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INVASIVE SPECIES III

Stress tolerance of two freshwater invaders exposed to *Microcystis aeruginosa* and microcystin-LR

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Abstract Invasion of freshwaters by non-native animal species is a major threat to global aquatic systems. Asian clams (*Corbicula fluminea*) and quagga mussels (*Dreissena rostriformis bugensis*) are two highly invasive bivalves in North America that share life histories that facilitate invasion; however, they may experience different stress tolerances which can influence local population distribution. We compare the sensitivity of these two bivalves to the environmental stressors *Microcystis aeruginosa* and its associated toxin, microcystin. In laboratory assays, we exposed both species to *M. aeruginosa* and measured oxygen consumption and filtration rate. Sensitivity to microcystin was evaluated through two oxidative stress biomarkers, catalase, and lipid peroxidation. Quagga mussels exhibited elevated physiological responses, consuming more oxygen with cyanobacteria exposure, at an approximately doubled rate compared to Asian clams. Both species had increased filtration rates when fed *M. aeruginosa* with quagga mussels filtering at an overall higher rate. Oxidative stress was variable in both species. These results indicate that quagga mussels may be more sensitive to *M. aeruginosa* and microcystin than Asian clams, possibly due to physiological differences associated with invasion histories. The findings from these studies can be used to predict range expansion and lead to more effective control of nuisance populations.

Keywords Oxidative stress · Quagga mussel · Asian clam · Filtration · Respiration

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Introduction

Invasion of freshwater habitats by non-native animal species is a major threat to global aquatic systems (Strayer, 2010). While few introduced species become invasive, those that are successful may thrive in new habitats through plastic responses or broad physiological tolerances (Lee & Gelembiuk, 2008). Mollusks represent a significant proportion of aquatic invasive species in North America and typically thrive in areas

of poorer water quality than native species (Karatayev et al., 2009). The Asian clam, *Corbicula fluminea* (Müller, 1774), and quagga mussel, *Dreissena rostriformis bugensis* (Andrusov, 1897), are two bivalves introduced to a wide distribution of freshwater systems outside of their native ranges. Asian clams and quagga mussels pose a serious threat to native biodiversity and ecosystem functioning, with the potential to devastate food webs, nutrient cycling, and freshwater economies (Sousa et al., 2008; Higgins & Vander Zanden, 2010; Strayer, 2010). Both species generally prefer habitats that are frequently disturbed, often invading areas prone to human use (McMahon, 1982; Lee & Gelembiuk, 2008). Both Asian clams and quagga mussels are globally invasive species with overlapping distribution in many colonized areas (Karatayev et al., 2007). While Asian clams and quagga mussels can behave similarly as invaders, they also have distinct life history traits that may result in different tolerances to stress which can influence local population distribution.

Asian clams originate from freshwaters in Asia, Africa, and Australia and may have been intentionally introduced to North America as a food resource (Sousa et al., 2008). The first North American specimens of Asian clams were found in British Columbia, Canada, in 1924 as empty shells, and live collections were retrieved from the Columbia River in Washington, USA, in 1938 (Mills et al., 1993). By the 1960s, Asian clams had spread throughout the United States via connected waterways and human transport (McMahon, 1982) and by the 1980s, the distribution of the genus *Corbicula* included South America and Europe (McMahon, 2002). Successful invasion and dispersal of Asian clams is closely linked with human activities and life history characteristics, such as rapid growth and high fecundity (Sousa et al., 2008).

Like Asian clams, quagga mussels have become globally invasive in many freshwater systems. Byssate bivalves, such as zebra and quagga mussels, which secrete strong threads (byssus) used for attachment to substrate or objects, are extremely uncommon in freshwater systems, with quagga and zebra mussels being rare exceptions (Karatayev et al., 2009). Quagga mussels began their range expansion out of the Ponto-Caspian region in the 1940s, and in 2004, they were discovered in Romania, initiating their expansion into Western Europe aided by human vectors and connected water bodies (bij de Vaate et al., 2013). In 1991, quagga mussels were first reported in Lake Ontario, unintentionally transported via ballast water, but may have appeared in the Great Lakes – St. Lawrence River drainage years earlier (Mills et al., 1993; Ricciardi & MacIsaac, 2000). Although zebra mussels, *Dreissena polymorpha* (Pallas, 1771), a congeneric species, were the first documented dreissenid invaders to freshwaters of North America, quagga mussels have been displacing existing North American zebra mussel populations in the large lakes and are expanding their range into deeper, colder bodies of water (Ram et al., 2012). Quagga mussels can tolerate a range of brackish salinities (up to 8 psu; Ram et al., 2012), which may have aided in their transport and invasion in North America (Ricciardi & MacIsaac, 2000).

Potentially toxic cyanobacteria can act as an environmental stressor for many freshwater organisms. Harmful cyanobacteria blooms, which can produce toxins, are becoming more frequent and severe in freshwater systems worldwide (Paerl & Paul, 2012). Toxin-producing cyanobacteria can cause public health crises, illness, and even death in some animals (Carmichael & Boyer, 2016). Furthermore, various cyanobacteria species tend to be deficient in essential lipids and fatty acids to filter feeding organisms compared to other types of phytoplankton (Vanderploeg et al., 1996) and can lead to reduced growth rates in both Asian clams and quagga mussels (Wright et al., 1996; Basen et al., 2011). Cyanobacteria are demonstrably less desirable than other types of phytoplankton for Asian clams and quagga mussels. Thus, cyanobacteria can be useful as an experimental stressor to examine metabolic and physiological response differences between quagga mussels and Asian clams.

The objective of this study was to compare physiological responses of Asian clams and quagga mussels to the cyanobacteria, *Microcystis aeruginosa* (Kützing, 1846), and its associated toxin, microcystin-LR, two environmental stressors. We examined the differential response in oxygen consumption, filtration rate, and two oxidative stress biomarkers, catalase and lipid peroxidation of quagga mussels and Asian clams. We hypothesized that quagga mussels would experience higher stress than Asian clams due to their associated metabolic and physiological adaptations related to their origins from more brackish waters and more recent invasion into North America. Previous literature has demonstrated a cost of tolerance

(Kashian et al., 2007) that coincides with adaptation to other stressors. Increased knowledge of stress tolerance can help resource managers make better predictions about range expansion for these two species and can provide greater insights into the dynamics of species invasions.

