Final Report
Ohio Lake Erie Commission Lake Erie Protection Fund
Small Grant SG 384-10 **Enhanced benthic algal growth from *Dreissena***

Christine M. Mayer (PhD)
Patricia M. Armenio (MS)

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Abstract

Dreissena spp. (zebra and quagga mussels) have greatly altered aquatic ecosystems since their invasion of the Great Lakes. *Dreissena* increase light to the benthos, provide hard structure for algal attachment, and may contribute a limiting nutrient to benthic algae, thereby facilitating blooms. The benthic cyanobacterium *Lyngbya wollei* (Farlow ex Gomont) Speziale and Dyck has recently become abundant in western Lake Erie and reaches nuisance levels. *Cladophora glomerata* (L.) Kütz., a green alga, has also been resurging in the Great Lakes and studies link this increase to *Dreissena*. Manipulative experiments showed that *L. wollei* had a significantly higher concentration of carbon, nitrogen, phosphorus, potassium, and sulfur in tanks with live *Dreissena*. *C. glomerata* had greater biomass in tanks with live *Dreissena*, but did not have significant increases in nutrient concentration as did *L. wollei*. *Dreissena* decreased calcium concentrations, a nutrient important for cell walls, in both algal species; although concentrations were still above the limiting level for growth. Neither algal species responded to structure as a resource for growth. These results suggest that *Dreissena* are providing nutrients and carbon to benthic algae and these added resources can increase the growth of benthic algae and productivity, and may promote blooms.

Activities and timelines and work products

P. Armenio successfully defended her MS. Thesis in the Department of Environmental Sciences at the University of Toledo in December 2010. Her thesis was based largely on work supported by this project. Ms. Armenio plans to pursue a career in aquatic natural resource management. She is submitting a manuscript to the Journal of the North American Benthological Society.

Demonstration of PAM flourometry to high school students.

Ms. Armenio developed a laboratory module of fluorometry was demonstrat at a local high school. Basic concepts of algae and specific species such as *Lyngbya* and *Cladophora* were discussed followed by a demonstration of a Pulse Amplitude Modulated (PAM) fluorometer. The students became more informed about algae (particularly algae in Lake Erie) and learned a scientific technique to measure the health of photosynthetic organisms.

Our time line was adjusted when we applied for a 6 month, no-cost extension. All proposed work has been completed. We will provide a copy of this report to the Ohio Phosphorus Task Force.

Main activities & outcomes; technical results

The technical results of this project follow in the form of a manuscript that we are finalizing for submission to the Journal of the North American Benthological Society. The manuscript is attached here.

Changes in project activities and hurdles experienced. Lessons learned.

Only minor changes in our experimental design were needed. The experiments described in the technical section of this report were slightly more detailed than originally described in the proposal for this project. Some analyses were added on the advice of the research committee of the graduate student (P. Armenio) who carried out this work.

The results of this project indicate that *Dreissena* do provide resources to benthic algae. This information is important when considering target levels of nutrients for the Great Lakes. The way in which *Dreissena* transfer nutrients from the water column to benthic algae means that nutrient levels may need to be lower than previously thought to prevent benthic algal blooms.

New resource contributions from *Dreissena* spp. to *Lyngbya wollei* and *Cladophora glomerata*

Patricia M. Armenio*, Christine M. Mayer and Scott Heckathorn

University of Toledo, Department of Environmental Sciences and the Lake Erie Center, 6200 Bayshore Rd. Oregon, Ohio 43616

*Corresponding Author

Patricia M. Armenio, University of Toledo, Department of Environmental Sciences and the Lake Erie Center, 6200 Bayshore Rd. Oregon, OH 43616

Email: pcope@rockets.utoledo.edu

Abstract

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Introduction

Dreissena spp. (zebra mussels, *Dreissena polymorpha* and quagga mussels, *Dreissena bugensis*) are invasive ecosystem engineers (Jones et al. 1994); they increase water clarity in freshwater lakes at a system-wide level (Leach 1993, Holland 1993, Mayer et al. 2002, Vanderploeg et al. 2002, Barbiero and Tuchman 2004). *Dreissena* thereby increase light to the benthic zone, which can increase benthic primary producer biomass (Lowe and Pillsbury 1995, Skubinna et al. 1995, Zhu et al. 2006), productivity (Fahnenstiel et al. 1995, Lowe and Pillsbury 1995, Cecala et al. 2008), and change nutrient stoichiometry (Qin et al. 2007). In addition to system-wide changes by increased water clarity, *Dreissena* change habitat locally and may increase the availability of macro or micronutrients, CO_2, and substrate thereby favoring increased benthic algal biomass and production. Although these effects are localized to *Dreissena*-colonized habitat, large geographic areas may be affected. For example, the nearshore shunt (Hecky et al. 2004) only occurs in proximity to *Dreissena*-colonized areas, even though this process may affect the over 10,000 miles of Great Lakes shoreline (Government of Canada and US EPA 1995) and is credited as the underlying cause of environmental problems such as benthic algal blooms (Hecky et al. 2004). In this study, we used a manipulative experiment to test the relative importance of different benthic algal resources (macro or micronutrients, CO_2, and substrate) that *Dreissena* may regulate at a small spatial scale. The direction and magnitude of these localized resource flows to benthic algae may influence the conditions that promote blooms at larger spatial scales.

Dreissena contribute nutrients to the benthic system (Lowe and Pillsbury 1995), such as nitrogen (N) and phosphorus (P), which are generally the limiting nutrients for algae. Blooms of *Cladophora* in the mid-1990s may have occurred

because of increased water clarity and phosphorus recycling from *Dreissena* (Higgins et al. 2008). *Dreissena* N and P excretion is an important source of nutrient recycling in the western basin of Lake Erie (Arnott and Vanni 1996, Conroy et al. 2005), suggesting that *Dreissena* can influence nutrient recycling over large areas. The excretion of feces and pseudofeces from *Dreissena* also add a source of nutrients to benthic algae, especially *Cladophora*, which prefer solid, rocky habitat (Hecky et al. 2004). Since *Dreissena* increase recycling and locally concentrate macronutrients, they may have a similar effect on micronutrients and may enhance algal growth by this mechanism. Micronutrient limitation can also slow growth and production of benthic algae; for example, iron (Fe) is important for cyanobacteria to reach maximum photosynthetic capacity (Trick et al. 1995, Albert et al. 2005) and silica (Si) can limit benthic algae in Lake Michigan (Carrick and Lowe 2007). The effects of *Dreissena* on micronutrient concentrations have not previously been studied. Macro or micronutrient input from *Dreissena* could therefore be important for the enhancement of benthic algal growth.

Another resource that *Dreissena* may contribute to benthic systems is CO_2. Compared to terrestrial environments, CO_2 is more likely to be depleted in aquatic systems and can sometimes be low in shallow freshwater environments (Stevenson 1988). Carbon can also be limiting in dense algal mats (Stevenson et al. 2004), which can restrict photosynthesis if the alga cannot catalyze HCO_3^- to CO_2 (Cheney and Hough 1983). The decomposition of fecal and pseudofecal materials from *Dreissena* is a new source of CO_2 to the benthic system (Hecky et al. 2004). Further, respiration by *Dreissena* contributes CO_2 as well, which at low levels can limit benthic algal photosynthetic rates (Hecky et al. 2004). *Dreissena* aggregations can have a very high density (Coakley et al. 2002); consequently their waste and respiration may be

substantial imports of carbon to the benthos. CO_2 can be limiting in the benthic zone, therefore its addition is another mechanism by which *Dreissena* may affect the growth of benthic algae.

Another possible way for *Dreissena* to increase benthic algae growth is by providing hard substrates with their shells. *Dreissena* have increased the structural complexity of the benthos, thereby increasing surface area available for colonization (Lowe and Pillsbury 1995). In general, mollusks can function as ecosystem engineers by providing shells as substrata for attachment (Gutiérrez et al. 2003). The increase of hard surface area by *Dreissena* may increase benthic algal attachment (Hecky et al. 2004). *D. bugensis* survives well on soft sediment (Dermott and Munawar 1993) and is the dominant species in many areas of the Great Lakes (Mills et al. 1993) and has recently expanded to inland lakes in the western United States (USGS 2009). *D. bugensis* qualitatively alters benthic habitat from soft to hard bottom, and may therefore have a particularly large affect. Further, the accretion of dead shells also adds hard surfaces to the bottom (Hecky et al. 2004); therefore, the ecological impact of an individual may last longer than its lifespan.

