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PRIMARY RESEARCH PAPER

Resource contributions from dreissenid mussels to the benthic algae *Lyngbya wollei* (Cyanobacteria) and *Cladophora glomerata* (Chlorophyta)

Patricia M. Armenio · Christine M. Mayer · Scott A. Heckathorn · Thomas B. Bridgeman · Sarah E. Panek

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Abstract *Dreissena* spp. (zebra and quagga mussels) are invasive to North America and increase light to the benthos, provide hard structure for algal attachment, and may contribute limiting nutrients to benthic algae, thereby facilitating algal blooms. We conducted experiments to determine how *Dreissena* affect nutrient stoichiometry and growth of *Lyngbya wollei* and *Cladophora glomerata*, two benthic algal species recently increasing in biomass in parts of the Laurentian Great Lakes, combined with a field survey to determine the likelihood of *L. wollei* co-occurrence with *Dreissena*. *L. wollei* had a significantly higher concentration of carbon, nitrogen, phosphorus, potassium, and sulfur when grown with live *Dreissena*. *C. glomerata* had greater biomass in tanks with live *Dreissena*, but did not have significant increases in nutrient concentration like *L. wollei* did. Neither algal species increased in growth due to the added structure of *Dreissena* shells. *L. wollei* biomass was greater in the presence of *Dreissena* during 1 year (of two) of our field survey. This field survey also showed that *L. wollei* and *Dreissena* are likely to co-occur. These results suggest that *Dreissena* provide several nutrients to benthic algae, and these added resources can promote algal growth and consequently blooms.

Keywords Nutrient · Phosphorus · Algal bloom · Quagga mussels · Zebra mussels · Lake Erie

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P. M. Armenio · C. M. Mayer · T. B. Bridgeman · S. E. Panek
Department of Environmental Sciences and the Lake Erie Center, University of Toledo, 6200 Bayshore Rd., Oregon, OH 43616, USA

Present Address:
P. M. Armenio (✉)
USGS Great Lakes Science Center, 1451 Green Road, Ann Arbor, MI 48105, USA
e-mail: pcope@rockets.utoledo.edu

S. A. Heckathorn
Department of Environmental Sciences, University of Toledo, 2801 W. Bancroft St., Toledo, OH 43606, USA

Introduction

Dreissena spp. (zebra mussels, *Dreissena polymorpha* Pallas and quagga mussels, *Dreissena rostriformis bugensis* Andrusov) are native to the Ponto-Caspian region and invaded North America in the 1980s (Hebert et al., 1989). *Dreissena* invasions can alter the ecosystem function within lakes at both lake-wide and at local spatial scales. For example, at the system-wide scale, these bivalves increase water clarity (Holland, 1993; Mayer et al., 2002; Vanderploeg et al., 2002; Barbiero & Tuchman, 2004) and, hence, levels of light are increased in lakes as well as the depth at which

light penetrates into the water column. A system-wide increase in light availability following *Dreissena* invasion can increase benthic primary producer biomass (Lowe & Pillsbury, 1995; Skubinna et al., 1995; Zhu et al., 2006), productivity (Fahnenstiel et al., 1995; Lowe & Pillsbury, 1995; Cecala et al., 2008), and change nutrient stoichiometry (Qin et al., 2007). Additionally, the large scale distribution of phosphorus (P) in lakes can also be affected by *Dreissena*, which can redirect P from the water column via their filter feeding to the nearshore (Hecky et al., 2004). The effect of *Dreissena* on P distribution has been termed the "nearshore shunt" (Hecky et al., 2004), and may operate over a large area; the Laurentian Great Lakes (where *Dreissena* are now abundant) have over 15,000 km of shoreline (Government of Canada & US EPA, 1995). Therefore, *Dreissena* can alter the system-wide distribution of light and important nutrients in a manner that may promote benthic over pelagic primary production and may further control where primary production will be greatest within a lake.

In addition to lake-wide effects, the invasion of *Dreissena* may specifically affect the benthic sub-systems of lakes in ways that contribute to blooms of benthic algae. *Dreissena* can contribute potentially limiting nutrients, such as nitrogen (N) and P to the benthic sub-system in lakes. For example, *Dreissena* are important in nutrient recycling within the western basin of Lake Erie (Arnott & Vanni, 1996; Conroy et al., 2005). The excretion of solid waste from *Dreissena* may especially benefit attached algae such as *Cladophora glomerata* (L.) Kütz. (Hecky et al., 2004); *Cladophora* blooms in the lower Great Lakes during the mid 1990s may have been caused by increased water clarity and P recycling from *Dreissena* (Higgins et al., 2008; Auer et al., 2010). *Dreissena* may also contribute CO_2 to benthic systems, and thereby potentially increase benthic photosynthesis. Shallow freshwater environments and areas of dense algal mats can become depleted in CO_2 (Stevenson, 1988; Stevenson et al., 2004), which can restrict photosynthesis if the alga cannot utilize HCO_3^- as well as CO_2 (Cheney & Hough, 1983). *Dreissena* aggregations can have a very high density (Coakley et al., 2002); consequently, CO_2 produced by their respiration and the decomposition of their waste may potentially increase CO_2 and soluble organic carbon within the benthos. Although studies have shown that *Dreissena* can promote benthic algal blooms through increased light, P, and N, the relative importance of potential flows of macronutrients and carbon (C) from *Dreissena* to algae on controlling benthic primary production has not been shown.

In the same way *Dreissena* can potentially provide macronutrients, micronutrients may be provided to benthic algae; but this question has received little study. However, it is known that micronutrients can limit primary production. For example, low iron (Fe) can restrict photosynthetic capacity in cyanobacteria (Trick et al., 1995; Albert et al., 2005), and silica (Si) can limit benthic diatoms in Lake Michigan (Carrick & Lowe, 2007). It is reasonable that *Dreissena* may concentrate micronutrients, and, thereby, provide an additional resource to benthic algae; however, little if any data are currently available on this potentially important interaction.

In addition to the chemical resources that *Dreissena* may provide to benthic algae, they may also provide a physical resource in the form of hard substrates for algal attachment (Lowe & Pillsbury, 1995). The quagga mussel survives well on soft sediment (Dermott & Munawar, 1993) and is the dominant dreissenid in many areas of the Great Lakes (Mills et al., 1993; Karatayev et al., 2014). The quagga mussel qualitatively alters the benthic habitat, changing it from soft to hard, and the accretion of its dead shells may also have a particularly large effect on the benthic community (Hecky et al., 2004). There is a lack of comparative studies that show the relative importance of various chemical and physical resources that *Dreissena* may provide to benthic algae and, thereby, possibly promote benthic primary production and algal blooms.

The growth form and physiological tolerances of benthic algal species may influence how *Dreissena* affect each algal species and under what conditions each algal species forms blooms. *Cladophora glomerata* is a green alga which, prior to the 1980s, formed blooms in many locations, probably due to eutrophication (Higgins et al., 2008). More recently, this species has become abundant in eastern Lake Erie and Lake Michigan, possibly due to high water clarity and an increase in P levels (Bootsma et al., 2005; Higgins et al., 2005b). *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck is an N fixing cyanobacterium that has recently become abundant in Lake Erie (Bridgeman & Penamon, 2010), due to an unknown cause.

Abundant *C. glomerata* can lead to problems such as decreased esthetic value and recreation, human health risks, and altered food web structure (Bootsma et al., 2005), while the same issues occur with *L. wollei*, including the capability of producing toxins (Carmichael et al., 1997). Unlike *C. glomerata*, *L. wollei* does not attach to hard substrates; it becomes partially covered in sandy or dreissenid shell substrate (Bridgeman et al., 2012). *L. wollei* also differs from *C. glomerata* in that it grows well in low light (Dyck, 1994). Little is known about the emerging *L. wollei* and the effect of dreissenids on it. However, field observations suggest *L. wollei* is often present near *Dreissena* in western Lake Erie (personal observation, Fig. 1). We observed *C. glomerata* at only a few locations in western Lake Erie; however, this species co-occurs with *Dreissena* in Lakes Michigan, Ontario, and eastern Lake Erie (Hecky et al., 2004; Higgins et al., 2005b; Depew et al., 2011). Hence, it is likely that greater benthic algal biomass will potentially occur where *Dreissena* are present, possibly mediated by increased physical structure or nutrients from *Dreissena*.

