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Use of primary production by harpacticoid copepods in a Louisiana salt-marsh food web

Abstract

We used stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) to examine temporal (quarterly sampling throughout a year) and interspecific variation in the use of primary producers by three harpacticoid copepod taxa (*Coullana* sp, *Pseudostenhelia wellsi*, and laophontids) in a low-salinity Louisiana salt marsh. Microphytobenthos (MPB), phytoplankton, and *Spartina alterniflora* are the major primary producers in this system. The natural $\delta^{13}C$ values of harpacticoids in summer suggested strong dependence on phytoplankton (*Coullana* sp.) or a mixture of phytoplankton and MPB (*P. wellsi* and laophontids). During fall, winter, and spring, however, $\delta^{13}C$ values of harpacticoids were significantly enriched relative to summer; the copepod isotope values were generally slightly more enriched than MPB, but intermediate between $\delta^{13}C$ values of *Spartina* and phytoplankton. Such intermediate values could be an indication of a mixed diet of *Spartina* and phytoplankton, a diet comprised primarily of MPB, or some combination of all three food sources. The dual-isotope approach ($\delta^{13}C$ and $\delta^{15}N$) did little to resolve this uncertainty because $\delta^{15}N$ values of primary producers were similar. Isotope-addition experiments were conducted at each sampling period in which MPB were labeled with additions of $NaH^{13}CO_3$. Uptake of added ^{13}C by harpacticoids was analyzed using a 3-source mixing model, which was based on ^{13}C uptake by MPB in combination with natural $\delta^{13}C$ values of *Spartina* and phytoplankton. Mixing-model results verified the importance of phytoplankton (61-71%) and MPB (15-37%) in summer; *Spartina* contributed only 1-14% to copepod diets. In winter and summer, phytoplankton contributed substantially, but to a lesser extent to copepod diets (36-59%). However, *Spartina* constituted > 50% of the diet of *P. wellsi*, and 22-48% of the diets of other copepods. During winter and summer, MPB contributed minimally (6-13%) to the diets of *P. wellsi* and *Coullana* sp. Collectively our data indicate strong temporal and interspecific variation in copepod diets; diets are dominated by phytoplankton in summer, but *Spartina* (presumably in the form of detritus) is an important component of the diet in other seasons. Our observations are in contrast with the prevailing dogma that MPB is the primary source of nutrition for salt-marsh invertebrates, and that *Spartina* contributes minimally to salt-marsh food webs.

Functioning of microphytobenthos in estuaries.
Royal Netherlands Academy of Arts and Sciences, 2006

Introduction

Relatively little is known about the food resources exploited by estuarine meiofaunal invertebrates (< 1 mm in size). ^{14}C-grazing studies suggest that meiofaunal grazing can have a significant impact on microphytobenthos (MPB) biomass and production in estuarine environments (Blanchard 1991; Montagna 1995; Carman *et al.* 1997; Pinckney *et al.* 2003), implying that MPB contribute significantly to meiofaunal diets. However, in addition to MPB, various studies indicate that some meiofauna may also consume phytoplankton (Decho 1986; Pace and Carman 1996; Buffan-Dubau and Carman 2000a) or vascular-plant (e.g., *Spartina alterniflora*) detritus (Couch 1989; Carman and Fry 2002). As currently employed, ^{14}C-grazing studies can be used to asses the consumption rate of a particular food source, such as MPB, but provide no information on the relative contributions of other potential food sources. Although poorly understood, it is clear that feeding strategies and dietary preferences vary substantially among meiofaunal taxa and individual species (*e.g.*, Carman and Thistle 1985; Pace and Carman 1996; Buffan-Dubau and Carman 2000a; Moens *et al.* 2002). Almost nothing is known about seasonal variation in the diets of meiofauna.

Stable isotopes (*e.g.*, ^{13}C and ^{15}N) can, in theory, be used to simultaneously determine the relative contributions of multiple food sources to consumer diets (Peterson and Fry 1987). Stable-isotope studies of estuarine salt-marsh food webs have led to the general conclusion that MPB primary production supports much of the secondary production by fish and macrofaunal invertebrates, and that production by vascular plants (*e.g.*, *Spartina alterniflora*) is a relatively minor source of food (*e.g.*, Sullivan and Moncreiff 1990; Currin *et al.* 1995; Currin *et al.* 2003). Such conclusions are consistent with observations that MPB are more labile and nutritious than detrital material derived from vascular plants (Miller *et al.* 1999). Similarly detailed information is lacking for meiofaunal-sized animals. Because of their small size, large numbers (100's to 1000's) of meiofaunal individuals were required to provide the biomass needed for a single determination of stable-isotope content (^{13}C and/or ^{15}N; Couch 1989; Riera *et al.* 1996; Middelburg *et al.* 2000), and thus only a few studies have attempted to use stable isotopes in the study of meiofaunal food webs. Couch (1989) concluded that *S. alterniflora* detritus was the primary source of nutrition for meiofaunal harpacticoid copepods and nematodes, but the few other stable-isotope studies of meiofauna have concluded that they rely heavily on MPB (Riera *et al.* 1996; Middelburg *et al.* 2002; Moens *et al.* 2002). Recent methodological developments allow for the accurate measurement of ^{13}C and ^{15}N on relatively small numbers (5-60) of meiofaunal animals (Carman and Fry 2002), which presents the opportunity to use stable isotopes for species-level analyses of meiofaunal diets.

