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Stable isotope addition reveals dietary importance of phytoplankton and microphytobenthos to saltmarsh infauna

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ABSTRACT: Despite the paradigm that *Spartina* spp. detritus is the basis for estuarine food webs, other primary producers may contribute to the diets of saltmarsh consumers. To determine the dietary contribution of primary producers to benthic infauna in the Plum Island Estuary, Massachusetts, USA, we examined natural abundance stable isotopes in 4 intertidal saltmarsh habitats and conducted an ^{15}N enrichment experiment in 2 habitats. Natural abundance isotope data suggested that *Spartina* spp. detritus was of limited dietary importance to infauna in all habitats (including *Spartina* spp. understory) and instead benthic algae and phytoplankton were the dominant food sources. ^{15}N enrichment was used to improve dietary resolution of benthic algae and phytoplankton sources that had similar natural abundance values. To label only benthic algae, ^{15}N-enriched $Na^{15}NO_3$ was applied daily for 14 d to sediment in mudflat and creek-wall habitats. Food-web incorporation of ^{15}N-labeled benthic algae was found in most species. However, label uptake in the polychaetes *Manayunkia aestuarina*, *Fabricia sabella* and *Streblospio benedicti* indicated that phytoplankton was the most important food source for these consumers. Label uptake in the polychaete *Nereis diversicolor* differed between habitats, suggesting a large dietary contribution of microphytobenthos (MPB) in mudflat and phytoplankton in creek wall. The oligochaete *Paranais litoralis* consumed both MPB and phytoplankton regardless of habitat. The harpacticoid copepod *Heterolaophonte* sp. consumed primarily epiphytic diatoms. Overall, infauna in this system relied on phytoplankton and benthic algae as dominant food resources, and dietary contributions from primary producers varied among species and habitats.

KEY WORDS: Food web · Stable isotopes · Saltmarsh · Isotope addition · Infauna · Microphytobenthos · Phytoplankton

INTRODUCTION

Estuaries and associated saltmarshes are among the most productive ecosystems in the world. Macrophyte marsh plants, phytoplankton and benthic algae, including macroalgae, filamentous algae, associated epiphytes and sediment-associated microphytobenthos (MPB), all contribute to high primary productivity. Each of these types of primary producers is a potential food source for estuarine consumers. Historically, food webs in saltmarshes were thought to be based on the detritus of macrophyte plants, primarily *Spartina alterniflora* and *S. patens* (Teal 1962). However, *Spartina* spp. detritus is low in nutritional value and is more refractory to food web use than other estuarine primary producers (Tenore 1988, Mann 2000). Bacteria may improve the nutritional value of macrophyte detritus by incorporating nitrogen from surrounding waters, but even so, bacterial biomass and production are probably too low to wholly support deposit feeders

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(Cammen 1980, Lopez & Levinton 1987). For example, van Oevelen et al. (2006a) found that bacteria contributed minimally to the diet of intertidal benthic infauna. Recent attention has been given to the dietary role of the less conspicuous MPB, macroalgae, filamentous algae and epiphytic algae (here collectively called benthic algae) that inhabit marsh mudflats and surrounding areas (Haines & Montague 1979, Kwak & Zedler 1997, Quiñones-Rivera & Fleeger 2005). Specifically, isotope studies have revealed the importance of MPB and other algae to the diet of saltmarsh infauna (Herman et al. 2000, Levin et al. 2006). Because they are relatively nutritious and easy to digest, algae may be a preferred food source for deposit- and suspension-feeding infauna even though they may live in a sediment matrix rich in *Spartina* spp. detritus (Lopez & Levinton 1987, Tenore 1988, Kreeger & Newell 2000, Sullivan & Currin 2000).

Infaunal invertebrates play an important role in the structure and function of saltmarsh ecosystems, especially because they are abundant across the marsh landscape from tidal creek to marsh platform. As herbivores and as prey for fish and shellfish, infauna are key intermediate consumers linking higher trophic levels to basal food resources. In addition, grazing by infauna may limit primary producer biomass (Carman et al. 1997). Infauna have a range of feeding modes, including surface- and subsurface deposit feeding as well as suspension feeding, which allow them to exploit an array of living or detrital primary producers (Fauchald & Jumars 1979). The type of feeding mode utilized may vary with season, tidal flow, habitat, presence of predators and phytoplankton abundance (Esselink & Zwarts 1989, Smith et al. 1996, Vedel 1998). As a result, the diet of infauna may change over space and time (Carman & Fry 2002, Maddi 2003). Similarly, food resources (i.e. phytoplankton, MPB and *Spartina* spp. detritus) may be spatially and temporally variable. For example, subsurface-deposit feeders such as oligochaetes are frequently assumed to be macrophyte detrital feeders due to their spatial segregation from surface microalgae. However, subsurface feeders may consume settled phytodetritus (Levin et al. 1999, Holmes et al. 2000, Hughes et al. 2000). In addition, some infauna possess feeding structures that may be placed above the sediment–water interface to suspension feed or on the sediment to surface-deposit feed (Fauchald & Jumars 1979). These organisms may switch feeding behavior based on availability as well as quantity and quality of resources. For instance, at low tide, infauna inhabiting aerially exposed mudflats cannot suspension feed. Conversely, the amount of phytoplankton, suspended MPB and sediment may vary with tidal stage in water at higher elevations (such as the marsh platform), which may in turn influence the type of feeding. Such natural variability and the small size of infauna have made it difficult to accurately determine basal resource contributions (Carman & Fry 2002). Nonetheless, the infaunal role in saltmarsh food webs as both prey and consumers is pervasive and warrants continued investigation.

The use of multiple natural abundance stable isotopes has become an important tool in investigating trophic interactions. Furthermore, improved techniques have facilitated isotope analysis of small organisms such as infauna (Carman & Fry 2002). However, natural abundance stable isotopes are most helpful in systems with few primary producers that each have distinct isotope values (Haines & Montague 1979, Moncreiff & Sullivan 2001). The utility of natural abundance stable isotopes is limited in resolving food-web questions in systems such as saltmarshes when primary producers have similar isotope values. One way to increase the power of stable isotope studies is to add isotope labels (Hughes et al. 2000, Carman & Fry 2002, Levin et al. 2006, van Oevelen et al. 2006a,b). When primary producers have similar natural isotope values, the goal of isotope additions is to enhance primary-producer isotope differences for more accurate determination of basal resource contributions in food webs.

We used the combination of a natural abundance stable isotope survey and experimental ^{15}N additions to assess small infauna–primary producer trophic linkages across the marsh landscape. Our null hypotheses were that (1) there is no difference in the relative contribution among primary producers to the benthic food web, and (2) dietary contributions from primary producers to benthic food webs do not change spatially from creek to marsh platform habitats. An alternative hypothesis is that 1 or 2 basal resources such as *Spartina* spp. detritus or benthic algae and phytoplankton were dominant in supporting infaunal consumers. The hypotheses were tested across the marsh landscape in mudflat, creek wall, *S. alterniflora* understory and *S. patens* understory habitats.

