CHAPTER 32

Role of Selective Grazing by Dreissenid Mussels in Promoting Toxic *Microcystis* Blooms and Other Changes in Phytoplankton Composition in the Great Lakes

Henry A. Vanderploeg, Alan E. Wilson, Thomas H. Johengen, Julianne Dyble Bressie, Orlando Sarnelle, James R. Liebig, Sander D. Robinson, and Geoffrey P. Horst

CONTENTS

ABSTRACT

We investigated the feeding response of zebra and quagga mussels to *Microcystis aeruginosa* strains from culture collection and from natural seston from Saginaw Bay (Lake Huron), western Lake Erie, and enclosures from Gull Lake, an inland lake in Michigan. These experiments were done to evaluate the roles of strain identity, toxin concentration (microcystin), colony size, and environmental phosphorus concentrations as they affect ingestion or selective rejection of *Microcystis* in pseudofeces and potential *Microcystis* bloom promotion through the selective-rejection process. A combination of traditional feeding experiments with mussels confined in beakers and videotaping of mussel behavior was used. We measured changes in *Microcystis* concentration in the feeding experiments using changes in chlorophyll and the toxin associated with *Microcystis* (microcystin) in small (<53 μm) and large (>53 μm) size fractions. In natural seston, most colonies fell within the large size fraction. Overall, there were complex interactions that could not be simply explained by microcystin concentration, colony size, or environmental P concentration. Experiments with toxic and nontoxic strains from culture collection indicated different reasons for rejection. In one nontoxic strain having colonies in both the small

and large fractions, small colonies were ingested, while large colonies were not. In another nontoxic strain, consisting only of large colonies, no colonies were ingested; however, when the colonies were broken apart by sonication, no small colonies or even single cells were ingested. Video observations showed that both of these strains were readily captured and rejected in pseudofeces after a large number were collected. Mussels fed upon the small colonies of a moderately toxic strain, whereas for another less toxic strain, no feeding occurred. When mussels were induced to feed on this latter strain by adding *Cryptomonas*—a favorite food of mussels—to the suspension, one of the mussels showed extreme sensitivity to *Microcystis* by rejecting each colony as they entered the incurrent siphon. Experiments with *Microcystis* having moderate microcystin concentration from both the low P (Saginaw Bay) and high P (Maumee Bay) sites in the Great Lakes were rejected. *Microcystis* from enclosures in Gull Lake was ingested despite having very high microcystin concentrations. Whether the selective-rejection process results in a *Microcystis* bloom depends on both mussel abundance and environmental P concentration as they affect mortality and growth rate of algae competing with *Microcystis*, as well as the composition of different *Microcystis* strains (genetic identities) that can coexist at the same time in the same water body. Questions for future research and research approaches to understand these complex interactions are outlined.

INTRODUCTION

Toxic Microcystis Blooms Return to Great Lakes

Noxious blooms of colonial cyanobacteria such as *Microcystis*, *Anabaena*, and *Aphanizomenon* are well-known symptoms of cultural eutrophication caused by excessive phosphorus (P) loading (Smith 1983, Sommer et al. 1986). Cyanobacterial blooms were common occurrences in Saginaw Bay (Bierman et al. 1984, Stoermer and Theriot 1985) and Lake Erie (Makarewicz 1993) during the 1960s and 1970s. With the control of P loading instituted during the mid-1970s, these blooms diminished. Therefore, it was a surprise that intense toxic *Microcystis* blooms occurred in both Saginaw Bay (Lake Huron) and Lake Erie during the mid- to late-1990s, 3–5 years after zebra mussels became established in these water bodies (Vanderploeg et al. 2001). In addition to the increase in *Microcystis* and its toxins in the Great Lakes, *Microcystis* abundance and its toxins increased in inland lakes in Michigan where zebra mussels became established and total phosphorus (TP) concentrations were <20 µg/L (Raikow et al. 2004, Knoll et al. 2008), suggesting these patterns and underlying mechanisms were not unique to large systems.

Microcystis and other cyanobacterial blooms have serious consequences to ecosystem function and health, to aesthetics, and to wildlife and human health. *Microcystis*, once thought to consist of five or more morphologically distinct species (morphospecies), likely consists of a single species, *M. aeruginosa*, that exhibits considerable intraspecific variation (genetic or strain variation) and phenotypic diversity within strains (Otsuka et al. 2001). A given strain, or genetically distinct population, can exhibit different traits such as colony morphology depending on environment conditions. We will refer to *Microcystis* available from culture collections as strains as these cultures are isolated from single cells or colonies. The genetic identities of some of these cultures, or strains, are described and some are not. *Microcystis* produces a potent diverse class of hepatotoxins (with >75 variants) and other secondary compounds that can poison aquatic organisms and wildlife, pets, and humans that drink the water (e.g., Sivonen and Jones 1999, Wiegand and Pflugmacher 2005). The main exposure pathway to aquatic animals is through ingestion of the cells (Lampert 1982, Nizan et al. 1986, Wiegand and Pflugmacher 2005). Although individual cells are small (~2–6 µm), *Microcystis* is a colonial cyanobacterium that can vary in size from a few cells to over 10,000 cells imbedded in mucilage of varying quantity and viscosity. Larger colonies can exceed 1000 µm. *Microcystis* is a typical dominant of the epilimnion of eutrophic lakes and its main selective advantages are its resistance to grazing and its buoyancy that prevents sedimentation and gives it the capacity to vertically migrate to take advantage of gradients of light and nutrients (Sommer et al. 1986, Reynolds 1997, 2006).

Dominance of *Microcystis* in the plankton can lead to inefficient food webs and shifts in zooplankton community structure. In many systems, zooplankton, including *Dreissena* larvae, can have very low ingestion rates of *Microcystis* colonies, because the colonies are often too large (>50 µm) to be handled by the mouthparts or are too large to fit in the mouth. Small colonies that are within the ingestible size range may be avoided because of toxicity or other taste factors. If ingested, *Microcystis* may lead to lowered survival and growth due for a variety of factors including toxic secondary compounds, such as microcystin, or the poor nutritional quality, for example, low concentration of n-3 polyunsaturated fatty acids (Fulton and Paerl 1987a,b, Vanderploeg et al. 1996, Wiegand and Pflugmacher 2005, Wilson et al. 2006, Ger et al. 2010). Vanderploeg et al. (2009) noted that mussel condition was negatively affected by *Microcystis*; therefore, mussels negatively affected their own populations by promoting *Microcystis* dominance.

Selective Rejection Paradigm

Vanderploeg et al. (1996, 2001) demonstrated that zebra mussels selectively reject *Microcystis* in pseudofeces and, given their high abundance and clearance rates, were likely responsible for promoting *Microcystis* blooms in Saginaw Bay and western Lake Erie. Important to the development of this concept were direct behavioral observations of zebra

mussels feeding on *Microcystis* and other seston collected during a *Microcystis* bloom in Lake Erie (Vanderploeg and Strickler 2013; see Sequence 1), shows mussels taking in *Microcystis* and other algae into the incurrent siphon and forcefully expelling pseudofeces containing *Microcystis* every few minutes. Vanderploeg et al. (2001) clearly demonstrated that *Microcystis* colonies (mostly found in the >53 μm screen-size fraction) in Saginaw Bay and Lake Erie were rejected or ingested at a low rate; in addition, both small (<53 μm) and large (>53 μm) colonies of a toxic *Microcystis* strain (LE-3) isolated from Lake Erie were rejected or ingested at a low rate. In contrast, they observed that other toxic (PCC 7820) or nontoxic (CCAP 1450/11) strains of colonial *Microcystis* were readily ingested. This followed observations of Baker et al. (1998) that showed that unicellular toxic (UTEX 2385) and nontoxic strains (UTEX 2386) of *Microcystis* were readily ingested. In addition, zebra mussels cleared the small colonies of *Microcystis* in the Hudson River at 25% of the rate of the preferred phytoplankton, cryptophytes, and unicellular *Microcystis* (Bastviken et al. 1998). Thus, at the outset, there was some controversy as to the importance and relevance of the selective rejection mechanism as the promoter of *Microcystis* blooms. Vanderploeg et al. (2001) reasoned that strain and colony size were major factors. In the case of the Hudson River, Vanderploeg et al. (2001) argued that the strength of selective pressure from mussels is likely reduced, relative to lakes, because of the shorter residence time of *Microcystis* population and lack of grazing selective pressure on *Microcystis* originating upstream of the mussels. Later, two modeling studies—using multiclass-phytoplankton water quality models—corroborated that selective rejection was a necessary condition for the proliferation of *Microcystis* that followed the invasion of mussels in Saginaw Bay (Bierman et al. 2005, Fishman et al. 2009).

Since the time of these early observations, more evidence has been collected that suggests strain identity of *Microcystis* is important to selective rejection, but most of these observations were made with *Microcystis* from culture collections. Dionisio Pires et al. (2005) reported modest clearance (∼3 mL/mg dry weight/h; compare with maximum of ∼40 mL/mg dry weight/h for a desirable alga; see succeeding text) rates on small (<60 μm) and large (>60 μm) size colonies of toxic (V40) and nontoxic (V131) *Microcystis* strains isolated from Lake Volkerak in The Netherlands. In contrast, Juhel et al. (2006) demonstrated that the unicellular toxic *Microcystis* strain CCAP 1450/10 caused distress to mussels and *Microcystis* was ejected in pseudofeces through the incurrent siphon and the pedal gape.

