Excretion of organic matter and nutrients from invasive quagga mussels and potential impact on carbon dynamics in Lake Michigan

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Abstract

The Laurentian Great Lakes have been infested with invasive quagga mussels resulting in unprecedented changes in foodweb structure and ecological function over the past decade. Nevertheless, impacts of quagga mussels on dynamics of carbon and nutrients in the Great Lakes remain poorly quantified. Here, we report yields, composition and size-spectra of dissolved organic matter (DOM) and nutrients excreted from quagga mussels and their potential impact on carbon dynamics in Lake Michigan. Clearance rates of different sized microparticles indicate that quagga mussel ctenidial fibers can efficiently retain particles as small as 0.5-µm. Smaller-sized mussels have higher DOM excretion rates (0.076±0.004 µmol-C/mg DW/d) compared to larger mussels (0.012±0.0002 µmol-C/mg DW/d). Nitrogen excretion rate was up to 0.24±0.01 µmol-N/mg DW/d, 3 times higher than dissolved organic carbon (DOC), while inorganic phosphorus excretion was only 0.0076±0.0030 µmol-P/mg DW/d. Excreted DOM was mostly chromophoric and high-molecular-weight in nature with a colloidal size spectrum centered at 1-5 kDa, had a low C/N but higher N/P ratio, and was comprised of up to 78% carbohydrates with high abundance of structural polysaccharides. Fluorescence EEMs and PARAFAC analysis identified two major fluorescent DOM components: a tryptophan-like and a UVC humic-like, suggesting that excreted DOM could be potentially labile. Compared with field measurements, only ~12% of organic matter consumed by quagga mussels is excreted/egested, and the vast majority is likely respired as CO_2, potentially contributing to an increase in CO_2 concentration in the water column and changes in carbon dynamics in Lake Michigan after the colonization of invasive quagga mussel.

Keywords: Invasive quagga mussel; dissolved organic matter; nutrient excretion; CDOM; colloidal size distribution; Lake Michigan

Introduction

Since their colonization in Lake Michigan, the invasive quagga mussel (*Dreissena rostriformis bugensis*) has caused unprecedented ecological and environmental changes including a decline in primary production and fish biomass, increasing water clarity, food web alterations, and changes to carbon and nutrient cycling (Binding et al. 2015; Bunnell et al. 2014; Cuhel and Aguilar 2013; Madenjian et al. 2015; Lin and Guo. 2016a,b). Quagga mussels couple the benthic and pelagic systems through filtering pelagic particulate organic matter (POM), including phytoplankton and zooplankton, and excreting/egesting nutrients in the benthos (Schindler et al. 2002). Specifically, quagga mussels have been shown to excrete both particulate and soluble inorganic and organic phosphorus species, ammonia, as well as increasing benthic oxygen consumption (Bootsma et al. 2012; Conroy et al. 2005; Ramcharan and Turner 2010; Mosley and Bootsma 2015). Excretion of phosphorus in the nearshore zone has even been linked to nuisance algal blooms that have forced costly beach closures and facilitated the spread of disease (Hecky et al. 2004; Whitman et al. 2003).

It is also evident that quagga mussels have interactions with different organic carbon (OC) pools, most obvious being the exhaustive consumption of POM resulting in increasing water clarity or Secchi depth (Binding et al. 2015; Kerfoot et al. 2010; Vanderploeg et al. 2010). Studies have suggested that quagga mussels have the capacity to consume over half of the annual net primary production in southern Lake Michigan which has contributed to a decline in forage fish (Egan 2008; Madenjian et al. 2015; Tyner et al. 2015). Although changes in food web structure and ecosystem function in Lake Michigan by quagga mussels and their potential interactions with OC pools are widely recognized, the quantitative role of quagga mussels in the uptake or excretion of dissolved and colloidal organic matter species are largely unknown.

Specific cycling pathways of organic matter and nutrient species, quantitative linkages between benthic and water column properties, and the ultimate fate of consumed organic matter by mussels remain elusive.

Dissolved organic matter (DOM) is the largest fraction of active OC in aquatic systems. Within the bulk DOM pool, the light absorbing fraction known as chromophoric DOM (CDOM) could have higher photoreactivity and has been widely used as a proxy for the bulk DOM pool (Coble 2007; Hur et al. 2006; Zhou et al. 2016). The source, chemical composition and size of DOM could significantly impact ecological function and biogeochemical cycling (Guo et al. 2002; Xu and Jiang, 2013). Further, the composition of DOM can affect heterotrophic metabolism (Lennon and Pfaff 2005). Despite their importance, knowledge of the dynamics of different carbon species in the Great Lakes remains scarce and changes in carbon cycling after the colonization of the invasive quagga mussel are largely unknown. Therefore, understanding the interactions between benthic quagga mussels and the DOM pool is vital for understanding changes in biogeochemical cycling of carbon and nutrient species and thus the impact of invasive species on the Laurentian Great Lakes ecosystem.

Major objectives of this study were to 1) examine filtration rates and retention efficiency of macromolecular organic matter by invasive quagga mussels using model compounds and/or nanoparticles; 2) determine the excretion/egestion rates of DOM by quagga mussels through incubation experiments; 3) characterize the size spectrum and organic composition of excreted-DOM from quagga mussels; and 4) provide insights into a better understanding of the role of invasive quagga mussels in regulating the carbon dynamics in the Great Lakes.

Methods

Sample collections

Quagga mussels were collected on October 1, 2014 onboard the R/V Neeskay with a ponar grab from stations with a water depth of ~50 m in southwestern Lake Michigan. Mussels were thoroughly rinsed with lake water, wrapped in dampened paper towels, placed in plastic bags, and kept in a cooler with ice, but not in direct contact with ice (Baldwin et al. 2002; Conroy et al. 2005). Once in the lab, mussels were kept at ~4°C until habituation for incubation experiments (within 24 h). Bottom water temperature at the collection site was 9.9°C.

Large volumes of surface lake water were collected from an open lake station (43º04.5000'N and 87º46.0230'W) for laboratory incubation experiments. Surface water was directly pumped peristaltically through a 0.4 µm polycarbonate cartridge into acid cleaned carboys. The 0.4 µm filtrate was further ultrafiltered through a 1 kDa ultrafiltration cartridge to remove any colloids and particles >1 kDa or 1.2 nm (Guo and Santschi 2007). The <1 kDa filtrate was then used for mussel excretion experiments.