However, in estuarine salt marshes, ^{13}C values of both MPB and many consumers (meiofaunal and macrofaunal) are typically intermediate between those of *Spartina* (which is more enriched with ^{13}C) and phytoplankton (which is more depleted). When three or more food sources are available, the diets of consumers with intermediate ^{13}C values cannot be unambiguously determined because of uncertainty as to whether consumers are using primarily MPB, a combination of *Spartina* and phytoplankton, or possibly all three food sources (Figure 1).

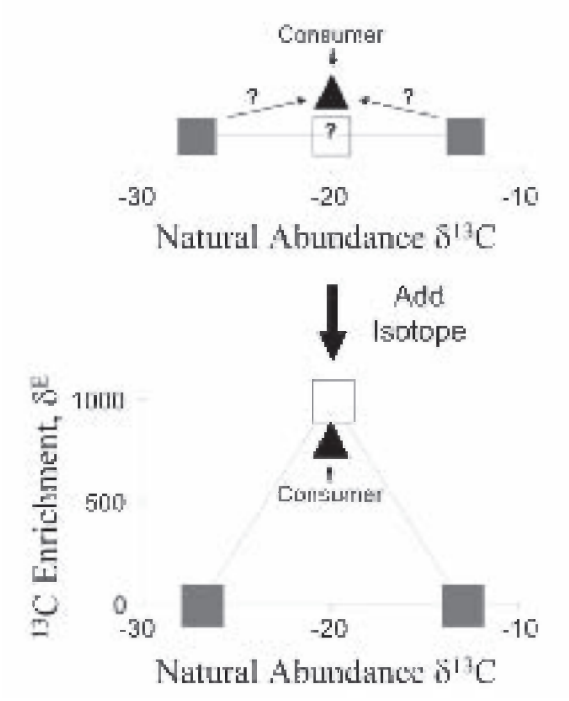

Figure 1. Stable-isotope mixing model. Determination of consumer food source(s) is uncertain when three or more food sources are available and consumer isotope values are intermediate between the most ^{13}C-enriched (*e.g.*, *Spartina*) and most ^{13}C-depleted (*e.g.*, phytoplankton) food sources. This uncertainty can be partially resolved by labeling the food source with the intermediate isotope value (*e.g.*, MPB). Uptake of label by consumers is proportional to the degree to which they feed on the intermediate food source.

In situ labeling of MPB with added ^{13}C has been used to study the dynamics of MPB consumption by meiofauna (Herman *et al.* 2000; Middelburg *et al.* 2000; Carman and Fry 2002; Moens *et al.* 2002). *In situ* labeling of MPB can, in principle, also be used to resolve uncertainty in food sources described above because it can strongly differentiate animals that consume MPB as food from those that do not (Figure 1).

In the present study, we measured natural stable-isotope (^{13}C and ^{15}N) values of three harpacticoid copepods and conducted simultaneous isotope-addition experiments ($NaH^{13}CO_3$) throughout the course of a year. These observations were used to address three questions: (1) Does meiofaunal consumption of MPB (and other sources of primary production) vary temporally and (2) among species, and (3) can ^{13}C labeling of the natural MPB assemblage be used to resolve uncertainty regarding meiofaunal consumption of MPB?

Methods and materials

The study site was an intertidal mud-flat surrounded by *Spartina alterniflora* cord grass located in Terrebonne Bay estuary (29° 15' N, 91° 21' W) near Cocodrie, Louisiana, USA. Tidal amplitudes are low (approximately 0.3 m), salinity ranges from 3-15 psu, and dissolved oxygen ranges from 3-8 mg L^{-1}. Samples to determine abundance and isotopic composition of phytoplankton, MPB, and meiofauna were

collected at low tide during summer (07.02.01), fall (10.19.01), winter (02.11.02) and spring (04.24.02). Two poles were placed on either end of a 10-m transect, approximately 2 m away from and parallel to the marsh edge. Four replicate cores were collected from the mudflat at randomly determined intervals along the transect. On each sampling date, a parallel $NaH^{13}CO_3$ tracer-addition experiment was performed to evaluate the role of MPB as food for meiofauna (described below).

Major food sources

Phytoplankton: Suspended particulate material (SPM) (consisting of a mixture of microalgae, zooplankton, microorganisms, and nonliving organic material) was used as a proxy for phytoplankton. Four replicate samples of surface water were collected using 1-L plastic bottles. Water (50-100 mL) was filtered using GF/F Whatman filters for pigment analysis. Phytoplankton abundance was determined from HPLC (High Performance Liquid Chromatography) analysis of Chl *a* (described below).

For pigment analysis, filters were extracted in 5 mL 100 % HPLC-grade acetone (Fisher Scientific). Acetone extracts were filtered using syringe filters (Sun International; diameter: 13 mm; pore size: 0.2 µm) twice to remove particulates. Extracts were diluted (66 µL sample + 44 µL water) with HPLC quality water (Fisher Scientific) to improve the sharpness of peaks, and photopigments were analyzed using HPLC (Wright *et al.* 1991).

For stable-isotope analysis, GF/F filters were pre-combusted (450 °C, 2 h) to remove excess carbon and SPM was concentrated until the filters clogged. Filters were dried and 4.5-mm circles were removed from each filter using a handheld punch. Six circles from each replicate were used for analysis of stable isotopes.

MPB: The top 1 cm of sediment from 3.2 cm i.d. butyrate cores was collected for analysis of meiofauna and MPB. Sediment was homogenized with a spatula, and a sub-sample (approximately 300 mg wet wt.) was collected for HPLC analysis of Chl *a* (Buffan-Dubau and Carman, 2000b). The remaining sample was fixed in 4 % formaldehyde for analysis of meiofauna.

