Mussel-derived stimulation of benthic filamentous algae: The importance of nutrients and spatial scale

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Abstract

The reoccurrence of benthic filamentous algal (FA) blooms in the Great Lakes, without associated increases in phosphorus loading, has stimulated renewed interest in determining the causes of Great Lakes benthic algal blooms. We investigated the potential roles of invasive mussels and nutrient limitation with experimental substrata within inner Saginaw Bay. FA abundance on live mussel substrata was typically significantly greater than that on inert (empty shell or rock) substrata. Nutrient addition (from an artificial source) significantly increased FA abundance on inert substrata. These results suggest that: 1) mussel nutrient excretion could be a primary stimulatory mechanism; 2) mussel-mediated stimulation may be even stronger in other, more oligotrophic, Great Lakes nearshore zones; and 3) increased nutrient loading to inner Saginaw Bay may exacerbate existing FA blooms. FA abundance on inert substrata was not affected, even in close proximity to mussels, indicating that the observed stimulatory effect of mussel-derived P on live mussels attenuated at very small spatial scales, on the order of centimeters or less.

Key words: Saginaw Bay, nutrient limitation, *Dreissena*, *Cladophora*

Introduction

Historically, excessive benthic algal growth and shoreline fouling events in the Laurentian Great Lakes have largely been a consequence of anthropogenic eutrophication (Auer, 1982; Bootsma et al., 2004; Herbst, 1969). Phosphorus (P) abatement programs ameliorated much of these historical problems (Auer et al., 2010; Bootsma et al., 2004; Higgins et al., 2008a), but now benthic filamentous algae (FA) blooms have returned to many areas of the Great Lakes, despite allochthonous P inputs remaining relatively steady (Bootsma et al., 2004, 2006; Cha et al., 2010; Malkin et al., 2010; Stow et al., 2014). Thus, the ecology underlying the causes of benthic algal blooms and associated wash-up of algal detritus requires attention (Auer et al., 2010; Bootsma et al., 2015; Hecky et al., 2004), as a firm understanding of ecology can indicate potential management solutions.

Modification of Great Lakes coastal ecosystems by invasive mussels may contribute to benthic algal blooms. In North America, dreissenid mussel invasion has greatly altered the ecology and biogeochemistry of aquatic ecosystems (Strayer, 2009). Benthic FA biomass commonly increases after dreissenid invasion (Higgins and Vander Zanden, 2010), and mussel-invaded aquatic ecosystems tend to shift from planktonic towards benthic production (i.e., the "benthification" of Mills et al., 2003).

The mussel-related habitat modifications of increased nutrient, light, and hard substratum availability are likely drivers of increased FA biomass caused by dreissenid mussels. Through their filter feeding and excretion, dreissenid mussels can move pelagic P to benthic habitats

(Hecky et al., 2004) and increase P bioavailability (Higgins et al., 2012). Stoichiometric FA surveys (Bootsma et al., 2004; Higgins et al., 2008a; Winslow et al., 2014), FA responses to point source nutrient inputs (Higgins et al., 2008b), and experimental P enrichments (Francoeur et al., 2015; Pillsbury et al., 2002) all indicate that FA biomass in the Great Lakes is often constrained by P availability; therefore mussel-derived P supplied to benthic habitats could increase FA growth. Mussel filter-feeding reduces phytoplankton abundance and abiotic turbidity, thereby increasing water clarity (see review by Higgins and Vander Zanden, 2010), which in turn increases light penetration to the benthos. Following dreissenid invasion of the Great Lakes, increased benthic light availability appears to have allowed benthic FA to grow at depths that were too poorly illuminated to support FA growth prior to dreissenid mussel invasion (e.g., Lowe and Pillsbury, 1995; Winslow et al., 2014). Mussels also add additional hard substrata (shells; Bially and MacIsaac, 2000; Vanderploeg et al., 2002) to the lakebed. Shells of living and dead dreissenid mussels now cover appreciable amounts of soft-sedimented lakebed within the Great Lakes (e.g., Berkman et al., 2000; Coakley et al., 1997; Dermott and Munawar, 1993). Because many FA taxa grow preferentially on hard substrata (John, 2003), dreissenid mussel shells could provide critical FA habitat in areas of soft lakebed sediments, analogous to the way native unionid mussel shells function as "islands" of suitable FA habitat in silty lakebeds (e.g., Francoeur et al., 2002). Whereas the above three effects of mussels are the most commonly cited, Armenio et al. (2016) have also proposed that mussel respiration may enhance FA growth by supplying additional inorganic carbon to FA.

The functioning and relative importance of aforementioned mussel-mediated processes in stimulating FA algal growth in Great Lakes nearshore zones is likely to be contextual and to vary

across spatial scales. For example, rapid (relative to phytoplankton doubling time) mixing of epilimnetic water spatially integrates the filtering action of individual mussels, as the water cleared by the filtering activity of an individual mussel does not remain directly above that mussel. Thus, mussel-induced increases in water clarity should reflect the aggregate of mussel filtering activity over large spatial scales, on the order of kilometers. However, rapid uptake of mussel-derived nutrients by adjacent FA likely results in a nutrient effect at small spatial scales (on the order of centimeters) surrounding individual or clumps of mussels, analogous to local nutrient supply by plumes of excreta from individual zooplankters to phytoplankton (Lehman and Scavia, 1982) and from chironomid larvae to adjacent benthic algae in streams (Mooney et al., 2014; Power, 1991). Similarly, the effects of mussel-derived hard substrata should act at small (cm) spatial scales, as the provision of FA habitat by the shell substratum is limited to the shell itself.

The functioning and importance of mussel-related factors affecting FA may also vary temporally over the course of a growing season. The magnitude and importance of mussel-derived nutrient supply and water clarity should change over time, as mussel filtering and excretion rates respond to temporal changes in factors such as temperature and food availability (e.g., Bootsma and Liao, 2014; Johengen et al. 2014), or as alternative sources of nutrients and turbidity cause seasonal shifts in nutrient and light availability (e.g., Fahnenstiel et al., 1995). The ability of FA to respond to favorable nutrient, light, or substratum conditions can also vary temporally, for example as altered temperature affects algal physiological performance (e.g., Graham et al., 1982).

Inner Saginaw Bay provides an ideal context in which to investigate the relative importance of mussel-derived mechanisms for stimulating FA abundance; in particular, the light-poor and P-rich conditions relative to many other Great Lakes nearshore habitats (Fig. 1) present an opportunity for a conservative test of the existence and importance of mussel-mediated P stimulation of FA. If mussel-derived nutrients are important in light-poor and P-rich inner Saginaw Bay, then they should be at least equally important in other, more oligotrophic Great Lakes nearshore habitats. Additionally, inner Saginaw Bay currently experiences benthic FA blooms and shoreline fouling (Bull, 2015; Francoeur et al., 2014; Stow et al., 2014), and should be a reasonable model for other shallow, relatively eutrophic areas of the Great Lakes (e.g., the western Basin of Lake Erie), allowing for both direct application of Saginaw Bay-specific results to an existing management challenge and straightforward extension of such results to other eutrophic Great Lakes habitats currently experiencing similar problems.

The goal of this study was to examine the mechanisms by which mussels may stimulate FA abundance by empirically investigating the importance of mussel-derived nutrient supply and shell habitat on benthic FA abundance at small (cm) spatial scales in a low-light, high-nutrient context. This study therefore does not address potential stimulatory effects at large spatial scales, in which beds of mussels may affect algal growth conditions over larger areas. Specifically, we examined if: 1) the type of hard substrata (live mussels vs. inert empty shells or rocks) affected FA growth, 2) additional nutrient supply stimulated FA growth, and 3) mussels provided sufficient nutrients to influence FA growth on themselves and adjacent substrata.

Methods

Study site.