MATERIALS AND METHODS

Study area. Our investigations were carried out in the Plum Island Estuary (PIE), Massachusetts, USA (42° 44′ N, 70° 52′ W). PIE has extensive saltmarshes; *Spartina alterniflora* and *S. patens* are the dominant macrophytes on the marsh platform. Salinities at the experimental site at the time of the experiment ranged from 8 to 28‰. The estuary experiences semi-diurnal tides with ~3 m tidal amplitude.

Within tidal creeks, steep, almost vertical, 2 m high creek walls are irregularly covered with macroalgae and filamentous algae. At the time of the addition

experiment, macroalgae were rare in and around the study site and were not observed in any algal collections. However, there was a nearly continuous, ~20 cm high band of *Rhizoclonium* spp. filamentous algae (consisting of long filaments up to 500 µm in diameter) near the top of the creek wall. At low tide within tidal creeks surrounding the marsh, gently sloped mudflats were aerially exposed.

Macroinfauna in PIE are distributed broadly throughout creek and marsh platforms with similar salinity and consist mostly of annelids (Johnson et al. 2007). Meiofaunal communities are dominated by nematodes and harpacticoid copepods (Fleeger et al. 2008). Potential predators on infauna include the killifish *Fundulus heteroclitus*, the green crab *Carcinus maenas* and the grass shrimp *Palaemonetes pugio* (Deegan et al. 2007).

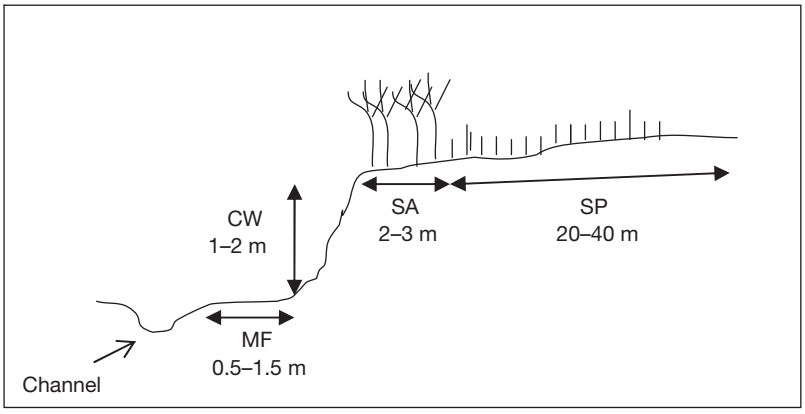

Fig. 1. Modified schematic (not to scale) from Johnson et al. (2007) of sampled saltmarsh habitats with approximate size ranges of habitats: mudflat (MF), creek wall (CW), *Spartina alterniflora* understory (SA) and *S. patens* understory (SP)

Collections. In order to determine the diet of infauna in different saltmarsh habitats, we collected primary producers and infauna from 4 habitats: mudflat, creek wall, *Spartina alterniflora* understory and *S. patens* understory. Epipelic or migrating diatoms served as a proxy for MPB and were collected from mudflat using 125 µm Nitex mesh (15.2 cm^2 in area). Nitex was placed directly on exposed mudflats, moistened with seawater filtered with precombusted (4 h at 480°C) Whatman GF/F filters with nominal 0.7 µm retention. Air bubbles under the Nitex mesh were removed by smoothing by hand. Nitex was retrieved after 1 h. In the laboratory, MPB samples were decanted 3 to 5 times to separate microalgae from denser detrital and sediment particles. Microscopic inspection of the purified samples indicated that pennate diatoms dominated collections. Samples were filtered on precombusted Whatman GF/F filters for isotope analysis. We also attempted to collect migrating MPB from creek-wall sediments using Nitex. Only minute amounts were collected and these were insufficient for isotope analysis. Creek wall may not be as hospitable for MPB as mudflat due to its relatively more compacted sediments that contain large volumes of *S. alterniflora* root tissue (Fig. 1). In addition, filamentous algae inhabiting creek wall form a canopy that may shade underlying sediments. Filamentous algae from creek wall were collected by hand and sonicated for 1 min to remove associated epiphytic diatoms. The resulting algae were inspected by microscopic examination, and only algae devoid of epiphytic diatoms were utilized for stable isotope analysis. Epiphytes (mostly diatoms) were removed by sonication and were filtered on precombusted Whatman GF/F for isotope analysis. Two replicate 1 l Nalgene bottles were submerged in the water column at high tide to collect suspended particulate organic matter (SPOM). SPOM samples were rinsed through a 63 µm sieve and fractions were examined microscopically. The portion of sample <63 µm visually contained a greater proportion of phytoplankton with fewer zooplankton. Sieved SPOM was filtered on precombusted Whatman GF/F filters for isotope analysis, and was used as a proxy for phytoplankton. Leaves of live *S. alterniflora* and standing dead *S. patens* were clipped from the marsh platform with garden shears. Leaves were cleaned of foreign debris, rinsed with distilled water and dried at 70°C. We used macrophyte leaves from live *S. alterniflora* as a proxy for *S. alterniflora* detritus. Currin et al. (1995) found no difference in $\delta^{13}C$ values between live and standing dead *S. alterniflora* but found lower $\delta^{15}N$ values in standing dead *S. alterniflora*.

For infauna collections, multiple large (6.5 cm diameter) and small (2.2 cm diameter) cores were taken in all 4 habitats. Large cores were taken to 5 cm in depth, and sediments were sieved through a 500 µm screen for macrofauna. Small cores were taken to 2 cm in depth, and sediments were sieved through a 63 µm screen for meiofauna. Eight large cores and 16 small cores were taken in the more detailed studies of both the mudflat and creek-wall habitats. In these habitats, infauna were pooled by species to obtain adequate sample mass for isotope analysis and to homogenize spatial variability within habitats. For *Spartina alterniflora* and *S. patens* habitats, replicate samples of pooled organisms were taken under the macrophyte canopy (2 per habitat). In an attempt to capture spatial variability within habitats, replicates were taken randomly within a

40 m^2 area of *S. alterniflora* and a 1000 m^2 area for *S. patens*. All samples, excluding macrophytes, were preserved in a 5% buffered formalin-Rose Bengal solution. Edwards et al. (2002) and Levin et al. (2006) both reported that short-term fixation in formalin has little effect on $\delta^{15}N$ and $\delta^{13}C$ values. After samples were sieved, infauna were sorted from remaining sediment and organic matter using a dissecting microscope. Gut contents were extruded from all infauna and organisms devoid of gut contents were rinsed with deionized water and dried at 70°C for 24 h for isotope analysis. All annelids were prepared for isotope analysis within 2 wk after fixation. Samples for isotope analysis were not acidified but were rinsed with deionized water to remove external sediment. Natural abundance $\delta^{15}N$ and $\delta^{13}C$ stable isotope values for primary producers and infauna were determined from samples taken within 1 wk prior to the start of the ^{15}N tracer addition and were treated as Time 0. Natural abundance $\delta^{15}N$ isotope values were used to determine trophic level, while $\delta^{13}C$ isotope values were used to determine food source contributions (Fry 2006).

