Absorption of dissolved organic substances and its importance to metabolic requirements of the zebra mussel, *Dreissena polymorpha*.

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Running head: DOC and *D. polymorpha*

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1 **Abstract** . The filter feeding zebra mussel, *Dreissena polymorpha*, succeeds in environments
2 even though there is not enough phytoplankton production to support it metabolically. One
3 source of energy and C that is not typically considered in the bioenergetics of zebra mussels is
4 the direct uptake of dissolved organic substances. We determined the rates at which *D.*
5 *polymorpha*, assimilated radiolabeled acetate, monosaccharides, amino acids and fatty acids at
6 environmental concentration levels. The mussels exhibited an ability to incorporate all of the
7 substances presented to them. Much of the ^{14}C labeled substrate that was taken up was respired
8 to $^{14}CO_2$, indicating that the substrates were used for metabolic purposes. Non-acidic amino
9 acids and fatty acids were taken up fastest, with absorption efficiencies (AE = percent of filtered
10 substrate removed) of 13% and 86%, respectively. The AEs for monosaccharides (1.5 %),
11 acetate (0.2%) and the acidic amino acid, glutamic acid (0.79%), were much lower. Among the
12 non-acidic amino acids, non-polar forms (AE = 19.5%) were preferred over basic and polar
13 neutral forms (AE = 9.3%). Based on direct measurements of free amino acid concentrations and
14 literature based estimates of free sugars, acetate and short chain fatty acids in surface waters, we
15 estimate that direct uptake of these monomers amounts to about 0.18% of the zebra mussel C d^{-1},
16 or 19-22% of the zebra mussel maintenance ration. Our results indicate that the direct uptake of
17 dissolved organic matter may be metabolically significant to zebra mussels.

Introduction

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 filtration 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 μg Chl *a* L^{-1} 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 by 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 may 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 DOC is of allochthonous origin, it is also not subject to the negative feedbacks inherent to typical predator-prey relationships. Thus, direct uptake of DOC by zebra mussels may 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 *D. polymorpha*, may 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 DOC directly from solution (Stephens 1968; Jorgensen 1976; Wright and Pajor 1989). Uptake of amino acids, in particular the neutral and basic forms, appears to be particularly efficient (Preston 1993; Wright and Pajor 1989). Significant uptake of fatty acids has also been observed in some marine invertebrates (Jaeckle 1995). Competitive inhibition experiments indicate that several distinct transport channels with high affinities for specific classes of amino acids are used for transport into the organisms (Wright 1985). Concomitant production of reduced waste products, such as NH_4 and CO_2, suggest metabolism of the absorbed molecules (Manahan et al. 1982). However, unless the organism is small, as in the case of lecithotrophic larvae (Jaeckle 1995; Manahan et al. 1983a), or the concentrations of dissolved organic molecule are very high as within sediments (Jorgensen 1976), such uptake is rarely metabolically significant.

Marine bivalves accomplish net uptake of amino acids against very steep gradients. Intracellular and haemolymph concentrations of amino acids can be 10^7 M higher than in the external environment (Preston 1993; Wright and Pajor 1989). In marine systems, the energy required to drive uptake across such gradients derives largely from the co-transport of several Na+ ions from the ambient media into the organisms, where Na^+ ion activity is 10 times lower at typical seawater concentrations (Preston 1993; Wright and Pajor 1989). Marine bivalves exposed to lower osmolarities 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). In this way, marine bivalves regulate their internal free amino acid concentrations as a means of maintaining a healthy water balance within the cells (Pierce and Greenberg 1972; Rice and Stephens 1988). In contrast,

1 freshwater bivalves typically use only inorganic ions in osmoregulation. Possibly as a
2 consequence, they possess very low concentrations of dissolved organic osmolytes (Deaton and
3 Greenberg 1991; Gainey and Greenberg 1977).