Three in-situ experiments were performed from 2010-2012 within inner Saginaw Bay, approximately 2.7 km offshore from Linwood, Michigan, USA, adjacent to a previously-established survey site (the 3 m-deep station on survey transect 13, see Skubinna et al. 1995 and Francoeur et al. 2014). The study site (43°44'34.6" N 83°54'58.0" W) was ~ 3 m deep, with a sandy (~50-60%) rocky (~25-30%) lakebed. FA (mean 9.26 g DM m^{-2}, maximum 50 g DM m^{-2}; predominantly *Cladophora*, *Oedogonium*, *Spirogyra*, *Mougeotia*, and *Zygnema*), charophyte algae, and dreissenid mussels (~1600 mussels/m^2) were common at this site (Francoeur et al., 2014), and FA were often observed growing upon mussels.

Survey of environmental conditions.

On each site visit, we measured Secchi depth, downwelling irradiance (Li-Cor LI-192) and water temperature. In 2011, water samples were collected from ~0.5m below the surface, filtered (0.7 μm glass fiber) and frozen in acid-washed bottles for later analysis of PO_4, NO_3, and NH_4, using a Seal Discrete Nutrient Analyzer and EPA-approved methods (Seal Analytical 2015). Water samples for TP measurements were collected and frozen without filtering, then digested with potassium persulfate prior to analysis.

In order to characterize benthic irradiance and estimate the potential for FA light saturation, we obtained hourly solar flux (J m^{-2} h^{-1}) measurements from the nearby Linwood, Michigan, meteorological station (http://www.agweather.geo.msu.edu/mawn/) for the 21-day period immediately prior to each sampling event. We converted hourly solar flux to irradiance

(μmol m^{-2} s^{-1}), and applied the light extinction coefficient (k_d, calculated from downwelling irradiance measured over the 0-2 m interval) from the corresponding sampling date to calculate benthic irradiance (see Winslow et al. 2014). We then compared the maximum hourly benthic irradiance for each day (daily peak irradiance) to the lowest saturating irradiance (I_k) value reported for FA from inner Saginaw Bay (101 μmol m^{-2} s^{-1}; Winslow et al. 2014) to determine if FA potentially experienced saturating irradiance for at least 1 h of that day. We then calculated the number of days with potential light saturation and the average daily peak irradiance for the 21-day period preceding each sampling event, in order to give more information on benthic light availability and a reasonable indication of whether FA could have been light-saturated in the period immediately preceding our experimental sampling.

Experiment 1: Substratum comparison

The substratum comparison experiment was designed to: a) test the hypothesis that live mussels fostered additional FA growth on the mussels themselves relative to other hard substrata, and b) to test if any factor intrinsic to mussel shell substrata fostered FA growth relative to rock substrata. We compared FA abundance on live mussels, empty shells, and rock substrata. Comparison of FA abundance on live mussels to two types (rock and empty shell) of inert substrata elucidated whether mussel activity (e.g., nutrient release) stimulated FA growth at a local level (i.e., beyond any large spatial scale background stimulation attributable to being in an area with many mussels). Comparing growth on empty shells to that on rocks tested whether any increased FA abundance on live mussels could be due to some factor intrinsic to mussel shells as a substratum. Sampling this experiment multiple times during the growing season allowed us to assess whether the strength of mussel-related FA growth stimulation varied temporally.

Three treatments ("rock", "empty shell", and "live mussel") were used to examine relative growth of FA on different substrata. Forty-five experimental substrata (n = 15 in each treatment) were constructed. Each substratum consisted of two 10.8 x 10.8 cm ceramic tiles affixed glazed-side down (Z-spar A-788 epoxy putty) to a concrete brick, with rocks, empty shells or live mussels affixed to the tiles (Z-spar A-788 epoxy putty). Each "rock" substratum had 8-12 rocks, each "empty shell" substratum had 50 shells, and each "live mussel" substratum had 50 mussels (2143 mussels/m^2, similar to ambient mussel density at this site). Only a small portion of one valve was epoxied in place, so the mussels could continue to filter and feed normally. Rocks (3-4 cm diameter) and mussels were collected from Saginaw Bay. Mussels for the "live mussel" treatment were carefully removed from natural habitats by cutting byssal threads. To generate empty shells, some mussels were frozen and thawed, and then mussel tissue was removed from the shells. Prior to attachment, rocks, empty shells, and live mussels were scrubbed clean with a toothbrush dipped in a 5% bleach solution to remove any attached material, thus providing a clean surface for adhesion of epoxy and colonization of FA. Live mussels were kept moist during processing; time from mussel collection to deployment of experimental substrata was less than 36 h.

On 24 August 2010, the "rock" and "empty shell" treatments were deployed at the study site. On 25 August 2010, poor weather prevented the precise deployment of the "live mussel" treatment at the study site, so this treatment was temporarily deployed nearby (< 20 m from other treatments, in an area of equal depth and similar lakebed composition). On 10 October 2010, the "live mussel" treatment was relocated to the study site, and all substrata were repositioned so that

the replicates of all three treatments were randomly interspersed (< 5cm between adjacent experimental substrata). Substrata then overwintered at the study site prior to sampling the following summer.

We visited this experiment on three occasions (12 May 2011, 7 July 2011, and 9 September 2011), employing visual estimates of FA cover. Visual estimates of FA cover has been used to estimate FA abundance in the Great Lakes (e.g., Higgins et al., 2005; Depew et al. 2009), and percent cover of FA is strongly related to other measures of FA abundance, such as dry mass, when percent covers spans a wide range of possible values. For example, *Cladophora* percent cover (range 0 – 80%) was strongly related to *Cladophora* dry mass ($r^2 = 0.70$, $p < 0.001$, $n = 11$) in a New Hampshire estuary (Cianciola and Burdick, 2014).

For the May and September samplings, 4-7 replicates per treatment (Table 1) were carefully removed from the lakebed by divers. Substrata were individually placed into resealable plastic bags and then returned to the surface. Substrata were handled gently to avoid dislodging FA. Individual substrata were placed into a tub of water, and the percent cover of FA on the experimental surface was assessed visually by viewing vertically downward and noting the amount of each substratum covered by FA attached to that substratum. When mussels colonized the experimental surface, the percent cover of algae growing upon colonizing mussels was not included in the total. Visual assessments of each substratum were made by two independent observers; reported data are the consensus estimates of the two observers. At the July sampling date, the percent cover estimates were made in-situ by individual divers, without removal of substrata from the Bay. Within each sampling date, differences in the percent cover of FA on the

different substrata types were assessed with Kruskal-Wallis tests and Bonferroni-corrected multiple comparisons (Zar, 2010); non-parametric statistical procedures were employed because of underlying non-normality in the data. The seven "live mussel" substrata collected in September were placed in a cooler, returned to the laboratory, and opened to assess survivorship of mussels in the live mussel treatment.

Experiment 2: Spatial scale of mussel influence

This experiment was designed primarily to examine and quantify the fine-scale (0-5 cm) spatial extent of live mussel influence. Secondary goals were to confirm the results of the substratum comparison experiment regarding effects of nutrient excretion and mussel shells as a substratum, examine temporal variation in the strength of mussel-induced FA stimulation, and examine the influence of mussel density on FA stimulation. Materials were procured, prepared, and assembled into experimental substrata as previously described for the substratum comparison experiment, with the exceptions that: each experimental unit consisted of a single 10.8 x 10.8 cm ceramic tile which supported all three substratum types (live mussels, empty shells, and rocks); substratum types were positioned in a different spatial arrangement upon the tiles (see below); and rock substrata (1-1.5 cm diameter) were purchased at a local garden center.

Sixty experimental units (n = 30 in each experimental treatment) were constructed, as shown in Figure 2. The 30 "1 live" units were constructed by affixing 3 rocks and 3 empty shells attached in two separate lines (one line of rocks, one line of shells) along adjacent sides of the tile. The line of rocks and the line of shells intersected at one corner of the tile. A single live mussel was epoxied at the interior junction of the line of rocks and the line of shells. A second

line of three rocks and a second line of three shells were affixed near the opposite edges of the tile, equidistant (4-5 cm) to the live mussel. Offsetting the live mussel from the center of the tile facilitated the positioning of matched sets of inert substrata at two different distances from the live mussel. Thirty "9 live" units were constructed identically to the "1 live" units, except that nine live mussels were epoxied at the interior junction of the line of rocks and the line of shells instead of a single live mussel. On 7 October 2011, all units were deployed (treatments randomly oriented and interspersed, with ~ 20 cm between individual units) at the study site, where they overwintered prior to sampling the following summer.

