Natural abundance stable isotopes and dual isotope tracer additions help to resolve resources supporting a saltmarsh food web

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A B S T R A C T
We used a combination of natural abundance stable isotopes (NASI) and multiple isotope additions to examine the importance of local and tidally imported basal resources to infauna in a tidal, saltmarsh creek within the Plum Island Estuary, Massachusetts, U.S.A. NASI analysis was conducted on primary producers and infauna prior to isotope additions. While algal resources as a group had distinct NASI values compared to Spartina spp. resources, various algal resources [sediment-associated algae (local) and phytoplankton (tidally imported)] had similar NASI leading to low dietary resolution with mixing models. To separate primary producer isotope values, we conducted a large-scale, 42 day 15N-addition to the whole saltmarsh creek, which enriched tidally imported (i.e., phytoplankton) and local algal resources. We also conducted small-scale additions of enriched 13C to 1-m2 plots within the creek receiving the 15N addition to separately label local resources. Enriched 15N and 13C were taken up by filamentous algae and associated epiphytic diatoms and benthic microalgae creating distinct isotope values in local algal resources. In addition, changes in 14N-tracer uptake by phytoplankton during the first week of the 15N-addition provided a unique enrichment pattern that was followed through the food web. Both NASIs and isotope additions indicated that Spartina spp. was not an important basal food resource to infauna, lending credence to the notion that saltmarsh food webs don’t often rely on macrophyte detritus. Isotope additions indicated that various types of algae, both local and tidally imported, were important to the diets of infauna, including polychaetes, tanaids and harpacticoid copepods. Isotope additions increased diet resolution and revealed less accurate basal resource contributions determined with NASI alone. While many food web studies rely on NASI, we feel that the combination of NASI and isotope additions is needed in systems with similar primary producer values to more accurately determine resource contributions, to increase resolution of dietary contributions and to determine the dietary importance of local basal resources.

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1. Introduction

Tidal salt marshes have a variety of primary producers that may support secondary production. Local production of edaphic algal resources including benthic microalgae (BMA) and filamentous algae as well as resources imported with tides, (i.e., phytoplankton and detrital macrophytes) may be important to the diets of aquatic and intertidal organisms. As herbivores and prey for algae as well as resources imported with tides, (i.e., phytoplankton...)
BMA helped to change the emphasis of food web studies from tidal imports (Spartina detritus) to local resources. Yet, the limitations of the single isotope approach in resolving dietary sources were quickly revealed when attempting to resolve the diet of consumers that utilized several basal resources (primary producers) or when basal resources had similar isotope values. Soon after, researchers demonstrated the utility of using multiple natural abundance stable isotopes (δ^{13}C, δ^{15}N and δ^{2}H) to distinguish between the dietary importance of multiple primary producers (Peterson et al., 1985; Peterson et al., 1986; Peterson and Howarth, 1987). Today, this approach is commonly used in food-web studies and has further illuminated the importance of microalgae in estuarine food webs (Choy et al., 2008; Curran et al., 1995; Kang et al., 2007; McMahon et al., 2005; Sullivan and Moncreiff, 1990; Wainright et al., 2000).

Although the use of multiple natural abundance stable isotopes has helped to further our understanding of resource use in estuaries, this approach is still limited in systems that have multiple species of primary producers with similar or highly variable isotope values (Cloern et al., 2002). For example, Galván et al. (2008) found similar natural abundance stable isotope values for the polychaete, N. diversicolor from two adjacent habitats, and mixing model results suggested consumption of benthic microalgae in both locations. However, the addition of δ^{15}N-enriched tracer elucidated the dietary importance of phytoplankton in one habitat that natural abundance stable isotopes alone could not. Therefore, while the use of natural abundance stable isotopes is common in food-web studies, natural abundance stable isotopes may be misleading especially in systems with similar primary producers isotope values.

One way to increase the power of stable isotopes is to use natural abundance stable isotopes in combination with isotope additions (Carman and Fry, 2002; Hughes et al., 2000; Levin et al., 2006; Maddi et al., 2006; Middelburg et al., 2000). The goal of isotope additions is to enhance natural differences in primary producer isotope values by creating a distinct isotope label in specific primary producers. The label can then be followed through the food web via consumption of a labeled primary producer. Moreover, isotope additions may be conducted in such a way as to target uptake in a specific primary producer(s). In combination with natural abundance stable isotope analysis, isotope additions allow for better resolution of basal resource contributions to food webs. This method has been applied to estuaries and has revealed the importance of phytoplankton to the diets of both benthic and water column consumers (Hughes et al., 2000), a shift from an algae-based system to a primarily detrital-based system with the invasion of a Spartina hybrid (Levin et al., 2006) and the importance of microphytobenthos to the diet of nematodes (Moens et al., 2002) and other benthic fauna (Herman et al., 2000). The chemical composition of the isotopes added is chosen according to the targeted food source; enriched or depleted forms of δ^{13}C and δ^{15}N are commonly used in food web studies. For example, the importance of heterotrophic bacteria in carbon flow from basal resources has been addressed using δ^{13}C-labeled glucose (Rossi et al., 2009; van Oevelen et al., 2006).

Our objectives for this study were to examine the importance of basal resources (i.e., local resources as well as resources imported with the tide) to infauna in a saltmarsh food web. Given the complexities of infauna diets and the diversity of primary producers in estuaries, a combined approach of natural abundance stable isotopes and isotope additions is well suited to more accurately determine basal resource contributions to the food web. Specifically, we conducted a large-scale addition of 15N-enriched NO_3^- to the water column of a saltmarsh creek for 42 days to target uptake in resources imported with the tide (i.e., phytoplankton) as well as in situ benthic primary producers in the creeks. Natural abundance stable isotopes were measured just prior to the isotope addition. To label local resources (BMA and filamentous algae), we conducted a simultaneous 2 week addition of 13C-enriched NaHCO_3 in small-scale plots in targeted habitats within the 15N-addition creek on day 27 of the water column 15N-addition. This approach (i.e., multiple isotope tracers) helped to further separate primary producer isotope values and to determine if localized resources were consumed thus, improving dietary resolution that natural abundance stable isotopes and large scale isotope additions alone could not. We hypothesized that infauna will have isotope values that more closely reflect the use of local resources because the habitats addressed in this study are exposed at low tide and thus have only intermittent access to tidally imported resources.

