VARIATION WITHIN A POPULATION OF TOXIC, BLOOM-FORMING PHYTOPLANKTON (*MICROCYSTIS AERUGINOSA*) DRIVES VULNERABILITY TO GRAZING

By

Jeffrey White

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Fisheries and Wildlife

2011

ABSTRACT

VARIATION WITHIN A POPULATION OF TOXIC, BLOOM-FORMING PHYTOPLANKTON (*MICROCYSTIS AERUGINOSA*) DRIVES VULNERABILITY TO GRAZING

By

Jeffrey White

Genetically-based trait variation and size variation within a single population (Gull Lake, Michigan) of the toxic, bloom-forming phytoplanker, *Microcystis aeruginosa*, are explored as drivers of grazing vulnerability to the invasive zebra mussel (*Dreissena polymorpha*). Laboratory feeding experiments were used in which preference for *M. aeruginosa* was assessed relative to a high-quality alga (*Ankistrodesmus falcatus*). Chapter 1 demonstrates that some *M. aeruginosa* genotypes from the same population are maximally edible, whereas others are not ingested by mussels. Thus, the range in vulnerability of sympatric *M. aeruginosa* clones to mussels is equal to that reported across all phytoplankton species. Mussel selectivity was not related to microcystin toxin quota and is most likely driven by variation in expression of a genetically-based factor. In Chapter 2, the mortality rate of *M. aeruginosa* to 16-21 mm mussels is quantified as a function of colony size. Mussels were fed a single, highly palatable clone in size fractions. Colonies ≥ 111 μm were not consumed, representing a critical size threshold of invulnerability. Smaller colonies and single cells were consumed non-selectively. Field surveys of the Gull Lake *M. aeruginosa* population revealed that colony size consistently decreases during the summer, from above to less than or equal to the vulnerability threshold, which could allow for major shifts in the overall susceptibility of the population to mussel grazing. Intraspecific variation (genetic and phenotypic) in ecological traits could thus influence the promotion or control of noxious blooms of toxigenic cyanobacteria.

ACKNOWLEDGMENTS

I am indebted to my advisor, O. Sarnelle, and my committee members, S. Hamilton and P. Soranno, for their superior guidance, patience, and expertise in overseeing my Master's program. Portions of this work were done in collaboration with R. Kaul, A. Wilson, and L. Knoll, and their contributions greatly enhanced the quality and scope of this thesis. The faculty, graduate students, and post-docs in the 'Limnology Lab' at Michigan State University are extraordinary mentors, colleagues, peers, and friends. I thank the 'Sarnelle Lab' technicians, K. Lincoln and M. Iadonisi, for their hard work assisting with the experiments and field work. The long-term study of Gull Lake has involved numerous 'generations' of graduate students and assistants, who deserve acknowledgment for leaving behind a legacy of sample bottles for me to process: J. Berry, J. Chiotti, J. Comtois, L. Dillon, B. Hanna, G. Horst, M. Iadonisi, C. Kissman, K. Lincoln, E. Milroy, J. Northrop, D. Raikow, N. Sarnelle, T. Sarnelle, A. Schuerer, T. Toda, and A. Wilson. N. Consolatti, M. Williams, and S. Bassett of the Kellogg Biological Station were instrumental in providing logistical support for the sampling of Gull Lake, whether the boat was submerged by a storm or the keys were locked in the lab truck with the windows rolled up.

I also must acknowledge the faculty in the Department of Biology at Saint Michael's College, Vermont who fostered my initial curiosity in limnology and encouraged me to pursue my graduate studies. Finally but importantly, I must thank my family—especially my parents, D. and K. White, and my fiancé, J. Comtois—for their encouragement and unyielding support.

Funding for this thesis research was provided by the Environmental Protection Agency (ECOHAB/2004-STAR-C1, project RD83170801), the National Science Foundation (DEB-0841864), and a summer fellowship from Michigan State University.

PREFACE

This thesis was prepared in manuscript format with both chapters to be submitted as separate papers to peer-reviewed journals. Therefore, 'we' is used in place of 'I' throughout to reflect the contribution of all co-authors to this work, including conceptualization, data collection and analyses, and feedback on earlier drafts. Co-authors on Chapter 1 are: RajReni Kaul, Lesley Knoll, Alan Wilson, and Orlando Sarnelle. Orlando Sarnelle is a co-author on Chapter 2. Chapter 1 synthesizes two related projects, one carried out in 2001 and the second in 2010. Experiments conducted in 2001 were performed by Alan Wilson and Lesley Knoll. Genetic analyses were conducted in 2010 by RajReni Kaul.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROLOGUE

VARIATION WITHIN A POPULATION OF TOXIC, BLOOM-FORMING PHYTOPLANKTON (*MICROCYSTIS AERUGINOSA*) DRIVES VULNERABILITY TO GRAZING

By

Jeffrey White

In ecology, emphasis is often placed on the relative vulnerability of different producer species to grazing, because variation in producer vulnerability can modify the outcome of species interactions and trophic cascades, regulate the flow of energy in food webs, and affect community composition. For example, across a broad range of phytoplankton species, factors such as cell size, morphology, digestibility, and chemical deterrents play a role in phytoplankton resistance to consumption by grazers. However, large within-species variation in fundamental ecological traits, like grazing vulnerability, could also be important. Such intraspecific variation might be manifest as phenotypic plasticity (trait expression varies with the environment); it could be genetically-based (different genotypes constitutively vary in the expression of a trait); or, even still, it could be a function of ontogeny, such as age or size class. Inducible phenotypic variation, including in cell digestibility and morphology driven by resource levels or grazer presence, has received considerable attention with respect to its role in phytoplankton grazing vulnerability. Although large genetic and morphological variation is documented within and among some phytoplankton populations, comparatively less is known about how such variation might mediate grazing vulnerability.

In this thesis, genetically-based trait variation and colony size-class variation within a single population of the toxic, bloom-forming phytoplanker, *Microcystis aeruginosa*, are explored as potential drivers of grazing vulnerability to the invasive zebra mussel (*Dreissena*

polymorpha). *M. aeruginosa* typically forms large colonies of microcystin toxin-producing cells, and is therefore generally regarded to be 'inedible' to grazers. Although tremendous spatial and temporal variation in morphology and genetic composition has been documented among and within populations of *M. aeruginosa*, an understanding of the ecological consequences of this variation is still emerging. Furthermore, the interaction between *D. polymorpha* and *M. aeruginosa* is of ecological and social interest because *D. polymorpha* has had dramatic and variable effects (ranging from positive to negative) on the abundance of this harmful phytoplankter in different environments.

M. aeruginosa is commonly assumed to be inedible to grazers, due to the formation of large colonies and the production of toxins and other deterrent compounds, but this assumption may not always be valid. The range in vulnerability of sympatric *M. aeruginosa* clones to mussels quantified in Chapter 1 is essentially equal to that typically measured across all phytoplankton species. In addition, Chapter 2 quantifies the relationship between colony size of *M. aeruginosa* and vulnerability to *D. polymorpha* – information necessary to produce more sophisticated models of its size-structured populations. Variation in clone vulnerability and population size structure may help to explain highly-variable effects of *D. polymorpha* on the dynamics of this harmful phytoplankter within and across habitats. More insight into the ecological role of this potentially important intraspecific variation in traits (genetic and phenotypic) is needed, because it could thus influence the promotion or control of noxious blooms of toxigenic cyanobacteria.

CHAPTER 1

LARGE VARIATION IN VULNERABILITY TO GRAZING WITHIN A POPULATION OF THE COLONIAL PHYTOPLANKTER, *MICROCYSTIS AERUGINOSA*

Introduction

Vulnerability to grazing is an ecological trait that critically influences the effect of consumers and trophic cascades on phytoplankton biomass, production and species composition (Carpenter et al. 1985; Carpenter et al. 1995; Bell 2002). 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 (McCauley and Briand 1979; 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; Porter 1977; Webster and Peters 1978; Fulton and Paerl 1987*a*). 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 (Bell 2002). 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 (Butler et al. 1989; Lürling and Van Donk 1997; Van Donk 1997; Fialkowska and Pajdak-Stós 2002; 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*a*,*b*; 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 (Strayer 2009). 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 (Saker et al. 2005; Kardinaal et al. 2007; Dyble et al. 2008; Rinta-Kanto et al. 2008; Bozarth et al. 2010) and across (Wilson et al. 2005; Wilson et al. 2006*b*; Martins et al. 2009; Tanabe et al. 2009; Sabart et al. 2010) 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 co-existing genotypes and could thus influence the composition and toxic properties of local *M. aeruginosa* populations (Dyble et al. 2008; van

Gremberghe et al. 2009*b,c*), 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?

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*), 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 0.5X WC-S growth medium, a half-strength formulation of Stemberger's (1981) modified WC 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 0.5X 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 less than four years (Table 1.1).

Table 1.1. 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	0.5X WC-S	2010
2008C	25 Jul 2008	0.5X WC-S	2010
2009A, 2009C	23 Jul 2009	0.5X WC-S	2010

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 L suspension (2001) or for 1 h on a 0.9 L suspension (2010). Mussel shell length did not statistically differ across treatments within any experiment (analysis of variance, ANOVA, $p >$ 0.20; 2001 range: 16.7-17.6 mm, 2010 range: 17.9-19.1 mm). All experiments were conducted in 1 L 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'; chlorophyll *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 chlorophyll *a* L^{-1}, which is within the natural range encountered by mussels in Gull Lake (J. White unpubl.).

Beakers were sampled for algal cell counts (preserved in 1% Lugol's iodine), and in some cases also for chlorophyll *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 re-suspend 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 4 replicate beakers with mussels (in two cases, only 2 and 3 beakers were employed) and 2 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 5 replicates with mussels and 1 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; Dionisio Pires et al. 2005). 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, 12, 24, 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 0.5X 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 8 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 chlorophyll *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

Mussel filtering rates were measured via the particle depletion method (Omori and Ikeda 1984). 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 200X on an inverted microscope (Nikon Eclipse TE2000-S; Lund et al. 1958; Sandgren and Robinson 1984). The subsample was then treated with 1 mol L^{-1} sodium hydroxide (Reynolds and Jaworski 1978) to dissolve the colony matrix of *M. aeruginosa*, re-settled, and individual *M. aeruginosa* cells counted at 400X. Species-specific filtering rates (L $individual^{-1}$ d^{-1}) were calculated for each beaker as:

$$F = \left(\frac{\ln C_i - \ln C_f}{t} \right) \frac{V}{N}$$, 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 chlorophyll *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* (Jacobs 1974; Sterner 1989).

Biovolume of cultured algae was determined via cell counts and measures of cell dimensions (Hillebrand et al. 1999), 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.). Chlorophyll *a* was measured via dark extraction of filters in cold 95% ethanol for 24 h, followed by fluorometric analysis (Turner Designs; Welschmeyer 1994).

