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Trophic position of zebra mussel veligers and their use of dissolved organic carbon

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Abstract

We evaluated by stable isotope analysis the trophic structure of an estuarine transition zone (ETZ) food web and the role of an invasive species, the veliger stage of the zebra mussel *Dreissena polymorpha.* In the St. Lawrence ETZ, where zebra mussel veligers are now the dominant zooplankton in summer, $\delta^{13}C$ ranged from −31.2‰ (seston) to −16.1‰ (adult fish) and $\delta^{15}N$ ranged from 2.6‰ to 17.4‰. Isotopic analysis of samples indicated that the overall food web was largely supported by autochthonous phytoplankton rather than by allochthonous terrestrial carbon. Large differences among the isotopic signals of veligers, cladocerans, and copepods suggested the use of different proportions of food items, and the isotopic values of fish larvae indicated no significant assimilation of veligers. The $\delta^{13}C$ signature of the veligers was in a range consistent with feeding on free-living bacteria and dissolved organic carbon (DOC) or both, and freshwater algae incubated in situ. To investigate the possibility of DOC uptake by the veligers, we incubated veligers on ^{14}C-labelled algal lysates. There was rapid uptake of DOC and incorporation into biomass, equivalent to 6% of the soft tissue dry weight per hour. Zebra mussel veligers are likely using autochthonous DOC as an alternate food source, and they occupy an exotic trophic position in which there is little direct interaction with other major components of the ETZ food web.

Estuarine transition zones (ETZs), where riverine waters first mix with seawater, are typically rich in both allochthonous (terrestrial) and autochthonous (in situ photosynthetic) organic matter. They also contain abundant consumer populations, but little attention has been given to the sources and pathways of carbon flow. Some studies have argued for a dominant role of allochthonous carbon in sustaining food webs in rivers (McCallister et al. 2004), whereas other authors have stressed the greater importance of autochthonous sources (Martineau et al. 2004 and references therein). The St. Lawrence River ETZ has long been recognized as supporting high biological productivity and an important larval fish nursery, with large standing stocks of phytoplankton and zooplankton (Frenette et al. 1995; Winkler et al. 2003). In spite of the ecological importance of this ETZ, there has been no analysis of its overall food web structure.

Like many waterways in North America and elsewhere, the St. Lawrence River has recently been invaded by the zebra mussel, *Dreissena polymorpha*, and since July 1994 large concentrations of zebra mussel larvae (veligers) have been registered in the river and ETZ. These veligers are now recognized as the dominant zooplankton component, constituting between 52% and 90% of the total zooplankton counts in summer (Winkler et al. 2005). The larvae are likely retained and concentrated in the ETZ by the same physical mechanisms that cause the high turbidity (Frenette et al. 1995). There is now an extensive literature on the effects of adult zebra mussels in aquatic ecosystems; however, the ecological role of their abundant veliger stage has rarely been addressed.

Our previous distributional analysis of veligers and microbial food web components in the St. Lawrence ETZ indicated that there was little effect of this invasion on the

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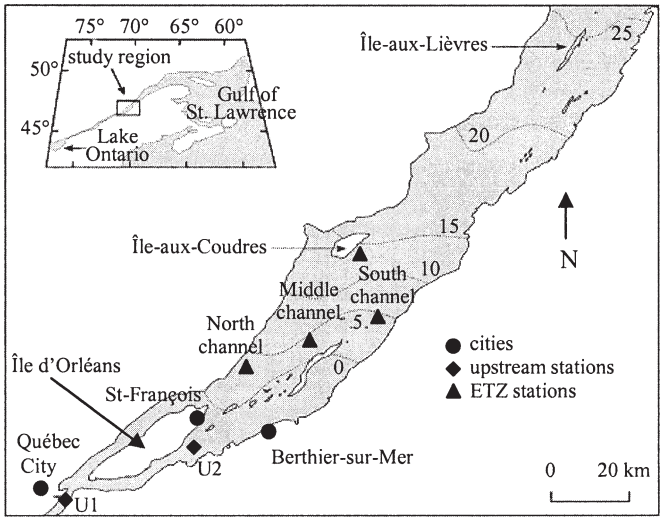

Fig. 1. Sampling sites in the estuarine transition zone (ETZ) of the St. Lawrence River. Pelagic organisms and microbial components were collected in the three main stations (North, Middle, and South). Berthier-sur-Mer and St-François were the sites of adult mussel collection and Île-aux-Coudres was the location where the adult fishes were caught. Microbial components were also collected at the freshwater (U2) station. The upstream site (U1) was located in front of Québec City. Dotted lines indicate isohalines. Algal incubations were at U2 and North Channel.

pelagic microbial community, but we also drew attention to the need to analyze potential impacts both on primary consumers that may utilize similar resources and on higher trophic levels that could potentially feed on veligers (Barnard et al. 2003). Laboratory experiments have shown that veligers feed on particles ≤ 10 μm, including bacteria, protists, and detritus (Sprung 1993; Wacker et al. 2002); however, there is increasing evidence that adult zebra mussels are able to use dissolved organic carbon (DOC) as an alternate carbon source (Roditi et al. 2000). Recent measurements of DOC uptake in combination with chemical analysis of monomers such as acetate and amino acids in natural DOC showed that adult zebra mussels could potentially obtain 10–25% of their metabolic needs for carbon from DOC in river water (Baines et al. 2005). To our knowledge, similar experiments have not been conducted on zebra mussel veligers, although active DOC uptake is known for the larval stage of some marine invertebrates, including bivalves (Manahan et al. 1982).

The aims of the present study were to determine: (1) the trophic position of zebra mussel veligers in an ETZ ecosystem, the St. Lawrence River and upper estuary; (2) the potential interactions between these organisms and other food web components; (3) the primary carbon source for the overall ETZ food web and the carbon sources used by veligers; and (4) the capacity for DOC uptake by veligers. We applied stable isotope techniques (δ^{13}C and δ^{15}N) to the ETZ and the upstream freshwater community during the summer period of maximum veliger abundance, and combined these measurements with experimental analysis of DOC uptake.

Materials and methods

Study area—The St. Lawrence River is the third largest river on the North American continent, extending 500 km from the Great Lakes to the sea. The turbid waters of the estuary extend 150 km from eastern tip of Île d'Orléans to the Saguenay River mouth, with a maximum turbidity zone located 30–45 km downstream of Québec City. Salinity in this freshwater–saltwater transition zone ranges from 0.2 to 10 and turbidity reaches several hundred NTU. There is a shift in the bacterial community across the ETZ, from free-living to attached cells (Painchaud et al. 1995), and the food web structure changes substantially. Amphipods (*Gammarus* spp.) and cladocera (*Bosmina longirostris)* are the predominant zooplankton in the freshwater part of the estuary and in the south channel of the ETZ, shifting further into the ETZ to estuarine mysids (*Neomysis americana* and *Mysis stenolepis*), copepods (notably *Eurytemora affinis*) and the sand shrimp *Crangon septemspinosa*. The high primary production and zooplankton biomass sustain large stocks of fish larvae, with concentrations of 85 *Osmerus mordax* (rainbow smelt) and 153 *Microgadus tomcod* (Atlantic tomcod) per 100 m^3 (Winkler et al. 2003 and references therein).

