Limnol. Oceanogr., 49(4), 2004, 1144–1151

Tissue carbon, nitrogen, and sulfur stable isotope turnover in transplanted *Bathymodiolus childressi* mussels: Relation to growth and physiological condition

S. Dattagupta[1], *D. C. Bergquist,*[2] *and E. B. Szalai*[3]
Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

S. A. Macko
Department of Environmental Sciences, University of Virginia, Charlottesville, Virginia 22903

C. R. Fisher
Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802

Abstract

The growth and physiological condition of the methanotrophic hydrocarbon seep mussel *Bathymodiolus childressi* reflects the habitat quality of the sites it occupies on the seafloor of the upper Louisiana slope of the Gulf of Mexico. Here, tissue stable isotope compositions, growth, and physiological health of *B. childressi* mussels transplanted between different seep sites changed within a year to reflect conditions at the new environment. Tissue stable carbon and nitrogen isotope turnovers, although substantial, were not complete at the end of the 1-yr transplant period; they were strongly correlated with each other, and the extent of turnover of both varied by site and was related to the growth of the mussels. Carbon and nitrogen isotopic turnovers caused by metabolic tissue turnover were about 35% per year. This slow isotopic turnover in *B. childressi* is presumably due to its relatively slow growth and low metabolic rate. On the other hand, tissue stable sulfur isotope turnover was not correlated with either stable carbon or nitrogen isotope turnover or growth and was higher in the site with higher levels of sulfide in the environment. This indicated that tissue stable sulfur isotope turnover in these mussels is influenced by sulfide detoxification activities.

The study of tissue stable isotope turnover is a powerful approach to analyzing dietary and environmental factors that can affect an organism's metabolic processes, such as tissue growth and replacement. In general, tissue stable isotope ratios of an animal reflect its nutritional resources, although small changes can occur without changes in diet as a result of seasonal variation in metabolism (Lorrain et al. 2002). Changes in diet resulting from migration (reviewed in Hobson [1999]), availability of prey (MacAvoy et al. 2001), or ontogenetic shifts (Herzka et al. 2001) can lead to concomitant changes in tissue stable isotope composition, provided the different diets have distinct stable isotope signatures. When an animal switches its diet, its tissue stable isotope composition begins to reflect its new diet at a rate proportional to its growth and metabolic turnover (Fry and Arnold 1982; Herzka et al. 2001; MacAvoy et al. 2001). Consequently, factors such as food availability or habitat conditions, which affect rates of tissue growth and replacement, are also likely to influence rates of tissue stable isotope turnover.

Bathymodiolus childressi is a widely distributed and numerically dominant mussel species at hydrocarbon seep sites on the seafloor of the upper Louisiana slope (ULS) of the Gulf of Mexico. The gills of this mussel contain methanotrophic endosymbionts (Childress et al. 1986) that provide it with the bulk of its nutrition (Fisher and Childress 1992; Streams et al. 1997). In fact, it is capable of growing in the presence of methane as a sole carbon and energy source (Cary et al. 1988), and based on its tissue carbon isotope value, most of its carbon is methane derived (Brooks et al. 1987). However, *B. childressi* is also able to filter feed, which might be important for acquiring essential organic nutrients not supplied by its endosymbionts (Page et al. 1990) and perhaps for obtaining bulk nitrogen (Pile and Young 1999). In addition to acquiring nitrogen through filter feeding, *B. childressi* can utilize other nitrogen resources, such as free amino acids, inorganic nitrates, or ammonia (Lee et al. 1992; Lee and Childress 1995, 1996). So far, dietary sulfur sources of *B. childressi* have not been investigated in detail. However, a study by Brooks et al. (1987) showed that the tissue $\delta^{34}S$ values of some *B. childressi* mussels from the ULS are more similar to the +20‰ $\delta^{34}S$ value of seawater sulfate than to the −12 to +2‰ $\delta^{34}S$ values of sulfide-utilizing tubeworms and clams living in the same area. This indicated that seawater sulfate might be an important source of sulfur for *B. childressi*.

[1] Corresponding author (szd103@psu.edu).
[2] Present address: University of Florida, Department of Fisheries and Aquatic Sciences, 7922 NW 71st Street, Gainesville, Florida 32653.

Acknowledgments

We thank the captain and crew of the Research Vessel (RV) *Edwin Link* as well as the crew and pilots of the Johnson Sea Link submersible (Harbor Branch Oceanographic Institution). We also thank R. W. Lee for helpful discussions. This manuscript has benefited significantly from suggestions made by S. E. MacAvoy, H. Sahling, and an anonymous reviewer. The Minerals Management Service, Gulf of Mexico Regional OCS office, through contract number 1435-01-96-CT30813, the NOAA National Undersea Research Program at the University of North Carolina, Wilmington, and National Science Foundation grant OCE0117050 supported this work.

The hydrocarbon seep environment on the ULS is patchy and variable, and *B. childressi* mussels at various sites experience a range of habitat conditions. Three sites inhabited by *B. childressi* are Brine Pool NR-1 (BP), GC234, and Bush Hill (BH). Whereas BH and GC234 are separated by less than a kilometer and are habitat-wise rather similar, BP is about 4 km away and is environmentally distinct from both BH and GC234. The pool of brine at BP is saturated with methane and lacks sulfide. Levels of sulfide and long-chain hydrocarbons are generally higher at GC234 and BH than at the most densely occupied part of the mussel bed at BP, whereas methane levels are generally higher at BP (Nix et al. 1995; Smith et al. 2000). These environmental parameters likely contribute to the larger maximal size, better health, and greater densities of *B. childressi* mussels at BP than at GC234 and BH (Nix et al. 1995; Smith et al. 2000). Moreover, the δ^{13}C value of bubbles of methane gas at BP is about −64‰, whereas it is about −49‰ at GC234 and −44‰ to −46‰ at BH (MacDonald et al. 1990; Sassen et al. 1999). Since the tissue δ^{13}C signatures of *B. childressi* mussels reflect their methane source (Brooks et al. 1987), the tissue δ^{13}C values of mussels at BP and GC234 (or BH) are very different (Kennicutt et al. 1992).

