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Uptake of dissolved organic matter (DOM) and its importance to metabolic requirements of the zebra mussel, *Dreissena polymorpha*

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Abstract

We determined the rates at which *Dreissena polymorpha* assimilated radiolabeled acetate, monosaccharides, amino acids, and fatty acids at environmental concentration levels. The mussels incorporated all of the substances presented to them. Much of the ^{14}C-labeled substrate that was taken up was respired to $^{14}CO_2$, indicating that the substrates were used for metabolic purposes. Nonacidic amino acids and fatty acids were taken up fastest, with absorption efficiencies (AE, percentage of filtered substrate removed) of 13% and 85%, respectively. The AEs for monosaccharides (1.5%), acetate (0.2%), and the acidic amino acid glutamic acid (0.79%) were much lower. Among the nonacidic amino acids, nonpolar forms (AE = 19.5%) were preferred over basic and polar neutral forms (AE = 9.3%). On the basis of direct measurements of free amino acid concentrations and literature estimates of free sugars, acetate, and short-chain fatty acids in surface waters, we estimated that direct uptake of these monomers amounts to ~10–25% of the zebra mussel maintenance ration. Direct uptake of dissolved organic matter might be metabolically significant to zebra mussels.

Invasive zebra mussels are capable of causing significant and persistent alterations to freshwater ecosystem structure and function by consuming a large fraction of the available phytoplankton production (MacIsaac 1996; Strayer et al. 1999). Moreover, the mussel populations and their effects can persist even though their high densities and clearance rates allow them to reduce their food resources to low levels. For example, filtration by zebra mussels in the freshwater portions of the Hudson River has reduced the average summertime algal biomass by 85% from 30 to <5 μg Chl *a* L^{-1} (Caraco et al. 1997). In turn, the planktonic primary production in the Hudson has been reduced to one third of the 120–150 g C m^{-2} yr^{-1} required to support zebra mussel production (Strayer et al. 1996). Zebra mussels can filter bacteria to some degree, but the importance of this source of C is probably not large enough to account for a large fraction of their metabolism (Frischer et al. 2000). Other sources of C and energy are necessary to fuel zebra mussel growth and reproduction in the Hudson and, presumably, in other ecosystems as well.

One way that zebra mussels might persist in the face of low particulate food concentrations is through the direct assimilation of dissolved organic substances from water (Roditi et al. 2000). Because most of the bulk dissolved organic matter (DOM) is of allochthonous origin, it also is not subject to the negative feedbacks inherent in typical predator–prey relationships. Thus, direct uptake of DOM by zebra mussels might constitute the kind of unregulated trophic subsidy that can stabilize populations of generalist predators at relatively high abundances, making it possible for them to cause prey levels to remain consistently low or to go extinct without endangering the health of the predator population (Polis et al. 1997). Obviously, competing consumers, such as zooplankton in the case of *Dreissena polymorpha*, might be adversely affected as a result, with significant consequences for the rest of the ecosystem (Pace et al. 1998).

Many marine invertebrates (with the notable exception of crustaceans) actively take up dissolved organic compounds directly from solution (Stephens 1968; Jorgensen 1976; Wright and Pajor 1989). Efficient uptake of amino acids, in particular the neutral and basic forms, appears to be common (Wright and Pajor 1989; Preston 1993), and significant uptake of fatty acids has also been observed (Jaeckle 1995). Marine bivalves transport amino acids across cell membranes against concentration gradients of $1:1 \times 10^7$ mol L^{-1}, with the energy derived from the down-gradient cotransport of several Na^+ ions into the organisms (Wright and Pajor 1989; Preston 1993). In brackish water or freshwater, in which down-gradient cotransport of Na^+ is impossible because ambient concentrations of Na^+ are similar to or less than those in the organism, marine bivalves significantly reduce their gross uptake of amino acids (Matsushima and Hayashi 1988; Matsushima and Yamada 1992) and even temporarily excrete them (Heavers and Hammen 1985; Rice and Stephens 1988), presumably to maintain a healthy intracellular water balance (Pierce and Greenberg 1972; Rice and Stephens 1988). Unless the organism is small, as in the case of lecithotrophic larvae (Manahan et al. 1983*a*; Jaeckle 1995), or the concentrations of dissolved organic molecules are high, as within sediments (Jorgensen 1976), uptake of

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organic substances also is rarely considered to support a large proportion of the organism's metabolic needs.

In contrast to their marine counterparts, DOM uptake by freshwater bivalves has rarely been reported, perhaps because freshwater bivalves typically use only inorganic ions in osmoregulation (Gainey and Greenberg 1977; Deaton and Greenberg 1991). When such uptake is observed, it is often presumed to serve as a means of chemically locating food (Thomas et al. 1984). However, *D. polymorpha* recently has been shown to take up radiolabeled dissolved algal lysates at rates that could be metabolically significant (Roditi et al. 2000). Rough calculations suggested that up to 50% of a mussel's maintenance ration for C could be supplied by such uptake. However, as Roditi et al. (2000) point out, these estimates were based on several assumptions that have not been tested. One such assumption is that the natural DOM will be taken up at the same rate as the algal lysates. This assumption might not be correct because most natural DOM is refractory to biological breakdown, as indicated by bioassays with bacteria (Tranvik 1988), whereas algal lysates are readily utilized by bacteria (Cole et al. 1982). Consequently, the calculations might overestimate the potential importance of DOM–to–zebra mussel metabolism. On the other hand, DOM uptake rates might have been underestimated by Roditi et al. (2000) if particularly labile compounds were completely utilized before the end of their short (2 h) experiments, which might not happen in situ if supply is continuous. Because the depletion of individual compounds was not tracked during the algal lysate experiments, it is impossible to know how important this last source of bias was.

In this paper, we address some of these issues by quantifying the degree to which zebra mussels take up a range of defined radiolabeled dissolved organic biomolecules at environmentally realistic concentrations. Simple laboratory experiments were used to determine whether zebra mussels efficiently remove amino acids, simple sugars, acetate, and fatty acids from solution; whether the mussels prefer certain classes of compounds over others; and whether any of the assimilated organic matter is respired by the mussels. By combining the radiotracer data and clearance rate data for individual mussels, we determined the absorption efficiencies for classes of organic molecules. With the use of these data and dissolved concentrations of these substances, which were either measured or taken from the literature, we then explored whether this pathway of organic matter acquisition could contribute significantly to zebra mussel metabolism.

Methods

Collection and storage of mussels and water—Water was collected from the Hudson River at either North Germantown or Poughkeepsie, New York, near the Mid-Hudson bridge, transported in 20-liter carboys to the Stony Brook campus, and filtered through 0.2-μm Millipak 200 canister filters (Millepore Corp.) into triple deionized-water-rinsed 20-liter carboys. The filtered water was then stored at 4°C in the dark until use. Zebra mussels also were collected from North Germantown in June 2002 and May 2003 and maintained in long-term feeding chambers (Roditi et al. 1996) at 16°C in vigorously aerated 0.2-μm-filtered Hudson River water until used in the experiment. Mussels were fed either rehydrated 40-μm-filtered dried *Chlorella* powder or living *Chlorella vulgaris* cells that had been cultured at 23°C in WCL-1 media (Guillard 1975).

Feeding experiments—Mussels with shell lengths of 2.2–2.4 cm were cleaned thoroughly to remove biota that might contribute to uptake of organic matter. The mussels were then transferred into 100 ml of Hudson River water that had been filtered through 0.2-μm Millipak 200 canister filters and allowed to acclimate for 1 d at room temperature (23°C) without food. After the acclimation/starvation period, nonfiltering mussels were discarded. Because we were primarily interested in assessing DOM uptake into soft tissues, our uptake experiments were designed to disturb the organisms as little as possible, while allowing easy observation of their behavior and quick replacement of nonfiltering animals. Consequently, the experiments were not closed to the atmosphere and were thus subject to loss of respired $^{14}CO_2$. *C. vulgaris* was added to beakers containing mussels to achieve initial cell concentrations of ~20,000 cells ml^{-1}, or about 30 μg C L^{-1}. These values are lower than the incipient limiting concentrations above which clearance rates can be reduced (Sprung 1995). Samples (5 ml) for cell counts were removed immediately and at 10-min intervals thereafter for 1 h and preserved with Lugol's solution. Cell number and cell size in the samples were then analyzed with a Coulter Counter Multisizer II system.