Incubation experiments to determine clearance rate

Prior to incubation, mussels were habituated to room temperature (22°C) for ~ 24 hours. Mussels were then thoroughly scrubbed with a toothbrush to remove microbes and rinsed with the <1 kDa ultrafiltered lake water. Individual mussels were then placed in beakers filled with 200 ml of ultrafiltered lake water spiked with 2 ppm fluorescently tagged, carboxylate modified polystyrene microspheres (Life Technologies), including 20, 100, 200, and 500 nm, and 1 and 2 µm, one at a time (Table 1), and were covered with aluminum foil to prevent contamination.

A separate incubation experiment combining both 20 and 500 nm microspheres (representing the lower and upper limits of the operationally defined dissolved phase) with different characteristic excitation-emission (Ex/Em) wavelengths was also conducted to examine whether mussels will selectively remove a specific sized particle when multiple sizes were

available (Fig. 1). This was designed to insure differences in clearance rates were a result of particle size, and not due to other factors. Incubations of each microsphere size were done in triplicate with a control with no mussel to assess any potential effects of coagulation and precipitation as well as potential adsorptive loss to the beaker wall.

Incubations were carried out in a dark room to mimic benthic conditions as well as to prevent photobleaching of the fluorophore tags on microspheres. Time series samples were collected at 0, 0.5, 1, 2, 4, 8, and 24 h and began once mussels started filtering. Before sampling, air was gently bubbled into the beaker to homogenize the solution and ensure the beakers had sufficient dissolved oxygen. Microsphere concentration in each sample was determined fluorometrically with a Horiba Fluoromax4 spectrofluorometer targeting the specific Ex/Em wavelength of the fluorescent tag (Table 1). Removal rates were calculated by assuming first-order removal (i.e. $A=A_o e^{(-\lambda t)}$). Removal residence time was taken as $1/\lambda$, where λ represents F/V (F = volume/time in L hr^{-1} and V = volume in L) (Haye et al. 2006). Regressions were performed to generate λ values. An ANOVA analysis was performed to determine any difference in clearance rates between microsphere size classes. Statistical parameters are presented in Table 2.

Excretion of DOM and nutrients

All excretion experiments were carried out in 600 ml beakers filled with 500 ml of ultrafiltered lake water and covered with foil. Cleaned mussels were sorted into three different size classes: namely 5-15, 15-25, and >25 mm. Two replicates of the 5-15 mm class with 20 mussels per beaker, 6 replicates of the 15-25 mm class with 10 mussels per beaker, and 2 replicates of the >25 mm class with 10 mussels per beaker were sampled for a total of 10 replicates. A control with no mussels was also sampled, and no significant differences were found for all measured parameters throughout the incubation. The number of replicates for each

size category was determined based on the size distribution and quantity of collected mussels. Samples for the measurements of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), carbohydrates (CHO), UV-visible absorbance, and fluorescence were filtered through a 0.7 µm GF/F syringe filter (Whatmann). Samples for phosphorus (P) analysis were filtered through a 0.45 µm polycarbonate syringe filter (Whatmann). Dissolved organic carbon and TDN samples were collected at 0, 2, 4, 8, 24, and 48 h, transferred into pre-combusted glass vials, acidified with concentrated HCl to pH ≤2. Samples for carbohydrates, UV-vis absorbance, and fluorescence analysis were collected at 0, 0.5, 1, 2, 4, 8, 24, and 48 h, transferred to acid washed centrifuge tubes. Samples for P were collected at 0, 2, 4, 8, 24, and 48 h, transferred to acid washed, 30 ml HDPE bottles (Nalgene). All samples were stored either in a refrigerator (for DOC and CDOM samples) or a freezer (for nutrient samples) until analysis, usually within one week.

After the incubation, the remaining solution from each beaker was combined, homogenized, and filtered through a pre-combusted 0.7 µm GF/F filter (Whatman) for the analysis of particulate OC (POC), and through a 0.45 µm polycarbonate filter (Millipore) for total suspended solids (TSS), respectively. The filtrate was stored in an acid washed bottle (Nalgene) in a refrigerator for the analysis of DOM size distribution using flow field-flow fractionation.

Excretion rates were converted to mass-specific excretion rates (i.e. µmol/mg DW/ d) using a length-weight relationship from the same region of Lake Michigan (Mosley and Bootsma 2015): $DW = 0.0035 \times L^{2.78}$ where DW denotes dry weight (mg) and L denotes length (mm), which was applied to the average mussel length for each replicate. Removed volumes were accounted for by converting each measured concentration into an inventory (concentration × volume in beaker

when sample was taken) for each time series sample. Thus, plots used to calculate clearance rates consisted of time (h) vs. inventory (μmol).

Measurements of UV-visible absorbance and fluorescence EEMs

UV-visible absorption spectra were measured with an Agilent 8453 spectrophotometer using a 1 cm path-length quartz cuvette over a wavelength range of 190-1,100 nm with 1 nm increments. The absorbance of all samples was less than ≤0.02 at 260 nm and therefore did not require dilution to minimize inner-filtering effects (Zhou et al. 2013). The water blank was subtracted and the refractive index effect was corrected by subtracting the averaged absorbance between 650 and 800 nm (Stedmon et al. 2000). Absorption coefficients at wavelength λ ($a_{(\lambda)}$ in m^{-1}) were calculated as $a_{(\lambda)} = 2.303\ A(\lambda)/L$, where A(λ) is the absorbance at wavelength λ, and L is the cuvette path-length in m. Specific UV absorbance at 254 nm ($SUVA_{254}$) was calculated as $A_{254}/L/DOC$ (DOC concentration in mg-C L^{-1}), resulting in a dimension of L/m/mg-C or m^2/g-C (Zhou and Guo 2012). The linear spectral slope between 350-400 nm ($S_{350-400}$) was calculated to provide information on DOM molecular weight (Helms et al. 2008).

Fluorescence excitation-emission matrices (EEMs) were measured with a Horiba Fluoromax-4 spectrofluorometer. Samples were scanned from excitation wavelength 220-480 nm at 2 nm intervals and emission wavelength 240-600 nm at 1 nm intervals. A 1 cm path-length, quartz cuvette was used for the analysis. A water blank was scanned each day of analysis and EEM regions affected by Rayleigh and Raman scattering peaks were eliminated.