^{15}N tracer addition. An isotope addition experiment was carried out in four 1 m^2 plots within 2 habitats in Sand Creek within PIE from July 21 to August 4, 2004. Two plots were placed in mudflat habitat and 2 in creek-wall habitat. Plots were marked at the corners with PVC poles (30 cm in length). We added 0.29 g of 10% ^{15}N-enriched $NaNO_3$ daily at low tide directly to sediment in all plots for 14 d. The ^{15}N-enriched $NaNO_3$ was dissolved in Whatman GF/F filtered seawater and was applied using a common garden sprayer. Due to the vertical nature of creek wall, the exposure time to the enriched isotope may have differed from mudflat. However, the canopy-like quality of filamentous algae in creek wall enhanced the retention of ^{15}N-enriched water. Based on nutrient concentrations typical of tidal creeks in PIE (Deegan et al. 2007), the amount of ^{15}N-enriched nitrate added did not significantly alter ambient concentrations and is therefore considered a tracer addition and not a fertilizer addition. Furthermore, ^{15}N enrichment in phytoplankton and *Spartina* spp. detritus is unlikely because of dilution/advection of the isotope signal when the tide returns and because non-living detrital material cannot take up the isotope label. Bacteria that use *Spartina* spp. detritus as a carbon source may take up enriched ^{15}N, but $\delta^{13}C$ natural abundance stable isotopes of this detritus and its consumers should reflect the $\delta^{13}C$ values of *Spartina* spp. detritus (Kreeger & Newell 2000).

To determine changes in $\delta^{15}N$ isotope values for primary producers and infauna over the 14 d ^{15}N addition, samples were collected by the methods described above from enrichment plots on Days 3, 7, 9 and 14 (last day of addition). Samples were taken prior to the daily ^{15}N addition and samples were collected on Day 21, 1 wk after the addition stopped. Filamentous and associated epiphyte algal samples from Day 14 were lost in transit to Louisiana State University (LSU). Heavy rainfall prevented collections of MPB on Day 21 because mudflats did not become aerially exposed at low tide.

The small size of infauna and the requirements for sample mass for stable isotope analysis made replicate collections of infauna problematic. To compensate, in our natural abundance study, we pooled creek samples from individual habitats (mudflat and creek wall; see 'Materials and methods — Collections') into composites from several samples but replicated marsh platform samples (*Spartina alterniflora* and *S. patens* understories). Multiple individuals of infaunal species (25 to 60), as described above, were pooled for analysis. We compared natural abundance stable isotope values from mudflat and creek-wall habitats in Sand Creek to replicate (3 per habitat) samples in an adjacent creek and found $\delta^{13}C$ values differed by <1.0‰ in the mudflat habitat for *Streblospio benedicti* and *Paranais litoralis*. $\delta^{13}C$ values for *S. benedicti* and *P. litoralis* in the adjacent creek were –19.6, –18.4 and –19.5‰, and –20.6, –20.5 and –19.7‰, respectively. In the creek-wall habitat, $\delta^{13}C$ values for *Manayunkia aestuarina* differed by <0.5‰ in the adjacent creek and were –21.7, –21.4 and –21.0‰. The similar isotope values suggest that our values closely represent true mean isotope values of these consumers and therefore represent the true diet. The enrichment study was conducted in only small 1 m^2 plots and this small scale also limited the faunal biomass available for sampling. Although replication was low, patterns of enrichment over the 21 d experiment were consistent and showed either high enrichment or low enrichment.

Gut content analysis. Gut contents of *Nereis diversicolor* and *Streblospio benedicti* were extracted to study ingested material. Infauna were bisected and ingested material was excised with forceps. In addition, *Paranais litoralis* (mudflat, *Spartina alterniflora* understory), *Manayunkia aestuarina* (creek wall) and *Fabricia sabella* (creek wall) were digested whole in HCl acid (Azovsky et al. 2005). Gut contents and digestion remains from Time 0 were examined microscopically to supplement diet information obtained from stable isotope analysis.

Mass spectrometry. Most samples were analyzed at the Isotope Facility at the University of California, Davis, using a continuous flow isotope ratio mass spectrometer. Some samples were analyzed at LSU using an elemental analyzer-stable isotope ratio mass spectrometer system following the protocol of Carman & Fry (2002). Samples were reported relative to the stan-

dards, atmospheric N_2 and Vienna PeeDee Belemnite (VPDB) carbon. Stable isotope values are reported in δ notation:

$$\delta^{13}C \text{ or } \delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 10^3$$

where R is respectively $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Mixing models and trophic enrichment factors (TEFs). A 3-source mixing model was used to determine possible contributions of primary producers to the diet of infauna from natural abundance data at Time 0 ($\delta^{13}C$ and $\delta^{15}N$; 4 habitats) and from the results of the ^{15}N-enrichment experiment (mudflat and creek wall). The mixing model is based on 3 mass-balance equations (Fry 2006):

$$f_1 + f_2 + f_3 = 1 \quad (1)$$

where subscripts 1 to 3 denote the 3 sources and f is the contribution of each source;

$$f_1 \times \delta^{13}C_1 + f_2 \times \delta^{13}C_2 + f_3 \times \delta^{13}C_3 \quad (2)$$

is the natural abundance $\delta^{13}C$ of the sample, and

$$f_1 \times \delta^{15}N_1 + f_2 \times \delta^{15}N_2 + f_3 \times \delta^{15}N_3 = \delta^{15}N \text{ or } \delta^{E} \quad (3)$$

where $\delta^{15}N$ is the natural abundance stable isotope values for samples collected prior to the ^{15}N addition (Time 0) from mudflat, creek wall, *Spartina alterniflora* and *S. patens* understories (henceforth referred to as the natural abundance model), or $\delta^{15}N = \delta^{E}$ where δ^{E} = highest $\delta^{15}N$ observed during the 21 d experiment – natural abundance $\delta^{15}N$ from Time 0 (henceforth referred to as the enrichment model; see Table 1). We used the highest observed $\delta^{15}N$ enrichment because we assumed tissue turnover was rapid and that all infauna reached C and N isotope steady-state equilibrium with the new labeled diets. Generally, interspecific differences in $\delta^{15}N$ enrichment over the 21 d experiment may be attributed to differences in tissue turnover times or the importance of unlabeled dietary food source(s). Consumers with a larger body size are expected to reach tissue equilibrium more slowly than smaller organisms and may assimilate less ^{15}N label over a short-term (14 d) experiment such as ours. We based our assumption of tissue equilibrium on the larger size but high level of enrichment in *Nereis diversicolor* during the experiment (mudflat; see Table 2) and rapid tissue turnover times reported (Doi et al. 2007) for both C and N in deposit-feeding chironomids of a mass similar to the annelids studied here. In the Doi et al. 2007 study, isotope equilibrium was reached at 12 d. To determine source contributions in the enrichment model, we used the natural abundance $\delta^{13}C$ values averaged over the 21 d experiment plus δ^{E}.