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 non-negative 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 the 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, re-suspended in sterile deionized water, then pelleted and dried before storing at -20°C. To extract deoxyribonucleic acid (DNA), pelleted cells were re-suspended 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 polymerase chain reaction (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 (Cat. 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 (UPGMA) 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 chlorophyll *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. Since *M. aeruginosa* colony morphology ranges from nearly spherical to highly dissected, we also calculated a development-ratio index (designed for application to lake shorelines, Wetzel and Likens 1991) 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 five weeks; mean microcystin quota for each clone was determined over three weeks of exponential growth. Microcystin was extracted from cells collected on A/E filters using 75% methanol (Harada et al. 1999). Extracts were diluted 1:30 with deionized water before loading onto the ELISA plate. Along with microcystin quota, we quantified the chlorophyll *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. 1.1a) 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; 1-tailed *t*-test, F_m:F_a significantly less than 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. 1.1b,c), 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. 1.1). For these clones, selectivity was unrelated to microcystin production, since 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

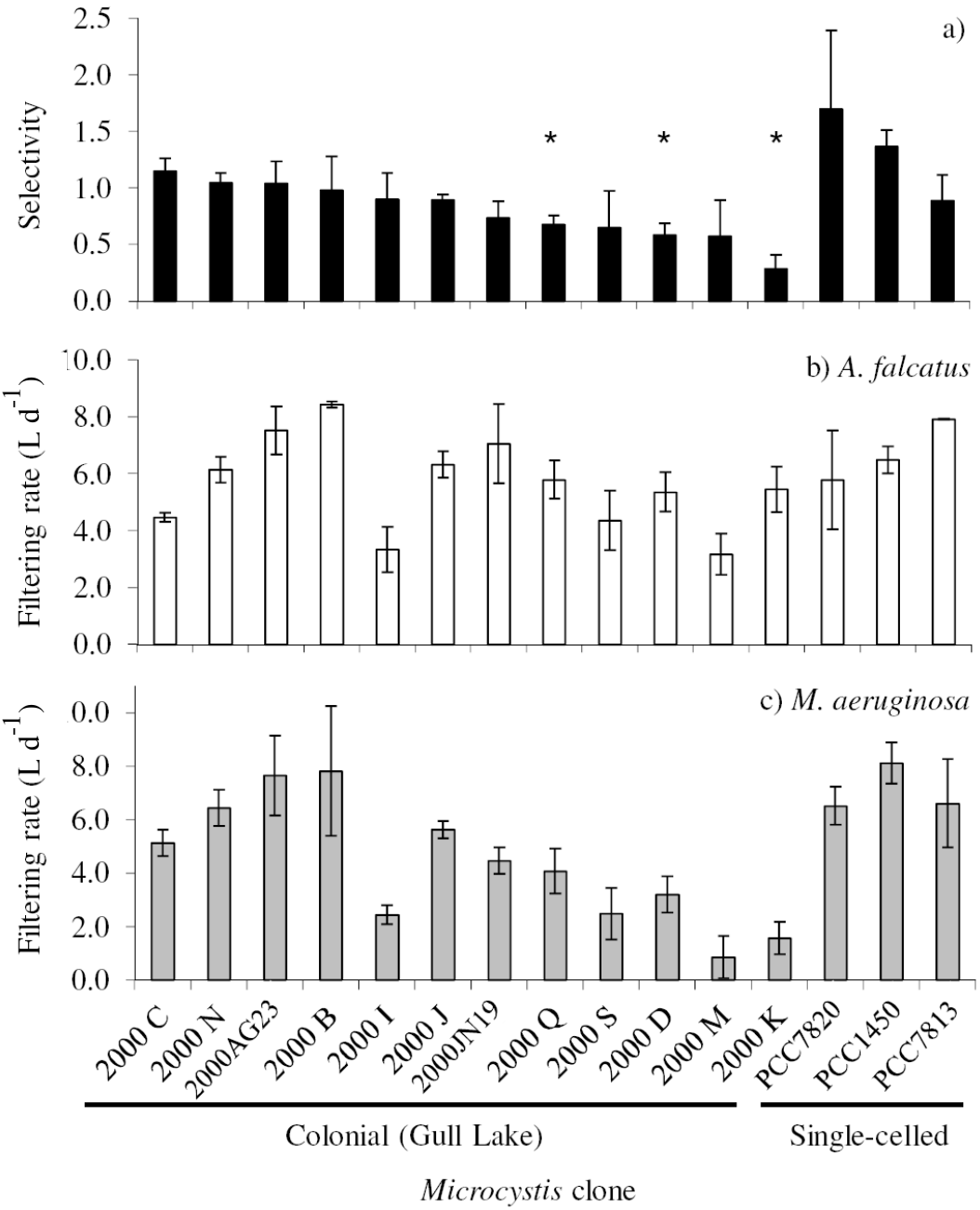


Figure 1.1. (a) Selectivity by zebra mussels for different clones of *Microcystis aeruginosa* in the 2001 particle-choice experiments. All treatments contained colonial Gull Lake clones (n = 48), except for the three single-celled clones (CC7820, CC1450, and CC7813) which were included for comparative 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 ± 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.

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. 1.2a). Mussels exhibited significant selection against clones 2008C and 2006A (1-tailed *t*-test, F_m:F_a significantly less than 1, $t = -3.4$, df = 4, $p < 0.03$), and in fact, filtering rates on these two clones of *M. aeruginosa* were not significantly greater than 0 (1-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. 1.2b,c). 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* (~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* (Sarnelle and Wilson 2008), 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).

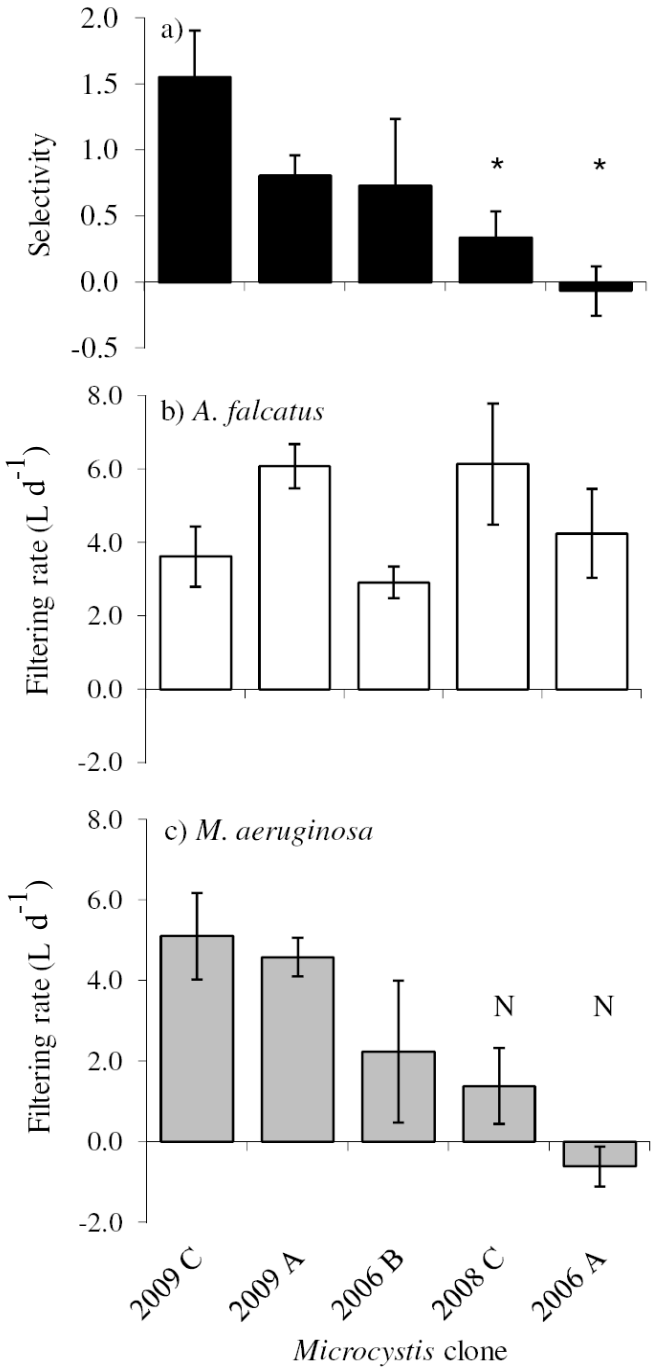


Figure 1.2. (a) Selectivity by zebra mussels for different colonial clones of Gull Lake *Microcystis aeruginosa* in the 2010 particle-choice experiment (n = 23). Selectivity was calculated as in Fig. 1.1. Bars are mean selectivity ± SE. Asterisks indicate clones for which significant avoidance was detected (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 non-detectable filtration of *M. aeruginosa* (filtering rate not significantly greater than 0).

We checked for a relationship between time since initial clone isolation from Gull Lake (varying from 1 to 4 years) 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; 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 at least 20% dissimilar) based upon sequencing of five housekeeping loci (Fig. 1.3). 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 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

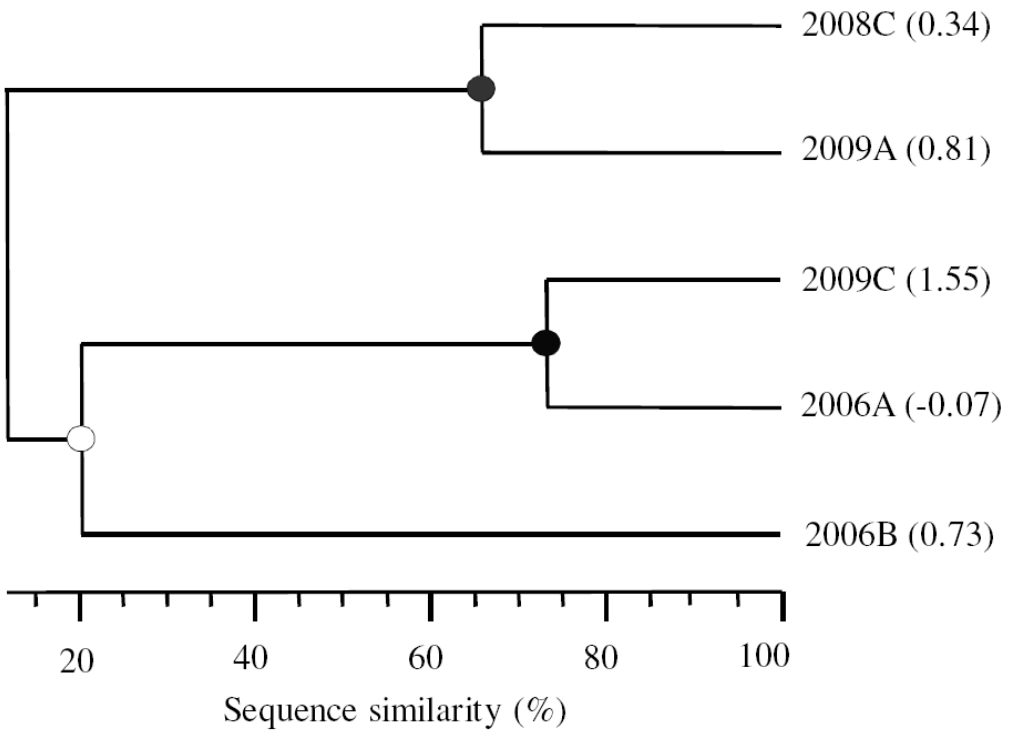


Figure 1.3. Phylogenetic tree of *Microcystis aeruginosa* clones used in the 2010 particle-choice experiment, based on 5 concatenated housekeeping genes. Tree nodes are placed along the *x*-axis based on nucleotide polymorphism; allelic divergence between clones is proportional to branch lengths to the right of the node. Closed nodes have significant (>90) statistical support based on re-sampling 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 parentheses) does not cluster according to multilocus sequence typing phylogenetic relationships.

species. However, clones did not cluster according to mussel selectivity using MLST phylogenetic relationships (Fig. 1.3).