Similar to the substratum comparison experiment, increased FA abundance on live mussels vs. inert substrata would support the hypothesis that nutrients from mussels stimulated FA growth on a very local scale (i.e., beyond any stimulatory effect of mussel beds operating more uniformly on a larger spatial scale). Examination of FA abundance on inert substrata incubated at known (0 to ~5 cm) distances from live mussels allowed us to quantify how far the zone of stimulation extended beyond the mussels themselves. Using both rocks and empty mussel shells allowed (as in the substratum comparison experiment) examination of the influence of mussel shell substratum, independent of any nutrient effect, on algae growth. Sampling this experiment multiple times during the growing season allowed us to assess whether the strength of mussel-related FA growth stimulation varied temporally. Varying the number of mussels used as experimental substrata allowed us to investigate the influence of mussel abundance on FA stimulation for the lowest possible mussel abundance (a single mussel) and for a small group of mussels at a reasonable density (9 mussels, 4000 individuals/m^2).

At each collection date (6 June 2012, 29 June 2012, and 31 July 2012) 2-4 units from each treatment were removed from the lake bed by divers, placed individually into resealable plastic bags, then returned to the surface (Table 1). Relatively low replication at each sampling date was a consequence of large overwinter losses of experimental units (i.e., occasional failures of the epoxy bond between tiles and anchoring bricks and apparent disturbance of experimental site by a dragging anchor resulted in many detached and overturned experimental substrata). After return to the surface, individual units were placed into a tub of water, and the percent cover of FA was visually assessed for each of the experimental substrata types (live mussels, empty shells, or rocks) and locations (lines of rock and shell near to/far from live mussels) on each unit as previously described. In order to improve differentiation of FA abundance between substrata with similar FA percent cover, filament length was also assessed (e.g., Higgins et al. 2005). When filaments were present, a ruler was used to measure the lengths of three different filaments on each combination of substratum type and location. In some instances of sparse algal abundance, fewer than three filaments were present; in these cases, fewer than three filaments were measured. Care was taken to avoid dislodging FA during collection, transport, and handling. Any mussels colonizing the experimental surfaces were quantified; the percent cover of algae growing upon colonizing mussels was not included in the total nor used for filament length measurements. FA percent cover was later re-scored from digital images of 20% of the substrata by a third observer. Re-scorings were very similar to the original scores (mean difference between original and re-scores = 6 percentage points; $r = 0.95$, $p < 0.001$, $n = 20$) indicating good reproducibility. The experimental units collected at each date were placed in a cooler and returned to the laboratory, where mussels were opened to assess survivorship.

Differences in the percent cover of FA and mean filament lengths (integrated over the entire experimental duration and within each individual sampling date) on the different substratum types/locations were assessed with Friedman's tests and Bonferroni-corrected multiple comparisons (Zar 2010) on specific contrasts of interest (live mussel vs. each other substratum type and near vs. far locations within each substratum type). Since each "1 live" or "9 live" unit contained all substratum types/locations, each experimental unit was treated as a block in this statistical analysis. Differences between the "1 live" and "9 live" units were assessed using Mann-Whitney U tests, as these comparisons were not experimentally paired. The specific comparisons examined were the percent cover of FA and mean filament lengths of: 1) FA growing only upon live mussels (i.e., considering only the 1 live mussel and 9 live mussels substratum types) and 2) the pooled mean values from FA growing on all non-live mussel substrata (rocks and empty shells) between the "1 live" and "9 live" units. These comparisons could only be made on the 6 June data, as sample sizes on the other dates were too low (i.e., power of Mann-Whitney U test = 0 for 29 June and 31 July comparisons).

Experiment 3: Nutrient enrichment.

This experiment tested the hypothesis that FA at our site were nutrient-limited in order to better understand the mechanism of mussel-mediated FA stimulation. If FA on inert substrata were already nutrient-saturated, then increased FA abundance on mussels cannot be attributed to mussel-derived nutrients.

Sixty experimental substrata (n = 30 in "control" and "fertilized" treatments) were constructed in a similar fashion as the spatial scale experiment, except no live mussels were

attached to these substrata, and rocks and empty shells were attached in two separate lines (one line of 3 rocks, one line of 3 shells) near opposite edges of the tile. Comparison of FA abundance on "control" and "fertilized" treatments provided a direct test of FA nutrient limitation, and examination of FA abundance on both rock and shell substrata would allow test of the hypothesis that there was an interactive effect of substratum type (rock or shell) and nutrient supply on FA growth.

Nutrient enrichment experiment substrata were deployed at the same time and site as substrata for the spatial scale experiment. After in-situ overwintering of substrata, divers attached mesh fertilizer packets to the "fertilized" treatment substrata on 1 May 2012. Each packet contained 15.4 g of Osmocote 14-14-14 slow-release fertilizer; a preliminary study was conducted to verify that nutrients would be released from fertilizer pellets over the duration of this experiment (see Electronic Supplement Material (ESM Fig. S1). Fertilizer packets were oriented along the center of the "fertilized" tiles. Collection of nutrient enrichment experiment substrata occurred on the same dates as the spatial scale experiment (6 June 2012, 29 June 2012, and 31 July 2012), but due to extensive overwinter losses of nutrient enrichment experiment substrata, the "control" and "fertilized" substrata collected on 6 June were cleaned with a nylon bristle brush, reset on the lake bottom on 29 June, and re-sampled on 31 July. Prior to re-set, used fertilizer packets were replaced with fresh packets.

FA percent cover and mean filament length were quantified as previously described, except the entire 10.8 cm x 10.8 cm experimental surface was assessed, due to highly-abundant FA on fertilized substrata completely obscuring the attached rocks and shells. Re-scoring 20% of

FA percent cover estimates indicated good reproducibility (mean difference between original and re-scores = 4 percentage points; $r = 0.99$, $p < 0.001$, $n = 6$). Within each sampling date, differences in the percent cover and mean length of FA were assessed with Kruskal-Wallis tests.

Algal P content.

Filamentous algae were removed from the "fertilized" and "control" tiles, as well as from groups of live mussels in the "9 live" treatment (6 June, 29 June) or from "fertilized" tiles, a single group of live mussels in the "9 live" treatment, and adjacent lakebed (31 July), and assayed for phosphorus content analysis (method of Winslow et al., 2014). Differences in FA P content between treatments at each date were assessed with one-way ANOVAs and Tukey multiple comparisons for each sampling date.

Results

Environmental Conditions.

Within each field season, temperature increased from May to July, while water clarity decreased by late July (Table 2). Calculated benthic light levels and the potential for FA light saturation were generally greatest in mid-summer (June and early July); and least in late summer (late July and September) during both 2011 and 2012 (Table 2). In contrast, calculated benthic light and the potential for light saturation varied between years during early spring (May); in 2011, light appeared to be scarce and light saturation unlikely, but in 2012, light was more abundant and light saturation was likely. Measured Secchi depths and nutrient concentrations

were within the ranges normally observed in inner Saginaw Bay (see Stow et al. 2014). Colonizing mussels were noted on 48% of 2011 experimental substrata (mean = 2.6 mussels/experimental unit, range 0-20); two "live mussel" substrata collected in September had an abundance (16 and 20) of very small colonizing mussels, somewhat skewing the overall mean. Mussel colonization of 2012 experimental substrata was similar (mean = 1.4 mussels/experimental unit, range 0-7), and 48% of the units were colonized. Macroscopic observations indicated that firmly-attached algal filaments lacking substantial extracellular polysaccharide coatings (e.g., *Cladophora* or *Oedogonium*) were the predominant FA taxa colonizing experimental substrata.

Experiment 1: Substratum comparison.