Parallel factor (PARAFAC) analysis was applied to EEM data (a total of 80 samples) using MATLAB software and the DOMFluor toolbox (Stedmon and Bro 2008). Sample matrices were calibrated, corrected, and normalized to maximum fluorescence intensity after the removal of

water scattering peaks. A non-negativity outlier test was performed, and a split-half analysis was performed for model validation.

Measurements of bulk DOC, TDN and carbohydrates

Concentrations of DOC and TDN were measured with a Shimadzu TOC-L analyzer equipped with a TNM-L nitrogen analyzer, using the high temperature combustion method (Guo and Santschi 1997). Three to five replicate measurements of 150 μL each were made for each sample with a coefficient of variance of ≤2%. DOC blanks, including water and instrument blanks, were typically ≤2 μM and the detection limit was ≤1 μM. Calibration curves were produced before each analysis and ultrapure water, internal standards, and certified DOC standards or consensus reference deep ocean seawater (from University of Miami) were measured every eight samples for quality assurance (Zhou and Guo 2012). Total dissolved nitrogen was measured with the 720°C catalytic thermal decomposition/chemiluminescence method. Similar quality assurance/control was applied to TDN analysis. The detection limit was <1 μM. Precision was indicated by a coefficient of variance of ≤2%.

Carbohydrate (CHO) species including monosaccharides (MCHO), dilute acid-hydrolysable carbohydrates (HCl-CHO) and total carbohydrates (TCHO) were measured using a modified version of the 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method (Hung et al. 2001). The dilute acid-hydrolysable polysaccharides (HCl-PCHO) were determined as the difference between dilute acid-hydrolysable CHO (HCl-CHO) and MCHO (i.e. HCl-PCHO = HCl-CHO – MCHO). Dilute acid-resistant polysaccharides (HR-PCHO) were determined as the difference between TCHO and HCl-PCHO (i.e. HR-PCHO = TCHO – HCl-PCHO) (Lin and Guo 2015).

Analysis of dissolved organic and inorganic P species

Total dissolved phosphorus (TDP) samples were digested with persulfate solution at 95° C for 24 h (Lin et al. 2012) and were subsequently measured using the standard phosphomolybdenum blue method using a spectrophotometer (Agilent 8453) and a 5 cm cuvette at a wavelength of 882 nm. Dissolved inorganic phosphorus (DIP) was directly measured without digestion. Dissolved organic phosphorus (DOP) was determined as the difference between TDP and DIP (i.e. DOP = TDP – DIP).

Analysis of DOM size distribution

The size distribution of DOM excreted by quagga mussels was determined with an asymmetrical flow field-flow fractionation (FlFFF) system (AF2000, Postnova) coupled online with two fluorescence detectors (Shimadzu RF20A, with Ex/Em at 275/340 nm and 350/450 nm to track protein-like and humic-like DOM components, respectively) and a UV-vis detector (Postnova SPD20A, set at 254 nm). FlFFF is capable of simultaneous separation and characterization of colloids and macromolecules. Detailed calibration and procedures are described in Zhou and Guo (2015). Briefly, a 300 Da ultrafiltration membrane was used in the FlFFF system, allowing any material >300 Da to be analyzed. The relationship between FlFFF retention time and diffusion coefficient (D) was determined graphically using protein standards with known D (Stolpe et al. 2010; Zhou and Guo 2015). The hydrodynamic diameter was calculated from D based on the Stokes relation. Diffusion coefficient was also converted to molar mass (MW) by calibration with polystyrene sulfonate (PSS) standards (Stolpe et al. 2010).

Results

Clearance rates of macromolecules and microparticles by quagga mussels

Clearance rates derived from the 20, 100, and 200 nm spherical particles, which are in the operationally defined dissolved phase, were significantly lower than rates derived from micro-

particles at the larger colloidal size (500 nm) and particulate phase at 1 - 2 µm (Fig. 2a and Table 2). However, similar retention efficiency was observed for the colloidal and particulate sized spheres with no significant difference (ANOVA - $F = 0.868$, $p = 0.452$). Observed clearance rates were within the ranges of previously observed in situ and laboratory-derived rates for quagga mussels (e.g. Baldwin et al. 2002; Bootsma et al. 2012).

Interestingly, the 100 nm sized spheres did not display first order removal kinetics (i.e. the *p*-value of the exponential decay was >0.05) as observed with the 20 nm, 200 nm, 500 nm, 1 µm, and 2 µm spheres (Fig. 2b and Table 2) indicating that filtration rate was not related to particle concentration for these specific colloidal sized particles. Particle concentration actually increased between 8 and 24 h suggesting particles may have initially been retained within the gill filaments and slowly released over time as opposed to being egested as pseudofeces effectively removing them from solution. Therefore, clearance rates were calculated differently by dividing the grazing rate over the 24 h incubation (mg/h) by the concentration of particulate material (mg/L) to achieve dimensions of L /mussel/h. This is a common method of calculating clearance rates (Bootsma et al. 2012) but would be a gross underestimate for 20 and 200 nm spheres and larger sizes, as the majority of removal took place within the first 2 h (Fig. 2b). Clearance rates determined with 100 and 200 nm particles were lower than with 20 nm particles, although the differences were not significant (all $p>0.05$). This suggests that the space between ctenidial filaments of quagga mussels, or their apparent effective "pore size cutoff", is 200 - 500 nm and the low retention efficiency for the 20, 100, and 200 nm spheres was largely due to decreased probability of contact with gill filaments and cilia due to the small size of these spherical particles.

Carbohydrate composition of excreted DOM

As shown in Fig. 3a, the majority of excreted DOM, or 78% of the bulk DOC, consisted of carbohydrates after the 48-h excretion experiment. Within the total dissolved CHO pool (Fig. 3b), polysaccharides including acid-hydrolyzable and acid-resistant PCHO dominated with 60% HR-PCHO and 24% HCl-PCHO. Monosaccharides, on the other hand, comprised only 16% of the total dissolved CHO pool suggesting they are more metabolically important or more easily assimilated by quagga mussels. Conversely, PCHO, especially HR-PCHO which are mostly structural CHO, could be difficult to assimilate by quagga mussels, resulting in a high abundance in mussel excreted-DOM.