Radiotracer uptake experiments—All glassware in these experiments was autoclaved to reduce overall microbial activity. Hudson River water used in the experiments was sterile-filtered through an autoclaved 0.2-μm Millipak 200 filter (hereafter referred to as sterile-filtered Hudson River water, SFHRW). The mussels were starved for 1 d before the radiotracer experiments to reduce the amount of feces produced during the experiment. To start the experiments, 110 ml of SFHRW was first pipetted into each flask. The mussels were removed from their holding beakers and cleaned thoroughly. Each of the mussels was then transferred to an experimental beaker and allowed to acclimate for at least 1 h before the addition of the radiolabel. Mussels were not used if they did not begin filtering within this time.

In the first experiment, the uptake and transformation of ^{14}C-labeled alanine were followed over the course of 8 h. Uniformly ^{14}C-labeled alanine was added to the chambers with the mussels to a final experimental concentration of 50 nmol L^{-1}. Periodically thereafter, three mussels were removed at a time from the experimental beakers and placed in 250-ml beakers containing 200 ml of unlabeled SFHRW to flush the mussels' pallial space of radiolabeled fluid. After 10 min, the mussels were removed and immediately frozen in liquid nitrogen and stored at −4°C until dissection (<1 week). Two 1-ml aliquots were also taken at each of the time points from the three flasks that contained mussels for the entire 8-h period of the experiment. One of these was placed in a 20-ml vial containing 3 ml of 1 mol L^{-1} NaOH and the other was dispensed into a vial containing 1 ml of 1 mol L^{-1} HCl to volatilize inorganic ^{14}C. Particulate organic

Table 1. Summary of the radiolabeled organic substrates, their specific activities and their concentrations in the experiments.

Class	Compound	Solvent	Specific activity (GBq mmol^{-1})	Concentration (nmol L^{-1})
Nonpolar amino acids	L-alanine	Aqueous, 2% EtOH	5.99	50
	L-phenylalanine	Aqueous, 2% EtOH	17.35	50
	L-proline	Aqueous, 2% EtOH	8.95	50
Polar neutral amino acids	Glycine	Aqueous, 2% EtOH	3.74	50
	L-tyrosine	Aqueous, 2% EtOH	16.91	50
	L-glutamine	Aqueous, 2% EtOH	9.03	50
Basic amino acids	L-lysine	Aqueous, 2% EtOH	11.77	50
	L-arginine	Aqueous, 2% EtOH	12.88	50
Monosaccharides	D-glucose	Aqueous, 3% EtOH	11.32	50
	D-fructose	Aqueous, 3% EtOH	10.47	50
Carboxylic acid	Acetate	Aqueous	2.11	250
Saturated fatty acid	Palmitic acid	Toluene	30.4	1.0
Unsaturated fatty acid	Linoleic acid	Toluene	2.04	20
Acidic amino acid	L-glutamic acid	Aqueous, 2% EtOH	8.81	50

^{14}C was assayed in each beaker after the mussels had been removed by filtering 10-ml aliquots through 25-mm-diameter 0.2-μm polycarbonate membrane filters (Poretics Corp.). The filters were then placed into 20-ml scintillation vials containing 1 ml of 1 mol L^{-1} HCl. After degassing all acidified samples for >12 h, they were alkalized with 3 ml of 1 mol L^{-1} NaOH.

In the other set of experiments, mussels were exposed to a number of radiolabeled organic substrates for ~1 h (Table 1). Uniformly labeled organic substrates (Amersham Biosciences) were used in all cases, except for linoleic acid, which was labeled only at the C-1 position. Radioisotope additions were conducted in the same manner as those for the 8-h alanine experiments, except that the volume of SFHRW in which the isotope was initially dissolved was only 250 ml. The final concentration in all but three cases was 50 nmol L^{-1}. For acetate, low specific activities of the stock solution and the probability of high ambient concentrations led us to use a higher concentration. The final concentrations in the fatty acid experiments were set lower to approximate natural concentrations as closely as possible. Three mussels were assayed for each substrate. The water was sampled following the protocol used in the 8-h experiments, except that dissolved samples were taken at 10-min intervals over the course of 1 h. Mussels were harvested only at the end of the 1-h exposure. To assess the effect of adsorption to glass and of biota brought into the experimental beakers by the zebra mussels, we also included three control beakers for each substrate from which mussels were removed just before addition of the isotope.

We also directly measured adsorption of the two fatty acids onto shell in separate experiments by exposing empty shells to the radiolabeled solutions. To be certain that only the external parts of the shell were exposed to the solution, the space between the shell valves was filled with silicon caulking. These joined valves were prepared in the same way as the whole mussels and exposed to the radiolabeled solution for 1 h, after which they were rinsed in a beaker of 100 ml of SFHRW and placed in a scintillation vial. The shells were then soaked in 5 ml of acetone for 1 d, after which the acetone was evaporated in a hood and scintillant added before counting. Adsorption of fatty acids to the walls was determined by rinsing with SFHRW three times to remove labeled liquid then rinsing three times with acetone. The acetone rinse was collected in a scintillation vial and evaporated before adding scintillant.

Frozen mussels were dissected by allowing the shells to thaw slightly at room temperature before removing them from the still frozen soft tissues. Soft tissues were placed in tared glass 20-ml scintillation vials and allowed to thaw. Excess fluid was removed, and the vials were reweighed. A tissue solubilizer (3 ml, Solvable, Packard Instruments) was added to the tissue samples, which were then kept at 60°C overnight, after which nonsoluble remnants (small parts of shell and byssal threads) were removed, and the solution was cleared with 100 μl of 30% hydrogen peroxide.

Aliquots (10 m) of scintillant (Optima Gold, Packard Instruments) were added to all scintillation vials. The vials were mixed vigorously and allowed to sit in the dark for 1 d before analysis. Samples were then assayed for beta emissions with a Tri-Carb 2100TR (Packard Instruments). Quench correction was by the external standards method. Samples were counted for 15 min, or until the count standard deviation was <1.5% of the count. Counting blanks for the water samples consisted of equal volumes of SFHRW; for filters, they consisted of vials with unused filters; and for mussels, they consisted of dissolved mussel tissue unexposed to the radiotracer. In every case, blanks were at or near detection limits (<30 dpm) and constituted a <1% fraction of the total counts, with the exception of the fatty acid experiments, in which radioactivity was as low as 200 dpm at the end of the uptake period.

Amino acid analysis—Samples for amino acid analysis were collected on 2 October 2002 from three stations in the tidal freshwater Hudson River, New York: Castleton, Hudson, and Poughkeepsie. These stations are 42 km north, 13 km north, and 52 km south of the zebra mussel sampling station, respectively. Samples were filtered in the field through pre-ashed Whatman GF/F filters and collected into glass vials that had been washed in HCl and then ashed at 400°C. Samples were stored frozen until analysis by high-

performance liquid chromatography using methods outlined in Lindroth and Mopper (1979), as modified by Kuznetsova and Lee (2002).

Data treatment—Apparent instantaneous clearance rates (CR, ml h^{-1}) were calculated by multiplying the slope of the linear regression of the natural logarithm of cell concentration (cells ml^{-1}) on time (h) by the number of milliliters in the feeding solution. Instantaneous net radioisotope absorption rates (AR, ml h^{-1}) were calculated, assuming that loss from solution followed first-order loss kinetics. Under this assumption,

$$AR = \ln(\Delta Ac_t/Ac_0 - \Delta Ae_t/Ae_0) \times V \times t^{-1}, \quad (1)$$

where ΔAe_t and ΔAc_t are the change in dissolved organic activity in the experimental and control beakers at time t, Ae_0 and Ac_0 are the initial radioactivities associated with DOM in the experimental and control beakers, V is the initial volume (ml) of the experimental container, and t is time (h). Where the final time points deviate from the exponential model because of substrate depletion (i.e., for palmitic acid), we used only the first four time points to calculate uptake. Equation 1 accounts for ^{14}C lost to the air, to the sides of the container, and to respiration by microorganisms. The calculation assumes that all loss from the system was due to degassing of $^{14}CO_2$. This assumption seems reasonable because other fates of ^{14}C, such as adsorption to shell material, were largely determined to account for <1% of the inventory (see Results). Absorption efficiency, AE (%), of ^{14}C was calculated as 100 × AR/CR. Carbon in soft tissues was estimated by multiplying the soft tissue wet weight by 0.086, the fraction of dry weight after freeze-drying tissues to the wet weight, determined for a set of 20 mussels collected from the same site in the Hudson River. This value was then multiplied by 0.45 to get C in each mussel. The respired fraction, RF, after 1 h is defined as the ratio of respired ^{14}C to the total amount of ^{14}C taken up during the experiment. The respired ^{14}C is calculated by subtracting the activity found in the mussels from the total amount lost from the organic phase over the course of the experiment.