Because tissue stable carbon isotope compositions of *B. childressi* mussels from BP and GC234 (or BH) are distinct, we predicted that transplanting mussels between the different sites would enable us to measure site-specific, in situ tissue stable isotope turnover of *B. childressi*. By measuring growth rates in the same animals, we were able to separate the component of change in tissue stable isotope values due to tissue growth from that attributable to metabolic tissue turnover. Since mussels at BP are generally in better physiological condition than mussels at GC234 or BH, they are likely to have greater rates of tissue growth and repair. Therefore, we hypothesized that mussels transplanted from GC234 (or BH) to BP would undergo greater tissue stable isotope turnover than reciprocally transplanted mussels. In addition to providing new insights on the biology of *B. childressi*, this study adds substantially to our understanding of stable isotope turnover in general and to the limitations of tissue stable isotope analyses in hydrothermal vent and cold seep trophic studies (Tieszen et al. 1983; MacAvoy et al. 2001; Reilly and Hecky 2002).

Materials and methods

Study sites—BP is located at ~650 m depth on the ULS of the Gulf of Mexico (27°43.4′N, 91°16.5′W). This hypersaline (salinity = 121 g kg^{-1}) pool is ~22 m long and ~16 m wide and is surrounded by a large (3-7-m wide) continuous bed of *B. childressi* mussels, the inner rim of which is elevated a few centimeters above the surface of the brine (MacDonald et al. 1990). For this transplant study, we used mussels from the inner margin of this bed (inner BP), where concentrations of methane are high (42–626 μmol L^{-1}) and where sulfide is normally not detectable among the mussels (Smith et al. 2000). The salinity at siphon level of these mussels is rarely above 39 g kg^{-1} (Smith et al. 2000). It is important to note here that higher sulfide levels have been reported at the outer margin of the BP mussel bed (Smith et al. 2000), but we did not sample in that portion of the bed. The mussel bed at inner BP is densely packed with relatively fast-growing and healthy individuals (Smith et al. 2000).

The GC234 site is located at a depth of ~540 m on the ULS (27°44.7′N, 91°13.3′W). This site covers an area of several square kilometers and is dominated by large tubeworm aggregations. Mussel beds, when present, are associated with one or more tubeworm aggregations. Methane levels among mussels in these beds are variable (nondetectable to 10.7 mmol L^{-1}; Nix et al. 1995) and can sometimes be even higher than those found at the inner BP (Smith et al. 2000). Sulfide concentrations as high as 8 mmol L^{-1} have been detected among these mussels, and these values are associated with the high methane concentrations mentioned above (Nix et al. 1995). Moreover, oil globules are often released when mussel beds are disturbed at GC234 (Fisher pers. obs.). Previous studies found that the mussels at this site had lower physiological condition indices than inner BP mussels and did not grow as large as the BP mussels (Nix et al. 1995; Smith et al. 2000).

BH is located at a depth of ~540–580 m on the ULS (27°46.96′N, 91°30.46′W). Although much smaller in extent, this site is geochemically very similar to GC234, with relatively high sulfide levels (nondetectable to 56 μmol L^{-1}; Nix et al. 1995) and presence of oil in the mussel beds (Fisher pers. obs.). Previous studies found that the mussels at this site had very low physiological condition indices when compared to both BP and GC234 mussels and did not grow as large as the BP mussels (Nix et al. 1995; Smith et al. 2000).

Transplants—Two sets of reciprocal transplants of *B. childressi* mussels were conducted: one between mussel beds at GC234 and inner BP and another between mussel beds at BH and inner BP. Throughout this paper, the native site of the mussels is referred to as origin and the site to which they were transplanted as host. In September 1994, mussels were collected from the inner edge of the BP mussel community, a bed in GC234 (about 120 mussels each), and a bed in BH (75 mussels) using the Johnson Sea Link I manned submersible (Harbor Branch Oceanographic Institution). The mussels were transported to the surface in a temperature-insulated box attached to the front of the submersible. They were kept in chilled seawater (8°C) and processed in a cold room (8°C) on board the ship. The length of each mussel shell was measured to the nearest 0.1 mm using calipers and a commercial, color-coded, numbered larval fish tag was glued to its umbo for identification (Nix et al. 1995). Within 24 h after collection, the tagged mussels were transported in temperature-insulated containers filled with chilled seawater to the host site and deployed at the designated mussel bed. The mussels were retrieved 336 d later (August 1995) using the collection methods described above. The shell length of each mussel was measured, and marked individuals of a representative size range were subsampled from each collection. Tissue from subsampled mussels was processed for determination of physiological condition. Three indices of condition were determined: water content (as a percentage of wet weight), condition index (CI; a ratio of ash-free dry tissue mass–to-shell volume), and glycogen content (as a

percentage of wet weight) following the procedures of Smith et al. (2000). A subset of the tissue homogenates used for physiological condition analyses was used to determine stable isotope composition.

Control manipulations were not conducted during the same time period as the transplant experiments. Instead, control mussels were collected from inner BP and the mussel bed GC234 in June 1993 using the Johnson Sea Link I submersible. They were measured and tagged as described above for the transplant mussels and were deployed back to their original mussel bed. They were collected 456 d later (September 1994), and their tissues were processed in the same manner as the transplant mussels. The quality of these controls for site-to-site comparisons of growth and physiological condition is somewhat compromised, as small yearly differences in condition index and growth of *B. childressi* mussels from a single mussel bed have been observed (Smith et al. 2000). However, these yearly differences are of a much smaller magnitude than the differences between sites. To confirm that the tissue stable isotope compositions of mussels at our study sites did not change over the course of our study, we also determined the tissue stable isotope compositions of unmanipulated mussels collected from inner BP and GC234 in August 1995. We compared the stable isotope compositions of these two sets of control mussels (manipulated and unmanipulated) and found no significant difference between their tissue $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ values. Therefore, we used the mean values of tissue stable isotope compositions of the two sets of controls for further analyses. No control manipulations were performed on mussels from BH. Therefore, for BH controls, we analyzed tissue stable isotope compositions and physiological condition indices of unmanipulated mussels retrieved from BH in August 1995. No growth data were available for the BH controls.