$\delta^{13}C$ values for MPB were determined from acetone extracts of sediment that were dried on precombusted 4.5 mm GF/F filters. Acetone extracts were used because they include an enrichment of photosynthetic pigments; however, they also include photosynthetic pigments from both living and dead (detrital) algal material, as well as other moderately polar organic material. Therefore, while the $\delta^{13}C$ values of acetone extracts are considered as a proxy for MPB values, they do not represent a pure MPB sample. To determine bias associated with analysis of $\delta^{13}C$ in acetone extracts, a separate experiment was carried out for two seasons (winter and spring) using water collected for phytoplankton. Surface water was filtered on pre-combusted GF/F filters, which were dried and cut in half. One half of the filter was used to measure $\delta^{13}C$ in non-acetone-extracted material, and the second half was used to measure $\delta^{13}C$ in acetone extracts. The average difference between the extracted and un-extracted $\delta^{13}C$ values (4 ‰) was added to $\delta^{13}C$ values of the acetone extract to obtain an estimated $\delta^{13}C$ value of MPB.

Spartina alterniflora: Fresh leaf blades of *S. alterniflora* were collected along the marsh edge. The leaf blades were washed and oven dried and finely powdered using a Wig-L-Bug grinder before analyzing for $\delta^{13}C$ and $\delta^{15}N$.

Meiofauna

Sediment fixed in 4% formaldehyde was used for extraction of meiofauna used in the isotope analyses. In the laboratory sediment samples were washed through a 63-μm sieve and stained with Rose Bengal. Nematodes, ostracods, *Streblospio benedicti*, *Tanypus clavatus* (a chironomid larva), and three harpacticoid taxa (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontid copepods) were separated for stable-isotope analysis. Laophontids at this site consist of two species, *Onychocamptus mohammed*, (approximately 80%) and *Paronchocamptus huntsmani* (approximately 20%) (J. Fleeger, personal communication), the identities of which can be determine only with detailed microscopic analysis. Here, we focus on stable-isotope analyses of the three harpacticoid taxa. Based on the observations of Carman and Fry (2002), the necessary number of animals (10-20 individuals) were handpicked picked using a tungsten-wire probe and cleared of attached debris. Copepods were transferred to small tin cups (3 x 5 mm), dried, and analyzed as described below.

Tracer-addition experiment

A parallel tracer-addition experiment was performed in summer and winter to determine the short-term uptake of added $NaH^{13}CO_3$. Eight cores (7.5 cm i.d.) were collected at random locations on the 10-m transect. Overlying water was removed without disturbing the surface sediment. Twenty milliliters of GF/F-filtered marsh water containing 72 mg of $NaH^{13}CO_3$ (Middelburg *et al.* 2000) was added to each core. Four of the eight cores were covered with aluminum foil and used as dark controls to measure uptake of ^{13}C through non-photosynthetic processes. All the cores were incubated for 4 h in ambient sunlight. After 4 h, the top 1 cm of sediment was harvested and homogenized with a spatula; a sub-sample (approximately 5 mg) was collected to measure MPB ^{13}C incorporation and pigment analysis (HPLC). The remaining sediment was fixed with 4% formaldehyde and processed as described to determine ^{13}C incorporation by meiofauna.

Stable-isotopic analysis

Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ using a Carlo Erba NA 1500 elemental analyzer linked to a Finnigan Delta Plus ratio mass spectrometer. The elemental analyzer was modified for small-biomass samples as described by Carman and Fry (2002). Glycine and bovine liver were used as reference standards in combustion analysis. These standards as well as procedural blanks were used to correct for background values of C and N in the samples (Fry *et al.* 1992).

Isotope ratios were expressed as δ values (‰):

$$\delta^{13}C,\ \delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$$

where R = $^{13}C/^{12}C$ or $^{15}N/^{14}N$. Peedee Belemnite and atmospheric nitrogen were used as carbon and nitrogen isotope standards, respectively. For mixing-model calculations, consumer isotopic values were adjusted for fractionation by subtracting 0.5‰ for carbon, and 2.2‰ for nitrogen (McCutchan *et al.* 2003).

Values from tracer-addition experiments were expressed as ^{13}C enrichment (δ^E) by correcting $\delta^{13}C$ values from samples incubated in the light ($\delta^{13}C_{light}$) with the average values of samples incubated in the dark ($\delta^{13}C_{dark}$) to account for non-photosynthetic uptake of ^{13}C by copepods:

$$\delta^E = (((\delta^{13}C_{light} + 1000)/ (\delta^{13}C_{dark} + 1000))-1) * 1000$$

Mixing-model

The percent contributions of *Spartina*, MPB, and phytoplankton were calculated by using a 3-source mixing model that included natural $\delta^{13}C$ and $\delta^E{}^{13}C$ from tracer-addition experiments. Interpretations of the 3-source mixing model were based on the following assumptions:

1. In tracer-addition experiments, uptake of ^{13}C by meiofauna was only through consumption of MPB and ^{13}C-uptake by MPB was $\geq$ uptake by consumers.
2. Uptake of ^{13}C by *Spartina* and phytoplankton was zero in tracer-addition experiments.
3. *Spartina*, MPB, and phytoplankton were the major food sources available to meiofauna. Thus, the $\delta^{13}C$ and δ^E values of copepods were within the range of these three food sources.