To estimate the importance of DOM uptake to zebra mussels in the Hudson River, rates of dissolved substrate uptake were estimated from the experimental results; measurements of ambient free amino acid concentrations in the Hudson River; and literature values for dissolved fatty acids, monosaccharides, and acetate. In these calculations, we assumed that experimental and ambient concentrations of dissolved organic substances were far below the half-saturation constants for uptake of those substances and thus linearly related to ambient substrate concentrations. Half-saturation coefficients are >1 μmol L^{-1} amino acids and fatty acids (Thomas et al. 1984; Preston 1993; Jaeckle 1995), whereas our additions were always <250 nmol L^{-1} and usually ~50 nmol L^{-1} (Table 1). Ambient concentrations of amino acids, sugars, and short-chain fatty acids are usually in the low nanomolar range and below. Only acetate concentrations typically approach or surpass 1 μmol L^{-1}, but half-saturation constants for acetate also tend to be >1 mmol L^{-1} (Thomas et al. 1984).

Because the relationship between uptake of a substance, U, and its ambient concentration, $[X]$, is nearly linear under our assumptions, we can calculate total uptake by

$$U = AE \times CR \times [X], \quad (2)$$

where AE is the absorption efficiency for a substance and CR is the clearance rate of an individual mussel. It is important to note that this calculation represents a conservative estimate of the actual substrate uptake in situ. Enzyme kinetics requires that AE will, if anything, be lower in our experiments than in nature because adding substrate increases concentrations. However, we estimate this conservative bias should be <10%. Substances were grouped into six categories for the calculation: nonacidic polar amino acids, acidic amino acids, non-polar amino acids, monosaccharides, carboxylic acids, and fatty acids. Measured concentrations of nonacidic amino acids in the Hudson River were used for the amino acid uptake calculations. Uptake of monosaccharides, fatty acids, and carboxylic acids was estimated with the use of data from the literature for similar systems. All r^2 values reported are adjusted for degrees of freedom (adj. r^2; Draper and Smith 1998).

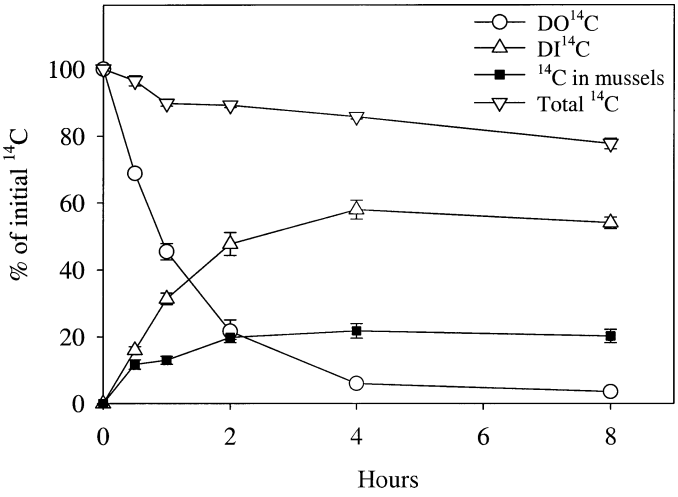

Fig. 1. The percentage of radiolabel in different fractions versus time in the 8-h uptake experiments with alanine. Each point represents the average of three animals. The error bars represent ±1 SE.

Results

Alanine was efficiently removed from solution by zebra mussels in the 8-h experiments. Only 45% of the organic radiolabel remained in solution after 1 h, 20% after 2 h, and 5% after 4 h (Fig. 1). This rate of removal corresponded to an instantaneous clearance rate of 81 ± 4 ml h^{-1} $mussel^{-1}$ according to an exponential model fit to the data (adj. $r^2 = 0.99$, $p < 0.0001$). By the end of the experiment, about 20% of the added isotope was found in the soft tissue of the zebra mussels. Three times more radioactivity ended up in the dissolved inorganic carbon fraction. The overall budget for ^{14}C in the beakers indicated that another 20% of the radiolabel was lost from the system over the course of the 8-h experiment. The amount lost in the rinse phase must have been

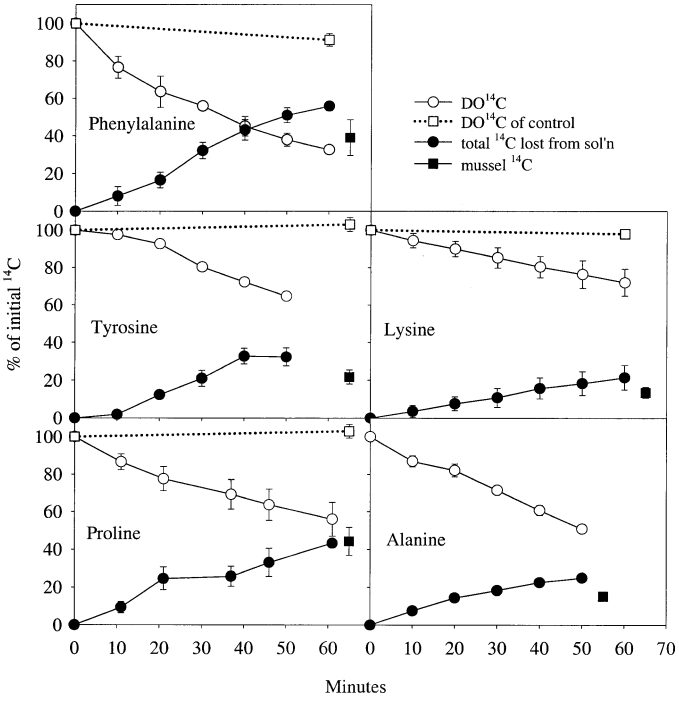

Fig. 2. Uptake of select amino acids by mussels and time series of loss from solution in 1-h experiments. The percentage of all radiolabel lost from solution is calculated by subtracting the percentage of radioactivity that remains in solution from 100. Differences between the percentage of ^{14}C in mussel soft tissues at the end of the experiments (filled squares) and the final percentage of total ^{14}C lost from solution (filled circles) reflect losses of radiotracer from the system. Controls for alanine were lost. Each point represents the average of three animals. The error bars represent ± 1 SE.

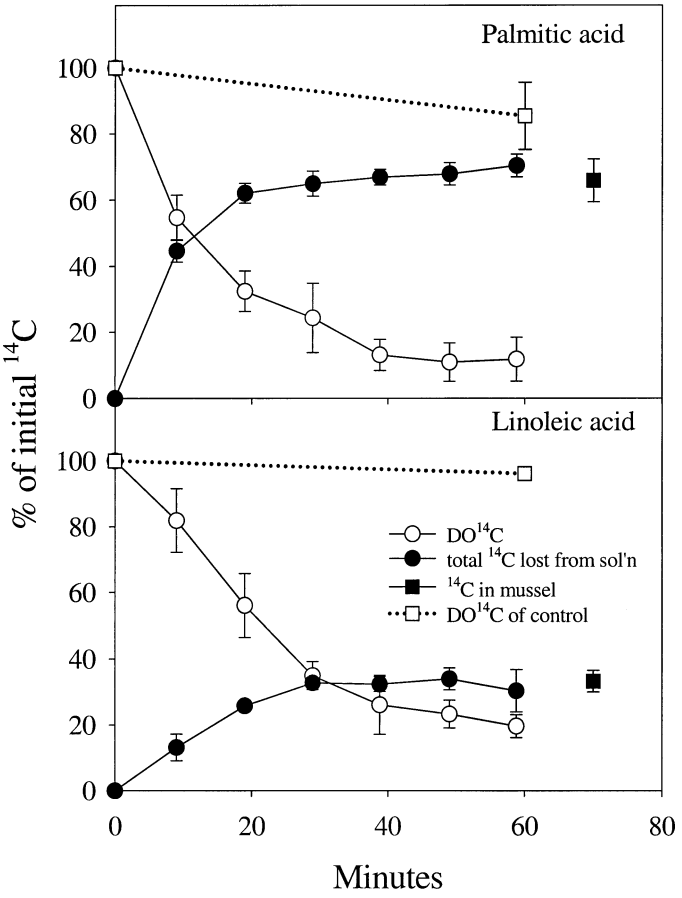

Fig. 3. Uptake of radiolabeled fatty acids by mussels and time series of loss from solution in 1-h experiments. See caption for Fig. 2 for the calculation of percentage lost. Each point represents the average of three animals. The error bars represent ± 1 SE.

