

Site-specific and ontogenetic variations in nutrition of mussels (*Bathymodiolus* sp.) from the Lucky Strike hydrothermal vent field, Mid-Atlantic Ridge

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Abstract

Lucky Strike mussels (*Bathymodiolus* sp.) support two metabolically distinct (methanotrophic and thiotrophic) prokaryotic endosymbionts in their gills. Differences in source inorganic carbon isotope ratios and in carbon fixation pathways between these two symbionts typically result in organic carbon with distinctive $\delta^{13}\text{C}$ values. Site-specific differences in isotopic compositions of host mussels may therefore reflect differences in sulfide and methane availability. Large differences in mean carbon and nitrogen isotopic compositions were observed in adult mussels collected from two chemically distinct vents at Lucky Strike (Sintra: $\delta^{13}\text{C} = -21.3\text{‰}$, $\delta^{15}\text{N} = -4.5\text{‰}$; Eiffel Tower: $\delta^{13}\text{C} = -30.7\text{‰}$, $\delta^{15}\text{N} = -10.5\text{‰}$). These values are consistent with the hypothesis that Sintra mussels are more dependent on methanotrophy than are Eiffel Tower mussels. Relative abundances of the two types of endosymbionts in mussel gill tissues (Sintra: mean = 15% methanotrophs; Eiffel Tower: mean = 6% methanotrophs) provide further support of this hypothesis, suggesting that Lucky Strike mussels express a nutritional response to environmental variations. Within sites, there were small but significant correlations between isotopic composition and mussel size over the shell lengths of 15–80 mm, but these shifts are so small that they are attributable to factors other than ontogenetic shifts in nutritional strategy. In contrast, large nitrogen isotope differences were observed between larval and adult stages. Based on $\delta^{15}\text{N}$ values, mussel larvae appear to rely very little on photosynthetically derived organic material. Observations of the demersal nature of mussel larvae and isotopic similarities between Sintra and Eiffel Tower larvae and Sintra adults suggest that the potential of stable isotopes as useful tracers of larval sources within the Lucky Strike vent field should be considered.

The Lucky Strike hydrothermal vent field is dominated by dense beds of mussels belonging to an undescribed species of the genus *Bathymodiolus* (Craddock et al. 1995; Van Dover 1995; Van Dover et al. 1996; Langmuir et al. 1997). As in other vent communities, Lucky Strike trophic ecology is based on bacterial chemosynthesis rather than photosynthesis, and the mussels depend on internal bacterial symbionts as a significant source of nutrition (Van Dover et al. 1996). Whereas Pacific vent bathymodiolid mussel species usually house only sulfide-oxidizing symbionts (Fisher 1990), Lucky Strike and other Atlantic basin bathymodiolid species exhibit an intracellular dual symbiosis of both sulfide-oxidizing and methanotrophic symbionts (Fiala-Médioni et al. 1986; Cavanaugh et al. 1987, 1992; Fisher et al. 1993; Distel et al. 1995). Dual symbioses allow host mussels to exploit a wider range of chemical environments (Distel et al. 1995) and are assumed to be indicative of exposure to both sulfide and methane substrates over coevolutionary time scales. Because of the difference in chemical substrates used for chemosynthesis by the two symbiont types, a biological response to

heterogeneous substrate availability within a vent field may be reflected in the relative or absolute abundance of each symbiont species.

Unlike other endosymbiont-dependent vent animals that have reduced digestive systems (e.g., adult vestimentiferans and pogonophorans lack mouth and gut; Jones 1981; Southward 1982), filter-feeding capability has been demonstrated in bathymodiolid mussels (e.g., Page et al. 1991). The importance of this heterotrophic nutritional pathway to these mussels has not yet been quantified. Heterotrophic uptake most likely includes some combination of free-living chemoautotrophic bacteria and sedimentary detritus (detritus ultimately derived from surface photosynthetic processes), although selective filtration is possible. Lucky Strike mussels thus have the potential to exploit thiotrophic, methanotrophic, or photosynthetic energy sources, and feeding strategies and the relative importance of nutritional sources may change during growth and development of these mussels. The symbiont–host relationship of at least one species of bathymodiolid mussel (*Bathymodiolus thermophilus*) is thought to be propagated by vertical transmission, with symbionts passing directly from adults to larvae in eggs (Cary and Giovannoni 1993). This mode of transmission presumably offers larval mussels immediate access to symbiotic energy sources. Histological analysis of the reproductive biology of *B. thermophilus* (Berg 1985) suggests that they exhibit planktotrophic (yolkless, feeding larvae) development, but it is unknown how long this developmental stage remains in the larval pool or from where their energy is derived.

We chose to investigate whether Lucky Strike mussels use a consistent energy source throughout their life or if they switch trophic modes depending on resources available during development and growth. As an a priori model, we hy-

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pothesized that larvae drift away from vent fields and rely on filter-feeding as their initial nutritional source, implicating a significant contribution of photosynthetically derived carbon. As postmetamorphic juveniles settle at vents, nutrition would switch quickly to reliance primarily on symbiotic chemoautotrophic energy sources. By this model, larvae would not be dependent on vent fluids to sustain symbiotic nutrition, and widespread dispersal could be routine. An alternative model suggests that larvae instead depend wholly on their symbionts or maternally derived reserves for energy. Unless the larvae can survive for long periods on stored resources, this strategy would presumably restrict larval dispersal primarily to within areas of active venting. A combination of these strategies is also possible, incorporating both facultative use of photosynthetic and symbiotic energy sources as resources become available.