Methods

Organism collection and culture maintenance

Quagga mussels and Asian clams were collected in Wayne County, Michigan, from two distinct sampling locations 48 km apart. Both species were collected between February 2016 and January 2017. Quagga mussels (15–35 mm length) were retrieved from the Detroit River on Belle Isle State Park, Detroit, MI (42° 21′ 0.62″ N, 82° 58′11.5″ W) where they were scraped from a seawall at depths of 1–3 m below the surface. The site water at time of collection ranged from 9.5 to 16.0°C and had an average dissolved oxygen of 10.9 ± 1.5 mg/l, pH of 7.4 ± 0.2, and conductivity of 491 ± 201 µS/cm.

Asian clams (15–35 mm length) were gathered by hand from the Rouge River in Plymouth, MI (42 ° 22′ 14.2″ N, 83° 26′ 21.1″ W). Asian clams were collected from predominantly pebble mixed with cobble substrates at depths of 1–2 m. Live, adult specimens of both species were transported back to the laboratory in a cooler containing site water. The site water at time of collection ranged from 12.8 to 15.5°C and had an average dissolved oxygen of 11.1 ± 0.3 mg/l, pH of 7.7 ± 0.2, and conductivity of 661 ± 299 µS/cm.

At the laboratory, specimens were immediately cleaned of excess debris under running tap water and stored separately by species in aerated aquaria filled with dechlorinated tap water. Animals were maintained at a density of 1 bivalve per 50 ml of water. Once per week, the aquaria were cleaned, dead animals removed, and the water refreshed. Mortality during the acclimation period was minimal and stocks of quagga mussels and Asian clams were only used if ≥ 90% survived acclimation. Animals were acclimated in laboratory aquaria for a minimum of two weeks prior to use in experiments, in 18 ± 1°C environmental chambers with a 16:8 light:dark cycle. An elevated mesh substrate was provided for animals in aquaria for waste settlement. Animals were fed three times per week with 4.0×10^6 cells/mL of laboratory cultured *Ankistrodesmus falcatus* (Ralfs, 1848) (UTEX #748), a green alga commonly used for maintenance of aquatic filter feeding animals (Boegehold et al., 2018). *A. falcatus* was also used as the control algae in experiments.

Two phenotypically distinct strains of *M. aeruginosa* were evaluated for adverse impacts on quagga mussels and Asian clams. One strain had been continuously cultured and was unicellular without visual production of extracellular polymeric substances (UTMS; purchased from the University of Texas Culture Collection of Algae) and the other was isolated from Lake Erie in 2013 and maintained a colonial nature with visible extracellular polymeric substance production (obtained from National Oceanic and Atmospheric Administration's Great Lakes Environmental Research Lab). Neither strain produced microcystins at the time of experiments but may nevertheless be a stressor because of poor nutritional quality and the production of other metabolites which have elicited stress responses from other filter feeding organisms (Sadler and von Elert, 2014). Both cyanobacteria isolates were cultured in laboratory-prepared WC media (Guillard & Lorenzen, 1972) and maintained in an environmental chamber at 19 ± 1°C with 16:8 light/dark cycle at 60 µE m^{-2} s^{-1} light intensity. *A. falcatus* was grown in COMBO media (Kilham et al., 1998) under a light intensity of 120 µE m^{-2} s^{-1}.

Oxygen consumption experiments

Oxygen consumption in quagga mussels and Asian clams was measured in the presence of two strains of *M. aeruginosa*, using *A. falcatus* as the control. To determine oxygen consumption rates for each species, ten animals of the same species were placed in 1 l Erlenmeyer flasks ($n = 5$ flasks per treatment for each species). Within each treatment, one flask was prepared without animals to correct for any oxygen production or consumption by the phytoplankton. Flasks were filled with 1 l of appropriate solution to minimize airspace in the container while maintaining a low animal density (100 ml/animal). Flasks were wrapped in foil to restrict photosynthesis and were kept in ambient conditions. Experimental solutions consisted of 50 µg/l chlorophyll-*a* phytoplankton mixed with dechlorinated tap water that was aerated

for at least 24 h prior to experiment. Chlorophyll-*a* was used as a proxy for phytoplankton culture densities instead of cell densities to account for the difference in cell shapes and sizes between species and cultures. Dissolved oxygen (DO, mg/l) was measured in each flask following methods adapted from Fernandez-Sanjuan et al. (2013). Oxygen measurements were taken with a YSI ProDO optical DO probe. Prior to experiments, the DO probe was calibrated and DO measurements were recorded once the meter maintained a reading for 10 s. Dissolved oxygen in each flask was measured at the initiation of the experiment (t = 0 h). After initial measurement, flasks were immediately covered with Parafilm to prevent gas exchange in and out of the flask. Subsequent DO concentrations were recorded at 24, 48, 72, and 96 h after t = 0. Upon termination of the experiment, the soft tissues of quagga mussels and Asian clams were removed and dried in a 60°C oven for 24 h and weighed to determine the oxygen consumption per dry weight of animals (g). Oxygen consumption was measured as differences in DO between time points during the exposure period. Oxygen measurements in the flasks without animals were used as a correction factor for the appropriate treatments, where any oxygen differences that occurred in the phytoplankton only flasks were subtracted from flasks containing animals. Variation in DO within these flasks was minimal, indicating no strong influence on DO concentration from factors other than the experimental animals.