To address the gap in knowledge of the relative importance of various chemical and physical resources that *Dreissena* may provide to benthic algae, we combined a manipulative experiment and a field survey to investigate how the presence of *Dreissena* affect the nutrient concentration and stoichiometry of several mineral nutrients, as well as the growth of benthic algae. The goals of this study were to (1) test if *Dreissena* can facilitate algal growth of two benthic algal species with different growth modes, (2) determine what resources *Dreissena* contribute to algae (nutrients vs. substrate surface), and (3) determine if *L. wollei* (the dominant benthic nuisance alga in western Lake Erie) is more likely to occur when *Dreissena* are present. We hypothesized that *Dreissena* would increase growth in both benthic algal species, primarily by affecting either macro- or micronutrients. Further, we hypothesized that *L. wollei* would occur more frequently where *Dreissena* were present in western Lake Erie due to higher nutrient resources being available. This manipulative experiment allowed us to compare the response of benthic algal growth and stoichiometry to the chemical and physical resources that *Dreissena* can provide to benthic algae, but its small scale is a simplification of a dynamic, natural system. Therefore, examining the spatial relationship between *L. wollei* and *Dreissena* in a large lake helps determine whether outcomes observed in controlled laboratory experiments are likely to also occur in a larger dynamic habitat.

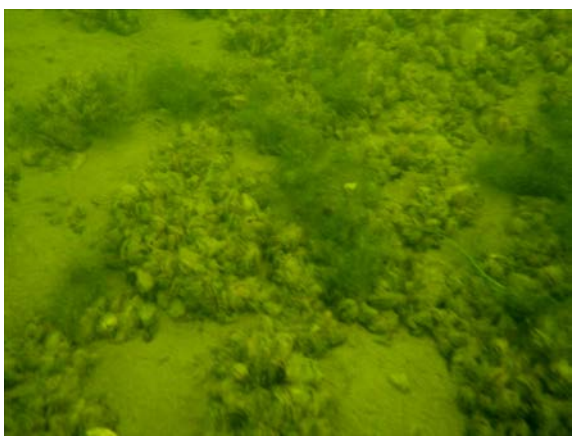

Fig. 1 Photo of *Dreissena* and *L. wollei* in western Lake Erie. Taken by P. Bichier

Materials and methods

Experimental set-up and design

In order to determine what resources *Dreissena* may provide to benthic algae, *L. wollei* and *C. glomerata* were grown under four treatments: (1) live *Dreissena*, (2) empty *Dreissena* shells, (3) pottery shards, and (4) sand only ($N = 10$ for each treatment and each algal species). The live *Dreissena* treatment could potentially provide macronutrients, micronutrients, or C to the algae through respiration or excretion of waste. The empty shell treatment could potentially leach macro- or micronutrients, and both the empty shell and pottery shards could alter algal growth by providing a hard substrate for attachment. We used sand as a control substrate because lake sediment could have provided the algae with some nutrients. Algae were grown in clear plastic 6 l tanks for 1 week, after which photosynthetic efficiency, wet biomass, dry biomass, pigment content, and tissue elemental concentrations were measured. We measured the increase in algal biomass as an index of growth and algal elemental composition to determine which resources affect the algae among these treatments. The short duration of the experiment was intended to reduce confounding

contamination of the tanks by other algae. Tanks were placed on shelves illuminated with hanging fluorescent lights controlled by timers to provide a 12-h photoperiod. Water temperature in tanks was not controlled directly, but was maintained by room temperature: for the *C. glomerata* experiment, it ranged from 28 to 30°C and for *L. wollei*, room temperature was 21°C because the experiments for the two algal species were run on different dates. Tanks of each treatment type were interspersed with two complete sets of treatments (i.e., substrate type) on each shelf to prevent spatial bias.

For the live *Dreissena* treatment, *Dreissena* were collected from sediment in western Lake Erie near Pelee Island (4145.806, −8246.198) at a depth of 8.4 m using a benthic trawl, and they were kept in stock tubs with lake water for 1–4 months until needed for experimentation. These samples were dominated by quagga mussels, as is now common across the Great Lakes (Karatayev et al., 2014). Unfiltered lake water with naturally occurring plankton was refreshed about twice a week in the stock tubs of *Dreissena* to provide food and other resources. The quantity of undamaged *Dreissena* added to the experimental tanks represented the density of *Dreissena* on soft sediments in Lake Erie (i.e., 3,400 individuals m^{-2} (Coakley et al., 2002) or 116 individuals/tank). Empty *Dreissena* clusters were made by attaching dead *Dreissena* shells together using small beads of non-toxic glue to provide the same physical structure as live *Dreissena*, without any contribution of resources from active metabolism. For the pottery shard treatment, clay pots were broken into pieces and then soaked in deionized water for at least one week prior to use; these pottery shards served as a comparison to the empty *Dreissena* shells, in case *Dreissena* shells provide important mineral nutrients via leaching, since any nutrients leaching from clay pots would be different from *Dreissena* shells. The volume of empty *Dreissena* and pottery shards was the same as live *Dreissena*: ca. 125 ml. All tanks contained 1.5 cm of sand (instead of lake sediment); the sand was rinsed and sieved with a 243 µm mesh so that organic nutrient background contributions would be minimal.

Both species of benthic algae were collected from western Lake Erie and washed in reverse osmosis water several times to remove invertebrates and detritus, while larger invertebrates and debris were removed with tweezers. As a baseline measurement, macro- and micronutrient concentrations of the algae were measured before culturing (Supplementary Material 1, lake; see "Measurements" below for methods). Prior to the experiment, algae were kept in WC media (Guillard & Lorenzen, 1972), a common nutrient solution containing all required mineral nutrients, which was refreshed every week. Two weeks prior to experimentation, the medium was changed to half concentration to prevent nutrient storage in the algae. The cultures were on a 12-h photoperiod with the same light levels as during the experiment (see below). The fresh biomass of *L. wollei* added to each tank was 230 g m^{-2}, which is similar to densities in western Lake Erie (Bridgeman & Penamon, 2010). The fresh biomass of *C. glomerata* added to each tank was 160 g m^{-2}, similar to biomass recorded in western Lake Erie (Higgins et al., 2008). *C. glomerata* was collected from rock substrate and placed in the tanks; the alga was detached from its original substrate using a sharp blade. We removed the *C. glomerata* in order to quantify initial biomass. Further, we were also testing whether hard substrate alone (pottery shards and empty *Dreissena* shells) affected algal growth; therefore, leaving the alga attached would have confounded these treatments. We did not expect the alga to reattach, since that was our experience during preliminary experiments. All tanks with *L. wollei* were shaded using window screening, due to its low light requirement. The average light level in the tanks with *L. wollei* was 29.29 ± 5.87 µmol m^{-2} s^{-1} PAR (photosynthetically active radiation). *L. wollei* tanks were gently aerated to keep *Dreissena* alive, but not disturb the alga. The average light level for tanks with *C. glomerata* was 65.32 ± 15.37 µmol m^{-2} s^{-1} PAR, and the tanks were vigorously aerated because this species occurs in shallow high wave action waters in western Lake Erie. Walls of the *C. glomerata* tanks were scraped on day 5 of the experiment to remove any periphyton growth; tanks with *L. wollei* did not need to be scraped.