2. Materials and methods

2.1. Study area

This investigation was carried out in the Plum Island Estuary (PIE) Massachusetts, USA (42°44′N, 70°52′W). PIE has extensive salt marshes; Spartina alterniflora and Spartina patens are the dominant macrophytes on the marsh platform. Within tidal creeks, steep, almost vertical, 2-m high creek walls are irregularly covered with macroalgae and filamentous algae. At the time of the experiment, macroalgae were visually less dominant than filamentous algae in and around the study creek. There was a nearly continuous, ~20 cm high bank of horizontal algae consisting of Rhizoclonium spp. and other 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. Salinities at the time of the experiment ranged from 15 to 28‰. The estuary experiences semi-diurnal tides with approximate 3-m tidal range. PIE is a low diversity consumer ecosystem, to the north of the distributional range of many abundant species (e.g., 

2.2. Natural abundance stable isotopes

Natural abundance stable isotope values were used to examine the importance of basal resources; they also served as a baseline for the isotope additions. Primary producers and infauna were collected from two habitats, the mudflat and creek wall (Fig. 1), in a saltmarsh creek on July 20 and 21, 2005. Creek dimensions are given in Drake et al. (2009). Organisms were collected from both habitats over a stretch of ~200 m (see collections below).

2.3. 15N-tracer additions

To label tidally imported algal resources (i.e., phytoplankton) as well as in situ benthic primary producers in the creek, 15N-tracer solution was added to the water column during every rising tide for 42 days beginning on July 23, 2005. We enriched a 300 m linear stretch of ~200 m (see collections below).
K\(^{15}\)NO\(_3\) was added over a 42 day period. The addition of \(^{15}\)N-enriched NO\(_3^-\) was not considered a fertilizer because the amount of K\(^{15}\)NO\(_3\) added did not significantly alter average ambient nutrient concentrations throughout the 42-day addition (Drake et al., 2009). This current study is focused on food-web incorporation of \(^{15}\)N-tracer by infauna. Details on other fates and transformations of added \(^{15}\)NO\(_3^-\) in this system are addressed in Drake et al. (2009).

Primary producers and consumers were collected ~24 h after the start of the \(^{15}\)N-addition and multiple times each week thereafter from the mudflat and creek-wall habitat. Additional samples were taken on day 42. Due to the patchy nature of infauna (Johnson et al., 2007, personal observations) not all taxa were abundant enough at each collection time for stable isotope analysis.

2.4. \(^{13}\)C-tracer additions

To determine if local primary producers (BMA and filamentous algae and associated epiphytic diatoms) were important to infauna diets, a \(^{13}\)C-isotope addition experiment was carried out in 8, 1-m\(^2\) plots within two habitats in the same marsh creek receiving \(^{15}\)N-enriched tracer additions. \(^{13}\)C-additions ran from August 19 to September 2, 2005 beginning on day 27 of the \(^{15}\)N-tracer addition. Four plots were placed in mudflat habitat to target uptake by BMA and four plots were placed in creek-wall habitat to target uptake by filamentous algae and associated epiphytes. Plots were marked at the corners with PVC poles (30 cm in length). Baseline samples of primary producers and infauna were collected within 1 week prior to the start of \(^{13}\)C-additions to determine natural abundance \(^{13}\)C values. A \(^{13}\)C-enriched \(\text{NaHCO}_3\) was dissolved in Whatman GF/F filters and was applied using a common garden sprayer. \(^{13}\)C enrichment in phytoplankton and \(\text{Spartina}\) spp. detritus is unlikely because of tidal-induced dilution and advection of the isotope signal and because non-living detrital material cannot take up the isotope label. Bacteria that use \(\text{Spartina}\) spp. detritus as a carbon source may take up the enriched \(^{13}\)N. However, baseline \(^{13}\)C values of consumers that utilize \(\text{Spartina}\) detritus and associated microbes should reflect the \(^{13}\)C values of \(\text{Spartina}\) spp. detritus (Kreeger and Newell, 2000).

Primary producers and infauna were collected 2 weeks after the start of the \(^{13}\)C-addition (1 day after the last addition of \(^{15}\)N and \(^{13}\)C tracer) to determine foodweb incorporation of local algae.

2.5. Collections

Epipelic or migrating diatoms in sediments were the most abundant primary producers comprising the BMA community and thus served as a proxy for BMA (Galván, unpublished data). BMA were collected from mudflats using 125 \(\mu\)m nitex mesh (15.2 cm\(^2\) in area). Nitex was placed directly on exposed mudflats, moistened with seawater filtered through precombusted (4 h at 480 °C) GF/F Whatman filters that have nominal 0.7 \(\mu\)m retention. Air bubbles through the nitex mesh were removed by smoothing by hand. Nitex was retrieved after 1 h. In the laboratory, BMA samples were decanted 3–5 times to separate microalgae from denser detrital and sediment particles. Microscopic inspection of the more purified samples indicated that pennate diatoms dominated collections. Samples were filtered on pre-combusted, Whatman GF/F filters for isotope analysis. BMA were not collected from the creek-wall habitat because previous attempts at collecting migrating diatoms from creek walls revealed only minute amounts that were insufficient for isotope analysis. Filamentous algae from the creek wall were collected by hand and sonicated for 1 min to remove associated epiphytic diatoms. Filamentous algae were inspected by microscopic examination, and only filamentous algae devoid of epiphytic diatoms were utilized for stable isotope analysis. After filamentous algae were removed from the sonicated sample, the remaining epiphytic diatom slurry was repeatedly decanted to separate sediments and debris from diatoms. Larger epiphytic diatoms that were not easily decanted off were removed from sediment and debris with a sorting loop and added to the decanted epiphyte sample. The resulting epiphytic samples (visual estimate was >95% diatoms) were filtered on pre-combusted Whatman GF/F for isotope analysis. Suspended particulate organic matter (SPOM) was sampled using a 70-\(\mu\)m phytoplankton tow. The phytoplankton tow was tossed approximately 15 ft per toss for a total of ~20 tosses. The samples were rinsed through a 63-\(\mu\)m sieve to remove larger zooplankton. The fraction of sample that passed through the sieve and the retained material were both repeatedly decanted in an attempt to achieve a more purified phytoplankton sample. Both fractions were examined microscopically. Zooplankton, primarily calanoid copepods, were removed with forceps from both fractions of the sieved SPOM samples. SPOM samples were filtered on pre-combusted, Whatman GF/F filters for isotope analysis, and was used as a proxy for phytoplankton. Leaves of live \textit{S. alterniflora} and standing dead \textit{S. patens} were clipped with garden shears from the marsh platform. Leaves were cleaned of foreign debris, rinsed with distilled water and dried at 70 °C. We used macrophyte leaves from live \textit{S. alterniflora} as a proxy for \textit{S. alterniflora} detritus. Currin et al. (1995) found no difference in \(^{13}\)C values between live and standing dead \textit{S. alterniflora} but found lower \(^{15}\)N values in standing dead \textit{S. alterniflora}.