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 1.2). Selectivity in the 2010 particle-choice experiment was independent of colony size, colony development ratio, microcystin quota, and chlorophyll *a* quota (linear regressions, $R^2 < 0.10$, $p > 0.60$). Mussels exhibited significant selection against and non-significant 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 not different from 0, indicating non-detectable consumption (Figs. 1.1, 1.2). Mussels were able to select against unpalatable clones without ceasing to feed altogether (Figs. 1.1, 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,

Table 1.2. Morphological and chemical characterization of the five Gull Lake *Microcystis aeruginosa* clones used in the 2010 particle-choice experiment. Reported values are means with SE in parentheses, 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)

selectivity was found to differ among clones isolated from Gull Lake on the same date (Fig. 1.1, 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, Baker 1998; 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 (Butler et al. 1989). 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. 1.1, 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 non-selective feeding by mussels on some clones of *M. aeruginosa* from Gull Lake and non-detectable 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. 1.1, 1.2). This same basic result was obtained in both series of experiments, regardless of mussel acclimation to experimental feeding suspensions (0 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 (Lürling and Van Donk 1997; Fialkowska and Pajdak-Stós 2002; van Gremberghe et al. 2009*a*; 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 (Saker et al. 2005; Wilson et al. 2006*a,b*; Martins et al. 2009). We quantified colony morphology with metrics that describe both particle size (equivalent diameter, 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*a*), 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. 1.1), 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 (DeMott et al. 1991; Vanderploeg et al. 2001; Wilson et al. 2006*a*; van Gremberghe et al. 2009*c*).

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 (Harada et al. 1999), is neither able to distinguish between microcystin variants nor detect all potentially-inhibitory compounds known to be produced by *M. aeruginosa* (Saker et al. 2005; 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,c*).

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) and Baker et al. (1998) found that Hudson River mussels exhibited a strong preference for *Microcystis*, although culture collection clones were used. 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 (Saker et al. 2005; Kardinaal et al. 2007; Rinta-Kanto et al. 2008; Bozarth et al. 2010) and space (Wilson et al. 2005; Tanabe et al. 2009; Sabart et al. 2010). 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 (Chase et al. 2000) and experimental data (Agrawal 1998; Steiner 2003; Yoshida et al. 2004) 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,c*), 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,c*) found that in the presence of *Daphnia,* toxic clones of *M. aeruginosa* can facilitate non-toxic 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; Bell 2002). 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,c*).

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 1.2; 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; Wilson and Hay 2007). Although many studies have found *M. aeruginosa* to be inedible and even lethal to many zooplankters (Fulton and Paerl 1987*a,b*; DeMott et al. 1991; Ghadouni et al. 2004; Wilson et al. 2006*a*; Tillmanns et al. 2008), there is evidence that *Daphnia* are able to locally tolerate and even suppress toxic *Microcystis* in nature (Hairston et al. 2001; Sarnelle and Wilson 2005; Sarnelle 2007). 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*a*; Tillmanns et al. 2008). 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 (Watson et al.1992; Watson et al. 1997), may also conceal potentially important complexities and in some instances impede our understanding of grazer-phytoplankton interactions in nature.

CHAPTER 2

SIZE-DEPENDENT VULNERABILITY OF THE COLONIAL PHYTOPLANKTER, *MICROCYSTIS AERUGINOSA,* TO GRAZING BY ZEBRA MUSSELS (*DREISSENA POLYMORPHA*)

Introduction

Particle size is an important trait limiting the handling and ingestion of phytoplankton by filter-feeding grazers (Burns 1968; Porter 1977; Webster and Peters 1978; Geller and Müller 1981; Defossez and Hawkins 1997). The size distributions of phytoplankton over which different species of filter-feeding zooplankton effectively graze are well documented, and are largely a function of grazer body size (Burns 1968; Geller and Müller 1981; Bogdan and Gilbert 1984; Hansen et al. 1994). Estimates are therefore available for grazing impacts on many species and size-classes of phytoplankton (Vanderploeg 1981; Lehman and Sandgren 1985; Knisely and Geller 1986; Jarvis et al. 1987, 1988). Based on these studies, phytoplankton taxa that form large colonies, filaments, or have linear cell dimensions > 35-50 μm are routinely classified as 'inedible' to most zooplankton grazers (McCauley and Briand 1979; Kalff 2002; Steiner 2003). Although this single dichotomous characterization has proved highly useful in plankton ecology, a more comprehensive quantification of relative mortality rates based on phytoplankton and grazer size would enable parameterization of more sophisticated models of phytoplankton population dynamics.

Many phytoplankton species also exhibit intraspecific variation in cell or colony size (Reynolds and Rogers 1976; Pentecost 1983; Duarte et al. 1990), which may have a genetic basis (Rico et al. 2006; Vanormelingen et al. 2009), or may be induced by the environment (Lürling and Van Donk 1997; Van Donk 1997; Van Donk et al. 2011). For many colonial species, colony size is largely a function of the number of cell divisions undergone and is thus an integrated

function of time and environment. In this case, substantial variation in colony size may be independent of genetic variation (Rico et al. 2006). Variation in size (e.g., colony dimensions and the number of cells per colony) within species can lead to differential vulnerability of size classes to grazers (Jarvis et al. 1987, 1988; Vanderploeg et al. 1988).

Many studies that have quantified particle size-specific grazing rates have used plastic beads (Bern 1990; Lei et al. 1996; Wissing 1997) or have substituted phytoplankton 'species' for 'size class' (e.g., by presenting grazers with whole or size-fractioned natural seston, or a different-sized species in each particle size class: Ten Winkel and Davids 1982; Sprung and Rose 1988; Bern 1994; Roditi et al. 1996; Dionisio Pires et al. 2004). Studies that investigate the variation in vulnerability across the natural range of sizes produced by a single phytoplankton species are less common (but, *see* Jarvis et al. 1987, 1988; Vanderploeg et al. 1988).

Microcystis aeruginosa, a common cyanobacterium in the phytoplankton community of eutrophic freshwaters, is widely characterized as inedible due to the formation of mucilaginous colonies that can span several orders of magnitude in size, and the production of putative chemical deterrents (Agrawal 1998; Fulton and Paerl 1987*a,b*; Jungmann and Benndorf 1994; Bierman et al. 2005; Zhang et al. 2008). Indeed, *M. aeruginosa's* toxicity and propensity to form dense surface blooms are of great interest due to the eutrophication of many inland waters and the health concerns associated with such 'harmful algal blooms' (HABs). Populations of this prokaryotic species show marked variation in colony size within and across lakes, and through time (Reynolds and Rogers 1976; Reynolds et al. 1981; Wilson et al. 2006*b*). Colony size is highly variable among clones, showing little genetic correlation to cell dimensions (Rico et al. 2006; Wilson et al. 2006*b*). Although great attention is paid to the population dynamics of *M. aeruginosa* due to its status as a HAB species, many studies investigating its vulnerability to

grazing have employed clones that are single-celled or produce small colonies < 53 μm (Baker 1998; Dionisio Pires and Van Donk 2002; Dionisio Pires et al. 2004), despite the fact that *M. aeruginosa* usually occurs as larger colonies in nature (Reynolds et al. 1981).

Grazer selectivity for size-fractioned *Microcystis* colonies has been investigated in *Daphnia* (Jarvis et al. 1987, 1988), with the highest filtration rates observed on colonies 40-60 μm but no consumption of colonies > 100-150 μm (Jarvis et al. 1987). These studies used a population of naturally-occurring *Microcystis*. Recent evidence indicates, however, that different genotypes of *M. aeruginosa* from the same lake can vary maximally in their vulnerability to grazing independent of colony size (White et al. in press), suggesting that genotype could potentially confound size effects if multiple genotypes with different palatablities are present in a feeding experiment.

The ongoing infestation of North American inland lakes by the invasive, filter-feeding zebra mussel (*Dreissena polymorpha*) is leading to marked changes in invaded ecosystems (Strayer 2009; Higgins and Vander Zanden 2010), including increases in undesirable *M. aeruginosa* and microcystin toxins in some systems (Vanderploeg et al. 2001; Raikow et al. 2004; Knoll et al. 2008). Zebra mussels are selective feeders (Baker et al. 1998; Bastviken et al. 1998; Vanderploeg et al. 2001; Dionisio Pires et al. 2004) and, despite their comparatively small body size, are capable of efficiently removing particles larger than those consumed by other filter-feeders, including native unionid mussels (Wissing 1997). *D. polymorpha* 9-25 mm in length have been shown to prefer particles with maximum dimensions of ~10-50 μm and reject particles > 50-80 μm (Ten Winkel and Davids 1982; Roditi et al. 1996; Wissing 1997; Naddafi et al. 2007) on the one hand and filter phytoplankton fractions ranging from 10-150 μm at equal rates (Horgan and Mills 1997) on the other hand, suggesting potentially varied ability to

consume colonial *M. aeruginosa* in nature. A clearer understanding of *D. polymorpha* grazing impact on *Microcystis* is still needed, particularly as a function of *Microcystis* colony size.

Given the aforementioned uncertainties of earlier studies, in addition to the need to accurately quantify the size-specific mortality of *M. aeruginosa* to *D. polymorpha*, we pose two questions. First, what is the relationship between colony size of *M. aeruginosa* and vulnerability to grazing by *D. polymorpha,* and second, how does this relationship relate to colony size distributions of *M. aeruginosa* in nature? Size-selective grazing by *D. polymorpha* on *M. aeruginosa,* if intense, might tend to shift the size distribution of *M. aeruginosa* toward larger, less edible colonies as smaller, more edible colonies are consumed. Knowledge of size-specific grazing rates on *M. aeruginosa* will improve our understanding of the complex interaction with *D. polymorpha* and the role of intraspecific variation in colony size in the population dynamics of this HAB species (Burkholder and Glibert 2009).