Stable isotope analyses—Gill and mantle tissues of individual mussels were homogenized to a 10× dilution in distilled, deionized water using a Brinkman PT3000 Polytron tissue homogenizer. Approximately 1-ml samples of these homogenates were incubated in an oven (60°C) until dry and were then ground into a fine powder with a mortar and pestle. The powder was transferred into tin capsules (1 mg of powder for $\delta^{13}C$ and $\delta^{15}N$ analyses, 6 mg of powder for $\delta^{34}S$ analyses) and the samples were converted to CO_2, N_2, and SO_2 for isotope analyses using a Carlo Erba Elemental Analyzer (EA) coupled to an OPTIMA stable isotope ratio mass spectrometer (Micromass). Carbon and nitrogen isotopic compositions were determined on a single combustion using a dual furnace system composed of an oxidation furnace at 1,020°C and a reduction furnace at 650°C. Using the EA, the samples for sulfur were separately pyrolyzed at 1,050°C using a single furnace combination oxidation and reduction system. The resulting gases were chemically dried and directly injected into the source of the mass spectrometer using continuous flow. The stable isotopic ratio is reported as follows:

$$\delta^{M}E = [R_{sample}/R_{standard} - 1] \times 10^{3} \quad (‰) \qquad (1)$$

where M is the atomic mass of the heavy isotope of the element E, and R is the abundance ratio of the heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$) of that element.

The standards used for carbon, nitrogen, and sulfur are PeeDee Belemnite limestone (PDB), atmospheric N_2 (air), and Canyon Diablo Triolite (CDT), respectively. For sulfur and carbon, the values are corrected for the influence of oxygen isotopes. The reproducibility of the measurement for these elements is typically better than ±0.2‰ using the continuous flow interface on the OPTIMA. In the laboratory, the samples are commonly measured against tanks of carbon dioxide, nitrogen, and sulfur dioxide gases that have been calibrated against NBS 22, atmospheric N_2, and NBS 127, respectively.

Data analyses—To ensure normality, all measurements for condition were transformed using an arcsine function ($y' = 2 \arcsin[y^{1/2}]$) before statistical analysis (Neter et al. 1996). Significant differences between transplanted and control mussels from either the origin or the host site were tested using analysis of covariance, with length as a covariate (SAS Version 6.07, Proc GLM). If the model was significant ($P < 0.05$), pairwise comparisons were made using Tukey's correction for multiple comparisons (SAS Version 6.07, Proc GLM).

Differences in growth parameters between transplant and control populations were tested using a linear regression with the indicator variables in a Ford Walford plot (Walford 1946). Two methods were used in order to adjust for the different recapture intervals between control and experimental animals prior to applying the Ford Walford plots. In the first method, which assumes equal recapture intervals, no adjustment was made to the length data. This method overestimates the difference between the control and transplant regression lines because it assumes that the group with the shorter recapture interval would have achieved no additional growth if its recapture interval had been lengthened to match the other group. Growth calculated by this method is referred to as unadjusted growth. In the second method, the final shell length of all mussels was standardized to 365 d by assuming equal growth per day for each individual. This method may underestimate the difference between the control and transplant regression lines if periods of slow growth coincide with the time period missed by the group with the shorter recapture interval. Growth calculated by this method is referred to as adjusted growth. Agreement between the results of the two correction methods increases our confidence in the conclusions.

Significant differences between stable isotope compositions of the two sets of control mussels and the transplant and control mussels were tested using two-tailed Student's *t*-tests at a significance level of 0.05 (Minitab Release 12.21, Minitab). Where multiple comparisons were made, the significance level was adjusted using the sequential Bonferroni method (Rice 1989). For each transplant mussel, amount of change in tissue stable isotope value of element E ($\Delta\delta^{M}E$) was calculated as

$$\Delta\delta^{M}E = |(\delta^{M}E \text{ of transplant mussel}) - (\text{mean } \delta^{M}E \text{ of origin control mussels})| \qquad (2)$$

The relationship between $\Delta\delta^{M}E$ and growth was tested by a linear regression between $\Delta\delta^{M}E$ values and values of per-

Table 1. Mean (standard deviation) of final shell length, condition index (CI), glycogen content, and water content of transplant and control mussels. n = sample size. Values with different letters indicate a significant difference ($P < 0.05$) between them (statistical tests were performed on the arcsine-transformed values).

Mussel type	n	Length (mm)	CI (g ml^{-3})	% glycogen	% water
BP controls	24	63 (24)	0.10 (0.03)^{a}	1.7 (0.6)^{a}	84.3 (3.2)^{a}
BP to GC234	6	60 (19)	0.06 (0.02)^{b}	0.2 (0.2)^{b}	88.8 (1.5)^{b}
GC234 to BP	17	62 (11)	0.07 (0.02)^{a}	0.3 (0.3)^{c}	83.5 (3.6)^{a}
GC234 controls	6	51 (20)	0.06 (0.02)^{b}	0.1 (0.2)^{b}	87.5 (2.7)^{b}
BP to BH	6	70 (28)	0.04 (0.01)^{b}	0.1 (0.2)^{b}	90.9 (1.7)^{b}
BH to BP	5	60 (10)	0.08 (0.01)^{a}	0.3 (0.1)^{c}	84.6 (1.8)^{a}
BH controls	6	58 (16)	0.04 (0.01)^{b}	0.04 (0.01)^{b}	92.2 (1.6)^{b}

cent change in shell length (%CL). %CL values were used as indicators of size-specific growth, and were calculated as

$$\%\mathrm{CL} = \left(\frac{\text{change in shell length}}{\text{initial shell length}}\right) \times 100 \qquad (3)$$

Here, values of shell growth were standardized to 365 d, assuming a constant daily growth rate. In order to correct for any potential size-related effects, relationships between tissue δ^{13}C, δ^{15}N, or δ^{34}S and final shell lengths of transplant and control mussels were investigated. No such relationship was found.

To estimate extents of isotopic turnover in populations of transplant mussels, the amount of change in mean δ^{M}E ($\Delta\delta^{M}E_{mean}$) of the transplant population was calculated as a percent of the total difference ($\Delta\delta^{M}E_{total}$) between the mean δ^{M}E of the origin and the host site controls. Here,

$$\Delta\delta^{M}E_{mean} = |(\text{mean } \delta^{M}E \text{ of transplant mussels}) - (\text{mean } \delta^{M}E \text{ of origin control mussels})| \qquad (4)$$

and

$$\Delta\delta^{M}E_{total} = |(\text{mean } \delta^{M}E \text{ of host control mussels}) - (\text{mean } \delta^{M}E \text{ of origin control mussels})| \qquad (5)$$

Percent change in mean δ^{M}E ($\%\Delta\delta^{M}E$) of each transplant population was calculated as