For infauna collections, sediment was collected by hand from mudflats and creek wall. Sediments were preserved in a 5% buffered formalin solution for a minimum of 2 days before being sieved. Edwards et al. (2002) and Levin et al. (2006) both report short-term fixation in formalin has little effect on \(^{15}\)N and \(^{13}\)C values. Sediment samples were sieved through a 500-\(\mu\)m sieve for macrofauna and through a 63-\(\mu\)m sieve for meiofauna. Infaunas were removed from remaining sediment and organic matter using a dissecting microscope. Infaunas were pooled within each habitat by species to obtain adequate sample mass for isotope analysis and to homogenize spatial variability within habitats. The number of individuals per pooled sample depended on body size; for the copepods, at least 75 individuals per isotope sample were analyzed. For annelids, 10–30 individuals were pooled per sample. Gut contents were extruded using forceps from all infauna. Tissues devoid of gut contents were rinsed with deionized water and dried at 70 °C for 24 h for isotope analysis. Excluding copepods and the tanaid, \textit{Hargertia rapax}, infauna samples for isotope analysis were not acidified but were rinsed with deionized water to remove external sediment. Copepods and \textit{H. rapax} were acidified with 1 M HCl acid. Natural abundance \(^{15}\)N and \(^{13}\)C stable isotope values for primary producers and consumers were determined from samples taken within 1 week prior to the start of the \(^{15}\)N-tracer addition and were treated as baseline isotope values for the \(^{15}\)N-tracer addition. Within 1 week prior to the \(^{13}\)C-addition, sediment samples were collected from mudflat and creek wall for natural abundance \(^{13}\)C stable isotope values and were treated as baseline values for the \(^{13}\)C-addition.
2.6. Isotope analysis

Samples were analyzed at the University of California Davis Stable Isotope Facility and at the Marine Biological Laboratory in Woods Hole. At the former, isotope values were determined using a PDZ Europa Automated Nitrogen and Carbon Analyser Gas–Solid–liquid elemental analyzer interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon). At the Marine Biological Laboratory in Woods Hole, MA, isotope values were determined by a continuous flow isotope ratio mass spectrometer (CF-IRMS). Samples were reported relative to the standards, atmospheric N2 and Vienna Pee Dee Belemnite (VPDB) carbon. Stable isotope values are reported in δ notation:

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

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

2.7. Mixing model and trophic enrichment factors (TEFs)

Multiple primary producers including marsh macrophytes, phytoplankton, filamentous algae and associated epiphytes and BMA have the potential to contribute to the saltmarsh benthic food web. However, linear mixing models do not determine unique dietary contributions when the number of potential food resources exceeds \( n+1 \), where \( n \) is the number of isotopes used (\( \delta^{13}C \) and \( \delta^{15}N \)). Therefore, we used IsoSource, a mixing model that uses all possible resource combinations to determine a range of possible source contributions (Phillips and Gregg, 2003). While IsoSource may not reveal unique dietary contributions as found with linear mixing models, results can be informative. For example, low maximum dietary contributions indicate when the number of potential food resources exceeds \( n+1 \), the potential to contribute to the saltmarsh benthic food web. How above baseline natural abundance isotope values and was calculated using the following equation:

\[ \delta^{\text{carbon}} \text{ or } \delta^{\text{nitrogen}} = \delta^{\text{observed}} - \delta^{\text{baseline}} \]

where X is \( ^{13}C \) or \( ^{15}N \). For the \( ^{12}C \)-tracer, observed values are isotope values of organisms collected 1 day after the last isotope addition. Because \( ^{13}C \)-additions were conducted in small, 1 m² plots, we assume that only infauna from the \( ^{13}C \)-addition plots were exposed to \( ^{13}C \)-labeled primary producers. Infauna and primary producers collected to address uptake of enriched \( ^{15}N \) resources only (with natural abundance \( ^{13}C \)) were always collected from sites > 8 m from plots receiving enriched \( ^{13}C \). In a previous study (Galván et al., 2008) within a PIE saltmarsh creek, tissue turnover time for infauna was estimated at 3 weeks. Thus, it is unlikely that infauna tissue reached equilibrium during the 14-day \( ^{13}C \)-additions. As a result, \( ^{13}C \) carbon values were not used in mixing models but were instead used to confirm results from the mixing models and to ensure the dietary importance of local primary producers. TEFs ranging from 1 to 2% were used for \( \delta^{15}N \) while a TEF of 0.5% was used for \( \delta^{13}C \). These values were chosen based on a previous isotope study involving infauna in the Plum Island Estuary (Galván et al., 2008).

3. Results

A total of 19 species of annelids and 38 species of copepods were found in quantitative studies of infauna in habitats across the tidal inundation gradient in PIE (Fleeger et al., 2008; Johnson et al., 2007); infaunas were most abundant in creek-wall habitat. In the present study, approximately 100 pooled samples of infauna were analyzed for \( \delta^{13}C \) and \( \delta^{15}N \); analysis was conducted on the more abundant infaunal species. Infauna from the mudflat included the polychaetes N. diversicolor (minimum of 30 individuals) and S. benedicti (minimum of 35 individuals), the oligochaetes, Cernosvitovia immota (minimum of 45 individuals) and Paranais litoralis (minimum of 28 individuals) and the meiobenthic harpacticoid copepods, Scottolana canadensis (minimum of 40 individuals) and Nannopus palustris (minimum of 85 individuals). Infauna from the creek-wall habitat included the polychaete, Manayunkia aestivalixa (minimum of 42 individuals from creek wall); the oligochaete, P. litoralis (minimum of 33 individuals); the meiobenthic harpacticoid copepod, N. palustris (minimum of 85 individuals) and the tanaid, Leptochelia savignyi (minimum of 25 individuals from creek wall). Although replication was low per faunal collection, many individuals were pooled per sample and collections were concentrated over a relatively short time (< 2 months) that would minimize temporal changes in diet. In addition, the variability among replicates was low (Table 1), suggesting our \( ^{13}C \) and \( ^{15}N \) values more accurately represent true means.

3.1. Natural abundance stable isotopes — July (baseline)

Natural abundance stable isotope values of S. alterniflora, S. patens and phytoplankton for \( ^{13}C \) ranged were 6.3 ± 0.6‰, 2.1 ± 0.8‰ and 12.0 ± 1.0‰, respectively (Table 1). \( ^{13}C \) values for these primary producers ranged from −21.0‰ for phytoplankton to −13.2 ± 0.1‰ for S. alterniflora. In mudflats, \( ^{15}N \) and \( ^{13}C \) values for BMA were 5.7 ± 1.0‰ and −19.7 ± 0.4‰, respectively. All mudflat consumers including N. diversicolor, S. benedicti, C. immota, P. litoralis, S. canadensis and N. palustris had relatively depleted \( ^{13}C \) natural abundance values compared to S. alterniflora (Table 1, Fig. 2). C. immota had the most enriched \( ^{13}C \) values which were intermediate between Spartina spp. and all algal resources. Excluding C. immota, all infauna from the mudflat had \( ^{13}C \) values > 4‰ lower than Spartina spp (Table 1). \( ^{13}N \) values for mudflat consumers ranged from 6.0 ± 0.1‰ for C. immota to 12.0‰ for the copepod, S. canadensis (Table 1).