Methods

M. aeruginosa colony size-selectivity by *D. polymorpha* was assayed via a 'particle-choice' or feeding-selectivity experiment in which eight size classes of a colonial clone of *M. aeruginosa* were individually presented to mussels in a mixture containing *Ankistrodesmus falcatus*. The latter alga was employed as a high-quality particle against which selectivity for *M. aeruginosa* size fractions could be standardized (*sensu* White et al. in press). To relate the experimental data to the size-structured *M. aeruginosa* population our mussels encounter in nature, we also measured the size distribution of *M. aeruginosa* colonies in Gull Lake, Michigan, from which the experimental mussels and the *M. aeruginosa* clone originated.

Collection and maintenance of organisms

Gull Lake, Michigan, (Barry and Kalamazoo Counties) is a low-nutrient lake (TP ~10 μg L^{-1}, mixed layer chlorophyll *a* ~3.5 μg L^{-1}) in which *M. aeruginosa* increased dramatically in abundance after zebra mussels invaded in 1994 (Sarnelle et al. 2005). Gull Lake has been the site of large-scale *in situ* mesocosm experiments (Sarnelle et al. 2005; Sarnelle et al. submitted) aimed at understanding the complex effects of mussels on *M. aeruginosa*.

Mussels were collected via Eckman dredge and immediately separated from substrata (macrophytes and small rocks). We selected animals with shell lengths of 16-21 mm for the experiment to help interpret *D. polymorpha* effects on *M. aeruginosa* from *in situ* mesocosm experiments in Gull Lake (Sarnelle et al. 2005; Sarnelle et al. submitted), which employed the same mussel size class. A preliminary assessment of the filtering impact of different size classes of mussels in Gull Lake (mussel density and size distribution data collected in 1999; *see* Wilson and Sarnelle 2002) indicated that 16-21 mm mussels impose a major, disproportionate fraction (33%) of the total filtering impact (L m^{-2} d^{-1}; Kryger and Riisgård 1988) on phytoplankton in the 0-5 m depth stratum of Gull Lake. This stratum contains 83% of the benthic habitat available to mussels within the summer mixed layer (J. White, unpubl.) and is therefore where mussel contact with *M. aeruginosa* is greatest, since *M. aeruginosa* is present in the water column at appreciable densities only during the stratified season (J White, pers. obs.). The largest mussels commonly found in invaded inland lakes also generally fall within this size class (Horgan and Mills 1997; Idrisi et al. 2001; Naddafi et al. 2007). Once collected, mussels were gently scrubbed free of attached material, acclimated to room temperature in the lab, and fed a diet of *A. falcatus* (~4 μg L^{-1} chlorophyll *a*).

We used a colonial clone of *M. aeruginosa* isolated from Gull Lake in 2009 (clone 2009C) that mussels filtered at rates similar to the high-quality alga, *A. falcatus,* in previous experiments (White et al. in press). The clone was maintained in batch cultures of 0.5X WC-S (recipe modified from Stemberger 1981), with an inoculum transferred to fresh, sterile medium every 4-8 weeks. *A. falcatus* was grown in semi-continuous culture in full-strength WC-S medium, with gentle aeration and stirring. All phytoplankton were grown on a 12:12 h light:dark cycle under fluorescent lights (70 μmol m^{-2} s^{-1}) at 20 °C.

A total of 25 L of clone 2009C was grown in 1 and 2 L batch cultures of 0.5X WC-S, under the same growth conditions described above. The large culture volume was required to provide sufficient biomass in all size classes for the feeding experiment. Bottle position was randomized every 2 d to reduce heterogeneity in light conditions during growth. Cultures were harvested for the experiment after 40 d and were still growing exponentially (J. White pers. obs.).

Twenty-four hours prior to the experiment, the *M. aeruginosa* cultures were pooled into a barrel and mixed thoroughly. Whole culture was set aside for use in controls and for mussel pre-acclimation (24 h) to the *M. aeruginosa* clone (White et al. in press). The remaining ~23 L of culture was sequentially passed through sieves of decreasing pore size (from 200 to 8 μm) to concentrate and establish 8 size classes, the smallest being composed of single cells (Table 2.1). Material trapped on the mesh was thoroughly rinsed with and then re-suspended in filtered Gull Lake water (1 μm nominal pore size; hereafter, 'filtered lake water'). Size-fractioned *M. aeruginosa* was stored in the dark overnight before the experiment.

Table 2.1. Establishment and composition of *Microcystis aeruginosa* size classes used in the particle-choice experiment. Both size metrics, equivalent diameter (ED) and maximum linear dimension (MLD), are reported to facilitate comparisons to similar studies (*see* text for a description of the metrics). All particles (single-cells and colonies) were produced by a single clone of *M. aeruginosa*. Standard errors of means are reported in parentheses.

	Equivalent diameter (μm)		Maximum linear dimension (μm)	
Mesh pore sizes (lower, upper; μm)	Mean	Median	Mean	Median
--, 8*	4.9 (0.08)	4.8	4.9 (0.08)	4.8
24, 35	22.4 (0.34)	21.5	31.8 (0.62)	29.6
35, 45	30.8 (0.56)	30.3	42.8 (0.91)	41.5
45, 53	34.6 (0.57)	34.8	48.7 (0.97)	46.8
53, 63	44.7 (0.75)	44.3	61.2 (1.33)	56.7
63, 80	62.3 (1.08)	62.6	80.3 (1.99)	75.4
100, 150	77.8 (2.15)	80.1	111.2 (3.44)	109.3
150, 200	88.5 (4.22)	87.7	124.1 (6.91)	120.8

***** Treatment composed of single cells.

Particle choice experiment

Feeding suspensions contained targeted levels of size-fractioned *M. aeruginosa* plus *A. falcatus* in filtered lake water (mean total chlorophyll *a* = 3.9 μg L^{-1}). The total chlorophyll target was selected to approximate summer mixed-layer conditions in Gull Lake (J. White unpubl.). We employed 4 replicate beakers with mussels for each of eight size class treatments, and 3 control beakers containing un-fractioned *M. aeruginosa* but lacking mussels. Mussels were held in filtered lake water for 3 h immediately prior to the experiment to cleanse their guts of assimilated food material and then allocated in pairs to 1 L glass beakers containing 0.9 L of feeding suspension. Beakers were gently aerated, which kept phytoplankton in suspension throughout the experiment.

The feeding period began once mussels were actively filtering (siphons fully extended), which generally occurred within 5 min of placement into beakers. Mussels were removed from beakers after 1 h to terminate the experiment. Beakers were sampled for algal biomass (preserved in 1% Lugol's iodine) and chlorophyll *a* (filtered onto Pall A/E glass-fiber filters and frozen) immediately before mussels were added (initial) and immediately after mussels were removed (final). Beakers were stirred before taking all samples.

Sample processing and data analysis

Subsamples from each preserved initial and final sample were settled in 10 mL phytoplankton chambers. *A. falcatus* cells were counted at 200X with an inverted microscope (Nikon Eclipse; Lund et al. 1958; Sandgren and Robinson 1984). Cell densities of *M. aeruginosa* were estimated by measuring colony surface areas from two-dimensional digital micrographs (SPOT, Diagnostic Instruments), and converting these areas into cell densities via a regression developed for Gull Lake *M. aeruginosa* (Sarnelle et al. submitted). Equivalent

diameter (ED) was calculated by solving for the diameter of a circle with surface area equivalent to that of the measured colony; the maximum linear dimension (MLD) of the colonies was directly measured. Depending upon the size treatment, measurements of *M. aeruginosa* colonies were made at either 100X or 200X; *M. aeruginosa* cells in the single-cell treatment were counted and measured at 400X.

We calculated species-specific filtering rates (L individual^{-1} d^{-1}) using the particle depletion method (Omori and Ikeda 1984), $F = \left(\frac{\ln C_i - \ln C_f}{t} \right) \frac{V}{N}$, 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 feeding suspension (L) and N is the number of mussels per beaker. This equation does not correct for changes in cell densities in control beakers, a simplification validated by the lack of any measured change in either cell densities or chlorophyll *a* in control beakers during the experiment (paired t-tests, $p > 0.4$). Mussel selectivity was determined using the ratio F_m:F_a, where F_m and F_a are the mean filtering rates on the *M. aeruginosa* size class and *A. falcatus,* respectively (Jacobs 1974; Sterner 1989). We determined phytoplankton biovolume from measurements of cell dimensions (Hillebrand et al. 1999), with subsequent conversion to dry biomass assuming a specific gravity of 1.0 and a wet-to-dry biomass ratio of 0.4 (O. Sarnelle unpubl.). Chlorophyll *a* was measured via dark extraction of A/E filters in cold 95% ethanol for 24 h, followed by fluorometric analysis with a Turner Model 10-AU-005 fluorometer (Welschmeyer 1994).

We tested for differences in mean mussel selectivity across *M. aeruginosa* size classes with a one-way ANOVA. In the event that the ANOVA was significant, we performed 1-tailed

t-tests to check for significant avoidance of *M. aeruginosa* (selectivity < 1) within each treatment. For treatments where mussels exhibited significant selection against *M. aeruginosa*, we further tested whether mussel consumption of *M. aeruginosa* was detectable (filtering rate on *M. aeruginosa* > 0). 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 non-negative filtering rates.

Gull Lake M. aeruginosa *colony size distribution*

Phytoplankton was sampled from the mixed layer of Gull Lake biweekly from July-August in 2001 and 2005-2008, and from June-September in 2009-2010, via two pooled hauls of an integrating tube sampler. Samples were collected from a near-shore station (depth = 13 m) during 2001 and 2005-2008. In 2009-2010, samples were collected from the near-shore station as well as a central station (depth = 25 m). Subsamples were preserved in 1% Lugol's iodine and settled in 10 mL phytoplankton chambers. Dry biomass and ED of *M. aeruginosa* colonies were determined as described above for the particle-choice experiment. Median colony size did not significantly differ between sampling stations in 2009 (paired *t*-test, $t = 0.87$, df = 7, $p > 0.4$) or 2010 (paired *t*-test, $t = 0.39$, df = 8, $p > 0.7$); therefore, data were pooled across stations for those two years.

Results

Particle-choice experiment

Size fractionation of the *M. aeruginosa* culture was effective at establishing a range of colony sizes spanning more than an order of magnitude across 8 treatments. Mean colony MLD in each fraction corresponded better to the sieve mesh sizes employed than did mean ED (Table 2.1), although we report both to facilitate comparisons across studies using different metrics.

Total algal biomass did not significantly differ across treatments (ANOVA, > 0.1, $n = 31$) and mean total chlorophyll *a* differed no more than 0.72 μg L^{-1} between any two treatments. There were also no significant differences in mean mussel size across treatments (ANOVA, $p > 0.09$, $n = 31$; range of shell lengths = 16.5-21.3 mm). Mussel selectivity in one beaker was found to be a large but unexplained outlier (studentized residual = 3.97) and this datum was omitted from all analyses, leaving a total $n = 31$ for the experiment.