At the May 2011 sampling date, experimental substrata types differed significantly in FA percent cover (Fig. 3, $p < 0.001$). Only the "live mussel" treatment had appreciable FA percent cover (~ 60%), and it differed from both the "rock" and "empty shell" treatments (p always < 0.004). FA percent cover on the "rock" and "empty shell" treatments were low (< 5%) and statistically indistinguishable ($p = 0.622$). FA percent cover was high (60-100%) on all substrata at the July 2011 sampling date. The "live mussel" treatment tended to have somewhat greater FA cover, but FA percent cover did not differ significantly among experimental substrata types ($p = 0.066$). At the September 2011 sampling date, FA percent cover was greatly reduced on all substrata (~ 20%) and experimental substrata types were statistically indistinguishable ($p = 0.353$). Sixty-eight percent (range 46 – 90% on individual substrata) of experimental mussels on the "live mussel" substrata collected in September 2011 were alive. There was no relationship between the number of surviving mussels and FA percent cover ($r = 0.34$, $n = 7$, $p > 0.45$) on the

“live mussel” substrata, suggesting that the observed range of mussel mortality did not confound our results for the September sampling date.

Experiment 2: Spatial scale of mussel influence.

First we consider differences of FA abundance on different substrata within each mussel abundance treatment. Neither FA percent cover nor filament length differed significantly among substrata within the “1 live” units (Fig. 4 A, C), regardless of whether the data were integrated over the entire experimental duration (p always > 0.13) or considered at individual sampling dates (p always > 0.26). In contrast, the “9 live” units displayed consistent patterns in FA percent cover and filament length. Both FA percent cover and filament length differed significantly among substrata within the “9 live” units when data were integrated over the experimental duration (Fig. 4 B, D, p always < 0.001). Both mean percent cover (~20-40%) and filament length (1-4 cm) were significantly greater on groups of 9 live mussels than all other rock or empty shell substrata (always $< 5\%$ and < 1 cm, p always ≤ 0.008, Bonferroni-adjusted $\alpha = 0.0083$). Neither percent cover nor filament length on rocks (p always > 0.65) or empty shells (p always > 0.31) differed with respect to distance from live mussels. Considered at each individual sampling date, differences in FA percent cover and filament length among substrata within the “9 live” units approached statistical significance on 6 June ($p = 0.089$ and 0.060, respectively) and 29 June ($p = 0.061$ in both cases), and were non-significant on 31 July ($p = 0.114$ and 0.406, respectively).

Comparing FA between the two mussel abundance treatments, FA percent cover and filament length tended to be greater on the live mussels of the “9 live” units than the "1 live"

units (i.e. greater on groups of 9 mussels than on individual mussels). On 6 June, filament length, but not percent cover, was significantly greater on live mussels ($p = 0.032$ and 0.075, respectively) on "9 live" units than on "1 live" units. In contrast, pooled FA percent cover on inert substrata appeared to be greater on the "1 live" than the "9 live" units, especially on 31 July, but there was no statistical support for this apparent pattern. On 6 June, neither filament length nor percent cover differed significantly ($p > 0.05$) between inert substrata on "1 live" and "9 live" units. Low sample size ($n = 2$-4, Table 1) limited statistical power of all individual-date comparisons, and prevented statistical analysis of the 29 June and 31 July data. Much of this apparent pattern was due to a single "1 live" experimental unit with very high (30% and 80%, respectively) coverage on close rock and far shell substrata on 31 July. Excluding this possible outlier changes the mean 31 July percent coverage on the "1 live" close rock and far shell substrata (12% to 2.5% and 40% to 20%, respectively), greatly reducing the apparent difference in FA abundance on inert substrata between the "9 live" and "1 live" units.

All experimental mussels remained attached to the tiles for the duration of the study. All mussels on the "1 live" units survived to the time of collection. A few mussels on the "9 live" units died prior to sampling (mean number of live mussels per replicate = 7.6, range = 7-9). There was no relationship between the number of surviving "9 live" mussels and FA percent cover ($r = -0.06$, $n = 8$, $p > 0.5$) or mean filament length ($r = -0.04$, $n = 8$, $p > 0.5$) on the "9 live" mussels, suggesting that this low amount of mussel mortality did not confound our results.

Experiment 3: Nutrient enrichment.

Both FA percent cover and filament length responded strongly to nutrient addition, typically displaying 3 - 7 fold increases at various sampling dates (Fig. 5). Filament length was significantly greater in the nutrient-enriched treatment on all sampling dates (6 June, $p = 0.021$; 29 June, $p = 0.021$; 31 July, $p = 0.038$). FA percent cover was significantly greater in the nutrient-enriched treatment on the two June sampling dates (6 June, $p = 0.019$; 29 June, $p = 0.019$), and neared statistical significance on 31 July ($p = 0.074$).

Algal P content

The P content of FA on fertilized substrata was more than double that of FA on control substrata (6 and 29 June, $p < 0.05$) and FA collected from the adjacent lakebed (31 July, $p < 0.05$) (Table 3). On 6 June, the P content of FA collected from groups of 9 live mussels was intermediate between that of fertilized and control substrata, and indistinguishable from either ($p > 0.05$). By 26 June, P content of FA collected from groups of 9 live mussels was significantly less than that of fertilized substrata ($p < 0.05$) and indistinguishable from control substrata ($p > 0.05$). Statistical comparisons could not be made for the P content of FA from groups of 9 live mussels on 31 July, due to only a single FA sample being collected from a group of live mussels, however, the single value measured was nearly identical to the mean value of lakebed FA P content (Table 3).

Discussion

Our *in situ* study demonstrated that dreissenid mussels can facilitate FA growth via benthic nutrient enrichment even in a eutrophic Great Lakes nearshore zone, and that the strength of this facilitation varies greatly over small (cm) spatial scales. Epizoic algae were stimulated on

live mussels, but the stimulatory effect did not extend to immediately adjacent empty shells or rocks. Although suitable hard substrata were needed for attachment, FA were not abundant on experimental substrata without additional nutrient augmentation (except for the 7 July 2011 sampling date). The fact that nutrient addition increased FA growth, despite the relatively high ambient nutrient concentrations and low light availability within inner Saginaw Bay (Fig. 1), indicates that mussel-derived nutrients may stimulate FA abundance even in turbid, nutrient-rich nearshore habitats. This finding suggests that nutrients likely constrain FA abundance on hard substrata in many areas of the Great Lakes. Because the relatively turbid water of inner Saginaw Bay rapidly attenuates light, our results (based on experiments conducted at a depth of 3 m) likely underestimate the importance of nutrient addition in shallower habitats within inner Saginaw Bay, where maximal FA biomass (up to 200 g DM m^{-2} at 2 m) typically occurs (Francoeur et al. 2014). The congruence of our results with those from a similar experiment in western Lake Erie (Stewart et al. 1998), suggests that stimulation of benthic algal abundance by nutrient excretion from living dreissenid mussels could be a common phenomenon in the Great Lakes, even in relatively light-poor and nutrient-rich regions.

Nutrient excretion as a stimulatory mechanism.

Our results support the hypothesis that mussel-mediated nutrient supply, and not CO_2 supply from mussel respiration or other mechanisms, caused the increased FA abundance on live mussels in our experiments (Figs. 3 & 4). FA abundance and P content greatly increased in response to nutrient addition from artificial fertilization (Fig. 5, Table 3), showing that nutrient supply increased FA growth at our site. These latter results are in line with the

stoichiometrically-based study of Winslow et al. (2014) that showed FA within inner Saginaw Bay are P-limited.

Because there were sampling dates in which FA grew much more abundantly on live mussels than on rocks or empty shells, we can ascribe the FA growth directly to the nutrient release from live mussels. These results mirror those of Stewart et al. (1998) for benthic algal biomass accrual in a similar experiment using tiles at Put-In-Bay, Lake Erie. Other studies have found FA occurring in association with dreissenid mussels more than other substrata in Lake Huron (Barton et al. 2013) and Lake Erie (Armenio et al., 2016), and Davies and Hecky (2005) observed that the FA (*Cladophora*) growing at Lake Erie sites with mussels appeared "healthier" than that growing at sites lacking mussels. In our study, artificial fertilization led to growth on all substrata (rock, shell, and ceramic tile), indicating that they were all suitable for FA. Further, we found substantial FA growth on all substrata at only a single date (7 July 2011) in our study, further indicating that despite all substrata being suitable for FA, ambient nutrient levels were typically too low to support abundant FA growth on inert substrata.