Filtration rate experiments

Methods for filtration rate experiments were modified from White & Sarnelle (2014). Filtration rate of Asian clams and quagga mussels was measured in response to two isolates of *M. aeruginosa,* using *A. falcatus* as the control. Five animals of the same species were placed in 500 ml Erlenmeyer flasks (n = 5 flasks per treatment for each species) filled with 500 ml of phytoplankton solution containing dechlorinated tap water. The concentration of phytoplankton was approximately 50 µg/l chlorophyll-*a* for each replicate. Chlorophyll-*a* was used as a proxy for phytoplankton culture densities instead of cell densities to account for the difference in cell shapes and sizes between species and cultures. For each treatment and the control, a separate flask was prepared without animals to adjust for any settlement or photosynthesis of phytoplankton that could influence chlorophyll-*a* measurements. Filtration rate experiments were performed in ambient conditions.

Aerators were placed in each flask to maintain aerobic conditions and homogeneity of solutions. Immediately after addition of algae, 50 ml of solution was filtered through 47 mm Whatman GF/F filter and chlorophyll-*a* was extracted and measured using hot ethanol extraction techniques (Biggs & Kilroy, 2000). This was repeated at 3 h after the initial algae addition. The animals remained in the flask in algae solution overnight. At t = 24, 48, 72, and 96 h after initiation of experiment, animals were placed in a clean flask with 500 ml fresh solution and the filtration measurement process was repeated. At the end of the experiment, soft tissue was removed from the animals and dried at 60° C for 24 h to measure dry weight (g). Filtration rate was calculated using the formula $F = (V/nt) \times \ln(C_i - C_f)$, where C_i and C_f are initial and final chlorophyll-*a* concentrations (µg/l), respectively, t is time (h), V is the volume of phytoplankton solution (l), and n is the combined dry weight (g) of the five animals in each flask (White & Sarnelle, 2014; Vanderploeg et al., 2001). Chlorophyll-*a* measurements taken from the flasks without animals were used as a correction factor, where any differences in chlorophyll-*a* from these flasks were subtracted from the flasks containing animals.

Oxidative stress response

Neither of the strains of *M. aeruginosa* used in the filtration rate and oxygen consumption studies produce cyanotoxins, and thus, we wanted to analyze the cellular effects of the purified toxin, microcystin-LR, on quagga mussels and Asian clams. To examine the effects of microcystin-LR, two oxidative stress biomarkers, catalase (CAT) and lipid peroxidation (LPO), were measured in quagga mussels and Asian clams exposed to the toxin for 96 h. These two biomarkers were chosen because they are widely conserved in the animal kingdom and well-studied (Faria et al., 2009; Nowicki & Kashian, 2018). Catalase is an enzyme that reduces reactive H_2O_2 to water, and lipid peroxidation is a marker of tissue damage caused by reactive oxygen species (Faria et al., 2009). Both quagga mussels and Asian clams were exposed to microcystin-LR (Beagle Bioproducts,

Columbus, Ohio, USA; > 95% purity by high performance liquid chromatography) at concentrations of 100 µg/l and 400 µg/l. These concentrations were chosen because they are in the higher range of environmental microcystin concentrations recorded globally (Carmichael & Boyer, 2016). Each experimental treatment consisted of 800 ml glass jars filled with 500 ml of solution ($n = 10$ jars) in static solution. At the beginning of the experiment, each jar contained ten animals of the same species. Animals were fed prior to oxidative stress biomarker experiments, but experimental solutions did not contain any phytoplankton. Dried microcystin was reconstituted using a small volume of acetone, and an equivalent volume of acetone was placed in the control jars to ensure that the solvent did not impact oxidative stress levels (maximum amounts of 1 ml/l acetone). Aerators were placed in each treatment jar to maintain aerobic conditions throughout the experiment. Oxidative stress response was measured at $t = 0$ h as a baseline response of oxidative stress biomarkers prior to experimental exposure and again at 24, 48, 72, and 96 h from initiation. Animal tissue was analyzed following the methods from Nowicki & Kashian (2018) and is described here briefly. Five animals from each treatment were randomly sacrificed from replicate jars within each treatment for analysis at each time point, resulting in gradual reduction of the number of animals in each experimental jar. Soft tissue from quagga mussels and Asian clams were dissected, weighed, and frozen in liquid nitrogen and stored at − 80°C until analysis. Prior to analysis, tissues were homogenized and digested in phosphate buffer. Lipid peroxidation was quantified by measuring malondialdehyde (MDA), a stable byproduct of LPO, using an OxiSelect thiobarbituric acid reactive substances (TBARS) assay kit (ThermoFisher Scientific). Samples were run in duplicate and analyzed against a standard curve. Results for each sample are expressed as the average of the duplicate values in units of MDA per gram of wet tissue weight. Catalase was measured as a decrease in absorbance of H_2O_2 consumption and standardized with total protein from each sample, quantified with Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific). Samples were run in duplicate and standardized by analyzing each sample for total protein quantification using a Coomassie Plus (Bradford) Assay Kit (Thermo Fisher Scientific).

Data analysis

Oxygen consumption data were tested for normality using a Shapiro–Wilk Test ($W = 0.911$, $P < 0.001$) and was log-transformed prior to analysis to reduce heterogeneity of variances. A Levene's Test determined that all data were homoscedastic ($F = 1.768$, $P = 0.194$). Data were then analyzed using an ANOVA with Tukey post hoc tests. These tests were used to compare both *M. aeruginosa* treatments to the *A. facaltus* control within species and across time points. Data were also analyzed to compare both quagga mussels and Asian clams in same treatments across time periods.

Filtration rate data were tested for normality using a Shapiro–Wilk test ($W = 0.908$, $P < 0.001$) and for homoscedasticity using Levene's Test ($F = 4.135$, $P = 0.044$) and data were log-transformed prior to analysis. Data were analyzed using a 2-way ANOVA to test effects of the two *M. aeruginosa* treatments with increasing exposure time. The 2-way ANOVA was tested individually for each species and a Tukey post hoc test was used to examine the differences within treatments between time points. ANOVA tests with Tukey post hoc tests were also used to evaluate the differences between species in the same treatments at each time point and to compare filtration rates in each species in the *M. aeruginosa* treatments to the *A. facaltus* control at each time point.

