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Isotope enrichment in mangrove forests separates microphytobenthos and detritus as carbon sources for animals

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Abstract

Microphytobenthos (MPB) and mangrove detritus were labeled with a carbon isotope (^{13}C) in separate experiments to quantify their contributions to the nutrition of major faunal components within a mangrove forest. Within 7 d of MPB labeling, crabs (*Parasesarma erythrodactyla* and *Australoplax tridentata*) and foraminifera (*Ammonia beccarii* and *Trochammina inflata*) were enriched. *A. tridentata* became more enriched (e.g., hepatopancreas, 522‰) than *P. erythrodactyla* (110‰), and *A. beccarii* (245‰) became more enriched than *T. inflata* (12‰). Addition of labeled mangrove detritus (−11.5‰ final enrichment vs. −28.8‰ for controls) to sediment resulted in enrichment of *P. erythrodactyla* (hepatopancreas, −21.2‰ vs. −26.6‰ for controls), *A. tridentata* (hepatopancreas, −24.2‰ vs. −27.1‰) and *A. beccarii* (−21.0‰ vs. −25.1‰) within 7 d. Compartment modeling showed that MPB contributed 93% of the nutrition for *A. tridentata* and 33% of the nutrition for *P. erythrodactyla* and that MPB provided more nutrition to *A. beccarii* (14%) than to *T. inflata* (minimal). There was a complementary estimated contribution of mangrove detritus to the diets of *P. erythrodactyla* (80%), *A. beccarii* (97%), and *A. tridentata* (minimal), although these estimates should be viewed with caution, due to low initial enrichment and the apparent short temporal persistence of ^{13}C-labeled detritus added to sediments. *T. inflata* was barely enriched in either experiment and may rely on a carbon source not considered. The combination of isotope labeling and compartment modeling is relatively new to ecology and shows potential for revealing differences in the patterns of use of algae and macrophyte detritus by consumers.

Coastal areas are valued as sources of nutrition (i.e., carbon) supporting food webs. Within coastal habitats, there are generally two potential carbon sources: microalgae and macrophytes. Whereas microalgae offer a labile source of carbon for consumers (Herman et al. 2000), macrophyte material is typically a poor-quality food resource (Skov and Hartnoll 2002). It has been proposed that macrophyte material could be important as the basis of a detritus-based pathway (Odum and Heald 1975) following microbial degradation that improves its quality (Werry and Lee 2005). However, limitations of existing techniques have largely prevented resolution of the importance of macrophyte detritus and microalgae to consumers.

In the tropics and subtropics, mangrove forests are one of the major coastal habitats for maintaining fisheries productivity. Mobile consumers such as fish and birds utilize invertebrates within mangrove forests, including meiofauna (Gee 1989) and crabs (Sheaves and Molony 2000), as a food source. However, the source of carbon supporting these invertebrates remains subject to debate. Crabs often dominate the mangrove fauna, and they derive much of their carbon from mangrove material, although some species appear to be more reliant upon microphytobenthos (MPB; Bouillon et al. 2008). Broadly, grapsid crabs (family Grapsidae) are considered to feed on leaf litter (Lee 1998) whereas ocypodid crabs (family Ocypodidae) are thought to rely on bacteria (Dye and Lasiak 1986), MPB (Rodelli et al. 1984), or plant matter (Hsieh et al. 2002). Meiofauna are less frequently studied, most likely due to their small size, but may serve an important role in carbon cycling within mangrove forests. Foraminifera, for example, are abundant throughout shallow-water sediments (e.g., up to 410 per g of wet sediment, Ellison 1984) and can respond rapidly to additions of phytodetritus (Moodley et al. 2002). Studies aiming to establish the primary sources of nutrition (i.e., the primary producers supporting the base of the food web) for consumers in mangrove forests have commonly used natural abundance stable isotopes.

These studies have suggested that some consumers assimilate significant mangrove carbon but that algal carbon is a more important source. Limitations of natural abundance studies, however, mean that the extent of contribution of MPB and mangrove carbon to consumers remains uncertain.

In natural abundance isotope studies, producers must have distinct carbon signatures that can be used in mixing models to determine a feasible combination of sources that could produce the observed consumer difference in ^{13}C, or $\delta^{13}C$ value. Where there are more than two alternative food sources, a consumer signature similar to that of either mangroves or MPB may be produced by a diet derived from a combination of other producers within the system, or derived from sources transported into the forest. For example, Guest et al. (2004) found that carbon signatures

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of crabs within a mangrove forest in southeast Queensland, Australia, were more similar to those of MPB than those of mangroves, but cautioned that these signatures may also result from assimilation of carbon derived from a combination of mangroves and saltmarsh plants, which may be transported into the habitat, with or without an MPB contribution. Phytoplankton (Bouillon et al. 2008) and material transported inshore (Connolly et al. 2005) can provide additional sources of primary production but are often not incorporated into mixing models. Inclusion of these producers may give alternative solutions to mixing models and thus different estimates of the contribution of mangroves to consumers (Melville and Connolly 2005).

The addition of a substrate enriched in a heavy, rare isotope (e.g., ^{13}C) generates a distinct signature for the producer of interest, and this has been shown to transfer to consumers that are directly or indirectly (via trophic transfer) reliant upon the producer in seagrass meadows (Winning et al. 1999), on mudflats (Herman et al. 2000; Middelburg et al. 2000), and in the upper reaches of estuaries (Hughes et al. 2000; Gribsholt et al. 2005).

Although enrichment experiments solve some of the problems associated with natural abundance stable isotope studies, they have some of their own problems (Hamilton et al. 2004). Enrichment experiments generally show which consumers utilize a particular source, but they are unable to quantify the extent of utilization. Quantifying relies on the consumer of interest attaining equilibrium with its food source, but enrichment experiments are rarely run for sufficient time for this to occur (Herman et al. 2000; Moens et al. 2002). In other cases, the degree of producer enrichment fluctuates temporally or spatially (Raikow and Hamilton 2001), preventing equilibrium from being reached. Hamilton et al. (2004) solved these issues in a freshwater system using a compartment-modeling approach that avoids the need for consumers to reach equilibrium with their diet, thus allowing for variable enrichment over time. Compartment models are often used to study biological systems and are particularly appropriate where an isotope label is used to trace movement of material through compartments of a defined system (Wastney et al. 1999). The model used by Hamilton et al. (2004) describes compartments relating to a freshwater stream as the biological system of interest and defines compartments as 1) the consumer of interest, 2) the producer of interest, and 3) all other potential food sources. The model structure also includes definition of the rates of exchange of carbon among the compartments. Having first defined a compartment model to describe the system, an equation-solving program (WinSAAM: the Windows version of Simulation, Analysis and Modeling) compares consumer signatures to those of producers over the time of the experiment and iteratively seeks the parameters giving the best fit of the model to the observed consumer values (according to fractional standard deviations, FSDs). Ideally, different body tissues should be analyzed, and the one with the fastest turnover should be used for modeling (Hamilton et al. 2004).