Based on assumption 1, the δ^E values of MPB were adjusted according to the isotopically most-enriched consumer in each season. Source contributions were calculated from simultaneous solution of three mass-balance equations:

(1) $f_1 + f_2 + f_3 = 1$
(2) $f_1N_1 + f_2N_2 + f_3N_3 =$ N of consumer
(3) $f_1E_1 + f_2E_2 + f_3E_3 =$ E of consumer

Where,

f = Fractional contribution of a food source
N = Natural carbon isotope value ($\delta^{13}C$)
E = Enriched carbon isotope value (δ^E)
Subscripts 1-3 refer to food sources 1-3

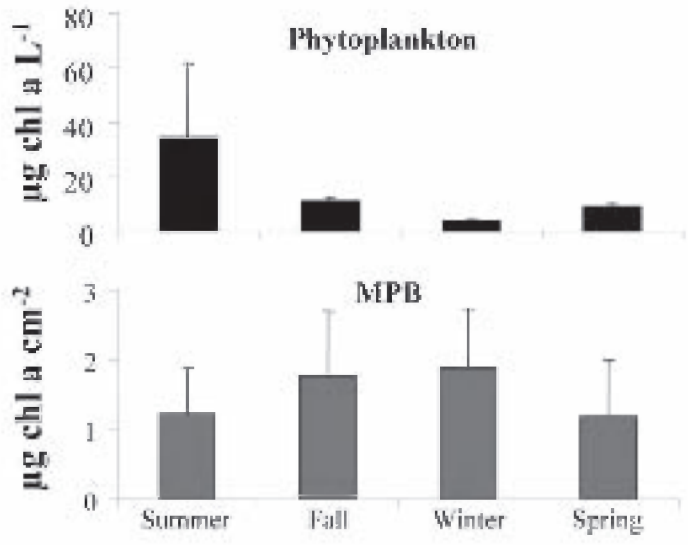

Figure 2. Temporal variation in the biomass of phytoplankton and MPB. Bars are means + 1 SD (N=4).

Results

Microalgal abundances

Although temporal variation was not significant (ANOVA, $p = 0.49$), MPB biomass as estimated from Chl *a* was highest in fall and winter when mud-flats are exposed during the day (Figure 2). MPB Chl *a* concentrations ranged from a minimum of 1.2 ± 0.8 (spring) to a maximum of 1.9 ± 0.8 µg cm^{-2} (winter). Phytoplankton abundance varied significantly among seasons (ANOVA, $p = 0.03$) and was approximately 3x higher in summer (34.6 ± 27.1 µg Chl *a* L^{-1}) than in other seasons (Figure 2).

Isotopic compositions of potential food sources

Natural $\delta^{13}C$ values varied among food sources and seasons (Figure 3, Table 1). Throughout the year, *Spartina* was consistently most enriched in ^{13}C, and phytoplankton was most depleted in ^{13}C. Isotopic values of MPB were more ^{13}C-enriched than phytoplankton in all seasons except fall, and the $\delta^{13}C$ of *Spartina* values were relatively constant throughout the year (~-13‰). Both phytoplankton and MPB showed similar temporal variation in $\delta^{13}C$, with most enriched values in winter and most depleted values in spring.

$\delta^{15}N$ values were available only for phytoplankton and *Spartina*. No significant differences were found between $\delta^{15}N$ values of *Spartina* and phytoplankton at any time of the year, and seasonal variation was small (Table 1).

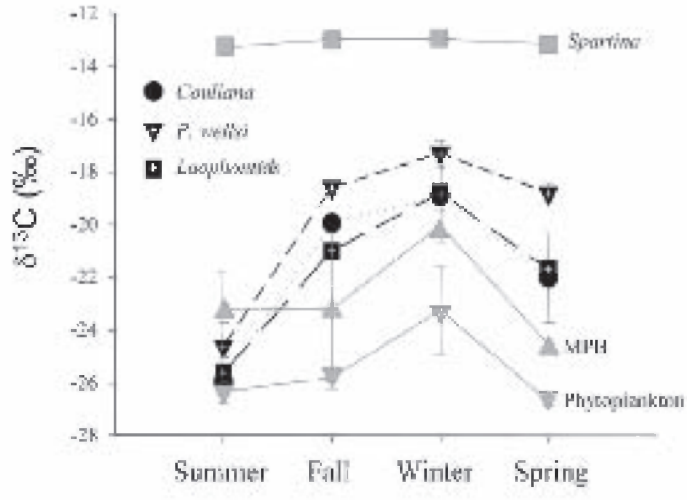

Figure 3. Temporal variation in the $\delta^{13}C$ of primary producers (*Spartina*, MPB, and phytoplankton) and three harpacticoid copepod taxa (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontids). Values are means ± 1 SD (N=4).

Table 1. Natural $\delta^{13}C$ and $\delta^{15}N$ values of harpacticoid copepods (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontids) and primary producers (MPB, phytoplankton, and *Spartina*) in four seasons. Values are means (SD), N=4.

	Summer 2001		Fall 2001		Winter 2002		Spring 2002	
Consumers	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$
Coullana sp.	-25.4 (0.5)	8.8 (1.1)	-19.4 (0.3)	7.4 (0.2)	-18.4 (1.2)	8.3 (0.8)	-21.5 (1.7)	7.6 (0.5)
P. wellsi	-24.1 (0.9)	9.1 (1.2)	-18.1 (0.2)	7.6 (0.6)	-16.8 (0.5)	7.6 (0.3)	-18.3 (0.4)	7.5 (0.5)
Laophontids	-25.1 (0.6)	11.4 (0.8)	-20.5 (0.2)	ND	-18.3 (0.3)	8.9 (0.8)	-21.2 (0.1)	ND
Food Sources								
MPB	-27.2 (1.4)	ND	-27.2 (3.0)	ND	-25.3 (0.6)	ND	-27.2 (0.2)	ND
Phytoplankton	-26.3(0.4)	6.7 (0.4)	-25.8 (0.2)	6.7 (0.8)	-23.3 (1.7)	5.2 (0.3)	-26.7 (0.2)	5.5 (0.9)
Spartina	-13.3 (0.0)	6.7 (0.2)	-13.0 (0.1)	5.8 (0.1)	-13.0 (0.1)	6.3 (0.3)	-13.2 (0.1)	6.0 (0.1)