Chlamydomonas reinhardtii served as food for *Dreissena* (Baldwin et al., 2002) during the experiment. The phytoplankton was also cultured in WC media (Guillard & Lorenzen, 1972) and relatively dense mature cultures were used. Mussels were fed *C. reinhardtii* starting the day before the experiment began, when *Dreissena* needed for the experiment were placed in a separate bucket with lake water and

15 ml *C. reinhardtii* per 3,400 individuals *Dreissena* m^{-2} (Nichols, 1993) was added. The addition of the phytoplankton continued throughout the experiment by giving the same amount (15 ml) of *C. reinhardtii* stock to each tank (even ones without *Dreissena*) every other day during the experiment, except the last day. The addition of *C. reinhardtii* in all tanks created a small increase in nutrients making laboratory conditions more closely resemble the lake.

Nearshore water from western Lake Erie was used for the experiment, which was pre-treated with 30 mg l^{-1} alum (aluminum sulfate) to remove excess P following Chaffin (2009), due to extremely high P concentrations in western Lake Erie. Water was sieved through a 243 μm mesh and kept aerated in 128 l bins until alum was added, then aeration was turned off to allow precipitation. Flock was siphoned from the bottom of the bins the following day. The lake water before and after alum treatment was analyzed for concentration of all elements detected by the ICP-OES analyzer (see methods below): boron (B), calcium (Ca), copper (Cu), Fe, potassium (K), magnesium (Mg), sodium (Na), P, sulfur (S), Si, and zinc (Zn), but our focus was P concentration. Four liters of the water was added to each experimental tank (18.5 × 18.5 × 17.5 cm). One liter of alum-treated lake water was added half way through the experiment to all tanks to compensate for evaporation. Water samples from each tank showed that alum treatment decreased P levels in the water before experimentation from an average of 320 to 80 μg l^{-1}.

Experimental measurements

For both benthic algal species, dissolved oxygen concentration and temperature in tanks were measured using a YSI 5000 dissolved oxygen meter (Yellow Springs Instruments) on the first and last day of the experiment. Light above the tanks and in the tanks was measured using a Loggerhead 2100 (Biospherical Instruments) light probe. As an indicator of the physiological health of the algae, photosynthesis was monitored via chlorophyll fluorescence using a Walz DIVING-PAM (pulse amplitude modulated) fluorometer on the first and last day of the experiment to assess initial and final conditions. Photosynthetic performance was assessed as steady-state light-adapted in situ quantum yield of photosystem II electron transport (Φ_{et}) and apparent relative rate of electron transport (ETR), i.e., relative due to use of assumed light absorption coefficient of 0.85 (Campbell et al., 1998).

All of the algae in experiments were rinsed in DI water before and after the experiment. Three samples of each type of algae were collected immediately prior to the experiment for determination of initial nutrient concentrations in algal tissues. Fresh mass of algal samples was determined after blotting the algae on a paper towel. The algal samples were dried to a constant weight at 65°C and then ground to a powder using mortar and pestle. Wet biomass and dry biomass of both *L. wollei* and *C. glomerata* were significantly related to each other ($r^2 = 0.80$, $P < 0.0001$; $r^2 = 0.69$, $P < 0.0001$, respectively), indicating that the procedure used to portion wet mass into each tank at the beginning of the experiment was a good estimate of dry biomass. The percentage of *Dreissena* still alive at the end of the experiment was noted as well. Phycocyanin (PC), the accessory light-harvesting pigment of cyanobacteria such as *L. wollei*, was extracted from 5 mg of dried algae in 0.1 M phosphate buffer pH 6.8 (Furuki et al., 2003; Sampath-Wiley & Neefus, 2007). The algae were ground to a powder in liquid nitrogen using mortar and pestle, and then ground in 10 ml of buffer. Samples were centrifuged for 10 min at 3800 rpm and then supernatant was collected and stored at −80°C until pigment quantification. PC concentration was quantified from PC fluorescence (10-AU Turner Design fluorometer with P/N 103-80 filters), using purified PC standards to generate a standard curve.

We measured chlorophyll (chl) *a*, the light-harvesting pigment of all photosynthetic organisms, as an indicator of algal biomass. Chlorophyll *a* and *b* were extracted from an average of 70 mg and 30 mg of fresh biomass tissue in 3.5 ml and 3 ml of 100% dimethyl sulphoxide (DMSO) for *L. wollei* and *C. glomerata*, respectively. The samples were heated in a 65°C water bath for 45 min, cooled to room temperature, and stored at −20°C for 2 days until quantification (Barnes et al., 1992). The samples were thawed to room temperature and centrifuged at 21,000×*g* for 10 min. Chlorophyll *a* and *b* concentrations were quantified from absorbance (UV-1650 PC Shimadzu spectrophotometer), using the equations from Barnes et al. (1992).

C and N concentrations were determined by gas chromatography following combustion (HCNO/S analyzer Perkin-Elmer 2400 series II) using 2.5–3 mg of dried algae tissue. A combination of standards was used including acetanilide, spinach leaves, and peach leaves with a deviation from standards of −0.075% for C and −0.143% for N, i.e., complete combustion. Micronutrients, P, and other macronutrients were determined by first digesting 150 mg of dried tissue in a microwave digester (MARS; CEM Corp.) using a modified EPA method (EPA method 3051; HNO_3 digestion at 200°C with an additional peroxide step). Then nutrient content was determined with inductively coupled plasma optical emission spectroscopy (ICP-OES Model IRIS Intrepid II), as in Krug et al. (2009) using the same standards as above. Nutrient concentrations as well as total nutrient content are shown because it can be hard to interpret biomass and concentrations alone when they have changed at the end of the experiment. Molar ratios from Kahlert (1998) were converted into mass ratios and used for an assessment of nutrient deficiencies. A C:P ratio less than 143 indicates P sufficiency in freshwater benthic algae and a C:N ratio above 9.4 indicates N deficiency (Kahlert, 1998). Total nutrient content in the water of only the *C. glomerata* experiment was also quantified using ICP-OES. The macro- and micronutrient contents of *Dreissena* tissue were measured at the end of the *C. glomerata* experiment (Supplementary Material 1, *Dreissena*, tissue).

Experimental data analysis

One-way non-parametric Kruskal–Wallis tests were used to determine the effects of *Dreissena* on *L. wollei* and *C. glomerata* in regards to biomass, pigments, and nutrients ($N = 10$ for each of the four treatments per algal species) using SAS (version 9.1; SAS Institute, Cary, North Carolina) with an alpha level of 0.05. Nemenyi tests, a non-parametric analog to Tukey's tests, were used to identify the significant differences among treatments when Kruskal–Wallis tests indicated a significant difference existed. Kruskal–Wallis tests, the non-parametric version of analysis of variance (ANOVA), were done because the data were not normal and, in most cases, highly skewed. A conservative Bonferroni correction for the larger number of multiple comparisons of nutrients ($N = 12$ nutrients, $N = 3$ nutrient ratios) was done, decreasing the alpha level from 0.05 to 0.0033. Original results of the Kruskal–Wallis tests were given along with results after the Bonferroni correction. T-tests with Satterthwaite correction for no assumption of equal variance compared initial values ($N = 3$) to all samples across treatments at the end of the experiment ($N = 40$); this corrected test adjusts standard error and degrees of freedom (Zar, 1999). The non-parametric version of the t test, the Mann–Whitney U test, was not used because it is not possible to account for heteroscedasticity with this test. We considered the need to correct for uneven variance more important than avoiding an assumption of normality because variance differed substantially among treatments. Linear regression tests were conducted to estimate the relationship between the wet and dry biomass of the algae at the end of the experiment and the relationship between dry biomass and photosynthetic efficiency.