Sampling sites—Zooplankton and ichthyoplankton were sampled in each of the three main channels of the ETZ (Fig. 1) in summer 2000 (*Stizostedium canadense* [sauger]) and 2001 (all taxa). *Dreissena bugensis* (quagga mussels) are also present in the St. Lawrence River, but are much less abundant than *Dreissena polymorpha* and represent less than 2% of the *Dreissena* population near Québec City (De LaFontaine pers. comm.). Quagga and zebra mussel veliger larvae can be readily distinguished because quagga veligers are kidney-shaped whereas zebra mussel veligers are D-shaped (Johnson 1995). The weak proportion of quagga veligers is consistent with the known preference of their adult stage for deep waters (>20 m) in the Great Lakes, and the paucity of such deep habitats in the St. Lawrence River upstream of Québec City. Adult zebra mussels were sampled 30 km upstream of the ETZ (Québec City) at site U1, and at two sites within the ETZ (Berthier-sur-Mer and St. François). Fish samples were collected near Île Madame (*S. canadense* from 2000 sampling) and from a trap on the southern bank of Île-aux-Coudres (*O. mordax* and *M. tomcod*) at the downstream end of the ETZ. Larval zebra mussels (D-stage veligers) were collected within the ETZ and at U1. Dissolved inorganic carbon (DIC), DOC, and microbial components (seston fractions and bacteria) were sampled from 60 liters of water collected during July and August 2003 at U2 and at the North Channel station in the ETZ. Algal cultures were incubated in situ at the latter stations to estimate the isotopic signature of the phytoplankton.

DIC and fractionation estimates—To measure the δ^{13}C and concentration of DIC, amber bottles fitted with gas-tight septa (cat # 15900-024, VWR) were filled with water obtained from tangential flow filtration with fluorocarbon polymer filters of 0.45-μm pore size (Pellicon system,

Millipore). For the purpose of comparing with the $\delta^{13}C$ of algae incubated in situ, we used DIC samples obtained during the incubation in August 2003. One sample at U2 and two samples at North Channel (low and high tide) were kept cool and immediately sent to the G.G. Hatch Isotope Laboratories (Ottawa) for analysis using a TOC-Analyser (Model 1010; OI Analytical) interfaced with a Delta Plus (Thermo Finnigan) isotope ratio mass spectrometer (IRMS).

The $\delta^{13}C$ of algae was predicted from the $\delta^{13}C$ of dissolved CO_2, (CO_2(d)), the DIC species that is mainly fixed enzymatically by phytoplankton (Rau et al. 1996), and the expected fractionation by phytoplankton. In the St. Lawrence estuary during the summer, the average pH is 7.84, with a range of 7.6–8.2 (Hélie et al. 2002). The principal species of DIC is therefore bicarbonate, by several orders of magnitude. Equilibrium isotopic fractionation (ε_d) between bicarbonate and CO_2(d) was calculated as (Mook et al. 1974):

$$\varepsilon_d = -(9.866 \pm 0.23) \times 10^3/T + (24.12 \pm 0.78)‰ \quad (1)$$

where T = temperature in degrees Kelvin. For the North Channel station, the average temperature between high and low tide was used.

Algal fractionation (ε_a) was calculated using CO_2(d) with the following equation from Laws et al. (1998):

$$\varepsilon_a = (\delta^{13}C_{DIC} - \delta^{13}C_a)/(1 + (\delta^{13}C_a/1000)) \quad (2)$$

where $\delta^{13}C_{DIC}$ = $\delta^{13}C$ value of CO_2(d) and $\delta^{13}C_a$ = $\delta^{13}C$ value of the algae.

Seston—At 0.5–1 m from the surface, 10-liter water samples were collected in acid-washed polyethylene containers and kept cool in the dark. For seston isotopic ratios, the samples were passed through Nitex filters to obtain the following size fractions: <10 μm (n = 11), 10–64 μm (n = 14) and >64 μm (n = 14), which were filtered onto precombusted (4 h at 450°C) GF/F filters and stored at −80°C prior to stable isotope analysis.

Bacteria—Upon arrival at the laboratory, particle-containing water >0.45 μm in 40 liters of water was concentrated to 500 mL by tangential flow filtration with fluorocarbon polymer filters (Pellicon, Millipore). The remaining 500 mL was passed through a 1.0 μm filter (n = 8) and this 0.45–1.0 μm fraction containing free bacteria was frozen at −80°C. For bacteria in aggregates (n = 7), which constitute a substantial fraction of total bacterial biomass in the maximum turbidity zone (Painchaud et al. 1995), the >10-μm fraction of the 500 mL concentrate was sonicated (100 W for 30–60 s) and then passed through a 1-μm filter and stored frozen at −80°C.

Algae—To complement the prediction of algal $\delta^{13}C$ using ε, algae were incubated in situ to obtain their isotopic signatures. The use of total seston for estimation of the phytoplankton isotopic signature is inappropriate in the ETZ because terrestrial-derived detritus and aggregates are dominant components of the seston and thus obscure the signal of autochthonous carbon (Martineau et al. 2004). Three protist species representing genera that are abundant in the St. Lawrence ETZ were obtained from the University of Toronto Culture Collection (UTCC): estuarine diatom UTCC 624 *Thalassiosira pseudonana*, freshwater mixotroph UTCC 336 *Cryptomonas* sp., and freshwater diatom UTCC 520 *Cyclotella* sp. (for detailed methods see Web Appendix 1 at http://www.aslo.org/lo/toc/vol_51/issue_3/1473al.pdf). The protists were incubated in situ at sites U2 and North Channel in Fisherbrand™ regenerated cellulose dialysis tubing (12,000–14,000 Dalton MWCO). After a growth period of 7 d, the algae were filtered onto GF/F filters for isotopic analysis and stored frozen at −80°C. Several tests were conducted to evaluate any boundary layer effects, DOC release from the tubes, and the isotopic equilibration time of the algae (Web Appendix 1).

Zooplankton and ichthyoplankton—Trawl sampling in the ETZ was carried out using two mesh sizes of net: 64-μm for zooplankton and 500-μm for fish larvae. A centrifugal pump was used to obtain the veliger larvae (flow of 20 L min^{-1}) between 2 and 8 m depth at all stations. Five to ten adult zebra mussels were collected by hand at low tide at St. Francois, Berthier-sur-Mer, and Québec City on rocky substrata near the shore. All organisms were preserved on ice until laboratory processing and then sorted by hand, washed with deionized water, freeze-dried, and kept in a desiccator. The larger samples were ground using an agate mortar and pestle.

Laboratory preparation of veligers for isotopic analysis—The presence of high concentrations of sediment, detritus, and microalgae created major difficulties for the sorting of small organisms, especially the veligers. We developed and applied an improved concentration protocol that yielded 20 composite samples with enough material (50–100 animals per sample) to give excellent resolution of the mass spectrometry peaks. For this revised protocol we used the colloidal silica matrix Ludox AM (specific gravity of 1.2) to separate veligers from heavier seston, as previously employed for studies of other bivalve larvae (Tremblay et al. 1987), and centrifugation in water to eliminate lighter detritus and microalgae from the veliger samples. An acid treatment was used to remove carbonate shell material (Pennington and Hadfield 1989) that could have masked the dietary carbon signal.