The growth form and physiological tolerances of benthic algal species may influence how *Dreissena* affect algae and what species form blooms under different conditions. *Lyngbya wollei* is a nitrogen fixing cyanobacterium that has recently become abundant in Lake Erie (Bridgeman and Penamon 2010), Florida (Cowell and Botts 1994) and other southeastern states (Speziale et al. 1991). Field observations suggest *L. wollei* is often present near *Dreissena* in Lake Erie (personal observation, Fig. 1). *Cladophora glomerata* is a green alga which formed blooms prior to the 1980s in many locations, probably due to eutrophication (Higgins et al. 2008). *C. glomerata* has more recently become abundant in eastern Lake Erie (Higgins et al.

2005b) and Lake Michigan (Bootsma et al. 2005) possibly due to high water clarity, as *C. glomerata* grows well in high light (Graham et al. 1982). Abundant *C. glomerata* can lead to problems such as decreased aesthetic value and recreation, human health risks, and altered food web structure (Bootsma et al. 2005), with the same issues occurring with *L. wollei* (Albert et al. 2005). Unlike *C. glomerata*, *L. wollei* does not attach to hard substrates; it becomes partially covered in sandy or dreissenid shell substrate. *L. wollei* also differs from *C. glomerata* in that it grows well in low light (Dyck 1994). The relative importance of the multiple mechanisms by which *Dreissena* affect different species of benthic algae may influence the best management actions to reduce blooms.

The goal of this study was to (1) test if *Dreissena* can facilitate algal growth for two benthic algal species with different growth modes, and (2) determine what resources *Dreissena* may be giving the algae to promote its growth. Manipulative experiments were conducted in which *Lyngbya wollei* and *Cladophora glomerata* were grown under four treatments: (1) live *Dreissena,* (2) empty *Dreissena* shells, (3) pottery shards, and (4) sand only. The live *Dreissena* treatment could provide macronutrients or micronutrients or carbon to the algae through metabolic excretion. The empty shell treatment could 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 sediment from the lake could have also provided the algae with nutrients. We measured increase in biomass as an index of growth and elemental composition to determine among these treatments which resource affect the algae. Additionally, we labeled *Chlamydomonas reinhardtii* with ^{13}C and ^{15}N to serve as food for *Dreissena* and to confirm if it can release carbon or nitrogen from food consumption to the benthic algae. Our

hypothesis is that *Dreissena* will increase growth of both benthic algal species by contributing either macro- or micronutrients. We also expect that the benthic algae will have greater ^{13}C and ^{15}N with live *Dreissena* through the consumption of *C. reinhardtii* and excretion of these nutrients by *Dreissena*, which *L. wollei* and *C. glomerata* will take up.

Methods

In order to determine what resources *Dreissena* may provide to benthic algae, *Lyngbya wollei* and *Cladophora 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). Algae were grown in clear plastic 6 L tanks for one week, after which photosynthetic efficiency, wet mass, dry mass, pigment content, elemental concentrations, and content of ^{13}C and ^{15}N stable isotopes were measured. Tanks were placed on shelves with hanging fluorescent lights on timers to provide a 12-hour photoperiod. Treatments were interspersed, with their location alternated on shelves.

Dreissena were collected from sediment in western Lake Erie near Pelee Island using a benthic trawl and were kept in tubs with lake water until needed for experimentation. The quantity of *Dreissena* added to the tanks represented the density of *Dreissena* on soft sediments in Lake Erie; i.e., 3400 individuals/m^2 (Coakley et al. 2002). Macro- and micronutrients were measured from *Dreissena* tissues at the end of the *C. glomerata* experiment (Table 3, D. tissue; see "Measurements" below for methods). Empty *Dreissena* clusters were made by gluing dead *Dreissena* shells together to provide the same physical structure as live *Dreissena*, without any contribution of macronutrients from active metabolism.

Pottery shards were made by breaking small clay pots into pieces which were soaked in deionized water for at least one week prior to use; these served as a structure that were not expected to contribute any type of nutrients, and can serve as a comparison to empty *Dreissena* shells which may contribute micronutrients. The volume of empty *Dreissena* and pottery shards was the same as live *Dreissena*. All tanks contained 1.5 cm of sand, rinsed and sieved with a 243 µm mesh, instead of lake sediment, so 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 micronutrients were measured from the algae before culturing (Table 3, lake; see "Measurements" below for methods). Prior to the experiment, algae were kept in WC media (Guillard and 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 fresh biomass of *L. wollei* added to each tank was 230 g/m^2, which is similar to densities in western Lake Erie (Bridgeman and 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; this original biomass was unable to reattach during the week long experiment. 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 quanta m^{-2} s^{-1}. *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

quanta m^{-2} s^{-1}, which were vigorously aerated because this species occurs in shallow, high wave action waters. 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.

Lake Erie water was used for the experiment. Excess P was precipitated with 30 mg/L Alum (Aluminum sulfate, Toledo Pools, Toledo, Ohio) (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. Four liters of the water were added to 18.5x18.5x17.5 cm containers. One liter of lake water was added half way through the experiment to all tanks due to evaporation.

^{13}C and ^{15}N stable isotopes

The ^{13}C and ^{15}N labels were used to directly measure if *Dreissena* were affecting the availability of C or N to the benthic algae via consumption and excretion of nutrients in food. *Chlamydomonas reinhardtii* was kept in an altered WC liquid medium (Guillard and Lorenzen 1972); sodium hydrogen carbonate ($NaHCO_3$) and sodium nitrate ($NaNO_3$) were replaced with sodium hydrogen carbonate 98% atom ^{13}C and potassium nitrate 98% atom ^{15}N, respectively. *C. reinhardtii* was grown in these stable isotopes and served as food for *Dreissena* (Berg et al. 1996, Horgan and Mills 1997, Baldwin et al. 2002) during the experiment, with the expectation that the mussels would eat it and excrete the isotope labels, which the benthic algae would then take up. Feeding of labeled algae began the day before the experiment was to begin, when *Dreissena* needed for the experiment were placed in a separate bucket

with lake water and 15 ml *C. reinhardtii* per 3400 individuals *Dreissena*/m^2 (Nichols 1993) was added. Feeding of labeled algae continued throughout the experiment by giving the same amount of labeled *C. reinhardtii* to each tank every other day during the experiment, except the last day (N=4). The addition of *C. reinhardtii* in all tanks creates a small increase in making laboratory conditions more closely resemble the lake. After harvest, the benthic algae were rinsed with DI water to remove any loose label before analyses were conducted. After 24 and 48 hours of the experiment, an average of 335 mg of *C. glomerata* fresh tissue was extracted from five replicates of each treatment to determine stable isotope content. *Dreissena* tissue at the end of the *C. glomerata* experiment was also analyzed for stable isotope content. For stable-isotope analysis of ^{13}C and ^{15}N, 3 mg of dried tissue was sent to University of California, Davis Stable Isotope Facility, where an isotope ratio mass spectrometer (IRMS) was used to determine delta values relative to international standards, Vienna PeeDee Belemnite (V-PDB) and Air for C and N, respectively.

Measurements

For both benthic algal species, dissolved oxygen and temperature in tanks were measured using a YSI 5000 (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. Photosynthesis was monitored via chlorophyll fluorescence using a Walz DIVING-PAM (pulse amplitude modulated) fluorometer on the first and last day of the experiment as indicators of the physiological health of the algae. Steady-state light-adapted *in situ* quantum yield of photosystem II electron transport (Φ_{et}) and apparent relative rate of electron transport (ETR) (Campbell et al. 1998) were measured as these indicators (relative due to use

of assumed light-absorption coefficient of 0.85). The percentage of *Dreissena* still alive at the end of the experiment was noted as well. All of the algae used were rinsed in DI water before and after the experiment. Three samples of each type of algae were conserved for initial values, not used in the experiment. At the end of the experiment, all algal samples were dried to a constant weight at 65º C and ground using mortar and pestle.

