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Seston quality drives feeding, stoichiometry, and excretion in zebra mussels

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SUMMARY

1. Seston availability and quality can affect the condition, nutrient stoichiometry and nutrient excretion of dreissenid mussels and other aquatic consumers. Nutrient excretion by dreissenid mussels may affect phytoplankton community composition by altering nitrogen:phosphorus (N:P) ratios of the water and may be an important accessory factor leading to increased *Cladophora* and toxic *Microcystis* blooms in mussel-invaded lakes.
2. We manipulated phosphorus enrichment levels (no (L), moderate (M), and high (H)) and zebra mussel concentrations (1, 2, and 4 g dry mass m^{-2}) to produce a total of 9 treatment combinations, each one held in a 31 m^3 enclosure in an oligotrophic lake. We measured zebra mussel condition, carbon:nitrogen:phosphorus (C:N:P) tissue stoichiometry, feeding rate, and nutrient excretion and egestion as related to varying conditions of chlorophyll *a* (Chl), particulate phosphorus (PP), particulate organic nitrogen (PON) and seston C:N:P ratios at three time periods: 5-7, 18-20, and 32-34 d subsequent of adding mussels to the enclosures.
3. Consistent with approximate homeostatic control of N and P, there were only modest differences in C:N:P ratios in mussel soft tissue despite greatly different seston C:N:P ratios among enrichment treatments. Mussel condition (mass per unit length) decreased with increased seston N:P, C:P and C:N ratios and percent composition of Cyanobacteria, and increased with percentage composition of cryptophytes and other flagellates.
4. Assimilation rates of Chl and calculated potential assimilation rates of N and P linearly increased ($P < 0.05$) with increasing seston Chl, PON, and PP concentrations.
5. P excretion measured as soluble reactive phosphorus (SRP) significantly decreased in exponential fashion by two orders of magnitude as C:P ($R^2 = 0.71$) and N:P ratios ($R^2 = 0.66$) increased by a factor of 4. P excretion was significantly correlated with seston PP concentration, which varied over a 19-fold range; however, there was much scatter in the relationship ($R^2 = 0.29$). In contrast, NH_4-N excretion significantly decreased ($R^2 = 0.31$) with N:P ratio by a factor of 2 over this same N:P range, and was not significantly correlated with PON concentration. Soluble P excretion was significantly correlated with potential P assimilation, whereas NH_4 excretion was not significantly correlated with potential N assimilation. The ratio of N:P excreted showed a significant exponential increase with seston N:P ratio.

6. P and N egestion rates were higher than corresponding P and N excretion rates from the same trials; however, the fate of this egested material—whether recycled by resuspension or remaining in the benthos—is not known.

7. Mussel excretion and its impacts are highly context dependent, varying with algal composition, seston stoichiometry, and mussel abundance and feeding rate. The low P excretion but high N excretion observed when mussel feeding stops implies that under poor feeding conditions typical of summer seston, mussels excrete little P but continue excreting N, which would slow production rate of producers such as *Cladophora* and *Microcystis* in low-P systems. In contrast, NH_4 excretion by mussels may prolong *Microcystis* blooms as nitrate is used up by the bloom in moderate-P systems.

Introduction

The Ponto-Caspian zebra mussel (*Dreissena polymorpha*, Dreissenidae) and quagga mussel (*Dreissena rostriformis bugensis*) have spread throughout much of Europe and North America, and when reaching high abundances, have radically reengineered the physical habitat (substrate and water clarity), altered trophic and spatial interactions of the food web, and reengineered nutrient recycling (Vanderploeg *et al.*, 2002, Hecky *et al.*, 2004; Higgens & Vander Zanden, 2010; Ozersky, Evans, & Ginn, 2015; Waajen et al., 2016). Dreissenid mussels are thought to have promoted harmful blooms of the toxic cyanobacterium *Microcystis* (Microcystaceae) in the Saginaw Bay and Lake Erie as well as in low-nutrient inland lakes they have invaded (Vanderploeg *et al*., 2001; Raikow *et al*., 2004; Knoll *et al*., 2008), yet in other systems, such as hypereutrophic ponds in the Netherlands, grazing and mesocosm experiments have shown they readily grazed on and reduced *Microcystis* along with other components of the algal community (e.g., Waajen et al., 2016).

Experimental (Vanderploeg *et al*., 2001, 2013) and modelling (Bierman *et al*., 2005) evidence argued that selective rejection of *Microcystis* in pseudofaeces post

capture—which is sensitive to strain and toxicity of *Microcystis*—was a necessary mechanism in the promotion of *Microcystis* dominance after the dreissenid mussels invaded. In contrast, Zhang *et al*. (2011)—using a mathematical model and weight-specific excretion rates of Conroy et al. (2005)— argued that nutrient fertilization was much more important than selective feeding by mussels for promoting *Microcystis* blooms in Lake Erie. They posited that mussels are in a boundary layer on the bottom and are not connected with phytoplankton in the water column, so feeding and selective grazing will be limited, but that excretion will continue in these food-deprived mussels thereby promoting algal growth. Their model used N and P excretion rates that were independent of feeding-rate and were derived from experiments with mussels under highly starved condition. Mussels were kept in damp cold (4ºC) storage (not in water) for 3-4 days before being placed in containers of filtered lake water where SRP and NH_4 accumulation was measured for 6 h at 22-24ºC (Conroy *et al*., 2005).

Increased abundance of macrophytes and nuisance blooms of the attached macroalga *Cladophora* (Cladophoraceae)—proliferating in many nearshore regions and fouling beaches of the Great Lakes—are thought to be dually promoted by mussel filtration via increasing light availability and mussel nutrient excretion (e.g., Vanderploeg *et al*., 2002; Hecky et al., 2004; Higgins & Vander Zanden, 2010: Bootsma *et al*., 2015). Dreissenid mussels are thought to have decimated the spring phytoplankton bloom in middle depth regions of Lake Michigan through their filtering activities (Vanderploeg *et al*., 2010; Rowe *et al*., 2015), but the dual roles of filtering and nutrient recycling on phytoplankton and nutrient dynamics in the lake as a whole are unclear.

Despite the important role that dreissenid mussels play in selective filtering and reengineering nutrient cycling—with potentially important effects to the food web—in the Great Lakes and other systems, there is not much useful quantitative information to understand and predict N and P excretion of mussels living and feeding under a broad variety of trophic conditions (e.g. Arnott and Vanni, 1996; Johengen *et al*., 2013; Bootsma & Liao, 2013; Mosley & Bootsma, 2015). This lack of understanding is a serious information gap, since the only study explicitly relating nutrient excretion to mussel feeding rate showed that feeding rate can be an important driver of nutrient excretion (Johengen *et al.*, 2013).

Vanderploeg e*t al*. (2002) proposed that nutrient excretion in mussels was likely to follow the rules for homeostatic maintenance of constant nutrient content in the consumer body, as has been observed in zooplankton (Sterner 1992; Sterner & Elser, 2002). This paradigm proposes that nutrients consumed in excess of needs would be excreted, implying that P excretion would increase relative to N excretion when they ingested particles having high P content and low N:P ratios, and conversely would decrease when ingesting particles with high N:P ratios, such as in regions receiving low P loading. This hypothesis was consistent with observations of Johengen *et al*. (2013) from P-poor Saginaw Bay and P-rich Lake Erie, however, in that study the clustering of results and many non-detectable values of P excretion prevented detailed analysis of excretion rates. Also, patterns in C:N:P ratios in mussel tissues were not measured so that homeostasis implied by constancy of tissue C:N:P ratios under different seston C:N:P ratios could not be examined. Recent studies have presented evidence that C:N:P ratios in mussel tissues vary among lakes and that relaxation of stoichiometric constraints of constant tissue C:N:P ratios give the invading zebra mussels an advantage over other species (Naddafi *et al*., 2009, 2012; González *et al*., 2010).

The study of Horst et al. (2014) examining the roles of zebra mussel abundance and nutrient concentrations in experimentally manipulated enclosures gave us an opportunity to simultaneously measure zebra mussel tissue C:N:P ratios, feeding, and N and P excretion across a broad gradient of P loading and mussel abundance. We report here the results of a study to examine the roles of seston stoichiometry and algal food quality in driving the feeding and nutrient excretion responses.

Although our primary goal was to examine soluble nutrient excretion, our experimental design also allowed us to estimate N and P egestion so that we could contrast soluble nutrient excretion rates with those for N and P egestion. Part of the captured material—be it sediment, detritus, or undesirable phytoplankton—may be egested (i.e., discharged) as pseudofaeces without ingestion, i.e., not entering the mouth and digestive tract. Of the material ingested, a portion may be egested as faeces containing different amounts of C, N, and P (Gergs *et al*. 2009; Mosley & Bootsma, 2015).

Methods

151

152 *Study site*

153 Experiments were conducted with mussels and water taken from large enclosures
154 (~31,000 L: 2-m diameter × 10 m deep) in oligotrophic Gull Lake at the same time as the
155 experiment described by Horst *et al*. (2014) in which both zebra mussel dry mass (DM)
156 concentrations and nutrient concentrations were manipulated. Mussels were kept in mesh
157 baskets at mid-depth of the epilimnion in the enclosures (Horst et al., 2014). We
158 examined feeding and nutrient excretion in 9 enclosures having 3 nutrient enrichment
159 levels and 3 levels of mussels stocked at 1, 2, and 4 g DM m^{-2}. Although nutrient
160 addition alone would be expected to be a major driver of stoichiometry, it was necessary
161 to also use different mussel treatment levels because different levels of selective feeding
162 could affect algal composition with subsequent feedback into the feeding (Vanderploeg *et*
163 *al*., 2009) and nutrient excretion responses.

164 Total phosphorus (TP) levels in the enclosures of this lake, which had a nitrate
165 concentration of ~ 300 µg L^{-1}, were experimentally manipulated as follows: low (L),
166 receiving no added P to simulate oligotrophic conditions (target TP ~ 8-10 µg L^{-1});
167 medium (M) receiving enough P to simulate mesotrophic conditions (target TP ~ 15 µg
168 L^{-1}); and high (H) receiving enough P to simulate mildly eutrophic conditions (target TP
169 ~ 25 µg L^{-1}) (Horst *et al*. 2014). These concentrations were set up by dripping a solution
170 of NaH_2PO_4 over a 7 d period (June 29-July 5, 2007) to reach initial target levels and
171 then maintaining levels by subsequent weekly monitoring of TP and addition of
172 NaH_2PO_4 as needed (Horst *et al*., 2014). Mussels were added to the enclosures on July 5
173 (time 0 [t_0]). Throughout the text we refer to the combination of treatments with the letter
174 designations of L, M, and H for nutrient levels followed by the number designations of 1,
175 2, and 4 indicating nominal mussel biomass concentrations in g DM m^{-2} added to the
176 enclosures: L1, L2, L4, M1, M2, M4, H1, H2, and H4. Experiments were conducted with
177 mussels and water taken during three time periods subsequent of first adding mussels to
178 the enclosures: round #1 (July 10-12; $t_5 - t_7$, i.e., days 5-7), round #2 (July 24-26; $t_{18} -$
179 t_{20}), and round #3 (August 7-9; $t_{32} - t_{34}$). Due to the failure of a freezer in which the
180 nutrient excretion samples were stored, we lost excretion data from the first round.

181 *Handling of mussels*

Mussels to be used in the experiments were briefly removed from enclosures on the day before the experiments to clean and make them readily available for rapid transfer from enclosures to feeding and nutrient excretion trials on the next day. Specifically, we removed a basket of mussels from each of three enclosures in the afternoon (14:00 – 16:00 EDT) and placed each basket of mussels in a 20-L bucket filled with lake water for brief transport (5 min) to a lakeside laboratory. There, 20 mussels from each basket were detached from the bottom of the basket by cutting byssal threads with a sharp razor blade. Loose periphyton was removed from the mussel shells with a toothbrush and razor blade, and any film of living periphyton remaining on the shells was killed by rubbing with a cotton swab dipped in a commercial bleach solution diluted to 5% in distilled water. Cleaned mussels were then immediately rinsed with lake water to remove any residual bleach solution. Mussels were completely closed up during cleaning, and the return of normal mussel siphoning shortly after bleach treatment showed that the cleaning treatment had no long-term effect on mussel behavior. The sorting and cleaning took about 0.5-1.0 h per basket, and 20 cleaned mussels were placed in clean baskets and returned to respective enclosures immediately after cleaning, for re-acclimation to the conditions in the enclosures. Except during the very brief brushing and cleaning (few minutes), all mussels were kept in lake water to minimize stress.