Stable isotope enrichment techniques have been applied to a variety of environments, but only one study (van Oevelen et al. 2006) has utilized compartment modeling in a marine environment. No published studies have enriched MPB within mangrove forests, and, although it is possible to alter the isotopic composition of mangrove leaves (e.g., with ^{14}C, Newell et al. 1995), no published studies have added enriched mangrove detritus to sediments to monitor and quantify its assimilation by consumers. The current study uses the compartment-modeling approach of Hamilton et al. (2004), in combination with ^{13}C-enrichment of MPB and mangrove detritus, to quantify the importance of MPB and mangrove detritus as carbon sources for consumers (crabs and meiofauna) within mangrove forests.

Methods

Experiments to label MPB and mangrove detritus were done in small clearings in a mangrove forest at Coombabah Lake, southeast Queensland, Australia (27°54′50S, 153°21′22E). MPB- and detritus-labeling experiments were both done during the Australian spring, but were done at separate times. MPB labeling was done in September 2005, and the mangrove detritus labeling experiment was done in November 2005. For the MPB-labeling experiment, and again for the mangrove-detritus–labeling experiment, 18 circular plots (1 m in diameter) were randomly assigned to one of three treatments: 1) enriched, 2) procedural control, or 3) control (six plots per treatment). Plots were separated by at least 5 m and were positioned at similar heights on the tidal gradient to minimize potential transfer of labeled material among sites by tidal movement. Control plots were used to test for movement of ^{13}C among plots, and they also provided background values to compare against $\delta^{13}C$ values of consumers, MPB, and detritus in enriched plots. Procedural controls were compared to controls to verify that changes in consumer isotope ratios were due to experimental treatment.

Preparation of labeled material and plots—MPB was labeled in enriched plots using ^{13}C-enriched sodium bicarbonate ($NaH^{13}CO_3$, > 99% ^{13}C, 0.8 g m^{-2}) dissolved in filtered seawater and sprayed evenly over the sediment surface on day 0, day 7, and day 14 of the study.

Detritus of the dominant mangrove species at the experimental site (*Avicennia marina*) was enriched by enclosing juvenile trees (< 1 m tall) in plastic chambers with transparent tops (80 cm [length] × 60 cm [width] × 40 cm [height]). Chambers were pushed 5 cm into the sediment to form a seal. Sediment around the perimeter of the chambers was cut to a 20-cm depth to prevent plants obtaining carbon from outside of the chambers via aerial roots. ^{13}C-enriched CO_2 (30–40 mL every second day) was injected into chambers via septa. After 14 d, leaves were collected, combined, and dried at 60°C. The $\delta^{13}C$ of this leaf material (detritus) was determined by analyzing a homogenized subsample using a Carlo Erba NA1500 Carbon, Nitrogen and Sulfur analyzer interfaced via a Conflo II to a Finnigan Mat Delta S isotope ratio mass spectrometer (EA-IRMS) operating in continuous flow

mode. Combustion and oxidation were achieved at 1090°C, and reduction was achieved at 650°C. Leaf material had a δ^{13}C value of 220‰.

Labeled detritus was ground to obtain a composition similar in size to naturally occurring detritus (determined for three replicate samples from the study site). On average, sediment from the site contained 49 g dry weight of detritus retained on a 2-mm sieve per m^2. This was dominated by the 1–2-mm fraction (68%). A total of 3.2 g m^{-2} of detritus was added to sediments, accounting for approximately 6% of the detritus available to consumers.

Fresh mangrove leaves typically are not very nutritious, having high C : N ratios, and contain tannins that make them less palatable to consumers. Therefore, the detritus produced in the current study from green leaves may not be an exact mimic of the detritus that is naturally available to consumers. The leaves of the mangrove species we used (*A. marina*), however, have a relatively low C : N ratio and low tannin content (Micheli et al. 1993), so this is less of an issue. Degradation of leaves through drying, a method routinely applied to produce detritus in feeding experiments (*see* review by Tenore et al. 1982), in combination with natural degradation processes operating upon mangrove material after its addition to sediments, would have further reduced any effect of using fresh, rather than degraded, leaves.

Procedural controls were treated similarly to enriched plots, except that either nonenriched sodium bicarbonate (MPB enrichment) or nonenriched mangrove leaves (detritus enrichment) were used. Control plots were not treated at all.

Sample collection—For both MPB- and detritus-enrichment experiments, one plot of each treatment type was destructively sampled at time 0, prior to labeling of enriched plots, then 7 d, 14 d, 21 d, 28 d, and 35 d after enrichment. For determination of δ^{13}C of MPB and mangrove detritus, subsamples of surface sediment (upper 5 mm) were pooled from across each plot. Consumers considered in this study were the most abundant: crabs (grapsid crab, *Parasesarma erythrodactyla*, and ocypodid crab, *Australoplax tridentata*) and foraminifera (*Ammonia beccarii* and *Trochammina inflata*). Crabs of each species with carapace widths from 8 mm to 13 mm were collected within 20 cm of the plot center. For the duration of the 35-d study, the plot diameter was sufficient to incorporate the entire home range of crabs with burrows toward the plot center (Guest et al. 2006), so no confinement of crabs was required. As suggested by Hamilton et al. (2004), we analyzed a variety of tissue types for crabs (i.e., gill, muscle, and hepatopancreas) and based our modeling on the hepatopancreas because it had the highest carbon turnover rate. Foraminifera were handpicked from cores (5 cm deep × 15 cm in diameter) collected at the center of each plot. To provide sufficient material for isotope analysis, approximately 30 individuals were required. A single, pooled sample was obtained at each sampling time by pooling individuals collected from all sites of each treatment type. Samples of foraminifera were acid washed (5% HCl, Moodley et al. 2002) to remove inorganic carbon.

