiments, we detected no changes in cell densities or Chl *a* in control beakers over the course of the feeding incubations, validating our use of a calculation that ignores changes in the control. Selectivity was calculated for each beaker as $F_m : F_a$, where F_m and F_a are the respective filtering rates on the *M. aeruginosa* clone and *A. falcatus*.

Biovolume of cultured algae was determined via cell counts and measures of cell dimensions, and converted to dry biomass assuming a specific gravity of 1.0 and a wet-to-dry biomass conversion factor of 0.4 (O. Sarnelle unpubl.). Chl *a* was measured via dark extraction of filters in cold 95% ethanol for 24 h, followed by fluorometric analysis (Turner Designs).

We tested for differences in mean feeding selectivity across *M. aeruginosa* clones within each of the two series of experiments with one-way ANOVAs. If the ANOVA revealed significant differences among clones, we then compared mean selectivities for each clone to the null mean of 1 (indicating no preference) using one-tailed *t*-tests. For clones showing significant selection against *M. aeruginosa* ($F_m : F_a < 1$), we further tested whether mussel filtering rates on *M. aeruginosa* were > 0 using one-tailed *t*-tests. We used one-tailed tests because the a priori expectations are for mussels to prefer *A. falcatus* when they are selective ($F_m : F_a \leq 1$), and to have nonnegative filtering rates. Two beakers from two different treatments in the 2010 particle-choice experiment were omitted from data analyses. In one case, mussels were not actively filtering during the experiment. The other case was an unexplained but severe outlier relative to the other 23 beakers (studentized residual > 6).

Characterization of M. aeruginosa *clones used in the 2010 experiments*—Genetic characterizations were conducted to determine whether all five clones of *M. aeruginosa* (as defined by microscopy) employed in the 2010 particle-choice experiment (1) conformed to existing molecular-based definitions of this genus, (2) possessed the *mcyB* gene needed for the biosynthesis of microcystin, and (3) were genetically unique. Samples from each *M. aeruginosa* clone in culture were pelleted for molecular analysis in 0.2-mL polymerase chain reaction (PCR) tubes, resuspended in sterile deionized water, then pelleted and dried before storing at −20°C. To extract deoxyribonucleic acid (DNA), pelleted cells were resuspended in 10 μL Lyse-N-Go PCR reagent (Pierce Chemical) and heated to 95°C for 5 min. Clones were screened with the *Microcystis*-specific 16S rDNA primer pair Micr184F/431R (Neilan et al. 1997). The cyanobacterial-specific 16S rDNA primer pair 27F/809R was used as a positive control (Jungblut et al. 2005). Toxigenic potential was determined by positive amplification of the *mcyB* gene (Dyble et al. 2008). All PCR products were visualized using 1.5% agarose gels. Cultures were typed using five housekeeping loci (*ftsZ*, *glnA*, *pgi*, *gltX*, and *gyrB*) for a multilocus-like sequence approach. Loci were amplified using primers and PCR conditions described by Tanabe et al. (2007). Multilocus sequence typed (MLST) PCR products were purified using a Qiagen QIAquick PCR purification kit (Catalogue No. 28104) and sequenced bidirectionally using a 3730 Genetic Analyzer (Applied Biosystems). Consensus sequences were assembled using Sequencher 4.10.1 (Gene Codes Corporation). Aligned concatenated sequences of the housekeeping loci were used to create an Unweighted Pair Group Method

with Arithmetic Mean clustered similarity matrix and corresponding tree (Bionumerics version 6.5, Applied Maths).

We also characterized the 2010 clones with respect to morphology, microcystin quota, and Chl *a* quota. Morphological traits were measured by capturing images of $>$ 30 Lugol's-preserved *M. aeruginosa* colonies and then single cells (following dissolution of the colonies, *see* above) per clone with a digital camera connected to an inverted microscope. Colony surface area, colony perimeter, and cell diameters were obtained from the two-dimensional images using digital imaging software (Spot Advanced, Diagnostic Instruments). Colony size was expressed as equivalent diameter (ED), a more relevant measure than surface area with respect to size-selective feeding, by solving for the diameter of a circle with surface area equal to that of the measured colony. *M. aeruginosa* colony morphology ranges from nearly spherical to highly dissected; therefore, we also calculated a development-ratio index to quantify the extent to which the colony perimeter deviated from that of a circle with equivalent surface area (e.g., a metric for colony 'shape'). Microcystin quota (mg g^{-1}dry biomass) was quantified during exponential growth of batch cultures by enzyme-linked immunosorbent assay (ELISA; Envirologix QuantiPlate for Microcystins) and concomitant measurement of dry biomass. Samples were taken from cultures once per week for 5 weeks; mean microcystin quota for each clone was determined over 3 weeks of exponential growth. Microcystin was extracted from cells collected on A/E filters using 75% methanol. Extracts were diluted 1 : 30 with deionized water before loading onto an ELISA plate. Along with microcystin quota, we quantified the Chl *a* quota (mg g^{-1} dry biomass) for each clone, using the fluorometric analysis described above.

Results

2001 experiments—Microcystis ranged from 74.4% (SE = 0.75) to 93.8% (SE = 0.42) of total algal biomass in the feeding suspensions containing colonial Gull Lake clones, but selectivity for the colonial clones was independent of percent *M. aeruginosa* biomass (linear regression, $R^2 <$ 0.01, $p = 0.98$). Filtering rates on *M. aeruginosa* clones in the 2001 experiments tended to be similar to filtering rates on *A. falcatus*, but mean selectivity did vary significantly among the 12 Gull Lake clones (ANOVA, $p < 0.05$, $n =$ 48). Mean selectivity for the Gull Lake clones varied from 1.15 (SE = 0.12, not different from 1, $p > 0.10$) for clone 2000C to 0.29 (SE = 0.12, not different from 0, $p > 0.45$; Fig. 1) for clone 2000K, clones that were isolated from the lake on the same date. Three clones were significantly selected against by mussels (2000Q, 2000D, and 2000K; one-tailed *t*-test, $F_m : F_a$ significantly < 1, $t = -4.0$, df = 3, $p < 0.02$), with low selectivities driven by relatively low filtering rates on *M. aeruginosa*, not unusually high filtering rates on *A. falcatus* (Fig. 1), although mean filtering rates on the two algal species were positively correlated (Pearson correlation coefficient = 0.78, $p < 0.001$). Mussels filtered single-celled culture collection clones at rates comparable to rates for *A. falcatus* ($F_m : F_a$ not different from 1, $p >$ 0.06; Fig. 1). For these clones, selectivity was unrelated to microcystin production, because PCC7813 and PCC7820 produce microcystin, whereas PCC1450 does not (Vanderploeg et al. 2001).