We conducted three simultaneous trials of the feeding and nutrient excretion experiments each day between 08:00 – 15:00 EDT—each a different treatment— using water from the enclosures and mussels cleaned the previous day. This process was continued for three consecutive days so that a total of nine experimental trials, covering all 9 treatments, were conducted during each round. Water was collected from the upper 7 m of the enclosure (the epilimnion) with a tube and put into 25-L carboys for transport to the lakeside lab. Mussel baskets were retrieved from the enclosures and transported to the laboratory in respective 20-L buckets of enclosure water, where they continued to feed, until the experiment was set up, shortly after returning to the lab.

Feeding experiment set-up

Feeding rate of mussels was determined from changes in chlorophyll a (Chl). Chl was measured in different size fractions (< 53 µm and > 53 µm) but since we were primarily interested in feeding rate on the total phytoplankton assemblage and not individual size

fractions, feeding rate was examined from measurements of total Chl (sum of size fractions) following methods, formulas, and calculations developed by Vanderploeg *et al*. (2001, 2009) and adapted for total Chl (i.e., a single size fraction) by Tang et al. (2014; equations 1-5). Water in the carboy from a given treatment enclosure was uniformly distributed among beakers to be used in the feeding experiment. Feeding experiments were conducted in 2-L beakers filled with 1.8 L of water held in dim light (< 10 μmol quanta m^{-2} s^{-1}) at ambient lake temperature in large water baths. Each feeding trial for a treatment included three replicate experimental beakers (each containing six mussels, 14-17 mm long) and two control beakers without mussels. All beakers were gently aerated to assure mixing. Water remaining in the carboys after set-up was sampled for nutrients (TP, particulate P [PP], particulate organic C [POC] and particulate organic N [PON]), phytoplankton composition to characterize initial conditions of the feeding suspension (further described below).

We took three water samples (200-mL) during the course of each 1.5-h feeding trial from both experimental and control beakers. An initial sample was taken from each beaker just before adding mussels. A final "water-column sample" was taken by sampling water above the bottom in both control and experimental beakers using a large bore pipette as described by Vanderploeg et al. (2001, 2009). After removing the mussels, each control and experimental beaker was mixed and this "mixed-beaker sample" was used to capture remaining water-column and settled Chl. In the case of the experimental beakers, the settled Chl also included material egested by the mussels.

This sampling scheme allowed us to do a mass balance of mussel-induced changes to estimate the amount of Chl removed from the water column for calculation of gross clearance rate (*F*) and associated Chl capture rate [*CR*(Chl)] as well as net clearance rate (F_A) and assimilation rate[*A*(Chl)] (= Chl destroyed; Vanderploeg et al. (2001, 2009). Unless there was significant viable gut passage of algae with Chl unaffected like other cellular constituents, *A*(Chl) for practical purposes is equivalent to ingestion rate (e.g., Bundy *et al*., 2005). Since we are primarily interested in clearance rate associated with ingestion we do not report out *F*. All rates were normalized to dry mass (DM).

We also calculated capture rate of N [*CR*(N)] and P [*CR*(P)] and potential assimilation rate of N [*A*(N)] and P [*A*(P)] from the product of respective values of Chl capture and assimilation and PON/Chl and PP/Chl ratios (Johengen *et al*., 2013). Capture rate properly accounts for all material captured, that is, all material removed from the water column. However, we use the expression "potential assimilation" because we recognize the approach could represent an upper bound for assimilation, because some C, N, and P in seston will be associated with detritus and could have lower assimilation efficiency relative to Chl or not be ingested but rejected as pseudofaeces (e.g. Johengen *et al*., 2013). Although different algal, microbial, and zooplankton communities along with detritus would be expected to form over time in the different enclosures, the epilimnetic seston would be expected to consist of high proportion of living material because of sedimentation of detrital material formed in place, lack of input of allochthonous material, and lack of resuspended sediment in the unstirred enclosures (e.g., Sterner & Elser, 2002). Egestion rates of Chl, N, and P were estimated as the difference between respective capture and assimilation rates.

Nutrient excretion

To determine excretion rate of soluble reactive P (SRP) and NH_4, we examined excretion rate of mussels placed in 0.2-µm filtered (0.2-µm Pall capsule filter, Pall Corp. Port Washington, NY, USA) enclosure water (from the same carboy used to set up the feeding experiment) immediately following each feeding trial after the methods of Johengen *et al.* (2013). The 6 mussels from each of the 3 feeding trials replicates were placed in respective capped bottles filled with 120 mL of filtered lake water for 2 h. Two additional bottles without mussels served as controls. Excretion rates were measured from the differences between initial and final SRP and NH_4 in the bottles (methods described below). Since there were no measureable differences in nutrient concentrations between initial and final samples in the controls, we assumed changes in the bottles with mussels were caused by mussel excretion.

After taking water for nutrient analyses and removing the mussels from the excretion bottles the end of the experiment, the remaining water was preserved in 1% Lugol solution for later qualitative examination under a binocular scope (10 to 100×) to look at biodeposits and their contents.

Analytical methods—nutrients and microcystin

For POC and PON, we filtered triplicate 300-600 mL samples of water through Whatman (G.E. Healthcare Sciences Pittsburg, PA, USA) GF/F type filters of 25 mm diameter that had been combusted for 4 hours at 400 °C and stored frozen. Just prior to analysis, filters were acidified with 1N hydrochloric acid, sufficient to drive off inorganic CO_2 when dried in an oven at 60°- 65°C. Analyses were done on an Elantech EA1100 CHN Analyzer (CE Elantech, Inc., Lakewood, NJ, USA).

Particulate P (PP) was sampled in triplicate by filtering 50 to 200 mL of water through a 47-mm-diameter, 0.2-µm pore-size polycarbonate membrane filters (Nuclepore, GE Healthcare, Pittsburgh, PA, USA). Particulate phosphorus filters were stored frozen. For analysis, PP filters were suspended in 50 mL of deionized distilled water and digested as for TP described below. Total P samples were taken by sampling 50 mL of whole water, which was stored cold (~ 3°C) in the refrigerator. Both sample types were digested for 35 minutes in an autoclave with 10 ml of 5% potassium persulfate solution (Johengen *et al*., 2013). Following digestion, P concentrations were measured as orthophosphate on a Technicon Auto Analyzer II (Seal Analytical, Mequon, WI 53092), using the ascorbic acid method (Johengen *et al*., 2013). Dissolved P (DP) was estimated from the difference between TP and PP.

Particulate microcystin concentration was determined in triplicate using ELISA kits (Envirologix Inc. Portland ME, USA) after extracting in 75% MeOH and water with sonification as described by Vanderploeg et al. (2013).

For nutrient excretion experiments, water was sampled at the beginning and the end of 2-h incubation and filtered through a 0.2-µm nylon G.E. Cameo syringe filter (G.E. Healthcare Sciences Pittsburg, PA, USA) into 14-mL test tubes that were frozen until analysis. SRP and NH_4/NH_3 were measured using automated colorimetric methods on a Technicon Auto Analyzer II (Johengen *et al*., 2013).

At the conclusion of each set of experiments, mussels were frozen in glass vials for later estimates of condition and C, N, and P concentrations. Shell length of all mussels was measured to the nearest 0.1 mm, and tissue from all mussels within a single replicate was composited, dried at 60-65 °C overnight, weighed, and ground with mortar and pestle. Mussel condition was estimated as mussel soft tissue ash-free dry mass

(AFDM) per shell length (cm) (Nalepa *et al.*, 1993; Vanderploeg *et al*., 2009). AFDM was not directly measured in the Gull Lake experiments; therefore, we used a conversion factor of 0.88 (Nalepa *et al*., 1993) to covert DM to AFDM. A weighed subsample was taken for POC and PON analysis as described above, and a second was taken for P analysis. The subsamples for P analysis were transferred into glass tubes and combusted at 500 °C for 2 hours. The combusted mussel-tissue was oxidized to orthophosphate with 25 ml of 1N HCl in a hot water bath (95-99 ° C) for 60 minutes, adapted from Andersen (1976), and after dilution to 0.019 N analyzed by the ascorbic acid method as described above.

Phytoplankton enumeration

Phytoplankton enumeration followed methods used previously by Vanderploeg et al. (2001). Lake-water samples for phytoplankton enumeration were preserved in 1% Lugol's solution, and typically 20 mL subsamples were filtered onto membrane filters for permanent mounting on slides and counted at 100 × for larger cells and colonies and 1000 × for small phytoplankton (Fahnenstiel *et al*., 1998). Cell dimensions of the different taxa were converted to cell carbon for reporting biomass using the method of Fahnenstiel *et al*. (1998).

Statistical methods

Simple correlation analysis, regression analysis, and multiple regression analysis were used to examine relationships among variables ($\alpha = 0.05$). In examining the variables in the enclosures affecting mussel condition and stoichiometry, we restricted our analysis to the last two rounds of experiments to allow mussel tissues time to adjust to the different nutrient and seston regimes set up in the enclosures. Results were pooled from these rounds.

Results

Conditions in enclosures—water and mussels

Total phosphorus (TP) concentrations in the enclosures roughly approximated target TP concentrations of 8, 15, and 25μg L^{-1} for respective L, M, and H treatment levels (Table 1). As expected, P addition stimulated primary production within the enclosures as witnessed by higher concentrations of PP, PON, POC, and Chl across treatments.

However, the magnitude of response varied by nutrient parameter, with PP varying across enclosures by a 19-fold range (2.5-45.6 μg L^{-1}), PON over a 7-fold range (0.041-0.273 mg L^{-1}), and POC over a 5-fold range (0.40-2.04 mg L^{-1}) (Table 1). Chl varied over an 11-fold range (1.1-12.0 μg L^{-1}) (Fig. 1; Table 1). Significant correlations ($P< 0.05$) between TP and each of these variables were seen: PP ($r =0.949$), PON ($r=0.822$), POC ($r= 0.805$), and Chl ($r= 0.840$). Although PP and TP concentrations were highly correlated across treatments, at times an appreciable fraction of TP was in the dissolved phase (Table 1). Temperatures stayed within the narrow range of 24 to 27°C (Table 1).

A broad range of Chl and *Microcystis* concentrations as well as microcystin concentration (MC) and microcystin to Chl ratio (MC/Chl) provided a relatively wide spectrum of feeding conditions for the zebra mussels in terms of *Microcystis* abundance and toxicity (Fig. 1). Trends in total phytoplankton concentration (Fig. 2a) generally mirrored those for Chl (Fig. 1a) with increased levels in H and M treatments, especially during the second round, and all treatments converging to relatively low or intermediate values in the third round (Fig. 2a). Phytoplankton concentration was significantly correlated with TP ($r=0.440$) and Chl ($r=0.682$). There was considerable variation in *Microcystis* concentrations among mussel and nutrient treatments (Fig. 1b). Percent composition of Cyanobacteria often increased with time in L and M treatments (Fig 2b), and *Microcystis* often made up the majority of cyanobacterial biomass since Cyanobacterial and *Microcystis* percent compositions were often similar. The M2 treatment in round #1 had the highest *Microcystis* concentration (Fig. 1b). High relative (>33%) concentrations were found at times in the L4, M1, M2, and H4 treatments; the M2 treatment had high relative concentrations at all times (Fig. 2c).

Particulate microcystin (MC) concentration and Chl normalized concentration (MC/Chl)—measures of total and relative toxicity of the phytoplankton community—varied similarly to *Microcystis* concentration and composition (Figs. 1 and 2). MC concentration was significantly correlated with *Microcystis* concentration ($r = 0.680$), and *Microcystis* percent composition was significantly correlated with MC/Chl ($r = 0.544$). Relatively high MC concentrations (> 0.4 μg/L) were seen in many of the treatments irrespective of nutrient level or mussel density; e.g., L1 of round #2, M2 round #2, M4

rounds #2 and 3, and H1 round #2, and H4 round #2 (Fig. 1c). However, elevated (> 0.20) MC/Chl ratios were only seen in all L treatments and the M4 treatment (Fig 1d).