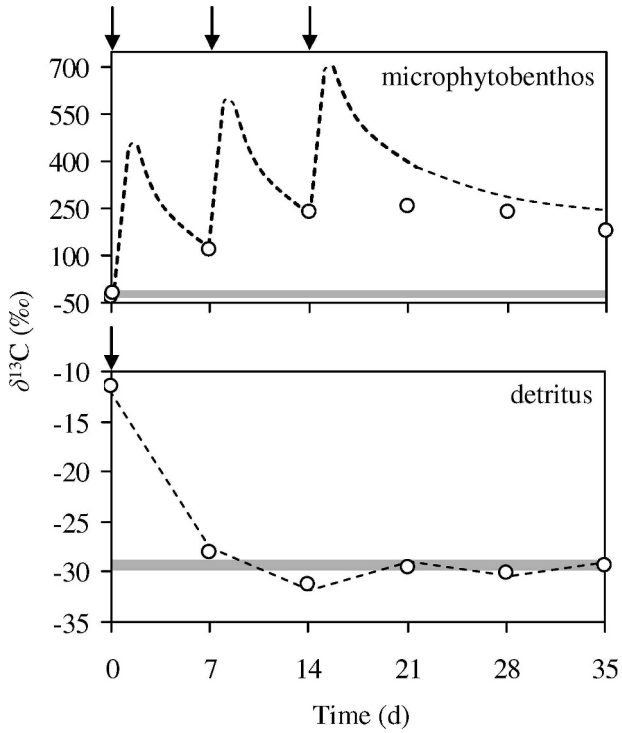

Fig. 1. Observed δ^{13}C values (triangles) for MPB and mangrove detritus (1000–2000-μm size fraction) in enriched plots throughout the experimental period. Dashed line indicates predicted δ^{13}C values. Times of ^{13}C-labeling are indicated by arrows. Grey bar = δ^{13}C values for mangrove detritus and MPB in control plots (mean, width = ±SE).

Foraminifera and crab tissues were prepared for analysis on an EA-IRMS, as described for mangrove detritus. Samples were analyzed in duplicate and had a reproducibility of ~ 0.2‰. For the MPB enrichment experiment, sediment samples and cores for meiofauna (but not crabs) were also collected 4 h after initial treatment, using only a small area of a randomly selected plot of each treatment type. For the detritus-enrichment experiment, a small sample of surface sediment was also obtained from a randomly selected enriched plot just after the addition of enriched detritus to sediments.

Determination of MPB and mangrove detritus δ^{13}C—Due to rapid uptake and depuration, δ^{13}C of MPB would ideally be monitored constantly throughout the experimental period. Because this was not possible, data from a pilot study run over 18 d at the same site, using the same application of enriched sodium bicarbonate, was used to determine rates of uptake (20.325‰ h^{-1}, described by a linear equation) and depuration (0.009‰ h^{-1}, described by an exponential equation) of label by MPB. These rates were then used to estimate the response of MPB to enrichment over time. To verify these predictions, samples of MPB collected during the experiment were compared to those predicted (Fig. 1). These values were estimated based on the δ^{13}C value of the compound phytol as described by Oakes et al. (2005). With the exception of day 7, all estimates were within 100‰ of observed MPB δ^{13}C values.

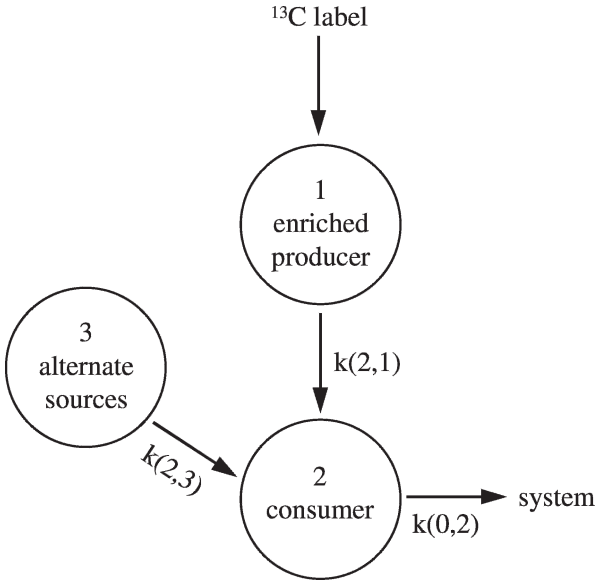

Fig. 2. Schematic diagram depicting the relationships among compartments in the model. Numbers within circles indicate compartment numbers as referred to in the text. k = rate constant for transfer of ^{13}C into and out of compartments, in the direction indicated by arrows. Numbers in brackets indicate the transfer that rate constants refer to, e.g., k(2,3) = rate of transfer of ^{13}C to compartment 2 from compartment 3.

A similar method was used by Hamilton et al. (2004) to predict $\delta^{15}N$ of algae and microbes in an isotope addition experiment in a freshwater stream.

Mangrove detritus was separated from sediment using a combination of sieving and manual removal of contaminants, then mangrove detritus was analyzed on an EA-IRMS, as described above. Values of $\delta^{13}C$ for detritus at each sampling time were obtained by pooling detritus from all plots of each sampling type (i.e., no replicates for each time).

Modeling—Modeling was done separately for the MPB and mangrove detritus–enrichment experiments and for each consumer species within these experiments using the Windows version of Simulation Analysis and Modeling (WinSAAM 3.0.7, an equation-solving program developed by the U.S. National Institute of Health; *see* www.winsaam.com). Compartments within the model were these: 1) the ^{13}C-enriched primary carbon source (MPB or mangrove detritus), 2) the consumer of interest, and 3) all alternate, unknown carbon sources (Fig. 2). The producer $\delta^{13}C$ values used were those predicted for MPB and those measured for mangrove detritus. These values were used as a forcing function, and parameters defining the exchange rates among the compartments were varied between 0 and 1 (0 to 100% of material transferred between compartments per unit of time). The transfer rates of interest were from producer (^{13}C-enriched MPB or mangrove detritus) to consumer (k[2,1]), from alternate carbon sources to the consumer (k[2,3]), and out of the consumer to the system in general (k[0,2]; Fig. 2). The contribution of MPB or detritus to the nutrition of each consumer of interest was determined from the ratio of k(2,1) to k(0,2). These transfer rates were determined through modeling with WinSAAM, which sought a generalized, least-squares fit of the compartment model to the consumer $\delta^{13}C$ values. The higher enrichment that was achieved for MPB means that there is greater sensitivity for detecting uptake of MPB-derived carbon. Transfer rates from the consumer to the system (k[0,2]), essentially the turnover rate of consumer tissue) that were determined for MPB were therefore imposed as fixed parameters in the models to determine the contribution of detritus to consumers. To further constrain the model, standard deviations of the mean $\delta^{13}C$ values of crabs at each sampling time were used to assign weights to individual data points during the data fitting process. Statistical weights assigned to datum by WinSAAM were proportional to the inverse of the square of the standard deviation, meaning that greater weights were assigned to observations with smaller standard deviations. For foraminifera, however, individual weighting was not possible because pooling of samples meant that no standard deviations were determined. In this case, equal weightings were assigned to the observations. The best fit of the model to the data was determined to occur when feasible parameters were obtained that gave the smallest fractional standard deviations (FSDs). FSDs provide an estimate of statistical precision similar to the standard error used in inferential statistics, and they equate with the standard deviation divided by the mean (Pawlosky et al. 2001). Low FSD values indicate that model parameters are well defined and that estimates made using the model are robust and give realistic values. FSDs < 0.5 are considered to indicate a good fit of the model to the data (Stefanovski et al. 2003).