Three-way multivariate analysis of variance (MANOVA) using the Pillai's Trace test statistic was used to determine the significance of overall oxidative stress measured by the two dependent variables (CAT and LPO) in response to the independent variables (species, time, toxin concentration). The MANOVAs identified significance between species, among treatments, over time, and in independent variable interactions (e.g., species × toxic concentration, or species × time). One-way independent measure analysis of variance (ANOVA) followed by Student–Newman–Keuls (SNK) post hoc comparison tests were used to compare the differences among time points, and the individual significance of lipid peroxidation levels and catalase response among treatments and between species. Prior to analysis, a Levene's test was used to determine the variance homoscedasticity among groups (CAT $F = 10.211$, $P = 0.002$; LPO $F = 0.604$, $P = 0.439$) and a Box's M test was used to determine the homogeneity of covariance

(χ^2 = 42.847, df = 3, $P < 0.001$). Both LPO and CAT data were log-transformed prior to analysis. All statistical analyses were performed using R statistical software using $\alpha = 0.05$ to determine the significance in statistical analysis (Version 3.3.1; R Core Team, 2015).

Results

Oxygen consumption

Quagga mussels exhibited an overall higher respiration rate than Asian Clams. Quagga mussels respired at rates that were at least approximately 0.5 mg/l per 24 h per gram dry weight higher than Asian clams over the course of 96 h when exposed to either *M. aeruginosa* strains (LEMS $P < 0.001$, UTMS $P = 0.002$) and in the *A. facaltus* control ($P = 0.017$) (Table S1, Fig. 1). Oxygen consumption in quagga mussels varied depending on exposure length and which *M. aeruginosa* strain they were exposed to, while Asian clams were not impacted by either strain of *M. aeruginosa* at any time period. Over the course of 96 h, quagga mussels consumed on average 0.5 mg/l per 24 h per gram dry weight more oxygen in the LEMS treatment compared to the control (Table S1, Fig. 1; $P = 0.011$). There was no difference in oxygen consumption by Asian clams in either of the *M. aeruginosa* treatments compared to the control over the course of the experiment (Table S1, Fig. 1; $P > 0.05$).

Filtration rate

Filtration rate in both species was variable in both *M. aeruginosa* treatments and trends were dependent on the duration of exposure (Table S2, Fig. 2; $P < 0.05$). Both species maintained consistent rates of filtration in the *A. facaltus* control throughout the experiment (Fig. 2; $P > 0.05$). Filtration rate between the quagga mussels and Asian clams only differed on $t = 96$ h when quagga mussels filtered double the amount of *M. aeruginosa* (UTMS) than Asian clams (Fig. 2; $P =$

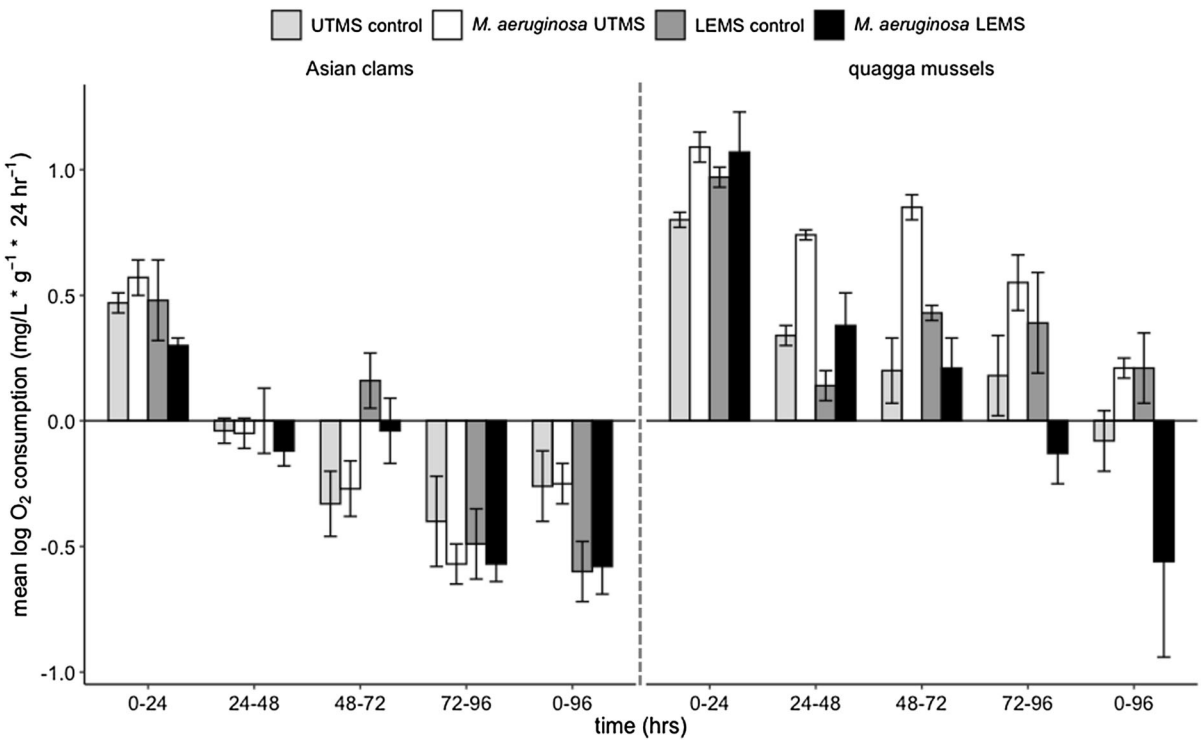

Fig. 1 Mean oxygen consumption rates (± SE, log-transformed) over a 96-h period for Asian clams and quagga mussels exposed to two strains of *Microcystis aeruginosa* (UTMS from University of Texas Culture Collection of Algae and LEMS collected from Lake Erie) and the respective *Ankistordesmus facaltus* controls run alongside each *M. aeruginosa* strain