$<1\%$ because the volume within the shells was typically ~ 1 ml. The rate of loss from the system corresponded roughly to the amount of labeled dissolved inorganic carbon ($DI^{14}C$) present in the beakers, consistent with the notion that much of the radioisotope loss was from escape of $^{14}CO_2$ to the atmosphere. Adding this 20% of ^{14}C lost from the system to the 60% found in the DIC pool indicates that four times more C in the alanine was oxidized through respiration than assimilated into tissues.

In the shorter term experiments, many of the organic substrates were efficiently removed from solution by the zebra mussels. For the nonacidic amino acids, only 75–35% of the added substrate remained in solution after 1 h of exposure to the mussels (Fig. 2). For the fatty acids, the results were more striking, with $<20\%$ remaining after 1 h of exposure (Fig. 3). Usually the amount of loss from the control beakers was not statistically significant, indicating that almost all of the loss could be attributed to the presence of the zebra mussels. In two instances, however, there were large losses of labeled organic matter, phenylalanine (10%), and acetate (17%). Adsorption of the two fatty acids to glass and shell material accounted for $<1\%$ of the loss of radiolabel from solution. In contrast to the results for the fatty acids and the nonacidic amino acids, $>85\%$ of the radiolabeled organic molecules remained in solution in the beakers containing glucose, fructose, acetate, and glutamic acid. In every case except for the fatty acids, $<1\%$ of the radiolabel inventory could be captured on a 0.2-μm filter. For linoleic acid, 13% of the radiolabel was retained on a 0.2-μm filter and 9% was retained on a 1-μm filter, whereas 30–35% of the palmitic acid was retained on a 0.2-μm filter. In neither case did this ratio change over 1.5 h of exposure to the mussels.

For every substrate, measurable amounts of radiolabel were found associated with mussel soft tissues at the end of the exposures. The percentage of added radiolabel associated with mussel tissues ranged from $<1\%$ for acetate, fructose, glucose, and glutamic acid to $>30\%$ for the fatty acids (Table 2; Figs. 3, 4). The same percentage for nonacidic amino acids ranged from 7.3% for glycine to 24.5% for phenylalanine (Fig. 2). In every case except acetate, a significant fraction of the added radiolabel was also recovered as $DI^{14}C$. In every case except phenylalanine, in which there was much interreplicate variability, the sum of radiolabel incorporated into mussel soft tissues and remaining in solution as either organic or inorganic C was within 10% of the added radiolabel (Figs. 2–4). Because (1) the fatty acid experimental budgets were nearly closed, (2) adsorption of fatty acids to shell was negligible, and (3) adsorption of amino acids and sugars onto shells of freshwater organisms was likely to be

Table 2. Summary of average values for uptake experiments. The numbers in italics below the averages are the SEs.

	Total wet wt (μg)	Soft tissue wet wt (μg)	Length (mm)	Initial activity (dpm)	% ^{14}C in mussel (%)	% DO^{14}C lost (%)	Respired fraction (1 h, %)	Uptake rate (ml h^{-1} mussel^{-1})	Clearance rate (ml h^{-1} mussel^{-1})	Absorption efficiency (%)
Acetate	1,317	430	23.0	6,376	0.77	0.77	na	0.8	396	0.21
	99	*39*	*0.4*	*18*	*0.10*	*0.10*		*0.1*	*66*	*0.05*
Fructose	1,377	316	22.8	26,870	0.42	3.73	88.8	3.8	388	1.05
	122	*15*	*0.2*	*96*	*0.10*	*0.56*	*2.3*	*0.6*	*56*	*0.24*
Glucose	1,299	389	22.8	124,670	0.64	5.80	88.4	6.0	354	1.74
	53	*16*	*0.2*	*511*	*0.04*	*0.67*	*1.4*	*0.7*	*41*	*0.28*
Alanine	1,337	421	23.0	15,004	13.35	49.06	72.9	81.1	537	15.12
	76	*15*	*0.2*	*89*	*1.07*	*1.70*	*1.3*	*4.0*	*30*	*0.54*
Arginine	1,511	408	22.2	42,039	19.80	40.04	50.2	52.1	490	10.96
	320	*63*	*0.4*	*171*	*2.37*	*5.76*	*1.6*	*10.0*	*107*	*1.14*
Glutamic acid	1,305	370	22.7	29,325	0.45	0.91	49.2	1.8	188	0.85
	94	*53*	*0.3*	*299*	*0.26*	*0.53*	*0.0*	*1.0*	*26*	*0.40*
Glutamine	1,393	412	23.2	27,801	10.61	33.08	67.9	40.2	593	6.82
	49	*41*	*0.2*	*137*	*0.77*	*0.31*	*2.3*	*0.5*	*40*	*0.38*
Glycine	1,309	335	22.5	12,712	7.35	14.95	49.2	16.3	291	5.59
	85	*129*	*0.4*	*257*	*1.44*	*2.92*	*0.0*	*3.4*	*24*	*1.03*
Lysine	1,260	378	22.5	34,147	13.60	23.06	38.5	26.9	347	7.54
	52	*16*	*0.2*	*170*	*2.90*	*6.27*	*4.6*	*8.1*	*26*	*2.05*
Phenylalanine	1,444	555	23.7	54,284	39.04	66.78	41.8	110.6	436	25.35
	112	*130*	*0.5*	*4,604*	*9.52*	*2.09*	*13.3*	*6.3*	*4*	*1.25*
Proline	1,280	392	22.7	24,125	28.99	51.75	45.2	75.5	456	17.98
	56	*14*	*0.2*	*59*	*6.80*	*9.93*	*3.6*	*18.9*	*58*	*5.68*
Tyrosine	1,304	361	23.0	51,042	21.77	29.84	27.7	42.6	319	15.77
	44	*25*	*0.5*	*1,846*	*3.70*	*2.05*	*8.4*	*3.5*	*88*	*4.99*
Linoleic acid	1,448	434	23.1	493	30.8	71.1	61.6	234.3	345	73.1
	142	*43*	*0.3*	*9*	*1.9*	*7.06*	*1.8*	*18.9*	*26*	*16.0*
Palmitic acid	1,247	374	22.8	3,053	65.4	88.9	26.5	330.5	320	94.8
	66	*20*	*0.2*	*536*	*4.4*	*5.8*	*0.5*	*42.9*	*20*	*7.7*