Chromophoric DOM and optical properties

In addition to bulk DOC, UV-absorbance and derived optical properties including $SUVA_{254}$ and $S_{350\text{-}400}$, which are related to DOM aromaticity and molecular weight, respectively, were quantified to demonstrate the relationship between bulk DOC and optical properties during quagga mussel excretion. Excretion kinetics of CDOM (in terms of a_{254}) are depicted in Fig 4a for three mussel size classes. As shown in Fig. 4b, bulk DOC was significantly correlated to a_{254}. Based on their relationship, an average non-chromophoric DOC concentration of 13 µM can be estimated for the excreted DOM pool. Although the uncertainties here are large, with the 95% confidence interval ranging from 37 µM to virtually 0, this non-chromophoric DOC comprised only an average of 12% of the bulk DOC pool with a range of 0-34%. In other words, the majority of excreted DOM (88%) was chromophoric. The high percentage of CDOM or lack of non-CDOM components excreted from quagga mussels is somewhat surprising since non-chromophoric DOC in Great Lake waters is normally in the range of 50-60% (Zhou et al. 2016), and aquagenic DOM usually has a lower aromaticity compared to terrestrial DOM (e.g. Gueguen et al. 2005). However, glucose has been shown to contribute to UV absorbance. Given the high

abundance of excreted carbohydrates observed in the present study, it is possible they are responsible for the high CDOM abundance. Regardless, this suggests a potential for quagga mussel excrement to affect optical properties in the overlying water column.

As seen in Fig. 4c, there was no significant change in the aromaticity of excreted DOM over the course of the incubation. However, spectral slope values decreased during excretion, and the spectral slope-derived DOM molecular weight increased at a similar (non-linear) rate as bulk DOC excretion (Fig. 4d). This trend indicates that low molecular weight (LMW)-DOM seems to be assimilated preferentially or does not represent a significant fraction of excreted DOM while high molecular weight (HMW)-DOM was consistently being excreted.

Fluorescent DOM components excreted by quagga mussels

The fluorescence characteristics of excreted DOM are shown in Fig. 5. After 48 h, there was a prominent tryptophan-like peak which has been linked to intact or less degraded, labile peptide material and can be of autochthonous origin or from terrestrial and microbial DOM sources (Fellman et al. 2010). Less apparent are peaks A and C, which are both UVC humic-like, commonly linked to terrestrial sources, and are also the major DOM components in Great Lake waters (DeVilbiss et al. 2016; Zhou et al. 2016). This suggests the majority of fluorescent DOM excreted from quagga mussels is of autochthonous origin and is potentially labile.

PARAFAC analysis was run using EEM data of quagga mussel excrement to generate major fluorescent DOM components (Fig. 5). A split-half analysis validated a 2 component model, with the protein-like component, component 1 (C1, peak Ex/Em = 275/324 nm) being the most abundant, followed by humic-like component, component 2 (C2, peak Ex/Em = 260/450 nm). There was no significant trend of C1/C2 over time indicating that while C1 was approximately twice as abundant as C2, the relative abundance of each component did not significantly change

over time (Fig 6). There appears to be a slight decrease in C1/C2 ratio between 0 and 8 h, and a slight increase after 8 h, however standard deviations were high. This agrees with the lack of change in $SUVA_{254}$ values and indicates that, while DOM increased in quantity during excretion, the composition of optically active DOM, both CDOM and FDOM, excreted by mussels remained relatively constant over time.

Size spectra of excreted DOM

The majority of excreted DOM was found to have a colloidal size range between 1 and 5 kDa, including UV-derived chromophoric DOM and fluorescent DOM in humic-like and protein-like components (Fig. 7). In addition to the 1-5 kDa DOM components, chromophoric DOM also contained a significant amount of the <1 kDa DOM. Excreted humic-like and protein-like DOM, on the other hand, also contained a significant amount of DOM with a colloidal size between 1-100 kDa (Fig. 7). Collectively, DOM excreted by quagga mussels seems to have a normal size distribution pattern with the 1-5 kDa DOM fraction in the summit. Also, the majority of excreted fluorescent DOM expressed protein-like fluorescence indicating that it is potentially less degraded and labile which is in agreement with fluorescence EEM data and PARAFAC analysis.

Carbon and nutrient (N and P) yields during quagga mussel excretion

Organic carbon species, including bulk DOC and TCHO, displayed non-linear excretion kinetics, with an initial rapid release within the first 4 h followed by a slower, steady rate (Figs. 8a and 8b). On the other hand, nutrient species (i.e. TDN and TDP) remained highly linear over the entire 48 h incubation (Figs. 8c and 8d). It is possible that the sharp initial increase in DOC and TCHO concentrations represents excretion during high food conditions, and once mussels have purged what they filtered and retained from the lake prior to collection, excretion rates

decreased representing low food conditions. This would also indicate that the stoichiometry of mussel excrement differs during high and low food conditions, with less OC being excreted compared to nutrients (N and P), perhaps reflecting the metabolic importance of OC during times of low food conditions. However, it is also possible that the steep initial increase in DOC and TCHO concentration was a result of interstitial fluid release, which should have a DOC concentration similar to lake water but higher than the ultrafiltered (<1 kDa) lake water used for the incubation. If this is the case, then the steep initial increase in concentration was a combination of both excretion and interstitial fluid release and the slower rate following 4 h represents a net OC excretion rate.

In order to test this hypothesis, the amount of excess DOC in the interstitial fluid was estimated. The difference in DOC concentrations between lake water (150 µMC) and ultrafiltered water used for the incubation (90 µM-C) is 60 µMC. The volume of interstitial fluid for each size class of the mussels can be estimated based on their average length, width, and height. Assuming 10% of the volume was occupied by interstitial fluid, we estimated that between 3.3 and 19 nmoles of DOC could have been released from interstitial fluid during excretion experiments. This DOC quantity is too small to explain the observed trend, indicating excretion is responsible for the trend in DOC increase (Fig. 8). Regardless, the average of the steep initial rate and slower subsequent rate was used when calculating excretion rates. This trend may not have been observed for nutrients because the difference in the concentrations of nutrient species between lake water and ultrafiltered water are much less than for DOC.