Results

MPB labeling—The maximum MPB enrichment during the experimental period was calculated to be 720‰, shortly after the final addition of ^{13}C on day 14 (Fig. 1). Throughout the experimental period there was no evidence of MPB or consumer enrichment in either control or procedural control plots, for which $\delta^{13}C$ values were similar (-25.5 ± 1.0‰), confirming that there was no movement of the ^{13}C label among plots and that additional carbon did not alter the extent of use of MPB by consumers (i.e., no shift in natural abundance $\delta^{13}C$ values of consumers could be detected).

In treatment plots, consumers responded rapidly to enrichment. Within 4 h of initial label addition, the foraminifera *A. beccarii* showed evidence of enrichment (38.7‰ compared with -25.1‰ for control plots) and, for both crab species, all tissues were enriched within 7 d, with the hepatopancreas tissue becoming most enriched, followed by gill tissue, then muscle tissue (Table 1). Hamilton et al. (2004) recommended that the tissue type with most rapid turnover be used, so modeling was done using the hepatopancreas values for both crab species.

The hepatopancreas of *A. tridentata* was enriched to 231 $\pm$ 35‰ by day 7 and attained a maximum enrichment of

Table 1. Mean natural abundance (control) and maximum $\delta^{13}C$ (±SE, except for pooled samples) for MPB, mangrove detritus and consumers, and transfer rates and % contributions of MPB and mangrove detritus to consumers determined through modeling, where applicable. FSDs in brackets. Fixed = defined value; — = no model generated; k(2,1) = transfer from producer to consumer; k(0,2) = transfer from consumer to system.

		MPB enrichment			Mangrove detritus enrichment			% contribution of MPB	% contribution of detritus
Compartment	Natural $\delta^{13}C$ (‰)	Max. $\delta^{13}C$, ‰	k(2,1)	k(0,2)	Max. $\delta^{13}C$, ‰	k(2,1)	k(0,2)		
Microphytobenthos	−25.5±1.0	720.0							
Mangrove detritus	−28.8±0.5				−11.5				
A. tridentata									
Hepatopancreas	−27.1±0.4	521.6±73.4	0.0128 (0.39)	0.0137 (0.36)	−24.2±0.1	—	—	93	—
Gill	−23.6±0.3	231.2±31.0			−22.2±0.4				
Muscle	−22.8±0.4	88.7±23.3			−21.6±0.1				
P. erythrodactyla									
Hepatopancreas	−26.6±0.6	110.5±39.6	0.0030 (0.55)	0.0090 (0.17)	−21.2±2.3	0.0072 (0.20)	0.0090 (fixed)	33	80
Gill	−24.0±0.4	108.7±52.4			−19.0±3.3				
Muscle	−23.0±0.2	50.0±30.8			−21.3±1.3				
A. beccarii	−25.1±0.2	245.3	0.0144 (0.30)	0.1029 (0.18)	−21.0	0.1000 (0.97)	0.1029 (fixed)	14	97
T. inflata	−24.7±0.2	11.7	—	—	−24.7	—	—	—	—

521 ± 73‰ at day 28. The response of *P. erythrodactyla* to MPB enrichment was less pronounced, with the hepatopancreas enriched to 55 ± 32‰ by day 7 and a maximum enrichment of 110 ± 40‰ at day 35 (Table 1, Fig. 3). Parameter values for *A. tridentata* gave a good fit of the model to the observed data (as indicated by FSD values $<$ 0.5, Stefanovski et al. 2003), but parameters were not as well defined for *P. erythrodactyla* (FSD for k[2,1] = 0.55, Table 1). However, modeling was able to confirm that *A. tridentata* derives a far greater proportion of its nutrition from MPB (~ 93%) than does *P. erythrodactyla* (~ 33%; Table 1).

Both foraminifera species showed evidence of enrichment, but there was a marked difference in their reliance on MPB (Fig. 3). *A. beccarii* attained a $\delta^{13}C$ value of 38.7‰ after 7 d, and a maximum enrichment of 245‰ at day 28. *T. inflata*, however, showed very little evidence of enrichment until day 28, when an enriched sample (11.7‰) was obtained. There was no feasible fit of a model to the observed data for *T. inflata*, but the enrichment clearly observed relative to control values indicates some, though apparently minimal, uptake of MPB carbon by this species. Parameters for *A. beccarii* were well-defined (FSDs $<$ 0.5) and indicated that *A. beccarii* derives 14% of its carbon from MPB.

Detritus labeling—The most enriched $\delta^{13}C$ value (−11.5‰) obtained for the 2-mm size fraction of detritus was for sediment collected immediately after addition of detritus. One week following addition of detritus, however, $\delta^{13}C$ values for 2-mm detritus in enriched plots were approximately 1‰ enriched relative to control plots (average $\delta^{13}C$ throughout experimental period = −28.8 ± 0.5‰). Thereafter, $\delta^{13}C$ values of detritus in enriched and nonenriched plots were indistinguishable (Fig. 1). Throughout the experiment $\delta^{13}C$ values of detritus and consumers within control plots did not vary. Similar $\delta^{13}C$ values for consumers in control and procedural control plots also show that addition of detritus, and the processes involved, did not alter the diet of consumers in a way that altered their isotope signatures. The consistent values for controls and procedural controls also indicate that there was no significant transfer of label among treatment types.

The tissues of *A. tridentata* became only marginally enriched relative to control plots during the experiment (Table 1, Fig. 3). However, all tissues of *P. erythrodactyla* were enriched within 7 d of the addition of enriched detritus, when the peak in enrichment was observed (Table 1, Fig. 3). Muscle tissue became only marginally enriched (−21.3 ± 2.3‰ at 7 d, compared with −23.0 ± 0.2‰ in controls). Although gill and hepatopancreas tissues responded similarly to enrichment (−19.0 ± 3.3‰ and −21.2 ± 2.3‰ at 7 d, respectively), the difference between mean $\delta^{13}C$ in control and treatment plots at this time was smaller for gill (5.0‰) than for hepatopancreas (5.4‰), confirming the greater turnover rate of the hepatopancreas (Table 1), as observed following MPB enrichment. For both tissue types, a second, lower peak in ^{13}C-enrichment was evident (−22.6 ± 1.3‰ and −24.6 ± 0.4‰, respectively) at day 21.