Field survey

Field surveys of the presence or absence of *L. wollei* and *Dreissena* were conducted from June–August 2009 ($N = 140$) and 2010 ($N = 178$) in western Lake Erie (see Panek, 2012). The depths ranged from 0.5 m to 9.5 m, with a variety of substrate types (clay, mud, gravel, sand, etc.,). At each site, five replicate Ekman grabs were collected, and *L. wollei* in these samples was dried at 70°C to a constant weight and averaged across the five replicates to obtain biomass represented as g m^{-2} (Bridgeman et al., 2012). During 2009, dreissenid shells found in the samples were not categorized as dead or alive. However, in 2010, *Dreissena* presence was separated as live *Dreissena*, empty shells, or both. In cases where both live *Dreissena* and empty shells were present, their counts were combined in both the live *Dreissena* category and *Dreissena* shell category, rather than having a separate category for each. Dreissenid biomass was not measured in either year.

A Pearson's χ^2-test for independence was used to test the count data by comparing the observed presence/absence field data of *L. wollei* and *Dreissena* to the calculated expected frequencies in 2009 and 2010. A Yates' correction was employed for the 2010 data to prevent overestimation of significance since the expected frequency was less than 5 in more than 20% of the categories. Our hypothesis was that *L.*

wollei and *Dreissena* presence were not independent and for both years we would find: (1) more instances of *L. wollei* present when *Dreissena* were also present, and (2) fewer instances of *L. wollei* present when *Dreissena* were absent. Further, a one-tailed t-test assuming unequal variances was used to compare *L. wollei* biomass when *Dreissena* were present and absent in both years.

Results

Experiment: *Lyngbya wollei*

Overall, *Dreissena* did not have a significant effect on *L. wollei* biomass. *L. wollei* wet biomass increased above the initial level in every treatment, but there was no difference among treatments in percent increase in mass (Kruskal–Wallis: $\chi^2 = 0.84$, $P = 0.84$). Final wet and dry biomass also did not differ among treatments (Fig. 2a, Kruskal–Wallis: $\chi^2 = 0.73$, $P = 0.87$, wet mass; $\chi^2 = 3.28$, $P = 0.35$, dry mass).

Despite the lack of response in algal biomass among treatments, *L. wollei* nutrient tissue concentrations were different in the live *Dreissena* treatment compared to the others. C, N, P, K, and S concentrations of *L. wollei* were significantly higher in tanks with live *Dreissena* and Ca concentration of *L. wollei* was significantly lower in tanks with live *Dreissena* (Fig. 3a–c; Table 1) relative to the other treatments, i.e., empty *Dreissena*, pottery shards, and sand only. Total P and N contents of *L. wollei* showed the same trend as the algal P and N concentrations, but were not significantly different among treatments (Fig. 3d, e, Kruskal–Wallis: $\chi^2 = 6.25$, $P = 0.10$; Kruskal–Wallis: $\chi^2 = 3.67$, $P = 0.30$, respectively). Tissue N, P, S, B, Cu, and Mn concentrations of *L. wollei* were significantly lower at the end of the experiment relative to the initial concentration (Table 2); however, total C content increased at the end of the experiment (Fig. 2b, $t = 3.75$, $P = 0.03$) accounting for the biomass increase. Despite the increase in total C and decrease in P concentration, no *L. wollei* in any treatment exhibited a P deficiency (C:P <143). However, *L. wollei* in every treatment except live

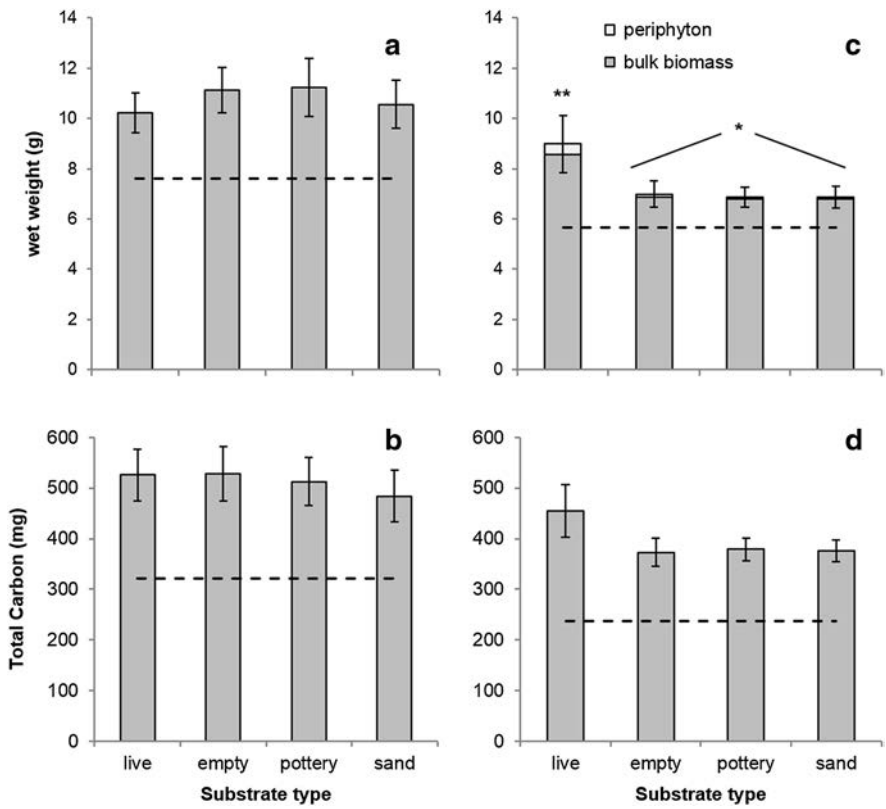

Fig. 2 **a** Wet biomass of *L. wollei* at the end of the experiment on four substrates: (1) live *Dreissena*, (2) empty *Dreissena*, (3) pottery shards, and (4) sand. **b** Total carbon content of *L. wollei*. **c** Wet biomass of *C. glomerata* separated between floating (bulk) *C. glomerata* and periphyton. *Error bars* represent 1 standard error of total wet biomass. **d** Total carbon content of *C. glomerata*. *Dotted line* represents initial values at the start of the experiment. *Error bars* represent 1 standard error. *Asterisks* represent statistical significance. $N = 10$ per treatment