Sources were spatially distinct among habitats, and we therefore varied the primary producers used in mixing models depending on habitat. Mixing models included either (1) SPOM, the local dominant *Spartina* spp. and epiphytes for the creek-wall habitat, or (2) SPOM, MPB and the local dominant *Spartina* spp. for all other habitats. MPB was not abundant in the creek wall and was therefore excluded from creek-wall mixing models. We used MPB isotope values from the mudflat as a proxy for MPB under the *Spartina* spp. canopy. However, isotope values for the same organisms may change over space and time, and it is possible that different species of MPB are found in different habitats. Thus, isotope composition of MPB in the marsh platform may have differed from that of the mudflat. In the following year, we subsequently observed that MPB isotope values from samples collected under the *S. alterniflora* canopy were slightly more enriched in $\delta^{13}C$ (–17.1‰) than their mudflat counterparts (–19.2‰ ± 0.2; K. Galván unpubl.). If MPB isotope values on the marsh proper were more enriched than mudflat values in our study year, the mixing model would yield even smaller dietary contributions from *Spartina* spp.

Live filamentous algae were excluded from mixing models based on large algal size (up to 500 µm diameter) relative to the morphology of infauna and because the addition experiment and gut content analysis did not suggest ingestion. In creek wall, infauna may feed on living or detrital filamentous algal particles. However, PIE lacks a number of intermediate algal and detrital shredders (i.e. crabs) found in more southern saltmarshes that could provide detrital material for consumers. The green crab *Carcinus maenas* and mud crabs in the family Xanthidae are found in PIE; however, the nature of the vertical wall generally restricts these consumers to mudflats and subtidal areas.

Generally, consumers' natural abundance stable isotope values differ predictably from their food source values. This difference, fractionation or trophic enrichment factor ($TEF = \delta_{consumer} - \delta_{food\ source}$), is used in natural abundance stable isotope models to determine diet. For $\delta^{13}C$, a TEF of 0.5‰ was used for infauna in mixing models (Fry 2006). For $\delta^{15}N$, many studies use an average TEF of 3.4‰ (Minagawa & Wada 1984); however, McCutchan et al. (2003) and Vanderklift & Ponsard (2003) reported relatively smaller (<3.4‰) ^{15}N TEFs for marine organisms, detritivores and invertebrates. To better assess this ^{15}N TEF in our study animals, we first determined diet through the addition of enriched ^{15}N and then used this to determine species-specific TEFs with natural abundance stable isotopes. When feasible, these TEFs were used in mixing models to more accurately determine basal resource contributions.

RESULTS

A total of 19 species of annelids and 38 species of copepods were found in quantitative studies across

habitats in PIE (Johnson et al. 2007, Fleeger et al. 2008). Isotope analyses were conducted on the more abundant infaunal species, including the annelids *Nereis diversicolor* (mudflat and creek wall), *Streblospio benedicti* (mudflat), *Paranais litoralis* (mudflat, *Spartina alterniflora* and *S. patens* understories), *Manayunkia aestuarina* (creek wall, *S. alterniflora* and *S. patens* understories), *Fabricia sabella* and *Pygospio elegans* (both creek wall), and the abundant harpacticoid copepod *Heterolaophonte* sp. (creek wall).

Natural abundance

Natural abundance stable isotope values of primary producers at the beginning of the addition experiment for $\delta^{15}N$ ranged from 5.3 to 6.4‰, with the exception of SPOM, which was 8.9 ± 3.4‰. $\delta^{13}C$ values showed a wider range (Table 1) with lowest values for SPOM (–23.7 ± 1.3‰), highest values for *Spartina alterniflora* (–13.2‰) and intermediate values for benthic algae (Table 1).

Table 1. Natural abundance stable isotope (NA) $\delta^{13}C$ and $\delta^{15}N$ values of primary producers and infauna from the following habitats: mudflat (MF), creek wall (CW), *Spartina alterniflora* (SA) and *S. patens* (SP) understories. Using natural abundance stable isotopes only, primary producer dietary contributions (%) were calculated for all 4 habitats (see 'Materials and methods—Mixing models and trophic enrichment factors'). For mudflat and creek wall, δ^E (highest observed enrichment minus background natural abundance isotope values) over the 21 d experiment and an average $\delta^{13}C$ natural abundance value from the 21 d experiment were used to calculate percent dietary contributions. The 3 sources used in mixing models varied depending on habitat. For mudflat, *S. alterniflora* and *S. patens* habitats, local *Spartina* spp. (reported under % *Spartina* spp.), suspended particulate organic matter (SPOM) and microphytobenthos (MPB) are sources in the mixing model. In creek-wall habitat, *S. alterniflora*, SPOM and epiphytes were used. FA: filamentous algae, P: polychaete, O: oligochaete, C: copepod, SF: suspension feeder, SDF: surface-deposit feeder, SSDF: subsurface-deposit feeder, na: not applicable. Single values represent pooled samples; other values are means ± SD of pooled samples (number of replicates in parentheses). Values reported are measured values, not corrected for trophic fractionation. The high natural abundance $\delta^{15}N$ values for *Pygospio elegans* indicate possible predation; rather than infer multiple trophic-level fractionation and lag enrichment, we chose not to calculate possible source contributions