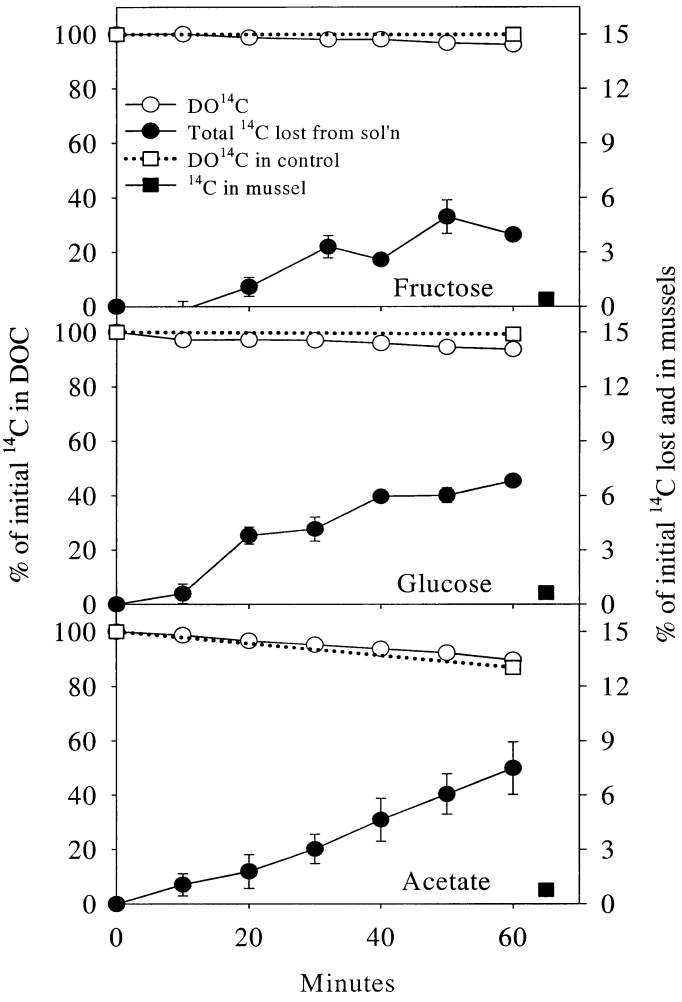

Fig. 4. Uptake of radiolabeled acetate and monosaccharides by mussels and time series of loss from solution in 1-h experiments. See caption for Fig. 2 for calculation of percentage lost. Each point represents the average of three animals. The error bars represent ±1 SE.

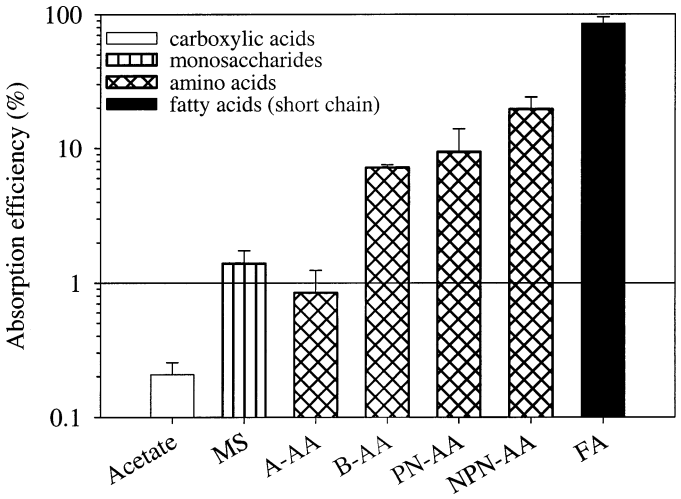

Fig. 5. Averages of absorption efficiencies for the classes of organic molecules assayed in this paper. Values are averages of the means for each substance. MS, monosaccharides; A-AA, acidic amino acids; B-AA, basic amino acids; PN-AA, polar neutral amino acids; NPN-AA, nonpolar neutral amino acids; FA, fatty acids. Error bars represent either ±1 SE ($n = 3$) or the range of observations ($n = 2$). For acetate, the standard error for the replicate flasks is used.

even less given their high solubility in water (Thomas et al. 1984), the <10% of missing ^{14}C probably resulted from loss of aqueous CO_2 into the gas phase. Thus, the total amount of $^{14}CO_2$ produced by the mussels was determined by adding the total loss of ^{14}C from the system to the $DI^{14}C$ measured at the end of the experiment.

The 1-h respired fraction (RF) for amino acids averaged 54% (±13%), with a range between 39% (tyrosine) and 73% (alanine and glutamic acid; Table 2). The RFs for glucose (92.8% ± 1.4%) and fructose (89.3% ± 1.7%) were substantially higher than those for the amino acids. Palmitic acid, which is a saturated fatty acid, had an RF of 26% (±0.5%), which was significantly less than the RF of 62% ± 3.4% for linoleic acid, an unsaturated fatty acid ($p < 0.0001$; two-tailed *t*-test). Because of a tendency for a greater fraction of the less assimilated compounds to be respired, variability in the AR (ml h^{-1} $mussel^{-1}$) was less than that of radiolabel uptake into mussel tissues. The percentage uptake into mussel tissue varied 31-fold between amino acids and sugars, for instance, but the AR for sugars was only 10-fold less, at 5 ml h^{-1}, than the 55 ml h^{-1} AR for amino acids. Still, absorption rates varied widely, ranging from ~1 ml h^{-1} $mussel^{-1}$ for glutamic acid and acetate to nearly 300 ml h^{-1} $mussel^{-1}$ for the fatty acids. Differences between the nonacidic amino acids also are apparent. The AR for nonpolar amino acids averaged 89 ± 9 ml h^{-1}, or more than twice the value for the basic and the nonpolar neutral amino acids at 39 and 33 ± 8.4 ml h^{-1}, respectively. Absorption efficiencies were 1–2% for the sugars, 5.6–25% for the nonacidic amino acids, and 73–95% for the fatty acids (Table 2; Fig. 5). Glutamic acid had the lowest absorption efficiency at 0.78%. The absorption efficiency including respiration for acetate could not be determined because the loss of organic radiolabel from the experimental beakers did not differ from the loss observed in the control beakers.

Total free amino acid concentrations at Castleton, Hudson, and Poughkeepsie were 152, 240, and 172 nmol L^{-1} (9.0, 10.9, and 8.1 μg L^{-1}), respectively. Riverwide averages of total dissolved free amino acids (DFAA), acidic DFAA, and nonacidic DFAA were 188 ± 38, 21.6 ± 4.3, and 167 ± 33 nmol L^{-1} (9.2 ± 1.1, 1.1 ± 0.21, and 8.1 ± 0.9 μg L^{-1}), respectively. Although there were longitudinal shifts in the predominance of certain amino acids, the balance among the main groups of amino acids was rather consistent. Nonpolar neutral amino acids dominated the DFAA pool throughout the river, making up >75% of the DFAA. The next most important class was the polar neutral amino acids, followed by the basic and acidic forms.

Discussion

The observed uptake of DOM by zebra mussels is not likely to result from artifacts associated with the radiotracer

technique. Adsorption to shell and glass in the fatty acid control experiments was minimal. Excretion of DON or amino acids by marine bivalves is typically undetectable when incubation times are short (Manahan et al. 1982). Marine bivalves generally have impermeable membranes and release organic substances only when reductions in ambient salinity require some control of cell volume (Preston 1993), although *D. polymorpha* appears to have membranes that are more permeable with respect to inorganic ions at least (Dietz et al. 1997). Consequently, uptake rates of radiolabeled organic substances usually agree well with measurements of net uptake (Manahan et al. 1983*a*,*b*; Stephens 1988). The radiotracer uptake of the amino acids, acetate, and sugars also was not a result of ingestion of particles (e.g., bacteria) that had incorporated the radiolabel. The amount of radiolabel captured onto 0.2-μm filters for these compounds was always $<1\%$ of the total dissolved inventory. Even presuming a 100% filtration efficiency for all particles regardless of size, potential particle uptake was always $<10\%$ of observed uptake. Other workers who used radiography have also shown that ^{14}C-labeled organic compounds are taken up into the epidermal tissues of bivalves and not into bacteria living on tissue surfaces (Jordan and Valiela 1982; Manahan et al. 1982; Siebers and Winkler 1984). Abiotic adsorption onto mucous surfaces is also very unlikely given that dead freshwater organisms do not accumulate organic substances at rates approaching those of live organisms (Testerman 1972; Thomas et al. 1984).

