TRAIT AND ENVIRONMENTAL VARIATION MEDIATE THE INTERACTION BETWEEN A HARMFUL PHYTOPLANKTER AND AN INVASIVE GRAZER

By

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ABSTRACT

TRAIT AND ENVIRONMENTAL VARIATION MEDIATE THE INTERACTION BETWEEN A HARMFUL PHYTOPLANKTER AND AN INVASIVE GRAZER

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Phytoplankton that form harmful algal blooms (HABs) can foul water with unpleasant odors and tastes, accumulate as visible surface scums, and produce potentially dangerous toxins. The cyanobacterium *Microcystis aeruginosa* is the most widespread of the freshwater HAB-forming species, and this dissertation explores the influences of variation in environmental drivers (biotic, abiotic) and variation in biological traits (colony size, growth rate) on its ecology—in particular, the interaction with a facilitator species, the invasive zebra mussel (*Dreissena polymorpha*).

Chapter 1 quantifies the vulnerability of *Microcystis* to grazing by zebra mussels as a function of large variation in both *Microcystis* colony size (5-88 μm equivalent diameter) and zebra mussel body size (8-28 mm shell length), and relates the findings to their size distributions in the primary study lake, Gull Lake, Michigan. Based on colony size alone, the *Microcystis* population in Gull Lake can vary widely in its vulnerability to grazing within single growing seasons, and the range of ingestible colonies ($\leq$ 80 μm equivalent diameter) is greater for zebra mussels than published ranges for other dominant filter-feeding grazers (e.g., *Daphnia*). Following a mass mortality event (~100%) of zebra mussels on epilimnetic sediments in Gull Lake during a relatively warm summer, evidence from a combination of *in situ* monitoring and experiments presented in Chapter 2 demonstrates a causal relationship between chronic, accumulated heat exposure (> 25 °C) and elevated zebra mussel mortality. Though these temperatures are lethal to zebra mussels, they are within the optimal range for *Microcystis*.

Results from a long-term (13-year) study of the Gull Lake *Microcystis* population are presented in Chapter 3, the first long-term analysis of *Microcystis* dynamics in a low-nutrient lake—an uncharacteristic niche for this species strongly facilitated by zebra mussels. *Microcystis* biomass and microcystin toxin were significantly higher and peak biomass occurred significantly earlier in warmer summers, consistent with climate change projections. However, the heat-induced mass mortality event of zebra mussels (Chapter 2) resulted in a 2-year collapse of the *Microcystis* population during the warmest period in the time series, highlighting the need to understand how these two strongly interacting species will respond together to climate warming. Lastly, Chapter 4 returns to the importance of large intraspecific trait variation for the ecology of *Microcystis,* to further understand its niche expansion into low-nutrient lakes. Laboratory growth assays of 18 colonial strains, recently isolated from 11 Michigan inland lakes spanning the entire productivity gradient (7.6-196 μg L^{-1} total phosphorus), show that *Microcystis* strains from high-nutrient lakes grow significantly faster (up to ~7 fold) than those from low-nutrient lakes, which may indicate the presence of an ecological trade-off enabling local adaptation to these widely disparate habitats. Possibly as a result of their faster growth rates, strains from high-nutrient lakes are also more likely to cease colony formation in culture sooner, which has implications for the design and interpretation of lab studies of *Microcystis.*

Taken together, the chapters within this dissertation demonstrate important ecological consequences of the biological trait variation inherent within and among populations, and illustrate how that diversity might interact with other biotic and abiotic factors, improving our understanding of species' responses to complex global change.

To my wife and parents,
who gave and sacrificed so much to support me in this endeavor.

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Lincoln, Megan Schuetz, and Dave Weed. The staff at the W.K. Kellogg Biological Station, especially Tyler Brownell, Nina Consolatti, Andy Fogiel, and Mark Williams were instrumental in ensuring all seven of my field seasons on Gull Lake ran smoothly. There are many other people and organizations that deserve recognition and a big 'thank you,' and they are acknowledged at the conclusion of each chapter for which they made specific contributions.

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To the 547 students I had the privilege to work with in class: thank you for your enthusiasm for learning, your contributions that made me a better educator, and for affirming my decision to be a life-long educator and learner.

The Biology faculty at Saint Michael's College inspired me to pursue graduate school and a career in undergraduate education, two major life goals that have now been realized as a result of huge leaps they helped and encouraged me to take.

Last but not least, I thank my wife Jenny, parents David and Kathy, and my other family and friends for their love, kindness, and unyielding support. I could not have done this without you.

PREFACE

This dissertation was prepared in manuscript format with each chapter submitted or 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 project conceptualization, data collection and analyses, and feedback on earlier written drafts. Co-authors are: Chapter 1, Orlando Sarnelle; Chapter 2, Stephen Hamilton and Orlando Sarnelle; Chapter 3, Stephen Hamilton and Orlando Sarnelle; Chapter 4, Orlando Sarnelle.

At the time this dissertation was written, Chapters 1 and 2 were already published in the peer-reviewed literature exactly as they appear here. Complete bibliographic information for these two publications is as follows.

Chapter 1:

White, J. D. and Sarnelle, O. 2014. Size-structured vulnerability of the colonial cyanobacterium, *Microcystis aeruginosa*, to grazing by zebra mussels (*Dreissena polymorpha*). Freshwater Biology **59**:514-525.

Chapter 2:

White, J. D., Hamilton, S. K., and Sarnelle, O. 2015. Heat-induced mass mortality of invasive zebra mussels (*Dreissena polymorpha*) at sublethal water temperatures. Canadian Journal of Fisheries and Aquatic Sciences. Pagination not final.

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KEY TO SYMBOLS AND ABBREVIATIONS

°C	degree Celsius
μeq	microequivalent
μg	microgram
μm	micrometer
μmol	micromole
AIC	Akaike information criterion
ANCOVA	analysis of covariance
ANOVA	analysis of variance
C	cell density
Chl-*a*	chlorophyll-*a*
CV	coefficient of variation
d	day
df	degrees of freedom
DIC	dissolved inorganic carbon
DIN	dissolved inorganic nitrogen
ED	equivalent diameter
F	filtering rate
g	gram
h	hour
ha	hectare
HAB	harmful algal bloom
id	inner diameter

k	light extinction coefficient
KBS	W. K. Kellogg Biological Station
K_m	half-saturation constant
L	liter
ln	natural logarithm (base e)
log	logarithm (base 10)
LTER	Long Term Ecological Research
m	meter
max	maximum
mg	milligram
MiCorps	Michigan Clean Water Corps
min	minimum
mL	milliliter
MLD	maximum linear dimension
MLSA	Michigan Lake and Stream Associations
mm	millimeter
N	population size
n	sample size
N	nitrogen
NH_4^+	ammonium
NO_3^-	nitrate
p	p-value
P	phosphorus

r	Pearson correlation
R^2	coefficient of determination
RM	repeated measures
r_{max}	maximum intrinsic growth rate
SE	standard error
SRP	soluble reactive phosphorus
t	t-value
TDP	total dissolved phosphorus
TP	total phosphorus
V	volume
W	watt
Z_{epi}	epilimnion (mixed layer) depth
Z_{SD}	Secchi disk transparency

PROLOGUE

The quality of freshwater ecosystems is of paramount social and economic concern given their importance as drinking water sources, as well as their aesthetic and recreational values. Yet, these systems are increasingly vulnerable to and impacted by numerous anthropogenic stressors associated with global change—particularly cultural eutrophication, climate warming, and the spread of invasive species (Carpenter et al. 1992, Ricciardi and MacIsaac 2000, Rigosi et al. 2014). Each of these stressors is known to promote species of nuisance phytoplankton that can form harmful algal blooms (HABs) (Schindler 1974, Raikow et al. 2004, Paerl and Huisman 2008, Elliott 2012), which foul water with unpleasant odors and tastes, accumulate as visible surface scums (Fig. 1), and produce potentially dangerous toxins that can poison humans, livestock, and pets (Carmichael 1994, Chorus and Bartram 1999, Huisman et al. 2005, Cronberg and Annadotter 2006). The colonial, toxin-producing cyanobacterium *Microcystis aeruginosa* (Fig. 2a) is the most common of the freshwater HAB-forming species in nutrient-polluted (eutrophic) lakes, and represents a major global threat to water quality and human health (Visser et al. 2005).

Despite the fact that *M. aeruginosa* is an asexual, prokaryotic organism, its populations are known to harbor a surprising extent of genetic (Wilson et al. 2005, Kardinaal et al. 2007a, Tanabe et al. 2007, Dyble et al. 2008) and phenotypic (Otsuka et al. 2000, Martins et al. 2009, Horst et al. 2014, White and Sarnelle 2014) variation, which could strongly influence its ecology, human impacts, and response to global change (Burkholder and Glibert 2009). However, understanding of the ecological implications of this large biological variation within *M. aeruginosa,* and species in general, is still limited (Bolnick et al. 2011, White et al. 2011, Violle et al. 2012).

Surprisingly, *M. aeruginosa* has also expanded its niche into low-nutrient (oligotrophic) lakes, where it would not otherwise achieve appreciable abundance (Watson et al. 1997, Downing et al. 2001), as a direct result of facilitation by an invasive bivalve grazer, the zebra mussel (*Dreissena polymorpha;* Fig. 2b) (Vanderploeg et al. 2001, Raikow et al. 2004, Sarnelle et al. 2005). *Microcystis* biomass and associated microcystin toxin concentrations are significantly elevated in these otherwise high-quality lakes, as compared to similar but non-invaded lakes (Knoll et al. 2008, Sarnelle et al. 2010). Though this pattern has been experimentally validated (Sarnelle et al. 2012), little else is known about the ecology of *M. aeruginosa* in these uncharacteristic low-nutrient habitats. In particular, the consequences of ongoing climate change for *Microcystis* populations in low-nutrient lakes are unknown.

This dissertation explores the influences of variation in environmental drivers (e.g., zebra mussels, temperature) and variation in biological traits (e.g., colony size, growth rate) on the ecology of *Microcystis,* with an emphasis on the ecological interaction with zebra mussels. In addition to improving knowledge of the dynamics of harmful cyanobacteria, the presented results have broader implications for our understanding of population dynamics and species interactions, and for predicting the responses of ecosystems to complex global change.

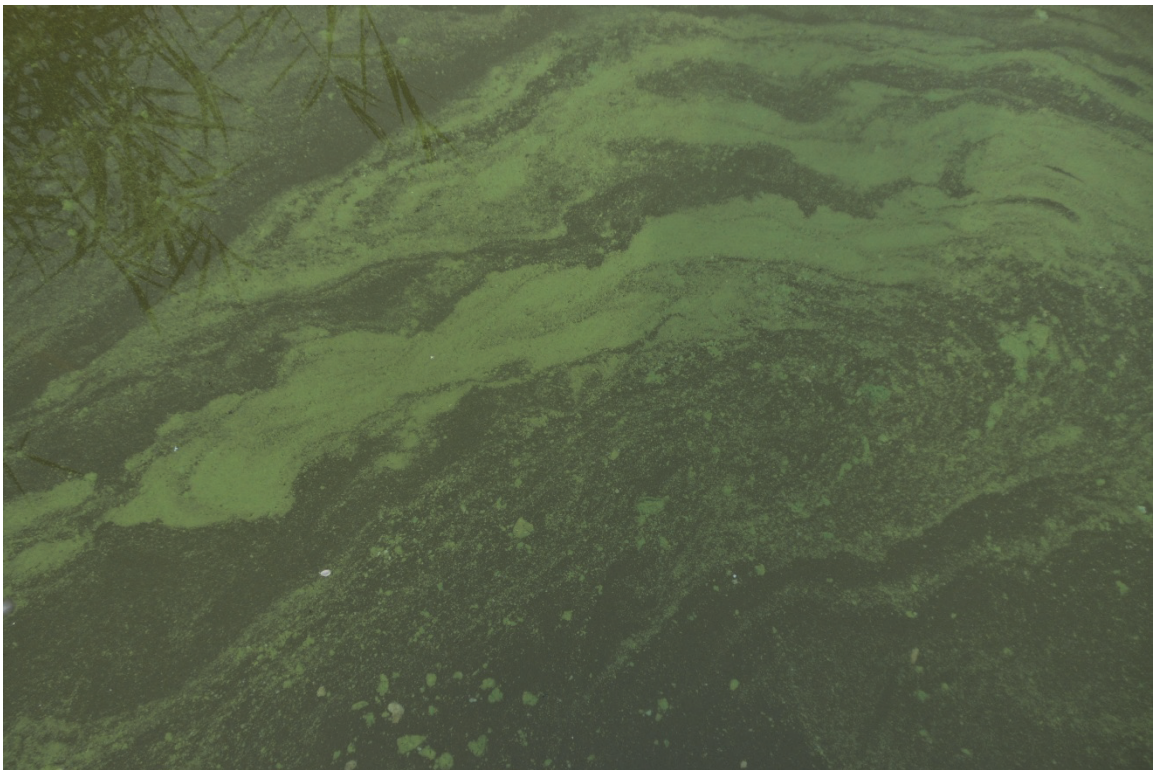
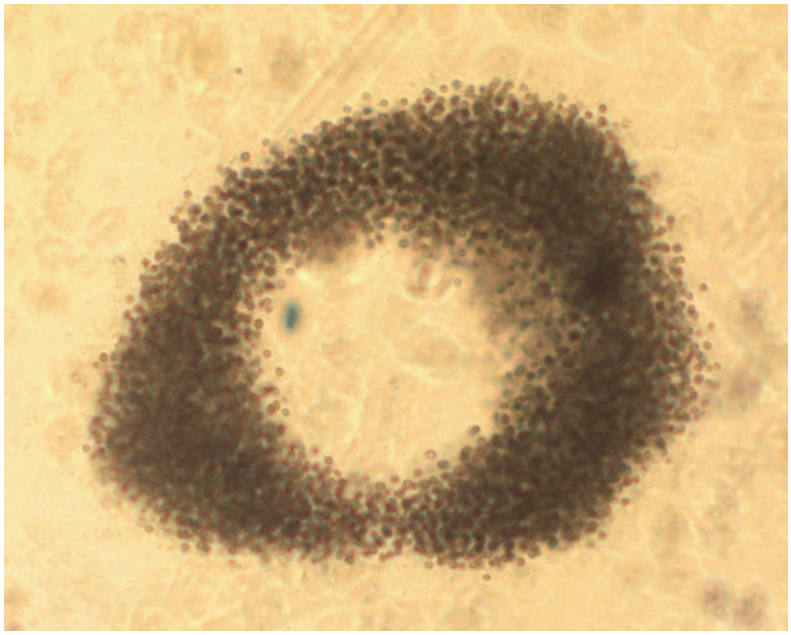


Figure 1. Formation of a surface scum during a mid-August bloom of toxic *Microcystis aeruginosa* in a hyper-eutrophic shallow lake in Michigan. Photo credit: Jeffrey D. White.

A)



B)

Figure 2. (A) *Microcystis aeruginosa* colony from Gull Lake, Michigan, photographed under a light microscope at 100×. The cells appear brown due to preservation in Lugol's iodine. (B) Zebra mussels (*Dreissena polymorpha*) filter-feeding in Gull Lake at a depth of 1 m. Photo credits: Jeffrey D. White.

CHAPTER 1

SIZE-STRUCTURED GRAZING VULNERABILITY OF THE COLONIAL CYANOBACTERIUM, *MICROCYSTIS AERUGINOSA,* TO ZEBRA MUSSELS (*DREISSENA POLYMORPHA*)

Abstract

We quantified the vulnerability of colonies of the bloom-forming cyanobacterium, *Microcystis aeruginosa,* to grazing by the invasive filter feeding zebra mussel (*Dreissena polymorpha*) as a function of size in both organisms with laboratory feeding experiments. In one experiment, size-selectivity of 16-21 mm mussels was assessed for a single *M. aeruginosa* strain across a wide size range (~5-88 μm median equivalent diameter, ED). Consumption of colonies ≥ 80 μm median ED (109 μm median maximum linear dimension, MLD) was undetectable, indicating a size threshold of grazing invulnerability. Smaller colonies and single cells were consumed at rates similar to a highly-palatable alga (*Ankistrodesmus*). In a second experiment, the size-selectivity of three size classes of mussels (8-11, 17-20, and 25-28 mm shell length) was assessed across three size classes of *M. aeruginosa* (~32-75 μm median ED). There were no systematic differences in the abilities of the different mussel size classes to consume the largest colonies within this size range. An 8-year field survey of the *M. aeruginosa* population in Gull Lake, MI, the source of the experimental organisms, revealed that median colony size consistently decreased during each summer, from above to below the size threshold of effective mussel feeding we identified, which suggests major within-season shifts in the overall vulnerability of the *M. aeruginosa* population to mussel grazing. Variation in the size structure of *M. aeruginosa* may help explain highly-variable effects of *D. polymorpha* on the dynamics of this harmful phytoplankter within and across systems.

Introduction

Microcystis aeruginosa, a colonial cyanobacterium common in eutrophic freshwaters, poses a major water quality threat due to the production of toxic, harmful algal blooms (HABs) that degrade drinking water and impede recreational use. Better understanding of the ecology of this species is critical for mitigating these negative effects. In this paper, we focus on the ability of grazers to consume and potentially control *M. aeruginosa* populations in nature by quantifying size-selective feeding behavior by the filter-feeding zebra mussel, *Dreissena polymorpha,* within a context of natural size variation in *M. aeruginosa*.

Due to the formation of large, mucilaginous colonies and the production of toxins and other putative chemical deterrents (Fulton and Paerl 1987b, a, Jungmann and Benndorf 1994, Agrawal 1998), *M. aeruginosa* is widely characterized as 'inedible,' although recent studies have documented high rates of consumption and control of this species by some grazers (White et al. 2011, Chislock et al. 2013). Populations exhibit marked variation in colony size (spanning several orders of magnitude) within and across lakes (Reynolds and Rogers 1976, Reynolds et al. 1981, O'Brien et al. 2004, Wilson et al. 2006). Colony size is also highly variable within and among genotypes, showing little genetic correlation (Rico et al. 2006, Wilson et al. 2010). Despite the potential importance of size structure in *Microcystis*-grazer interactions, many studies investigating the grazing vulnerability of *M. aeruginosa* have employed cultured strains that are either single-celled or produce only small colonies (Baker et al. 1998, Dionisio Pires and Van Donk 2002, Dionisio Pires et al. 2004).

Grazer selectivity for size-fractioned *Microcystis* has been investigated in *Daphnia* (Jarvis et al. 1987, 1988). These studies used naturally-occurring colonial *Microcystis*, thus avoiding the aforementioned limitations and providing valuable information about the size-

structured grazing vulnerability of the particular *Microcystis* population under scrutiny. However, we recently reported that different strains of *M. aeruginosa* isolated from the same population can vary maximally in their vulnerability to grazing independent of colony size (White et al. 2011). Consequently, factors other than colony size that vary among co-occurring genotypes (e.g., cellular chemistry) could confound attempts to delineate size-vulnerability relationships in feeding experiments using natural seston. Thus, there is a need for further investigation as to how colony size affects grazing vulnerability in the absence of such confounds.

The ongoing infestation by the invasive, filter-feeding zebra mussel (*Dreissena polymorpha*) has led to marked changes in North American freshwater ecosystems (Strayer 2008, Higgins and Vander Zanden 2010), including unexpected increases in *M. aeruginosa* biomass in low-nutrient lakes (Vanderploeg et al. 2001, Raikow et al. 2004, Knoll et al. 2008, Sarnelle et al. 2010). Furthermore, the effect of *D. polymorpha* on *M. aeruginosa* biomass is highly variable across invaded ecosystems, exhibiting strong sensitivity to total phosphorus concentration (Sarnelle et al. 2012). Better understanding of this interaction is needed, and large natural variation in *M. aeruginosa* colony size may help explain these variable effects.

Dreissena are selective feeders (Baker et al. 1998, Bastviken et al. 1998, Vanderploeg et al. 2001, Dionisio Pires et al. 2004), but there is great variation in the size ranges of ingestible particles reported for *D. polymorpha*. For example, *D. polymorpha* has been shown to reject particles > 50-80 μm in some studies (Ten Winkel and Davids 1982, Roditi et al. 1996, Naddafi et al. 2007), K. Wissing, unpubl. data) yet filter phytoplankton up to 150 μm at equal rates in another (Horgan and Mills 1997). Often, particle size-classes in feeding studies are created by varying phytoplankton species composition, either by using different laboratory-cultured species

or by fractionating natural seston (Sprung and Rose 1988, Bern 1994, Horgan and Mills 1997, Dionisio Pires et al. 2004). This introduces a confound between particle size and other factors (such as chemical composition) that vary across phytoplankton species, and it has been established that factors other than particle size have a strong influence on *Dreissena* feeding (Ten Winkel and Davids 1982, Bastviken et al. 1998, Naddafi et al. 2007, White et al. 2011), leaving lingering uncertainty about their size selectivity.

Previous studies have provided a valuable starting point with which to specifically assess size-selectivity of *D. polymorpha* for *M. aeruginosa*, but the upper limit to ingestion has not been determined. For example, studies have fractionated pure *Microcystis* cultures into coarse 'small' versus 'large' size classes about an arbitrary cutoff (e.g., 53 or 60 μm (Vanderploeg et al. 2001, Dionisio Pires et al. 2005) and observed efficient clearance by mussels on the larger fraction. We can thus expect the upper limit to ingestion to be greater than 53-60 μm. However, more precisely estimating the upper size limit of effective grazing requires employing many more size fractions that each contains a very narrow range of colony sizes.

In addition to the above, we are not aware of any study quantifying the relative mortality imposed by different size classes of *Dreissena* on different size classes of phytoplankton (Naddafi et al. 2007). Body size is of fundamental importance in determining the size spectrum of ingestible particles for filter-feeding zooplankton: larger animals are capable of consuming larger particles (Burns 1968, Geller and Müller 1981, Bogdan and Gilbert 1984, Hansen et al. 1994). *Dreissena polymorpha* inhalant siphon diameter increases with body size, leading to the prediction that larger mussels should be able to ingest larger particles (MacIsaac et al. 1991, Naddafi et al. 2007). Bridoux et al. (2010), quantifying phytoplankton biomarkers in Lake Erie *D. polymorpha* tissue, found evidence for increased importance of larger phytoplankton taxa in

the diet of larger mussels. Yet, MacIsaac *et al.* (1995) found no systematic differences among size classes of *D. polymorpha* >10 mm in their ability to ingest microzooplankton up to 89 μm, though these results may not be directly comparable to phytoplankton. An assessment of the relative size-selectivities of different size classes of *D. polymorpha* for phytoplankton, and more specifically for *Microcystis,* is still needed.

Our study addresses two basic questions. Firstly, how does the vulnerability of *M. aeruginosa* to grazing by *D. polymorpha* vary with colony and mussel size? Secondly, how do these size-vulnerability relationships compare to size distributions of *M. aeruginosa* colonies in nature? The latter comparison may help to explain variable effects of *D. polymorpha* invasion on *M. aeruginosa* biomass. Size-selective grazing by *D. polymorpha* on *M. aeruginosa,* if intense, might also shift the size distribution of *M. aeruginosa* colonies toward less vulnerable size classes. This could lead to *M. aeruginosa* populations dominated by large colonies, which should be more likely to accumulate at the surface as a scum (possibly elevating exposure risk to more concentrated doses of toxins) due to faster vertical migration velocities (Visser et al. 1997). In general, knowledge of grazing impacts on *M. aeruginosa* as a function of both colony and mussel size would improve our understanding of the complex interaction with *D. polymorpha* and the role of large variation in colony size in the population dynamics of this HAB species (Burkholder and Glibert 2009, Pitcher 2012).

Methods

Size-selectivity of *D. polymorpha* was assayed via a 'particle- choice' experiment in which eight size classes of a single, palatable strain of *M. aeruginosa* (2009C) were individually presented to mussels along with *Ankistrodesmus falcatus* (mean cell dimensions: 38.5 × 2.5 μm).

A. falcatus was employed as a high-quality alga against which selectivity for *M. aeruginosa* size fractions could be assessed (White et al. 2011). Size selectivity as a function of both colony and mussel size was measured in a second experiment in which three size classes of mussels were fed three size classes of a single, palatable strain of *M. aeruginosa* (G11-08). In the second experiment, we used a simpler 'no choice' design because filtration rates on *M. aeruginosa* were found to be strongly correlated with selectivity (*M. aeruginosa* versus *A. falcatus*) in previous experiments (White *et al*., 2011; this study). To relate measured size-selectivities to a natural population of *M. aeruginosa*, we also report the results of extensive monitoring of *M. aeruginosa* size distributions in Gull Lake, MI (2001-2011), the source of the experimental mussels and *M. aeruginosa* strains.

Collection and maintenance of experimental organisms

Gull Lake is a large (area: 822 ha, max depth: 33 m), low-nutrient lake (TP ~10 μg L^{-1}, mixed layer chlorophyll *a* ~1-6 μg L^{-1}) in southwest MI (Barry and Kalamazoo Counties). The biomass of *M. aeruginosa* increased dramatically after zebra mussels invaded in 1994 (Sarnelle et al. 2005). Gull Lake has been the site of large-scale enclosure experiments (Sarnelle et al. 2005, Sarnelle et al. 2012) aimed at understanding the complex interaction between *D. polymorpha* and *M. aeruginosa*.

Mussels were collected with an Ekman grab and immediately separated from substrata (macrophytes and small rocks). 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 selected animals with shell lengths of 16-21 mm for the first experiment because this size class was used in the aforementioned enclosure experiments. Based on a survey

of the *D. polymorpha* population conducted in 1999 (Wilson and Sarnelle 2002), this size class accounts for ~30% of the total *Dreissena* filtering impact on phytoplankton in the mixed layer (mean depth ~7.5 m) of Gull Lake during summer stratification (Fig. 3). More generally, the 16-21 mm size class is representative of the larger individuals commonly found in invaded inland lakes (Horgan and Mills 1997, Idrisi et al. 2001, Naddafi et al. 2007). For the second experiment, we sorted mussels into three size classes (8-11, 17-20, and 25-28 mm shell length) that collectively account for ~70% of the total filtering impact in the mixed layer of Gull Lake (Fig. 3).

We selected two strains of *M. aeruginosa* isolated from Gull Lake that were: 1) still forming colonies in culture, and 2) shown to be highly palatable to mussels (i.e., elicited filtering rates comparable to *A. falcatus* in previous experiments (White et al. 2011), unpublished data). The strains were maintained in batch cultures of 0.5× WC-S medium, with an inoculum transferred to fresh, sterile medium every 4-8 weeks (White et al. 2011). *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.

Before each experiment, we grew 25 L of *M. aeruginosa* in 1 and 2 L bottles of 0.5× WC-S, under the same growth conditions described above. The large volume was required to yield sufficient biomass in all size classes for the feeding experiments. Bottle position was randomized every 2 d to reduce heterogeneity in light conditions during growth. Cultures were harvested for the experiment 30 d after inoculation, at which time they were still growing exponentially (J. White, pers. obs.).

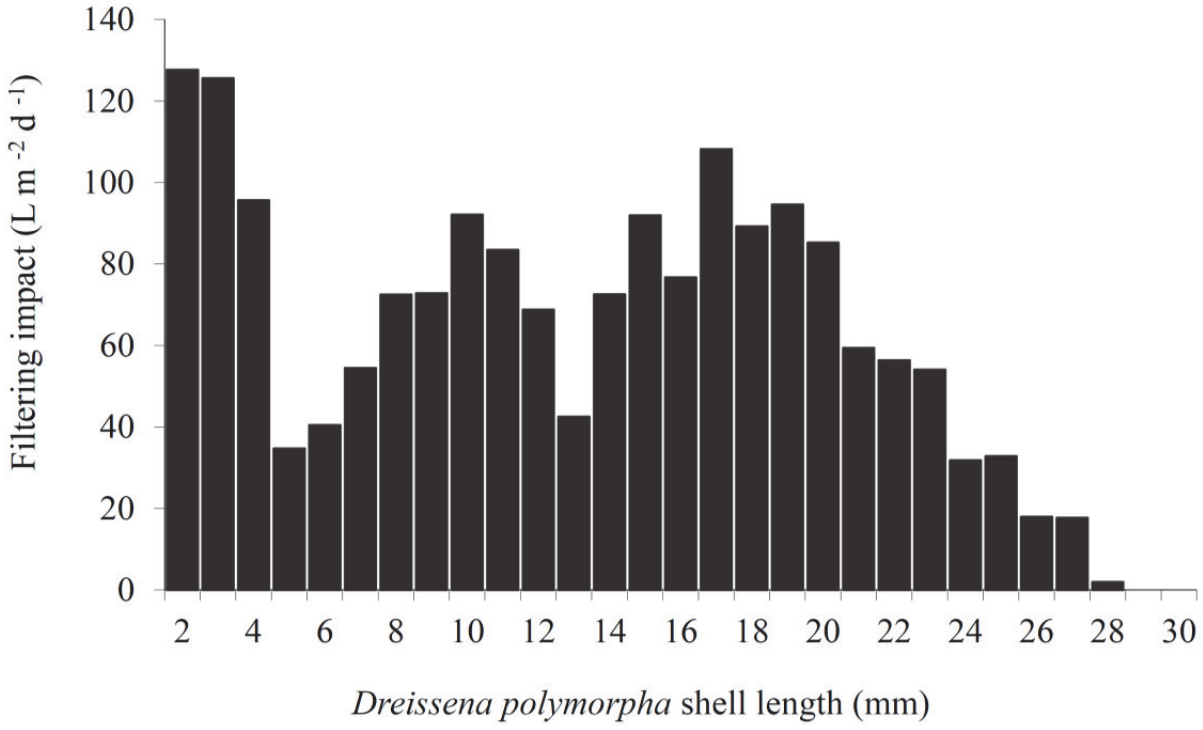


Figure 3. Estimated *Dreissena polymorpha* filtering impact on phytoplankton in the mixed layer of Gull Lake (0-7.5 m depth). Size distribution data were obtained from Wilson and Sarnelle (2002). Filtering impact was calculated using the *D. polymorpha* length-filtering rate regression of Kryger and Riisgård (1988).

Twenty-four hours prior to an experiment, the *M. aeruginosa* cultures were pooled into a single container and mixed thoroughly. Two liters were set aside for controls and mussel pre-acclimation (24 h) to the *M. aeruginosa* strain (White et al. 2011). The remaining ~23 L was sequentially passed through sieves of decreasing pore size to gently size-fractionate the colonies (Table 1). Material collected was thoroughly rinsed from each sieve and 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 experiments.

Design of feeding experiments

Each feeding suspension contained one size class of *M. aeruginosa* plus *A. falcatus* in filtered lake water (first experiment: mean total chlorophyll *a* = 3.9 μg L^{-1}) or just one size class of *M. aeruginosa* (second experiment: mean chlorophyll *a* = 2.3 μg L^{-1}). Chlorophyll levels were within the range of mixed-layer conditions in Gull Lake. In the first experiment, we employed 4 replicate beakers with mussels for each of eight size-class treatments, and a total of 3 control beakers containing un-fractionated *M. aeruginosa* without mussels. The second experiment consisted of three levels of mussel size (8-12, 17-20, and 23-27 mm shell length) crossed with three size classes of *M. aeruginosa* (Table 2), with three replicate beakers of each treatment combination. We also employed two replicate beakers per mussel size class of an *A. falcatus* suspension (mean chlorophyll *a* = 3.2 μg L^{-1}).

Table 1. Median sizes of *Microcystis aeruginosa* colonies (Gull Lake strain 2009C) in the eight feeding suspensions used in first experiment. See text for a description of the size metrics.

Mesh sizes (lower, upper; μm)	Median equivalent diameter (μm)	Median maximum linear dimension (μm)
--, 8*	4.8	4.8
24, 35	21.5	29.6
35, 45	30.3	41.5
45, 53	34.8	46.8
53, 63	44.3	56.7
63, 80	62.6	75.4
100, 150	80.1	109.3
150, 200	87.7	120.8

***** Treatment composed of single cells.

Table 2. Median sizes of *Microcystis aeruginosa* colonies (Gull Lake strain G11-08) in the three feeding suspensions used in the second experiment. See text for a description of the size metrics.

Mesh sizes (lower, upper; μm)	Median equivalent diameter (μm)	Median maximum linear dimension (μm)
35, 53	31.5	38.9
53, 100	51.7	76.1
100, --	75.1	100.6

Mussels were held in filtered lake water for 3 h immediately prior to the experiments 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 (Fig. 4). Beakers were gently aerated, which kept phytoplankton in suspension throughout the experiments. The feeding period began once mussels were actively filtering (siphons fully extended), which generally occurred within 5 minutes of placement into beakers. Mussels fed for 1 h (first experiment) or 1.5 h (second experiment). Beakers were mixed thoroughly and then sampled for chlorophyll *a* (filtered onto Pall A/E glass-fiber filters and frozen) and algal counts (first experiment only, preserved in 1% Lugol's iodine) immediately before mussels were added (initial) and immediately after mussels were removed (final). In the second experiment, each feeding suspension was sampled for measurements of colony size (preserved in 1% Lugol's iodine) prior to allocation to beakers.

Sample processing and data analysis

For the first experiment, Lugol's-preserved subsamples were settled in 10 mL phytoplankton chambers. *Ankistrodesmus* cells were counted at 200× with an inverted microscope (Nikon Eclipse; (Lund et al. 1958, Sandgren and Robinson 1984). The surface area and maximum linear dimension (MLD) of each *M. aeruginosa* colony ($n \sim$ 25-50 colonies per sample) were measured (at 100× or 200×, depending on size class) from digital micrographs (SPOT, Diagnostic Instruments). Total surface area in a sample was converted to cell density via a regression developed for Gull Lake *M. aeruginosa* (Sarnelle et al. 2012). The equivalent diameter (ED) of each colony was also determined by calculating the diameter of a circle with surface area equivalent to that of each measured colony. *Microcystis* cells in the single-cell treatment were counted and measured at 400×.

