Vol. 240: 85–92, 2002 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published September 12

Small-sample methods for $\delta^{13}C$ and $\delta^{15}N$ analysis of the diets of marsh meiofaunal species using natural-abundance and tracer-addition isotope techniques

Kevin R. Carman[1,*], Brian Fry[2]

[1]Department of Biological Sciences, Louisiana State University, Baton Rouge, Louisiana 70803, USA
[2]Department of Oceanography and Coastal Studies, Coastal Ecology Institute, Louisiana State University, Baton Rouge, Louisiana 70803, USA

ABSTRACT: Modifications to a conventional elemental analyzer-stable isotope ratio mass spectrometer system (EA-MS system) are described that allow the analysis of ^{13}C and ^{15}N in small samples (≥1 µg N and 2 µg C). This system was used to analyze $\delta^{13}C$ and $\delta^{15}N$ of meiofaunal species from a coastal marsh using pooled samples of 5 to 60 individuals. In a conventional field survey, ^{13}C and ^{15}N isotope values indicated that (1) harpacticoid copepod species, nematode species, and ostracods differentially exploited phytoplankton detritus, *Spartina alterniflora* detritus, and benthic microalgae, and (2) all taxa showed a general shift toward *S. alterniflora* isotope values in winter relative to summer. In a field experiment, benthic microalgae were labeled *in situ* by addition of ^{13}C and ^{15}N to 1 m^2 sediment plots. Two nematode species with apparently similar primary food resources (*S. alterniflora* detritus) based on natural isotopic values differed dramatically in their uptake of ^{13}C and ^{15}N in labeled plots, indicating differences in feeding strategies that were not indicated by natural isotope values. A combination of natural-abundance isotope surveys and isotope-addition experiments appears to be a powerful approach for investigating both average patterns and interspecific variability in resource exploitation.

KEY WORDS: $\delta^{13}C$ · $\delta^{15}N$ · Meiofauna · Harpacticoids · Nematodes · Ostracods · Benthic microalgae · *Spartina*

INTRODUCTION

Carbon and nitrogen stable isotopes are commonly used to examine the structure of food webs in freshwater (e.g. del Giorgio & France 1996, Keough et al. 1998) and marine (e.g. Sullivan & Moncreiff 1990, Currin et al. 1995) ecosystems (see reviews by Peterson & Fry 1987, Peterson 1999). Because analytical methods typically require milligram quantities of biomass for reliable detection of isotopic compositions, most studies have focused on larger animals, vascular plants, and/or plankton 'fractions' that can be concentrated via filtration. Smaller organisms such as meiofauna have been the subject of relatively few stable-isotope studies, primarily because meiofauna have low biomass and large numbers of them must be collected to obtain sufficient material for analysis (Couch 1989, Riera et al. 1996, Middleburg et al. 2000). Meiofaunal grazing, particularly by copepods, may control benthic microalgal biomass (Montagna 1995, Carman et al. 1997), and meiofauna are important prey for larger animals such as juvenile fish, shellfish and infauna (Gee 1989). In spite of their potential importance in marine food webs, relatively little is known about interspecific differences or seasonal changes in meiofaunal feeding strategies. Because of their ubiquitous distribution and high abundance, meiofauna can be

*E-mail: zocarm@lsu.edu

analyzed and experimentally manipulated on relatively small spatial scales with good replication, and thus present opportunities for logistically feasible ecosystem-level analyses of food-web structure and dynamics. However, the small size (and thus small biomass) of meiofaunal organisms makes it technically challenging to perform stable-isotope investigations of the feeding ecology of individual species. Thus, better methods are needed for small sample work. Here, we describe a procedure in which analysis of $\delta^{13}C$ and $\delta^{15}N$ can be reliably performed with pooled samples of 5 to 60 individual meiofauna. This enhanced sensitivity allows for species-level stable-isotopic analysis of meiofaunal-sized organisms. Winter and summer field surveys of natural $\delta^{13}C$ and $\delta^{15}N$ values were used to determine major sources and seasonal variation of nutritional sources (suspended particulate material, benthic microalgae, and *Spartina alterniflora*) in selected meiofaunal species and ostracods. A field-manipulative experiment involving the addition of $NaH^{13}CO_3$ and $^{15}NH_4Cl$ to exposed mudflats was used to determine if nematode species with similar natural-isotope signatures differ in their exploitation of benthic microalgae.