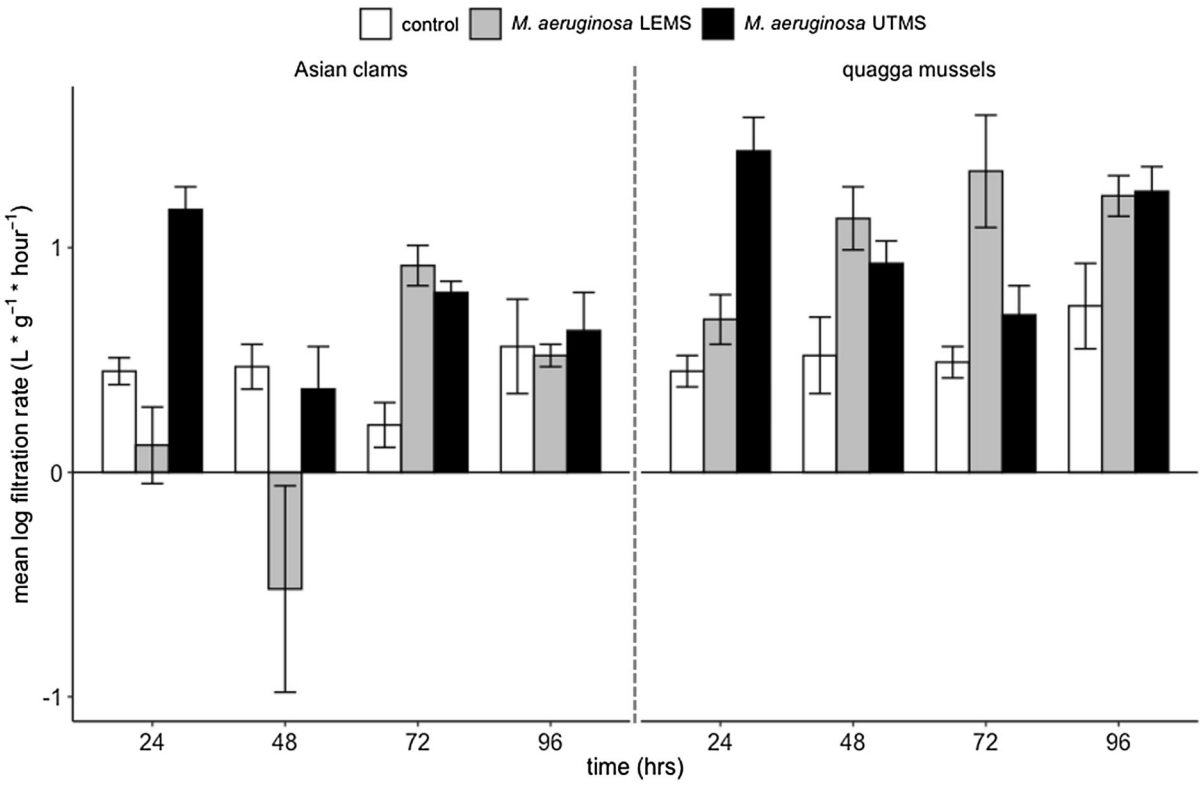

Fig. 2 Mean filtration rate (± SE, log-transformed) of Asian clams and quagga mussels exposed to two strains of *Microcystis aeruginosa* (UTMS from University of Texas Culture Collection of Algae and LEMS collected from Lake Erie) and *Ankistrodesmus facaltus* (control) over a 96-h period

0.029). Asian clams filtered a higher amount of both LEMS ($P = 0.003$) and UTMS ($P = 0.005$) on $t = 24$ h and a higher amount of UTMS ($P = 0.009$) and LEMS ($P = 0.039$) on $t = 72$ h (Fig. 2). Asian clams filtered a consistent amount of *A. facaltus* throughout the 96 h experiment (Table S2, Fig. 2; $P > 0.05$). However, filtration rate was impacted by both *M. aeruginosa* strains. Filtration rate in the UTMS treatment was lower on $t = 48$ h compared to $t = 24$ h (Fig. 2; $P = 0.047$). Asian clams exposed to LEMS experienced lower filtration rates on $t = 48$ h ($P < 0.001$) and $t = 96$ h ($P = 0.011$) compared to $t = 24$ h and had a slight decrease in filtration rates from $t = 24$ h to $t = 72$ h (Fig. 2; $P = 0.05$). Quagga mussels also filtered a consistent amount of *A. facaltus* throughout the experiment (Fig. 2; $P > 0.05$). While quagga mussel filtration rates did not vary with the exposure to UTMS ($P > 0.05$), filtration rates in the LEMS treatment decreased from $t = 24$ h to $t = 48$ h ($P = 0.022$) and from $t = 24$ h to $t = 72$ h (Fig. 2; $P = 0.003$).

Oxidative stress

Quagga mussels maintained higher levels of oxidative damage (LPO) and antioxidant response (CAT) than Asian clams throughout the experiment and across treatments (MANOVA, $P < 0.001$; Tables S3–S5, Fig. 3). Quagga mussels also had a higher baseline ($t = 0$ h) level of CAT than Asian clams (ANOVA, $P = 0.002$), but there was no difference in baseline levels of LPO (ANOVA, $P > 0.05$). Treatment was also a factor that resulted in a change of oxidative stress levels (MANOVA, $P < 0.001$; Table S3 and S4, Figs. 3 and 4); however, the trend was contrary to our hypothesis. LPO response in quagga mussels was highest in the control, where the control was significantly higher than the 400 µg/l microcystin-LR treatment throughout the experiment (SNK, $P = 0.009$; Table S4, Fig. 4). Catalase response in quagga mussels did not differ between the control and either concentration of microcystin-LR ($P > 0.05$; Table S4, Fig. 3). Cumulative CAT response in Asian clams was

