Limnol. Oceanogr., 52(1), 2007, 70–78

Dissolved organic matter and persistence of the invasive zebra mussel (*Dreissena polymorpha*) under low food conditions

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Abstract

To determine whether the ability of zebra mussels (*Dreissena polymorpha*) to absorb and metabolize a variety of dissolved organic compounds allows them to persist when food levels are too low to sustain them, we compared how quickly starving mussels lost weight when kept in water with and without natural dissolved organic matter (DOM). Mussels fed a starvation ration of algae were maintained either in filtered Hudson River water or in ultraviolet-treated deionized water with major ions added at concentrations equivalent to those in the Hudson. Both types of water were continually filtered. Zebra mussels lost weight two- to fivefold faster in the absence of natural DOM, consistent with the idea that the uptake of DOM provides a substantial metabolic subsidy to these organisms. Ingestion of bacteria could not account for this effect. We calculate that the DOM subsidy amounted to more than half of the zebra mussel respiratory requirement under the experimental conditions, which is enough to double the length of time that zebra mussels can survive in the complete absence of particulate food. The effect of DOM on zebra mussel metabolism is 1.5- to fourfold larger than that predicted solely from estimates of amino and fatty acid uptake in the Hudson River. Because Hudson River DOM is largely allochthonous in origin, this subsidy could give zebra mussels a distinct advantage over other organisms competing for phytoplankton resources. We postulate that zebra mussels will have the largest effects on freshwater ecosystems that receive substantial inputs of organic matter from the surrounding watershed.

The zebra mussel, *Dreissena polymorpha*, often causes profound changes to the river and lake ecosystems it invades (MacIsaac 1996; Strayer et al. 1999). In particular, their intense filter-feeding can cause substantial reductions in phytoplankton biomass (Caraco et al. 1997). These reductions can negatively affect other species that depend either directly or indirectly on phytoplankton. Conspicuous among these are native filter-feeding bivalves, which have declined precipitously after zebra mussel invasions in several instances (Strayer 1999). Additionally, experiments and observational studies suggest that certain planktivorous zooplankton and fish might also be affected (Pace et al. 1998; Strayer et al. 2004). These effects are reminiscent of those caused by invasions by other filter-feeding bivalves, such as *Potamocorbula amurensis* and *Corbicula fluminea* (Alpine and Cloern 1992; Strayer et al. 1999; McMahon 2002). Although the rapid spread of zebra mussels across North America and Europe can be explained by their free-swimming larval stage, high reproductive potential, and human dispersal vectors (Carlton 1993), it is their ability to drive down phytoplankton biomass while still persisting at high densities that enable zebra mussels and other invasive bivalves to cause long-term structural changes to ecosystems.

Zebra mussels are notoriously resistant to starvation, a trait that undoubtedly contributes to their persistence (Chase and McMahon 1995). To date, this trait has been attributed largely to their metabolic flexibility and effective use of energy stored in tissues (McMahon 1996). However, mass balance considerations suggest that zebra mussels must also have a hitherto unappreciated source of nutrition. Zebra mussels in the Hudson River, for example, have reduced average annual phytoplankton biomass by 85% from 30 μg chlorophyll *a* (Chl *a*) L^{-1} to 5 μg Chl *a* L^{-1} (Caraco et al. 1997) and system-wide primary productivity to only one third of the 120–150 g C m^{-2} yr^{-1} required to support observed zebra mussel production (Strayer et al. 1996). Particulate nutritional sources other than phytoplankton, such as bacteria and detrital particulate organic matter, cannot make up this deficit (Schneider et al. 1998; Frischer et al. 2000). Compounding the problem is the fact that zebra mussels assimilate far less energy from food when forced to filter and sort the high volumes of low-quality suspended matter that typically exist in rivers (Madon et al. 1998).

One potential resource that has received little consideration is dissolved organic matter (DOM), which constitutes by far the largest pool of organic matter in most aquatic ecosystems (Wetzel 1984). Because DOM in lakes and rivers is derived primarily from terrestrial sources (Wetzel 1992), its uptake could represent an external energy subsidy to zebra mussels that allows them to persist despite having depleted their particulate food. Marine bivalves are well

Acknowledgments

P. Lam, S. Palma, R. Stein, B. Rhau, and F. Gallery provided laboratory assistance. C. Graham and three anonymous reviewers provided helpful comments on the manuscript. This research was funded by a grant from the Hudson River Foundation. This is contribution number 1322 of the Marine Sciences Research Center and a contribution to the Institute of Ecosystem Studies.

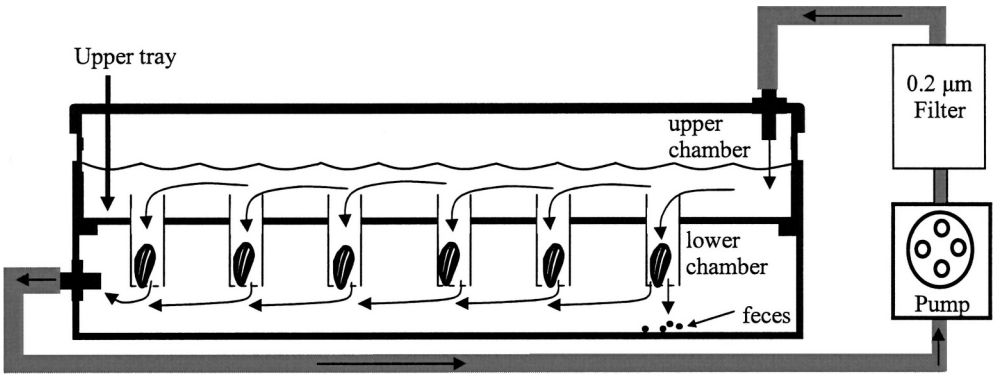

Fig. 1. Schematic of the experimental chambers. Arrows indicate the direction of water flow.