It is possible that the fatty acids in these experiments were not truly dissolved and that some uptake of these substances was by filtration of particulate matter from the water column. The water solubilities of longer chain fatty acids are low, especially at low temperatures. Recently, the spontaneous production of micelles in the 0.4–0.8-μm size range from the hydrophobic portion of sterilized DOM has been observed (Kerner et al. 2003). Crystallization of fatty acids from the toluene carrier solution might also have occurred after addition. We found that $\sim$35% of the labeled palmitic acid and $\sim$13% of the linoleic acid in our experiments were removed by 0.2-μm filters, and marginally smaller fractions by 1.0-μm filters. Studies with serially aligned 0.2-μm filters indicated that this fraction could not be removed by filtration, suggesting that the radiolabeled substances were either adsorbing to the filters or quickly reforming ''particles'' during the few seconds between passage through the two filters. In the unlikely event that this filterable fraction represents particles that could be filtered by zebra mussels with 100% efficiency, filtration of this material could account for 18% of the uptake we observed for linoleic acid and $<37\%$ of the uptake of palmitic acid, resulting in corrected absorption efficiencies of about 60% for both fatty acids. However, because many of these particles were probably <2 μm in diameter, and thus filtered at $<100\%$ efficiency by the zebra mussels (Lei et al. 1996), filtration of particulate fatty acid probably accounted for less of the total uptake. In any case, it is also worth noting that ingestion of fatty acids in micelles or nonliving precipitates is an unrecognized source of organic matter and energy to zebra mussels and other filter feeders (Kerner et al. 2003).

The efficient removal of amino acids by zebra mussels is surprising given what we know about the mechanisms of amino acid uptake in marine invertebrates. This uptake occurs against a $>10^7:1$ concentration gradient (Wright and Pajor 1989; Preston 1993). Manipulations of internal and external [Na^+] appear to indicate that the energy needed to fuel this uptake is derived from the cotranslocation of Na^+ down the $>10:1$ concentration gradient that exists across cell membranes in marine habitats (Wright and Pajor 1989; Preston 1993). In freshwater habitats, however, the gradient in Na^+ across the membrane goes in the reverse direction, suggesting that this exact mechanism cannot support organic substrate transport in zebra mussels. Indeed, the brackish water clam *Corbicula japonica* essentially stops transporting alanine across the gill surface when exposed to near-freshwater conditions (Matsushima and Hayashi 1988; Matsushima and Yamada 1992). Clearly, another mechanism would be needed to explain uptake of amino acids at such low ambient concentrations of Na^+ in low-salinity waters. Such a mechanism might not be limited to the zebra mussel. The uptake of amino acids, acetate, glucose, and fatty acids have been noted in pulmonate snails (Thomas et al. 1984), tubifex worms (Hipp et al. 1986*a*,*b*; Hoffmann and Wulf 1993), and the freshwater clam *Pisidium* (Efford and Tsumura 1973). What separates the zebra mussel from these other organisms is the ability to pump large volumes of water past the gill surfaces that are responsible for organic matter absorption.

The ability of zebra mussels to take up both lysine (a basic amino acid) and alanine (an alpha neutral amino acid) suggests that zebra mussels, like *Mytilus*, are utilizing a range of uptake enzymes to allow for uptake of a wide range of amino acids (Wright 1985). Zebra mussels seem to be somewhat less efficient at removing amino acids from water that passes their gills than are some marine bivalves, however. Comparisons of amino acid concentrations in intake and expelled water of the mussel *Mytilus californicus* indicate that 36–94% of the amino acids aspartic acid, glycine, and serine were removed after a single pass through the mantle cavity (Manahan et al. 1982). The highest absorption efficiency for amino acids observed in our study was about 25%. It is possible that our lower estimates of uptake efficiency were caused by reduced clearance rates under the experimental conditions in which no food particles were present. Zebra mussels can reduce filtration and pumping activity when exposed to very low or zero ambient food concentrations, presumably to save energy (Horgan and Mills 1997). However, all the mussels observed in this study were pumping actively, or they were not used.

Zebra mussels take up fatty acids from the ambient environment more efficiently than they do amino acids. Uptake of free fatty acids from the ambient environment has rarely been assayed. Jaeckle (1995) also observed that larvae of the sponge *Tedania ignis* took up palmitic acid from a 1-μmol L^{-1} solution at much faster rates than they took up alanine at the same concentration. The uptake of these fatty acids might be facilitated by their hydrophobic character, which could cause the fatty acids to be more particle reactive and more easily transported through the cell membranes of the animal. It is apparent from the amount of absorbed fatty acid that was respired ($>25\%$) that the molecules did not remain in the membrane but were available for respiratory

Table 3. Calculations of net C uptake by zebra mussels and importance to metabolism in C units. AE = absorption efficiency. Maintenance ration was determined for a 15.5 mg C mussel according to Walz (1978).

Substrate	AE (%)		Concentration (μg C L^{-1})		C uptake (μg d^{-1})		Contribution (% of maintenance ration)	
	Low	High	Low	High	Low	High	Low	High
Acetate	0.2		2.4	240	0.03	3.4	0	2.8
Monosaccharides	1.5		7.2		0.8		0.7	
Acidic amino acids (cationic)	1.0		1.2		0.09		0.1	
Basic amino acids (anionic)	10		0.8		0.56		0.5	
Polar neutral amino acids	10		2.5		1.8		1.5	
Nonpolar amino acids	20		5.4		7.8		6.5	
Fatty acids	60	85	0.5	2.5	2.2	15.3	1.8	12.8
Total					13.3	29.8	11.1	24.9

metabolism. Past work has shown saturable kinetics for fatty acid uptake by invertebrates (Testerman 1972; Thomas et al. 1984; Jaeckle 1995). Because many fatty acids have a higher caloric content than amino acids, uptake of fatty acids could be important metabolically to mussels, even though free fatty acid concentrations in situ are typically lower than those of amino acids. It is uncertain whether uptake of fatty acids bound to glycerides would be taken up as readily as free fatty acids.

Much of the added radiolabel was recovered as $DI^{14}C$, indicating respiration of the adsorbed organic substrate. Except in the case of acetate and phenylalanine (where interreplicate variability was high), the production of $DI^{14}C$ was significantly greater than observed in control beakers without mussels, indicating that mussels caused the production of $DI^{14}C$ in every case. This was most evident for the substrates that were most efficiently absorbed (i.e., the nonacidic amino acids and the two fatty acids). The long-term uptake experiment with alanine indicated that this respiration of organic radiolabel mostly occurred within the first 2 h of uptake. Over the remaining 6 h, little of the radiolabel remaining in the animal was respired. No measurements were made of radiolabel incorporation into protein. However, in marine bivalves, ^{14}C from amino acids directly absorbed from water is often found throughout the body of the mussel after a couple hours of exposure (Rice and Stephens 1987). It is likely that the radiolabel that remained in the mussel during the long-term alanine experiment was also incorporated into proteins and, therefore, less available as a substrate for respiration. Consequently, little production of respired ^{14}C was found after the absorption phase.

Contribution to zebra mussel metabolism—Although we have not determined uptake for the entire range of compounds making up the DOM, we have tested a wide range of labile forms likely to be subject to uptake across the gills. On the basis of these results, we estimated the potential importance of uptake of these substances for zebra mussel metabolism. Some simplifications are required to make such calculations. First, we assume that the average uptake rates that we measured for the various organic substrates are representative of uptake of all substances from their corresponding classes of organic substrate. These classes include (1) acidic amino acids, (2) basic amino acids, (3) polar neutral amino acids, (4) nonpolar amino acids, (5) monosaccharides, and (6) short-chain ($<C_{20}$) nonvolatile fatty acids. We also assume a clearance rate of 400 ml h^{-1} $mussel^{-1}$ from our own measurements (Table 2). This rate agrees well with a prediction of 388 ml h^{-1} from a regression of clearance rate on shell length from the data of Horgan and Mills (1997) but is somewhat higher than predicted by the equations of Kryger and Riisgard (1988) on the basis of dry body weight. This minor discrepancy might result from our use of a freeze-dryer rather than an oven to dry the soft tissues, resulting in a lower dry weight. We have also assumed a filtration period of 18 h d^{-1} (Nichols 1993).

To calculate uptake rates by Eq. 2, we must also have estimates of ambient concentrations of the substrate classes (Table 3). For our purpose here, which is to simply assess if direct uptake of known labile components of ambient DOM could potentially support a significant fraction of zebra mussel metabolism in the Hudson River and elsewhere, we need only approximate concentrations of these substances. Amino acids were measured directly at three stations spanning much of the river inhabited by *D. polymorpha.* For an upper estimate of monosaccharides in the river, we used Yu's (1999) high estimate for glucose concentrations (100 nmol L^{-1}) at Haverstraw Bay, New York, a site that ranges seasonally from brackish to freshwater depending on the flow regime, to calculate uptake of monosaccharides. Yu also provided two independent and very different measurements of acetate in Haverstraw Bay. Standard gas chromatographic analyses of acetate indicated concentrations ranging between 1 and 15 μmol L^{-1}. However, bioassay approaches indicate that the bioavailable pool of acetate was typically $\ll$100 nmol L^{-1}. This discrepancy might reflect a large fraction of acetate bound to dissolved matter that is not bioavailable (Yu 1999). We have taken 10 μmol L^{-1} to represent a high value for acetate and 10 nmol L^{-1} to represent the low estimate.