MATERIALS AND METHODS

Meiofauna collection and sample processing. Stable-isotope analyses were performed on meiofaunal taxa resident in the mudflat of a salt marsh in Terrebonne Bay estuary near LUMCON (Louisiana Universities Marine Consortium) in the northern Gulf of Mexico (29° 15′ N, 91° 21′ W). The marsh is dominated by *Spartina alterniflora* (Loisel.) (hereafter '*Spartina*'). Tidal range is ~0.3 m and salinity ranges from 1 to 20 psu. Sediments consist of fine mud, and have a median grain size of 38 µm (primarily silts [41%] and clays [17%]), and an organic carbon content of ca. 2.5%. Sediment samples were collected from an intertidal mudflat approximately 1 m from the *Spartina* border using butyrate cores (10 cm^2 cross-sectional area). Three replicate cores were collected at random positions along a 50 m transect. The top 1 cm of sediment was extruded, collected, preserved in 4% formaldehyde and stained with Rose Bengal.

Field surveys of the isotopic compositions of 2 harpacticoid copepod species (*Pseudostenhelia wellsi* [Coull and Fleeger] and *Coullana* sp.), 2 nematode species (*Ptycholaimellus pandispiculatus* [Hopper] and *Daptonema* sp.), and mixed ostracod species were performed in June 1999 and January 2001. In addition, *Spartina* leaves, suspended particulate material (SPM), and benthic microalgae samples were collected (n = 3 for each). *Spartina* leaves were dried and powdered using a Wig-L-Bug grinder prior to analysis. SPM samples were collected by filtering 250 ml of marsh water onto pre-combusted GF/F filters. Filters were dried and fumigated with concentrated HCl, and then a subsample was taken using a paper punch. SPM samples were collected as an indication of phytoplankton stable-isotope content, but detrital material and zooplankton were also collected on the filters. Benthic microalgae were concentrated by placing 2 layers of 63 µm Nitex mesh netting onto the sediment surface during low tide. Motile diatoms migrated through the lower layer of mesh and were captured with minimal sediment contamination in the top layer. Diatoms were removed from the mesh by gentle sonication in sterile artificial seawater, concentrated by centrifugation, and transferred to 3 × 5 mm tin cups (Costech Analytical Technologies) for analysis. The presence of diatoms in mesh samples was confirmed microscopically; no animals, and only small quantities of detritus were observed.

In January 2001, ^{13}C and ^{15}N were added to exposed mudflats for the purpose of tracing consumption of benthic microalgae by meiofauna. Six replicate 1 m^2 mudflat plots were marked with PVC stakes. Each plot was separated by a linear distance of 1 m and oriented parallel to and 1 m from the marsh grass edge. Three plots were sprayed during a morning low tide with 250 ml of a $NaH^{13}CO_3/^{15}NH_4Cl$ solution in 10 psu artificial seawater (ASW) (18.5 mg $^{15}NH_4Cl$ m^{-2}; 4 g $NaH^{13}CO_3$ m^{-2}; Sigma Aldrich) (cf. Middleburg et al. 2000). The remaining 3 control plots (each adjacent to a treated plot) were sprayed with 250 ml unlabeled ASW. A 10 cm^2 sediment core was collected from the center of each plot immediately prior to and 3 d after isotope application, and the nematode species *Ptycholaimellus pandispiculatus* and *Daptonema* sp. were sorted from sediment samples and assayed for $\delta^{13}C$ and $\delta^{15}N$.

Laboratory gloves were worn at all times during meiofaunal processing to minimize C and N contamination. Meiofauna were manually removed from field-sediment samples and transferred to a clean Petri dish containing deionized water. Any debris adhering to meiofauna was carefully removed. Ostracods were transferred to a vial containing 1.2 M HCl and incubated overnight to remove carbonates associated with their exoskeleton. A small drop (~5 µl) of deionized water was placed in small tin cups (3 × 5 mm), and meiofauna were transferred to the cups using a tungsten-wire probe. The tin cups were arranged in a 96-well plate and dried at 60°C. The cups were crimped and manually compacted to minimize internal air space, then analyzed for $\delta^{13}C$ and $\delta^{15}N$ as described below.