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 and Neefus 2007). The algae were ground to a powder in liquid nitrogen using mortar and pestle, and then in 10 ml of buffer. Samples were centrifuged for 10 minutes at 3800 rpm. Supernatant was then stored at -80º C until ready for quantification. A 10-AU Turner Design fluorometer with P/N 103-80 filters was used to record PC fluorescence. A standard curve of C-PC standards was used to quantify PC.

Chlorophyll (chl) *a*, the light harvesting pigment of all photosynthetic organisms, is often used as an indicator of biomass. Chlorophyll *a* and b were extracted from an average of 70 mg and 30 mg of fresh weight tissue in 3.5 ml and 3 ml of 100% dimethylsulphoxide (DMSO) for *L. wollei* and *C. glomerata*, respectively. The samples were heated in a 65ºC water bath for 45 minutes, cooled to room temperature, and stored at -20ºC for 2 days until quantification. The samples were thawed to room temperature and centrifuged at 21,000 *g* for 10 minutes. A UV-1650 PC Shimadzu spectrophotometer and equations from Barnes et al. (1992) were used to determine chl *a* and b concentration.

Carbon (C) and nitrogen (N) concentrations were determined by gas-chromatography following combustion (HCNO/S analyzer Perkin-Elmer 2400 series

II) using 2.5 to 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 inductively coupled plasma optical emission spectroscopy (ICP-OES) on 150 mg of dried tissue (Frantz et al. 2008). Molar ratios from Kahlert (1998) were converted into mass ratios and used as indication of nutrient deficiencies. Total nutrient content in the water of the *C. glomerata* experiment were also quantified using ICP-OES.

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 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 compare significant differences among treatments when Kruskal-Wallis tests were significant. Kruskal-Wallis tests, the non-parametric version of analysis of variance (ANOVA), were done so the assumptions of normality were not violated. Unequal variance t-tests compared initial values to the end of the experiment. The Satterthwaite correction of the unequal variance t-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 the assumption of equal variance was considered more important than normality. The mixed-linear-model procedure was used for repeated measures tests with *C. glomerata*, to compare tissue nutrient concentrations after one day, two days and seven days. Repeated harvest of algal tissue was not done for *L. wollei*.

Results

Lyngbya wollei

Wet weight and dry weight of *L. wollei* were significantly correlated (r^2 = 0.80, p <0.0001), indicating that the procedure used to portion wet mass into each tank at the beginning of the experiment was a good estimate of dry weight. *L. wollei* wet weight biomass increased above the initial level in every treatment, but there was no difference among treatments in percent increase in mass (Kruskal-Wallace: χ^2 = 0.84, p = 0.84). Final wet and dry weight biomass also did not differ among treatments (Kruskal-Wallace: χ^2 = 0.73, p = 0.87; χ^2 = 3.28, p = 0.35, respectively) (Fig. 2A).

Photosynthetic efficiency (Φ_{et}), with an overall average of 0.40, and ETR did not differ among treatments. However, photosynthetic efficiency was significantly negatively related to dry weight (r^2 = 0.40, p <0.0001) indicating that *L. wollei* became less efficient when biomass was greater. *L. wollei* did not contain any chl b, indicating 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-Wallace: χ^2 = 18.32, p = 0.0004) (Fig. 3). The large difference in PC numbers among treatments is consistent with findings from Chaffin (2009) for other cyanobacteria.

Carbon, phosphorus, nitrogen, potassium, and sulfur concentration of *L. wollei* were significantly higher in tanks with live *Dreissena* and calcium concentration of *L. wollei* was significantly lower in tanks with live *Dreissena* (Table 1, Fig. 4A-C). Total P and N content of *L. wollei* mirrored the concentrations, but were not significantly different among treatments (Fig. 4D, E). Tissue C, N, P, K, S, B, Cu, Fe,

and Mn concentration of *L. wollei* significantly decreased at the end of the experiment compared to the initial concentration (Table 2); however total C content increased at the end of the experiment ($t = 3.75$, $p = 0.03$) (Fig. 2B) accounting for the biomass increase. Magnesium concentration significantly increased at the end of the experiment compared to the initial values (Table 2).

No *L. wollei* in any treatment exhibited a P deficiency; however, *L. wollei* in every treatment except live *Dreissena* were deficient in N (Fig. 5A, B). A C:P ratio less than 143 indicates P sufficiency (Kahlert 1998). *L. wollei* could have stored enough P before the experiment so that it did not become deficient. A C:N ratio above 9.4 indicates N deficiency (Kahlert 1998). The live *Dreissena* treatment had no N deficiency, whereas the other treatments were N deficient because *L. wollei* tissue N concentration was higher in the live *Dreissena* treatment.

^{13}C concentration in *L. wollei* was significantly lower in tanks with live *Dreissena* (Kruskal-Wallace: $\chi^2 = 16.99$, $p = 0.0007$) but there was no difference among the treatments for ^{15}N. It is likely that *Dreissena* were eating the *C. reinhardtii*, therefore making ^{13}C less available than in the other treatments. The other treatments had a significantly larger amount of ^{13}C than the live *Dreissena* treatment because the *C. reinhardtii* was present and respiring C.

Cladophora glomerata

As with *L. wollei*, wet weight and dry weight of *C. glomerata* had a significant positive relationship ($r^2 = 0.69$, $p < 0.0001$) indicating pre-experiment wet mass was a good estimate of dry weight. 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 with the highest final biomass in live *Dreissena* tanks, but no statistically significant difference among treatments (Kruskal-Wallace: $\chi^2 = 2.09$, $p = 0.55$) (Fig. 2C). However, when the other three treatments were grouped and compared to live *Dreissena*, there was a marginally significant difference with total wet weight ($t = 1.79$, $p = 0.05$) (Fig. 2C). The bottom-associated mass was also larger in tanks with live *Dreissena*, but there was no statistically significant difference among treatments (Kruskal-Wallace: $\chi^2 = 2.35$, $p = 0.50$). It was difficult to retrieve all the bottom-attached algae, especially when attached to the mussels, which contributed to high variability. The total dry mass did not differ among treatments (Kruskal-Wallace: $\chi^2 = 1.56$, $p = 0.67$).

Photosynthetic efficiency and ETR did not have a significant difference among treatments but was lower at the end of the experiment ($t = 12.77$, $p < 0.0001$; $t = 4.45$, $p < 0.0001$, respectively) indicating that *C. glomerata* was physiologically stressed. The average photosynthetic efficiency (Φ_{et}) of *C. glomerata* at the beginning of the experiment was 0.729, which is greater than field values found in summer (Ensminger et al. 2000); while the average at the end was 0.586 indicating the alga became less efficient as biomass accumulated. However, the experiment was meant to have the alga under low nutrient conditions in order to detect any effects from *Dreissena*. Chl *a* and b did not differ among treatments, nor did the chl *a*:chl b ratio.

Carbon, nitrogen, phosphorus, potassium, sulfur, and zinc tissue concentration of *C. glomerata* were all highest in the live *Dreissena* treatment, but there were no statistically significant differences due to high variability (Table 3, 4, Fig. 6). However, total N and P content of *C. glomerata* were significantly higher with live *Dreissena* (Fig. 6A, B). Total C content increased at the end of the experiment (t =

5.08, $p = 0.007$) (Fig. 2D) producing the increase in biomass. Calcium and iron concentration were the lowest in the live *Dreissena* treatment, showing a marginally significant difference (Kruskal-Wallace: $\chi^2 = 7.78$, $p = 0.05$; Kruskal-Wallace: $\chi^2 = 7.84$, $p = 0.05$, respectively) (Fig. 6C). Potassium tissue concentration showed a marginally significant decrease at the end of the experiment compared to initial values ($t = 3.90$, $p = 0.06$) and copper had a marginally significant increase ($t = 2.99$, $p = 0.06$) (Tables 3, 4). The repeated measures ANOVA showed that nutrient concentrations were significantly different between days 1, 2, and 7, but there was no difference between treatment or day by treatment interaction and therefore only the last day is represented. No treatments exhibited a P deficiency, with a C:P ratio less than 143 (Kahlert 1998), with the lowest C:P ratio in the live *Dreissena* treatment. This shows that *Dreissena* are contributing P to the alga, even though P tissue concentration was not statistically higher in that treatment and the alga in this treatment would provide the best food source for grazing invertebrates. All treatments had a N deficiency with a C:N ratio greater than 9.4 (Kahlert 1998). Although *C. glomerata* were categorized as N deficient according to the criterion of Kahlert (1998), the high P and N tissue concentration of the alga suggests that it was not deficient.