Natural abundance flora and fauna	Feeding mode	Habitat	$\delta^{13}C$ (n)	$\delta^{15}N$ (n)	% *Spartina* spp.	% SPOM	% MPB or epiphytes
Spartina alterniflora		SA understory	–13.2	7.4	–	–	–
Spartina patens		SP understory	–13.8	4.9	–	–	–
FA		CW	–18.0	6.1	–	–	–
Epiphytic algae		CW	–18.3	5.3	–	–	–
SPOM		Creek water	–23.7 ± 1.3 (2)	8.9 ± 3.4 (2)	–	–	–
MPB		MF	–19.2	6.0	–	–	–
Nereis diversicolor (P)	SF, SDF, SSDF	MF	–19.8	8.7	1	6	93
Streblospio benedicti (P)	SF, SDF	MF	–19.2	6.1	0	10	90
Paranais litoralis (O)	SDF, SSDF	MF	–20.6	7.0	4	4	91
Nereis diversicolor (P)	SF, SDF, SSDF	CW	–20.1	7.9	1	44	55
Pygospio elegans (P)	SF, SDF	CW	–17.2	11.9	–	–	–
Manayunkia aestuarina (P)	SF, SDF	CW	–21.4 ± 0.0 (2)	8.8 ± 1.3 (2)	1	69	30
Fabricia sabella (P)	SF, SDF	CW	–20.8	6.8	17	72	11
Heterolaophonte sp. (C)	SDF	CW	–17.6	6.5	5	3	92
Paranais litoralis (O)	SDF, SSDF	SA	–18.5 ± 1.4 (2)	8.5 ± 0.8 (2)	13	11	76
Manayunkia aestuarina (P)	SF, SDF	SA	–21.7 ± 0.8 (2)	8.6 ± 0.5 (2)	2	68	30
Paranais litoralis (O)	SDF, SSDF	SP	–19.4 ± 2.1 (2)	8.6 ± 0.6 (2)	23	29	48
Manayunkia aestuarina (P)	SF, SDF	SP	–20.8 ± 0.0 (2)	9.8 ± 0.9 (2)	12	49	39

^{15}N-enriched flora and fauna	Habitat	Average NA $\delta^{13}C$	Highest δ^E	% *Spartina* spp.	% SPOM	% MPB or epiphytes
MPB	MF	–19.1	1050	–	–	–
Nereis diversicolor (P)	MF	–18.9	890	4	11	85
Streblospio benedicti (P)	MF	–19.3	180	29	53	17
Paranais litoralis (O)	MF	–19.0	360	22	43	34
FA	CW	–17.3	1740	–	–	–
Epiphytic algae	CW	–18.1	660	–	–	–
Nereis diversicolor (P)	CW	–19.6	2	34	66	0
Pygospio elegans (P)	CW	–17.2	130	na	na	na
Manayunkia aestuarina (P)	CW	–21.5	55 ± 7.0 (2)	12	80	8
Fabricia sabella (P)	CW	–21.0	20	19	78	3
Heterolaophonte sp. (C)	CW	–17.4	500	16	8	76

Mudflat habitat

At Time 0, *Streblospio benedicti*, *Paranais litoralis* and *Nereis diversicolor* had relatively depleted $\delta^{13}C$ natural abundance values compared to *Spartina alterniflora* (Table 1, Fig. 2). All 3 had $\delta^{13}C$ values that differed from *S. alterniflora* by a relatively large amount, ≥3.9‰ (Table 1). Results from the natural abundance model for *N. diversicolor, S. benedicti* and *P. litoralis* indicate that MPB dominated diets at 93, 90 and 91% respectively (Table 1).

Creek-wall habitat

At Time 0, the sabellid polychaetes *Manayunkia aestuarina* and *Fabricia sabella* had relatively low $\delta^{13}C$ values of −21.3 ± 0.2‰ and −20.8‰, and $\delta^{15}N$ values of 8.2 ± 0.4‰ and 6.8‰, respectively. *Pygospio elegans*, a spionid polychaete, and *Heterolaophonte* sp., a harpacticoid copepod, had $\delta^{13}C$ values that were close to those of filamentous algae and epiphytic diatoms (Table 1). Natural abundance $\delta^{13}C$ and $\delta^{15}N$ values for *Nereis diversicolor* were respectively −20.1 and 7.9‰ and were similar to mudflat values. All of the infauna had $\delta^{13}C$ values that again differed from *Spartina alterniflora* or *S. patens* by a relatively large amount, ≥3.9‰ (Table 1). Results from the natural abundance model indicate that *Spartina* spp. contributed little (at most 17%) to the diet of creek-wall infauna (Table 1); instead, the diet consisted primarily of benthic and pelagic algae (83 to 99%). The natural abundance model indicated that epiphytes contributed most to the diets of *N. diversicolor* and *Heterolaophonte* sp. at 55 and 92% respectively, while SPOM contributed most to the diets of both *M. aestuarina* and *F. sabella* at 69 and 72% respectively.