$\delta^{13}C$ and $\delta^{15}N$ analysis. Most modern laboratories use a conventional elemental analyzer-stable isotope

ratio mass spectrometer system (EA-MS system) to analyze both carbon and nitrogen isotopes from single samples of animals, plants, or soils (Barrie & Prosser 1996). In the present study, the EA-MS system consisted of a Carlo Erba NA1500 elemental analyzer linked to a Finnigan Delta Plus isotope-ratio mass spectrometer. In these systems, samples are combusted at high temperature in the elemental analyzer, water is removed via an adsorption trap, and the resulting dried CO_2 and N_2 gases are carried to the mass spectrometer in a He stream for the final analysis of peak areas corresponding to sample C and N mass, and for analysis of ion-beam ratios corresponding to isotopic compositions ($\delta^{13}C$ and $\delta^{15}N$). To analyze small meiofauna samples, we primarily worked with the elemental analyzer, reducing background isotope values in blank samples and miniaturizing the high-temperature reactors as follows.

Background values of $\delta^{15}N$ were influenced mostly by air leakage during valve and autosampler switching, and were reduced by using He instead of compressed air to power valves in the elemental analyzer, and using high He flow rates (200 ml min^{-1}) to purge the AS 200 autosampler. Also, the autosampler was operated in a quick-delivery fashion (1 s between on and off commands) to reduce blank values.

Carbon blanks were the result of carbon contained in walls of nominally pure tin cups, with larger cups (5 × 9 mm) having higher carbon blanks. Using smaller cups was the simplest way to reduce carbon blanks (e.g. 5 × 9 mm cups had ~1 µg C cup^{-1} and 3.5 × 5 mm cups ~0.5 µg C cup^{-1}).

We also miniaturized the high-temperature-reactor system in the elemental analyzer that normally consists of separate quartz oxidation and reduction tubes held at 1000 and 650°C, respectively. Quartz reactors with smaller (8 mm) internal diameter (ID) were substituted for the normal tubes that are 14 to 18 mm ID, and reactors were filled with the normal oxidation packings (chromic oxide and silvered cobaltous-cobaltic oxide) and reduction packings (elemental Cu, 0.3 mm) specified by the elemental analyzer manufacturer. We also experimented with a further simplification of the reactor system, collapsing the oxidation and reduction functions into a single tube, similar to that commonly employed for sulfur analysis (e.g. Fry et al. 1992). Trials with single reactors were made at 800°C, and single reactors were filled halfway with elemental copper. The 800°C temperature is hot enough to ensure flash combustion of small meiofaunal samples, but cool enough so that the Cu did not melt and block flows. Before loading into the single reactor, the active surfaces of Cu particles were exposed by processing in a Wig-L-Bug grinder for 10 s. Flow rates were 40 ml min^{-1} for the single reactor and 65 ml min^{-1} for the 2-reactor system. Other conditions in the elemental analyzer were those used in normal CN runs (Barrie & Prosser 1996), e.g. a 1.5 or 2 m CN column was used to separate CO_2 and N_2 gases produced in the elemental analyzer, and a magnesium perchlorate trap was used to dry the gases. Especially by employing the reduced-volume reactors, peak heights were increased 3 to 5× and peak widths decreased 3 to 5×, thus improving signal:noise ratios in the elemental analyzer output.

Careful examination of magnified mass spectrometer traces showed that the baseline in front of nitrogen sample peaks was typically disturbed by subtle pressure waves from valve switching associated with sample introduction. Prior to sample introduction, a long sample run (675 s) was used to produce a steady baseline. Portions of this steady baseline were used in calculating peak areas, and using a steady baseline was important for obtaining consistent nitrogen-isotope results.