2010 experiments—Two preliminary no-choice feeding assays in 2010 identified colonial Gull Lake clones for which mussel filtering rates varied significantly (ANOVA, p < 0.001, $n = 30$ and $n = 25$) and ranged from near maximal (mean = 4.51 L d^{-1}, SE = 0.34; i.e., similar to filtering rates on *A. falcatus*) to near 0 (mean = -0.10 L d^{-1}, SE = 0.29). These results informed our selection of clones for the particle-choice experiment.

M. aeruginosa constituted between 78.2% (SE = 0.91) and 91.9% (SE = 0.91) of total algal biomass, on average, in the particle-choice experiment, but again selectivity was not related to percent *M. aeruginosa* biomass in the feeding suspensions (linear regression, $R^2 = 0.11$, $p > 0.50$). Selectivity differed significantly across clones in the particle-choice experiment (ANOVA, $p < 0.03$, $n = 23$; Fig. 2). Mussels exhibited significant selection against clones 2008C and 2006A (one-tailed *t*-test, $F_m : F_a$ significantly < 1, $t = -3.4$, df = 4, $p < 0.03$), and in fact, filtering rates on these two clones of *M. aeruginosa* were not significantly > 0 (one-tailed *t*-test, $t = -1.2$, df = 4, $p >$ 0.1). Examination of the filtration rates for each species revealed that variation in selectivity among *M. aeruginosa* clones was driven by variation in mussel filtering rates on *M. aeruginosa*, not *A. falcatus* (Fig. 2). Filtering rates on *M. aeruginosa* differed across treatments (ANOVA, $p <$ 0.01, $n = 23$), whereas filtering rates on *A. falcatus* did not (ANOVA, $p > 0.1$, $n = 23$). Further, filtering rates on *A. falcatus* ($\sim$ 4–6 L individual^{-1} d^{-1}) were similar to reported maximum filtration rates for mussels of the size used in our experiment (Kryger and Riisgård 1988).

Using chlorophyll : carbon ratios determined for *M. aeruginosa* (Raps et al. 1983) and *A. falcatus* (O. Sarnelle unpubl.), we estimate total mean algal carbon to have been 52 μg C L^{-1} in the 2010 particle-choice experiment. This food quantity falls well within the linear range of the zebra mussel functional response to increasing food concentrations ($<$ 2.0 mg C L^{-1}; Walz 1978).

We checked for a relationship between time since initial clone isolation from Gull Lake (varying from 1 yr to 4 yr) and mussel selectivity to rule out legacy effects that might vary among clones due to the different lengths of time spent in culture in the laboratory, such as those potentially imposed by more recent contact with grazers (Lakeman et al. 2009). The relationship was not significant (linear regression, $R^2 = 0.43$, $p > 0.20$).

Characterization of M. aeruginosa *clones used in the 2010 experiments*—All of our colonial clones conformed to existing morphological criteria for *M. aeruginosa*. However, the existence of large morphological plasticity within and low nucleic acid diversity among many morphologically defined species of *Microcystis* (as determined by 16S rDNA hybridization, which is normally used to define prokaryotic species of the *Microcystis* genus) has prompted their unification under *M. aeruginosa* (Kondo et al. 2000;