In the creek wall, filamentous algae and epiphytic diatoms had \( ^{15}N \) values of 6.0 ± 0.2‰ and 6.1 ± 0.0‰ and \( ^{13}C \) values of −18.2 ± 2.2‰ and −19.4 ± 0.4‰, respectively. In the creek-wall habitat, all taxa sampled had relatively low \( ^{13}C \) values at −21.4 ± 0.4‰ for M. aestuaria, −18.4 ± 0.5‰ for P. litoralis, −20.3 ± 0.4‰ for N. palustris and −19.8 ± 0.8‰ for H. rapax. All infauna from the creek-wall habitat had \( ^{13}C \) values > 4‰ lower than Spartina spp. \( ^{13}C \) values for P. litoralis, H. rapax and N. palustris were intermediate \( ^{13}C \) values for filamentous algae and epiphytic diatoms (Fig. 2). \( ^{15}N \) values were relatively similar for infauna and ranged from of 7.0 ± 0.1‰ to 8.6‰ respectively (Table 1).

3.2. IsoSource (baseline)

\( ^{13}C \) isotope values for phytoplankton were more enriched than the suspension-feeding polychaete, M. aestuaria. However, M. aestuaria is a known phytoplankton feeder (Galván, 2008; Galván et al., 2008). In two previous studies conducted in saltmarsh creeks within the same...
Table 1

δ¹³N and δ¹⁴C natural abundance stable isotope values (baseline) and maximum δ¹³N (highest observed enrichment minus background natural abundance isotope values) in organisms over the 42-day ¹⁵N-tracer addition. δ¹³C is the enrichment of ¹³C addition minus background natural abundance isotope values for primary producers and infauna after 14 days of ¹³C-addition. Natural abundance values reported are means ± (1 sd). Pelagic and benthic copepods were not replicated. The dash (−) indicates samples were not analyzed. Abbreviations include mudflat (MF), creek wall (CW), S. patens understory (SU), stunted S. alterniflora (SSA).

<table>
<thead>
<tr>
<th>Common name</th>
<th>Species</th>
<th>Habitat</th>
<th>Baseline</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary producers</td>
<td></td>
<td></td>
<td>δ¹³N (%)</td>
<td>δ¹⁴C (%)</td>
</tr>
<tr>
<td>Smooth cordgrass</td>
<td>Tall Spartina alterniflora</td>
<td></td>
<td>6.3 ± 0.6 (2)</td>
<td>−13.7 ± 0.2 (2)</td>
</tr>
<tr>
<td></td>
<td>Stunted S. alterniflora</td>
<td></td>
<td>2.3 ± 0.9 (2)</td>
<td>−13.2 ± 0.1 (2)</td>
</tr>
<tr>
<td></td>
<td>S. patens</td>
<td></td>
<td>2.1 ± 0.8 (2)</td>
<td>−13.7 ± 0.7 (2)</td>
</tr>
<tr>
<td>Phyttoplankton</td>
<td></td>
<td></td>
<td>12.0 ± 1.0 (4)</td>
<td>−21.0 ± 0.0 (4)</td>
</tr>
<tr>
<td>Filamentous algae</td>
<td>Rhiolocion spp.</td>
<td>CW</td>
<td>6.0 ± 0.2 (2)</td>
<td>−18.2 ± 2.2 (2)</td>
</tr>
<tr>
<td>Epiphytic diatoms</td>
<td></td>
<td>CW</td>
<td>6.1 ± 0.0 (2)</td>
<td>−20.9 ± 1.9 (2)</td>
</tr>
<tr>
<td>BMA</td>
<td>Mixed diatom species</td>
<td>MF</td>
<td>5.7 ± 1.0 (2)</td>
<td>−19.4 ± 0.4 (2)</td>
</tr>
</tbody>
</table>

| Consumers | | | δ¹⁵N (%) | δ¹⁴C (%) |
| Benthic copepods | Scototolina canadensis | MF | 12.1 | −21.4 |
| | Nannopodus palustris | MF | 8.2 | −19.3 |
| | Nereis diversicolor | MF | 8.6 | −20.3 |
| Spionid polychaete | Streblosia benedicti | MF | 8.0 ± 1.0 (2) | −20.6 ± 0.4 (2) |
| Oligochaetes | Cernosvitovia immota | MF | 6.0 ± 0.1 (2) | −16.0 ± 0.6 (2) |
| | Paranais litoralis | MF | 7.9 ± 1.1 (2) | −19.8 ± 0.8 (2) |
| Sabellid polychaete | Manayunkia aestuarina | CW | 7.1 ± 0.5 (2) | −18.4 ± 0.5 (2) |
| Tanaid | Hargreia rapax | CW | 7.0 ± 0.1 (2) | −19.8 ± 0.8 (2) |

wetland, δ¹³C and δ¹⁵N stable isotopes values and gut content analysis determined that phyttoplankton was the most important food resource for M. aestuarina (Galván, 2008; Galván et al., 2008). In addition to M. aestuarina, the ribbed mussel, Geukensia demissa, is also known phyttoplankton feeder in the PIE (Galván, 2008). At the time of this study, G. demissa had δ¹³C and δ¹⁵N values of −22.5 ± 0.4 and 7.3 ± 0.0 (Galván, 2008). These values were slightly more depleted than phyttoplankton isotope values. Because baseline phyttoplankton δ¹³C values were more enriched than the known phyttoplankton feeders in PIE and because water samples were not pure phyttoplankton (see methods), M. aestuarina δ¹³C and δ¹⁵N isotope values from this study and G. demissa isotope values from Galván (2008) served as a proxy for phyttoplankton in mixing models. Isotope values used in mixing models for all other primary producers were actual measured values from this study.

In the mudflat habitat, IsoSource revealed large uninformative ranges of algal dietary contributions for most consumers (Table 2). Ranges of Spartina spp. dietary contributions were generally small with relatively low maximum potential contributions and minimum contributions of 0% (Table 2). An exception was N. palustris from the mudflat habitat; the range of potential dietary contributions from S. alterniflora for N. palustris was 15–26%. In addition, Spartina contributions to the diet of C. immota ranged from 0 to 61%. Maximum combined Spartina spp. dietary contributions for N. diversicolor were <10% indicating Spartina spp. was not important to the diet of this consumer. However, BMA contributions to the diet of N. diversicolor ranged from 46 to 81% and phyttoplankton contributed a minimum of 19% with maximum potential contributions being substantially higher (Table 2). Phyttoplankton contributed substantially to the diet of N. palustris (31–70%).