Isotopic analysis—Samples were freeze-dried (zooplankton and bacteria) or air-dried at 60°C (algae and seston) for 24 h and any carbonate was removed by exposure to HCl vapor. All isotopic analyses, except for bacteria and DOC, were carried out with an elemental analyzer NC 2500 (CE Instruments) coupled with an IRMS (VG Prism III, Fisons Instruments) at Commission Géologique du Canada, Quebec. Bacteria were analyzed at the G.G. Hatch Laboratory (Ottawa) using a Finnigan MAT DeltaPlus IRMS with an online elemental analyzer. DOC samples were analyzed at the same laboratory using a TOC-Analyser (Model 1010, OI Analytical) interfaced with

a Delta Plus IRMS (Thermo Finnigan). Stable isotope ratios were expressed in delta (δ) notation (‰) according to the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000 \quad (3)$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio, $^{13}C : {}^{12}C$ or $^{15}N : {}^{14}N$. The reference standards for ^{13}C and ^{15}N were PeeDee Belemnite and atmospheric N_2 respectively. The analytical error (SD) was 0.23‰ for carbon and 0.28‰ for nitrogen.

Trophic level (TL) for each sample was estimated by assuming a $\delta^{15}N$ trophic enrichment factor of 3.4‰. This value when applied to an entire food web with multiple trophic pathways and many species is a widely used average (reviewed in Post 2002). The natural variability in source N was corrected for by subtracting the value of $\delta^{15}N$ in local zebra mussels, assumed to occupy TL position 2 (Cabana and Rasmussen 1996):

$$TL = [(\text{mean organism } \delta^{15}N - \text{mean mussel } \delta^{15}N)/3.4] + 2 \quad (4)$$

Radiolabelled DOC uptake—We obtained radiolabelled DOC by incubating an algal culture with ^{14}C-bicarbonate (Roditi et al. 2000). In brief, 370 kBq of $NaH^{14}CO_3$ was added to 500 mL of an exponentially growing culture of the euryhaline diatom *Thalassiosira pseudonana* (UTCC 624). After 5 d and an increase in algal biomass from 8.6×10^3 to 7.9×10^4 mg m^{-3}, the cells were filtered onto 1.0-μm filters and lysed in 500 mL of 0.2-μm filtered freshwater from Lake St. Pierre (Canada) in which naturally occurring DOC had been photo-oxidized (<0.10 mg DOC L^{-1}) using a 450-W Hanovia UV lamp (Armstrong et al. 1966). The final concentration of the radioactive DOC (filtered through a 0.2-μm membrane) used in the uptake experiments was 4.15 mg DOC L^{-1} with an associated radioactivity of 1.5×10^5 kBq, after dilution with DOC-free water. This concentration was similar to naturally occurring concentrations at the site where the veligers were collected (2.8 ± 0.5 mg DOC L^{-1}), downstream of Lake St. Pierre, where concentrations averaged 5.0 ± 1.5 mg DOC L^{-1}.

Veligers were collected on the south shore of the St. Lawrence River at the outlet of fluvial Lake St. Pierre using slow-moving horizontal 63-μm zooplankton net hauls. The veligers were gently transferred to 2-liter polyethylene bottles that were filled to the rim with water (<63 μm) to minimize sloshing and mechanical damage to the animals during their immediate transport to the laboratory. The veligers were kept at a temperature of 22 ± 2°C at all times, similar to natural conditions. Upon arrival, they were left undisturbed for 45–60 min and then filtered between 150 and 63 μm to ensure uniformity in size (length = 102 ± 5 μm, width 78 ± 4 μm; $n = 50$). Veligers were then placed under a dissecting scope equipped with cross-polarization (Johnson 1995). Actively swimming veligers were isolated with a micropipette (10 μL) to minimize uptake of other particles and were transferred to DOC-free water a minimum of three times to minimize carryover effects. One hundred veligers were placed into 2 mL of DOC-free water in modified glass scintillation vials that had 63-μm screens across the top and screw caps with holes (diameter 1.4 cm). Veligers were left undisturbed for 45 min to decrease stress and to standardize their nutritional status. Veliger activity was then evaluated by inspection under the dissecting scope. Blanks were prepared with veligers that were killed by being kept in water at 60°C for at least 1 h.

Fifteen milliliters of freshly filtered (0.2-μm) radioactive DOC was added to each vial for the following time intervals: 5, 10, 20, 30, 40, 50, 60, and 120 min. Three trials were conducted with 100 live and dead veligers at each time interval. For blanks, the time intervals were 0, 10, 30, 50, and 120 min. When time was up, solution was sucked out of the vial through the 63-μm mesh. The veligers were thoroughly rinsed at least 3 times with DOC-free water. To dissolve the shell, 5 mol L^{-1} HCl (40 μL) was added for 2 min. The pH was then neutralized with 5 mol L^{-1} NaOH (45 μL). Veliger soft tissues were solubilized with 1 mL Hyamine Hydroxide (ICN Biomedicals) for 6 h at 60°C. The vials were allowed to cool, filled with the scintillation cocktail CytoScint ES™ (ICN Biomedicals), shaken and placed in the dark for a minimum period of 7 d before reading by scintillation counter. All samples were counted in a Wallac 1409 liquid scintillation counter, with the counts corrected for quenching by external standards. To test for the effect of DOC on veliger behavior, 9 veligers were isolated and kept in each DOC-containing and DOC-free water for a time series of 0, 44, 103, 223, and 403 min. Swimming activity, ciliate movement, and tissue contraction were noted from microscopic observations at each time interval.

Uptake calculations—Clearance rate (CR) and net DOC uptake rate (U) were calculated as follows, modified from Baines et al. (2005):

$$CR = \ln(((^{14}C_{initial} - {}^{14}C_{veligers} \text{ at time } t) \times [DOC])/{}^{14}C_{initial}) \times \text{mL solution/number of veligers} \quad (5)$$

$$U = CR \times \text{ng DOC mL}^{-1} \text{ in solution} \quad (6)$$

Similar rates were also obtained using a linear net-uptake model.

Autochthonous to allochthonous ratios of DOC—The DOC used in our uptake experiment was composed of autochthonous carbon derived from algal lysis. This type of DOC is considered labile, whereas allochthonous matter, originating from terrestrial sources, tends to be more recalcitrant and less suitable as a biological carbon source. In the natural river environment, both sources are likely to be present, and the extent of DOC utilization by veligers may depend on their relative concentrations. As one approach toward assessing the proportions of each type of DOC, we applied a synchronous fluorescence index (Belzile et al. 2002 and references therein) to 57 and 56 samples collected in 2001 at U2 and North Channel