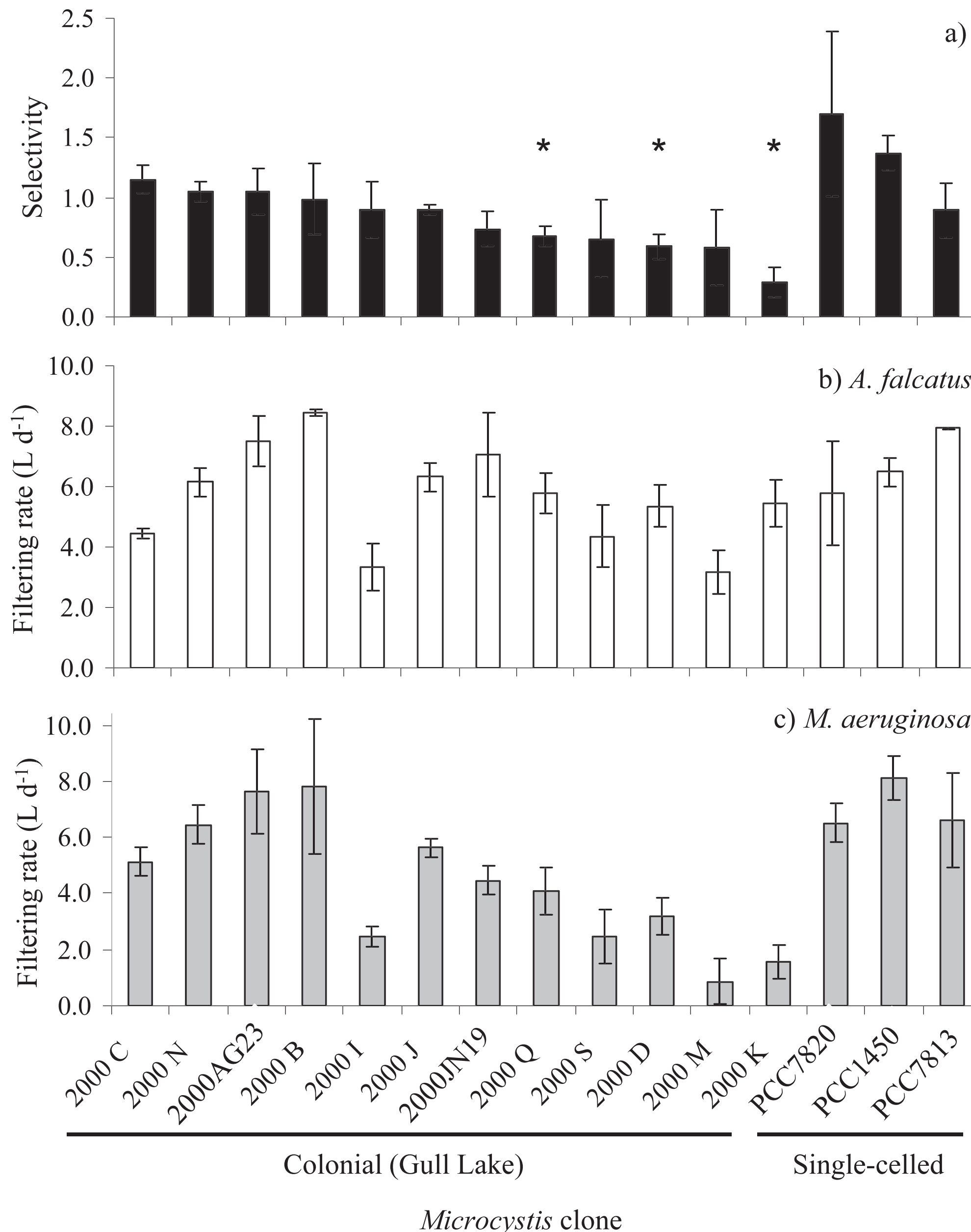

Fig. 1. (a) Selectivity by zebra mussels for different clones of *Microcystis aeruginosa* in the 2001 particle-choice experiments. All treatments contained colonial Gull Lake clones (n = 48), except for the three single-celled clones (CC7820, CC1450, and CC7813), which were included for comparative purposes. Selectivity was calculated as the ratio of the filtering rate on *M. aeruginosa* and the filtering rate on the standard food alga, *Ankistrodesmus falcatus.* Bars are mean selectivity $\pm$ standard error (SE). Asterisks indicate clones for which significant avoidance was detected (selectivity significantly < 1). (b, c) Comparison of the filtering rates (L d^{-1} per individual mussel) on *A. falcatus* and *M. aeruginosa*, respectively; bars are mean $\pm$ SE. Filtering rates were calculated from algal cell counts before and after incubation with mussels.

Otsuka et al. 2001). Therefore, species classifications based on both molecular and morphological criteria are more robust. All clones used in the 2010 particle-choice experiment were identified as *Microcystis* from 16S rDNA PCR amplification according to methods outlined in Neilan et al. (1997). All clones were found to be genetically unique (all were $\geq$ 20% dissimilar) based upon sequencing of five housekeeping loci (Fig. 3). Sequences from these five genes (*ftsZ*, *glnA*, *gltX*, *gyrB*, and *pgi*) were deposited in GenBank under accession numbers HQ847833–HQ847857. All clones produced positive amplification of the *mcyB* gene and were, therefore, genetically capable of synthesizing microcystin. Clones employed in the 2010 experiments are highly related to *M. aeruginosa* multilocus-typed

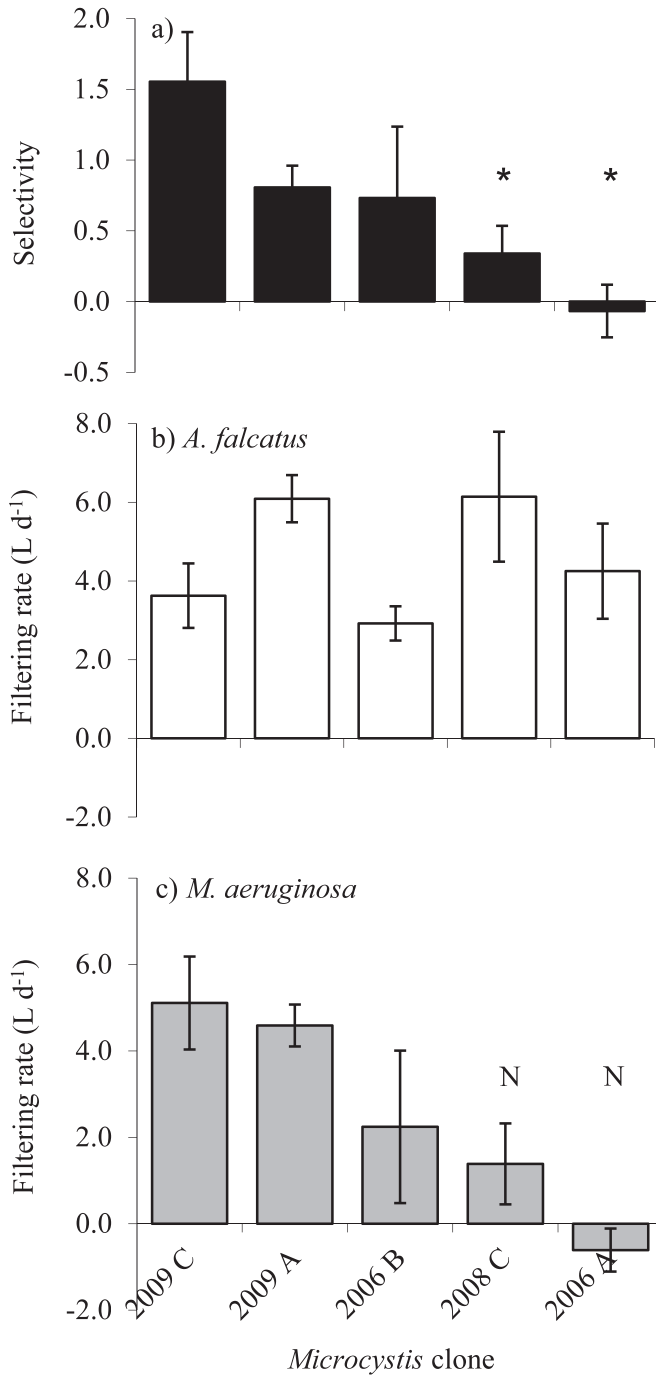

Fig. 2. (a) Selectivity by zebra mussels for different colonial clones of Gull Lake *Microcystis aeruginosa* in the 2010 particle-choice experiment ($n = 23$). Selectivity was calculated as in Fig. 1. Bars are mean selectivity ± standard error (SE). Asterisks indicate clones for which significant avoidance was detected (selectivity significantly < 1). (b, c) Comparison of the filtering rates (L d^{-1} per individual mussel) on *A. falcatus* and *M. aeruginosa*, respectively; bars are mean ± SE. Filtering rates were calculated from algal cell counts before and after incubation with mussels. An N indicates nondetectable filtration of *M. aeruginosa* (filtering rate not significantly different from 0).