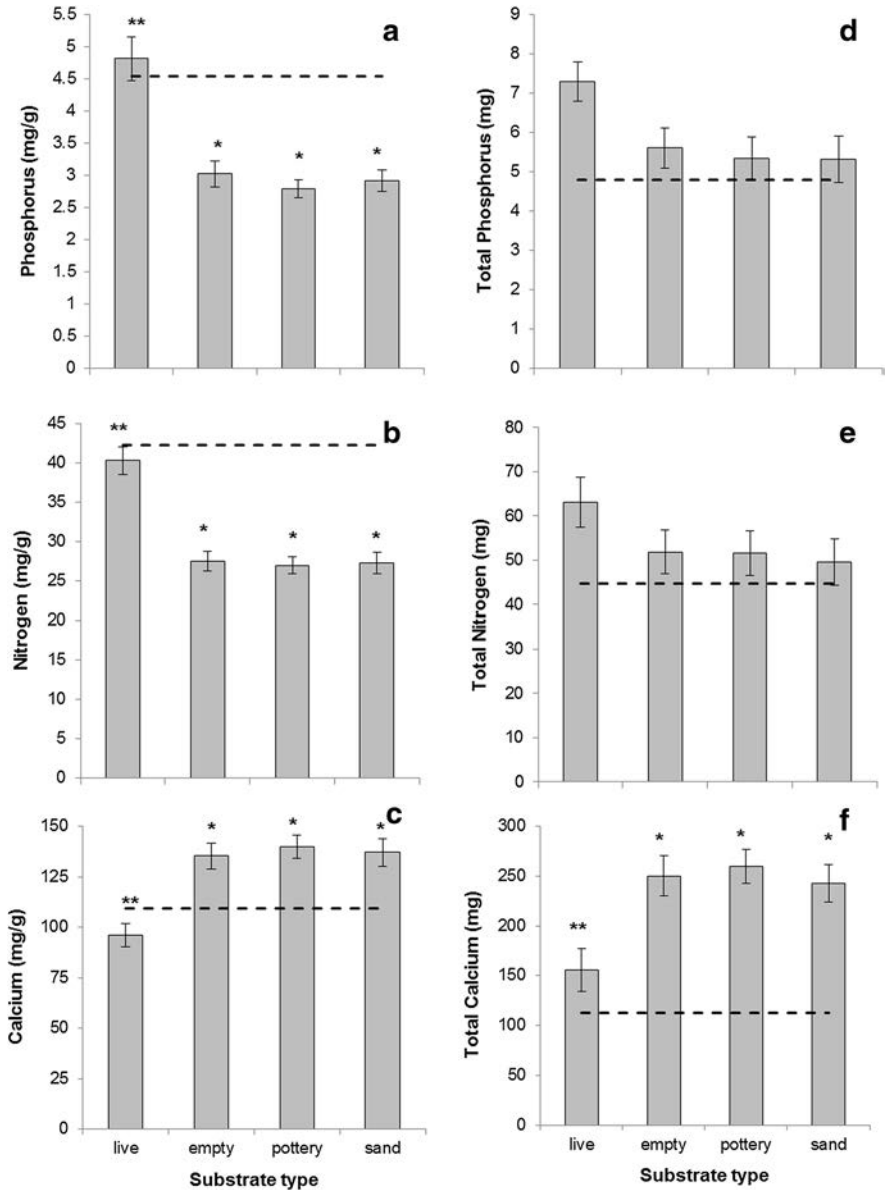

Fig. 3 **a** Phosphorus, **b** nitrogen, and **c** calcium concentrations of *L. wollei* at the end of the experiment. **d** Total phosphorus, **e** nitrogen, and **f** calcium contents of *L. wollei* at the end of the experiment. *Dotted line* represents initial values at the start of the experiment. *Error bars* represent 1 standard error. *Asterisks* represent statistical significance. $N = 10$ per treatment

Dreissena were deficient in N (Fig. 4a, b); the C:N ratio of *L. wollei* with live *Dreissena* was 18.8% lower than the other treatments. Mg concentration of the alga significantly increased 17% at the end of the experiment compared to the initial values (Table 2).

Photosynthetic efficiency (Φ_{et}), with an overall average of 0.40, and ETR did not differ among treatments. Photosynthetic efficiency was also not significantly different from the beginning and at the end of the experiment ($t = 0.80$, $P = 0.4263$). However, Φ_{et} at the end of the experiment was significantly negatively related to dry biomass ($r^2 = 0.40$, $P < 0.0001$) indicating that *L. wollei* had lower efficiency when biomass was greater. *L. wollei* did not contain any chl *b*, indicating that it was not contaminated with green algae. Chl *a* concentration did not differ among treatments, but PC was significantly higher in the live *Dreissena* treatment (Kruskal–Wallis: $\chi^2 = 18.32$, $P = 0.0004$) than the empty shells and pottery shards treatments, but not the sand treatment. There was no difference in water light level among the treatments (Kruskal–Wallis: $\chi^2 = 0.1713$, $P = 0.9821$),

Table 1 Results of Kruskal–Wallis tests of nutrient concentrations of *L. wollei* and *C. glomerata* at the end of the experiment comparing the four treatments: live *Dreissena*, empty *Dreissena*, pottery shards, and sand

Nutrients	*Lyngbya wollei*		*Cladophora glomerata*	
	χ^2	*P*	χ^2	*P*
Carbon	20.37	**0.0001**	2.30	0.5126
Nitrogen	20.26	**0.0001**	0.71	0.8694
Phosphorus	19.98	**0.0002**	1.95	0.5827
Potassium	20.28	**0.0001**	1.31	0.7279
Calcium	19.16	**0.0003**	7.78	0.0507
Magnesium	3.09	0.3774	2.39	0.4955
Sulfur	18.20	**0.0004**	0.78	0.8549
Boron	15.91	**0.0012**	0.91	0.8237
Copper	3.05	0.3834	0.76	0.8598
Iron	2.41	0.4917	7.84	**0.0494***
Manganese	1.51	0.6789	1.87	0.5995
Zinc	8.79	**0.0322***	3.96	0.2657
C:N	13.75	**0.0033**	0.14	0.9864
C:P	12.79	**0.0051***	1.51	0.6796
C:N:P	19.65	**0.0002**	0.89	0.829

Bold represents statistical significance. Asterisk represents values no longer significant after Bonferroni correction. Degrees of freedom = 3 for all tests

meaning there was no increase in water clarity in the tanks with live *Dreissena*.

Experiment: *Cladophora glomerata*

Unlike the *L. wollei* experiment, there was noticeable periphyton growth on the bottom of the tanks; this material was weighed separately from the bulk of the *C. glomerata* biomass to determine if there was a difference among treatments. The unattached *C. glomerata* biomass at the end of the experiment was highly variable but increased in every treatment. The highest final biomass occurred in live *Dreissena* tanks, but there was no significant difference among treatments (Fig. 2c, Kruskal–Wallis: $\chi^2 = 2.09$, $P = 0.55$). However, when the other three treatments were grouped and compared to live *Dreissena*, the wet biomass was 26.2% higher with live *Dreissena* (Fig. 2c, $t = 1.79$, $P = 0.05$). The bottom-associated mass was also larger in tanks with live *Dreissena*, but there was no significant difference among treatments (Kruskal–Wallis: $\chi^2 = 2.35$, $P = 0.50$). It was difficult to retrieve all the bottom-attached algae because of the large amount of very small strands, especially when attached to the mussels. The total dry mass did not differ among treatments (Kruskal–Wallis: $\chi^2 = 1.56$, $P = 0.67$).

Table 2 Results of *t*-table of tissue nutrient concentrations comparing *L. wollei* and *C. glomerata* before and after the experiment

Nutrients	df	*t* statistic	*P*	Change
Lyngbya wollei				
Carbon	9	2.64	**0.0269***	–
Nitrogen	16	8.56	**<0.0001**	–
Phosphorus	13	5.37	**0.0001**	–
Potassium	8	2.36	**0.0459***	–
Calcium	3	1.89	0.1554	+
Magnesium	40	3.74	**0.0006**	+
Sulfur	7	6.41	**0.0004**	–
Boron	12	9.79	**<0.0001**	–
Copper	7	7.06	**0.0002**	–
Iron	3	5.86	**0.0099***	–
Manganese	4	9.66	**0.0006**	–
Zinc	4	0.97	0.3878	+
C:N	5	7.16	**0.0008**	+
C:P	29	6.72	**<0.0001**	+
C:N:P	41	8.49	**<0.0001**	+
Cladophora glomerata				
Carbon	2	1.56	0.2598	–
Nitrogen	2	2.63	0.1191	–
Phosphorus	2	1.85	0.2050	–
Potassium	2	3.90	0.0600	–
Calcium	2	0.91	0.4605	+
Magnesium	2	2.44	0.1347	+
Sulfur	2	3.14	0.0880	–
Boron	2	2.69	0.1150	–
Copper	3	2.99	0.0583	+
Iron	2	1.68	0.2352	–
Manganese	2	2.01	0.1822	–
Zinc	8	0.37	0.7204	–
C:N	4	4.99	**0.0076***	+
C:P	4	3.41	**0.0270***	+
C:N:P	5	4.43	**0.0068***	+

Bold represents statistical significance. Asterisk represents values no longer significant after Bonferroni correction. Degrees of freedom represent unequal variances

Dreissena did not significantly alter algal nutrient concentrations in *C. glomerata*. C, N, P, K, S, and Zn tissue concentrations of *C. glomerata* tended to be greater in the live *Dreissena* treatment, but there were