4 As might be expected given this background, DOM uptake by freshwater animals has
5 rarely been reported. When such uptake is observed, it is often presumed to serve as a means of
6 chemically locating food (Thomas et al. 1984). However, *D. polymorpha* has recently been
7 shown to take up radiolabeled dissolved algal lysates at rates that may be metabolically
8 significant (Roditi et al. 2000). Rough calculations suggested that up to 50% of the mussel's
9 maintenance ration for C could be supplied by such uptake. However, as Roditi et al. point out,
10 these estimates were based on several assumptions that have not been tested. One such
11 assumption is that the natural DOC will be taken up at the same rate as the algal lysates. This
12 assumption may not be correct since most natural DOC is refractory to biological breakdown as
13 indicated by bioassays using bacteria (Tranvik 1988), whereas algal lysates are readily utilized by
14 bacteria (Cole et al. 1982). Consequently, the calculations may overestimate the potential
15 importance of DOC to zebra mussel metabolism. On the other hand, DOC uptake rates may
16 actually have been underestimated by Roditi et al. if particularly labile compounds were
17 completely utilized before the end of their short (2 h) experiments, as might not happen in situ if
18 supply is continuous. Since the depletion of individual compounds were not tracked during the
19 algal lysate experiments, it is impossible to know how important this last source of bias was.

20 In this paper, we address some of these issues by quantifying the degree to which zebra
21 mussels take up a range of defined radiolabeled dissolved organic biomolecules at
22 environmentally realistic concentrations. Simple laboratory experiments were used to determine
23 if zebra mussels efficiently remove amino acids, simple sugars, acetate and fatty acids from

1 solution, if the mussels prefer certain classes of compounds over others, and if any of the
2 assimilated organic matter is respired by the mussels. By combining the radiotracer data and
3 filtration rate data for individual mussels, we can then establish absorption efficiencies for
4 classes of organic molecules. Based on both direct and literature estimates of ambient
5 concentrations of these substances, we then try to determine the possible contribution that uptake
6 of these specific substances make to zebra mussel metabolism.
7
8 **Methods**
9 *Collection and storage of mussels and water.* The water used in these experiments was
10 collected from the Hudson River at either the North Germantown, NY boat launch, or the city of
11 Poughkeepsie boat launch near the Mid-Hudson bridge. Water was transported in 20 L carboys
12 to the Stony Brook campus and filtered into triple DI rinsed 20 L carboys through 0.2 μm
13 Millipak 200 cannister filters (Millepore Corp.). The filtered water was then stored at 4°C in the
14 dark until use. Zebra mussels were also collected from the North Germantown boat launch in
15 June of 2002 and May of 2003. Entire rocks with encrusted mussels were removed from the
16 water and placed into 30 L coolers filled with ambient Hudson River water and then transported
17 back to the Stony Brook University campus. In the lab, mussels were removed from the rocks by
18 carefully cutting off byssal threads with dissecting scalpels. The organisms were then placed in
19 long-term feeding chambers (Roditi et al. 1996), and maintained at 16°C in vigorously aerated
20 0.2 μm-filtered Hudson River water until used in the experiment. Mussels were fed either
21 rehydrated 40 μm filtered dried *Chlorella* powder or living *Chlorella vulgaris* cells that had been
22 cultured at 23°C in WCL-1 media (Guillard 1975).
23 *Feeding experiments.* Only actively filtering mussels with shell lengths of 2.2-2.4 cm

1 were used in these experiments. Once selected, mussels were cleaned thoroughly by carefully
2 scraping and scrubbing the shells to remove biota on the shells that might contribute to uptake of
3 organic matter. The mussels were then transferred to 250 ml beakers containing 100 ml of
4 Hudson River that had been filtered through 0.2 µm Millipak 200 canister filters. Mussels were
5 then allowed to acclimate for 1 d in this water at room temperature (23° C) without food. After
6 the acclimation/starvation period the mussels were centered in the beakers and checked for
7 filtering behavior. Non-filtering mussels were discarded.