Based on data shown in Fig. 8, mass-normalized excretion rates by different mussel size classes were calculated and are summarized in Table 3 and depicted in Fig. 9 for different carbon and nutrient species including DOC, TDN, MCHO, HCl-PCHO, HR-PCHO, DIP, and DOP. For

all measured species, mass-normalized excretion rates decreased with increasing mussel size, with the 5-15 mm mussel size class having the highest mass-specific rates, followed by the 15-25 mm class and lastly the >25 mm class. This trend is seemingly contradictory, but similar results have also been reported in previous studies and indicate that smaller mussels have higher metabolic rates than larger ones (Mosley and Bootsma 2015; Tyner et al. 2015). Among all measured chemical species, TDN, bulk DOC, and CHO species, including MCHO, HCl-PCHO and HR-PCHO were excreted at the highest rates, with TDN being excreted at a rate 3 times faster than DOC for the smallest size class, while DIP and DOP had the lowest excretion rates (Table 3 and Fig. 9). The excreted DOC/TDN ratios are surprisingly low, averaging 1.1 ± 0.1, whereas the TDN/TDP ratios averaged 32.9 ± 3.8, which is higher than the Redfield ratio but lower than the averaged N/P ratios of lake POM samples (41±13, Peng Lin pers. comm.). Both DOC/TDN and TDN/TDP ratios were significantly lower than the <1 kDa lake water, which averaged 3.3±0.3 and 60±7, respectively (p<0.0001). Since OC could be metabolized and released in the form of CO_2 while N and P converted from DOM will mostly remain in solution, a low DOC/TDN ratio suggests that most of the OC being consumed is being metabolized and respired as CO_2.

Mass specific excretion rates followed a power function relationship (Fig. 10) which has been observed in previous studies (Mosley and Bootsma, 2015; Bootsma, 2009). This highlights the importance of mussel size when inferring the effects of mussel excretion on carbon and nutrient cycling. There have been no reported studies measuring excretion rates of DOC, TDN, and CHO species for comparisons. Recently, Mosley and Bootsma (2015) reported a DIP mass specific rate of $y = 0.001DW^{-0.343}$ for profundal quagga mussels in Lake Michigan, which can be compared with our rate of $y = 0.014DW^{-0.631}$ where DW denotes dry weight in mg.

Discussion

Possible retention and uptake of DOM by quagga mussels

It is well known that filter feeding bivalves have the ability to retain particles smaller than the space between ctenidial filaments as a result of the low angle of approach of particles which increases the rate and likelihood of particle contact with filaments, movement of latero-frontal cirri which produce currents that redirect particles towards the frontal surfaces of ctenidial filaments, as well as cilia that extend out from the gills (Jørgensen 1984; Ward et al. 1998). Therefore, it is possible the space between ctenidial filaments is larger than our observed cutoff of 500 nm. For comparison, studies have demonstrated that particle retention efficiency in the congeneric zebra mussels (*Dreissena polymorpha*) began to significantly decrease at particle sizes of 1.5 µm and lower, suggesting that quagga mussels are more efficient than zebra mussels at retaining small material which may further explain their ability to outcompete zebra mussels in Lake Michigan (Jørgensen et al. 1984; Lei et al. 1996).

There is a possibility that when food is sufficient as in the laboratory, quagga mussel feeding physiology such as increased mucus production or increased ciliary and cirri beat rates are altered to optimize capture rates. Conversely, when only small particles (≤0.2 µm) are present, mussels may have a more difficult time detecting the presence of food due to decreased contact with gill fibers, so mucus production and ciliary beat rates may not increase, resulting in lower capture efficiency as observed. However, there are confounding studies on the plasticity of filter feeding physiology in bivalves (Rosa et al., 2015; Jorgensen, 1996 and references therein).

Studies have demonstrated the ability of zebra mussels to directly assimilate DOM (Baines et al. 2005; Baines et al. 2007; Wang and Guo 2000). This behavior is likely the case with quagga mussels in Lake Michigan, especially under conditions with increasing water clarity and

decreasing phytoplankton biomass and food availability (Binding et al. 2015; Cuhel and Aguilar 2013; Madenjian et al. 2015), but it has yet to be tested. Uptake is an active process dependent on external variables such as salinity, sodium concentrations, or food conditions (Baines et al. 2005, Siebers and Winkler 1984). Despite abundant evidence that dreissenids have the ability to directly assimilate DOM across cell membranes, microspheres used in the present study were inert. Uptake in the microsphere incubations would not occur this way and therefore indicates the ability of quagga mussel ctenidial filaments to retain, to a certain degree, DOM and other dissolved materials, regardless of particle quality.

Organic matter metabolism by quagga mussels

Carbohydrates have been shown to be the most abundant DOM component in natural waters (Hung et al. 2001; Lin and Guo 2015). Main sources consist of phytoplankton excretion and release from ruptured cells following herbivorous grazing. It seems quagga mussel filtration, metabolism, and excretion may be a source of dissolved carbohydrates, especially polysaccharides, as filtration, digestion, and subsequent excretion facilitates their release from cells of the phytoplankton they graze. This is further supported by the large fraction of carbohydrates comprised of HR-PCHO. Acid-resistant PCHO are thought to be comprised mainly of structural polysaccharides such as cellulose which comprises cell walls of certain phytoplankton like dinoflagellates.

The combination of structural polysaccharides and protein-like fluorescence characteristics in quagga mussel excrement suggested that extracellular polymeric substance (EPS) comprised a significant fraction of the excreted DOM. It is possible that quagga mussels lack the endogenous enzymes required to break down the polysaccharides and glycoproteins in EPS, which has been

shown to be biologically refractory (Zhang et al. 2015). Although some studies have reported endogenous cellulase activity in mussels including the zebra mussel indicating their ability to metabolize cellulose, the utilization of cellulose as a carbon source has been observed to be low (<10%); and there are no studies on quagga mussel carbohydrase activity (Langdon and Newell 1990; Palais et al. 2010). This would suggest that a large fraction of ingested structural PCHO is not assimilated and is excreted back into the water column.