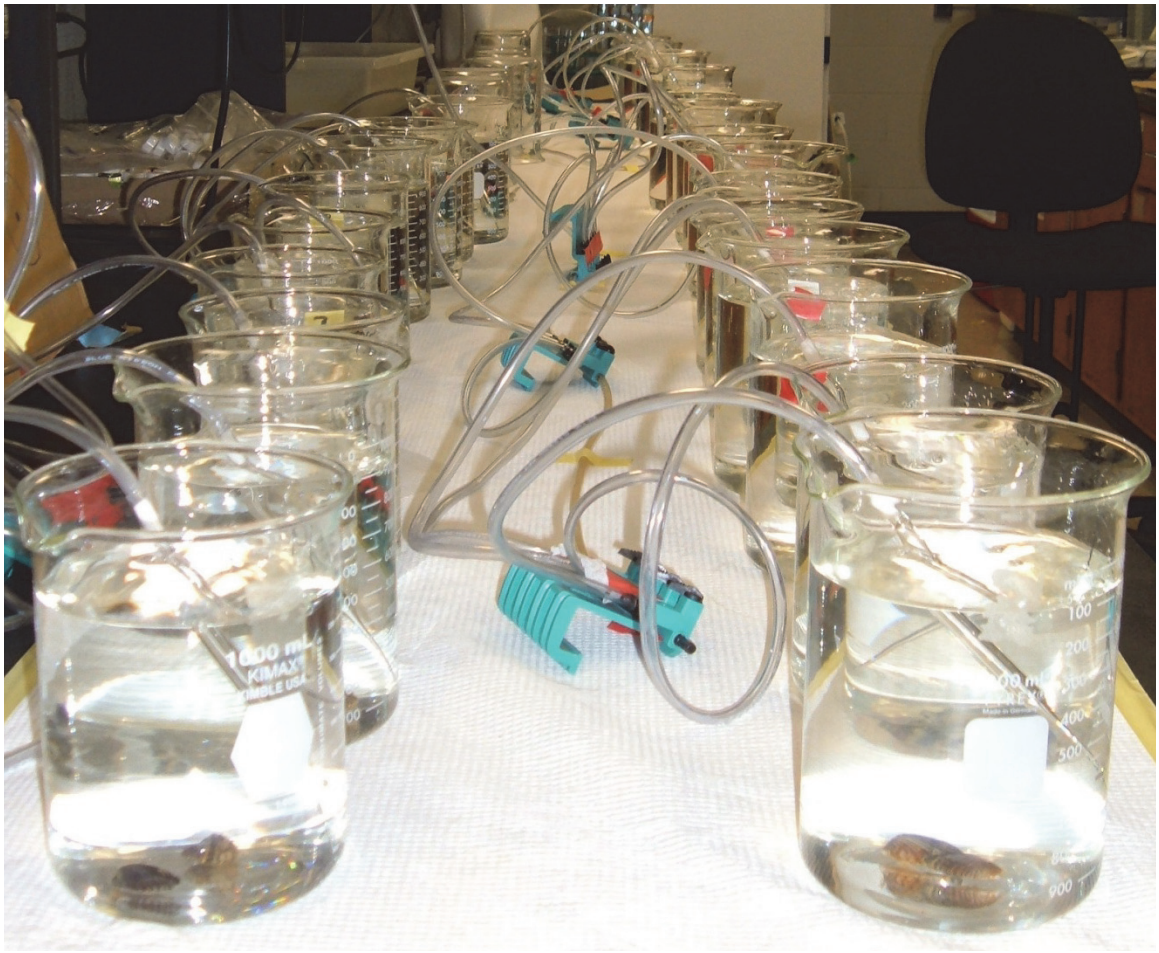


Figure 4. View of a laboratory feeding experiment with zebra mussels. Photo credit: Jeffrey D. White.

For the first experiment, we calculated species-specific filtering rates (L individual^{-1} d^{-1}; i.e., mortality rates due to mussel grazing) 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. No correction for changes in the control beakers was needed because no changes in cell density occurred during these short incubations (paired t-tests, $p > 0.4$). Mussel selectivity was determined for each beaker as F_m/F_a, where F_m and F_a are the filtering rates on the *M. aeruginosa* size class and *A. falcatus,* respectively (Jacobs 1974, Sterner 1989).

For the second experiment, initial colony size of *M. aeruginosa* in each of the three suspensions was determined as above. Filtering rates on the unialgal suspensions were measured as the rate of chlorophyll *a* depletion over time as above. 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).

Statistical analyses were performed with R version 3.0.1 (R Foundation for Statistical Computing). We tested for differences in mean mussel selectivity across *M. aeruginosa* size classes in the first experiment with a one-way ANOVA. In the event that this ANOVA was significant, we performed 1-tailed *t*-tests to check for significant avoidance of *M. aeruginosa* (selectivity < 1) for each size class. For treatments where mussels exhibited significant selection against *M. aeruginosa*, we further tested whether mussel consumption of *M. aeruginosa* was > 0 (1-tailed *t*-tests). We used one-tailed tests because the *a priori* expectations are for mussels to prefer *A. falcatus* when they are selective (since *A. falcatus* is consumed at maximal rates), and to have non-negative filtering rates (White et al. 2011). In the second experiment, we tested for a

significant interaction between mussel body size and colony size of *M. aeruginosa* on mussel filtering rate using a two-way ANOVA. Since per capita filtering rate increases with body size in *D. polymorpha* (Kryger and Riisgård 1988), filtering rates were standardized to mussel mass (Wilson and Sarnelle 2002). Mass-specific filtering rates were log-transformed to reduce heterogeneity of variances.

Lake sampling

Phytoplankton were sampled from the mixed layer of Gull Lake biweekly from July-August in 2001 and 2005-2008, and from June-September in 2009-2011, via two pooled casts of a depth-integrating tube sampler. Samples were collected from a near-shore station (depth = 13 m) during 2001 and 2005-2008. In 2009-2011, samples were collected from the near-shore station as well as a central station (depth = 30 m). Subsamples were preserved in 1% Lugol's iodine and settled in 10 mL phytoplankton chambers. Colony ED of *M. aeruginosa* was determined as described above. Median colony size did not significantly differ between sampling stations in 2009 (paired *t*-test, $t = 0.87$, df = 7, $p > 0.4$), 2010 (paired *t*-test, $t = 0.39$, df = 8, $p > 0.7$) or 2011 (paired *t*-test, $t = 0.0019$, df = 4, $p > 0.9$); therefore, data were pooled across stations for those three years.

Results

Experiment 1: colony size-selectivity experiment

Size fractionation of the *M. aeruginosa* culture produced a range of colony sizes spanning more than an order of magnitude across 8 treatments. Median MLD in each fraction corresponded to the mesh sizes employed more than median ED (Table 1), although we report

both to facilitate comparisons across studies using different metrics. Initial total algal biomass did not significantly differ across treatments (ANOVA, $F_{(7,23)} = 1.58$, $p = 0.190$). Mussel selectivity in one beaker was a large, 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 among all 8 size treatments (ANOVA, $F_{(7,23)} = 4.85$, $p = 0.002$; Fig. 5a). *Dreissena* exhibited significant selection against *M. aeruginosa* (selectivity < 1; 1-tailed *t*-tests, $t < -2.45$, $df = 3$, $p < 0.05$) in the two treatments having median colony sizes ≥ 80.1 μm ED (109.3 μm MLD), whereas selectivity for *M. aeruginosa* within the remaining, smaller fractions was not different from 1 (indicating non-selective feeding), consistent with a previous study that found strain 2009C to be highly palatable to *D. polymorpha* (White et al. 2011). Congruent with the selectivity results, mussel filtering rates on *M. aeruginosa* varied significantly across treatments (ANOVA, $F_{(7,23)} = 4.59$, $p = 0.002$; Fig. 5c) and were not significantly greater than 0 in treatments with colonies ≥ 80.1 μm median ED (1-tailed *t*-tests, $t > 0.21$, $df = 3$, $p > 0.09$). Conversely, filtering rates on *A. falcatus* did not significantly vary among treatments (ANOVA, $F_{(7,23)} = 0.34$, $p = 0.929$; Fig. 5b). All *Microcystis* colonies observed in the post-feeding samples appeared healthy and exhibited no signs of damage as a possible result of collection and rejection by *D. polymorpha*.

Experiment 2: mussel size × colony size experiment

There was no mussel size × colony size interaction (2-way ANOVA, $F_{(4,18)} = 2.13$, $p = 0.119$; Fig. 6), and no effect of *M. aeruginosa* colony size on filtering rate for any mussel size class over the tested range of colony sizes ($F_{(2,18)} = 2.46$, $p = 0.114$). Thus, we found no evidence for size-selective feeding by any of the three mussel size classes in this experiment.

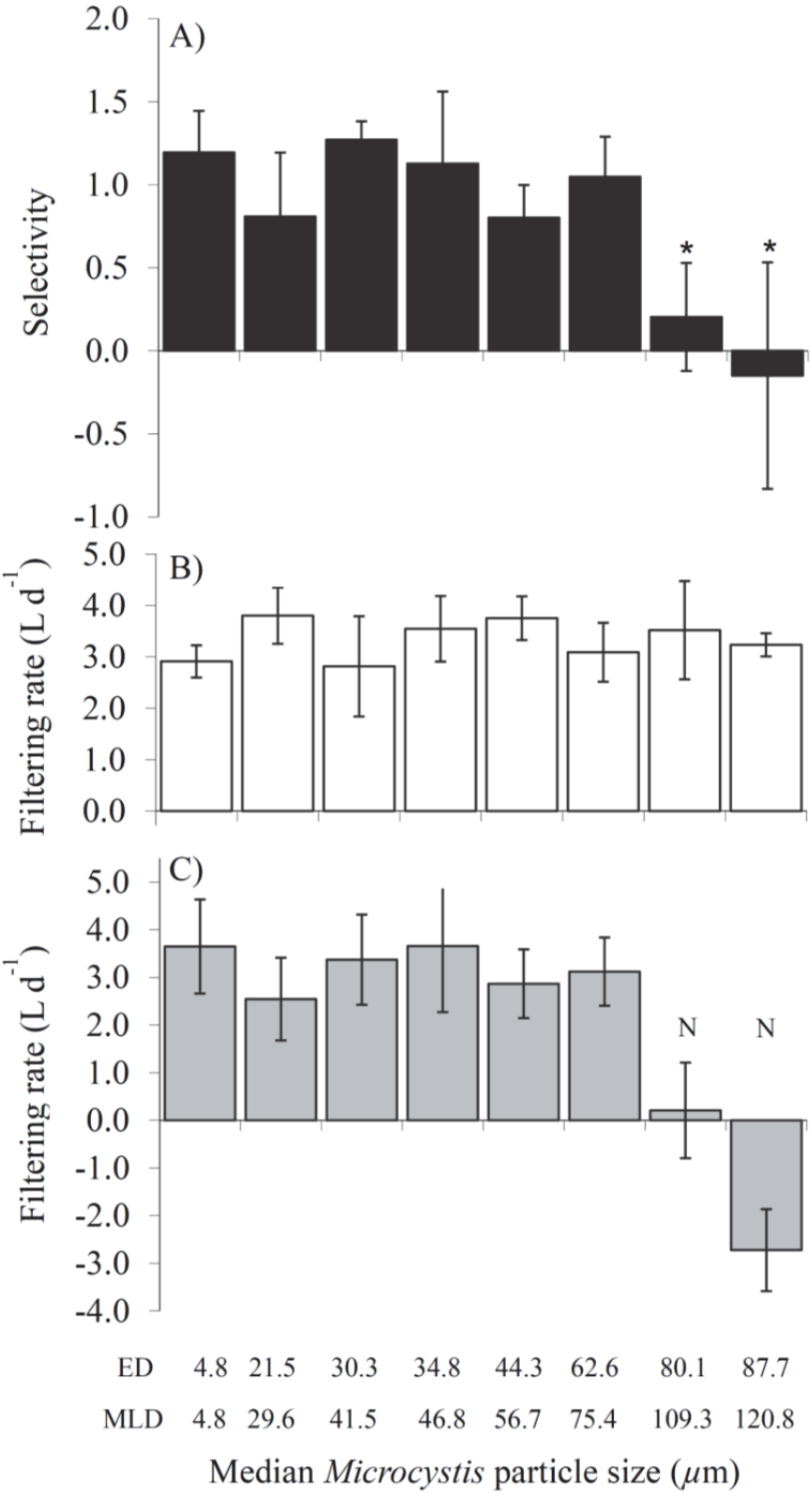


Figure 5. Results from experiment 1, the colony size-selectivity experiment.

Figure 5. (cont'd)

(A) Mean selectivity of *Dreissena polymorpha* across size classes of a single strain (2009C) of colonial *Microcystis aeruginosa* (n = 31). Selectivity was calculated as filtering rate on *M. aeruginosa* divided by filtering rate on the high-quality food alga, *Ankistrodesmus falcatus.* Error bars represent ± 1 SE. Asterisks indicate size fractions eliciting significant avoidance (selectivity significantly < 1). (B, C) Comparison of mean filtering rates on *A. falcatus* and *M. aeruginosa,* respectively. Error bars represent ± 1 SE. An N indicates non-detectable filtration (filtering rate not significantly > 0). Median *Microcystis* size is reported as both equivalent diameter (ED) and maximum linear dimension (MLD).

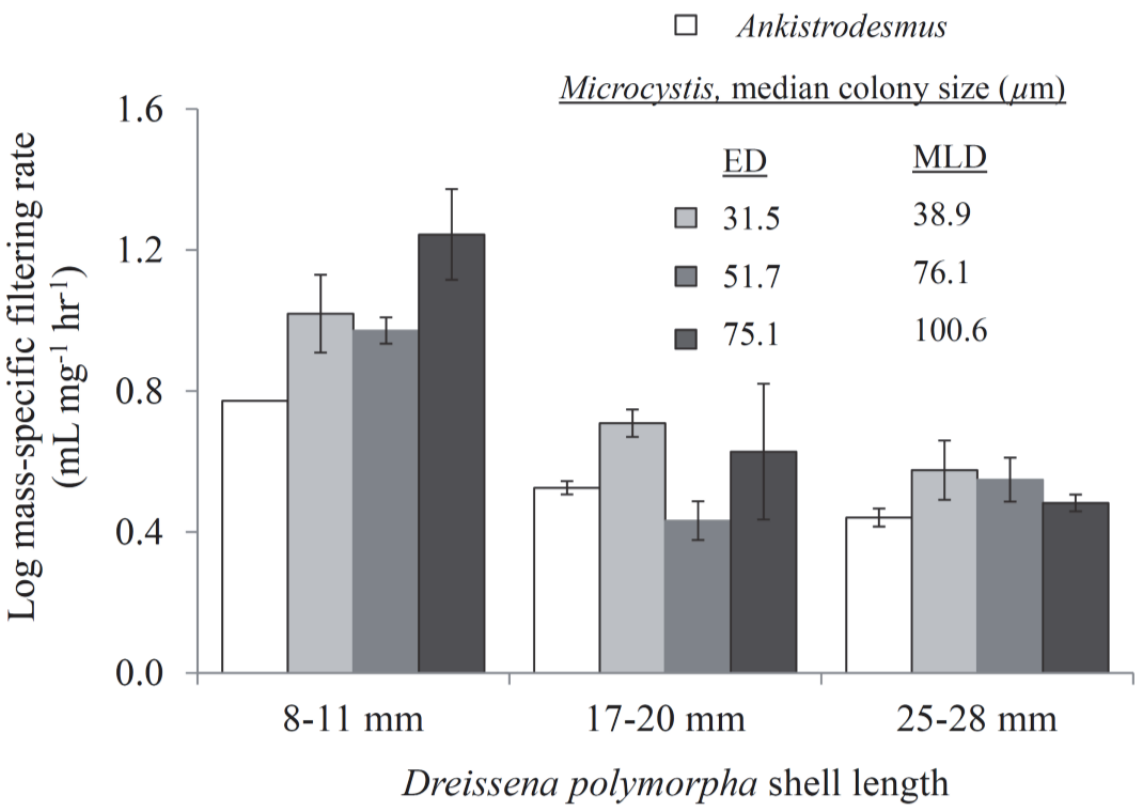


Figure 6. Results from experiment 2, the mussel size × colony size experiment. Mean mass-specific filtering rates of three common size classes of *Dreissena polymorpha* (shell length ranges given in mm) feeding on a size-fractioned strain of *Microcystis aeruginosa* (*n* = 27). Error bars represent ± 1 SE. Median *M. aeruginosa* colony size is reported as both equivalent diameter (ED) and maximum linear dimension (MLD).

Dreissena filtering rates on *M. aeruginosa* were similar to or greater than those on *A. falcatus* (Fig. 6), so the lack of evidence for size-selectivity was not due to lack of overall filtering on this *M. aeruginosa* strain. Small mussels have higher mass-specific filtering rates than larger mussels due to allometric scaling and so, not surprisingly, we also observed a significant effect of mussel size on mass-specific filtering rate ($F_{(2,18)} = 41.26$, $p < 0.001$). One *A. falcatus* replicate (within the 8-11 mm mussel class) was omitted from analysis because mussels did not feed in that beaker.

Lake sampling

The overall distribution of Gull Lake colony sizes, pooled from 8 summers of data, was right-skewed with a median of 79.5 μm ED (Fig. 7, $n = 3{,}035$). However, there was considerable inter- and intra-annual variability in colony size within the Gull Lake population. In fact, median colony size decreased from July through August in every year, by as much as 27%, with July median ED being significantly greater than August median ED across all 8 years (paired *t*-test, $p < 0.001$; Fig. 8). In 2009- 2011, years where sampling was extended, the decrease in colony size over the summer was especially apparent (Fig. 9). Most importantly, median colony ED tended to be above the mussel selectivity threshold identified in the colony size-selectivity experiment (~80 μm) early in the summer, but decreased to less than or equal to the threshold later in the summer, with the overall median tending to almost exactly the same size across years (Figs. 7, 8, 9).

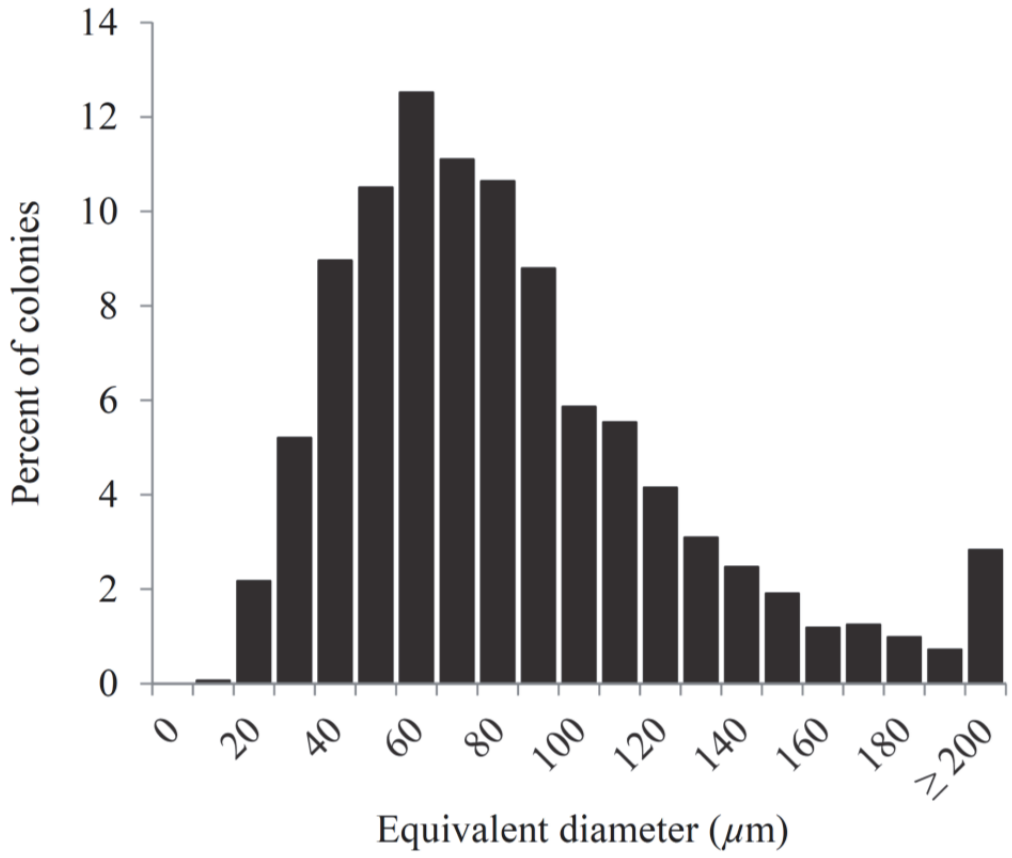


Figure 7. Size structure of the *Microcystis aeruginosa* population in Gull Lake: overall colony size distribution (equivalent diameter, ED) obtained from pooling 8 summers of measurements (n = 3,035).

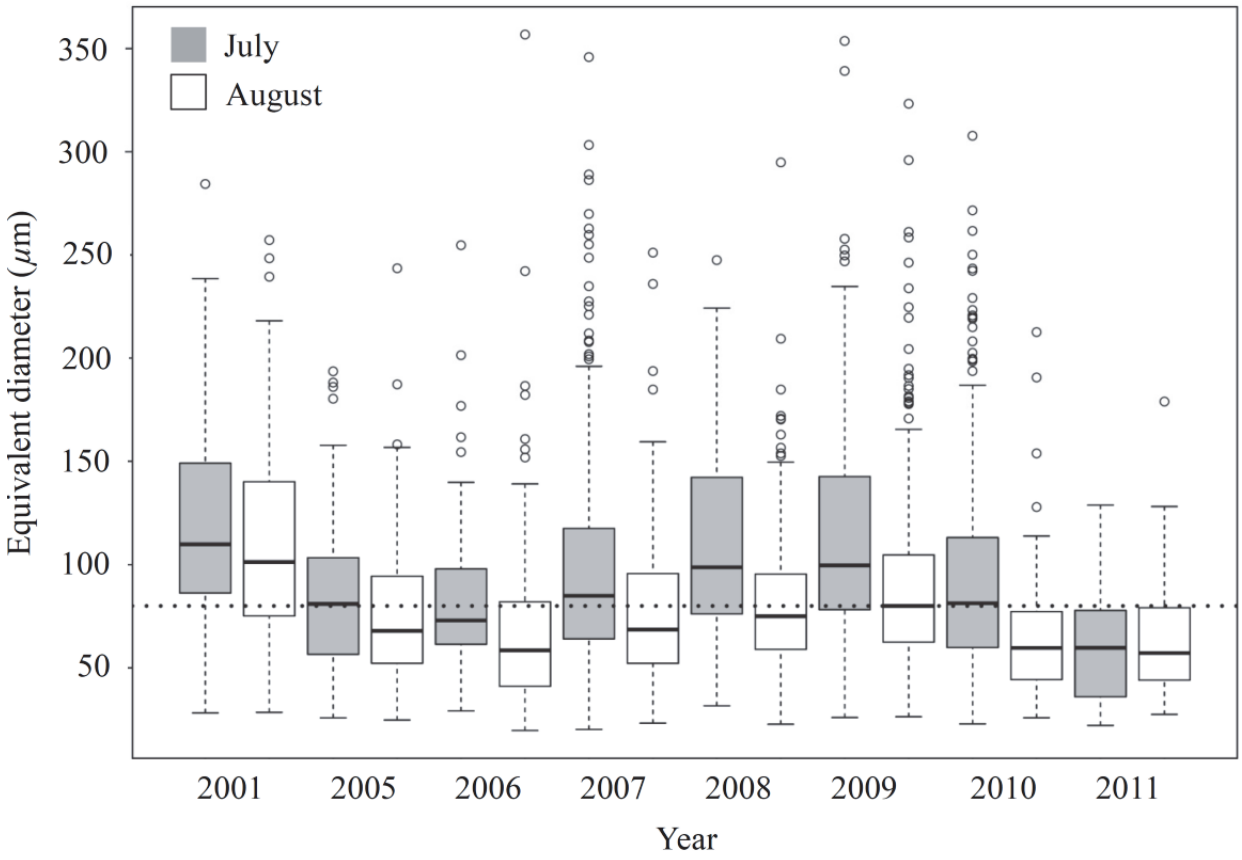


Figure 8. Size structure of the *Microcystis aeruginosa* population in Gull Lake: comparison of July and August colony size distributions by year. The horizontal dotted line at 80 μm ED is the approximate threshold above which *M. aeruginosa* is largely invulnerable to grazing by *Dreissena polymorpha* (*see* Fig. 5).

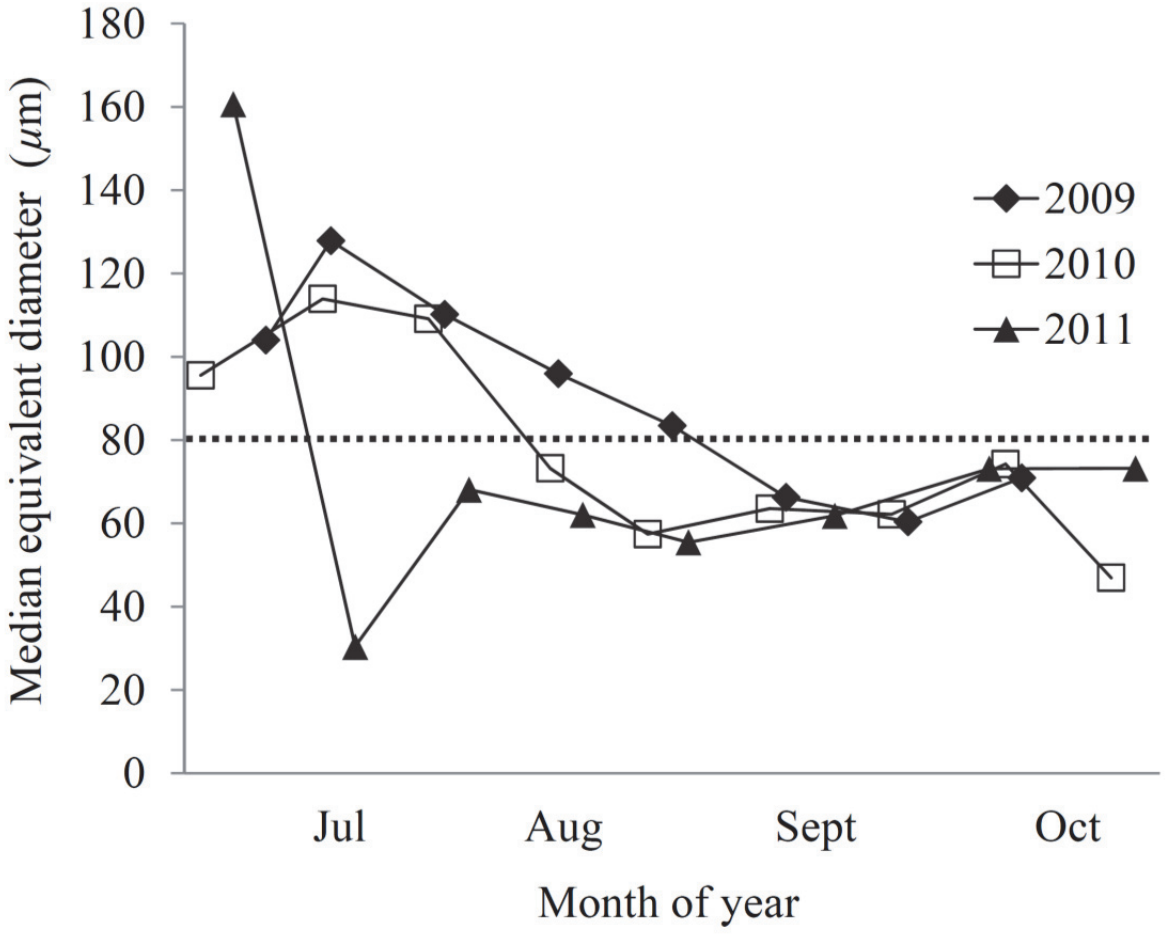


Figure 9. Seasonal change in median *Microcystis aeruginosa* colony size (equivalent diameter, ED) in Gull Lake during three consecutive summers. The horizontal dotted line at 80 μm ED is the approximate threshold above which *M. aeruginosa* is largely invulnerable to grazing by *Dreissena polymorpha* (*see* Fig. 5).

Discussion

We identified a *Microcystis* colony-size threshold of ~80 μm median ED (~109 μm median MLD) for 16-21 mm *D. polymorpha,* where filtration rates essentially fell to zero. Smaller colonies and single cells were consumed non-selectively and at maximal rates (Kryger and Riisgård 1988, White et al. 2011); Fig. 5). Previous experiments measuring the grazing of *D. polymorpha* on *M. aeruginosa* have employed single-celled strains (Baker et al. 1998, Dionisio Pires and Van Donk 2002, Dionisio Pires et al. 2004) or coarse 'small' versus 'large' size fractions (Vanderploeg et al. 2001, Dionisio Pires et al. 2005), and so do not clearly identify an upper size threshold for effective *D. polymorpha* feeding. In the latter case, where mussel filtering rates were assessed on aggregate fractions greater than and less than an arbitrary size (e.g., 53 and 60 μm, respectively), a significant proportion of the colonies in the larger fraction may still have been within the edible range, given that the size distributions of colonies in culture are generally highly right-skewed (J. White, pers. obs.). This may explain the lack of differences observed between filtering rates on colonial *Microcystis* in the < 60 μm and > 60 μm fractions (overall range: 41-722 μm) in Dionisio Pires *et al.* (2005).

Jarvis et al. (1987, 1988) quantified the size-dependent mortality imposed on a natural *M. aeruginosa* population by *Daphnia,* among the few previous grazing studies on *M. aeruginosa* to more systematically account for the effect of natural size variation. Our study goes further in eliminating all biological properties that could be confounded with size by utilizing single, highly palatable strains of *M. aeruginosa* grown under a single set of environmental conditions. Thus, the variation in selectivity and filtering rate that we observed (Fig. 5) can be unequivocally attributed to variation in colony size.

Our results are similar to some previous studies of *D. polymorpha* size-selective feeding, although there is uncertainty in comparing our results to the literature because of the aforementioned species-for-size confound in many studies. *D. polymorpha* (15-30 mm) have been shown to clear particles as small as 5 μm, equivalent in size to single cells of *M. aeruginosa* (Sprung and Rose 1988, Dionisio Pires et al. 2004). Also congruent with our study, Ten Winkel and Davids (1982) observed preferential ingestion by *D. polymorpha* (25 mm) of diatoms averaging 10-40 μm, but rejection of larger diatoms and chrysophytes > 80 μm. However, mussels (12-20 mm) were observed to largely reject phytoplankton > 50 μm and < 7 μm in another study (Naddafi et al. 2007); in still another study, mussels (18-22 mm) preferred natural seston in a 30-100 μm fraction over the 2-30 μm fractions (Dionisio Pires et al. 2004). Furthermore, Horgan and Mills (1997) found no differences in filtering rates of small mussels (9-11 mm) across size classes of natural phytoplankton ranging from 10-150 μm MLD, considerably higher than the threshold *M. aeruginosa* colony size identified for 16-21 mm mussels in the present study. Perhaps the rather inconsistent picture of *D. polymorpha's* size selectivity that emerges from the literature is in part a function of the use of different phytoplankton species as surrogates for variation in particle size.

With respect to models of size-structured grazing vulnerability, quantification of mortality rates imposed on different size fractions of *M. aeruginosa* is also more useful, compared to measurements of the maximum particle size collected by an individual *D. polymorpha,* since mortality rates are needed for predicting population dynamics. Though *D. polymorpha* has been shown to collect phytoplankton as large as 0.9-1.2 mm, these particles are usually rejected and expelled shortly thereafter (Horgan and Mills 1997) and so are likely to experience negligible grazing mortality (particle 'collection' is not synonymous with particle

'ingestion'). Collected phytoplankton that are expelled by *D. polymorpha* in this manner (as pseudofeces) generally remain viable and can return to the water column (Vanderploeg et al. 2001).

Dreissena also filtered the same strain (2009C) employed in our first experiment at near maximal rates in a previous study (White et al. 2011), although the strain was not size-fractionated in that experiment. Median colony size in that experiment was 85.3 μm ED, slightly higher than the threshold size for consumption found in the present study. This apparent discrepancy may arise because a large fraction of the colonies in the previous experiment were within the edible size range, since all sizes below 85 μm were present. The range of colony sizes within each size fraction in the present study was very narrow by design—critical for precisely quantifying grazer size-selectivity. *Dreissena* also consumed colonies (75.1 μm median ED) during the second experiment near to, though still below, the size threshold identified in the first experiment (80.1 μm ED; Figs. 5, 6). However, the *M. aeruginosa* strains employed in the two experiments were different and so may have differed subtly in morphology. In addition, we note that the variability in *D. polymorpha* selectivity and filtering on *M. aeruginosa* was relatively large for the two largest size classes in the first experiment (Fig. 5). This high variability may signal that minor variations in size distributions near the size threshold may have major impacts on filtering rate. Thus, our estimate of the upper size threshold should be interpreted as somewhat fuzzy, although it is considerably more definitive than can be gleaned from the existing literature, especially with respect to this important HAB species.

Counter to previous expectations based on shell length-siphon diameter relationships for *D. polymorpha* (MacIsaac et al. 1991, Naddafi et al. 2007), we found no evidence for an effect of mussel size on *M. aeruginosa* vulnerability to grazing (Fig. 6). Inhalant siphon diameter in 16

mm *D. polymorpha* is generally 20 times wider than the *M. aeruginosa* colonies that were too large to be consumed in our first experiment (MacIsaac et al. 1995)(Fig. 5), suggesting that siphon diameter is not an important determinant of size selectivity. Our experiment appears to be the only direct measurement of phytoplankton size-based vulnerability to grazing as a function of *D. polymorpha* body size (Naddafi et al. 2007), and we employed a fairly wide range of body sizes (8-11 to 25-28 mm) relative to the body-size variation present in a natural system (Fig. 3). Consistent with our results, MacIsaac et al. (1995) found no systematic differences among *D. polymorpha* size classes (range: 10-22 mm) in their ability to ingest microzooplankton (mean size: 89 μm). Conversely, Bridoux et al. (2010), quantifying phytoplankton biomarkers in Lake Erie *D. polymorpha* tissue, found indirect evidence for increased importance of larger phytoplankton taxa in the diet of larger mussels.