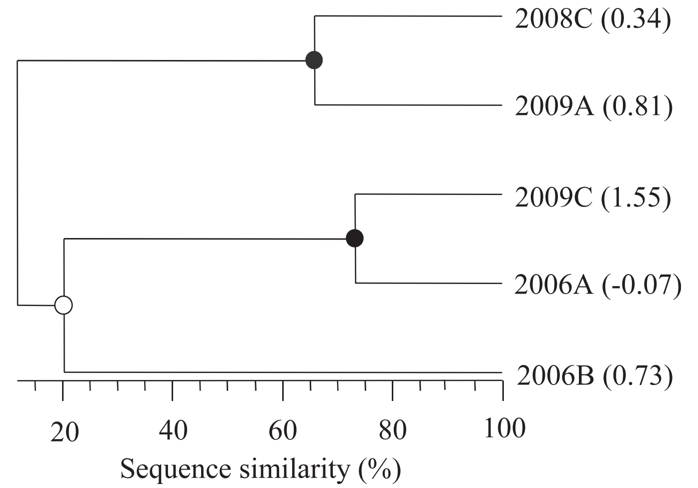

Fig. 3. Phylogenetic tree of *Microcystis aeruginosa* clones used in the 2010 particle-choice experiment, based on five concatenated housekeeping genes. Tree nodes are placed along the x-axis based on nucleotide polymorphism; allelic divergence between clones is proportional to branch lengths to the right of the node. Closed nodes have significant (> 90) statistical support based on resampling of variable sites. Similarity of clones was well within our detection limit of 97% similarity based on polymorphic sites. Clone vulnerability to zebra mussel grazing (denoted by the selectivity index in parenthesis) does not cluster according to multilocus sequence typing phylogenetic relationships.

cultures; in addition, genetic distances measured among these five clones were an order of magnitude smaller than those observed in previous studies of *M. aeruginosa* (Tanabe et al. 2007). Thus, as best as can be determined, all five clones of *M. aeruginosa* that we fed to mussels in the 2010 experiments belong to a single species. However, clones did not cluster according to mussel selectivity using MLST phylogenetic relationships (Fig. 3).

Clones differed significantly in mean colony ED (ANOVA on log-transformed data, $p < 0.001$) and cell diameter (ANOVA, $p < 0.001$), but the latter differences were small (Table 2). Selectivity in the 2010 particle-choice experiment was independent of colony size, colony development ratio, microcystin quota, and Chl *a* quota (linear regressions, $R^2 < 0.10$, $p > 0.60$). Mussels exhibited significant selection against and nonsignificant filtering rates on two clones with median colony sizes that were larger (2008C) or smaller (2006A) than the clone mussels preferred most (2009C).

Discussion

We observed large variation in selectivity by *D. polymorpha* fed different colonial clones of *M. aeruginosa* isolated from the same lake. This result was repeatable across two sets of experiments employing different sets of recently isolated clones, indicating that the functional diversity observed within *Microcystis* is a robust and persistent characteristic of the lake population. Selectivity for many clones was not significantly different from 1, indicating no avoidance of *M. aeruginosa* by mussels, whereas selectivity and filtering rates for other clones were

Table 2. Morphological and chemical characterization of the five Gull Lake *Microcystis aeruginosa* clones used in the 2010 particle-choice experiment. Reported values are means with standard errors (SE) in parenthesis, except for equivalent diameter, which is reported here as the median because of highly right-skewed distributions. Quotas are mg g^{-1} dry biomass.

Clone	Colony equivalent diameter (μm)	Colony development ratio	Cell diameter (μm)	Microcystin quota (mg g^{-1})	Chlorophyll quota (mg g^{-1})
2009C	85.3	1.52(0.093)	4.1(0.138)	1.55(0.38)	22.0(0.34)
2009A	50.7	1.47(0.042)	4.1(0.092)	3.71(2.77)	37.8(21.2)
2006B	46.8	1.39(0.032)	3.9(0.102)	3.80(0.20)	28.6(2.77)
2008C	101.7	1.48(0.069)	4.7(0.134)	1.48(0.13)	15.2(0.41)
2006A	65.3	1.58(0.152)	4.1(0.109)	2.98(0.59)	27.7(4.53)

not different from 0, indicating nondetectable consumption (Figs. 1, 2). Mussels were able to select against unpalatable clones without ceasing to feed altogether (Figs. 1, 2). Genetic and morphological analyses in 2010 and in Wilson et al. (2005) confirmed our assumption that all clones isolated from Gull Lake are *M. aeruginosa*, demonstrating large variation in vulnerability to grazing within a single population of phytoplankton. Further, selectivity was found to differ among clones isolated from Gull Lake on the same date (Fig. 1, clones 2000C and 2000K), indicating that contemporaneous genotypes can also vary greatly in their vulnerability to grazing mortality and that selectivity is not related to time since isolation (Lakeman et al. 2009).

Dreissena are known to be selective feeders and can sort among particles drawn in through the incurrent siphon, expelling some material as pseudofeces while ingesting the rest (Horgan and Mills 1997; Vanderploeg et al. 2001). Although clone-specific feeding responses by mussels have been reported previously for *M. aeruginosa*, these studies tended to examine variation in mussel response across culture collection genotypes, which typically occur as single-cells or small colonies (< 53 μm; Vanderploeg et al. 2001; Dionisio Pires and Van Donk 2002) and do not naturally co-occur with the tested mussels. In nature, *M. aeruginosa* occurs predominantly as large colonies (Gull Lake median colony size generally $\geq$ 65 μm; J. White unpubl.) and our focus on *M. aeruginosa* clones isolated from the same lake as the mussels provides a natural context in which to interpret the selectivity results.