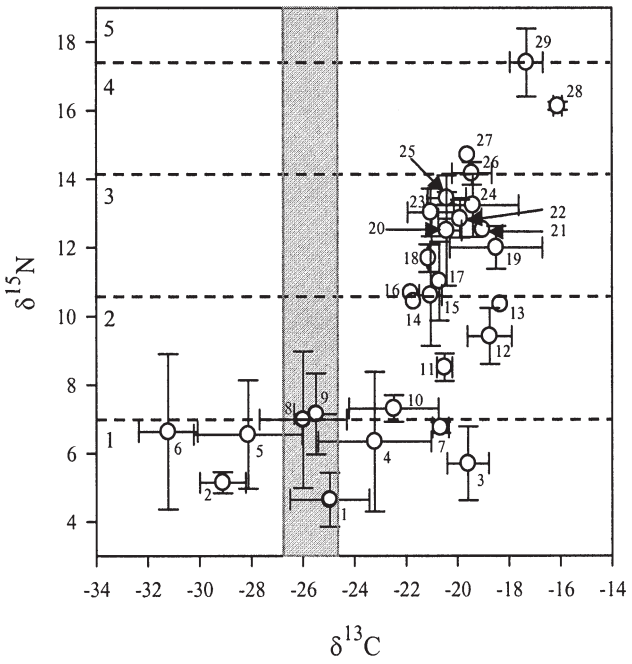

Fig. 2. Dual isotopic plot for all food web components in the St. Lawrence River ETZ. Sampling was conducted in summer 2001, with the exception of *S. canadense* (summer 2000) and microbial components (summer 2003). All data are averages ± SD. δ^{13}C of DOC is represented by the gray rectangle. Dotted lines delimit trophic levels 1–5. (1) Free bacteria, (2) aggregate bacteria, (3) estuarine algae, (4) *D. polymorpha* veligers, (5) seston 10–64 μm, (6) seston 0–10 μm, (7) *Keratella* sp., (8) freshwater algae, (9) seston >64 μm, (10) *D. polymorpha* adults, (11) *B. longirostris*, (12) *Gammarus* sp., (13) *C. commersoni*, (14) *A. sapidissima*, (15) *E. affinis*, (16) *A. pseudoharengus*, (17) Nauplii larvae, (18) *E. curticorne*, (19) *C. septemspinosa*, (20) Mysids, (21) *M. tomcod* juvenile, (22) *M. tomcod* larvae, (23) *O. mordax* larvae, (24) *G. aculateus*, (25) *M. villosus* larvae, (26) *O. mordax* adults, (27) *S. canadense* juvenile, (28) *M. tomcod*, (29) *S. canadense* adults.

respectively. Synchronous fluorescence scans (SFS) were recorded with a Cary Eclipse spectrofluorometer. The instrument was used in synchronous mode with a slit width of 5 nm on both the excitation and emission side and a difference of 14 nm ($\Delta\lambda$) between both light beams, and the results were corrected for the inner-filter effect based on spectrophotometric measurements of the same water. The SFS waveband ratio 265–335 nm to 336–500 nm was used as a guide to the autochthonous : allochthonous DOC ratio, modified from Belzile et al. (2002).

Results

Overall trophic structure—The ETZ food web components were ranked according to their carbon and nitrogen isotopic ratios (Figs. 2, 3; Table 1). The δ^{13}C values ranged from −32.5‰ (seston <10 μm) to −15.9‰ (adult *M. tomcod*) and δ^{15}N ranged from 2.6‰ (seston <10 μm) to 18.1‰ (adult *S. canadense*). There was no obvious clustering of species into functional trophic groups (primary consumers versus predators, for example).

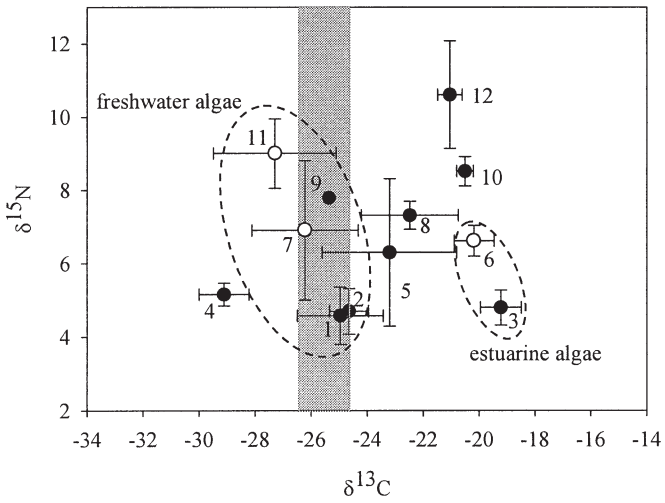

Fig. 3. Dual isotope plot for microbial components and primary consumers in the St. Lawrence River system. Values are means ± SD. δ^{13}C of DOC is represented by the gray rectangle. Black dots (North Channel): (1) Free bacteria (mean for U2 and North Channel), (2) *Cyclotella* sp., (3) *Thalassiosira* sp., (4) aggregate bacteria (mean for U2 and North Channel), (5) *D. polymorpha* veligers (mean for U2 and North Channel), (6) *Thalassiosira* sp., (7) *Cyclotella* sp., (8) *D. polymorpha* adults, (9) *Cryptomonas* sp. (10) *Bosmina* sp., (11) *Cryptomonas* sp., (12) *Eurytemora* sp.; white dots (U2): Values in parentheses below are the results from paired *t*-tests between the veligers' carbon signature (mean of ETZ) and individual potential carbon sources, for U2 and ETZ (North Channel) respectively. *Cyclotella* sp. (t = 1.96, df = 19, p = 0.065; and t = 0.67, df = 18, p = 0.511); *Cryptomonas* sp. (t = 2.28, df = 18, p = 0.035; and NA [n = 1]); *Thalassiosira pseudonana* (t = −2.64, df = 19, p = 0.016; and t = −3.975, df = 20, p < 0.001); free bacteria (t = 1.19, df = 20, p = 0.25); aggregate bacteria (t = 5.86, df = 22, p < 0.001); DOC (t = 0.79, df = 18, p= 0.439; and t = 2.04, df = 20, p = 0.055).

DOC and microbial food web—DOC, free bacteria and freshwater diatoms (*Cyclotella* sp.) and mixotrophs (*Cryptomonas* sp.) had overlapping δ^{13}C values, ranging from −30.2‰ to −18.6‰ (Fig. 3). The lowest values were for aggregate bacteria and the highest for the estuarine alga *Thalassiosira pseudonana*. Free bacteria had very similar δ^{13}C to that of DOC (Table 1) whereas the δ^{13}C of aggregate bacteria was about 4‰ lower and very close to that of the <10-μm and 10–64-μm seston fractions (Fig. 2). Large differences in δ^{13}C were observed between the freshwater and estuarine algae (Fig. 3). There was much overlap in the δ^{15}N at this lowest trophic level, ranging between 3.4‰ and 9.7‰, with the highest values obtained for the mixotrophs and the lowest for the free bacteria (Fig. 3).

DIC and fractionation estimates—Measured δ^{13}C of DIC and calculated δ^{13}C of CO_2(d) are shown in Table 2. These were within the range of previously obtained values in the St. Lawrence upper estuary (Hélie et al. 2002).

Seston and primary consumers—For seston, means for July and August and high and low tides were calculated. Seston δ^{13}C for the <10-μm fraction was well below the

Table 1. Trophic position and stable isotope data for each food web component sampled in the St. Lawrence estuarine transition zone. For the algae, values are means from U2 and North Channel.