Taken together, our two experiments indicate that *D. polymorpha* between ~8 and ~28 mm in shell length consume particles non-selectively from 5-75 μm ED. This higher upper threshold relative to filter-feeding zooplankton (e.g., *Daphnia*) indicates that standard demarcations of 'edible' versus 'inedible' in phytoplankton studies (McCauley and Briand 1979, Cyr and Pace 1992) are not suitable for assessing phytoplankton vulnerability to *Dreissena* grazing. Rather, given that *D. polymorpha* can filter particles as small as 0.4-1.5 μm (Cotner et al. 1995, Lei et al. 1996) and the majority of freshwater phytoplankton biomass typically falls below 75 μm (Sprules et al. 1983), size may be generally less important relative to other factors in determining overall phytoplankton vulnerability to *Dreissena* grazing, as compared to *Daphnia*.

By putting our experimental data (Figs. 5, 6; White et al. 2011) into context and comparing them to the size distribution of *M. aeruginosa* in nature (Figs. 7, 8, 9), it seems

apparent that much of the *M. aeruginosa* population in Gull Lake is vulnerable to *D. polymorpha* grazing. If anything, the consistent seasonal decline in median colony size of *M. aeruginosa* from above to below 80 μm ED (Figs. 8, 9) indicates an overall increase in vulnerability during the growing season on the basis of size, and is contrary to the expectation that intense size-selective grazing by *D. polymorpha* would increase the proportion of large, less edible colonies. However, the relative abundance of different genotypes and chemotypes within *M. aeruginosa* populations is also known to shift within a season (Saker et al. 2005, Kardinaal et al. 2007a, Welker et al. 2007, Rinta-Kanto et al. 2009, Bozarth et al. 2010), and different genotypes of *M. aeruginosa* from Gull Lake can vary maximally in their vulnerability to mussel grazing irrespective of colony size within the edible range (White et al. 2011). Thus, the late-summer population, though composed of smaller colonies, may be dominated by genotypes that are grazing resistant due to other factors (e.g., chemical inhibitors). Clearly, 'vulnerability' should always be considered in relative terms; it is a potentially variable and dynamic attribute of *M. aeruginosa* populations. Although small colonies of the two Gull Lake strains we employed in our experiments were fully vulnerable to grazing, the lake population consists of other strains (White et al. 2011) and size fractions (this study) that are invulnerable. Variation in both colony size distributions and genotypic composition within and among *M. aeruginosa* populations could 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, Knoll et al. 2008, Sarnelle et al. 2010).

Size is a 'master trait' (Litchman and Klausmeier 2008) driving phytoplankton population processes and also factors into important ecological trade-offs. Large phytoplankton, including large colonies of *M. aeruginosa,* generally have lower per capita growth rates than

smaller cells or colonies (Kruk et al. 2010, Wilson et al. 2010), and are also less efficient at assimilating resources (Reynolds 2006). Large size can also confer benefits—for example, faster migration rates for buoyant *Microcystis* (Visser et al. 1997, Reynolds 2006) and, of course, lower vulnerability to consumption. Since variation in colony size is a characteristic trait of *M. aeruginosa* of clear ecological importance, models of *M. aeruginosa* population dynamics should incorporate 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.

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CHAPTER 2

HEAT-INDUCED MASS MORTALITY OF INVASIVE ZEBRA MUSSELS (*DREISSENA POLYMORPHA*) AT SUBLETHAL WATER TEMPERATURES

Abstract

We observed a massive die-off of zebra mussels (*Dreissena polymorpha*) on the epilimnetic sediments of Gull Lake (Michigan, USA) during the relatively warm summer of 2010, even though water temperatures were below widely-reported lethal temperatures of ≥ 30 °C. We followed up this observation with four years of *in situ* monitoring of caged mussels stocked across a depth-temperature gradient in Gull Lake. Mortality of caged *D. polymorpha* was largely explained by accumulated degree hours > 25 °C, a temperature threshold that is considerably lower than laboratory-derived lethal temperatures for *D. polymorpha*. We also assessed both the acute and chronic thermal tolerance of Gull Lake *D. polymorpha* with laboratory experiments, which confirmed higher acute tolerance (up to 32 °C) under otherwise ideal conditions but high susceptibility to prolonged exposure to "sublethal" temperatures (exceeding 1,700 degree hours > 25 °C) as occurred in Gull Lake during the die-off. Our results indicate that the thermal tolerance of *D. polymorpha* under natural conditions may be lower than has been reported from laboratory studies. Lower temperature tolerance may have major implications for the dynamics, impacts, and management of this invasive species given future climate change scenarios.

Introduction

Zebra mussels (*Dreissena polymorpha*) are among the most successful and impactful aquatic invasive species in North America and Western Europe (Strayer 2008, Higgins and

Vander Zanden 2010). Great effort and expense are invested to understand, predict, and prevent their spread. Habitats that are most susceptible to invasion have been identified on the basis of environmental conditions and known physiological tolerances of mussels, with temperature playing an important role in delineating the potential range of *D. polymorpha* (Strayer 1991, Drake and Bossenbroek 2004). Given the profound impacts *D. polymorpha* has in invaded habitats, understanding its thermal sensitivity is important for present-day management, as well as to predict responses under future climate change scenarios (Thorp et al. 1998, Griebeler and Seitz 2007, Rahel and Olden 2008). Climate forecasts include more frequent extreme temperature events and a 2 °C increase in mean air temperature for much of temperate North America by 2050 (Romero-Lankao et al. 2014). Observations of temperate lakes have already identified responses to shorter- and longer-term climatic variation in the past few decades, including warmer water temperatures and increased intensity and duration of thermal stratification (Schindler et al. 1990, Jankowski et al. 2006).

Temperature tolerance of North American *D. polymorpha* has been extensively studied in the laboratory, but reported upper limits and requisite exposure times are highly variable. Lethal temperature thresholds for *D. polymorpha* have been reported between 29-32 °C (Garton et al. 2014), with the time to death decreasing from 4 days to 5 minutes as temperature increases from 30 to 43 °C (Iwanyzki and McCauley 1993, McMahon et al. 1994, McMahon and Ussery 1995, Elderkin and Klerks 2005, Beyer et al. 2011). In general, 30 °C is widely accepted as the "upper incipient lethal temperature" for *D. polymorpha* in North America (Iwanyzki and McCauley 1993). These temperature thresholds have been obtained largely from short-duration (minutes-days) laboratory studies that expose mussels to temperature regimes that are extreme relative to nature, typically to identify suitable, acutely lethal temperatures for rapidly purging industrial

intakes and boat hulls of fouling mussels. In contrast, mussels have been shown to survive as long as 14-35 days at 30-32 °C when gradually acclimated (McMahon et al. 1994, Aldridge et al. 1995, Spidle et al. 1995). However, over prolonged periods (> 30 days) at elevated temperatures, *D. polymorpha* exhibit metabolic imbalance and negative somatic growth, even under otherwise ideal laboratory conditions (Aldridge et al. 1995).

We observed a rapid and large (approaching 100%) mortality event of all size classes of *D. polymorpha* on epilimnetic sediments in Gull Lake, Michigan (USA) in early August 2010, during a relatively warm summer (Fig. 10) although water temperatures never exceeded 29 °C at 1 m depth. Mussels survived at greater depths (lower epilimnion and metalimnion) where temperatures were ≤ 25 °C. Other variables of known importance to *D. polymorpha* (e.g., chlorophyll-*a,* dissolved oxygen, calcium, and pH) (Garton et al. 2014) were all within their typical ranges for the 16 years since *D. polymorpha* appeared in Gull Lake (J. White, unpubl.). Though the summer of 2010 was warm, it was neither the only warm summer nor the warmest since *D. polymorpha* first invaded the lake (Fig. 11), yet no mass-mortality events of this magnitude had been observed in Gull Lake previously. This suggested that if temperature played a role in the 2010 die-off, its effect was likely more complex than would be predicted by simple consideration of median or maximum temperatures.

A)

B)

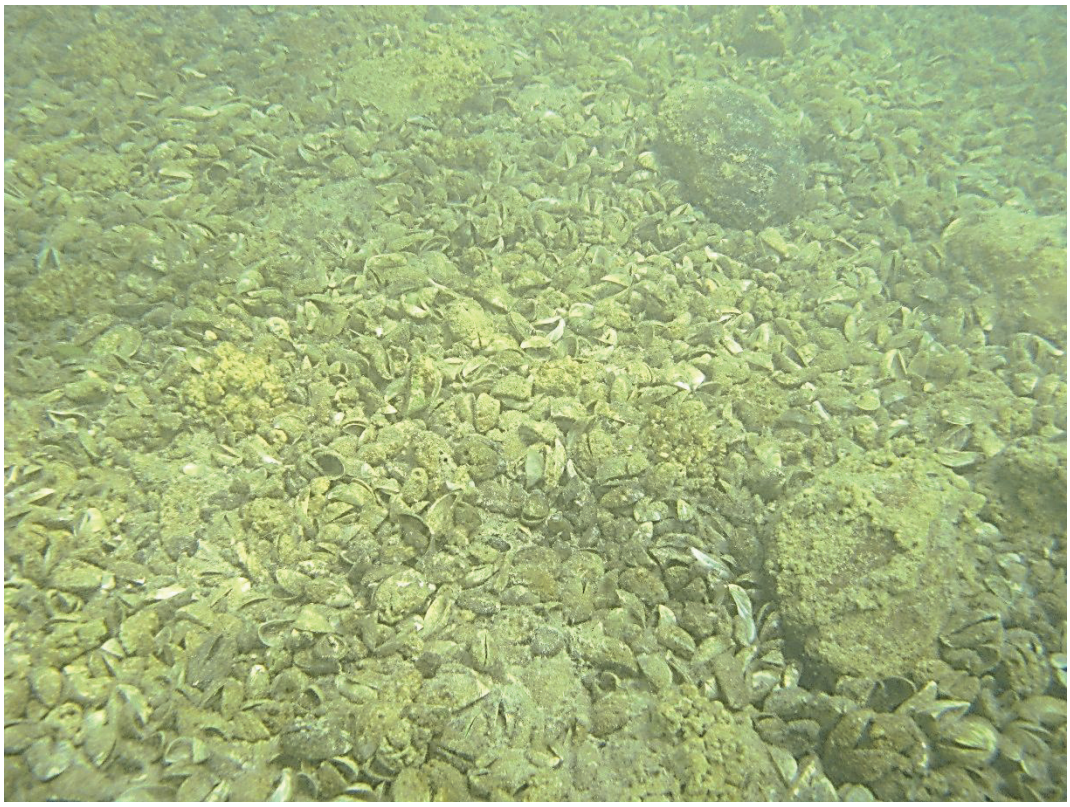


Figure 10. (A) Recently expired *Dreissena polymorpha* retrieved from the nearshore area of Gull Lake immediately following the 2010 epilimnetic mass die-off event. (B) Shells of dead *D. polymorpha* cover the bottom of Gull Lake at a depth of 2 m in 2011, following the 2010 mass die-off event. Photo credits: (A) Stephen K. Hamilton; (B) Jeffrey D. White.

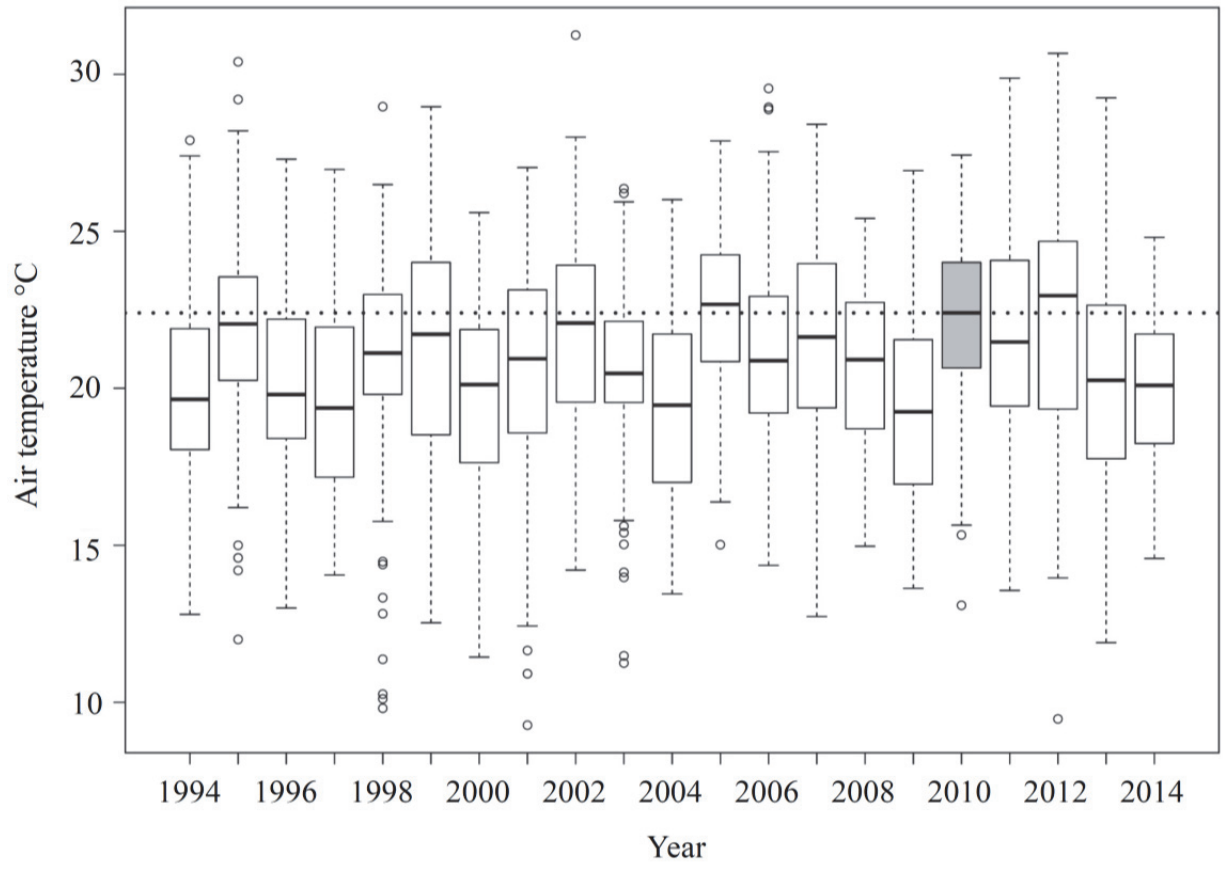


Figure 11. Box plots of mean daily June-August air temperatures recorded at the Kellogg Biological Station, adjacent to Gull Lake, for the years after *Dreissena polymorpha* invaded Gull Lake. The die-off occurred during the shaded summer (2010), and the dotted line indicates the median air temperature (22.4 °C) for that summer. Whiskers extend to the lowest and highest non-outliers; open circles indicate outliers.

There are other reports in the literature of large *D. polymorpha* mortality events that have been associated with high water temperatures, though to our knowledge these observations have been exclusive to the southernmost limits of their range. In the southern Mississippi River (Baton Rouge, Louisiana), water temperatures peak at 29-30 °C for several weeks during summer, with mortality of adult *D. polymorpha* reaching 60% *in situ* (Allen et al. 1999). Large die-offs have also been documented in Oklahoma reservoirs when maximum summer water temperatures reached 30 °C (Boeckman and Bidwell 2014). These *in situ* observations are consistent with the aforementioned experimentally-derived tolerance estimates, though tolerance is known to vary geographically, with populations in the southern Mississippi River drainage being more tolerant of warmer water temperatures than north-temperate populations (Elderkin and Klerks 2005), possibly explaining why Gull Lake mussels might be vulnerable at relatively lower temperatures. In any case, the observed die-off in Gull Lake is not consistent with either existing lab-derived acute mortality data or field observations from southern populations.

Based on these preliminary observations from Gull Lake, we hypothesized that sustained temperatures that are below the range of published acute lethal temperatures lead to high mortality of zebra mussels. To assess the influence of these "sublethal" temperatures on *D. polymorpha* mortality, we 1) conducted four years of *in situ* monitoring of caged *D. polymorpha* in Gull Lake (2011-2014), 2) analyzed *D. polymorpha* mortality data from enclosure experiments conducted in Gull Lake (2005-2008), and 3) performed laboratory experiments testing both the acute and chronic thermal tolerance of Gull Lake *D. polymorpha*.

Methods

Study site

Gull Lake is a large (822 ha), deep (33 m maximum depth, 7.7 m mean mixed-layer depth), oligotrophic hardwater lake located in Barry and Kalamazoo Counties in southwestern Michigan. *Dreissena polymorpha* was first observed in Gull Lake in 1994. A mean density in the littoral zone (2.5-7 m depth) of 2,193 individuals m^{-2} was estimated in 1999 (Wilson and Sarnelle 2002, White and Sarnelle 2014). We made annual observations of littoral *D. polymorpha* during their post-establishment period in Gull Lake, and we did not observe any mass-mortality events prior to 2010 (S. Hamilton, pers. obs.). However, one enclosure experiment in 2005 was compromised by unacceptably high mortality of enclosed mussels (> 30%).

In situ mortality: caged mussels

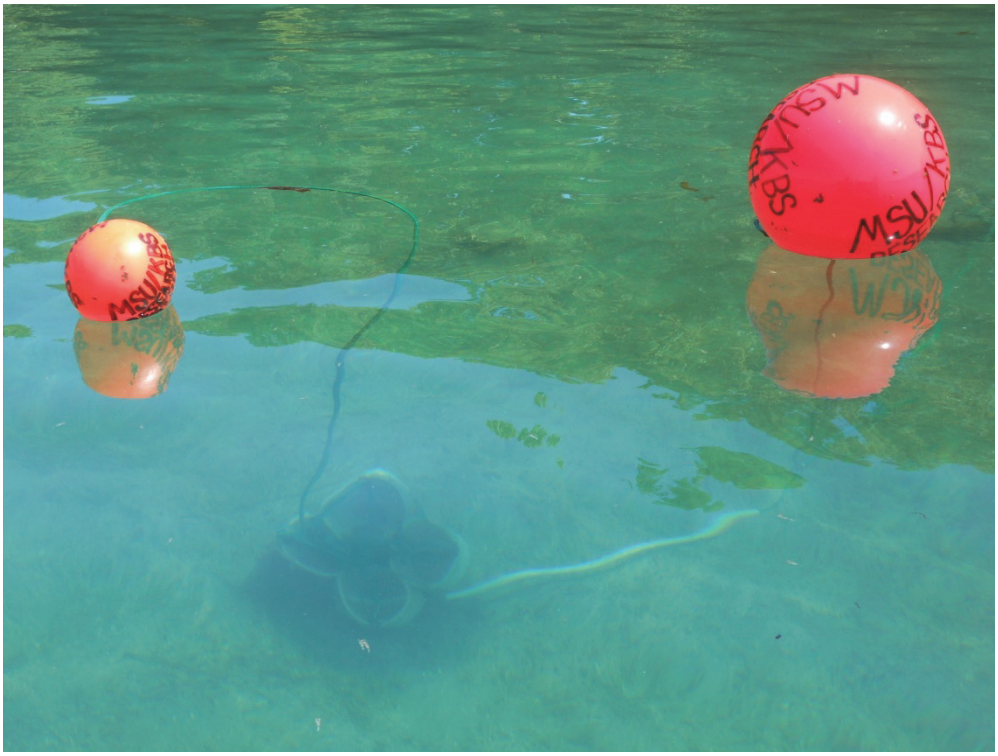
We measured mortality of *D. polymorpha* in each of the four consecutive years (2011-2014) following the initial die-off event in Gull Lake. Mussels were harvested in early May with an Ekman grab from the lower epilimnion (5 m, where they survived) and stocked into cages built of rigid plastic mesh (16 cm diameter, 13 cm tall, mesh size 3.5 mm). In all years, large mussels (16-30 mm) were stocked into every cage, with either 20 (2011-2012) or 12 (2013-2014) individuals per cage. In 2012-2014, small mussels (8-15 mm) were also stocked, either into separate cages (20 individuals each, 2012) or together with the large mussels (12 individuals each, 2013-2014). Cages were deployed in replicates of four at three depths: 2 m (upper epilimnion), 5 m (lower epilimnion), and 9 m (metalimnion). These depths span the range where *D. polymorpha* had previously been abundant in Gull Lake and were selected based upon inspection of temperature profiles, to provide a range of ambient temperatures. Cages were

anchored at the bottom and tethered to a surface buoy (Fig. 12a). Submersible loggers (Onset Corporation) recorded water temperatures at hourly intervals just above the sediments at each depth. Cages were briefly pulled to the surface approximately weekly for inspection of stocked *D. polymorpha*. Cages were retrieved in October and a final mortality assessment was made. Storms in 2011 and 2014 dragged and filled the 5 m cages with sediment, resulting in substantial mortality of enclosed mussels, so data from these cages were omitted from all analyses.

In situ mortality: mussels in enclosures

To assess whether mortality of Gull Lake *D. polymorpha* varied as a function of the wide range of temperature conditions present in the years prior to the 2010 die-off (Fig. 11), we analyzed mortality data from the summers of 2005-2008 collected during a series of enclosure experiments in Gull Lake. These experiments were designed to test the interactive effects of *D. polymorpha* and nutrients on the biomass of the harmful cyanobacterium, *Microcystis.* Experimental details can be found elsewhere (Hamilton et al. 2009, Sarnelle et al. 2012, Horst et al. 2014). In all cases, Gull Lake mussels were harvested and stocked into the same plastic cages as described above and suspended within large, tubular polyethylene enclosures (diameter = 2 m, depth = 8 m, volume = 25,000 L; Fig. 12b) at a depth of 2.5 m (always within the mixed layer). Experiments ran for 27-45 days during July and August, and concluded with an assessment of mussel mortality within the enclosures. We found no effect of nutrient treatments on mortality (ANOVA, $p = 0.96$, $n = 21$), so data from all enclosures were pooled for each experiment. Continuous water temperature data are not available for Gull Lake during this time period. Instead, we used continuous KBS LTER air temperature data (http://lter.kbs.msu.edu/datatables/7, datatable KBS002-001.27) as a rough proxy for mixed layer

A)



B)

Figure 12. Monitoring of *in situ* zebra mussel mortality in Gull Lake, Michigan. (A) View of tethered cages in position on the lake bottom at a depth of 2m. (B) View of the 2008 enclosure experiment. Mussels were suspended within the enclosures inside cage trees similar to those visible on the platform at center right. Photo credits: Jeffrey D. White.

water temperature, since daily water temperature at a depth of 2 m in Gull Lake is positively related to air temperature during summer months (Pearson correlation = 0.80).

Experiment 1: acute temperature tolerance

We conducted a laboratory experiment during the summer of 2012 to assess the short-term, acute thermal tolerance of Gull Lake *D. polymorpha.* Six 9.5 L aquaria containing filtered (1 μm nominal pore size) Gull Lake water were nested individually within 38 L aquaria containing deionized water. Submersible aquarium heaters (300 W, Marineland) were installed in 3 of the outer aquaria to create a water bath (heated treatments). All aquaria were housed within an incubator maintained at 23 ± 1 °C (control temperature) and a 12:12 hour light:dark cycle (Fig. 13). Temperature was recorded at hourly intervals by submersible loggers (Onset Corporation) in the 9.5 L aquaria. All aquaria were kept vigorously aerated throughout the experiment.

Zebra mussels were harvested for the experiment as described above, gently scrubbed clean of periphyton, and maintained in the lab as described in White and Sarnelle (2014). Twelve individuals from each of two size classes (8-15 mm and 16-25 mm shell length) were randomly allocated to each inner aquarium. Mussels were allowed to acclimate for 48 hours prior to the start of the temperature manipulation. Each day thereafter, temperature was increased 1 °C day^{-1} in heated treatments (n = 3); controls were continually maintained at 23 ± 1 °C (n = 3). Mussels were fed a satiating ration (~10-15 μg L^{-1}) of a high-quality, palatable green alga (*Ankistrodesmus falcatus*) daily (White and Sarnelle 2014). Mortality was monitored daily; mussels were considered dead when gentle probing failed to elicit a shell closure response (Iwanyzki and McCauley 1993, Spidle et al. 1995), and were immediately removed without

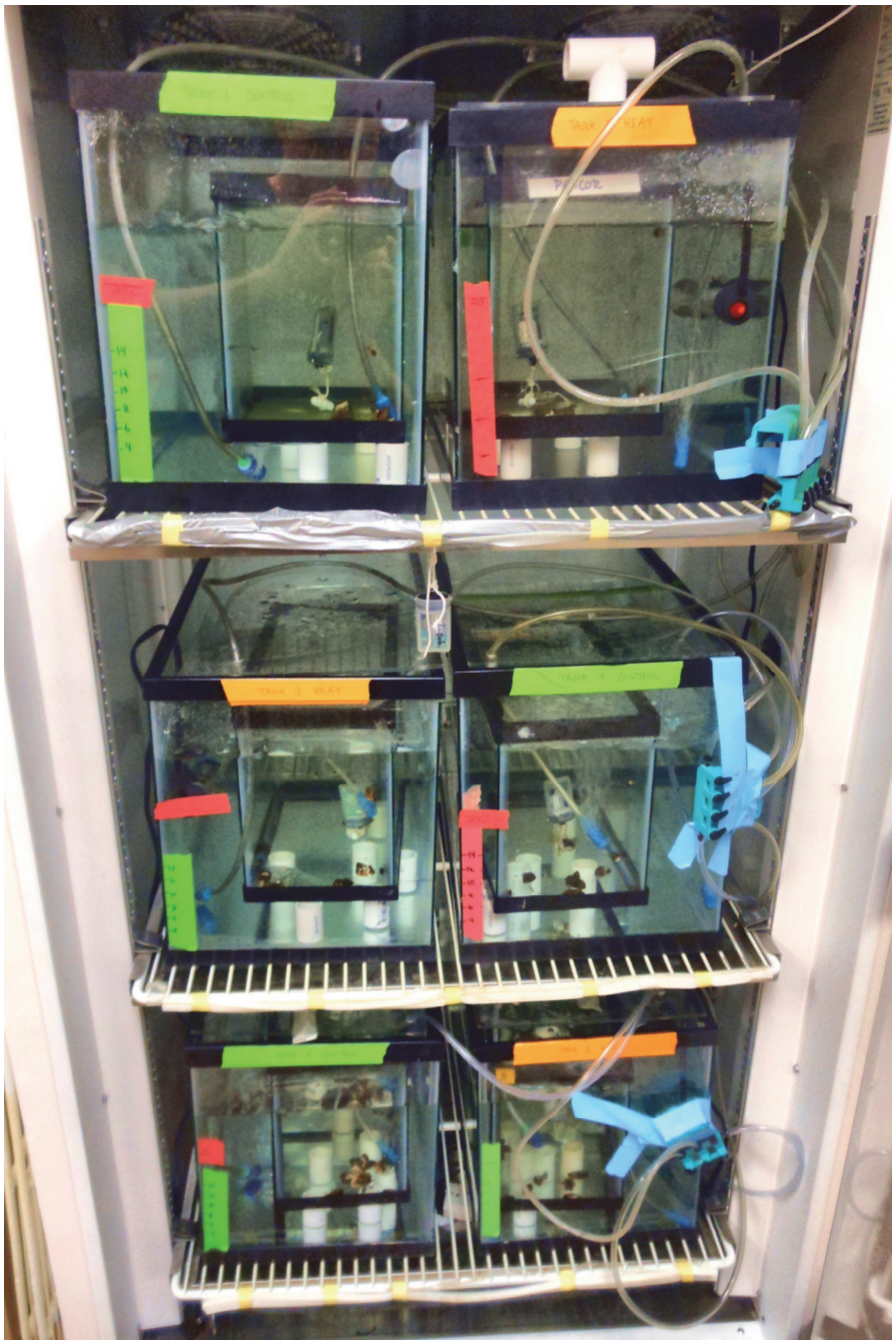


Figure 13. View of the zebra mussel acute temperature tolerance experiment, showing the nested aquarium water bath design. The entire experiment was housed within an incubator. Photo credit: Jeffrey D. White.

replacement. The experiment was concluded when mortality reached 100% in heated aquaria (day 16).

Experiment 2: chronic temperature tolerance

In an effort to experimentally re-create temperature conditions during the mass mortality event of 2010, we exposed Gull Lake *D. polymorpha* to prolonged elevated temperature in the summer of 2014 using a flow-through system housed in a lakeshore laboratory. The experiment consisted of ten 32 L polyethylene trays (46 × 66 × 15 cm), each fitted with inflow and outflow spigots and a submersible temperature logger (Onset Corporation). A 38 L glass aquarium was nested within each tray, and a submersible aquarium heater (300 W, Marineland) was installed in five of these aquaria to create a water bath (heated treatments, $n = 5$). Fresh lake water from Gull Lake was pumped into two 1,100 L holding tanks every 3-4 days. This lake water, containing the natural Gull Lake phytoplankton community, was continually supplied to the experimental trays with a peristaltic pump (50 L day^{-1} flow rate, Ismatec/Cole-Parmer). All holding tanks and experimental trays were kept vigorously aerated throughout the experiment (Fig. 14).

Mussels were harvested as described previously, and individuals with a shell length of 16-18 mm were used in the experiment. Eleven mussels were then allocated randomly to each experimental tray. This density was selected such that the collective *D. polymorpha* filtering rate (Kryger and Riisgård 1988) balanced the inflow rate of lake water to the trays. Mussels were allowed to acclimate to experimental trays for 24 hours prior to the start of the temperature manipulation (day 0). On day 1, the temperature in heated trays was gradually increased from 22.0 to ~27.0 °C over 12 hours. Unheated trays were maintained at ambient room temperature (mean = 22.8, range = 20.4 - 24.9 °C). Mortality was monitored as described above. The

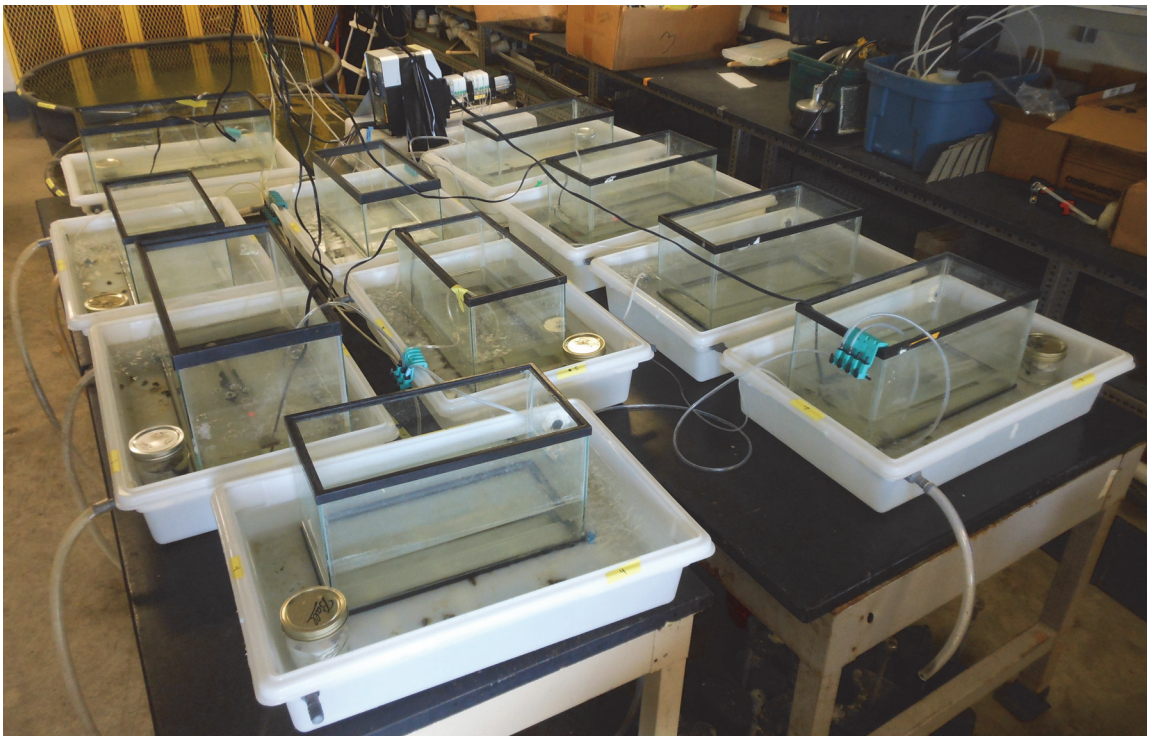


Figure 14. View of the zebra mussel chronic temperature tolerance experiment. Experimental trays with nested water baths are in the foreground; lake water holding tanks are visible in the background. The peristaltic pump is at top center. Photo credit: Jeffrey D. White.

experiment was ended on day 23, once the number of degree hours achieved in heated treatments was comparable to that observed in Gull Lake during the 2010 *D. polymorpha* die-off event and periods of high mortality during *in situ* monitoring (*see* Results).

Statistical analyses

To explore the influence of accumulated heat exposure on *in situ* mussel mortality from the caged mussel study, we employed a degree hour approach. To identify the most-likely temperature threshold driving mortality from prolonged heat exposure, we performed a series of linear regressions of mortality versus degree hours above a threshold temperature. We started with a threshold of 23 °C, and then sequentially increased the threshold temperature by 1 °C. We assessed the relative fit of each regression model with the Akaike Information Criterion (AIC), where a lower AIC indicates a better fit, and then computed ΔAIC for each model with respect to the model with the lowest AIC. Models having $\Delta AIC \leq 2$ were considered to have equal statistical support as the model with the lowest AIC (Burnham and Anderson 2001). This procedure was performed separately for both the large and small size classes of caged mussels.