All *M. aeruginosa* clones used in the experiments were grown under the same conditions of nutrients and light, so it is reasonable to infer that the large differences in grazing vulnerability that we observed have a genetic basis. Although all clones were harvested for use in experiments during exponential growth, it is possible that cultures could have differed in algal density at the time of harvest (as a result of variation in growth rate; Wilson et al. 2010), which might affect cellular quotas and, thus, vulnerability to mussels. However, growth-rate variation among the 2010 clones was relatively small (0.17–0.21 d^{-1}) under growth conditions identical to those used for the experiment, suggesting that any density effects on cellular quotas were probably negligible.

The filtration rates we observed on *A. falcatus* (~ 4–8 L d^{-1}; Figs. 1, 2) correspond well to maximal rates reported for *D. polymorpha* of this size (2001 size range = 16.7–17.6 mm; 2010 size range = 17.9–19.1 mm) feeding on other high-quality algae (e.g., ~ 4–5 L d^{-1} on *Chlorella*; Kryger and Riisgård 1988); therefore, our observations of nonselective feeding by mussels on some clones of *M. aeruginosa* from Gull Lake and nondetectable consumption of other clones from Gull Lake show that different genotypes from the same population can be maximally edible or fully inedible in the same ecosystem. Thus, the range in vulnerability to dreissenid grazing within the *M. aeruginosa* population of Gull Lake is essentially equivalent to the range observed across all taxonomic groups of phytoplankton.

Differences in mussel selectivity across clones were largely driven by mussels altering their consumption of *M. aeruginosa*, rather than mussels ceasing to feed (Figs. 1, 2). This same basic result was obtained in both series of experiments, regardless of mussel acclimation to experimental feeding suspensions (0 h vs. 24 h). These two acclimation regimes roughly bracket typical acclimation schedules employed in similar studies (Vanderploeg et al. 2001; Dionisio Pires and Van Donk 2002). We can also rule out particle density as a causal factor, because the proportion of each algal species in the feeding suspensions was unrelated to selectivity. Furthermore, a clone-specific induced response—a form of intraspecific variation that has received considerable attention with respect to its role in grazing vulnerability (Van Donk et al. 2011)—is highly unlikely given the short exposure time to mussels in our experiments (minimally 30 min in 2001) and the inability of *M. aeruginosa* to rapidly adjust its phenotype (Jang et al. 2007; van Gremberghe et al. 2009*a*).

Two traits of *M. aeruginosa* that are likely to influence grazer selectivity are colony morphology and chemical composition, both of which are known to vary within and among populations (Wilson et al. 2006; Martins et al. 2009). We quantified colony morphology with metrics that describe both particle size (ED) and particle shape (colony development ratio). However, selectivity was not related to either of these metrics in the 2010 particle-choice experiment. Particle size has been shown to be of major importance in driving the selectivity of filter feeders (Geller and Müller 1981); however, the range of colony sizes encountered by mussels in the 2010 experiment (median colony ED = 46.8–101.7 μm) falls within that of particles efficiently cleared by *D. polymorpha* in another study (10–150 μm; Horgan and Mills 1997). Given the restricted range in morphological characteristics of the clones we employed, our results do not imply that colony size or shape is

unimportant as a factor driving mussel selectivity in general. Further experiments with a wider range of sizes are needed.

Cellular chemical content is another variable trait of *M. aeruginosa* routinely argued to affect grazer preference. Our data provide no support for the hypothesis that microcystin toxin, the most widely quantified compound produced by *M. aeruginosa* (Wilson et al. 2006), drives variation in *M. aeruginosa* palatability to grazers (Vanderploeg et al. 2001). The range in microcystin quota across clones was fairly large in 2010 (1.48–3.80 mg g^{-1}), yet we found no relationship between *M. aeruginosa* microcystin quota and mussel selectivity. Likewise, in the 2001 experiments, mussels filtered a toxic, single-celled clone at rates similar to or exceeding those for *A. falcatus* and another single-celled clone producing no microcystin (Vanderploeg et al. 2001; Fig. 1), suggesting that in the absence of particle size differences, microcystin content alone does not affect selectivity. The role of microcystin as a factor regulating *M. aeruginosa* vulnerability to grazers remains controversial and equivocal (Vanderploeg et al. 2001; Wilson et al. 2006; van Gremberghe et al. 2009*b*).

Microcystis produces other compounds that inhibit zooplankton feeding, which have been variously termed '*Daphnia* toxic compound' (Jungmann and Benndorf 1994), 'dissolved metabolites' (Haney et al. 1994), and simply 'bad taste factor' (Ghadouani et al. 2004). The toxin assay we used, although routinely employed to measure the dominant microcystin variants, is neither able to distinguish between microcystin variants nor detect all potentially inhibitory compounds known to be produced by *M. aeruginosa* (Martins et al. 2009). Thus, particular variants of microcystin, or other chemical factors, could underlie the large differences in palatability of clones to *D. polymorpha* that we observed.

Interestingly, although we detected significant selectivity by mussels in 2001, filtration rates on the two algal species were positively correlated in those experiments, suggesting that there was a possible inhibitory effect of *Microcystis* on overall filtering rates that was clone-dependent. The presence of inhibitory clones could reduce mussel consumption rates of less defended clones or other co-occurring phytoplankton in nature. Recent studies have found that *Daphnia* feeding on more grazing-vulnerable *Microcystis* clones could be inhibited in the presence of toxic clones that were unpalatable to *Daphnia,* providing a refuge for the susceptible clones (van Gremberghe et al. 2009*b*).

Our results may help explain large variation in the response of *Microcystis* biomass to *Dreissena* invasion. *M. aeruginosa* increased in invaded Michigan inland lakes having relatively low productivity, but not in lakes with higher nutrients (Raikow et al. 2004; Knoll et al. 2008). Vanderploeg et al. (2001) found that *M. aeruginosa* was largely rejected by Lake Huron mussels feeding on the natural phytoplankton assemblage, evidence for a causal link to the observed increase in *M. aeruginosa* throughout the Laurentian Great Lakes. In contrast, *Microcystis* abundance plummeted following mussel invasion of the Hudson River (Smith et al. 1998). Furthermore, mussels had positive and negative effects on *M. aeruginosa* biomass in Gull Lake in different years, when mussel biomass was manipulated within enclosures (Sarnelle et al. 2005). These conflicting observations suggest that local *Microcystis* populations may be generally inedible to mussels in some environments and edible in others. Identification of the critical characteristic(s) of *M. aeruginosa* driving mussel preference, and a quantification of how they vary across clones and with the environment, may greatly enhance our understanding of the *Dreissena–Microcystis* interaction.