Seston molar ratios of C:P and N:P reflected effects of P addition, and C:P ratios for H enclosures generally approximated the Redfield ratio (C:P = 106:1; N:P = 16:1; C:N = 6.6:1 in molar units) suggesting P sufficiency had been reached, while larger P-deplete ratios were observed for M and L enclosures (Table 1). The C:P values for the L enclosures (350-400) as well as N:P ratios (34-41) were suggestive of severe P deficiency as defined by criteria of Healy & Hendzel (1979) used by Hecky, Campbell, & Hendzel (1993) to characterize condition in surveyed lakes, but moderate-high deficiency based on more recent observations of C:P ratios > 600 in many lakes (Sterner & Elser, 2002; Hessen, 2006). C:P ratios for the M enclosures are suggestive of moderate P limitation by the Hecky et al. (1993) criteria. C:N ratios (7.3 – 12.5) for seston suggested no or moderate nitrogen deficiency for all treatments. Soluble nutrient measurements indicated that the outlying high C:P and N:P ratios seen for the H2 treatment in round #1 were a result of not enough P added to this treatment at first.

Mussels in enclosures—all of relatively uniform length ($\bar{X} \pm SD$ = 15.8 ± 0.6)—exhibited varying condition (AFDM length^{-1}) that ranged between 2.9 and 6.0 mg AFDM cm^{-1} (Fig. 3) with $\bar{X} \pm SD$ = 4.5 ± 0.8. Mussel condition was generally highest in H treatments, lower in M treatments, and lowest in L treatments throughout the experiment (Fig. 3). Although there were large differences in mussel condition, mussels had fairly constant C, N, and P content per unit dry mass. C content ranged between 0.488 and 0.522 with $\bar{X} \pm SD$ = 0.503 ± 0.009 mg C mg DM^{-1}; N content ranged between 109 and 136, with $\bar{X} \pm SD$ = 122.9 ± 6.4 μg N mg DM^{-1}; and P content ranged between 8.96 and 11.6, with $\bar{X} \pm SD$ = 10.12 ± 0.72 μg P mg DM^{-1}.

Mussel C:P, N:P, and C:N ratios, fell within a relatively narrow range and were generally reflective of P additions to enclosures. Higher C:P ratios and N:P ratios tended to occur in the L treatments compared to intermediate values found in the M treatments and lower values in the H treatments. C:N ratios followed the opposite trend (Fig. 3). Reflecting these trends, tissue N:P ratio was negatively (r =-0.685) correlated and tissue C:N ratio was positively correlated (r = 0.548) with mussel condition.

Mussel condition and mussel C:N:P ratios showed varying responses to seston C:N:P ratio, PP and algal composition (Figs. 4-5). Mussel condition was significantly positively correlated with PP, concentration of cryptomonads and flagellates (CryptoFlag), and was significantly negatively correlated with percent Cyanobacteria and seston C:P, C:N, and N:P ratios, with the last having the highest correlation of all variables (Fig. 4). Despite the negative correlation of condition with Cyanobacteria there was no significant correlation with *Microcystis*.

Mussel C:N:P ratios showed varying responses to seston C:N:P ratios (Fig. 5). Mussel C:N was significantly negatively correlated with seston C:N (Fig. 4). Mussel C:P ratio was not significantly correlated with seston C:P, showing very little variance about a nearly constant C:P ratio. Mussel N:P was significantly correlated with seston N:P, showing a modest slope (Fig. 5).

Feeding rates in enclosures

F_A plotted against initial Chl concentration showed high scatter among treatments (Fig. 6a) and was not significantly correlated with any environmental variable except MC and the correlation coefficient was low (r = -0.39). In contrast, *A*(Chl), was positively correlated to initial Chl concentration (R^2 = 0.65; Fig. 6b), and positively correlated with additional measures of algal abundance including Chl, total phytoplankton concentration and diatom concentration (Table 2), as well as to particulate nutrients (POC, PON, PP) and TP concentrations. Significant negative correlations to *A*(Chl) were seen with MC/Chl, C:P, and N:P ratios. Overall Chl concentration was the most important driver of *A*(Chl) based on the strength of the correlations.

The patterns of correlations for *A*(N) and *A*(P) with seston variables were similar to those for *A*(Chl) (Table 2). The relations of *A*(P) and *A*(N) with their respective particulate nutrient concentrations mirrored the relationship for *A*(Chl) and Chl concentration (Fig. 6b-f). *A*(N) had slightly higher correlations with Chl than with PON concentration, whereas *A*(P) had a higher correlation coefficient with PP than with Chl. Although the highest nutrient ingestion rates (Fig.6b-f) associated with highest nutrient concentrations were important to the strength of regressions, all were significant with removal of these points. The very low value of *A*(P) at of 31 µg L^{-1} in the *A*(P) vs. PP

relationship (Fig. 6c) matches up with the lowest F_A value seen Fig. 6a. Removal of this outlier increases R^2 to 0.81.

Relation of nutrient excretion to nutrient assimilation and stoichiometry

Seston stoichiometry and P ingestion appeared to be important drivers of SRP excretion. SRP excretion decreased over two orders of magnitude as seston C:P ratio or N:P ratio decreased by a factor of 4 (Fig. 7a,b). High correlations were seen for exponential plots for both variables. SRP excretion was significantly correlated with PP, which varied over a broad range (Fig. 7c), but there was considerable scatter about the relationship, and R^2 (0.29) was low compared to R^2 of N:P (0.66) and C:P ratios (0.71). There was no significant correlation with PON or POC over this broad range of values (Fig. 7d,e). Multiple linear regression was used to examine for possible improvement in the ln P excretion relationship by adding PP or ln PP as an additional variable. No improvement was found. SRP excretion was significantly positively correlated with *A*(P) with R^2 = 0.50 (Fig. 7f) and CryptoFlag (r=0.493) and negatively correlated with % Cyanobacteria (r=-0.577) and MC/Chl (r=-0.509).

In contrast to SRP excretion, NH_4 excretion decreased with increasing seston N:P ratio by only a factor of ~ 2 over this same range of seston N:P ratios, and although the correlation was significant, R^2 was low (0.31; Fig. 8). NH_4 excretion was not significantly correlated with PON or with *A*(N). Due mostly to changes in P excretion, N:P excretion ratio significantly (R^2 = 0.62) increased by two orders of magnitude (11.9 – 2521) with seston N:P ratio, which varied over a 4-fold range (11 – 41).

Egestion compared with excretion

Egestion rates of nutrients were greater than excretion rates of nutrients. P excretion and P egestion $\overline{X} \pm SDs$ were 0.0086 ± 0.0092 and 0.045 ± 0.039 µg P mg DM^{-1} h^{-1}, respectively, and the mean ratio of P excretion to ingestion was 0.393 ±0.988 for the 18 experimental trials conducted during rounds 2 and 3. N excretion and egestion $\overline{X} \pm SDs$ were respectively 0.103 ± 0.024 and 0.373 ± 0.216 µg N mg DM^{-1} h^{-1}, with the ratio of N excretion to N egestion equal to 0.54 ±0.84. Microscopic analysis of settled material in the incubation bottles indicated that the biodeposits—most likely pseudofaeces—consisted of intact phytoplankton such as colonial *Microcystis*, other large phytoplankton, or small zooplankton such as *Bosmina*. Little detrital material was seen.

458

459 **Discussion**

460 To our knowledge, this is the first study in which mussel soft tissue nutrient
461 stoichiometry, feeding rates, and nutrient excretion were measured simultaneously across
462 a broad spectrum of seston stoichiometry corresponding to a TP range characteristic of
463 oligotrophic to moderately eutrophic lakes. This spectrum was accomplished by creating
464 an artificial gradient of C:N:P ratios and mussel abundance in large enclosures with
465 subsequent measurements of feeding and nutrient excretion following quickly upon
466 removal of mussels from their enclosures. These results extend the observations of
467 Johengen *et al*. (2013), who looked at excretion and feeding at two extremes of seston
468 N:P ratios represented by Lake Erie (typically 10-18) and inner Saginaw Bay (typically
469 32-40), which correspond with our H and L treatments, respectively.

470

471 *Feeding rates*

472 Feeding rates varied greatly among enclosures and were likely an important factor in
473 driving ingestion or assimilation of C, N, and P by mussels. Consistent with expectation,
474 feeding rate, expressed as *A*(Chl), increased significantly with Chl concentration. The 10-
475 fold range of F_A over a Chl concentration range of only 1-3 µg L^{-1} (Fig. 6a) implied that
476 food quality rather than food quantity was driving F_A. The low F_A values were
477 consistent with dreissenid feeding on a summer phytoplankton assemblage containing
478 undesirable species such as coccoid cyanobacteria fed upon at low rates (e.g.,
479 Vanderploeg *et al*., 2009, 2013; Tang *et al*., 2014).

480

481 *Condition and nutrient stoichiometry of mussel tissues*

482 Although there were large differences in seston C:N:P ratios associated with different
483 levels of P addition, there were only modest differences in C, N, and P content and ratios
484 in mussel tissues despite large changes in mussel condition. Consistent with expectation,
485 mussel condition was negatively related to seston C:N, C:P, and N:P and %
486 Cyanobacteria; whereas it was positively correlated with PP, TP, CryptoFlag and %
487 CryptoFlag. The condition response to CrypoFlag and Cyanobacteria variables may
488 reflect a time-integrated response to feeding on algae of different food quality. The low

values of condition in L and M enclosures match the mid to lower end of the range of observed for Saginaw Bay mussels in the inner bay, which varied between a high of 7 mg AFDM cm^{-1} in spring under relatively good feeding conditions to a low of 2.5 mg AFDM cm^{-1} in summer under extremely poor feeding conditions with little ingestion (Vanderploeg *et al*., 2009).

A major source of variation in tissue P content and C:N:P ratios in invertebrates is the association of P with ribosomal RNA, which increases under high growth rate and adequate P in the diet (Sterner & Elser 2002; Elser *et al*., 2003). Invertebrates with higher growth (including reproductive output) would be expected to have higher P concentrations and lower N:P and C:P ratios in their tissues (Sterner & Elser, 2002; Elser *et al*. 2003).

Variation in C:N:P ratios in mussel soft tissue associated with changes in seston C:N:P ratios among lakes and seasons within lakes has been taken as evidence that zebra mussels have the capacity to modify their tissue P concentration in relation to lake trophic state (Naddafi *et al*., 2009, 2012; González *et al*., 2010). Naddafi et al. (2009) posited that this capacity may be important to their invasion success and competition with native mussels. Our study included TP levels in the oligotrophic range in addition to the mesotrophic and eutrophic conditions in the lakes studied by Naddafi *et al*. (2009, 2012). At the low TP levels associated with our L1, L2, and L4 treatments, seston C:P ratios (~400) and N:P ratios (~40) were extremely high compared to the studies of Naddafi *et al.* (2009, 2012) with C:P ratios ~200 and N:P ratios ~30. The insignificant correlation of mussels soft tissue C:P ratio and modest response of tissue N:P with large changes in respective seston C:P and N:P ratios seen in our study would suggest mussels do not have great capacity to escape stoichiometric constraints in low TP environments by functioning with higher tissue C:P or N:P ratios.

The negative correlation of mussel C:N ratio with seston C:N ratio would at first seem surprising. High C:N ratios in seston are usually thought of as an indicator of N deficiency. Only moderate deficiency as defined by the Healy and Hendzel criterion (N:P = 8.3-14.6) was observed in some of the treatments, and seston C:N ratio was positively significantly correlated with seston C:P ($r = 0.79$) and N:P ($r = 0.63$) ratios, measures indicative of potential P limitation rather than N limitation. High tissue C:N ratios are

associated with C-rich, N-poor lipids and glycogen (Sterner & Elser, 2002) found in mussels with a high condition index (Nalepa et al., 1993). These prior findings match up with the positive correlation between condition and tissue C:N ratio observed in our experiments. Thus, the positive correlation between condition and tissue C:N ratio and positive correlation of C:N seston ratio with seston C:P and N:P ratios likely explains the negative rather than expected positive slope of the tissue C:N ratio vs. seston C:N ratio. Likewise the lack of significant correlation between mussel C:P and seston C:P may reflect a simultaneous increase in both P and C content with high growth and high condition.