In the creek-wall, ranges of dietary contributions of filamentous algae and associated epiphytes were large and uninformative. However, resource contributions from phyttoplankton were substantial for multiple consumers including N. palustris (33–86%) and M. aestuarina (65–94%). Phyttoplankton contributed a minimum of 13% to the diet of H. rapax; maximum potential contributions were substantially higher (Table 2). Similar to the mudflat, ranges of Spartina spp. dietary contributions in the creek-wall habitat were generally small with relatively low maximum potential contributions and minimum contributions of 0% (Table 2). For example, maximum combined Spartina spp. dietary contributions for M. aestuarina were ≤20% indicating Spartina spp. was not important to the diet of this consumer.

3.3. ¹⁵N-tracer addition

Water-column ¹⁵NO₃⁻ additions were targeted at 1000‰. However during the first week of the ¹⁵N-tracer addition, background NO₃⁻ concentrations were unexpectedly lower (~1.3 μM) than time 0 concentrations (~1.7 μM) (time-0 measurements were used to calculate ¹⁵N-addition amounts necessary to achieve the targeted goal), resulting in water column δ¹⁵NO₃⁻ of −1500‰ (Drake et al., 2009). During the second week, background NO₃⁻ concentrations increased to 14–20 μM and remained at these elevated levels for the duration of the ¹⁵N-addition period (42 days) resulting in water column δ¹⁵NO₃⁻
of 20–300‰. As a result of fluctuating background NO₃ concentrations (see Drake et al., 2009), phytoplankton δ¹⁵N was high during the first 4 days of the addition reaching a maximum δ¹⁵N of ~325‰ on day 3 (Fig. 3; Table 1). By day 5, phytoplankton δ¹⁵N decreased to 0–5‰ and stayed at these low levels for the remainder of the addition (Fig. 3). BMA δ¹⁵N reached 25‰ on day 3 and fluctuated between 6 and 35‰ throughout the 42-day addition. Peak tracer enrichment (δ¹⁵N) was reached on day 10 at ~70‰ and day 14 at ~50‰ for filamentous algae and associated epiphytic diatoms, respectively (Fig. 3). On day 42, live leaf tissue of S. alterniflora and S. patens was enriched above background values at ~11‰ and ~9‰ respectively (Table 1).

All consumers were enriched in ¹⁵N above background levels over the addition period (Figs. 4a and c and 5). However, ¹⁵N-enrichment in some consumers was very low. Consumer δ¹⁵N differed among species and habitats.

In the mudflat habitat, maximum δ¹⁵N was reached on day 42 for N. diversicolor and was 44% of the maximum δ¹⁵N of BMA, the dominant primary producer found in the mudflats (Fig. 4a). Maxima δ¹⁵N found in S. benedicti, C. immota, P. litoralis and S. canadensis were 65‰, 22‰, 33‰ and 25‰ that of BMA, respectively (Fig. 4a). δ¹⁵N in N. patulus reached 100% that of BMA. Natural abundance δ¹³C values for mudflat consumers changed little over the 42 days indicating no change in the use of basal food resources (Fig. 4b).

In the creek-wall habitat, δ¹⁵N in the suspension feeder, M. aestuariina, was highest during the first 10 days of the addition but quickly dropped off to low levels of enrichment for the remainder of the addition (Fig. 4c). After the first 10 days, δ¹⁵N in M. aestuariina did not exceed 7‰. Mean peak δ¹⁵N, the average δ¹⁵N over the last 3 weeks of the ¹⁵N-addition, for H. rapax, N. patulus and P. litoralis was 34‰, 52‰ and 43‰, respectively after 42 days. Filamentous algae and associated epiphytes were the dominant labeled resources available to creek-wall consumers. After day 10, δ¹⁵N of filamentous algae (~60‰ to 70‰) remained relatively constant for the remainder of the addition. Average peak δ¹⁵N was 35‰ for epiphytic diatoms; however, epiphytes reached a maximum δ¹⁵N of ~50‰ on day 14. Excluding H. rapax, natural abundance δ¹³C values for all primary producers and consumers in the creek wall changed little over the 42 days indicating no diet change (Fig. 4d).

### Table 2

<table>
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<th>Organism</th>
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<th>SA</th>
<th>SP/SSA</th>
<th>PP</th>
<th>FA</th>
<th>Epiphytes</th>
<th>BMA</th>
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### 3.4. IsoSource (42 days of ¹⁵N-addition)

Using IsoSource, ranges of potential resource contributions were determined for infauna from the mudflat and creek-wall habitat using natural abundance δ¹³C and δ¹⁵N values where both were averaged from samples collected over the last 4 weeks of the ¹⁵N-addition for primary producers and from the last 3 weeks of the ¹⁵N-addition for consumers. Exceptions included Spartina spp.: Spartina spp. requires an aging process before it is available to the consumers analyzed in this study. We assumed that all Spartina detritus consumed within the 42 day ¹⁵N-addition was of an older, unlabeled source. As a result, 0‰ was used for δ¹³C in ¹⁵N of Spartina spp. in mixing models.

Dietary resolution increased for multiple consumers in the mudflat after 42 days of ¹⁵N-addition including S. benedicti, P. litoralis and C. immota. Prior to the ¹⁵N-addition, IsoSource results revealed large uninformative ranges of dietary contributions from BMA and phytoplankton for S. benedicti and P. litoralis. However, IsoSource results after the ¹⁵N-addition revealed a much narrower range of algal resource use. Specifically, the range of resource contributions from Mudflat and Creek Wall.
BMA was 70–75% for *S. benedicti*, while phytoplankton contributions to the diet of *P. litoralis* ranged from 45 to 61%. For *C. immota*, natural abundance stable isotope values revealed large, uninformative ranges of resource use with IsoSource. However, IsoSource results after the 15N-addition indicate *S. patens* contributed 44–68% of its diet. Prior to the 15N-addition, ranges of phytoplankton contributions to the diet of *N. palustris* in the mudflat were 31–70%. However, the level of 15N-enrichment in *N. palustris* suggests BMA was important to this consumer. IsoSource results after the 15N-addition revealed phytoplankton contributions for *N. palustris* from the mudflat were 0 while BMA contributions were 91%. The similarity in natural abundance δ13C values for *N. palustris* at the start and end of the δ15N addition in combination with substantially different IsoSource results after the 15N-addition indicates that natural abundance stable isotopes revealed less accurate resource contributions with mixing models for this consumer.