Phytoplankton $\delta^{13}C$ values (-26.7 to -23.3‰) were consistent with values from a previous study at this site (Carman and Fry 2002). Our phytoplankton $\delta^{13}C$ values were lower than many estuarine literature values (*e.g.*, Currin *et al.* 1995; Riera *et al.* 1996), but similar to those reported in low-salinity systems (Deegan and Garritt 1997; Wainright *et al.* 2000). Our $\delta^{15}N$ phytoplankton values were consistent with published values (*e.g.*, Currin *et al.* 1995; Fogel *et al.* 1989). Our *Spartina* values $\delta^{13}C$ (-13.3 to -13.0‰) and $\delta^{15}N$ (5.8 - 6.7‰) values were consistent with literature values (*e.g.*, Couch 1989; Peterson and Howarth 1987; Sullivan and Moncreiff 1990; Currin *et al.* 1995). The proxy $\delta^{13}C$ values of MPB measured in this study were

lighter (-24.6 to -20.0‰) than most existing literature values for salt marshes (e.g., -16 to -18‰: Haines 1976; Currin *et al.* 1995), but similar to those reported by Sullivan and Moncreiff (1990) in a Mississippi salt marsh (-20.6‰). $\delta^{13}C$ values of MPB in winter were enriched by ~4‰ relative to other seasons, and temporal variation of MPB $\delta^{13}C$ values resembled that observed for phytoplankton.

Isotopic compositions of consumers

The $\delta^{13}C$ values of copepods varied temporally, and all three copepod taxa showed similar temporal variation (Figure 3, Table 1). Copepod $\delta^{13}C$ values were most depleted in summer and most enriched in winter. Although *Coullana* sp., *P. wellsi*, and laophontids showed similar seasonal trends, *P. wellsi* values were slightly heavier than those of the other copepods. In summer, the $\delta^{13}C$ values of copepods were most similar to those of phytoplankton, while in other seasons they were intermediate among food sources.

$\delta^{15}N$ values are not discussed separately, but are presented in dual-isotope plots with $\delta^{13}C$ (Figure 4). $\delta^{15}N$ were available in all seasons only for *Coullana* sp. and *P. wellsi*. Because $\delta^{15}N$ values were not measured for MPB in this study, we used published values for this study site as an estimate (Carman and Fry 2002).

In summer, the dual-isotope composition of *Coullana* sp. was closely aligned with phytoplankton (Figure 4). Laophontid copepods and *P. wellsi* isotope compositions were distinctly different from each other, but generally intermediate between MPB and phytoplankton isotope compositions. Summer $\delta^{13}C$ values of copepods contrasted markedly with those from other seasons; in all other seasons, copepod $\delta^{13}C$ values were intermediate between the $\delta^{13}C$ values of *Spartina* and phytoplankton.

Tracer-addition experiments

Uptake of ^{13}C by copepods in tracer-addition experiments varied among seasons and among taxa (Table 2; data were unavailable for fall). Uptake of ^{13}C by *Coullana* sp. was highest in summer and lowest in winter; uptake by *P. wellsi* was highest in summer and spring, and lowest in winter; uptake by laophontids was highest in winter and spring and lowest in summer.

As described in Methods and Materials, data from uptake of ^{13}C by copepods in tracer-addition experiments were used in a 3-source mixing model to determine the relative contributions of phytoplankton, MPB, and *Spartina*. Results in Table 2 are shown incorporated in the 3-source mixing models of Figure 5. Results of the model calculations (Figure 6) support the inference from natural $\delta^{13}C$ (Figure 3) that copepods depended heavily on phytoplankton (61-71%) and secondarily on MPB (15-37%) in summer; *Spartina* contributed only1-14% to copepod diets in summer. In winter, phytoplankton contributed substantially, but to a lesser extent to copepod diets (36-54%). However, *Spartina* constituted > 50% of the diet of *P. wellsi* in winter, and comprised 26-40% of the diets of *Coullana* and laophontids. During winter, MPB contributed minimally (6%) to the diets of *P. wellsi* and *Coullana* sp., but comprised a greater fraction of laophontid diets (38%).

