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Trophic relationships among invertebrates at the Kairei hydrothermal vent field (Central Indian Ridge)

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Abstract Exploration of hydrothermal vent systems in locations remote from well-studied sites allows ecologists to determine the degree of site-specific variation in trophic relationships among communities. A preliminary outline of the trophic structure of the Kairei hydrothermal vent community on the Central Indian Ridge (25°19.23'S; 70°02.42'E) is provided here, based on analysis of collections from an April 2001 expedition. Invertebrate biomass at Kairei is dominated by organic carbon with a $\delta^{13}\text{C}$ isotopic value of about -13‰ , due to the abundance of primary consumers (shrimp: *Rimicaris* aff. *exoculata*) and secondary consumers (anemones: *Marianactis* n. sp.) with this isotopic composition. Filamentous thiotrophic episymbionts on shrimp have been interpreted to be the major diet items of the shrimp and hence are the dominant primary producers within the community. Free-living autotrophic microorganisms are implicated as the dietary base for other invertebrate species. Four trophic groups are identified within the Kairei invertebrates based on carbon- and nitrogen-isotope ratios, but these groups do not always define discrete trophic levels. Ontogenetic shifts in diet are documented for *R.* aff. *exoculata* and brachyuran crabs (*Austinograea* n. sp.). Diets of symbiont-bearing mussels (*Bathymodiolus* aff. *brevior*) and two species of gastropods are isotopically constant throughout the range of sizes analyzed. There is a consistent but unexplained pattern of increasing nitrogen isotopic composition with increasing carbon isoto-

pic composition in vent communities from geographically disjunct oceanic regions. Given the assumptions associated with interpretations of isotopic data, there remains a missing pool of carbon (presumably unsampled bacterial biomass) that contributes to the maintenance of the ^{13}C - and ^{15}N -enriched primary consumers in these ecosystems. Electronic supplementary material to this paper can be obtained by using the Springer LINK server located at <http://dx.doi.org/10.1007/s00227-002-0865-y>.

Introduction

More than two decades have passed since the initial discovery of invertebrate–bacterial trophic relationships in hydrothermal-vent tubeworms (Cavanaugh et al. 1981; Felbeck 1981). Despite the chemoautotrophic base of vent food webs, trophic structure in vent communities is comparable in many ways to food webs of photosynthetically based, shallow-water ecosystems (Van Dover 2000). In addition to free-living and symbiotic microbial primary producers, there are a variety of primary and secondary consumer types among vent invertebrate species, including grazers, suspension-feeders, deposit-feeders, predators, scavengers, and parasites. Regional differences in vent food webs reflect the species composition of the community and the functional nature (thiotrophic and/or methanotrophic) of the primary producers. On the East Pacific Rise, for example, tubeworms, clams, and mussels are typical biomass dominants. They are host to endosymbiotic bacteria that oxidize sulfide (i.e. are thiotrophic) to generate a cellular energy source for fixation of an inorganic carbon source (CO_2) into organic carbon. On the Mid-Atlantic Ridge, shrimp or mussels are typical biomass dominants. The shrimp lack endosymbionts; instead they rely at least in part on thiotrophic, filamentous episymbionts that grow in dense mats on the carapace that lines their branchial chambers (Gal'chenko et al. 1989; Casanova et al. 1993; Polz et al. 1998). The Mid-Atlantic Ridge mussel species

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(*Bathymodiolus azoricus* and *B. puteoserpentis*) host two types of autotrophic symbionts (thiotrophic and methanotrophic). While the biomass of East Pacific Rise and Mid-Atlantic Ridge vent communities is dominated by organic material produced by symbiotic bacteria, stable-isotope studies suggest that many of the primary and secondary consumer invertebrates associated with biomass dominants rely on an alternative resource, inferred to be free-living autotrophic bacteria (Van Dover and Fry 1994).