The sensitivity and precision of the instrumentation was tested by assaying glycine standards ranging from 0 to 12 µg. Solutions containing known amounts of glycine were prepared, pipetted into tin cups and dried at 60°C in a convection oven prior to analysis. Measured amounts and isotope values were corrected for C and N values in blanks as described previously (Fry et al. 1992).

RESULTS AND DISCUSSION

Using the small-volume reactors in the 1- or 2-tube configuration, isotopic determinations of normal precision (±0.3‰ SD or better) could be made for samples containing a minimum of 2 µg N and 4 µg C (Fig. 1). Use of a single reactor, rather than 2 reactors, approximately halved these lower-sample limits to 1 µg N and 2 µg C (Fig. 1). In addition to the isotope assays, the chemical analyses yielded C and N contents for meiofaunal taxa (Table 1). Calculations showed that when using the single-reactor system operating near its lower limits, a minimum of 1 to 12 individuals (depending upon the species) can be assayed if one is interested only in $\delta^{13}C$; for coupled determinations of $\delta^{13}C$ and $\delta^{15}N$ from the same sample, a minimum of 2 to 28 individuals is required. We used pooled samples of approximately 5 to 60 individuals to assure that handling and procedural blanks for both $\delta^{13}C$ and $\delta^{15}N$ were relatively small. Given that typical meiofaunal abundance in estuarine and coastal sediments is ~1000 ind. 10 cm^{-2}, sufficient numbers of animals can be obtained from standard meiofaunal samples. Further reductions in sample size are possible for the EA-MS system by using slower flow rates (Fry et al. 1996) and closed-atmosphere autosamplers (available from

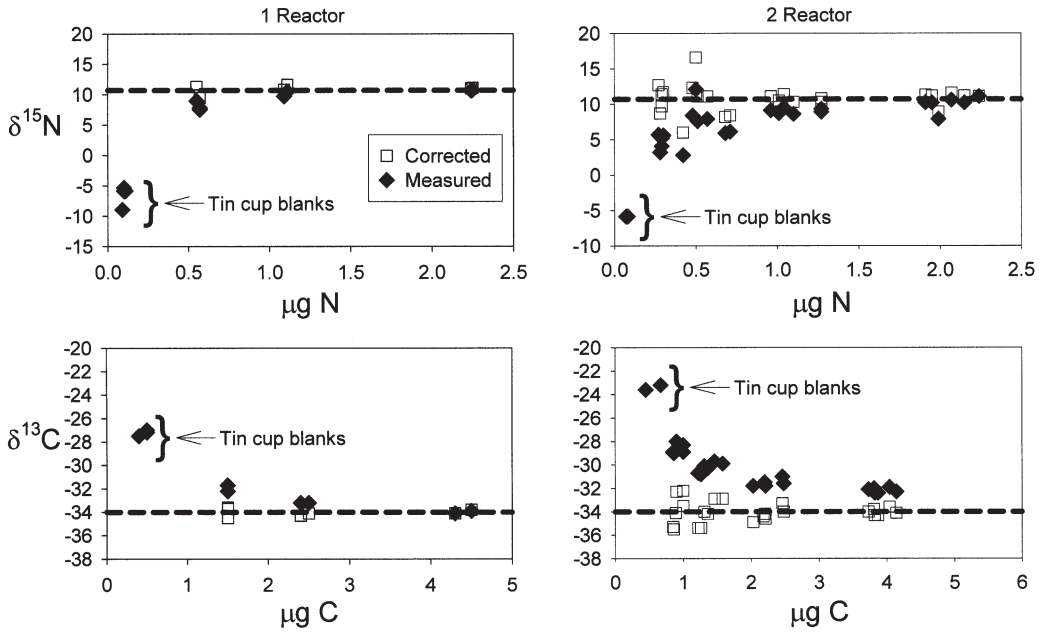

Fig. 1. $\delta^{13}C$ and $\delta^{15}N$ values for small samples of glycine measured with 1- and 2-reactor setups for the elemental analyzer, as described in the text. 'Measured' values (♦) are raw values not corrected for blank values measured from empty tin cups; blank-corrected values (□) approximate the true values of the glycine standard (10.7‰ $\delta^{15}N$ and –34.0‰ $\delta^{13}C$; shown as dashed reference lines)

Costech Analytical), and may be necessary for the analysis of smaller taxa.