One problem with *Microcystis* from culture collections is that most of these cultures tend to be axenic and lose their colonial integrity during serial subculture (Otsuka et al. 2001), a possible result of loss of critical co-occurring heterotrophic bacteria that are thought to stimulate mucilage and colony formation (Shen et al. 2011). It is worth noting that the colonial laboratory strains (CCAP 1450/11, LE-3, and PCC 7820) examined by Vanderploeg et al. (2001) and those of Dionisio Pires et al. (2005) did not form particularly large colonies or have a heavy investment of mucilage. In fact, Dionisio Pires et al. (2005) described the colonies of his cultures as cells stuck together without mucilage.

Possible Interactions with Phosphorus

One can hypothesize that rejected *Microcystis* would get a boost from nutrient excretion from mussels that had processed the nutrient competitors of *Microcystis.* Others have argued that mussel nutrient excretion alone can explain the shift to *Microcystis* dominance by excreting more P relative to N and thereby shifting nutrient concentration to lower N:P ratios that favor cyanobacteria (Arnott and Vanni 1996, Bykova et al. 2006, Zhang et al. 2011). However, this advantage would not apply to *Microcystis* since it, unlike other species such as *Anabaena* and *Aphanizomenon*, is not an N-fixer. Although a detailed discussion of nutrient excretion is beyond the scope of this chapter, it is worth considering that the N:P ratio of excreted nutrients varies with seston stoichiometry and algal composition, and *Microcystis* has been reported to increase in mussel-invaded systems with low TP conditions in the seston. As noted by Vanderploeg et al. (2002) and Johengen et al. (2013), N:P ratio by definition would be high in low TP lakes, and the N:P ratio would be further skewed higher by mussels homeostatically retaining P under low TP conditions.

Using a flow cytometer, Dionisio Pires et al. (2005) observed that *Microcystis* consisting of unicells and small colonies (typically <20 μm) were ingested at the same modest-to-moderate rates as other phytoplankton in Lake IJsselmeer, The Netherlands. As a result of observations like these, Pires et al. (2005) advocated use of *Dreissena* as a biomanipulation tool to suppress cyanobacteria and increase water clarity. To increase mussel impact, they recommended the addition of hard substrate to increase zebra mussel populations. Both Lake IJsselmeer and the Hudson River are eutrophic systems having TP concentrations >50 μg/L. The potential role of TP concentration is also underscored by an increase in *Microcystis* in enclosures with zebra mussels fertilized to contain TP ≈ 9 μg/L (Sarnelle et al. 2005) and a decrease when TP = 40 μg/L (Sarnelle et al. 2012). These observations, plus observed increases of *Microcystis* in mussel-invaded lakes with low TP concentrations, have led to the current consensus—or paradigm—that *Dreissena* filtering will lead to *Microcystis* dominance in lakes of low TP. Is the observed lack of *Microcystis* dominance in mussel-invaded lakes with high TP concentrations a result of characteristics of the strains from high TP lakes, or a result of other interactions associated with P concentrations, such as differential growth rates of different algae? Our main focus is the selective rejection mechanism; however, we also consider our results in the context of possible nutrient interactions.

Overview of Experimental Approach and Objectives

To get further insight into the selective rejection mechanism and the role of mussels in promoting *Microcystis* dominance, we used a variety of approaches. First, we did feeding experiments and direct observations of mussels feeding on different laboratory strains of *Microcystis*, which retained their natural colonial form. The strains exhibited a range of toxicity and originated from four inland lakes in Michigan with zebra mussels (Gull Lake, Bear Lake) and without zebra mussels (Gilkey Lake, Hudson Lake). The strains were of known genetic identity and date of isolation (Wilson et al. 2005). These experiments were coupled with video observations to observe the mussel's behavioral response to the different strains.

Second, we examined feeding with natural seston and mussels from outer Maumee Bay, a high TP site in western Lake Erie associated with high TP inflow from the Maumee River, and with natural seston and mussels from inner Saginaw Bay, Lake Huron, which was a low TP site. Experiments were done with both zebra mussels and with quagga mussels, which have now effectively displaced zebra mussels in the Great Lakes. Quagga mussels have an even greater potential for affecting algal composition because they have attained abundances greater than zebra mussels in many nearshore regions and, unlike zebra mussels, are able to colonize deep, offshore regions (Nalepa et al. 2010). Do quagga mussels also selectively reject *Microcystis*?

Lastly, we report on results of a few experiments using water from Gull Lake and results of two enclosure experiments (mesocosm experiments) also in Gull Lake. Gull Lake is considered to be oligotrophic (Knoll et al. 2008) and provides a contrast to eutrophic western Lake Erie and inner Saginaw Bay. The enclosure experiments were designed to explore *Microcystis*–mussel interactions across a trophic gradient of N and P addition to help understand why *Microcystis* is promoted by mussels in some systems but not others (Raikow et al. 2004, Knoll et al. 2008, Sarnelle et al. 2012). Overall, our purpose here is to show a few contrasting results from different lakes or conditions.

After discussing the selective rejection mechanism, we explore the conditions under which *Microcystis* dominance can be promoted in natural systems and the future research that must be done to unravel interactions between mussels and naturally occurring *Microcystis* strains.

METHODS

All experiments, whether with natural seston or laboratory cultures, followed the same protocol: (1) all mussels were typically 14–16 mm in shell length, and (2) mussels were acclimated or re-acclimated to the particular feeding suspension for a minimum of 8 h. The acclimation period was designed to make sure the mussels were familiar with the particular feeding suspension and hence promote feeding and digestive equilibrium with their food prior to measurements. Setup of experiments and calculations of grazing followed standard methods described by Vanderploeg et al. (2001, 2009). In brief, we measured changes in chlorophyll concentration (Chl) in three control beakers without mussels and four experimental beakers with mussels. Samples for Chl were taken from beaker water at the beginning and end of the experiment and from mixed beaker contents at the end of the experiment. This allowed us to calculate both net and gross feeding rate variables; however, we report only net clearance rate (F_A). We typically used five mussels per 2 L beaker, and the duration was typically 2–3 h. Gentle mixing of the beaker water (but not settled material) was provided by bubbling in air through a pipette. We determined feeding on small (<53 μm) and large (>53 μm) size fractions of Chl by collecting seston on a sequential filtering apparatus that collected seston on a 53 μm Nitex screen and seston that passed through the screen to a GF/F filter (Vanderploeg et al. 2001). Summing the material from both fractions allowed us also to do a total mass balance of the constituent Chl collected on the filters. Given algae of moderate to high quality, ~30%–70% of the Chl would be removed with this experimental duration and mussel abundance.

Previous experience with seston from Lake Erie and Saginaw Bay had shown that most *Microcystis* was found in the large size fraction and was often the dominant phytoplankter when present. Clearance rates (mL/h) of mussels were expressed on a per unit ash-free dry weight (AFDW) (mL/mg/h) (Vanderploeg et al. 2001). Water taken from initial control and experimental bottles was preserved with 1% Lugol's solution for later identification of phytoplankton composition (Vanderploeg et al. 2001). Clearance rates were tested to see if they were significantly different ($P < 0.05$) from zero using a two-tailed t-test.

Location for collection of mussels and water for Saginaw Bay experiments with natural seston is the same 3.8 m deep inner bay Site SB5 (43.8953°N, −83.8605°W) we used in earlier studies (Vanderploeg et al. 2001, 2009). For Lake Erie, we collected water and mussels from a 3 m deep station on outer Maumee Bay (41.7417°N, −83.3555°W) because of its high nutrient concentration associated with P loading from the Maumee River.

In experiments evaluating the response to laboratory strains of *Microcystis*, we ran the same experiments with mussels collected at the same time with the cryptophyte, *Cryptomonas ozolini*, or the green alga, *Scenedesmus obliquus*. Clearance rates on these small high-quality algae served as a benchmark for evaluating the clearance rates on *Microcystis* (e.g., Vanderploeg et al. 2001, 2009). Vanderploeg et al. (2001) noted that clearance rates on *Cryptomonas* were somewhat higher than observed by Kryger and Riisgård (1998) for the green alga *Chlorella*; results of the latter study are often used as a benchmark for comparison with other

studies. Culture methods for *Microcystis* and algae followed methods described by Vanderploeg et al. (2001, 2009, 2010). Algae were suspended in 0.2 µm filtered lake or river water to create the feeding suspension. In these experiments, we used zebra mussels collected in the Huron River at Argo Dam (near Ann Arbor, Michigan). We generally sought to keep initial Chl at or below 4 µg/L to keep algal concentration low enough to avoid a significant clearance rate drop above the incipient limiting concentration (Vanderploeg et al. 2001, 2009, 2010).

To get further insight on the selective-rejection process with natural seston, we conducted long-term feeding experiments immediately following some of the short-term (2 h) feeding experiments using the same mussels and water used to set up the short-term experiment. Long-term experiments were aimed at obtaining better estimates of low clearance rates for less preferred particles such as *Microcystis*. Long-term experiments, which lasted 16 h with much of the time in the dark, were set up identically to short-term experiment, except beaker contents were mixed every 4 h with a spoon to make sure mussels were always exposed to *Microcystis* during the long duration of the experiment. In these experiments, samples for initial and final Chl concentrations were collected after beaker contents were thoroughly mixed.

