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Seston quality drives feeding, stoichiometry and excretion of 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 nine 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 days 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

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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.

Keywords: *Dreissena*, Ecological stoichiometry, lakes, nitrogen excretion, phosphorus excretion

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 (substratum and water clarity), altered trophic and spatial interactions of the food web and reengineered nutrient recycling (Vanderploeg *et al*., 2002; Hecky *et al*., 2004; Higgins & 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, Culver & Boegman (2011) – using a mathematical model and weight-specific excretion rates of Conroy *et al*. (2005) – argued that nutrient fertilisation 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 (4 °C) 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 22–24 °C (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; Ozersky *et al*. (2009); 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 & Vanni, 1996; Bootsma & Liao, 2013; Johengen, Vanderploeg & Liebig, 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 *et 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, Elser & Hessen, 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, Eklov & Pettersson, 2009; Gonzalez *et al.*, 2010; Naddafi *et al.*, 2012).

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, for example, 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, Rinke & Rothhaupt, 2009; Mosley & Bootsma, 2015).

Methods

Study site

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

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

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 hours 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 behaviour. 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 minimise stress.

We conducted three simultaneous trials of the feeding and nutrient excretion experiments each day between 08:00–15:00 hours 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 nine 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* (hereafter, Chl). Chl was measured in different size fractions (<53 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 characterise 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 normalised 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 recognise 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) 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 six mussels from each of the three 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 measurable 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 (10–100×) 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) GF/F type filters of 25-mm-diameter that had been combusted for 4 h at 400 °C and stored frozen. Just prior to analysis, filters were acidified with 1 N 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).

Particulate P (PP) was sampled in triplicate by filtering 50–200 mL of water through a 47-mm-diameter, 0.2-μm pore-size polycarbonate membrane filters (Nuclepore, GE Healthcare, Pittsburgh). Particulate phosphorus filters were stored frozen. For analysis, PP filters were suspended in 50 mL of deionised 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 (*c.* 3 °C) in the refrigerator. Both sample types were digested for 35 min 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), 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) 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) 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 h. The combusted mussel tissue was oxidised to orthophosphate with 25 mL of 1N HCl in a hot water bath (95–99 °C) for 60 min, adapted from Andersen (1976), and after dilution to 0.019 N analysed 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 25 μg 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 24–27 °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 normalised 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^{-1}) were seen in many of the treatments irrespective of nutrient level or mussel density; for example, 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 Healey & Hendzel (1979) used by Hecky, Campbell & Hendzel (1993) to characterise 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 ($\bar{X} \pm SD = 15.8 \pm 0.6$) – exhibited varying condition (AFDM $length^{-1}$) that ranged between 2.9 and 6.0 mg AFDM cm^{-1} (Fig. 3) with $\bar{X} \pm SD = 4.5 \pm 0.8$. 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

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 *a* (Chl) concentrations with SEs (n = 3) of enclosure water used for experiments. Enclosure treatments are designated by letters labelled 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.