known to take up simple organic compounds, which compose a small proportion of the total DOM pool (Wright and Manahan 1989). However, most freshwater bivalves take up such compounds far less efficiently, if at all—a fact often attributed to the need for a high ambient dissolved sodium concentration to make cross-membrane transport energetically favorable (Wright and Manahan 1989). However, recent work clearly shows that zebra mussels are unique among the freshwater organisms that have so far been studied in this regard; they can take up dissolved free amino and fatty acids very efficiently at natural concentrations (Baines et al. 2005). Significant DOM uptake is also exhibited by larval zebra mussels (Barnard et al. 2006). Furthermore, the estimated amount of DOM taken up by adults is equivalent to 10–50% of the typical ration required for zebra mussels to maintain constant weight (Roditi et al. 2000; Baines et al. 2005).

Accurately assessing the importance of natural DOM to aquatic organisms is more difficult than demonstrating their ability to take up specific compounds in the laboratory. Typically, models based on the uptake of specific organic substances in laboratory experiments are combined with field measurements of those same substances to estimate total DOM uptake rates; these estimates are then compared with an organism's metabolic demands (Wright and Manahan 1989). This approach has produced valuable insight but cannot unambiguously be extrapolated to natural DOM, which comprises a vast number of compounds, many of which remain poorly characterized and quantified. In addition, radiotracer uptake experiments cannot exclude the possibility that any gain via uptake is offset by losses of unlabeled compounds or that uptake is mediated by microbial consortia associated with gill or mantle surfaces and does not benefit the mussels per se.

To better assess the role of natural DOM in zebra mussel metabolism, we determined the effect of exposure to naturally occurring DOM from the Hudson River on an ecologically important physiological endpoint: the ability of zebra mussels to maintain their weight when starved of food. Weight loss by starving mussels maintained in river water containing DOM was compared with that of mussels maintained in artificially produced water that lacked DOM. The differences were then used to deduce the metabolic importance of DOM uptake to adult mussels and to infer potential effects of DOM use on ecosystem dynamics.

Methods

Our study design included two separate experiments. The first experiment was designed to determine whether the experimental setup was adequate to detect differences in zebra mussel condition indices resulting from exposure to different concentrations of DOM. We also wanted to determine whether the condition indices in the different treatments changed linearly over time. Because the results from the first experiment confirmed the adequacy of our experimental design and indicated that the zebra mussels lost weight at a consistent rate over the course of a month, we ran a second experiment using a simpler endpoint design (beginning and ending zebra mussel measurements) that allowed us to double the number of experimental chambers and coincidentally make other measurements. The two experiments used animals and water collected at different times and different experimental chambers, so the combination of experiments gave us statistically independent assessments of the effect of DOM on zebra mussels.

Rocks encrusted with zebra mussels were collected from the Hudson River at the North Germantown, New York, boat landing and were transported immediately back to Stony Brook, New York, in coolers filled with ambient water. In the laboratory, the mussels were carefully removed from the rocks, the shells were thoroughly cleaned, individuals were weighed, and shell lengths were measured. To reduce variability, only individuals with shell lengths of 1.7–2.3 cm were used. Individuals were assigned to one of three to five (depending on the experiment) groups with similar average wet weight. One of the groups (~25 individuals) was sacrificed to determine initial dry weight : wet weight ratios, the contribution of shell to total weight, soft tissue water content, glycogen content, and elemental composition. Before the experiment, mussels were maintained at 19°C and fed on dried *Chlorella* powder (Glenbrook Farms—Herbs and Such) for 3 d.

During the experiment, the mussels were kept in 15-liter polypropylene exposure chambers that were designed to ensure unidirectional flow of water past the experimental animals, to separate the mussels from their feces, and to

Table 1. Concentrations of major ions in Hudson River water and synthetic water.

Ion	Concentration (μmol L^{-1})	
	Hudson River	Synthetic water
Ca	700	700
Na	210	210
Mg	160	180
SO_4	180	180
Cl	600	612

allow the water to be continually and rapidly filtered (Fig. 1). The container was split horizontally into upper and lower compartments, and the mussels were held individually in polypropylene cylinders situated between these compartments. The bottoms of these cylinders were covered with 5-mm polypropylene mesh that retained the mussels within the cylinders and allowed feces to fall away from the mussels into the bottom compartment. Unidirectional flow of water downward past the mussels was achieved by recirculating this water from the lower chamber through a return pipe back into the upper chamber at a rate of $\sim$2.0 L min^{-1}. To ensure that suspended particles in the exposure chambers were kept to a minimum throughout the experiment, the water being returned to the upper compartments was forced through high-throughput 0.22-μm canister filters (Opticap 10″ [25 cm] hydrophilic Durapore polycarbonate, Millipore Corp.). At a pumping rate of $\sim$2.0 L min^{-1}, this system filtered 15 times the volume of the exposure chambers each hour. Experiments were conducted at 19°C.