In the long-term experiments, we determined feeding rates on *Microcystis*, not only by determining changes in Chl but also by measuring particulate microcystin concentration. Hence, in the latter case, we were measuring feeding on the toxic *Microcystis*. To quantify toxicity and compare it with our results from natural systems, we normalized microcystin concentration to Chl concentration. Microcystin was determined from colonies and cells collected on similar filters in the same filtration system as used for Chl, extracted in 75% MeOH and water, broken apart using a Tekmar probe sonifier, vortexed to remove remaining cellular debris from filters, and centrifuged to segregate fiber filters. The supernatant was analyzed by enzyme-linked immunosorbent assay (ELISA) kits (Envirologix Inc.) read on a Stat Fax 3200 spectrophotometer plate reader (Awareness Technology Inc.) following the Envirologix protocol. For the two experiments with Gull Lake enclosures, we measured zebra mussel feeding on seston from two low-P (no P addition) enclosures having stocked mussel dry-weight densities of 1 g/m^2 and 4 g/m^2 as dry tissue mass (Sarnelle et al. 2005) 1 week after the addition of mussels.

Methods of videotaping mussels followed those of Vanderploeg et al. (2001, 2009). We were particularly concerned about documenting the flow of *Microcystis* into the mussel incurrent siphon and expulsion as pseudofeces (Vanderploeg et al. 2001, 2009) in order to gain further insights into selective rejection mechanisms (further details are given in Vanderploeg and Strickler 2013).

In some experiments, we manipulated the size of the colonies by breaking them apart with a sonic probe. We used this approach with both laboratory cultures and natural colonies of *Microcystis* concentrated with a plankton net (100 µm mesh). The concentrated *Microcystis* was suspended in 80 mL of modified WC media (Vanderploeg et al. 2001), sonicated with several 1 s bursts of a sonic probe, and poured through a 25 µm screen to remove larger colonies not broken apart by sonication. By this method, we were able to produce unicells of *Microcystis* or colonies containing a few cells. The minimum number (typically 20) of bursts necessary to break down the colonies varied with strain and sonic probe used. Microscopic examination of the sonicated cells showed that although the colonies were broken apart, a halo of mucilage was found around the cells. After sonication, the cells were suspended in 0.2 µm filtered lake water. To create a mixture of small and large colonies, sonicated and unsonicated colonies were combined.

RESULTS

Strains Isolated from Inland Lakes

The four strains of *Microcystis* used in the feeding experiments had different toxicity, average colony size, and previous exposure to zebra mussels (Table 32.1), and also came from lakes with average TP concentrations ranging between 8 and 66 µg/L. As noted, strains from Bear Lake (strain: Bear AC) and Gull Lake (strain: Gull 8/23/00) were exposed to zebra mussels, and strains from Gilkey Lake (strain: Gilkey L) and Hudson Lake (strain: Hudson BD) were not exposed to zebra mussels (Table 32.1). Chlorophyll-specific microcystin (MC) concentrations (µg/µg Chl) were highest in Bear AC (0.202) and Gull Lake 8/23/00 (0.190) strains, intermediate in the Gilkey Lake L strain (0.099), and effectively zero in the Hudson BD strain (Table 32.2). The latter strain totally lacked the microcystin gene. The finding of microcystin in the Bear AC strain was a surprise, since it was reported to lack the microcystin gene (Wilson et al. 2005). All strains at the time of our experiments had significant representation in small and large size fractions except for the Hudson BD strain, which had colonies almost exclusively in the >53 µm fraction. The Hudson BD strain also differed from the others in its very heavy investment of mucilage around the cells (Figure 32.1).

Overall, experiments showed an extreme range of mussel clearance rates and behavioral responses that were not necessarily a function of microcystin concentration but rather other properties of the cells, such as mucilage or colony size. Small colonies of the toxic Bear AC strains were cleared at moderate rates—roughly 50% of that observed for the ideal food, *Cryptomonas*; however, large colonies of the Bear AC strain were not cleared at all (Figure 32.2). Both small and large colonies of the toxic Gull 8/23/00 strain were ingested at modest rates (Figure 32.3). Thus, larger colonies of the Gull 8/23/00 strain did not offer protection from grazing by mussels, in contrast

Table 32.1 Characteristics of Colonial *Microcystis* Strains and Lake of Origin of Strains Used in the Zebra Mussel Feeding Experiments with Laboratory Cultures. Strains Were Isolated from the Various Inland Lakes in Michigan by Wilson et al. (2005). Mussels for All Experiments Came from the Huron River, Except That Gull Lake Mussels Were Used for the Experiment with Sonicated Hudson BD Strain of *Microcystis*. TP Is the Total Phosphorus Concentration of the Lake of Origin. Also Listed Is the Control Alga (*Scenedesmus Obliquus* or *Cryptomonas ozolini*) That Was Given to Other Mussels to Serve as a Benchmark of What Feeding Would Be on a Desirable alga. Results of Video Observations on the Mussels Used in the Experiments Are Also Described. Microcystin (MC, µg/L) Was Measured by the ELISA Technique and Normalized to Chlorophyll Concentration (Chl, µg/L). The Gull Lake Strain Was Isolated in August 2000, and All Others in August 2002. The Date Given Is the Date the Experiments Were Conducted; All Experiments Were Run at 20°C

				MC/Chl		
Date	**Lake of Origin**	**Strain/Treatment**	**TP (µg/L)**	**Mean**	**SE**	**Video Observations**
12/20/05	Gull Lake	Gull 8/23/00	8	0.190	0.008	No observations
	Dreissenids present	Toxin gene: yes				
12/20/05		*Scenedesmus*				
1/13/2006	Gilkey Lake Dreissenids absent	Gilkey L Toxin gene: yes	17	0.099	0.015	Mussels exhibited a weak feeding current and stopped feeding. When *Cryptomonas* was added to the *Microcystis* suspension, the mussels resumed feeding and appeared to reject individual colonies of *Microcystis* immediately after they were siphoned.
1/13/2006		*Cryptomonas*				
2/3/2006	Hudson Lake	Hudson BD	49	0.003	0.0001	Strong filtering current with later forceful (group) rejections of large pseudofeces
	Dreissenids absent	Toxin gene: no				
2/3/2006		*Cryptomonas*				
3/10/2006	Bear Lake	Bear AC	66	0.202	0.014	Strong filtering current and (group) rejections as large pseudofeces
	Dreissenids present	Toxin gene: no?				
		Mussels in lake				
3/10/2006		*Cryptomonas*				
6/22/07	Hudson Lake Dreissenids absent	Hudson BD sonicated Toxin gene: no	49	—	—	No observations

Table 32.2 Conditions for the Short-Term Feeding Experiments in Saginaw Bay (SB) and Lake Erie (LE) during August 2004 and 2005. Temperature (T), Microcystin (MC; µg/L), and Chlorophyll (Chl; µg/L) Were Measured at Time of Experiment. Total Phosphorus (TP) Was Derived from Literature. MC/Chl, Microcystin to Chlorophyll Ratio; ZM, Zebra Mussels; and QM, Quagga Mussel

				MC/Chl	
Date	**Treatment/Dominant Alga**	**T (°C)**	**TP (µg/L)**	**Mean**	**SE**
8/24/2004	ZM & SB seston: *Microcystis* in >53 µm fraction	20	12 ± 4[a]	0.355	0.003
8/24/2004	QM & SB seston: *Microcystis* in >53 µm fraction	20	12 ± 4[a]	0.355	0.003
8/31/2005	QM & LE seston: *Aulacoseira* in >53 µm fraction	24	50–90[b]	—	—

[a] Stow (GLERL/NOAA, unpublished data) mean ± SD (N = 14) 2008–2010.
[b] Chaffin et al. (2011), range of 6 sites in western Lake Erie in 2008.

to the Bear AC strain, which did. We do not have detailed size distributions to evaluate whether the Bear AC strain colonies were just too large for ingestion. That clearance rates for the Gull 8/23/00 strain were the same as seen for the control algae *Scenedesmus* should not be interpreted as a very strong feeding rate response because *Scenedesmus* was presented at a very high concentration (Figure 32.3), thus leading to a considerably lower clearance rate (7 mL/mg/h) on this alga compared to higher clearance values seen on the control *Cryptomonas* (ranging between 27–50 mL/mg/h). Net clearance rates observed with the toxic Gilkey L strain and nontoxic Hudson BD strains were zero or not significantly different from zero (Figure 32.2). In the case of the Gilkey L strain, colonies were available

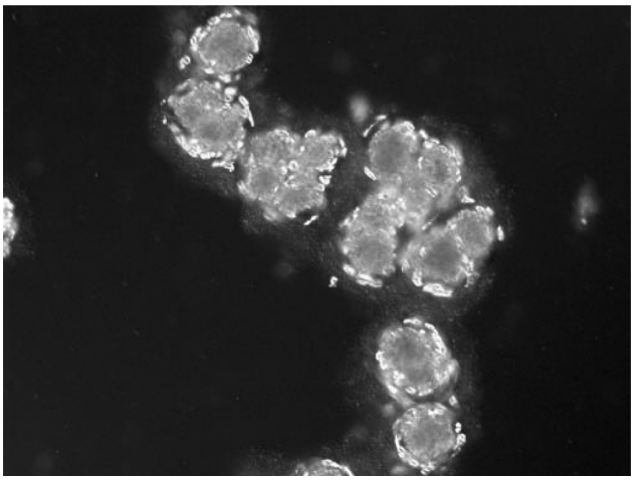

Figure 32.1 The Hudson BD strain of *Microcystis aeruginosa*. The halo around the cells is mucilage.

in both size fractions, whereas in the case of the Hudson BD strain, there were a few small colonies. Note the high negative clearance rate in the <53 μm size fraction is likely a result of particle production (Vanderploeg et al. 1984), that is, the breakdown of larger colonies into colonies falling in the smaller size fraction, which had few colonies to begin with.