Seston stoichiometry and feeding as drivers of nutrient excretion

Our finding that P excretion was strongly associated with seston C:N:P ratios and assimilation variables, whereas N excretion was not, may be related to different functions of P and N in animal physiology. Since most P is associated with RNA in consumers, it can be uncoupled from C and N excretion in the sense that it is not used as an energy source for respiration (e.g., Anderson *et al*., 2005).

The high correlation of SRP excretion with C:P and N:P ratios contrasted to the lower correlation with *A*(P), and the lowest correlation with PP concentration point to interesting interactions among stoichiometry, algal composition, and feeding in our experiments. First, whether any "waste" P is available for excretion from P assimilated depends on seston P content relative to C or N content, whether it is used for maintenance or for both maintenance and growth. Second, low N:P or C:P seston ratios at the same time often captured food quality both in terms of high P content and desirable phytoplankton composition leading to high *A*(P) values. The similar correlations for *A*(P) and SRP excretion with algal composition imply both are affected by feeding rate mediated by algal composition. Lastly, not all high concentrations of PP led to high *A*(P) values, which could explain in part the weak correlation of SRP excretion with PP.

The shallow slope and high intercept of NH_4 excretion vs. *A*(N) regression implies that at low feeding rates we are observing a relatively high basal catabolism of N, whereas at higher feeding rates modest additional NH_4 excretion was likely associated with specific dynamic action of ingested food. Our NH_4 excretion rates at low feeding

551 rates are similar to the basal rates observed by Aldridge *et al.* (1995) at the same
552 temperatures. They noted that at temperatures between 25 and 28ºC there was a large
553 drop in the oxygen consumption:NH_4 excretion ratio as mussels shifted from lipid and
554 carbohydrate catabolism to protein catabolism.
555 Consistent with the stoichiometry paradigm, N:P ratio excreted by mussels was
556 strongly correlated with seston N:P ratio (Sterner 1990, Sterner *et al*., 2002, Vanderploeg
557 *et al*., 2002; Anderson *et al*. 2005; Johengen *et al.*, 2013). Interestingly, the logarithmic
558 relationship between N:P excretion ratio and seston N:P ratio showed N:P excretion
559 equaled seston N:P at N:P seston ratio of 11, a value considerably lower than the N:P
560 ratio of mussel soft tissue (22-30) and even the Redfield ratio (16) indicating P
561 sufficiency of phytoplankton. This result contrasts with Bootsma & Liao (2013) who
562 predicted that the transition zone would occur between N:P ratios of 22 and 30. For the
563 summer conditions under which we conducted our experiment, this meant that over a
564 broad range of seston N:P ratios, N excretion relative to P excretion was much higher.
565 This result could be related to the high endogenous N excretion at high temperatures,
566 regardless of feeding rates, as well as the extreme sensitivity of P excretion to feeding
567 and P ingestion rates, which can be very low during summer owing to dominance of
568 grazing resistant phytoplankton. Since P and N excretion are affected by temperature
569 (Bootsma and Liao, 2013; Johengen *et al*., 2013), the relation we observed could vary
570 with temperature.
571 Our results spanned a broad range of N:P ratios in seston—10 to 40. Johengen *et*
572 *al*., (2013) saw many similar patterns in feeding and nutrient excretion from the pooled
573 results from Lake Erie and Saginaw Bay that demonstrated both the importance of
574 stoichiometry and feeding rate. Across all seasons (April – October), *A*(Chl) was
575 negatively correlated with C:P and N:P ratios, and P excretion was positively correlated
576 with *A*(Chl) and *A*(P). Both *A*(P) and P excretion were positively correlated with
577 %CryptoFlag. When feeding stopped during a *Microcystis* bloom, P excretion was zero
578 and NH_4 excretion rate was high.
579
580 *Ecosystem implications*

The differing behavior of N and P excretion in response to feeding and C:N:P ratios has potentially important consequences to nutrient availability and phytoplankton succession. With a 4-fold increase in seston N:P ratio, P excretion decreased and N:P excretion increased by a factor of ~100. In the case of our experiments, when seston N:P ratio was above a relatively low N:P ratio of 11, mussel excretion could theoretically exacerbate P limitation. However, low ingestion rate of P due to poor feeding conditions likely was an important accessory factor defining this relationship.

The extreme sensitivity of P excretion to *A*(P) has important implications to understanding and modeling summer succession of phytoplankton, including *Microcystis*, as well as proliferation of the *Cladophora* in the nearshore zone. If mussels find themselves decoupled from the phytoplankton in the water column owing to poor mixing and stratification in the scenario proposed by Zhang *et al*., (2011), P excretion will be greatly reduced; therefore, the hypothesis that mussels will promote *Microcystis*—or any other phytoplankter—by P excretion in non-feeding mussels (sensu Zhang *et al*., 2011) would seem unlikely. This decoupling could also play a role in mussel-*Cladophora* interactions. As *Cladophora* proliferates around mussels (e.g., Bootsma *et al*., 2015), access to phytoplankton decreases, which would in turn create a feedback to reduce mussel P excretion and reduce its availability to *Cladophora* growth as well. Likewise, when feeding rate stops during a *Microcystis* bloom (Johengen *et al.*, 2013), P excretion is greatly reduced, while N excretion continues.

The balance between grazing and nutrient excretion impacts is context dependent, including nutrient loading and mussel abundance, which itself can be a function of TP concentration in the lake. Vanderploeg *et al*., (2002, 2013) argued that under low P conditions (assuming no or low *Microcystis* mortality) grazing by a sufficiently high biomass of mussels is able to control slowly growing non-*Microcystis* algal species; conversely, as P concentration increases, phytoplankton growth relative to mortality increases. Therefore, the argument could be made that under low TP conditions, mussel P excretion will be a relatively weak force in affecting community algal growth rate and grazing would be the dominant force. However, the community could be affected by a further shift to greater N:P seston ratios and exacerbation of P limitation. Also, recent work (reviewed in Gobler *et al.*, 2016) suggests that *Microcystis* may have a competitive

612 advantage over other phytoplankton under low-P conditions, and that under these high
613 N:P ratios, MC production will be high. This would be consistent with both higher
614 *Microcystis* and MC concentrations (Raikow *et al*., 2004; Knoll *et al*., 2008) found in
615 low-TP mussel-invaded inland lakes. We could also argue that mussel N excretion—by
616 coming late in the bloom cycle in moderate N:P lakes—could prolong toxic *Microcystis*
617 blooms by providing N necessary for MC production to *Microcystis* as nitrate is used up
618 by the bloom.

619 In the Great Lakes, dreissenid mussels will have greatest direct impacts in warm,
620 shallow areas such as Saginaw Bay (Lake Huron) and western Lake Erie, where
621 *Microcystis* can grow and mussels are abundant enough to influence phytoplankton
622 mortality and nutrient recycling. In offshore regions, mussels on the bottom will be
623 decoupled from surface epilimnetic water and will have little immediate impact to
624 *Microcystis*. However, this is not to say that P sequestration in tissue, shells, and
625 associated benthic community will not have an effect over the longer term.

626 The large potential nutrient reservoir in egested material observed in our
627 experiments, those of Mosley & Bootsma (2015), and Vanderploeg *et al*. (2009) could be
628 a source of P for the benthos and loss from the pelagic zone. However, the nature of the
629 biodeposits and turbulence in the system will affect whether the egested material is
630 resuspended or becomes part of the sediment (Vanderploeg *et al.*, 2002). Furthermore,
631 we must distinguish between nutrients egested in pseudofaeces and those in faeces. We
632 were not able to do this, since our examination of biodeposits was done long after
633 preservation thereby making discernment between faeces and pseudofaeces inexact.
634 Pseudofaeces, often consisting of loosely consolidated seston, can be quite fragile and
635 living phytoplankton in them can remain viable and be resuspended by gentle water
636 motion (Vanderploeg *et al*., 2001, 2002, 2009). Depending on the contents of
637 pseudofaeces (algae and suspended sediment) they can have differing buoyancies—
638 including positive buoyancy (Vanderploeg *et al.,* 2013; Vanderploeg and Strickler, 2013).
639 Therefore, seston composition, including algal species composition and abundance of
640 suspended sediment, combined with turbulence in the system are important factors
641 driving input to the benthos or return to the water column. In our experiments we used
642 gentle mixing, which allowed pseudofaeces to settle in experimental containers. High

mixing rates would have led to breakdown of pseudofaeces and release of their contents to the water column above.

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References

Aldridge D.W., Payne B.S. & Miller A.C. (1995) Oxygen consumption, nitrogenous excretion, and filttration: rates of *Dreissena polymorpha* at acclimation temperatures between 20 and 32°C. *Canadian Journal of Fisheries and Aquatic Sciences,* **52,** 1761-1767.

Andersen J.M. (1976) An Ignition Method for Determination of Total Phosphorus in Lake Sediments. *Water Research,* **10,** 329-331.

Anderson T.R. & Hessen D.O. (2005) Threshold elemental ratios for carbon versus phosphorus limitation in *Daphnia*. *Freshwater Biology,* **50,** 2063-2075.

Arnott D.L. & Vanni M.J. (1996) Nitrogen and phosphorus recycling by the zebra mussel (*Dreissena polymorpha*) in the western basin of Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences,* **53,** 646-659.

Bierman V.J., Kaur J., Depinto J.V., Feist T.J. & Dilks D.W. (2005) Modeling the role of zebra mussels in the proliferation of blue-green algae in Saginaw Bay, Lake Huron. *Journal of Great Lakes Research,* **31,** 32-55.

Bootsma H.A. & Liao Q. (2013) Nutrient cycling by dreissenid mussels: controlling factors and ecosystem response, pp. 555-574 (Chapter 35). In: *Quagga and zebra mussels: biology, impacts, and control.* (Eds. T.F. Nalepa & D.W. Schloesser). Taylor and Francis, Boca Raton, Florida.

Bootsma H.A., Rowe M.D., Brooks C.N. & Vanderploeg H.A. (2015) The need for model development related to *Cladophora* and nutrient management in Lake Michigan. *Journal of Great Lakes Research,* **41 (Supplement 3)**, 7-15.

673 Bundy M.H., Vanderploeg H.A., Lavrentyev P.J. & Kovalcik P.A. (2005) The
674 importance of microzooplankton versus phytoplankton to copepod
675 populations during late winter and early spring in Lake Michigan. *Canadian*
676 *Journal of Fisheries and Aquatic Sciences,* **62,** 2371-2385.
677 Conroy J.D., Edwards, W.J., Pontius R.A., Kane D.D., Zhang H.Y., Shea J.F., Richey J.N. &
678 Culver D.A. (2005) Soluble nitrogen and phosphorus excretion of exotic
679 freshwater mussels (Dreissena spp.): potential impacts for nutrient
680 remineralisation in western Lake Erie. *Freshwater Biology,* **50,** 1146-1162.
681 Elser J.J., Acharya K., Kyle M., Cotner J., Makino W., Markow T., *et al.*. (2003) Growth
682 rate-stoichiometry couplings in diverse biota. *Ecology Letters,* **6,** 936-943.
683 Fahnenstiel G.L., Krause A.E., Mccormick M.J., Carrick H.J. & Schelske C.L. (1998) The
684 structure of the planktonic food-web in the St. Lawrence Great Lakes. *Journal*
685 *of Great Lakes Research,* **24,** 531-554.
686 Gergs R., Rinke K. & Rothhaupt K.-O. (2009) Zebra mussels mediate benthic-pelagic
687 coupling by biodeposition and changing detrital stoichiometry. *Freshwater*
688 *Biology,* **54,** 1379-1391.
689 Gonzalez A.L., Kominoski J.S., Danger M., Ishida S., Iwai N. & Rubach A. (2010) Can
690 ecological stoichiometry help explain patterns of biological invasions? *Oikos,*
691 **119,** 779-790.
692 Gobler C.J., Burkholder J.M., Davis T.W., Harke M.J., Johengen T., *et al.* (2016) The
693 dual role of nitrogen supply in controlling the growth and toxicity of
694 cyanobacterial blooms. *Harmful Algae,* **54,** 87-97.
695 Healey F.P. & Hendzel L.L. (1979) Indicators of phosporus and nitrogen deficiency in
696 5 algae in culture. *Journal of the Fisheries Research Board of Canada,* **36,**
697 1364-1369.
698 Hecky R.E., Campbell P. & Hendzel L.L. (1993) The stoichiometry of carbon,
699 nitrogen, and phosphorus in particulate matter of lakes and oceans.
700 *Limnology and Oceanography,* **38,** 709-724.
701 Hecky R.E., Smith R.E.H., Barton D.R., Guildford S.J., Taylor W.D., Charlton M.N. &
702 Howell T. (2004) The nearshore phosphorus shunt: a consequence of

ecosystem engineering by dreissenids in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **61,** 1285-1293.