The exposure chambers were filled with one of two kinds of water. First, Hudson River water (HRW) that had been collected at the same time as the mussels was filtered through 0.22-μm–pore size filter canisters (Milli-pak 200, Millipore Corp.). This water was then stored in the dark at 4°C and filtered again just before use. Filtered HRW contained 3.5–4.0 mg L^{-1} dissolved organic carbon (DOC) and represented the natural DOM exposure. For the exposure treatment without natural DOM, we used synthetic river water (SRW), which consisted of Milli-Q water to which salts were added to reproduce the pH and major ion concentrations typical of HRW (*see Table 1* for additions and target concentrations). The water purification included an ultraviolet (UV) irradiation step that reduced DOC concentrations in the SRW to $<$0.4 mg L^{-1} (measured with a Shimadzu 5000 TOC analyzer). Before use in experiments, the SRW was also passed though 0.2-μm–pore size canister filters. The experimental chambers were filled with 8 liters of either HRW or SRW.

Zebra mussels, like most bivalves, will reduce pumping activity by the gills in the absence of suspended particulate food (Horgan and Mills 1997). Because DOM uptake by bivalves occurs through the gills (Wright and Manahan 1989), such reductions in pumping could bias our results. To keep the mussels filtering to the greatest extent possible, they were fed a daily starvation ration of C*hlorella vulgaris* that had been grown in WCL-1 medium (Guillard 1975). Algal cells were added to polyethylene containers filled with 8 liters of synthetic river water to produce a cell density of 8 $\times$ 10^4 cells mL^{-1}. Mussels were then exposed to the feeding suspension by transferring the top portion of the experimental chamber with the cylinders containing the mussels into the feeding chamber. The mussels were allowed to feed for 4 h, after which the tray holding the mussels was removed from the feeding chamber and rinsed with clean synthetic water before being placed back in the maintenance chambers. The mussels typically cleared half of the algae that were added. We calculated an average ration in units of C of $\sim$0.18% d^{-1}, which is more than fourfold lower than the maintenance ration of 0.77% d^{-1} estimated by Walz (1978*b*).

The first of the two experiments conducted was designed to determine how several condition indices varied over time within and between two treatments: HRW (high DOM) and SRW (low DOM). Water in this first experiment was changed two to three times weekly. This interval allowed the mussels to filter, on average, a volume of water 25–30 times larger than the 8 liters of water in the exposure chamber. This value is similar to the number of times (5–60) that a parcel of water in the lower freshwater Hudson River passes through zebra mussel gills during its 10–120-d transit from Albany to the saltwater front (Roditi et al. 1996). Every week for 7 weeks, four to six mussels were removed for weighing, dissection, and tissue glycogen analysis. Total wet weight (TWW) was measured after blotting mussels dry and subtracted from initial wet weights to determine the individual change in TWW (ΔTWW). Mussels were then frozen in liquid nitrogen, and after thawing briefly, shells were removed from the still frozen soft tissues. The soft tissues were then allowed to thaw and were weighed after wicking away extra fluid to get soft tissue wet weight (STWW). They were then refrozen in liquid N_2, immediately freeze-dried, and weighed again to get soft tissue dry weight (STDW). Shells were weighed after drying for at least 24 h at 60°C. Aliquots of the freeze-dried soft tissues were analyzed for glycogen content (Naimo et al. 1998). Bacterial concentrations were measured weekly with 4′,6-diamidino-2-phenylindole (DAPI) staining and UV epifluorescence at a magnification of $\times$1,000 (Porter and Feig 1980).

Results from this first experiment were analyzed by analysis of covariance, with time as a continuous variable and DOM treatment as the discrete variable. The test of the interaction between main effect terms was used as the test of the null hypothesis that weight loss or condition factors changed at different rates in the two treatments. To determine instantaneous daily loss rates, weight-related variables were regressed against time with exponential decay models fitted with an iterative Marquardt–Levenberg search technique in Sigma Plot 7.1 (SPSS Inc.).

The second experiment used only TWW loss at the end of the exposure period as the response variable. By allowing us to conduct more replicates, this simplification allowed us to address two new issues. First, we wanted to determine whether feeding and filtration behavior differed among DOM treatments in a way that could explain any of the patterns observed. Second, we explored the effect of water replacement frequency on weight loss by the zebra mussels.

On the basis of a measured clearance rate of ~2 L mussel d^{-1} in our chambers (*see below*), the 36 mussels in each chamber could have filtered a volume of water ninefold greater than that of the exposure chamber every day. At that rate, the mussels should have removed >80% of the entire free amino acid pool, excluding glutamate and aspartate, less than a day after each change of water (Baines et al. 2005). Such a depletion is unlikely in situ, where zebra mussels typically filter a fraction of their ambient environment daily (Strayer 1999) and free amino acid pools are quickly turned over through the ectohydrolytic activity of microbes (Taylor et al. 2003). If mussels in our experiments were in fact depleting these labile DOM pools, then the difference in growth rate between the high DOM and low DOM treatments should be greater when the water is replaced more frequently.