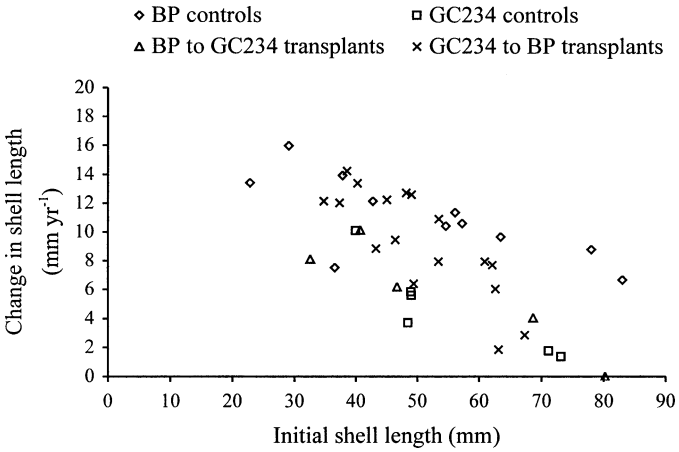

Fig. 1. Change in shell length standardized to one year (adjusted shell growth) versus initial shell length of transplant and control mussels from the reciprocal transplant between BP and GC234.

$$\%\Delta\delta^{M}E = (\Delta\delta^{M}E_{mean}/\Delta\delta^{M}E_{total}) \times 100 \qquad (6)$$

The above values of $\%\Delta\delta^{M}E$ estimate isotopic turnover of transplant animals caused by both shell (and tissue) growth and metabolic tissue turnover. To estimate the amount of isotopic turnover due to metabolic tissue turnover alone (i.e., in the absence of growth), the mean value of $\Delta\delta^{M}E$ in the absence of shell growth ($\Delta\delta^{M}E_{O}$) was calculated for transplant populations as

$$\Delta\delta^{M}E_{O} = \text{‘y’-intercept of line relating } \Delta\delta^{M}E \text{ and \%CL} \qquad (7)$$

To allow direct comparisons between different transplant populations, the ‘y’-intercept was determined from the regression lines made in the %CL range of 0% to 25%, the %CL range common to all transplant populations for which there was a correlation between $\Delta\delta^{M}E$ and %CL. Percent change in $\delta^{M}E$ in the absence of shell growth ($\%\Delta\delta^{M}E_{O}$) was then calculated as:

$$\%\Delta\delta^{M}E_{O} = (\Delta\delta^{M}E_{O}/\Delta\delta^{M}E_{total}) \times 100 \qquad (8)$$

Results

Growth and condition indices—BP control mussels had significantly higher condition index (CI) and glycogen content and significantly lower water content than either BH or GC234 control mussels (Table 1), which is consistent with previously reported results (Nix et al. 1995; Smith et al. 2000). They also grew significantly more than GC234 control mussels (Fig. 1; $P < 0.001$ for adjusted and unadjusted growth).

Growth and physiological condition of transplanted mussels tended to resemble those of their host populations. All three physiological condition indices of mussels transplanted from BP to either GC234 or BH were not significantly different from those of their host populations but were significantly different from those of their origin populations (Table 1). Growth of BP to GC234 transplant mussels was not significantly different from that of their host population ($P = 0.117$ and 0.349 for adjusted and unadjusted growth, respectively), but was significantly different from that of their origin population ($P < 0.001$ for both adjusted and unadjusted growth; Fig. 1). Similarly, all three physiological condition indices of mussels transplanted from either GC234 or BH to BP were significantly different from those of their origin populations, and all variables except glycogen content of these mussels were not significantly different from those

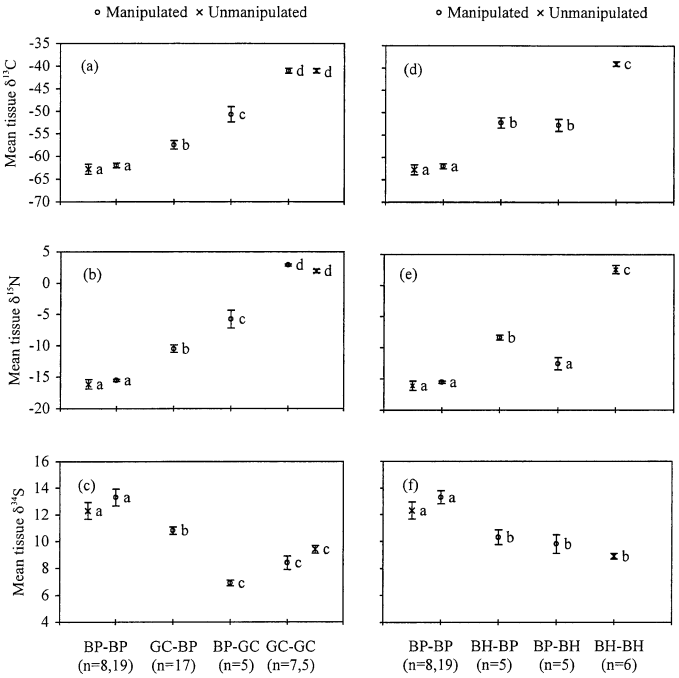

Fig. 2. Mean values of tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of transplant and control mussels from the reciprocal transplant between (a–c) BP and GC234 and (d–f) BP and BH. Notations for transplants and controls have the name of the origin site first, followed by the name of the host site. For example, BP-BP represents BP controls, and GC-BP represents GC234 to BP transplants. Controls were of two types: manipulated and unmanipulated (*see* text for details). n = sample size. Error bars represent standard error. Different letters indicate a significant ($P < 0.05$) difference between data points.