Hessen D.O. (2006) Determinants of seston C : P-ratio in lakes. *Freshwater Biology,* **51,** 1560-1569.

Higgins S.N. & Vander Zanden M.J. (2010) What a difference a species makes: a meta-analysis of dreissenid mussel impacts on freshwater ecosystems. *Ecological Monographs,* **80,** 179-196.

Horst G.P., Sarnelle O., White J.D., Hamilton S.K., Kaul R.B. & Bressie J.D. (2014) Nitrogen availability increases the toxin quota of a harmful cyanobacterium, Microcystis aeruginosa. *Water Research,* **54,** 188-198.

Johengen T.H., Vanderploeg H.A. & Liebig J.R. (2013) Effects of algal composition, seston stoichiometry, and feeding rate on zebra mussel (*Dreissena polymorpha)* nutrient excretion in two Laurentian Great Lakes. In: *Quagga and Zebra Mussels: Biology, Impacts, and Control, Second Edition.* (Eds. T.F. Nalepa & D.W. Schloesser), pp. 445-459 (Chapter 27). CRC Press, Taylor and Francis Group, Boca Raton, FL

Knoll L.B., Sarnelle O., Hamilton S.K., Kissman C.E.H., Wilson A.E., Rose J.B. & Morgan M.R. (2008) Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **65,** 448-455.

Morehouse R.L., Dzialowski A.R. & Jeyasingh P.D. (2013) Impacts of excessive dietary phosphorus on zebra mussels. *Hydrobiologia,* **707,** 73-80.

Mosley C. & Bootsma H.A. (2015) Phosphorus recycling by profunda quagga mussels (*Dreissena rostriformis bugensis*) in Lake Michigan. *Journal of Great Lakes Research,* **41 (Supplement 3)**, 38-48.

Naddafi R., Eklov P. & Pettersson K. (2009) Stoichiometric constraints do not limit successful invaders: Zebra mussels in Swedish lakes. *Plos One,* **4**, 1-8.

Naddafi R., Goedkoop W., Grandin U. & Eklov P. (2012) Variation in tissue stoichiometry and condition index of zebra mussels in invaded Swedish lakes. *Biological Invasions,* **14,** 2117-2131.

Nalepa T.F., Cavaletto J.F., Ford M., Gordon W.M. & Wimmer M. (1993) Seasonal and annual variation in weight and biochemical content of the zebra mussel, *Dreissena polymorpha*, in Lake St. Clair. *Journal of Great Lakes Research,* **19,** 541-552.

Ozersky T., Malkin S.Y., Barton D.R. & Hecky R.E. (2009) Dreissenid phosphorus excretion can sustain C. glomerata growth along a portion of Lake Ontario shoreline. *Journal of Great Lakes Research,* **35,** 321-328.

Ozersky T., Evans D.O. & Ginn B.K. (2015) Invasive mussels modify the cycling, storage and distribution of nutrients and carbon in a large lake. *Freshwater Biology,* **60,** 827-843.

Raikow D.F., Sarnelle O., Wilson A.E. & Hamilton S.K. (2004) Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low-nutrient lakes is associated with exotic zebra mussels. *Limnology and Oceanography,* **49,** 482-487.

Rowe M.D., Obenour D.R., Nalepa T.F., Vanderploeg H.A., Yousef F. & Kerfoot W.C. (2015) Mapping the spatial distribution of the biomass and filter-feeding effect of invasive dreissenid mussels on the winter-spring phytoplankton bloom in Lake Michigan. *Freshwater Biology,* **60,** 2270-2285.

Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry,* Princeton University Press, Princeton, New Jersey.

Sterner R.W., Elser J.J. & Hessen D.O. (1992) Stoichiometric relationships among producers, consumers an nutrient cycling in pelagic ecosystems. *Biogeochemistry,* **17,** 49-67.

Tang H., Vanderploeg H.A., Johengen T.H. & Liebig J.R. (2014) Quagga mussel (Dreissena rostriformis bugensis) selective feeding of phytoplankton in Saginaw Bay. *Journal of Great Lakes Research,* **40,** 83-94.

Vanderploeg H.A., Johengen T.H. & Liebig J.R. (2009) Feedback between zebra mussel selective feeding and algal composition affects mussel condition: did the regime changer pay a price for its success? *Freshwater Biology,* **54,** 47-63.

Vanderploeg H.A., Liebig J.R., Carmichael W.W., Agy M.A., Johengen T.H., Fahnenstiel G.L., *et al*. (2001) Zebra mussel (*Dreissena polymorpha*) selective filtration

promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences,* **58,** 1208-1221.

Vanderploeg H.A., Liebig J.R., Nalepa T.F., Fahnenstiel G.L. & Pothoven S.A. (2010) *Dreissena* and the disappearance of the spring phytoplankton bloom in Lake Michigan. *Journal of Great Lakes Research,* **36,** 50-59.

Vanderploeg H.A., Nalepa T.F., Jude D.J., Mills E.L., Holeck K.T., Liebig J.R., *et al*. (2002) Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences,* **59,** 1209-1228.

Vanderploeg, H.A. & Strickler, J.R. (2013). Video Clip 6: Behavior of zebra mussels (*Dreissena polymorpha*) exposed to *Microcystis* colonies from natureal seston and laboratory culture. In: *Quagga and Zebra Mussels: Biology, Impacts, and Control, Second Edition*. (Eds. T.F. Nalepa & D.W. Schloesser), pp. 757-758. CRC Press, Taylor and Francis Group, Boca Raton, FL

Vanderploeg H.A., Wilson, A.E. , Johengen, T.H. , Dyble Bressie, J., Sarnelle, O., Liebig, J.R., *et al*. (2013) Role of selective grazing by dreissenid mussels in promoting toxic *Microcystis* blooms and other changes in phytoplankton composition in the Great Lakes. In: *Quagga and Zebra Mussels: Biology, Impacts, and Control, Second Edition.* (Eds T.F. Nalepa & D.W. Schloesser), pp. 509-523 (Chapter 32). CRC Press, Taylor and Francis Group, Boca Raton, FL

Waajen G., Van Bruggen N.C.B., Pires L.M.D., Lengkeek W. & Lurling M. (2016) Biomanipulation with quagga mussels (Dreissena rostriformis bugensis) to control harmful algal blooms in eutrophic urban ponds. *Ecological Engineering,* **90,** 141-150.

Zhang H., Culver D.A. & Boegman L. (2011) Dreissenids in Lake Erie: an algal filter or a fertilizer? *Aquatic Invasions,* **6,** 175-194.

Figure legends

794 Figure 1. Chlorophyll a (Chl) concentration, *Microcystis* concentration, microcystin
795 concentration and microcystin/Chl ratio in enclosures at time of experiments (±SE,
796 n = 3). Enclosure treatments are designated by letters L (no), M (medium) and H
797 (high) nutrient additions followed by 1, 2, or 4 indicating nominal mussel dry mass
798 concentrations (g m^{-2}) stocked in the enclosure.
799
800 Figure 2. Phytoplankton abundance and composition in enclosures. CrypFlag is the
801 combined category of cryptophytes and flagellates. Enclosure treatments are
802 designated by letters L (no), M (medium) and H (high) nutrient additions followed
803 by 1, 2, or 4 indicating nominal mussel concentrations (g m^{-2}) stocked in the
804 enclosure.
805 Figure 3. Mussel condition and C:N:P ratios (±SE, n = 3) in enclosures. Enclosure
806 treatments are designated by letters L (no), M (medium) and H (high) nutrient
807 additions followed by 1, 2, or 4 indicating nominal mussel dry mass concentrations
808 (g m^{-2}) stocked in the enclosure.

809

810

811 Figure 4. Relationship of mussel condition (expressed as ash-free dry mass per unit
812 length) to particulate P and important food-quality (stoichiometry and algal
813 composition) variables in enclosures during rounds #2 and #3 after addition of
814 mussels to enclosures. CryptoFlag is combined abundance of cryptomonads and
815 flagellates. All regressions are significant at the P< 0.05 level.

816

817 Figure 5. Mussel C:N:P ratios as function of seston C:N:P ratios during rounds #2
818 and #3 after addition of mussels to enclosures. Regressions are significant at the P<
819 0.05 level.

820

821 Figure 6. Net clearance rate (F_A), assimilation rate (A) of Chl, and potential
822 assimilation of P and N as linear functions of Chl and particulate N and P.
823 Regressions are significant at the P< 0.05 level.

824

825

826 Figure 7. Soluble reactive P excretion as a function of: seston C:P and N:P ratios;
827 seston particulate phosphorus (PP), particulate organic N (PON), and particulate
828 organic C (POC) concentrations; and potential assimilation rate of P (A).
829 Regressions are significant at the P <0.05 level.

830

831 Figure 8. NH_4 excretion as a function of seston N:P ratio, particulate organic N
832 (PON) concentration, and potential assimilation rate (A) of N; and N:P excretion as a
833 function of seston N:P ratio. Regressions are significant at the P <0.05 level.

Table 1. Temperature, nutrient concentrations (total phosphorus [TP], dissolved phosphorus [DP], particulate phosphorus [PP], particulate organic nitrogen [PON], particulate organic carbon [POC]), C:P, C:N, and C:P ratios of particulate material, and chlorophyll (Chl) concentrations with SEs (n = 3) of enclosure water used for experiments. Enclosure treatments are designated by letters labeled L (no), M (medium) and H (high) nutrient additions followed by 1, 2, or 4 indicating nominal mussel concentrations (g dry mass m^{-2}) stocked in the enclosure. Round (R) 1, 2, and 3 refer respectively to experiments performed during the time periods 9 - 12 July, 23-27 July, and 6-9 August 2007. DP was calculated from the difference of TP and PP measurements.

