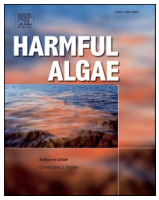
Harmful Algae 115 (2022) 102236

Contents lists available at ScienceDirect

Harmful Algae

journal homepage: www.elsevier.com/locate/hal



Original Article

Hepatotoxic shellfish poisoning: Accumulation of microcystins in Eastern oysters (*Crassostrea virginica*) and Asian clams (*Corbicula fluminea*) exposed to wild and cultured populations of the harmful cyanobacteria, *Microcystis*

Nora R.W. Straquadine [a], Raphael M. Kudela [b], Christopher J. Gobler [a,*]

[a] School of Marine and Atmospheric Sciences, Stony Brook University, 239 Montauk HWY, Southampton, NY 11968, USA
[b] University of California Santa Cruz, 1156 High Street Santa Cruz, CA 95064, USA

ARTICLE INFO

Keywords:
Microcystin
Microcystis
Bivalves
Oysters
Hepatotoxic shellfish poisoning
Asian clams

ABSTRACT

The Asian clam (*Corbicula fluminea*) and eastern oyster (*Crassostrea virginica*) are important resource bivalves found in and downstream of waterways afflicted with cyanobacterial harmful algae blooms (CHABs), respectively. This study examined the potential for *C. fluminea* and *C. virginica* to become vectors of the hepatotoxin, microcystin, from the CHAB *Microcystis*. Laboratory experiments were performed to quantify clearance rates, particle selection, and accumulation of the hepatotoxin, microcystin, using a microcystin-producing *Microcystis* culture isolated from Lake Erie (strain LE-3) and field experiments were performed with water from *Microcystis* blooms in Lake Agawam, NY, USA. Clearance rates of *Microcystis* were faster ($p<0.05$) than those of *Raphidocelis* for *C. fluminea,* while *C. virginica* cleared *Microcystis* and *Tisochrysis* at similar rates. For both bivalves, clearance rates of bloom water were slower than cultures and clams displayed significantly greater electivity for green algae compared to wild populations of cyanobacteria in field experiments while oysters did not. In experiments with cultured *Microcystis* comprised of single and double cells, both bivalves accumulated >3 μg microcystins g^{-1} (wet weight) in 24 – 72 h, several orders of magnitude beyond California guidance value (10 ng g^{-1}) but accumulated only up to 2 ng microcystins g^{-1} when fed bloom water dominated by large *Microcystis* colonies for four days. For Asian clams, clearance rates and tissue microcystin content decreased when exposed to toxic *Microcystis* for 3 – 4 days. In contrast, eastern oysters did not depurate microcystin over 3 - 4-day exposures and accumulated an order of magnitude more microcystin than clams. This contrast suggests Asian clams are likely to accumulate minor amounts of microcystin by reducing clearance rates during blooms of *Microcystis*, selectively feeding on green algae, and depurating microcystin whereas oysters are more likely to accumulate microcystins and thus are more likely to be a vector for hepatotoxic shellfish poisoning in estuaries downstream of *Microcystis* blooms.

1. Introduction

Cyanobacterial harmful algae blooms (CHABs) largely form due to anthropogenic nutrient pollution (O'Neil et al., 2012) and can disrupt ecosystems by causing hypoxic conditions and introducing toxins into both drinking water and aquatic food webs (Chen et al., 2009). The most common CHAB-forming cyanobacteria genus is *Microcystis*, which usually occurs in freshwater systems but can tolerate brackish waters (10–15 PSU) when it is flushed into estuaries (Preece et al., 2017). *Microcystis* sp. can produce multiple congeners of the hepatotoxin, microcystin (Harke et al., 2016). To date, more than 240 different microcystins have been identified (Zhang et al., 2022), and the most abundant and studied variant in water samples is microcystin-LR (Li and Xie, 2021). Acute effects of microcystin-LR for humans include skin rashes, gastrointestinal illness, and respiratory issues (Massey et al., 2018). In an extreme case in 1996, the water used for dialysis treatments in Caruaru, Brazil, was contaminated with microcystin-LR and led to an outbreak of acute liver failure and death in 26 people (Carmichael et al., 2001). Microcystins have also contributed to mortality in other mammals, including African elephants (Wang et al., 2021), sea otters (Miller et al., 2010), cattle (Mez et al., 1997) and dogs (Lawton et al., 1995; Backer et al., 2013; Hilborn and Beasley, 2015).

Invasive to the United States, the edible Asian clam (*Corbicula fluminea*) can be found in the some of the same waterways that are afflicted

* Corresponding author.
E-mail address: Christopher.gobler@stonybrook.edu (C.J. Gobler).

https://doi.org/10.1016/j.hal.2022.102236
Received 26 January 2022; Received in revised form 5 April 2022; Accepted 8 April 2022
Available online 18 April 2022

with CHABs whose range is projected to increase this century (Chapra et al., 2017). These small (<5 cm) freshwater clams are native to South Asia and are a popular food around the world. The clams were introduced into North America in the 1930s and can now be found in 44 of the U.S. states (NY Invasive Species Information, 2019). These clams have been noted to have a high physiological tolerance for fluctuations in temperature, pH, dissolved oxygen, and emersion (Sousa et al., 2008). In the U.S., the clam is generally sold canned or smoked in Asian markets and large quantities of fresh clams harvested from the Potomac River have been found to be sold in New York fish markets (Phelps, 1994).

Eastern oysters (*Crassostrea virginica*) can be found along the eastern seaboard of North America, as well as in the Gulf of Mexico. These shellfish live in brackish zones of estuaries, where they can be exposed to brackish-tolerant freshwater cyanobacteria (Vareli et al., 2013). More so than Asian clams, oysters are an important commercial shellfishery in the United States, valued over $200 million in 2018 (NMFS, 2018). Gibble et al. (2016) reported that oysters (*Crassostrea* sp.) and mussels (*Mytilus californianus*) off the coast of California had microcystin levels in their tissue that were above the World Health Organization's recommended total daily intake (TDI) limit of 0.04 μg kg^{-1} body weight and the California guidance value of 10 μg kg^{-1} (WHO, 1998; Chorus and Bartram, 1999).

Suspension feeding bivalves remove particles from the water column and sort them using special feeding structures that move particles along the ctenidia for particle capture and waste removal (Atkins, 1937; Yonge, 1923). The cilia and cirri transport the particles to food grooves along parts of the gills, which in turn move the particles to the mouth. Particles are further sorted using the labial palps, which are paired structures around the mouth (Yonge, 1923). *Corbicula* spp. can filter bacteria more efficiently than native mussels due to more complex feeding structures, mainly more cilia and cirri (Vaughn and Hakenkamp, 2001; Bolam et al., 2019). Oysters also sort particles on the ctenidium (Beals, 2004) and are known to filter particles greater than 5 μm with high efficiency (Riisgaard, 1988). Removal of particulate matter from the water column, either through ingestion of particles or by pseudofeces-production (ejecting mucus packets of rejected particles), can significantly influence water quality (zu Ermgassen et al., 2013).

Bivalve clearance rates specifically refer to the volume of suspended particles in water cleared per unit time (Riisgard, 2001). The clearance rates and particle selection mechanisms leading up to accumulation of microcystin are poorly understood for *C. fluminea* and, to a limited extent, for *C. virginica* (Ward et al., 1997; Mafra et al., 2009). Liu et al. (2009) found a statistically significant lower clearance rate when *C. fluminea* were exposed to a microcystin-containing *Microcystis* compared to nontoxic *Microcystis*, and, more recently, Silva et al. (2020) observed that the strain of *Microcystis* offered to *C. fluminea* in their study was removed at a slower rate than a control alga, *Raphidiopsis subcapitata*. To date, no studies have quantified the rate at which *C. fluminea* or *C. virginica* accumulate microcystin-LR when exposed to bloom concentrations of freshwater cyanobacteria, while considering how quickly the bivalves could clear *Microcystis* from blooms. Freitas et al. (2014) exposed Asian clams to 1×10^5 cells mL^{-1} of *Microcystis* for four days and documented an accumulation of microcystin-LR, but did not report on the dynamics of accumulation nor on clearance rates of algae. More recently, Boegehold and Kashian (2021) conducted filtration experiments with *C. fluminea* and quagga mussels (*Dreissena rostriformis bugensis*) using unicellular and colonial strains of *Microcystis aeruginosa* and found that filtration rates increased for both species during the experiment, though the *Microcystis* strains used did not produce microcystins at the time of the experiments.

This study was conducted to provide a understanding of the ability of bivalves to filter feed on the CHAB *Microcystis* and the potential health risks posed by CHAB-exposed shellfish. To understand interactions between freshwater microcystin-producing cyanobacteria and shellfish, experiments were conducted with a freshwater bivalve that is present where CHABs begin (*C. fluminea*) and an estuarine bivalve present in estuaries and coastal zones where CHABs are eventually discharged (*C. virginica*). Laboratory experiments were performed exposing the bivalves to differing levels of cultured *Microcystis* as a monoculture and within a mixed culture, and clearance rates were quantified (Table 1). Field experiments were performed to assess the ability of the bivalves to clear cyanobacterial blooms of differing densities (Table 1). For both types of experiments, bivalve tissues were analyzed to assess the accumulation of microcystins during the experimental exposures (Table 1). We hypothesized that Asian clams and eastern oysters exposed to toxic *Microcystis aeruginosa*, both cultured and wild, would accumulate microcystins at rates proportional to the rates at which cyanobacteria were cleared from the water.

2. Materials and methods

2.1. Collection and maintenance of shellfish

Asian clams (30 – 50 mm) were collected from Lake Peconic, NY, USA (40.913, −72.716), and added to a freshwater recirculating system with sand substrate at the Stony Brook - Southampton Marine Science Center. The clams were fed ad libitum the green microalga, *Raphidocelis* sp., (formerly known as *Selenastrum*). Eastern oysters (30 – 50 mm) were obtained from the Aeros shellfish hatchery (Southold, NY, USA) and fed ad libitum a mixed diet of *Tisochrysis sp*. and *Tetraselmis sp*.. The oysters were acclimated to 15 PSU (from ~30 PSU in hatchery waters) over 12 days (Thompson et al., 2012) and then maintained at 15 PSU with weekly water changes. All bivalves were maintained with constant aeration. Bivalves were starved 24 h prior to experiments to ensure that any feces or pseudofeces produced were a result of the experiment and not a relic of earlier feeding (Leverone et al., 2007; Cranford et al., 2011). Both clams and oysters had no previous exposure to *Microcystis* and microcystin in the lake or hatchery.

2.2. Microalgal cultures

Microcystis aeruginosa strain LE-3 (Brittan et al., 2000), isolated from Lake Erie, and *Raphidocelis subcapitata* were cultured in BG-11 media (Waterbury and Stanier, 1981). Cultures were maintained at 21 °C within exponential growth phase with light supplied on a 14:10 h cycle at 100 μmol photons $m^{-2} s^{-1}$. *Tisochrysis* sp. and *Tetraselmis* sp. were grown under the same conditions in f/2 media without silicic acid (Guillard, 1975). Cultures were grown to $>1 \times 10^6$ cells mL^{-1} and diluted as needed. Aliquots of the *Microcystis* culture were saved for microcystin analysis and levels were quantified using a Eurofins Abraxis ELISA method as described in Harke et al. (2015).

2.3. Laboratory experiments

2.3.1. Monoculture clearance rate experiments

The laboratory studies for the clams and oysters were conducted in 250 mL beakers on four, 15-spot stir plates. The clams sat on raised mesh pedestals with magnetic stir bars underneath to keep the algal cells homogenized and suspended. The stir bars did not re-homogenize any excrement that was produced during the experiments. Three concentrations of *M. aeruginosa* were investigated ranging from a dense bloom to more moderate bloom conditions: 1) 1×10^6 cells mL^{-1}, 2) 5×10^5 cells mL^{-1}, and 3) 2.5×10^5 cells mL^{-1} (experiment 3.1.1; Table 1). *R. subcapitata* was offered to bivalves at the *M. aeruginosa* biovolume equivalent (2 *Microcystis* cells = one *Raphidocelis* cell; experiment 3.1.1; Table 1). Maintenance cultures were concentrated via centrifugation at 3000 g for 10 min, quantified, and added to each treatment with dilutions made with filtered (1 μm) seawater and spring water to bring the experimental volume to 200 mL. There were six experimental replicates and three no-bivalve controls of each algal concentration. Initial samples from all beakers for cell density quantification were preserved with 1% formalin and analyzed using a Beckman Coulter CytoFLEX flow

Table 1
Summary of the 10 experiments conducted during this study outlining algae used, bivalve used, experiment type, experiment duration, and the section within the results within which each experimental result is described.

Experiment	Type	Treatment	Duration (h)	Bivalve	cHAB species	Control algae
3.1 Laboratory Experiments						
3.1.1	Clearance Rate	Monoculture	1	Asian clam	*Microcystis aeruginosa* LE3	*Raphidocelis subcapitata*
3.1.2	Clearance Rate	Monoculture	1	Eastern oyster	*Microcystis aeruginosa* LE3	*Tisochrysis lutea*
3.1.3	Clearance Rate	Mixed	1	Asian clam	*Microcystis aeruginosa* LE3	*Raphidocelis subcapitata*
3.1.4	Clearance Rate	Mixed	1	Eastern oyster	*Microcystis aeruginosa* LE3	*Tisochrysis lutea*
3.1.5	Clearance rate & accumulation	Monoculture	72	Asian clam	*Microcystis aeruginosa* LE3	–
3.1.6	Clearance rate & accumulation	Monoculture	72	Eastern oyster	*Microcystis aeruginosa* LE3	–
3.2 Natural bloom clearance rate experiments						
3.2.1	Clearance Rate	Bloom	1	Asian clam	Lake Agawam *Microcystis* sp.	Lake Agawam green algae
3.2.2	Clearance Rate	Bloom	1	Eastern oyster	Lake Agawam *Microcystis* sp.	Lake Agawam green algae
3.2.3	Clearance rate & accumulation	Bloom	96	Asian clam	Lake Agawam *Microcystis* sp.	–
3.2.4	Clearance rate & accumulation	Bloom	96	Eastern oyster	Lake Agawam *Microcystis* sp.	–

cytometer and Nikon Eclipse TS100 microscope. Once all stir plates with the appropriate algal cell densities were established, bivalves were added and the moment when clams opened and stuck out their siphons to feed was recorded (Jacobs et al., 2015). Similarly, the time when oysters opened was also noted. During initial experiments, cell samples were collected initially every 20 min for one hour. Given that analyses revealed no significant difference between 20-min, 40 min, and 60-min clearance rates, during subsequent experiments final samples were taken after one hour. The oyster experiments differed from clam experiments in that the oysters were placed in 250 mL beakers with a mixture of spring water and filtered seawater to achieve a final salinity of 15 PSU (Alexander et al., 2008; experiment 3.1.2; Table 1). There was the same number of experimental and control replicates for the oysters, but *T. lutea* replaced the *R. subcapitata* as the control algae (one *Microcystis* cells = one *Tisochrysis* cell; experiment 3.1.1; Table 1). Final samples were taken after an hour and processed as described above. At the end of the experiments, bivalves were measured, shucked, and weighed before and after being placed in a 60 °C drying oven for 48 h.