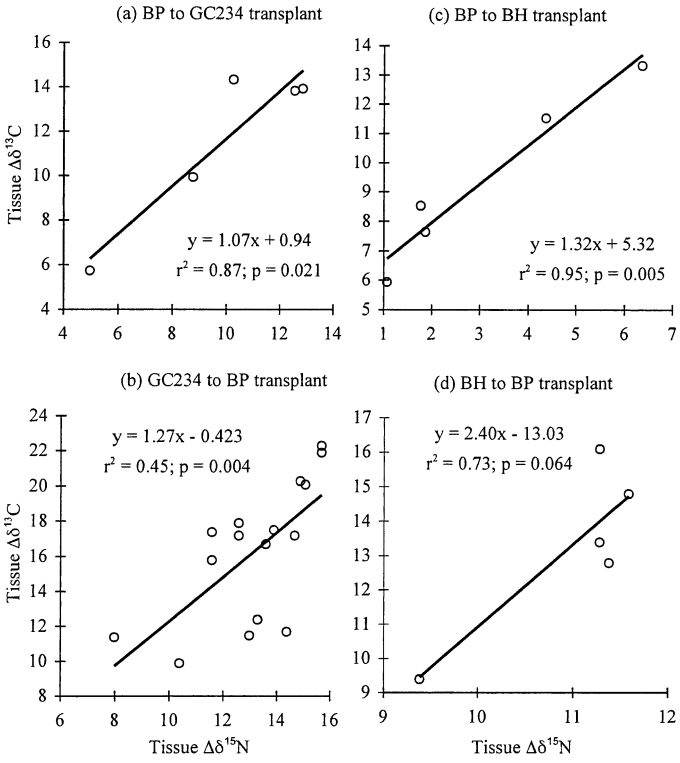

Fig. 3. Relationships between tissue $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ values of individual mussels belonging to the transplants between BP and GC234 or BH.

of their host populations (Table 1). Growth of GC234 to BP transplant mussels was intermediate between and significantly different from that of both their host and origin populations (Fig. 1). Growth of mussels transplanted from BP to BH and from BH to BP was significantly lower than that of BP control mussels ($P < 0.001$ for both adjusted and unadjusted growth; data not shown), and very little growth was observed for BP to BH transplant mussels. No growth data were available for BH control mussels, so comparisons could not be made.

Stable isotope analyses—The respective tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of BH and GC234 control mussels were not significantly different. However, tissue $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of BP control mussels were all significantly different from those of BH and GC234 mussels. The mean tissue $\delta^{13}C$ values of BP, BH, and GC234 control mussels (−62‰, −39‰, and −41‰, respectively; Fig. 2a,d) reflected the $\delta^{13}C$ values of methane sources at the three sites (Brooks et al. 1987; Kennicutt et al. 1992), with tissue $\delta^{13}C$ values of BP control mussels being significantly more depleted than those of either BH or GC234 control mussels ($t = 32.58$; df = 14; $P < 0.00001$; and $t = 36.3$; df = 36; $P < 0.00001$, respectively). Whereas mean tissue $\delta^{15}N$ values of BH and GC234 control mussels were +2.6‰ and +2.9‰, respectively, BP control mussels had a mean tissue $\delta^{15}N$ value of −15.5‰, which was significantly more depleted than both ($t = 26.26$; df = 6; $P < 0.00001$; and $t = 52.7$; df = 32; $P < 0.00001$, respectively; Fig. 2b,e). Conversely, BH and GC234 control mussels had significantly ($t = -7.56$; df = 29; $P < 0.0001$; and $t = 6.87$; $df = 35$; $P < 0.0001$, respectively) more depleted mean tissue $\delta^{34}S$ values (+8.9‰ and +8.4‰, respectively) than that of BP control mussels (+13.1‰; Fig. 2c,f).

All three stable isotope ratios of transplanted mussels resembled those of their host populations more than those of their origin populations (Fig. 2), indicating that tissue stable isotope turnover occurred in all transplants between BH or GC234 and BP. The mean tissue $\delta^{13}C$ and $\delta^{15}N$ values of transplanted mussels were intermediate between and significantly different ($P < 0.05$) from those of both their host and origin populations, except tissue $\delta^{15}N$ values of the BP to BH transplant mussels, which were not significantly different from those of their origin population. While the mean tissue $\delta^{34}S$ values of GC234 or BH to BP transplant mussels followed a similar pattern, the mean $\delta^{34}S$ values of BP to GC234 or BH transplant mussels were not significantly different from those of their host populations (BP to GC234: $t = 2.35$; df = 3; $P = 0.10$; BP to BH: $t = 1.35$; df = 3; $P = 0.27$).

Changes in stable carbon isotope ratios ($\Delta\delta^{13}C$; Eq. 2) of individual mussels transplanted between BP and GC234 were significantly ($P < 0.05$) correlated with changes in stable nitrogen ratios ($\Delta\delta^{15}N$) (Fig. 3). However, changes in sulfur stable isotope ratios ($\Delta\delta^{34}S$) of these mussels were not significantly correlated with either $\Delta\delta^{13}C$ or $\Delta\delta^{15}N$ values

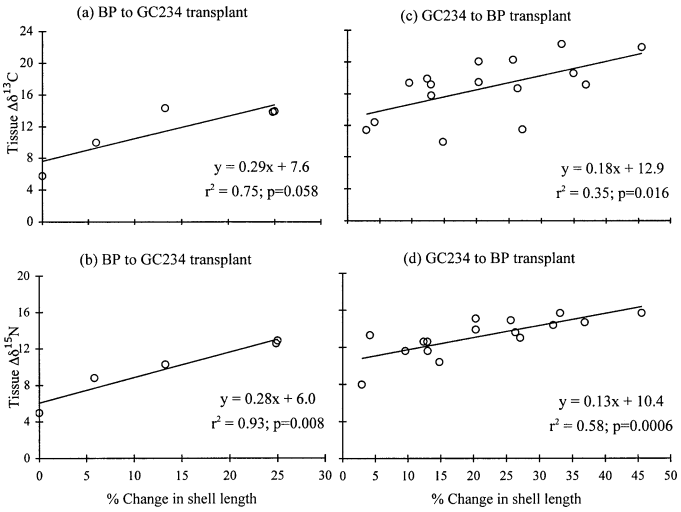

Fig. 4. Relationships between changes in carbon or nitrogen stable isotope values and shell growth represented by percent change in shell length (%CL) for BP to GC234 transplant mussels and GC234 to BP transplant mussels. a, c) Tissue $\Delta\delta^{13}$C versus %CL; and (b, d) tissue $\Delta\delta^{15}$N versus %CL.

(BP to GC234: $P = 0.108$ and 0.290; GC234 to BP: $P = 0.525$ and 0.900 for relationships with $\Delta\delta^{13}$C and $\Delta\delta^{15}$N, respectively). Further, a strong correlation was found between $\Delta\delta^{13}$C and $\Delta\delta^{15}$N values of transplant mussels and size-specific shell growth (%CL; Fig. 4), and this relationship was significant ($P < 0.05$), except in the case of $\Delta\delta^{13}$C of BP to GC234 transplant mussels ($P = 0.058$). $\Delta\delta^{34}$S and %CL were not significantly correlated for either transplant (BP to GC234: $P = 0.346$; GC234 to BP: $P = 0.601$).