Mussel excretion is the most likely mussel-derived nutrient source. Invertebrate nutrient excretion can stimulate planktonic (Lehman and Scavia, 1982) and benthic (Mooney et al., 2014; Power, 1991) algae. Nutrient excretion from benthic mussels stimulated benthic algal biomass in freshwater riverine mesocosms (Vaughn et al., 2007) and marine tide pools (Pfister, 2007). Temporal trends of dissolved nutrients and O_2 concentrations within in-situ benthic chambers suggested that mussel-derived nutrients stimulated FA photosynthesis in Lake Erie (Davies and Hecky 2005). We also observed that epizoic FA was often localized near the excurrent siphon of

the mussels in our experiments (authors' personal observation), suggesting excretion as the source of nutrients. Nevertheless, other mussel-associated nutrient supply pathways are possible, including: mussel filter feeding causing localized water motion that can speed nutrient transport to algal cells (e.g., Riber and Wetzel, 1987; Whitford and Schumacher, 1964), and nutrient remineralization arising from increased organic matter (including feces and pseudofeces) deposition and macroinvertebrate densities associated with live mussels relative to areas covered by empty shells (Stewart et al. 1998). Further study will be required to evaluate the relative importance of these nutrient supply pathways.

Decay of dead mussel tissue could also provide nutrients, so unnatural mussel mortality due to experimental handling could potentially introduce an experimental artifact. We minimized handling-induced mussel mortality by keeping emersion duration short (< 36 h) and keeping mussels moist during emersion. Dreissenid mussels generally survive several days of emersion, as long as they are kept moist (see Garton et al., 2014). We also deployed substrata in the autumn, so that any mussels that died due to handling would have several months in which to decay prior to spring algal colonization and sampling. Observed mussel mortality (32% and 16% in the substratum comparison and spatial scale experiments, respectively) was similar to expected natural annual mortality rates (Garton et al., 2014). Thus, unnatural mussel mortality and the resulting nutrient supply did not confound our experimental results.

Spatial extent of mussel-induced FA stimulation.

The spatial scale experiment indicated that the zone of live mussel stimulation of FA was quite localized in spatial extent, being restricted to the mussels themselves. FA length and

abundance on empty shells and rocks immediately adjacent to live mussels displayed no increases, despite abundant epizoic FA on the mussels (Fig. 4 B, D). Such observations are consistent with rapid dilution of nutrient-rich mussel excreta, uptake of released nutrients by FA epizoic on mussels, or the combined action of both of these phenomena.

A likely explanation for the spatial extent of mussel-induced stimulation being limited to epizoic algae is rapid dilution of nutrient-rich mussel excreta by mixing with nutrient-poor lake water. A cross-system comparison suggests that this phenomenon is plausible. In flowing streams, stimulation by invertebrate nutrient excretion was limited to epizoic algae (Mooney et al. 2014), but in isolated tide pools (Pfister, 2007) and static-water mesocosms (Vaughn et al., 2007) invertebrate excretion stimulated algal growth on substrata adjacent to mussels. Enrichment of SRP due to mussel excretion occurred at > 10 cm above a mussel-covered Lake Michigan lakebed during quiescent conditions; however, during turbulent conditions, spatial SRP gradients were undetectable due to rapid mixing of excreta and lakewater (Dayton et al. 2014). Similarly, the Lake Michigan benthic nutrient cycling model of Bootsma and Liao (2014) indicated that water motion can determine whether mussel-derived nutrients remain available for local benthic FA uptake or are rapidly diluted by mixing. Thus, at our relatively shallow (3 m) experimental site, wind-induced wave action and the resulting mixing of benthic water could have limited the spatial extent of mussel-induced stimulation; a larger stimulation zone might be expected at deeper, less turbulent sites. Uptake of mussel-derived nutrients by epizoic FA should also reduce the spatial extent of the zone of stimulation. Dayton et al.'s (2014) documentation of elevated SRP concentrations above a mussel-covered lakebed, despite the presence of modest amounts of FA, indicates that mussel excretion can outpace FA uptake, at least under some circumstances.

A small, localized zone of strong FA stimulation by mussel-derived nutrients is consistent with previous experiments and observations in the Great Lakes. Barton et al. (2013) reported that FA occurred more commonly on dreissenid mussels than inert substrata in the nearshore zone of southeastern Lake Huron, and Stewart et al. (1998) found significantly less benthic algal biomass accrual on inert substrata relative to mussel-covered tiles, despite all inert substrata being only 10s of cm from the mussel-covered lakebed at Put-In-Bay, Lake Erie.

The size and density of mussel beds may also affect the likelihood and spatial scale of FA responses. Clearly, FA on an inert substratum seem less likely to be stimulated if only a few live mussels are nearby, then if surrounded by an extensive bed of mussels. We examined small-scale spatial patterns, and employed only small (1, 9, or 50 mussels) to moderate density (440, 4000, or 2311 mussels/m^2) beds of mussels in our study. Our experiments found frequent mussel-mediated stimulation of epizoic FA on beds of 9 and 50 mussels, but no "off mussel" stimulation of FA. One might expect more "off mussel" FA response to larger nutrient releases from more extensive mussel beds, as sufficiently large mussel-mediated nutrient supply rates could influence FA some distance away from mussel beds, especially if dilution of excreted nutrients is an important mechanism preventing stimulation of remote FA. For example, Francoeur et al. (2015) suggested that immediately post-invasion, very high mussel densities and the resulting abundance of P-rich mussel excreta in the benthos may have temporarily saturated benthic algal P demand, even on mussel-free experimental substrata ~10 cm away from lakebed mussels in outer Saginaw Bay. The generally low FA abundance observed on our inert experimental substrata suggests that little P was supplied to these substrata by the surrounding mussel

population at our experimental site; however, FA were quite abundant on inert substrata at one sampling date (7 July 2011) of the substratum comparison experiment, indicating that P was temporarily sufficient to support abundant FA growth. Surrounding lakebed mussels (< 10 cm distant from experimental substrata) are one potential source for this P, but other sources (run-off/river inputs, sediment release, etc.) are also quite possible. Similarly, other potentially stimulatory factors may also be related to the size of mussel beds. For example, if nutrient supply from sediments, feces, and pseudofeces trapped within crevices between mussels is an important resource, then FA on groups of mussels will be advantaged. The mussel-induced elevation of SRP concentrations at distances of 10 – 20 cm from mussel beds under quiescent conditions (Dayton et al. 2014) may suggest a maximum size for the zone of stimulation. Nevertheless, an assessment of the potential for larger-scale stimulation of FA by mussel-derived nutrients is required to fully understand the effects of mussels.

Temporal patterns in mussel-induced stimulation.

The strength of mussel-induced stimulation of FA growth varied temporally, at least in 2011. In the substratum comparison experiment (2011), the greatest FA response to live mussels was observed in mid-May. In July and September of 2011 there was no detectable difference in FA abundance amongst the substrata, but for opposite reasons; in July 2011 FA were abundant on all treatments, but in September 2011 all treatments had low FA abundance. In contrast, the experiment in 2012 displayed a more consistent pattern of greater FA abundance on the "9 live" mussel treatment, but the stimulatory effect of live mussels again appeared to weaken by late summer (Fig. 4 B, D). The unfortunate destruction of many experimental substrata led to low

sample sizes and low statistical power at the individual sampling dates in the spatial scale experiment, and weakened the inferences possible from this experiment.