Proportional mortality data were arcsine square-root transformed before statistical analysis, but this transformation had no substantive effect on any result, so we present untransformed mortality data for ease of interpretation. We performed linear regressions of mortality on degree hours, maximum water temperature, and depth for the *in situ* cage study, and of mortality on median air temperature for the enclosure data (combined from all years). Residual plots revealed no systematic violations of model assumptions. Because our predictors of mortality (degree hours, maximum temperature, and depth) are highly correlated, we compared the relative fits of the individual univariate models with AIC. We performed analysis

of variance (ANOVA) to test for differences in mortality of caged mussels as a function of stocking density. To determine whether the relationship between mortality and degree hours differed between large and small mussels in the *in situ* cage study, we conducted a homogeneity of slopes test, followed by analysis of covariance (ANCOVA) to test for differences in intercepts in the event the former test was not significant. To assess the effects of temperature and time on mortality in the acute and chronic temperature tolerance experiments, we performed repeated measures multivariate analysis of variance (RM-ANOVA). Statistical analyses were performed with R (version 3.0.1, The R Foundation for Statistical Computing).

Results

In situ mortality: caged mussels

We detected no differences in mortality among cages stocked with different sizes or total biomass of mussels (ANOVA, $p = 0.21$, $n = 10$). As assessed by ΔAIC, mortality of small mussels was explained equally well by threshold temperatures of 23-26 °C and mortality of large mussels was explained equally well by threshold temperatures of 23-25 °C ($\Delta AIC \leq 2$, Table 3). Model fit progressively diminished (AIC increased) as the threshold temperature was adjusted beyond these ranges (Table 3). For ease of interpretation, we chose 25 °C as the threshold temperature of best fit, since this is the highest temperature having maximal statistical support across both size classes of mussels. Given our hypothesis that mussels have a lower temperature tolerance than previously reported, choosing the highest threshold temperature that best fits the data is a conservative approach with respect to our understanding of chronic temperature stress in zebra mussels.

Table 3. Selection of an accumulated degree hour threshold temperature that best explains mortality of caged large (16-30 mm, n = 10) and small (8-15 mm, n = 7) *Dreissena polymorpha* in Gull Lake. Results are for linear regressions. Model fit was assessed with AIC, where a lower AIC indicates a better fit. ΔAIC was then computed for each model with respect to the model with the lowest AIC. Models having ΔAIC ≤ 2 (underlined) were considered to have equal statistical support as the model with the lowest AIC.

	Large mussels				Small mussels			
Temperature threshold, °C	AIC	ΔAIC	R^2	p	AIC	ΔAIC	R^2	p
> 23	0.77	<u>0.87</u>	0.67	0.004	-0.52	<u>0.87</u>	0.73	0.015
> 24	-0.11	<u>0.00</u>	0.70	0.003	-1.39	<u>0.00</u>	0.76	0.011
> 25	0.95	<u>1.05</u>	0.67	0.004	-1.27	<u>0.11</u>	0.76	0.011
> 26	2.28	2.38	0.62	0.007	-0.64	<u>0.74</u>	0.73	0.014
> 27	4.35	4.45	0.53	0.017	1.08	2.47	0.66	0.027
> 28	7.71	7.82	0.34	0.077	2.99	4.38	0.55	0.056
> 29	10.53	10.64	0.12	0.317	3.59	4.98	0.51	0.071

Natural temperature variation across years and cage depths provided a large range of accumulated heat exposure (Table 4). Mortality of caged *D. polymorpha* in Gull Lake was strongly related to accumulated degree hours > 25 °C for both large (Fig. 15a; linear regression, $p = 0.004$, $n = 10$) and small (Fig. 15b; linear regression, $p = 0.011$, n = 7) individuals. We found no difference in degree hours versus mortality regression slopes (homogeneity of slopes test, $p = 0.86$) or intercepts (ANCOVA, $p = 0.67$) between the two size classes of mussels, indicating similar susceptibility. Maximum water temperature and cage depth, which are both correlated with degree hours > 25 °C (r = 0.74 and -0.70, respectively), were poorer predictors of mortality of large individuals (linear regressions, $p \leq 0.04$, AIC > 5.0, $n = 10$) and were not significant predictors of mortality of small individuals (linear regressions, $p \geq 0.15$, AIC > 5.4, $n = 7$). Notably, the highest levels of mortality were observed at exposures comparable to that during the die-off (> 1,500 degree hours > 25 °C, Fig. 15).

In situ mortality: mussels in enclosures

Mortality of *D. polymorpha* within experimental enclosures in Gull Lake (July-August, 2005-2008) was positively correlated with median air temperature during the experiment (r = 0.87, Fig. 16), in the absence of a continuous water temperature record. Notably, the highest mortality (33%) was observed during the 2005 experiment (8 July- 4 August), when the median summer air temperature was similar to that in 2010 during the mass-mortality event in Gull Lake (Fig. 11).

Table 4. Accumulated heat exposure (degree hours > 25 °C) across years and depths in the study of caged *Dreissena polymorpha* in Gull Lake.

	Depth (m)		
Year	2	5	9
2011	1,909	1,440	0
2012	2,804	--	526
2013	1,248	770	0
2014	153	--	2

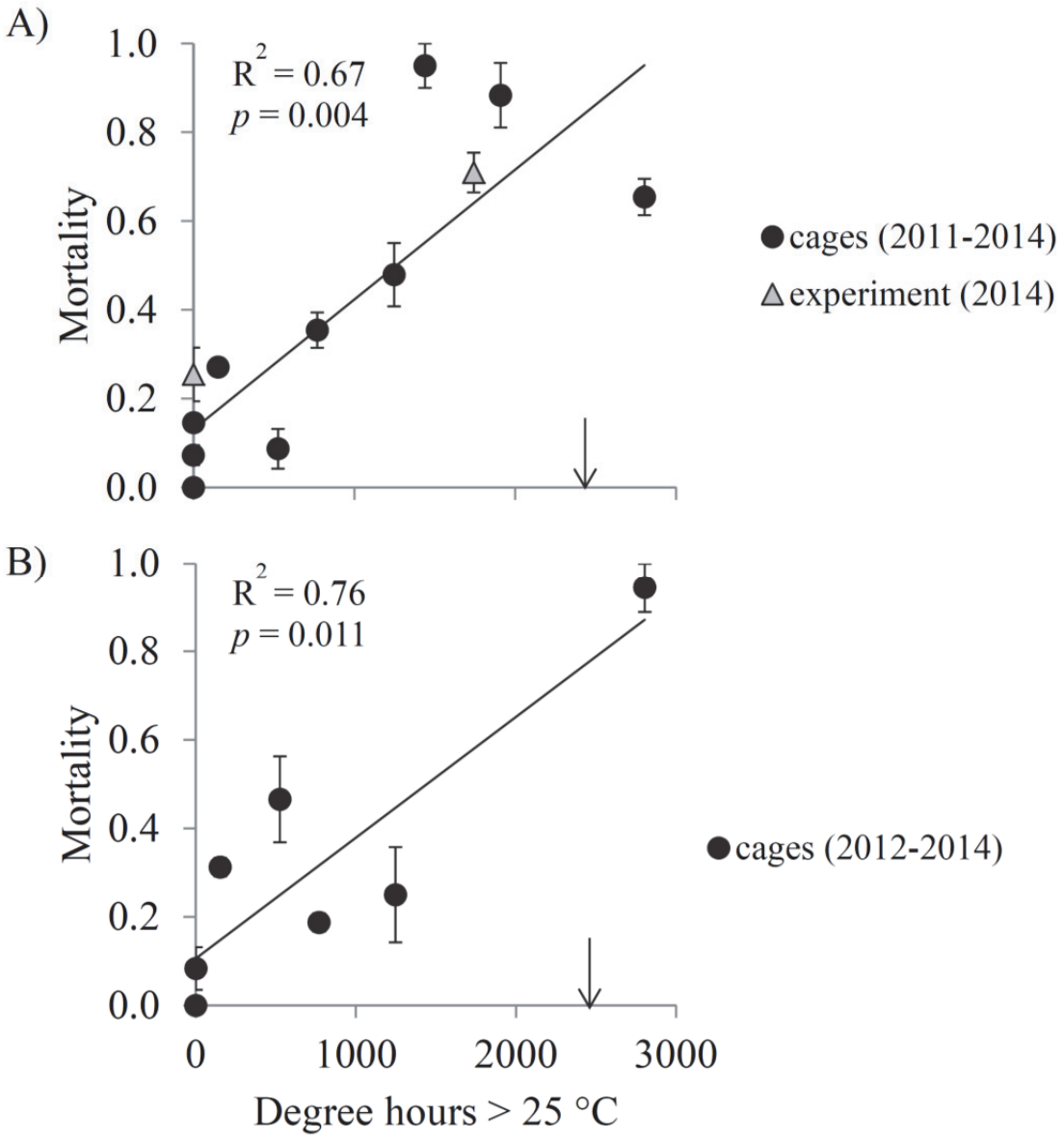


Figure 15. Mortality of caged *Dreissena polymorpha* in Gull Lake (May-October, 2011-2014) for (A) large (16-30 mm) and (B) small (8-15 mm) individuals as a function of lake degree hours > 25 °C. Results from the chronic temperature tolerance experiment (*see* Fig. 18) are indicated for comparison. The regression lines (significant at $p < 0.05$) are for caged *D. polymorpha* only. Mortality is given as the proportion of dead individuals. Bars are ± SE. The arrows denote accumulated degree hours in Gull Lake during the initial mass die-off event in 2010.

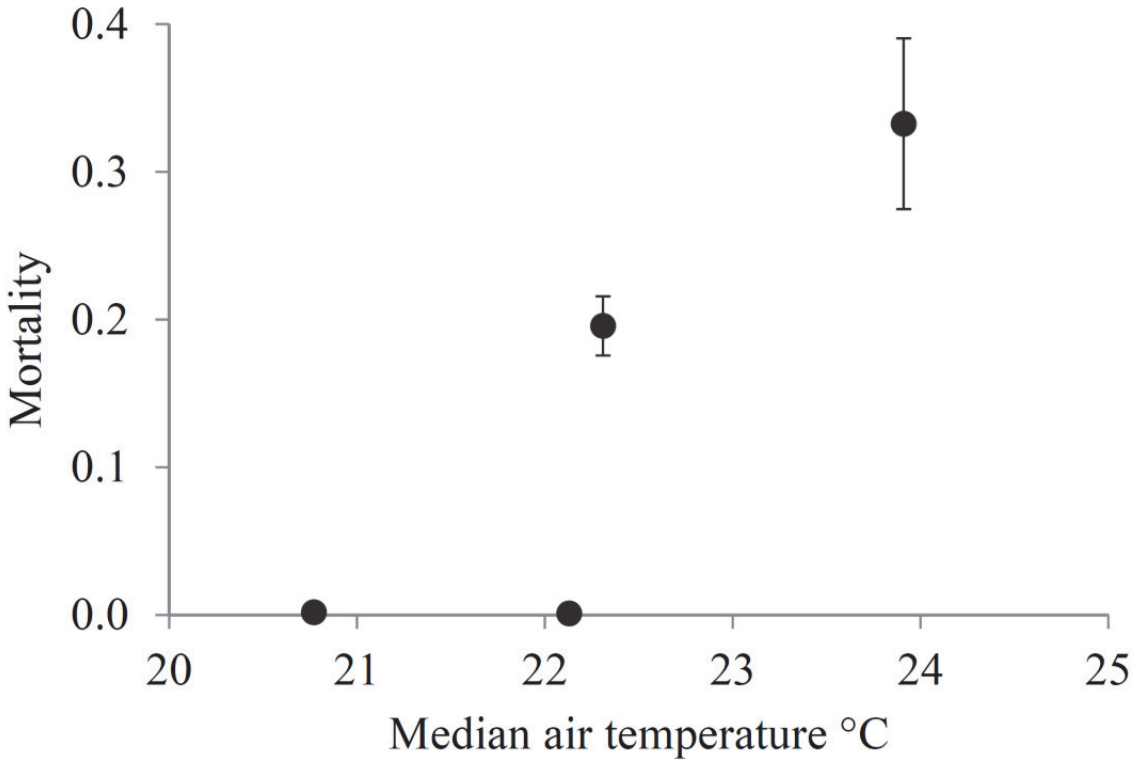


Figure 16. Mortality of Gull Lake *Dreissena polymorpha* in four enclosure experiments conducted in Gull Lake (July-August, 2005-2008), as a function of median air temperatures recorded nearby. Mortality is given as the proportion of dead individuals. Bars are ± SE.

Experiment 1: acute temperature tolerance

The effect of temperature on mussel mortality was significant, and this effect varied over time (RM-ANOVA, $p < 0.001$, $n = 6$) for both size classes of mussels. No mortality occurred in heated treatments until temperature reached 32 °C (day 12), at which point mortality increased rapidly (Fig. 17a). Over the first 14 days, there was zero mortality in the 23 °C controls (Fig. 17b). Temperature was further increased to 33 °C, the maximum attainable with our heaters, and maintained at 33 °C during the last 3 days of the experiment. Smaller individuals (8-15 mm) appeared to be more tolerant of elevated temperature than larger individuals (16-25 mm): mortality exceeded 90% for large mussels after less than 24 hours at 33 °C (day 13), significantly higher than smaller mussels despite the same exposure time (ANOVA, $p = 0.04$, $n = 6$, Fig. 17a). This same level of mortality was not observed in smaller mussels until nearly 2 days at 33 °C (day 15). Mortality was ~100% for both size classes after 3 days at 33 °C, at which point the experiment was ended. Average mortality over the entire experiment was < 3% in controls maintained at 23 °C (Fig. 17b).

Experiment 2: chronic temperature tolerance

The effect of elevated temperature (> 25 °C versus ~ 23 °C in controls) on mussel mortality also varied over time in this experiment (RM-ANOVA, $p < 0.001$, $n = 10$). Mortality of mussels in the heated treatment was low and similar to controls up to day 10 (600 degree hours > 25 °C), then steadily increased relative to controls (Fig. 18). By the end of the experiment (1,700 degree hours > 25 °C), mortality was 2.7 times higher in heated trays (71% ± 0.045 SE) compared to unheated trays (26% ± 0.060 SE; t-test: $t = 6.06$, $df = 8$, $p < 0.001$). Notably, these mortality rates are highly congruent with the mortality versus heat exposure relationship

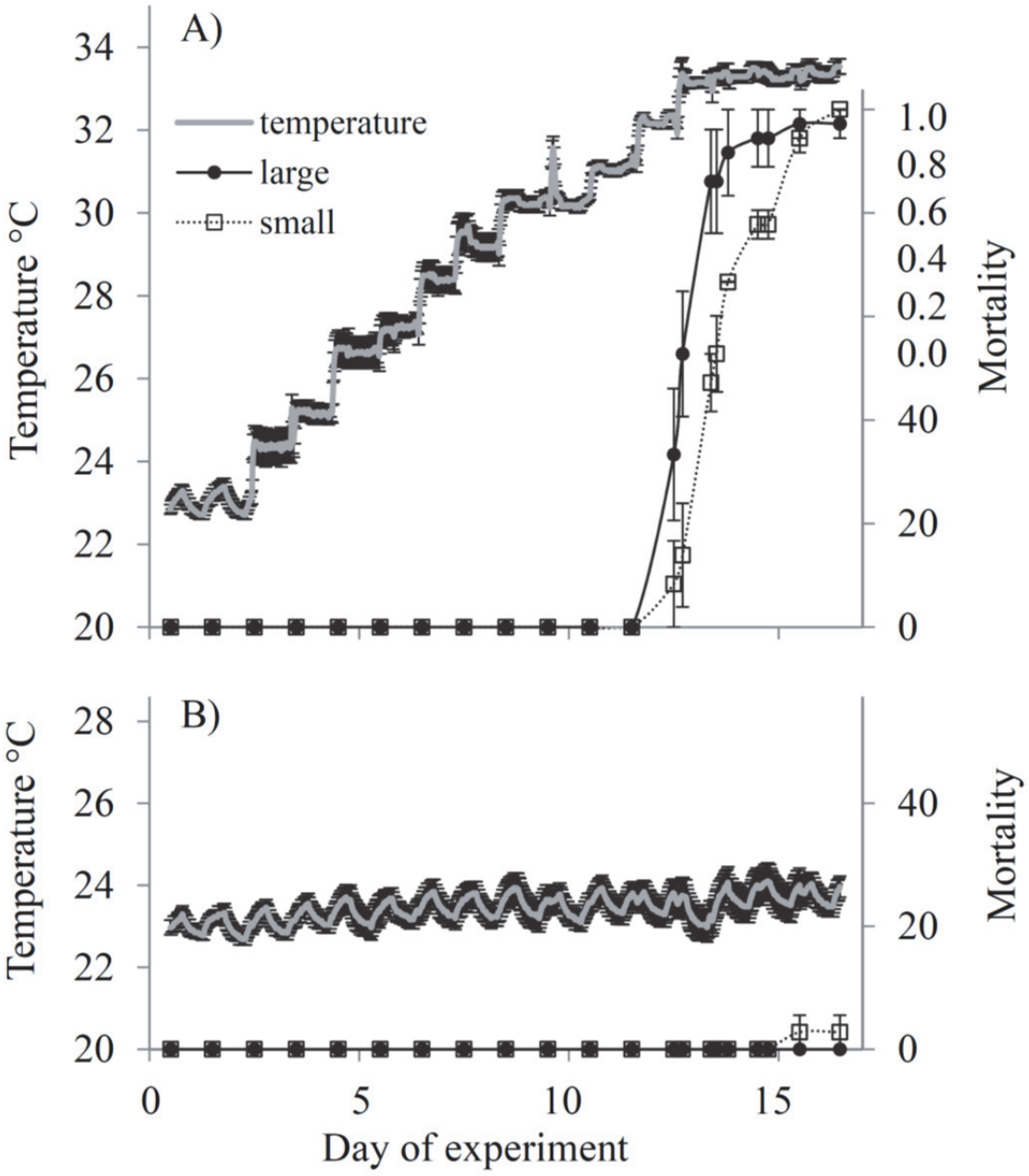


Figure 17. Water temperatures and mortality of large (16-25 mm) and small (8-15 mm) Gull Lake *Dreissena polymorpha* in the acute temperature tolerance experiment for (A) heated and (B) control treatments. Mortality is given as the proportion of dead individuals. Bars are ± SE.

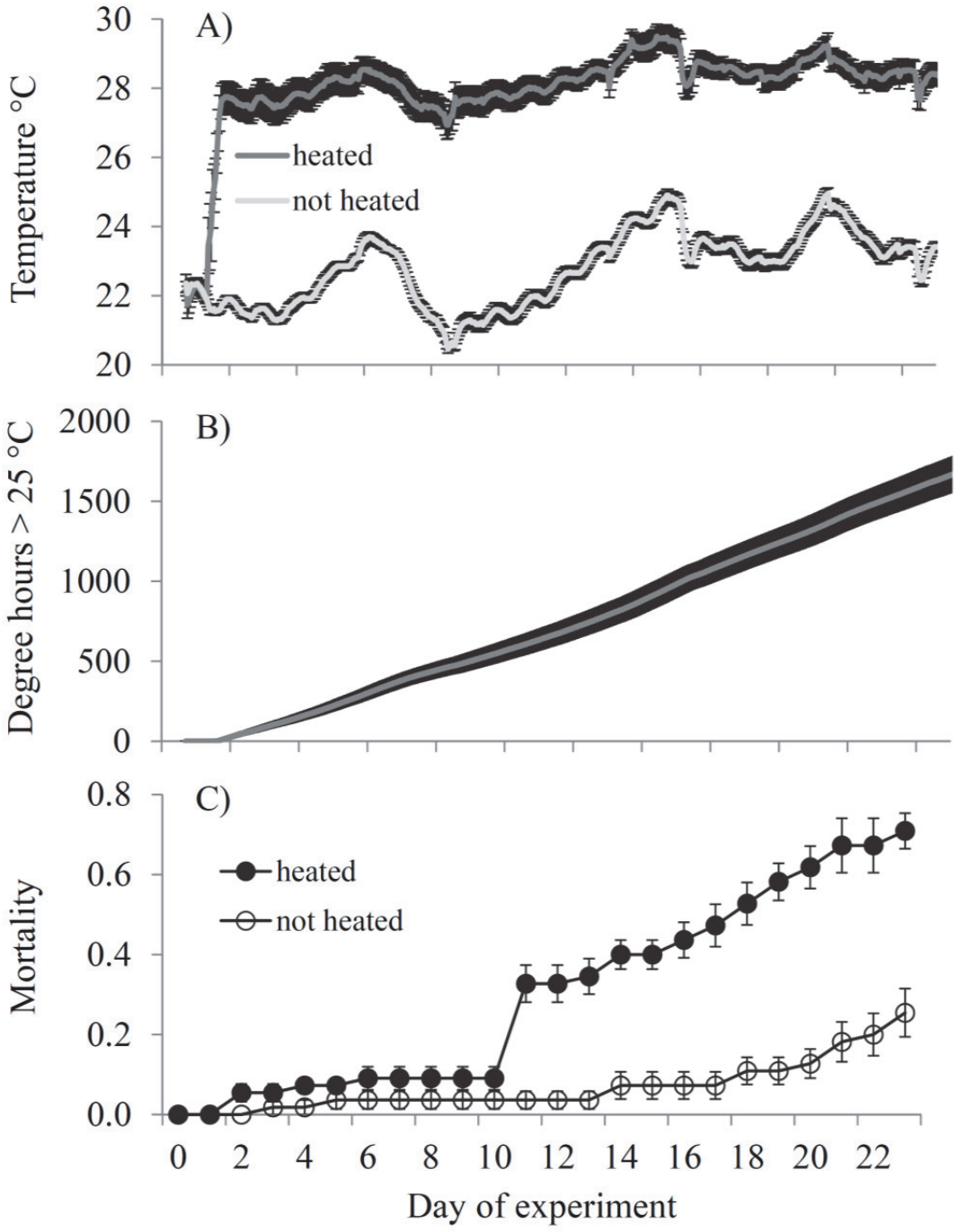


Figure 18. Results from the chronic temperature tolerance experiment: (A) temperature, (B) accumulated degree hours > 25 °C, and (C) mortality of large (17 mm) Gull Lake *Dreissena polymorpha* in heated and control treatments. Mortality is given as the proportion of dead individuals. Bars are ± SE.

observed for caged mussels in Gull Lake (Fig. 15a). Chlorophyll-*a* concentrations (mean = 1.28 μg L^{-1}) were within the range found in the lake (J. White, unpubl.) and did not differ between heated and unheated trays (paired t-test: t = 1.06, df = 7, p = 0.33).

Discussion

A massive die-off of *Dreissena polymorpha* occurred in Gull Lake, Michigan in 2010 after 2,500 accumulated degree hours > 25 °C, despite the fact that temperatures never reached the widely-accepted, acutely lethal threshold of ~30 °C (Iwanyzki and McCauley 1993, Garton et al. 2014). In our *in situ* caged mussel study in which year and lake depth provided natural temperature variation, seasonal mortality of Gull Lake mussels was largely explained by accumulated degree hours > 25 °C (Fig. 15), a "sublethal" threshold. Furthermore, using accumulated exposure times and temperatures similar to those observed in Gull Lake during the 2010 die-off, our experimental results demonstrate a causal link between this level of exposure and mortality, while simultaneously ruling out depth as a confounding factor. Earlier observations of mortality of experimental mussels in Gull Lake enclosures (Fig. 16) are also consistent with temperature being the likely driver. Populations of *D. polymorpha* are known to exhibit a variety of different dynamics, including boom-bust, though the influence of large interannual variation in temperature has generally either not been previously investigated or not found to be important in explaining these population fluctuations of *D. polymorpha* (Ramcharan et al. 1992, Strayer et al. 2011). We identified that periods of high temperatures were likely to have had a direct role in causing a large decline in *D. polymorpha* density, and such declines may become increasingly important given future climate change scenarios.

The distinction between measuring acute and chronic thermal tolerance is critical, since the acute lethal temperature will generally be higher than the chronic lethal temperature (Spidle et al. 1995), as we found in this study. Like many previous laboratory studies that report high survival of North American *D. polymorpha* at higher temperatures than those observed in Gull Lake and our chronic tolerance experiment (McMahon et al. 1994, Aldridge et al. 1995, Spidle et al. 1995), we also found high tolerance of Gull Lake mussels for relatively short-term exposure to high temperatures ≥ 30 °C in the laboratory (Fig. 17). However, many previous laboratory assessments of *D. polymorpha's* thermal tolerance, upon which widely reported lethal thresholds of > 30 °C are largely based, were specifically designed to identify the acutely lethal threshold suitable for rapid extermination (Iwanyzki and McCauley 1993, McMahon et al. 1995, McMahon and Ussery 1995, Beyer et al. 2011); therefore, these studies necessarily employ acclimation and treatment temperature regimes that are almost always extreme relative to nature, and therefore may not necessarily predict tolerance *in situ*. Indeed, we found that maximum water temperature was a relatively poor predictor of *in situ* mortality, presumably because the maximum temperatures observed in Gull Lake during our studies (≤ 30.5 °C) were typically transient and not acutely lethal to *D. polymorpha*.

With acclimation, north-temperate *D. polymorpha* have been shown to survive as long as 14-35 days at 30-32 °C in the laboratory (McMahon et al. 1994, Aldridge et al. 1995, Spidle et al. 1995), which exceeds the level of exposure we identified as driving Gull Lake *D. polymorpha* mortality. However, in laboratory studies of thermal tolerance like these, conditions are generally otherwise ideal including feeding mussels high-quality, cultured phytoplankton. In our *in situ* and chronic temperature tolerance studies, mussels fed on natural Gull Lake seston, and, in the case of the *in situ* study, were also subject to the full range of environmental conditions present

in Gull Lake. Time to death at a given temperature is known to increase with food quality for *D. polymorpha* (Stoeckmann and Garton 2001). Factors such as food quantity and quality may therefore influence the response to chronic temperature stress.

Accumulated exposure time is clearly important for driving mortality of *D. polymorpha* at sublethal temperatures. For example, in our chronic tolerance experiment, mussels were initially acclimated to experimental conditions by increasing the temperature 5 °C over 12 hours without any observable consequence. In fact, there was no discernable effect of temperature treatment on mortality for the first 10 days and 600 degree hours > 25 °C of the experiment, which is consistent with studies that report a rapidly increasing survival time (from hours to days) as temperatures are dropped even as little as 1-2 °C below the acutely lethal threshold (Iwanyzki and McCauley 1993, McMahon et al. 1994) and mussels have survived at these temperatures for the entirety of relatively short-term (< 14 days) incubations (Spidle et al. 1995). Physiologically, chronic exposure to sublethal temperatures in this range results in a metabolic imbalance whereby feeding cannot compensate for energy expenditures (Aldridge et al. 1995), which could eventually result in starvation and death.

Summer die-offs have major implications for *D. polymorpha* population dynamics, and these implications may vary by region. To our knowledge, all previously published observations of large-scale, apparently temperature-driven (≥ 30 °C) mortality occurring in nature have been restricted to warmer, southern ecosystems where *D. polymorpha* thermal tolerance is already known to be greater (e.g., perhaps as a result of local adaptation to warmer temperature regimes) (Allen et al. 1999, Elderkin and Klerks 2005, Boeckman and Bidwell 2014), suggesting that we should expect north-temperate populations to be vulnerable to temperatures < 30 °C in nature. Given the thermal preferences of *D. polymorpha* (spawning and growth occur at 12-24 °C),

spring-summer is the period when mussels in temperate lakes reproduce and achieve maximal somatic growth, whereas most growth in warmer habitats occurs in the cooler months (Karatayev et al. 1998, Allen et al. 1999, Garton et al. 2014). Summer die-offs are expected in southern populations, as temperatures are more likely to exceed thermal limits; however, these populations might be able to recover more quickly by allocating resources to growth and reproduction at other times of the year, which may not be possible for north-temperate populations (Allen et al. 1999). As a result, north-temperate populations may have longer lag times to recovery following summer die-offs, resulting in prolonged effects.

The existence of a thermal refuge should also influence the rate of population recovery from a die-off. It required nearly three years for *D. polymorpha* to successfully re-establish (persist through two consecutive growing seasons) on the epilimnetic sediments of Gull Lake following the initial die-off, and densities are still substantially lower than historical levels (J. White, pers. obs.). This is despite the fact that cooler, deeper waters provided a thermal refuge for *D. polymorpha* in Gull Lake where they persisted during the die-off and periods of high mortality in epilimnetic cages (Fig. 17, Table 4), presumably expediting recovery in shallower waters. Populations in shallow, well-mixed lakes that lack a thermal refuge may be more vulnerable to local, temperature-driven extirpations and could also experience longer lags to recovery with an increased dependence on exogenous dispersal for re-establishment.

Population size structure may also interact with temperature to determine population resilience, since larger mussels, if anything, were more vulnerable to higher temperatures than smaller mussels (Fig. 17, Table 3). This is in agreement with previous studies of *D. polymorpha* both *in situ* (Mississippi River) (Allen et al. 1999) and in the lab (McMahon et al. 1994, Elderkin

and Klerks 2005). Populations composed of a greater proportion of older, larger individuals might be more likely to experience mass mortality at elevated temperatures.

Given the well-documented effects of this invasive species on lake ecology, large-scale die-offs of *D. polymorpha* should have concomitantly large effects on lake ecosystems. Based on literature values for N and P tissue content of *D. polymorpha* (0.36 and 0.015 % of dry tissue mass, respectively)(Arnott and Vanni 1996) and a biomass of ~6 g m^{-2} in Gull Lake (Wilson and Sarnelle 2002), we estimate that the sudden demise of the population resulted in a pulse of ~21 mg N and ~1 mg P m^{-2} into the mixed layer of oligotrophic Gull Lake. Such a synchronized pulse of nutrients previously sequestered by mussels could have important consequences for the lake foodweb (Strayer 2014). For example, mixed layer chlorophyll-*a* concentrations in Gull Lake during the month immediately following the 2010 *Dreissena* die-off were at their highest observed levels for the period of record from 1998-2014 (maximum of 8.65 μg L^{-1}, relative to the 16-year summer mean of 3.72 μg L^{-1}; J. White, unpubl.), suggesting this nutrient pulse stimulated phytoplankton growth. In shallower, well-mixed systems where filter-feeding *D. polymorpha* exert stronger control on phytoplankton biomass (Higgins and Vander Zanden 2010), temperature-induced die-offs could reverse *Dreissena's* large effects on chlorophyll concentrations and transparency (Higgins et al. 2011, Cha et al. 2013). Die-offs of *Dreissena* could also result in marked shifts in the composition of the associated benthic invertebrate community (Ward and Ricciardi 2007, Gergs and Rothhaupt 2015).

Dreissenids are also strong promoters of the toxic cyanobacterium *Microcystis aeruginosa,* especially in low-nutrient lakes (Vanderploeg et al. 2001, Raikow et al. 2004, Knoll et al. 2008, Sarnelle et al. 2012). Cyanobacteria, including *Microcystis,* prefer warm temperatures (growth optimized at 28 °C) and are expected to increase worldwide with climate

change (Zehnder and Gorham 1960, Paerl and Huisman 2008). However, in low nutrient lakes like Gull Lake where the success of *Microcystis* depends on zebra mussels (Sarnelle et al. 2005, Sarnelle et al. 2012), *Microcystis* biomass and associated toxin might actually decrease if temperatures increase above the thermal tolerance of *D. polymorpha*. A complex interaction between *D. polymorpha, Microcystis,* and temperature may lead to non-monotonic responses of *Microcystis* to a warming climate in low nutrient lakes.

Mass-mortality events, local extirpations, and range contractions have been reported in marine ecosystems (Australian coast: canopy-forming seaweeds; Mediterranean Sea: gorgonians, sponges) in response to recent, extreme heat waves (Coma et al. 2009, Garrabou et al. 2009, Smale and Wernberg 2013). Given documented and anticipated increases in temperatures of inland waters as a result of climate change (Schindler et al. 1990, Jankowski et al. 2006), such events might also become more common in freshwaters. Specifically, our results suggest that summer declines or die-offs of invasive *D. polymorpha* may become more frequent in north-temperate lakes in the future. Therefore, it is critical to have estimates of environmental tolerances that accurately reflect processes in nature when forecasting the combined influences of species invasions and climate change on ecosystems. Given that highly successful invasive species like *D. polymorpha* will likely continue to adapt to their changing environmental template, understanding and management of these species will likewise need to be responsive to ongoing global change.

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Supplemental Information

For additional results pertinent to this chapter, *see* the Appendix (Survey of zebra mussel *[Dreissena polymorpha]* status in other Michigan inland lakes during recent warm summers).

CHAPTER 3

OPPOSING RESPONSES OF STRONGLY INTERACTING SPECIES TO ELEVATED TEMPERATURES SUPPRESS THE HARMFUL PHYTOPLANKTER *MICROCYSTIS*

Abstract

Climate change has already resulted in large changes in the spatial and temporal distributions of species, with significant consequences for individual populations. However, the community- and ecosystem-level implications may be complex and challenging to anticipate due to the cascading effects of disrupting the interactions among species. Toxic, bloom-forming cyanobacteria like *Microcystis aeruginosa* are expected to increase worldwide with climate change, due in part to their relatively high temperature optima for growth. Facilitation by invasive zebra mussels (*Dreissena polymorpha*) has also resulted in increases of *Microcystis* in low-nutrient (oligotrophic) lakes, an uncharacteristic habitat for this harmful phytoplankter. We conducted a 13-year study of a *M. aeruginosa* population in a low-nutrient lake invaded by zebra mussels. In 11 of the 13 years, there was a significantly positive relationship between *M. aeruginosa* biomass and mean spring-summer water temperature, which is consistent with climate change forecasts. Surprisingly, we observed very low *Microcystis* biomass and microcystin toxin concentrations during one of the warmest years in the time series following a heat-induced mass mortality event of zebra mussels, which resulted in low mussel densities for two consecutive summers. Upon elimination of its facilitating species, the positive relationship between *Microcystis* biomass and temperature decoupled. Thus, predicting the response of a species to climate change may require, at minimum, quantification of temperature responses of both the focal species and species that strongly interact with it. Consequently, monitoring of intact communities with respect to climatic variables seems essential.