Four groups of 35 mussels were uniquely identified and weighed before being placed in four separate chambers. There were three treatments: SRW changed weekly (two chambers), HRW changed weekly (one chamber), and HRW changed daily (one chamber). Mussels were fed a starvation ration for a week before making initial TWW measurements. After 34 d, all mussels were sampled to determine TWW, ΔTWW, STWW, STDW, and shell weight. The instantaneous daily loss rate for TWW was then estimated as $\ln(TWW_t/TWW_0)/t$, where TWW_t is TWW at time t and TWW_0 is initial TWW. Analyses were also conducted for C and N content of soft tissues. Mussel clearance rate (CR, mL h^{-1}) was measured regularly to determine whether it and, by extension, ingestion differed among treatments. On 15 separate days throughout the experiment, we used a Turner AU-10 fluorometer (Turner Designs) to measure the decline in in vivo fluorescence over the course of the feeding period. Ingestion rate (mg C $mussel^{-1}$ d^{-1}) was then calculated by multiplying the amount of algae added to the feeding chamber by the fraction removed. This calculation assumes that no pseudofeces are produced, which concurs with our observations and is reasonable for zebra mussels at the food concentrations used (<0.3 mg L^{-1}; Schneider et al. 1998). Clearance rate was calculated by multiplying the volume of the feeding chamber (V) by the instantaneous rate at which fluorescence (F) declined, $CR = V \times \ln(F_t/F_0)/t$.

We could not use analysis of variance to analyze raw results from the second experiment because of nonnormally distributed residuals ($p < 0.0001$; Shapiro–Wilk test) and unequal variances among the groups ($p = 0.04$; Levene's test for unequal variances). Transforming the data did not solve this problem. Because bootstrapping methods are insensitive to nonnormality and nonequality of variances, we used them to characterize the uncertainty in the estimates of the mean for each of the four experimental chambers (Efron and Tibshirani 1993). We took 10,000 random bootstrap samples, calculated the means for each, and used the 2.5th and 97.5th percentiles of this population of means to denote the 95% confidence intervals around the mean estimates for each chamber. Closer inspection of the data from the second experiment revealed the presence in each of the chambers of one to five individuals that had lost an unusual amount of weight (21–37% of STDW regardless of treatment), presumably because they were dead or dying at the end of the experiment. Weight loss after death is a process unrelated to weight loss from starvation. Moreover, observations of dying individuals disproportionately affected the means of those treatments with slower weight loss. To characterize weight loss related solely to starvation, dead or dying individuals were removed by excluding all points whose residuals were >3.0 times the quartile intervals away from the median. These data were then analyzed by the same bootstrap methodology described above for the unmodified data.

Across the two experiments, there were a total of three replicates for both the high- and low-DOM treatments. Consequently, we were also able to conduct a simple t-test on the mean instantaneous wet weight loss rates for each chamber to test for a consistent effect of DOM across all experimental chambers.

Results and discussion

The experimental mussels in all treatments were clearly starving during and possibly before the experiments. Over the course of the first experiment, STDW declined significantly ($p = 0.01$), as did STWW ($p = 0.017$) and glycogen content ($p = 0.01$; Fig. 2). However, because these variables could only be measured once for an individual mussel (i.e., when it was harvested), it was not possible to correct for initial differences among individuals. Consequently, these variables were prone to high variability that obscured differences between the DOM treatments; the coefficients of variation for STWW and STDW were 24% and 27%, respectively. In contrast, TWW could be measured on an individual both at the beginning of the experiment and at the time of harvest, allowing us to calculate an individual specific rate of weight loss that was not affected by initial differences among individuals. The coefficient of variation for ΔTWW calculated in this way was >10-fold lower, at 1.8%. Because this lower variability afforded us the statistical power to detect the rather small (<10%) but biologically significant differences that we expected to see between the treatments, we will focus most of the remaining discussion on patterns in ΔTWW.

Mussels in the low-DOM SRW treatments always exhibited greater declines in TWW than did those maintained in high-DOM HRW treatments, consistent with the hypothesis that uptake of natural DOM contributes to mussel metabolism (Fig. 3). On the basis of the fitted exponential decay models, the rate of wet weight loss in the low-DOM SRW during the first experiment was 0.17% d^{-1} ($p > 0.0001$, $r^2 = 0.49$), or 3.3-fold higher than the loss rate in the high-DOM HRW, which was 0.051% d^{-1} ($p = 0.02$, $r^2 = 0.14$). In the second experiment, loss rates were indistinguishable between the two low-DOM containers ($p > 0.12$; Fig. 4), averaging 0.14% d^{-1} when dead or dying individuals were excluded. These rates were almost twice as fast as the rate observed in the high-DOM HRW chamber with weekly water replacement (0.08% d^{-1}; $p < 0.0001$; Fig. 4). The effect of DOM on weight loss was even more