Treat.	R	Temp (°C)	TP (µg L^{-1})	DP (µg L^{-1})	PP (µg L^{-1})	PN (mg L^{-1})	POC (mg L^{-1})	C:P	C:N	N:P	Chl (µg L^{-1})
L1	1	25.0	5.2 ± 0.5	2.4	2.8 ± 0.0	0.043 ± 0.002	0.42 ± 0.00	384.5 ± 7.2	11.45 ± 0.32	33.7 ± 1.4	1.21 ± 0.04
L1	2	24.2	3.7 ± 0.2	1.0	2.7 ± 0.2	0.048 ± 0.001	0.43 ± 0.01	409.2 ± 25.0	10.39 ± 0.18	39.4 ± 2.5	1.62 ± 0.08
L1	3	26.2	3.5 ± 0.0	1.0	2.5 ± 0.1	0.046 ± 0.001	0.40 ± 0.01	413.1 ± 21.3	10.04 ± 0.17	41.2 ± 0.21	1.64 ± 0.03
L2	1	24.6	5.2 ± 0.5	2.3	2.9 ± 0.2	0.041 ± 0.001	0.44 ± 0.01	378.2 ± 29.4	12.48 ± 0.21	31.1 ± 2.3	1.14 ± 0.05
L2	2	23.6	4.6 ± 0.1	1.2	3.4 ± 0.0	0.063 ± 0.001	0.56 ± 0.01	416.6 ± 9.7	10.28 ± 0.18	40.5 ± 0.8	1.97 ± 0.07
L2	3	26.0	4.5 ± 0.1	1.0	3.5 ± 0.1	0.059 ± 0.000	0.52 ± 0.01	385.3 ± 13.1	10.29 ± 0.12	37.4 ± 1.2	2.37 ± 0.05
L4	1	25.4	4.7 ± 0.1	1.9	2.8 ± 0.1	0.044 ± 0.002	0.43 ± 0.01	390.2 ± 21.2	11.26 ± 0.14	34.7 ± 2.1	1.09 ± 0.05
L4	2	23.3	4.9 ± 0.3	1.7	3.2 ± 0.1	0.051 ± 0.000	0.51 ± 0.01	402.6 ± 17.4	11.48 ± 0.24	35.1 ± 1.3	1.41 ± 0.03
L4	3	25.0	5.2 ± 0.1	1.5	3.7 ± 0.1	0.063 ± 0.001	0.49 ± 0.00	341.0 ± 7.6	9.03 ± 0.11	37.8 ± 1.0	3.00 ± 0.07
M1	1	25.0	7.0 ± 0.2	1.8	5.2 ± 0.1	0.062 ± 0.001	0.49 ± 0.01	243.1 ± 6.5	9.20 ± 0.09	26.4 ± 0.5	1.51 ± 0.02
M1	2	24.0	12.1 ± 0.1	0.6	11.5 ± 0.6	0.130 ± 0.004	0.99 ± 0.01	223.2 ± 11.7	8.90 ± 0.17	25.1 ± 1.5	3.96 ± 0.05
M1	3	25.6	9.7 ± 0.1	2.2	7.5 ± 0.2	0.066 ± 0.002	0.50 ± 0.00	171.2 ± 5.4	8.85 ± 0.16	19.4 ± 0.8	2.31 ± 0.03

M2	1	24.4	7.9 ± 0.2	2.4	5.5 ± 0.2	0.069 ± 0.002	0.60 ± 0.02	281.7 ± 14.5	10.24 ± 0.03	27.5 ± 1.4	2.07 ± 0.03
M2	2	23.6	15.1 ± 0.4	0.4	14.7 ± 0.5	0.103 ± 0.003	0.78 ± 0.02	136.3 ± 6.2	8.85 ± 0.01	15.4 ± 0.7	4.37 ± 0.02
M2	3	25.8	11.2 ± 0.2	2.9	8.3 ± 0.6	0.084 ± 0.002	0.56 ± 0.01	173.9 ± 13.3	7.75 ± 0.06	22.5 ± 1.7	1.96 ± 0.06
M4	1	25.8	5.9 ± 0.1	1.6	4.3 ± 0.2	0.054 ± 0.001	0.48 ± 0.02	285.3 ± 18.0	10.36 ± 0.28	27.5 ± 1.4	1.70 ± 0.03
M4	2	23.5	12.8 ± 0.2	1.5	11.3 ± 0.6	0.112 ± 0.002	0.89 ± 0.02	203.7 ± 11.9	9.27 ± 0.01	22.0 ± 1.3	2.47 ± 0.03
M4	3	25.2	14.1 ± 0.2	3.7	10.4 ± 0.1	0.134 ± 0.007	0.97 ± 0.03	240.0 ± 7.8	8.49 ± 0.17	28.3 ± 1.5	3.72 ± 0.02
H1	1	25.4	14.9 ± 0.2	2.2	12.7 ± 0.4	0.112 ± 0.001	0.78 ± 0.00	158.1 ± 5.0	8.12 ± 0.10	19.5 ± 0.6	3.14 ± 0.01
H1	2	24.4	61.9 ± 3	15.3	46.6 ± 0.1	0.273 ± 0.012	2.04 ± 0.09	113.1 ± 5.1	8.75 ± 0.11	12.9 ± 0.6	12.00 ± 0.06
H1	3	26.8	32.0 ± 2.3	18.7	13.3 ± 0.4	0.078 ± 0.001	0.68 ± 0.02	132.5 ± 5.3	10.25 ± 0.19	12.9 ± 0.4	5.69 ± 0.40
H2	1	24.7	12.9 ± 0.3	3.1	9.8 ± 0.3	0.153 ± 0.003	1.14 ± 0.02	299.3 ± 11.2	8.68 ± 0.05	34.5 ± 1.3	6.21 ± 0.08
H2	2	24.0	21.9 ± 1.2	0.2	21.7 ± 0.4	0.112 ± 0.002	0.74 ± 0.02	88.4 ± 3.2	7.73 ± 0.12	11.4 ± 0.3	4.54 ± 0.13
H2	3	26.5	14.9 ± 0.9	8.3	6.6 ± 0.4	0.043 ± 0.002	0.27 ± 0.01	104.7 ± 7.4	7.31 ± 0.18	14.3 ± 1.0	1.17 ± 0.03
H4	1	26.6	15.8 ± 0.0	1.3	14.5 ± 1.1	0.114 ± 0.006	0.83 ± 0.03	148.1 ± 12.2	8.55 ± 0.25	17.4 ± 1.6	4.55 ± 0.16
H4	2	24.7	37.7 ± 2.1	6.7	31 ± 0.1	0.159 ± 0.004	1.08 ± 0.02	89.8 ± 1.7	7.94 ± 0.07	11.3 ± 0.3	3.40 ± 0.24
H4	3	26.3	21.9 ± 0.6	12.7	9.2 ± 0.5	0.067 ± 0.001	0.49 ± 0.01	136.6 ± 7.4	8.44 ± 0.06	16.2 ± 0.9	2.69 ± 0.05

Table 2. Correlations of feeding and nutrient ingestion rate variables with seston nutrient stoichiometry and algal composition expressed as biomass in carbon units or percent of total carbon biomass (N=26). Abbreviations are: PON (particulate organic N), PP (particulate P), POC (particulate organic C), Chl (chlorophyll a), and MC (particulate microcystin). All significant correlations ($p < 0.05$) are in bold.

Variable	F_A	*A(Chl)*	*A(N)*	*A(P)*
Chl	-0.096	**0.833**	**0.721**	**0.792**
POC	-0.267	**0.632**	**0.619**	**0.672**
PON	-0.233	**0.634**	**0.641**	**0.705**
PP	-0.151	**0.621**	**0.594**	**0.809**
TP	0.110	**0.621**	**0.537**	**0.770**
molar C:N	-0.147	-0.356	**-0.435**	**-0.494**
molar C:P	0.140	**-0.450**	**-0.465**	**-0.675**
molar N:P	-0.131	**-0.412**	**-0.412**	**-0.679**
MC/L	**-0.395**	-0.043	0.035	-0.004
MC/Chl	-0.271	**-0.534**	**-0.447**	**-0.498**
Cyanobacteria	-0.063	0.301	0.385	0.194
Diatoms	-0.002	**0.570**	**0.620**	**0.453**
Greens	-0.072	0.089	0.053	0.046
CryptoFlag	0.106	0.340	0.303	**0.422**

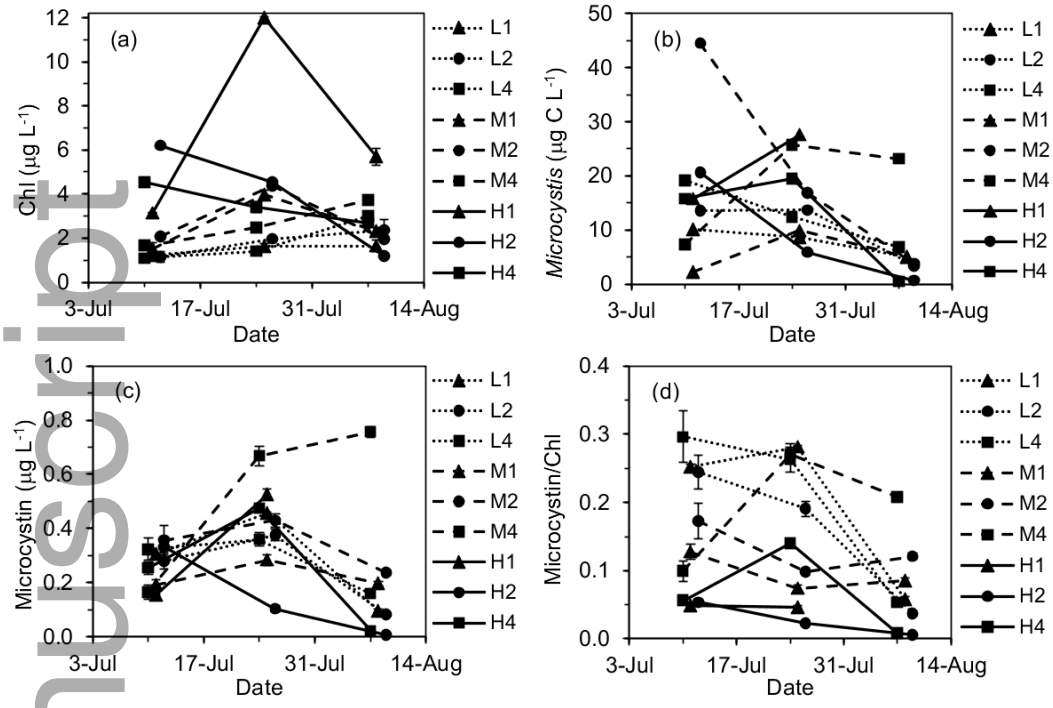

Figure 1. Chlorophyll a (Chl) concentration, *Microcystis* concentration, microcystin concentration and microcystin/Chl ratio in enclosures at time of experiments (±SE, n = 3). Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2, or 4 indicating nominal mussel concentrations (g m^{-2}) stocked in the enclosure.

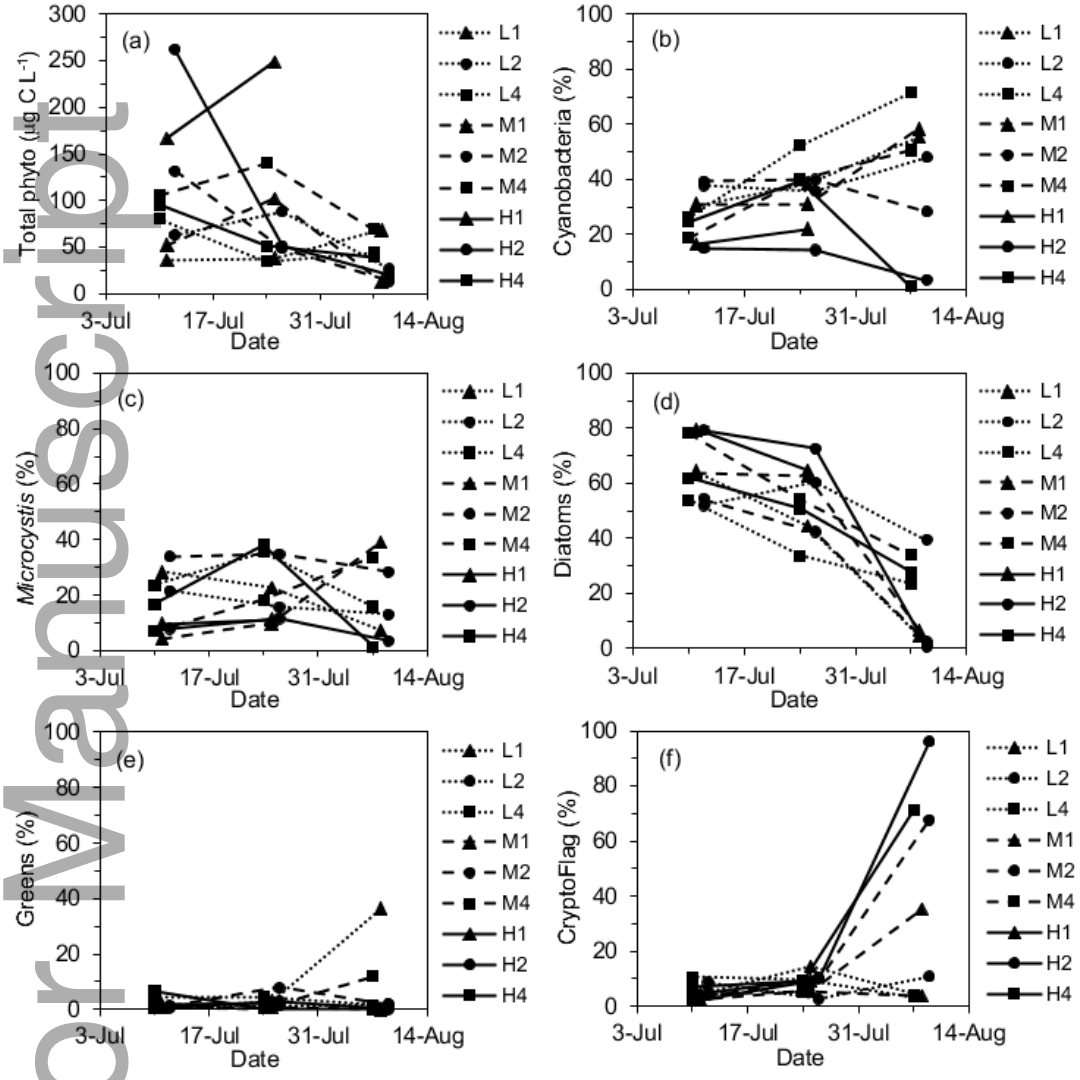

Figure 2. Phytoplankton abundance and composition in enclosures. CrypFlag is the combined category of cryptophytes and flagellates. Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2, or 4 indicating nominal mussel concentrations (g m^{-2}) stocked in the enclosure.