The repeated measures ANOVA for stable isotope showed that there was a significant difference between days 1, 2, and 7 with the amount of label increasing with day, and there was a significant difference with the day by substrate interaction for ^{15}N. The live *Dreissena* treatment had a significantly lower ^{15}N concentration than the other treatments on the last day (Kruskal-Wallace: $\chi^2 = 19.59$, $p = 0.0002$) (Fig. 7 A, B). ^{13}C concentration of *C. glomerata* was also significantly different between the days, but it was not significantly different among treatments. Figure 7

(C, D) shows a preliminary trial of this experiment with *C. glomerata* which lasted two weeks, and this shows the same result as found for only one week.

Water samples from each tank showed that Alum did decrease P in the water before experimentation ($t = 10.22$, $p = 0.0002$) from an average of 320 µg/L to 80 µg/L. Water nutrients B, Ca, Fe, K, Mg, Na, P, S, and Si were not significantly different among treatments at the end of the experiment. Calcium and phosphorus water concentrations significantly decreased at the end of the experiment compared to initial values ($t = 15.96$, $p < 0.0001$; $t = 5.75$, $p = 0.01$, respectively) (Fig. 8). Calcium concentration increased in the alga tissue and was taken up by *Dreissena*, and therefore decreased from the water. *C. glomerata* was also taking up P, decreasing the water concentrations while using the P for growth at the same time, creating a decrease in tissue P concentration at the end of the experiment. Sodium and sulfur water concentrations significantly increased at the end of the experiment ($t = 4.55$, $p = 0.0002$; $t = 2.98$, $p = 0.005$, respectively), possibly due to evaporation.

Discussion

Our results suggest that *Dreissena* act as ecosystem engineers and can alter the availability of resources to other organisms. *Dreissena* are likely to substantially alter ecosystem processes at lake-wide and local levels (Mayer et al. 2002). The goal of this study was to test the relative importance of specific resources that *Dreissena* may provide to benthic algae, thereby contributing to nuisance level blooms. The results of this experiment suggest that *Dreissena* increase the availability of carbon and macronutrients in two species of bloom-forming algae. Knowing what potentially limiting resources *Dreissena* provide benthic algae can inform management decisions to help reduce algal blooms.

Biomass and carbon

Only *C. glomerata* had increased biomass with live *Dreissena*, although *L. wollei* may experience increased growth near *Dreissena* because this species had higher PC and nutrient concentrations with live *Dreissena*. Experimental conditions were appropriate for growth because both species grew up to twice their starting weight and had an equivalent or better photosynthetic efficiency (Φ_{et}) than found in literature; the average Φ_{et} for *L. wollei* was 0.40, which was higher than values found for *L. majuscula* (Hewson et al. 2001, Watkinson et al. 2005). Higher growth for *C. glomerata* in the live *Dreissena* treatment matched our expectation. Photosynthetic pigment concentrations did not differ among treatments for *C. glomerata*, possibly because biomass with live *Dreissena* diluted pigments and nutrients. Contrary to our expectation, *L. wollei* did not show increased growth with *Dreissena*, possibly due to time constraints (Carrick and Lowe 2007). However, *L. wollei* in the live *Dreissena* treatment had a larger concentration of PC and looked darker and healthier; in the long-term this increased health could lead to greater biomass accumulation.

Total C mirrored the biomass pattern of the two species; total C content increased in all treatments at the end of the experiment as did biomass (Fig. 2). Carbon 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 (Table 3) and *L. majuscula* (Watkinson et al. 2005), further indicating that conditions for growth were appropriate. Live *Dreissena* increased C concentration in both species tested and total C content and biomass in *L. wollei* indicating that *Dreissena* do increase C availability

to benthic algae; this resource contribution may contribute to the formation of algal blooms.

Nutrients

Our results suggest that *Dreissena* can elevate algal tissue concentrations of N, P, and other macronutrients above other treatments, as predicted. Nutrient P, K, N and S concentrations in *L. wollei* tissue declined relative to initial in all treatments except live *Dreissena* as the alga increased in biomass, although this did not translate into increased growth in that treatment during the time span of this experiment. Similarly, *L. wollei* growth did not respond positively to N or P addition (Cowell and Botts 1994) and cyanobacterial abundance may not change with nitrogen or phosphorus availability (Thacker and Paul 2001). The lack of growth with *L. wollei* could have also been because it was laden with N before the experiment and had to use the N before growth could occur. Alternatively, *C. glomerata* did not exhibit statistically significant differences among treatments for nutrients but it did show the same trend as *L. wollei*. Further, the higher biomass achieved by this species in the live *Dreissena* treatment would have diluted nutrient concentrations. Our results suggest that while only some benthic algal species may increase in biomass near *Dreissena*, these algae are similar or possibly increased in food quality (i.e. nutrient content) relative to algae not in proximity to *Dreissena*; therefore species that do not increase in biomass are likely to present a highly nutrient-dense food source to invertebrates.

Even though *Dreissena* elevated several nutrients above other treatments in *L. wollei*, it significantly decreased Ca. Calcium is important for *L. wollei* to stimulate the formation of its polysaccharide sheath (Foerster 1964, Robbins et al. 1998,

Camacho and Thacker 2006). *C. glomerata* also has a relatively high Ca requirement; we measured an average tissue concentration of 65.3 mg Ca/g 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, although the levels in all experimental treatments where high relative to *C. glomerata* collected directly from Lake Erie; *Dreissena* needs the Ca for its shell, and likely outcompetes the algae. Low Ca could hinder growth of *L. wollei* or *C. glomerata* if Ca levels fall below the critical level. However it is unlikely that this decrease would stunt the growth of either species in natural lakes, especially habitats such as the Great Lakes, which often have high Ca supplied due to underlying geology.

Tissue nutrient concentrations of *L. wollei* and *C. glomerata* from this experiment were within the range of those seen in natural populations (Adams and Stone 1973, Gerloff and Fitzgerald 1976, Sikes 1977, Watkinson et al. 2005, Higgins et al. 2008) or within the lake (Table 3, 4). According to C:N and C:P ratios (Kahlert 1998), *C. glomerata* had a N deficiency in all treatments at the end of the experiment but had no P deficiency. *L. wollei* had a N deficiency in all treatments except with live *Dreissena*, and no P deficiency in any treatment. Algae with live *Dreissena* had lower C:N and C:P ratios due to the added N and P making the algae a higher food quality for grazers. Whether or not there was a N or P deficiency according to nutrient ratios, both algal species studied had the highest C, N, and P nutrient concentration when grown with live *Dreissena*.

The two algal species responded differently in the experiment in regards to some nutrients. Tissue Fe concentrations decreased at the end of the experiment for both species, but was highest in the live *Dreissena* treatment for *L. wollei* and lowest

in the live *Dreissena* treatment for *C. glomerata*. The marine *L. majuscula* is known to have high Fe requirements (Gross and Martin 1996, Kuffner and Paul 2001, Ahern et al. 2007) and *L. wollei* also shows high concentrations of Fe, therefore *L. wollei* may have taken up the needed Fe, whereas *C. glomerata* does not need it. Tissue Cu concentration decreased at end of experiment for *L. wollei*, but increased for *C. glomerata*, possibly because the initial Cu concentration for *C. glomerata* was low compared to *C. glomerata* from the lake (Table 4), and the lake water provided Cu for the alga to take up. Each type of alga is different in needs and will respond to the environment depending on the alga's requirements.