Differences in the degree of dependence on methanotrophic and thiotrophic microorganisms, such as those observed between Mid-Atlantic Ridge and East Pacific Rise vent systems, point to the potential for food-web variations in other regions. Discovery of vent communities in the Indian Ocean at the Kairei hydrothermal field (Hashimoto et al. 2001) provided an opportunity to look for additional trophic novelties within vent ecosystems. Invertebrate biomass within the Kairei field is dominated by swarms of shrimp (*Rimicaris* aff. *exoculata*) on the surfaces of black-smoker chimneys (10–20°C) and by anemone beds in a lower-temperature peripheral zone (1–2°C; Hashimoto et al. 2001; Van Dover et al. 2001). A variety of other invertebrates occur in a narrow transitional zone (1–2 m) between the base of the shrimp swarms and the proximal boundary of the anemone beds (see Appendix 1 at <http://dx.doi.org/10.1007/s00227-002-0865-y>, electronic supplementary material, for a photographic overview of these faunal zones). Many of the species found at this Indian Ocean vent field have evolutionary affinities to western Pacific vent faunas, but the ecologically dominant shrimp closely resembles its Mid-Atlantic Ridge counterpart (Hashimoto et al. 2001; Van Dover et al. 2001).

An outline of potential trophic interactions or patterns within a community can be obtained rapidly through a survey of stable-isotope compositions. Stable-isotope methods are not the only tool for assessment of trophic relationships among species within a community, and they are subject to site- and species-specific violation of some underlying assumptions regarding trophic shifts in isotopic composition. Nevertheless, isotopic methods have proven valuable in generating or constraining hypotheses about trophic relationships in systems where direct observations of feeding interactions and samples are difficult to obtain, as at deep-sea hydrothermal vents (Conway et al. 1994). Carbon and nitrogen stable-isotope compositions have been used to advantage in a variety of marine settings (e.g. Rau and Hedges 1979; Rau et al. 1992; Van Dover et al. 1992; Wainright et al. 1993). Characterization of patterns of isotopic compositions among individuals and among species at vents permits development of hypotheses regarding trophic issues such as changes in diet during growth within species (Pond et al. 1997, 2000; Polz et al. 1998; Rieley et al. 1999), site-specific differences in diet within species (Trask and Van Dover 1999; Colaço et al. 2002), and trophic interactions among species (Van Dover and Fry 1989, 1994; Fisher et al. 1994;

Southward et al. 1994; Vereshchaka et al. 2000; Colaço et al. 2002). Isotopic techniques have also been used to infer the importance of free-living chemoautotrophic microorganisms in the diet of heterotrophic invertebrates within vent communities (Van Dover and Fry 1994).

Interpretations of trophic interactions among Kairei invertebrates using isotopic compositions are based on empirical and experimental evidence for fidelity between $\delta^{13}\text{C}$ values of diet items and direct consumers (Peterson and Fry 1987), a 3–4‰ shift toward more-positive $\delta^{15}\text{N}$ values from diet items to direct consumers (Minagawa and Wada 1984), and the potential for mixing of two or more isotopically distinct dietary sources to yield intermediate isotopic values of carbon and nitrogen in consumers (Fry and Sherr 1984). These assumptions are valid only where isotopic compositions of inorganic resources do not vary significantly within sites and where species-specific fractionations of carbon and nitrogen isotopes are not anomalous. In this report, isotopic data are used in conjunction with other information regarding nutritional modes (including presence or absence of symbionts, behavior, morphology of feeding structures and digestive systems, and gut contents) to characterize a species as either a symbiont-bearing primary consumer, a non-symbiont-bearing primary consumer (e.g. suspension feeder or grazer), or an omnivore. Strict predatory or scavenging behaviors within vent invertebrates are difficult to distinguish with confidence. Many top invertebrate consumers are likely to have mixed diets that include microorganisms, primary consumers, and other omnivores.