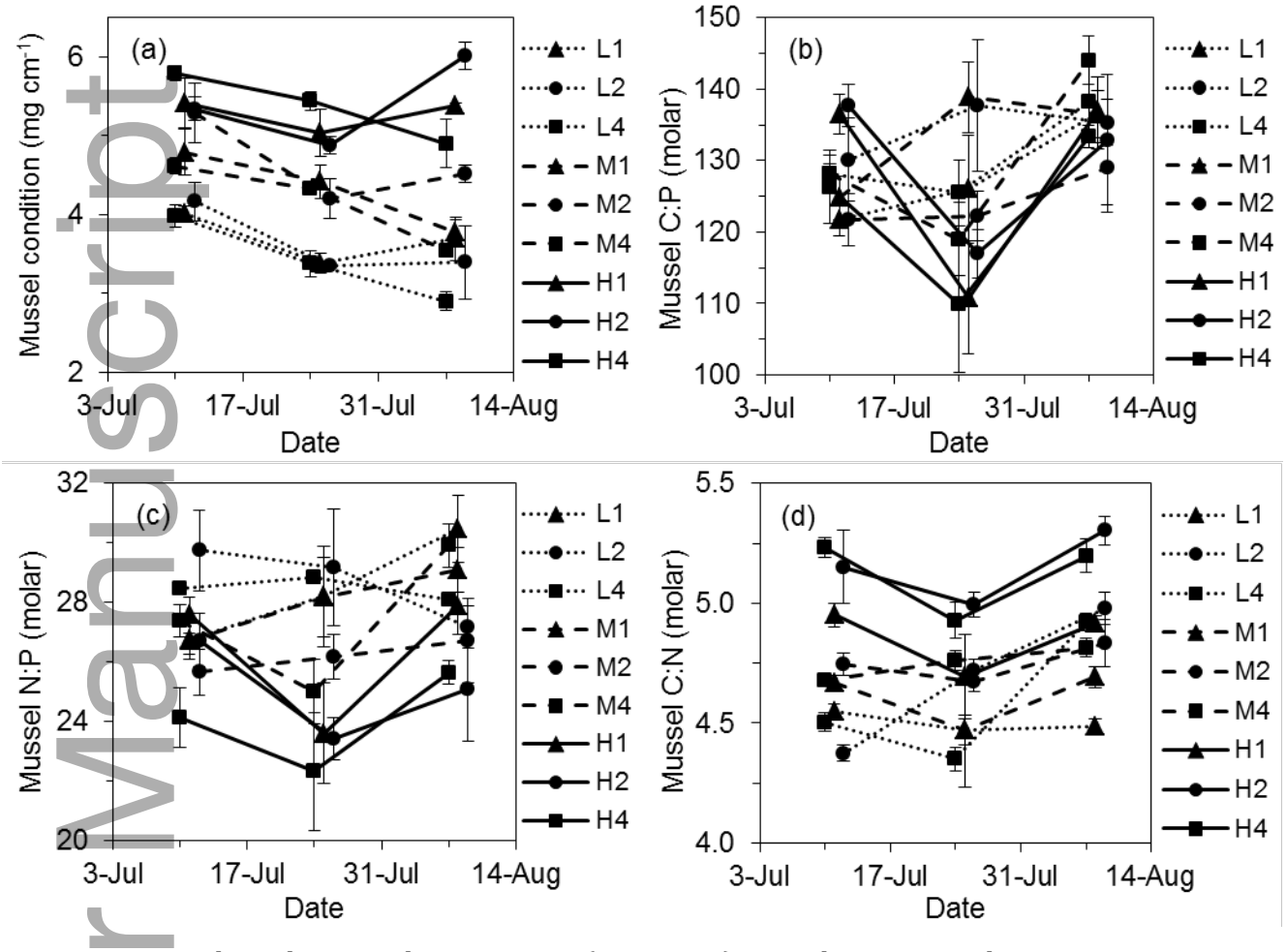

Figure 3. Mussel condition and C:N:P ratios (±SE, n = 3) in enclosures. Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2, or 4 indicating nominal mussel dry mass concentrations (g m^{-2}) stocked in the enclosure.

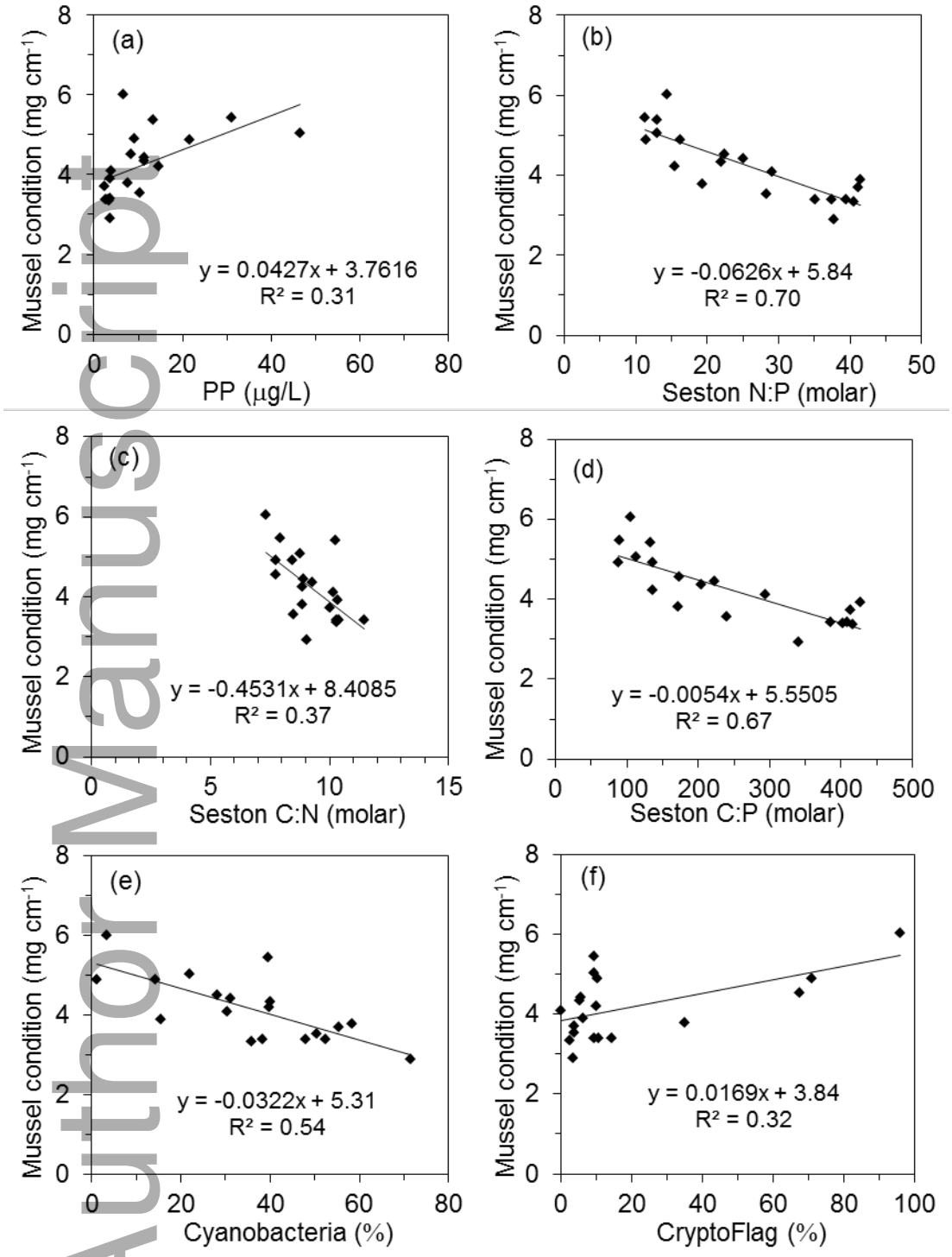

Figure 4. Relationship of mussel condition (expressed as ash-free dry mass per unit length) to particulate P and important food-quality (stoichiometry and algal composition) variables in enclosures during rounds #2 and #3 after addition of

mussels to enclosures. CryptoFlag is combined abundance of cryptomonads and flagellates. All regressions are significant at the P< 0.05 level.

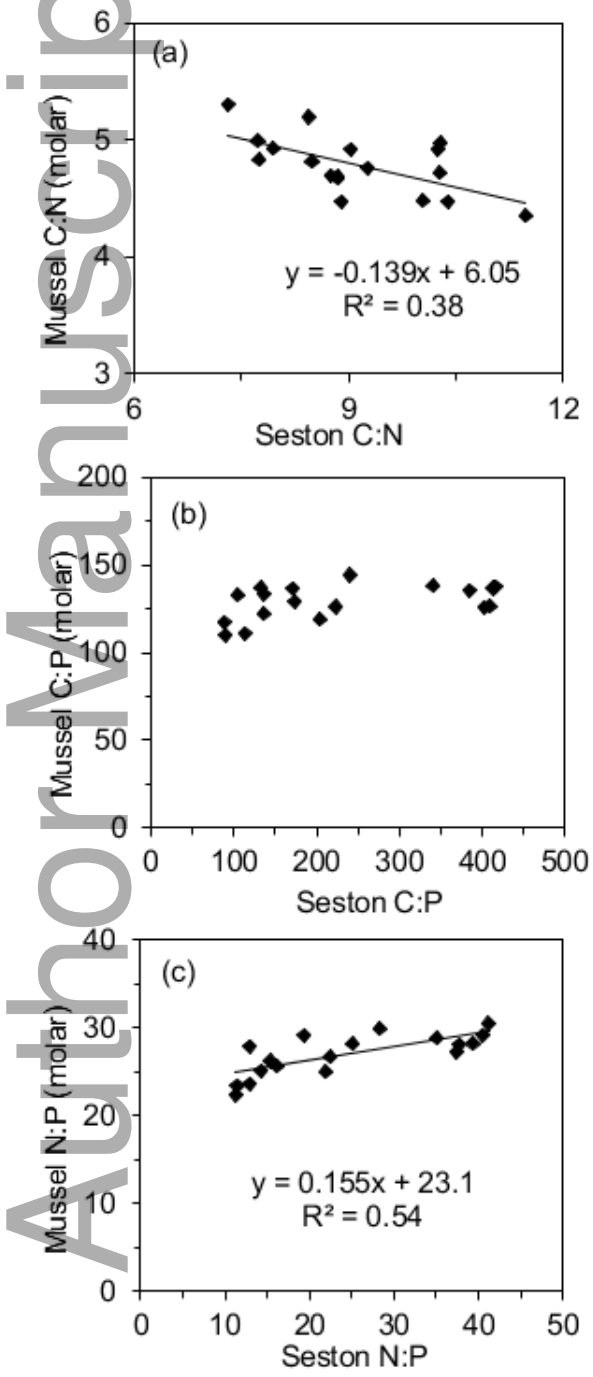

Figure 5. Mussel C:N:P ratios as function of seston C:N:P ratios during rounds #2 and #3 after addition of mussels to enclosures. Regressions are significant at the P< 0.05 level.