Species	Trophic position	δ^{13}C (‰)	Range	δ^{15}N (‰)	Range
Stizostedion canadense	5.0	−17.3	−16.9 to −17.8	17.4	16.7 to 18.1
Microgadus tomcod	4.6	−16.1	−16.3 to −15.9	16.1	16.0 to 16.3
Osmerus mordax	4.0	−19.4	−19.9 to −17.7	14.2	13.7 to 14.6
Mallotus villosus larvae	3.8	−20.4	−21.5 to −19.9	13.4	13.2 to 13.5
O. mordax larvae	3.7	−21.0	−22.3 to −19.7	13.0	11.8 to 14.4
M. tomcod larvae	3.7	−19.9	−21.6 to −18.7	12.9	12.0 to 13.6
Mysids	3.6	−20.2	−21.1 to −18.9	12.6	10.6 to 18.4
Crangon septemspinosa	3.4	−18.5	−20.4 to −15.7	12.0	11.3 to 12.8
Ectinosoma curticorne	3.3	−21.1	−22.2 to −21.1	11.7	11.4 to 12.0
Nauplii larvae	3.1	−20.7	−21.0 to −20.6	11.0	10.2 to 11.8
Eurytemora affinis	3.0	−21.0	−21.8 to −20.3	10.6	9.2 to 12.5
Gammarus sp.	2.6	−18.7	−20.4 to −17.6	9.4	8.5 to 11.0
Bosmina longirostris	2.4	−20.5	−20.9 to −20.1	8.5	8.2 to 8.8
Cryptomonas sp.	2.4	−26.6	−28.8 to −25.4	8.6	7.8 to 9.7
Dreissena polymorpha	2.0	−22.5	−23.9 to −20.5	7.3	6.1 to 7.7
Keratella sp.	1.8	−20.7	−20.9 to −20.2	6.8	6.5 to 6.9
D. polymorpha veliger	1.7	−23.2	−24.6 to −19.9	6.3	3.7 to 8.6
Cyclotella sp.	1.6	−25.6	−28.3 to −24.2	6.0	4.3 to 8.2
Thalassiosira sp.	1.5	−19.6	−20.9 to −18.6	5.7	4.3 to 6.9
Bacteria (aggregates)	1.4	−29.1	−30.2 to −27.8	5.1	4.8 to 5.5
Bacteria (free cells)	1.2	−25.0	−27.2 to −22.1	4.6	3.4 to 5.8
>64-μm seston	—	−25.5	−27.7 to −24.3	7.1	5.6 to 9.5
10–64-μm seston	—	−28.1	−31.2 to −23.8	6.6	5.0 to 7.9
0–10-μm seston	—	−31.21	−32.5 to −29.7	6.6	2.6 to 10.5
DOC	—	−25.5	−26.7 to −24.7	—	—

values of all components of the food web, including the primary consumers *Dreissena polymorpha*, *Keratella* sp., *Bosmina longirostris*, and *Eurytemora affinis*. The δ^{13}C increased with increasing size fraction, averaging −31.21‰, −28.1‰, and −25.5‰ for the fractions <10, 10–64, and >64 μm respectively (Table 1). The four primary consumer species in the ETZ ranged in δ^{13}C from −22.5‰ to −20.5‰ with a mean of −21.0 ± 0.9‰, well above the seston values. There was no overlap in δ^{13}C of these four consumers. The disparity between the seston and the primary consumers was even more striking for the <10-μm fraction, which contributes 55–80% of total seston carbon, with δ^{13}C averaging about 11‰ below the primary consumers. The δ^{15}N for the seston fractions averaged 6.6 ± 2.3‰, 6.6 ± 1.6‰, and 7.1 ± 1.2‰ respectively for the fractions <10, 10–64, and >64 μm, whereas primary consumers averaged 8.5 ± 1.9‰, with significantly higher values for the copepod *E. affinis*. The consumers of lowermost trophic rank, the zebra mussel veligers, had δ^{13}C intermediate between freshwater and estuarine diatoms incubated in situ in the river and were not significantly different from freshwater *Cyclotella* sp., DOC, and free bacteria (Fig. 3). Much δ^{15}N variability was observed with the veligers, and these values overlapped with those of bacteria (free-living and aggregated), seston fractions, adult zebra mussels, and the rotifer *Keratella* sp.

Application of a 3-end-member mixing model (the MIXMODEL of Phillips and Gregg [2003]) to these isotopic data indicated that DOC could provide 26–33% of food sources for the veligers, with the remainder contributed by algae and free-living bacteria. For this analysis, we assumed fractionation by the veligers of 1‰ for ^{13}C and 3.4‰ for ^{15}N.

Zebra mussel veligers and adults showed similar average isotopic values and trophic ranking, implying a similar diet and carbon source (Table 1). There was no difference in the isotopic composition of *D. polymorpha* veligers between the upstream and ETZ areas, nor was there any difference between adult populations from the two regions. The veligers showed a gradual, linear enrichment in their ^{13}C content throughout the summer ($y = 0.0961x - 42$, $n = 14$, $r^2 = 0.59$). The δ^{13}C ranged from −26.8‰ to −19.3‰ with no significant difference between upstream U1 and ETZ samples ($t = -0.0456$, $n = 14$, df = 12, $p = 0.96$). Upstream veligers averaged a lower δ^{15}N (1.6‰) relative to those in the ETZ (6.3‰), but given the large variability within each population this difference was not significant ($t = 3.0$; $n = 6$; df = 4; $p = 0.13$). For the adults, there was no significant difference in carbon isotopic ratios between ETZ and upstream individuals ($t = 0.573$; $n = 8$; df = 6; $p = 0.59$). However, the average δ^{15}N was higher (7.3‰) in the ETZ mussel tissues relative to upstream mussels (6.6‰; $t = 2.54$; $n = 8$; df = 6; $p = 0.04$).

Bosmina longirostris had a higher trophic position (2.4) relative to adult *D. polymorpha* (2.0) and veligers (1.7). Adult *D. polymorpha* had isotope ratios that were closer to those of *B. longirostris* for both carbon (depleted by 1.97‰) and nitrogen (depleted by 1.20‰) than veliger larvae, which were depleted by 2.71‰ and 2.17‰ respectively for carbon and nitrogen relative to *B. longirostris*. Statistical analysis of these results for adult *D. polymorpha* and *B. longirostris* confirm that δ^{13}C enrichment was greater than 1‰ (t =

Table 2. Physical variables in the St. Lawrence estuarine transition zone. Station locations are shown in Fig. 1.

Station	δ^{13}C DIC (‰)	δ^{13}C CO_2(d) (‰)*	[DIC] (mg L^{-1})	Temperature (°C)†	Salinity†
U2	−2.95	−9.33	16.55	21.78	0.12
North Channel					
High tide	−2.02	−9.62††	17.25	17.73	7.35
Low tide	−5.05		15.00	20.00	1.12

* Calculated value (see Materials and methods).
† Average from surface to 2 m.
†† Average for high and low tide in the transition zone.

5.05, $n = 30$, df = 28, $p < 0.0001$) but δ^{15}N enrichment was smaller than 3.4‰ ($t = -4.6097$; $n = 18$; df = 16; $p = 0.0002$), indicating an indistinguishable trophic level for these organisms according to the criteria of Post (2002). The differences in isotopic ratio between *B. longirostris* and veliger larvae were statistically greater than 1‰ for carbon ($t = 3.978$, $n = 20$, df = 18, $p = 0.0008$) and equal to 3.4‰ for nitrogen ($t = -0.994$, $n = 12$, df = 10, $p = 0.34$).

Secondary consumers—*Gammarus* sp. averaged −18.7‰ for δ^{13}C and 9.4‰ for δ^{15}N (Table 1). *Eurytemora affinis* and its larval nauplii had similar isotopic values (*t*-test: $t = -0.115$, df = 17, $p = 0.91$ for carbon; Mann–Whitney rank sum test: T = 60.5, $n_{\text{nauplii}} = 6$, $n_{E.\ affinis} = 14$, $p = 0.87$ for nitrogen) and thus occupied the same trophic level (Table 1). The sand shrimp *Crangon septemspinosa* averaged a relatively high trophic position (3.4) with a wide range of δ^{15}N suggesting large variations in diet among individuals. The mysids (*Mysis stenolepis* and *Neomysis americana*) averaged a trophic position of 3.6, just below that of the fish larvae *O. mordax* and *M. tomcod*, with δ^{13}C ranging between −21.1‰ and −18.9‰ (Table 1).