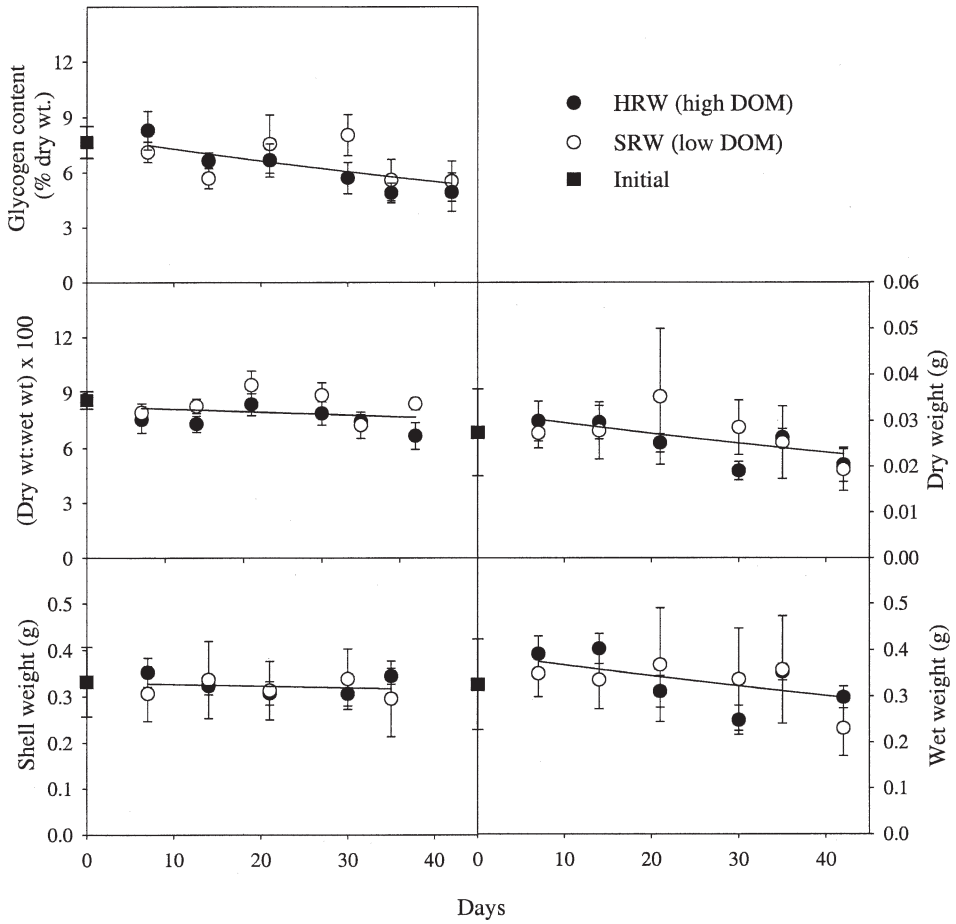

Fig. 2. The change in glycogen content, water content, soft tissue wet (STWW) and dry weight (STDW), and shell weight (SW) over time during the first experiment. Symbols represent means of four to six individuals (±SE). Lines are fitted by regression analysis to the equation $Y = ae^{-bt}$. Individual observations rather than weekly means were used in fitting regressions. p values presented are for H_0: b = 0. None of the slopes differed signifnantly between the treatments ($p > 0.05$).

pronounced in the high-DOM treatment with daily water replacement, with weight loss averaging fivefold less than in the low-DOM chambers (Fig. 4; Table 2). These loss rates did not differ significantly from those in the high-DOM treatment with weekly water replacement ($p = 0.08$; Fig. 4) unless dead or dying individuals were excluded ($p < 0.0001$; Fig. 4).

The observed differences in ΔTWW between treatments implied even larger and more biologically significant differences in soft tissue loss over the course of the experiments. Because shell weight remained constant (Fig. 2) and soft tissues made up on average 37.5% of total mussel wet weight in the first experiment, our results suggest that soft tissues declined at rates of 0.17/0.375 = 0.45% d^{-1} in the low-DOM SRW and 0.0051/0.375 = 0.14% d^{-1} in the high-DOM HRW (Table 2). These rates amount to a total loss over the course of the experiment of 5.6% of soft tissues in the HRW and 17% in the SRW. In the second experiment, loss rates for soft tissues were 0.30–0.32% d^{-1} in the two SRW chambers with weekly water replacement, 0.17% d^{-1} for the HRW treatment with weekly replacement, and 0.06% d^{-1} for the HRW treatment with daily replacement (Table 2). These values amount to total soft tissue losses over the course of the experiment of 9.2% for the low-DOM chambers with weekly replacement, and 5.4% and 2.0% for the high-DOM treatments with weekly and daily replacement of HRW, respectively.

Because of logistic constraints, the two experiments used only one or two independent chambers each for the low- and high-DOM treatments. Hence, it is possible that container-related effects, such as toxicity because of contamination, could lead to the false conclusion that DOM affects weight loss. Two considerations argue strongly against this interpretation. First, measurements of ingestion rate in the second experiment do not indicate systematic differences in mussel activity among treatments, as might be expected if differences in toxicity were causing the perceived differences among treatments (Fig. 4). Second, the results were very consistent between the two experiments, even though they used animals and water collected at different times, used completely new experi-

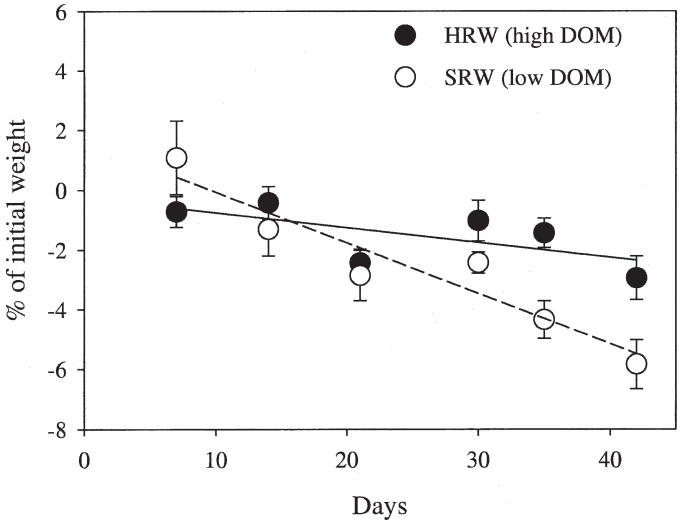

Fig. 3. Percent change in total wet weight (TWW) for individual mussels over the course of the first experiment. Each point is the mean of four to six individuals (±SE). The lines represent best fits of the data to the equation $Y = ae^{-bt}$, with the solid line representing the high-DOM HRW treatment and the broken line representing the low-DOM SRW treatment. Observations from individual mussels were used to generate the regression lines. The slopes are significantly different (ANCOVA $p = 0.0017$).