I identified isotopically distinct groups within the Kairei invertebrate community and inferred the dietary resources and trophic relationships of these species. For five Kairei species, I examined the relationship between individual size and isotopic composition to determine and interpret shifts in diet during growth. Comparisons of carbon and nitrogen isotopic ratios for invertebrates of Indian Ocean, Atlantic, and Pacific vents allow evaluation of trophic similarities and contrasts in biogeographically disparate vent systems.

Materials and methods

Individuals of 15 invertebrate species (Table 1) were collected from the Kairei hydrothermal field (25°19.23'S; 70°02.42'E; 2415–2460 m depth) on the Central Indian Ridge using the remotely operated vehicle (R.O.V.) "Jason" in April 2001. Voucher specimens of undescribed species have been retained in an archival collection at The College of William & Mary. In situ observations of invertebrate distributions and behavior were documented by digital video, recorded during extended periods of exploration and sampling of the biological communities at the Kairei site (> 72 h). Both the video and detailed video logs have been archived at Woods Hole Oceanographic Institution. Observations reported here were recorded as the video was prepared.

Tissues were acid-fumed to remove contaminating carbonates, dried (60°C), and powdered prior to isotopic analysis. Gill and mantle tissues were analyzed separately for mollusk species known to host chemoautotrophic endosymbionts in their gills. The

Table 1. Major groups and species of Kairei invertebrates analyzed for carbon- and nitrogen-isotope composition, including familial identification, habitat, and numbers of individuals (*n*). The central region of the Kairei Field is characterized by black smokers, warm (> 10°C) diffuse flows, and swarming shrimp. The lower boundary of the shrimp swarms is sharply defined and forms the top of a 1–2 m wide transition zone of lower-temperature (< 10°C) sulfides. A second sharp boundary exists at the base of the transition zone, where dense, peripheral populations of anemones occur on sulfides in ambient seawater. The peripheral zone extends as much as tens of meters away from the base of the black smoker complexes in some areas

| Species | Family | Habitat | <i>n</i> |
|--|---------------------------------|-------------------------------|----------|
| Symbiont-bearing mollusks | | | |
| <i>Bathymodiolus</i> aff. <i>brevior</i> | Bathymodiolid mussel | Transition | |
| Mantle | | | 15 |
| Gill | | | 15 |
| <i>Alviniconcha</i> n. sp. | Provannid gastropod | Transition | |
| Mantle | (hairy gastropod) | | 10 |
| Gill | | | 9 |
| Potential symbiont-bearing mollusk | | | |
| Scaly-footed vetigastropod | Neomphalid gastropod | Transition | |
| Mantle | | | 10 |
| Gill | | | 9 |
| Other mollusks | | | |
| <i>Desbruyeresia</i> n. sp. | Provannid gastropod | Transition | 3 |
| <i>Phymorhynchus</i> n. sp. | Turrid gastropod | Transition, periphery | 5 |
| <i>Lepetodrilus</i> n. sp. | Lepetodrilid limpet | Transition | 9 |
| Polychaetes | | | |
| <i>Branchiopolynoe</i> n. sp. | Polynoid (commensal) polychaete | Transition | 4 |
| <i>Branchinotogluma</i> n. sp. | Polynoid polychaete | Transition | 6 |
| <i>Archinome</i> cf. <i>rosacea</i> | Archinomid polychaete | Transition, periphery | 15 |
| <i>Amphisamytha</i> n. sp. | Ampharetid polychaete | Transition | 10 |
| Crustaceans | | | |
| <i>Rimicaris</i> aff. <i>exoculata</i> | Alvinocarid shrimp (> 13 mm) | Center | 7 |
| <i>Austinograea</i> n. sp. | Bythograeid crab | Center, transition, periphery | 10 |
| <i>Neolepas</i> n. sp. | Eolepadid barnacle | Transition, periphery | 12 |
| Other | | | |
| Nemertea | Unknown | Transition | 15 |
| <i>Marianactis</i> cf. <i>bythios</i> | Actinostolid anemone | Periphery | 10 |