Video observations showed different behavioral responses to the strains. Zebra mussels showed strong filtering currents and vigorous rejection of pseudofeces for both Hudson BD and Bear AC strains (Table 32.1; see Sequences 2 and 3 in Vanderploeg and Strickler 2013). This result is particularly interesting for the Hudson BD strain because there was no significant overall net clearance on colonies. In contrast to observations for other strains, mussels showed a strong negative behavioral response to the Gilkey L strain (Table 32.1). Siphons were only partially extended, and feeding currents and expulsion of *Microcystis*-containing pseudofeces were weak (see Sequence 4 in Vanderploeg and Strickler 2013). After *Cryptomonas* was added to the feeding suspension, the mussels exhibited normal feeding behavior (siphons fully extended with a strong feeding current), and it was observed that individual *Microcystis* colonies were rejected immediately after entering the filter chamber (see Sequence 5 in Vanderploeg and Strickler 2013).

To investigate whether the lack of ingestion of the Hudson BD strain was a result of colonies simply being too large for ingestion, we created a mixture of sonicated and unsonicated colonies (Table 32.1; Figure 32.2). Overall, there was no net clearance on either small or large colonies of this nontoxic strain. It is possible that the response was related to some property of the mucilage, since a small halo of mucilage was seen around the sonicated cells and colonies. Also, we cannot rule out other cellular constituents not identified in the present study.

Experiments with Natural Seston

The standard (short-term) clearance-rate experiments conducted in the summers of 2004 and 2005 showed that when *Microcystis* dominated (found in >53 μm fraction) in Saginaw Bay seston, there was no significant feeding by either zebra or quagga mussels; that is, clearance rates for total Chl were not significantly different from zero (Table 32.2; Figure 32.4). The considerable negative clearance rates on the <53 μm fraction suggested breakage of larger colonies into smaller colonies. These experiments represent the only time we were able to collect both quagga and zebra mussels at the same site for comparison when *Microcystis* was dominant. In the experiment of August 31, 2005, with Lake Erie seston, quagga mussels readily fed on the large size fraction dominated by the colonial diatom *Aulacoseira*, demonstrating that other large colonial algae were readily ingested. We do not have details on the upper size range to know if the colonies were really large and beyond some ingestible size range.

Experiments with *Microcystis* colonies from Gull Lake showed that mussels did not feed on either large colonies or small colonies derived from the large colonies by sonication (Table 32.3 and Figure 32.3). In the experiment in which the (unsonicated) colonies collected by net tow were mixed with *Cryptomonas* (August 11, 2005), zebra mussels readily fed on the *Cryptomonas* (present in the <53 μm fraction), but no *Microcystis* colonies (>53 μm fraction) were removed. In both experiments with mixtures of sonicated and unsonicated cells, small colonies of *Microcystis* were removed, but overall, there was no significant ingestion. In the experiment in August, there was a clear large negative clearance rate for the large (>53 μm) size fraction, consistent with particle production most likely in the form of pseudofeces. These results are in marked contrast to the modest feeding seen on both size categories of the Gull 8/23/00 strain. These results are consistent with the much lower microcystin to Chl (MC:Chl) concentration of the Gull 8/23/00 strain (0.190) relative to natural colonies collected in July (0.433) and August (0.777) (Tables 32.1 and 32.3).

Combined Long-Term/Short-Term Experiments with Natural Seston

Both experiments with Saginaw Bay and Lake Erie seston (Table 32.4) clearly showed that no *Microcystis* was ingested in the short- (2–3 h) or long-term (16 h) experimental time periods. There was no change in chlorophyll in either size fraction. The microcystin results show that most microcystin, and by extension toxic *Microcystis*, was found in the >53 μm fraction as expected (Vanderploeg et al. 2001). Microscopic analysis showed that the *Microcystis* colonies were large and would have been present in the larger size fraction. Clearance rates for

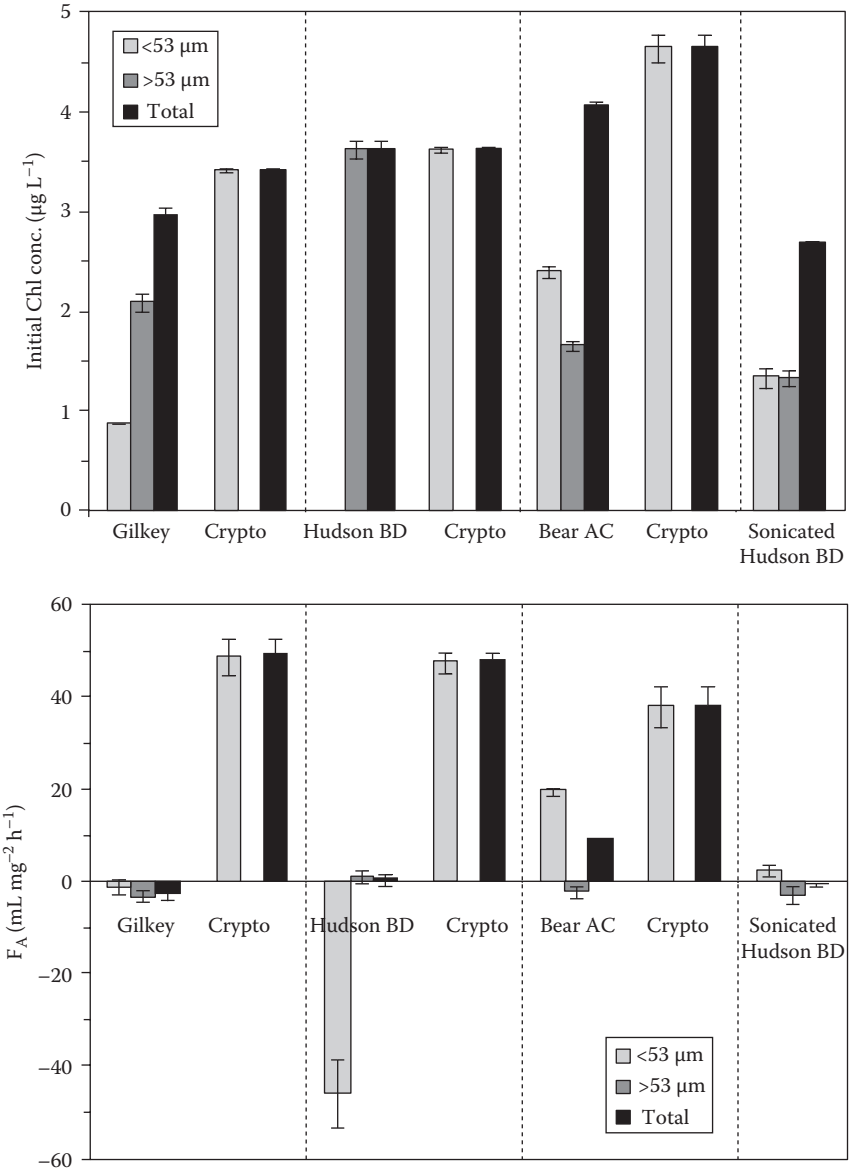

Figure 32.2 Feeding experiments with algal cultures isolated from inland Michigan lakes except for the Gull Lake strain (shown in Figure 32.3). To help put results in context of potential clearance rates, clearance rates on *Cryptomonas*, an ideal food for mussels, from the same collection are compared. Error bars are ±1 SE. Initial chlorophyll concentrations (Chl; upper panel) and net clearance rates (F_A; lower panel) of zebra mussels in feeding experiments designed to determine feeding response to different size fractions (<53 µm, >53 µm) of various *Microcystis* strains. The strains were isolated from various inland lakes in Michigan: Gilkey L strain from Gilkey Lake, Hudson BD strain from Hudson Lake, and Bear AC strain from Hudson Lake. Results for the Gull L strain from Gull Lake are given in Figure 32.3. The Hudson BD strain was sonicated to change the size structure of the colonies. To put clearance rates on the various strains in perspective, clearance rates on *Cryptomonas*, an ideal food, are also given. Error bars are ±1 SE.

microcystin in both these experiments were very low and not significantly different from zero (Table 32.4).

In contrast to results for Saginaw Bay and Lake Erie, results of the enclosure experiments with natural seston in Gull Lake point to modest feeding, including ingestion of *Microcystis* colonies that were codominant with diatoms. MC:Chl ratios were considerably higher in the Gull Lake mesocosms than in the Saginaw Bay and Lake Erie experiments, even though these ratios would be diluted by Chl in co-occurring diatoms, especially in the case of the experiment in mesocosm GL#7, where diatom biomass was greater than *Microcystis* biomass (Vanderploeg, unpublished data). In Saginaw Bay and Lake Erie experiments and in mesocosm GL#13, much of the microcystin and highest MC:Chl ratios were in the >53 µm fraction, whereas microcystin concentrations and