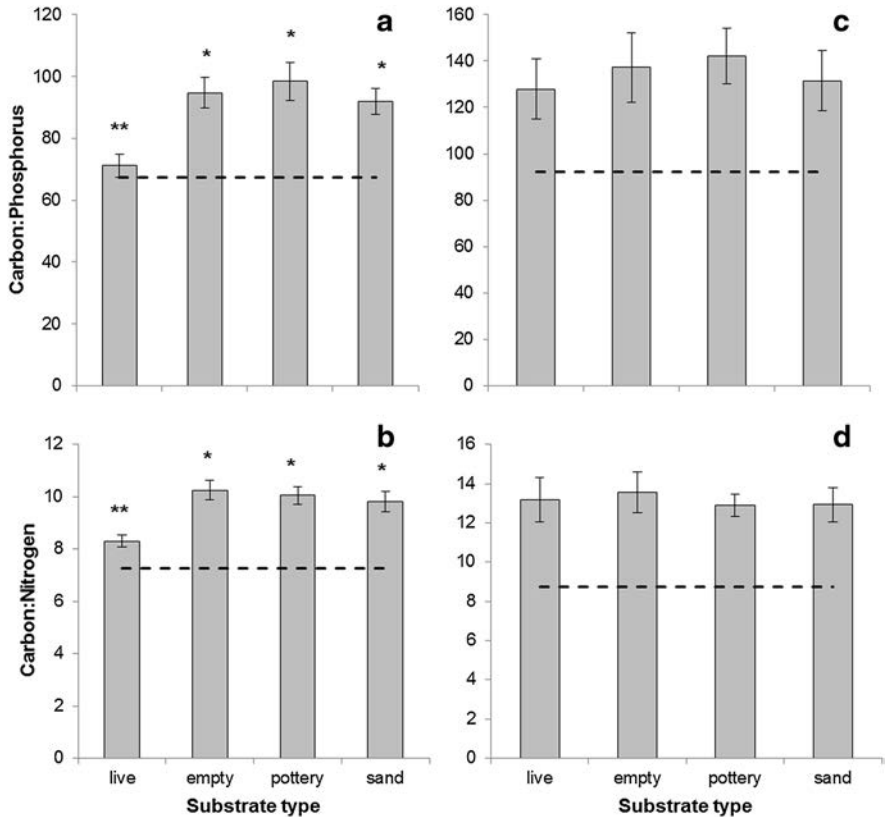

Fig. 4 **a** Carbon-to-phosphorus and **b** carbon-to-nitrogen ratios for *L. wollei* at the end of the experiment. **a** Carbon-to-phosphorus and **b** carbon-to-nitrogen ratios for *C. glomerata* at the end of the experiment. *Dotted line* represents initial values. *Error bars* represent 1 standard error. *Asterisks* represent statistical significance. $N = 10$ per treatment

no significant differences (Supplementary Material 1, Table 1; Fig. 5). Total N and P contents of *C. glomerata* were higher with live *Dreissena* (Fig. 5a, b, Kruskal–Wallis: $\chi^2 = 7.68$, $P = 0.05$; Kruskal–Wallis: $\chi^2 = 9.95$, $P = 0.02$, respectively), but this P value was no longer significant after the correction for multiple tests; the total amount of N and P reflects both the concentration and the higher biomass (Fig. 2c) with live *Dreissena*. Total C tissue content increased 66.5% at the end of the experiment (Fig. 2d, $t = 5.08$, $P = 0.007$) consistent with the increase in biomass. Ca and Fe concentrations tended to be lower in the live *Dreissena* treatment relative to the other treatments, yet this was not a significant change (Fig. 5c; Table 1, Kruskal–Wallis: $\chi^2 = 7.78$, $P = 0.05$; Kruskal–Wallis: $\chi^2 = 7.84$, $P = 0.05$, respectively). K tissue concentration showed a decrease of 52% at the end of the experiment compared to initial values ($t = 3.90$, $P = 0.06$) and Cu had an increase of 40.6% (Supplementary Material 1, Table 2, $t = 2.99$, $P = 0.06$), but neither was significantly different. The live *Dreissena* treatment had the lowest C:P ratio, but there was no significant difference among treatments (Fig. 4c; Table 1, Kruskal–Wallis: $\chi^2 = 1.51$, $P = 0.68$). There was no significant difference nor trend among treatments for the C:N ratio of *C. glomerata* (Fig. 4d; Table 1, Kruskal–Wallis: $\chi^2 = 0.14$, $P = 0.99$).

Photosynthetic efficiency (Φ_{et}) and ETR of *C. glomerata* did not have a significant difference among treatments, but was 19.6 and 24.7% lower at the end of the experiment, respectively ($t = 12.77$, $P < 0.0001$; $t = 4.45$, $P < 0.0001$, respectively). Chl *a* and *b* did not differ among treatments, nor did the chl *a*:chl *b* ratio. Similar to the *L. wollei* experiment, there was no difference in water light level among the treatments (Kruskal–Wallis: $\chi^2 = 0.3209$, $P = 0.9560$), meaning there was no increase in water clarity in the tanks with live *Dreissena*.

Most water nutrient concentrations tested did not significantly change during the experiment. The B, Ca, Fe, K, Mg, Na, P, S, and Si water nutrient concentrations (mg l^{-1}) were not significantly different among treatments at the end of the experiment. Ca and P water concentrations decreased 44.6 and 80.7%, respectively, at the end of the experiment from all