8 The feeding rate experiments were begun by adding aliquots of *Clorella vulgaris* to
9 achieve initial cell concentrations of about 20,000 cells ml^{-1}, or about 30 µg C L^{-1} assuming a C
10 per $µm^3$ conversion of 0.2. These values are lower than the incipient limiting concentrations
11 above which filtration rates can be reduced (Sprung 1995). Five-ml samples for cell counts were
12 removed immediately after addition and at 10 min intervals thereafter for 1 h. These samples
13 were preserved with Lugol's solution. Cell number and cell size in the samples was then
14 analyzed using a Coulter Counter Multisizer II system fitted with a 70 µm aperture. NaCl was
15 added to the samples and the electrolyte solution to achieve a final concentration of 2 ‰ so as to
16 achieve sufficient electrical conductivity to analyze the samples. Past work has shown that this
17 addition of NaCl results in little cell shrinkage and no loss of cells for preserved samples of this
18 algal species. Only cell numbers for cells >2 µm in size were analyzed since zebra mussels may
19 not be effective at filtering out smaller particles (Lei et al. 1996).

20 *Radiotracer uptake experiments.* Microbial uptake of radiolabeled organic molecules
21 represented a potentially important artifact in these experiments. Therefore, all glassware in
22 these experiments was autoclaved before use. The Hudson River water used in the experiments
23 was also sterile filtered through an autoclaved 0.2 µm Millipak 200 canister filter, and is

1 hereafter referred to as sterile-filtered Hudson River Water (SFHRW) and all transfers were done
2 with sterile autoclaved graduated cylinders or pre-sterilized 50 ml pipettes. Since zebra mussel
3 gut passage times have been measured at ~1 d (Roditi and Fisher 1999), the mussels were starved
4 for 1 d prior to the radiotracer experiments to reduce the amount of feces produced during the
5 experiment. To start the experiments, 75 ml of SFHRW was first pipetted into each flask. The
6 mussels were removed from their holding beakers and scrubbed and rinsed with SFHRW to
7 remove as much microbiota as possible from the shells. Each of the newly cleaned mussels was
8 then transferred to a separate beaker and allowed to acclimate for at least an hour prior to
9 addition of the radiolabel. Mussels were not used if they did not begin filtering within this time.
10 In the first experiment, the uptake and transformation of ^{14}C labeled alanine were
11 followed over the course of 8 h. Uniformly ^{14}C-labeled alanine was first added to 500 ml of
12 SFHRW to achieve a final concentration of 200 nM. Then, 25 ml of this working stock solution
13 was added to each experimental beaker to achieve a final alanine concentration of 50 nmol L^{-1},
14 using the water flow to mix the solution as much as possible while limiting disturbance to the
15 mussels. Periodically, 3 mussels were removed from the experimental beakers and placed in
16 250 ml beakers containing 200 ml of unlabeled SFHRW to flush the mussels' pallial space of
17 radiolabeled fluid. After 10 min., the mussels were removed and immediately frozen in liquid
18 nitrogen and stored at -4°C until dissection (< 1 week). Two 1-ml aliquots were also taken at
19 each of the time points from the three flasks that contained mussels for the entire 8 h period of
20 the experiment. At each time point, both aliquots were transferred to 20 ml plastic scintillation
21 vials, one of which contained 3 ml of 1 N NaOH to help retain any inorganic ^{14}C and the other 1
22 ml of 1N HCl.to volatilize inorganic ^{14}C. Particulate organic ^{14}C was assayed in the radiolabeled
23 working stocks and in each beaker after the mussels had been removed by filtering 10 ml aliquots