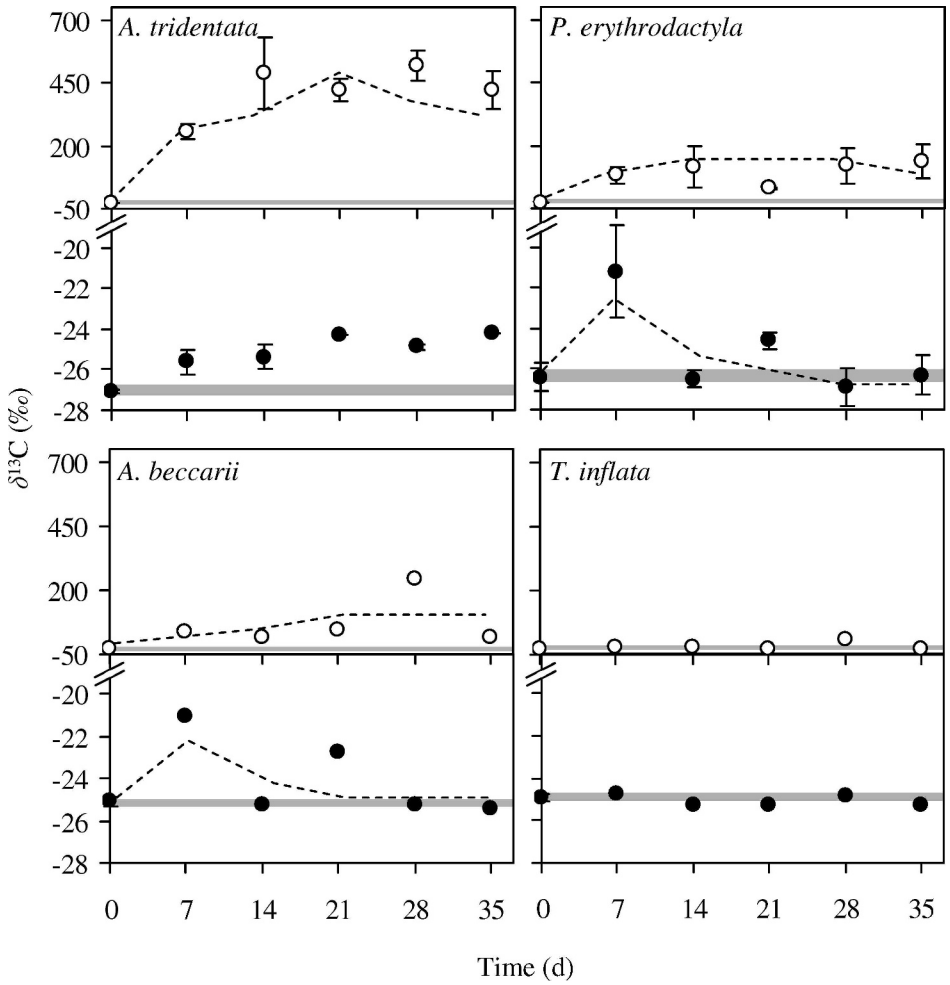

Fig. 3. Mean observed $\delta^{13}C$ values ($\pm$SE) for hepatopancreas tissue of crabs and for foraminifera following enrichment of MPB (open circles) and mangrove detritus (closed circles). Grey bars represent $\delta^{13}C$ values for similar samples from control plots (mean, width = $\pm$SE). Dashed lines show model that best described the observed $\delta^{13}C$ values (omitted where no model could be fitted).

Of the foraminifera, only *A. beccarii* became enriched. There was no evidence of enrichment for *T. inflata* throughout the experimental period with $\delta^{13}C$ values for individuals in enriched plots (−24.7‰) remaining similar to values for *T. inflata* in control plots (average $\delta^{13}C$ throughout experimental period = −24.7 ± 0.2‰; Fig. 3). *A. beccarii* attained a maximum $\delta^{13}C$ of −21.0‰, noticeably enriched compared to control plots (−25.1 ± 0.2‰) at day 7, and had a second, lower peak in ^{13}C-enrichment (−22.7‰) at day 21.

Compartment modeling was applied to the consumers that became enriched during the experimental period (*P. erythrodactyla*, *A. tridentata* and *A. beccarii*; Table 1). No feasible model fit could be obtained for *A. tridentata*, most likely due to the low contribution of detritus to this species combined with the low initial enrichment of detritus. For *P. erythrodactyla*, however, WinSAAM modeling using $\delta^{13}C$ of the hepatopancreas showed that 80% of the nutrition of *P. erythrodactyla* was derived from mangrove detritus. The FSD value associated with the k(2,1) parameter indicates a good fit of the model to the observed data and yields a high degree of confidence in the estimate of detritus contribution to the nutrition of this consumer.

For *A. beccarii*, modeling showed that 97% of its nutrition is derived from mangrove detritus. However, the FSD value for the parameter k(2,1) was high (0.97), indicating a poor fit of the model and showing that the error associated with this estimate of detritus contribution is higher than that for *P. erythrodactyla*.

Discussion

Labeling of MPB with ^{13}C and application of ^{13}C-enriched mangrove detritus to sediments effectively demonstrated the transfer of carbon from these sources to macroinvertebrates and meiofauna within mangrove forests and showed that both MPB and mangrove detritus can be important sources of nutrition for consumers. MPB, detritus, and consumers in procedural control plots did not become enriched, indicating that there was no contamina-

tion among plots and that addition of carbon did not affect the use of different sources by consumers enough to alter naturally abundant isotope values. The enrichment of consumers in treatment plots therefore demonstrates that they obtain at least some of their carbon from MPB and/or detritus.

We were able to quantify the contribution of MPB and/or detritus to most consumers, although higher initial enrichment and the resulting better characterization of MPB enrichment means that there was greater sensitivity for detecting uptake of MPB-derived carbon than detritus-derived carbon. Results for MPB are therefore more certain. The level of enrichment achieved for total mangrove detritus in sediments was relatively low (−11.5‰), and there was rapid loss of label from surface sediments over the first 7 d of the experiment, most likely due to either resuspension or burial of detritus. This resulted in a decreased ability to detect uptake of detritus-derived carbon. It was therefore particularly important to obtain accurate δ^{13}C values for the detritus material. Based on a two-source mixing model combining 3.2 g of labeled detritus (220‰) with 49 g of unlabeled detritus (−28.8‰), the δ^{13}C of total detritus in the enriched plots was expected to be approximately −12.5‰. Close agreement between this value and the δ^{13}C measured for detritus in labeled plots (−11.5‰) shows that measured δ^{13}C values were accurate.