Similar results were obtained for mussels transplanted between BP and BH. $\Delta\delta^{13}$C values of transplant mussels were correlated with $\Delta\delta^{15}$N values (BP to BH: $P = 0.005$; BH to BP: $P = 0.064$; Fig. 3). Again, no correlation was found between $\Delta\delta^{34}$S values and either $\Delta\delta^{13}$C or $\Delta\delta^{15}$N values of these mussels (BP to BH: $P = 0.367$ and 0.503; BH to BP: $P = 0.481$ and 0.407 for relationships with $\Delta\delta^{13}$C and $\Delta\delta^{15}$N, respectively). $\Delta\delta^{13}$C and $\Delta\delta^{34}$S values of BH to BP transplant mussels were not significantly correlated with %CL values ($P = 0.837$ and 0.329 for C and S, respectively), but the correlation between $\Delta\delta^{15}$N of these mussels and %CL was significant ($P = 0.045$). Growth of BP to BH transplant mussels was almost always negligible, and changes in tissue stable isotope compositions and %CL values were not significantly correlated for these mussels.

The relative amounts of tissue stable isotope turnover of transplant mussels were compared using values of percent changes in mean stable isotope ratios (%$\Delta\delta^{13}$C, %$\Delta\delta^{15}$N, %$\Delta\delta^{34}$S; Eq. 6). Mussel populations transplanted from either GC234 or BH to BP had higher total %$\Delta\delta^{13}$C and %$\Delta\delta^{15}$N and lower total%$\Delta\delta^{34}$S than the respective BP to GC234 or BH transplant populations (Table 2). Mussel populations transplanted between BP and GC234 had greater tissue stable isotope turnovers than those transplanted between BP and BH.

Tissue stable carbon and nitrogen isotope turnover resulting from metabolic tissue turnover (Table 2) was calculated using the 'y'-intercepts of lines relating $\Delta\delta^{13}$C or $\Delta\delta^{15}$N to %CL (Eq. 8). The 0% to 25% range of %CL values was common to all transplant populations. Thus, the 'y'-intercepts of regression lines corresponding to the GC234 to BP and BH to BP transplants were calculated using a subset of data points in this %CL range (GC234 to BP: $\Delta\delta^{13}$C vs. %CL: $y = 34x + 11.3$; $P = 0.03$; $r^2 = 0.46$; $\Delta\delta^{15}$N vs. %CL: $y = 21x + 9.5$; $P = 0.02$; $r^2 = 0.50$; BH to BP: $\Delta\delta^{15}$N vs. %CL: $y = 0.25x + 9.74$; $P = 0.045$; $r^2 = 67.4\%$). These calculations indicate that about 34–56% of carbon and nitrogen isotopic turnover of these mussels was due to metabolic tissue replacement. Tissue stable sulfur turnover due to metabolic tissue replacement was not estimated, as the relation between $\Delta\delta^{34}$S and %CL was not significant for any of the transplants ($P > 0.3$).

Discussion

Transplanted mussels underwent significant changes in physiological health and isotopic composition within a year (Table 1; Fig. 2). Growth and condition data for BP, GC234, and BH control mussels were consistent with that of earlier studies and confirm that the inner edge of BP is more favorable for *B. childressi* mussels than either GC234 or BH (Nix et al. 1995; Smith et al. 2000). When transplanted to BP, the physiological condition of both GC234 and BH mussels improved significantly (Table 1) to mirror their host site. GC234 to BP transplant mussels also grew significantly more compared to their origin population (Fig. 1). On the other hand, BP mussels significantly deteriorated in physiological condition (Table 1) when transplanted to either GC234 or BH, and BP to GC234 transplant mussels grew

Table 2. Percent change in the tissue stable isotope ratios of transplant mussels in the presence and absence of shell growth during the study period of about 1 yr. Values were calculated using Eqs. 6 and 8.

	Average values			At zero shell growth	
Transplant	%$\Delta\delta^{13}$C	%$\Delta\delta^{15}$N	%$\Delta\delta^{34}$S	%$\Delta\delta^{13}C_o$	%$\Delta\delta^{15}N_o$
BP to GC234	54.2	55.0	156.7	35.9**	33.6
GC234 to BP	77.8	71.1	51.3	53.3	52.6
BP to BH	40.5	17.9	76.3	—*	—*
BH to BP	57.3	63.6	36.8	—†	56.3

* These values could not be calculated as above, as growth of all BP to BH transplant mussels was negligible (<0.1 %CL).
† This value was not calculated, as the line relating $\Delta\delta^{13}$C and %CL of BH to BP transplant mussels was not significant ($P = 0.8$; $r^2 = 0.01$).
** The *P*-value for the line relating $\Delta\delta^{13}$C and %CL of BP to GC234 transplant mussels was 0.058.

significantly less than their origin population (Fig. 1). The change in physiological condition indices upon transplantation to a less favorable habitat is reminiscent of the deterioration in condition of hydrothermal vent mussels when transplanted away from hydrothermal flow (Smith 1985). In addition to improving physiological condition, GC234 to BP and BH to BP transplant mussels experienced greater tissue carbon and nitrogen stable isotope turnover than BP to GC234 and BP to BH transplant mussels, respectively (Table 2). Thus, the amount of tissue carbon and nitrogen stable isotope turnover in *B. childressi* mussels is likely to be linked to conditions at the host site through changes in growth and physiological condition.

In this study, a correlation between tissue $\Delta\delta^{13}C$ and $\Delta\delta^{15}N$ of individual transplant mussels was observed (Fig. 3), indicating similar and perhaps coupled dynamics for carbon and nitrogen turnover in *B. childressi* tissue. The extent of an organism's tissue isotopic turnover is a function of both tissue growth and metabolic tissue replacement (Fry and Arnold 1982; Herzka et al. 2001; MacAvoy et al. 2001). The significant correlation between $\Delta\delta^{13}C$ or $\Delta\delta^{15}N$ and shell growth of mussels transplanted between BP and GC234 illustrates the relation between growth and tissue carbon and nitrogen stable isotope turnover in *B. childressi.* The slopes of the lines ($\Delta\delta^{13}C$ or $\Delta\delta^{15}N$ vs. %CL) corresponding to the BP to GC234 and GC234 to BP transplants were somewhat similar, indicating that growth-induced carbon and nitrogen stable isotope turnovers were similar at the two sites.