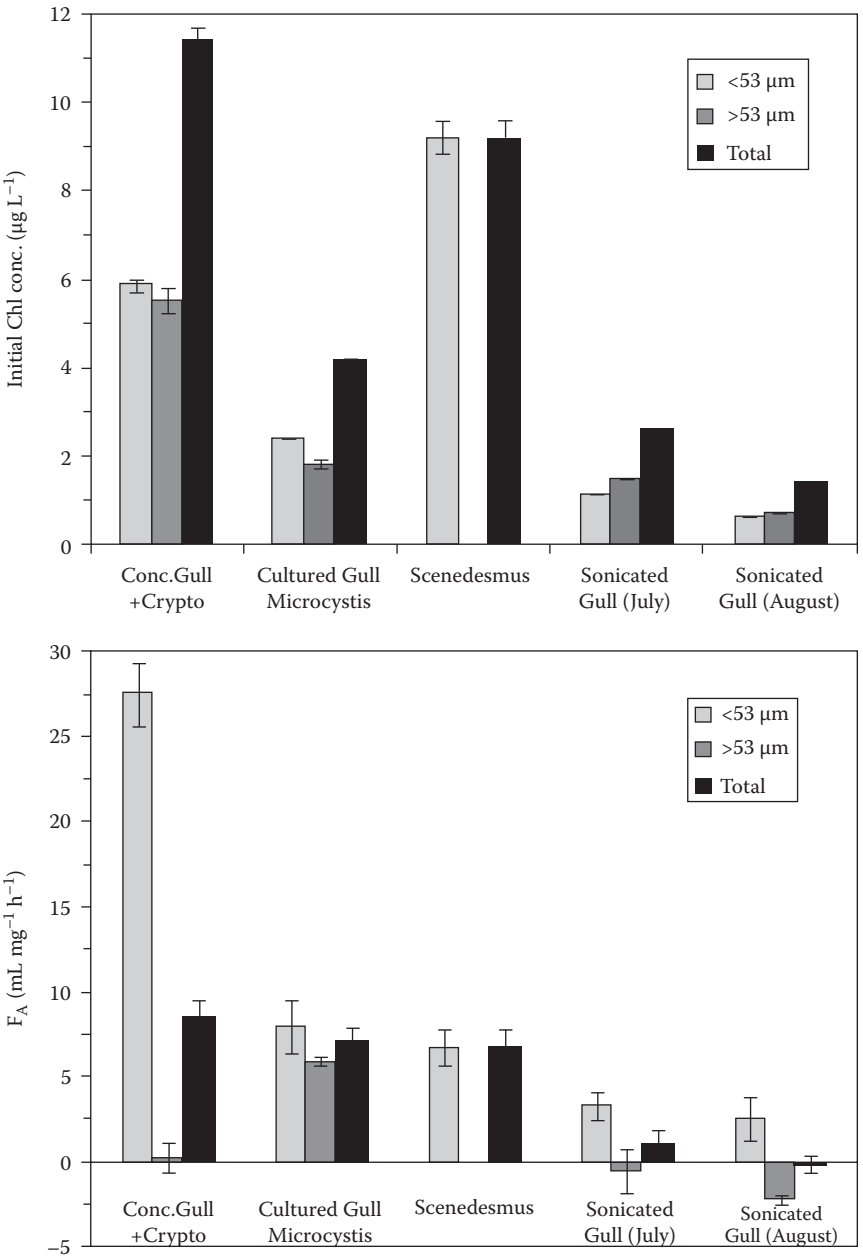

Figure 32.3 Initial chlorophyll concentrations (Chl; upper panel) and net clearance rates (F_A; lower panel) of zebra mussels in feeding experiments designed to determine feeding response to different size fractions (<53 µm, >53 µm) of the Gull 8/23/00 strain from Gull Lake and to different size fractions of *Microcystis* colonies collected in Gull lake with a plankton net. From left to right: *Microcystis* colonies mixed with cultured *Cryptomonas*, the 8/23/00 strain contrasted with results for *Scenedesmus* control culture, and mixtures of sonicated and unsonicated natural colonies from experiments in July and August 2007. Error bars are ± 1 SE. See Table 32.1 for details on the Gull L 8/23/00 strain, and Table 32.3 for details on experiments with naturally occurring Gull Lake *Microcystis* colonies.

M:Chl ratios were more evenly balanced between the two fractions in mesocosm GL#7. In the short-term experiments, we cannot specify whether anything in the >53 µm fraction was ingested because clearance rates had high standard errors (SEs). Moderate clearance rates were seen for the <53 µm fraction and for total Chl overall. Both microcystin and Chl in the long-term treatments were cleared at low, albeit statistically significant rates (2.2–4.7 mL/mg/h). Clearance rates on toxic *Microcystis* (microcystin) in the <53 µm size category were slightly higher than for the >53 µm fraction. Note that we would expect the long-term Chl clearance to be dominated in

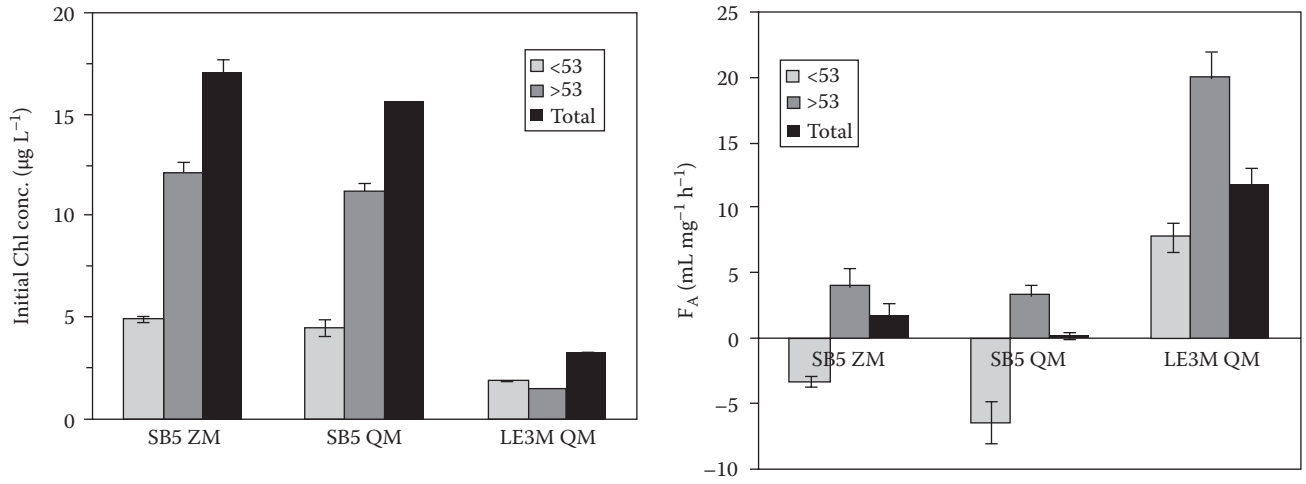

Figure 32.4 Initial chlorophyll concentrations (Chl; upper panel) and net clearance rates (F_A; lower panel) of zebra mussels (ZM) and quagga mussels (QM) in short-term feeding experiments designed to determine feeding response to different size fractions (<53 μm, >53 μm) of seston collected in Saginaw Bay (SB) and western Lake Erie (LE). The >53 μm fraction of the Saginaw Bay seston was dominated by *Microcystis*, and the >53 μm fraction of the Lake Erie seston was dominated by *Aulacoseira*. See Table 32.2 for conditions at the two collection sites. Error bars are ± 1 SE.

Table 32.3 Conditions of Experiments with *Microcystis* Colonies Collected with 100 μm Mesh Plankton Net in Open Water (11 m Depth Contour) of Gull Lake. Treatments as Indicated and Suspended in 0.2 μm Filtered Lake Water. Temperature (T), Microcystin (MC, μg/L), and Chlorophyll (Chl, μg/L) Were Measured at Time of Experiment. MC/Chl = Microcystin to Chlorophyll Ratio. Experiments Conducted with Zebra Mussels

			MC/Chl	
Date	**Treatment**	**T (°C)**	**Mean**	**SE**
8/11/2005	*Microcystis* colonies mixed with cultured *Cryptomonas*	27	—	—
7/16/2007	Mixture of sonicated and unsonicated colonies	20	0.448	0.021
8/10/2007	Mixture of sonicated and unsonicated colonies	26	0.777	0.088

the long-term experiment by *Microcystis* kinetics because the only Chl left over the longer term would likely come from *Microcystis*.

DISCUSSION

Differences in Grazing Vulnerability of Microcystis among Strains and Lakes

Overall, clearance-rate experiments and visual observations reinforce the notion that rejection of *Microcystis* varies with strain as observed with both laboratory cultures and natural seston. We observed extreme variance of zebra mussel response to the four laboratory strains that could not be simply related to microcystin concentration or colony size. Our results reflect observations of extreme variance seen among other strains noted earlier, but our study also showed high variance for cultures that maintained colonial integrity at the time of experiments. Observations with the Gilkey L strain showed complete shutdown of the feeding response when presented with a sole food source, with feeding being resumed only when this *Microcystis* strain was mixed with *Cryptomonas*. This is in contrast to high feeding (enthusiastic filtering) responses to the Bear AC strain, which was ingested, and to the Hudson BD strain that was not ingested whether presented as large colonies or small sonicated colonies. In both Hudson BD and Bear AC strains, there was periodic rejection of larger pseudofeces. The immediate rejection of the Gilkey L colonies as they were captured likely indicated a secondary compound and not microcystin (or at least the microcystin measured by the ELISA method) irritated the mussels. The rejection response was so striking even though microcystin concentrations were only at modest levels.