The clearance rates of monoculture experiments were quantified following the methods of Coughlan (1969) and then standardized to bivalve dry weight (Eq. (1))

$$CR = \frac{V \times \left[\frac{ln\ (C_o)\ -ln\ (C_t)}{t}\right] - A}{W_d} \quad (1)$$

where CR is clearance rate, V is volume of suspension, Co is initial, Ct is final, A is the average of controls, and Wd is dry weight (Coughlan, 1969). All clearance rates for Asian clams and oysters were standardized to the dry weight of the bivalve and were compared using a two-way ANOVA for each bivalve where algal food and cell densities were the main effects with post-hoc Tukey tests used to resolve differences among cell densities. Data were arcsine transformed as needed to meet normality and homogeneity of variance assumptions. When these assumptions could not be met after transformation, non-parametric tests like ANOVA on ranks or Mann-Whitney Rank Sum were used. Clearance rates during hepatotoxin accumulation experiments were determined every 12 h, and dry weights were estimated from a linear dry weight to wet weight regression derived from measurements of >100 bivalves used in the monoculture experiments.

2.3.2. Mixed culture clearance rate experiments

Mixed culture experiments were performed with *Microcystis* and the control algae *Raphidocelis* or *Tisochrysis* to assess clearance rate and particle selection by *C. fluminea* and *C. virginica*. Asian clams were placed in beakers of 100:0, 75:25, 50:50, 25:75, or 0:100 *Microcystis* and *Raphidocelis* at *Microcystis* concentrations of 1×10^6 cells mL^{-1}, 5×10^5 cells mL^{-1}, and 2.5×10^5 cells mL^{-1} (experiment 3.1.3; Table 1) while oysters were placed in beakers of *Microcystis* and *Tisochrysis* using equivalent percentages (experiment 3.1.4; Table 1). Densities of the control alga were set to match the CHAB species in biovolume. There were six experimental replicates and three controls for each ratio. Clearance rates samples were collected one hour after the bivalves opened and samples for Beckman Coulter CytoFLEX flow cytometer and light microscope were preserved with 1% formalin and Lugol's, respectively, with bivalve excrement collected as it was produced. The clearance rates for each of the five ratios were compared a two-way ANOVA with factors being cell densities and percentage of *Microcystis* in treatment.

2.3.3. Natural bloom clearance rate experiments

To understand the effects of wild cyanobacterial blooms on clearance rates by *C. fluminea* and *C. virginica*, experiments were performed with bloom water dominated by *Microcystis* (50 – 90% of algal biomass) collected from Lake Agawam, Long Island, NY, USA (40.881° N, 72.392° W). Levels of total chlorophyll *a* and the relative contribution of different phytoplankton groups to total chlorophyll *a* were quantified with a BBE Moldenke Fluoroprobe which estimates abundances of cyanobacteria, green algae, brown algae (e.g., diatoms, dinoflagellates, raphidophytes, and haptophytes) and cryptophytes based on differential fluorescence of photosynthetic accessory pigments (Beutler et al. 2002; Harke et al. 2015; Jankowiak et al., 2019). These trials had six experimental replicates and three controls per treatment. The treatments were 100% bloom water, 50% bloom water, and 25% bloom water for Asian clams. The treatments for oysters were 75% bloom water (10 PSU), 50% bloom water (15 PSU), and 25% bloom water (20 PSU), mimicking the gradual dilution of a freshwater bloom within an estuary. A 100% treatment was excluded because it was ecologically unrealistic for oysters to be exposed to 0 PSU water. The Asian clam treatments were diluted with phytoplankton-free spring water and the oyster treatments were diluted with a mixture of phytoplankton-free spring water and filtered seawater. BBE fluoroprobe cyanobacteria levels for the Asian clam bloom water experiment was 142 μg chl-aL^{-1} (10/2/2020) and the average diluted densities of the experiment were 110 μg chl-aL^{-1}, 94 μg chl-aL^{-1}, 69 μg chl-aL^{-1}, and 33 μg chl-aL^{-1} (experiment 3.2.1; Table 1). The oyster bloom water experiment occurred during one of the densest blooms of the year with the lake cyanobacteria levels reading 4816 μg chl-aL^{-1} (10/24/2020), and the diluted densities averaged 1681 μg chl-aL^{-1}, 1096 μg chl-aL^{-1}, and 542 μg chl-aL^{-1} (experiment 3.2.2; Table 1). Selectivity indexes were quantified by measuring biomass levels of each group using a BBE Fluoroprobe (Vanderploeg et al., 2001; Jankowiak et al., 2019). Clearance rates of different algal groups measured on the Fluoroprobe were quantified via a two-way ANOVA whereby percent dilution and the algal group were the main effects. Differences among groups were analyzed with post-hoc Tukey multiple comparisons tests. The clearance rates for the accumulation experiments were standardized to expected dry weight by a linear regression of $W_d = W_w a$, where W_d is dry weight, W_w is the wet weight, and *a* is the coefficient derived from the measurement of 100 individuals measured during lab clearance rate experiments.

2.3.4. Electivity index

The calculation for particle electivity was based on the Vanderploeg and Scavia (1979) electivity index (E_i; Eq. (2)).

$$E_i = \frac{W_i - \frac{1}{n}}{W_i + \frac{1}{n}} \quad (2)$$

where W_i is the selection coefficient ($W_i = F_i / \Sigma F_i$), that was calculated using the clearance rate on *Microcystis* (F_i) and the sum of grazing rates of both *Microcystis* and the control (ΣF_i). n was the total number of prey types (Vanderploeg and Scavia, 1979). The index is determined by comparing the clearance rates of two algal prey species when both are present simultaneously and ranges from −1 to 1. A positive number closer to 1 means preferential ingestion, while a negative number closer to −1 means rejection of algal species. The electivity indexes for the mixed experiments and bloom water dilutions were compared using Student's *t*-test and Mann-Whitney Rank Sum tests.

2.3.5. Hepatotoxin accumulation experiments

For hepatotoxin accumulation experiments, bivalves were exposed to Lake Agawam, NY bloom water for four days (experiments 3.2.3 and 3.2.4; Table 1) and were exposed to 10^6 cells mL^{-1} of cultured *Microcystis* for three days (experiment 3.1.5 and 3.1.6; Table 1). The clam experiment was carried out October 8–11, 2020, with a BBE fluoroprobe initial cyanobacteria average of 118 ± 15 μg chl-aL^{-1} (microcystin: 38 ± 3 $\mu g\ L^{-1}$; experiment 3.2.3; Table 1). The oyster experiment occurred October 27–30, 2020 (experiment 3.2.4; Table 1), with a BBE fluoroprobe cyanobacteria average of 71 ± 12 μg chl-aL^{-1} (microcystin: 29 ± 10 $\mu g\ L^{-1}$) after a 50% dilution with filtered seawater (final salinity of 15 PSU). Both clams and oysters were kept in one-liter beakers and the temperature, dissolved oxygen, and pH were recorded at the beginning and end of each 12-hour period (Freitas et al., 2014). Cell densities were monitored, and cells (for lab experiment) and water (for Lake Agawam experiment) was replenished every 12 h, as the cells were consumed. Clearance rates were quantified every 12 h. Field-based clearance rates were compared using a two-way ANOVA between time point and algal class. For the culture experiments, there were 12 experimental replicates and three controls, and four bivalves were removed for hepatotoxin analysis after days 1, 2, and 3. For bloom water experiments, there were 20 experimental replicates with three controls and five bivalves from each treatment were removed for hepatotoxin analysis after days 1, 2, 3, and 4. After sampling, the bivalves were weighed, shucked, and tissue was frozen. Hepatotoxins were measured from homogenized bivalves since that is what humans typically ingest (Chen and Xie, 2007; Adamovsky et al., 2017).

For tissue extraction, triplicate shellfish from each date were shucked and the muscle tissue homogenized in a glass blender prior to extraction in acidified MeOH with probe sonication followed by bath sonication. The supernatant was then collected via centrifugation and SPE-cleaned using Bakerbond C18 6cc 500 mg columns, generally following Mekebri et al. (2009) with modifications as described. The columns were conditioned using 100% MeOH and 100% acidified water prior to loading the sample diluted to less than 10% MeOH with acidified water (0.1% Formic Acid, 0.05% Trifluoroacetic Acid). The sample was dried for 5 min on the column and then eluted with the Acid-MeOH. As an additional purification step, 600–1000 µl of the shellfish extract was extracted using STRATA-X 100 mg cartridges conditioned with MeOH and water. The sample was then loaded onto the column and rinsed with 10% MeOH, prior to elution with 100% MeOH mixed with 2% Formic acid.

Microcystin content in the shellfish tissue was analyzed using liquid chromatograph mass spectrometry (LCMS) on an Agilent 1200 HPLC system coupled to an Agilent 6150 MS using Selected Ion Monitoring (SIM) with JetSpray technology. Analysis was conducted following well-established protocols, developed by Mekebri et al. (2009) and subsequently refined and modified to accommodate specific sampling needs (Miller et al., 2010; Kudela 2012; Gibble and Kudela 2014; Gibble et al., 2016; Peacock et al. 2018). Analysis was conducted with ESI in positive mode and SIM, using external certified reference material (NRC Canada, Sigma-Aldrich, and Abraxis Eurofins) as standards. Levels of the microcystin congeners MC-LR, MC-RR, MC-YR, MC-LA, and MC-LF were measured along with desmethyl-microcystin (dmLR). A two-way ANOVA with a covariate being cell densities and days of exposure was used to analyze the treatment groups measuring response variable microcystin content. Differences among groups were analyzed with post-hoc Tukey multiple comparisons tests.

3. Results

3.1. Laboratory experiments

3.1.1. Asian clam and Microcystis monoculture clearance rate experiment

In the culture-based monoculture clearance rate experiment conducted with Asian clams, the clams cleared the *Microcystis* at higher rates than the *Raphidocelis* ($p<0.05$, Two-way ANOVA). Specifically, the clearance rates of the clams were significantly lower for *Raphidocelis* than *Microcystis* at the 1×10^6 cells mL^{-1} level ($p<0.05$; Tukey test; Fig. 1). While not significantly different, there was evidence of a dose dependent algal preference. At the two lowest algal densities (2.5×10^5 cells mL^{-1} and 5×10^5 cells mL^{-1}) the toxic *Microcystis* and nontoxic *Raphidocelis* were cleared at similar rates of ~3 L h^{-1} g^{-1} and ~2 L h^{-1} g^{-1} respectively. At the highest algal density treatments (1×10^6 cells mL^{-1}) the *Microcystis* was cleared at an average of 3.97 L h^{-1} g^{-1}, three times higher than the *Raphidocelis* (1.23 L h^{-1} g^{-1}; Fig. 1).

3.1.2. Eastern oyster and Microcystis monoculture clearance rate experiment

In the monoculture clearance rate experiments with eastern oysters, clearance rates were not significantly different between algal species or across cell densities ($p>0.05$, Two-way ANOVA). For the 1×10^6 and 2.5×10^5 cell mL^{-1} densities, the clearance rates of the control algae *Tisochrysis* were ~33% less than *Microcystis* (Fig. 2). For the 5×10^5 cell mL^{-1} density, the clearance rates of *Microcystis* were 74% lower than the control algae. The *Microcystis* clearance rates were 5.16 ± 1.5 L h^{-1} g^{-1}, 0.99 ± 0.64 L h^{-1} g^{-1}, 3.75 ± 0.75 L h^{-1} g^{-1} for 2.5×10^5, 5×10^5, and 1×10^6 cell mL^{-1} respectively, with *Tisochrysis* at 3.36 ± 0.94 L h^{-1} g^{-1}, 3.92 ± 1.27 L h^{-1} g^{-1}, and 2.50 ± 0.43 L h^{-1} g^{-1} (Fig. 2).

3.1.3. Asian clam and Microcystis mixed culture clearance rate experiments

For the 1×10^6 cell mL^{-1} density there was a significant difference between the treatments and between the algal type ($p<0.05$ for both; Two-way ANOVA; Fig. 3A). For the 5×10^5 cell mL^{-1} density there was only a significant difference between algal type ($p<0.05$, Two-way ANOVA; Fig. 3B) and for the 2.5×10^5 cell mL^{-1} density it was only for treatments ($p<0.05$, Two-way ANOVA; Fig. 3C). For 1×10^6 cell mL^{-1}, the 75% and 50% *Microcystis* treatment had significantly higher clearance rates than the 25% *Microcystis* treatments ($p<0.05$, Tukey test). During the same experiment, *Microcystis* was cleared significantly faster than *Raphidocelis* ($p<0.05$, Tukey), in the monoculture treatments (100% vs 0%) and in the 25% and 75% treatments ($p<0.05$, Tukey). *Raphidocelis* was cleared significantly faster than *Microcystis* in the 25% *Microcystis* treatment in the 5×10^5 cell mL^{-1} density experiment ($p<0.05$, Tukey). Lastly, the 25% treatment had significantly slower clearance rates compared to the 75% *Microcysts* treatment in the 2.5×10^5 cell mL^{-1} ($p<0.05$, Tukey). In all Asian clam mixed experiments, there was a trend of increasing clearance rates as the percentage of *Microcystis* in the water increased, which usually peaked at the 75% treatment before dropping in the 100% treatment. Precise clearance rates appear in Fig. 3 and Supplemental Table 1. The Asian clams did not produce pseudofeces in the *Microcystis* mixed trials indicating that the

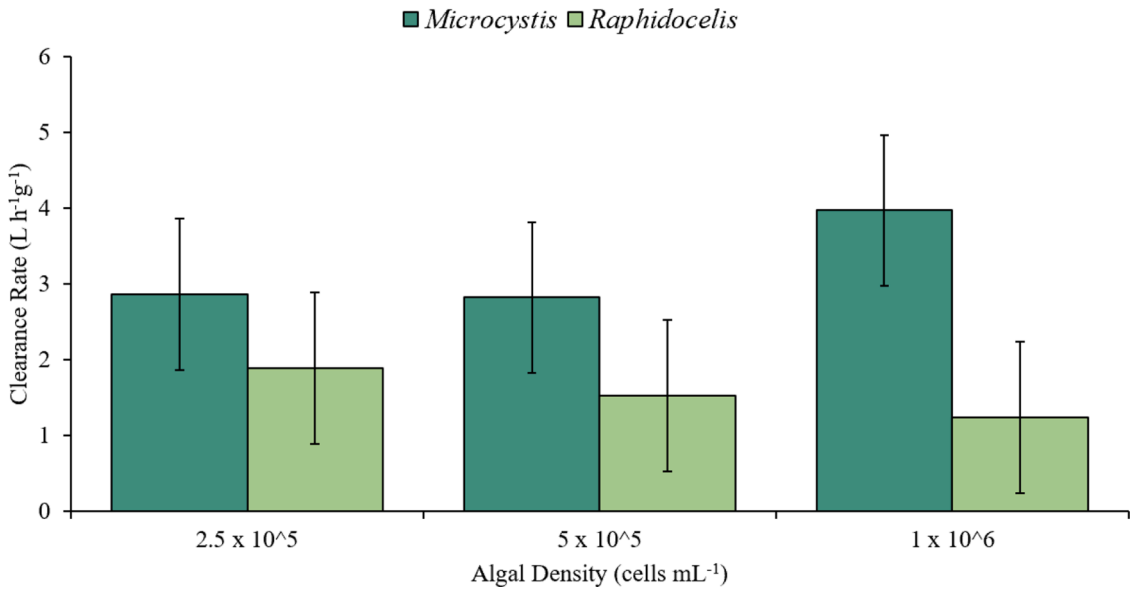


Fig. 1.. Clearance rates of monocultures of the toxic cyanobacterium *Microcystis* and nontoxic green algae *Raphidocelis (*formerly *Selenastrum)* by the freshwater Asian clam. Clams were incubated in cultures of three densities 1×10^6, 5×10^5 and 2.5×10^5 cell mL^{-1}. The clearance rates of the clams were significantly lower for *Raphidocelis* than *Microcystis* at the 1×10^6 cells mL^{-1} level ($p < 0.05$; Tukey test). Error bars indicate standard error.