A first-order assessment of the relative importance of chemoautotrophic production in the nutrition of vent mussels throughout their life history can be made using stable isotope analyses. Differences in isotopic fractionation of carbon during metabolism result in distinct variations in isotope ratios of photosynthetically and chemosynthetically derived organic carbon. Carbon isotope values are relatively conservative in trophic interactions—marine food webs typically express <1‰ increases in $\delta^{13}\text{C}$ per trophic level (DeNiro and Epstein 1978). This carbon isotope fidelity between trophic levels allows one to implicate specific food resources in the diet of a consumer when putative foods are isotopically distinct. Differences in nitrogen isotope ratios can help resolve trophic relationships because there is typically a 2–4‰ increase in $\delta^{15}\text{N}$ per trophic level (Minagawa and Wada 1984; Rau 1985).

Given large differences in the isotopic compositions of putative nutritional resources of Lucky Strike mussels, we undertook a survey approach to the study of their nutrition, examining both size- and site-related differences in isotopic composition. We also analyzed the mussel commensal polychaete *Branchipolynoe seepensis* to explore the fidelity of commensal and host isotopic composition between sites. Significant differences in stable isotope ratios between mussel populations at Lucky Strike suggested site-specific differences in endosymbiont associations. To assess the degree of variation in relative abundance of methanotrophic and sulfide-oxidizing symbionts in the Lucky Strike mussel population, we used transmission electron microscopy to determine relative abundances of gill-housed symbionts at each site.

Methods

Study site—The Lucky Strike hydrothermal vent area is located at 37°17.5'N, 32°16.5'W on the Mid-Atlantic Ridge (Fig. 1). The vent field comprises several areas of active venting following a roughly north–south line across the summit of the Lucky Strike Seamount (Langmuir et al. 1997). Samples in this study were obtained from two vent sites: Eiffel Tower, a 20-m high tapered spire in the southern area of the field (1,687 m), and Sintra, a 5-m high spire in the northern area (1,618 m). Both vents are similar in structure,

although Eiffel Tower exhibits higher fluid temperatures (Table 1; Langmuir et al. 1997; Von Damm et al. 1998). Differences in fluid chemistry (e.g., chlorinity, iron concentration) indicate that each site is fed by different hydrothermal sources, although they are separated by a distance of only ~400 m (Von Damm et al. 1998).

Collection of specimens—All mussel specimens were collected in July 1996 during several ROV (remotely operated vehicle) *Jason* dives on the LUSTRE '96 cruise to the Lucky Strike hydrothermal vent field. Mussel clumps were harvested from the substrate with the manipulator claw of *Jason* and placed in discrete collection buckets until the mussel volume reached the 2-liter mark, at which point the bucket was closed to retain sample integrity. Five samples were collected from both Sintra and Eiffel Tower. Samples were retrieved via free-ascent elevator to minimize the time between collection and fixation. Clumps of mussels were rinsed with filtered seawater over a 63- μm sieve to collect organisms living within interstitial spaces. These washings were preserved in 10% seawater–formalin and then transferred to 70% ethanol for storage. Mussel larvae and post-larvae were sorted from these preserved washings.

Adult mussels were sorted by size, and a 1-in-*k* systematic sample (where *k* represents the number required to obtain subsamples of 10–15 mussels) was taken from each ordered replicate. Maximum shell length was measured for each mussel, and foot (muscle) and gill tissues were removed and frozen at -70°C at sea. Commensal worms (e.g., *Branchipolynoe seepensis*), if present, were also removed, measured, and frozen.

Preparation of samples for mass spectrometry—All frozen mussel subsamples from each site were pooled and sorted according to size. Because of small sample sizes, tissue pairs from all mussels under 30 mm were analyzed to uniformly represent the entire size range of mussels collected. A systematic sample was taken from the larger (ordered) mussels to obtain a total sample of 24 tissue pairs from each site. Mussel tissues and whole commensal worms were dried in a 65°C oven and finely ground using a WIG-L-BUG grinder (Crescent Dental Corp.).

Of two distinct sizes of larvae observed (~300 μm and 500 μm), only the larger group was abundant enough to analyze. We distinguished mussel larvae and post-larvae using morphological and functional characteristics (Baker and Mann 1997). The developmental stage referred to here as a larva has a pink larval shell (prodissoconch) with no evidence of a dissoconch (adult shell). Based on shell morphology alone, we considered it to be a late larval (or early postlarval) prodissoconch stage. The first millimeters of dissoconch shell growth are readily distinguished by a yellow color in what is referred to here as a postlarva. Nascent gills are evident in the larger, postmetamorphic dissoconch stage postlarvae but are difficult to discern in histological sections of the prodissoconch stage. The benthic larval stages observed are referred to here as plantigrade larvae (sensu Baker and Mann). More detailed histological studies are necessary to determine if this dissoconch stage is pre- or postmetamorphic.