Treatment	R	Temperature (°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

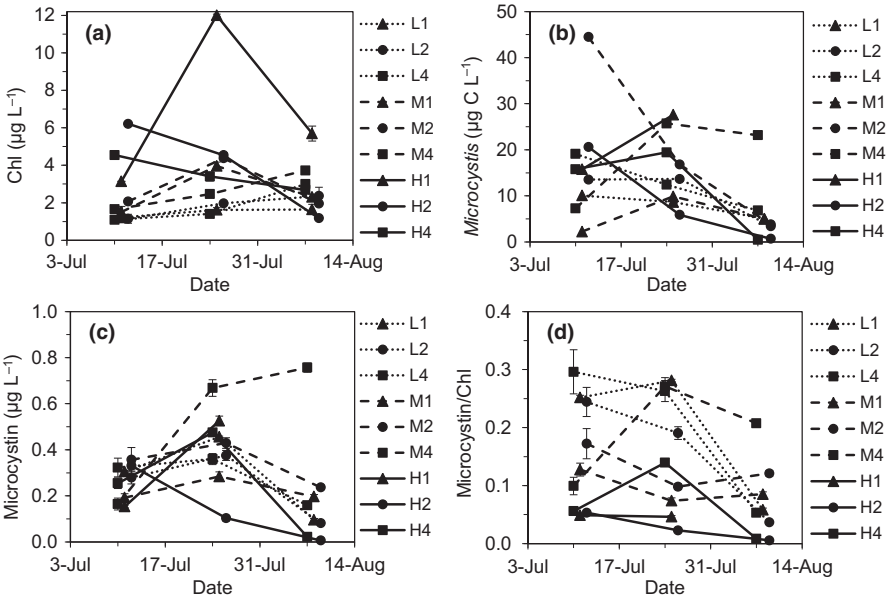

Fig. 1 Chlorophyll *a* (Chl) concentration, *Microcystis* concentration, microcystin concentration and microcystin/Chl ratio in enclosures at time of experiments (±SE, $n = 3$). Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2 or 4 indicating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

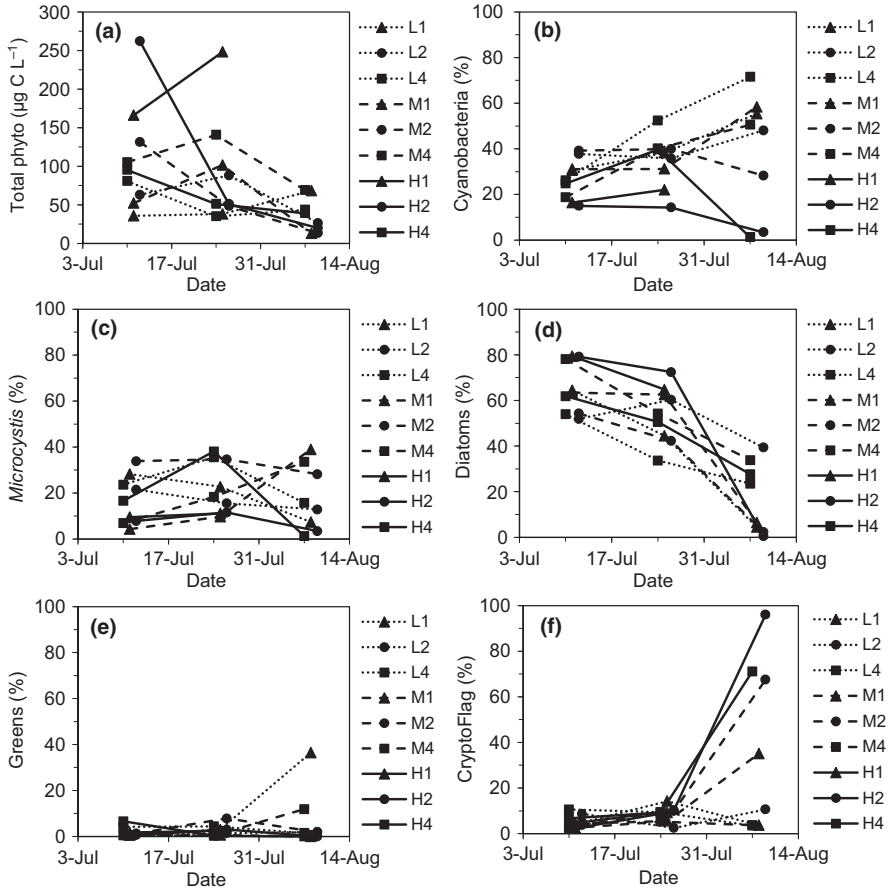

Fig. 2 Phytoplankton abundance and composition in enclosures. CrypFlag is the combined category of cryptophytes and flagellates. Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2 or 4 indicating nominal mussel concentrations (g m^{-2})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