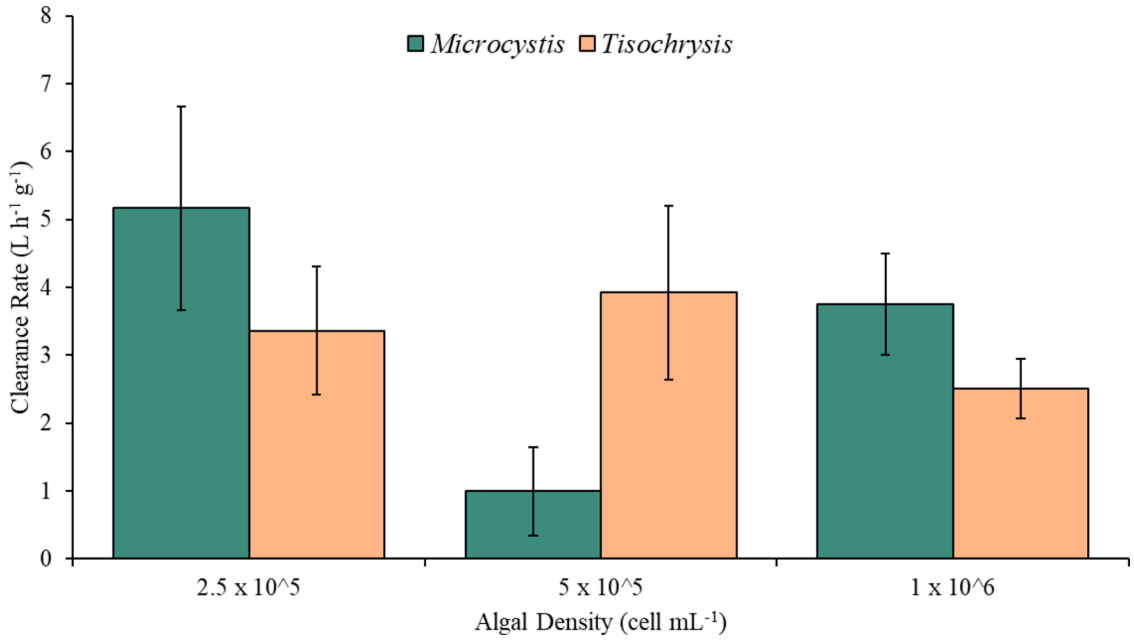


Fig. 2.. Clearance rates of monocultures of the toxic cyanobacterium *Microcystis* and nontoxic algae *Tisochrysis* by the eastern oyster. Oysters were incubated in cultures of three densities 1×10^6, 5×10^5 and 2.5×10^5 cell mL^{-1}. Clearance rates were not significantly different between algal species or across cell densities ($p > 0.05$, Two-way ANOVA). Error bars indicate standard error.

clams were ingesting the cells. Across all experiments, clams had a significantly high electivity index for *Microcystis* (0.027±0.028) compared to *Raphidocelis* (−0.120±0.044) for ($p < 0.05$, Student's *t*-test, Fig. 3D; Supplemental Table 3).

3.1.4. Oyster and Microcystis mixed culture clearance rate experiments

There was no significant difference between algal type or treatments in the mixed culture-based oyster experiments ($p > 0.05$, Two-way ANOVA). Precise clearance rates appear in Fig. 4 and Supplemental Table 2. The combined electivity index for all the mixed culture oyster experiments showed there were no significant differences in the electivity indexes for *Tisochrysis* and *Microcystis* (−0.161±0.073 and −0.046 ±0.073, respectively; $p > 0.05$, Wilcox on Signed Rank Fig. 4D; Supplemental Table 3).

3.1.5. Three-day Asian clam clearance rates and hepatotoxin accumulation

During the three-day clearance rate and hepatotoxin accumulation experiment with Asian clams, clearance rates significantly decreased by 99% over the three-day period ($p < 0.001$; One-way ANOVA; Fig. 5A) dropping from 0.97 L h^{-1} g^{-1} to 0.08 L h^{-1} g^{-1}. Specifically, the 12 and 24 h clearance rates were significantly higher than the 36, 48, and 60 h clearance rates, and after the initial drop in feeding the 72-hour clearance rates were significantly higher than the 48- and lower 60-

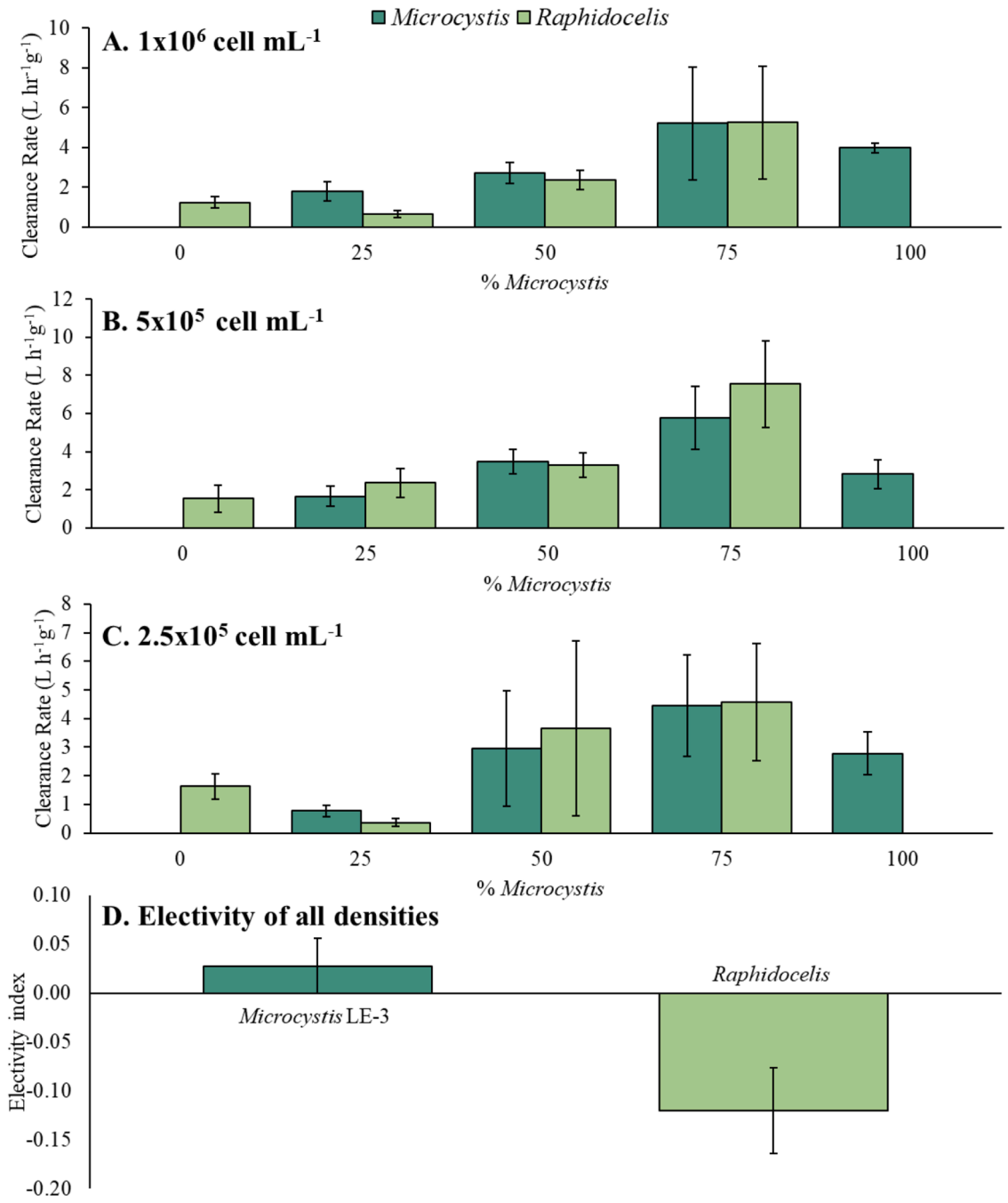


Fig. 3.. Clearance rates of mixed treatments of the toxic cyanobacterium *Microcystis* and nontoxic green algae *Raphidocelis (Selenastrum)* by the freshwater Asian clam. Clams were incubated in five ratios of mixed cultures at three densities A) 1×10^6, B) 5×10^5 and C) 2.5×10^5 cell mL^{-1}, where each bar represents average clearance rates and error bars indicate standard error. The electivity index (D) for *Microcystis* LE-3 was significantly higher than for *Raphidocelis.*

hour clearance rates ($p<0.05$, Tukey). The microcystin concentrations in the clam tissue (ng g^{-1}) generally tracked clearance rates, going from 3668 ng g^{-1} after 24 h to 1625 ng g^{-1} after 48 h and finally to 719 ng g^{-1} after 72 h (Fig. 5B). The amount of microcystin in the clams at 24 h was significantly higher than the levels after 72 h ($p<0.05$, One-way ANOVA, $p<0.05$, Tukey). For the congeners, LR was significantly higher between day 1 and days 2 and 3 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), LF between day 1 and 3 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), dmLR between day 1 and days 2 and 3 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), and LY between day 1 and 2 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey).

3.1.6. Three-day oyster clearance rates and hepatotoxin accumulation

In contrast to the clams, oyster clearance rates varied little over the three-day experiment, averaging 0.11 ± 0.02 L h^{-1} ($p>0.05$, One-way ANOVA; Fig. 6A). The microcystin in the oyster tissue (ng g^{-1}) increased over time from 2627 ng g^{-1} to 1873 ng g^{-1} and to 4905 ng g^{-1} after 24, 48, and 72 h, respectively ($p<0.05$, One-way ANOVA) (Fig. 6B). Beyond the total amount of microcystin increasing significantly by the end of the experiment, the levels of each microcystin congener also increased significantly each day ($p<0.05$, One-way ANOVA; Fig. 6B). Specifically, LR was different between day 3 and day 1 and 2 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), LF between day 2 and 3 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), dmLR for day 3 and day 1 and 2 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey), and for LY

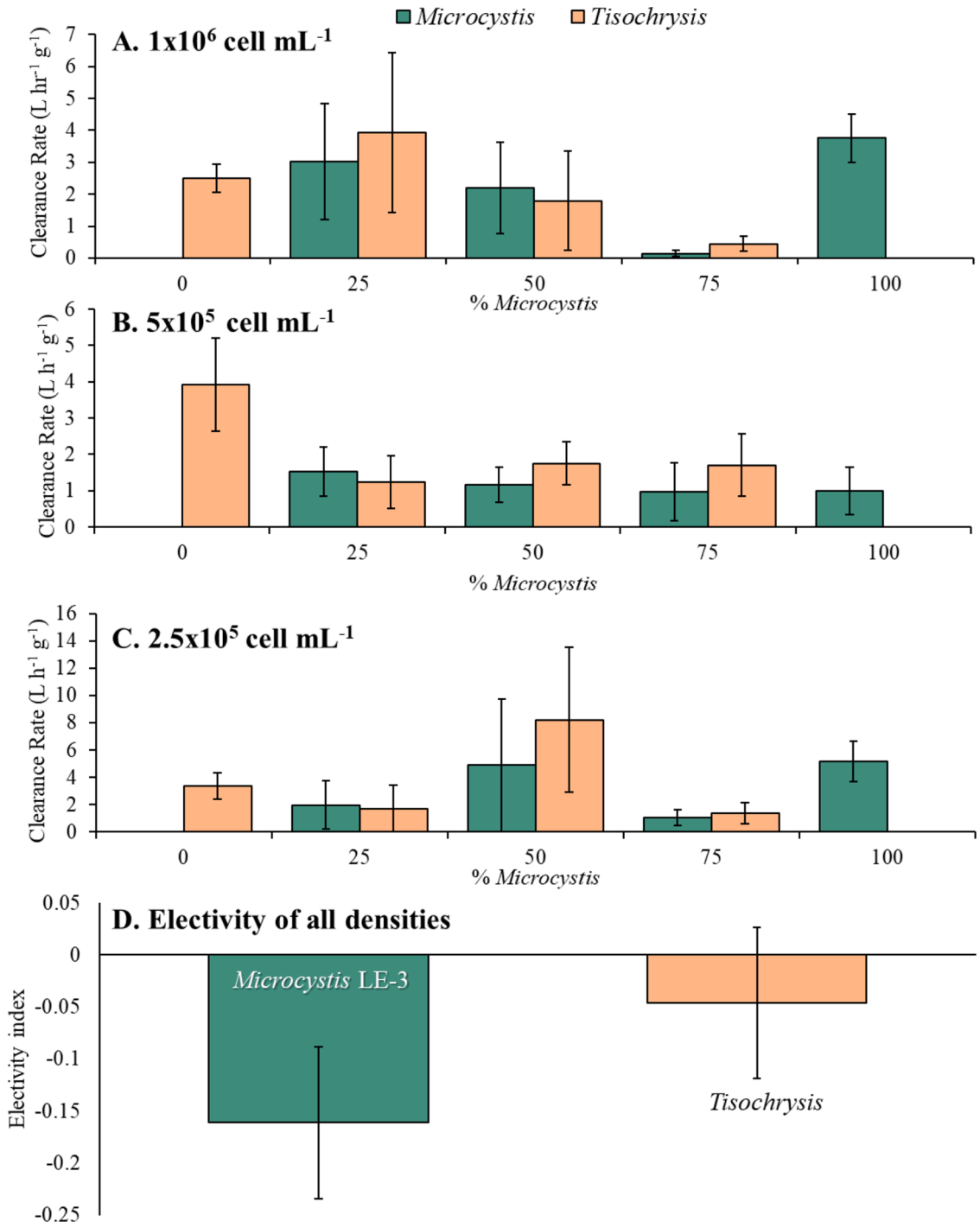


Fig. 4.. Clearance rates of mixed treatments of the toxic cyanobacterium *Microcystis* and nontoxic algae *Tisochrysis* by the eastern oysters. Oysters were incubated in five ratios of mixed cultures at three densities A) 1×10^6, B) 5×10^5 and C) 2.5×10^5 cell mL^{-1}. There was no significant difference between algal type or treatments in the mixed culture-based oyster experiments ($p>0.05$, Two-way ANOVA). Each bar represents average clearance rate standardized to dry oyster weight over the 60-minute exposure and error bars indicate standard error. There was a negative electivity index for *Microcystis* and *Tisochrysis* (D).

between day 2 and 3 ($p<0.05$, One-way ANOVA, $p<0.05$ Tukey).