1 through 25 mm diameter 0.2 μm polycarbonate membrane filters (Poretics Corp.). The filters
2 were then placed into 20 ml scintillation vials with 1 ml of 1N HCl.
3 In the other set of experiments, mussels were exposed to a number of radiolabeled
4 organic substrates for approximately 1 h (Table 1). Uniformly labeled organics were used in all
5 cases except for linoleic acid which was only labeled at the 1-C position. Three mussels were
6 assayed for each substrate. The water was sampled for dissolved and particulate constituents
7 using the protocol used in the long-term alanine experiments, except that dissolved samples were
8 taken at each 10-min interval over the course of the hour. Mussels were harvested only at the
9 end of the 1 h exposure. We also included 3 control beakers for each substrate that were treated
10 identically to the other beakers except that the mussels were removed just prior to addition of the
11 isotope. These controls allowed us to assess the impact of biota brought into the experimental
12 beakers by the zebra mussels on the fate of the radiolabeled compounds.
13 The acidified scintillation vials (both fluid and filter samples) were left open in a hood for
14 >12 h with occasional swirling to help the exchange of ^{14}C labeled gaseous CO_2, then 3 ml of 1 N
15 NaOH was added to produce the alkaline conditions required by the scintillant. Frozen mussels
16 were dissected by allowing the shells to thaw slightly at room temperature before removing them
17 from the still frozen soft tissues. Soft tissues were placed in tared glass 20 ml scintillation vials
18 and allowed to thaw. Excess fluid was then wicked away with a Kimwipe, taking care not to
19 remove tissues, and the vials were then weighed. Three ml of a tissue solubilizer (Solvable,
20 Packard Instruments Corp) was added and the scintillation vials were kept at 60° C overnight
21 with periodic swirling to aid tissue breakdown. Non-soluble remnants (small parts of shell and
22 byssal threads) were then removed and the solution cleared with 100 μl of 30% hydrogen
23 peroxide before adding 10 ml of Optima Gold scintillant (Packard Instruments). 10 ml of

1 scintillant were added to all scintillation vials and the resulting solutions mixed vigorously and
2 allowed to sit in the dark for 1 d before analysis. All samples were then assayed for beta
3 emissions using a Tri-Carb 2100TR (Packard Instruments). Quench correction was by the
4 external standards method. Samples were counted for 15 min, or until the count SD was $< 1.5\%$
5 of the count. To get wet weights, weights of the scintillation vials with the mussel tissue were
6 corrected for the contribution of removed shell fragments and weights of the empty vials. The
7 shell fragments from the scintillant vials were then added to the shell material removed during
8 dissection and weighed.

9 *Amino acid analysis.* Samples for amino acid analysis were collected from 3 stations in
10 the tidal freshwater Hudson River in NY: Castleton, Hudson and Poughkeepsie. Samples were
11 filtered in the field trhough pre-ashed Whatman GF/F filters and collected into glass vials which
12 has been washed in HCl and then ashed at 400°C. Samples were stored frozen until analysis, by
13 HPLC (CINDY REF).

14 Data treatment. Apparent instantaneous filtration rates (FR, ml hr^{-1}) were calculated by
15 multiplying the slope of the linear regression of the natural logarithm of cell concentration (cells
16 ml^{-1}) on time (h) by the number of ml in the feeding solution. The respiratory quotient, RQ, after
17 1 h is defined as the ratio of respired ^{14}C to the total amount of ^{14}C taken up during the
18 experiment. Respiratory ^{14}C includes that lost from the system as well as that found as $DI^{14}C$.
19 The respired ^{14}C was added to that found in the mussel tissues to determine the total uptake of
20 C^{14}. Instantaneous net radioisotope absorption rates (AR, ml h^{-1}) were calculated assuming that
21 loss from solution followed first order loss kinetics. Under this assumption, $AR = \ln((\Delta Ac_t/Ac_0$ -
22 $\Delta Ae_t/Ae_0) \cdot V \cdot t^{-1}$, where ΔAe_t and ΔAc_t are the change in dissolved activity in the experimental
23 and control beakers at time t, Ae_0 and Ac_0 are the initial activities in the experimental and control

1 beakers, V is the initial volume in ml of the experimental container and t is time in hours. By
2 depending on the radioactivity remaining after acidification and correcting for loss from the
3 control beakers, this equation accounts for ^{14}C lost to the air, to the sides of the container and to
4 respiration by microbial organisms. Absorption efficiency, AE (%), was calculated as 100 ·
5 AR÷FR. C in soft tissues were estimated by multiplying the soft tissue wet weight by 0.086, the
6 fraction of dry weight after freeze drying tissues to the wet weight as determined for a set of 20
7 mussels collected from the same site in the Hudson River. This value was then multiplied by
8 0.45, the empirically determined C content relative to dry weight of the zebra mussels, to get C in
9 each mussel.