The potential for large, genetically based variation in grazing vulnerability is at least consistent with other studies that have investigated genetic variation in *M. aeruginosa* populations. Naturally occurring *M. aeruginosa* populations are composed of a mosaic of different genotypes, with the genetic composition of the population varying through time (Kardinaal et al. 2007) and space (Wilson et al. 2005; Tanabe et al. 2009). Thus, the presence of large within-species genetic variation may be a predictor for which algal species might exhibit large trait-based variation, such as grazing vulnerability. Knowledge of the extent of both genetic and genetically based trait variation in the phytoplankton is surprisingly limited, however. Where intraspecific genetic variation has been documented, it has tended to be large and present in a diversity of taxa, including dinoflagellates (Logares et al. 2009), diatoms (Rynearson and Armbrust 2000; De Bruin et al. 2004), and cyanobacteria (Wilson et al. 2005; Tanabe et al. 2009). This variation can have functional importance. Recently, genetically based trait variation has been documented as an adaptation to an environmental gradient of grazing pressure in a green alga (Vanormelingen et al. 2009), and different co-occurring genotypes of a freshwater diatom show variability in their susceptibility to infection by parasitic fungi (De Bruin et al. 2004). Given the identification of both large genetic and genetically based trait variation within species of phytoplankton, additional research linking the two is needed.

Theory and experimental data readily identify tradeoffs between vulnerability to grazing and other traits, such as growth rate and competitive ability, as well as differential competitive ability among different species of phytoplankton for limiting resources (Tilman et al. 1986). If such dynamics also occur to some extent between different genotypes of the same species (van Gremberghe et al. 2009*b*), different environments may favor dominance by characteristic genotypes, potentially leading to complex interactions between the phytoplankton population and grazers; such tradeoffs could also explain how edible and inedible genotypes are able to coexist at some level in the same lake. For example, van Gremberghe et al. (2009*b*) found that in the presence of *Daphnia,* toxic clones of *M. aeruginosa* can facilitate nontoxic clones in mixtures, whereas competition between clones is generally intensified in the absence of grazers. Our data provide one example of the raw variation required within a species to potentially drive such phenomena in nature.

Given that we identified edible and inedible genotypes of the same species, traditional edible–inedible groupings of phytoplankton based on taxonomy may be too simplistic

(Long and Hay 2006). Specifically, we propose that prey edibility should be defined and understood in terms of a gradient, both across and within species, rather than as a dichotomy between two extremes (Agrawal 1998). For example, accurate models of algal dynamics are needed for taxa that form HABs, including *M. aeruginosa*, particularly in light of global change. Models developed for the Laurentian Great Lakes have assumed that *M. aeruginosa* experiences zero grazing mortality (Bierman et al. 2005; Zhang et al. 2008). Such an assumption, however, may not be appropriate for all systems, and could lead to the development of models with poor predictive power if assumptions regarding prey edibility are not validated in the specific system of interest. Indeed, recent evidence points to intraspecific variation playing a significant role in the ecology of HAB species such as *M. aeruginosa* (Burkholder and Glibert 2009; van Gremberghe et al. 2009*b*).

Our finding that the same population of phytoplankton is both edible and inedible to a single species of grazer is not likely to be a special case limited to *D. polymorpha*. If anything, zooplankton selectivity would be more likely to respond to the range of colony sizes among the clones we employed (Table 2; Geller and Müller 1981). Chemical factors associated with *M. aeruginosa* are also likely to affect zooplankton selectivity (Jungmann and Benndorf 1994; Ghadouani et al. 2004). Although many studies have found *M. aeruginosa* to be inedible and even lethal to many zooplankters (DeMott et al. 1991; Wilson et al. 2006), there is evidence that *Daphnia* are able to locally tolerate and even suppress toxic *Microcystis* in nature (Sarnelle and Wilson 2005). Such contradictory data are consistent with the idea that the edibility of a particular phytoplankton species may be highly variable and context-dependent—not unlike the case with *Microcystis* and *Dreissena.*

We are aware of only one study on phytoplankton that demonstrated similarly large intraspecific variation in vulnerability to herbivory (Long and Hay 2006), and none that have measured such variation across co-occurring genotypes by isolating and testing many clones from the same system. The reliance of many previous experimental studies on single-celled genotypes from culture collections may limit their application to nature (Wilson et al. 2006). Indeed, variation in the vulnerability of different Gull Lake *M. aeruginosa* clones to *D. polymorpha* is equivalent to the range in vulnerability across phytoplankton species, which is consistent with the conclusion that intraspecific variation can rival interspecific variation in grazing susceptibility (Long and Hay 2006). The use of simple dichotomous classifications such as edible and inedible to differentiate among phytoplankton taxonomic groups or size-classes, while useful in many cases, may also conceal potentially important complexities and in some instances impede our understanding of grazer–phytoplankton interactions in nature.

Acknowledgments

We thank K. Lincoln for assistance in the field and lab, and G. Horst, S. Hamilton, and P. Soranno for suggesting improvements to the experimental design, methodology, and analyses. Comments from two anonymous reviewers helped improve the manuscript. Funding for this research was provided by the Environmental Protection Agency (Ecology and Oceanography of Harmful Algal Blooms/2004-Science to Achieve Results-C1, project RD83170801), the National Science Foundation (Division of Environmental Biology-0841864), and Michigan State University (Summer Fellowship to J. White).