3.2. Natural bloom clearance rate experiments

3.2.1. Asian clam and bloom water clearance rate experiment

From March to May the green algae class was most abundant in Lake Agawam (mean = 84 μg green algae L^{-1}), but for the rest of the year, and during experiments that were all performed during the fall, cyanobacteria were the dominant class ranging from 64 – 5000 $\mu g\ L^{-1}$ with all other groups being <10 $\mu g\ L^{-1}$ (Fig. 7). Microscopic evaluation indicated that *Microcystis* was the dominant algal species for all blooms, although green algae, diatoms, and other cyanobacteria species were also present. Levels of cyanobacteria during the hour-long clearance rate experiment with Asian clams (average of 142 μg cyanobacteria L^{-1} on 10/2/20) were similar to levels during the four-day accumulation experiment with Asian clams (average of 117 μg cyanobacteria L^{-1} from 10/8/20 to 10/11/20; Fig. 7).

Asian clams exposed to whole (100% = 142 μg chl-*a* L^{-1}) and

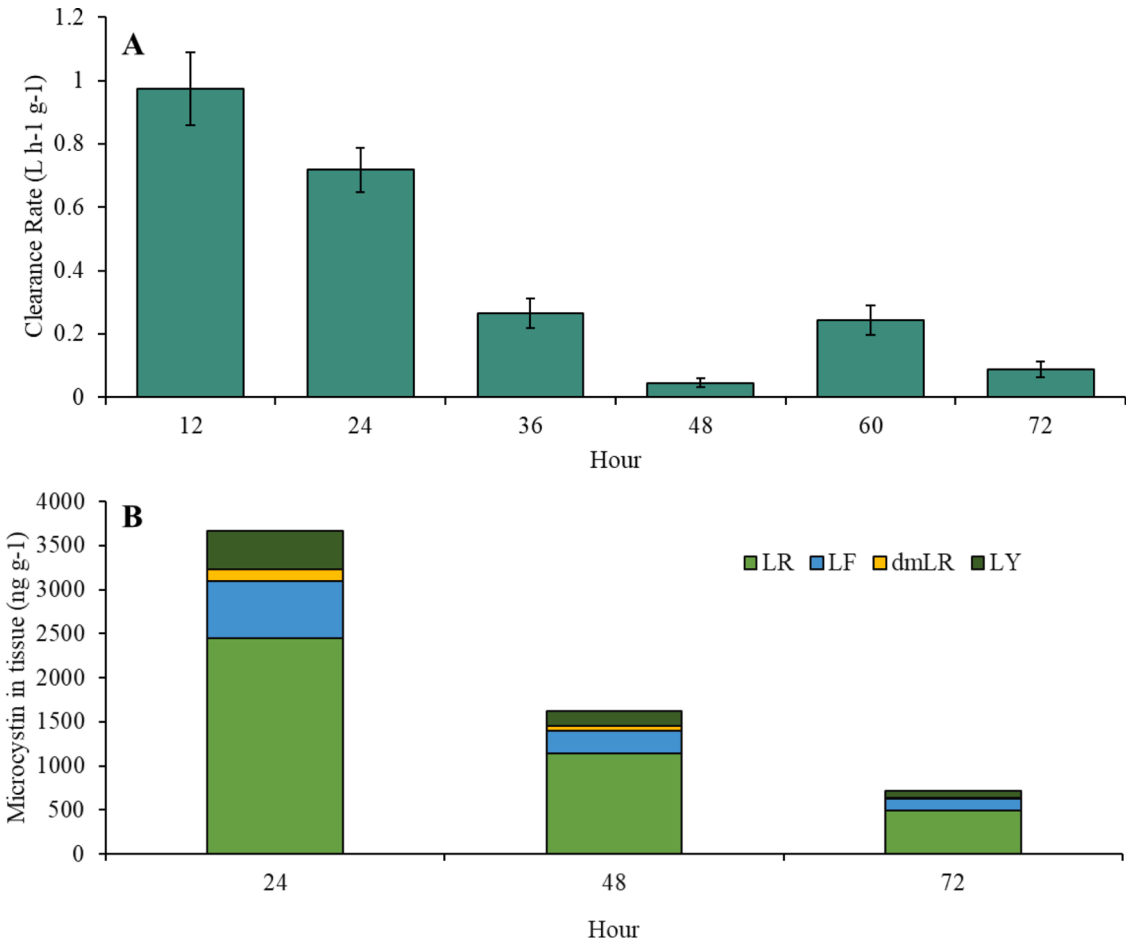


Fig. 5.. A. Clearance rates and B. microcystin accumulation of Asian clams during exposure to *Microcystis* LE3 over three days. Bars are means and error bars indicate standard error. Clearance rates and tissue toxins decreased significantly over the three days ($p<0.001$; One-way ANOVA).

diluted (25%, 50%, 75% = 35, 71, 106 μg chl-*a* L^{-1}) natural bloom water from Lake Agawam, NY, had significantly higher clearance rates in more dilute bloom water and had faster clearance rates for green algae compared to cyanobacteria ($p<0.05$ for both; two-way ANOVA; Fig. 8A). An important point here is that green algae levels were only ~4 μg chl-*a* L^{-1} in the 100% treatment, so in no way was biomass matched between algal classes. Clearance rates were 0.59 L h^{-1} g^{-1} for cyanobacteria and 1.71 L h^{-1} g^{-1} for green algae in the 25% treatment, 1.33 L h^{-1} g^{-1} for cyanobacteria and 2.26 L h^{-1} g^{-1} for green algae in the 50%, 0.09 L h^{-1} g^{-1} for cyanobacteria and 1.05 L h^{-1} g^{-1} for green algae in the 75%, and 0.13 L h^{-1} g^{-1} and 1.09 L h^{-1} g^{-1} for green algae in whole bloom water (Fig. 8A). Tukey tests indicated significant differences across levels ($p<0.05$; Tukey Test) between algal types in all treatments except for 50% and for the 50% treatment compared to the 100% and the 75% treatment for the blue green data. Clams did not produce pseudofeces during the 60-minute exposure. Clams overall had a significantly higher electivity index for green algae compared to cyanobacteria (−0.412±0.063 and 0.107±0.055, $p<0.05$, Mann-Whitney Rank Sum test, Fig. 8B;; Supplemental Table 3).

3.2.2. *Oyster and bloom water clearance rate experiment*

The oyster hour-long clearance rate experiment was performed with the densest bloom of the year (whole water reading of ~5000 μg chl-*a* L^{-1} on 10/24/20. Oysters incubated in bloom water from Lake Agawam, NY, had similar clearance rates for cyanobacteria across the three concentrations: 25%, 50%, and 75% (543, 1096, 1681 μg chl-*a* L^{-1} after dilution; $p>0.05$, ANOVA on ranks; Fig. 9); levels of green algae were below detection limited during this experiment. Oysters produced some pseudofeces, but they were too difficult to collect and quantify. Attempts at collection caused the pseudofeces to disperse and associated mucous that was collected stuck inside pipette tips.

3.2.3. *Four-day bloom Asian clam clearance rates and hepatotoxin accumulation*

During the four-day experiment with Asian clams using Lake Agawam bloom water (mean cyanobacterial biomass = 118±15 μg chl-*a* L^{-1} from 10/8/20 to 10/11/20), clams cleared green algae at an average of 40% higher rates than cyanobacteria (Fig. 10A). There were no significant differences in the clearance of green algae and cyanobacteria over the four days ($p>0.05$; ANOVA on ranks). The clearance rates of green algae went from 0.75 L h^{-1} g^{-1} to 0.16 L h^{-1} g^{-1} on the final day. The cyanobacteria clearance rates were 0.50 L h^{-1} g^{-1} on the first day and dropped to 0.12 L h^{-1} g^{-1} on the final day. The total microcystin found in the tissues was low and did not differ significantly over time being 0.06 ng g^{-1}, 0.23 ng g^{-1}, 0.17 ng g^{-1}, and 0.035 ng g^{-1} after 24, 48, 72, and 96 h ($p>0.05$, One-way ANOVA). Again, green algae levels on average were much lower than cyanobacteria (4.6 ± 0.23 μg chl-*a* L^{-1}). Clams had a significantly higher electivity index for green algae compared cyanobacteria (−0.084±0.014 and 0.032±0.020, respectively, $p<0.05$, Mann-Whitney Rank Sum test, Fig. 10B; Supplemental Table 3).

3.2.4. *Four-day bloom oyster clearance rates and hepatotoxin accumulation*

During the four-day experiment with oysters (mean cyanobacterial biomass = 71±12 μg L^{-1} from 10/27/20 to 10/30/20), the clearance

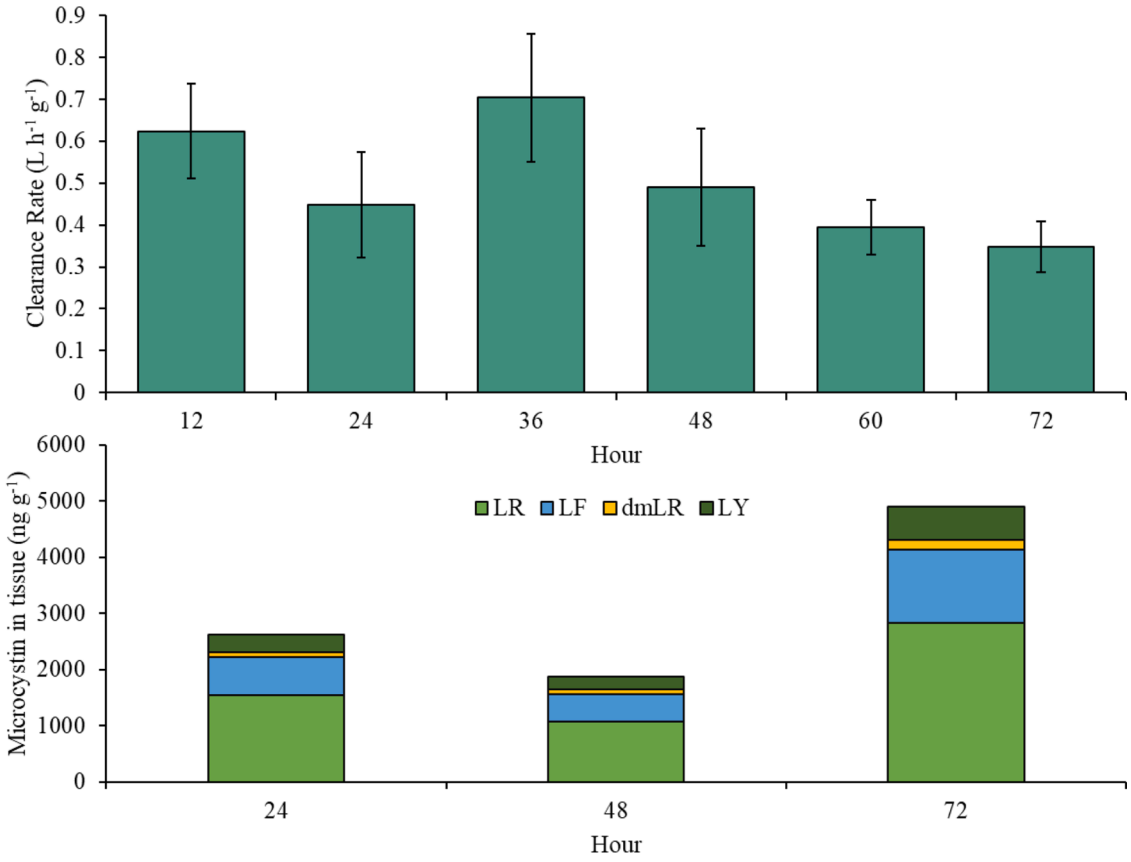


Fig. 6.. A. Clearance rates and B. microcystin accumulation of eastern oysters during exposure to *Microcystis* LE3 over three days. Bars are means and error bars indicate standard error. Clearance rates decreased slightly over the three days ($p>0.05$, One way-ANOVA while tissue toxins increased ($p<0.05$, One-way ANOVA).