Table 32.4 Mean (± SE) Clearance Rates (F_A) of Zebra Mussels Feeding on Large (>53 μm) and Small (<53 μm) Size Fractions of Seston in Short-Term (2–3 h) and Long-Term (~16 h) Experiments. Seston Was Collected from Inner Saginaw Bay (SB) and Western Lake Erie (LE). Also Given Are Clearance Rates on Seston in the Early Phase (within 1 Week of Setup) of Enclosure Experiments Conducted in Gull Lake. Clearance Rates Based on Changes in Chlorophyll and Microcystin (MC) during the Experimental Period. T = Temperature

			Initial Concentration (μg/L)			F_A (mL/mg/h)		
						Short Term	Long Term	
Experiment	T (°C)	Size Fraction	Chl	MC	MC/Chl	Chl	Chl	MC
SB; 7/18–19/2006	26	>53 μm	8.19	1.04	0.127	–1.83 ± 0.17	0.19 ± 0.11	–0.28 ± 0.12
Microcystis dominant in >53 μm fraction		<53 μm	7.74	0.03	0.004	0.45 ± 0.57	0.04 ± 0.07	0.26 ± 0.84
		Total	14.81	1.07	0.072	–0.57 ± 0.29	0.11 ± 0.09	–0.27 ± 0.10
LE;8/29–30/2006	24	>53 μm	16.12	2.97	0.170	0.19 ± 0.11	0.38 ± 0.12	0.11 ± 0.19
Microcystis dominant in >53 μm fraction		<53 μm	4.95	0.30	0.058	–1.20 ± 0.55	–0.11 ± 0.10	–0.48 ± 0.09
		Total	21.08	3.27	0.155	–0.37 ± 0.15	0.26 ± 0.10	0.05 ± 0.17
GL #13 7/10–11/2007	27	>53 μm	0.361	0.194	0.576	7.13 ± 5.81	2.78 ± 0.78	2.82 ± 0.22
4 g/m²; low P; *Microcystis* and diatoms dominant		<53 μm	1.102	0.072	0.065	6.77 ± 1.38	2.74 ± 0.11	4.72 ± 0.81
		Total	1.463	0.266	0.183	7.28 ± 3.61	2.78 ± 0.20	3.20 ± 0.10
GL #7 7/11–12/2007	27	>53 μm	0.318	0.110	0.351	5.04 ± 5.24	1.64 ± 0.08	2.16 ± 0.24
1 g/m²; low P		<53 μm	0.917	0.221	0.245	12.02 ± 1.32	1.98 ± 0.26	2.64 ± 0.61
Diatoms and *Microcystis* dominant		Total	1.235	0.331	0.273	9.47 ± 1.62	1.88 ± 0.18	2.44 ± 0.46

Short-term experiments with natural *Microcystis* colonies or with unmodified lake seston also point to mussels selectively rejecting the colonies or shutting down feeding responses. In short-term experiments with Saginaw Bay seston, no significant overall (total) clearance rate was observed for zebra and quagga mussels when *Microcystis* was dominant in the >53 μm size fraction. Likewise, Gull Lake colonies captured in plankton net from the open lake—whether presented to zebra mussels alone or as a combination of sonicated and unsonicated colonies—were not ingested. This was in marked contrast to the Gull 8/23/00 strain that was readily ingested by zebra mussels. The natural Gull Lake colonies had extremely high microcystin levels in contrast to the isolated strain. In the combined long- and short-term experiments, using *Microcystis* from Saginaw Bay and Lake Erie, zebra mussels did not ingest *Microcystis* or any phytoplankton, whether clearance rate was measured from Chl or microcystin content. Experiments in the two enclosures showed that toxic *Microcystis* (measured by microcystin) was cleared at low rates in the long-term experiments. This was also reinforced by observed ingestion of Chl.

There can be multiple *Microcystis* strains in lakes that vary in terms of both colony size and form, and toxicity; these strains can occur at different times or simultaneously in the same lake (e.g., Dionisio Pires et al. 2005, Wilson et al. 2005, van Gremberghe et al. 2009, White et al. 2011). White et al. (2011) used various *Microcystis* strains isolated from Gull Lake, including two isolated on the same date, to study selectivity responses of zebra mussels. Relative to the green alga *Ankistrodesmus*, strain selectivity ranged from one (equal preference relative to *Ankistrodesmus*) to zero.

As noted earlier, the question of whether selective grazing by mussels can promote *Microcystis* dominance in different systems—including those with different TP concentrations—hinges on two issues. First, will the mussels ingest or reject the strains found in a given system? Second, is mussel filtering impact large enough to induce significant mortality on phytoplankton relative to their growth rate? With regard to the first question, strains isolated from both low and high TP lakes can have low or high vulnerability to grazing by mussels. As noted earlier, Dionisio Pires et al. (2005) observed moderate clearance rates on both toxic and nontoxic strains isolated from a high TP lake in The Netherlands. For the strains from Michigan inland lakes, the results were highly variable. Gull 8/23/00 and Gilkey L strains were both from lakes with TP concentrations of <20 μg/L, and clearance rates were high and low, respectively. Bear AC and Hudson BD strains were both from lakes having high (49–66 μg/L) TP concentrations, and clearance rates were high and low, respectively. The strongly rejected LE-3 strain (Brittain et al. 2000) used by Vanderploeg et al. (2001) had a MC:Chl ratio of 0.66 and came from Hatchery Bay of South Bass Island (eastern portion of the western basin of Lake Erie), where TP concentration was 35 μg/L (Holland et al. 1995). As noted earlier, White et al. (2011) noted large variations in zebra mussel grazing across *Microcystis* strains in Gull Lake (TP = 8 μg/L). Also, results of all these experiments show there is no apparent general relationship of grazing response with microcystin concentration.

Despite earlier observations that *Microcystis* in natural seston was readily ingested in high TP systems (e.g., Bastviken et al. 1998, Dionisio Pires et al. 2005), our experiments with seston from Saginaw Bay (TP = 12 ± 4 μg/L) and

from western Lake Erie near Maumee Bay (TP = 50–90 µg/L) and previous experiments with seston from western Lake Erie near Hatchery Bay (TP = 35 µg/L) (Vanderploeg et al. 2001) suggest a variable vulnerability of *Microcystis* to mussel grazing that cannot simply be related to TP concentration. Clearance rates in TP-rich western Lake Erie were essentially zero in our experiments and in the experiments of Vanderploeg et al. (2001). Likewise, our experiments with whole and sonicated colonies from the open waters of Gull Lake showed *Microcystis* (derived from plankton net concentrate) was not ingested. These large net-collected colonies presumably survived despite the biomass of mussels in the lake. In this regard, it is worth noting that the concentration of mussels in the lake (~6 g/m^2) was higher than in the enclosures, a necessity because of low survival of mussels stocked at concentrations higher than 4 g/m^2. Interestingly, the combination of short- and long-term feeding experiments did point to an overall low feeding rate on the assemblage of *Microcystis* found in the Gull Lake enclosures. As in the experiments with cultures, no correlation could be made with microcystin concentration.

It is theoretically possible that, in addition to strain effects of *Microcystis*, there can be genetic differences or even phenotypic adaptations among mussel populations to *Microcystis* found in different water bodies. For example, *Daphnia* clones from eutrophic environments were less sensitive to the negative impact of ingestion of a unicellular toxic *Microcystis* strain (Sarnelle and Wilson 2005). Can this same mechanism apply to dreissenid mussels? As mentioned, Dionisio Pires et al. (2005) found that clearance rates of zebra mussels feeding on small colonies of *Microcystis* were similar to rates on other phytoplankton in a eutrophic lake in The Netherlands. Is it possible that zebra mussels in this lake were genetically or phenotypically adapted to the relatively small colonies *Microcystis* found there? Interestingly, although such a phenomenon has not been observed for *Microcystis*–dreissenid interactions, there are reports of population-specific responses by the marine mussel *Mytilus edulis* to the toxic dinoflagellate *Protogonyaulax tamarensis* (Shumway and Cucci 1987). The GT429 strain of this dinoflagellate, which produces a potent neurotoxin, was readily ingested by *M. edulis* from Maine (USA) with no impacts, yet ingestion led to shell closure and death of mussels from New Jersey (USA) and Spain.

Projecting Mussel Impact on Phytoplankton Community

Zebra mussels (and quagga mussels) have clearance rates on a per unit weight basis that are about the same as the filter-feeding zooplankter *Daphnia* (Dionisio Pires et al. 2005). The potential of mussels to affect major changes in water clarity and change phytoplankton composition depends on their ability to develop large populations (Nalepa et al. 2010) relative to other potential grazers (Vanderploeg et al. 2001, 2002, 2009, 2010) and on the ability to feed on a broad size range of particles. In contrast, zooplankton are constrained to feeding on small phytoplankton (typically <30 µm) (e.g., Vanderploeg et al. 1988, Vanderploeg 1994, Dionisio Pires et al. 2005).

The carrying capacity of dreissenids for a given water body can be affected by nutrient loads and calcium concentrations. Wilson and Sarnelle (2002) observed a significant correlation between mussel abundance and TP, but the amount of variation in mussel biomass explained was relatively low (24%). The risk of dreissenid invasion and establishment increases as calcium concentrations increase above a certain threshold (Whittier et al. 2008). With the expansion of energetically efficient quagga mussels, which can utilize soft substrates as well as hard substrates including those found in the hypolimnion of lakes, it is possible that much larger populations can develop, leading to even greater impacts (Nalepa et al. 2010, Vanderploeg et al. 2010). In Lake Michigan, for example, large enough quagga mussel populations developed so as to decimate the spring phytoplankton bloom and leave behind a phytoplankton community with a higher proportion of cyanobacteria than before quagga mussels expanded (Fahnenstiel et al. 2010, Nalepa et al. 2010, Vanderploeg et al. 2010). Note that we would not expect *Microcystis* to be dominant in Lake Michigan because of relatively cool water temperatures in this large, deep lake. The question remains as to how mussel carrying capacity is related to nutrient loading and phytoplankton production, which obviously will dictate mussel impacts.

An important advantage mussels have over zooplankton in affecting phytoplankton communities is their long life span and lower vulnerability to predation. Dreissenids can grow and increase in biomass during spring and winter periods when food quality is good and survive during summer when food quality is poor (e.g., Vanderploeg et al. 2002, 2009). In contrast, *Daphnia* have short life spans and typically occur in spring and summer and then decline as phytoplankton food quality decreases and planktivorous fish populations increase (e.g., Sommer et al. 1986). However, mussels are not immune to predation, particularly in the Great Lakes, where significant predation can occur from round gobies (*Neogobius melanostomus*), which is another invader from the Ponto-Caspian region (Vanderploeg et al. 2002).