References

Agrawal, A. A. 1998. Algal defense, grazers, and their interactions in aquatic trophic cascades. Acta Oecol. **19:** 331–337, doi:10.1016/S1146-609X(98)80037-4

Bierman, Jr., V. J., J. Kaur, J. V. DePinto, T. J. Feist, and D. W. Dilks. 2005. Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. J. Great Lakes Res. **31:** 32–55, doi:10.1016/S0380-1330(05)70236-7

Burkholder, J. M., and P. M. Glibert. 2009. The importance of intraspecific variability in harmful algae—preface to a collection of topical papers. Harmful Algae **8:** 744–745, doi:10.1016/j.hal.2009.03.006

De Bruin, A., B. W. Ibelings, M. Rijkeboer, M. Brehm, and E. van Donk. 2004. Genetic variation in *Asterionella formosa* (Bacillariophyceae): Is it linked to frequent epidemics of host-specific parasitic fungi? J. Phycol. **40:** 823–830, doi:10.1111/j.1529-8817.2004.04006.x

DeMott, W. R., Q. X. Zhang, and W. W. Carmichael. 1991. Effects of toxic cyanobacteria and purified toxins on the survival and feeding of a copepod and three species of *Daphnia.* Limnol. Oceanogr. **36:** 1346–1357, doi:10.4319/lo.1991.36.7.1346

Dionisio Pires, L. M., and E. Van Donk. 2002. Comparing grazing by *Dreissena polymorpha* on phytoplankton in the presence of toxic and nontoxic cyanobacteria. Freshw. Biol. **47:** 1855–1865, doi:10.1046/j.1365-2427.2002.00933.x

Dyble, J., G. Fahnenstiel, R. W. Litaker, D. F. Millie, and P. A. Tester. 2008. Microcystin concentrations and genetic diversity of *Microcystis* in the lower Great Lakes. Environ. Toxicol. **23:** 507–516, doi:10.1002/tox.20370

Fulton, III, R. S., and H. W. Paerl. 1987. Effects of colonial morphology on zooplankton utilization of algal resources during blue green algal (*Microcystis aeruginosa*) blooms. Limnol. Oceanogr. **32:** 634–644, doi:10.4319/lo.1987.32.3.0634

Geller, W., and H. Müller. 1981. The filtration apparatus of Cladocera: Filter mesh-sizes and their implications on food selectivity. Oecologia **49:** 316–321, doi:10.1007/BF00347591

Ghadouani, A., B. Pinel-Alloul, K. Plath, G. A. Codd, and W. Lampert. 2004. Effects of *Microcystis aeruginosa* and purified microcystin-LR on the feeding behavior of *Daphnia pulicaria.* Limnol. Oceanogr. **49:** 666–679, doi:10.4319/lo.2004.49.3.0666

Haney, J. F., D. J. Forsyth, and M. R. James. 1994. Inhibition of zooplankton filtering rates by dissolved inhibitors produced by naturally occurring cyanobacteria. Arch. Hydrobiol. **132:** 1–13.

Horgan, M. J., and E. L. Mills. 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): Implications for freshwater lakes. Can. J. Fish. Aquat. Sci. **54:** 249–255, doi:10.1139/f96-276

Jang, M. H., J. M. Jung, and N. Takamura. 2007. Changes in microcystin production in cyanobacteria exposed to zooplankton at different population densities and infochemical concentrations. Limnol. Oceanogr. **52:** 1454–1466, doi:10.4319/lo.2007.52.4.1454

JUNGBLUT, A., I. HAWES, D. MOUNTFORT, B. HITZFELD, D. R. DIETRICH, B. P. BURNS, AND B. A. NIELAN. 2005. Diversity within cyanobacterial mat communities in variable salinity meltwater ponds of McMurdo Ice Shelf, Antarctica. Environ. Microbiol. **7:** 519–529, doi:10.1111/j.1462-2920.2005.00717.x

JUNGMANN, D., AND J. BENNDORF. 1994. Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins. Freshw. Biol. **32:** 13–20, doi:10.1111/j.1365-2427.1994.tb00861.x

KARDINAAL, W. E. A., AND OTHERS. 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. Aquat. Microb. Ecol. **48:** 1–12, doi:10.3354/ame048001

KNISELY, K., AND W. GELLER. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. Oecologia **69:** 86–94, doi:10.1007/BF00399042

KNOLL, L. B., O. SARNELLE, S. K. HAMILTON, C. E. H. KISSMAN, A. E. WILSON, J. B. ROSE, AND M. R. MORGAN. 2008. Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. Can. J. Fish. Aquat. Sci. **65:** 448–455, doi:10.1139/f07-181

KONDO, R., T. YOSHIDA, Y. YUKI, AND S. HIROISHI. 2000. DNA–DNA reassociation among a bloom-forming cyanobacterial genus, *Microcystis.* Int. J. Syst. Evol. Microbiol **50:** 767 770, doi:10.1099/00207713-50-2-767

KRYGER, J., AND H. U. RIISGÅRD. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. Oecologia **77:** 34–38, doi:10.1007/BF00380921

LAKEMAN, M. B., P. VON DASSOW, AND R. A. CATTOLICO. 2009. The strain concept in phytoplankton ecology. Harmful Algae **8:** 746–758, doi:10.1016/j.hal.2008.11.011

LEHMAN, J. T., AND C. D. SANDGREN. 1985. Species-specific rates of growth and grazing loss among freshwater algae. Limnol. Oceanogr. **30:** 34–46, doi:10.4319/lo.1985.30.1.0034

LOGARES, R., A. BOLTOVSKOY, S. BENSCH, J. LAYBOURN-PARRY, AND K. RENGEFORS. 2009. Genetic diversity patterns in five protest species occurring in lakes. Protist **160:** 301–317, doi:10.1016/j.protis.2008.10.004

LONG, J. D., AND M. E. HAY. 2006. When intraspecfic exceeds interspecific variance: Effects of phytoplankton morphology and growth phase on copepod feeding and fitness. Limnol. Oceanogr. **51:** 988–996, doi:10.4319/lo.2006.51.2.0988

MARTINS, J., M. L. SAKER, C. MOREIRA, M. WELKER, J. FASTNER, AND V. M. VASCONCELOS. 2009. Peptide diversity in strains of the cyanobacterium *Microcystis aeruginosa* isolated from Portuguese water supplies. Appl. Microbiol. Biotechnol. **82:** 951–961, doi:10.1007/s00253-009-1877-z

NEILAN, B. A., D. JACOBS, T. DEL DOT, L. L. BLACKALL, P. R. HAWKINS, P. T. COX, AND A. E. GOODMAN. 1997. rRNA sequences and evolutionary relationships among toxic and nontoxic cyanobacteria of the genus *Microcystis*. Int. J. Syst. Bacteriol. **47:** 693–697, doi:10.1099/00207713-47-3-693