Data on total dissolved ($<C_{20}$) fatty acids in freshwater rivers are rare, and studies have varied significantly in the analytical methods used and the operational definition of particulate and dissolved fractions (Mannino and Harvey 1999). Moreover, there is little known about seasonal variations in dissolved fatty acids, although there is consensus that the dissolved fatty acid pool is dominated by saturated

molecules ranging between C_{14} and C_{20} in length. Jaffe et al. (1995) reported dissolved (<1.2 μm) fatty acid concentrations ranging from 412 to 2,179 ng L^{-1} (mean = 1,347 ng L^{-1}) for a number of rivers in the Orinoco basin. The mainstem Orinoco site was most similar to the Hudson in dissolved organic carbon (DOC) concentration (4.6 compared with 3.9 mg L^{-1}; Findlay et al. 1998) and exhibited a fatty acid concentration of 2,166 ng L^{-1}. Mannino and Harvey (1999) measured concentrations of fatty acids within the 1-kDa to 0.2-μm size fraction at two freshwater stations in the Delaware ranging from 547 to 2,339 ng L^{-1} (mean 1,096 ng L^{-1}). We have adopted a low value for dissolved fatty acid concentrations of 500 ng L^{-1} and a high value of 2,500 ng L^{-1} to bracket the range of possibilities in our calculations of fatty acid uptake. These estimates might overestimate the available free fatty acids because the analytical methods employed by both Jaffe et al. (1995) and Mannino and Harvey (1999) include fatty acids bound to glycerides.

The substrates that potentially contribute most to the C budget of *D. polymorpha* are nonacidic amino acids, lipids, and acetate. Uptake of amino acids generally amounts to about 10 μg C d^{-1}. Uptake of the nonpolar amino acids constitutes >75% of this total. If the gas chromatography estimate is used for acetate, uptake of this substance could account for another 4 μg C d^{-1}, even without knowing how much acetate was respired by the mussels. If we assume that the bioavailable fraction of acetate is much smaller, however, this value falls to 0.03 μg C d^{-1}. Fatty acid uptake could also account for a significant amount of uptake, in part because of the ability of zebra mussels to take up such compounds very efficiently and in part because of the large amount of C within each fatty acid molecule. A fatty acid concentration of 2.5 μg L^{-1} and an absorption efficiency of 85% uptake of these compounds would be about 15.3 μg C d^{-1}. The uptake is only 2.2 μg C d^{-1} assuming an AE of 60% and a fatty acid concentration of 500 ng L^{-1}, indicating how sensitive our conclusions are to initial assumptions. Direct uptake of the acidic amino acids and the sugars were unimportant sources of C to the mussels, contributing <1 μg C d^{-1}.

The uptake of DOM might contribute a significant fraction to basal zebra mussel metabolism (Table 3). We have estimated a maximum potential flux of 29.8 μg C d^{-1} into the zebra mussels from direct absorption of DOM. The mussels used in this experiment contained about 15.5 mg C each, so our estimates of uptake amount to about 0.19% of the mussel C per day. This is approximately half the value estimated by Roditi et al. 2000. Because clearance rate in zebra mussels, unlike most other mussels, scales very nearly proportionally with size (Sprung 1995), this fraction is likely to be similar or a little larger for smaller mussels. Walz (1978) estimated that the zebra mussel zero-growth ration essentially scales proportionally with body size, averaging about 0.77% d^{-1} of mussel dry wt. Our estimates of C-specific uptake of organic substrates amount to ~10–25% of this value.

Uptake of DOM might allow zebra mussels to persist even though they have depleted their particulate food resources to levels that should result in starvation. Indeed, weight loss and mortality are much lower when zebra mussels are maintained in particle-free Hudson River water containing >3 mg L^{-1} of DOC than when they are maintained in DOM-free water that contains only inorganic ions (Baines et al. unpubl. data). Whether the direct uptake of DOM can have this effect depends on two things. The first is the amount of C and energy that can be taken up via this pathway. At present, we have shown that direct uptake of organic substances can account for a significant fraction, but only a fraction, of the zebra mussel maintenance ration. Better information is needed regarding uptake of more complex substrates, and near-bottom concentrations of free amino acid and fatty acids over time are needed to improve these estimates. Second, the source of the DOM needs to be determined. All of the substances tested in this study are readily produced by phytoplankton, which are often significantly reduced after zebra mussel invasions. If these substances are also supplied by nonphytoplankton sources, the direct uptake of DOM could free zebra mussels of the typical constraints of predator–prey systems. Answers to these questions could very well prove to be specific to the habitat in question and thus critical to predicting where the effects of zebra mussels will be greatest.

References

CARACO, N. F., J. J. COLE, P. A. RAYMOND, D. L. STRAYER, M. L. PACE, S. E. G. FINDLAY, AND D. T. FISCHER. 1997. Zebra mussel invasion in a large, turbid river: Phytoplankton response to increased grazing. Ecology **78:** 588–602.

COLE, J. J., G. E. LIKENS, AND D. L. STRAYER. 1982. Photosynthetically produced dissolved organic-carbon—an important carbon source for planktonic bacteria. Limnol. Oceanogr. **27:** 1080–1090.

DEATON, L. E., AND M. J. GREENBERG. 1991. The adaptation of bivalve molluscs to oligohaline and fresh waters: Phylogentic and physiological aspects. Malacol. Rev. **24:** 1–19.

DIETZ, T. H., S. J. WILCOX, R. A. BYRNE, AND H. SILVERMAN. 1997. Effects of hyperosmotic challenge on the freshwater bivalve *Dreissena polymorpha*: Importance of K+. Can. J. Zool. **75:** 697–705.

DRAPER, N. R., AND H. SMITH. 1998. Applied regression analysis, 3rd ed. Wiley.

EFFORD, I. E., AND K. TSUMURA. 1973. Uptake of dissolved glucose and glycine by *Pisidium*, a freshwater bivalve. Can. J. Zool. **51:** 825–832.

FINDLAY, S., R. L. SINSABAUGH, D. T. FISCHER, AND P. FRANCHINI. 1998. Sources of dissolved organic carbon supporting planktonic bacterial production in the tidal freshwater Hudson River. Ecosystems **1:** 227–239.

FRISCHER, M. E., S. A. NIERZWICKI-BAUER, R. H. PARSONS, K. VATHANODORN, AND K. R. WAITKUS. 2000. Interactions between zebra mussels (*Dreissena polymorpha*) and microbial communities. Can. J. Fish. Aquat. Sci. **57:** 591–599.

GAINEY, L. F., AND M. J. GREENBERG. 1977. Physiological basis of species abundance salinity relationship in mollusks—speculation. Mar. Biol. **40:** 41–49.

GUILLARD, R. R. L. 1975. Culture of phytoplankton for feeding marine invertebrates, p. 29–60. *In* W. L. Smith and M. H. Chanley [eds.], Culture of marine invertebrate animals. Plenum.

HEAVERS, B. W., AND C. S. HAMMEN. 1985. Fate of endogenous free amino-acids in osmotic adjustment of *Crassostrea-virginica* (Gmelin). Comp. Biochem. Physiol. A **82:** 571–576.

HIPP, E., U. BICKEL, T. MUSTAFA, AND K. H. HOFFMANN. 1986*a*.

Integumentary uptake of acetate and propionate (VFA) by *Tubifex* sp, a fresh-water oligochaete. 2. Role of VFA as nutritional resources and effects of anaerobiosis. J. Exp. Zool. **240:** 299–308.

———, T. MUSTAFA, U. BICKEL, AND K. H. HOFFMANN. 1986*b*. Integumentary uptake of acetate and propionate (VFA) by *Tubifex sp.*, a fresh-water oligochaete. 1. Uptake rates and transport kinetics. J. Exp. Zool. **240**: 289–297.