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

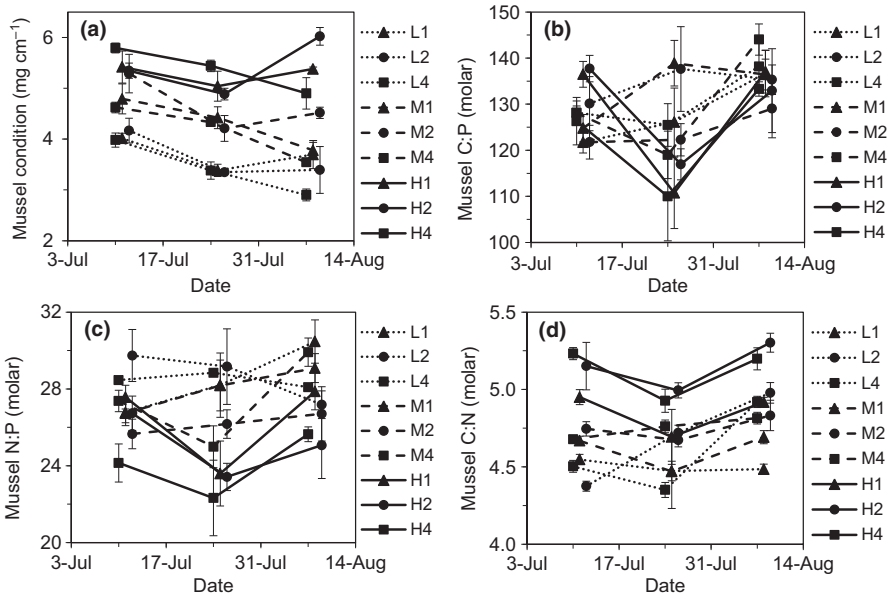

Fig. 3 Mussel condition and C:N:P ratios (±SE, n = 3) in enclosures. Enclosure treatments are designated by letters L (no), M (medium) and H (high) nutrient additions followed by 1, 2 or 4 indicating nominal mussel dry mass concentrations (g m^{-2}) stocked in the enclosure.

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 and 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 with 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) versus 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