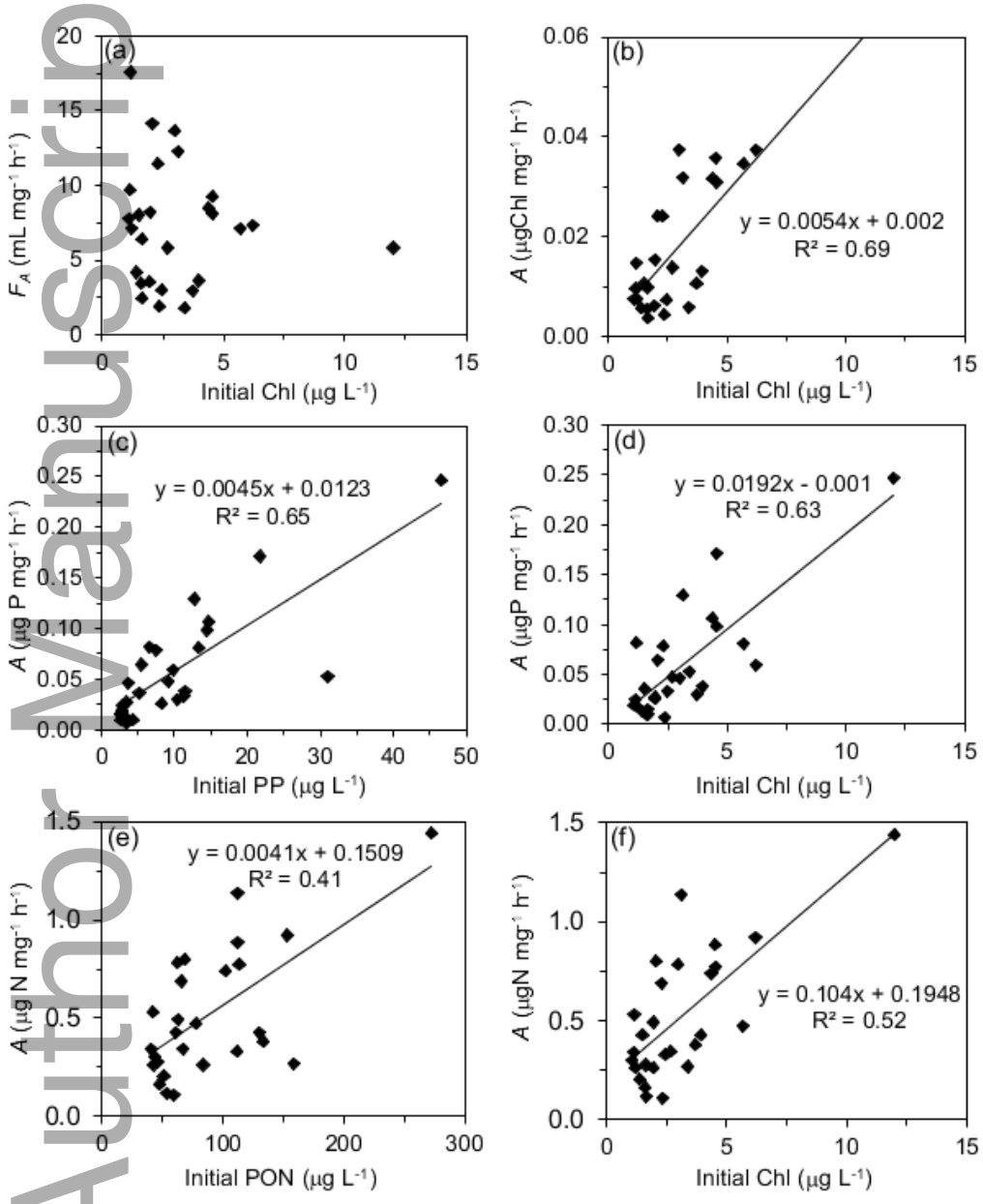

Figure 6. Net clearance rate (F_A), assimilation rate of Chl, and potential assimilation of P and N as linear functions of Chl and particulate N and P. Regressions are significant at the P< 0.05 level.

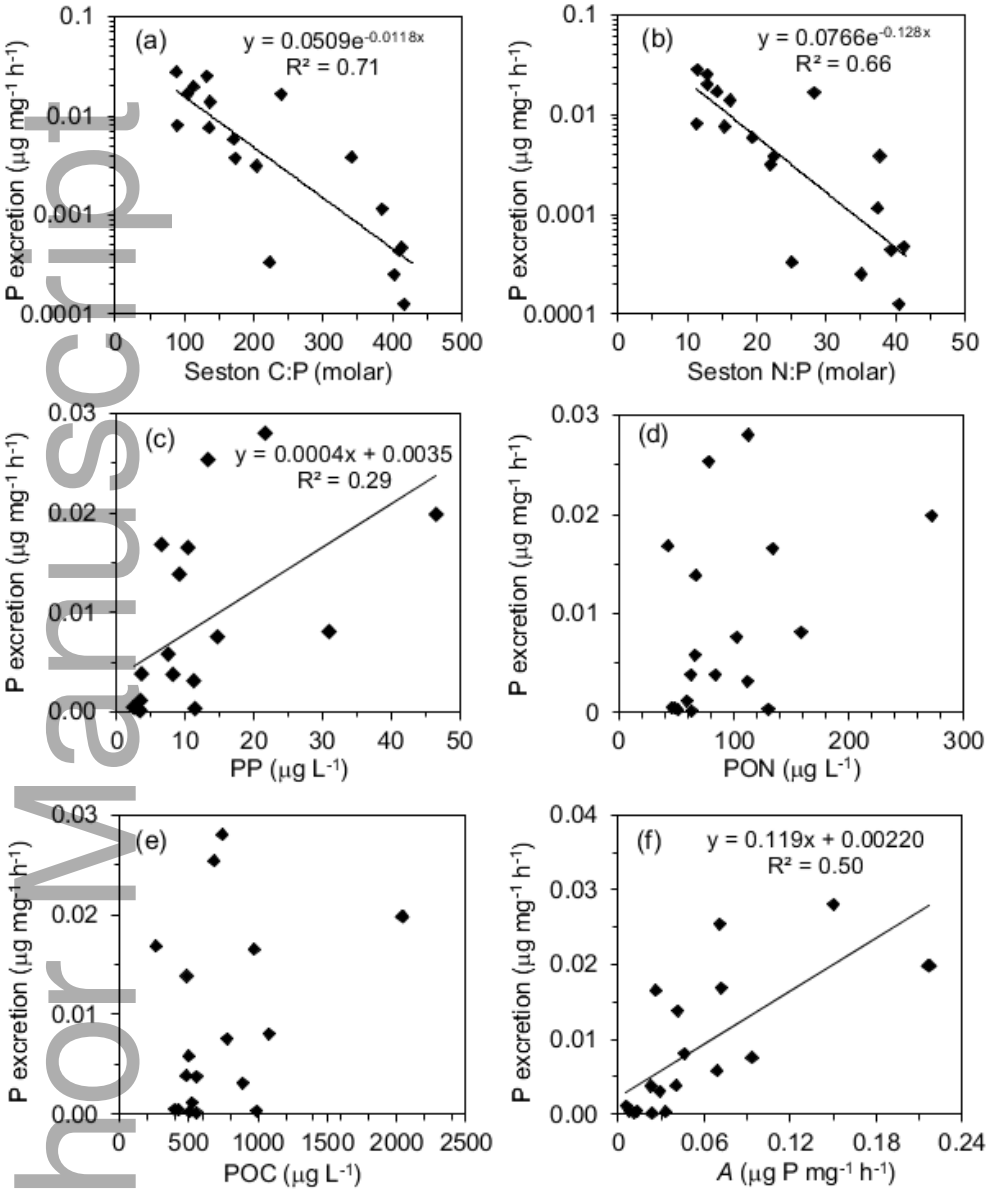

Figure 7. Soluble reactive P excretion as a function of: seston C:P and N:P ratios; seston particulate phosphorus (PP), particulate organic N (PON), and particulate organic C (POC) concentrations; and potential assimilation rate of P (*A*). Regressions are significant at the $P < 0.05$ level.

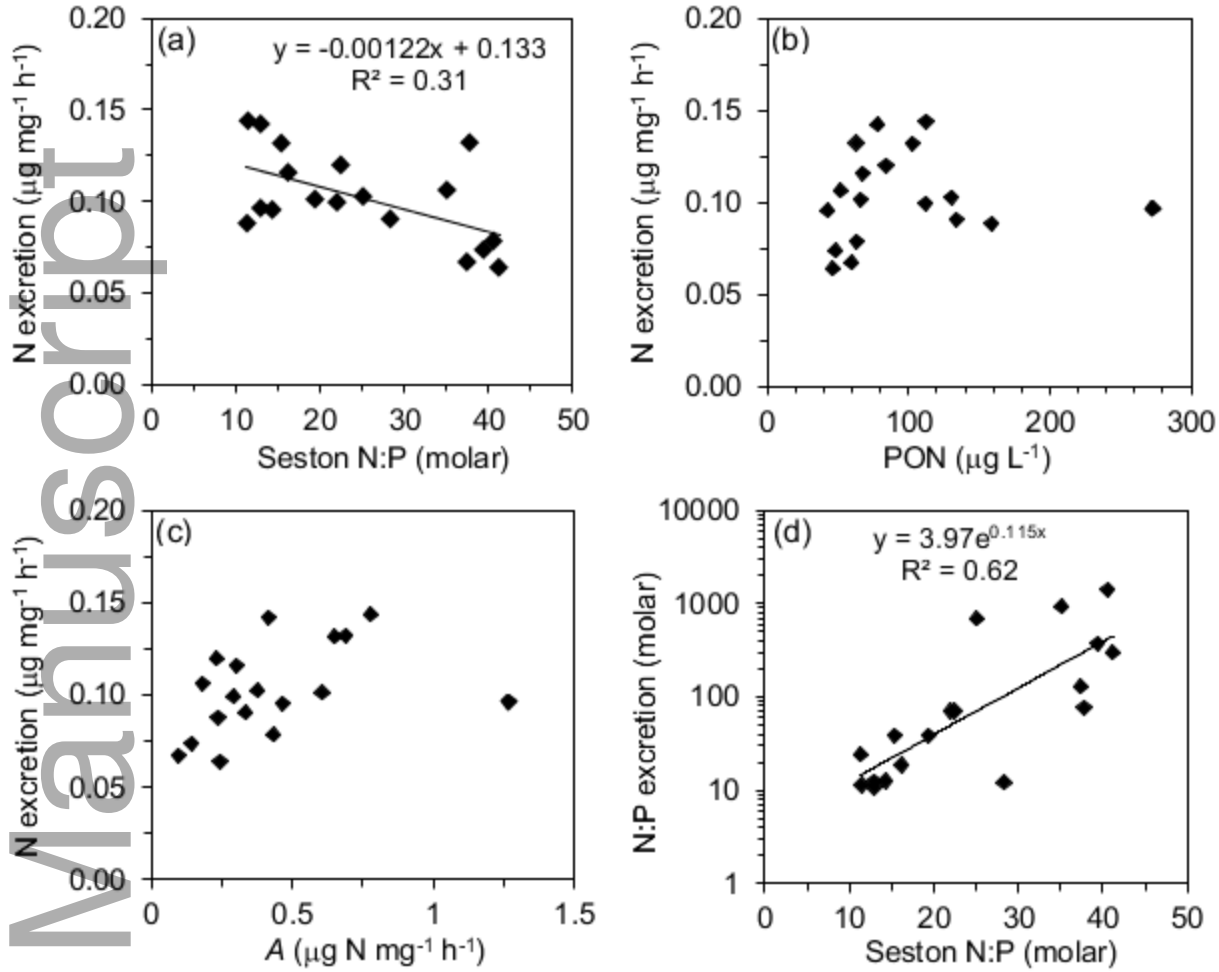

Figure 8. NH_4 excretion as a function of seston N:P ratio, particulate organic N (PON) concentration, and potential assimilation rate (*A*) of N; and N:P excretion as a function of seston N:P ratio. Regressions are significant at the $P < 0.05$ level.