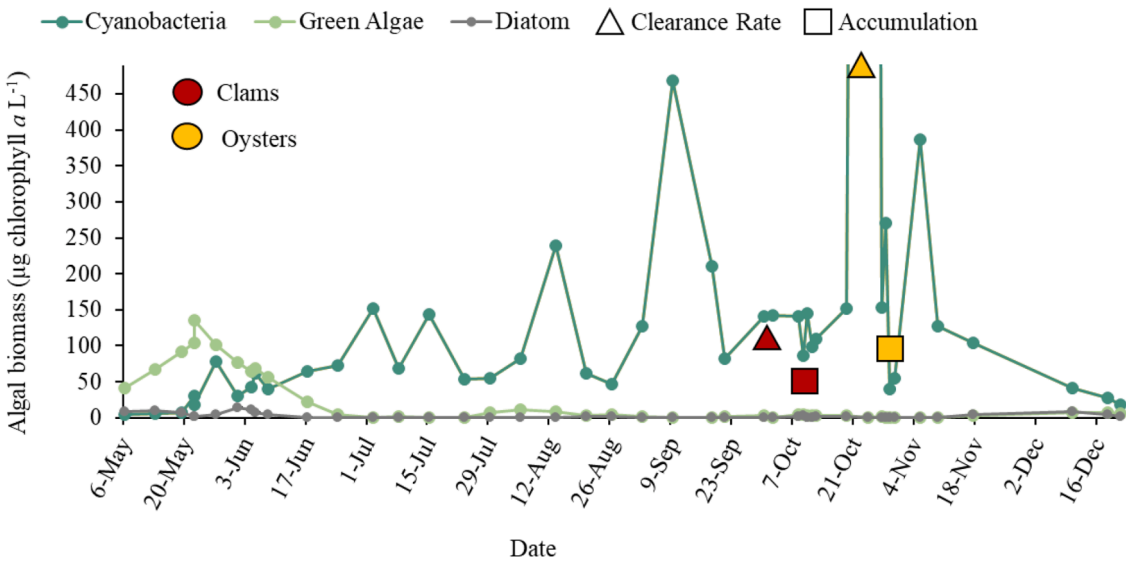


Fig. 7.. Dynamics of cyanobacteria, green algae, and diatoms in Lake Agawam, NY, USA, May to December 2020. Triangles indicate the date of clearance rate experiments while squares denote dates of microcystin accumulation experiments. Clam experiments are red symbols whereas oyster experiments are yellow symbols.

rates standardized to dry weights for cyanobacteria started at 0.51 ± 0.07 L h^{-1} g^{-1}, peaked at 0.79 ± 0.15 L h^{-1} g^{-1} at 36 h, and then dropped to 0.16 ± 0.07 L h^{-1} g^{-1} for the 96-hour time point. The oyster's clearance rates of green algae were nearly an order of magnitude lower than those of cyanobacteria (0.08 ± 0.03 L h^{-1} g^{-1}; Fig. 11), and oyster clearance rates of cyanobacteria declined over the course of the experiment ($p<0.05$; ANOVA on ranks). Clearance rates at 12-hour and 36-hour time points were significantly higher than rates for the 48-, 60-, 72-, 84-, and 96-hour time points ($p<0.05$ Tukey). The total microcystin found in the tissues increased 20-fold during the experiment but did not differ significantly over time, at 0.09 ng g^{-1}, 0.68 ng g^{-1}, 1.94 ng g^{-1}, and 1.45 ng g^{-1} after 24, 48, 72, and 96 h ($p>0.05$, One-

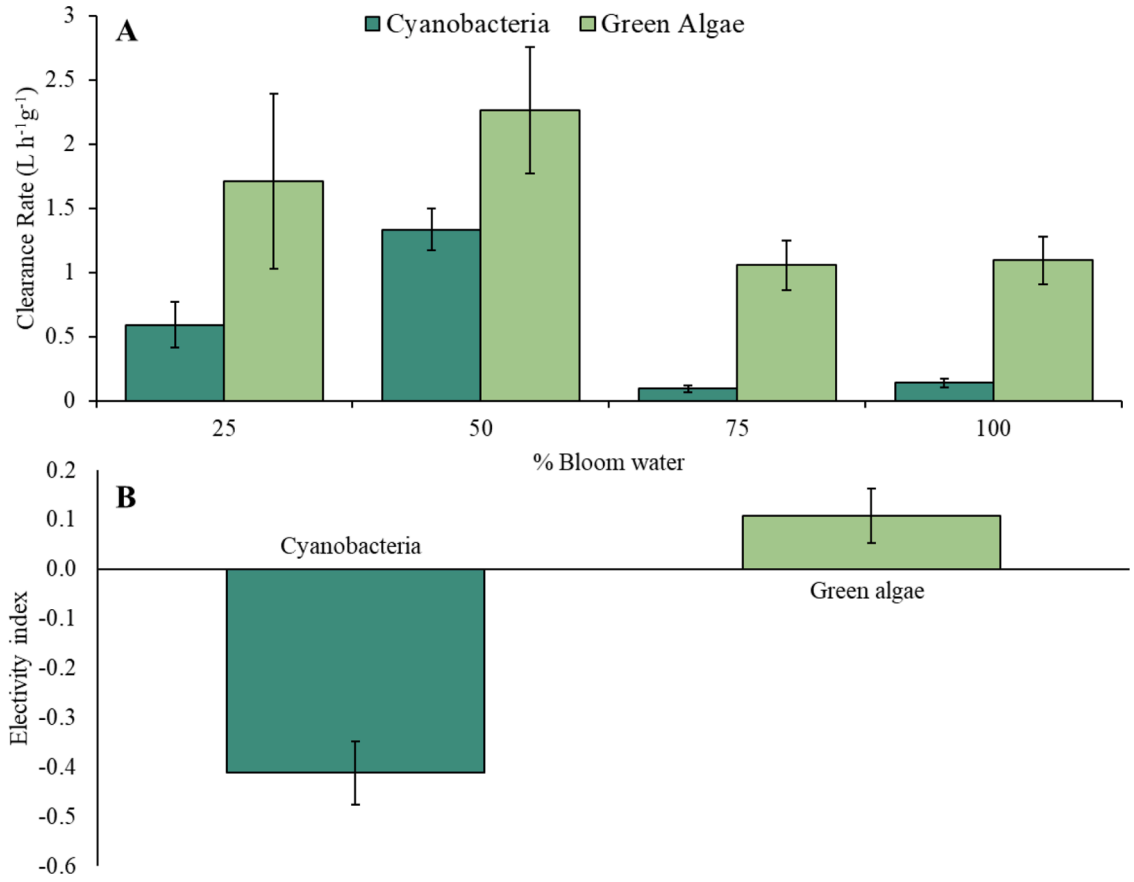


Fig. 8.. A. Asian clam clearance rates of cyanobacteria and green algae during a *Microcystis*-dominated bloom within Lake Agawam, NY, USA. B. Electivity index of clams for cyanobacteria and green algae. Whole bloom water (100%) was progressively diluted with filtered lake water at proportions of 75, 50, and 25% lake water (25, 50, and 75% filtered water). Clams has significantly lower electivity index for cyanobacteria compared to green algae ($p<0.05$, Mann-Whitney Rank Sum Test). Error bars indicate standard error.

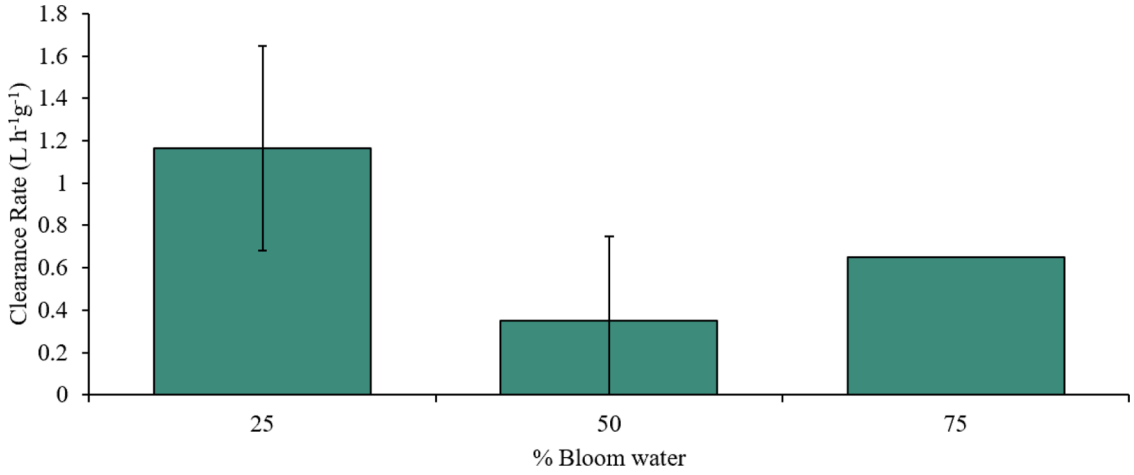


Fig. 9.. Clearance rate of eastern oysters during a *Microcystis*-dominated bloom in Lake Agawam, NY, USA. Whole bloom water (100%) was progressively diluted with filtered lake water at proportions of 75, 50, and 25% lake water (25, 50, and 75% filtered water). The oysters had similar clearance rates for cyanobacteria across the three concentrations ($p>0.05$, ANOVA on ranks). Error bars indicate standard error. The monospecific nature of the bloom prohibited the determination of electivity indexes.

way ANOVA). Oysters produced pseudofeces during this field experiments which were added to BG11 media in a 21 °C incubator but did not produce vegetative cells after two weeks. Two oysters died over the course of this four-day experiment.

4. Discussion

This study was conducted to assess the ability of bivalves to filter feed *Microcystis* and to determine whether or not they accumulated microcystin, in order to gain insight regarding how bivalves may be affected by freshwater blooms that migrate into estuaries. While Asian

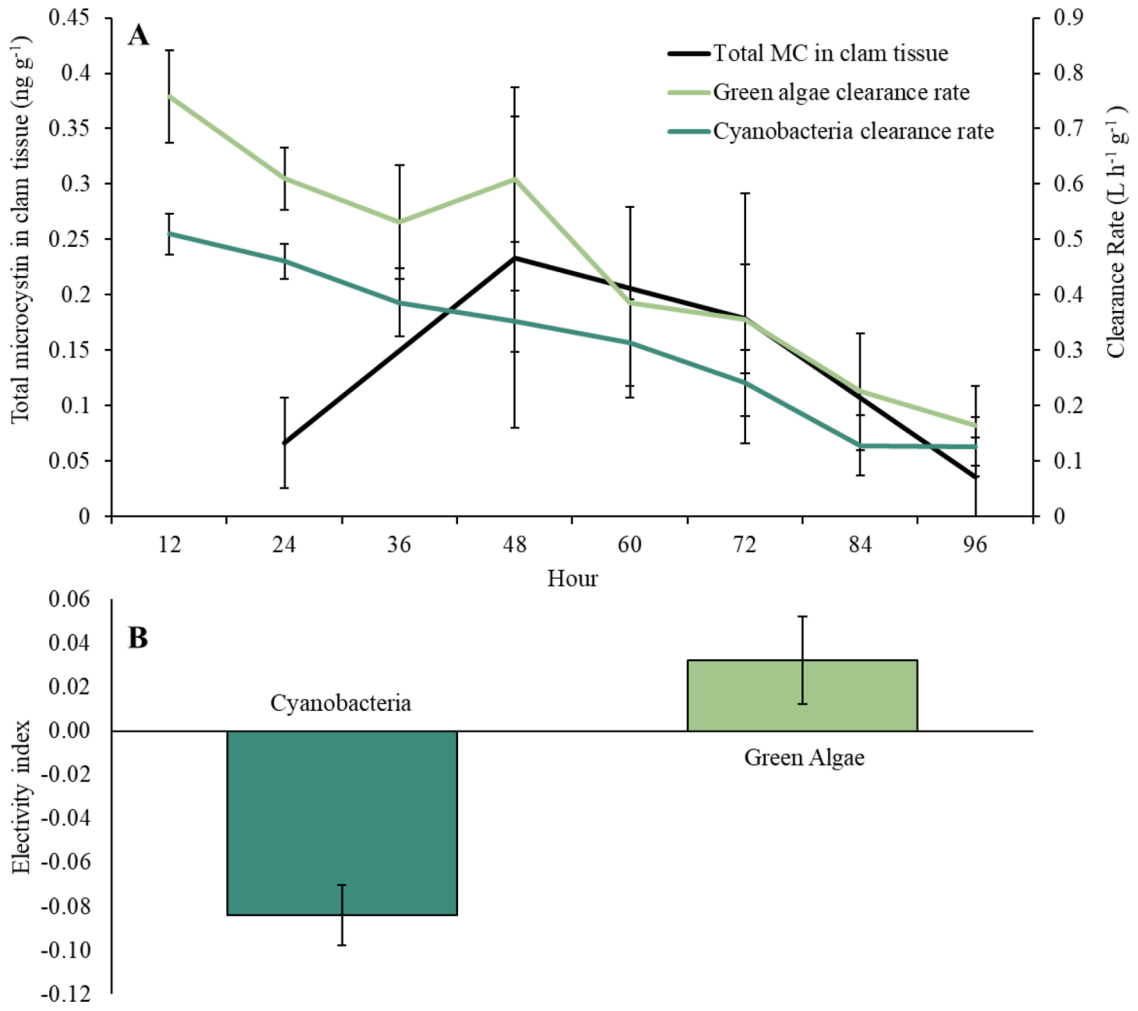


Fig. 10.. A. Clearance rates and microcystin accumulation of Asian clams a during four-day exposure to Lake Agawam bloom water. B. Electivity index of clams for cyanobacteria and green algae. Clams had significantly higher electivity index (B) for green algae compared to cyanobacteria ($p<0.05$, Mann-Whitney Rank Sum test). Error bars indicate standard error.

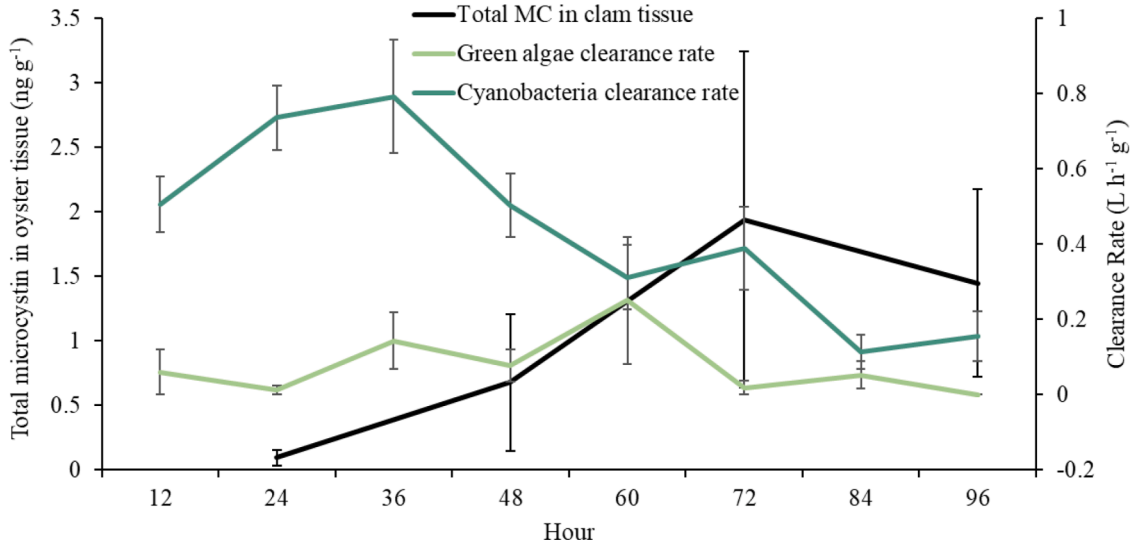


Fig. 11. Clearance rates and microcystin accumulation of eastern oysters during a four-day experiment with water from Lake Agawam. Clearance rates of cyanobacteria significantly declined over the experiment ($p<0.05$, ANOVA on ranks), and the microcystin in oyster tissue increased but not significantly ($p>0.05$, One-way ANOVA). The monospecific nature of the bloom prohibited the determination of electivity indexes.

clams readily cleared *Microcystis* and accumulated microcystin in culture, their filtration rates slowed after short exposure periods (days), resulting in net hepatotoxin depuration. In addition, Asian clams cleared natural bloom populations of *Microcystis* significantly slower than cultures and preferentially cleared green algae over *Microcystis* blooms and, consequently, accumulated lower microcystin amounts when exposed to blooms compared to cultures of *Microcystis*. While eastern oysters also cleared *Microcystis* from cultures at a faster rate than field bloom populations, they displayed no depuration of microcystin over multi-day exposures and accumulated greater amounts of microcystin compared to clams when exposed to field bloom conditions. Collectively, these observations bring new insight into the prospect of microcystin accumulation in bivalves.