The analytical system described here can be used to determine $\delta^{13}C$ and $\delta^{15}N$ on samples containing as little as 2 µg C and 1 µg N. With some loss of precision, smaller samples can also be analyzed, but attention must be paid to details of sample sorting to minimize blank values. For example, blank tin cups carried through the sorting process using only water droplets (and no meiofauna) typically increased in C content by 0.2 to 1 µg, but did not influence N content. In a practical sense, reducing the sample-size requirements of the EA-MS system must be made in conjunction with improvements in cleanliness of sample handling, because an ultra-sensitive EA-MS analysis system is of little benefit if background contamination is high. Nevertheless, the system outlined here involves relatively few and simple changes to existing EA-MS systems. Although it is adequate for the analysis of many meiofaunal species, small taxa, such as many nematode species, will still require hours of sorting to obtain an adequate number of animals (Table 1).

Table 1. Carbon and nitrogen content (mean ± SD, n = 3) of representative copepod and nematode species, and ostracods as a taxon, and the resultant number of individual animals required for $\delta^{13}C$ and $\delta^{15}N$ analysis. Minimum: the smallest number of animals that can be analyzed under optimal conditions; Normal: the sample size needed for C + N isotope measurement, with blank-corrected isotope values at a precision of ±0.3‰ or better

	µg N ind.$^{-1}$	µg C ind.$^{-1}$	C, ind. needed per sample		N, ind. needed per sample	
			Minimum (2 µg)	Normal (10 µg)	Minimum (1 µg)	Normal (2 µg)
Copepods						
Pseudostenhelia wellsi	0.78 ± 0.14	0.18 ± 0.12	3	13	6	11
Coullana sp.	2.21 ± 0.51	0.54 ± 0.13	1	5	2	4
Microarthridion littorale	1.12 ± 0.41	0.22 ± 0.06	2	9	6	10
Nematodes						
Daptonema sp.	0.80 ± 0.16	0.17 ± 0.03	3	13	6	12
Ptycholaimellus pandispiculatus	0.17 ± 0.03	0.04 ± 0.01	12	58	28	55
Ostracods	0.71 ± 0.21	0.16 ± 0.06	3	14	6	13

Kaehler & Pakhomov (2001) observed that formaldehyde preservation decreased $\delta^{13}C$ by 0.5 to 1.5‰ but did not affect $\delta^{15}N$ in fish, octopus, and kelp. While we found no evidence of artifacts in $\delta^{13}C$ or $\delta^{15}N$ associated with formaldehyde preservation and staining of meiofaunal-sized animals (data not shown), a more general examination of the effects of preservation methods as they relate to species, body size, type of preservative, and length of storage would seem prudent.

Our field data illustrate that $\delta^{13}C$ and $\delta^{15}N$ analyses can be performed on meiofaunal species collected from the field. Meiofaunal species differed substantially in their isotope signatures, and thus their sources of nutrition (Fig. 2). Using the guidelines that animals have similar carbon isotopic compositions to their diets and are typically enriched in ^{15}N by 3.4‰ versus their diets (Fry & Sherr 1984, Peterson & Fry 1987), data from July 1999 suggest that species may gain their nutrition mainly from phytoplankton (*Pseudostenhelia wellsi*), benthic microalgae (*Coullana* sp. and ostracods), or *Spartina* detritus (the nematodes *Daptonema* sp. and *Ptycholaimellus pandispiculatus*).

Pseudostenhelia wellsi is an obligate sediment dweller that resides in tubes (Chandler & Fleeger 1984). Little is known about its feeding biology, but gut-pigment analyses suggested that detrital (dead and decomposing) phytoplankton and/or benthic microalgae may contribute to its diet (Pace & Carman 1996, Buffan-Dubau & Carman 2000). The July 1999 $\delta^{13}C$ data suggest that the detrital algae that *P. wellsi* consumes comes primarily from phytoplankton (Fig. 2).