Stable isotopes

The carbon and nitrogen isotopes that were used to detect if *Dreissena* were directly excreting C or N to benthic algae did not show up in the benthic algae in the given time period. We hypothesized that *Dreissena* would filter the labeled *C. reinhardtii* and excrete the C and N, which would be taken up by the algae. *Dreissena* did eat *C. reinhardtii* (*Dreissena* tissue isotope was greater at the end of the experiment after being fed labeled *C. reinhardtii* than initially), but results showed that Dreissena did not release the ^{13}C and ^{15}N back to the benthic algae after the one (or two) week period. ^{13}C and ^{15}N concentration of *L. wollei* are similar to values found in Silver Glen Springs, FL (Sickman et al. 2007); however *L. wollei* results from the stable isotope cannot fully be explained. Alternatively, results from *C. glomerata* showed that *Dreissena* may be removing competition for nutrients by eating *C. reinhardtii*. Once *C. reinhardtii* is eaten, it is no longer present to take up the nutrients and the benthic alga, *C. glomerata*, is then able to take up the nutrients in

the treatments without live *Dreissena*. Perhaps this is yet another way for *Dreissena* to benefit benthic algae, by eliminating the algae's competitors.

Structure

Neither alga tested responded to the structure treatments; neither species showed biomass or nutrient concentration differences among the empty shell, pottery shard or sand alone treatments suggesting that the addition of structure is not an important mechanism for *Dreissena* to increase 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 to *C. glomerata* which was detached from its original surface when it was collected from the field. During the experiment, the bulk of this biomass was not able to reattach to the substrates in the experiment minimizing the likelihood of observing a structure effect if one existed. There was a greater amount of attached algae with live *Dreissena*, but it was not only on the mussels themselves, it was throughout the tank further suggesting that *Dreissena* were providing some other resource such as carbon or nutrients. *L. wollei* does not have a mechanism to attach to substrate, and would only be expected to have an effect if it became entangled in the substrate. The results indicate that because *Dreissena* significantly enhanced macronutrient concentrations of the algae but did not increase growth by its structure, nutrient contributions should be more of a concern for management of blooms of benthic algae.

Implications

This study showed that *Dreissena* resulted in a significant increase in algal biomass for one of two algal species studied and that *Dreissena* can contribute several important nutrients to benthic algae, agreeing with assumptions of the nearshore shunt hypothesis. 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 algae could store these nutrients from *Dreissena* which can be helpful in a time of need when the nutrient availability is low. *C. glomerata* becomes nutrient stressed in late summer (Higgins et al. 2005a,b), and these added nutrients could create a longer period of presence for this alga. 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* help benthic algae to acquire C. Depending on the relative importance of C verses 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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Table 1. Kruskal-Wallis table of nutrient concentrations of *L. wollei* at the end of the experiment comparing the four treatments: 1) live *Dreissena*, 2) empty *Dreissena*, 3) pottery shards, 4) sand. Bold represents statistical significance.

Nutrient	df	χ^2	*p*
Carbon	3	20.37	**0.0001**
Nitrogen	3	20.26	**0.0001**
Phosphorus	3	19.98	**0.0002**
Potassium	3	20.28	**0.0001**
Calcium	3	19.16	**0.0003**
Magnesium	3	3.09	0.3774
Sulfur	3	18.20	**0.0004**
Boron	3	15.91	**0.0012**
Copper	3	3.05	0.3834
Iron	3	2.41	0.4917
Manganese	3	1.51	0.6789
Zinc	3	8.79	**0.0322**
C:N	3	13.75	**0.0033**
C:P	3	12.79	**0.0051**
C:N:P	3	19.65	**0.0002**

Table 2. T-table of tissue nutrient concentrations comparing *L. wollei* before and after the experiment. Bold represents statistical significance.

Nutrient	df	t statistic	t critical	*p*	change
Carbon	9	2.64	1.83	**0.0269**	-
Nitrogen	16	8.56	1.75	**<0.0001**	-
Phosphorus	13	5.37	1.77	**0.0001**	-
Potassium	8	2.36	1.86	**0.0459**	-
Calcium	3	1.89	2.35	0.1554	+
Magnesium	40	3.74	1.68	**0.0006**	+
Sulfur	7	6.41	1.89	**0.0004**	-
Boron	12	9.79	1.78	**<0.0001**	-
Copper	7	7.06	1.89	**0.0002**	-
Iron	3	5.86	2.35	**0.0099**	-
Manganese	4	9.66	2.13	**0.0006**	-
Zinc	4	0.97	2.13	0.3878	+
C:N	5	7.16	2.57	**0.0008**	+
C:P	29	6.72	2.05	**<0.0001**	+
C:N:P	41	8.49	2.02	**<0.0001**	+

Table 3. Macronutrient concentration (mg/g) of *C. glomerata* (C.) and *L. wollei* (L.) from western Lake Erie (lake), initial values before experiment (initial), and treatments from the experiment (live, empty, pottery, sand). D. tissue is *Dreissena* tissue from the end of the experiment. Values ± standard deviation.

		mg/g						
	n	C	N	P	K	Ca	Mg	S
C. lake	5	240.72 ± 11.37	27.10 ± 2.31	2.38 ± 0.22	26.77 ± 0.19	65.33 ± 9.09	4.40 ± .021	14.60 ± 0.66
C. initial	3	314.50 ± 45.56	36.87 ± 9.86	3.59 ± 1.27	27.13 ± 6.04	83.83 ± 52.72	3.33 ± 1.20	18.49 ± 2.96
C. live	10	285.77 ± 17.54	23.16 ± 6.44	2.46 ± 0.84	15.73 ± 8.32	100.36 ± 24.64	5.28 ± 1.76	13.70 ± 4.16
C. empty	10	259.81 ± 41.76	20.11 ± 5.07	2.09 ± 0.68	11.94 ± 5.91	101.13 ± 24.02	4.55 ± 1.44	12.20 ± 3.03
C. pottery	10	269.17 ± 31.20	21.16 ± 3.71	2.01 ± 0.58	12.20 ± 5.64	122.30 ± 23.95	5.12 ± 1.47	12.62 ± 3.62
C. sand	10	276.73 ± 23.86	22.46 ± 6.05	2.30 ± 0.75	12.24 ± 3.82	122.79 ± 22.14	5.54 ± 1.29	13.01 ± 2.14
L. lake	15	196.96 ± 47.67	29.37 ± 10.08	2.22 ± 0.89	2.32 ± 1.01	96.28 ± 24.90	6.30 ± 1.57	2.59 ± 0.59
L. initial	3	306.37 ± 8.99	42.30 ± 1.35	4.54 ± 0.22	2.92 ± 0.16	109.21 ± 14.85	4.23 ± 0.10	3.39 ± 0.16
L. live	10	331.96 ± 20.52	40.36 ± 5.57	4.82 ± 1.07	3.26 ± 0.50	96.15 ± 17.97	5.10 ± 1.04	3.18 ± 0.70
L. empty	10	278.97 ± 24.26	27.54 ± 4.13	3.03 ± 0.64	2.40 ± 0.27	135.43 ± 20.26	4.79 ± 1.14	2.39 ± 0.33
L. pottery	10	269.15 ± 27.74	26.99 ± 3.46	2.80 ± 0.44	2.24 ± 0.21	139.98 ± 18.87	5.33 ± 1.52	2.36 ± 0.20
L. sand	10	263.77 ± 25.35	27.27 ± 4.27	2.92 ± 0.51	2.51 ± 0.76	137.27 ± 21.85	4.56 ± 0.92	2.46 ± 0.28
D. tissue	10	437.59 ± 12.58	112.54 ± 3.83	10.35 ± 0.91	2.22 ± 0.10	38.46 ± 10.42	1.21 ± 0.06	7.59 ± 0.26

Table 4. Micronutrient concentration (mg/g or µg/g) of *C. glomerata* (C.) and *L. wollei* (L.) from western Lake Erie (lake), initial values before experiment (initial), and treatments from the experiment (live, empty, pottery, sand). D. tissue is *Dreissena* tissue from the end of the experiment. Values ± standard deviation.