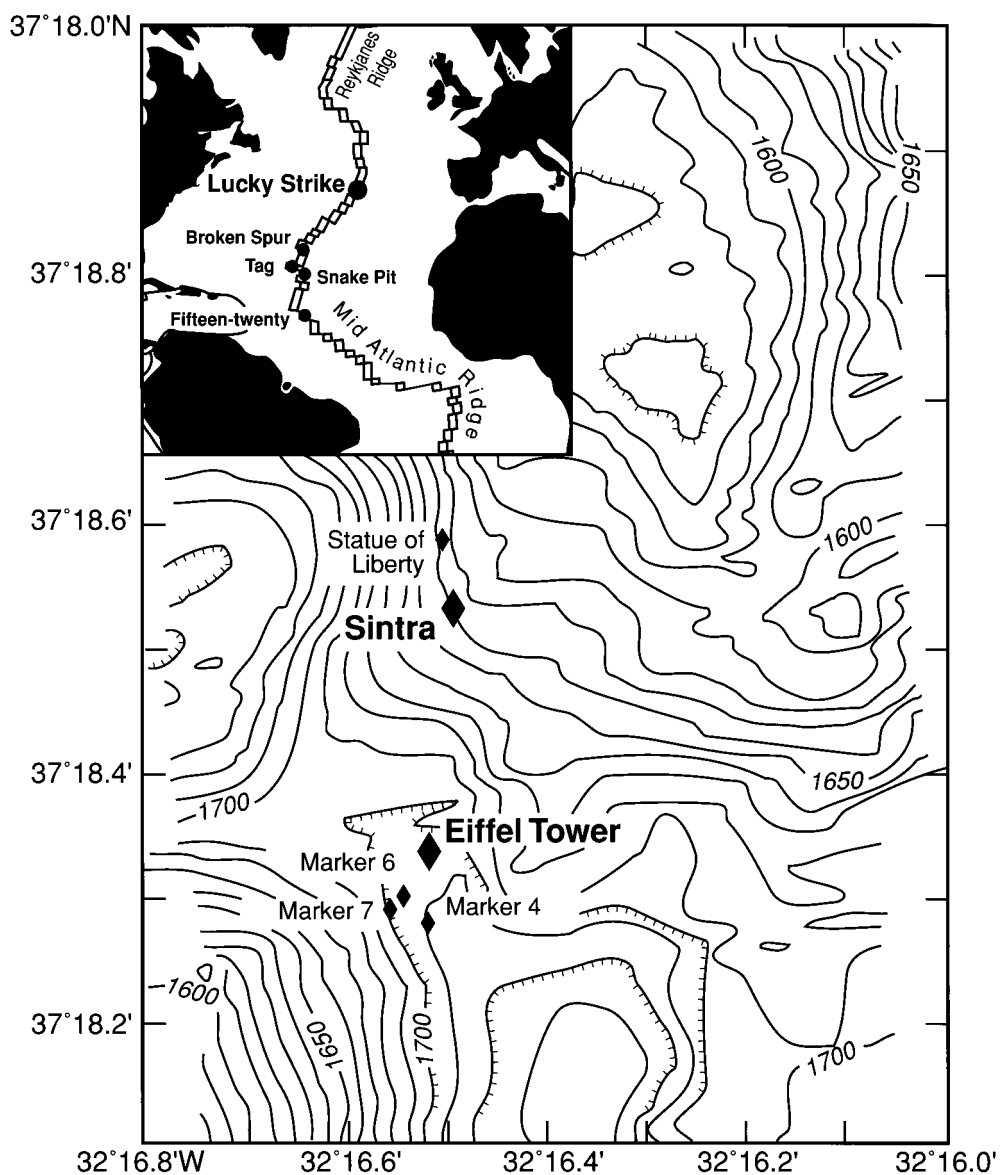


Fig. 1. Lucky Strike hydrothermal vent field. Mussels for this study were collected at Sintra and Eiffel Tower. *Inset*: Location of Lucky Strike relative to other known vent fields on the Mid-Atlantic Ridge.

Table 1. Fluid temperatures and chemistry, 1996, from two sites at the Lucky Strike hydrothermal vent field (Von Damm et al. 1998). Methane concentrations from M. Lilley (unpub.).

	Sintra	Eiffel Tower
Temperature (°C)	222	323
H ₂ S (mmol/kg)	1.96 ± 0.10	1.78 ± 0.09
CH ₄ (mmol/kg)	0.5	0.4
Chlorinity (mmol/kg)	532 ± 3	441 ± 2
Fe (μmol/kg)	191 ± 2	595 ± 6

Larvae were combined to make three pooled samples, each of approximately 1 mg dry weight (about 100–150 individuals). One sample (A) consisted of exclusively Sintra larvae, one sample (B) was exclusively Eiffel Tower larvae, and one sample (C) was a mixture from the two sites. Post-larvae ranged from 0.7 to 1.4 mm and were similarly combined into three 1-mg samples, i.e., one Sintra sample (A), one Eiffel Tower sample (B), and one mixed sample (C) (~50–75 individuals each). To remove calcium carbonate in the minute larval and postlarval shells, samples were washed in 10% HCl until evidence of reaction was no longer visible. Samples were then rinsed and dried overnight at 65°C. Larvae and postlarvae were not ground because of the small sample sizes available.

Mussel postlarvae (~1,700 μm; *Mytilus galloprovinci-*

alis) (Coast Seafoods) were used to analyze the effect of formalin preservation on stable isotope composition. Post-larvae were separated into two treatment groups and then split into three samples within each treatment. Samples in one treatment were fixed in 10% formalin and transferred to 70% ethanol, and samples in the other treatment were preserved directly in 70% ethanol. All samples were acid washed to remove calcium carbonate, dried in a 65°C oven overnight, and finely ground using a WIG-L-BUG grinder.