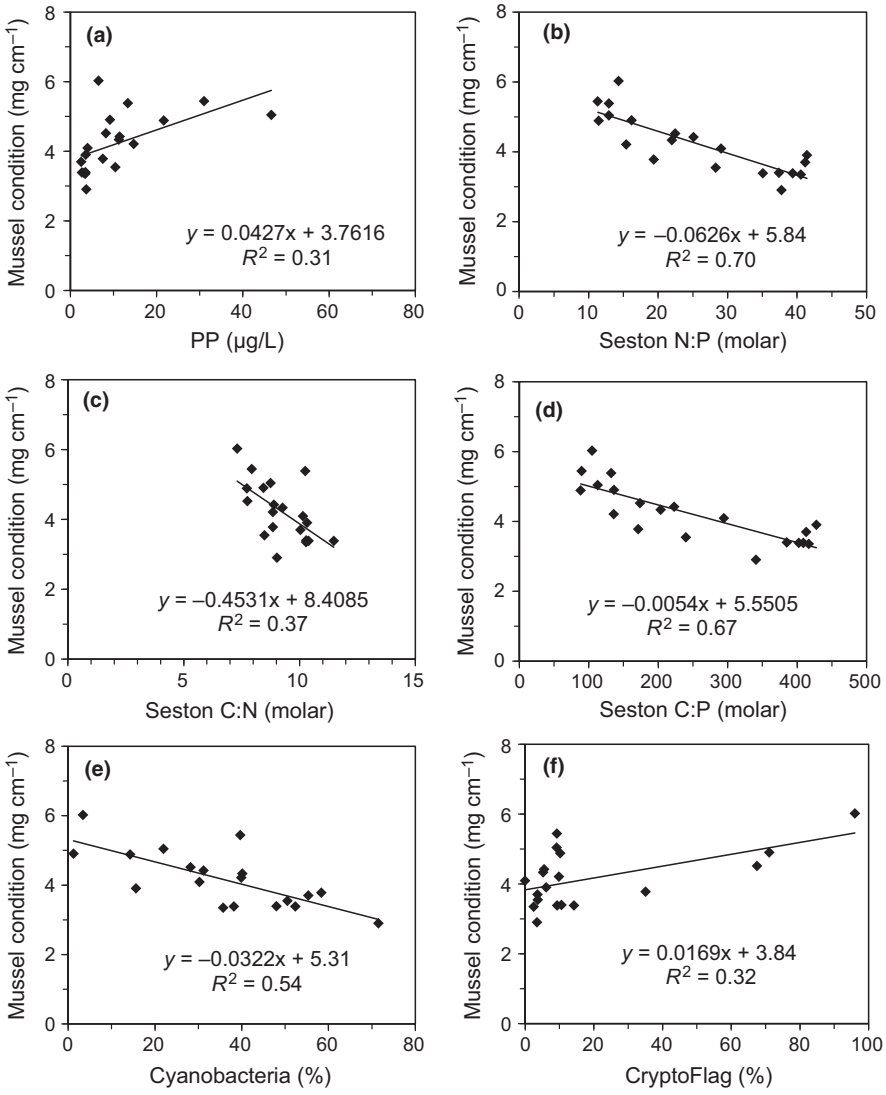

Fig. 4 Relationship of mussel condition (expressed as ash-free dry mass per unit length) to particulate P and important food quality (stoichiometry and algal composition) variables in enclosures during rounds #2 and #3 after addition of mussels to enclosures. CryptoFlag is combined abundance of cryptomonads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

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 *c*. 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 $\bar{X} \pm SD$ 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 $\bar{X} \pm SDs$ 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

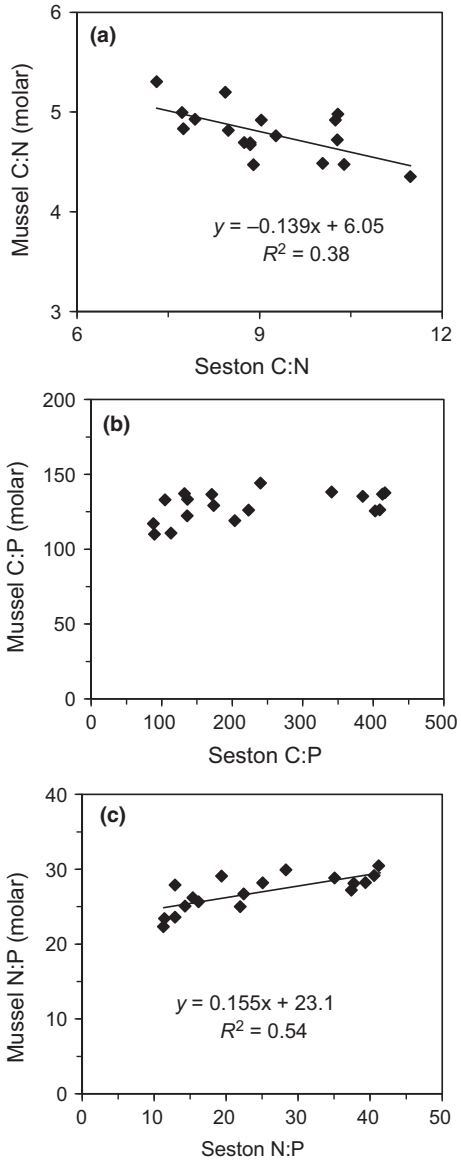

Fig. 5 Mussel C:N:P ratios as function of seston C:N:P ratios during rounds #2 and #3 after addition of mussels to enclosures. Regressions are significant at the $P < 0.05$ level.

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.

Discussion

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

Feeding rates

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

Condition and nutrient stoichiometry of mussel tissues

Although there were large differences in seston C:N:P ratios associated with different levels of P addition, there were only modest differences in C, N and P content and ratios in mussel tissues despite large changes in mussel condition. Consistent with expectation, mussel condition was negatively related to seston C:N, C:P and N:P and % Cyanobacteria; whereas it was positively correlated with PP, TP, CryptoFlag and % CryptoFlag. The condition response to CrypoFlag and Cyanobacteria variables may 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