		mg/g	µg/g			
	n	Fe	B	Cu	Mn	Zn
C. lake	5	9.63 ± 0.83	99.46 ± 3.78	26.96 ± 9.99	655.35 ± 82.06	59.71 ± 8.94
C. initial	3	3.51 ± 1.41	319.41 ± 78.15	14.28 ± 3.08	601.40 ± 266.48	27.72 ± 1.92
C. live	10	1.56 ± 0.97	191.65 ± 51.03	20.55 ± 5.92	272.28 ± 184.50	29.07 ± 6.67
C. empty	10	2.37 ± 1.15	190.19 ± 50.50	20.71 ± 4.96	294.27 ± 132.47	23.85 ± 7.57
C. pottery	10	2.23 ± 0.46	204.25 ± 56.99	18.76 ± 3.50	276.89 ± 97.95	27.52 ± 5.69
C. sand	10	2.31 ± 0.89	199.46 ± 43.05	20.30 ± 5.68	313.02 ± 145.68	28.05 ± 9.25
L. lake	15	10.53 ± 4.32	11.93 ± 9.92	31.78 ± 9.15	401.66 ± 120.69	62.93 ± 20.05
L. initial	3	5.83 ± 0.64	9.50 ± 0.36	38.56 ± 1.72	140.88 ± 8.78	46.29 ± 5.40
L. live	10	3.81 ± 0.97	7.42 ± 1.31	31.90 ± 7.23	92.06 ± 32.31	55.62 ± 15.39

L. empty	10	3.42 ± 0.97	6.99 ± 1.68	28.34 ± 6.56	81.60 ± 16.90	51.65 ± 11.52
L. pottery	10	3.31 ± 0.96	5.55 ± 1.10	26.85 ± 4.51	75.23 ± 14.92	39.89 ± 9.68
L. sand	10	3.50 ± 0.60	4.68 ± 1.26	28.66 ± 4.62	79.67 ± 15.25	52.59 ± 12.42
D. tissue	10	0.88 ± 0.19	0.84 ± 0.36	12.88 ± 1.56	141.14 ± 53.50	65.03 ± 3.08

Figure Legends:

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

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

Figure 3. Phycocyanin (mg/g) concentration of *L. wollei* at the end of the experiment. Error bars represent 1 standard error. N=10 per treatment.

Figure 4. (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 content 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. N=10 per treatment.

Figure 5. (a) Carbon to phosphorus and (b) carbon to nitrogen ratios for *L. wollei* at the end of the experiment. Dotted line represents initial values. Error bars represent 1 standard error. N=10 per treatment.

Figure 6. (a) Phosphorus, (b) nitrogen, and (c) iron concentration of *C. glomerata* at the end of the experiment. Initial values for phosphorus = 3.6 mg/g; nitrogen = 36.9 mg/g; iron = 3.5 mg/g. (d) Total phosphorus, (e) nitrogen, and (f) iron content 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. N=10 per treatment.

Figure 7. (a) Delta ^{13}C and (b) delta ^{15}N (permil) of *C. glomerata* at the end of the experiment (one week, N=10 per treatment). Initial values for $\delta\ ^{13}C = -19$; $\delta\ ^{15}N = 16.2$. (c) Delta ^{13}C and (d) delta ^{15}N (permil) of *C. glomerata* at the end of a two week trial experiment (N=3 per treatment). Initial values for $\delta\ ^{13}C = -19$; $\delta\ ^{15}N = 0.37$. Error bars represent 1 standard error.

Figure 8. Calcium (mg/l) concentration of the lake water at the beginning and end of the *C. glomerata* experiment. Error bars represent 1 standard error. N= 40.

Figure 1

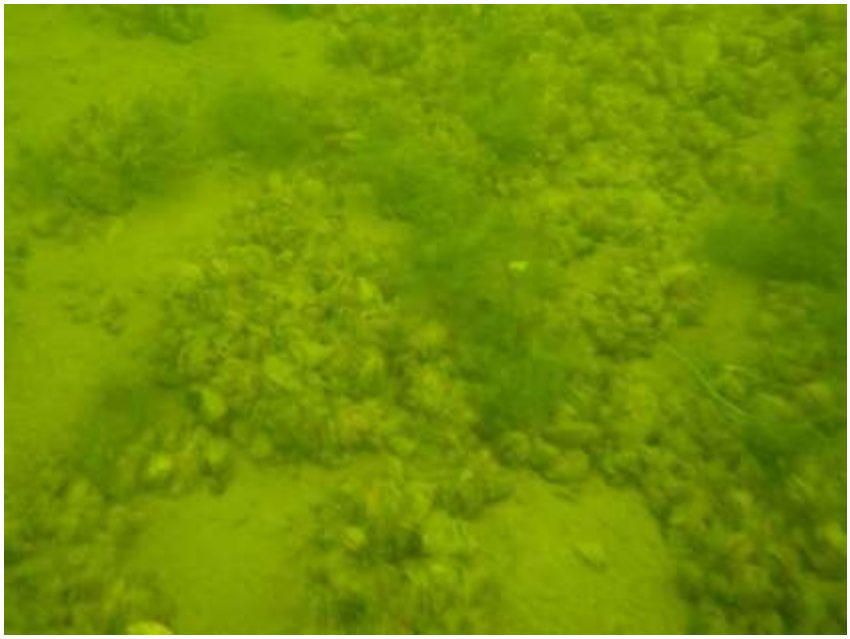

Figure 2

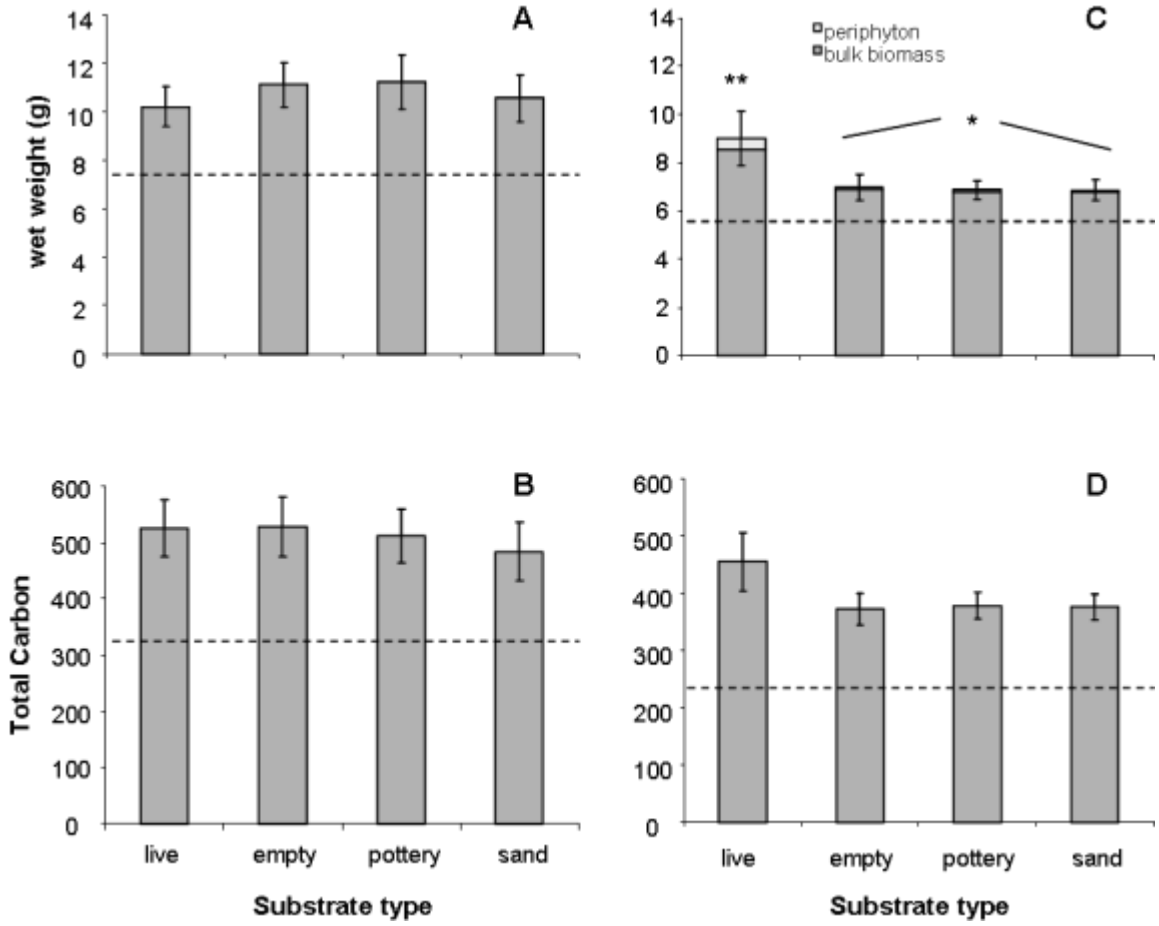
A
B
C
D
wet weight (g)
Total Carbon
periphyton
bulk biomass
**
*
live
empty
pottery
sand
Substrate type
Substrate type