10 Rates of dissolved substrate uptake in situ for comparison to zebra mussel metabolic
11 requirements were estimated from the experimental results, measurements of ambient amino acid
12 concentrations. We assumed in these calculations that experimental and ambient concentrations
13 of dissolved organic substances were far below the half saturation constants for uptake of those
14 substances. This assumption is based on observations that half saturation coefficients are > 1
15 $\mu mol\ L^{-1}$ amino acids and fatty acids (Jaeckle 1995; Preston 1993; Thomas et al. 1984), whereas
16 our additions were always <250 $nmol\ L^{-1}$ and usually ~50 $nmol\ L^{-1}$ (Table 1). Ambient
17 concentrations of amino acids, sugars and short chain fatty acids are usually in the low
18 nanomolar range. Only acetate concentrations typically approach or surpass 1 $\mu mol\ L^{-1}$, but half
19 saturation constants for acetate also tend to be > 1 $mmol\ L^{-1}$(Thomas et al. 1984). Since the
20 relationship between uptake of a substance and its ambient concentration is nearly linear under
21 our assumptions, we can calculate total uptake by

22 $$U = AE_i * FR * [X], \quad (1)$$

23 where AE is the absorption efficiency for substance *i* and FR is the filtration rate of an individual

1 mussel. Substances were grouped into 5 categories for the calculation: Non-acidic amino acids,
2 acidic amino acids, neutral amino acids, monosaccharides, carboxylic acids and fatty acids.
3 Measured concentrations of non-acidic amino acids in the Hudson River were used for the amino
4 acid uptake calculations. Uptake of monosaccharides, fatty acids and carboxylic acids was
5 estimated using data from the literature on similar systems.
6
7 **Results**
8 Alanine was efficiently removed from solution by zebra mussels in the 8 hr experiments.
9 Only 45% of the organic radiolabel remained in solution after 1 hr, 20% after 2 hr and 5% after 4
10 hr (Fig.1). This rate of removal corresponded to an instantaneous clearance rate of 81 ± 4 ml h^{-1}
11 $mussel^{-1}$ according to an exponential model fit to the data (adj. $r^2 = 0.99$, $p<0.0001$). By the end
12 of the experiment about 20% of the added isotope was found in the soft tissue of the zebra
13 mussels. Three times more radioactivity ended up in the dissolved inorganic carbon fraction.
14 The overall budget for ^{14}C in the beakers indicated that another 20% of the radiolabel was lost
15 from the system over the course of the experiment. The rate of loss corresponded roughly to the
16 amount of $DI^{14}C$ present in the beakers indicating that much of the radioisotope loss was due to
17 evasion of $^{14}CO_2$ to the atmosphere. Adding this 20% of ^{14}C lost from the system to the 60%
18 found in the DIC pool indicates that 4x more C in the alanine was oxidized through respiration
19 than assimilated into tissues.
20 In the shorter term experiments, many of the organic substrates were efficiently absorbed
21 by the zebra mussels. For the non-acidic amino acids, only 75 to 35% of the added substrate
22 remained in solution after an hour of exposure to the mussels (Fig 2). For the fatty acids, the
23 results were more striking, with <20% remaining after 1 h of exposure (Fig 3). Usually the

1 amount of loss from the control beakers was not statistically significant, indicating that almost all
2 of the loss could be attributed to the presence of the zebra mussels. In two instances, however,
3 there were large losses of labeled organic matter, phenylalanine (10%) and acetate (17%). In
4 contrast to the results for the fatty acids and the non-acidic amino acids, >85% of the radio-
5 labeled organic molecules remained in solution in the beakers containing glucose, fructose,
6 acetate and glutamic acid.