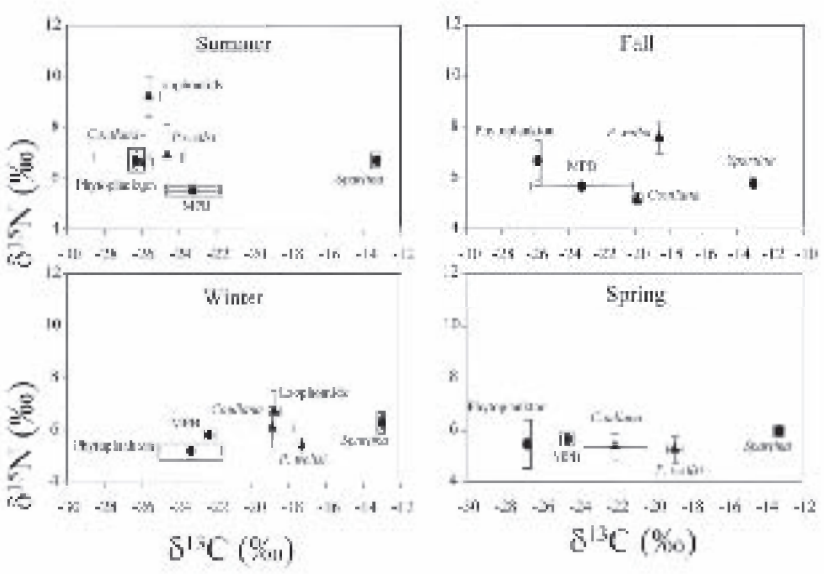

Figure 4. Dual-isotope plots of $\delta^{13}C$ v. $\delta^{15}N$ for primary producers (*Spartina*, MPB, and phytoplankton) and three copepod taxa (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontids). Separate plots are shown for each season. $\delta^{15}N$ values of MPB were not measured in this study, but were estimated from a previous study (Carman and Fry 2002). Values are means ± 1 SD (N=4).

Table 2. Uptake of ^{13}C by consumers and MPB in isotope-addition experiments conducted in summer and winter. Values are mean (SD). Although only uptake by copepods is discussed in this paper, several other taxa were examined in the broader experiment. The taxon with the maximum uptake (highest δ^E values; shown in bold) in a season was used as the MPB value in the 3-source mixing model (see text). Maximum uptake in summer was observed in *Tanypus clavatus* (a chironomid larva), and maximum uptake in winter was observed in ostracods (mixed assemblage of species).

Consumers	**Summer 2001** δ^E	**Winter 2002** δ^E
Coullana sp.	247 (133)	65 (21)
P. wellsi	137 (21)	51 (10)
Laophontid	177 (37)	403 (132)
Ostracod	133 (175)	**897 (190)**
T. clavatus	**531 (19)**	336 (135)
MPB	184 (137)	414 (308)

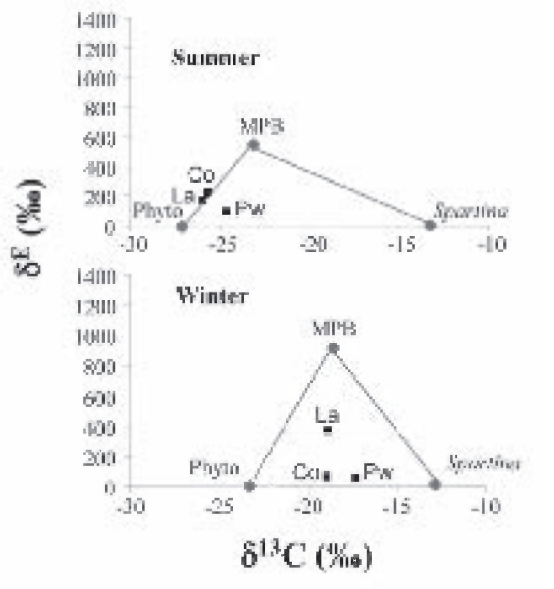

Figure 5. Three-source mixing models used to estimate contributions of MPB, phytoplankton (Phyto), and *Spartina* to the diets of three copepod taxa (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontids). The Y-axis depicts ^{13}C values in copepods and food sources in isotope-enrichment experiments. 'MPB' enrichment values were taken as the maximum value observed in all grazers examined (see text for explanation; values shown in bold in Table 2). The X-axis shows the natural $\delta^{13}C$ values for phytoplankton (Phyto) and *Spartina*. 'Co' = *Coullana* sp., 'Pw' = *Pseudostenhelia wellsi*, and 'La' = laophontids.

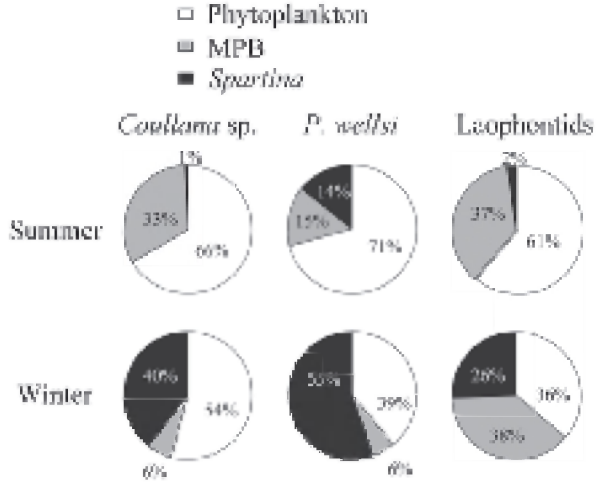

Figure 6. Results of 3-source mixing model showing calculated relative contributions of phytoplankton, MPB, and *Spartina* to the diets of three copepod taxa (*Coullana* sp., *Pseudostenhelia wellsi*, and laophontids) in three seasons. The mixing model is graphically illustrated in Figure 5, and calculations are described in the text.

Discussion

A major limitation in the use of stable isotopes in food-web studies lies in the interpretation of consumer-isotope values that are intermediate between three or more potential food sources. When this situation occurs, it is difficult to determine the contribution of each food source because the isotopic values of consumers may reflect consumption of a single food source or a mixture of two or more food sources. In the current study, the $\delta^{13}C$ isotopic values of potential food sources were generally well separated, with the exception of MPB and phytoplankton in fall. In summer, both single-isotope ($\delta^{13}C$) and dual-isotope ($\delta^{13}C$ v $\delta^{15}N$) plots strongly indicated that phytoplankton was the primary source of nutrition for *Coullana* sp. Natural-isotope values for *P. wellsi* and laophontids also indicated a significant dietary contribution from phytoplankton; however, natural-isotope compositions indicated that the diets of *P. wellsi* and laophontids also consisted of either a substantial amount of MPB or a relatively small amount of *Spartina*. During other seasons, natural $\delta^{13}C$ and $\delta^{15}N$ compositions indicated that the diets of all copepods differed substantially from summer; however, neither single- nor dual-isotope values provided conclusive evidence of copepod diets. Natural-isotope values did, however, indicate that *Spartina* biomass contributed significantly to the diets of all copepods during fall, winter, and spring.