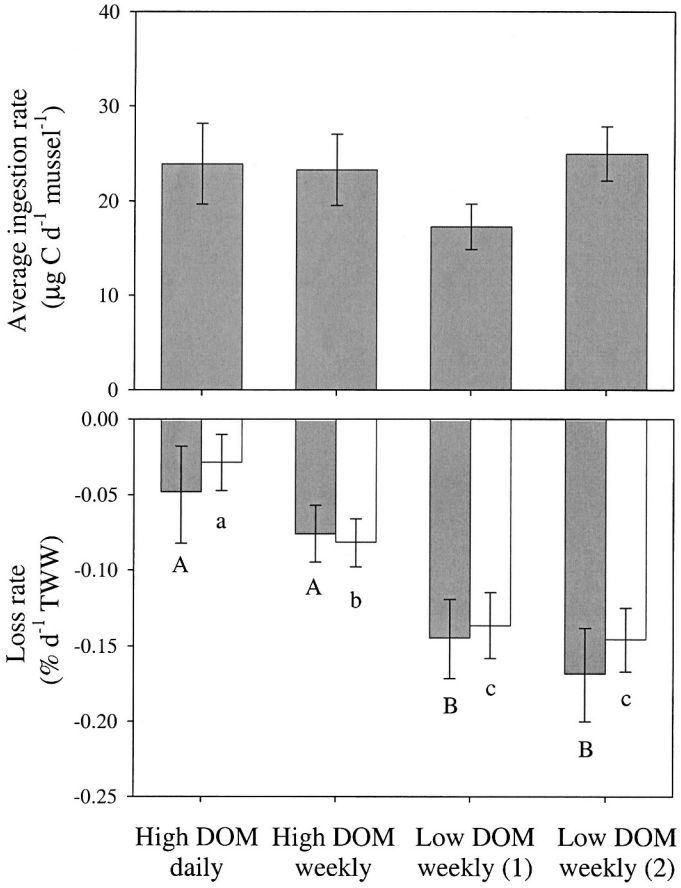

Fig. 4. Differences in ingestion rate (IR) and exponential rate of total wet weight change (ΔTWW) in the second experiment. Results from the two duplicate chambers with low DOM are presented separately. Solid bars on the lower panel represent the data before removing dead or dying outliers from the data. Open bars represent data with dead or dying outliers removed. Error bars are the 95% confidence intervals. Letters in the lower panel indicate significantly different groupings ($p < 0.05$). Uppercase letters refer to tests with the original data, and lowercase letters to tests with dead or dying outlier data removed.

mental chambers, and were conducted more than 6 months apart. Combining the results from both experiments provides us with enough degrees of freedom to make this point statistically. A one-tailed *t*-test on the mean soft tissue weight loss for each container indicates a significant effect of DOM on the rate of soft tissue weight loss ($p = 0.009$), even though differences in experimental conditions among the HRW treatments almost certainly resulted in relatively high background variability and, therefore, a higher chance of falsely rejecting the null hypothesis.

Differences in the abundance of bacteria between the high- and low-DOM treatments also could not have caused the observed differences in soft tissue and total weight loss. In the first experiment, bacteria averaged 4×10^4 cells mL^{-1} across all treatments and were 3.5×10^4 cells mL^{-1} more concentrated on average in the HRW than in the SRW (paired *t*-test $p = 0.07$), probably because of faster growth of bacteria in the presence of DOM. Given a measured mussel clearance rate of ~2 L ind^{-1} d^{-1}, a typical average bacterial cell diameter of 0.6 μm, a 10% filtration efficiency on particles <1 μm in diameter (Frischer et al. 2000), a bacterial C content of 0.26 pg C μm^{-3} (Bratbak 1985), and a maximum carbon assimilation efficiency of 80% (Schneider et al. 1998), we calculate that the average C assimilated by each mussel from bacteria differed between treatments by only 1.7 μg C d^{-1}. Thus, daily assimilation of C from bacteria differed by 0.004% of the initial average C content of the experimental mussels per day. By comparison, the difference between soft tissue loss rates in the HRW and SRW was 0.30% d^{-1}, or 75 times larger. Intertreatment differences in ingestion of algae during the feeding periods were also not responsible for observed differences in weight loss. Ingestion rates for the second experiment determined during the daily 4-h feeding periods were uniform across the treatments ($p = 0.8$) and amounted to 0.18% of mussel biomass on a carbon basis daily across all treatments (Fig. 4; Table 2).