Although detritus δ^{13}C values were well-defined, there remains some uncertainty regarding the loss of δ^{13}C-labeled detritus throughout the experiment. For modeling purposes, it was assumed that loss of label was linear throughout the first 7 d of the experiment. However, it is possible that the enriched δ^{13}C value recorded for detritus at day 7 represented the upper limit of naturally occurring detritus δ^{13}C values rather than remnant enriched detritus; ^{13}C-enriched detritus may have been removed from the sediment at a faster rate than was assumed here. This uncertainty is a further consequence of the low initial enrichment of mangrove detritus and means that output of the detritus modeling should be interpreted with caution.

Addition of detritus that was more highly labeled would improve the sensitivity of our method. In the current study, however, assuming that the δ^{13}C value of detritus at day 7 represents remnant ^{13}C-enriched detritus, labeling was sufficient to allow quantification of the use of detritus to *P. erythrodactyla*. This was most likely assisted by the large contribution of detritus to this consumer. For those species where model fits were poor or not possible, but enrichment clearly demonstrated some use of detritus-derived carbon, more effective labeling of the detritus source would improve the ability to quantify detritus contributions.

With the exception of *T. inflata*, modeling indicated that some combination of MPB and mangrove detritus provided all of the carbon required by the consumers studied. However, where contributions of both MPB and detritus were able to be quantified, the total contribution added to over 100%. This demonstrates that there is some degree of error associated with the estimates of contribution. Individuals of a species are likely to vary in their use of resources due to differences in size and/or access to carbon sources, and this is likely to be reflected in the modeling results, accounting for some error in estimates of contribution. Temporal variation in resource use might also introduce error to estimates of contribution in the current study, given that MPB and detritus-enrichment experiments were done 2 months apart. These sources of error could be reduced through greater replication to better characterize variations within a population, through greater definition of a smaller range of sizes for individuals, and by running labeling studies of alternate carbon sources concurrently. The shortcomings of the detritus-labeling experiment, as discussed previously, might also have contributed to some error in estimates of MPB and detritus-carbon assimilation. Despite potential errors, however, the current study appears to provide a good indication of the relative contributions of MPB and mangroves to consumers.

The current study clearly demonstrated that the extent of uptake of ^{13}C from MPB and detritus varied among taxa. Whereas almost all the carbon for the ocypodid crab, *A. tridentata*, was derived from MPB, the grapsid crab, *P. erythrodactyla* relied upon mangrove detritus for the majority of its nutrition. Feeding experiments and field observations have indicated a similar pattern in the use of detritus and algae by grapsid and ocypodid crabs (Rodelli et al. 1984; Lee 1998). However, field observations show that ingestion, rather than assimilation, of carbon sources and feeding experiments are typically laboratory based, without alternative carbon sources available. The current study provides the first unequivocal evidence that ocypodid crabs derive the majority of their nutrition from MPB. It also shows, however, that grapsid crabs are not entirely reliant upon leaf litter, with *P. erythrodactyla* deriving approximately a third of its carbon from MPB. Bouillon et al. (2002) similarly found that, although the grapsid crab *P. asperum* is typically considered to depend upon leaf litter, discrepancies with mangrove carbon signatures indicated that another source contributed to the diet of this species. The current study shows that MPB is a feasible alternative source of carbon for grapsids, such as *P. asperum*, that do not entirely rely upon mangrove carbon.

Naturally abundant stable isotope ratios of carbon are often used to investigate food webs; however, the conclusions of the current study could not have been obtained using natural δ^{13}C values alone. For *P. erythrodactyla*, δ^{13}C of muscle and gill tissues was depleted compared with these tissues in *A. tridentata*, indicating greater use of a more depleted carbon source such as mangrove leaf material. In contrast, hepatopancreas tissues of *A. tridentata* had δ^{13}C values that were more depleted than those of *P. erythrodactyla*. Natural δ^{13}C values in consumers are expected to be enriched by approximately 1–2‰, but this does not appear to be true in the system we studied. *A. beccarii*, in particular, has a signature more similar to that of MPB than detritus, despite modeling indicating that this species probably relies upon mangrove detritus for the majority of its carbon. Such differences in carbon isotope signature may relate to the use of additional sources of carbon that were not considered in the current study. Assimilation of a combination of mangrove detritus and

material from nearby saltmarsh plants, for example, could account for a $\delta^{13}C$ similar to MPB (Guest et al. 2004). However, given that modeling indicated that MPB and detritus together account for all, or almost all, of the carbon utilized by both crab species and *A. beccarii*, it seems more likely that discrepancies between the observed natural $\delta^{13}C$ values of consumers and their modeled carbon sources represent selective uptake and assimilation of organic matter during uptake and assimilation of carbon. Consumers do not assimilate all components of food items they ingest and in the system in the current study it appears that consumers are probably assimilating components of their food source that are relatively enriched compared to bulk cell material (e.g., monosaccharides; van Dongen et al. 2002).

In the current study, stable-isotope enrichment was found to be a suitable method for discerning the diet of foraminifera. Although a stable-isotope enrichment experiment by Moodley et al. (2002) demonstrated rapid uptake of phytoplankton-derived carbon by foraminifera, their study artificially increased the quantity of algal material available. In the current study, labeling of MPB in situ, and labeling of mangrove detritus through the addition of minimal quantities of detritus relative to that already within sediments, allowed us to study the nutrition of foraminifera that only had access to naturally occurring carbon sources. The sources of carbon available to the foraminifera in the study by Moodley et al. (2002), which looked at deep-sea sediment, would also differ from those available to foraminifera in mangrove forests such as the one studied here. The carbon source for foraminifera within mangrove forests has not previously been studied, but it was found that *A. beccarii* derived the bulk of its carbon from mangrove detritus, with a small contribution from MPB.

However, the source of carbon for the foraminifera *T. inflata* not determined. It is likely that this species either utilizes a carbon source not considered in the current study or did not have access to the labeled material during the experimental period. Infaunal foraminifera such as *T. inflata* may not migrate to upper sediment layers to feed (Heinz et al. 2001), so it is possible that *T. inflata* did not have access to the labeled material during the 7 d for which enrichment was maintained in surface sediments. Given that there was no evidence that *T. inflata* utilized detritus-derived carbon, if this species relies on mangrove material transported to deeper sediments, the 35 d for which we monitored label uptake may not have allowed sufficient time for labeled material to be transported downward to *T. inflata*, or else the level of enrichment in mangrove material was too low to detect this. Alternatively, detritus may have been lost from surface sediments via resuspension before burial could occur. *T. inflata*, however, most probably obtains its carbon from older, degraded material from MPB, mangrove detritus, or another source in deeper sediments.