Introduction

Anthropogenic global climate change is causing rapid and large shifts in the environmental template of species, which is very likely to continue given additional increases in global mean temperatures forecasted by the end of the century (IPCC 2014). Responses of individual species to recent climatic variation have already been documented, including range shifts (Perry et al. 2005, Chen et al. 2011), changes in the phenology of critical life history stages (Edwards and Richardson 2004), mass-mortality events and local extirpations (Garrabou et al. 2009, Smale and Wernberg 2013, White et al. 2015), and potentially extinctions (Pounds et al. 2006) across a broad range of taxonomic groups (algae, plants, invertebrates, amphibians, birds) and habitats (terrestrial, marine, freshwater). Since not all species will respond in the same manner, this information is valuable for understanding and predicting the response of different populations, and for guiding appropriate management and conservation decisions. However, because species are embedded in communities composed of complex networks of interactions, the response of an individual species to climate warming could result in unexpected, cascading effects on ecosystems by modifying the predicted responses of other interacting species, with the net result of these interactions potentially even negating the direct effects of climate (Suttle et al. 2007, Post and Pedersen 2008, Tylianakis et al. 2008, Van der Putten et al. 2010). The emphasis that has generally been placed to date on assessing the responses of individual species in isolation may therefore result in the failure to accurately predict community- and ecosystem-level responses to climate change (Gilman et al. 2010). Information about how closely interacting species respond together to large climatic variation in natural systems, particularly in direct response to elevated temperatures, is currently limited (Gilman et al. 2010, Van der Putten et al. 2010).

Rising water temperatures, longer growing seasons, and increases in the duration and stability of thermal stratification have been reported recently from north-temperate lakes (Schindler et al. 1990, Winder and Schindler 2004, Jankowski et al. 2006, Austin and Colman 2008, Dobiesz and Lester 2009, Schneider and Hook 2010, Rösner et al. 2012), changes that are expected to exacerbate the growth of toxic, bloom-forming cyanobacteria like *Microcystis aeruginosa* in freshwaters worldwide with climate change (Paerl and Huisman 2008, Paul 2008, Carey et al. 2012b, Elliott 2012, Paerl and Paul 2012, Reichwaldt and Ghadouani 2012). Harmful cyanobacteria like *Microcystis* can present significant human health concerns and impair use of drinking and recreational waters (Carmichael 1994, Pilotto et al. 1997, Falconer 1999). Although there is a consensus that climate change will promote these nuisance phytoplankton in general, models that make predictions for specific harmful cyanobacteria species are less developed (Pitcher 2012). Furthermore and not surprisingly, attention has almost exclusively been focused on phosphorus-polluted (eutrophic) lakes (Carey et al. 2008), since the biomass and relative abundance of cyanobacteria and their toxins generally increase with lake total phosphorus (TP) concentration (Smith 1985, Trimbee and Prepas 1987, Watson et al. 1997, Downing et al. 2001, Giani et al. 2005, Kotak and Zurawell 2007, Bigham et al. 2009).

However, facilitation by the strongly interacting, invasive zebra mussel (*Dreissena polymorpha*) has resulted in significant increases (3.6-fold in Michigan, USA) of toxic *Microcystis* in low-nutrient (oligotrophic) lakes—an uncharacteristic habitat for a harmful phytoplankter typically associated with high-nutrient habitats (Vanderploeg et al. 2001, Raikow et al. 2004, Knoll et al. 2008, Sarnelle et al. 2010). Consequently, *Microcystis* dynamics in these lakes are unlikely to be predicted well using existing models based solely on nutrient concentrations, nutrient ratios, and other variables related to lake productivity (Smith 1983,

1986, Smith et al. 1987, Canfield et al. 1989, Downing et al. 2001). Zebra mussels, highly successful and impactful invasive species, also continue to expand their range across North America and Europe (Strayer 2008, Higgins and Vander Zanden 2010). This ongoing species invasion presents an additional case of global change impacting *M. aeruginosa* and water quality, and it is currently unknown how *Microcystis* will respond to climatic warming in these socially and economically valuable low-nutrient lakes where its success is highly dependent on *Dreissena*.

Low-nutrient Gull Lake, Michigan, where *Microcystis* promotion following zebra mussel invasion has been extensively studied (Sarnelle et al. 2005, Sarnelle et al. 2012, Horst et al. 2014), experienced an unprecedented, heat-induced mass-mortality event of zebra mussels on epilimnetic sediments during the warm summer of 2010, followed by two years (2011-2012) of failed recolonization when water temperatures continued to exceed their chronic thermal tolerance (> 25 °C, with maximum temperatures of 29-30.5 °C; *see* Chapter 2) (White et al. 2015). These elevated temperatures, though lethal to its facilitating species, are within the range of laboratory-derived optimal temperatures for *Microcystis* (~25-32 °C) (Zehnder and Gorham 1960, Robarts and Zohary 1987, Nalewajko and Murphy 2001, Imai et al. 2009), which may lead to unexpected responses of *Microcystis* to increasing temperatures in low-nutrient lakes.

Microcystis is now the only toxin-producing cyanobacterium that regularly achieves appreciable biomass in Gull Lake (Sarnelle et al. 2005), making it an ideal habitat for investigating the response of *Microcystis* to climatic variation under low-nutrient conditions. First, given the aforementioned high temperature optima reported for cyanobacteria and *Microcystis,* which are expected to make them superior competitors at elevated temperatures (McQueen and Lean 1987, Fujimoto et al. 1997, Kosten et al. 2012, Rigosi et al. 2014), we

predicted that inter-annual variation in *Microcystis* biomass in Gull Lake is primarily driven by water temperature. Second, however, since *D. polymorpha* is already known to promote *Microcystis* and microcystin in Gull Lake (Raikow et al. 2004, Sarnelle et al. 2005, Knoll et al. 2008, Sarnelle et al. 2010, Sarnelle et al. 2012), we anticipated *a priori* that the mass-mortality event of *Dreissena* would have significant negative impacts on *Microcystis*, which might lead to non-monotonic responses of *Microcystis* to elevated temperatures in low-nutrient lakes. Third, we predicted that microcystin toxin concentrations in Gull Lake would be positively related to *Microcystis* biomass and available nitrogen, since microcystin cell quota is constrained by nitrogen availability (Horst et al. 2014). We analyzed 13 years of physical, chemical, and biological data from Gull Lake to test these predictions.

Methods

Study site

Gull Lake is a large (822 ha), deep (33 m maximum depth, 12 m mean depth), oligotrophic hardwater lake located in Barry and Kalamazoo Counties in southwestern Michigan (Fig. 19). The lake is phosphorus-limited (Moss 1972b), and summer TP concentrations in the mixed layer average 7.59 μg L^{-1} with a DIN:TP ratio of ~42:1 (Table 5). Details of the lake's geologic and climatic setting are summarized in Moss (1972a) and Tessier and Lauff (1992). Like other low-nutrient lakes, *Microcystis* biomass increased dramatically (from ~0% to >15%

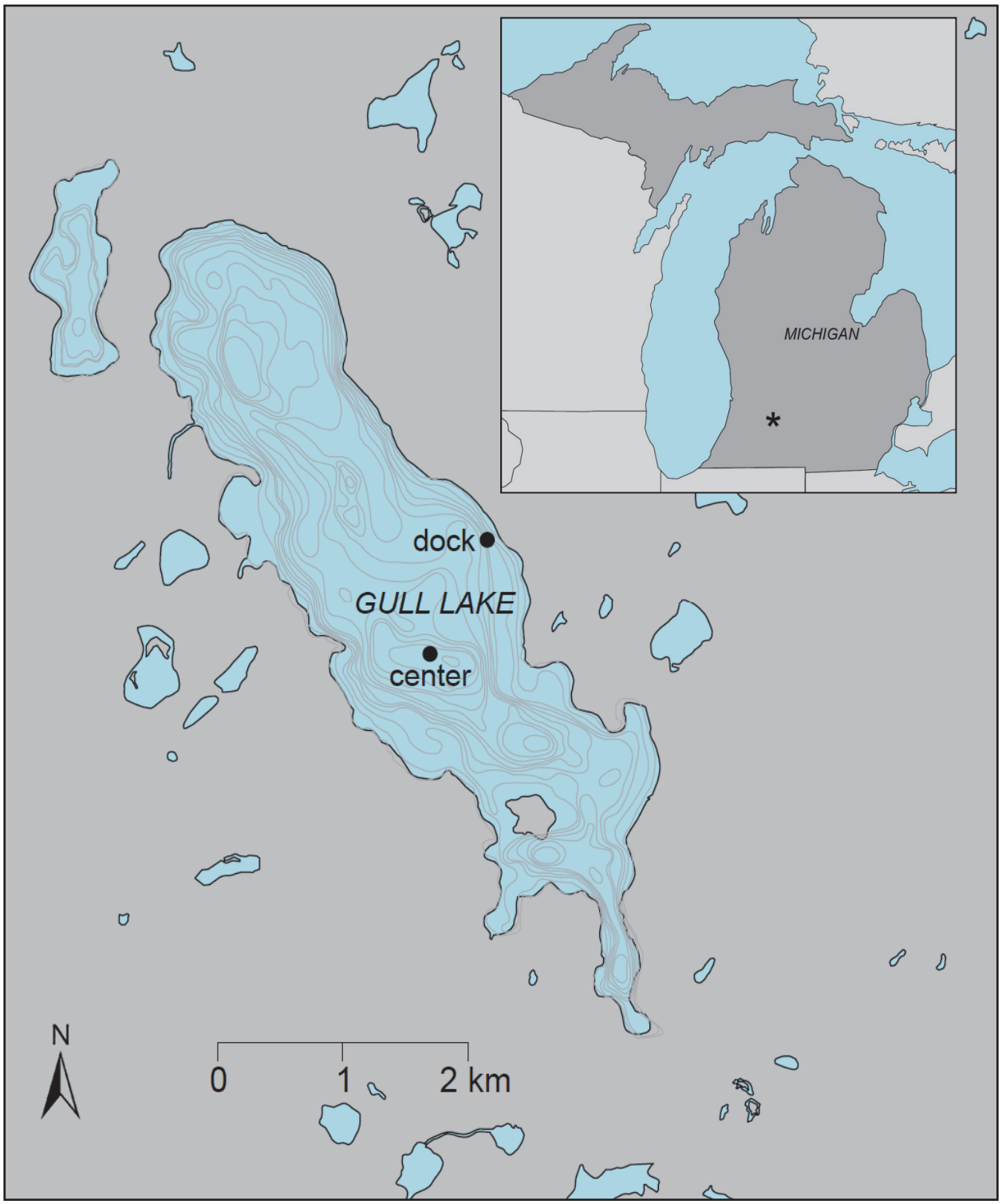

Figure 19. Map of Gull Lake, Michigan, indicating the locations of the nearshore ("dock") and central ("center") sampling stations. Depth contours are in increments of 3 meters.

Table 5. Summary of limnological characteristics of Gull Lake, Michigan (1998-2014, mixed layer during summer stratification). SE = standard error; 10^{th} = 10^{th} percentile; 90^{th} = 90^{th} percentile; *n* = number of observations; Chl-*a* = chlorophyll-*a*; Secchi = Secchi disk transparency; Z_{epi} = epilimnion depth; Alk = alkalinity; TP = total phosphorus; TDP = total dissolved phosphorus; SRP = soluble reactive phosphorus; NO_3^- = nitrate; NH_4^+ = ammonium; DIN:TP = ratio of [NO_3^- + NH_4^+] to TP.

Parameter (units)	Mean (SE)	10^{th}	90^{th}	*n*
Chl-*a* (μg L^{-1})	3.86 (0.12)	1.40	6.10	231
Secchi (m)	4.18 (0.11)	2.53	6.43	242
Z_{epi} (m)	7.71 (0.13)	5.50	10.00	226
pH	8.53 (0.02)	8.15	8.85	184
Alk (μeq L^{-1})	3,107 (45.63)	2846	3353	80
TP (μg L^{-1})	7.59 (0.12)	5.53	9.68	245
TDP (μg L^{-1})	4.76 (0.14)	3.14	6.76	182
SRP (μg L^{-1})	1.29 (0.06)	0.42	2.72	255
NO_3^- (μg L^{-1})	277 (7.68)	116	410	223
NH_4^+ (μg L^{-1})	22.09 (1.20)	6.67	38.41	230
DIN:TP	42 (1.53)	15	72	211

relative abundance) in Gull Lake following invasion by *D. polymorpha* in 1994 (Sarnelle et al. 2005), including a visible surface scum that occurred shortly thereafter (A. Tessier, pers. comm.). Prior to 1994, *Microcystis* was essentially absent from the phytoplankton community, although it was observed sporadically during a period of cultural eutrophication in the 1960s-1970s when surface blooms of *Anabaena* were reported (Moss 1972a, Moss et al. 1980). This eutrophication trend was reversed with the installation of a sanitary sewer around the lake in 1984, after which TP quickly decreased (Tessier and Lauff 1992) and has continued to decline to present-day oligotrophic levels (S. Hamilton, unpubl.).

Limnological sampling

Weekly sampling of Gull Lake was conducted from June-August in 1998-2001 and 2005-2008, and from June-September in 2009-2014. During 2000-2001 and 2009-2011, samples were collected from a near-shore station ("dock"; depth = 13 m) as well as a central station ("center"; depth = 30 m; Fig. 19). Only the near-shore station was sampled during 2005-2008, and only the central station was sampled during 1998-1999 and 2012-2014. None of the response variables (paired *t*-tests, $n = 21\text{-}29$, all $p \geq 0.24$) or predictor variables (paired *t*-tests, $n = 15\text{-}29$, all $p \geq 0.14$) significantly differed between sampling stations when both sites were sampled in parallel, except for ammonium (paired *t*-test, $n = 22$, $p = 0.002$), which was significantly higher at the near-shore station. However, the mean difference in ammonium concentration between sampling stations was only 2.78 μg L^{-1}, a relatively small difference when compared to the observed range in ammonium in Gull Lake (Table 5). Therefore, data were averaged across both stations for all variables and pooled for all analyses.

A vertical lake profile was taken from the surface to the bottom at 1 m intervals for temperature, dissolved oxygen, conductivity, and pH using a multiparameter sonde (Hydrolab Surveyor and Datasonde 4a). The temperature profile data was solely used to identify the epilimnion in the field, and to later calculate the thermocline depth and Schmidt water column stability (Idso 1973) using the rLakeAnalyzer package in R (Read et al. 2011). We measured water transparency (Z_{SD}) with a 20-cm Secchi disk, and estimated the light extinction coefficient, k, as 1.7 / Z_{SD} (Wetzel 2001). Lake water was collected from the epilimnion via two pooled casts of a depth-integrating tube sampler (12 m length × 2.5 cm i.d.). After thorough mixing and within 1 hour of collection, subsamples were taken for phytoplankton identification and enumeration (unfiltered water preserved in 1% Lugol's iodine), total phosphorus (unfiltered water), chlorophyll-*a* and particulate microcystin (retained on Pall A/E glass fiber filters, 1.0 μm nominal pore size), and dissolved nutrients (filtrate passed through A/E filters: soluble reactive phosphorus, ammonium, nitrate, and major cations). Filters were frozen immediately, and water samples for nutrients were either analyzed promptly or frozen for later analysis.

Lab analyses

Chlorophyll-*a* was measured fluorometrically following 12-hour dark extraction of frozen A/E filters in 10 mL of cold 95% ethanol (Welschmeyer 1994, White et al. 2011). Particulate microcystin was measured by ELISA (enzyme-linked immunosorbant assay; Envirologix QuantiPlate Kit for Microcystins visualized with a LabSystems Multiskan Microplate Reader) following three pooled 45 min, 10 mL extractions of A/E filters in 75% methanol (Harada et al. 1999, White et al. 2011). Soluble reactive phosphorus (SRP; molybdenum-blue method) and ammonium (indophenol-blue method) were analyzed with

standard colorimetric techniques and long-pathlength spectrophotometry (Murphy and Riley 1962, Solórzano 1969, Aminot et al. 1997). Total phosphorus was analyzed as above for SRP following persulfate digestion of organic material in an autoclave (Menzel and Corwin 1965). Nitrate was analyzed by Dionex membrane-suppression ion chromatography (Hamilton et al. 2009).

Lugol's preserved subsamples were settled in 10 mL tubular plankton chambers (Hydro-Bios), followed by counting and measuring of sedimented phytoplankton via the Utermöhl inverted microscope method (Lund et al. 1958, Hasle 1978). *Microcystis* biomass was quantified from measurement of two-dimensional surface areas of all colonies in the chamber using digital image analysis software (Spot Advanced) at 100×. These measurements were converted to dry biomass using a regression ($\log_{10}[y] = 1.05\log_{10}[x] - 1.32$; $R^2 = 0.94$) that estimates cell density from colony surface area for Gull Lake *Microcystis;* cell density was converted to biovolume and ultimately to dry biomass using measured cell dimensions, and assuming a specific gravity of 1.0 and a wet-to-dry biomass conversion factor of 0.4 (Sarnelle et al. 2012, Horst et al. 2014). Colony size was measured as the equivalent diameter (ED), by calculating the diameter of a circle with surface area equivalent to that of each measured colony (White and Sarnelle 2014).

Full phytoplankton community counts were performed on 8 dates (4 per year in 2 different years), selected to represent the typical range of total phytoplankton biomass for Gull Lake as assessed from chlorophyll-*a* (~1.5-5.5 μg L^{-1}). Phytoplankton were identified to genus, and cell counts were made as above at a variety of magnifications (100, 400, 1000×) to accurately assess densities of both large and small taxa. Approximately 50 random fields were viewed in each of two chamber regions (outer and inner, of nearly equal surface area) to account for non-random settling of cells (Sandgren and Robinson 1984). Cell volumes (exclusive of

spines, horns, and sheaths) were determined from digitized measurements of geometric dimensions (Hillebrand et al. 1999, Sarnelle et al. 2005) and converted to dry biomass as above. We then performed a linear regression of log-transformed phytoplankton dry biomass on chlorophyll-*a* ($y = 0.141x + 1.801$, $n = 8$, $R^2 = 0.62$, $p = 0.021$; Fig. 20) to enable more rapid estimation of relative *Microcystis* biomass.

Temperature data

Since variation in water temperature is likely to cause a lagged response of *Microcystis* biomass as a function of time-integrated conditions, we considered annual water temperature effects at three seasonal scales: spring (April-May), summer (June-August), and the combination of the two (spring-summer). Water temperature data were averaged separately for each of these three seasonal scales to assess their relative explanatory power.

Furthermore, because instantaneous water temperatures observed at the time of weekly sampling do not necessarily reflect prevailing conditions, we collected high-frequency (hourly) water temperature data by installing a vertical chain of data loggers (Onset Corp.) through the mixed layer of Gull Lake from April-August in 2010-2014, which was used to compute a daily mixed layer average. Since this high-frequency temperature record is unavailable for Gull Lake prior to 2010, we constructed empirical models to predict pre-2010 daily mixed layer water temperatures from daily air temperature records obtained for the entire duration of study from the Long Term Ecological Research (LTER) site at the Kellogg Biological Station (KBS, http://lter.kbs.msu.edu/datatables/12, datatable KBS002-006.27), which is adjacent to Gull Lake. We first confirmed that KBS LTER air temperature is a reasonable approximation of air

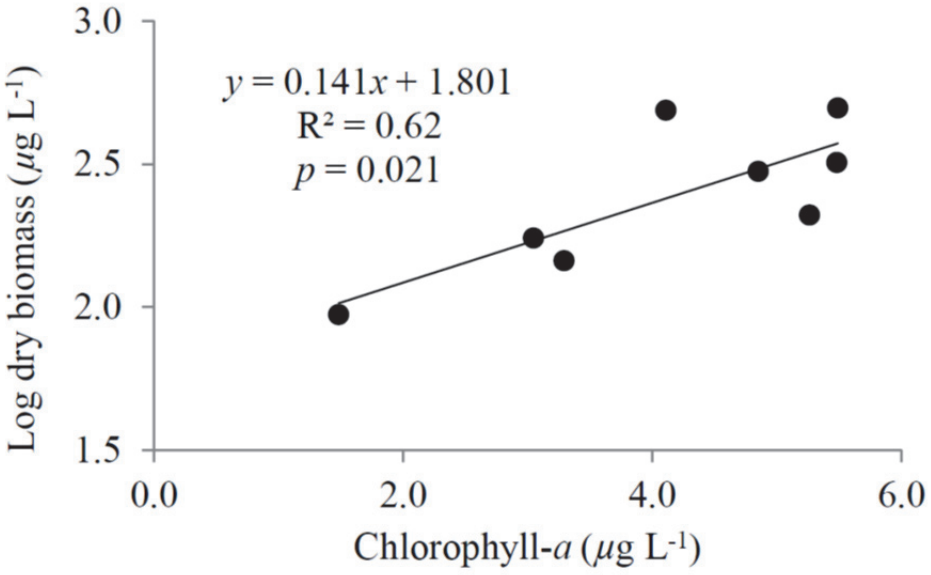

Figure 20. Prediction of total phytoplankton dry biomass from chlorophyll-*a* concentration in the epilimnion of Gull Lake (n = 8).

temperatures over Gull Lake using nearshore data recorded in 2010 and 2012 (linear regression; $n = 273$, $R^2 = 0.86$, $p < 0.001$, Fig. 21).

To account for lags in the response of water temperature to variation in air temperature when performing the conversion, we performed a series of linear regressions where we varied the time over which air temperatures were averaged leading up to and including the day of water temperature observation, starting with no lag and sequentially increasing the lag time by 1 day. We continued to increase the lag time until model fit was maximized and/or began to diminish again. We assessed the relative fit of each regression model with the Akaike Information Criterion (AIC), where a lower AIC indicates a better fit, and then computed ΔAIC for each model with respect to the model with the lowest AIC. Models having $\Delta AIC \leq 2$ were considered to have equal statistical support as the model with the lowest AIC (Burnham and Anderson 2001). Since the slope of the air-water temperature relationship differed by season (homogeneity of slopes test, $p < 0.001$), we performed this procedure separately for the spring and summer seasons (*see* above). Twenty-two days was the shortest lag having maximal statistical support ($\Delta AIC \leq 2$) for both the spring ($y = 1.587x + 5.955$, AIC = 392, $R^2 = 0.84$, $n = 105$, $p < 0.001$; Fig. 22a) and summer ($y = 0.870x + 6.403$, AIC = 1474, $R^2 = 0.72$, $n = 458$, $p < 0.001$; Fig. 22b) seasons. Therefore, these are the two models we used to estimate daily Gull Lake mixed layer water temperatures in the absence of direct, continuous observations prior to 2010.

Statistical analyses

The effect of interannual variation in water temperature in driving variation in *M. aeruginosa* biomass, relative biomass, timing of the maximum biomass, median colony size, net population growth rate, and particulate microcystin concentration were analyzed individually

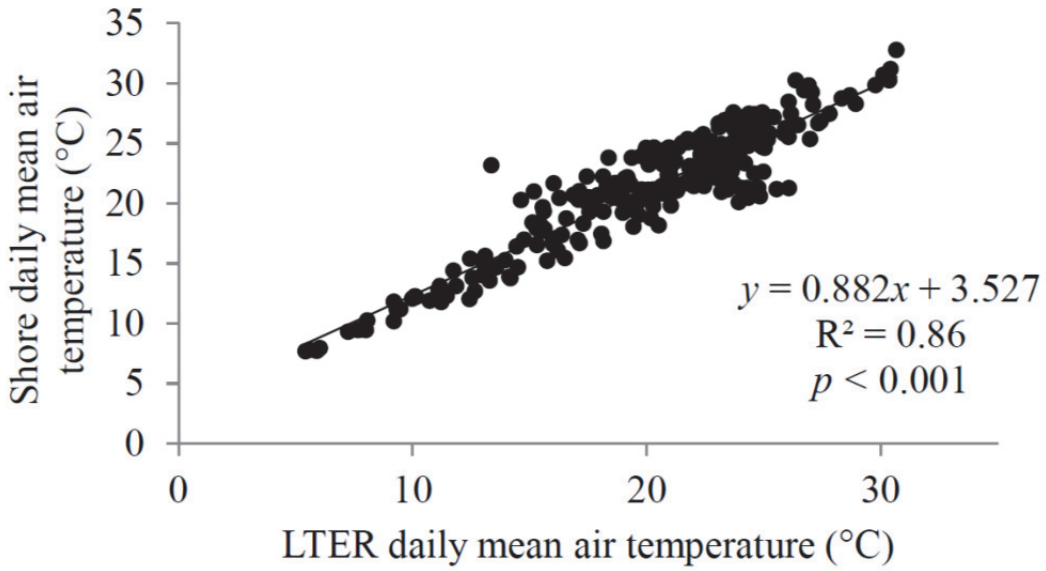

Figure 21. Relationship between daily mean air temperatures recorded at the Long-term Ecological Research (LTER) site at the Kellogg Biological Station, and at a lakeshore laboratory on Gull Lake (n = 273).

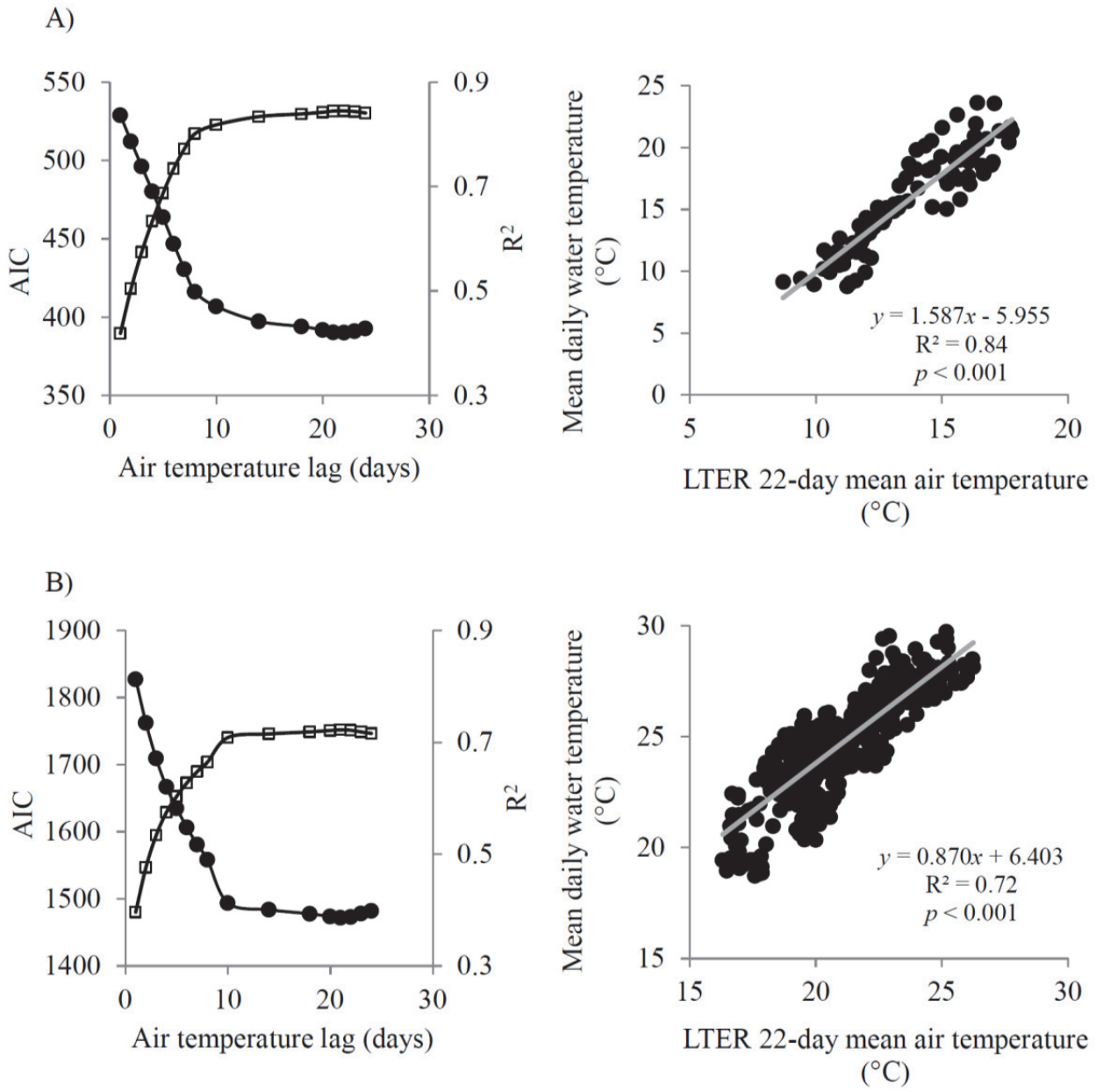

Figure 22. Conversion of KBS-LTER air temperature data to epilimnetic water temperature for Gull Lake, by season.

Figure 22. (cont'd)

(A) Spring (April-May, $n = 105$) and (B) summer (June-August, $n = 604$). Left panels illustrate the selection of the best-fitting lag time between air and water temperatures via R^2 (squares) and AIC (circles). Right panels show the relationship between observed epilimnetic water temperatures in Gull Lake (2010-2014) and the best-fitting air temperature lag of 22 days. The 22-day mean air temperature leading up to and including the day of water temperature observation was the shortest lag having maximal statistical support ($\Delta AIC < 2$; *see* text).

with linear regressions. Given the anticipated consequences of the mass-mortality event of *D. polymorpha* on *Microcystis* (*see* Introduction), we decided *a priori* to perform all statistical analyses separately for all years (n = 13) versus excluding 2011-2012 (n = 11), during which time *D. polymorpha* was largely absent from the epilimnetic sediments of Gull Lake (White et al. 2015).

To explore whether including other typical drivers and correlates of *Microcystis* biomass reported from eutrophic lakes in the literature (TP, Schmidt stability, and light availability) could explain any of the remaining variation, we performed stepwise multiple regression using both forward and backward variable selection techniques. The influence of *Microcystis* biomass and available nitrogen (as NO_3^-) on particulate microcystin concentrations was analyzed with multiple regression.

Due to the varied duration of sampling programs prior to 2009, the declining phase of the *Microcystis* population was not fully observed in each year. Therefore, mean biomass was computed from the time of first detection through the population maximum. Mean biomass was then log-transformed to stabilize the variance. Relative biomass (as a proportion) was arcsine-squareroot transformed prior to analysis to meet distributional assumptions. However, this data transformation had no substantiative effect on any result, and so we report results for the raw data for ease of interpretation. All predictor variables, with the exception of water temperature (*see* above) were averaged from June-August annually. All statistical analyses were performed with R version 3.1.3 (R Foundation for Statistical Computing) and SYSTAT version 12.

Results

Microcystis biomass, relative biomass, timing of maximum biomass, median colony size, and particulate microcystin all exhibited substantial intra- and inter-annual variation in Gull Lake over the period of observation from 1998-2014 (Fig. 23). *Microcystis* generally first appears in the water column in June, increases to a maximum in July or August, and then declines (Fig. 24). The timing of the population maximum varied by as much as one month over the period of observation, from 26 July to 27 August. Biomass at the time of the population maximum ranged from 6 μg L^{-1} to 135 μg L^{-1}, corresponding to ~3% and 80%, respectively, of the total phytoplankton dry biomass (Fig. 23). On average, *Microcystis* constituted ~2-25% of the total annual phytoplankton dry biomass during the summer in Gull Lake. Annual net population growth rates ranged from 0.04 day^{-1} to 0.11 day^{-1}, and median colony size ranged from 69 μm to 138 μm equivalent diameter. Annual mean particulate (presumably cell-bound) microcystin concentrations ranged from 0.02 μg L^{-1} to 0.34 μg L^{-1}, and no individual observation exceeded 0.45 μg L^{-1} (Fig. 23).