Specimens were analyzed using a stable isotope analyzer (Europa Scientific Model 20–20). All results are reported using parts per mil (‰) notation, where

$$\delta^{13}\text{C}(\text{‰}) = \left\{ \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{SAMPLE}}}{(^{13}\text{C}/^{12}\text{C})_{\text{STANDARD}}} \right] - 1 \right\} \times 10^3$$

and

$$\delta^{15}\text{N}(\text{‰}) = \left\{ \left[\frac{(^{15}\text{N}/^{14}\text{N})_{\text{SAMPLE}}}{(^{15}\text{N}/^{14}\text{N})_{\text{STANDARD}}} \right] - 1 \right\} \times 10^3.$$

The laboratory standard used was a reference peptone, and data are reported relative to the Pee Dee Belemnite (PDB) standard ($\delta^{13}\text{C}$) and atmospheric nitrogen ($\delta^{15}\text{N}$).

Transmission electron microscopy—Gill tissues were removed from five adult mussels from each site, fixed in 10% seawater–formalin, and transferred to 70% ethanol for storage. Tissues were rehydrated to water through a graded ethanol series. Samples were then postfixed in 2% osmium tetroxide in 0.1 M cacodylate buffer, rinsed in water, and stained in 7% uranyl acetate in 50% methyl alcohol for 30 min. Samples were rinsed in water again and dehydrated through a graded ethanol series. Tissues were embedded in Epon 812 embedding medium and polymerized at 60°C for 48 h. Thin sections were cut on an ultramicrotome (Reichert Jung Ultracut E), stained with lead citrate, and examined on a transmission electron microscope (Zeiss 109). Mosaic series of eight micrographs were taken from the gill filaments of each mussel between the distal end of the filament and midway down the filament length. Area of gill tissue in each micrograph was determined using image-analysis software (Sigma Scan Pro). Two distinct morphological types of endosymbionts were visible on micrographs of gill filaments. Size and structure of these cells were consistent with descriptions of chemoautotrophic sulfur-oxidizing and methanotrophic symbionts (Fisher et al. 1993). All endosymbionts on each micrograph were counted, and average symbiont proportions were calculated for each mussel.

Data analysis—One-way analyses of variance and associated *F*-tests were used to compare means of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for adult mussels. Student's *t*-tests were used to assess differences between larval and postlarval isotope values, to compare these groups with adult mussels, and to determine the formalin effect. Spearman rank-order correlation was used to assess the relationship between length and isotopic composition of juveniles and adults. Paired *t*-tests were used to examine differences between mantle and gill tissues and between commensal and host tissue. Holm's simultaneous test (Holm 1979) was used for hypothesis tests for all comparisons. Bootstrapped 95% confidence intervals (CIs) and a permutation test of the hypothesis were used to analyze

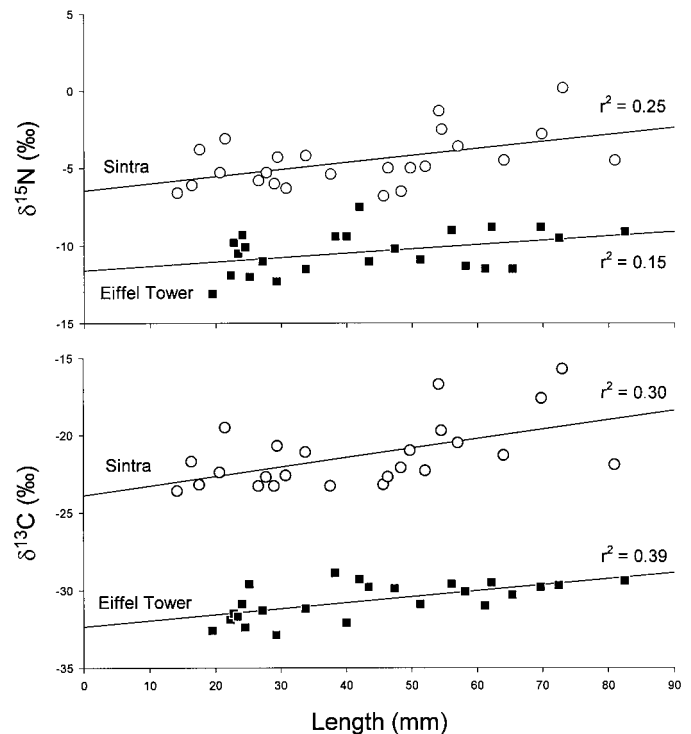


Fig. 2. Relationship between length and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for adult mussels (15–80 mm).

symbiont populations and to assess differences in symbiont proportions between sites.

Results

Formalin effect on isotopic composition of mussel larvae—Student's *t*-tests showed a significant difference between $\delta^{13}\text{C}$ values of formalin- and non-formalin-preserved larval samples; values for samples preserved in formalin were $\sim 1.5\text{‰}$ lower ($P < 0.001$) than those of samples preserved in ethanol. A marginally significant difference was indicated for nitrogen ratios, with formalized samples $\sim 0.5\text{‰}$ lower than those preserved in ethanol. Larval and postlarval isotope ratios were corrected for this formalin effect, and subsequent data are presented as corrected values.