From this study it can be seen that consumers with the same available carbon sources can selectively utilize either MPB or mangrove detritus as their primary source of carbon. This feeding selectivity is clearly evident in the different label-uptake levels observed for the two crab species and for the two foraminifera in the current study. Of the crabs, *A. tridentata* relied almost solely upon MPB and *P. erythrodactyla* obtained most of its carbon from mangrove detritus. In the detritus-labeling experiment, the different use of mangrove detritus by the two crab species is evident from the spike in enrichment at day 7 for *P. erythrodactyla*. This indicates rapid uptake of mangrove detritus, reflecting the greater use of this carbon source by *P. erythrodactyla*. Although it has not been so convincingly demonstrated as in the current study, other studies have also concluded that many ocypodid crabs rely upon MPB (Rodelli et al. 1984) and that grapsid crabs upon mangrove leaf litter (Lee 1998). This highlights that feeding selectivity may be a common phenomenon among crabs and suggests that there may be an advantage for these crabs to rely upon different diets rather than competing for the more palatable, less refractory MPB. Similarly, the difference in carbon sources observed for the two foraminifera species in the current study is not unique. Moodley et al. (2002) and Moodley et al. (2000) also reported marked differences in carbon uptake by groups of benthic foraminifera following application of labeled phytodetritus. It has been suggested that such trends provide evidence of resource partitioning by foraminifera (Moodley et al. 2000). A fundamental theory of community ecology is that, in order to coexist, similar species must have minimal overlap of their niches. By differing in their requirements, species are able to share resources, thereby avoiding competitive exclusion. The selective feeding of the two crab species and the two foraminifera species studied here may be a strategy that allows the species to coexist (Kristensen 2008).

With the exception of *T. inflata*, the consumers studied derived all of their carbon from locally available carbon sources (either MPB, or mangrove detritus, or some combination of the two). Rapid enrichment of consumers (within 4 h of MPB enrichment for *A. beccarii*) and disappearance of labeled material (within 7 d for mangrove detritus) indicates that uptake of this carbon can occur rapidly. The rapid assimilation of carbon from MPB and mangrove detritus by foraminifera and crabs, both of which are abundant in mangrove forests, indicates that these consumers may play an important role in carbon cycling within mangrove forests. Many studies have provided evidence that benthic invertebrates, including foraminifera (Chong and Sasekumar 1984) and crabs (Salini et al. 1990), are important prey items for fish and crustaceans that utilize estuarine habitats when they are inundated by high tides. Any locally derived carbon utilized by prey species would be transferred to the fish that consume them, providing scope for considerable export of locally derived carbon to offshore systems where it may support higher-order consumers. The extent to which benthic invertebrates utilize MPB and macrophytes therefore determines the contribution of these sources to the nutrition of higher consumers. This type of scenario should be imbedded, ultimately, within the concept of trophic relay (Kneib 1997); the possibility that a series of predator–prey interactions results in a net transfer of organic matter from intertidal to deeper waters in the bodies of animals.

The current study is the first to add ^{13}C-labeled vascular detritus to coastal sediments to trace transfer of carbon to consumers. It also demonstrates the application of a combination of techniques (^{13}C-enrichment and compartment modeling) used to quantify the importance of microalgae and macrophyte detritus as carbon sources. Another feature of this study was the enrichment of the entire home range of consumers, preventing interference with natural foraging behaviors that may occur with laboratory experiments (Abed-Navandi et al. 2005) or where consumers are confined by barriers in the field (Herman et al. 2000). Application of the described method at different times of year, and for different consumers, would assist in the development of a broader understanding of the carbon sources used by consumers in mangrove forests. The techniques nevertheless show promise to potentially resolve similar debates regarding the contribution of detritus and microalgae to food webs in other systems such as seagrass beds and saltmarsh.

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References

Abed-Navandi, D., H. Koller, and P. C. Dworschak. 2005. Nutritional ecology of thalassinidean shrimps constructing burrows with debris chambers: The distribution and use of macronutrients and micronutrients. Mar. Biol. Res. **1:** 202–215.

Bouillon, S., R. M. Connolly, and S. Y. Lee. 2008. Organic matter exchange and cycling in mangrove ecosystems: Recent insights from stable isotope studies. J. Sea Res. **59:** 44–58.

———, N. Koedam, A. V. Raman, and F. Dehairs. 2002. Primary producers sustaining macroinvertebrate communities in intertidal mangrove forests. Oecologia **130:** 441–448.

Chong, V. C., and A. Sasekumar. 1981. Food and feeding habits of the white prawn *Penaeus merguiensis*. Mar. Ecol. Prog. Ser. **5:** 185–191.

Connolly, R. M., J. S. Hindell, and D. Gorman. 2005. Seagrass and epiphytic algae support nutrition of a fisheries species, *Sillago schomburgkii*, in adjacent intertidal habitats. Mar. Ecol. Prog. Ser. **286:** 69–79.

Dye, A. H., and T. A. Lasiak. 1986. Microbenthos, meiobenthos, and fiddler crabs: Trophic interactions in a tropical mangrove sediment. Mar. Ecol. Prog. Ser. **32:** 259–267.

Ellison, R. L. 1984. Foraminifera and meiofauna on an intertidal mudflat, Cornwall, England: Populations, respiration and secondary production, and energy budget. Hydrobiologia **109:** 131–148.

Gee, J. M. 1989. An ecological economic review of meiofauna as food for fish. Zoo. J. Linn. Soc. **96:** 243–261.

Gribsholt, B., and others. 2005. Nitrogen processing in a tidal freshwater marsh: A whole-ecosystem ^{15}N labeling study. Limnol. Oceanogr. **50:** 1945–1959.

Guest, M. A., R. M. Connolly, S. Y. Lee, N. R. Loneragan, and M. J. Breitfuss. 2006. Mechanism for the small-scale movement of carbon among estuarine habitats: Organic matter transfer not crab movement. Oecologia **148:** 88–96.

———, ———, and N. R. Loneragan. 2004. Carbon movement and assimilation by invertebrates in estuarine habitats at a scale of metres. Mar. Ecol. Prog. Ser. **278:** 27–34.

Hamilton, S. K., J. L. Tank, D. F. Raikow, E. R. Siler, N. J. Dorn, and N. E. Leonard. 2004. The role of instream vs. allochthonous N in stream food webs: Modeling the results of an isotope addition experiment. J. N. Am. Benthol. Soc. **23:** 429–448.