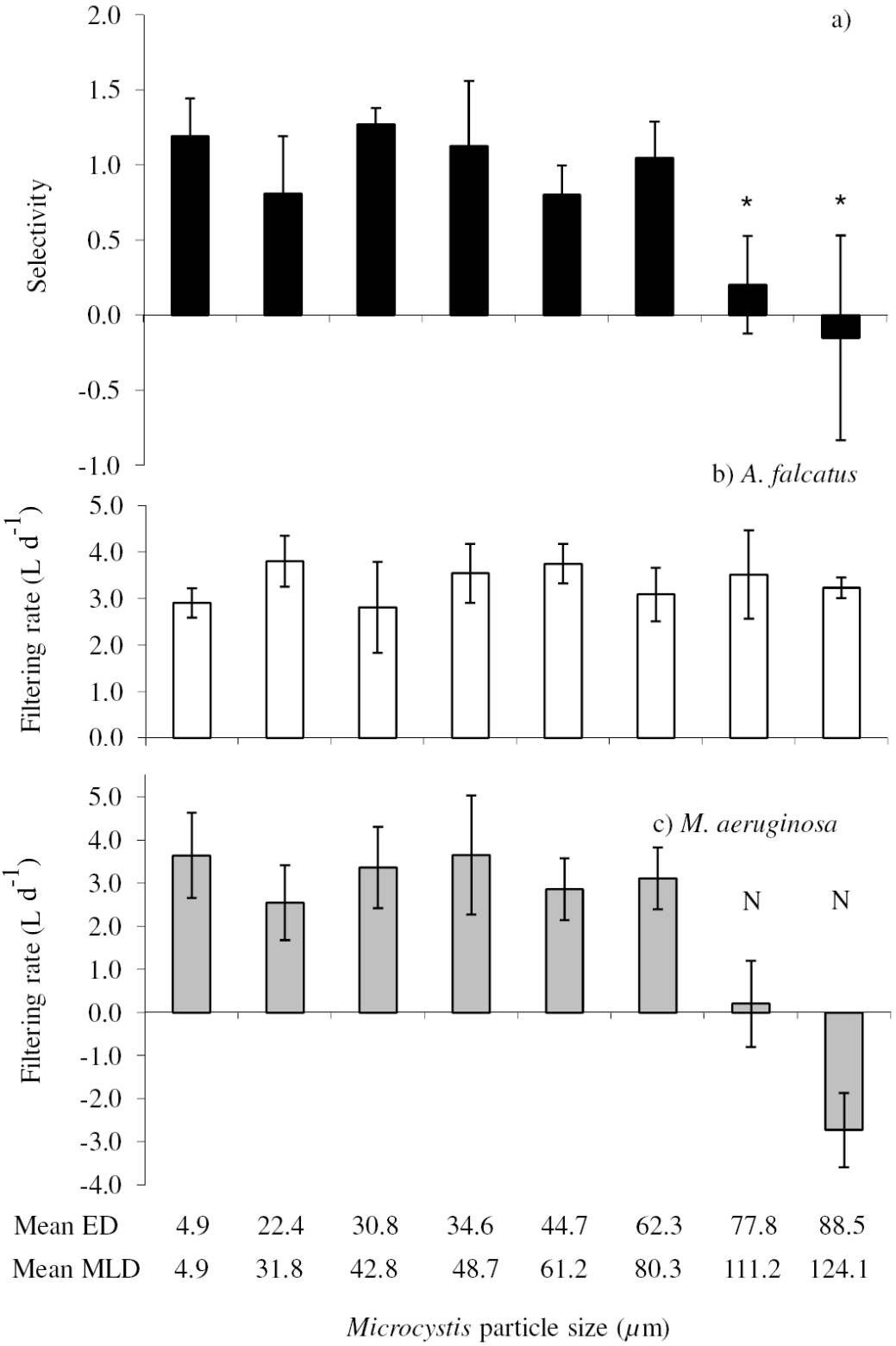
Mussel selectivity for *M. aeruginosa* differed significantly over the 8 size treatments (ANOVA, $p = 0.002$, $n = 31$; Fig. 2.1a). Mussel filtering rates on *M. aeruginosa* also varied significantly across the size class treatments (ANOVA, $p = 0.002$, $n = 31$; Fig. 2.1c), whereas filtering rates on *A. falcatus* did not (ANOVA, $p > 0.8$, $n = 31$; Fig. 2.1b). The latter were similar to maximal filtering rates of mussels of this size (Kryger and Riisgård 1988; White et al. in press). Mussels exhibited significant selection against *M. aeruginosa* (selectivity < 1; 1-tailed *t*-tests, $p < 0.05$) in the two treatments having mean colony sizes ≥ 77.8 μm ED (111.2 μm MLD). In addition, filtering rates on *M. aeruginosa* in these two treatments were not significantly greater than 0 (1-tailed *t*-tests, $p > 0.05$). Mussel selectivity for *M. aeruginosa* within the remaining, smaller size fractions was not different from 1 (indicating non-selective feeding), consistent with a previous study that found clone 2009C to be maximally edible by mussels (White et al. in press).

Gull Lake M. aeruginosa *colony size distribution*

M. aeruginosa biomass in Gull Lake generally increases in the water column during July, peaks in early-mid August, and then declines (J. White unpubl.). Analysis of the lake samples revealed considerable interannual variability in median colony size (~68 to > 110 μm ED) within the Gull Lake population across the seven summers of sampling. Median colony size decreased

Figure 2.1. (a) Selectivity by zebra mussels for different particle size classes of a single, colonial clone of *Microcystis aeruginosa* in a particle-choice experiment (n = 31). 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 ± SE. Asterisks indicate colony size fractions 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. An N indicates non-detectable filtration of *M. aeruginosa* (filtering rate not significantly greater than 0).

Figure 2.1. (Cont'd)



from July to August in every year, by as much as 27%, with July median ED being significantly greater than August median ED across all years (paired *t*-test, $p < 0.001$; Fig. 2.2a). In 2009-2010, years where more data are available, the gradual decreasing trend in colony size during the summer is especially apparent (Fig. 2.2b). Interestingly, median colony ED tended to be above the mussel selectivity threshold identified in our particle-choice experiment (~80 μm) early in the summer, but decreased to less than or equal to the threshold later in the summer (Figs. 2.1, 2.2).

Discussion

Filter-feeding grazers can select phytoplankton on the basis of particle size, and ingestion is mechanically constrained at some upper limit (Webster and Peters 1978; Geller and Müller 1981), yet studies that systematically quantify how natural variation in particle size translates into differential rates of grazing mortality within a single phytoplankton species are relatively uncommon (Jarvis et al. 1987, 1988; Vanderploeg et al. 1988). Our study goes still further in eliminating all biological properties that could be confounded with size by utilizing a single, highly palatable clone of *M. aeruginosa* grown under a single set of environmental conditions. Thus, the variation in selectivity and filtering rate that we observed (Fig. 2.1) can be unequivocally attributed to variation in colony size. Many previous studies have quantified grazer size selectivity using plastic beads (Burns 1968; Bern 1990; Lei et al. 1996; Wissing 1997) or testing different algal species in each size-fraction (Bern 1994; Horgan and Mills 1997). Substituting species for size class can confound attempts to quantify grazer size-selectivity, since preference can vary across different algal taxa of similar size (Ten Winkel and Davids 1982; Bastviken et al. 1998). Likewise, the use of inert particles is only applicable to the subset of grazers that will eat them (DeMott 1995). Furthermore, in the case of *M. aeruginosa*, many

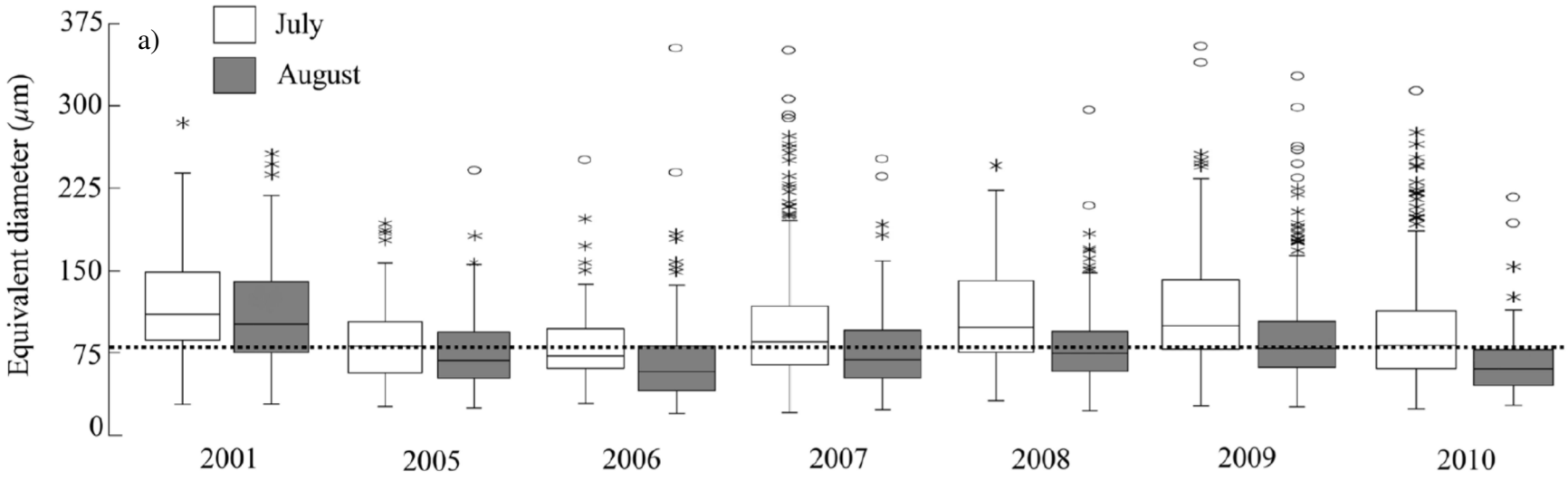
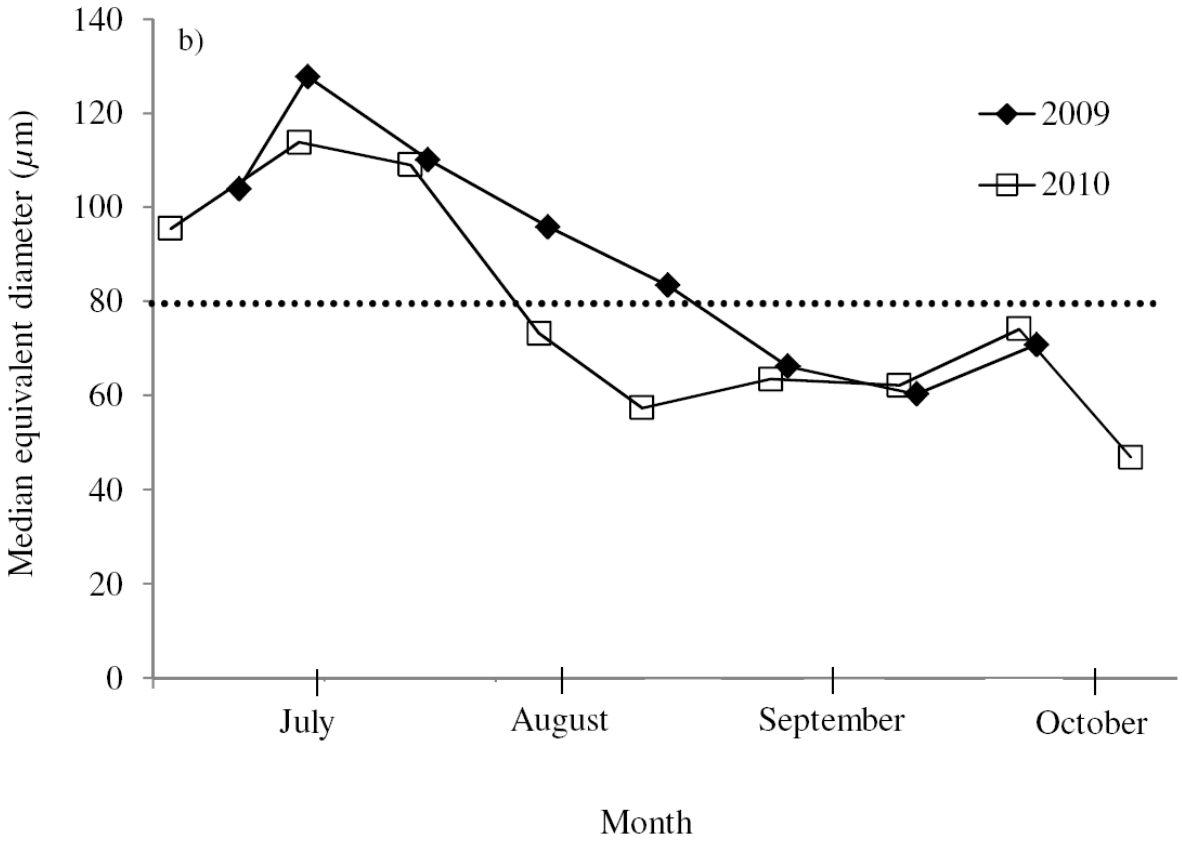