Ontogenetic variation in isotopic composition—Stable isotope compositions very gradually increased to more positive values with increasing size of mussels. Both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were positively correlated with length ($P < 0.001$ for all tests; Fig. 2). Regressions for both carbon and nitrogen isotope values versus length at both sites resulted in at least marginally significant coefficients (range: $P = 0.001$ [Eiffel Tower $\delta^{13}\text{C}$] to $P = 0.061$ [Eiffel Tower $\delta^{15}\text{N}$]). No site difference was observed in either larval or postlarval $\delta^{13}\text{C}$ values (Fig. 3), but small sample sizes (one sample from each site) prevented testing of statistical significance. Larvae (mean = -19.5‰) and postlarvae (mean = -18.2‰) exhibited comparable $\delta^{13}\text{C}$ values, but Sintra adult mussels (mean = -21.3‰) and Eiffel Tower adults (mean = -30.7‰) were significantly more negative than larvae and

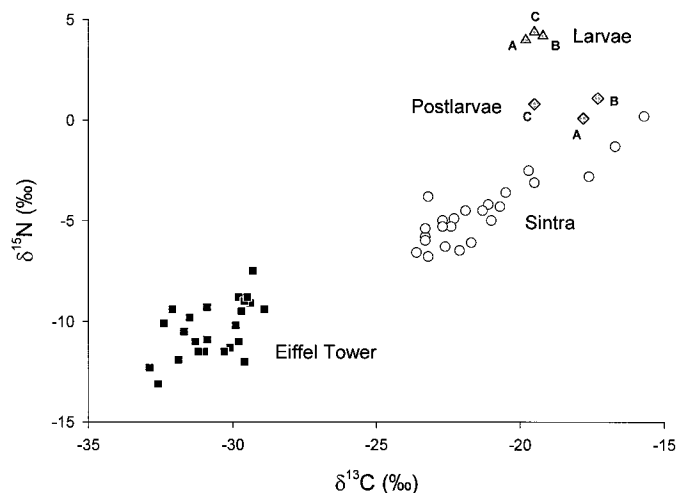


Fig. 3. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ for all mussel groups. Larval and postlarval pooled samples: A = Sintra; B = Eiffel Tower; C = mixed Sintra–Eiffel Tower.

postlarvae (Tables 2, 3; postlarvae vs. Sintra $P = 0.015$; all other comparisons $P < 0.001$).

Nitrogen isotope ratios of larvae and postlarvae differed significantly (Tables 2, 3; Fig. 3), with larvae more positive (mean = $+4.2\text{‰}$) than postlarvae (mean = $+0.7\text{‰}$; $P = 0.007$). Postlarvae were significantly more positive ($P < 0.001$) than adult mussels from Sintra (mean = -4.5‰) or Eiffel Tower (mean = -10.5‰).

Muscle versus gill tissue isotopic composition in juveniles and adults—Paired t -tests for differences in tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ indicated that gills had consistently lower values than muscle tissue for both isotopes ($P < 0.001$; Fig. 4). Gill $\delta^{13}\text{C}$ averaged $\sim 0.5\text{‰}$ lower (range -2.0‰ to 2.1‰), and gill $\delta^{15}\text{N}$ values were $\sim 1.0\text{‰}$ lower (range -1.0‰ to 2.2‰) than those values for muscle tissue ($P < 0.001$). To simplify discussion, all site- and size-related comparisons among adults (15–80 mm) are reported here using only muscle tissue $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values.

Site-specific variation in adults—Adult mussels from Eiffel Tower exhibited carbon and nitrogen isotope ratios that were significantly different from those of Sintra mussels (Tables 2, 3; Fig. 3). $\Delta\delta^{13}\text{C}$ values between Sintra and Eiffel Tower averaged $+9.5\text{‰}$ ($P < 0.001$); mean $\Delta\delta^{15}\text{N}$ was $+6.0\text{‰}$ ($P < 0.001$). Counts of methanotrophs and thiotrophs via transmission electron microscopy showed that proportions of these two symbiont species in gill tissues varied between sites (Table 4). Symbiont communities in the gills of Eiffel Tower mussels were 1–10% methanotrophs (mean = 6%; 95% CI = 3%, 9%), and Sintra mussel gill symbiont communities contained 9–20% methanotrophs (mean = 15%; 95% CI = 12%, 19%). Site differences in symbiont proportions in mussels was significant ($P = 0.004$).

Commensal isotopic composition—Paired t -tests showed no significant difference between carbon isotope values of

Eiffel Tower commensals and host mantle tissue but indicated a significant difference between Sintra commensals and hosts ($P < 0.001$), with commensal $\delta^{13}\text{C}$ 2.0‰ lower on average than corresponding host $\delta^{13}\text{C}$ (Table 3, Fig. 5). Differences in $\delta^{13}\text{C}$ between hosts and commensals at Sintra ranged from approximately -5.0‰ to -0.5‰ , and those at Eiffel Tower ranged from approximately -1.0‰ to 0.5‰ . Commensals at both sites exhibited a $\sim 2.5\text{‰}$ enrichment in $\delta^{15}\text{N}$ relative to the host mussels ($P < 0.001$; Table 3, Fig. 5).