Tracer-addition experiments were designed to assess meiofaunal consumption of MPB, and to help resolve ambiguities in the interpretations of natural-isotope compositions. ^{13}C uptake by copepods in tracer-addition experiments reflect feeding activity over a relatively short (4-h) time, whereas natural $\delta^{13}C$ (and $\delta^{15}N$) values reflect integrated dietary preferences over a longer period (days to weeks). Thus, it is possible that the use of data from tracer-addition experiments in the 3-source mixing model results in a bias toward short-term feeding activity that may not be representative of longer-term feeding habits. Although it is known that meiofaunal feeding activity may vary throughout a diel period (Buffan-Dubau and Carman 2000a), it is not known if meiofauna qualitatively change the type of food that they are consuming over short time scales. Adult *Coullana* females feed most actively at noon (Buffan-Dubau and Carman 2000a), but *Coullana* is negatively phototactic (Harris 1977). It is therefore possible that *Coullana* were not feeding at maximum rates at the time of tracer-addition experiments (midday) due to high light exposure to the cores. However, we note that low ^{13}C uptake in summer relative to winter correlated well with that of natural-isotope values, which indicated a strong dependence on phytoplankton in summer. Uptake of ^{13}C in tracer-addition experiments was assumed to be related to feeding on live, photosynthetically active MPB. The generally low uptake of ^{13}C observed for *P. wellsi* is consistent with the conclusion of Pace and Carman (1996) that *P. wellsi* feeds primarily on detrital algae (based on gut-pigment analysis).

Future studies would benefit from better characterization of $\delta^{13}C$ and δ^{E} values for MPB. As noted in Methods, acetone extracts of sediment include a variety of organic materials that are not from MPB, and thus $\delta^{13}C$ values obtained by this method are almost certainly not an accurate representation of MPB ^{13}C content. Better physical separation of MPB is needed.

Acetone extracts of sediment yielded δ^E values that were lower than the δ^E values of several grazers (Table 2). This observation is contrary to food-web tracer theory, which predicts that isotope concentration in algae that incorporate label must be greater than isotope concentration in consumers that ingest the labeled algae (Daro 1978). We compensated for this problem by using the maximum δ^E values observed in grazers as an estimate of δ^E values in MPB. However, determination of δ^E values for MPB, and thus the accuracy of the 3-source mixing model, would be improved by better separation of MPB.

Also, we generally observed high variability among replicates in the enrichment experiments. To gain statistical confidence in final estimates of food source contributions (e.g., Figure 6), the use of 8-10 replicates would seem advisable in future studies.

Nevertheless, our observations indicate that copepod diets do change substantially over the course of a year. In winter, laophontids showed greater ^{13}C uptake than did *Coullana* sp. and *P. wellsi*,, and the mixing model indicated that MPB contributed more to laophontid diets (38 % of total diet) than to the diets of *P. wellsi* or *Coullana* sp. (6 % of total diet). For all three copepods, our observations indicated a significant shift from predominant use of microalgal (phytoplankton and possibly MPB) food in summer, to diets that had significant contributions from *Spartina* in other seasons. Thus, we conclude that the diets of copepods do vary during the course of the year. This observation indicates that food-web studies conducted at a single time (typically summer) should be interpreted with caution.

Based on habitat preference and feeding mechanisms, four feeding types of harpacticoid copepods have been identified (Marcotte 1983). *Coullana* sp. belongs to a group that feeds by sorting 3-dimensional food particles (*e.g.*, clusters of diatoms) from the sediment; laophontids belong to a feeding type that feeds on the edges of food particles such as grass blades or sediment particles; and *P. wellsi* belongs to a group that gleans food particles from the surface of detritus and clay floccules. Although, the three harpacticoid copepods technically belong to different feeding groups, our observations do not indicate major differences in their feeding strategies.

However, natural stable-isotope analyses and results of the 3-source mixing model indicate subtle, but potentially important dietary differences among copepods. For example, natural $\delta^{13}C$ values of *P. wellsi* were consistently more enriched than those of other species, and mixing-model results indicated that *P. wellsi* relied more on *Spartina* detritus than did other species.

Conclusions

Copepods exhibited strong temporal variability in their utilization of food sources as well as inter-specific differences in diet. While our observations suggest that algae contribute significantly to the diets of all three copepod taxa, MPB is not necessarily the principal source of algal food. In summer in particular, phytoplankton appears to be a principal source of food. Preliminary mixing-model results suggested that MPB

never contributed more than 38 % to the diets of copepods. Further, our data suggest that *Spartina* (presumably in the form of detritus) contributes significantly to copepod diets during much of the year. The latter observation contrasts with various food-web studies of estuarine macrofauna, which conclude that primary production from MPB is the principal source of nutrition for primary consumers.