Similar to IsoSource results prior to the 15N addition, *Spartina* spp. contributions were relatively small for most consumers in the mudflat. Maximum combined *Spartina* contributions were ~20% for *N. palustris*, *N. diversicolor*, and *S. benedicti*. Two exceptions include *P. litoralis* and *C. immota*; dietary contributions from SP/SSA ranged from 23 to 29% and 44 to 68%, respectively. In the creek-wall habitat, dietary resolution increased for *P. litoralis* and *H. rapax* after 42 days of 15N-addition. Prior to the 15N-addition, IsoSource revealed wide, uninformative ranges of resource contributions from filamentous algae and associated epiphytes for these two consumers. However, IsoSource results after the 15N-addition revealed filamentous algae contributed 48–72% of the diet of *P. litoralis* and 25–48% of the diet of *H. rapax*. Phyttoplankton was less important for *N. palustris* than natural abundance stable isotopes had suggested and minimum contributions from filamentous algae and epiphytes were higher at 21 and 27%, respectively. Similar to IsoSource results prior to the 15N addition, *Spartina* spp. contributions were relatively small for most creek-wall consumers.

**Fig. 4.** a) δ15N (nitrogen) over the 42-day 15N-addition for mudflat infauna and b) natural abundance δ13C values for mudflat infauna over the 42-day experiment, c) δ15N (nitrogen) over the 42-day 15N-addition for creek-wall infauna and d) natural abundance δ13C values for over the 42-day 15N-addition for creek-wall infauna. For mudflat infauna, BMA supplied local labeled resources while filamentous algae and associated epiphytes were labeled in creek-wall habitat.

**Fig. 5.** A dual isotope plot of observed averaged δ15N (nitrogen) and averaged natural abundance δ13C value (see methods). Excluding copepods, values reported are means. Consumer isotope values are corrected for trophic fractionation.
3.5. Dual $^{15}$N- and $^{13}$C-tracer addition

The $^{13}$C-enriched label was taken up by all targeted benthic algal primary producers and overall, results from the $^{13}$C-addition support findings from the $^{15}$N-addition. In the mudflat, after 2 weeks of daily $^{13}$C-additions beginning on day 27 of the $^{14}$N-addition, BMA was enriched in both $^{13}$C and $^{15}$N above background values where $\delta^{13}C$ was 37% and 20‰, respectively (Fig. 6). N. diversicolor, S. benedicti, and N. palustris all became enriched in both $^{13}$C and $^{15}$N above background values (Fig. 6) indicating consumption of locally labeled BMA. After day 42, $^{15}$N-addition and a simultaneous 2 week $^{13}$C-addition, $\delta^{15}N$ in N. diversicolor, S. benedicti and N. palustris was 17‰, 8‰, and 35‰ for $^{13}$C, respectively and was 9‰, 13‰, and 20‰ for $^{15}$N, respectively (Fig. 6). Uptake of both labels was minimal for S. canadensis indicating the importance of an unlabeled food resource (i.e., phytoplankton).

In the creek-wall habitat, filamentous algae and associated epiphytic diatoms were enriched in both $^{13}$C and $^{15}$N where $\delta^{13}C$ was −21‰ for both algal groups and $\delta^{15}N$ was 70‰ and 35‰, respectively. P. litoralis, H. rapax and N. palustris became enriched in both $^{13}$C and $^{15}$N above background values indicating consumption of locally labeled filamentous algae and/or epiphytic diatoms. $\delta^{13}C$ in $^{15}$N for P. litoralis was 18‰ and 32‰, respectively. H. rapax had the highest $\delta^{15}N$ in $^{13}$C at 38‰ with a $^{15}$N $\delta^{15}N$ of 34‰. Based on the level of enrichment in H. rapax and P. litoralis, it is likely that epiphytic diatoms were important to the diet of these two consumers. N. palustris was enriched in $^{13}$C and $^{15}$N above background values by 15‰ and 50‰ respectively (Fig. 6). The high level of $^{15}$N-enrichment in N. palustris suggests that filamentous algae and/or epiphytic diatoms were an important dietary resource for this copepod in the creek-wall habitat (Fig. 6).

4. Discussion

We used a combination of multiple natural abundance stable isotopes and isotope tracers (enriched $^{13}$C and $^{15}$N) to determine dietary contributions of local and tidally imported basal resources to infauna. Both natural abundance stable isotopes and isotope additions revealed Spartina spp. was relatively unimportant to the diets of most mudflat and creek-wall infauna, while the isotope additions helped to better resolve the dietary contributions of algal resources, determine local resources important to the saltmarsh food web and reduce erroneous conclusions found with natural abundance stable isotopes alone.

Dietary resolution was relatively low using natural abundance stable isotopes and primarily revealed resources that were not important to diets. Specifically, natural abundance stable isotope values revealed Spartina spp. was not important to the diets of infauna but could not reveal the dietary importance of specific algal resources. However, the addition of enriched $^{14}$N and $^{13}$C created distinct primary producer isotope values that allowed us to determine resource contributions from specific algal resources. The addition of enriched $^{15}$N resulted in highly labeled filamentous algae (~70‰); moderately labeled epiphytic diatoms (~35‰) and lower levels of $^{13}$N-tracer in BMA (~20‰). $^{15}$N-tracer uptake in phytoplankton changed with unanticipated changes in background NO$_3^−$ concentrations. Low levels of NO$_3^−$ at the start of the $^{15}$N-addition resulted in high phytoplankton $\delta^{15}N$. The large increases in background NO$_3^−$ that followed lowered the $^{15}$N-tracer concentration and reduced the $\delta^{15}N$ in phytoplankton to 0−5‰ for the remainder of the addition. Changes in $^{15}$N-tracer uptake by phytoplankton during the first week of the $^{15}$N-addition provided a unique but short-term enrichment pattern that was followed through the food web (i.e., M. aestuariina and S. canadensis).