Discussion

Isotope values and diets—a priori expectations—Based on empirical data, mussels hosting only sulfide-oxidizing symbionts are expected to have $\delta^{13}\text{C}$ values centered around -33‰ and $\delta^{15}\text{N}$ values $< 0\text{‰}$ (e.g., Rau and Hedges 1979; Fisher et al. 1988; Van Dover and Fry 1994). If mussels rely primarily on sedimentary detritus, their carbon and nitrogen isotopic compositions should approach those of detritus-feeding deep-sea invertebrates collected from nonvent environments ($\delta^{13}\text{C} = -17\text{‰}$, $\delta^{15}\text{N} > 11\text{‰}$; Van Dover and Fry 1989). The isotopic composition of organic carbon fixed by methanogenic symbionts from Lucky Strike is expected to be in the range of -20 to -28‰ (Pond et al. 1998). The published value for bulk tissues collected from mussels at the Statue of Liberty site at Lucky Strike was -24‰ (Van Dover et al. 1996). Carbon isotope composition of thermogenic methane available to chemosynthetic organisms has been measured from Pacific vents at -16‰ (Welhan and Craig 1983) and from Atlantic vents at -13‰ (Radford-Knoery et al. 1998).

A persistent ambiguity regarding the relative importance of free-living (e.g., suspended in the water column) versus symbiotic bacteria in the nutrition of mussels exists because $\delta^{13}\text{C}$ values of the free-living bacteria are rarely determined. Furthermore, in instances where $\delta^{13}\text{C}$ values of free-living bacteria have been determined (i.e., in chemoautotrophic bacterial mats), they vary widely, from -16‰ to -41‰ (Trager and DeNiro 1990; Van Dover and Fry 1994).

Ontogenetic variation in isotope compositions at Lucky Strike—High densities of larval and postlarval mussels were found among adults in 10 2-liter samples of mussel clumps (e.g., 1,276 larvae versus 1,438 adults, total counts). The presence of so many larvae and postlarvae within the vent environment offered the opportunity to test for chemosynthetic nutrition of these dispersive stages using isotope techniques. Although we cannot resolve ecological details of the dispersive phases of mussels in this study, Lucky Strike may prove a particularly amenable site for investigation of mussel larval biology if high larval abundances are normal there.

Larvae and postlarvae were enriched, as compared with adults, in ^{15}N , regardless of the vent site from which they were collected (Fig. 3). This finding implies that these developmental stages are likely to use a nutritional strategy that is in some way distinct from that of the adults. Larval and postlarval $\delta^{13}\text{C}$ values (corrected for the formalin effect) in samples collected from Sintra overlap with $\delta^{13}\text{C}$ values of adults from the same site. Curiously, $\delta^{13}\text{C}$ values of Eiffel

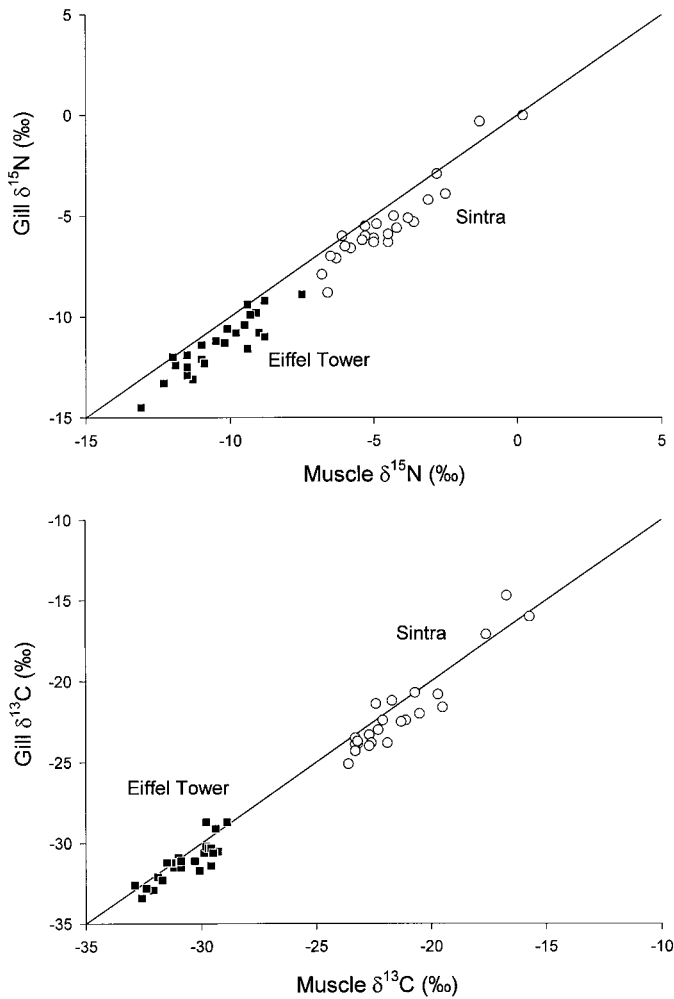


Fig. 4. Muscle (foot) versus gill $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Diagonal lines represent a 1:1 relationship. Samples falling below the line have gills that are enriched with the lighter isotope.

Tower larvae and postlarvae also match those of Sintra adult mussels rather than Eiffel Tower adults (Fig. 3). One hypothesis consistent with these results is that the cohort of larvae and postlarvae sampled in 1996 at Sintra and Eiffel Tower originated from Sintra (or some other isotopically related population), retaining the site-specific carbon isotopic signature of their maternally derived nutritional reserves. An alternative hypothesis is that larval and postlarval carbon isotope compositions may indicate greater dependence on the methanotrophic symbiotic relationship at early stages of development. The mechanism of symbiont transmission has not been determined for Lucky Strike mussels, and it is possible that only the methanotroph is transmitted vertically in this species or that the functionality of the dual symbioses develops unequally.