Information on temporal changes in mussel biomass in areas of the Great Lakes where thermal (high water temperature) and nutrient characteristics would support *Microcystis* growth during summer is rather limited. However, we know, for example, that abundances of dreissenids in Saginaw Bay are now lower than during the 1990s, when round gobies were not present (Nalepa, unpublished). Round goby abundance in turn is affected by predation from piscivorous fishes (e.g., Madenjian et al. 2011), and the habitat characteristics that promote high removal of gobies is not well defined. Thus, it is difficult to predict what dreissenid biomass will be in the future; likewise, we cannot with certainty predict future impacts.

Microcystis Affects Filtering Intensity

We observed that mussels can selectively reject *Microcystis* and simultaneously feed at high rates on high-quality algae when available. However, it is obvious that once *Microcystis* becomes a major component of the phytoplankton, pumping rates of the mussels decreases—at least for some *Microcystis* strains—as observed in experiments here and those reported by Vanderploeg et al. (2001, 2009). Hence, once *Microcystis* becomes a dominant component of the phytoplankton, mussels are no longer a factor in the promotion of *Microcystis*. This phenomenon has been reported by van Gremberghe et al. (2009) for *Daphnia–Microcystis* interactions. This feeding shutdown may be responsible for the existence of grazing-resistant and grazing-vulnerable *Microcystis* colonies in Gull Lake (White et al. 2011). Thus, at least for some environments, such as Saginaw Bay, promotion of *Microcystis* blooms by dreissenids likely occurs early in the summer season when *Microcystis* first develops. Interestingly, we have seen *Microcystis* blooms quite late in the summer or early fall on Lake Erie (Vanderploeg et al. 2001). This would be consistent with mussels removing palatable algae that would normally start to succeed summer species like *Microcystis*, as waters begin to cool down.

Nutrients and Phytoplankton Growth Rate

For a given *Microcystis* strain having a defined vulnerability to grazing by mussels or zooplankton, nutrient concentrations can potentially affect competitive outcomes between *Microcystis* and other phytoplankton species because grazing impact relative to phytoplankton growth rate will vary with nutrient concentration and mussel density. Thus, unless the fraction of the water column cleared by mussels (FC) is an appreciable fraction of phytoplankton community growth rate, selective rejection of *Microcystis* will not affect the competitive outcome between *Microcystis* and other species (Vanderploeg et al. 2001). Typical growth rates of phytoplankton in nutrient-limited systems range between 0.2 and 0.5/d, with a maximum between 1 and 2/d in systems where nutrients are not limited (Reynolds 1997). Measured growth rates in Saginaw Bay during summer were 0.20–0.25/d. *Microcystis* colony growth is relatively slow (e.g., Fahnenstiel et al. 1995, Reynolds 1997), and large colonies (within and across clones) grow more slowly than small colonies (e.g., Wilson et al. 2010). Wilson et al. (2010) observed considerable variation in growth of *Microcystis* strains under nutrient-saturating conditions, but most values fell between 0.2 and 0.5/d. One can imagine that under high nutrient conditions or during a nutrient-enrichment experiment in an enclosure that competitors of *Microcystis* with high growth rates could overcome effects of selective rejection, unless *Microcystis* rejection rates were high and FC was high enough to overcome the growth rate of the competitors, which could be on the order of 0.5–1/d. Note that other variables such as mixing, temperature, and light can affect the competitive outcome as well. *Microcystis* has the capacity to regulate its position in the water column with gas vacuoles and, as noted earlier, it does best in warm water temperatures and grows better under high light regimes (e.g., Reynolds 1997).

Future Directions

Variable responses of dreissenids to *Microcystis* as observed in the laboratory and field reflect similar responses of zooplankton to *Microcystis* (e.g., van Gremberghe et al. 2009). This variable response means that laboratory experiments with strains available from culture collections, even from the same lake, may sometimes have little meaning for understanding *Microcystis*–grazer interactions in the field, especially when strains lack naturally occurring characteristics such as colony formation. For example, note our contrasting results with the cultures from Gull Lake and colonies captured freshly from the same lake. We observed that the cultures did change over time, and at this writing, none of the colonial *Microcystis* used in the described experiments retained its colonial form. The question may also be asked as to whether the problem of cultures changing over time also applies to secondary compounds that deter grazing. An example is the LE-3 strain, a strain isolated from a bloom in Lake Erie that did not have much colonial integrity, but maintained a chemical deterrent.

Another problem is that many strains of *Microcystis* can co-occur in lakes and this makes generalizations based on a few isolates difficult. Relevant in this regard were observations of White et al. (2011) who showed different responses to strains isolated from Gull Lake, including two isolated on the same day.

It is also possible that *Microcystis*–grazer interactions will not be stable over time as populations adjust to each other. This interplay could include a shift in dominance of particular strains (*Microcystis* and mussels), phenotypic response to changing environmental conditions (including each other), as well as genetic adaptation that can be relatively rapid in the case of *Microcystis* (e.g., Rouco et al. 2011). Understanding important mechanisms associated with dreissenid–*Microcystis* interactions is more urgent than for zooplankton–*Microcystis* interactions since dreissenids are relatively unconstrained by phytoplankton size and can function either to promote harmful cyanobacteria or to control them.

Understanding dreissenid–*Microcystis* interactions in the field has presented difficult experimental challenges. First, there is an enormous amount of work involved in identifying and counting phytoplankton in all the replicates of an experiment, including initial concentrations, concentrations in the water column, and concentrations of settled material of control and experimental beakers (e.g., Vanderploeg et al. 2001). This is especially pertinent to experiments with seston, since taxonomic expertise and estimates of biomass for each of the

diversely shaped species are required. *Microcystis* presents the added difficulty of having to estimate the number of cells in colonies of varying sizes and shapes. That is why in many of our experiments, we looked at Chl concentrations, including the larger size fraction, which would account for large colonies. For the same reason we used microcystin—which also can be easily measured—as a surrogate for toxic *Microcystis*. In Gull Lake, microcystin concentration is well correlated with *Microcystis* biomass ($R^2 = 0.87$; Sarnelle et al. 2012). We note that imaging instruments such as flow cytometers (Dionisio Pires et al. 2005) and digital imaging flow cytometers (FlowCAM; Fluid Imaging, Inc) may help automate counting and sizing of phytoplankton and distinguishing them from *Microcystis*. Ultimately, we would like to measure feeding on different strains in nature. In principle, this could be done by examining changes in the genetic material in a feeding experiment.

REFERENCES

Arnott, D. L. and M. J. Vanni. 1996. Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. *Can. J. Fish. Aquat. Sci*. 53: 646–659.

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

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

Bierman, V. J., D. M. Dolan, and R. Kasprzyk. 1984. Retrospective analysis of the response of Saginaw Bay, Lake Huron to reductions in phosphorus loadings. *Environ. Sci. Technol*. 18: 23–31.

Bierman, V. J., J. Kaur, J. V. DePinto, T. J. Feist, and D. W. Dilks. 2005. Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. *J. Great Lakes Res*. 31: 32–55.

Brittain, S. M., J. Wang, L. Babcock-Jackson, W. W. Carmichael, K. L. Rinehart, and D. A. Culver. 2000. Isolation and characterization of microcystins, cyclic hepatotoxins from a Lake Erie strain of *Microcystis aeruginosa*. *J. Great Lakes Res*. 26: 241–249.

Bykova, O., A. Laursen, V. Bostan, J. Bautista, and L. McCarthy. 2006. Do zebra mussels (*Dreissena polymorpha*) alter lake water chemistry in a way that favours *Microcystis* growth? *Sci. Total Environ*. 371: 362–372.

Dionisio Pires, L. M., B. W. Ibelings, M. Brehm, and E. Van Donk. 2005. Comparing grazing on lake seston by *Dreissena* and *Daphnia*: Lessons for biomanipulation. *Microb. Ecol*. 50: 242–252.

Fahnenstiel, G. L., T. B. Bridgeman, G. A. Lang, M. J. McCormick, and T. F. Nalepa. 1995. Phytoplankton productivity in Saginaw Bay, Lake Huron: Effects of zebra mussel (*Dreissena polymorpha*) colonization. *J. Great Lakes Res*. 21: 465–475.

Fahnenstiel, G., S. Pothoven, H. Vanderploeg, D. Klarer, T. Nalepa, and D. Scavia. 2010. Recent changes in primary production and phytoplankton in the offshore region of southeastern Lake Michigan. *J. Great Lakes Res*. 36: 20–29.

Fishman, D. B., S. A. Adlerstein, H. A. Vanderploeg, G. L. Fahnenstiel, and D. Scavia. 2009. Causes of phytoplankton changes in Saginaw Bay, Lake Huron, during the zebra mussel invasion. *J. Great Lakes Res*. 35: 482–495.

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

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

Ger, K. A., S. J. Teh, D. V. Baxa, S. Lesmeister, and C. R. Goldman. 2010. The effects of dietary *Microcystis aeruginosa* and microcystin on the copepods of the upper San Francisco Estuary. *Freshwat. Biol*. 55: 1548–1559.

van Gremberghe, I., P. Vanormelingen, K. Van der Gucht et al. 2009. Influence of *Daphnia* infochemicals on functional traits of *Microcystis* strains (Cyanobacteria). *Hydrobiologia* 635: 147–155.

Holland, R. E., T. H. Johengen, and A. M. Beeton. 1995. Trends in nutrient concentrations in Hatchery Bay, Western Lake Erie, before and after *Dreissena polymorpha*. *Can. J. Fish. Aquat. Sci*. 52: 1202–1209.