In addition to greater dietary resolution, the isotope additions revealed incorrect resource contributions using natural abundance stable isotopes and mixing models. For example, $\delta^{15}N$ values changed little over time for the harpacticoid copepod N. palustris (and most other infauna) from both habitats indicating there was no temporal change in the diet of consumers. However, ranges of resource contributions as determined by natural abundance stable isotopes (baseline $\delta^{13}C$ and $\delta^{15}N$) greatly differed from ranges of resource contributions with the $^{14}$N-addition (mudflat only). Specifically, using natural abundance stable isotopes, phytoplankton comprised 31−70% of the diet of N. palustris. After the $^{14}$N-addition, the mixing model estimated that BMA comprised 91% of the diet of N. palustris and phytoplankton contributions equaled 0%. Prior to the addition of enriched isotopes, $\delta^{13}C$ values for BMA and phytoplankton were similar, likely resulting in both low dietary resolutions as well as less accurate ranges of resource contributions. As concluded by Phillips and Gregg (2003) and confirmed in this study, although the ranges of dietary contributions provided by IsoSource can be useful, the utility of this approach in resolving dietary contributions greatly decreases in situations where basal resources have similar isotope values. Similar conclusions can be drawn from natural abundance isotope studies in a variety of ecosystems including a riparian forest (Scott et al., 2009), coastal shoals (Grippo et al., 2011), a mangrove (Oakes et al., 2010) and salt marshes (Galván et al., 2008; Winemiller et al., 2007). Stable isotope additions in these systems could increase our understanding of food web interactions (see Oakes et al., 2010) and nutrient cycling and may reveal past erroneous conclusions in studies that used natural abundance stable isotopes alone. While the intertidal nature of the current study system, which exposed mudflats and creek-wall areas at low tide for spraying, allowed us to target not only specific areas, but specific food resources, it may be difficult in other ecosystem to enrich target species in the field. These studies may need a similar approach to Levin et al. (1999) where resources were isotopically labeled in the lab and placed in the field.

4.1. Basal resource contributions

In the mudflat, BMA was an important food resource for the meio-benthic copepod N. palustris. Prior to this study, little was known about the diet of N. palustris; however, studies suggest that grazing diatoms are a common harpacticoid feeding strategy (Azovsky et al., 2005; Carman et al., 1997). N. palustris is a poor swimmer (Fleeger,
personal observations), and is not known to enter the water column to feed (Fleeger et al., 1984) as with some other copepods (e.g., S. canadensis). The uptake of $^{13}$C and $^{15}$N label in this study indicate that N. palustris is a specialist, feeding on BMA. The moderate $^{15}$N and $^{13}$C label found in N. diversicolor and S. benedicti relative to BMA (and to N. palustris) indicates that these consumers likely relied on a mixed diet of BMA and phytoplankton. N. diversicolor lives in intracuraceous burrows within the sediment matrix and has been shown to have a variety of feeding modes including selective surface-deposit feeding, subsurface feeding and suspension feeding. The type of feeding mode utilized by N. diversicolor may depend on the type and availability of primary producers as well as the threat of predation (Harley, 1950; Smith et al., 1996; Vedel, 1998). N. diversicolor has been found in the gut contents of killifish from PIE and may be an important trophic link between primary producers and higher trophic levels (Galván, 2008).

Similar to N. diversicolor, S. benedicti has flexible feeding modes that include selective surface deposit feeding and suspension feeding. S. benedicti is a spionid polychaete and the type of feeding mode utilized by spionids has been shown to depend on the (water) flux of organic matter where these polychaetes are thought to surface deposit feed at low fluxes but suspension feed at higher fluxes (Taghon, 1983; Taghon et al., 1980). More recent work (Shimeta, 2009) however, suggests that spionids do not feed well at high flow. Thus, high flow rates may reduce suspension feeding while increasing surface deposit feeding. While the diet of S. benedicti in PIE appears to be a mix of BMA and phytoplankton, BMA was substantially more important to its diet. S. canadensis, P. litoralis and C. imnota were only slightly enriched in $^{15}$N above background levels and were not enriched in $^{13}$C (C. imnota was not analyzed from $^{13}$C plots), indicating the importance of an unlabeled food resource. S. canadensis is a meiobenthic copepod that, similar to close relative Cauillona sp., migrates up into the water column to feed on phytoplankton (Decho, 1986; Lonsdale and Levinton, 1986; Maddi et al., 2006). The low level of $^{15}$N-enrichment and lack of $^{13}$C-enrichment together with low natural abundance $^{15}$N values indicated that basal resources of S. canadensis were almost exclusively phytoplankton. In addition, S. canadensis followed a similar pattern of $^{15}$N-enrichment found in phytoplankton further suggesting the dietary importance of phytoplankton. However, high natural abundance $^{15}$N values indicate the importance of a trophic intermediate, most likely water-column dwelling protozoans or the nauplii of pelagic copepods (Heinle et al., 1977). The low levels of tracer incorporation and relatively low natural abundance $^{15}$N values for P. litoralis indicated that this oligochaete most likely relied on a mixture of detrital (unlabeled) BMA and settled phytoplankton. In contrast, the low enrichment in $^{15}$N and relatively high natural abundance $^{15}$N values for C. imnota indicate that this oligochaete likely relied on a mixture of Spartina detritus and algal resources. Specifically, Spartina contributed substantially more to the diet of C. imnota than any other infauna comprising a minimum of 44% of its diet. Oligochaetes such as C. imnota and P. litoralis have traditionally been considered subsurface deposit feeders that consume sediment organic matter as they move through the sediment matrix (Faucihd and Jumars, 1979). Surface and water column algae were previously thought to be a food resource unavailable for subsurface feeders (Lopez and Levinton, 1987); however, recent studies have shown that surface and settled phytoplankton may be an important dietary resource for subsurface deposit feeders (Holmes et al., 2000; Hughes et al., 2000; Levin et al., 1999).

Detrital BMA and phytoplankton may be available to subsurface deposit feeders through the burrows of other infauna (Papasyprou et al., 2006) or when surface deposits are drawn down to sediment depths through the activity of other infauna (Josefson et al., 2002). In the creek-wall habitat, filamentous algae and associated epiphytes were differentially labeled in tracer $^{15}$N, where maximum average $^{15}$N in epiphytic diatoms was 66% that of filamentous algae. The difference in tracer uptake likely increased diet resolution in this habitat with mixing models. Mixing models indicated filamentous algae were important to the diet of only one investigated consumer, the meiobenthic copepod, N. palustris. N. palustris has reduced mouthparts compared to amphipods and larger crustaceans, suggesting it may use detrital algae or more likely epiphytic diatoms that varied in $^{15}$N-enrichment. The lack of specialized mouthparts for shredding algae most likely limits the use of filamentous algae for many infauna species. However, epiphytic diatoms on filamentous algae are abundant in the creek-wall habitat, may be a more suitable food source due to size and have been found in the guts of numerous infaunas from PIE (Galván, 2008). Furthermore, enrichment in $^{13}$C indicates that N. palustris consumed local algal resources which were most likely epiphytic diatoms. Of the creek-wall infauna, epiphytic diatoms were important for the oligochaete P. litoralis, and the tanaid, H. rapax; both consumers relied almost exclusively on a diet of epiphytes. Although lacking external feeding structures, P. litoralis likely consumes epiphytes loosely attached and shaken loose by tidal flow or some other action such as that of another consumer. Gut-content analysis has previously revealed consumption of epiphytic diatoms by P. litoralis (Galván, 2008). Due to their small size and difficulty in collecting a pure sample, epiphytic diatoms are often overlooked in food-web studies. However, a food web study conducted in a Japanese estuarine lagoon, also revealed the dietary importance of epiphytic diatoms to the amphipod, Amphithoe valida and the polychaete, Capitella sp. (Kanaya et al., 2007). H. rapax is an abundant, yet patchy, tube-building tanaid found primarily in the creek-wall habitat in the PIE. Stable isotope values from this study strongly suggest H. rapax relies primarily on a diet of epiphytic diatoms. In addition, microscopic examination of living specimens from PIE revealed H. rapax partially emerges from its tube to feed on epiphytic diatoms. The suspension feeder M. aestuaria reached peak $^{15}$N enrichment within the first 2 weeks of the $^{15}$N-addition. However, enrichment decreased quickly thereafter. The pattern of enrichment found in M. aestuaria was similar to $^{15}$N enrichment in phytoplankton indicating the dietary importance of phytoplankton (Fig. 4c). M. aestuaria has previously been reported to feed on phytoplankton in creek habitats as well as from sediments on the marsh platform in the PIE (Galván et al., 2008).