Coullana sp. lives in U-shaped burrows (Chandler & Fleeger 1987) and can feed on phytoplankton (Decho 1986, Pace & Carman 1996), but is also capable of consuming benthic microalgae (Buffan-Dubau & Carman 2000). The July 1999 stable-isotope data for *Coullana* sp. show a typical problem for natural-abundance isotope surveys. The carbon isotope values for *Coullana* sp. are consistent with a strong reliance on benthic microalgae, but values are also consistent with a mixed diet of phytoplankton and *Spartina* detritus. In such cases, which are quite common for isotope field surveys (Fry & Sherr 1984), use of multiple natural isotopes (Sullivan & Moncreiff 1990, Riera et al. 1996, Peterson 1999) or isotope-addition experiments (Middleburg et al. 2000) are often necessary to resolve diets. Adding labels to several foods in parallel or sequentially, along with estimates of animal growth rates and label turnover (Raikow & Hamilton 2001), may be necessary in pulse-addition experiments to firmly establish food sources for marsh meiofauna.

Major shifts in the $\delta^{13}C$ content of field-collected animals from July 1999 to January 2001 (Fig. 2) indicate the likelihood of seasonal shifts in the nutritional sources of meiofaunal consumers from algae (in summer) toward a greater proportion of *Spartina* detritus (in winter). Clearly this hypothesis will require more careful study, but the approaches described here make such a study feasible. Previous work at this site using ^{14}C grazing methods indicated that meiofaunal grazing on benthic microalgae was much lower in January than in the summer (Goldfinch & Carman 2000). This observation could result from lower meiofaunal feeding in winter or a shift to an alternative food source; the stable-isotope data presented here suggest that the latter explanation is plausible.

Morphologies of mouthparts are commonly used to assign nematodes to 'feeding groups' (Wieser 1953, Moens & Vincx 1997). Based on this classification, *Ptycholaimellus pandispiculatus* is an epistrate feeder and *Daptonema* sp. is a non-selective deposit feeder. Although the 2 species differed substantially in their $\delta^{15}N$ signatures, $\delta^{13}C$ values suggested that both depend heavily on *Spartina* detritus for their nutrition (Fig. 2). Prior to addition of $NaH^{13}CO_3$ and $^{15}NH_4Cl$ to sediment plots, the natural-isotope signatures of *P. pandispiculatus* and *Daptonema* sp. were comparable and similar to *Spartina* values (Fig. 2; January 2001 values shown in Fig. 3 are individual replicates of the mean values shown in Fig. 2). Three days after addition of isotopes, the isotope signatures of *P. pandispiculatus* ($\delta^{15}N = 7.5 \pm 0.5$; $\delta^{13}C = -14.6 \pm 0.3$: mean ± 1 SD)

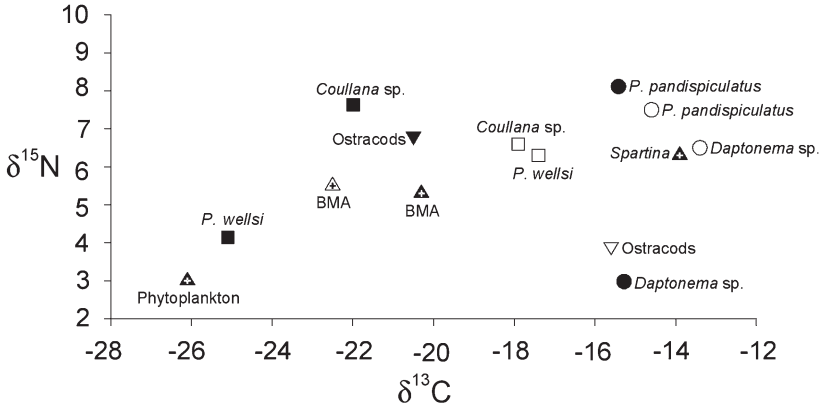

Fig. 2. Seasonal variation of $\delta^{13}C$ and $\delta^{15}N$ values in ostracods (inverted triangles), 2 harpacticoid copepod species (squares), 2 nematode species (circles), and 3 major sources of primary production (triangles with crosses; phytoplankton, benthic microalgae [BMA], and *Spartina alterniflora*). July 1999 data: filled symbols; January 2001 data: open symbols. Each point represents the mean of 3 replicates. See text for full genus names