OTSUKA, S., S. SUDA, S. SHIBATA, H. OYAIZU, S. MATSUMOTO, AND M. M. WATANABE. 2001. A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kützing *ex* Lemmermann 1907 under the Rules of the Bacteriological Code. Int. J. Syst. Evol. Microbiol. **51:** 873–879, doi:10.1099/00207713-51-3-873

PORTER, K. G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature **244:** 179–180, doi:10.1038/244179a0

RAIKOW, D. F., O. SARNELLE, A. E. WILSON, AND S. K. HAMILTON. 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. Limnol. Oceanogr. **49:** 482–487, doi:10.4319/lo.2004.49.2.0482

RAPS, S., K. WYMAN, H. W. SIEGELMAN, AND P. G. FALKOWSKI. 1983. Adaptation of the cyanobacterium *Microcystis aeruginosa* to light intensity. Plant Physiol. **72:** 829–832, doi:10.1104/pp.72.3.829

RYNEARSON, T. A., AND E. V. ARMBRUST. 2000. DNA fingerprinting reveals extensive genetic diversity in a field population of the centric diatom *Ditylum brightwellii.* Limnol. Oceanogr. **45:** 1329–1340, doi:10.4319/lo.2000.45.6.1329

SARNELLE, O., AND A. E. WILSON. 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol. Oceanogr. **50:** 1565–1570, doi:10.4319/lo.2005.50.5.1565

———, ———, S. K. HAMILTON, L. B. KNOLL, AND D. F. RAIKOW. 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha,* and the harmful phytoplanker, *Microcystis aeruginosa.* Limnol. Oceanogr. **50:** 896–904, doi:10.4319/lo.2005.50.3.0896

SMITH, T. E., R. J. STEVENSON, N. F. CARACO, AND J. J. COLE. 1998. Changes in phytoplankton community structure during the zebra mussel (*Dreissena polymorpha*) invasion of the Hudson River (New York). J. Plankton Res. **20:** 1567–1579, doi:10.1093/plankt/20.8.1567

TANABE, Y., F. KASAI, AND M. M. WATANABE. 2007. Multilocus sequence typing (MLST) reveals high genetic diversity and clonal population structure of the toxic cyanobacterium *Microcystis aeruginosa*. Microbiology **153:** 3695–3703, doi:10.1099/mic.0.2007/010645-0

———, ———, AND ———. 2009. Fine-scale spatial and temporal genetic differentiation of water bloom-forming cyanobacterium *Microcystis aeruginosa*: Revealed by multilocus sequence typing. Environ. Microbiol. Reports **1:** 575–582, doi:10.1111/j.1758-2229.2009.00088.x

TILMAN, D., R. KIESLING, R. STERNER, S. S. KILHAM, AND F. A. JOHNSON. 1986. Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Arch. Hydrobiol. **106:** 211–223.

VANDERPLOEG, H. A., J. R. LIEBIG, W. W. CARMICHAEL, M. A. AGY, T. H. JOHENGEN, G. L. FAHNENSTIEL, AND T. F. NALEPA. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Can. J. Fish. Aquat. Sci. **58:** 1208–1221, doi:10.1139/f01-066

VAN DONK, E., A. IANORA, AND M. VOS. 2011. Induced defenses in marine and freshwater phytoplankton: A review. Hydrobiologia **668:** 3–19, doi:10.1007/s10750-010-0395-4

VAN GREMBERGHE, I., P. VANORMELINGEN, K. VAN DER GUCHT, A. MANCHEVA, S. D'HONDT, L. DE MEESTER, AND W. VYVERMAN. 2009*a*. Influence of *Daphnia* infochemicals on functional traits of *Microcystis* strains (cyanobacteria). Hydrobiologia **635:** 147–155, doi:10.1007/s10750-009-9907-5

———, ———, B. VANELSLANDER, K. VAN DER GUCHT, S. D'HONDT, L. DE MEESTER, AND W. VYVERMAN. 2009*b*. Genotype-dependent interactions among sympatric *Microcystis* strains mediated by *Daphnia* grazing. Oikos **118:** 1647–1658, doi:10.1111/j.1600-0706.2009.17538.x

VANORMELINGEN, P., W. VYVERMAN, D. DE BOCK, K. VAN DER GUCHT, AND L. DE MEESTER. 2009. Local genetic adaptation to grazing pressure of the green alga *Desmodesmus armatus* in a strongly connected pond system. Limnol. Oceanogr. **54:** 503–511, doi:10.4319/lo.2009.54.2.0503

WALZ, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance I: Pattern of activity, feeding, and assimilation efficiency. Arch. Hydrobiol. Suppl. **55:** 83–105.

WILSON, A. E., R. B. KAUL, AND O. SARNELLE. 2010. Growth rate consequences of coloniality in a harmful phytoplankter. PLoS

One **5:** 1–8, doi:10.1371/annotation/5452a5f9-9d97-4be3-a4ca-bca4122b10fc

———, O. Sarnelle, B. A. Neilan, T. P. Salmon, M. M. Gehringer, and M. E. Hay. 2005. Genetic variation of the bloom-forming cyanobacterium *Microcystis aeruginosa* within and among lakes: Implications for harmful algal blooms. Appl. Environ. Microbiol. **71:** 6126–6133, doi:10.1128/AEM.71.10.6126-6133.2005

———, ———, and A. R. Tillmanns. 2006. Effects of cyanobacterial toxicity and morphology on the population growth of freshwater zooplankton: Meta-analyses of laboratory experiments. Limnol. Oceanogr. **51:** 1915–1924, doi:10.4319/lo.2006.51.4.1915

Zhang, H., D. A. Culver, and L. Boegman. 2008. A two-dimensional ecological model of Lake Erie: Application to estimate dreissenid impacts on large lake plankton populations. Ecol. Model. **214:** 219–241, doi:10.1016/j.ecolmodel.2008.02.005

Associate editor: Luc De Meester

Received: 15 December 2010
Accepted: 05 May 2011
Amended: 02 June 2011