7 For every substrate, measurable amounts of radiolabel were found associated with mussel
8 soft tissues at the end of the exposures. The percentage of added radiolabel associated with
9 mussel tissues ranged from <1 % for acetate, fructose, glucose and glutamic acid to > 30% for
10 the fatty acids (Table 2; Figs. 3, 4). The same percentage for non-acidic amino acids ranged from
11 7.3 % for glycine to 24.5 % for phenylalanine (Fig. 2). In almost every case a significant amount
12 of the radiolabel was found in the inorganic fraction and in all but one case the mineralization of
13 the organic label was much higher in the beakers containing mussels than in the control beakers
14 (Figs. 2 - 4). The exception to this rule was acetate that was lost from acidified samples at
15 similar rates in the experimental and control beakers, making it impossible to determine the
16 amount of acetate respired in those experiments (Fig. 4). Because a significant fraction of ^{14}C
17 often left the experimental vessels via loss of aqueous CO_2 into the gas phase, we determined the
18 total amount of $^{14}CO_2$ produced by the mussels by adding the total loss of ^{14}C from the system to
19 the $DI^{14}C$ measured at the end of the experiment. The 1 h respiratory quotient, RQ, for amino
20 acids averaged 54 (± 13) % with a range between 39 % (tyrosine) and 73 % (alanine and glutamic
21 acid; Table 2). The RQs for glucose, 92.8 (±1.4) %, and fructose, 89.3 (±1.7) %, were
22 substantially higher than those for the amino acids. Palmitic acid, which is a saturated fatty acid,
23 had an RQ of 63.2 (± 0.3) % that was significantly less than the RQ of 80 (±1.1) % for linoleic

1 acid, an unsaturated fatty acid.

2 Because there was a tendency for a greater fraction of the less assimilated compounds to
3 be respired, variability in the absorption rate, AR (ml h^{-1} $mussel^{-1}$), was less than that of
4 radiolabel uptake into mussel tissues. The percentage uptake into mussel tissue varied by 31-fold
5 between amino acids and sugars, for instance, but the AR for sugars was only 10-fold less at 5 ml
6 h^{-1} than the 55 ml h^{-1} AR for amino acids. Still, absorption rates varied widely, ranging from ~1
7 ml h^{-1} $mussel^{-1}$ for glutamic acid and acetate, to nearly 300 ml h^{-1} $mussel^{-1}$ for the fatty acids.
8 Differences between the non-acidic amino acids also are apparent. The AR for non-polar amino
9 acids averaged 89 ± 9 ml h^{-1}, or more than twice the value for the basic and the non-polar neutral
10 amino acids at 39 ml ml h^{-1} and 33 ± 8.4 ml h^{-1}, respectively.

11 Absorption efficiencies were 1-2 % for the sugars, 5.6 - 25 % for the non-acidic amino
12 acids, and 76 - 95 % for the fatty acids (Table 2). Even after correcting for respiration of
13 radiolabel, glutamic acid had the lowest absorption efficiency at 0.78%. Among the amino acids,
14 the non-polar neutral forms were absorbed with twice the efficiency (20%) on average of the
15 polar neutral and basic amino acids (10%, Fig. 5). The absorption efficiency including
16 respiration for acetate could not be determined because the loss of organic radiolabel from the
17 experimental beakers did not differ from the loss observed in the control beakers. Consequently,
18 we have adopted a rate based solely on accumulation of radioactivity in the mussel tissue for this
19 substance.

20 Total free amino acid concentrations at Castleton, Hudson and Poughkeepsie were 152
21 nmol L^{-1} (9.0 μg L^{-1}), 240 nmol L^{-1} (10.9 μg L^{-1}), and 172 nmol L^{-1} (8.1 μg L^{-1}), respectively (Fig.
22 6). Riverwide averages of total DFAA, acidic-DFAA and non-acidic DFAA were 188 ± 38 nmol
23 L^{-1} (9.2 ± 1.1 μg L^{-1}), 21.6 ± 4.3 nmol L^{-1} (1.1 ± 0.21 μg L^{-1}), and 167 ± 33 nmol L^{-1} (8.1 ± 0.9

μg L^{-1}). Although there were longitudinal shifts in the predominance of certain amino acids, the balance between the main groups of amino acids was rather consistent. Non-polar neutral amino acids dominated the DFAA pool throughout the river, making up > 75% of the DFAA. The next most important group were the polar neutral amino acids, followed by the basic and acidic forms.