Johengen, T. H., H. A. Vanderploeg, and J. R. Liebig. 2013. Effects of algal composition, seston stoichiometry, and feeding rate on zebra mussel (*Dreissena polymorpha*) nutrient excretion in two Laurentian Great Lakes. In *Quagga and Zebra Mussels: Biology, Impacts, and Control*. 2nd Edn., T. F. Nalepa and D. W. Schloesser, eds., pp. 445–459. Boca Raton, FL: CRC Press.

Juhel, G., J. Davenport, J. O'Halloran, S. Culloty, R. Ramsay, K. James, A. Furey, and O. Allis. 2006. Pseudodiarrhea in zebra mussels *Dreissena polymorpha* (Pallas) exposed to microcystins. *J. Exp. Biol*. 209: 810–816.

Knoll, L. B., O. Sarnelle, S. K. Hamilton et al. 2008. Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. *Can. J. Fish. Aquat. Sci*. 65: 448–455.

Kryger, J. and H. U. Riisgard. 1988. Filtration-rate capacities in 6 species of European fresh-water bivalues. *Oecologia*. 77: 34–38.

Lampert, W. 1982. Further studies on the inhibitory effect of the toxic blue-green *microcystis aeruginosa* on the filtering rate of zooplankton. *Archiv. Hydrobiol*. 95: 207–220.

Madenjian, C. P., M. A. Stapanian, L. D. Witzel, D. W. Einhouse, S. A. Pothoven, and H. L. Whitford. 2011. Evidence for predatory control of the invasive round goby. *Biol. Invasions* 13: 987–1002.

Makarewicz, J. C. 1993. Phytoplankton biomass and species composition in Lake Erie, 1970 to 1987. *J. Great Lakes Res*. 19: 258–274.

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

Nizan, S., C. Dimentman, and M. Shilo. 1986. Acute toxic effects of the cyanobacterium *Microcystis aeruginosa* on *Daphnia magna*. *Limnol. Oceanogr*. 31: 497–502.

Otsuka, S., S. Suda, S. Shibata, H. Oyaizu, S. Matsumoto, and M. M. Watanabe. 2001. A proposal for the unification of five species of the cyanobacterial genus *Microcystis* Kützing ex Lemmermann 1907 under the Rules of the Bacteriological Code. *Int. J. Syst. Evol. Microbiol*. 51: 873–879.

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

Raikow, D. F., O. Sarnelle, A. E. Wilson, and S. K. Hamilton. 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. *Limnol. Oceanogr*. 49: 482–487.

Reynolds, C. S. 1997. *Vegetation Processes in the Pelagic: A Model for Ecosystem Theory*. Oldendorf/Luhe, Germany: Ecology Institute.

Reynolds, C. S. 2006. *Ecology of Phytoplankton*. Cambridge, U.K.: Cambridge University Press.

Rouco, M., V. Lopez-Rodas, A. Flores-Moya, and E. Costas. 2011. Evolutionary changes in growth rate and toxin production in the cyanobacterium *Microcystis aeruginosa* under a scenario of eutrophication and temperature increase. *Microb. Ecol*. 62: 265–273.

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

Sarnelle, O., J. D. White, G. P. Horst, and S. K. Hamilton. 2012. Phosphorus addition reverses the positive effect of zebra mussels (*Dreissena polymorpha*) on the toxic cyanobacterium, *Microcystis aeruginosa. Water Res*. 46: 3471–3478.

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

Sarnelle, O., A. E. Wilson, S. K. Hamilton, L. B. Knoll, and D. F. Raikow. 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankton, *Microcystis aeruginosa. Limnol. Oceanogr*. 50: 896–904.

Shen, H., Y. Niu, P. Xie, M. Tao, and X. Yang. 2011. Morphological and physiological changes in *Microcystis aeruginosa* as a result of interactions with heterotrophic bacteria. *Freshwat. Biol*. 56: 1065–1080.

Shumway, S. E. and T. L. Cucci. 1987. The effects of the toxic dinoflagellate Progonyaulax tamarensis on the feeding and behavior of bivalve molluscs. *Aquat. Toxicol*. 10: 9–27.

Sivonen, K. and G. Jones. 1999. Cyanobacterial toxins. In *Toxic Cyanobacteria in Water*. I. Chorus and J. Bartram, eds., pp. 41–111. London, U.K.: E&F Spon.

Smith, V. H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science* 221: 669–671.

Sommer, U., Z. M. Gliwicz, W. Lampert, and A. Duncan. 1986. The PEG model of seasonal succession of planktonic events in fresh waters. *Archiv. Hydrobiol*. 106: 433–471.

Stoermer, E. F. and E. Theriot. 1985. Phytoplankton distribution in Saginaw Bay. *J. Great Lakes Res*. 11: 132–142.

Vanderploeg, H. 1994. Zooplankton particle selection and feeding mechanisms. In *The Biology of Particles in Aquatic Systems*. R. S. Wotton, ed., pp. 205–234. Boca Raton, FL: CRC Press.

Vanderploeg, H. A., T. H. Johengen, and J. R. Liebig. 2009. Feedback between zebra mussel selective feeding and algal composition affects mussel condition: Did the regime changer pay a price for its success? *Freshwat. Biol*. 54: 47–63.

Vanderploeg, H. A., J. R. Liebig, and W. W. Carmichael et al. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Can. J. Fish. Aquat. Sci*. 58: 1208–1221.

Vanderploeg, H. A., J. R. Liebig, and A. A. Gluck. 1996. Evaluation of different phytoplankton for supporting development of zebra mussel larvae (*Dreissena polymorpha*): The importance of size and polyunsaturated fatty acid content. *J. Great Lakes Res*. 22: 36–45.

Vanderploeg, H. A., J. R. Liebig, T. F. Nalepa, G. L. Fahnenstiel, and S. A. Pothoven. 2010. Dreissena and the disappearance of the spring phytoplankton bloom in Lake Michigan. *J. Great Lakes Res*. 36: 50–59.

Vanderploeg, H. A., T. F. Nalepa, and D. J. Jude et al. 2002. Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Can. J. Fish. Aquat. Sci*. 59: 1209–1228.

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

Vanderploeg, H. A., D. Scavia, and J. R. Liebig. 1984. Feeding rate of *Diaptomus sicilis* and its relation to selectivity and effective food concentration in algal mixtures and in Lake Michigan. *J. Plankton Res*. 6: 919–941.

Vanderploeg, H. A. and J. R. Strickler. 2013. Video Clip 6: Behavior of zebra mussels (*Dreissena polymorpha*) exposed to *Microcystis* colonies from natural seston and laboratory cultures. In *Quagga and Zebra Mussels: Biology, Impacts, and Control*, 2nd Edn., T. F. Nalepa and D. W. Schloesser, eds., pp. 757. Boca Raton, FL: CRC Press (this volume).

White, J. D., R. B. Kaul, L. B. Knoll, A. E. Wilson, and O. Sarnelle. 2011. Large variation in vulnerability to grazing within a population of the colonial phytoplankter, *Microcystis aeruginosa. Limnol. Oceanogr*. 56: 1714–1724.

Whittier, T. R., P. L. Ringold, A. T. Herlihy, and S. M. Pierson. 2008. A calcium-based invasion risk assessment for zebra and quagga mussels (*Dreissena* spp.). *Front. Ecol. Environ*. 6: 180–184.

Wiegand, C. and S. Pflugmacher. 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. *Toxicol. Appl. Pharm*. 203: 201–218.

Wilson, A. E., R. B. Kaul, and O. Sarnelle. 2010. Growth rate consequences of coloniality in a harmful phytoplankter. *PLoS One* 5. DOI: 10.1371/journal.pone.0008679.

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

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

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

Zhang, H., D. A. Culver, and L. Boegman. 2011. Dreissenids in Lake Erie: An algal filter or a fertilizer? *Aquat. Invasions* 6: 175–194.

The opinions expressed in this book are solely those of the authors and do not reflect the policy or official opinions of NOAA, USGS, Department of Commerce or any other agency of the Federal Government.

Cover Image Credit: Chris Houghton, Rob Paddock, and John Janssen, University of Wisconsin-Milwaukee, School of Freshwater Sciences. This image of quagga mussels (profunda/deepwater morph) presents a composite of photos taken on the southern slope of Northeast Reef in Lake Michigan (43° 45.11′N, 87° 34.70′W) on April 25, 2012. Image photos were taken at a depth of 52 m with a deepwater ROV and clearly show the elongated siphons of the profunda morph.

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

Printed on acid-free paper
Version Date: 20130912

International Standard Book Number-13: 978-1-4398-5436-5 (Hardback)

Library of Congress Cataloging-in-Publication Data

Zebra mussels.
Quagga and zebra mussels : biology, impacts, and control / editors, Thomas F. Nalepa and Don Schloesser. -- Second edition.
pages cm
"A CRC title, part of the Taylor & Francis imprint, a member of the Taylor & Francis Group, the academic division of T&F Informa plc."
Includes bibliographical references and index.
ISBN 978-1-4398-5436-5 (hardcover : acid-free paper) 1. Zebra mussel. 2. Quagga mussel. 3. Zebra mussel--Control. 4. Quagga mussel--Control. 5. Zebra mussel--Environmental aspects. 6. Quagga mussel--Environmental aspects. I. Nalepa, T. F. II. Schloesser, Donald W. III. Title.

QL430.7.D8Z45 2013
594'.4--dc23 2013017601

Visit the Taylor & Francis Web site at
http://www.taylorandfrancis.com

and the CRC Press Web site at
http://www.crcpress.com