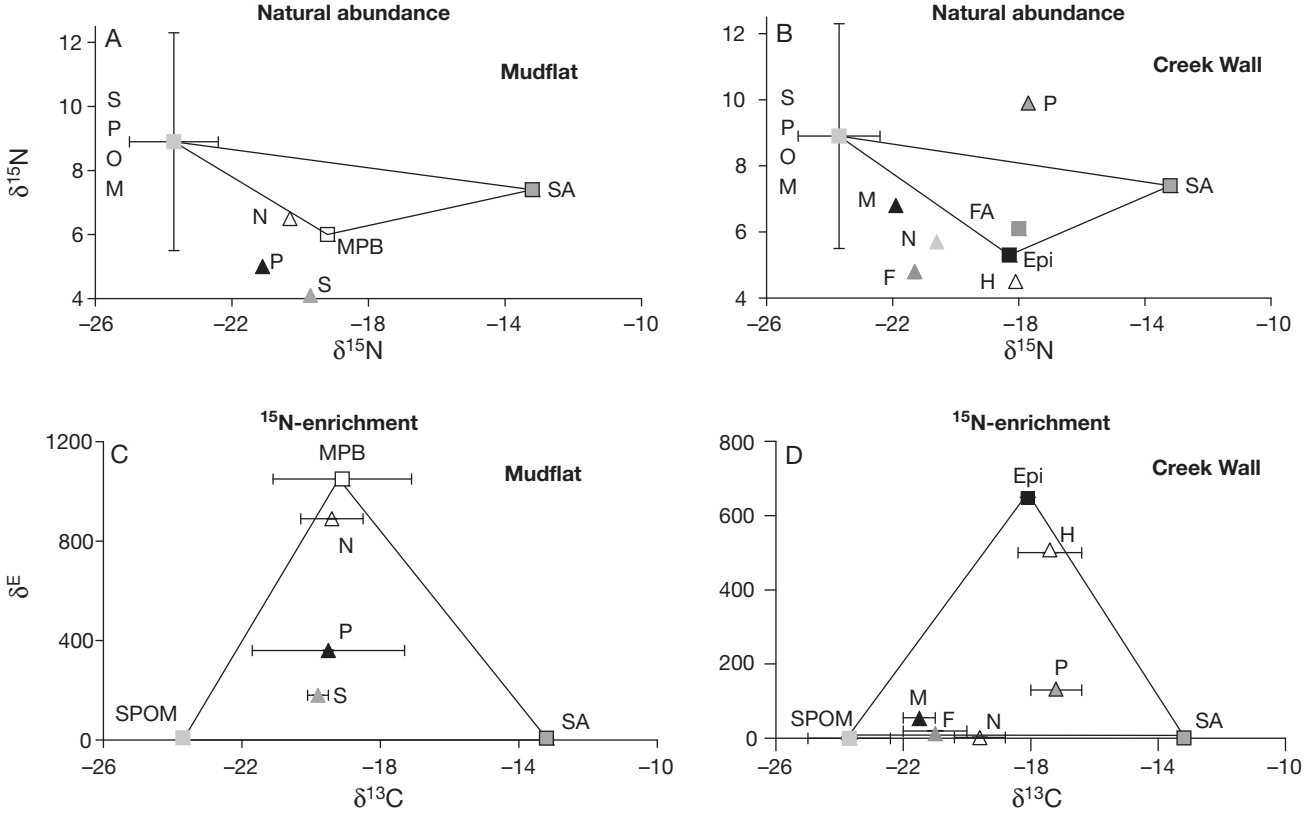

Fig. 2. Natural abundance stable isotope values ($\delta^{15}N$ and $\delta^{13}C$; prior to isotope additions) for (A) mudflat and (B) creek wall; and ^{15}N-enrichment (δ^{E}, highest) in primary producers and infauna during the 21 d experiment plotted against an average $\delta^{13}C$ found over the 21 d experiment in (C) mudflat and (D) creek-wall habitats. Isotope values are corrected for trophic enrichment (see 'Materials and methods—Mixing models and trophic enrichment factors'). Primary producers are squares; infauna are triangles. The polygons connect the 3 sources used in the mixing model. SPOM: suspended particulate organic matter, MPB: microphytobenthos, FA: filamentous algae, Epi: epiphytes, N: *Nereis diversicolor*, S: *Streblospio benedicti*, P in mudflat: *Paranais litoralis*, P in creek wall: *Pygospio elegans*, M: *Manayunkia aestuarina*, F: *Fabricia sabella*, H: *Heterolaophonte* sp. Consumers with values outside the mixing triangles were assumed to fall on the nearest mixing line, a possibility consistent with isotope variation in the source (apex) plant isotope values

Spartina understory

Manayunkia aestuarina and *Paranais litoralis* were collected from sediments on the marsh platform from 2 habitats: *Spartina alterniflora* and *S. patens* understories. Similar to creek habitats (mudflat and creek wall), both *S. alterniflora* and *S. patens* understories had annelids that were relatively depleted in ^{13}C compared to *S. alterniflora* (Table 1), indicating a diet based primarily on benthic and/or pelagic algae. Results from the natural abundance model indicate that MPB and SPOM contributed a combined 77 to 98% of the diet for both species on the marsh platform, while *Spartina* spp. contributed 2 to 23% (Table 1).

^{15}N tracer addition

The ^{15}N-enriched label was taken up by all targeted benthic algal primary producers: MPB, filamentous algae and associated epiphytes. Enrichment above background levels was observed on Day 3, the earliest sampling point after additions started (Table 2).

Mudflat habitat

$\delta^{15}N$ isotope values in MPB increased over the 14 d addition, with peak enrichment reaching a value of 1050‰ above natural abundance values (Table 2). All 3 annelids investigated became enriched in ^{15}N beyond natural abundance levels (Table 2). *Nereis diversicolor* was highly enriched and reached a value of 890‰ on Day 21 (7 d after isotope addition stopped); *Streblospio benedicti* and *Paranais litoralis* were enriched to 180 and 360‰ respectively (Table 2). Results from the enrichment model confirmed the dietary importance of MPB to *N. diversicolor*, comprising >84% of its diet (Table 1). The enrichment model refuted the dominance of MPB for *S. benedicti* and *P. litoralis*. Although the enrichment model indicated the importance of both SPOM and *Spartina alterniflora*, the natural abundance model indicated that *Spartina* spp. contributes little to the diet of either species (Table 1).

Creek-wall habitat

Filamentous algae became enriched in ^{15}N and reached values >1700‰ above natural abundance on Day 14; epiphytic diatoms reached 660‰ above natural abundance values on Day 7 (Table 2). $\delta^{15}N$ in filamentous algae decreased rapidly to approximately 500‰ above background levels 7 d after label addition ceased.

Uptake of the ^{15}N-enriched label was minimal for most infauna analyzed (Table 2). Label enrichment in *Manayunkia aestuarina* was highest on Day 21 but reached values of only ~55‰, while *Fabricia sabella* reached its highest enrichment of 20‰ on Day 14. On Day 21, *Pygospio elegans* reached its maximum $\delta^{15}N$ value of 130‰. Unlike in the mudflat habitat, *Nereis diversicolor* was not enriched in ^{15}N above natural abundance values in creek wall (Fig. 2). Highest enrichment was observed in *Heterolaophonte* sp. with values reaching 290‰ on Day 14 and 500‰ above natural abundance on Day 21 (7 d after addition stopped).

Table 2. ^{15}N enrichment and natural abundance $\delta^{13}C$ in primary producers and infauna in mudflat (MF) and creek-wall (CW) habitats over 21 d in the enrichment study. Day 14 was the last day of ^{15}N addition; Day 21 was 1 wk after cessation of ^{15}N addition. Enrichment above natural abundance $\delta^{15}N$ is reported (in ‰), where δ^E is the enriched $\delta^{15}N$ value for that time point minus natural abundance $\delta^{15}N$ from Time 0. *Manayunkia aestuarina* value on Day 21 is the mean ± SD of 2 replicate pooled samples. na: not applicable

	Day 3		Day 7		Day 14		Day 21	
	$\delta^{13}C$	δ^E	$\delta^{13}C$	δ^E	$\delta^{13}C$	δ^E	$\delta^{13}C$	δ^E
MF habitat								
MPB	–20.4	90	–20.1	120	–16.8	1050	na	na
Nereis diversicolor	–19.5	40	–19.0	90	–19.5	490	–17.5	890
Streblospio benedicti	–19.4	10	–19.4	30	–19.5	100	–18.9	180
Paranais litoralis	–21.4	3	–20.2	120	–16.9	360	–17.3	na
CW habitat								
Filamentous algae	–17.4	280	–16.6	1210	–16.9	1740	–18.4	520
Epiphytic diatoms	–18.1	270	–18.0	660	na	na	–18.3	510
N. diversicolor	–18.5	0	na	na	–20.0	2	–19.6	0
P. elegans	–19.6	30	–17.7	40	–18.2	80	–18.5	130
M. aestuarina	–21.0	0	–22.1	5	–21.6	30	–21.4	55 ± 7
Fabricia sabella	–20.7	1	–20.9	10	–21.3	20	–21.5	20
Heterolaophonte sp.	–16.9	5	–17.6	80	–17.6	290	–17.1	500

The enrichment model confirmed the importance of epiphytes as suggested by the natural abundance model as the primary basal resource utilized by *Heterolaophonte* sp., constituting ~75% of its diet (Table 1). The enrichment model refuted the importance of epiphytes for *N. diversicolor* indicated by the natural abundance model. Uptake of enriched ^{15}N by epiphytes and enriched filamentous algae and the subsequent lack of uptake by *N. diversicolor* in creek wall indicated that benthic algae contributed little if at all to the diet of *N. diversicolor*. Instead, the enrichment model indicated that SPOM comprised 66% of its diet. Due to limited uptake of the enriched primary producer, the dietary importance of *Spartina alterniflora* increased for *N. diversicolor* with the enrichment model. The enrichment model indicated that SPOM contributed the most to the diets of *M. aestuarina* and *F. sabella* (Table 1).