Figure 2.2. Field survey data on the size structure (equivalent diameter, ED) of the Gull Lake, Michigan *M. aeruginosa* population for the period 2001 and 2005-2010. (a) Comparison of July (white boxes) and August (gray boxes) colony size distributions by year. The horizontal dotted line has been placed at 80 μm ED, the approximate threshold size at which *M. aeruginosa* is invulnerable to grazing by *D. polymorpha* (*see* Fig. 2.1). (b) Changes in median colony size (ED) during the growing seasons of 2009 (black diamonds) and 2010 (white squares) observed in samples collected from two sampling stations. Data for the two stations have been pooled for each date (*see* Methods). The horizontal dotted line has been placed at 80 μm ED, the approximate threshold size at which *M. aeruginosa* is invulnerable to grazing by *D. polymorpha* (*see* Fig. 2.1).

Figure 2.2. (Cont'd)



grazing experiments have employed single-celled clones or dichotomous 'small' versus 'large' colony size fractions (Baker 1998; Vanderploeg et al. 2001; Dionisio Pires and Van Donk 2002; Dionisio Pires et al. 2004), precluding more sophisticated inferences about the consequences of natural variation in colony size (Wilson et al. 2006*a*; but, *see* Jarvis et al. 1987, 1988).

Quantification of grazing mortality rates imposed on different size classes of colonies is more informative, with respect to models of size-structured population dynamics, than are simpler measurements of the maximum particle size ingested. We identified a size-selectivity threshold of ~111 μm MLD (~78 μm ED) for 16-21 mm *D. polymorpha* feeding on colonial *M. aeruginosa,* above which mussel consumption declined sharply to zero. All *M. aeruginosa* particles ≤ ~80 μm MLD (~62 μm ED), including single-cells (diameter = 4.9 μm), were consumed non-selectively and at rates similar to reported maximal filtration rates for 16-21 mm *D. polymorpha* (Kryger and Riisgård 1988; Fig. 2.1). Thus, our data describe the entire function relating mortality inflicted by mussels to the natural size spectrum of *M. aeruginosa*.

D. polymorpha possesses multiple sites of potential pre-ingestive particle selection (e.g., inhalant siphon, ctenidia, and labial palps) which vary mechanically in their size-selective properties and according to feeding conditions (Morton 1969; Ward and Shumway 2004). Whereas retention of the smallest particles by *D. polymorpha* (0.4-1.5 μm, Cotner et al. 1995; Lei et al. 1996) is attributed to the 'capture net' formed by gill cirri (pore size 0.2-0.7 μm; Silverman et al. 1996), larger particles are swept across the gills by feeding currents and sorted by the ctenidia and labial palps (Morton 1969; Baker et al. 2000; Ward and Shumway 2004). Ultimately, particles that are drawn into the mantle but are too large to be ingested are swept into rejection currents, bound in mucus, and ejected through the inhalant siphon as pseudofeces (Morton et al. 1969; Baker et al. 2000).

Our results are generally congruent with previous studies of *D. polymorpha* size-selective feeding, with the added value of eliminating any uncertainty about confounds resulting from comparisons across phytoplankton taxa. Sprung and Rose (1988) measured high relative filtration rates by *D. polymorpha* (mean shell length = 15-30 mm) on particles as small as 5 μm, which is equivalent in size to single-cells of *M. aeruginosa*. Ten Winkel and Davids (1982) observed preferential ingestion by *D. polymorpha* (mean shell length = 25 mm) of centric and pennate diatoms with mean cell lengths of 10-40 μm, but rejection of larger pennate diatoms (*Asterionella*) and *Dinobryon* that were > 80 μm. Algae (*Cosmarium*, *Peridinium*) with diameters of ~50 μm were consumed non-selectively. On the other hand, Horgan and Mills (1997) did not detect significant differences in mussel filtering rates across particle size classes ranging from 10-150 μm MLD on natural phytoplankton, although filtering rates on filamentous and colonial cyanobacteria tended to be lower than on other taxa.

In a previous experiment, *D. polymorpha* filtered the same clone employed in the present study (2009C) at maximal rates, although the clone was not size-fractioned in that experiment (White et al. in press). Median colony size of the clone in that study was 85.3 μm ED (range = 17.5-273.1 μm), which was higher than the threshold size for consumption determined in the present study. This apparent inconsistency is likely a consequence of the fact that a large proportion of the colonies in the previous experiment were within the edible size range, whereas the range of colony sizes within each size fraction in the present study was very narrow.

Contrary to the expectation that intense size-selective grazing might increase the proportion of large, less edible colonies in the population, the annual decrease in median colony size of Gull Lake *M. aeruginosa* from above to below 80 μm ED observed during the summer (Fig. 2.2) suggests that the average vulnerability of the population to *D. polymorpha* grazing

varies sharply within the growing season. However, the relative abundance of different genotypes within the *M. aeruginosa* population might also shift during the summer (Saker et al. 2005; Kardinaal et al. 2007; Rinta-Kanto et al. 2008; Bozarth et al. 2010), and we have previously shown that different genotypes of *M. aeruginosa* in Gull Lake can vary maximally in their vulnerability to mussel grazing irrespective of colony size within the edible range (White et al. in press). Size and genotypic variation within and among *M. aeruginosa* populations could therefore help to explain the dramatic differences in response of *M. aeruginosa* biomass to *D. polymorpha* invasion across systems (Smith et al. 1998; Vanderploeg et al. 2001; Raikow et al. 2004; Sarnelle et al. 2005; De Stasio et al. 2008; Knoll et al. 2008).

Our study examines size-selectivity of a single, but important, size cohort of mussels in Gull Lake, although particle size selection may also be dependent upon mussel body size. The selectivity spectra of filter-feeding zooplankton (and therefore phytoplankton vulnerability) generally broaden with increasing grazer body size (Burns 1968; Geller and Müller 1981). Although variation in body size within zooplankton species is a good predictor of the largest particles that can be cleared from suspension (Burns 1968; Geller and Müller 1981; Bogdan and Gilbert 1984; Hansen et al. 1994), it may be irrelevant with respect to the capture of small particles (Bogdan and Gilbert 1984), which is largely a function of filtering mesh size and taxonomy (Gophen and Geller 1984; Brendelberger 1991). *D. polymorpha* tend to be larger on average (mean shell lengths > 8 mm; Custer and Custer 1997; Mills et al. 1999) in the Laurentian Great Lakes than *D. polymorpha* in Gull Lake (mean shell length = 3.4 mm; J. White, unpubl.). Furthermore, *D. polymorpha* are being replaced by the closely-related quagga mussel (*D. rostriformis bugensis*) throughout the Great Lakes (Nalepa et al. 2010), which have similar size-specific clearance rates to but can attain even larger body sizes than *D. polymorpha* (Mills et al.

1999; Baldwin et al. 2002). However, to our knowledge, the body size versus particle size-selectivity relationship has not been directly quantified for *Dreissena.* The diameter of the inhalant siphon in *D. polymorpha* does scale positively with mussel length ($r = 0.87$, MacIsaac et al. 1991), which has been posited to enable ingestion of larger particles (and by extension a larger range of *M. aeruginosa* colonies; MacIsaac et al. 1991). However, inhalant siphon diameter in *D. polymorpha* > 8 mm is generally at least 10-fold wider than the largest *M. aeruginosa* colonies consumed in our experiment (MacIsaac et al. 1991). Nevertheless, observations indicate that larger individuals within a species of filter-feeding bivalves, including *D. polymorpha*, are capable of ingesting larger particles and or clearing them more efficiently than are smaller individuals (James et al. 2001; Beck and Neves 2003; Bridoux et al. 2010).

The vulnerability of a phytoplankton species or size class is also dependent upon the composition of the grazer community (Bergquist et al. 1985; Cyr and Curtis 1999), due to differences in optimal prey size among grazers (Geller and Müller 1981; Hansen et al. 1994). Although *M. aeruginosa* typically occurs as colonies much greater in size than the widely-cited 35-50 μm threshold for edibility to most crustacean zooplankton, experimental manipulations of *Daphnia* have demonstrated substantial negative effects on *M. aeruginosa* biomass (Sarnelle 2007). Jarvis et al. (1987) observed intermediate selectivity (~0.5-0.6) by daphnids > 1 mm for *M. aeruginosa* colonies as large as 60-100 μm. Such data indicate the need to verify the edibility of the local phytoplankton community before any assumption is made.

Size is a critical regulator of other population processes in addition to grazing mortality, and also factors into many ecological tradeoffs. Larger phytoplankton, including larger colonies of *M. aeruginosa,* generally have lower intrinsic growth rates than smaller cells or colonies (Malone 1980; Kruk et al. 2010; Wilson et al. 2010), and are also less-efficient at assimilating

resources (Reynolds 1984). Large size can also be beneficial, due to greater migration rates for buoyant species such as *Microcystis* (Reynolds 1984; Visser et al. 1997) and, of course, lower vulnerability to consumption (this study; Jarvis et al. 1987; Vanderploeg et al. 1988; Steiner 2003; Visser et al. 2005). Models of *M. aeruginosa* population dynamics should therefore incorporate such size-structured growth and loss processes, in a way that is analogous to the age-structured models of animal populations, in order to more accurately forecast harmful algal blooms.