and *Daptonema* sp. ($\delta^{15}N = 6.5 \pm 0.7$; $\delta^{13}C = -13.4 \pm 0.6$) taken from control plots located adjacent to the enrichment plots did not differ from Day 0 values (Fig. 3), demonstrating that isotopes added to experimental plots did not contaminate adjacent sediments. In experimental isotope-addition plots, however, the $\delta^{13}C$ and $\delta^{15}N$ values of *P. pandispiculatus* were elevated dramatically 3 d after addition of isotopes, while those of *Daptonema* sp. were relatively unaffected (Fig. 3). Two-way ANOVAs (factors: species and time) on $\log_{10}$-transformed $\delta^{13}C$ and $\delta^{15}N$ data confirmed these observations. The main effects of species ($F = 61.8$ and 76.5 for $\delta^{15}N$ and $\delta^{13}C$, respectively) and time ($F = 43.6$ and 47.4 for $\delta^{15}N$ and $\delta^{13}C$, respectively) were highly significant ($p < 0.001$) for both $\delta^{13}C$ and $\delta^{15}N$. The species × time interactions were also significant for both $\delta^{13}C$ ($F = 46.8$; $p = 0.003$) and $\delta^{15}N$ ($F = 42.8$; $p = 0.004$). Tukey *a posteriori* comparisons of means within factors showed that neither $\delta^{13}C$ ($p = 0.21$) nor $\delta^{15}N$ ($p = 0.38$) content of *Daptonema* sp. and *P. pandispiculatus* differed significantly prior to isotope addition. Day 3 (post-isotope addition) *P. pandispiculatus* signatures were significantly enriched relative to Day 0 ($\delta^{13}C$ and $\delta^{15}N$, $p < 0.001$), but *Daptonema* sp. were not ($\delta^{13}C$, $p = 0.96$; $\delta^{15}N$, $p = 0.97$). And on Day 3, both $\delta^{13}C$ and $\delta^{15}N$ were significantly ($p < 0.001$) elevated in *P. pandispiculatus* relative to *Daptonema* sp.

Although the relatively elevated ^{13}C in *Ptycholaimellus pandispiculatus* could be from direct ingestion of benthic microalgae that photosynthetically fixed $NaH^{13}CO_3$, the natural isotope signature does not indicate that benthic microalgae were contributing significantly to its diet. Middleburg et al. (2000) observed that $NaH^{13}CO_3$ fixed by benthic microalgae in natural sediment plots was transferred quickly (after a few hours), presumably as ^{13}C-labeled organic material, to bacteria. Thus, enhanced uptake of ^{13}C by *P. pandispiculatus* after 3 d may represent consumption of ^{13}C-labeled bacteria. While understanding the precise trophic pathways involved with ^{13}C uptake will require further study, results from the isotope-addition experiment suggest that the diets of *P. pandispiculatus* and *Daptonema* sp. differ qualitatively, even though their natural isotope signatures are similar.

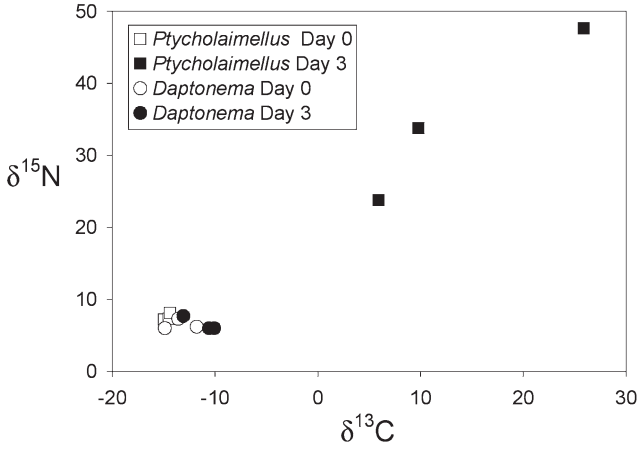

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ values of 2 nematode species, *Ptycholaimellus pandispiculatus* and *Daptonema* sp., before (Day 0) and after (Day 3) the addition of a $NaH^{13}CO_3/^{15}NH_4Cl$ solution to 3 replicate 1 m^2 plots of mudflat sediment in January 2001