Responses of Microcystis *biomass to interannual variation in water temperature*

On average, *Microcystis* biomass was significantly higher in Gull Lake during warmer years (linear regression, $n = 11$, $R^2 = 0.45$, $p = 0.025$; Fig. 25a), but this was only true for years when zebra mussels were present on epilimnetic sediments. While zebra mussels remained absent on the epilimnetic sediments during 2011-2012, the positive effect of temperature on *Microcystis* biomass decoupled (linear regression; $n = 13$, $R^2 = 0.22$, $p = 0.11$; Fig. 25a). Decoupling occurred despite the fact that this period of high mortality and failed recolonization of zebra mussels overlapped with the warmest summer in the time series—the mean

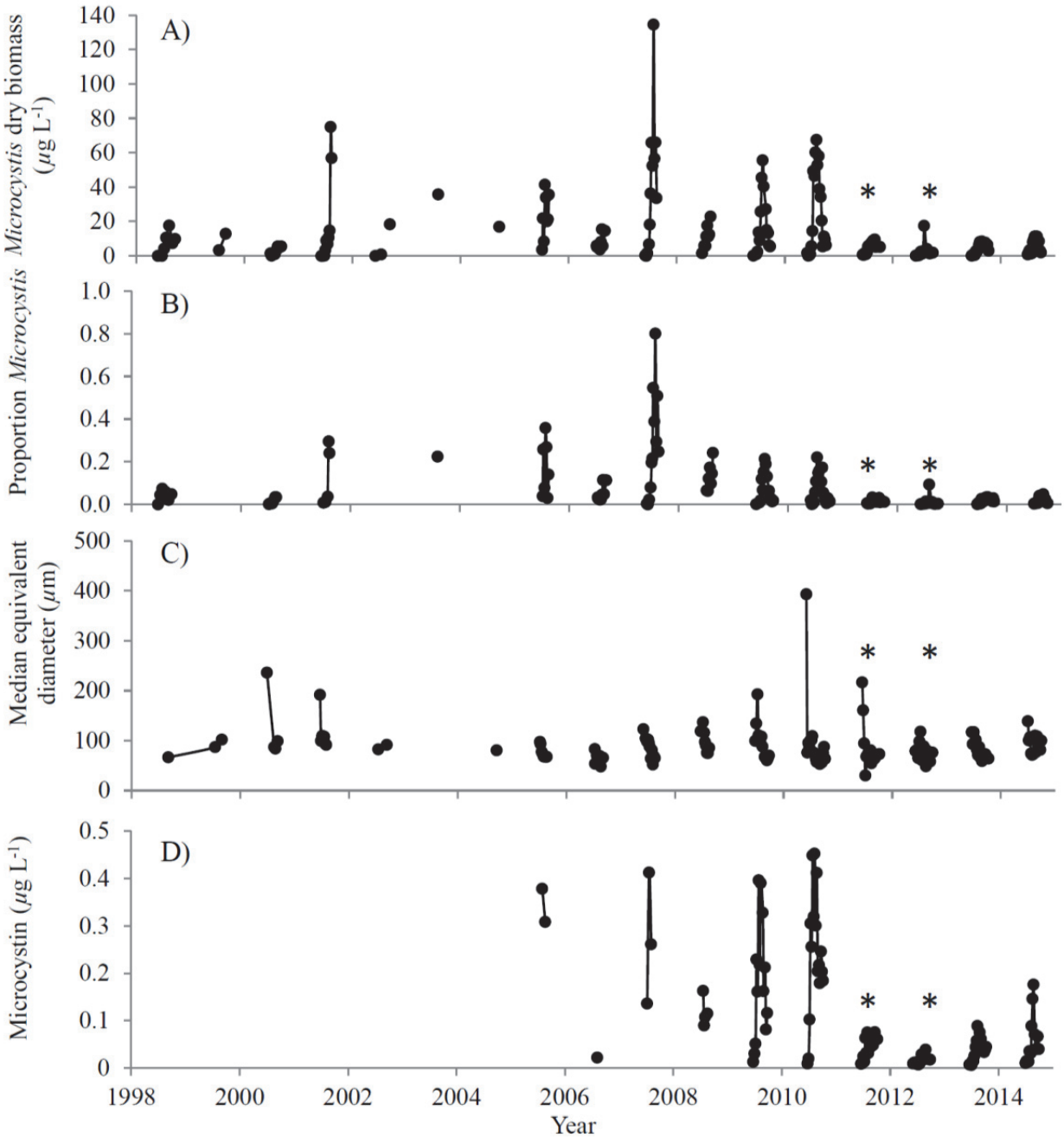

Figure 23. Time series plots of *Microcystis* parameters measured in Gull Lake, 1998-2014.

Figure 23. (cont'd)

(A) *Microcystis* dry biomass, (B) proportion of the total phytoplankton dry biomass as *Microcystis*, (C) median *Microcystis* colony size (as equivalent diameter), and (D) particulate (cell-bound) microcystin concentration. Microcystin was not monitored in Gull Lake until 2005, and sampling for all other response variables was conducted intermittently in 1999 and 2002-2004. Asterisks (*) indicate observations made during years in which zebra mussels were largely absent from the epilimnetic sediments of Gull Lake (due to heat-induced mass mortality, *see* text).

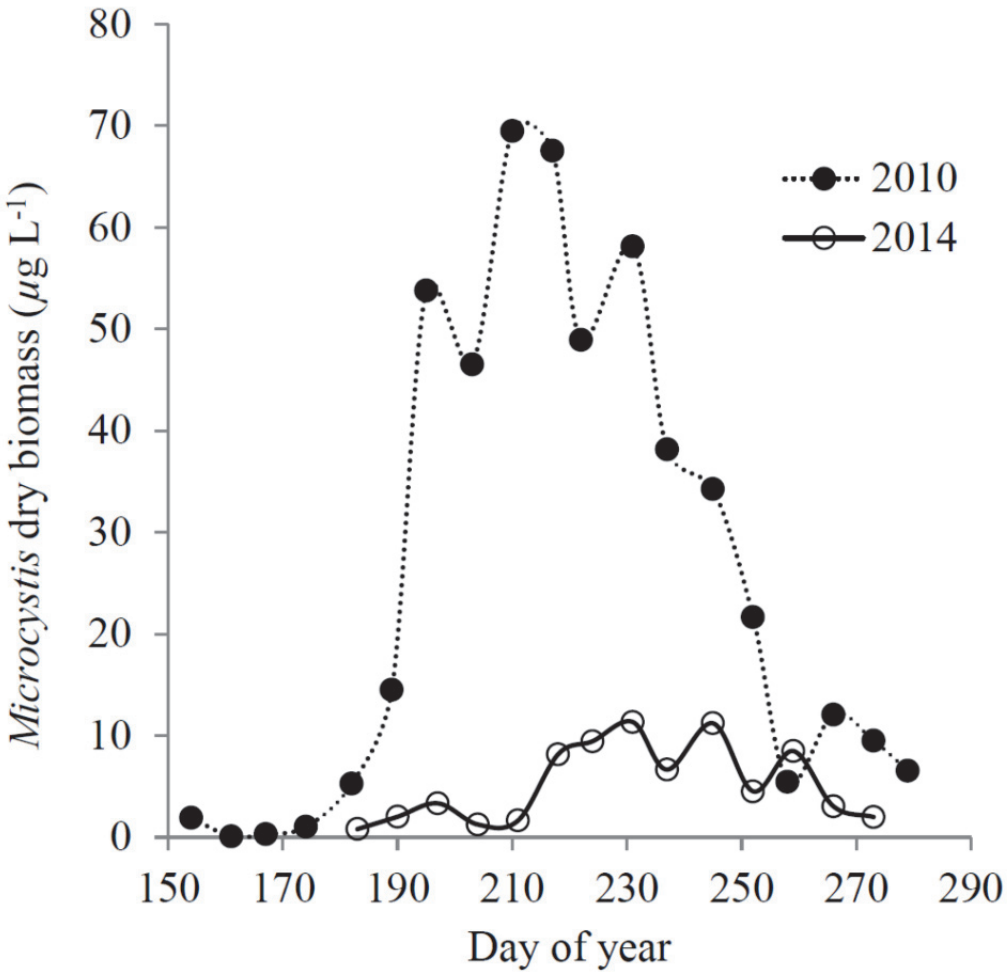

Figure 24. Seasonal dynamics of *Microcystis* biomass in Gull Lake during a representative warm year (2010; mean spring-summer water temperature = 21.41 °C, maximum biomass = 69.5 μg L^{-1} on 29 July) and a representative cool year (2014; mean spring-summer water temperature = 19.51 °C, maximum biomass = 11.4 μg L^{-1} on 19 August). The two series are plotted on a common time scale (day of year) to emphasize differences in the timing of first appearance and the timing and magnitude of the biomass maximum. The mass mortality event of zebra mussels occurred between days 209 and 215 in 2010.

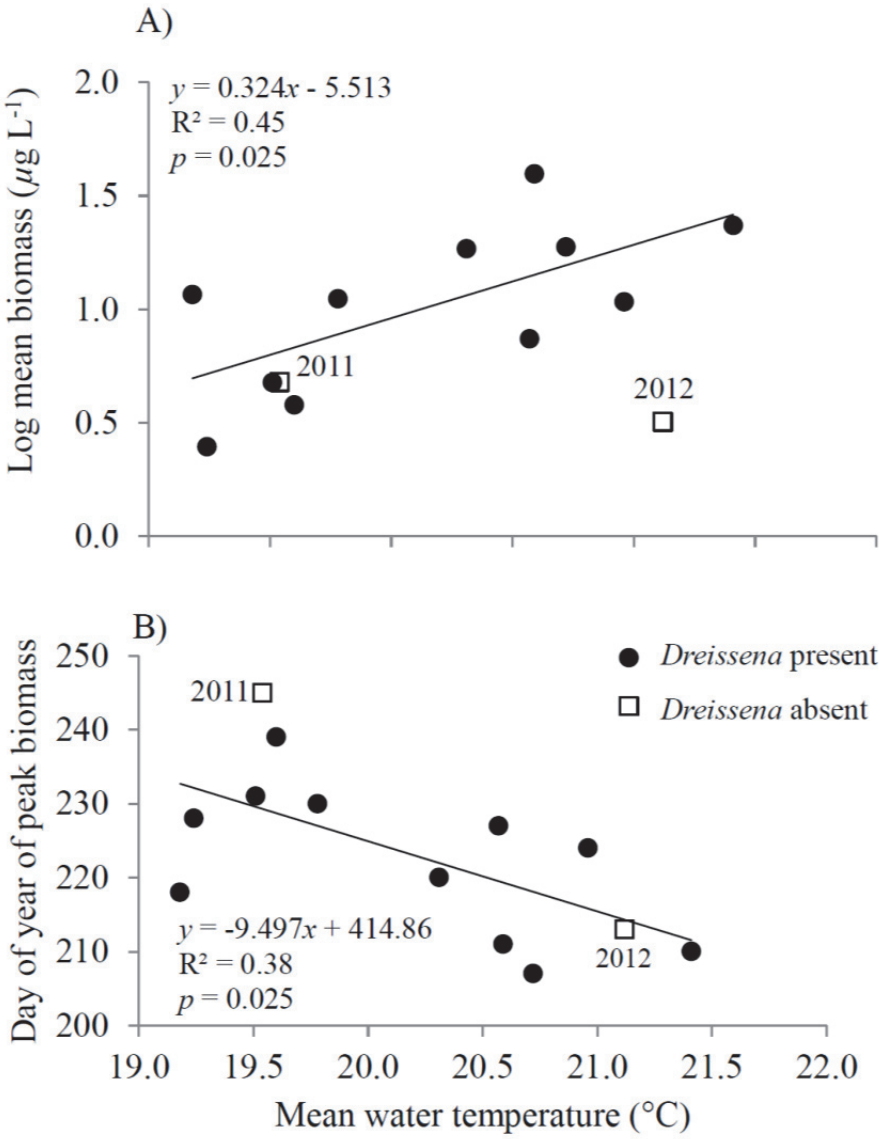

Figure 25. *Microcystis* biomass dynamics as a function of interannual variation in Gull Lake water temperatures: (A) annual mean *Microcystis* dry biomass, and (B) day of year of maximum *Microcystis* biomass. Years with zebra mussels (*Dreissena polymorpha*) present on epilimnetic sediments (circles, $n = 11$) are differentiated from years (2011 and 2012, squares) during which zebra mussels were largely absent on epilimnetic sediments as a result of heat-induced mass mortality (*see* text). Results from the linear regression analysis in (A) are for years with *Dreissena* only, whereas those in (B) are for all years (*see* text for explanation).

spring-summer water temperature was 21.1 °C in 2012, and Gull Lake reached a maximum epilimnetic temperature of 30.5 °C (Table 6). However, mean *Microcystis* biomass in 2012 (3.19 μg L^{-1}) was equivalent to that in a year with a predicted mean spring-summer water temperature of only 18.6 °C—cooler than any of the observed years. *Microcystis* biomass was reduced on average by 71% during 2011-2012, as compared to years when zebra mussels were present on epilimnetic sediments (Fig. 26a). The annual *Microcystis* population maximum also occurred significantly earlier in the growing season during warmer years, and this relationship was unaffected by the mass mortality event of zebra mussels (linear regression; $n = 13$, $R^2 = 0.38$, $p = 0.025$; Table 7, Figs. 24, 25b).

In all cases where significant temperature effects were found, spring-summer mean water temperature was a better predictor than either the spring or summer seasonal means alone (Table 7). We did not detect significant temperature effects on relative *Microcystis* biomass, median colony size, or net population growth rate at the annual scale (linear regressions; $n = 11$, $R^2 \leq 0.32$, $p \geq 0.082$; Table 7).

Responses of Microcystis *biomass to interannual variation in other potential drivers*

We found no influence of annual TP, Schmidt stability, or light availability (k) on *Microcystis* biomass or timing of maximum biomass (stepwise multiple regressions; p for all parameters > 0.05, irrespective of *Dreissena* presence). Forward and backward variable selection confirmed water temperature to be the best predictor of log mean biomass (only when *Dreissena* was present) and timing of maximum biomass (irrespective of *Dreissena* presence; *see* above) at the annual scale. The mean proportion of total phytoplankton dry biomass as *Microcystis* was

Table 6. Mean Gull Lake water temperatures (°C), computed annually for each seasonal time scale: spring (April-May), summer (June-August), and spring-summer.

Year	Spring	Summer	Spring-summer
1998	14.85	24.14	20.96
2000	11.98	23.03	19.24
2001	14.12	23.54	20.31
2005	11.98	25.29	20.72
2006	12.67	24.60	20.57
2007	12.18	24.97	20.59
2008	12.03	23.87	19.78
2009	11.56	23.16	19.18
2010	13.96	25.30	21.41
2011	10.09	24.57	19.54
2012	13.07	25.32	21.12
2013	10.82	24.18	19.60
2014	10.95	23.97	19.51

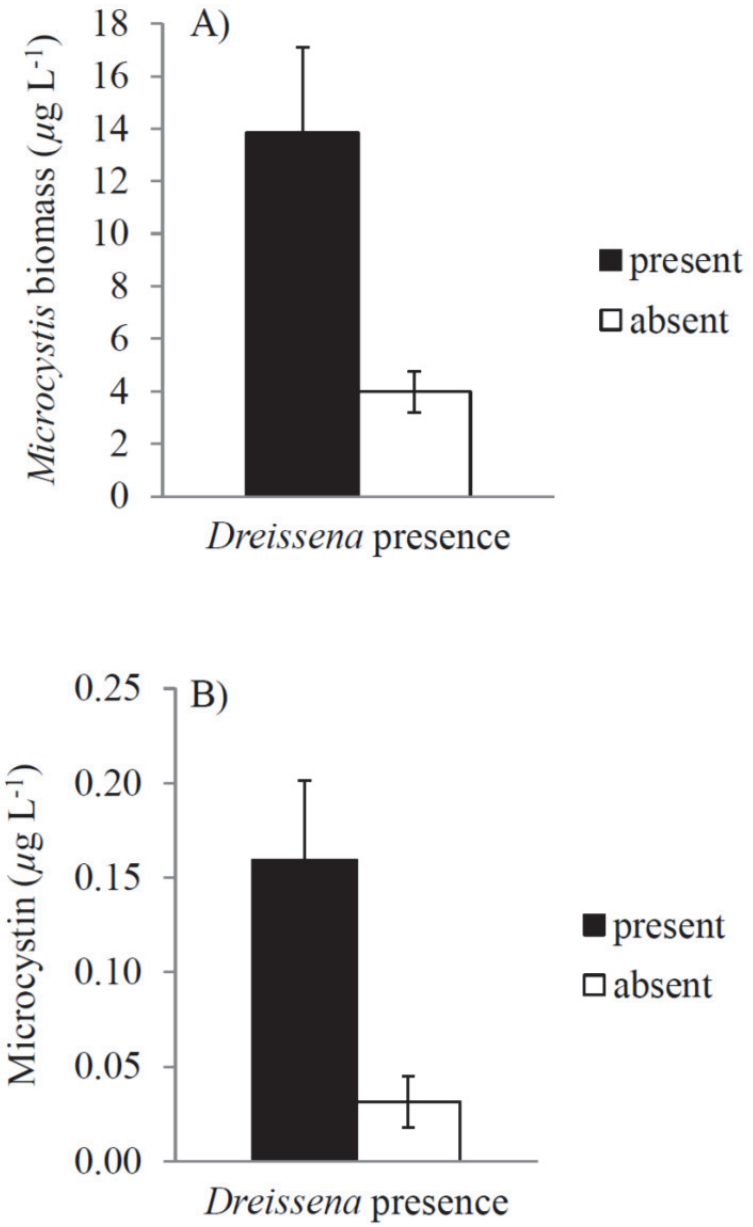

Figure 26. *Microcystis* biomass (A) and particulate (cell-bound) microcystin concentration (B) in Gull Lake in the presence (n = 11, black columns) versus absence (2011-2012; n = 2, open columns) of zebra mussels (*Dreissena polymorpha*) on the epilimnetic sediments. Zebra mussels were largely absent on epilimnetic sediments in 2011-2012 as a result of heat-induced mass mortality (*see* text). Error bars represent ± SE.

Table 7. Influence of water temperature on annual means for each response variable for years when zebra mussels were present in Gull Lake (excluding 2011-2012, *see* text).

Response	Predictor (seasonal scale)	*n*	R^2	*p*	AIC
Mean *Microcystis* biomass	Spring-summer	11	0.45	0.025	7.3
	Summer	11	0.33	0.063	9.3
	Spring	11	0.20	0.165	11.3
Day of peak *Microcystis* biomass	Spring-summer	11	0.36	0.050	82.1
	Summer	11	0.29	0.086	83.1
	Spring	11	0.14	0.257	85.3
Relative biomass	Summer	11	0.30	0.082	-15.1
	Spring-summer	11	0.25	0.113	-14.4
	Spring	11	0.05	0.512	-11.7
Colony size	Spring	10	0.32	0.086	91.4
	Spring-summer	10	0.06	0.485	94.7
	Summer	10	0.01	0.799	95.2
Mean particulate microcystin	Spring-summer	8	0.26	0.200	-8.8
	Summer	8	0.28	0.176	-9.1
	Spring	8	0.15	0.344	-7.8
Population growth rate	Spring-summer	11	0.03	0.644	-39.7
	Summer	11	0.04	0.576	-39.8
	Spring	11	0.00	0.864	-39.5

Table 7. (cont'd)

We also assessed the influence of the seasonal scale over which water temperatures were averaged on each response variable. The three seasonal time scales are: spring (April-May), summer (June-August), and spring-summer. Predictors are listed from the best to the worst fit, as assessed by AIC. *Microcystis* biomass was log-transformed prior to analysis. Significant ($p \leq 0.05$) results are underlined. All results are for linear regressions.

significantly, positively related to *Microcystis* dry biomass, irrespective of *Dreissena* presence (linear regression; $n = 13$, $R^2 = 0.74$, $p < 0.001$; Fig. 27a).

Responses of microcystin toxin

Annual particulate (presumably cell-bound) microcystin concentrations in Gull Lake were significantly, positively related to *Microcystis* biomass (linear regression; $n = 10$, $R^2 = 0.79$, $p < 0.001$; Fig. 27b). Although this relationship was unaffected by the mass mortality event of zebra mussels, particulate microcystin concentrations were reduced on average by 80% during 2011-2012, as compared to years when zebra mussels were present on epilimnetic sediments (Fig. 26b). Adding nitrate as a covariate in addition to *Microcystis* biomass did not improve model fit (ANOVA goodness-of-fit test, $p = 0.097$).

Discussion

In stark contrast to existing climate change projections that predict increases in harmful cyanobacteria as a result of positive temperature effects on growth physiology and water column stratification (Paerl and Huisman 2008, Paul 2008, Carey et al. 2012b), we observed a crash in *Microcystis* during an especially warm summer in low-nutrient Gull Lake. The primary driver of *Microcystis* in oligotrophic Gull Lake is unequivocally zebra mussels, as both pre-invasion (Sarnelle et al. 2005) and post-2010 heat-induced mass mortality data demonstrate (Fig. 26). *Microcystis* biomass increased with temperature in Gull Lake only when zebra mussels were present (Fig. 25a), implicating *Dreissena* as strong facilitators of *Microcystis* in low-nutrient lakes ($\leq$ 10-25 μg L^{-1} TP) (Raikow et al. 2004, Knoll et al. 2008). Zebra mussels remained absent

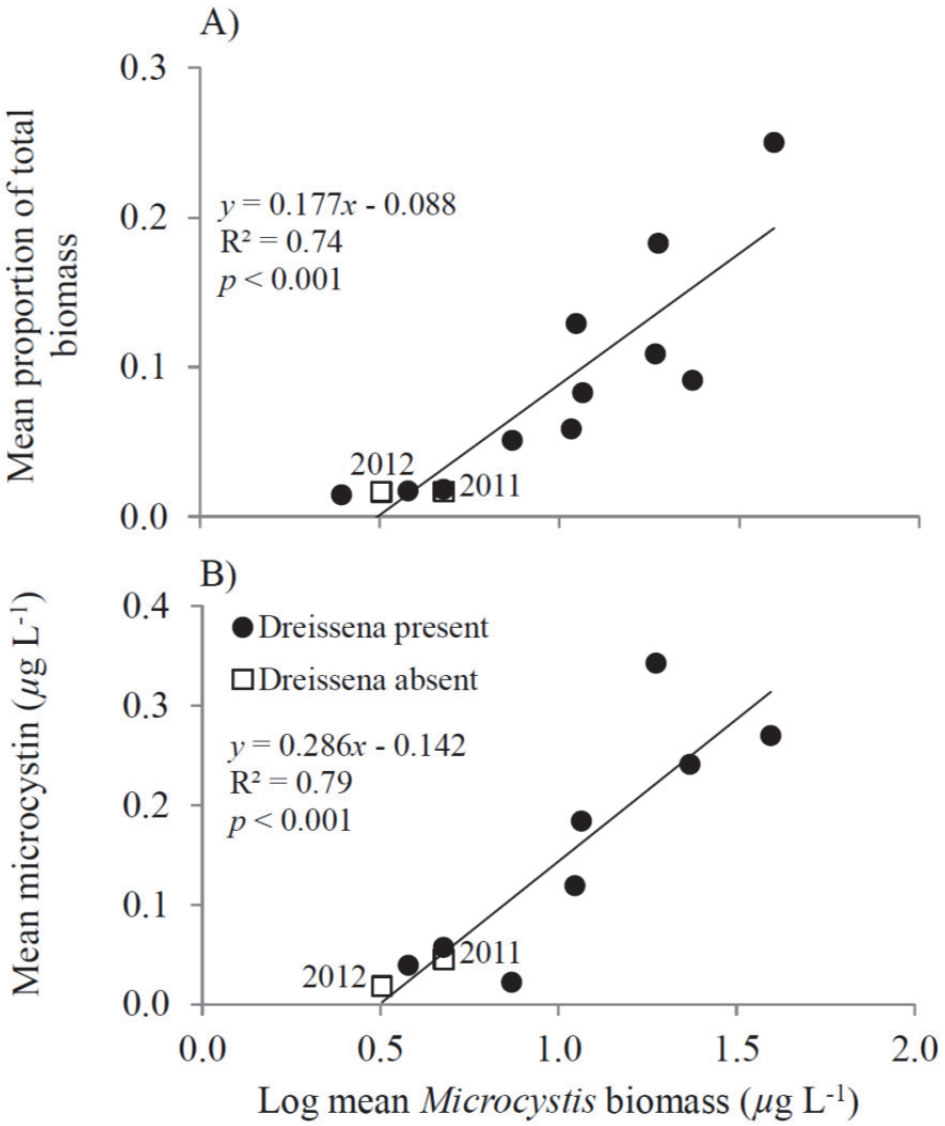

Figure 27. Relationships between mean *Microcystis* biomass in Gull Lake and (A) mean proportion of total algal dry biomass as *Microcystis* (n = 13) and (B) mean particulate (cell-bound) microcystin toxin concentration (n = 10). Years with zebra mussels (*Dreissena polymorpha*) present on epilimnetic sediments (circles) are differentiated from years (2011 and 2012, squares) during which zebra mussels were largely absent on epilimnetic sediments as a result of heat-induced mass mortality (*see* text). Results from linear regression analyses are for all years (*see* text for explanation).

from the epilimnetic sediments during 2011-2012 following the initial 2010 mortality event because prolonged temperatures > 25 °C (maximum = 30.5 °C) continued to exceed their chronic lethal threshold (White et al. 2015) (*see* Chapter 2). Despite the fact that these water temperatures were within the known optimal range for *Microcystis* growth and dominance (~25-32 °C) (Zehnder and Gorham 1960, Robarts and Zohary 1987, Imai et al. 2009), *Microcystis* dynamics during this period resembled those for the coolest years of observation (Figs. 23, 25a, 21). In fact, the magnitude of the responses of *Microcystis* and microcystin (3.5× and 5.1×, respectively; Fig. 26) to the *Dreissena* mortality event are highly congruent with comparative data from invaded and non-invaded lakes (3.6× and 3.3-8.0×, respectively) (Knoll et al. 2008, Sarnelle et al. 2010). Temperature, then, only modulates the actual biomass achieved in any given year in Gull Lake, since the promotion of *Microcystis* by zebra mussels in low-nutrient lakes is reversible upon elimination of zebra mussels, as shown in this study and in enclosure experiments (Sarnelle et al. 2005, Sarnelle et al. 2012). Thus, climate change might result in complex, non-monotonic responses of *Microcystis* to elevated temperatures in low-nutrient lakes by disrupting the critical interaction with its facilitator species, highlighting the need to consider species interactions in studies of ecological responses to climate change (Tylianakis et al. 2008, Van der Putten et al. 2010).

To date, most research on the effect of climate change on species interactions has emphasized spatial and temporal mismatches in the phenologies of critical life history events (e.g., migration, germination, emergence) in predator-prey and mutualistic systems where species differ in the timing, direction, and magnitude of their responses (Edwards and Richardson 2004, Winder and Schindler 2004, Both et al. 2006, Schweiger et al. 2008, Yang and Rudolf 2010). We observed maximally opposed responses (i.e., high mortality versus enhanced

growth) by strongly interacting, apparently commensal species to the same large climatic variation within a single lake ecosystem. Furthermore, the opposing response of the facilitator species completely negated the positive climatic effect on *Microcystis,* emphasizing that understanding how a species responds in isolation to climatic drivers, particularly in an experimental setting, does not necessarily predict the response of the community in nature (Suttle et al. 2007, Post and Pedersen 2008).

There are at least two non-mutually exclusive explanations for why zebra mussels promote *Microcystis* so strongly in low-nutrient lakes like Gull Lake: the selective feeding hypothesis, and the nutrient excretion hypothesis. Zebra mussels are highly selective feeders, and will reject unpalatable strains (White et al. 2011) and large colonies (White and Sarnelle 2014)(*see* Chapter 1) of *Microcystis* back into suspension, which can remain viable (Vanderploeg et al. 2001). Selective rejection of *Microcystis,* in concert with high mortality imposed on other competing phytoplankton, has been posited as the primary mechanism by which zebra mussels have promoted *Microcystis* in the Laurentian Great Lakes (Vanderploeg et al. 2001). However, zebra mussel excretion also diverts nutrients assimilated by phytoplankton from the open water column to the benthos (Hecky et al. 2004). Although the low N:P ratios evidenced to favor cyanobacteria in eutrophic lakes (Smith 1983) are generally not encountered in the open waters of low-nutrient lakes like Gull Lake (Table 5), zebra mussels excrete at low N:P (Arnott and Vanni 1996), which may alleviate nutrient limitation and facilitate *Microcystis* recruitment to the plankton from the sediments (Brunberg and Blomqvist 2003, Ståhl-Delbanco et al. 2003, Bykova et al. 2006). Interestingly, *Microcystis* still crashed in Gull Lake during the summers of 2011-2012 despite the fact that zebra mussels persisted at deeper, cooler depths below the thermocline throughout the entire period of high mussel mortality on the epilimnetic

sediments (White et al. 2015). This implies that *Microcystis* promotion in low-nutrient lakes requires the direct contact with *Dreissena* afforded within the epilimnion, which is congruent with both the selective rejection and nutrient excretion hypotheses.

Our study also constitutes the first long-term analysis of *Microcystis* dynamics in a low-nutrient inland lake, an uncharacteristic niche for this harmful algal bloom-forming species, and one of very few long-term (≥ 10 years) studies of natural populations of *Microcystis* in general (Utkilen et al. 1996, Liu et al. 2011). We found considerable interannual variation in *Microcystis* biomass, timing of maximum biomass, relative abundance, colony size, and microcystin in oligotrophic Gull Lake (Figs. 23, 24). The population maximum occurred significantly earlier during warmer years, irrespective of *Driessena* presence (Figs. 24, 25b), indicating that warmer temperatures expedite the seasonal succession of the phytoplankton community to *Microcystis.* Where we found significant temperature effects on *Microcystis* dynamics, spring-summer averages had better predictive power than either average spring or average summer temperatures alone (Table 7), suggesting that the influence of temperature is integrative and cumulative over the entire growing season. Dynamics of particulate (cell-bound) microcystin concentrations closely tracked *Microcystis* biomass (Fig. 23), and *Microcystis* biomass was a significant predictor of both relative *Microcystis* biomass and annual microcystin toxin concentrations in Gull Lake irrespective of *Dreissena* presence (Fig. 27). Therefore, in low-nutrient lakes where *Microcystis* is the only common producer of microcystin, *Microcystis* biomass may serve as a reliable proxy for water column microcystin concentrations. Large intra-annual variation, including a seasonal decline, in *Microcystis* colony size has been reported previously in Gull Lake (White and Sarnelle 2014) (*see* Chapter 1).

Our observations of *Microcystis* dynamics in low-nutrient Gull Lake in the presence of *Dreissena* are similar to some aspects of studies of *Microcystis* populations in eutrophic lakes. A long-term (11-year) monitoring study of *Microcystis* dynamics in eutrophic Lake Taihu, China also found that temperature was a primary driver of biomass, which increased markedly as water temperature exceeded ~20 °C, with peak biomass occurring during the summer months (Liu et al. 2011). The annual temporal pattern of *Microcystis* biomass and microcystin we observed in Gull Lake (population maximum in July-August) is also similar to that reported for eutrophic lakes in Germany and England (Reynolds 1973, Fastner et al. 1999), although the annual decline of the population in Gull Lake tended to occur earlier and more rapidly following the population maximum than is reported in eutrophic lakes (Reynolds and Rogers 1976, Reynolds et al. 1981, Bigham et al. 2009, Imai et al. 2009).

Our results contrast markedly, however, with respect to many other critical drivers of *Microcystis* reported from regional, comparative studies of eutrophic lakes. Cyanobacterial dominance of the phytoplankton community typically occurs at high total phosphorus concentrations (i.e., in eutrophic lakes) (Smith et al. 1987, Watson et al. 1997, Downing et al. 2001), yet we observed *Microcystis* achieving up to 80% dominance in a lake with a mean TP of only 7.59 μg L^{-1} (Fig. 23). Dominance by cyanobacteria in eutrophic lakes has been hypothesized to result from the competitive advantage of nitrogen-fixing species at low TN:TP (< 29:1) (Schindler 1977, Smith 1983). However, this open-water nutrient ratio hypothesis cannot explain the occasional dominance of *Microcystis* in oligotrophic Gull Lake, since *Microcystis* cannot fix nitrogen and the open-water DIN:TP ratio alone is ~42:1 (Table 5), which is consistent with other studies that argue TN:TP and nitrogen fixation are not adequate for explaining cyanobacterial dominance in lakes (Downing et al. 2001, Ferber et al. 2004). In fact, we did not

find any significant influence of TP on annual *Microcystis* biomass. A recent study identified nutrients over temperature as the primary driver of total cyanobacteria biovolume across lakes of all trophic states, although *Microcystis* biovolume specifically exhibited a substantially larger response to temperature than nutrients relative to other cyanobacteria (Rigosi et al. 2014), highlighting the need for additional species-specific data (Pitcher 2012).

Low light availability, depleted carbon dioxide concentrations, and elevated pH are also associated with or hypothesized to competitively favor cyanobacteria in eutrophic lakes (Smith 1986, Shapiro 1997, Bigham et al. 2009). However, annual light climate (k range: 0.32-0.54 m^{-1}) did not explain a significant proportion of the residual variation in *Microcystis* biomass. Dissolved inorganic carbon (DIC) limitation is also unlikely to explain years of high biomass given that Gull Lake always contains high concentrations of DIC (Hamilton et al. 2009) and, although the pH can be high (range: 8.1-9.0) and potentially selective for phytoplankton that can utilize bicarbonate, this capability is not exclusive to *Microcystis*. Our finding that most environmental variables that show significant influences at the regional scale had little or no predictive power in a single lake is perhaps not surprising, given that the among-lake variation in these studies is typically enormous relative to the inter-annual variation observed within an individual lake—let alone a lake of low productivity (Tillmanns and Pick 2011).

Of course, the relative importance of different environmental drivers can depend on the temporal and spatial scales of the analysis (Reynolds 2007, Tillmanns and Pick 2011), and our consideration of only annual means may conceal important relationships that exist at weekly or even monthly scales. For example, within a single eutrophic lake in Ontario, microcystin was not significantly related to any environmental variable (including TP, SRP, TN, NH_4^+, pH, transparency, and temperature) at the seasonal scale, but at monthly and 6-day timesteps

significant relationships emerged, and different variables were significant at different timesteps (Tillmanns and Pick 2011). Therefore, our results do not imply that environmental factors besides temperature are unimportant to *Microcystis* in low-nutrient lakes—only that they are relatively poor predictors of biomass at the annual scale.

Cyanobacteria toxins, like microcystin, are generally associated with eutrophic habitats, yet detectable levels have been documented by recent surveys of low-nutrient lakes and reservoirs across a broad spatial extent, including lakes that are not experiencing cultural eutrophication and also lack zebra mussels. However, detection is relatively rare (12-33% of lakes) and concentrations are generally always well below the World Health Organization's drinking water guideline of 1.0 μg L^{-1}(Giani et al. 2005, Bigham et al. 2009, Graham and Jones 2009, Sarnelle et al. 2010). Surprisingly, the colonial cyanobacterium *Gloeotrichia echinulata* has also been reported to be on the rise in oligotrophic and mesotrophic lakes that lack *Dreissena* in the northeastern United States, where it has produced detectable levels of microcystin (Carey et al. 2008, Carey et al. 2012a). Microcystin concentrations in low-nutrient Midwestern lakes invaded by zebra mussels are also significantly higher than predicted by TP (Knoll et al. 2008, Sarnelle et al. 2010). For example, the model of Giani et al. (2005) for uninvaded lakes spanning the productivity gradient predicts an average microcystin concentration of 0.003 μg L^{-1} for Gull Lake (7.59 μg L^{-1} TP), when average annual concentrations are actually as much as two orders of magnitude higher (0.022-0.343 μg L^{-1}). Though cyanobacteria toxins in the open waters of low-nutrient lakes are generally below levels triggering public health concerns, their occasional presence in these systems is still noteworthy since they are the lakes that are frequently associated with drinking and recreational uses.