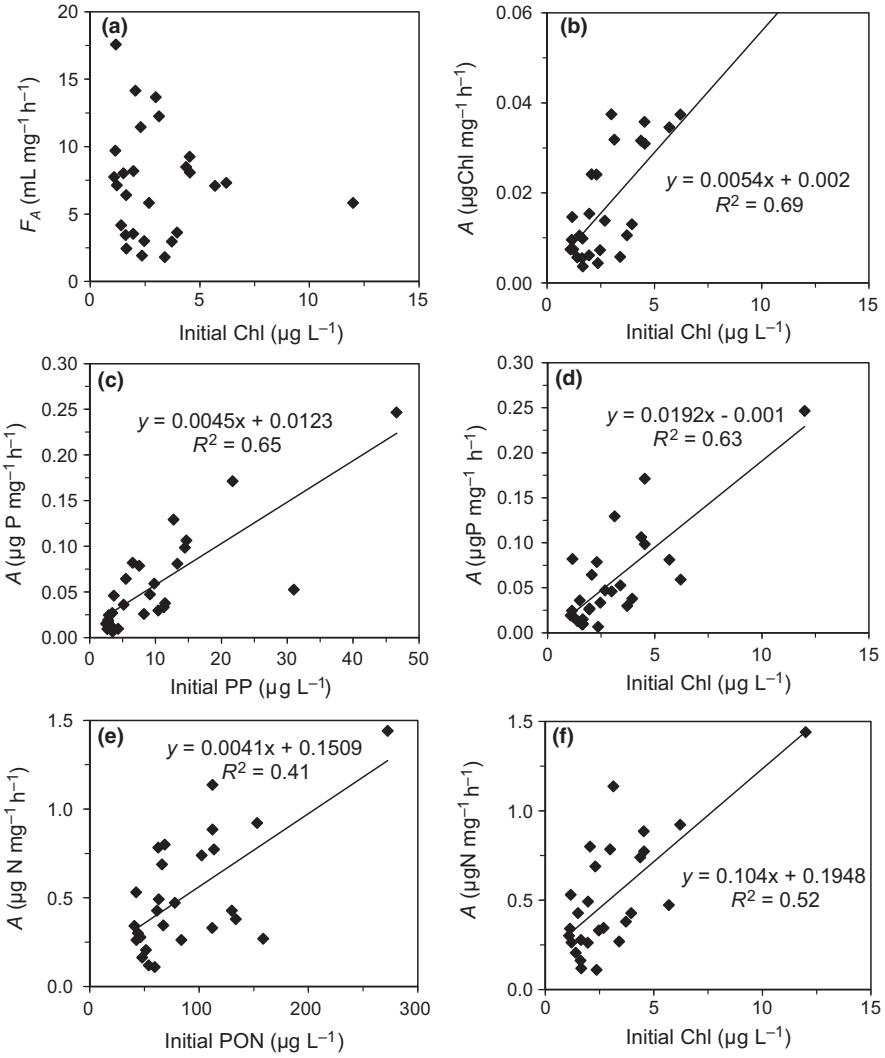

Fig. 6 Net clearance rate (F_A), assimilation rate (A) of Chl, and potential assimilation of P and N as linear functions of Chl and particulate N and P. Regressions are significant at the $P < 0.05$ level.

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; Gonzalez *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 (*c*. 400) and N:P ratios (*c*. 40) were extremely high compared to the studies of Naddafi *et al*. (2009, 2012) with C:P ratios *c*. 200 and N:P ratios *c*. 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

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). PON, particulate organic N; PP, particulate P; POC, particulate organic C; Chl, chlorophyll *a*; 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**

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 versus 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 & Hessen, 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 versus 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 rates are similar to the basal rates observed by Aldridge, Payne & Miller (1995) at the same temperatures. They noted that at temperatures between 25 and 28 °C there was a large drop in the oxygen consumption:NH_4 excretion ratio as mussels shifted from lipid and carbohydrate catabolism to protein catabolism.