Temporal patterns in the strength of mussel-induced stimulation may reflect benthic light availability. Low water clarity and concomitant low benthic light availability and lack of algal light saturation was coincident with weak stimulation of FA by nutrients and relatively low FA abundance on experimental substrata in both July 2012 and September 2011 (see Table 2, Figs.3, 4, & 5), suggesting that light limitation might have reduced the ability of algae to respond to additional nutrients later in the summer. In contrast, FA were strongly stimulated by live mussels in May 2011, despite relatively low calculated benthic light availability and a lack of algal light saturation. Several potential hypotheses might explain this apparent conundrum. Deficiency of either nitrogen or phosphorus can reduce algal photosynthetic performance (Senft 1978; Smith 1983; Geider et al. 1993), so perhaps mussel-derived nutrients helped offset algal light limitation in this instance. It is also possible that our estimate of saturating irradiance was too high, leading to underestimation of FA light saturation. Although we employed the lowest value of I_k reported for Saginaw Bay FA (101 μmol m^{-2} s^{-1}; Winslow et al. 2014) in order to account for algal photoacclimation to dimly lit habitats, I_k values < 100 μmol m^{-2} s^{-1} have occasionally been reported for Great Lakes FA (Higgins et al. 2008a). Lastly, our 12 May 2011 k_d measurement may not accurately reflect typical water clarity in the 21 days preceding the sampling date. Data from the Linwood, Michigan, meteorological station (http://www.agweather.geo.msu.edu/mawn/) indicate periods of moderate (21 km h^{-1}) northeasterly winds on 9 and 11 May (just before our measurements on May 12), which could have generated waves, suspended sediments, and reduced water clarity thereby causing an

underestimate of the actual benthic light availability for the 21 day period preceding 12 May 2011.

Temporal changes in the strength of mussel-induced stimulation could also result from temporal changes in mussel excretion (e.g., Bootsma and Liao, 2014; Johengen et al., 2014), ambient nutrient availability (e.g., Fahnenstiel et al., 1995; Johengen et al., 1995), or environmental factors such as water temperature (e.g., Fahnenstiel et al., 1995; Johengen et al., 1995) or near-bed hydraulic conditions that affect the ability of FA to respond to nutrients. In calm periods, one would expect that mussel-derived nutrients would become increasingly available to FA in near-bed water, instead of being diluted by mixing throughout the entire water column. Both the modeling exercise of Bootsma and Liao (2014) and the in-situ nutrient measurements of Dayton et al. (2014) show that water motion can determine whether mussel-derived nutrients remain available for local benthic FA uptake or are rapidly diluted by mixing. The relatively high FA percent cover on all substrata of the substratum comparison experiment on 7 July 2011 suggests that nutrients were sufficiently available to stimulate FA growth on all hard substrata at this date. In contrast, the relatively low FA percent cover on all substrata of the same experiment on September 2011 is consistent with environmental conditions being sufficiently poor to inhibit abundant FA growth, regardless of nutrient availability. Likewise, the somewhat weaker responses of FA to fertilization on 31 July 2012 in the nutrient-enrichment experiment (coincident with the weaker response to mussel substrata on the same date in the spatial scale experiment) suggested that conditions in late July 2012 were such that FA were less able to respond to nutrient supply. Benthic surveys in previous years (Francoeur et al. 2014) indicated that FA biomass within inner Saginaw Bay often begins a seasonal decline between

late July and late August, which supports the idea that environmental conditions could have been unfavorable for FA growth at the final sampling dates of 2011 and 2012.

Potential implications for other Great Lakes nearshore zones.

Our results from relatively turbid and nutrient-rich inner Saginaw Bay, combined with previously published experimental and observational data from the similarly light-poor and nutrient-rich western basin of Lake Erie (Fig. 1), suggest that that stimulation of benthic algal abundance by living dreissenid mussels may be a general phenomenon throughout the Great Lakes, where turbidity and background nutrient levels are typically lower. Logically, one would expect that the stimulatory strength of nutrient enrichment would be greatest at sites with high light availability and low ambient nutrient supply. Placing nearshore sites within the Great Lakes on axes of light availability and ambient nutrient supply (estimated by mean Secchi depth and mean TP concentration, respectively; Fig. 1), can be used to provide a context for extending inferences to other sites. Neither Secchi depth nor TP concentration perfectly correspond to light or nutrient supply; actual measures of benthic light availability and site-specific P loading would be ideal. However, such data are not available, so we used Secchi depth and TP concentration as readily-available proxies of these environmental characteristics. Nutrient enrichment experiments have confirmed P limitation of FA in both outer (Francoeur et al., 2015) and inner Saginaw Bay (this study), and stoichiometric analyses indicate FA P deficiency within both inner Saginaw Bay (Winslow et al., 2014) and shallow depths at the Lake Ontario nearshore sites of Fig. 1 (Higgins et al., 2012). Thus, we predict that FA at the lower-nutrient Lake Huron site (Fig. 1; but perhaps not the higher-nutrient Lake Huron site) would also be P-limited. Similarly, mussel-mediated stimulation of epizoic FA abundance at inner Saginaw Bay (this study) and at

Put-In-Bay, Lake Erie (Stewart et al., 1998) suggests that mussel-derived nutrient supply should stimulate FA abundance at the Lake Ontario nearshore sites and the lower-nutrient (but perhaps not the higher-nutrient) Lake Huron site. Nearshore sites generally exhibit great spatial & temporal variability in nutrients and water clarity, and the sites in Figure 1 are no exception (see Beeton et al., 1996; Higgins et al., 2012; Howell et al., 2014; Stow et al., 2014). Because FA can rapidly respond to naturally fluctuating light and nutrient availability, long-term environmental conditions are only predictive of the likelihood of FA responses, not the FA response at any particular time. Nevertheless, FA in Great Lakes nearshore zones with higher light and lower P would be expected to display even stronger and more temporally-consistent responses to mussels than those observed in our study.

Our results suggest that the mechanism driving increased FA presence at sites where mussels have colonized soft lakebed sediments (e.g., Francoeur et al. 2014) might not be solely the provision of hard substrata for FA attachment, but rather the simultaneous provision of hard substrata and local nutrient enrichment. The importance of this additive substratum + nutrient effect epizoic FA on mussels colonizing soft substrata may be related to the size of mussel clusters. In our experiment, we could not detect a stimulatory effect of individual mussels, but FA stimulation was consistently observed for groups of 9 mussels. Future investigations will be required to evaluate the generality of and mechanisms underlying this scaling effect.

Regulation of FA growth and management implications.

Despite nutrient reallocation by dreissenid mussels from pelagic to benthic habitats (Hecky et al., 2004), inert hard substrata in our experiments did not usually support maximal FA

biomass; direct nutrient fertilization caused large, persistent increases in FA abundance and P-content on such substrata. Thus increased P inputs to Saginaw Bay could exacerbate future FA blooms, even within the relatively turbid and nutrient-rich inner bay. The apparently greater ability of FA to respond more strongly to nutrient enrichment in May and June suggests that temporal shifts towards larger May P inputs to Saginaw Bay (Stow et al., 2014) may engender strong biological responses.

Our results highlight the continued need for P management within Saginaw Bay, and support the previous modeling-based recommendations for additional P management within Great Lakes littoral zones (i.e., Auer et al., 2010; Malkin et al., 2008). Modeling-based analyses have advocated a management target of 1 μg SRP/l for control of *Cladophora* growth in the Great Lakes (Tomlinson et al. 2010). Our empirical results from inner Saginaw Bay, as well as the results of in-situ nutrient enrichment experiments in outer Saginaw Bay (Pillsbury et al. 2002, Francoeur et al. 2015), and nutrient enrichment experiments in lotic systems (e.g., Horner et al. 1990) all indicate that FA biomass accrual can be P limited at SRP concentrations > 1 μg/l. Thus, P supply can still constrain FA biomass under these conditions, and P management within such habitats may still be of value for reducing FA proliferations, even if it is not possible to lower SRP concentrations below 1 μg/l. Our results also suggest that localized P supply by mussels may be an important driver of FA growth, and thus this pathway should receive consideration in algal growth models.