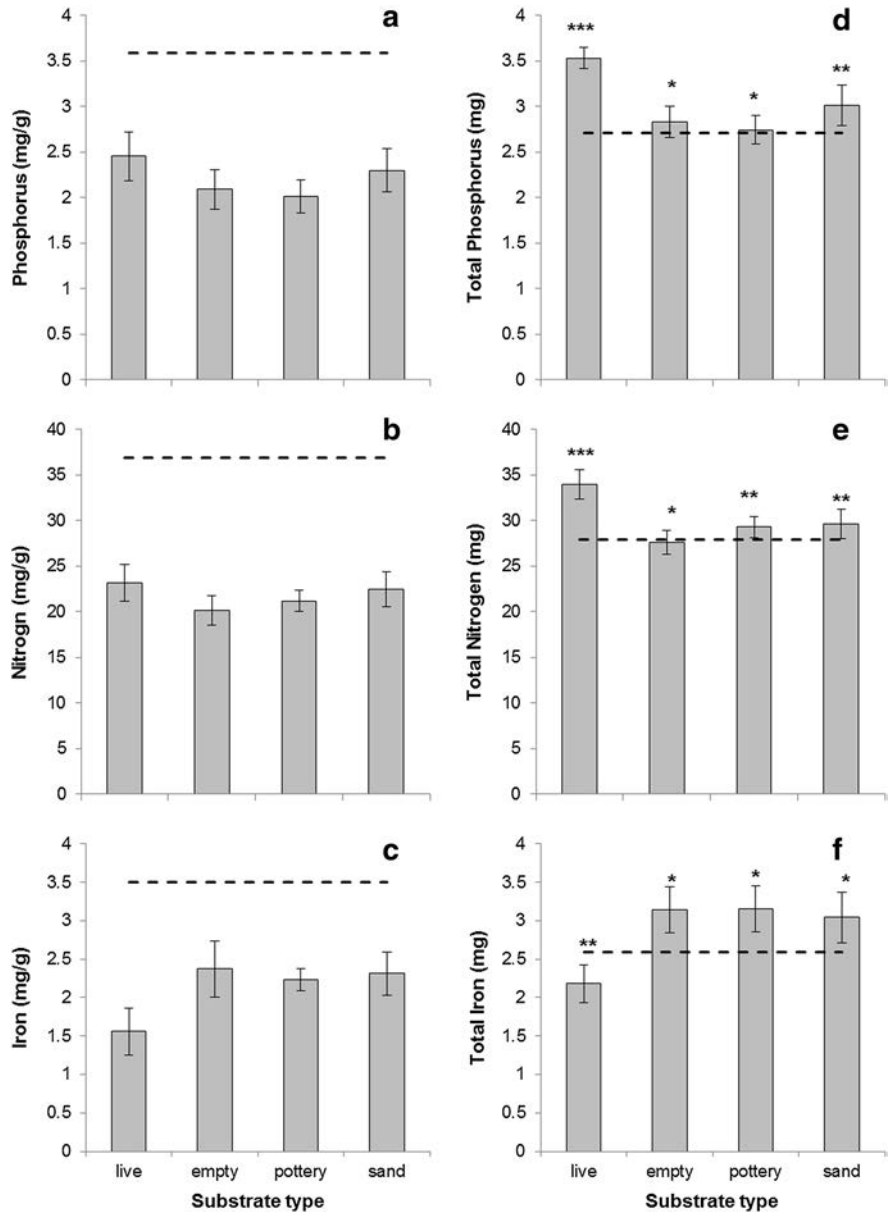

Fig. 5 **a** Phosphorus, **b** nitrogen, and **c** iron concentration of *C. glomerata* at the end of the experiment. **d** Total phosphorus, **e** nitrogen, and **f** iron contents of *C. glomerata* at the end of the experiment. *Error bars* represent 1 standard error. *Dotted line* represents initial values at the start of the experiment. *Asterisks* represent statistical significance. $N = 10$ per treatment

treatments combined compared to initial values ($t = 15.96$, $P < 0.0001$; $t = 5.75$, $P = 0.01$, respectively). Na and S water concentrations significantly increased 9.5 and 7.3%, respectively, at the end of the experiment ($t = 4.55$, $P = 0.0002$; $t = 2.98$, $P = 0.005$, respectively).

Field survey

There was a significant relationship between the presence of *Dreissena* and the presence of *L. wollei* in 2009 and 2010 ($\chi^2 = 17.99$, $P = 0.0004$; $\chi^2 = 14.05$, $P = 0.0009$, respectively). The observed occurrences of both *Dreissena* and *L. wollei* presence were greater than expected (Supplementary Material 2). Correspondingly, the observed count when neither *Dreissena* nor *L. wollei* was present was also greater than the calculated expected count in both 2009 and 2010 (Supplementary Material 2). In 2010, there were less occurrences of *L. wollei* present with *Dreissena* shells than expected (Supplementary Material 2). *L. wollei* biomass was not significantly different whether

Dreissena were present or absent in 2009, but *L. wollei* biomass was significantly greater in 2010 at the sites where *Dreissena* were present in western Lake Erie (Fig. 6, $t = 1.73$, $P = 0.22$; $t = 1.67$, $P < 0.0001$, respectively). *L. wollei* biomass in 2010 was not significantly different between live *Dreissena* and *Dreissena* shells ($t = 1.18$, $P = 0.2497$).

Discussion

Our results suggest that *Dreissena* alter the availability of resources to benthic algae, thereby documenting an additional way in which this invasive species alters energy flow and ecosystem structure in lakes. Our experiment showed that *Dreissena* can increase the availability of C and other macronutrients in two species of bloom-forming benthic algae. In both the laboratory experiment and the field survey, we showed that in some instances, the growth or presence of benthic algae increases in proximity to *Dreissena*. Mussels have similar effects in other large coastal ecosystems. For example, *Mytilus edulis* mussel beds influence nutrient cycling in estuaries (Dame et al., 1991) and fertilize the eelgrass *Zostera marina* through biodeposition (Reusch et al., 1994). Similarly, the seagrass *Thalassia testudinum* experienced increased N and P concentrations because of the effect of the bivalve *Modiolus americanus* (Peterson & Heck, 1999). The transfer of nutrients and C to benthic producers may therefore be a common trait of mussels. In the case of an invasive mussel such as *Dreissena*, this process may represent a new pathway funneling nutrients to benthic species that can form harmful blooms.

Biomass and carbon

In our experiment, only *C. glomerata* had increased biomass with live *Dreissena*, but there was no difference in chl *a* concentrations among treatments for *C. glomerata*, possibly because the increased biomass of this alga in the live *Dreissena* treatment resulted in lower concentrations of pigments and nutrients per unit biomass. Contrary to our expectation, *L. wollei* did not show increased growth with *Dreissena*, possibly due to time constraints of the 1 week long experiment. *L. wollei* in the live *Dreissena* treatment had a larger concentration of PC,

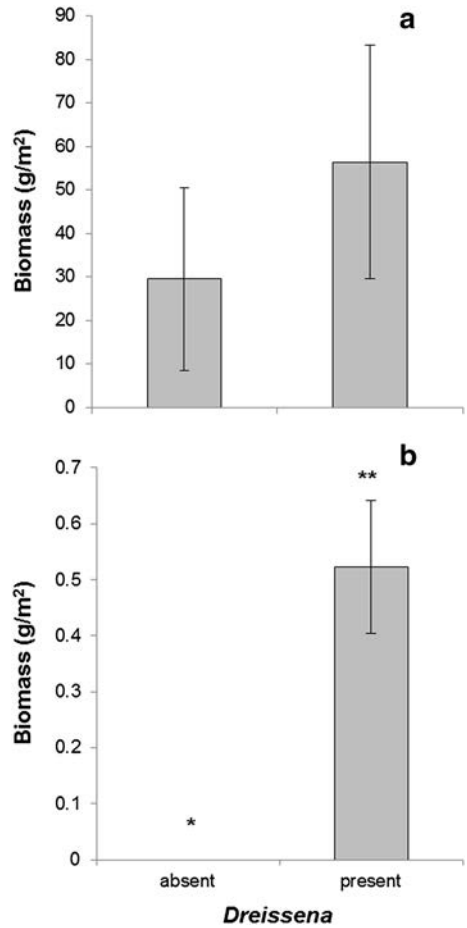

Fig. 6 **a** Biomass of *L. wollei* in 2009 and **b** 2010 in the presence and absence of *Dreissena*. *Error bars* represent 1 standard error. *Asterisks* represent statistical significance. For 2009, $N = 4$ for *L. wollei* presence without *Dreissena*, $N = 69$ for *L. wollei* presence with *Dreissena*. For 2010, $N = 0$ for *L. wollei* presence without *Dreissena*, $N = 71$ for *L. wollei* presence with *Dreissena*

possibly due to the increased N; in the long term, *L. wollei* may experience increased growth near *Dreissena* because this species has higher PC and nutrient concentrations with live *Dreissena*. Therefore, *Dreissena* can increase algal biomass quickly, as in the way of *C. glomerata*, or possibly have long-term effects by providing resources used for PC accumulation, as in the way of *L. wollei*, that may eventually lead to increased growth.

The average Φ_{et} of *C. glomerata* at the beginning of the experiment was 0.729, while the average at the end was 0.586 indicating the alga became less efficient photosynthetically as biomass accumulated. This experiment was meant to have the alga under lower

nutrient conditions in order to detect any effects from *Dreissena*, yet experimental conditions were appropriate for growth because both species grew to twice their starting weight. This suggests that our results are relevant to growth conditions experienced in natural systems.

Total C mirrored the biomass pattern of the two algae species; total C content increased in all treatments at the end of the experiment as did biomass (Fig. 2). Algal tissue C concentrations found in the experiment were similar to field values for *C. glomerata* (Higgins et al., 2008), but *L. wollei* tissue C concentration at the end of the experiment was higher than *L. wollei* found from Lake Erie (Supplementary Material 1). Live *Dreissena* increased C concentration in both species tested and total C content and biomass in *C. glomerata* indicating that *Dreissena* can increase C availability to benthic algae; this resource contribution may contribute to the formation of algal blooms.