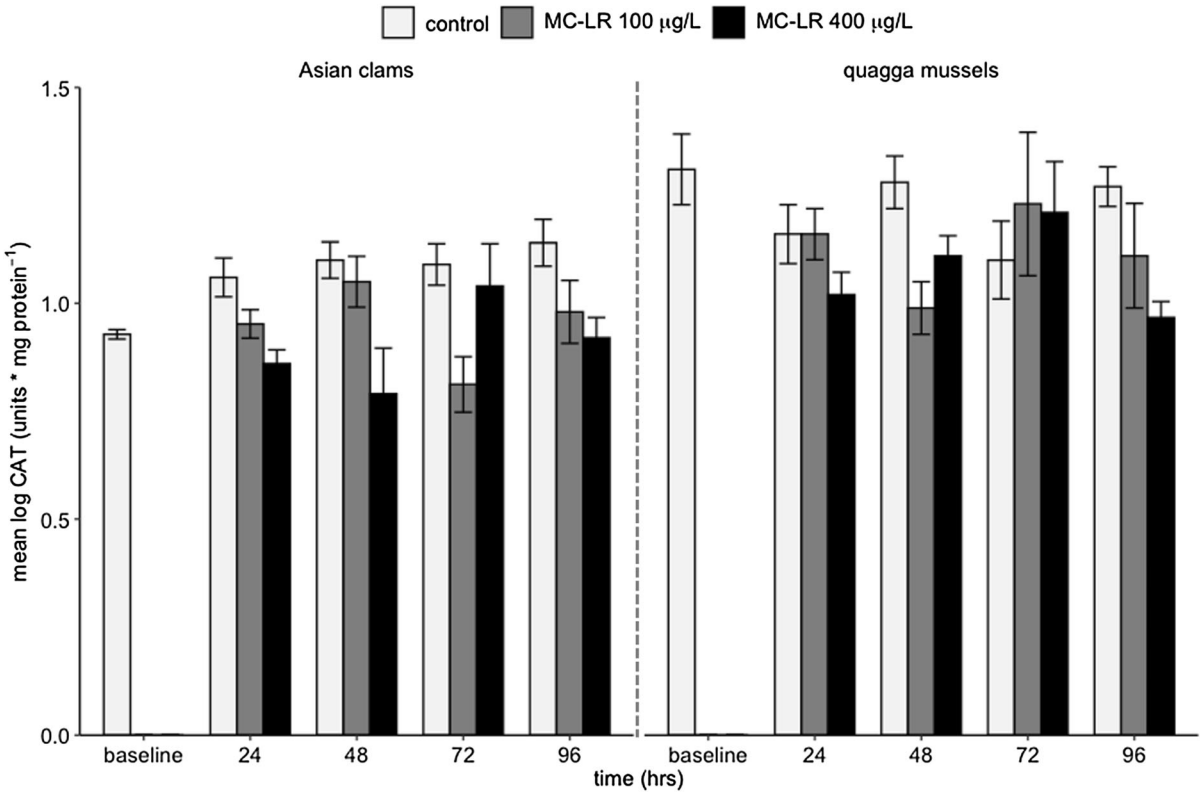

Fig. 3 Mean catalase response (± SE, log-transformed) of Asian clams and quagga mussels exposed to microcystin-LR (MC-LR) over a 96-h period

elevated in the control compared to the 100 µg/l treatment microcystin-LR (SNK, $P = 0.003$) and the 400 µg/l treatment (SNK, $P = 0.001$) (Table S4, Fig. 3). However, LPO damage in Asian clams did not vary among treatments (ANOVA, $P = 0.189$; Table S4, Fig. 4). While oxidative stress response in both treatments and the control was consistent throughout the study between $t = 24$ and $t = 96$ h (SNK, $P > 0.05$), the baseline LPO at $t = 0$ h was higher than $t = 24$ h ($P = 0.019$), $t = 48$ h ($P = 0.002$), and $t = 72$ h ($P = 0.003$) for Asian clams (Fig. 4).

Discussion

Although freshwater invasion by brackish species is becoming more common due to global anthropogenic activities (Lee et al., 2012), species invasion across salinity barriers is still uncommon and may help explain why quagga mussels exhibited higher rates of stress response than Asian clams. The ecological context of invasive Asian clam and quagga mussel populations may play a role in their sensitivity to environmental stressors in their invaded ranges (Kashian et al., 2007; Clements et al., 2012). Results from the current study demonstrate that quagga mussels have higher variability in oxygen consumption and filtration rate than Asian clams when exposed to *M. aeruginosa*. The organisms used in the current study were collected from waters with a high flow rate that do not experience regular cyanobacteria blooms, and thus, it is not expected that either test population would have any prior acclimation to *M. aeruginosa* or the toxin microcystin-LR.

Asian clams have an increased tolerance in hypoxia compared to zebra mussels, which supports our finding that oxygen consumption by Asian clams was less responsive to *M. aeruginosa* than quagga mussels; however, both Asian clams and quagga mussels can survive for at least four days under hypoxic conditions (Matthews & McMahon 1999; Nowicki & Kashian 2018). Stress response, including