4.1. Microcystis

4.1.1. Microcystis sp. blooms vs Microcystis aeruginosa LE-3 culture

While there were many differences between experiments with bloom water and cultures, one of the greatest was the distribution of particle sizes. *Microcystis aeruginosa* LE-3 is a single or double cell strain whereas the bloom population in Lake Agawam exist primarily as large colonies (>100 μm), as is the case for most field populations (Orr et al., 2004; Harke et al., 2016). A culture of 1×10^6 cells mL^{-1} *Microcystis aeruginosa* LE-3 was equivalent to ~30 μg chl-aL^{-1} using the BBE Fluoroprobe whereas experiments performed with bloom water had initial undiluted biomass levels of 143 and 4817 μg chl-aL^{-1} for clam and oyster 1-hour experiments and 118 and 129 μg chl-aL^{-1} averages for clam and oyster 4-day experiments. While these were diluted up to 25%, biomass levels and, likely *Microcystis* densities were higher during bloom experiments. Hence, the lower clearance rates during field experiments could have been caused by higher biomass levels (Riisgård, 1988, 2001) as well as the fact that *Microcystis* exists in dense colonies.

4.1.2. Microcystis nutritional values

Bivalve clearance rates can also be influenced by the nutritional qualities of the plankton on which they feed. Algae grown under different conditions, in this case Lake Agawam compared to nutrient-rich BG-11 media, can be biochemically different (Waterbury and Stanier, 1981; Wikfors et al., 1984). In long-term studies examining the growth of *C. fluminea* fed cyanobacteria, clams had positive somatic growth rates compared to starved clams but grew substantially slower compared to a diet of eukaryotic algae with a higher lipid content (Basen et al., 2011). *Microcystis* has a low concentration of sterols, an important biochemical nutrient, and when cyanobacterial diets are supplemented with sterols, clams grow significantly faster (Basen et al., 2012). Given the short-term nature of these experiments, direct growth effects on bivalves would be minimal, but nutritional content could have altered clearance rates during multi-day experiments as consumption and digestion of cells might have begun to influence the physiology of the bivalves (Capelle et al., 2021; Dutertre et al., 2017).

4.2. Clearance rates

4.2.1. Asian clam clearance rates

During one-hour exposures to *Microcystis*, clearance rates of Asian clam were significantly greater than clearance rates during multi-day exposures. Liu et al. (2009) had found a statistically significant lower hourly clearance rate when these clams were exposed to 1×10^6 *M. aeruginosa* cells mL^{-1} compared to a nontoxic alga of the same density. In contrast, during the present study, the clams fed *Microcystis* cleared cells significantly faster than the control algae at all three biomass levels. In addition, clams had higher clearance rates when fed mixtures of *Microcystis* and *Raphidocelis* compared to monoculture (100% *Microcystis* or *Raphidocelis*) treatments, though not significantly so. This difference has also been found for another freshwater invasive bivalve, the zebra mussel (*Dreissena polymorpha*), which had significantly higher clearance rates for *Microcystis* and *Scenedesmus* mixtures compared to monocultures of the algae or cyanobacteria (Dionisio Pires et al., 2004). Dionisio Pires et al. (2004) suggested this was due to 1) particle texture and taste and 2) the particle mixture has to be drawn into the clam first before being rejected on the gill surfaces (Vanderploeg et al., 2001). In the present study, the higher clearance rates in the mixture treatments might have been due to rapid sorting between the spherical *Microcystis* and the sickle-shaped *Raphidocelis* that was twice the biovolume. Clams were more selective than oysters, electing for *Microcystis* LE-3 over *Raphidocelis* in culture experiments and for green algae over cyanobacteria in field experiments.

4.2.2. Oyster clearance rates

In contrast to Asian clams, oysters discriminated less between *Microcystis* and other algae as there was no significant difference in their clearance of *Microcystis* and *Tisochrysis* in monoculture trials and there was no difference in electivity indexes of oysters for cultured *Microcystis* and *Tisochrysis*. Similarly, zebra mussels do not discriminate against cyanobacteria and graze effectively on small (4 μm) *Microcystis* cells (Dionisio Pires et al., 2004). In addition, unlike Asian clams that displayed an order of magnitude decline in clearance rates during multi-day exposures to *Microcystis* cultures, oyster feeding rates were unchanged over a 72-h exposure period. During bloom exposures, clearance rates by oysters were lower than the clearance rates for cultures and rates declined slightly over a 96-h exposure but remained at levels more than an order of magnitude greater than Asian clams throughout the experiment. While examining bivalve clearance indicates whether algal cells were initially ingested, pseudofeces production indicates rejection of food prior to digestion (Ward et al., 1994). Although Asian clams did not produce pseudofeces, oysters did so during field experiments indicating rejection of some *Microcystis*.

4.3. Particle size and biomass

4.3.1. Asian clams and particle size

As previously noted, differences in biomass levels and particle sizes could explain the differences in clearance rates between field and lab experiments. Asian clams are generally considered to be non-selective suspension feeders (Lauritsen 1986; Vaughn and Hakenkamp 2001), and particle sorting in bivalves has been found to be affected by the size and shape of suspended materials (Ward et al., 1998, Vanderploeg et al., 2001, Basen et al., 2011). Asian clams can clear particles ranging in size from 5 to 30,000 μm^2 (Wallace et al., 1977, Lauritsen 1983). Digital imaging flow cytometry has documented *Microcystis* colonies ranging from 579 μm^2 to 39,029 μm^2 in size (Park et al., 2019), suggesting that clams could theoretically physically manage some of the large colonies though the range in sizes of the *Microcystis* colonies from Lake Agawam are not known. Additionally, variation in gill structure between bivalves is thought to create differences in particle sorting ability (Ward et al., 1997; Basen et al., 2011). *Corbicula* gill structures have a greater surface area (more cilia and cirri) compared to other freshwater bivalves which allows them to be more efficient at removing particles from the water column, although particle size becomes more influential on filtration rate when particle concentration is low for some other bivalves (Vaughn and Hakenkamp, 2001; Bolam et al., 2019). Short term (1 h) clearance rates of Asian clam were lower when exposed to bloom water from Lake Agawam as compared to *Microcystis aeruginosa* LE-3 in culture, with culture-based clearance rates ranging from 1 – 5 L g^{-1} d^{-1} and bloom-water rates ranging from 0.1 – 1.3 L g^{-1} d^{-1} ($p<0.05$, ANOVA on Ranks). The *Microcystis* colonies may have been too large to be quickly sorted in the one-hour clearance rate experiment, while the ~4 μm (~50 μm^2) single cells of *M. aeruginosa* LE-3 and 2–8 μm *Raphidocelis* cells were likely easily drawn in by the clams. In experiments with zebra mussels, bloom water containing toxic *Microcystis* colonies >53 μm were filtered less efficiently than colonies <53 μm (Vanderploeg et al., 2001). In the Boegehold and Kashian (2021) experiment, *C. fluminea* had higher

filtration rates of the unicellular strain of *Microcystis aeruginosa* offered over the 96 h as compared to the colonial strain, which was likely due to size difference since no microcystins were present. Other *Corbicula* sp. have shown the ability to reject toxic *Microcystis* cells without metabolizing them (Pham et al., 2015), but this was not seen in these experiments as cells were consumed and pseudofeces were not produced during both short (one hour) and long-term experiments (four days). *C. fluminea* are capable of removing single cell, chain-forming and filamentous cyanobacteria (*Synechococcus elongatus, Anabaena variabilis, Aphanizomenon flos-aquae*), but typically produce more pseudofeces and have lower ingestion when exposed to these cyanobacteria (Basen et al., 2011, 2012). The lack of pseudofeces production when fed *Microcystis* indicates that *C. fluminea* did not reject the *Microcystis* in the lab or field but rather consumed cells, albeit at extremely low rates in the case of the field experiments, potentially due to the higher biomass present (Riisgård, 1988, 2001).

4.3.2. Oysters and particle size

Oysters are well-known for their rapid filtration rates (Newell, 2004; zu Ermgassen et al., 2013; Weissberger and Glibert, 2021) and are known to specifically filter particles greater than 5 μm with high efficiency (Riisgaard, 1988) and remove particles 2–4 μm more efficiently than 1 μm particles (Haven and Morales-Alamo, 1970). *Microcystis* LE-3 and *Tisochrysis* are near-spherical microalga with 4 μm and 5 μm diameters, respectively, and there was no significant difference between clearance rates in mixed trials for the similar sized algae. Oysters can preferentially ingest and reject organic material found in natural seston (Newell and Jordan 1983; Ward et al., 2019; Weissberger and Glibert, 2021) that travels across the gills by grooves and tracts with materials further sorted by cilia (Yonge, 1923; Ward et al., 1994). Using endoscopy, Ward et al. (1994) deduced that an increase in particle density led to a "slurry" of particles traveling across gill grooves and higher rate of intake. Oyster clearance rates were lowest in the one-hour bloom experiment, likely due to the high *Microcystis* density and/or the colony morphology as this experiment has nearly an order of magnitude higher biomass levels than other field experiments. When exposed to high concentrations of algae, the grooves in which particles travel in oysters produced excess mucus (Ward et al., 1994). This mucus might explain the species differences in clearance rates and needs more exploration. Despite the high biomass for short term (one hour) experiments for the oysters, the clearance rates were not the lowest seen for all oyster experiments (1.16±0.48 μg chl-aL^{-1} for 25% dilution, 0.35±0.39 for the 50% dilution, and 0.65±n/a for the 75% dilution). The oyster experiment with the lowest clearance rates was the 4-day bloom water experiment. In this case there was a much lower average density of cyanobacteria (129 μg chl-aL^{-1})) cleared which peaked at 0.79 L h^{-1} g^{-1} at 36 h and dropped to 0.1 L h^{-1} g^{-1} by 96 h, suggesting prolonged exposure to blooms has a greater inhibitory effect on clearance rates than high biomass levels.

4.4. Hepatotoxin accumulation

4.4.1. Hepatotoxin accumulation in Asian clams

During exposures to *Microcystis* (field and culture), clearance rates for Asian clams began high but then declined to low levels after 24 h suggesting an effect by the accumulation and exposure to *Microcystis* cells and associated compounds. As noted earlier, *Microcystis* is a poor nutritional source for bivalves (Basen et al., 2011; Basen et al., 2012). A recent study of Asian clams found that high concentrations of microcystin from *Microcystis* cells can reduce glutathione sulfur transferase activity which is a detoxification biomarker and thus could cause impairment and, ultimately, mortality (Chen et al., 2021). In addition, exposure to microcystin-producing *Microcystis* suppresses immune functions in zebra mussels (Juhel et al., 2015). Hence, short-term exposure to *Microcystis* may have compromised the physiology of Asian clams, contributing to lowered clearance rates. While assessing the chronic toxicity of microcystins was outside the scope of this study, Gene et al. (2019) observed that microcystins can exert toxic effects on juvenile freshwater mussels (*Lampsilis siliquiodea*) at environmentally relevant concentrations.

The temporally dynamic feeding by Asian clams and differences between culture and field-based clearance rates were reflected in microcystin accumulation and depuration. In both cases, clams accumulated higher levels of microcystin after 24 – 48 h compared to the end of experiments, evidence for net microcystin accumulation followed by net depuration. The absolute concentrations of microcystin accumulated provides insight into this process and potential mechanisms as well. In culture, clams contained ~4000 ng g^{-1} after only 24 h of exposure but decreased to 1600 and 700 ng g^{-1} after 48 and 72 h, respectively, indicating a rapid hepatotoxin accumulation and depuration, despite continued exposure and clearance of cells. Depuration rates over the final two days were linear at ~60 ng g^{-1} h^{-1}, a rate that, if continued, would have fully eliminated microcystin from tissues in another day. Bloom exposed clams only accumulated 0.2 ng microcystin g^{-1} after 48 h and depurated to < 0.1 ng microcystin g^{-1} after 96 h suggesting the lower clearance rates in the presence of colonies limited uptake of microcystin. The significant reduction in clearance rates despite low levels of microcystin accumulation in tissues suggests that reduced clearance rates in field populations were a function of food quality (e.g., colonies) or quantity rather than the accumulation of microcystin in tissues. While it appears that Asian clams can fully depurate microcystins from their tissues in a matter of days, longer experiments are needed to confirm this. Regardless, it is of note that in another study where *C. fluminea* were fed a culture of *M. aeruginosa* (10^5 cells L^{-1}), microcystin accumulated to 26 - 100 ng g^{-1} after four days (Freitas et al., 2014). While this study did not track microcystin levels over time, it is likely that clams in our experiment would have contained levels of microcystin within this range after four days had the decay rate observed during the first 72 h of the experiment continued.

4.4.2. Hepatotoxin accumulation in oysters

The findings of this study suggest that eastern oysters are likely to accumulate microcystins present in brackish water environments downstream of intense *Microcystis* blooms due to in high bioaccumulation and slow depuration. During a three-day clearance rate experiment, oysters filtered at constant rates and accumulated 3000 – 5000 ng microcystin g^{-1} after three days with no evidence of hepatotoxin depuration. In a study by Gibble et al. (2016), cultured oysters from the US West Coast (*Crassostrea sp.*) were exposed to a culture of ~1 × 10^6 cells mL^{-1} *Microcystis* water for 24 hour when they accumulated 5 ng microcystin g^{-1} and were moved to *Microcystis*-free water where they exponentially depurated more than half of the cyanotoxin in 12 h, but retained the remaining microcystin for four weeks and still had detectable levels after 8 weeks. This indicates that microcystin can persist in oyster tissue months even after a brief (24 hour) exposure to microcystin. For other algal toxins, like brevetoxins, eastern oysters depurated 78% after two weeks, but 22% brevetoxin and subsequent metabolites were still detectable in oysters after 82 days (Griffith et al., 2013). Given that depuration times of organic contaminants in oysters tend to depend on contaminant molecular weights (Sericano et al., 1996), it would be of interest to understand depuration of multiple microcystin congeners over time. Regardless, the results of this and prior studies suggest oysters can retain microcystin for weeks-to-month after exposure to blooms of *Microcystis* and thus transfer the cyanotoxin to humans if ingested.