^{15}N trophic enrichment factors (TEF)

The addition of enriched ^{15}N revealed that *Nereis diversicolor* and *Heterolaophonte* sp. fed almost exclusively on MPB (in mudflat) and epiphytes (in creek wall) respectively. Therefore, we calculated TEFs for both organisms by subtracting the natural abundance $\delta^{15}N$ value of MPB and epiphytes from the natural abundance $\delta^{15}N$ value of *N. diversicolor* and *Heterolaophonte* sp. respectively (see 'Materials and methods—Mixing models and trophic enrichment factors'). A TEF of 2.7‰ was found for *N. diversicolor* and 1.2‰ for *Heterolaophonte* sp. These TEFs allowed us to more accurately determine dietary contributions using mixing models. We averaged $\delta^{15}N$ TEFs for *N. diversicolor* and *Heterolaophonte* sp. to obtain a 2‰ TEF for other consumers. We recognize that an average TEF may not accurately depict TEFs in individual species but this average may more closely reflect actual TEFs and is similar to a 2.2‰ average TEF reported for invertebrates by McCutchan et al. (2003). After correction for trophic fractionation, consumers with values outside the mixing triangles were assumed to fall on the nearest mixing line, a possibility consistent with isotope variation in the source (apex) plant isotope values (see Fig. 2).

Gut content results

Gut contents were not quantified; only the presence of a basal resource was determined. Gut content analysis revealed that both pennate and centric diatoms were abundant as well as unidentifiable material in the guts of *Streblospio benedicti* (mudflat, n = 3) and *Nereis diversicolor* (mudflat, n = 3, and creek wall, n = 3). Tissue digestion of *Paranais litoralis* (mudflat), *Manayunkia aestuarina* (creek wall) and *Fabricia sabella* (creek wall) (n = 3 for each species) also revealed frustules of pennate and centric diatoms.

DISCUSSION

While $\delta^{13}C$ was primarily useful for indicating a low importance of *Spartina* spp. for all infauna, the addition of enriched ^{15}N to the sediment surface and subsequent uptake by benthic algae widened the primary producer isotope triangle (Fig. 2), thus improving resolution and our understanding of algal contributions to the benthic food web. The enrichment experiment confirmed the importance of MPB for some infauna and refuted conclusions from natural abundance data that MPB or epiphytes were dominant dietary source for other infauna. Enrichment studies allow for better food web resolution than natural abundance stable isotopes alone, particularly in systems where primary producer natural abundance stable isotope values are similar.

Mudflat habitat

High label uptake found in *Nereis diversicolor* confirmed surface deposit feeding on labeled MPB by this polychaete in the mudflat habitat. Both the natural abundance and enrichment mixing models indicated that MPB made up ~85% of its diet in this habitat. Label uptake in *Streblospio benedicti* and *Paranais litoralis* was relatively low compared to *N. diversicolor* suggesting a lesser dietary role for MPB in mudflat and the importance of an unlabeled food source. The unlabeled food source most likely was phytoplankton because consumer natural abundance values were similar to SPOM. Our enriched mixing models suggested that SPOM in the form of phytoplankton could have comprised 53 and 43% of the diet of *S. benedicti* and *P. litoralis* respectively (Table 1). In agreement with our findings, a similar isotope addition using enriched ^{13}C in a Louisiana saltmarsh found phytoplankton to contribute significantly to the diet of *S. benedicti* (Maddi 2003).

Oligochaetes, including *Paranais litoralis*, have traditionally been considered subsurface-deposit feeders that utilize detritus of an unknown age and origin by ingesting sediment in bulk as they move through sediments (Nilsson et al. 2000). However, recent studies classify *P. litoralis* as a surface-deposit feeder. Kelaher & Levinton (2003) found that abundances of *P. litoralis* increased with algal detritus enrichment and our results suggest consumption of both phytoplankton

and MPB. MPB and deposited phytoplankton may be accessed by surface feeding, by feeding on rapidly buried algae or by consuming algae that enters the burrows of *Nereis diversicolor* and other infauna (Papaspyrou et al. 2006) or when surface deposits are drawn down to sediment depths through the activity of subsurface-deposit feeders (Josefson et al. 2002).

Creek-wall habitat

$\delta^{15}N$ in filamentous algae and epiphytic diatoms increased rapidly over the 14 d addition in the creek-wall habitat. We excluded the possible consumption of filamentous algae by infauna (see 'Materials and methods—Mixing models and trophic enrichment factors') and therefore label uptake in consumers indicated dietary contributions from epiphytic diatoms for 4 of the 5 macrofaunal and meiofaunal taxa investigated (Table 2, Fig. 2D) in creek wall. *Heterolaophonte* sp. was most highly enriched in ^{15}N, confirming the trophic importance of epiphytic diatoms. Many studies suggest that grazing on diatoms is a common harpacticoid feeding strategy in shallow sediments (Carman et al. 1997, Azovsky et al. 2005). Both natural abundance and enrichment mixing models suggested that >75% of the diet potentially came from epiphytic algae.

Manayunkia aestuarina and *Fabricia sabella* are classified as selective surface- and suspension-feeding polychaetes and as a result have the ability to utilize a variety of primary producers (Fauchald & Jumars 1979). Our enrichment models indicated that contributions from *Spartina* spp. and labeled benthic algae to the diets of *M. aestuarina* and *F. sabella* were relatively small and instead suggest the greater importance of an unlabeled food source. Gut content analyses revealed the consumption of phytoplankton by both *M. aestuarina* and *F. sabella* and the enrichment model indicated that SPOM in the form of phytoplankton was the principal food source (Fig. 2).