LITERATURE CITED

LITERATURE CITED

Agrawal, A. A. 1998. Algal defense, grazers, and their interactions in aquatic trophic cascades. Acta Oecol. **19:** 331-337.

Baker, S. M., J. S. Levinton, J. P. Kurdziel, and S. E. Shumway. 1998. Selective feeding and biodeposition by zebra mussels and their relation to changes in phytoplankton composition and seston load. J. Shellfish Res. **17**: 1207-1213.

Baker, S. M., J. S. Levinton, and J. E. Ward. 2000. Particle transport in the zebra mussel, *Dreissena polymorpha* (Pallas). Biol. Bull. **199:** 116-125.

Baldwin, B. S., M. S. Mayer, J. Dayton, N. Pau, J. Mendilla, M. Sullivan, A. Moore, A. Ma, and E. L. Mills. 2002. Comparative growth and feeding in zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*): implications for North American lakes. Can. J. Fish. Aquat. Sci. **59:** 680-694.

Bastviken, D. T. E., N. F. Caraco, and J. J. Cole. 1998. Experimental measurements of zebra mussel (*Dreissena polymorpha*) impacts on phytoplankton community composition. Freshwater Biol. **39:** 375-386.

Beck, K., and R. J. Neves. 2003. An evaluation of selective feeding by three age-groups of the rainbow mussel *Villosa iris.* N. Am. J. Aquacult. **65:** 203-209.

Bell, T. 2002. The ecological consequences of unpalatable prey: phytoplankton response to nutrient and predator additions. Oikos **99:** 59-68.

Bergquist, A. M., S. R. Carpenter, and J. C. Latino. 1985. Shifts in phytoplankton size structure and community composition during grazing by contrasting zooplankton assemblages. Limnol. Oceanogr. **30:** 1037-1045.

Bern, L. 1990. Postcapture particle size selection by *Daphnia cucullata* (Cladocera). Limnol. Oceanogr. **13:** 923-926.

Bern, L. 1994. Particle selection over a broad size range by crustacean zooplankton. Freshwater Biol. **32:** 105-112.

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.

Bogdan, K. G., and J. J. Gilbert. 1984. Body size and food size in freshwater zooplankton. Proc. Natl. Acad. Sci. **81:** 6427-6431.

Bozarth, C. S., A. D. Schwartz, J. W. Shepardson, F. S. Colwell, and T. W. Dreher. 2010. Population turnover in a *Microcystis* bloom results in predominantly nontoxigenic variants late in the season. Appl. Environ. Microbiol. **76**: 5207-5213.

Brendelberger, H. 1991. Filter mesh size of cladocerans predicts retention efficiency for bacteria. Limnol. Oceanogr. **36:** 884-894.

Bridoux, M., M. Sobiechowska, A. Perez-Fuentetaja, and K. T. Alben. 2010. Algal pigments in Lake Erie dreissenids, pseudofeces and sediments, as tracers of diet, selective feeding and bioaccumulation. J. Great Lakes Res. **36:** 437-447.

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.

Burns, C. W. 1968. The relationship between body size of filter-feeding Cladocera and the maximum size of particle ingested. Limnol. Oceanogr. **13:** 675-678.

Butler, N. M., C. A. Suttle, and W. E. Neill. 1989. Discrimination by freshwater zooplankton between single algal cells differing in nutritional status. Oecologia **78:** 368-372.

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. Bioscience **35:** 634-639.

Carpenter, S. R., D. L. Christensen, J. J. Cole, K. L. Cottingham, X. He, J. R. Hodgson, J. F. Kitchell, S. E. Knight, M. L. Pace, D. M. Post, D. E. Schindler, and N. Voichick. 1995. Biological control of eutrophication in lakes. Environ. Sci. Technol. **29:** 784-786.

Chase, J. M., M. A. Leibold, and E. Simms. 2000. Plant tolerance and resistance in food webs: community-level predictions and evolutionary implications. Evol. Ecol. **14:** 289-314.

Cotner, J. B., W. S. Gardner, J. R. Johnson, R. H. Sada, J. F. Cavaletto, and R. T. Heath. 1995. Effects of zebra mussels (*Dreissena polymorpha*) on bacterioplankton: evidence for both size-selective consumption and growth stimulation. J. Great Lakes Res. **21:** 517-528.

Custer, C. M., and T. W. Custer. 1997. Occurrence of zebra mussels in near-shore areas of western Lake Erie. J. Great Lakes Res. **23:** 108-115.

Cyr, H. and J. M. Curtis. 1999. Zooplankton community size structure and taxonomic composition affects size-selective grazing in natural communities. Oecologia. **118:** 306 315.

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.

Defossez, J.-M., and A. J. S. Hawkins. 1997. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. Mar. Biol. **129:** 139-147.

DeMott, W. R. 1995. The influence of prey hardness on *Daphnia's* selectivity for large prey. Hydrobiologia. **307:** 127-138.

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.

De Stasio, B. T., M. B. Schrimpf, A. E. Beranek, and W. C. Daniels. 2008. Increased chlorophyll *a,* phytoplankton abundance, and cyanobacteria occurrence following invasion of Green Bay, Lake Michigan by dreissenid mussels. Aquatic Invasions. **3**: 21-27.

Dionisio Pires, L. M., B. M. Bontes, E. Van Donk, and B. W. Ibelings. 2005. Grazing on colonial and filamentous, toxic and nontoxic cyanobacteria by the zebra mussel *Dreissena polymorpha*. J. Plankton Res. **27**: 331-339.

Dionisio Pires, L. M., R. R. Jonker, E. Van Donk, and H. J. Laanbroek. 2004. Selective grazing by adults and larvae of the zebra mussel (*Dreissena polymorpha*): application of flow cytometry to natural seston. Freshwater Biol. **49:** 116-126.

Dionisio Pires, L. M., and E. Van Donk. 2002. Comparing grazing by *Dreissena polymorpha* on phytoplankton in the presence of toxic and nontoxic cyanobacteria. Freshwater Biol. **47:** 1855-1865.

Duarte, C. M., S. Agustí, and D. E. Canfield, Jr. 1990. Size plasticity of freshwater phytoplankton: Implications for community structure. Limnol. Oceanogr. **35:** 1846-1851.

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.

Fialkowska, E., and A. Pajdak-Stós. 2002. Dependence of cyanobacteria defense mode on grazer pressure. Aquat. Microb. Ecol. **27:** 149-157.

Fulton III, R. S., and H. W. Paerl. 1987*a*. Toxic and inhibitory effects of the blue-green alga *Microcystis aeruginosa* on herbivorous zooplankton. J. Plankton Res. **9:** 837-855.

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

Geller, W., and H. Müller. 1981. The filtration apparatus of Cladocera: filter mesh-sizes and their implications on food selectivity. Oecologia **49:** 316-321.

Gophen, M. and W. Geller. 1984. Filter mesh size and food particle uptake by *Daphnia.* Oecologia **64:** 408-412.

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.

Hairston, Jr., N. G., C. L. Holtmeier, W. Lampert, L. J. Weider, D. M. Post, J. M. Fischer, C. E. Cáceres, J. A. Fox, and U. Gaedke. 2001. Natural selection for grazer resistance to toxic cyanobacteria: evolution of phenotypic plasticity? Evolution **55:** 2203-2214.

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.

Hansen, B., P. K. Bjørnsen, and P. J. Hansen. 1994. The size ratio between planktonic predators and their prey. Limnol. Oceanogr. **39**: 395-403.

Harada, K., F. Kondo, and L. Lawton. 1999. Laboratory analysis of cyanotoxins, p. 369-405. *In* I. Chorus and J. Bartram [eds.], Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management. E & FN Spon and WHO.

Higgins, S. N., and M. J. Vander Zanden. 2010. What a difference a species makes: a meta analysis of dreissenid mussel impacts on freshwater ecosystems. Ecol. Monogr. **80:** 179 196.

Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollinger, and T. Zohary. 1999. Biovolume calculation for pelagic and benthic microalgae. J. Phycol. **35:** 403-424.

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.

Idrisi, N., E. L. Mills, L. G. Rudstam, and D. J. Stewart. 2001. Impact of zebra mussels (*Dreissena polymorpha*) on the pelagic lower trophic levels of Oneida Lake, New York. Can. J. Fish. Aquat. Sci. **58:** 1430-1441.

Jacobs, J. 1974. Quantitative measurement of food selection: a modification of the forage ratio and Ivlev's electivity index. Oecologia **14:** 413-417.

James, M. R., M. A. Weatherhead, and A. H. Ross. 2001. Size-specific clearance, excretion, and respiration rates, and phytoplankton selectivity for the mussel *Perna canaliculus* at low levels of natural food. N. Z. J. Mar. Freshwat. Res. **35:** 73-86.

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.

Jarvis, A. C., R. C. Hart, and S. Combrink. 1987. Zooplankton feeding on size fractioned *Microcystis* colonies and *Chlorella* in a hypereutrophic lake (Hartbeespoort Dam, South Africa): implications to resource utilization and zooplankton succession. J. Plankton Res. **9:** 1231-1249.

Jarvis, A. C., R. C. Hart, and S. Combrink. 1988. Cladoceran filtration rate-body length relations: model improvements developed for a *Microcystis*-dominated hypertrophic reservoir. J. Plankton Res. **10:** 115-131.

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.

Jungmann, D., and J. Benndorf. 1994. Toxicity to *Daphnia* of a compound extracted from laboratory and natural *Microcystis* spp., and the role of microcystins. Freshwater Biol. **32:** 13-20.

Kalff, J. 2002. Limnology. Prentice Hall.

Kardinaal, W. E. A., I. Janse, M. K. van Agterveld, M. Meima, J. Snoek, L. R. Mur, J. Huisman, G. Zwart, and P. M. Visser. 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. Aquat. Microb. Ecol. **48:** 1-12.

Knisely, K., and W. Geller. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. Oecologia **69:** 86-94.

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.

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.

Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lürling, C. S. Reynolds, and M. Scheffer. 2010. A morphological classification capturing functional variation in phytoplankton. Freshwater Biol. **55:** 614-627.

Kryger, J., and H. U. Riisgård. 1988. Filtration rate capacities in 6 species of European freshwater bivalves. Oecologia **77:** 34-38.

Lakeman, M. B., P. von Dassow, and R. A. Cattolico. 2009. The strain concept in phytoplankton ecology. Harmful Algae **8:** 746-758.

Lehman, J. T., and C. D. Sandgren. 1985. Species-specific rates of growth and grazing loss among freshwater algae. Limnol. Oceanogr. **30:** 34-46.

Lei, J., B. S. Payne, and S. Y. Wang. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha*. Can. J. Fish. Aquat. Sci. **53:** 29-37.

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.

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.

Lund, J. W. G., C. Kipling, and E. D. Le Cren. 1958. The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting. Hydrobiologia **11:** 143-170.