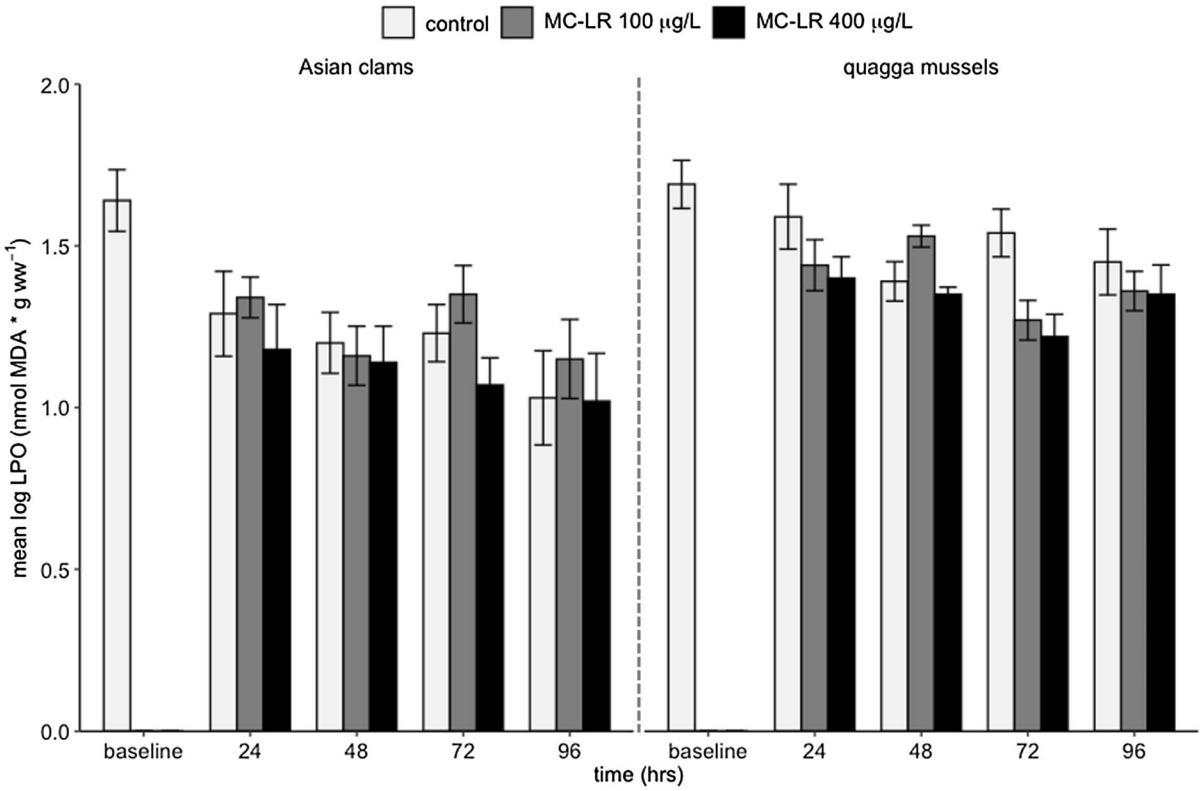

Fig. 4 Mean lipid peroxidation (± SE, log-transformed) of Asian clams and quagga mussels exposed to microcystin-LR (MC-LR) over a 96-h period

those from toxin exposure, is energetically expensive for organisms. For example, respiration rates in zebra mussels can increase during toxin exposure (Fernández-Sanjuan et al., 2013). In addition to stress associated with toxin exposure, physical disturbances can also elicit a stress response where increased turbidity can affect oxygen consumption in zebra mussels (Alexander et al., 1994). To our knowledge, the effects of *M. aeruginosa* exposure on oxygen have not been previously investigated in either species, and not much research exists on physiological endpoints in quagga mussels or Asian clams exposed to *M. aeruginosa*. Oxygen consumption of quagga mussels and Asian clams in the *A. falcatus* control was consistent with respiration rates reported for similar control populations (Tyner et al., 2015; Oliveira et al., 2018). In response to stressors, Vijayavel & Kashian (2019) found quagga mussel respiration was reduced in the presence of a chemical stressor, sodium phosphate, and Oliveira et al. (2018) measured reduced respiration in Asian clams with exposure to mercury. Contrary to these chemicals reducing respiration rates, the current study found increased respiration to quagga mussels exposed to cyanobacteria and changes in oxygen consumption in Asian clams were not observed. We did not account for oxygen demand created by the production of feces and pseudofeces, which is a consideration that should be taken into account for future studies; however, we did not observe noticeable waste produced by either species. As the oxygen consumption experiment proceeded in the current study, oxygen consumption decreased with a reduction in dissolved oxygen in the experimental chambers; however, respiration in quagga mussels was maintained at a higher rate than that of Asian clams, and oxygen consumption in quagga mussels varied with exposure to *M. aeruginosa* in response to the control, indicating that quagga mussels may be more prone to stress from cyanobacteria blooms.

Contrary to initial predictions, filtration rate was equal to or higher in the *M. aeruginosa* treatments compared to the *A. facaltus* control for both species

over the entire experiment suggesting that despite the varied life histories between the two species, filtration rates are naturally similar. While researchers initially thought dreissenid mussels preferentially rejected *M. aeruginosa* cells, some studies demonstrate that they will feed on the cyanobacteria, dependent on environmental conditions and phenotypic properties of cyanobacteria populations (Sarnelle et al., 2005; Vanderploeg et al., 2013; White & Sarnelle, 2014). A study published by White & Sarnelle (2014) reports that zebra mussels can filter *Ankistrodesmus* and *Microcystis* colonies up to 75 µm in diameter at an equal rate. Similarly, Asian clams feed on non-toxic strains of *M. aeruginosa* at the same rate as the green alga, *Scenedesmus obliquus* (Kützing 1883) (Liu et al., 2009) but can also filter green algae at higher rates than the cyanobacterium, *Planktothrix adardhii* (Anagnostidis & Komárek, 1988) (Marroni et al., 2014). Liu et al (2009) recorded higher filtration rate in Asian clams fed both toxic and non-toxic strains of *M. aeruginosa* compared to the *S. obliquus.* They also observed more waste products generated by Asian clams fed *M. aeruginosa*, indicating that it is not assimilated as efficiently as other phytoplankton and thus a high rate of filtration may not indicate it is a suitable food source. Pham et al. (2015) also observed higher filtration rates of *Corbicula leana* (Prime, 1867) in the presence of toxic and non-toxic *M. aeruginosa* than in the green alga, *Chlorella* sp. (Beijerinck, 1890). These studies, along with the results of the current study, indicate a complex relationship between filter feeding bivalves and potentially toxic cyanobacteria that is not completely explained by testing filtration rate alone and may be rooted in differential life histories between the species. To further complicate this relationship, a high level of variability in filtration rate was sometimes observed within the *M. aeruginosa* treatments of the same species (Fig. 2). Future studies should include additional test endpoints related to filtration feeding, such as food assimilation, waste production, and production of pseudofeces to determine the preference over phytoplankton species.