While oyster clearance rates were significantly lower during field exposures compared to experiments with culture, rates were an order of magnitude higher than Asian clams. Still, microcystin accumulation in oysters was three orders of magnitude lower during bloom experiments compared to laboratory experiments, suggesting that the lowered clearance rates as well as the presence of *Microcystis* colonies limited the uptake of microcystin. In addition, during the bloom water experiment,

oysters produced pseudofeces that would have also lowered cyanotoxin accumulation since some cleared cells and colonies were rejected as pseudofeces. Pseudofeces production by eastern oysters varies for harmful marine algae in 1×10^4 cells mL^{-1} densities, with *Heterosigma akashiwo* causing the oysters to produce no pseudofeces, *Alexandrium fundyense* causing small amounts of feces production, and *Prorocentrum minimum* causing both feces and pseudofeces production (Hegaret et al., 2007). In the present study, oysters removed some *Microcystis* cells and colonies but might have avoided some microcystins by producing pseudofeces.

During the four-day accumulation experiment, two oysters died, an outcome that did not occur during the 72-hour experimental exposure to *M. aeruginosa* cultures. The microcystin content for the bloom water was, on average 32 μg L^{-1}, compared to the concentrations in cultured algae at 114 μg L^{-1}, and the microcystin content of oysters during the field experiments was orders of magnitude lower than the culture experiment suggesting that microcystin was not solely responsible for the mortalities. As noted above, clearance rates were lower during the field experiment and progressively declined over the course of the experiment. Moreover, the production of pseudofeces and low sterol content of *Microcystis* (Basen et al., 2011, 2012) would have reduced nutritional intake for these animals even further. This lack of nutrition coupled with impairment of glutathione sulfur transferase activity (Chen et al., 2021) and immune function (Juhel et al., 2015) all likely contributed to the mortality of these oysters. It is likely a more prolonged exposure could have cause additional mortality in this experiment and that such an outcome in an ecosystem setting would also be plausible.

4.4.2.1. Salinity tolerance of Microcystis. The increasing prevalence of *Microcystis* blooms globally (Ho et al., 2019) coupled with the salinity tolerance of *Microcystis* (Orr et al., 2004; Preece et al., 2017) has increased the likelihood of *Microcystis* and therefore microcystin entering and persisting in estuaries. *Microcystis* cells can tolerate brackish waters of up to 15 PSU, although higher salinities cause cell-lysis and subsequent release of toxins (Orr et al., 2004; Preece et al., 2017). During this study, the single-celled *Microcystis* LE-3 did not lyse in 15 PSU while changes in colony morphology in the oyster bloom experiments was not noted. Gibble et al. (2016) noted that *Microcystis* cells in colonies can remain intact and viable for 24 h even at full salinity. Adult *C. virginica* can tolerate and even thrive at salinities less than 3 PSU where they have a refuge from disease and marine predators (Levinton et al., 2011) whereas juveniles grow optimally at 7–16 PSU (Heilmayer et al., 2008; Scharping et al., 2019). Salinities above 15 PSU would cause a break-up of *Microcystis* colonies and eventual decrease in total *Microcystis* cell concentrations as cells lyse over time, causing a subsequent increase in dissolved or abiotic particulate microcystin levels in the water (Gerard et al., 2005) which may be absorbed by oysters (Gibble et al., 2016). There are, therefore, multiple factors that make eastern oyster particularly vulnerable to microcystin exposure in estuaries hosting microcystin-producing CHABs in their headwaters.

4.5. HAB mitigation using bivalves

Bivalves, especially freshwater dreissenids, are often considered to be a phytoplankton bloom or even HAB control measure due to their prodigious filter feeding capacities (Officer et al., 1982; Waajen et al., 2016). This study documented the potential of bivalves to filter feed during intense and toxic blooms, at least during short term, monoculture experiments. Other findings, however, indicate that deploying Asian clams or eastern oysters to control *Microcystis* blooms would likely be ineffective. The Asian clams cleared cyanobacteria from the bloom water at a lower rate than they cleared green algae, suggesting that in an ecosystem setting, Asian clams could selectively promote cyanobacterial blooms or at least not remove enough cyanobacteria to control a bloom. Moreover, as an invasive species, Asian clams would ideally not be deployed in an ecosystem setting, where free-swimming larvae could easily invade other water bodies (Hassett et al., 2017). While oysters had similar clearance rates of cyanobacteria and green algae and cleared bloom populations more rapidly than clams, they are not found in lakes where these HABs occur. While they may effectively filter feed *Microcystis* blooms transported into estuaries, this scenario could create a human health threat if they are subsequently harvested.

4.6. Human health implication

Upon exposure to wild and cultured *Microcystis*, Asian clams accumulated 0.2 ng g^{-1} after 48-hour exposure to colony forming and 3668 ng g^{-1} after 24-hour exposure to a single and double celled strain, while accumulation in the oysters peaked at 1.9 ng g^{-1} and 4905 ng g^{-1} after 72 h of exposure. Oyster bloom water values were comparable to those found by Gibble et al. (2016; 5 ng g^{-1}) who also collected bloom water and mixed it with saltwater. The California Office of Environmental Health and Hazard Assessment sets the limit on consumption of seafood with microcystin levels at 10 ng g^{-1} (Gibble et al., 2016), meaning that the peak microcystin levels in tissue exposed to *Microcystis* cultures were 400-fold and 500-fold greater than the advisable limit for Asian clams and oysters, respectively. These extreme levels of cyanotoxins compared to the levels when exposed to large colonies suggests that blooms made up of individual cells or small colonies could lead to rapid accumulation of microcystin, again creating a public health threat. Certain biotic and abiotic conditions can favor unicellular *Microcystis* populations including the absence of other cyanobacterial species and zooplankton, high temperatures, high light levels, low nutrient input, and low levels of heterotrophic bacteria (Harke et al., 2017; Xiao et al., 2018). Clams were able to depurate microcystin faster than oysters and reduced their clearance rates rapidly when exposed to *Microcystis* blooms, while oysters did not, indicating oysters are more likely to accumulate and retain microcystin. The salinity tolerance of *Microcystis* has potential to cause colonies to lyse into smaller particles as they are transported into estuaries (Orr et al., 2004) potentially making them more easily assimilated by oysters. Colonies breakup into smaller particles could create a scenario where oysters are progressively exposed to smaller, easy to assimilate *Microcystis* cells / colonies in brackish locations and leading to the progressive accumulation of microcystin and trophic transfer to predators (Miller et al., 2010; Gibble et al., 2016; Preece et al., 2017). Given this potential, more information is needed regarding trophic transfer of microcystins, the presence of microcystins across food webs, and effects of continued CHAB exposure in seafood (Bukaveckas et al., 2017). More information is also needed regarding the transfer of freshwater cyanotoxins to marine shellfish, including the monitoring of toxins in locations where CHAB have the potential to discharge into marine environments, particularly in regions where oysters are harvested.

5. Conclusion

This research demonstrates that Asian clams that are suddenly exposed to blooms of single-celled *Microcystis* populations can rapidly accumulate microcystin at levels hundreds of times above public health advisory levels but also rapidly depurate microcystins in a matter of days, even when exposure to *Microcystis* and microcystin continues. In contrast, eastern oysters fed on *Microcystis* can accumulate microcystin faster than clams and accumulate greater microcystin levels when fed single- or double-celled *Microcystis* or large colonies from blooms and did not display cyanotoxin depuration. Given these observations, as well as prior studies of bivalves and microcystins (Miller et al., 2010; Gibble et al., 2016), it would seem Hepatotoxic Shellfish Poisoning (HSP) may be a common a shellfish toxicity syndrome emanating from CHABs that produce microcystins. This may be a cryptic yet common occurrence in brackish, estuarine regions downstream of intense *Microcystis* blooms.

Comprehensive field studies of longer duration that consider both uptake and depuration of microcystins are needed to better understand the prevalence of HSP in aquatic ecosystems.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was funded by NOAA's MERHAB program awards NA19NOS4780186 (to CJG) and NA19NOS4780186 (RMK) and is MERHB publication # 243. We acknowledge the support of Stony Brook Southampton Marine Science Center and of Gobler Lab members Jennifer Jankowiak, Craig Young, Ronojoy Hem, Jacob Flanzenbaum, Benjamin Kranmer, Michael Doall, Jennifer Goleski, Ann Marie Famularo, Brendan Hallinan, Christina Woodard, Ally Giza, Ginger Hughes, An Li Kreidler-Siwinski, Bradley McGuire, and Darren DeSilva in performing experiments.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hal.2022.102236.

References

Adamovsky, Ondrej & Blaha, Ludek. (2017). Extraction of microcystins from Animal Tissues. 10.1002/9781119068761.ch40.

Alexander, J.A., Stoecker, D.K., Merrit, D.W., Alexander, S.T., Padeletti, A., Johns, D., Glibert, P.M., 2008. Differential production of feces and pseudofeces by the oyster crassostrea ariakensis when exposed to diets containing harmful dinoflagellate and raphidophyte species. J. Shellfi. Res. 27 (3), 567–579.

Atkins, D., 1937. On the ciliary mechanisms and interrelationships of lamellibranches. Part III: types of lamellibranch gills and their food currents. Q. J. Microsc. Sci. 79, 375–420.

Beals, C.D. (2004). Clearance rates and particle selectivity in the Hard Clam, Mercenaria, from Warm Water Habitats.

Backer, L.C., Landsberg, J.H., Miller, M., Keel, K., Taylor, T.K., 2013. Canine cyanotoxin poisonings in the United States (1920s–2012): review of suspected and confirmed cases from three data sources. Toxins, 5 (9), 1597–1628.

Basen, T., Martin-Creuzburg, D., Rothhaupt, K.O., 2011. Role of essential lipids in determining food quality for the invasive freshwater clam Corbicula fluminea. J. N. Am. Bentholog. Soc. 30 (3), 653–664. https://doi.org/10.1899/10-087.1.

Basen, T., Rothhaupt, K.O., Martin-Creuzburg, D., 2012. Absence of sterols constrains food quality of cyanobacteria for an invasive freshwater bivalve. Oecologia 170 (1), 57–64. https://doi.org/10.1007/s00442-012-2294-z.

Boegehold, A.G., Kashian, D.R., 2021. Stress tolerance of two freshwater invaders exposed to Microcystis aeruginosa and microcystin-LR. Hydrobiologia 848 (9), 2369–2382. https://doi.org/10.1007/s10750-020-04511-8.

Bolam, B.A., Rollwagen-Bollens, G., Bollens, S.M., 2019. Feeding rates and prey selection of the invasive Asian clam, Corbicula fluminea, on microplankton in the Columbia River, USA. Hydrobiologia 833 (1), 107–123.

Bukaveckas, P.A., Lesutienė, J., Gasiūnaitė, Z.R., Ložys, L., Olenina, I., Pilkaitytė, R., Wood, J., 2017. microcystin in aquatic food webs of the Baltic and Chesapeake Bay regions. Estuarine, Coastal and Shelf Science, 191, 50–59. https://doi.org/10.1016/j.ecss.2017.04.016.

Carmichael, W.W., Azevedo, S.M.F.O., An, J.S., Molica, R.J.R., Jochimsen, E.M., Lau, S., Eaglesham, G.K., 2001. Human fatalities form cyanobacteria: chemical and biological evidence for cyanotoxins. Environ. Health Perspect. 109 (7), 663–668. https://doi.org/10.1289/ehp.01109663.

Capelle, J.J., Hartog, E., van den Bogaart, L., Jansen, H.M., Wijsman, J.W.M., 2021. Adaptation of gill-palp ratio by mussels after transplantation to culture plots with different seston conditions. Aquaculture 541 (March), 736794. https://doi.org/10.1016/j.aquaculture.2021.736794.

Chapra, S.C., Boehlert, B., Fant, C., Bierman, V.J., Henderson, J., Mills, D., Paerl, H.W., 2017. Climate Change Impacts on Harmful Algal Blooms in U.S. Freshwaters: a Screening-Level Assessment. Environ. Sci. Technol. 51 (16), 8933–8943. https://doi.org/10.1021/acs.est.7b01498.

Chen, J., Xie, P., 2007. Microcystin accumulation in freshwater bivalves from Lake Taihu, China, and the potential risk to human consumption. Environ. Toxicol. Chem. 26 (5), 1066–1073.

Chen, J., Xie, P., Li, L., Xu, J., 2009. First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage. Toxicol. Sci. 108 (1), 81–89. https://doi.org/10.1093/toxsci/kfp009.

Chen, C., Pang, X., Wang, Y., Kong, M., Long, L., Xu, M., Deng, S., 2021. Antioxidant responses and microcystins accumulation in Corbicula fluminea following the control of algal blooms using chitosan-modified clays. J. Soils Sediment. 21 (10), 3505–3514. https://doi.org/10.1007/s11368-021-03022-w.

Chorus, I., & Bartram, J. (1999). Toxic Cyanobacteria in Water: a guide to their public health consequences, monitoring and management. Filtration.

Coughlan, Jack., 1969. The estimation of filtering rate from the clearance of suspensions. Mar. Biol. 2, 356–358. https://doi.org/10.1007/BF00355716.

Cranford, P.J., Ward, J.E., & Shumway, S.E. (2011). Bivalve filter feeding: variability and limits of the aquaculture biofilter. In Shellfish Aquaculture and the Environment (pp. 81–124).

Dionisio Pires, L.M., Jonker, R.R., Van Donk, E., Laanbroek, H.J., 2004. Selective grazing by adults and larvae of the zebra mussel (Dreissena polymorpha): application of flow cytometry to natural seston. Freshwater Biol. 49 (1), 116–126. https://doi.org/10.1046/j.1365-2426.2003.01173.x.

Dutertre, M., Ernande, B., Haure, J., Barillé, L., 2017. Spatial and temporal adjustments in gill and palp size in the oyster Crassostrea gigas. J. Molluscan Stud. 83 (1), 11–18. https://doi.org/10.1093/mollus/eyw025.

Freitas, M., Azevedo, J., Carvalho, A.P., Campos, A., Vasconcelos, V., 2014. Effects of storage, processing, and proteolytic digestion on microcystin-LR concentration in edible clams. Food Chem. Toxicol. 66, 217–223.

Gene, S.M., Shahmohamadloo, R.S., Ortiz, X., Prosser, R.S., 2019. Effect of Microcystis aeruginosa–Associated Microcystin-LR on the Survival of 2 Life Stages of Freshwater Mussel (Lampsilis siliquoidea). Environ. Toxicol. Chem. 38 (10), 2137–2144. https://doi.org/10.1002/etc.4527.