Our results verify quagga mussels are highly metabolically efficient, and metabolize the majority of consumed OC into CO_2. This is further supported by the discrepancy between capture rate (CR) and settling rate of organic matter. Mosley and Bootsma (2015) determined that the mean areal CR (estimated as the sum of excretion and egestion) of P by quagga mussels was close to 11 times that of the passive settling rate determined by sediment traps indicating quagga mussels recycle a large amount of P to the water column. However, our calculated CR (estimated as the mean areal excretion + egestion rates for the southern basin, which accounts for mussel size distribution over varying depths, normalized to surface area (m^2), using methods in Mosley and Bootsma, 2015) for OC was only 15% of the passive sedimentation rate of carbon in Lake Michigan. However, when CR was calculated using excretion rates of DOC and POC per mussel and not per mg DW (dry weight) averaged over the southern basin, the results were significantly different. Assuming a mussel density of 10,000/m^2 and applying DOC and POC egestion rates of 0.3 and 1.3 µmol C /mussel/ d, respectively, OC-CR was estimated to be 15,700 µmolC /m^2/d, which is 1.3 times the passive settling rate. The large variability between methods can most likely be attributed to the fact that separate DOC excretion rates were calculated for each size category while only one POC rate was calculated and averaged over the total number

of mussels (n = 120) used in all replicates. Regardless, both estimates are significantly less than those observed for P.

A potential explanation for this observation is that while the capture rate of colloids or particles by quagga mussels is higher than the passive settling rate as determined by P estimates, this trend is not observed for OC because the vast majority of captured organic matter is metabolized and respired as CO_2, which was not measured in our incubation experiments. However, the vast majority of TDP, including dissolved organic and inorganic P, is excreted or egested and remains in solution with minimal amounts being allocated to growth and reproduction (Stoeckmann and Garton 1997). These findings also support the high observed TDN excretion rates, which is likely comprised predominately of ammonia, a metabolic byproduct (Stoeckmann and Garton 1997). Stoeckmann and Garton (1997) also determined that metabolism comprises >90% of zebra mussels energy budget. Further, ^{14}C tracer studies showed that a significant amount of DOC consumed by zebra mussels was respired as CO_2, and therefore must have been metabolized (Baines et al. 2005), all in support of our findings.

Potential impact of quagga mussels on carbon dynamics in the water column

To speculate on the importance of mussel grazing, metabolism, and excretion/egestion in Lake Michigan, mass specific DOC excretion rates (Table 3) for each mussel size class were used to estimate annual DOC excretion in southern Lake Michigan in order to compare to existing data and develop a broad sense of the potential effects of quagga musels on the Lake Michigan ecosystem. Specifically, mussel densities and size distributions divided into depth classes of 0-15 m, 16-30 m, 31-50 m, 51-90 m, and >90 m reported in Nalepa et al. (2010) and summarized in Table 4 were used to generate areal DOC fluxes (in µmoles $C/m^2/d$) which were then integrated across the southern basin of Lake Michigan and extrapolated to one year (Tyner

et al. 2015). Excretion of DOC was estimated at $5.9x10^7$ kg C/y in the southern basin, which accounts for ~4% of total OC consumption in southern Lake Michigan estimated using dissolved O_2 consumption (Tyner et al. 2015). Based on these results, 96% of consumed OC would either be respired as CO_2, egested as feces/pseudofeces, converted to biomass, or allocated to reproduction, all indicating a loss of OC from the water column. However, it is acknowledged that many variables were not accounted for in this estimate, and it was performed as a simple comparison to existing data. Similarly, a rough estimate of POC egestion verifies only 8% ($1.0x10^4$ kg-C yr^{-1}) of consumed organic matter is egested. This suggests the majority of consumed OC may be respired as CO_2, supporting observed high assimilation efficiencies which have been suggested as a reason allowing the persistence of quagga mussels under low food conditions in open Lake Michigan (Cuhel and Aguilar 2013). However, POC egestion rates were normalized over the average mass of all mussel size classes because egestion rates were not measured separately for each size class. Regardless, these estimations are consistent with previous studies and confirm quagga mussels have the potential to be a massive sink for OC but a source for CO_2 in Lake Michigan.

The high assimilation efficiency of OC and the release of CO_2 by benthic quagga mussels observed here are consistent with changes in CO_2 dynamics in Lake Michigan after the colonization of invasive mussels (Lin and Guo 2016b). Thus, invasive quagga mussels could have changed carbon dynamics in the water column through metabolizing and respiring the vast majority of their OC intake. Further studies are needed to better understand the specific pathways and mechanisms.

Conclusions

Invasive quagga mussels appear to have the ability to physically retain smaller dissolved and colloidal particles than zebra mussels. The composition of excreted DOM consisted mainly of carbohydrates, specifically structural polysaccharides, suggesting quagga mussels could have altered the composition of the DOM pool in Lake Michigan by selectively retaining and metabolizing fractions of DOM. The excreted DOM also displayed chromophoric and fluorescent properties with protein-like DOM being the major fluorescent-DOM component, suggesting they are susceptible to photodegradation or highly reactivite. The lack of change in aromaticity and the ratio of PARAFAC-derived DOM components over time indicated that while the abundance of CDOM and FDOM increased over time, the composition or quality did not change significantly. Spectral slope and size distribution data indicated that the excreted DOM had typical size spectra centralized at 1-5 kDa. In addition, the excreted DOM seemed mostly HMW-DOM with less LMW-DOM. High excretion rates of TDN compared to DOC, along with the discrepancy between the capture rate of DOC and P suggest that quagga mussels have high assimilation efficiencies and respire most consumed OC to CO_2. During incubation, only ~12% of consumed OC is excreted as DOC and egested as POC, with the majority of OC is being converted to CO_2.

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Table 1

Size and fluorescence excitation/emission wavelengths for microspheres used in clearance rate incubations. All microspheres were carboxylate modified, polystyrene spheres from Life Technologies™.

Microsphere Size (µm)	Color (Ex/Em wavelength (nm))	Cat #
0.02	Nile red (535/575) and red fluorescent (580/605)	F8784 F8887
0.1	Red fluorescent (580/605)	F8887
0.2	Red fluorescent (580/605)	F8887
0.5	Red fluorescent (580/605)	F8887
1	Red fluorescent (580/605)	F8887
2	Red fluorescent (580/605)	F8887

Table 2

Average clearance rates (Cl R, in L/mussel/ d), removal residence times (τ, in h), r^2 values, and *p* values ± 1 SD determined from exponential decay regressions of microsphere concentrations. Note, standard deviations on *p* values are the result of multiple incubations each with their own *p* value and were presented to provide a stronger sense of statistical significance.