At the time of our study, neither strain of *M. aeruginosa* was producing toxins, and as such, any changes in filtration rate of the exposed Asian clams and quagga mussels were due to factors other than toxins and could possibly be related to lower nutritional quality or other secondary metabolites produced by the cyanobacteria. Accordingly, Boegehold et al. (2019) found the two strains of non-toxin-producing *M. aeruginosa* used in the current study also caused increased mortality in dreissenid larvae. Foods with lower energetic or nutrition value might result in higher feeding rates as the individual needs to consume more to meet its metabolic needs. Further, cyanobacteria can produce additional metabolites that can be toxic to aquatic organisms. Along with cyanotoxins, the production of feeding deterrents and lack of essential nutrients are the main causes of the adverse effects cyanobacteria can induce in aquatic invertebrates (Asselman et al., 2014). For example, *M. aeruginosa* can produce the teratogenic retinoid compounds which can be harmful to zebrafish embryos (Pipal et al., 2020) and other secondary metabolites may be more consistently toxic to zebrafish embryos than the cyanotoxin, cylindrospermopsin (Berry et al., 2009). Despite the differences in oxygen consumption and oxidative stress responses, both Asian clams and quagga mussels responded similarly by increasing filtration rate with exposure to *M. aeruginosa*.

Regardless of food preference, filter feeding by invasive populations of both Asian clams and quagga mussels can reduce phytoplankton abundances, causing the two species to outcompete native mussels (Marescaux et al., 2016). Productive freshwater ecosystems with high quality food promote survival and development of brackish invaders while species originating from freshwater conditions are less dependent on high food concentrations for survival in introduced freshwater ranges (Lee et al., 2013). Ponto-Caspian species accustomed to brackish waters have voracious filter feeding capacities in freshwaters, possibly due to the additional challenge of having less ionoregulatory capacities than their freshwater counterparts (Lee, 2016). There is evidence that Asian clams are better adapted to low food conditions than dreissenids, where they are better able to regulate filtration during seasonal variations in temperature and phytoplankton abundance (Marescaux et al., 2016). This corresponds with our initial hypothesis that quagga mussels must maintain a higher base rate of filter feeding to tolerate freshwater systems.

Quagga mussels exhibited reduced catalase rates following microcystin exposure, which may have aided in the prevention of lipid peroxidation damage. Catalase is an enzyme that converts hydrogen

peroxide into water and oxygen and acts as a major source of protection against oxidative stress damage (Kurutas 2016). Lipid peroxidation is an indicator of oxidative stress damage to cells and tissues, and thus, a proper catalase response to reactive oxygen species could aid in preventing oxidative stress, as indicated by the lack of lipid peroxidation in the current study (Kurutas 2016). Nowicki & Kashian (2018) reported higher levels of baseline catalase and lipid peroxidation in quagga mussels compared with zebra mussels and are consistent with baseline values from the current study. Similar to Nowicki & Kashian (2018), the current study assessed catalase as a ubiquitous enzyme that reacts with hydrogen peroxide to form water and oxygen, thereby reducing reactive oxygen species. On the other hand, lipid peroxidation was used to determine the amount of oxidative damage that had occurred in quagga mussels and Asian clams. Increased environmental stressors, which produces reactive oxygen species, can sometimes act to *inhibit* enzymatic response to cellular stress in short-term studies (Kono & Fridovich, 1982; Faria et al., 2009). Observed reductions in catalase in quagga mussels and Asian clams may be indicative of the increase in reactive oxygen species due to oxidative stress. Bigot et al. (2011) exposed Asian clams to metals copper and cadmium and recorded a reduced catalase response, indicating that toxic levels of certain contaminants might hinder an organism's ability to detoxify. Burmester et al. (2012) reported that catalase activity was unaffected in zebra mussels (*D. polymorpha*) exposed to microcystin-LR, but the toxin did affect the antioxidant enzymes superoxide dismutase (SOD) and glutathione S-transferase (GST). There was not as much variation in oxidative stress biomarkers in the current study as hypothesized. Future studies should also incorporate the quantification of the antioxidant glutathione (GSH) and GST as they may be responsible for detoxifying and excreting microcystins (Pham et al., 2015).

While catalase response in Asian clams and quagga mussels was highly variable, lipid peroxidation damage did not increase with microcystin exposure. This indicated that the animals used in the current study did not experience cellular damage by exposure to microcystin. The lack of cellular damage could also be from efficient enzyme response which prevented oxidative damage from occurring. Other studies have demonstrated that lipid peroxidation and other oxidative stress biomarkers in invasive dreissenid mussels and Asian clams can be induced by several chemical contaminants. Lipid peroxidation in zebra mussels was found to be extremely sensitive to drugs such as ibuprofen, where concentrations as low as 1 μg/l reduced overall mussel condition (weight g/shell length mm) and increased LPO (André & Gagné, 2017). Faria et al. (2009) observed higher rates of oxidative stress corresponding with high metal concentrations (Pb, Zn, As) in zebra mussels. Furthermore, Asian clams also experienced an increase in lipid peroxidation when exposed to ibuprofen, and other drugs such as carbamazepine, novobiocin, and tamoxifen, but were not sensitive to caffeine (Aguierre-Martinez et al., 2015). Four-day exposure to microcystin did not induce lipid peroxidation damage in Asian clams or quagga mussels in the current study, indicating short-term cyanotoxin exposure may not cause cellular-level damage. Pham et al. (2015) indicate that *C. leana* experienced elevated oxidative stress levels when exposed to both a toxic and a non-toxic strain of *M. aeruginosa*, which further supports the results of the current study that a cyanobacterial factor other than microcystin may be the cause for physiological responses in filtration rate and oxygen consumption. A more thorough investigation on oxidative stress biomarkers in these invasive species, both in the presence of toxin- and non-toxin-producing cyanobacteria, is needed.

Differences in physiological tolerances to environmental stressors between quagga mussels and Asian clams could be used to help predict their distribution and community dynamics. It could be possible that in overlapping ranges of invasion, quagga mussels dominate in times of high phytoplankton production, but are ultimately thwarted by their voracity, consequently causing their own population crash, opening the opportunity for a population boom in Asian clams which can thrive with less food. Understanding physiological tolerances in invaders with few, if any predators is essential for predicting population cycles. Both species are highly successful invaders; however, it is pertinent to evaluate how rapidly changing ecosystems will affect the population dynamics of the species that enact such drastic changes.

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Compliance with ethical standards

Conflict of interest None.

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