Heinz, P., H. Kitazato, G. Schmiedl, and C. Hemleben. 2001. Response of deep-sea benthic foraminifera from the Mediterranean Sea to simulated phytoplankton pulses under laboratory conditions. J. Foramin. Res. **31:** 210–227.

Herman, P. M. J., J. J. Middelburg, J. Widdows, C. H. Lucas, and C. H. R. Heip. 2000. Stable isotopes as trophic tracers: Combining field sampling and manipulative labeling of food resources for macrobenthos. Mar. Ecol. Prog. Ser. **204:** 79–92.

Hsieh, H., C. Chen, Y. Chen, and H. Yang. 2002. Diversity of benthic organic matter flows through polychaetes and crabs in a mangrove estuary: $\delta^{13}C$ and $\delta^{34}S$ signals. Mar. Ecol. Prog. Ser. **227:** 145–155.

Hughes, J. E., L. A. Deegan, B. J. Peterson, R. M. Holmes, and B. Fry. 2000. Nitrogen flow through the food web in the oligohaline zone of a New England estuary. Ecology **81:** 433–452.

Kneib, R. T. 1997. The role of tidal marshes in the ecology of estuarine nekton. Oceanogr. Mar. Biol. Ann. Rev. **35:** 163–220.

Kristensen, E. 2008. Mangrove crabs as ecosystem engineers: With emphasis on sediment processes. J. Sea Res. **59:** 30–43.

Lee, S. Y. 1998. Ecological role of grapsid crabs in mangrove ecosystems: A review. Mar. Freshw. Res. **49:** 335–343.

Melville, A. J., and R. M. Connolly. 2005. Food webs supporting fish over subtropical mudflats are based on transported organic matter not in situ microalgae. Mar. Biol. **148:** 363–371.

Micheli, F. 1993. Feeding ecology of mangrove crabs in North Eastern Australia: Mangrove litter consumption by *Sesarma messa* and *Sesarma smithii*. J. Exp. Mar. Biol. Ecol. **171:** 165–186.

Middelburg, J. J., C. Barranguet, H. T. S. Boschker, P. M. J. Herman, T. Moens, and C. H. R. Heip. 2000. The fate of intertidal microphytobenthos carbon: An in situ ^{13}C-labeling study. Limnol. Oceanogr. **45:** 1224–1234.

Moens, T. C., C. Luyten, J. J. Middelburg, P. M. J. Herman, and M. Vincx. 2002. Tracing organic matter sources of estuarine tidal flat nematodes with stable carbon isotopes. Mar. Ecol. Prog. Ser. **234:** 127–137.

Moodley, L., H. T. S. Boschker, J. J. Middelburg, R. Pel, P. M. J. Herman, E. de Deckere, and C. H. R. Heip. 2000. Ecological significant of benthic foraminifera: ^{13}C labeling experiments. Mar. Ecol. Prog. Ser. **202:** 289–295.

———, J. J. Middelburg, H. T. S. Boschker, G. C. A. Duineveld, R. Pel, P. M. J. Herman, and C. H. R. Heip. 2002. Bacteria and foraminifera: Key players in a short-term deep-sea benthic response to phytodetritus. Mar. Ecol. Prog. Ser. **236:** 23–29.

Oakes, J. M., A. T. Revill, R. M. Connolly, and S. I. Blackburn. 2005. Measuring carbon isotope ratios of microphytobenthos through compound-specific stable isotope analysis of phytol. Limnol. Oceanogr. Meth. **3:** 511–519.

Odum, W. E., and E. J. Heald. 1975. The detritus-based food web of an estuarine mangrove community, p. 265–286. *In* L. E. Cronin [ed.], Estuarine research. Academic Press.

Newell, R. I. E., N. Marshall, A. Sasekumar, and V. C. Chong. 1995. Relative importance of benthic microalgae, phytoplankton, and mangroves as sources of nutrition for penaeid prawns and other coastal invertebrates from Malaysia. Mar. Biol. **123:** 595–606.

Pawlosky, R. J., J. R. Hibbeln, J. A. Novotny, and N. Salem. 2001. Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. J. Lipid Res. **42:** 1257–1265.

Raikow, D. F., and S. K. Hamilton. 2001. Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. Limnol. Oceanogr. **46:** 514–522.

Rodelli, M. R., J. N. Gearing, P. J. Gearing, N. Marshall, and A. Sasekumar. 1984. Stable isotope ratios as a tracer of mangrove carbon in Malaysian ecosystems. Oecologia **61:** 326–333.

Salini, J. P., S. J. M. Blaber, and D. T. Brewer. 1990. Diets of piscivorous fishes in a tropical Australian estuary, with special reference to predation on penaeid prawns. Mar. Biol. **105:** 363–374.

Sheaves, M., and B. Molony. 2000. Short-circuit in the mangrove food chain. Mar. Ecol. Prog. Ser. **199:** 97–109.

Skov, M. W., and R. G. Hartnoll. 2002. Paradoxical selective feeding on a low-nutrient diet: Why do mangrove crabs eat leaves? Oecologia **131:** 1–7.

Stefanovski, D., P. J. Moate, and R. C. Boston. 2003. WinSAAM: A Windows-based compartment modeling system. Metabolism **52:** 1153–1166.

Tenore, K. R., L. M. Cammen, S. E. Findlay, and N. W. Phillips. 1982. Perspectives of research on detritus: Do factors controlling the availability of detritus to macroconsumers depend on its source? J. Mar. Res. **40:** 473–490.

Van Dongen, B. E., S. Schouten, and J. S. S. Damste. 2002. Carbon isotope variability in monosaccharides and lipids of aquatic algae and terrestrial plants. Mar. Ecol. Prog. Ser. **232:** 83–92.

Van Oevelen, D., L. Moodley, K. Soetart, and J. J. Middelburg. 2006. The trophic significance of bacterial carbon in a marine intertidal sediment: Results of an in situ stable isotope labeling study. Limnol. Oceanogr. **51:** 2349–2359.

Wastney, M. E., B. H. Patterson, O. A. Linares, P. C. Greif, and R. C. Boston. 1999. Investigating biological systems using modeling: Strategies and software. Academic Press.

Werry, J., and S. Y. Lee. 2005. Grapsid crabs mediate link between mangrove litter production and estuarine planktonic food chains. Mar. Ecol. Prog. Ser. **293:** 165–176.

Winning, M. A., R. M. Connolly, N. R. Loneragan, and S. E. Bunn. 1999. ^{15}N enrichment as a method of separating the isotopic signatures of seagrass and its epiphytes for food web analysis. Mar. Ecol. Prog. Ser. **189:** 289–294.

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