Average ± SD	Microsphere Size					
	20 nm	100 nm	200 nm	500 nm	1 µm	2 µm
Cl R	0.144±0.106	0.017±0.005	0.035±.032	5.80±1.83	8.16±2.91	7.71±4.43
τ	94.1±118.0	312.8±123	216.6±132	0.981±0.591	0.658±0.393	0.0795±0.020
r^2	0.932±0.06	0.117±0.07	0.810±0.15	0.939±0.04	0.942±0.04	0.975±0.48
p	0.0015±0.0019	0.4748±0.14	0.0144±0.012	0.0032±0.0038	0.0044±0.0018	0.0003±0.0004

Table 3

Average excretion rates for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), monosaccharides (MCHO), dilute-HCl-hydrolysable polysaccharides (HCl-PCHO), dilute-HCl-resistant polysaccharides (HR-PCHO), dissolved inorganic phosphorus (DIP), and dissolved organic phosphorus (DOP) (all in µmol mg DW/day^{-1} ± standard deviation). Average C/N/P ratios were derived from DOC, TDN, and TDP concentrations after 48 h.

Size Class (mm)	DOC	TDN	MCHO	HR-PCHO	HCl-PCHO	DIP	DOP	C/N/P
5-15	0.076 (±0.004)	0.24 (±0.01)	0.025 (±0.002)	0.041 (±0.009)	0.048 (±0.020)	0.0076 (±0.003)	0.0011 (±0.001)	33/33/1
15-25	0.021 (±0.009)	0.076 (±0.010)	0.0077 (±0.004)	0.035 (±0.007)	0.014 (±0.003)	0.0027 (±0.0004)	$9.9x10^{-5}$ (±0.0002)	39/34/1
>25	0.012 (±0.0002)	0.044 (±0.01)	0.0073 (±0.006)	0.013 (±0.029)	0.0018 (±0.003)	0.0017 (±0.0020)	0.00016 (±0.00010)	32/29/1

Table 4

Density and size distribution of quagga mussels at different depth intervals in Lake Michigan and total lake bottom area for each depth zone for the southern basin of Lake Michigan. Data summarized from Nalepa et al. (2010).

Depth (m)	Total Area (m^2)	No. of mussels in different size classes			
		0-15 mm	15-25 mm	>25 mm	Total
0-15	$1.84x10^8$	458	4,250	58	4,766
16-30	$3.98x10^9$	8,680	70	0	8,750
31-50	$2.04x10^9$	6,016	1,920	64	8,000
51-90	$5.67x10^9$	14,640	345	15	15,000
>90	$5.91x10^9$	4,428	14	18	4,459

Figure Captions

Fig. 1. Clearance kinetics for the 0.02 and 0.5 µm particles. Both size particles were combined in the same experimental beaker and dotted lines represent the control with microspheres but no mussels. Details of the microspheres used are listed in Table 1.

Fig. 2. a) Average clearance rates (± 1 SD) of quagga mussels determined with 0.02, 0.1, 0.2 0.5, 1, and 2 µm polystyrene microspheres and b) clearance kinetics of 0.02, 0.1, 0.2, 0.5, 1, and 2 µm microspheres by quagga mussels used to generate clearance rates. Error bars represent 1 standard deviation.

Fig. 3. Percent composition of monosaccharides (MCHO), dilute-HCl-hydrolysable polysaccharides (HCl-PCHO), and dilute-HCl-resistant polysaccharides (HR-PCHO) in a) Bulk DOC and b) the total dissolved carbohydrate pool in treatments after 48 h of excretion. Non-CHO represents the fraction of bulk DOC not comprised of carbohydrates.

Fig. 4. a) Excretion kinetics of CDOM measured as the absorption coefficient at 254 nm (a_{254}), b) the relationship between a_{254} and bulk DOC (gray dashed lines represent upper and lower limits and red dashed lines represent the 95% confidence interval), c) change in specific UV absorbance at 254 nm ($SUVA_{254}$) over time, and d) change in the spectral slope between 350 and 400 nm ($S_{350-400}$) of excreted DOM by quagga mussels with three different body size ranges, including the 5-15 mm, 15-25 mm and >25 mm groups.

Fig. 5. Examples of fluorescence EEM spectra from excretion experiments with the 5-15 mm quagga mussels showing the DOM characteristics before excretion (0 h - left panel) and after 48 h of excretion (right panel) and the two PARAFAC-derived DOM components, including component 1 (bottom left panel) with Ex/Em = 275/324 nm and component 2 (bottom right panel) with Ex/Em = 260/450 nm. Note the scale is the same for both EEM plots. EEM spectra from all size classes were similar. The PARAFAC-derived components (lower panels) are representative of all DOM samples.

Fig. 6. Changes in the ratio of DOM Component 1 to Component 2 (C1/C2) during the incubation by quagga mussels with three different body size ranges, including the 5-15 mm, 15-25 mm and >25 mm groups.

Fig. 7. Size distributions of DOM excreted by quagga mussels determined by flow field-flow fractionation techniques showing a) chromophoric DOM derived from UV-absorbance, b)

humic-like DOM as quantified by fluorescence Ex/Em of 350/450 nm, and c) protein-like DOM detected by fluorescence Ex/Em=275/340 nm.

Fig. 8. Excretion kinetics of a) dissolved organic carbon (DOC), b) total dissolved carbohydrates (TCHO), c) total dissolved nitrogen (TDN), and d) total dissolved phosphorus (TDP), by quagga mussels with three different body size ranges, including the 5-15 mm, 15-25 mm and >25 mm groups. Linear regressions were not shown for DOC and TCHO in order to highlight the non-linear nature of their excretion kinetics.

Fig. 9. Mass-normalized excretion rates by mussel size class for dissolved organic carbon (DOC), total dissolved nitrogen (TDN), monosaccharides (MCHO), dilute-HCl-hydrolysable polysaccharides (HCl-PCHO), dilute-HCl-resistant polysaccharides (HR-PCHO), dissolved inorganic phosphorus (DIP), and dissolved organic phosphorus (DOP).