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Table 1. Sample size for all experiments, by sampling date. Note that sample size for the spatial scale experiment refers to the number of blocks (all substratum types were present within each block).

Experiment	Sampling Date	Sample size
Substratum comparison	12 May	live = 6, shell = 6, rock = 6
Substratum comparison	7 July	live = 2, shell = 3, rock = 3
Substratum comparison	9 Sept	live = 7, shell = 4, rock = 4
Spatial scale	6 June	1 live = 4, 9 live = 3
Spatial scale	29 June	1 live = 3, 9 live = 3
Spatial scale	31 July	1 live = 3, 9 live = 2
Nutrient enrichment	6 June	control = 4, fertilized = 4
Nutrient enrichment	29 June	control = 4, fertilized = 4
Nutrient enrichment	31 July	control = 4, fertilized = 4

Table 2. Environmental conditions during experiments. * indicates Secchi measurement limited by water depth, - indicates no measurement data available, n.d. indicates value below detection limit.

Experiment	Date	Water Temp. (°C)	Secchi Depth (m)	k_d (m^{-1})	# days with potential light saturation	Mean daily peak irradiance (μmol m^{-2} s^{-1})	SRP (μg/l)	NH_4-N (μg/l)	NO_3-N (μg/l)	TP (μg/l)
Substratum	12 May	14.7	1.70	1.21	0/21	39	2.6	18	810	14.9
comparison	7 Jul.	22.8	1.82	0.88	15/21	112	2.2	n.d.	270	3.2
	9 Sept.	-	1.11	1.28	0/21	30	2.7	n.d.	30	16.5
Spatial scale &	1 May	9.7	2.3	0.66	18/21	201	-	-	-	-
Nutrient enrichment	6 Jun.	17.5	2.7*	0.56	20/21	298	-	-	-	-
	31 Jul.	23.8	1	1.05	0/21	70	-	-	-	-

Table 3. FA P content (μg P g dry wt^{-1}) during the 2012 experiments. Superscripts denote significant differences within each date, n.d. = statistical difference not determined due to a lack of replication.

Date	Treatment	P content (mean ± 1 SD)	Sample size	p
6 June	Fertilized^{A}	2228 ± 1405	3	0.012
	Control^{B}	569 ± 232	3	
	9 Live mussels^{A,B}	906 ± 62	4	
29 June	Fertilized^{A}	1061 ± 66	4	< 0.001
	Control^{B}	381 ± 8	2	
	9 Live mussels^{B}	383 ± 83	3	
31 July	Fertilized^{A}	1362 ± 286	5	0.002
	Lakebed^{B}	567 ± 34	3	
	9 Live mussels$^{n.d.}$	560	1	

Figures.

Figure 1. Relationship of Secchi depth and total phosphorus at selected sites in the Great Lakes. Locations, sample sizes, time periods and data sources are: Inner and Outer Saginaw Bay, n = 32–95, May-October 2008-2010, Stow et al. (2014); two Lake Huron nearshore sites, n = 141-150, May-September 2010, Howell et al. (2014); seven Lake Ontario nearshore sites, n = 20-74, May-August 2008, Higgins et al. (2012); Put-in-Bay Lake Erie, n = 18, May-September 1995, Beeton et al. (1996). Values are means (Beeton et al., 1996; Stow et al., 2014), or weighted means (Higgins et al., 2012; Howell et al., 2014). Secchi depths for Lake Ontario and Lake Huron sites were estimated from published turbidity data using the "all lakes" regression equation of Koenings and Edmundson (1991).

Figure 2. Pre- and post- experiment photos, documenting experimental substratum design for the spatial scale of mussel influence experiment. "1 live" substrata are on the left, "9 live" substrata are on the right. See text for positioning of empty mussel shells and rocks.

Figure 3. Mean (+ 1 SE) filamentous algal percent cover on experimental substrata in the substratum preference experiment. Letters denote significant differences between substratum types.

Figure 4. Mean (+ 1 SE) filamentous algal percent cover and filament length on experimental substrata in the "1 live" treatment (panels A & C) and the "9 live" treatment (panels B & D) of the spatial scale experiment. Asterisks denote significant difference between the marked substratum and all other substratum types within the "1 live" or "9 live" units. Letters denote significant differences between substrata of the same type (either live mussels or all inert substrata combined) between the "1 live" and "9 live" units on 6 June.

Figure 5. Mean (+ 1 SE) filamentous algal percent cover and filament length on experimental substrata in the nutrient enrichment experiment. Asterisks denote significant differences between "control" and "fertilized" treatments on a specific date.

Figure 1.

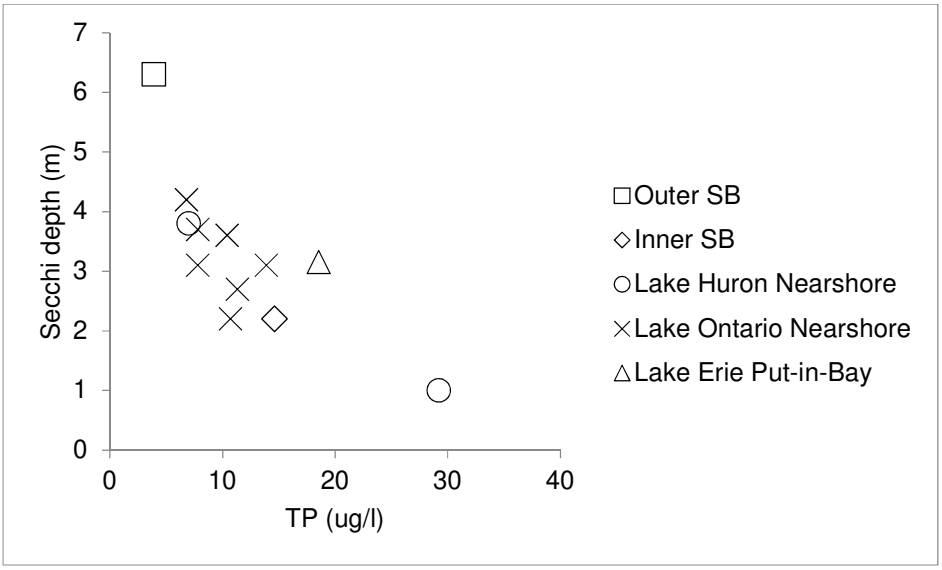

Secchi depth (m)
0
1
2
3
4
5
6
7
0
10
20
30
40
TP (ug/l)
Outer SB
Inner SB
Lake Huron Nearshore
Lake Ontario Nearshore
Lake Erie Put-in-Bay

Figure 2.

Figure 3.