Our results suggest that DOM could be even more important to the zebra mussels in the Hudson River than is apparent in our experiments. Given that the mussels filtered the entire volume of the experimental chamber nine times daily, they should have substantially depleted (>75%) the most bioavailable compounds within a day of exposure to fresh DOM. Hence, we expect that mussels should be able to maintain their weight better when the HRW is replaced more frequently. In accordance with this expectation, the ratio of weight loss in the high- and low-DOM treatments was greatest (5 : 1) when HRW was replaced daily, and lowest (1.7 : 1) when it was replaced

Table 2. Summary of weight loss and metabolic parameters calculated for the two starvation experiments. The number of observations used to calculate each mean is in parentheses. R^*, TD_{50} and Chl* were calculated with the use of mean values for I and L_{STWW}.†

DOM	Water replacement period (d)	STWW : TWW	L_{TWW} (% d^{-1})	L_{STWW} (% d^{-1})	I (% C d^{-1})	R^* (% C d^{-1})	TD_{50} Food (d)	TD_{50} No food (d)	Chl* (μg Chl *a* L^{-1})
Low	3.5	0.375(24)	0.17(36)	0.45(36)			266		
	7	0.46(27)	0.14(31)	0.30(31)	0.16(15)	0.43	401	279	1.9
	7	0.46(27)	0.15(34)	0.32(34)	0.19(15)	0.48	376	253	2.1
High	1	0.46(27)	0.03(35)	0.06(35)	0.18(15)	0.21	1,978	588	0.9
	3.5	0.375(24)	0.05(32)	0.14(32)			885		
	7	0.46(27)	0.08(33)	0.18(33)	0.19(15)	0.32	692	374	1.4

† L_{TWW}, specific loss rate of TWW; L_{STWW}, specific loss rate of STWW; I, measured daily C-specific ingestion rate for the whole chamber; R^*, estimated basal respiration rate calculated according to $R^* = L_{STWW} + (I \times 0.8)$, where 0.8 is the assimilation efficiency (AE) at low ingestion rates; TD_{50}, theoretical median time to death calculated according to $TD_{50} = \ln(0.3)/(L_{STWW}/100)$ in the presence of particulate food or $TD_{50} = \ln(0.3)/(R^*/100)$ in the absence of food (Chase and McMahon 1995); Chl*, the concentration of Chl *a* that should allow mussels to ingest enough C to maintain their weight in the Hudson River: Chl* = $[C_m(R^*/100)]/[CR \times AE(1 - PF) \times C : Chl\ a]$, where C_m is the C content per 20-mm-long mussel (12.6 mg in this study), CR is clearance rate (3.6 L d^{-1}; Roditi et al. 1996), AE is the assimilation efficiency at high particle loads (0.5; Walz 1978*a*; Schneider et al. 1998), PF is the fraction of filtered food rejected as pseudofeces under particle loads typical of the Hudson River (0.67; Schneider et al. 1998), and C : Chl *a* is the ratio for estuarine phytoplankton (50; Wienke and Cloern 1987).

weekly. During the first experiment, an intermediate replacement frequency of 2.5 d resulted in an intermediate weight loss ratio of 3.3 : 1 (Table 2). Replacing water more than once a day would probably better reflect in situ conditions in the Hudson River because the zebra mussel population there does not filter enough water to substantially deplete the most bioavailable dissolved organic compounds (Baines et al. 2005). Our results therefore suggest that weight loss in mussels exposed to DOM that is replaced that frequently would be at least fivefold lower than the weight loss in water without DOM.

By assuming that only monomers, such as amino acids, are available to zebra mussels, past calculations that were based on uptake of radiolabeled substances might have underestimated the overall uptake of DOM by these, and possibly other, bivalves. For example, Baines et al. (2005) estimated that zebra mussels in the Hudson River could take up organic monomers at rates amounting to 0.07–0.18% of the mussel C d^{-1}. We can estimate the specific assimilation rate of natural DOM by zebra mussels in experiments by comparing the rates of soft tissue weight loss in treatments with and without DOM. Such calculations produce conservative estimates of assimilation because they assume that no fraction of the DOM is depleted before water is replaced, that no DOM taken up at all in SRW, and that the energy density of zebra mussel tissue holds constant during starvation when it should in fact decline as stored lipids are used (Walz 1978*b*). We estimate specific DOM assimilation rates of 0.24–0.31% of mussel C d^{-1}, respectively. These rates are 1.5- and fourfold larger than our previously calculated rates of free amino and fatty acid uptake. Furthermore, they amount to 55–68% of the estimated daily respiration rate of 0.46% d^{-1} estimated from TWW loss and ingestion in the SRW treatment during the second experiment (Table 2).

The importance of DOM uptake in our experiments might also be underestimated because the starving mussels filtered less water than is typical for natural populations. Measured clearance rates in the second experiment averaged 78 mL ind^{-1} h^{-1}, or about half the value measured for zebra mussels feeding on natural Hudson River particles (150 mL ind^{-1} h^{-1}; Roditi et al. 1996) and one third the rate expected on the basis of weight-specific rates for zebra mussels fed on Illinois River seston (238 mL ind^{-1} h^{-1} for a 30-mg mussel [dry weight]; Schneider et al. 1998). It is also much lower than our previous observations of 400 mL ind^{-1} h^{-1} observed at 25°C for larger (22–24 mm) healthy zebra mussels collected from the Hudson River and fed living *Chlorella* (Baines et al. 2005). The discrepancies might reflect incomplete mixing of the feeding chambers in these experiments, which can result in the disproportionate "refiltration" of water already cleared of particles and, therefore, reduced ingestion rates. However, it is also possible that the mussels have reduced their filtering activity as a result of their poor physiological state or as an adaptation to the low concentrations of food particles to which they were exposed (Horgan and Mills 1997). In that case, healthier natural populations of mussels exposed to typical particle loads should filter more water than did our experimental organisms. If a fixed fraction of the DOM is removed from filtered water, zebra mussels in the wild should thus absorb more DOM than we infer from our experiments.