Microcystis strains that dominate populations in oligotrophic lakes are likely to differ markedly in their growth and resource-use physiology from those that dominate populations in eutrophic lakes, given that resource levels can vary several fold across the lake productivity gradient (Wetzel 2001). For example, strains from low-nutrient lakes, including Gull Lake, have lower maximum intrinsic growth rates than those from nutrient-rich lakes when grown in a common garden, indicating genetically based variation in growth (*see* Chapter 4). The half-saturation constant (K_m) for phosphorus-dependent growth of *Microcystis* isolated from nutrient-rich lakes is higher (5.9 μg L^{-1}) (Holm and Armstrong 1981, Nalewajko and Murphy 2001) than available phosphorus concentrations typically found in Gull Lake (Table 5) and other low-nutrient lakes, suggesting that *Microcystis* strains growing in low-nutrient lakes are either always growing well below maximal rates or possess a lower K_m conducive to growth in a chronically nutrient-poor habitat. Net population growth rates observed in Gull Lake (0.02-0.11 day^{-1}) are comparable to the median maximum intrinsic growth rate (0.15 day^{-1}; *see* Chapter 4) determined for 11 different Gull Lake *Microcystis* strains, lending support for the latter prediction. Large intraspecific variation in *Microcystis* has ecological consequences (White et al. 2011, White and Sarnelle 2014) (*see* Chapters 1, 4), and so there is precedent to expect it should facilitate adaptation to growth in low-nutrient lakes.

Freshwater ecosystems are increasingly vulnerable to and impacted by numerous anthropogenic stressors associated with global change—particularly cultural eutrophication and climate change—both of which are known to promote nuisance levels of toxic cyanobacteria (Carpenter et al. 1992, Carpenter et al. 1998, Paerl and Huisman 2008, O'Neil et al. 2012, Rigosi et al. 2014). Consistent with climate change forecasts, *Microcystis* biomass was generally elevated during warmer years in a low-nutrient lake, and microcystin concentrations closely

tracked biomass. However, *Microcystis* drivers and dynamics in low-nutrient Gull Lake differ in many fundamental respects from eutrophic systems, resulting in large part from strong interactions with invasive zebra mussels. Zebra mussel invasion of low-nutrient lakes represents yet another 'catalyst' for the global increase of harmful cyanobacteria (Paerl and Huisman 2009). Interactions among abiotic and biotic drivers, particularly temperature and invasive *Dreissena*, can result in complex responses by *Microcystis* in low-nutrient lakes that are not anticipated by current limnological paradigms or climate change forecasts. Thus, predicting the response of a species to climate change may require, at minimum, quantification of temperature responses of both the focal species and species that strongly interact with it. Consequently, monitoring of intact communities with respect to climatic variables seems essential for predicting the community- and ecosystem-level consequences of an era of unprecedented global change.

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CHAPTER 4

GROWTH VARIATION AMONG STRAINS OF THE HARMFUL CYANOBACTERIUM, *MICROCYSTIS AERUGINOSA,* ACROSS A LARGE PRODUCTIVITY GRADIENT OF LAKES

Abstract

The toxic cyanobacterium *Microcystis aeruginosa* now achieves non-trivial densities in many low nutrient (oligotrophic) lakes following invasion by zebra mussels (*Dreissena polymorpha*), which is unexpected given that this species is typically associated with high nutrient (eutrophic) lakes. We explored the extent of biological variation in growth traits within this species, which might enable it to succeed in these ecologically disparate habitats. Using common-garden laboratory growth assays, we quantified the maximum intrinsic growth rates of 18 colonial strains of *M. aeruginosa* recently isolated from 11 Michigan lakes spanning the entire productivity gradient, from oligotrophic to hyper-eutrophic (total phosphorus range: 7.6-196 μg L^{-1}). *Microcystis* strains possessed fixed variation in maximum growth rate as a function of source lake TP, with strains from eutrophic and hyper-eutrophic lakes growing up to ~7 times faster than strains from oligotrophic lakes. Strains from high-nutrient lakes also had a significantly greater probability of becoming single-celled during the first 1.0-1.5 years in lab culture, and strains grew faster as single-cells compared to their natural colonial growth habit, emphasizing that caution should be exercised when relating studies of single-celled lab strains to natural populations. Our results provide evidence that *M. aeruginosa* populations are genetically adapted to grow under specific local resource conditions, which may help to broaden the species' ecological niche and could strongly influence its response to global change.

Introduction

Harmful algal blooms (HABs) occur worldwide in nutrient-polluted waters, produce toxins and skin irritants, foul the taste and odor of drinking water, and can cause illness (Chorus and Bartram 1999, Huisman et al. 2005). HABs appear to be increasing (Hallegraeff 1993) and are projected to increase with climate warming and other facets of global change (Paerl and Huisman 2008, Paul 2008, Carey et al. 2012b, Elliott 2012, Paerl and Paul 2012). Recent work also emphasizes the importance of intraspecific trait variation among strains (genotypes) in explaining population and bloom dynamics for HAB-forming phytoplankton (Burkholder and Glibert 2009). For example, different strains of single HAB species have been shown to constitutively vary in their competitive ability for nutrients and light (Kardinaal et al. 2007b), nutrient uptake rate (Sinclair et al. 2009), vulnerability to grazing (White et al. 2011, White and Sarnelle 2014), size (Wilson et al. 2006), toxicity (Vezie et al. 1998, Mikalsen et al. 2003, Saker et al. 2005), and growth rate (Wilson et al. 2010, Calbet et al. 2011). Therefore, holistic traits of the population might reflect the relative abundance and respective phenotypes of the different strains present (Kardinaal et al. 2007a, Kardinaal et al. 2007b, van Gremberghe et al. 2009). Large biological variation within individual HAB species may thus play a key role in the extensive spatio-temporal variation observed in the traits of HAB populations. Whether these ecological traits vary predictably across populations of a single HAB species according to important ecological gradients is uncertain.

Cyanobacteria, characteristic of the phytoplankton community of phosphorus-enriched (eutrophic) lakes (Watson et al. 1997), are generally implicated in freshwater HABs. Numerous empirical and predictive models have been developed to account for variation in cyanobacteria biomass production, toxicity, and response to global change (Smith 1985, Trimbee and Prepas

1987, Downing et al. 2001, Kotak and Zurawell 2007, Bigham et al. 2009). However, models forecasting the abundance of particular HAB-forming cyanobacteria species are less developed, and better species-specific information is still needed (Pitcher 2012). Of the freshwater cyanobacteria, the colonial species *Microcystis aeruginosa* is most widely distributed and produces the most common class of toxins, the microcystins (Chorus and Bartram 1999). Large genetic variation has also been documented recently within *M. aeruginosa* (Wilson et al. 2005, Tanabe et al. 2007), though knowledge of the extent to which this raw genetic variation translates into ecologically relevant phenotypic variation, and its implications, is still limited (White et al. 2011).

Despite Federally-mandated phosphorus control programs in the United States, populations of *M. aeruginosa* have emerged in "unexpected" habitats—specifically, oligotrophic (low-nutrient) lakes where cyanobacteria are not otherwise predicted to be abundant, following invasion by zebra mussels (*Dreissena polymorpha*). Biomass of *M. aeruginosa* is ~4 fold and microcystins are ~3-8 fold higher in low-nutrient lakes (< 10-20 μg L^{-1} total phosphorus, TP) invaded by zebra mussels relative to similar, but non-invaded, lakes (Knoll et al. 2008, Sarnelle et al. 2010). Low-nutrient lakes are a fundamentally different habitat characterized by wholly different resource levels and phytoplankton communities from those currently assumed to be requisite for *M. aeruginosa*. Self-sustaining populations of *M. aeruginosa* in oligotrophic lakes indicate that this species has a much broader ecological niche than previously assumed, and perhaps this is a result of local adaptation facilitated by large intraspecific variation. Consistent with this idea, survivorship of *M. aeruginosa* strains isolated from low-nutrient lakes into nutrient-rich culture medium is much lower than for strains isolated from nutrient-rich lakes (Wilson et al. 2005), suggesting that strains might be adapted to grow under specific, local

nutrient regimes. *Microcystis aeruginosa* is therefore an ideal candidate for examining ecological trait variation within a species, and how such variation might mediate the responses of biodiversity to accelerating global change. Furthermore, better predictive models for *M. aeruginosa* can be constructed if variation in critical population parameters can be correlated to important ecological gradients (Rojo 1998).

Given that phosphorus availability can vary by more than 2 orders of magnitude across a lake productivity gradient from oligotrophic to hyper-eutrophic (Wetzel 2001), and the degree to which different strains of harmful phytoplankton are capable of exploiting different resources, we predicted that lake nutrient status structures *M. aeruginosa* populations by selecting for strains with a trait repertoire that is advantageous for the local environment (Litchman and Klausmeier 2008). Relative to strains from eutrophic lakes, we hypothesized that strains from oligotrophic lakes have lower maximal intrinsic growth rates (r_{max}), because their growth in nature is chronically nutrient-limited which might preclude synthesis of the cellular machinery required to sustain high rates of cell division (Klausmeier et al. 2004); whereas high r_{max} under eutrophic conditions should be advantageous to compensate for increased grazing pressure and competition for resources due to increased grazer and algal biomass at higher nutrients (Dillon and Rigler 1974, Hanson and Peters 1984). We tested this hypothesis using common-garden laboratory growth assays employing numerous strains isolated from lakes varying widely in TP, and monitored changes in growth characteristics of the strains over time in lab culture.

Methods

Isolation and maintenance of lab strains

Water samples were collected via two pooled casts of an integrating tube sampler (12 m length × 2.5 cm i.d.) from the mixed layer of 11 lakes distributed across southern Michigan between 5 July and 19 August, 2011, and from 14 lakes between 6 August and 12 September, 2013 (Fig. 28, Table 8). Eight lakes were sampled in both years. The lakes ranged widely in potential primary productivity from oligotrophic to hyper-eutrophic (7.9-196.8 μg L^{-1} TP, Table 8). *Microcystis* was isolated from these samples under a dissecting microscope (16×, Leica MS5) by pipetting individual colonies through a series of six washes in sterile 0.5× WC-S growth medium within a well plate (Corning, Inc.), prior to being transferred into individual 20 mL tubes of growth medium (White et al. 2011). For any given lake 27-100% of these isolates successfully established (i.e., grew and lacked other algal contaminants), and these were given unique strain designations identifying the originating lake, year, and strain number (e.g., F11-05; Table 8). We found no systematic variation in establishment success of strains as a function of source lake TP (linear regression of arcsine squareroot transformed proportions, $n = 25$, $p = 0.36$, $R^2 = 0.04$).

Once established, strains were maintained in 200 mL batch cultures of 0.5× WC-S at 23 °C and ~80 μmol m^{-2} s^{-1} on a 12:12 h light:dark cycle (Fig. 29), with an inoculum of culture transferred to fresh, sterile medium on a monthly basis. At the time of isolation, all strains conformed to current morphological criteria for *M. aeruginosa,* including the production of buoyant, mucilaginous colonies with cell diameters of ~4-6 μm (Otsuka et al. 2001, Wehr and Sheath 2003, Cronberg and Annadotter 2006). Thus, to the best of our ability, we identified all strains as belonging to the same species.

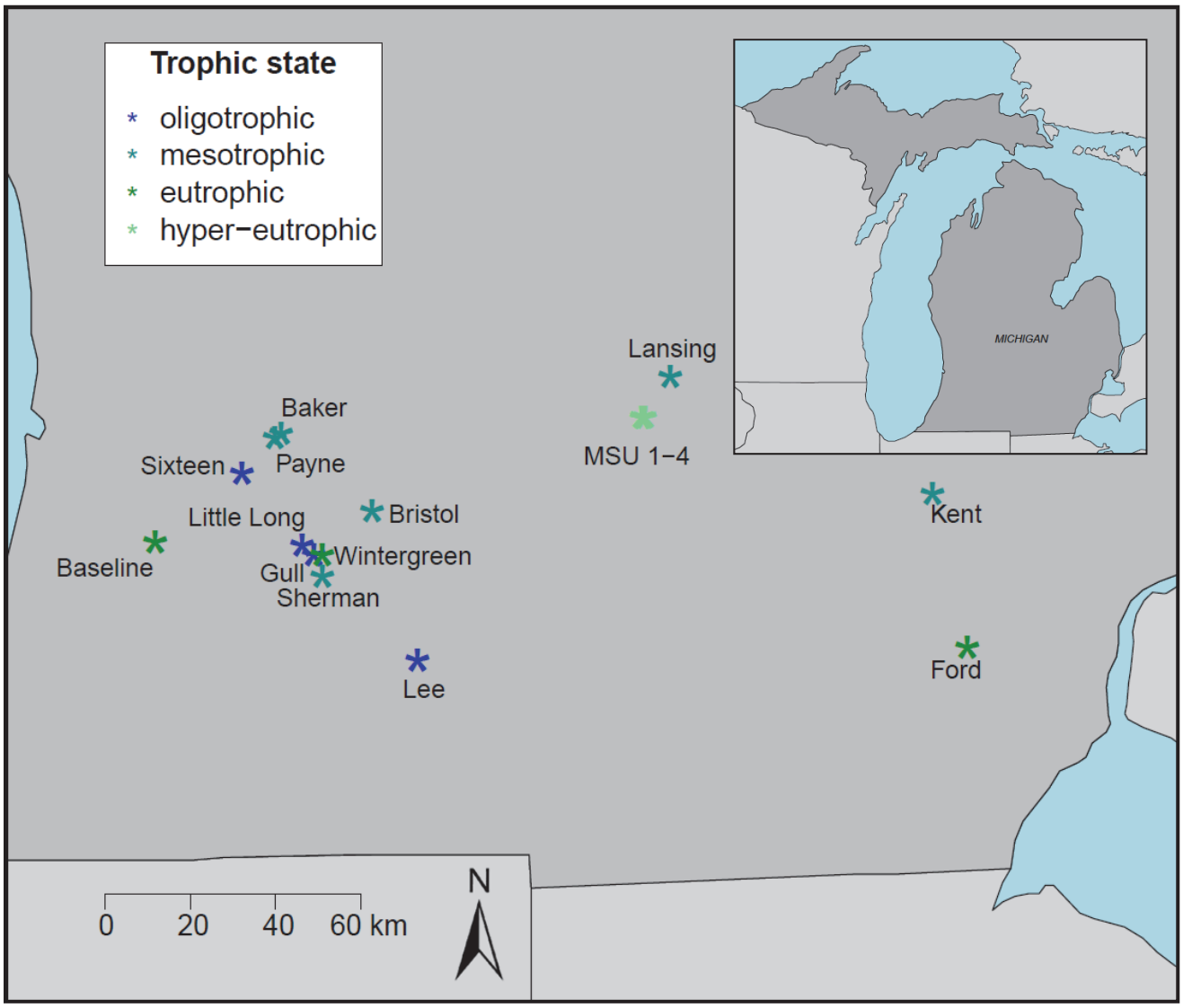

Figure 28. Map indicating the locations and trophic state (based on total phosphorus) of all 2011 and 2013 *Microcystis aeruginosa* source lakes.

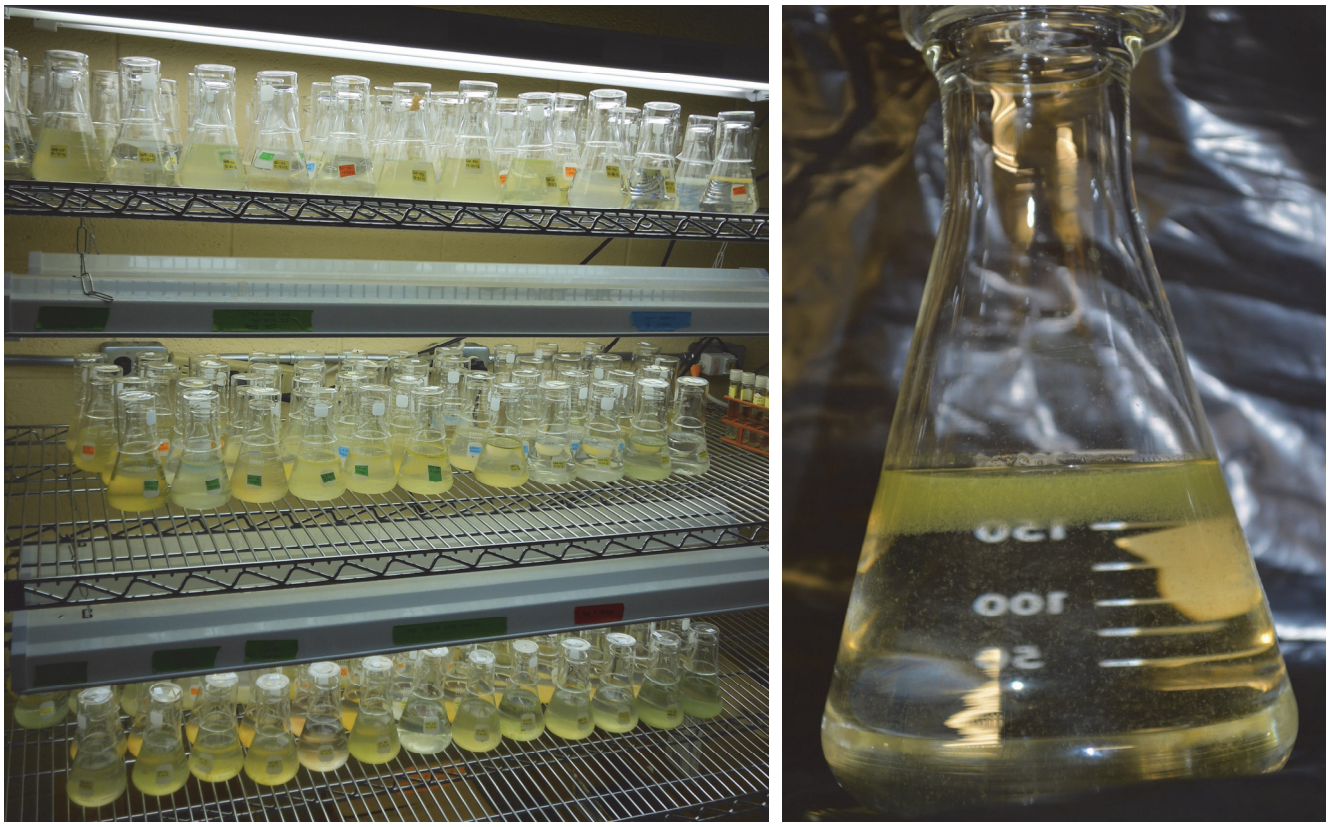

Figure 29. The *Microcystis aeruginosa* culture collection. The right panel illustrates the buoyant, colonial attributes of the recently isolated strains.

Table 8. Limnological data on the *Microcystis aeruginosa* source lakes, and a summary of the sampling, isolation, and establishment of all lab strains.

Lake (designation)	County (Michigan)	TP (μg L^{-1})	SRP (μg L^{-1})	Chl-*a* (μg L^{-1})	Secchi (m)	Sample date(s)	Colonies isolated	Strains established	% strains established
Baker (BK)	Barry	28.0	4.6	33.7	1.5	2011-08-08	7	6	86
						2013-08-07	10	3	30
Baseline (BS)	Allegan	36.1	3.6	47.1	1.0	2011-08-08	9	5	56
						2013-08-09	12	5	42
Bristol (BR)	Barry	13.8	1.1	7.3	1.5	2013-08-06	4	3	75
Ford (F)	Washtenaw	65.0	10.2	56.2	0.9	2011-08-01	9	5	56
						2013-08-15	14	4	29
Gull (G)	Kalamazoo	7.9	1.3	3.7	4.5	2011-07-05	11	7	64
						2013-08-08	12	10	83
Kent (K)	Oakland	23.6	3.3	23.1	2.2	2013-08-15	10	7	70
Lansing (LG)	Ingham	17.1	5.1	5.5	2.9	2011-08-05	7	5	71
						2013-08-14	15	7	47
Lee (LE)	Calhoun	9.0	1.9	4.0	3.1	2013-08-06	10	5	50
Little Long (LL)	Barry	8.0	1.0	4.1	4.6	2011-07-12	8	8	100
						2013-08-08	10	8	80
MSU1 (L1)	Ingham	163.5	155.8	12.2	2.1	2011-08-19	3	1	33
MSU2 (L2)	Ingham	196.8	7.3	240.8	0.4	2011-08-16	26	13	50
MSU3 (L3)	Ingham	128.7	4.1	53.3	0.6	2011-08-06	1	1	100
MSU4 (L4)	Ingham	124.7	8.4	99.2	0.5	2013-09-12	15	4	27
Payne (P)	Barry	11.2	1.4	7.5	3.0	2013-08-07	3	2	67
Sherman (S)	Kalamazoo	13.7	2.8	9.1	2.9	2011-08-08	7	6	86
						2013-08-07	10	3	30
Sixteen (SX)	Allegan	8.8	1.0	5.0	3.9	2013-08-07	12	10	83
Wintergreen (W)	Kalamazoo	47.8	2.7	21.4	2.4	2011-08-02	9	9	100
						2013-08-08	8	5	63

Table 8. (cont'd)

A strain was considered established if the isolate grew and the seed culture was devoid of any other algal contaminants. TP = total phosphorus; SRP = soluble reactive phosphorus; Chl-*a* = chlorophyll-*a*; Secchi = Secchi disk transparency.

Growth rate assays

Common-garden growth assays were conducted with the general design as follows. Fresh 20 mL cultures of strains were initiated 7 days prior to an assay to insure *M. aeruginosa* was exponentially growing. Individual *Microcystis* colonies were then inoculated via pipette (1 μL) into randomized, separate wells containing 0.5 mL sterile 0.5× WC-S within 8-well chambered slides (Nunc Lab-Tek II Chamber Slide System) (Wilson et al. 2010). Once inoculated (day 0), colonies were photographed every 2 days for 6 days at 100× using a light microscope interfaced with a digital camera (Fig. 30; Nikon Eclipse E600). Measurements, added to the images with computer software (Spot Advanced, Diagnostic Instruments), were made of colony surface area and depth (the straight line length perpendicular to the greatest linear dimension); colony volume (μm^3) was determined as the product of surface area and depth (Wilson et al. 2010). Growth rate was determined as the slope of the linear regression of natural logarithm-transformed colony volumes over time. Unless noted otherwise, one colony per strain was employed per treatment in a given experiment. Since coloniality is a characteristic trait of *M. aeruginosa* in nature (Wehr and Sheath 2003), all growth assays were performed using colonial strains that had been in culture for less than 1.5 years, unless noted otherwise. This contrasts with many previous lab studies of *M. aeruginosa* that have utilized old, single-celled culture collection strains. Furthermore, since all strains employed in a given experiment were the same age and were recently isolated, concerns arising from evolution in culture were minimized (Burkholder and Glibert 2009, Lakeman et al. 2009, Demott and Mckinney 2015).

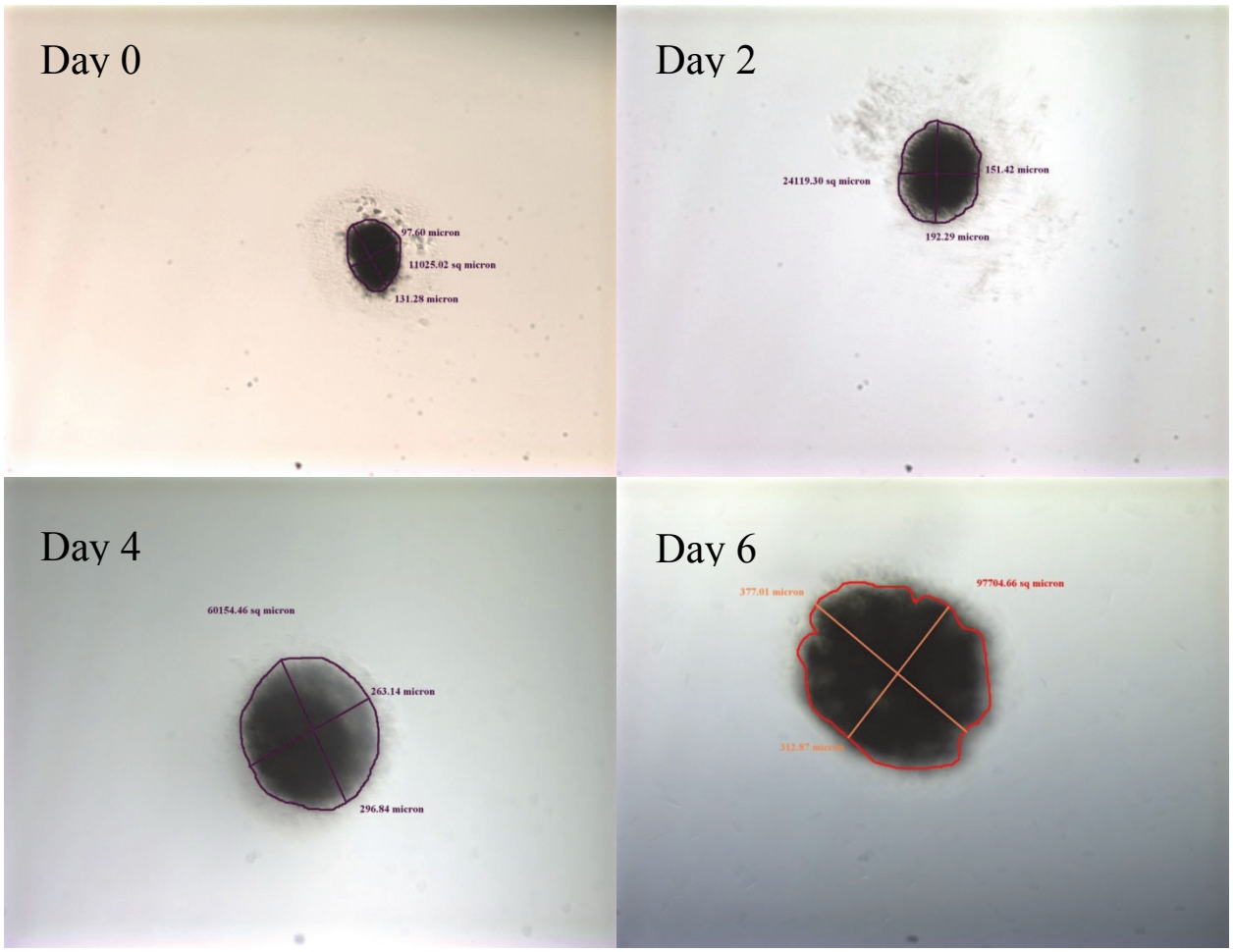

Figure 30. Sequence of digital micrographs depicting growth of an individual colony of *Microcystis aeruginosa* during the course of a 6-day growth assay. Colonies were photographed at 100× and all images are shown to scale. The strain pictured is F11-05, isolated in 2011 from eutrophic Ford Lake, Michigan. Photo credits: Jeffrey D. White.

Assaying growth of individual *M. aeruginosa* colonies is necessary and advantageous because, unlike batch culture assays, this permits controlling for colony size and inoculation density effects on growth rate, since small colonies grow faster than large colonies (Wilson et al. 2010). To further minimize the confounding effects of initial colony size and shape on growth rate, round colonies of approximately the same equivalent diameter were selected for each strain to the fullest extent possible using the microscope's ocular micrometer.

To quantify variation in maximum growth rate among strains, we performed a growth assay employing 19 colonial strains isolated from 11 lakes in 2011, selected in stratified random fashion according to source lake trophic state (TP range: 7.9-196.1 μg L^{-1}). Growth conditions were identical to those described for general culture maintenance, including saturating phosphate (480 μg L^{-1} SRP).

To validate the individual colony assay technique, a randomized subset of 8 of the above strains were grown in parallel 100 mL flasks of 0.5× WC-S (batch cultures) and chambered slides (individual colonies). All strains were grown in duplicate. The individual colony assay was conducted as above. Batch cultures were subsampled every 2 days for 8 days, starting on day 4, by filtering 15 mL of thoroughly-mixed culture onto A/E filters for chlorophyll-*a* analysis (*see* below). Growth rates of batch cultures were determined as above, using chlorophyll-*a*.

We conducted a second individual colony, maximum growth rate assay employing all the still-available colonial strains isolated in 2013 from Gull, Lansing, Kent, and Wintergreen Lakes to explore the range of maximum growth rates within individual populations from lakes of widely different productivity (oligotrophic to eutrophic, TP range: 7.9-47.8 μg L^{-1}). To capture as much of this biological variation as possible, we pooled the data with the first experiment for

the 2011 strains that originated from the same four lakes, to give a total sample size of n = 6-11 strains per *M. aeruginosa* population.

Monitoring changes in growth habit

Since *M. aeruginosa* typically loses the ability to form colonies over the first few years in lab culture (J. White pers. obs.)(Zhang et al. 2007), we made observations at 1.0 and 1.5 years post-isolation of the growth habit (colonial versus single-celled) for all strains isolated in 2013 (n = 73) at 100× under a light microscope. A strain was categorized as single-celled if and only if it was purely single-celled, since a transitioning strain will often produce a mixture of diffuse colonies and single cells (J. White, pers. obs.).

To assess any changes in maximal growth rates as a result of switching growth habits from colonial to single-celled, we re-assayed maximal growth rates for those 2011 and 2013 strains that had become single-celled after ~2.0 years in lab culture, and whose maximal growth rates were previously determined while still colonial. These assays were conducted in 2013 (2011 strains) and 2015 (2013 strains) using the batch-culture method described above. Results were then pooled from the two experiments.

Lab analyses

Subsamples of source lake water and growth medium were partitioned via filtration through A/E filters (Pall, 1.0 μm pore size) and then immediately frozen for later analysis. Analysis of available phosphorus (as SRP) was performed on the filtrate using the molybdenum-blue method and long-pathlength spectrophotometry (Murphy and Riley 1962). Total phosphorus

was analyzed on unfiltered samples as above for SRP, following persulfate digestion of organic material in an autoclave (Menzel and Corwin 1965).

Chlorophyll-*a* was measured fluorometrically following 12-hour dark extraction of frozen A/E filters in 10 mL of cold 95% ethanol (Welschmeyer 1994). Particulate microcystin was measured by ELISA (enzyme-linked immunosorbant assay; Envirologix QuantiPlate Kit for Microcystins visualized on a LabSystems Multiskan Microplate Reader) following three pooled 45 min, 10 mL extractions of A/E filters in 75% methanol (Harada et al. 1999, White et al. 2011). *Microcystis* biomass was quantified from measurement of 2-dimensional surface areas of colonies at 100× as above. These measurements were converted to dry biomass using a regression that estimates cell density from colony surface area (Sarnelle et al. 2012, Horst et al. 2014). Microcystin quotas (mg toxin mg^{-1} *Microcystis* biomass) were then calculated for lab strains.

Statistical analyses

We used source lake TP as the principal predictor for variation in *M. aeruginosa* growth traits, since TP is routinely used to classify lake trophic state (Wetzel 2001) and is also ecologically relevant to *M. aeruginosa* and HAB-forming cyanobacteria (Watson et al. 1997).

We performed linear regressions to test if maximum growth rates of *M. aeruginosa* strains (determined via the individual colony method) varied as a function of their source lake TP and initial colony size, and to test if strain microcystin quota varied as a function of either source lake TP or maximum growth rate. Microcystin quotas were log-transformed prior to analysis to meet normality requirements. Residual plots did not indicate any systematic departures from statistical model assumptions. We used the coefficient of variation (CV; ratio of the standard

deviation to the mean) to assess the relative extent of within-population variation in maximum growth rate. We used paired *t*-tests to compare growth rates determined using the individual colony versus batch culture assays, and to test for differences in maximum growth rate for strains that changed growth habit from colonial to single-celled over time in culture. To test whether strains from high-nutrient lakes were more likely to become single-celled during the first 1.0 and 1.5 years in culture, we performed logistic regressions. All statistical analyses were performed using R Version 3.1.3 (R Foundation for Statistical Computing).

Results

Growth rate assays

The two assay methods (individual colony versus batch culture) yielded similar results that were not significantly different (paired *t*-test, $n = 8$, df = 7, $p = 0.71$; Fig. 31), validating the individual colony method. Maximum intrinsic growth rates (r_{max}) of *M. aeruginosa* strains were significantly, positively related to their source lake TP (linear regression: $n = 18$, $p = 0.030$, $R^2 = 0.26$; Fig. 32). Observed growth rates ranged nearly 7-fold from 0.08 d^{-1} (source lake TP = 7.9 $\mu g\ L^{-1}$) to 0.55 d^{-1} (source lake TP = 196.1 $\mu g\ L^{-1}$). One colony (source lake TP = 16.6 $\mu g\ L^{-1}$) exhibited negative growth during the experiment and was omitted from analysis, leaving $n = 18$ for the experiment.