Fish populations—The abundant larval fish species of the ETZ, *O. mordax* and *M. tomcod,* had very similar isotopic ratios and consequently trophic rankings (Table 1). For the adults, *O. mordax*, *M. tomcod*, and *S. canadense* were statistically different in their isotopic ratios (Kruskal–Wallis one-way ANOVA: $H = 9.24$, df = 2, $p < 0.001$ for carbon and $H = 9.791$, df = 2, $p < 0.001$ for nitrogen). *S. canadense* occupied the highest trophic position (TL = 5).

DOC uptake and in situ composition—A mean net uptake rate of 0.031 ng (DOC) veliger^{-1} min^{-1} was calculated for the 2-h incubation of veligers on algal lysates, equivalent to 1.5% of the total dry weight of veligers and 6% of soft tissue dry weight per hour. The 2-h incubation gave a mean net uptake rate by veligers of 14.2 mg (DOC) g dry weight^{-1} h^{-1}. In the absence of DOC, the veligers contracted their tissues over time and were significantly smaller at 403 than at 0 min ($t = 3.93$. $n = 9$, df = 15, $p = 0.001$). Also, swimming activity eventually stopped and the cilia were completely contracted. However, in the presence of DOC, there was some initial contraction, then full recovery; swimming activity was maintained throughout the experiment, and there were no significant differences in size between the beginning and the end of the experiment ($t = 0.40$, $n = 9$, df = 15, $p = 0.69$). The SFS analysis of DOC during July–August 2001 showed that the mean (±SE) autochthonous: allochthonous ratio was 1.65 ± 0.13 for freshwater station U2 and 1.26 ± 0.06 for the ETZ North Channel station.

Discussion

Overall food web—The isotopic ranges observed in the St. Lawrence ETZ are similar to those in other estuarine ecosystems, with broad omnivory and an absence of distinct trophic levels (France et al. 1998). The pronounced δ^{13}C offset between consumers and seston and the relatively small δ^{15}N difference relative to primary consumers of lowest trophic rank were previously reported by Martineau et al. (2004). This disparity was interpreted as evidence both of weak trophic coupling between consumers and the bulk seston and of strong selectivity by the consumers for prey items that constitute only a minor fraction of the total seston. The more negative seston values for the transition zone likely reflect the high detrital and terrestrial influence in this maximum turbidity zone and are consistent with the values obtained by Martineau et al. (2004). The present study extends these observations to other animals, including veligers, and higher trophic levels, and shows that the entire ETZ food web is trophically decoupled from most of the particulate organic matter. This unusual feature sets the ETZ apart from many other types of aquatic ecosystems (see the intersystem comparison in Martineau et al. 2004). This was also observed in Bothnian Bay (Baltic Sea), where two separate trophic structures were noted. These included the smallest seston fractions (microbial components and terrigenous matter) with very negative δ^{13}C values and larger seston fractions (mesozooplankton) with higher δ^{13}C values, perhaps representative of the microbial-decomposer and the algal-grazer pathways (Rolff and Elmgren 2000) and consistent with our observations. Our data lend support to the contention of high prey selectivity. The δ^{13}C values of the ETZ consumers are intermediate between those of the freshwater protists, >10-μm seston, and estuarine algae, and consequently these consumers may rely on a combination of these sources. The observations are also consistent with a previous study that found that the maximum turbidity zone of the ETZ acted as a retention zone wherein diatoms and larger particles advected from upstream and produced in situ were concentrated, resulting in a rich feeding zone characterized by high diversity and productivity (Frenette et al. 1995). Aggregates and their associated organisms have been documented as an important food source for *Eurytemora* sp. (Zimmermann-Timm 2002). Because of the high δ^{13}C of the primary and secondary consumers of the ETZ relative to the freshwater algae, estuarine phytoplankton is likely to be a more important carbon source for the food web.

Zebra mussel carbon sources—The difference between veliger and <10-μm seston isotopic characteristics implies

that the veligers selected specific components of the total seston or that they used different carbon sources that were not well represented in our <10-μm seston samples. The carbon signature of bacteria in aggregates resembled that of the total seston, which is dominated by detrital material of largely terrigenous origin (Martineau et al. 2004). The isotopic data imply that bacteria in aggregates are not an important food source, but they do not exclude direct feeding by the veligers on DOC, free-living bacteria, or phytoplankton. There was much overlap between the δ^{15}N of the veligers and freshwater diatoms. Trophic δ^{15}N offsets are typically in the range of 2–4‰ (but with large variability between systems; Martineau et al. 2004), and the results imply that upstream phytoplankton populations were not a major nutritional source for the veligers. The large variability in δ^{15}N of the veligers, however, implies nitrogen uptake from diverse sources.

The isotopic characteristics of both zebra mussel life stages deviated greatly from the seston, especially the smaller seston size class containing particles they are likely to filter most efficiently (the size range 1–10 μm; Sprung 1993; Bernier 2003). As noted above, this disparity is representative of all ETZ animals and suggests a high selectivity for prey items. This feature is unusual relative to several other studies on mussels that have shown that the isotopic signature of the animals closely reflects that of the locally produced particulate organic matter (Thorp et al. 1998 and references therein). However, bivalve selectivity is known from other environments. For example, Raikow and Hamilton (2001) used both natural abundance and enrichment of ^{15}N to show that the freshwater bivalve *Sphaerium striatinum* preferentially assimilated the highly enriched living component of suspended and/or benthic organic matter, rather than assimilating the bulk material. Our results contrast markedly with those reported by Mitchell et al. (1996) who examined adult zebra mussels in Oneida Lake (New York). They observed highly depleted carbon ratios (−33.6‰ to −31.3‰) and concluded that the animals were using the entire seston resource rather than only one component, and that they were not feeding on sediment material (δ^{13}C = −26.2‰ to −25.6‰). The large variability in the δ^{15}N of veligers in this study may reflect the small number of samples, although each sample represented more than 50 individuals. Contamination of the samples is possible given the complex manipulations required to obtain a sufficient sample mass. However, it is likely that much of this variability is real and reflects broad omnivory by the larvae and variations in food composition.

Autochthonous $\delta^{13}C$—There were large differences in δ^{13}C among the three protist species incubated in situ. Given this interspecific variability, no additional precision was gained relative to using only DIC δ^{13}C and assuming a range of algal fractionation. However, the in situ incubation yielded algal biomass δ^{13}C that was substantially heavier than the seston (notably for the estuarine species), thereby corroborating the estimates based on fractionation. The in situ incubation data provided additional support for the contention that the isotope ratio of total seston is a poor indication of autochthonous sources, particularly in ecosystems that receive large inputs of allochthonous materials and detritus. The incubation data point to the differences in δ^{13}C that can occur among phytoplankton species even within the same physical and chemical environment, as well as the variability between sites for the same species. Such variability could not have been discerned from the fractionation estimates.