The mudflat and creek-wall communities of infauna have many surface deposit feeders capable of feeding on phytoplankton and BMA. Excluding P. litoralis and C. imnota, there are relatively few subsurface deposit feeders in the PIE. C. imnota is among the most abundant annelids found in sediments across the tidal gradient within saltmarsh creeks (Johnson et al., 2007) and is among the few consumers, including infauna, epifauna and nekton, in which Spartina spp. has been shown to be an important basal resource in the PIE (Galván, 2008).

Results from the present study corroborate findings from a food web study conducted the previous year in the PIE. As in the current study, Galván et al. (2008) found the diets of S. benedicti and P. litoralis were comprised of a mixture of BMA and phytoplankton and that BMA was important to the diet of N. diversicolor in the mudflat. The similarity in infauna diets between years illustrates the importance of algae as a basal food resource in salt marshes as well as isotopic continuity.

4.2. Food-web dynamics

Microalgae including BMA, epiphytic diatoms and phytoplankton were important to the saltmarsh food web. Although primary production of microalgae in salt marshes is generally lower than macrophyte production (Mann, 1988), microalgae are relatively more nutritious than macrophytes (Tenore, 1988). Microalgae, especially diatoms, are high in nitrogen and contain a wide spectrum of fatty acids important in secondary production (Tenore, 1988). Furthermore, because
microalgae can be directly consumed and do not require an aging process as found with Spartina spp., microalgae may be more readily available for consumption and ultimately assimilation. Food web studies from different geographic locations have found microalgae to have an important role in saltmarsh food webs (Curnin et al., 1995; Kwak and Zedler, 1997; Sullivan and Moncreiff, 1990).

By the end of the 42-day addition, relatively low levels of $^{15}$N-tracer were found in leaf tissue of S. alterniflora and S. patens. Because incorporation of macrophytes in the aquatic saltmarsh food web is primarily through a detrital pathway that requires a relatively long aging process, it is doubtful that any consumer $^{15}$N-enrichment occurred via consumption of Spartina spp. Instead, any food web incorporation of Spartina spp. throughout the addition of $^{15}$N-tracer was likely of an older, unlabeled source.

This study was conducted from mid July to early September. Although resources important to infauna were primarily algal, it is possible that Spartina contributions increase during other times of the year and in other habitats. A companion study examined resource contribution to epifauna on the marsh platform during the 42 day addition (Galván, 2008). Prior to the experiment, algal resources were important to the diet of epifauna. However, $^{6}$C natural abundance stable isotope values increased over the 42 days and mixing models revealed a change in diet to Spartina spp. During this time, sediment Chi concentrations, a proxy for sediment algal resources (e.g., BMA, filamentous algae), significantly decreased on the marsh platform under the grass canopy indicating a reduction in algal resources. Unlike marsh platform Chi concentrations, sediment Chi concentrations in creek habitats did not significantly change over time (May through September) and numerous infauna studied here had similar isotope values in May indicating little change in diet (Galván, 2008). However, it is possible that Spartina spp. dietary contributions increase in creek habitats at other times of the year when sediment algal resources decrease.

The killifish, F. heteroclitus, and the grass shrimp, P. pugio, numerically dominate saltmarsh creeks (>95%) along the northeastern U.S. Atlantic coast, including the PIE. Killifish and grass shrimp are abundant omnivores that consume benthic infauna, such as annelids and small crustaceans as well as plant and algal material (Cross and Stiven, 1999; Fleeger et al., 2008; Kneib, 1986, 1997). Manipulative studies suggest killifish and grass shrimp impact macroinfaunal densities (Beseres and Feller, 2007; Kneib and Stiven, 1982; Posey and Hines, 1991). Thus, our findings suggest algal resources are important in supporting secondary production in primary consumers as well as higher trophic levels.

5. Conclusions

We analyzed approximately 1000 infauna in over 8 species over a two month period for natural abundance and enrichment isotope values. Natural abundance stable isotopes revealed some resources (i.e., Spartina spp.) that were unimportant to the diets of most infauna but due to similar isotope values in algae could not reveal algal resources that were important to the saltmarsh food web. However, the enriched stable isotope additions created distinct isotope values in algal resources, which increased diet resolution with mixing models. Although Spartina spp. is the most visually dominant resource in the salt marsh and Spartina spp. detritus is abundant in sediments inhabited by these infaunas, it was the least important food resource for all but one infaunal species studied. Instead, algal resources including both local (BMA and epiphytic diatoms) and tidally imported sources (phytoplankton) contributed the most to diets. These results are similar to those found by Riera et al. (1999) in which diets did not reflect the availability of resources but instead indicate that many benthic consumers selectively feed on more nutritious algae.

Some consumers can be grouped based on resource use. Specifically, S. canadensis and M. aestuarina relied on phytoplankton as a basal resource. P. litoralis (creek-wall habitat), N. palustris (creek-wall habitat) and H. rapax relied on a diet of epiphytic diatoms. Some consumers relied on a mixed diet of phytoplankton and BMA including N. diversicolor, S. benedicti and P. litoralis (mudflat habitat) and some consumer diets differed by habitat (P. litoralis and N. palustris). C. immota was the only infauna consumer in which Spartina spp. contributed substantially to its diet. Infaunas are abundant primary consumers and prey for fish and shellfish and in the saltmarsh creeks of the PIE infauna almost exclusively link higher trophic level production to algal resources.

In addition to increasing dietary resolution, isotope additions revealed less accurate resource contributions determined with natural abundance stable isotopes alone. While many food web studies rely on natural abundance stable isotopes, we feel that the combination of natural abundance stable isotopes and multiple isotope additions (large- and small-scale) is needed in systems with similar primary producer values to more accurately determine resource contributions, increase resolution of dietary contributions and to determine the use of local basal resources.

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References