Lürling, M. and E. Van Donk. 1997. Morphological changes in *Scenedesmus* induced by infochemicals released in situ from zooplankton grazers. Limnol. Oceanogr. **42**: 783-788.

MacIsaac, H. J., W. G. Sprules, and J. H. Leach. 1991. Ingestion of small-bodied zooplankton by zebra mussels (*Dreissena polymorpha*): can cannibalism on larvae influence population dynamics? Can. J. Fish. Aquat. Sci. **48:** 2051-2060.

Malone, T. C. 1980. Algal size, p. 433-463. *In* I. Morris [ed.], The Physiological Ecology of Phytoplankton. Blackwell Scientific.

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.

McCauley, E., and F. Briand. 1979. Zooplankton grazing and phytoplankton species richness: Field tests of the predation hypothesis. Limnol. Oceanogr. **24:** 243-252.

Mills, E. L., J. R. Chrisman, B. Baldwin, R. W. Owens, R. O'Gorman, T. Howell, E. F. Roseman, and M. K. Raths. 1999. Changes in the dreissenid community in the lower Great Lakes with emphasis on southern Lake Ontario. J. Great Lakes Res. **25:** 187-197.

Morton, B. 1969. Studies on the biology of *Dreissena polymorpha* Pall. I. General anatomy and morphology. Proc. Malac. Soc. Lond. **38:** 301-321.

Naddafi, R., K. Pettersson, and P. Eklöv. 2007. The effect of seasonal variation in selective feeding by zebra mussels (*Dreissena polymorpha*) on phytoplankton community composition. Freshwater Biol. **52:** 823-842.

Nalepa, T. F., D. L. Fanslow, and S. A. Pothoven. 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. J. Great Lakes Res. **36:** 5-19.

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.

Omori, M., and T. Ikeda. 1984. Methods in marine zooplankton ecology. Wiley-Interscience.

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.

Pentecost, A. 1983. The distribution of daughter colonies and cell numbers in a natural population of *Volvox aureus* Ehrenb. Ann. Botany **52:** 769-776.

Porter, K. G. 1973. Selective grazing and differential digestion of algae by zooplankton. Nature **244:** 179-180.

Porter, K. G. 1977. The plant-animal interface in freshwater ecosystems. Am. Nat. **65:** 159-170.

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.

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.

Reynolds, C. S. 1984. The ecology of freshwater phytoplankton. Cambridge University Press.

Reynolds, C. S., and G. H. M. Jaworski. 1978. Enumeration of natural *Microcystis* populations. Br. Phycol. J. **13:** 269-277.

Reynolds, C. S., G. H. M. Jaworski, H. A. Cmiech, and G. F. Leedale. 1981. On the annual cycle of the blue-green alga *Microcystis aeruginosa* Kütz. Emend. Elenkin. Philos. Trans. R. Soc., B. **293:** 419-477.

Reynolds, C. S., and D. A. Rogers. 1976. Seasonal variations in the vertical distribution and buoyancy of *Microcystis aeruginosa* Kütz. Emend. Elenken in Rostherne Mere, England. Hydrobiologia **48:** 17-23.

Rico, M., M. Altamirano, V. López-Rodas, and E. Costas. 2006. Analysis of polygenic traits of *Microcystis aeruginosa* (cyanobacteria) strains by restricted maximum likelihood (REML) procedures: 1. Size and shape of colonies and cells. Phycologia. **43:** 237-242.

Rinta-Kanto, J. M., E. A. Konopko, J. M. DeBruyn, R. A. Bourbonniere, G. L. Boyer, and S. W. Wilhelm. 2008. Lake Erie *Microcystis:* relationship between microcystin production, dynamics of genotypes and environmental parameters in a large lake. Harmful Algae **8:** 665-673.

Roditi, H. A., N. F. Caraco, J. J. Cole, and D. L. Strayer. 1996. Filtration of Hudson River water by the zebra mussel (*Dreissena polymorpha*). Estuaries **19:** 824-832.

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.

Sabart, M., D. Pobel, E. Briand, B. Combourieu, M. J. Salençon, J. F. Humbert, and D. Latour. 2010. Spatiotemporal variations in microcystin concentrations and in the proportions of microcystin-producing cells in several *Microcystis aeruginosa* populations. Appl. Environ. Microbiol. **76**: 4750-4759.

Saker, M. L., J. Fastner, E. Dittmann, G. Christiansen, and V. M. Vasconcelos. 2005. Variation between strains of the cyanobacterium *Microcystis aeruginosa* isolated from a Portuguese River. J. Appl. Microbiol. **99:** 749-757.

Sandgren, C. D., and J. V. Robinson. 1984. A stratified sampling approach to compensating for non-random sedimentation of phytoplankton cells in inverted microscope settling chambers. Br. Phycol. J. **19:** 67-72.

Sarnelle, O. 2007. Initial conditions mediate the interaction between *Daphnia* and bloom forming cyanobacteria. Limnol. Oceanogr. **52**: 2120-2127.

Sarnelle, O., and A. E. Wilson. 2005. Local adaptation of *Daphnia pulicaria* to toxic cyanobacteria. Limnol. Oceanogr. **50**: 1565-1570.

Sarnelle, O., and A. E. Wilson. 2008. Type III functional response in *Daphnia.* Ecology **89**: 1723-1732.

Sarnelle, O., A. E. Wilson, 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.

Silverman, H., J. W. Lynn, and T. H. Dietz. 1996. Particle capture by the gills of *Dreissena polymorpha:* structure and function of latero-frontal cirri. Biol. Bull. **191:** 42-54.

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.

Sprung, M. and U. Rose. 1988. Influence of food size and food quantity on the feeding of the mussel *Dreissena polymorpha.* Oecologia. **77:** 526-532.

Steiner, C. F. 2003. Keystone predator effects and grazer control of planktonic primary production. Oikos **101:** 569-577.

Stemberger, R. S. 1981. A general approach to the culture of planktonic rotifers. Can. J. Fish. Aquat. Sci. **38:** 721-724.

Sterner, R. W. 1989. The role of grazers in phytoplankton succession, p. 107-170. *In* U. Sommer [ed.], Plankton ecology: Succession in plankton communities. Springer-Verlag.

Strayer, D. L. 2009. Twenty years of zebra mussels: lessons from the mollusk that made headlines. Front. Ecol. Environ. **7**: 135-141.

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.

Tanabe, Y., F. Kasai, and M. M. Watanabe. 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.

Ten Winkel, E. H., and C. Davids. 1982. Food selection by *Dreissena polymorpha* Pallas (Mollusca: Bivalvia). Freshwater Biol. **12:** 553-558.

Tillmanns, A. R., A. E. Wilson, F. R. Pick, and O. Sarnelle. 2008. Meta-analysis of cyanobacterial effects on zooplankton population growth rate: species-specific responses. Fundamental and Applied Limnology **171:** 285-295.

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. 1981. Seasonal particle-size selection by *Diaptomus sicilis* in offshore Lake Michigan. Can. J. Fish. Aquat. Sci. **38:** 504-517.

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.

Vanderploeg, H. A., G.-A. Paffenhöfer, and J. R. Liebig. 1988. *Diaptomus* vs. net phytoplankton: effects of algal size and morphology on selectivity of a behaviorally flexible, omnivorous copepod. Bull. Mar. Sci. **43:** 377-394.

Van Donk, E. 1997. Defenses in phytoplankton against grazing induced by nutrient limitation, UV-B stress and infochemicals. Aquat. Ecol. **31:** 53-58.

Van Donk, E., A. Ianora, and M. Vos. 2011. Induced defenses in marine and freshwater phytoplankton: a review. Hydrobiologia **668:** 3-19.

van Gremberghe, I, P. Vanormelingen, K. Van der Gucht, A. Mancheva, S. D'hondt, L. De Meester, and Wim Vyverman. 2009*a*. Influence of *Daphnia* infochemicals on functional traits of *Microcystis* strains (cyanobacteria). Hydrobiologia **635:** 147-155.

van Gremberghe, I., P. Vanormelingen, K. Van der Gucht, C. Souffreau, W. Vyverman, and L. De Meester. 2009*b.* Priority effects in experimental populations of the cyanobacterium *Microcystis.* Environ. Microbiol. **11:** 2564-2573.

van Gremberghe, I., P. Vanormelingen, B. Vanelslander, K. Van der Gucht, S. D'hondt, L. De Meester, and W. Vyverman. 2009*c*. Genotype-dependent interactions among sympatric *Microcystis* strains mediated by *Daphnia* grazing. Oikos. **118:** 1647-1658.

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.

Visser, P. M., B. W. Ibelings, L. R. Muir, and A. E. Walsby. 2005. The ecophysiology of the harmful cyanobacterium, *Microcystis:* Features explaining its success and measures for its control, p. 109-142. *In* J. Huisman, H. C. P. Matthijs, and P. M. Visser [eds.], Harmful Cyanobacteria. Springer.

Visser, P. M., J. Passarge, and L. R. Mur. 1997. Modelling vertical migration of the cyanobacterium *Microcystis.* Hydrobiologia **349:** 99-109.

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.

Ward, J. E., and S. E. Shumway. 2004. Separating the grain from the chaff: particle selection in suspension- and deposit-feeding bivalves. J. Exp. Mar. Biol. Ecol. **300:** 83-130.

Watson, S. B., E. McCauley, and J. A. Downing. 1992. Sigmoid relationships between phosphorus, algal biomass, and algal community structure. Can. J. Fish. Aquat. Sci. **49:** 2605-2610.

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

Webster, K. E., and R. H. Peters. 1978. Some size-dependent inhibitions of larger cladoceran filterers in filamentous suspensions. Limnol. Oceanogr. **23:** 1238-1245.

Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments. Limnol. Oceanogr. **39**: 1985-1992.

Wetzel, R. G., and G. E. Likens. 1991. Limnological analysis, 2nd ed. Springer-Verlag.

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

Wilson, A. E., and O. Sarnelle. 2002. Relationship between zebra mussel biomass and total phosphorus in European and North American lakes. Arch. Hydrobiol. **153:** 339-351.

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

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

Wilson, A. E., R. B. Kaul, and O. Sarnelle. 2010. Growth rate consequences of coloniality in a harmful phytoplankter. PLoS One **5:** 1-8.

Wilson, A. E., W. A. Wilson, and M. E. Hay. 2006*b*. Intraspecific variation in growth and morphology of the bloom-forming cyanobacterium *Microcystis aeruginosa*. Appl. Environ. Microbiol. **72:** 7386-7389.

Wissing, K. D. 1997. Particle size selection in unionid and zebra mussels: competitive overlap or niche separation? M.S. Thesis, Iowa State University, Ames, Iowa.

Yoshida, T., N. G. Hairston, Jr., and S. P. Ellner. 2004. Evolutionary trade-off between defence against grazing and competitive ability in a simple unicellular alga, *Chlorella vulgaris.* Proc. R. Soc. Lond. B. **271:** 1947-1953.

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.