Although carbon isotope information is confounded because of overlapping or unknown data on the isotopic composition of available suspended organic matter and photosynthetic carbon, nitrogen isotope ratios suggest that mussels do not rely heavily on photosynthetic energy sources as larvae, postlarvae, or adults. $\delta^{15}\text{N}$ values of photosynthetically

Table 4. Bacterial symbiont types in mussel gills expressed as mean number per micrograph (approximately $200\ \mu\text{m}^2$); based on eight micrographs per individual, five individuals per site.

Site	Methanotrophs (mean \pm SD)	Thiotrophs (mean \pm SD)	Methanotrophs :
			Thiotrophs (%, range)
Sintra	23 \pm 11	130 \pm 57	15 (9–20)
Eiffel Tower	11 \pm 9	184 \pm 82	6 (1–10)

derived organic material in deep water are generally $>6\text{‰}$ (reviewed by Michener and Schell 1994). When combined with a 2–4‰ trophic level enrichment usually seen in suspension feeders, $\delta^{15}\text{N}$ values of animals deriving their nutrition primarily from sinking detritus would have to be $>8\text{‰}$. The relatively low $\delta^{15}\text{N}$ values of the adult mussels (-13.1 to $+0.2\text{‰}$) indicate a primary reliance on a nondetrital nitrogen source. Larvae and postlarvae have somewhat higher

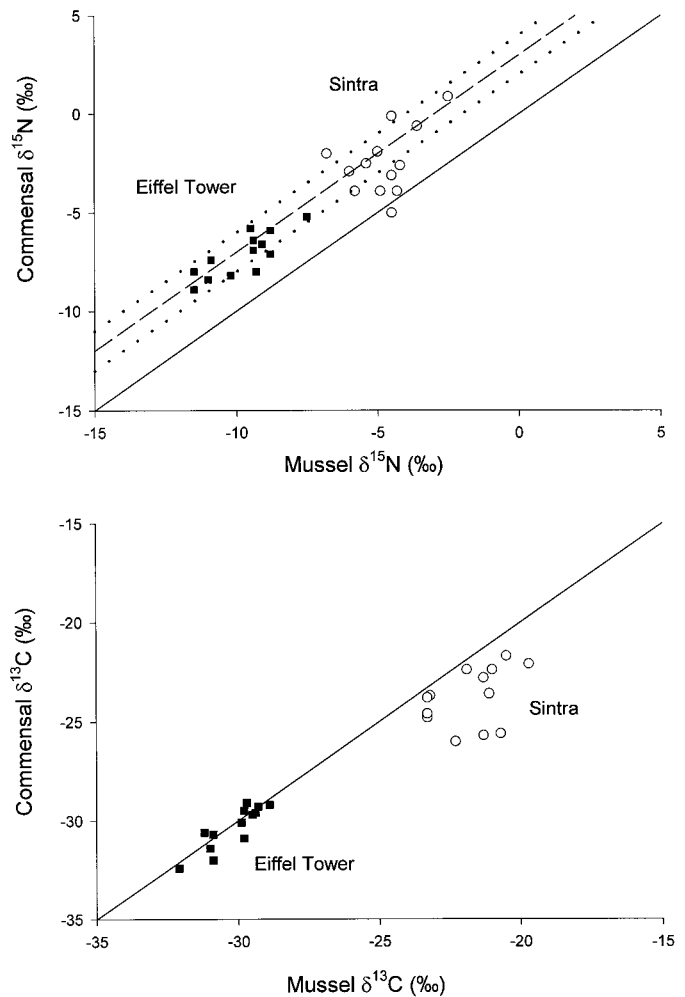


Fig. 5. Host (mussel) versus commensal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Solid diagonal lines represent a 1:1 relationship. Dashed line represents a 3‰ trophic enrichment; dotted lines represent 2‰ and 4‰ enrichments. Samples falling above the line indicate commensal tissues enriched with the heavier isotope.

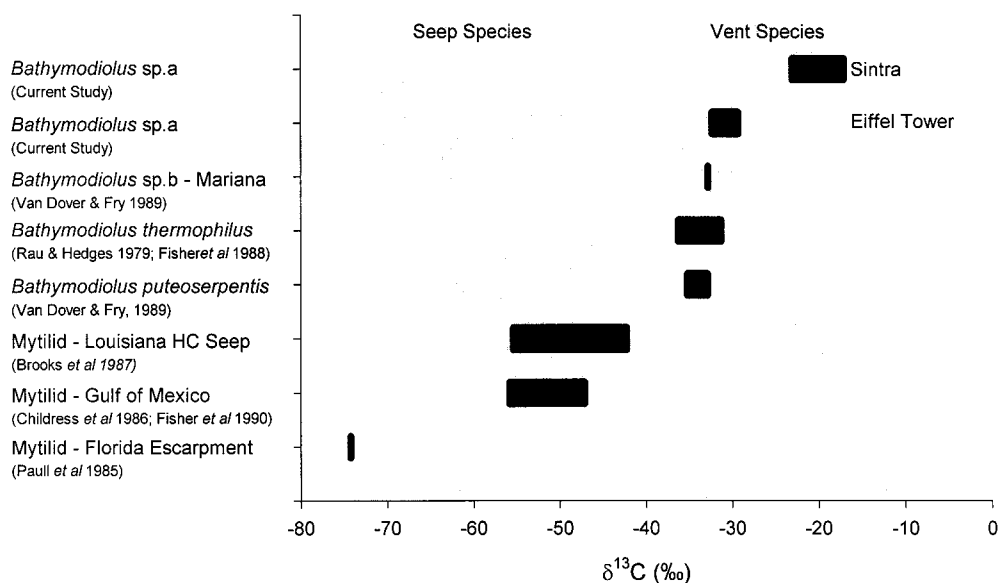


Fig. 6. Stable carbon isotope values from vent and seep mussels worldwide.