Gerard, C., Brient, L., & Le Rouzic, B. (2005). Variation in the Response of Juvenile and Adult Gastropods (Lymnaea stagnalis) to Cyanobacterial Toxin (microcystin-LR). Environ. Toxicol., 592–596. 10.1002/tox.20147.

Gibble, C.M., Kudela, R.M., 2014. Detection of persistent microcystin toxins at the land – sea interface in Monterey Bay, California. Harmf. Algae 39, 146–153.

Gibble, C.M., Peacock, M.B., Kudela, R.M., 2016. Evidence of freshwater algal toxins in marine shellfish: implications for human and aquatic health. Harmf. Algae 59, 59–66.

Griffith, A.W., Shumway, S.E., Volety, A.K., 2013. Bioaccumulation and depuration of brevetoxins in the eastern oyster (Crassostrea virginica) and the northern quahog (= hard clam, Mercenaria mercenaria). Toxicon 66, 75–81. https://doi.org/10.1016/j.toxicon.2013.01.016.

Guillard, R.R.L., 1975. Culture of phytoplankton for feeding marine invertebrates. In: Smith, W.L., Chanley, M.H. (Eds.), Culture of Marine Invertebrate Animals: Proceedings — 1st Conference on Culture of Marine Invertebrate Animals Greenport. Springer US, pp. 29–60. https://doi.org/10.1007/978-1-4615-8714-9_3.

Harke, M.J., Jankowiak, J.G., Morrell, B.K., Gobler, C.J., 2017. Transcriptomic responses in the bloom-forming cyanobacterium Microcystis induced during exposure to zooplankton. Appl. Environ. Microbiol. 83 (5), e02832–e028316.

Hassett, W., Bollens, S.M., Counihan, T.D., Rollwagen-Bollens, G., Zimmerman, J., Katz, S., Emerson, J., 2017. Veligers of the invasive Asian clam Corbicula fluminea in the Columbia River Basin: broadscale distribution, abundance, and ecological associations. Lake Reserv. Manage. 33 (3), 234–248. https://doi.org/10.1080/10402381.2017.1294218.

Haven, D.S., Morales-Alamo, R., 1970. Filtration of Particles from Suspension by the American Oyster Crassostrea virginica. Biol. Bull. 139 (2), 248–264.

Hégaret, H., Wikfors, G.H., Shumway, S.E., 2007. Diverse feeding responses of five species of bivalve mollusc when exposed to three species of harmful algae. J. Shellf. Res. 26 (2), 549–559, 10.2983/0730-8000(2007)26[549:DFROFS]2.0.CO;2.

Heilmayer, O., Digialleonardo, J., Qian, L., Roesijadi, G., 2008. Stress tolerance of a subtropical Crassostrea virginica population to the combined effects of temperature and salinity. Estuarine Coast. Shelf. Sci. 79 (1), 179–185. https://doi.org/10.1016/j.ecss.2008.03.022.

Hilborn, E.D., Beasley, V.R., 2015. One health and cyanobacteria in freshwater systems: animal illnesses and deaths are sentinel events for human health risks. Toxins 7 (4), 1374–1395. https://doi.org/10.3390/toxins7041374.

Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature 574 (7780), 667–670.

Jacobs, P., Troost, K., Riegman, R., van der Meer, J., 2015. Length- and weight-dependent clearance rates of juvenile mussels (Mytilus edulis) on various planktonic prey items. Helgol. Mar. Res. 69, 101–112. https://doi.org/10.1007/s10152-014-0419-y.

Jankowiak, J., Hattenrath-Lehmann, T., Kramer, B.J., Ladds, M., Gobler, C.J., 2019. Deciphering the effects of nitrogen, phosphorus, and temperature on cyanobacterial bloom intensification, diversity, and toxicity in western Lake Erie. Limnol. Oceanogr. 64 (3), 1347–1370. https://doi.org/10.1002/lno.11120.

Juhel, G., Ramsay, R.M., Davenport, J., O'Halloran, J.&., Culloty, S.C, 2015. Effect of the microcystin-Producing Cyanobacterium, Microcystis aeruginosa, on Immune Functions of the Zebra Mussel Dreissena polymorpha. J. Shellf. Res. 34 (2), 433–442. https://doi.org/10.2983/035.034.0227.

Lauritsen, D.D., 1986. Filter-feeding in corbicula fluminea and its effect on seston removal. J. N. Am. Bentholog. Soc. 5 (3), 165–172. https://doi.org/10.2307/1467704.

Lawton, L.A., Edwards, C., Beattie, K.A., Pleasance, S., Dear, G.J., Codd, G.A., 1995. Isolation and characterization of microcystins from laboratory cultures and environmental samples of Microcystis aeruginosa and from an associated animal toxicosis. Nat. Toxins 3 (1), 50–57. https://doi.org/10.1002/nt.2620030110.

Leverone, J.R., Shumway, S.E., Blake, N.J., 2007. Comparative effects of the toxic dinoflagellate Karenia brevis on clearance rates in juveniles of four bivalve molluscs

from Florida, USA. Toxicon 49 (5), 634–645. https://doi.org/10.1016/j.toxicon.2006.11.003.
Levinton, J., Doall, M., Ralston, D., Starke, A., Allam, B., 2011. Climate change, precipitation and impacts on an estuarine refuge from disease. PLoS One 6 (4), e18849.
Li, B., Xie, P., 2021. Research progress in the functionalization of microcystin-LR based on interdisciplinary technologies. Coord. Chem. Rev. 443, 214041.
Liu, Y., Xie, P., Wu, X.P., 2009. Grazing on toxic and non-toxic Microcystis aeruginosa PCC7820 by Unio douglasiae and Corbicula fluminea. Limnology 10 (1), 1–5.
Mafra, L.L.J., Bricelj, V.M., Ward, J.E., 2009. Mechanisms contributing to low domoic acid uptake by oysters feeding on Pseudonitzschia cells. II. Select. Reject. 6 (August), 213–226.
Mekebri, A., Blondina, G.J., Crane, D.B., 2009. Method validation of microcystins in water and tissue by enhanced liquid chromatography tandem mass spectrometry. J. Chromatogr. A 1216 (15), 3147–3155.
Mez, K., Hanselmann, K., Beattie, K., Codd, G., Hauser, B., Naegeli, H., Preisig, H., 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. Eur. J. Phycol. 32 (2), 111–117.
Miller, M.A., Kudela, R.M., Mekebri, A., Crane, D., Oates, S.C., Tinker, T.M., Jessup, D.A., 2010. Evidence for a novel marine harmful algal bloom: cyanotoxin (microcystin) transfer from land to sea otters. PLoS One 5 (9).
Newell, R., Jordan, S., 1983. Preferential ingestion of organic material by the American oyster Crassostrea virginica. Marine Ecol. Progr. Ser. 13 (January 1983), 47–53. https://doi.org/10.3354/meps013047.
Newell, R.I., 2004. Ecosystem influences of natural and cultivated populations of suspension-feeding bivalve molluscs: a review. J. Shellf. Res. 23 (1), 51–62.
NMFS, 2018. Report of Annual Fisheries Landings. National Marine Fisheries Service.
Officer, C., Smayda, T., Mann, R., 1982. Benthic filter feeding: a natural Eutrophication control. Marine Ecol. Progr. Ser. 9, 203–210. https://doi.org/10.3354/meps009203.
O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmf. Algae 14, 313–334.
Orr, P.T., Jones, G.J., Douglas, G.B., 2004. Response of cultured Microcystis aeruginosa from the Swan River, Australia, to elevated salt concentration and consequences for bloom and toxin management in estuaries. Mar. Freshwater Res. 55 (3), 277.
Park, J., Kim, Y., Kim, M., Lee, W.H., 2019. A novel method for cell counting of Microcystis colonies in water resources using a digital imaging flow cytometer and microscope. Environ. Eng. Res. 24 (3), 397–403. https://doi.org/10.4491/EER.2018.266.
Pham, T.L., Shimizu, K., Dao, T.S., Hong-Do, L.C., Utsumi, M., 2015. Microcystin uptake and biochemical responses in the freshwater clam Corbicula leana P. Exposed to toxic and non-toxic Microcystis aeruginosa: evidence of tolerance to cyanotoxins. Toxicol. Rep. 2, 88–98.
Preece, E.P., Hardy, F.J., Moore, B.C., Bryan, M., 2017. A review of microcystin detections in Estuarine and Marine waters: environmental implications and human health risk. Harmf. Algae 61, 31–45. https://doi.org/10.1016/j.hal.2016.11.006.
Riisgård, H., 1988. Efficiency of particle retention and filtration rate in 6 species of Northeast American bivalves. Marine Ecol. Progr. Ser. 45, 217–223. https://doi.org/10.3354/meps045217.
Riisgård, H.U., 2001. On measurement of filtration rates in bivalves—The stony road to reliable data: review and interpretation. Marine Ecol. Progr. Ser. 211, 275–291.
Scharping, R.J., Plough, L.V., Meritt, D.W., & North, E.W. (2019). Low Salinity Tolerance of Early-Stage Oyster Larvae from Mesohaline Estuary, 1–31.
Sericano, J.L., Wade, T.L., Brooks, J.M., 1996. Accumulation and depuration of organic contaminants by the American oyster (Crassostrea virginica). Sci. Total Environ. 179 (1–3), 149–160. https://doi.org/10.1016/0048-9697(96)90054-6.
Silva, C., Anselmo, A., Macário, I.P.E., de Figueiredo, D., Gonçalves, F.J.M., Pereira, J.L., 2020. The bad against the villain: suitability of Corbicula fluminea as a bioremediation agent towards cyanobacterial blooms. Ecol. Eng. 152 (May) https://doi.org/10.1016/j.ecoleng.2020.105881.
Sousa, R., Antunes, C., Guilhermino, L., 2008. Ecology of the invasive Asian clam Corbicula fluminea (Müller, 1774) in aquatic ecosystems: an overview. Annales de Limnologie 44 (2), 85–94. https://doi.org/10.1051/limn:2008017.
Thompson, E.L., Taylor, D.A., Nair, S.V., Birch, G., Coleman, R., Raftos, D.A., 2012. Optimal acclimation periods for oysters in laboratory-based experiments. J. Molluscan Stud. 78 (3), 304–307. https://doi.org/10.1093/mollus/eys012.
Vaughn, C.C., Hakenkamp, C.C., 2001. The functional role of burrowing bivalves in freshwater ecosystems. Freshwater Biol. 46 (11), 1431–1446.
Vanderploeg, H.A., & Scavia, D. (1979). Two electivity indices for feeding with special reference to zooplankton grazing. J. Fish. Res. Board Can.. 10.1139/f79-055.
Vanderploeg, H.A., Liebig, J.R., Carmichael, W.W., Agy, M.A., Johengen, T.H., Fahnenstiel, G.L., Nalepa, T.F., 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 (6), 1208–1221. https://doi.org/10.1139/cjfas-58-6-1208.
Vareli, K., Jaeger, W., Touka, A., Frillingos, S., Briasoulis, E., Sainis, I., 2013. Hepatotoxic seafood poisoning (HSP) due to microcystins: a threat from the ocean? Marine Drug. 11 (8), 2751–2768. https://doi.org/10.3390/md11082751.
Waajen, G.W.A.M., Van Bruggen, N.C.B., Pires, L.M.D., Lengkeek, W., Lürling, M., 2016. Biomanipulation with quagga mussels (Dreissena rostriformis bugensis) to control harmful algal blooms in eutrophic urban ponds. Ecol. Eng. 90, 141–150. https://doi.org/10.1016/j.ecoleng.2016.01.036.
Wallace, J.B., Webster, J.R., Woodall, W.R., 1977. The role of filter feeders in flowing waters. Archiv. Hydrobiol. 79 (4), 506–532.
Wang, H., Xu, C., Liu, Y., Jeppesen, E., Svenning, J.C., Wu, J., Xie, P., 2021. From unusual suspect to serial killer: cyanotoxins boosted by climate change may jeopardize megafauna. Innovation 2 (2), 100092. https://doi.org/10.1016/j.xinn.2021.100092.
Ward, J.E., Newell, R.I.E., Thompson, R.J., MacDonald, B.A., 1994. In vivo studies of suspension-feeding processes in the eastern oyster, Crassostrea virginica (Gmelin). Biol. Bull. 186 (2), 221–240. https://doi.org/10.2307/1542056.
Ward, J.E., Levinton, J.S., Shumway, S.E., Cucci, T., 1997. Site of particle selection in a bivalve mollusc. Nature 390 (6656), 131–132.
Ward, J.E., Zhao, S., Holohan, B.A., Mladinich, K.M., Griffin, T.W., Wozniak, J., Shumway, S.E., 2019. Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (Crassostrea virginica): implications for Using Bivalves as Bioindicators of Microplastic Pollution. Environ. Sci. Technol. 53 (15), 8776–8784. https://doi.org/10.1021/acs.est.9b02073.
Waterbury, J.B., Stanier, R.Y., 1981. Isolation and growth of cyanobacteria from marine and hypersaline environments. Prokaryotes 7, 221–223.
Weissberger, E.J., Glibert, P.M., 2021. Diet of the eastern oyster, Crassostrea virginica, growing in a eutrophic tributary of Chesapeake Bay, Maryland, USA. Aquacult. Rep. 20, 100655 https://doi.org/10.1016/j.aqrep.2021.100655.
Wikfors, G.H., Twarog, J.W.J., Ukeles, R., 1981. Influence of chemical composition of algal food sources on growth of juvenile oysters, Crassostrea Virginica. Biol. Bull. 167 (1), 251–263.
World Health Organization, Division of Operational Support in Environmental Health, 1998. Guidelines for drinking-water quality. Vol. 2, Health criteria and Other Supporting information: Addendum, 2nd ed. World Health Organization.
Xiao, M., Li, M., Reynolds, C.S., 2018. Colony formation in the cyanobacterium Microcystis. Biolog. Rev. 93 (3), 1399–1420. https://doi.org/10.1111/brv.12401.
Yonge, C.M., 1923. Studies on the comparative physiology of digestion. J. Exp. Biol. 1, 15–64.
Zhang, H., Li, B., Liu, Y., Chuan, H., Liu, Y., Xie, P., 2022. Immunoassay technology: research progress in microcystin-LR detection in water samples. J. Hazard. Mater. 424 (PB), 127406 https://doi.org/10.1016/j.jhazmat.2021.127406.
zu Ermgassen, P.S.E., Spalding, M.D., Grizzle, R.E., Brumbaugh, R.D., 2013. Quantifying the loss of a marine ecosystem service: filtration by the Eastern Oyster in US Estuaries. Estuaries Coast. 36 (1), 36–43. https://doi.org/10.1007/s12237-012-9559-y.