Natural abundance isotope results for *Nereis diversicolor* were very similar in mudflat and creek wall (Table 1) and the natural abundance mixing model suggested that epiphytes or MPB comprised >50% of the diet in both habitats. However, unlike results for mudflat *N. diversicolor*, creek-wall *N. diversicolor* did not become enriched above natural abundance $\delta^{15}N$ values (Table 2). These enrichment results allowed us to reject the importance of epiphytes in creek wall. Instead, the enrichment model suggested phytoplankton dominated the diet. *N. diversicolor* has been reported to have 3 different feeding modes: selective surface-deposit feeding, suspension feeding and predation (Harley 1950, Smith et al. 1996, Vedel 1998). *N. diversicolor* likely fed by different mechanisms in the 2 habitats. Various physical and chemical factors may govern feeding behavior in small infauna (Carman & Fry 2002, Maddi 2003). Vedel (1998) found that filter feeding in *N. diversicolor* is dependent on phytoplankton abundance and ceases at low concentrations. Position within the tidal gradient may also influence feeding mode. Suspended material from the sediment may lower the concentration of phytoplankton available to suspension feeders (Esselink & Zwarts 1989), especially at lower tidal elevation where tidally induced flow rates may exceed critical erosion velocity. In addition, suspension feeding may reduce the risk of predation to *N. diversicolor* by allowing worms to remain in burrows; worms must emerge to surface-deposit feed (Esselink & Zwarts 1989). Suspension feeding can be a primary feeding mode for *N. diversicolor* (Esselink & Zwarts 1989). *N. diversicolor* creates intricate burrows in the sediment to depths >15 cm (Davey 1994) where suspension feeding involves a mucous funnel that extends down into the burrow (Vedel 1998). Through undulations, the worm creates a current of water that brings in suspended particles including phytoplankton that are trapped by the funnel and later ingested (Harley 1950).

Pygospio elegans in the creek-wall habitat had natural abundance $\delta^{13}C$ values similar to filamentous algae and its epiphytic diatoms. This observation in combination with its relatively high natural abundance $\delta^{15}N$ values (Fig. 2) suggests *P. elegans* is likely an omnivore. During the ^{15}N addition, *P. elegans* reached a maximum $\delta^{15}N$ value of 130‰ (Table 2), which could be a result of feeding on enriched prey. Brey (1991) reported *P. elegans* was a predator. However, *P. elegans* may feed on both consumers and primary producers (Herman et al. 2000). *P. elegans* possesses feeding palps that may be used to suspension feed or selectively surface-deposit feed. Potential prey include sediment micrometazoans and/or zooplankton, but label uptake in *P. elegans* suggested that it fed on ^{15}N-enriched prey, perhaps meiofauna or small heterotrophic protists from the sediment rather than the water column.

Marsh platform habitats

Natural abundance mixing models indicated that benthic and pelagic algae contributed most to the diets of both *Manayunkia aestuarina* and *Paranais litoralis* in marsh platform habitats (Table 1). Furthermore, the dietary importance of *Spartina* spp. only slightly increased on the marsh proper relative to creek habitats for both consumers. These findings were surprising for a number of reasons. First, infaunal collections on the marsh proper were taken from underneath the *Spar-*

tina spp. canopy and therefore are in relatively closer proximity to macrophytes (and their detritus) than creek infauna. Second, shading by the *Spartina* spp. canopy is thought to limit algal biomass. HPLC analysis of PIE sediment revealed that benthic algal biomass on the marsh proper was on average lower than in the creek wall (Deegan et al. 2007, K. Galván unpubl.). Finally, tidal amplitude restricts availability of phytoplankton to the marsh proper, where *S. alterniflora* is flooded daily with high tide but *S. patens* is flooded only in spring tides, resulting in episodic but diminished exposure to phytoplankton.

Overall food web dynamics

Small suspension- and deposit-feeding infauna are found on shallow intertidal mudflats worldwide, with and without surrounding macrophytes. Most published isotope studies show that they feed primarily on MPB and phytoplankton regardless of presumed feeding group or the proximity of *Spartina* spp. (Herman et al. 2000, Hughes et al. 2000, Carman & Fry 2002, van Oevelen et al. 2006b); however, some studies have found evidence of macrophyte detrital consumption in the meiobenthos and some annelids (Carman & Fry 2002, Maddi 2003, Levin et al. 2006). Overall, these studies suggest that assimilation of *Spartina* spp. detritus seems limited, perhaps because of a scarcity of benthic and pelagic algae. Still, minimal dietary contributions from *Spartina* spp. detritus to infauna were unexpected given the close proximity to abundant macrophyte detritus within the sediment matrix. Furthermore, due to the limited volume of water in tidal creeks and the abundance of *Spartina* spp. detritus in the water column, phytoplankton in saltmarshes is usually considered to have a reduced importance in food webs. Overall, our observations suggest that infauna have flexible dietary needs and feeding strategies that may change over space and time and vary among species, making generalizations of the food-web position of infauna tenuous.

The refractory nature of macrophytes may be responsible for the lower uptake of detritus by infaunal consumers, although microbial colonization has been shown to enhance nutritional quality. Microbial decomposition of macrophyte detritus has been well documented and studies have illustrated the dietary use of such bacteria by infauna by microbial stripping, although van Oevelen et al. (2006a) found that bacterial trophic contributions were minimal on intertidal mudflats of the Scheldt Estuary. Nevertheless, isotope values of bacteria and subsequently infauna feeding on bacteria should reflect the original organic matter source (i.e. *Spartina* spp. detritus). One exception is chemosynthetic bacteria, which have highly depleted $\delta^{13}C$ values that reflect fixed CO_2 and not their original carbon and energy sources (Degens et al. 1968), but may be an important food source in saltmarshes (Peterson et al. 1980). However, to explain the –18 to –22‰ $\delta^{13}C$ values of PIE infauna, a large contribution of a third to a half of all carbon would have to derive from –30 to –40‰ chemosynthetic bacteria with the remainder from –13‰ *Spartina* spp. detritus. This would be a high contribution from chemosynthetic sources, especially because Boschker et al. (1999) found little evidence for an important role of chemosynthetic production in saltmarshes of Cape Cod, marshes similar to those studied here. It is possible that macrophyte detritus in PIE may be used by bacteria that do not contribute to higher trophic levels (i.e. act as a sink). *Littoraria irrorata*, the marsh periwinkle, is a principal macrophyte grazer in more southern marshes, but is not found in PIE; however, larger invertebrates found on the marsh proper such as amphipods (e.g. *Orchestia grillus*) and the coffee bean snail *Melampus bidentatus* may feed on macrophytes. Current research is examining this possibility in PIE (K. Galván et al. unpubl.).

Our results generally showed that natural abundance mixing models underestimated the importance of phytoplankton but overestimated the importance of MPB or epiphytes to infauna (Table 1). This highlights the difficulty of knowing diet with certainty when sources have a similar isotopic composition and shows that natural abundance data can lead to false conclusions about diet. However, it is possible that our addition studies overestimated the contribution from phytoplankton because we assumed that consumers had reached equilibrium (see 'Materials and methods—Mixing models and trophic enrichment factors') and because our labeled food source (MPB) was not consumed by some species. It is also possible that phytoplankton or algal detritus produced before the label addition contributed to the diet of some species. Cheng et al. (1993) found that *Paranais litoralis* uses sedimentary food resources and that nutritional quality varies over time, suggesting potential use of aged organic matter, and Levin et al. (1999) found that surface and subsurface-deposit feeders are both able to consume recently settled phytodetritus. The most unequivocal method would label each possible producer over longer time periods, creating unique isotopic compositions for all primary producers and their associated detritus.

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