In order to deduce the amount of isotopic turnover due to metabolic tissue replacement alone, we estimated stable isotope turnover in mussels with no growth. It is important to note that this estimate is based on shell growth, which is not necessarily a good predictor of tissue growth, as mussels may improve their physiological condition and increase tissue mass without any change in shell length. The physiological conditions of BP to GC234 transplant mussels deteriorated significantly on transplantation (Table 1), and so they are unlikely to have added tissue mass in the absence of shell growth. Therefore, the tissue stable isotope turnovers in BP to GC234 transplant mussels with no shell growth ($\%\Delta\delta^{M}E_{O}$; Eq. 8), which were 36% and 34% per year for carbon and nitrogen, respectively (Table 2), are estimates of tissue stable isotope turnover due to metabolic turnover alone, albeit in mussels of compromised condition. On the other hand, GC234 to BP transplant mussels increased their condition index on transplantation (Table 1), and so they may have increased their tissue mass without increasing their shell length. Thus, the $\%\Delta\delta^{M}E_{O}$ values for these mussels (53% to 54%, Table 2) are likely to be overestimates of tissue stable isotope turnover due to metabolic turnover alone. The close agreement between the independently derived estimates of $\%\Delta\delta^{13}C_{O}$ and $\%\Delta\delta^{15}N_{O}$ for a particular transplant argues for the validity of these parameters.

The pattern of sulfur stable isotope turnover in *B. childressi* mussels was distinctly different from that of carbon and nitrogen; $\Delta\delta^{34}S$ of individual transplant mussels was not correlated with either $\Delta\delta^{13}C$ or $\Delta\delta^{15}N$, and there was no apparent relationship between $\Delta\delta^{15}S$ and growth. The relative degree of sulfur stable isotope turnover in the transplant populations was also opposite to what was observed for carbon and nitrogen (Table 2). Although it was complete at the end of the transplant period in the BP to GC234 transplant population, it was only 50% complete for the GC234 to BP transplant population. Similarly, sulfur isotopic turnover was about 77% for the BP to BH transplant population and only about 37% for the BH to BP transplant population. These findings indicate that *B. childressi* mussels may incorporate sulfur through a process other than tissue growth and replacement. As mentioned earlier, mussels at both GC234 and BH are likely to be exposed to higher levels of sulfide compared to mussels on the inner margin of BP (Nix et al. 1995; Smith et al. 2000). Thus, mussels transplanted from BP to GC234 (or BH) may encounter high levels of sulfide in their new environment. *B. childressi* mussels are not known to utilize sulfide as an energy source; on the contrary, they produce sulfur-containing compounds such as hypotaurine in order to detoxify environmental sulfide (Pruski et al. 2000). Therefore, BP to GC234 (or BH) transplant mussels may start accumulating sulfide-detoxification compounds (Pruski et al. 2000), which may explain the relatively rapid sulfur isotope turnover observed in these mussels. The low rates of sulfur isotopic turnover in the reciprocal transplants may also be explained by remobilization of the sulfur from these detoxification compounds to meet metabolic sulfur demands.

As a result of the difficulty of collecting direct observational data, stable isotope analyses have been used extensively to obtain nutritional information on deep-sea hydrothermal vent and hydrocarbon seep organisms (Kennicutt et al. 1992; Conway et al. 1994; Fisher 1995). However, the study of trophic relationships using stable isotopes may be obscured by slow rates of stable isotope turnover (Tieszen et al. 1983). Reports of tissue stable isotope turnover rates are rare. Studies of red drum larvae (Herzka and Holt 2000), krill (Frazer et al. 1997), brine shrimp (Fry and Arnold 1982), and gerbils (Tieszen et al. 1983) have shown high rates of tissue stable isotope turnover associated with high growth and metabolic rates in these organisms. In contrast, studies of broad whitefish (Hesslein et al. 1993) and catfish (MacAvoy et al. 2001) found relatively slow tissue stable isotope turnover, which was attributed to slow metabolism and growth in these animals.

To the best of our knowledge, this article is the first to report tissue stable isotope turnover in a deep-sea organism. We found that the period of a year was not sufficient for the tissues of *B. childressi* to completely express the stable isotope signature of a new, isotopically distinct dietary source. This reflects a much slower isotopic turnover rate in tissues of *B. childressi* mussels than in tissues of some fast-growing organisms studied so far. For example, shrimp have shown carbon isotopic half-lives ranging between 4 and 19 d (Fry and Arnold 1982), larval krill have shown replacement of 22–29% of their carbon and 13–22% of their nitrogen at the end of 8–10 weeks (Frazer et al. 1997), and red drum larvae have shown almost complete carbon and nitrogen isotopic turnover at the end of 15 d (Herzka and Holt 2000). The slow isotopic turnover rate of *B. childressi* likely reflects its relatively slow growth and low metabolic rate, characteristics that are typical of a benthic bivalve living in a low-temperature (6–8°C) environment.

References

BROOKS, J. M., M. C. KENNICUTT II, C. R. FISHER, S. A. MACKO, K. COLE, J. J. CHILDRESS, R. R. BIDIGARE, AND R. D. VETTER. 1987. Deep-sea hydrocarbon seep communities: Evidence for energy and nutritional carbon sources. Science **238:** 1138–1142.

CARY, S. C., C. R. FISHER, AND H. FELBECK. 1988. Mussel growth supported by methane as sole carbon and energy source. Science **240:** 78–80.

CHILDRESS, J. J., C. R. FISHER, J. M. BROOKS, M. C. KENNICUTT II, R. BIDIGARE, AND A. E. ANDERSON. 1986. A methanotrophic marine Molluscan (Bivalvia, Mytilidae) symbiosis: Mussels fueled by gas. Science **233:** 1306–1308.

CONWAY, N. M., M. C. KENNICUTT II, AND C. L. VAN DOVER. 1994. Stable isotopes in the study of marine chemosynthetic-based ecosystems, p. 158–186. *In* K. Lajtha and R. H. Michener [eds.], Stable isotopes in ecology and environmental science. Blackwell Scientific.

FISHER, C. R. 1995. Toward an appreciation of hydrothermal-vent animals: Their environment, physiological ecology, and tissue stable isotope values. Geophys. Mono. **91:** 297–316.

———, AND J. CHILDRESS. 1992. Organic carbon transfer from methanotrophic symbionts to the host hydrocarbon-seep mussel. Symbiosis **12:** 221–235.