Discussion

We have observed uptake of a wide variety of dissolved organic substrates by zebra mussels. The observed uptake is not likely to result from artifacts associated with the radiotracer technique. 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 leakier 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. 1983a; Manahan et al. 1983b; Stephens 1988). The radiotracer uptake was also not due to ingestion of particles, such as bacteria, that had incorporated the radiolabel. The amount of radiolabel captured onto 0.2 μm filters 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 using 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 (Thomas et al 1984, Testerman 1972).

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. In the case of amino acids, this uptake occurs against a $>10^7$ to 1 M gradient in concentration (Preston 1993; Wright and Pajor 1989). Manipulations of internal and external $[Na^+]$ appear to indicate that the energy needed to fuel this uptake is derived from the co-translocation of Na^+ down the 10:1 M concentration gradient that exists across cell membranes in marine habitats (Preston 1993; Wright and Pajor 1989). 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 (Testerman 1972), 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 the organic matter absorption. Potentially, this could allow them to obtain enough dissolved substrate from their surroundings to be metabolically significant.

In all other ways, however, the uptake of exogenous organic compounds by zebra mussels resembles that exhibited by marine and brackish water mussels. First, the high affinity and velocity for uptake implied by the high absorption efficiencies for amino acids at low ambient

concentrations is similar to the high affinity (k_s = 1-4 μmol L^{-1}), high velocity uptake systems possessed by *Mytilus* (Preston 1993; Wright and Pajor 1989). Uptake of amino acids by marine mussels appears to be mediated by up to four enzyme systems that preferentially bind with amino acids of the same general structure (Wright 1985). 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 for a wide range of amino acids.

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 filtration 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 with remarkable efficiency. Nearly all of the fatty acid passing through the gills is absorbed by the mussels, which is >4 x more efficient than for alanine. Uptake of free fatty acids from the ambient environment has rarely been assayed previously. 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 may be facilitated by their hydrophobic character that may 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 fact that > 50% of the absorbed fatty acid was respired that the molecules did not remain in the membrane, but were available for respiratory metabolism. Past work has shown saturable kinetics for fatty acid uptake by freshwater invertebrates (Testerman 1972; Thomas et al. 1984). Since many fatty acids have a higher caloric content than amino acids, uptake of fatty acids may be important metabolically to mussels even though free fatty acid concentrations in situ are typically lower than those of amino acids.

In every case, most of the absorbed substrate was respired by the mussels, indicating use of the absorbed substrates for metabolic purposes. The long-term uptake experiment with alanine indicated that this respiration 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. While we have not determined uptake for the entire range of compounds making up the DOC, we have tested a wide range of labile forms most likely to be subject to uptake across the gills. Based on 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) non-polar amino acids, 5) monosaccharides and 6) short chain fatty acids. We also assume a filtration 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 filtration rate on shell length from the data of Horgan and Mills (1997), but is somewhat higher than predicted by the equations of Kryger and Riigaard (1988) based on dry weight of the body. This minor discrepancy may 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} (Nicholls 1993).

To calculate uptake rates using Equation 1, we must also know ambient concentrations of the substrate classes (Table 3). Amino acids were measured directly at three stations spanning much of the river inhabited by *D. polymorpha* (Fig. 6). For a high end estimate of monosaccharides in the river, we used Yu's (1999) upper estimate for glucose concentrations (100 nmol L^{-1}) at Haverstraw Bay, 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 <<100 nmol L^{-1}. This discrepancy may reflect a large fraction of acetate that is bound to dissolved ligand (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 free short chain (<20 C) fatty acids in rivers are rare.

1 For lack of any better information, we have used data on dissolved fatty acids from the Orinoco
2 River in Venezuela (Jaffe et al. 1995). It is uncertain to what degree the dissolved fatty acids
3 measured in these studies exist as monomers.