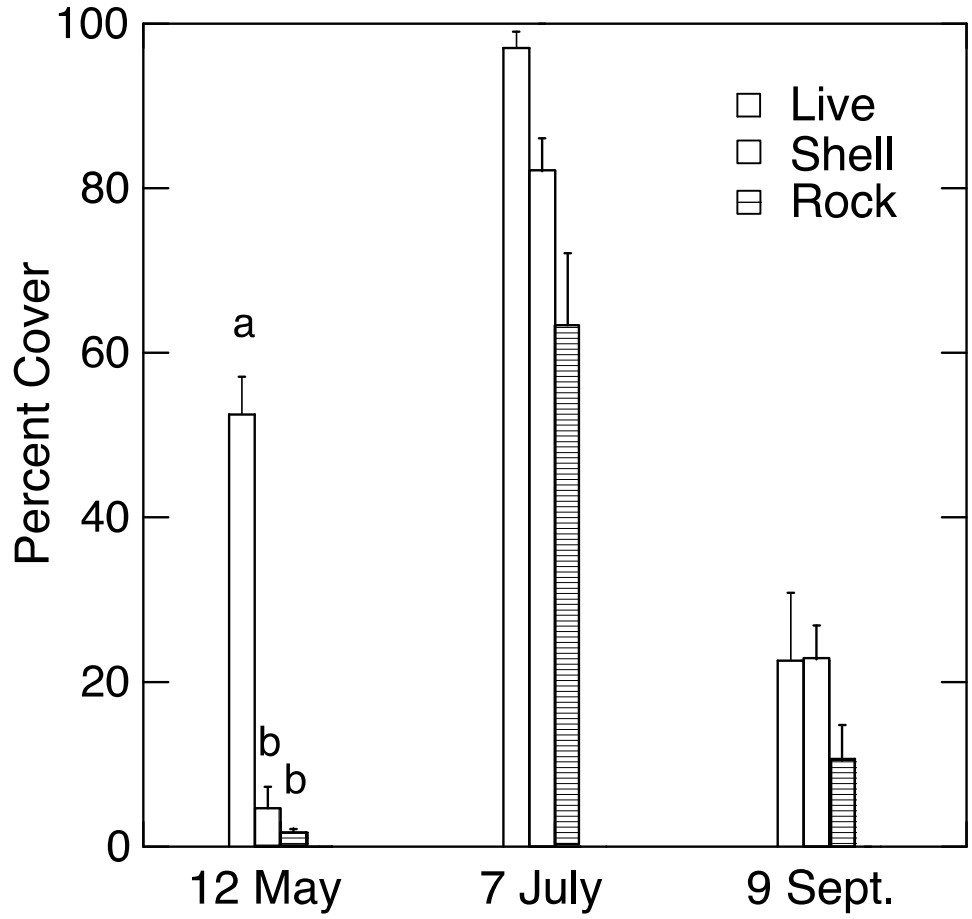

100
80
60
40
20
0
Percent Cover
Live
Shell
Rock
a
b
b
12 May
7 July
9 Sept.

Figure 4.

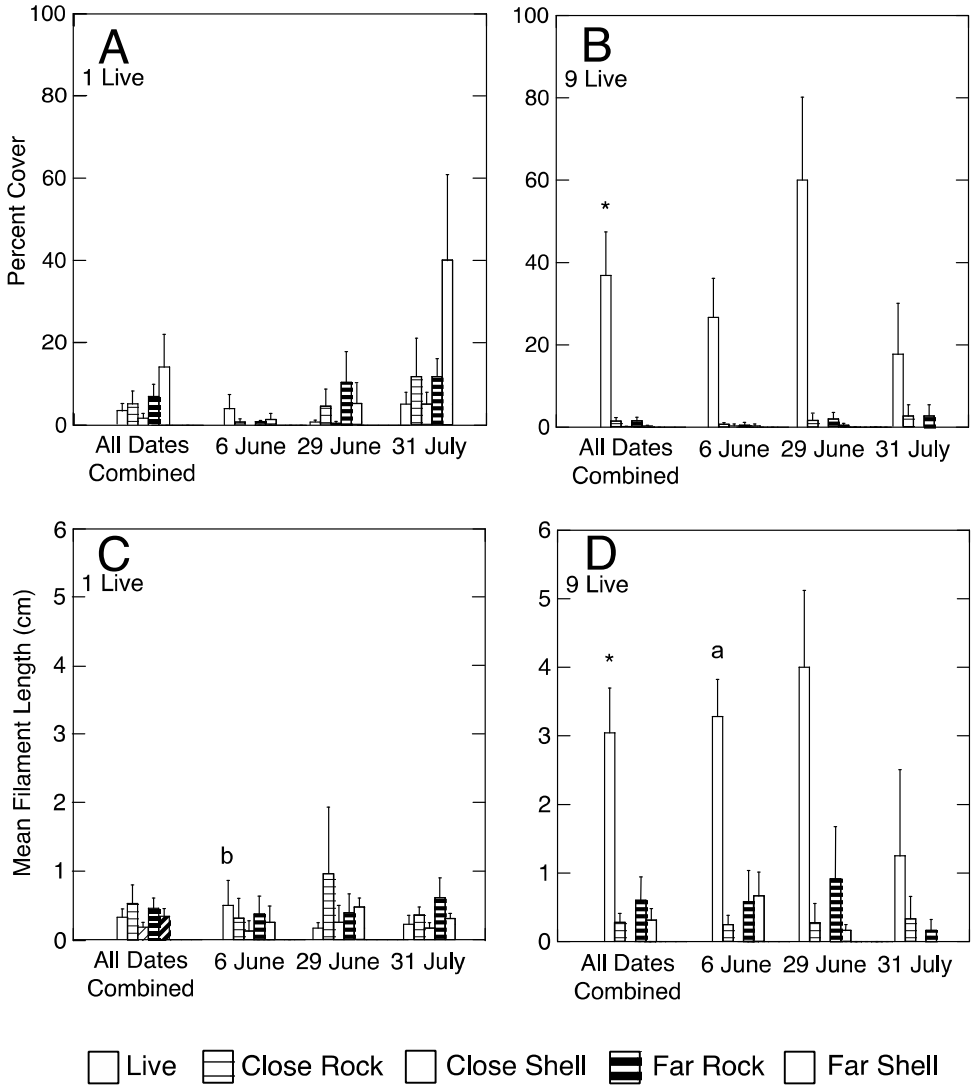
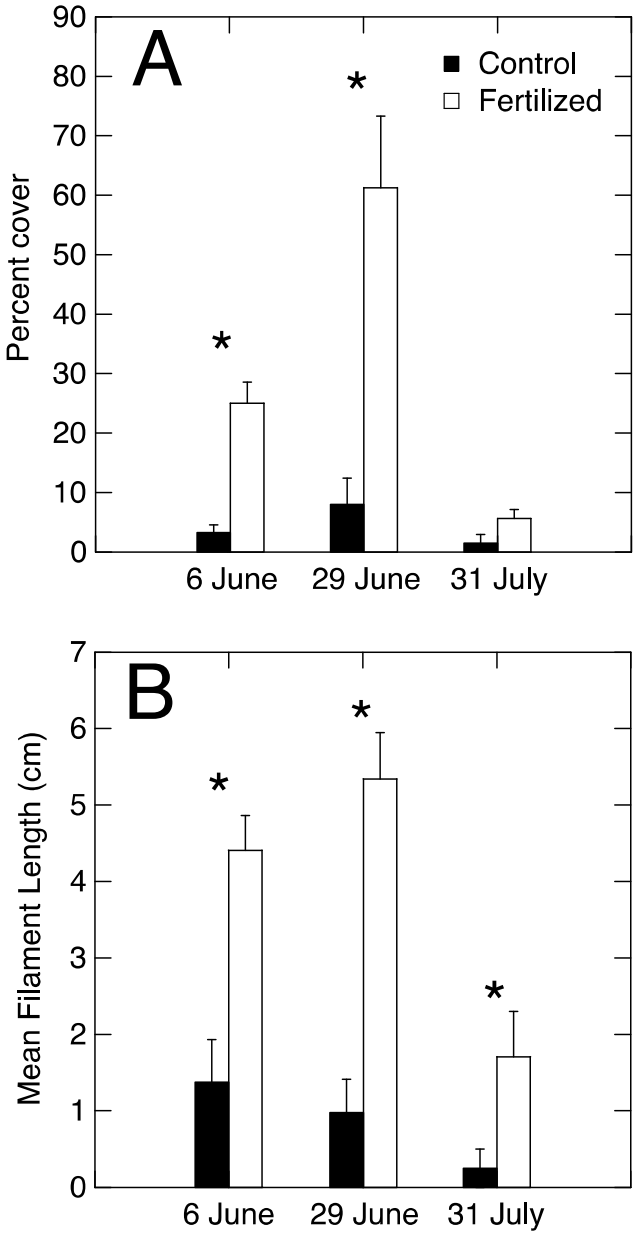

A
1 Live
B
9 Live
C
1 Live
D
9 Live
Percent Cover
Mean Filament Length (cm)
All Dates Combined
6 June
29 June
31 July
*
a
b
Live
Close Rock
Close Shell
Far Rock
Far Shell

Figure 5.


A
Control
Fertilized
Percent cover
90
80
70
60
50
40
30
20
10
0
*
*
6 June
29 June
31 July
B
Mean Filament Length (cm)
7
6
5
4
3
2
1
0
*
*
*
6 June
29 June
31 July