Figure 3

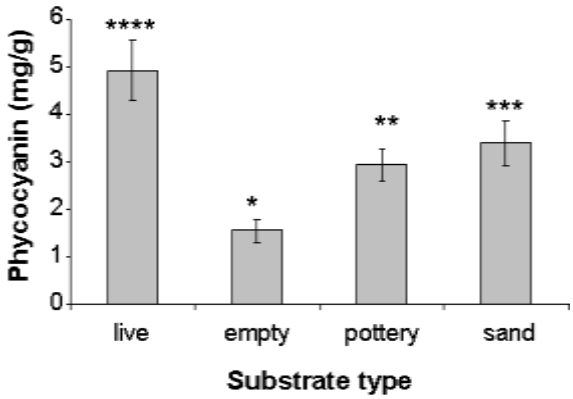
Phycocyanin (mg/g)
6
5
4
3
2
1
0
****
*
**
***
live
empty
pottery
sand
Substrate type

Figure 4

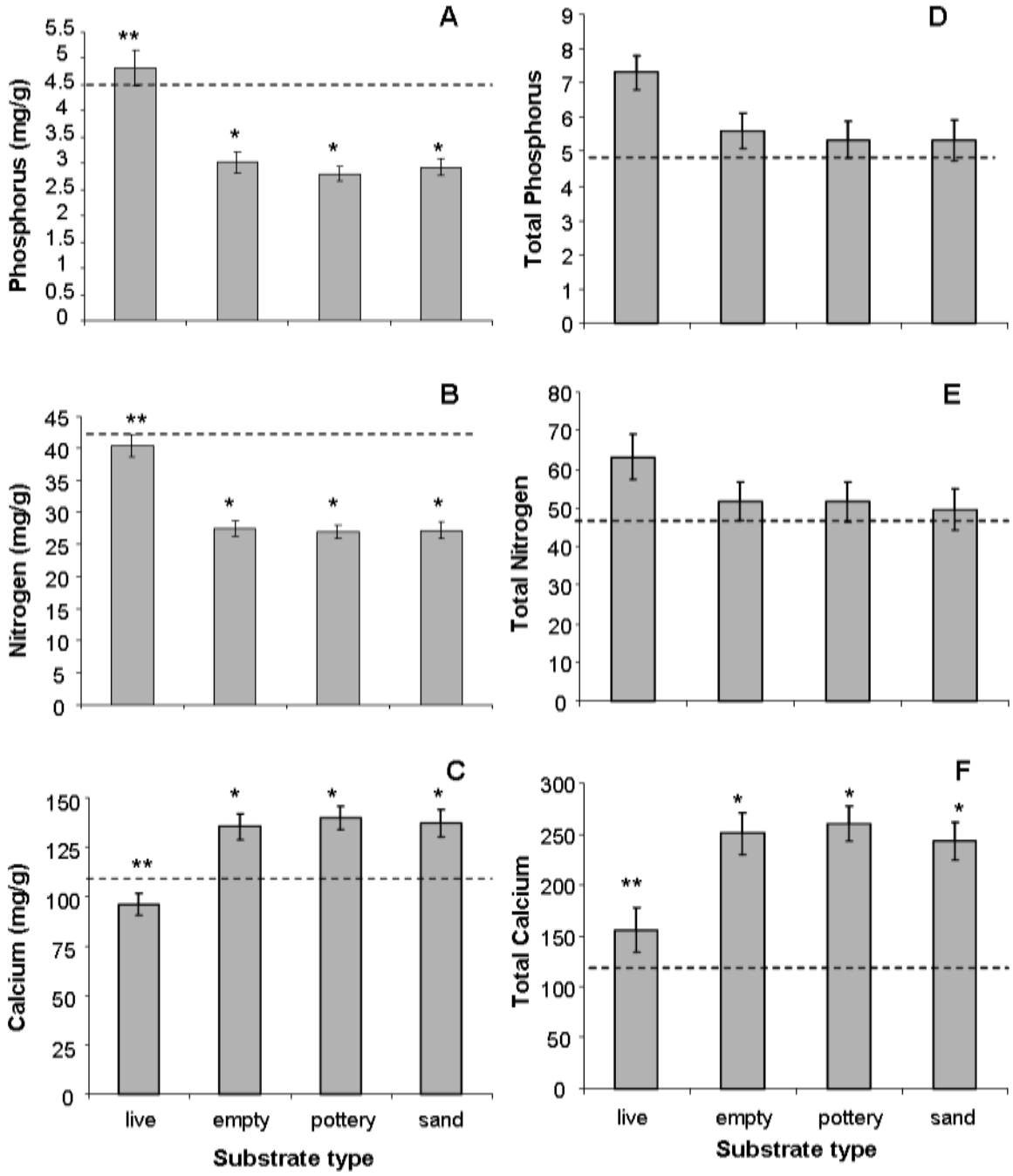
A
Phosphorus (mg/g)
B
Nitrogen (mg/g)
C
Calcium (mg/g)
D
Total Phosphorus
E
Total Nitrogen
F
Total Calcium
live
empty
pottery
sand
Substrate type

Figure 5

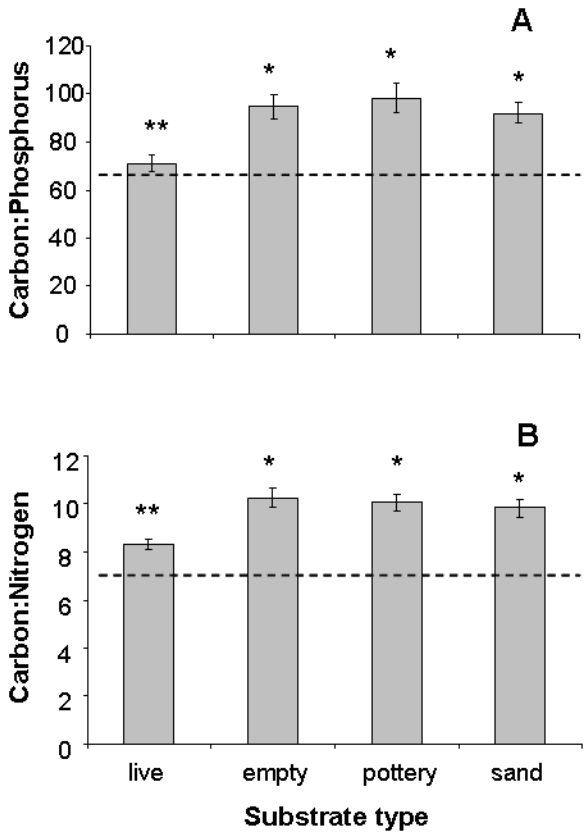
A
Carbon:Phosphorus
120
100
80
60
40
20
0
**
*
*
*
B
Carbon:Nitrogen
12
10
8
6
4
2
0
**
*
*
*
live
empty
pottery
sand
Substrate type