$\delta^{15}\text{N}$ values (-0.3 to $+4.0\text{‰}$), which suggests that a mixed chemosynthetic/photosynthetic diet (but heavily chemosynthetic) is plausible. Adult $\delta^{15}\text{N}$ of gill tissue is consistently lower than $\delta^{15}\text{N}$ of muscle tissue (Fig. 4), consistent with a nitrogen pool derived from symbionts rather than from sedimentary nitrogen (Rau 1981; Fisher et al. 1988).

The gradual increase in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ with mussel size (Fig. 2) suggests that there is no discrete change in nutritional strategy with increasing size. Although gradual changes in the relative importance of the different endosymbiont types could account for the change in isotopic compositions, we suggest that these numbers more likely reflect the steady accumulation of heavier isotopes in animal tissues during aging as lighter isotopes are metabolized preferentially. Alternative explanations include gradual changes in substrate $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in the vent environment or increasing diffusional path length between symbionts and dissolved carbon and nitrogen inorganic substrates as the mussels grow. Following settlement and metamorphosis, mussels at Lucky Strike sites probably express one nutritional strategy, without major ontogenetically or environmentally induced shifts within a population in the relative importance of energy sources, despite the accommodation of dual symbiont types.

Site-specific variation—The Lucky Strike hydrothermal vent field comprises two areas of active venting separated by only a few hundred meters yet fed by different hydrothermal fluid systems. The two vents sampled in this study are from the different venting areas and are chemically distinct based on chlorinity and other chemical measurements (Von Damm et al. 1998). Although differences in the chemistry of a vent environment are presumed to influence the nutritional structure of the biotic communities, the site-specific differences in isotope composition observed between Sintra and Eiffel Tower adult mussels are unprecedented. The possibility of cryptic species of mussels occupying each

site was entertained, but molecular genetic analyses of these animals indicated that only a single mussel taxon colonizes both Sintra and Eiffel Tower (T. Shank pers. comm.). The isotope difference observed between sites must then be attributable to differences in the nutritional ecology of these mussels.

Published values of bulk tissue $\delta^{13}\text{C}$ in mytilid mussels from vents and seeps range from -30‰ to -75‰ (Fig. 6). Within vent mussel species that host only sulfide-oxidizing endosymbionts, the range of carbon isotope values is relatively narrow and predictable (-30‰ to -37‰). Eiffel Tower mussels are mostly within the range of $\delta^{13}\text{C}$ values observed in sulfide-oxidizing symbiont relationships, but Sintra mussels have carbon isotope compositions that are significantly higher (-15‰ to -24‰). This ^{13}C enrichment at Sintra is consistent with greater reliance either on methanotrophic symbionts or on photosynthetic organic matter. Because nitrogen isotope ratios of Sintra mussels (mean = -4.6‰) are inconsistent with significant incorporation of photosynthetic organic matter ($\delta^{15}\text{N} > 11\text{‰}$), chemical differences in environmental fluid chemistry probably are responsible for the differences in the relative nutritional contributions of methanotrophic and thiotrophic symbionts within the mussels. Evidence from bacterial counts on transmission electron micrographs supports this hypothesis. At Sintra, the relative abundance of methanotrophs was on average twice as high as that in Eiffel Tower mussels (Table 4). Although end-member fluids do not show significant differences in CH_4 and H_2S compositions between the two sites (Table 1), biological responses indicate that some chemical difference may be present. Methane and sulfide concentrations present in low-temperature, diffuse-flow fluids encountered by mussel communities may be poorly represented by values in high-temperature end-member fluids (Lilley pers. comm.). Although a relationship between $\delta^{13}\text{C}$ and % methanotrophs was demonstrated, a regression study involving

isotope measurements and bacterial counts based on transmission electron micrographs from the same individuals is necessary to determine the statistical nature of this relationship.

A compelling follow-up experiment would be to conduct reciprocal transplants of mussels between Sintra and Eiffel Tower. This experiment would test whether nutritional strategies (as expressed by stable isotope compositions and symbiont abundance patterns) accommodate to the environmental differences between sites that are inferred here.

Carbon and nitrogen isotope compositions of commensal polychaetes hosted by mussels display the same site-specific differences as do the mussels themselves (Table 2, Fig. 5). The $\delta^{13}\text{C}$ values of Eiffel Tower commensals and their hosts are more closely matched than are those of the Sintra commensals and hosts. We tested and rejected the hypothesis that polychaetes in our Sintra samples were smaller and could perhaps more readily move between host mussels, thereby accounting for the reduced carbon isotopic fidelity between a particular host and commensal. Commensal nitrogen isotope values are higher than those of their hosts, consistent with a trophic level increase, which suggests that commensals are feeding on mussel tissue or mucus-rich pseudofeces rather than relying on a symbiotic energy source or consuming the bacteria present in and on the mussels.

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