Consistent with the stoichiometry paradigm, N:P ratio excreted by mussels was strongly correlated with seston N:P ratio (Sterner 1990, Sterner & Elser, 2002, Vanderploeg *et al.*, 2002; Anderson & Hessen, 2005; Johengen *et al.*, 2013). Interestingly, the logarithmic relationship between N:P excretion ratio and seston N:P ratio showed N:P excretion equalled seston N:P at N:P seston ratio of 11, a value considerably lower than the N:P ratio of mussel soft tissue (22–30) and even the Redfield ratio (16) indicating P sufficiency of phytoplankton. This result contrasts with Bootsma & Liao (2013) who predicted that the transition zone would occur between N:P ratios of 22 and 30. For the summer conditions under which we conducted our experiment, this meant that over a broad range of seston N:P ratios, N excretion relative to P excretion was much higher. This result could be related to the high endogenous N excretion at high temperatures, regardless of feeding rates, as well as the extreme sensitivity of P excretion to feeding and P ingestion rates, which can be very low during summer owing to dominance of grazing resistant phytoplankton. Since P and N excretion are affected by temperature (Bootsma & Liao, 2013; Johengen *et al.*, 2013), the relation we observed could vary with temperature.

Our results spanned a broad range of N:P ratios in seston – 10 to 40. Johengen *et al.* (2013) saw many similar patterns in feeding and nutrient excretion from the pooled results from Lake Erie and Saginaw Bay that

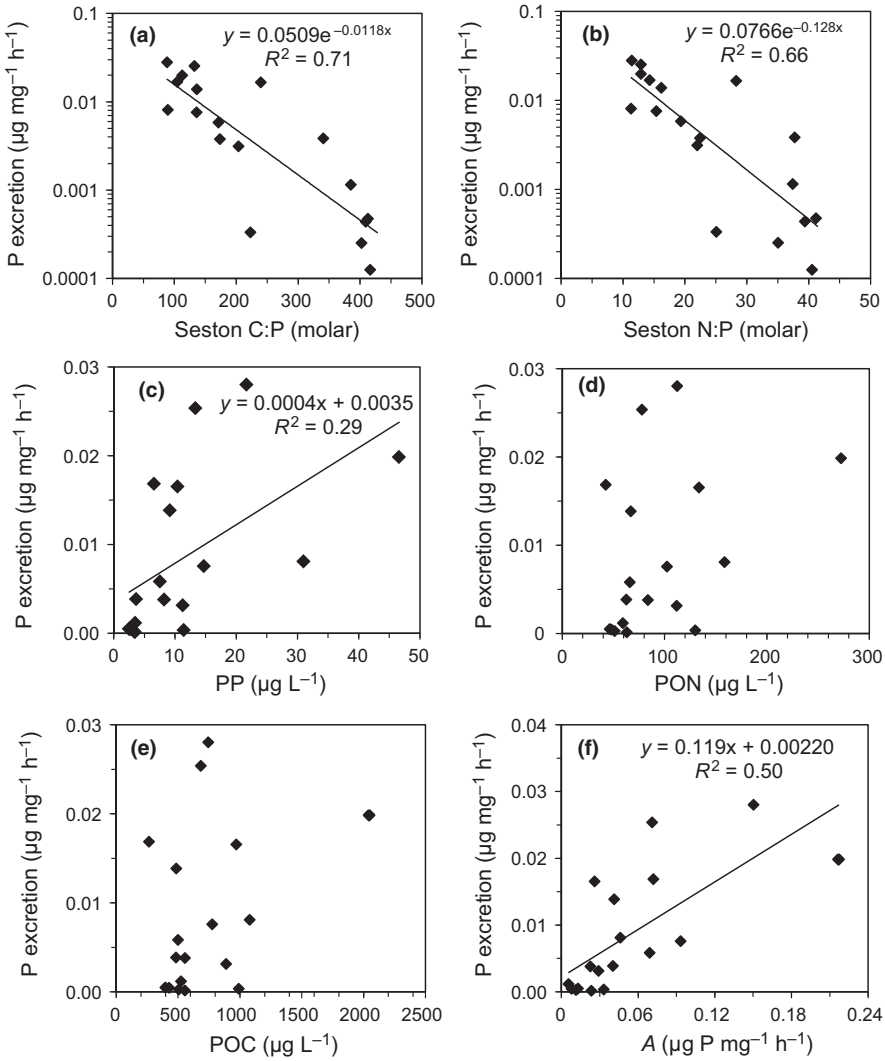

Fig. 7 Soluble reactive P excretion as a function of: seston C:P and N:P ratios; seston particulate phosphorus (PP), particulate organic N (PON) and particulate organic C (POC) concentrations; and potential assimilation rate of P (*A*). Regressions are significant at the $P < 0.05$ level.

demonstrated both the importance of stoichiometry and feeding rate. Across all seasons (April–October), *A*(Chl) was negatively correlated with C:P and N:P ratios, and P excretion was positively correlated with *A*(Chl) and *A* (P). Both *A*(P) and P excretion were positively correlated with %CryptoFlag. When feeding stopped during a *Microcystis* bloom, P excretion was zero and NH_4 excretion rate was high.