Nutrients

Our results suggest that live *Dreissena* can elevate algal tissue concentrations of N, P, and other macronutrients compared to treatments without *Dreissena*. Nutrient P, K, N, and S concentrations in *L. wollei* tissue declined relative to initial in all treatments except with live *Dreissena* as the alga increased in biomass, although growth was not higher in that treatment during the time span of this experiment. Similarly, *L. wollei* growth did not respond positively to N or P additions in a different preliminary field study (Cowell & Botts, 1994). Thacker & Paul (2001) found no significant relationship between the N fixing cyanobacteria studied (*Lyngbya*, *Tolypothrix*, and *Oscillatoria*) and N or P availability. However, with increased N and P from live *Dreissena*, enhanced *L. wollei* growth may be possible on a longer term. Additionally, *L. wollei* may not have had enough time or light in this experiment for N fixation (Phlips et al., 1992). The nutrient concentrations for Mg, Cu, and Mn were not significantly different among treatments for either species, suggesting that *Dreissena* do not influence these nutrients and they are not likely vital for algal growth. *C. glomerata* did not exhibit statistically higher nutrient concentrations with live *Dreissena*, but it did show the same trend as *L. wollei*. *C. glomerata* was increasing in biomass and, therefore, taking up P and, consequently, decreasing water P to 15 μg l^{-1}, possibly leading to P-limitation by the experiment. Further, the higher biomass achieved by this species in the live *Dreissena* treatment would have diluted nutrient concentrations. Additionally, some nutrient concentrations of *L. wollei* and *C. glomerata* were higher in the lake than our initial values for the experiment, indicating that the algae were cultured in a rich solution, thus minimizing an effect of dreissenid presence. The nutrient cycling from *Dreissena* to nearshore regions where *L. wollei* and *C. glomerata* reside increases benthic algal growth, keeping the nutrients nearshore, and, therefore, limiting resources available offshore (Hecky et al., 2004). Our results suggest that algae near *Dreissena* have potential for increased in food quality (i.e., nutrient content) relative to algae not in proximity to *Dreissena*. While many invertebrates feed on smaller epiphytic algae that grow on relatively large species such as *L. wollei* and *C. glomerata*, an increase in nutrient value may allow invertebrates with cutting mouth parts to also consume the macroalgae.

Tissue nutrient concentrations of *L. wollei* and *C. glomerata* from this experiment were within the range of those seen in natural populations (Adams & Stone, 1973; Gerloff & Fitzgerald, 1976; Sikes, 1977; Higgins et al., 2008) or within the lake (Supplementary Material 1). Based on C:N and C:P ratios (Kahlert, 1998), *C. glomerata* was N deficient in all treatments at the end of the experiment, but had no P deficiency. Although *C. glomerata* was categorized as N deficient (C:N > 9.4) according to the criterion of Kahlert (1998), both P and N tissue concentrations were high (Supplementary Material 1), suggesting neither nutrient limited growth. *L. wollei* had an N deficiency in all treatments except with live *Dreissena*, and no P deficiency in any treatment. *L. wollei* with live *Dreissena* had lower C:N and C:P ratios (Fig. 4a, b; Table 1), indicating that P and N were obtained from *Dreissena* at proportionally higher rates than C, making the algae a higher food quality for grazers. In addition to changes in nutrient ratios, both algal species studied displayed higher C, N, and P nutrient concentrations when grown with live *Dreissena*.

Even though the presence of dreissenids elevated several nutrients above other treatments in *L. wollei*, it significantly decreased Ca. Ca is important for *L. wollei* to stimulate the formation of its polysaccharide sheath (Foerster, 1964; Camacho & Thacker, 2006). *C.*

glomerata also has a relatively high Ca requirement; we measured an average tissue concentration of 65.3 mg Ca g^{-1} from algae taken directly from western Lake Erie, which is similar to the reported minimum tissue concentration to support maximum yield (Sikes, 1977). Both species had the lowest concentration of Ca in the live *Dreissena* treatment; *Dreissena* need Ca for their shell, and likely outcompetes the algae. The water Ca concentration in the *C. glomerata* experiment decreased likely from it being taken up by *Dreissena* and the alga tissue. Low Ca could hinder growth of *L. wollei* or *C. glomerata* if Ca levels fall below the critical level. However, it is unlikely that competition for Ca between *Dreissena* and benthic algae would stunt the growth of algae in natural lakes, especially habitats such as the Laurentian Great Lakes, which often have high Ca supplied due to underlying geology.

Structure

In this study, neither alga tested responded to the structure treatments in terms of biomass or nutrient concentration, suggesting that, at least in calm conditions, the addition of structure is not as important of a mechanism for *Dreissena* to increase the growth of benthic algae. The lack of a structure effect on benthic algae differs sharply from the effect of *Dreissena* on benthic invertebrates. Many invertebrates show elevated density when non-living shells are present (Botts et al., 1996), and invertebrates increase most when the background substrate is soft, making mussel shells the only available hard substrate (Mayer et al., 2002). Our experimental design may have underestimated the importance of structure, especially for *C. glomerata*, which was detached from its original surface when it was collected from the field. During the relatively short duration of the experiment, *C. glomerata* was not able to reattach to the substrates in the experiment, minimizing the likelihood of observing a structure effect if one existed. *L. wollei* does not attach to substrate, although the possibility of this filamentous algae becoming tangled on irregular substrate such as *Dreissena* beds, may be important under high wave conditions that are frequently present in the Laurentian Great Lakes. Small-scale experiments have limitations (Spivak et al., 2011), and our mechanistic laboratory experiment may not have been well suited to test an effect that may only appear under high wave conditions.

Lake observations

Complementing our manipulative experiment with a survey of *Dreissena* and *L. wollei* co-occurrence in western Lake Erie provided additional evidence that *Dreissena* may provide resources that promote bloom formation in benthic algae. *Dreissena* and *L. wollei* were more likely to co-occur in the western basin of Lake Erie than expected by chance. Further, *L. wollei* also had greater biomass where *Dreissena* were present in one study year. Similarly, *Cladophora* biomass has also been well documented to increase in proximity with *Dreissena* (Higgins et al., 2005b, 2008; Ozersky et al., 2009; Auer et al., 2010). Our laboratory results suggest that *Dreissena* can provide resources to benthic algae at least on a small spatial scale, whereas the observed pattern of co-occurrence in the lake suggests that the interaction can be important at the relevant ecological (in situ) scale. *L. wollei* does not attach to substrates, making it unlikely that *Dreissena* and *L. wollei* co-occur due to the physical attributes of specific sites. Further, the higher than expected frequency of *L. wollei* at sites with live *Dreissena* (Supplementary Material 2) suggests that the alga is responding to the mussel's biological activity and concentration of nutrients, not just the addition of hard substrate. Therefore, it is likely that *Dreissena* may contribute to the intensification of benthic algal blooms and influence the location of blooms.

Conclusion

This study showed that the presence of *Dreissena* can increase algal biomass and can contribute several important nutrients to benthic algae, consistent with the nearshore shunt hypothesis (Hecky et al., 2004). Further, *Dreissena* and *L. wollei* were spatially associated in western Lake Erie. Nutrient reduction policies have been put in place to help control phytoplankton and benthic algal blooms, but *Dreissena* aggregate N and P in a way that benefits benthic algae and may promote blooms despite reduced loading. The nutrients that *Dreissena* add may be particularly critical at certain times or extend the growing season, such as when *C. glomerata* becomes

nutrient stressed in late summer (Higgins et al., 2005a, b). Therefore, target nutrient levels may have to be lower than previously believed in order to reduce benthic algal blooms.

In addition to N and P, *Dreissena* can help some benthic algae to acquire C. Depending on the relative importance of C versus N or P limitations in various systems, and, therefore, the relative importance of these mechanisms, nutrient reductions may be ineffective in some systems because C provision is most important. *Dreissena* could promote the growth of benthic algae under certain conditions through nutrient enrichment, which can lead to increased grazers and increased nuisance algae fouling the lake and beaches.

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