We also note that, while *Ptycholaimellus pandispiculatus* was strongly labeled with both $\delta^{13}C$ and $\delta^{15}N$ in experimental plots, the degree of labeling was highly variable (e.g. $\delta^{13}C$ values in the 3 replicate plots were +5.9, +9.8, and +25.9‰; see Fig. 3), and much more variable than in untreated plots (e.g. $\delta^{13}C$ values in control plots were −14.4, −14.5, and −15.0‰). Factors that contributed to high variability in our pulsed-addition results may have included non-uniform application of label, variation in uptake of label by benthic microalgae, differential transfer of label to bacteria (Middleburg et al. 2000), and the known patchy distribution of meiofauna and their food resources (Lee et al. 1977, Findlay 1981). Future, more detailed pulse-addition experiments will be needed to separate these effects, and to develop a sampling strategy that minimizes these sources of variability relative to the intended treatment effect. One such strategy may be the use of much larger sediment samples from plots than the relatively small 10 cm^2 cores used in this study. Investigations of this nature may also lead to an improved understanding of patch dynamics in meiofaunal communities.

Very few field studies have examined dietary differences among meiofaunal species. These studies have focused on bacteria (Epstein & Shiaris 1992), benthic microalgae (Carman & Thistle 1985), and to a lesser extent on phytoplankton or phytodetritus (Pace & Carman 1996, Buffan-Dubau & Carman 2000) as potential sources of nutrition; to our knowledge, no previous field studies have examined species-specific variation in consumption of vascular plant detritus by meiofaunal species. Seasonal variation in meiofaunal feeding has also received relatively little attention (Montagna et al. 1983, Alongi 1988, Couch 1989, Goldfinch & Carman 2000). Our results suggest that detailed investigations of species-specific, seasonal, and geographic variation in meiofaunal diets will provide important insight into the structure of benthic food webs.

The label-addition experiments can be greatly expanded with the meiofaunal community, as 1 m^2 field plots contain abundant (10^6 ind. m^{-2}) and diverse communities, and the 1 m^2 scale allows relatively easy replication. Smaller-scale manipulations are also possible, and we have performed label-addition experi-

ments on intact sediment microcosms (8 cm diameter) in the lab over several weeks, and shorter-term (5 h) experiments on 15 cm diameter microcosms in the field (Carman & Fry unpubl.). The method described here makes feasible the species-level analysis of meiofaunal-sized animals. Our 2 field examples show (1) the utility of standard isotope field surveys to outline overall patterns of meiofaunal food-web dynamics in marine systems (Fig. 2), and (2) multiple-isotope addition experiments to test hypotheses arising from natural isotopic values (Fig. 3). The combination of small size, high abundances, and easy replication make meiofaunal communities suitable for studying and manipulating food-web patterns to assess such long-standing questions regarding the importance of detritus in coastal systems (Peterson & Jensen 1911, Coull 1999) or aspects of resource partitioning among co-occurring species (Buffan-Dubau & Carman 2000). The use of isotope studies adds the dimension of functional (food web) response to the structural (numbers and species) response commonly assayed in ecosystem experiments.

Acknowledgements. We thank R. Millward for nematode identification, and S. Hymel and T. Marshall for technical assistance. Comments from 3 anonymous reviewers significantly improved the manuscript. P. Maddi, A. Duplantis, T. Kang, and J. Zukowski assisted with sorting meiofauna. We thank the Institute of Pacific Islands Forestry, USDA Forest Service, Honolulu, for supporting B.F. during the writing of this paper. This work was supported by grants NOAA-870R0512 (CICEET 99-296) and NSF DEB-9815598 to B.F., and NSF OCE-9818453 to K.R.C.

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Editorial responsibility: William Stickle (Contributing Editor), Baton Rouge, Louisiana, USA

Submitted: December 10, 2001; Accepted: May 15, 2002
Proofs received from author(s): August 2, 2002