HOFFMANN, K. H., AND A. WULF. 1993. Comparative-studies of integumentary uptake of short-chain carboxylic-acids by freshwater oligochaetes. Comp. Biochem. Physiol. A **104:** 169–174.

HORGAN, M. J., AND E. L. MILLS. 1997. Clearance rates and filtering activity of zebra mussel (*Dreissena polymorpha*): implications for freshwater lakes. Can. J. Fish. Aquat. Sci. **54:** 249–255.

JAECKLE, W. B. 1995. Transport and metabolism of alanine and palmitic acid by field-collected larvae of *Tedania ignis* (Porifera, Demospongiae)—estimated consequences of limited label translocation. Biol. Bull. **189:** 159–167.

JAFFE, R., G. A. WOLFF, A. C. CABRERA, AND H. C. CHITTY. 1995. The biogeochemistry of lipids in rivers of the Orinoco basin. Geochim. Cosmochim. Acta **59:** 4507–4522.

JORDAN, T. E., AND I. VALIELA. 1982. A nitrogen budget of the ribbed mussel, *Geukensia demissa,* and its significance in nitrogen flow in a New England salt-marsh. Limnol. Oceanogr. **27:** 75–90.

JORGENSEN, C. B. 1976. Putter, Krough, and modern ideas on use of dissolved organic-matter in aquatic environments. Biol. Rev. Cambridge Phil. Soc. **51:** 291–328.

KERNER, M., H. HOHENBERG, S. ERTL, M. RECKERMANN, AND A. SPITZY. 2003. Self-organization of dissolved organic matter to micelle-like microparticles in river water. Nature **422:** 150–154.

KRYGER, J., AND H. U. RIISGARD. 1988. Filtration-rate capacities in 6 species of European fresh-water bivalves. Oecologia **77:** 34–38.

KUZNETSOVA, M. R., AND C. LEE. 2002. Dissolved free amino acids in nearshore seawater, sea surface microlayers and foams: Influence of extracellular hydrolysis. Aquat. Sci. **64:** 252–268.

LEI, J., B. S. PAYNE, AND S. Y. WANG. 1996. Filtration dynamics of the zebra mussel, *Dreissena polymorpha.* Can. J. Fish. Aquat. Sci. **53:** 29–37.

LINDROTH, P., AND K. MOPPER. 1979. High performance liquid chromotographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-ophthaldialdehyde. Anal. Chem. **51:** 1667–1674.

MACISAAC, H. J. 1996. Potential abiotic and biotic impacts of zebra mussels on the inland waters of North America. Am. Zool. **36:** 287–299.

MANAHAN, D. T., J. P. DAVIS, AND G. C. STEPHENS. 1983*a*. Bacteria-free sea-urchin larvae—selective uptake of neutral amino acids from seawater. Science **220:** 204–206.

———, S. H. WRIGHT, AND G. C. STEPHENS. 1983*b*. Simultaneous determination of net uptake of 16 amino acids by a marine bivalve. Am. J. Physiol. **244:** R832–R838.

———, ———, ———, AND M. A. RICE. 1982. Transport of dissolved amino acids by the mussel, *Mytilus edulis*—demonstration of net uptake from natural sea-water. Science **215:** 1253–1255.

MANNINO, A., AND H. R. HARVEY. 1999. Lipid composition in particulate and dissolved organic matter in the Delaware estuary: Sources and diagenetic patterns. Geochim. Cosmochim. Acta **63:** 2219–2235.

MATSUSHIMA, O., AND Y. S. HAYASHI. 1988. Uptake and accumulation of amino acids in the brackish-water bivalve *Corbicula japonica* prime during high salinity acclimation. J. Exp. Mar. Biol. Ecol. **123:** 201–210.

———, AND A. YAMADA. 1992. Uptake of L-alanine and D-alanine by a brackish-water bivalve, *Corbicula japonica,* with special reference to their transport pathways and the salinity effect. J. Exp. Zool. **263:** 8–17.

NICHOLS, S. J. 1993. Maintenance of the zebra mussel (*Dreissena polymorpha*) under laboratory conditions, p. 733–747. *In* T. F. Nalepa and D. W. Schloesser [eds.], Zebra mussels: Biology, impacts, and control. Lewis.

PACE, M. L., S. E. G. FINDLAY, AND D. FISCHER. 1998. Effects of an invasive bivalve on the zooplankton community of the Hudson River. Freshw. Biol. **39:** 103–116.

PIERCE, S. K., AND M. J. GREENBERG. 1972. Nature of cellular volume regulation in marine bivalves. J. Exp. Biol. **57:** 681–692.

POLIS, G. A., W. B. ANDERSON, AND R. D. HOLT. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Ann. Rev. Ecol. Syst. **28:** 289–316.

PRESTON, R. L. 1993. Transport of amino acids by marine invertebrates. J. Exp. Zool. **265:** 410–421.

RICE, M. A., AND G. C. STEPHENS. 1987. Uptake and internal distribution of exogenously supplied amino acids in the Pacific Oyster, *Crassostrea gigas* (Thunberg). Aquaculture **66:** 19–31.

———, AND ———. 1988. Influx, net flux and trans-epithelial flux of amino acids in the hardshell clam *Mercenaria mercenaria* (Linne)—influence of salinity. Comp. Biochem. Physiol. A **89:** 631–636.

RODITI, H. A., N. F. CARACO, J. J. COLE, AND D. L. STRAYER. 1996. Filtration of Hudson River water by the zebra mussel (*Dreissena polymorpha*). Estuaries **19:** 824–832.

———, N. S. FISHER, AND S. A. SANUDO-WILHELMY. 2000. Uptake of dissolved organic carbon and trace elements by zebra mussels. Nature **407:** 78–80.

SIEBERS, D., AND A. WINKLER. 1984. Amino acid uptake by mussels, *Mytilus edulis,* from natural sea-water in a flow-through system. Helgol. Meeresunters. **38:** 189–199.

SPRUNG, M. 1995. Physiological energetics of the zebra mussel *Dreissena polymorpha* in lakes. II. Food uptake and gross growth efficiency. Hydrobiologia **304:** 133–146.

STEPHENS, G. C. 1968. Dissolved organic matter as a potential source of nutrition for marine organisms. Am. Zoologist **8:** 95–105.

———. 1988. Epidermal amino-acid transport in marine-invertebrates. Biochim. Biophys. Acta **947:** 113–138.

STRAYER, D. L., N. F. CARACO, J. J. COLE, S. FINDLAY, AND M. L. PACE. 1999. Transformation of freshwater ecosystems by bivalves—a case study of zebra mussels in the Hudson River. Bioscience **49:** 19–27.

———, J. POWELL, P. AMBROSE, L. C. SMITH, M. L. PACE, AND D. T. FISCHER. 1996. Arrival, spread, and early dynamics of a zebra mussel (*Dreissena polymorpha*) population in the Hudson River estuary. Can. J. Fish. Aquat. Sci. **53:** 1143–1149.

TESTERMAN, J. K. 1972. Accumulation of free fatty-acids from seawater by marine invertebrates. Biol. Bull. **142:** 160–177.

THOMAS, J. D., P. R. STERRY, AND R. L. PATIENCE. 1984. Uptake and assimilation of short chain carboxylic-acids by *Biomphalaria glabrata* (Say), the fresh-water pulmonate snail host of *Schistosoma mansoni* (Sambon). Proc. R. Soc. Lond. Ser. B **222:** 447–476.

TRANVIK, L. J. 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. Microb. Ecol. **16:** 311–322.

WALZ, N. 1978. The energy balance of the freshwater mussel *Dreissena polymorpha* Pallas in laboratory experiments and in Lake Constance. III. Growth under standard conditions. Arch. Hydrobiol. (suppl.) **55:** 121–141.

WRIGHT, S. H. 1985. Multiple pathways for amino acid transport in *Mytilus* gill. J. Comp. Physiol. B **156:** 259–267.

———, AND A. M. PAJOR. 1989. Mechanisms of integumental amino acid transport in marine bivalves. Am. J. Physiol. **257:** R473.

YU, Y. 1999. Bacterial turnover of selected low molecular weight organic molecules in Hudson River and Long Island Sound estuaries. Ph.D. thesis, State Univ. of New York, Stony Brook.

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