As assessed with the coefficient of variation, variation in r_{max} within four *Microcystis* populations spanning the lake productivity gradient (oligotrophic to eutrophic, 7.9-47.8 $\mu g\ L^{-1}$) was generally similar (CV = 0.30-0.52), although strains from moderately productive Kent Lake (TP = 23.6 $\mu g\ L^{-1}$) exhibited the greatest variation (CV = 0.70; Table 9). Within a single oligotrophic lake population (Gull Lake, TP = 7.9 $\mu g\ L^{-1}$), r_{max} ranged from 0.08-0.37 d^{-1}

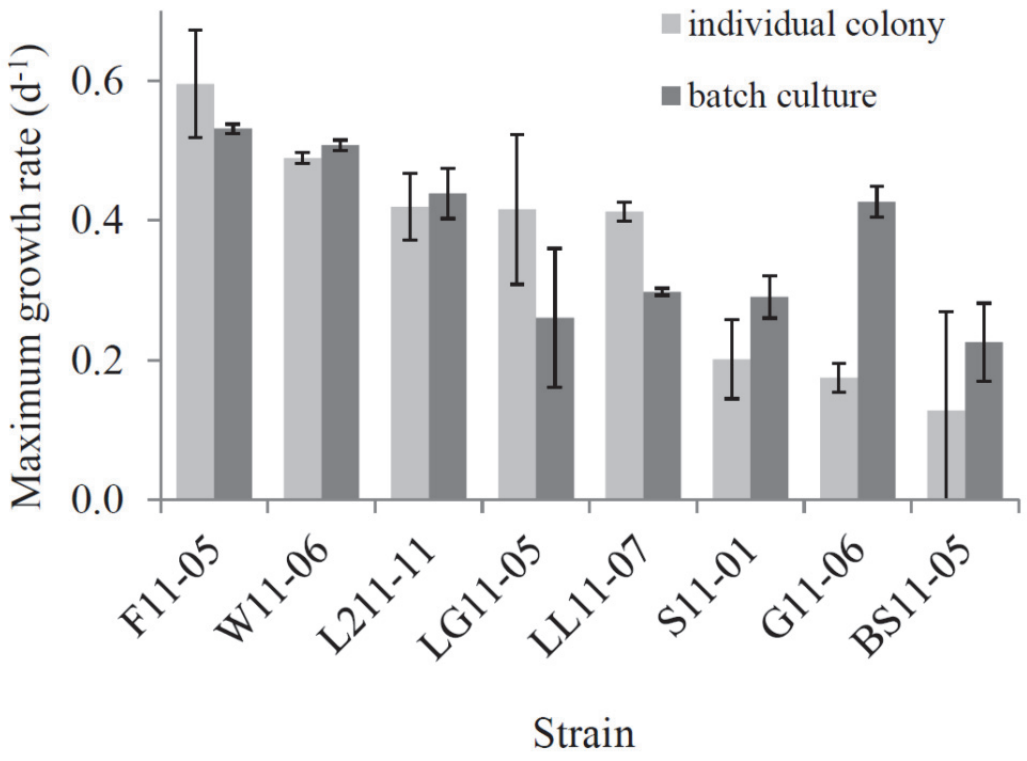

Figure 31. Comparison of maximum growth rates of eight strains of *Microcystis aeruginosa* as determined from two different methods: individual colony and batch culture assays. The two methods yielded growth rates that were not significantly different from each other. Error bars denote ± SE.

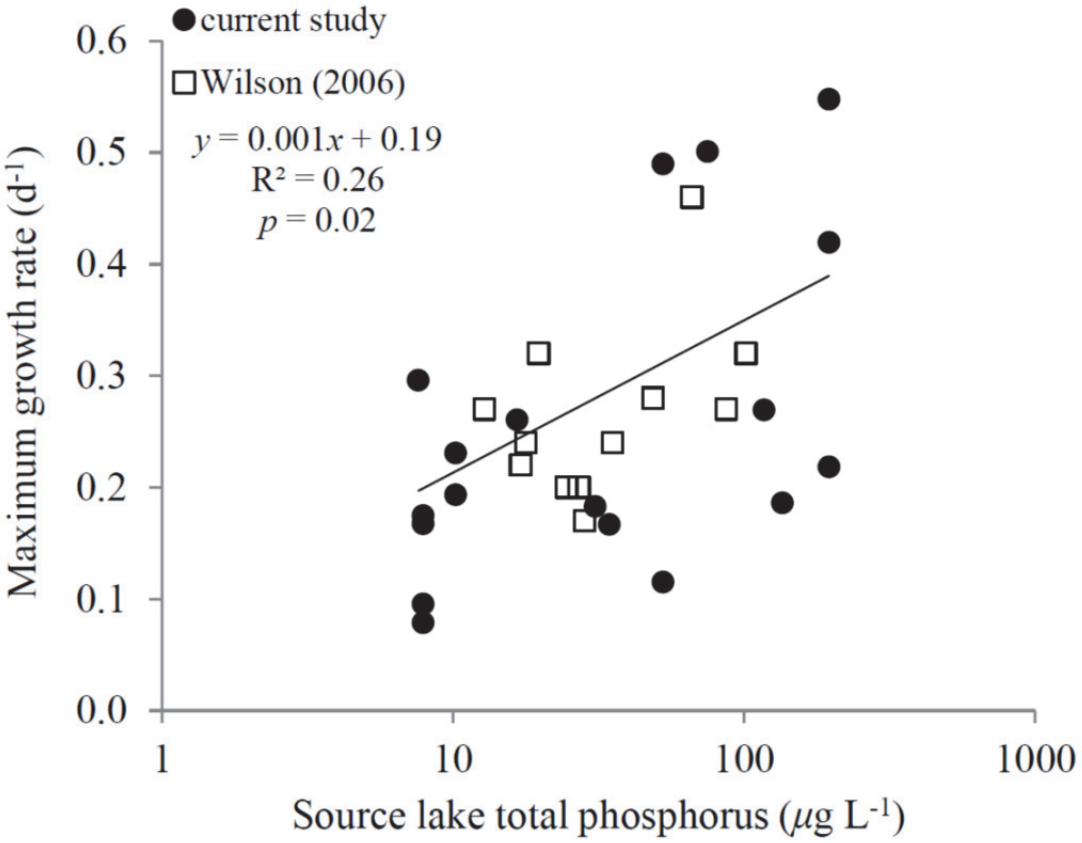

Figure 32. Variation in maximum intrinsic growth rate of *Microcystis aeruginosa* strains isolated from lakes spanning a large lake productivity gradient, from oligotrophic to hyper-eutrophic (as total phosphorus). Growth rates were determined from the change in volume of individual colonies during a 6-day growth assay with saturating nutrients (n = 18 strains). Growth rate data (batch culture assays of *M. aeruginosa*) from Wilson et al. (2006) are shown for comparison (squares). The linear regression is for the current study only. Note the log scale on the x-axis.

Table 9. Within-population variation in maximum intrinsic growth rate (d^{-1}) of *Microcystis aeruginosa,* as determined with individual colony growth assays. The four source lakes range from oligotrophic to eutrophic (7.9-47.8 μg L^{-1} total phosphorus) from top to bottom. Data were pooled for all 2011 and 2013 strains assayed from the given lake population. The coefficients of variation (CV) and sample sizes (*n*) are given.

Lake	Median (d^{-1})	Min (d^{-1})	Max (d^{-1})	CV	*n*
Gull	0.15	0.08	0.37	0.52	11
Lansing	0.22	0.14	0.30	0.30	6
Kent	0.21	0.01	0.46	0.70	6
Wintergreen	0.29	0.12	0.49	0.42	6

(n = 11 strains), and for a single eutrophic lake population (Wintergreen Lake, TP = 47.8 μg L^{-1}) from 0.12-0.49 d^{-1} (n = 6 strains; Table 9).

We found no relationship between microcystin quota and either source lake TP or r_{max} (linear regressions, n = 18, $p > 0.57$, $R^2 < 0.02$; Fig. 33) during exponential growth under saturating resources. Since we deliberately selected colonies of similar initial size, we also found no relationship between r_{max} and initial colony size in our individual colony experiment (linear regression: n = 18, p = 0.86, $R^2 < 0.01$), obviating the need to statistically control for the effects of initial colony size on growth rate.

Monitoring changes in growth habit

After 1.0 and 1.5 years in culture, *Microcystis* strains from more productive lakes were significantly more likely to have become purely single-celled, as compared to strains isolated from less productive lakes (data for 1.5 years: logistic regression, n = 73, p = 0.005, odds ratio = 1.04; Fig. 34). Of the 33 strains originating from oligotrophic (TP < 10 μg L^{-1}) lakes, 79% were still producing colonies after 1.5 years in culture, whereas of the 40 strains originating from more productive lakes (11.2 ≤ TP ≤ 124 μg L^{-1}), only 50% were still producing colonies.

Of the 18 strains isolated in 2011 that were initially assayed as colonies (Fig. 32), 6 became single-celled after a period of ~2 years years in culture; likewise, 6 of the 22 previously assayed strains isolated in 2013 (Table 9) became single-celled. When these strains were assayed again as single-celled organisms, r_{max} was significantly higher than when colonial. Since the result was the same for both sets of strains, results were pooled across the two growth assays (paired t-test, n = 12, df = 11, p = 0.005; Fig. 35).

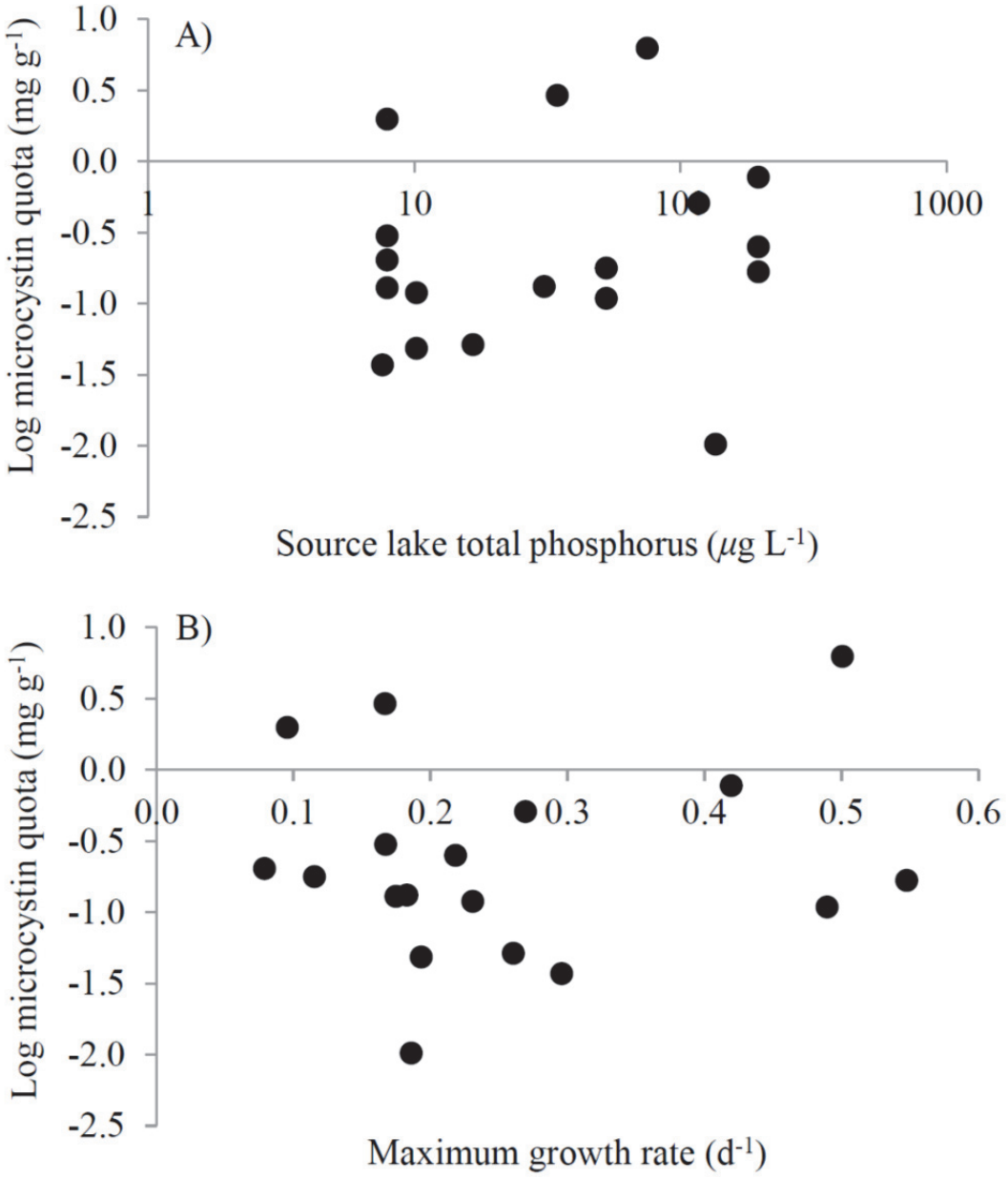

Figure 33. Variation in microcystin quota of the *Microcystis aeruginosa* strains (n = 18) assayed in Fig. 32 as a function of (A) source lake total phosphorus and (B) maximum intrinsic growth rate.

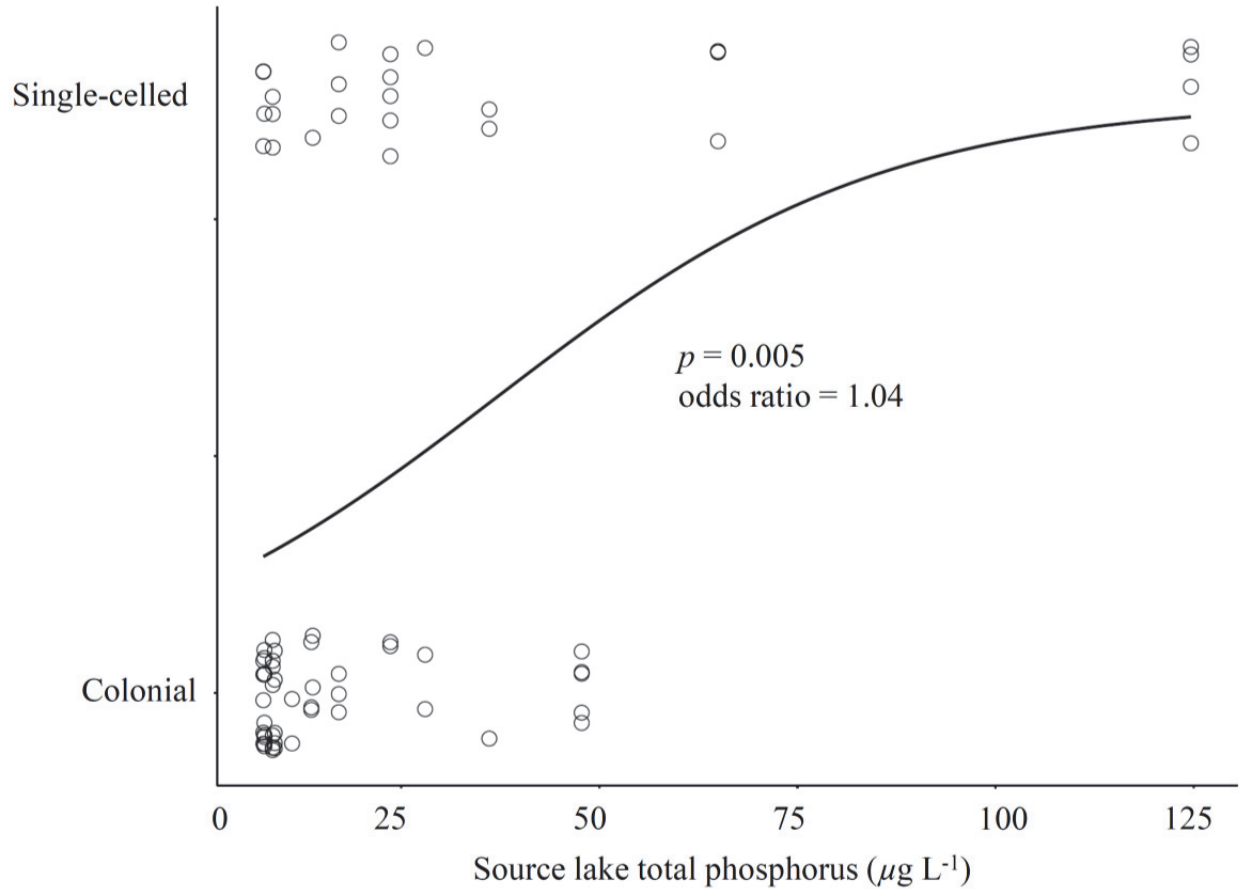

Figure 34. Logistic regression of growth habit (colonial or single-celled) of all *Microcystis aeruginosa* strains (n = 73) isolated in 2013 versus source lake total phosphorus, 1.5 years post-isolation into lab culture. All strains were colonial at the time of isolation. Data points are randomly jittered vertically to avoid over-plotting.

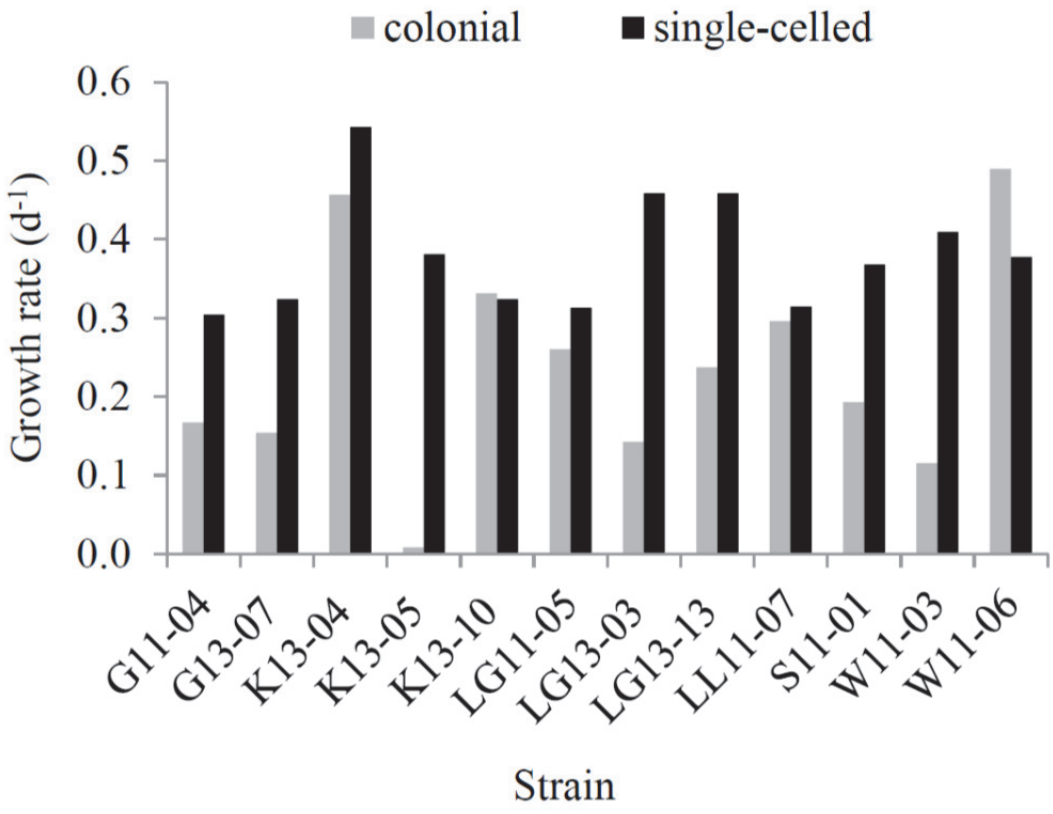

Figure 35. Comparison of maximum growth rates as a function of growth habit (colonial or single-celled) for twelve *Microcystis aeruginosa* strains. Strains were all assayed initially as colonies (*see* Fig. 32, Table 9), and then again after disaggregating following a period of ~2 years in lab culture.

Discussion

We identified extensive variation in maximum intrinsic growth rate (r_{max}) among *Microcystis aeruginosa* strains that were recently isolated from lakes spanning a large productivity gradient. In support of our hypothesis, strains isolated from eutrophic and hyper-eutrophic lakes grew up to ~7 times faster than strains originating from oligotrophic lakes under saturating nutrients (Fig. 32, Table 9), and these differences were not driven by colony size. Since all strains were grown in a common garden, these differences in maximal growth rates are assumed to be genetically based. Our results, obtained from testing colonial strains, are also more relevant to natural populations of *M. aeruginosa* since coloniality directly affects grazing vulnerability and migration velocity in addition to growth rate (Visser et al. 1997, Wilson et al. 2010, White and Sarnelle 2014); thus, coloniality could influence fitness and ecological trade-offs (Litchman and Klausmeier 2008). Furthermore, all strains employed in a given experiment were the same age, recently isolated, and shared the same transfer regimes, limiting the potential effects of evolution in culture (Lakeman et al. 2009, Demott and Mckinney 2015). Therefore, our study provides evidence for possible local adaptation by *M. aeruginosa* to ecologically disparate habitats and supports the conclusion that large, genetically based trait variation in this species has ecological consequences (White et al. 2011).

To our knowledge, only two previous studies (Wilson et al. 2005, Wilson et al. 2006) have explored trait variation among *M. aeruginosa* strains originating from lakes differing widely in productivity (12 Michigan lakes, 12.8-101.8 μg L^{-1} TP; strains isolated in 2002). Wilson et al. (2006) reported r_{max} for these strains ranging from 0.17-0.46 d^{-1} in batch culture, congruent with those reported here (Fig. 32, Table 9), although they did not test the influence of lake productivity on growth rate. Using the r_{max} data of Wilson et al. (2006, their Table 1) and

the source lake TP data of Wilson et al. (2005, their Table 1), we found a positive, but non-significant, relationship between r_{max} and source lake TP (linear regression: $n = 12$, $p = 0.1$, $R^2 = 0.24$). However, the sample size and source lake TP range in their study was smaller relative to the present study, and so the lack of statistical significance may be due to insufficient power. Maximum intrinsic growth rates for *M. aeruginosa* reported elsewhere under similar growth conditions [0.46 d^{-1}, Fujimoto et al. (1997); 0.48 d^{-1}, Reynolds (2006); ~0.30-0.50 d^{-1}, Wilson et al. (2010); 0.63 d^{-1}, Seip and Reynolds (1995); 0.88 d^{-1}, Nalewajko and Murphy (2001)] are consistent with those reported here for strains isolated from higher-nutrient lakes, which is expected given that most *M. aeruginosa* employed in growth studies originates from productive habitats.

Although possessing a fast growth rate should generally be advantageous for phytoplankton, the cellular machinery required to sustain high levels of growth in high-nutrient habitats may come at an energetic cost when available nutrients, particularly phosphorus, are chronically low (e.g., in oligotrophic lakes) (Klausmeier et al. 2004). Thus, a trade-off should exist to maintain the fitness of a slow-growing strain adapted to a low-nutrient habitat. In general, growth rates tend to increase with habitat productivity for freshwater phytoplankton, but with a concomitant decrease in affinity for phosphate and an increase in the half-saturation constant (K_m) for phosphate-dependent growth (Seip and Reynolds 1995, Spijkerman and Coesel 1998, Litchman and Klausmeier 2008), suggesting that slow growth may trade-off with an improved ability to glean nutrients and subsist at low resource levels. We predict that *M. aeruginosa* strains originating from oligotrophic lakes have lower K_m for phosphate and are therefore superior at maintaining positive somatic growth under conditions of chronic nutrient limitation, relative to strains originating from eutrophic and hyper-eutrophic lakes. *Microcystis*

has a reported K_m of 5.9 μg L^{-1} for phosphate-dependent growth (Holm and Armstrong 1981), a high resource concentration that is unlikely to be encountered in oligotrophic lakes (as SRP, Table 8), which suggests we should expect large intraspecific variation in K_m. The ability to make broad generalizations about these critical population parameters across the lake productivity gradient would be useful for modelling and lake management (Rojo 1998, Mieleitner and Reichert 2008), given the complex dynamics of local *Microcystis* populations at the strain level (Kardinaal et al. 2007a).

Other ecological trade-offs could also exist, including that between r_{max} and grazing resistance (Agrawal 1998, Demott and Mckinney 2015). These trade-offs could all have a genetic basis, although plastic responses to environmental gradients (such as N versus P limitation) are also common in phytoplankton (Van Donk 1997, Van Donk et al. 1997). Nonetheless, all of these traits and trade-offs are traditionally measured *between* species or across broad taxonomic groups, rather than among strains of the same species (White et al. 2011). Additional work is required to identify and measure these and other possible trade-offs in *M. aeruginosa*, particularly as a function of important ecological gradients like lake productivity.

Interestingly, we also found a relationship between the probability of a strain becoming single-celled during the first 1.5 years in culture and source lake productivity, with the log of the odds increasing by ~1.0 for a 1.0 μg L^{-1} increase in lake TP (Fig. 34). If the switch in growth habit from colonial to single-celled is triggered by mutation or is otherwise a function of the number of generations spent in culture, strains from higher-nutrient lakes may disaggregate sooner given their faster growth rates. We also found that maximal growth rates of individual strains were significantly higher for the single-celled growth habit than for colonies (Fig. 35), likely reflecting the growth costs incurred from being colonial (Wilson et al. 2010). These

observations reiterate the importance of characterizing traits of *M. aeruginosa* with recently isolated, colonial strains if the intention is to ultimately relate studies of laboratory strains to natural populations.

Using neutral genetic markers, recent studies have documented extensive genetic variation within *M. aeruginosa*, although these studies also report that within-lake genetic variation (which is different from ecological variation) can be similar in extent to among-lake genetic variation (Wilson et al. 2005, Tanabe et al. 2009). Yet, this may reflect the fact that a phylogenetic analysis across a large ecological gradient (e.g., oligotrophic to hyper-eutrophic lakes) has yet to be made with a sufficiently large number of strains and lakes, since most molecular studies include few, if any, *M. aeruginosa* strains from low-nutrient lakes (but, *see* Wilson et al. 2005). Therefore, molecular studies may still be underestimating the extent of genetic (and thus, perhaps, ecological) variation within *M. aeruginosa*.

We found no evidence for variation in microcystin quotas of *M. aeruginosa* strains, under conditions of saturating nutrients and exponential growth, as a function of either lake TP or r_{max}. This suggests that any observed variation in microcystin quota is primarily driven by environmental conditions (Van de Waal et al. 2009), since many laboratory strains of *M. aeruginosa,* including from both oligotrophic and eutrophic habitats, already possess the genetic capability of synthesizing microcystin (Dyble et al. 2008, White et al. 2011). Indeed, recent studies of both laboratory strains and natural populations of *M. aeruginosa* demonstrate the importance of nitrogen availability for regulating microcystin quota relative to other factors (Downing et al. 2005, Horst et al. 2014).

Ecologists have recently emphasized community- and ecosystem-level implications of the genetic and phenotypic diversity inherent within and among populations (Neuhauser et al.

2003, Whitham et al. 2003, Long and Hay 2006, Johnson and Stinchcombe 2007, Burkholder and Glibert 2009). Large genetic and phenotypic variation is obviously important from an evolutionary standpoint, as it represents adaptive potential for species like *M. aeruginosa*. The large selection pressures imposed by global change may cause complex responses of *M. aeruginosa* by interacting with the biodiversity it harbors. Recent studies have found that toxic strains of *M. aeruginosa* grow faster at elevated temperature and phosphorus compared to non-toxic strains (Davis et al. 2009), and *M. aeruginosa* strains artificially subjected to increasing temperature can evolve to grow at 35 °C (Huertas et al. 2011). Perennial *M. aeruginosa* populations emerging and persisting in oligotrophic lakes subsequent to zebra mussel invasion (Raikow et al. 2004, Knoll et al. 2008, Sarnelle et al. 2010) is another likely example. Understanding how different strains and populations of *M. aeruginosa* respond to variation in environmental conditions will enhance predictions for the response of this HAB species to global change; more generally, accounting for the diversity within and among ecological populations will be critical for conserving biodiversity and predicting population and community-level responses to rapid, large-scale changes in the environment.

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APPENDIX

APPENDIX

SURVEY OF ZEBRA MUSSEL *(DREISSENA POLYMORPHA)* STATUS IN OTHER MICHIGAN INLAND LAKES DURING RECENT WARM SUMMERS

This appendix describes a qualitative survey conducted in conjunction with the study of heat-induced zebra mussel mortality in Gull Lake, Michigan (Chapter 2). The survey results were not included in the published manuscript that resulted from that study.

Introduction and Methods

Since water temperatures in Gull Lake should covary with water temperatures in other inland lakes in the Lower Peninsula of Michigan, we would expect similar die-offs of *D. polymorpha* in other lakes during recent periods of warm summer temperatures if such temperatures are an important driver of mortality. To explore whether our observations of high *D. polymorpha* mortality in Gull Lake (*see* Chapter 2) represented an isolated case or a widespread occurrence, we surveyed lake associations and volunteer monitoring groups through the Michigan Lake and Stream Association (MLSA) and Michigan Clean Water Corps (MiCorps) list-serves and membership newsletters during the fall of 2013. We asked for any recent (beginning summer of 2010), qualitative observations of *D. polymorpha* in inland lakes, including declines, die-offs, or lack thereof. We conservatively scored a report as a "decline or die-off" if and only if the responder clearly described a very recent (summer 2010- fall 2013) and substantial (i.e., readily visible to the casual observer) change in *D. polymorpha* abundance, relative to prior years. If the reporter expressly noted otherwise, or if it was unclear from their description, the report was scored as a "no decline." We looked for a pattern between the nature and timing of the reports we received and the regional temperature conditions (Fig. 11) to assess the generality of our hypothesis.

Results

We received status reports on *D. polymorpha* from 43 inland lakes in the Lower Peninsula of Michigan. In 36 (84%) of these lakes, obvious declines or die-offs of *D. polymorpha* were independently observed during the time period from summer 2010 – fall 2013 (Fig. 36). These reports consistently included detailed descriptions of reduced mussel densities on dock pilings and mooring lines, and a dramatic reduction or absence of mussels on substrates at shallow depths where they had been observed previously. Sixty-eight percent of the reported declines or die-offs occurred or were first noticed during the period from 2010-2012, which are the same three consecutive warm summers (Fig. 11) during which the highest levels of mussel mortality were observed in Gull Lake (Table 4, Fig. 15). These qualitative, statewide observations suggest that the large decline of *D. polymorpha* we observed in Gull Lake was probably not an isolated occurrence, although we readily acknowledge that any further interpretation of these findings would be purely speculative given the nature of the information.

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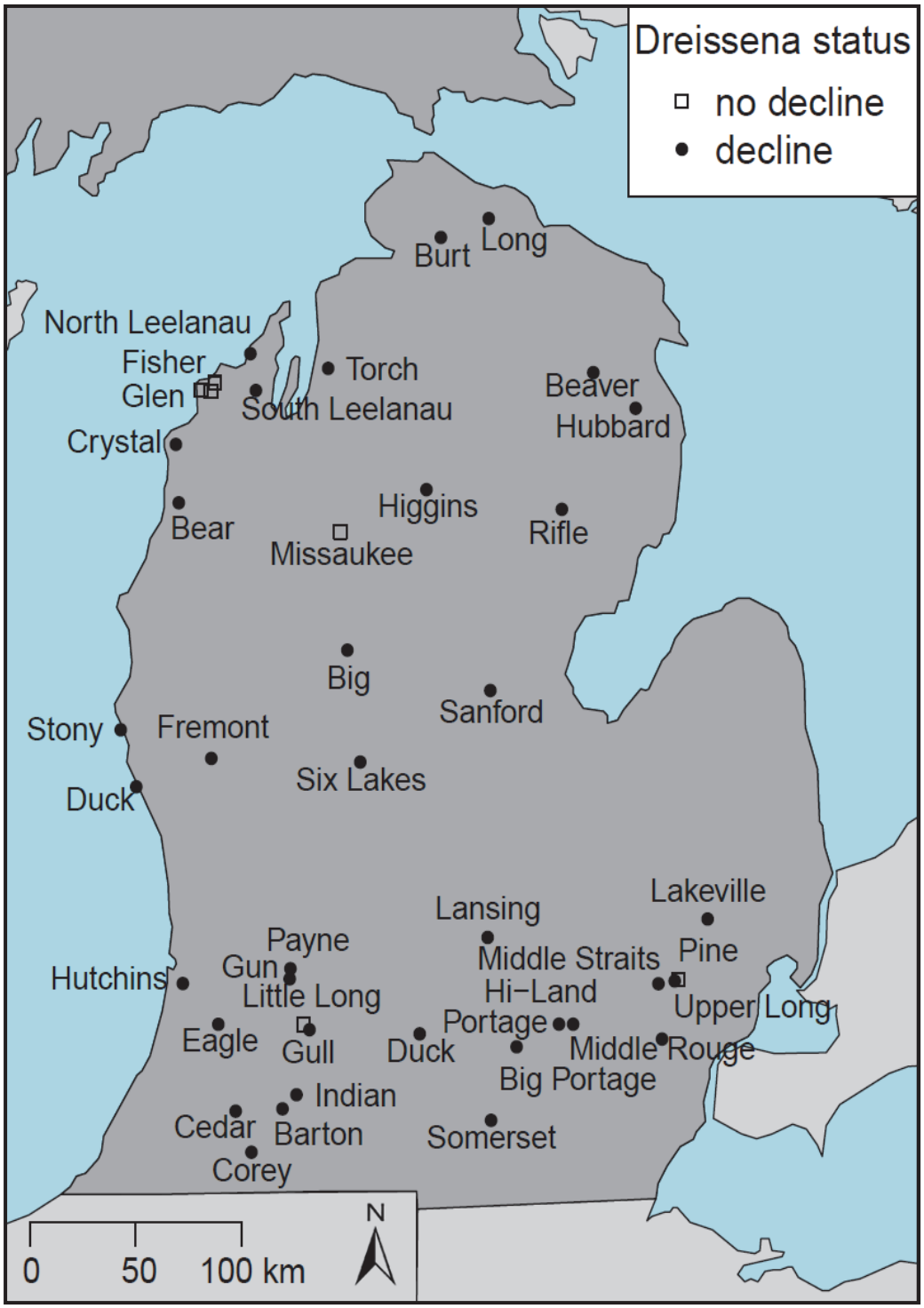

Figure 36. Locations of Michigan inland lakes for which recent *Dreissena polymorpha* status reports (decline or not during 2010-2013) were received during our qualitative survey of statewide lake associations.

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