More generally, our observations highlight the difficulties associated with interpreting food webs using natural-isotope analyses when three or more sources of primary production are available. It is relatively straightforward to determine the diets of consumers that have natural-isotope values similar to those of food sources with most enriched or depleted isotope values (phytoplankton or *Spartina* in this study). However, when consumer isotopic values are intermediate, food-source contributions cannot be determined with confidence. We show that labeling of one food source can help resolve ambiguity in interpreting natural-isotope compositions, and suggest that future studies should expand upon this approach by labeling multiple food sources. For example, experimental manipulations with isotopically enriched *Spartina* detritus would be useful for determining more conclusively whether or not *Spartina* contributes significantly to copepod diets, and possibly the diets of other consumers. We note, however, that data obtained from enrichment studies can be highly variable, which in turn will yield mixing-model results with broad confidence intervals. Increased replication would help to remedy this problem.

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Appendix 1. Notation and calculations for use with enriched samples.

a. Expressing enrichment as δ^E. Most ecologists calculate isotopic enrichment as $\Delta\delta$, subtracting a control δ value for an unenriched sample from the measured δ value for the enriched sample. However, the correct way to express the isotopic contrast between two samples is actually via the atom % notation (explained in section b, below), or using isotopic ratios of the two samples and a variant of the δ definition. Here we express ‰ enrichment as δ^E rather than $\Delta\delta$, with

$$\delta^E = \{[(\delta 1+1000)/(\delta 2+1000)]-1\}1000.$$

To give an example that shows the difference between enrichments calculated via δ^E vs. $\Delta\delta$, consider an isotope-enriched algal sample has a $\delta^{13}C$ value of 600 ‰ and a control $\delta^{13}C$ sample of this same species has a value of -21 ‰. The value for δ^E is 634 ‰, close to, but not the same as the incorrect $\Delta\delta$ enrichment value of 621 ‰ obtained by simple subtraction (600-(-21)) = 621 ‰).

The above formula for δ^E can be derived from the measured δ values of the two samples:

$$\delta 1 = [(R1/R) - 1]1000$$
$$\delta 2 = [(R2/R) - 1]1000$$

where R is the $^{13}C/^{12}C$ ratio in the standard, and R1 and R2 are the isotopic ratios in the samples. We define δ^E as:

$$\delta^E = [(R1/R2) - 1]1000$$

We can solve these equations for δ^E, first rearranging the above definitions to solve for R1 and R2:

$$R1 = R(\delta 1 + 1000)/1000$$
$$R2 = R(\delta 2 + 1000)/1000$$

The next step is to divide R1 by R2 and cancel R and 1000 values,

$$R1/R2 = (\delta 1 + 1000)/(\delta 2 + 1000)$$

Finally, to obtain δ^E, subtract 1 and multiply the entire result by 1000,

$$\delta^E = \{[(\delta 1 + 1000)/(\delta 2 + 1000)] - 1\}1000.$$

b. Atom % and mixing calculations for highly enriched samples. For samples with added isotope, δ values become increasingly unreliable in simple mixing equations. So, it is better to switch to atom percent that is a direct measure of "% isotope", and use the atom percent values in the normal mixing equations. Spreadsheets make it easy to convert δ values to atom percent values, starting from three equations:

1. the δ definition, $\delta = [(R_{SAMPLE}/R_{STANDARD}) - 1]1000$, where $R = {}^{H}F/{}^{L}F$, F = fractional abundance of the heavy isotope ${}^{H}F$ or light isotope ${}^{L}F$,
2. the sum of the fractions of the light and heavy isotope add to one: ${}^{H}F + {}^{L}F = 1$.
3. atom % heavy isotope = $100 * {}^{H}F$.

These equations can be solved for atom % percent of heavy isotope,

$$\text{atom \% } {}^{13}C = 100*(\delta + 1000)/[(\delta + 1000 + (1000/R_{STANDARD})]$$

where $R_{STANDARD}$ is the known isotope ratio of the standard, e.g., 0.0112372 for carbon isotopes.

Example: an aquatic insect from an isotope enrichment experiment has a $\delta^{13}C$ value of 350‰, and two potential foods measure 80 and 700‰. What are the source contributions for these two foods? Solution: convert the values to atom % values, and substitute the atom percent values for δ value into the normal two source mixing equation used for samples that are not enriched:

$$f = \text{fractional contribution of source 1} = (\delta_{SAMPLE} - \delta_{SOURCE2})/(\delta_{SOURCE1} - \delta_{SOURCE2}).$$

δ values for source 1, source 2 and the sample are 80, 700, and 350‰ respectively, and the corresponding atom % (%${}^{13}C$) values are 1.19907%, 1.87451% and 1.49435%. The solution is f = (1.49435-1.87451)/(1.19907-1.87451) = 0.5628, the fraction contributed by the 80‰ source 1, and the fraction contributed by the 700‰ source 2 is 1-f, or 0.4372.

We can compare this f = 0.5628 answer to that obtained with the original δ values, f = (350-700)/(80-700) = 0.5645, finding an almost identical result. So, in this case, using the δ values in the mixing equation is acceptable. However, in experiments with much higher isotope enrichments, differences between the two methods start to exceed 0.01 in the final fractional result (e.g., for the case when ${}^{13}C$-enriched values are 80, 3900 and 1711 for source 1, source 2 and the sample, f = 0.5628 when calculated with atom %, but f = 0.5730 when calculated with δ values; the difference in these f values = 0.0102). The results based on atom % are always the correct results, and especially as ${}^{13}C$ enrichments become larger than a few thousand ‰, calculations should be based on atom %. (Note: for nitrogen, the point at which differences in f start to exceed 0.01 is higher, about 11,500‰, with this higher limit due to a difference in the isotope value of the N vs. C standard used in the atom % equation above).

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