Our results indicate that exposure to natural DOM substantially improves the ability of zebra mussels to withstand starvation. Zebra mussels tend to die of starvation after losing 70–75% of their STDW (McMahon 1996). Given the weight loss rates we have observed, zebra mussels exposed to fresh natural DOM and fed a ration that is more than fourfold lower than the maintenance ration reported by Walz (1978c) should take 5.5 yr (~2,000 d) to reach critical weight (Table 2). Replacing the natural DOM seven times less frequently reduces the theoretical time needed to reach critical weight to 692 d, whereas in the absence of DOM, it would take a little more

than 1 yr (396 d). We can also estimate the hypothetical time to death by starvation in the complete absence of food by adding the daily specific rate of algal C assimilation, assumed to be half of the C-specific ingestion rate, to the specific weight loss rates in our experiments. The resulting estimates are 590 d in the high-DOM water with daily replacement, 374 d in the high-DOM water with weekly replacement, and 250–270 d in the low-DOM water with weekly replacement (Table 2). The last estimates are well within empirical observations of 118 and 352 d to death by starvation for mussels starved at 25°C and 15°C, respectively (Chase and McMahon 1995).

Looked at from a different perspective, our results suggest that zebra mussels in the presence of DOM can remain productive even when their phytoplankton food is at very low concentrations. On the basis of the results from the HRW treatment with daily replacement, a zebra mussel exposed to Hudson River DOM and ingesting 0.18% of its C mass in algae daily (80% of which is assimilated) need only assimilate another 0.06% of its weight in C as particulate food to maintain the status quo, assuming that the algal food and mussel tissue has similar energy density. Consequently, we estimate that in the presence of natural DOM, a zebra mussel in the Hudson need only assimilate $0.18\% \times 0.8 + 0.06\% = 0.21\%$ of its weight in C daily to maintain its body mass. Assuming that a typical 20-mm zebra mussel in the Hudson River discards 67% of the particles it filters from the water as pseudofeces (Madon et al. 1998; Schneider et al. 1998), has an organic C content of 12.6 mg, filters 150 mL h^{-1} over the course of a day (Roditi et al. 2000), and assimilates 50% of the algal C it ingests (Schneider et al. 1998; Walz 1978*a*), algal biomass need only reach 38 μg C L^{-1} to allow a zebra mussel to maintain its weight. On the basis of a C : Chl *a* ratio of 50 (Wienke and Cloern 1987), this concentration corresponds to a Chl *a* level of 0.9 μg L^{-1} (Table 2), which is typical of nutrient-poor freshwater environments and is more than threefold lower than the average open water concentrations that have been observed in the Hudson River after the zebra mussel invasion (Caraco et al. 1997). Consequently, zebra mussels in this ecosystem should be able to shunt most of the C and energy they assimilate from particulate food to growth and reproduction even after greatly reducing phytoplankton biomass.

Our results clearly show that zebra mussels lose weight more slowly in the presence of natural DOM than when it is absent. Coupled with studies proving that adult and larval zebra mussels are capable of efficiently absorbing specific dissolved organic compounds (especially amino acids and fatty acids) at environmentally realistic concentrations (Roditi et al. 2000; Baines et al. 2005; Barnard et al. 2006), our results suggest that zebra mussels gain significant metabolic benefits from absorbing DOM directly from the water. This study represents a distinct advance over previous work on the role of DOM in adult bivalve metabolism in that it directly measured the effect of DOM exposure on a physiological endpoint of great ecological importance. In addition, it assessed the effect of natural DOM in all its complexity, rather than focusing solely on those readily identified monomer compounds that typically constitute a small proportion of the DOM pool. It will be a challenge of future research to determine which components of this natural DOM pool that zebra mussels are primarily using.

The ecological consequences of DOM use by zebra mussels depend largely on the degree to which zebra mussels affect the supply of DOM. As in most rivers, the bulk of the DOM in the Hudson is derived from tributaries, runoff from the adjacent watershed, and decomposition of organic detritus from wetlands adjacent to the main stem (Findlay et al. 1998). Because supply of DOM from these sources is not directly affected by the activity of zebra mussels in the river proper, use of this DOM as a nutritional supplement might allow zebra mussels to significantly deplete their particulate food resources without experiencing large negative feedbacks. A similar prediction could be made with regard to many relatively unproductive lakes, in which terrestrial sources of C support much of the respiration and standing stock of consumers (del Giorgio and Peters 1993; Pace et al. 2004). It is unknown whether DOM uptake confers a unique competitive advantage on zebra mussels or on invasive bivalves generally. If so, then the uptake of DOM could help to explain the negative effects that these organisms have on native freshwater taxa. Alternatively, if an important metabolic role of DOM uptake is widespread among freshwater bivalve taxa, it would represent a previously unappreciated nutritional supplement to these organisms that might help explain distributional patterns and population dynamics. It is currently unknown to what degree other freshwater bivalves benefit substantially from uptake of DOM across their integuments. Future efforts should describe the distribution of DOM uptake in taxa that represent the major freshwater lineages, as well as taxa with distinct ecological habits.

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Received: 22 January 2006
Amended: 16 August 2006
Accepted: 3 September 2006