Fig. 10. Mass specific excretion rates for a) dissolved organic carbon, b) total dissolved nitrogen, c) monosaccharides, d) HCl-hydrolysable polysaccharides, e) dilute-HCl-resistant polysaccharides, f) dissolved inorganic phosphorus, and g) dissolved organic phosphorus. In e), the equation surrounded by the dashed lined box corresponds to the dashed line regression, which yielded a much stronger relationship than including the small size class replicates. Both are shown for comparison as they yield significantly different equations and r^2 values.

Table 1. Summary of Saffron cod and Arctic cod otolith samples used for age determination and growth curve estimation by region and year. Samples in 2012 were taken by bottom trawl (BT) and surface trawl (ST) during the 2012 Arctic Ecosystem Integrated Survey and in 1976-1979 in the NMFS base-line survey. Regions are separated in central and southern Chukchi Sea and northern Bering Sea.

Region	Year	Gear	Latitude Range	Average Latitude	Number Saffron cod	Number Arctic cod
S. Chukchi Sea	1976-79	BT	65.5 - 68.3	67.1	250	108
N. Bering Sea	1976-79	BT	63.1 - 65.5	64.2	433	316
C. Chukchi Sea	2012	BT, ST	68.3 - 72.0	69.9	150	605
S. Chukchi Sea	2012	BT, ST	65.5 - 68.3	66.9	170	622
N. Bering Sea	2012	ST,	63.1 - 65.5	63.6	301	233

Table 2. Growth parameters (von Bertalanffy) for saffron cod and Arctic cod by region and survey year. Samples in 2012 were taken by bottom trawl (BT) and surface trawl (ST) during the 2012 Arctic Ecosystem Integrated Survey and in 1976-1979 in the NMFS base-line survey. Regions are defined in Table 1.

Region	n	Parameter estimate (ASE) L_∞	K	t_0	r^2	s^2
			Saffron Cod			
1976-79						
Chukchi Sea	250	556	0.141	-0.947	0.86	530
		(410 – 701)	(0.090 – 0.202)	(-1.235 – -0.660)		
Northern Bering Sea	433	514	0.131	-1.612	0.87	505
		(398 – 675)	(0.088 – 0.180)	(-1.918 – -1.311)		
All regions		**560**	**0.121**	**-1.465**	0.86	546
		(446 – 396)	(0.081 – 0.159)	(-0.563 – -0.420)		
2012						
Chukchi Sea[1]	320	438[2]	0.262	-0.398	0.91	646
		(385 – 501)	(0.200 – 0.313)	(-0.470 – -0.333)		
Northern Bering Sea	301	438[2]	0.250	-0.944	0.73	704
		(385 – 501)	(0.172 – 0.483)	(-1.05 – -0.652)		
All regions		**363**	**0.378**	**-0.492**	0.86	675
		(330 – 396)	(0.312 – 0.444)	(-0.563 – -0.420)		
			Arctic Cod			
1976-79						
Southern Chukchi Sea	108	263	0.204	NE[3]	0.65	375
		(213 – 311)	(0.139 – 0.270)			
Northern Bering Sea	316	300	0.208	NE	0.75	418
		(262 – 335)	(0.169 – 0.247)			
All regions		**244**	**0.276**	NE	0.73	489
		(225 – 262)	(0.237 – 0.314)			
2012						
Central Chukchi Sea	605	197	0.324	-1.065	0.57	330
		(166 – 289)	(0.610 – 0.443)	(-1.371 – - 0.759)		
Southern Chukchi Sea	622	221	0.297	-0.895	0.71	346
		(189 – 253)	(0.212 – 0.381)	(-1.077 – -0.715)		
Northern Bering Sea	233	266	0.171	-1.174	0.73	361
		(182 – 450)	(0.051 – 0.393)	(-3.031 – -0.531)		
All regions		**209**	**0.312**	**-0.959**	0.71	351
		(187 – 226)	(0.251 – 0.382)	(-1.113 – -0.807)		

[1] Age data for central and southern Chukchi Sea combined.
[2] Parameter estimate L_∞ is shared between regions due to lack of data for ages 4+
[3] NE = Not estimated. Parameter t_0 set equal to 2012 for all regions due to lack of age 0 data in 1976-79.

Table 3. Approximate randomization tests comparing von Bertalanffy growth curves fit separately to each region for saffron cod and Arctic cod in the Chukchi (C = central Chukchi, S = southern Chukchi) and northern Bering Seas. Regions are defined in Table 1.

Year	Regional comparison	Test statistic[1] Observed: F_{obs}	Under null: $F_R(\alpha) = 0.01$[2]	*Probability* $P[F(x) \geq F_{obs}]$
		Saffron Cod		
1976-79	Chukchi Sea – N. Bering Sea	5.65	2.62	< 0.01
2012	Chukchi Sea[3] – Northern Bering Sea	36.4	5.23	< 0.0001
		Arctic Cod		
1976-79	Chukchi Sea – N. Bering Sea	26.9	5.76	< 0.0001
2012	C. Chukchi Sea - S. Chukchi Sea	4.50	1.90	< 0.005
	C. Chukchi Sea – N. Bering Sea	3.31	2.20	< 0.01
	S. Chukchi Sea N. Bering Sea	2.40	1.70	< 0.01

[1] $F = \frac{(SSE_r - SSE_f)/q}{SSE_f/(n-p)} = \frac{(SSE_r - SSE_f)/q}{MSE_f}$, subscripts *obs* and *R* refer to observed and randomized test statistics, respectively. The test statistic, *F*, is the difference in the residual sums of squares between the fit of the von Bertalanffy growth model to the pooled data set minus the residual sums of squares from the region-specific model. Hypothesis tests are constructed by comparing the observed test statistic, F_{obs}, to the empirical probability density function (pdf) of F_R generated under the null hypothesis by repeated randomization of the data to regions. Test outcomes are reported in terms of the probability, assuming the null hypothesis is true, that the test statistic would have been as large or larger than F_{obs}, i.e., $P[F(x) \geq F_{obs}]$.

[2] Comparison-wise error rate

[3] Central and southern Chukchi Sea age data combined.