The values in Table 3 for fractionation relative to CO_2(d) compare well with those of Fogel et al. (1992) for phytoplankton-dominated seston in the Delaware Estuary (pH from 7.0 to 8.4). Marine diatoms have been shown to directly take up bicarbonate without the energy-requiring steps of the enzyme carbonic anhydrase, which converts accumulated bicarbonate to CO_2, thereby reducing the overall carbon isotope fractionation (Korb et al. 1997; Tortell et al. 1997). This direct uptake mechanism is known for *T. pseudonana* (Laws et al. 1998), which may explain the lower carbon isotope fractionation observed in our study for this species relative to the freshwater taxa.

Veligers as competitors with indigenous species—Veligers and indigenous zooplankton species do not appear to be sharing the same carbon sources. The trophic position of veligers was well below that of *B. longirostris*, implying that dietary overlap is unlikely between these taxa. The nitrogen and carbon isotopic ratios of the adult mussels were more similar to those of *B. longirostris* than to those of the veligers, and therefore interspecific competition is likely to be greater between *B. longirostris* and the adults. However, no adults have been observed on the fluvial bed of the ETZ (A. Casper unpubl. data); hence, any effects on potential competitors should not be attributable to adult populations. Veligers had a trophic position (1.7) that was very close to that of the rotifer *Keratella* sp. (1.8), implying potential dietary overlap. However, their δ^{13}C differed, indicating some dietary difference. Veligers may be feeding less efficiently in the ETZ than upstream because of the stress induced by high turbidity, high turbulence, and changing salinity. This reduced feeding would be consistent with the lack of significant difference between their upstream and downstream carbon signatures.

Veligers as prey for indigenous species—The δ^{15}N values of the adult fishes occupying high trophic positions (*S. canadense, M. tomcod*, and *O. mordax*) were well above those of the veligers (more than one trophic position), indicating that the latter were not a major source of food for top predators. Consistent with our results, the stomach contents of *O. mordax* and *M. tomcod* larvae showed no evidence of predation on veligers by these species (H. Yoneyama unpubl. data). Based on this same isotopic argument, similar conclusions pertain to *M. villosus* larvae because their isotopic ratios were similar to those of other larval fish. Furthermore, the veliger nitrogen isotopic ratios were about two trophic levels below those of the fish larvae, indicating that they were not a major component of the larval fish diet.

Consumers dependent on veliger larvae should be found one trophic level higher in terms of δ^{15}N, and with a δ^{13}C

Table 3. Carbon isotope fractionation of algae (ε_a) based on uptake of CO_2(d) in the freshwater zone (U2) and North Channel in the transition zone. Values are means ± SD, with *n* indicated in parentheses.

Algae	U2	North Channel
Cryptomonas sp.	18.5 ± 2.3 (2)	16.1 (1)
Cyclotella sp.	17.3 ± 2.0 (3)	15.4 ± 0.7 (2)
Thalassiosira pseudonana	11.1 ± 0.7 (3)	9.7 ± 0.6 (4)

value close to that of veligers. Three fish species meet these criteria (Fig. 2): *A. sapidissima* (TL = 2.9), *C. commersoni* (TL = 2.9) and *A. pseudoharengus* (TL = 3.0). These species fed one trophic level higher than the veligers and showed similar δ^{13}C. However, *C. commersoni* is likely to be a benthic feeder, with high δ^{13}C values. *E. affinis* and nauplii larvae were also one trophic level higher than the veligers, had similar carbon isotopic ratios, and thus could be potential consumers. Amphipods (*Gammarus* spp.) were also one trophic level higher than the veligers, but had high δ^{13}C values consistent with their largely benthic habit. The isotope ratios of the mysids suggest little potential for veligers to serve as prey.

Prey of fish larvae—Consistent with previous studies (Winkler et al. 2005 and references therein), the isotopic data indicate that the zooplankton *B. longirostris* and *E. affinis* are important food sources for fish larvae. All the fish larvae analyzed in the present study (*M. villosus*, *M. tomcod*, and *O. mordax*) had very similar carbon and nitrogen isotopic signatures, suggesting their dependence on the same combination of prey items. Their δ^{13}C values suggest that *N. americana*, the copepods *E. affinis* and *E. curticorne*, nauplii larvae, and *B. longirostris* are likely to be the major prey items for fish larvae.

Secondary consumers—*Gammarus* sp. showed a large range of carbon and nitrogen isotopic ratios, implying omnivorous behavior, and their relatively high δ^{15}N likely reflects foraging on benthic invertebrates. Sand shrimp had a wide range of δ^{15}N, suggesting an omnivorous diet, consistent with studies on feeding habits by Wilcox and Jeffries (1974) that showed that this species fed on many crustacean taxa and other invertebrates as well as detritus.

Adult fish—The two planktivorous fishes sampled, *A. pseudoharengus* and *A. sapidissima*, are known to have very similar diets of cladocera, copepods, and insect larvae (Scott and Crossman 1974). Consistent with these previous observations, these two species had similar carbon and nitrogen isotopic ratios in the ETZ and occupied a similar trophic position (3.0). The adults of *C. commersoni* had δ^{13}C higher than either *Alosa* species, consistent with the known importance of benthic organisms in its diet, but δ^{15}N was similar (Scott and Crossman 1974). The highest trophic rankings in our analysis were for the three most abundant adult fishes in the ETZ: *O. mordax*, *M. tomcod*, and *S. canadense*. *S. canadense* occupied the highest trophic position. In the St. Lawrence estuary, they consume principally amphipods as juveniles and tomcod as adults (Dodson unpubl. data). *M. tomcod* typically preys on smaller fish (i.e., *Osmerus* sp. and *Gasterosteus* sp.) and many small crustacea such as shrimp and amphipods (Scott and Crossman 1974). Consistent with the partial feeding on small fish, the isotopic ratios of adult *M. tomcod* in the ETZ place it just above that of *O. mordax*. In the St. Lawrence estuary, smelt feed primarily on mysids, amphipods, *C. septemspinosa*, and nereids (Lecomte and Dodson 2005).

DOC uptake—Radiolabelled uptake experiments in the laboratory confirmed that veligers from the St. Lawrence River have the capacity to directly assimilate DOC from the water (Fig. 4). The 2-h incubation gave a mean net uptake rate by veligers of 14.2 mg (DOC) g dry weight^{-1} h^{-1}, whereas that for adults has been estimated as 0.03 mg (DOC) g dry weight^{-1} h^{-1} (Roditi et al. 2000). This difference may reflect the allometric relationship between specific growth rate and body size. This high rate for veligers is unlikely to be sustained for long periods of time, and although the algal lysates were supplied at concentrations similar to the DOC of the St. Lawrence River, they are likely to have been of much higher nutritional quality relative to a large fraction of DOC occurring naturally in the river. Furthermore, the veligers were preincubated in food-free water for 1 h prior to the start of the experiment, and this may have stimulated uptake rates. However, the mixing model supports our high uptake rates because it showed that DOC could represent an important carbon source for the veligers. The model's results may underestimate the importance of DOC for veligers if they discriminate against some phytoplankton cells, for example based on size, or differentially take up algal exudates in the dissolved organic matter pool that have a similar isotopic signature to algal carbon and nitrogen. The large DOC uptake capacity shown here points to algal exudates as an important carbon source that could meet much of the total growth and maintenance requirements of zebra mussel veligers. Our behavioral observations of the veligers with and without the algal lysates also implied a beneficial maintenance effect of DOC.