4 The substrates that potentially contribute most to the C budget of *D. polymorpha* are non-
5 acidic amino acids, lipids and acetate. Uptake of amino acids generally amounts to about 10 μg
6 C d^{-1}. Uptake of the non-polar amino acids constitutes >75% of this total. If the GC estimate is
7 used for acetate, uptake of this substance could account for another 4 μg C d^{-1} even without
8 knowing how much acetate was respired by the mussels. If we assume that the bioavailable
9 fraction of acetate is much smaller, however, this value falls to 0.03 μg C d^{-1}. Fatty acid uptake
10 could also account for a significant amount of uptake, in part because of the ability of zebra
11 mussels to take up such compounds very efficiently, and in part because of the large amount of C
12 within each fatty acid molecule. Based on a short-chain fatty acid concentration of 2 μg L^{-1},
13 uptake of these compounds would be about 12 μg C d^{-1}. By comparison, direct uptake of the
14 acidic amino acids and the sugars were unimportant sources of C to the mussels, contributing <1
15 μg C d^{-1}.

16 The uptake of dissolved substances may contribute a significant fraction to basal zebra
17 mussel metabolism (Table 3). We have estimated a potential flux of 28 μg C d^{-1} into the zebra
18 mussels from direct absorption of dissolved organic substances. The mussels used in this
19 experiment contained about 15.5 mg C each, so our estimates of uptake amount to about 0.18 %
20 of the mussel C per d. This is about half the value estimated by Roditi et al 1999. Since
21 filtration rate in zebra mussels, unlike most other mussels, scales very nearly proportionately
22 with size (Sprung 1995), this fraction is likely to be similar or a little larger for smaller mussels.
23 Walz (1978) estimated that zebra mussel zero-growth ration essentially scales proportionately

with body size, averaging about 0.77 % d^{-1} of mussel dry wt. Our estimates of C-specific uptake of organic substrates amount to about 20 - 25% of this value.

There are two major reasons to believe that uptake of dissolved organic substances may actually be more important than implied by these calculations. First, the dissolved organic concentrations to which zebra mussels are exposed may be significantly higher than is typical for surface waters because of diffusion from benthic sediments. Zebra mussels are known to significantly increase the organic content of the sediments near them (MacIsaac 1996; Strayer et al. 1999). Decomposition of phytoplankton from feces or pseudofeces could provide locally high concentrations of fatty acids, amino acids, sugars and carboxylic acids. Second, the classes of substances tested in our study represent < 10 % of the total DOC by C mass. Carboxylic acids, long-chain fatty acids, and glycollate are all examples of monomeric substances that may contribute substantially to DOC that we have not tested for uptake. Uptake of more complex combinations of these substances has yet to be tested. However, there is evidence for zebra mussels and other bivalves that metals associated with HMW and colloid fractions, can be assimilated to varying degrees (Roditi and Fisher 1999, Wang and Guo 2000, Pan and Wang 2002). If anything, therefore our results suggest that Roditi et al. (2000) may have underestimated the importance of DOC uptake to zebra mussels.

Our work may have important ecological and management implications. Uptake of dissolved organic substances may 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 maintained in particle free Hudson River water containing >3 mg L^{-1} of DOC, than when they are maintained in DOC-free water that containing only inorganic ions (Baines et al, in prep). Consequently, the ecosystem level effects of zebra

1 mussel invasion may also persist resulting in long-term transformation of energy and material
2 flow paths and organism abundances. Whether the direct uptake of dissolved organic substrates
3 can have this effect depends on two things. The first consideration is the amount of C and energy
4 that can be taken up via this pathway. At present, we have shown that direct uptake of organic
5 substances can account for a significant fraction, but only a fraction, of the zebra mussel
6 maintenance ration. Better information is needed regarding uptake of more complex substrates,
7 and near-bottom concentrations of free amino acid and lipids over time are needed to improve
8 these estimates. Second, the source of the dissolved organic substances needs to be determined.
9 All of the substances tested in this study are readily produced by phytoplankton. Reduction of
10 phytoplankton biomass by zebra mussels may decrease these substances as well, causing
11 negative predator-prey feedbacks on zebra mussel populations to come into play. If, however,
12 these substances are also supplied by sources not related to the phytoplankton – such as
13 advection, bacterial decomposition, or photolytic decay of allochthonous material – the direct
14 uptake of dissolved organic matter could free zebra mussels of the typical constraints imposed by
15 predator-prey systems. Answers to these questions could very well prove to be specific to the
16 habitat in question and thus critical to predicting where zebra mussel impacts will be greatest.
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