FRAZER, T. K., R. M. ROSS, L. B. QUETIN, AND J. P. MONTOYA. 1997. Turnover of carbon and nitrogen during growth of larval krill, *Euphausia superba* Dana: A stable isotope approach. J. Exp. Biol. Ecol. **212:** 259–275.

FRY, B., AND C. ARNOLD. 1982. Rapid $^{13}C/^{12}C$ turnover during growth of brown shrimp (*Penaeus aztecus*). Oecol. Berlin **54:** 200–204.

HERZKA, S. Z., AND G. J. HOLT. 2000. Changes in isotopic composition of red drum (*Sciaenops ocellatus*) larvae in response to dietary shifts: Potential applications to settlement studies. Can. J. Fish. Aquat. Sci. **57:** 137–147.

———, S. A. HOLT, AND G. J. HOLT. 2001. Documenting the settlement history of individual fish larvae using stable isotope ratios: Model development and validation. J. Exp. Mar. Biol. Ecol. **265:** 49–74.

HESSLEIN, R. H., K. A. HALLARD, AND P. RAMLAL. 1993. Replacement of sulfur, carbon and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$ and $\delta^{15}N$. Can. J. Fish. Aquat. Sci. **50:** 2071–2076.

HOBSON, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: A review. Oecologia **120:** 314–326.

KENNICUTT II, M. C., R. A. BURKE, JR., I. R. MACDONALD, J. M. BROOKS, G. J. DENOUX, AND S. A. MACKO. 1992. Stable isotope partitioning in seep and vent organisms: Chemical and ecological significance. Chem. Geol. (Isot. Geosci. Sect.) **101:** 293–310.

LEE, R. W., AND J. J. CHILDRESS. 1995. Assimilation of inorganic nitrogen by seep mytilid Ia, an undescribed deep-sea mussel containing methanotrophic endosymbionts: Fate of assimilated nitrogen and the relation between methane and nitrogen assimilation. Mar. Ecol. Prog. Ser. **123:** 137–148.

———, AND ———. 1996. Inorganic N assimilation and ammonium pools in a deep-sea mussel containing methanotrophic endosymbionts. Biol. Bull. **190:** 373–384.

———, E. V. THUESEN, J. J. CHILDRESS, AND C. R. FISHER. 1992. Ammonium and free amino acid uptake by a deep-sea mussel (*Bathymodiolus* sp., undescribed) containing methanotrophic bacterial symbionts. Mar. Biol. **113:** 99–106.

LORRAIN, A., P. YVES-MARIE, L. CHAUVAUD, N. SAVOYE, A. DONVAL, AND C. SAOUT. 2002. Differential $\delta^{13}C$ and $\delta^{15}N$ signatures among scallop tissues: Implications for ecology and physiology. J. Exp. Mar. Biol. Ecol. **275:** 47–61.

MACAVOY, S. E., S. A. MACKO, AND G. C. GARMAN. 2001. Isotopic turnover in aquatic predators: Quantifying the exploitation of migratory prey. Can. J. Fish. Aquat. Sci. **58:** 923–932.

MACDONALD, I. R., J. F. REILLY II, N. L. GUINASSO, JR., J. M. BROOKS, R. S. CARNEY, W. A. BRYANT, AND T. J. BRIGHT. 1990. Chemosynthetic mussels at a brine-filled pockmark in the Northern Gulf of Mexico. Science **248:** 1096–1099.

NETER, J., M. H. KUTNER, C. J. NACHTSHEIM, AND W. WASSERMAN. 1996. Applied linear statistical models, 4th ed. McGraw-Hill.

NIX, E. R., C. R. FISHER, J. VODENICHAR, AND K. M. SCOTT. 1995. Physiological ecology of a mussel with methanotrophic endosymbionts at three hydrocarbon seep sites in the Gulf of Mexico. Mar. Biol. **122:** 605–617.

PAGE, H. M., C. R. FISHER, AND J. J. CHILDRESS. 1990. Role of filter- feeding in the nutritional biology of a deep-sea mussel with methanotrophic symbionts. Mar. Biol. **104:** 251–257.

PILE, A. J., AND C. M. YOUNG. 1999. Plankton availability and retention efficiencies of cold-seep symbiotic mussels. Limnol. Oceanogr. **44:** 1833–1839.

POWELL, E. N., R. D. BARBER, M. C. KENNICUTT II, AND S. E. FORD. 1999. Influence of parasitism in controlling the health, reproduction and PAH body burden of petroleum seep mussels. Deep-Sea Res. I **46:** 2053–2078.

PRUSKI, A. M., A. FIALA- MÉDIONI, C. R. FISHER, AND J. C. COLOMINES. 2000. Composition of free amino acids and related compounds in invertebrates with symbiotic bacteria at hydrocarbon seeps in the Gulf of Mexico. Mar. Biol. **136:** 411–420.

RICE, W. R. 1989. Analyzing tables of statistical data. Evolution **43:** 223–225.

SASSEN, R., S. JOYE, S. T. SWEET, D. A. DEFREITAS, A. V. MILKOV, AND I. R. MACDONALD. 1999. Thermogenic gas hydrates and hydrocarbon gases in complex chemosynthetic communities, Gulf of Mexico continental slope. Org. Geochem. **30:** 485–487.

SMITH, E. B., K. M. SCOTT, E. R. NIX, C. KORTE, AND C. R. FISHER. 2000. Growth and condition of seep mussels (*Bathymodiolus childressi*) at a Gulf of Mexico Brine Pool. Ecology **81:** 2392–2403.

SMITH, K. L., JR. 1985. Deep-sea hydrothermal vent mussels: Nutritional state and distribution at the Galapagos Rift. Ecology **66:** 1067–1080.

STREAMS, M. E., C. R. FISHER, AND A. FIALA-MÉDIONI. 1997. Methanotrophic symbiont location and fate of carbon incorporated from methane in a hydrocarbon seep mussel. Mar. Biol. **129:** 465–476.

TIESZEN, L. L., T. W. BOUTTON, K. G. TESDAHL, AND N. A. SLADE. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: Implications for $\delta^{13}C$ analysis of diet. Oecol. Berlin **57:** 32–37.

WALFORD, L. A. 1946. A new graphic method of describing the growth of animals. Biol. Bull. Mar. Biol. Lab. Woods Hole **90:** 141–147.

Received: 23 September 2003
Accepted: 5 March 2004
Amended: 12 March 2004