Figure 6

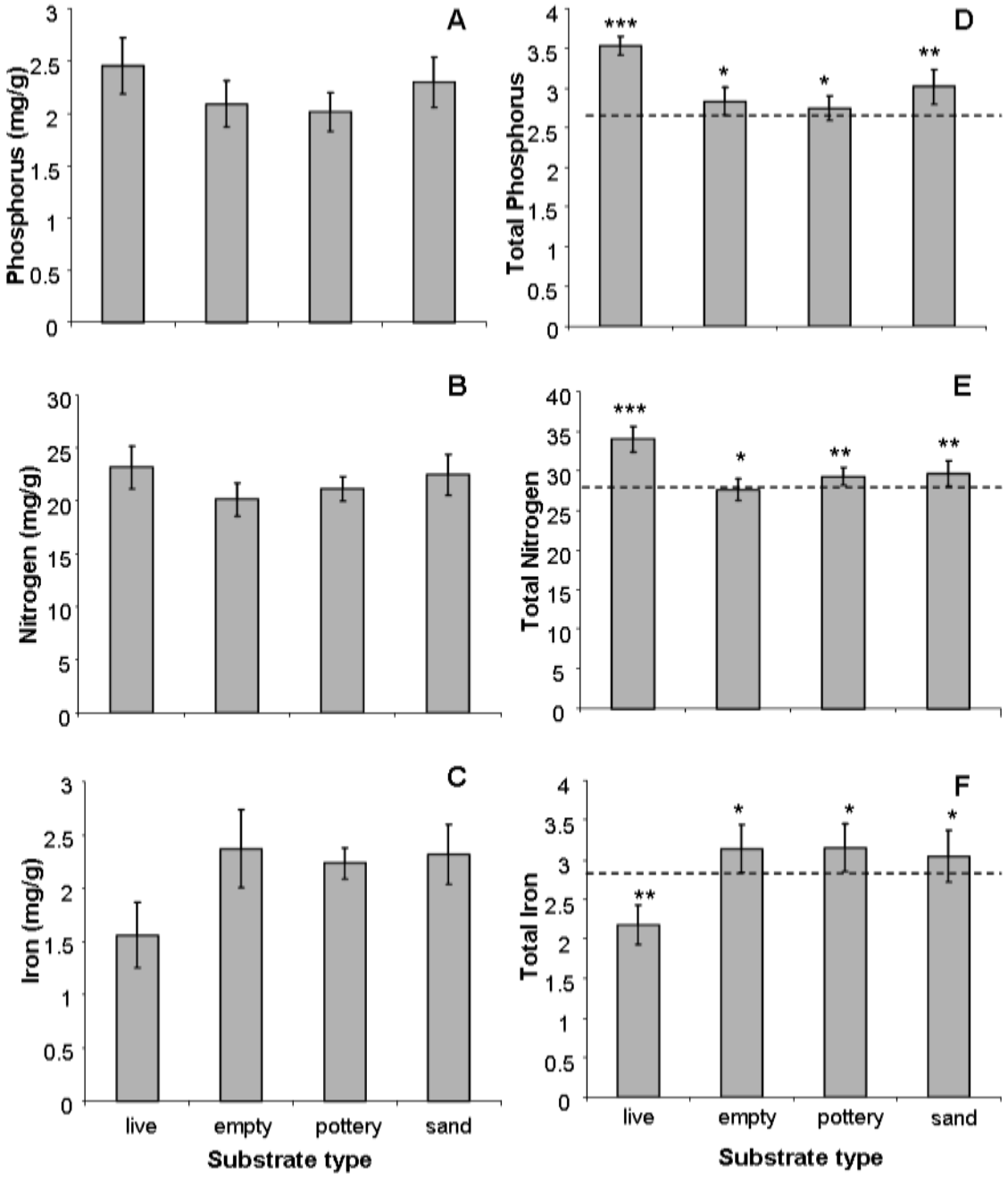
A
Phosphorus (mg/g)
3
2.5
2
1.5
1
0.5
0
D
Total Phosphorus
4
3.5
3
2.5
2
1.5
1
0.5
0
***
*
*
**
B
Nitrogen (mg/g)
30
25
20
15
10
5
0
E
Total Nitrogen
40
35
30
25
20
15
10
5
0
***
*
**
**
C
Iron (mg/g)
3
2.5
2
1.5
1
0.5
0
F
Total Iron
4
3.5
3
2.5
2
1.5
1
0.5
0
**
*
*
*
live
empty
pottery
sand
Substrate type
live
empty
pottery
sand
Substrate type

Figure 7

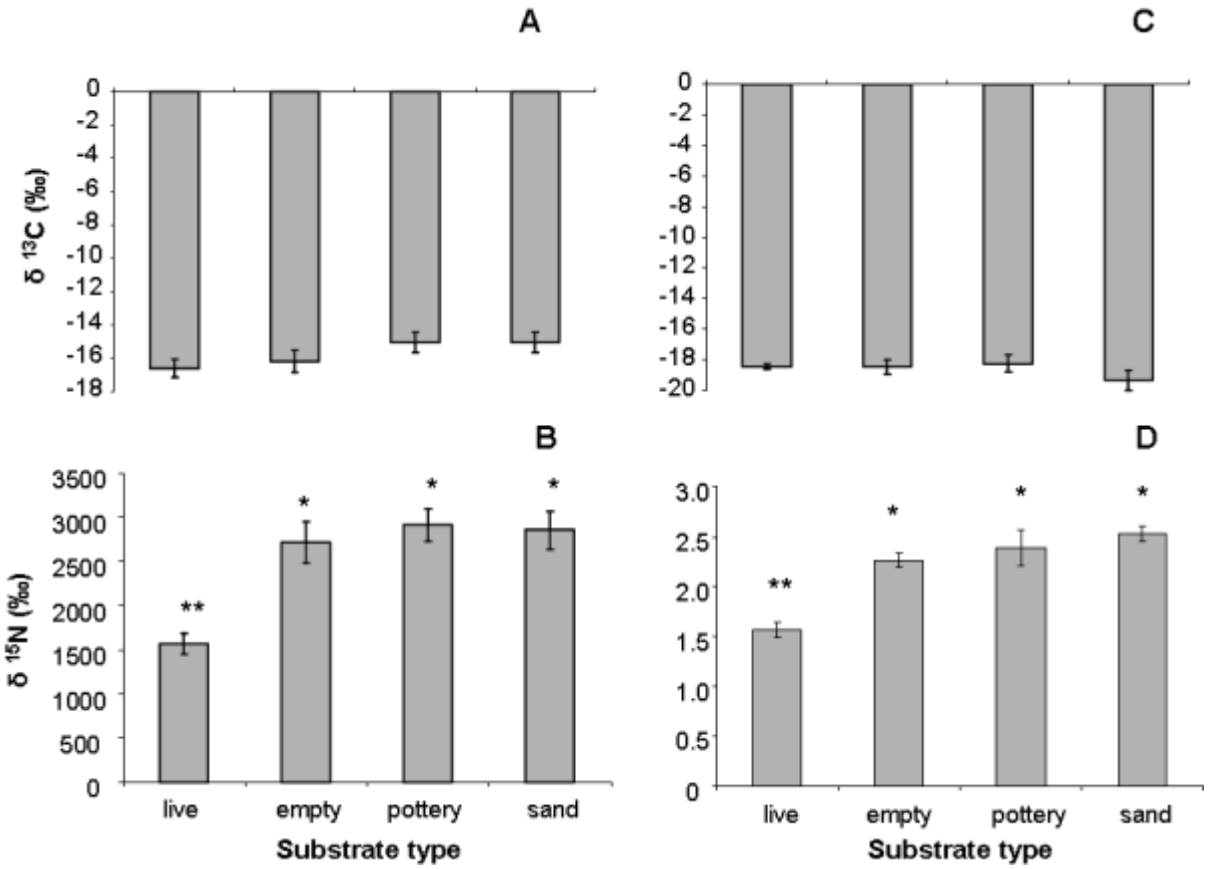
A
C
B
D
δ 13C (‰)
δ 15N (‰)
0
-2
-4
-6
-8
-10
-12
-14
-16
-18
-20
3500
3000
2500
2000
1500
1000
500
3.0
2.5
2.0
1.5
1.0
0.5
*
**
live
empty
pottery
sand
Substrate type

Figure 8