Ecosystem implications

The differing behaviour 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 *c*. 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 modelling 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

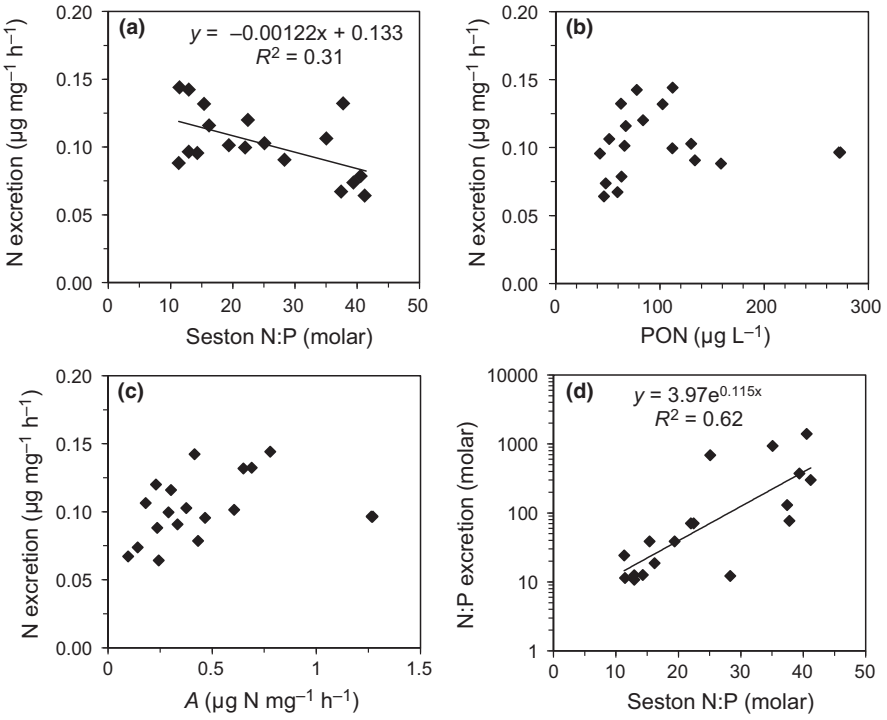

Fig. 8 NH_4 excretion as a function of seston N:P ratio, particulate organic N (PON) concentration, and potential assimilation rate (*A*) of N; and N:P excretion as a function of seston N:P ratio. Regressions are significant at the $P < 0.05$ level.

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

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

The large potential nutrient reservoir in egested material observed in our experiments, those of Mosley & Bootsma (2015), and Vanderploeg *et al.* (2009) could be a source of P for the benthos and loss from the pelagic zone. However, the nature of the biodeposits and turbulence in the system will affect whether the egested material is resuspended or becomes part of the sediment (Vanderploeg *et al*., 2002). Furthermore, we must distinguish between nutrients egested in pseudofaeces and those in faeces. We were not able to do this, since our examination of biodeposits was done long after preservation thereby making discernment between faeces and pseudofaeces inexact. Pseudofaeces, often consisting of loosely consolidated seston, can be quite fragile and living phytoplankton in them can remain viable and be resuspended by gentle water motion (Vanderploeg *et al*., 2001, 2002, 2009). Depending on the contents of

pseudofaeces (algae and suspended sediment) they can have differing buoyancies – including positive buoyancy (Vanderploeg & Strickler, 2013; Vanderploeg *et al*., 2013). Therefore, seston composition, including algal species composition and abundance of suspended sediment, combined with turbulence in the system are important factors driving input to the benthos or return to the water column. In our experiments, we used 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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