Previous work on bivalves in marine systems has similarly shown a large difference in DOC uptake capacity between life history stages. Specifically, *Mytilus edulis* veligers and pediveligers have up to an order of magnitude faster uptake rate of DOC relative to the adults, and this may confer an advantage on the larval stages given that the energy reserves provided by the adults are minimal (Manahan et al. 1982). Furthermore, the reorganization of tissues during metamorphosis from veliger to adult may interrupt particulate feeding for a period of several days, and DOC uptake may at that time provide the sole mechanism of carbon acquisition; it has been noted, for example, that during metamorphosis, *Mytilus edulis* veligers can absorb [^{3}H] glycine through developing gill buds (Manahan et al. 1982). Recent evidence has shown that the larvae of *Crassostrea gigas* can survive long feeding delays while maintaining a constant rate of metabolism, and the

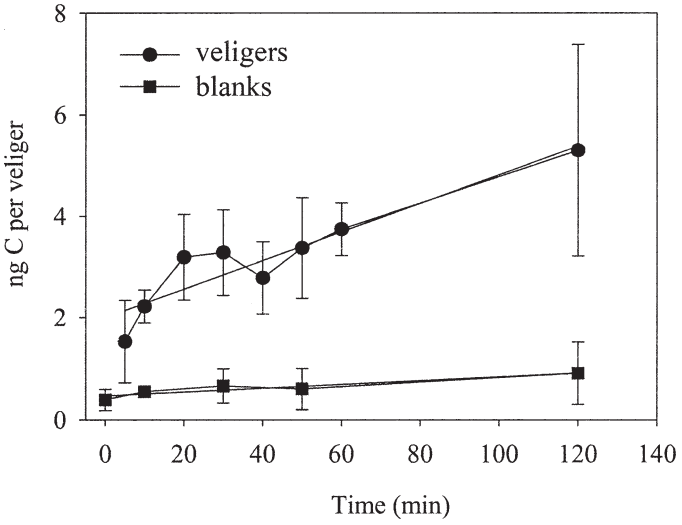

Fig. 4. Uptake of DOC by zebra mussel veligers. Each value is the mean of triplicates ± SD, with 100 veligers per replicate. Uptake from live veligers was significantly different from that of blanks (ANCOVA, $F_{1,36} = 131.56$, $n = 39$, $p < 0.0001$). The linear regression lines are for untransformed data ($r^2 = 0.84$, $n = 8$, $p = 0.001$; blank regression: $r^2 = 0.88$, $n = 5$, $p = 0.02$).

authors suggested that the larvae feed on detrital matter or DOC, which would fuel their maintenance metabolism for extended periods equivalent to four times the predicted lifespan (Moran and Manahan 2004). Previous studies have drawn attention to the high metabolic demands of zebra mussel veligers that seem incompatible with their low particle uptake rates (Sprung 1993). The direct uptake of DOC as observed here could satisfy this apparent imbalance in energy supply and demand. Polis et al. (1997) have suggested that the use of terrestrial subsidies by organisms could stabilize food webs. This could be the case with the veligers, the dominant zooplankton component, using an alternative subsidy, thereby not directly disturbing food web structure.

DOC: in situ composition—The autochthonous : allochthonous ratios of the natural DOC as determined by SFS analysis were >1, implying that labile carbon, perhaps algal-derived, makes a large contribution to the total DOC pool of the river and transition zone during the summer, consistent with previous biogeochemical analyses (Hélie 2004). Similarly, in large northeastern United States rivers, the composition of DOC was similar to that of algal exudates (Roditi et al. 2000). In the Mississippi River plume, elevated DOC concentrations were probably from direct release by the high phytoplankton biomass or from grazing-mediated processes during spring and summer (Dagg et al. 2004). High concentrations of combined dissolved carbohydrates provided molecular evidence for the production of DOC at midsalinities in that plume (Benner and Opsahl 2001). In the present study, the $\delta^{13}C$ for DOC and free bacteria was closer to values inferred for phytoplankton rather than the total seston, the latter more depleted and more strongly influenced by terrestrial organic matter and detritus (Martineau et al. 2004). This is consistent with of the findings of Chin-Leo and Benner (1992) who found that plankton-derived organic matter supported 68% of bacterial production in the Mississippi River during the summer. Nevertheless, the uptake of some allochthonous materials by veligers cannot be ruled out. Adult zebra mussels are known to take up humic acids in addition to algal lysates (Voets et al. 2004), but the $\delta^{13}C$ of humics is likely to reflect their terrestrial origins and the nonterrestrial signature of zebra mussel veligers in the St. Lawrence River implies that this is not a primary source in their diet.

DOC uptake mechanisms—Apart from the observational evidence presented here, there are also mechanistic reasons for considering DOC as a food source for zebra mussel veligers. Bivalves are known to take up amino acids by substrate-specific transporters located across the membranes of epithelial cells in their gills (Wright and Manahan 1989). No bacterial associations have been identified in conjunction with the uptake of DOM in adult and veliger bivalves (Manahan et al. 1982). Rapid, intense labeling of the gill epithelium of *Mytilus edulis* was revealed by autoradiograms of adults exposed for brief periods to radiolabeled amino acids (Manahan et al. 1982), indicative of direct assimilation rather than via any intermediate steps associated with symbiotic bacteria.

Although veligers have distinctive feeding mechanisms by comparison with other invertebrates, it may be that their rapid rates of DOC uptake are also to be found in other groups of freshwater taxa. Early studies gave conflicting results on the capacity of marine crustaceans to assimilate DOC (McWhinnie and Johanneck 1966; Anderson and Stephens 1969), yet they nonetheless resulted in the premature generalization that crustaceans cannot take up DOC. Studies on larval insects have shown the capacity for DOC uptake (Ciborowski et al. 1997), and this requires further investigation in other arthropod taxa. In some strongly heterotrophic systems, terrestrial matter seems to be the principal carbon source via bacterioplankton and their subsequent consumption, yet this bacterial carbon is inefficiently transferred to consumers, and is also insufficient in biomass to meet the carbon requirements for higher consumers. A recent study indicated that terrestrial matter was an important source of carbon for a lentic food web (Pace et al. 2004), yet imbalances in the carbon budget were noted because the sum of particulate organic carbon (POC) from both terrestrial sources and in-lake production could not meet the carbon requirements of the food web. It was speculated that the aggregation of DOC into microparticles and the subsequent ingestion of POC could possibly account for some of this carbon. Given that DOC is generally 10 times more abundant than POC (Wetzel 2001), the direct transfer of DOC to primary consumers and subsequently higher trophic levels deserves closer attention.

These results define the ETZ food web as a complex network in which omnivory dominates. Although this ecosystem is likely to be net heterotrophic (Painchaud et al. 1995), autochthonous sources appear to dominate the

trophic supply of carbon to ETZ animals, and the productive food web seems largely decoupled from decomposer pathways based on allochthonous organic matter. Despite their high abundance in the ETZ, zebra mussel veligers were not sharing resources with native zooplankton and did not appear to serve as important carbon sources for higher trophic levels. Veliger carbon sources likely include free bacteria, phytoplankton, and DOC. Uptake experiments resulted in relatively high uptake rates of algal lysates, implying that autochthonous DOC represents an alternate food source for this invasive zooplankton. Exotic invaders including adult zebra mussels often occupy unusual niches that have no parallel in the native community (Ricciardi and Rasmussen 1998). The larval stage of zebra mussels appears to follow this pattern and to neither compete with nor act as a major food source for other major food web components in the ETZ ecosystem.

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