scaly-footed vetigastropod was a candidate for a symbiotic association; its gill and mantle tissues were also analyzed separately. Whole bodies of other species were analyzed, with the exception of the crab and shrimp, where only muscle tissue was used. Shrimp were categorized into four groups (I–IV) based on similarities in size and shape (especially the degree of inflation of the carapace). The intent was to discriminate molt stages, but resolution of stages by this simple method is only approximate and should not be taken as a true indication of the actual number of molts. Shrimp carapace lengths were measured along the dorsal mid-line from the frontal margin to the posterior margin after the groups were segregated.

Stable-isotope analyses were conducted by R. Petty (Analytical Laboratory, Marine Science Institute, University of California, Santa Barbara) using a Europa Tracermass, continuous-flow, stable-isotope ratio, mass spectrometer, coupled with a Europa Roboprep elemental analyzer. Isotope ratios are reported using the delta notation:

$$\delta X = [(R_{\text{sample}} - R_{\text{reference}}) / R_{\text{reference}}] \times 10^3$$

where *X* (in ‰) is ¹³C or ¹⁵N, *R* is ¹³C/¹²C or ¹⁵N/¹⁴N, and reference materials are the Pee Dee Belemnite standard or air, respectively.

Significant differences in isotopic compositions of species were determined using one-way ANOVAs for each isotope. Because standard parametric assumptions were not met with natural-log- or square-root-transformed data, non-parametric Kruskal–Wallis tests were used to validate the ANOVAs. Post hoc Tukey's tests (error rate of 0.1) identified species with similar isotopic compositions. Paired *t*-tests were used to evaluate the significance of differences between mantle and gill tissues in mussels, hairy gastropods, and scaly-footed vetigastropods. Pearson product-moment correlations were used to evaluate the relationship between carbon and nitrogen isotopic values and between size and isotopic composition. All statistical tests were calculated using Minitab release 13.30.

Results and discussion

Behavioral observations (see Appendices 2–12 <http://dx.doi.org/10.1007/s00227-002-0865-y>, electronic supplementary material for video clips of observations described here)

As at Mid-Atlantic Ridge vents, shrimp (*Rimicaris* aff. *exoculata*) swarmed over surfaces of sulfide chimneys in warm water, presumably to maintain their episymbionts in concentrations of inorganic resources (CO₂, O₂, H₂S) necessary for chemoautotrophy. Anemones (*Marianactis* cf. *bythios*) narcotized and ingested shrimp that had been disturbed by R.O.V. “Jason” manipulations. Bathymodiolid mussels (closely related or identical to *Bathymodiolus brevior* first described from Mariana vents; Van Dover et al. 2001) and hairy gastropods (*Alviniconcha* n. sp.) occurred in sedentary aggregations of up to hundreds of individuals at the base of shrimp swarms. Several bythograeid crabs (*Austinograea* n. sp.) were observed consuming shrimp (probably *Rimicaris* aff. *exoculata*, based on the high densities of this species, but possibly *Chorocaris* n. sp.). Avoidance of crabs by shrimp was suggested by the presence of a shrimp-free halo of several centimeters around a crab in the middle of a shrimp aggregation. Archinomid polychaetes (*Archinome* cf. *rosacea*) occurred in a variety of settings, but individuals sampled for stable-isotope analyses were

from among accumulations of *Rimicaris* aff. *exoculata* molts, suggesting a nutritional role of shrimp exuviae in the diet of this polychaete. Large (~6 cm length) white polynoid polychaetes (*Branchinotogluma* n. sp.) roamed sulfide surfaces throughout the transition zone between anemones and shrimp. Red nemerteans occurred as rare, globular clusters of hundreds of individuals. The turrid gastropod (*Phymorhynchus* n. sp.) is mobile and was observed in the transition zone and in peripheral anemone beds, occasionally in clusters of several individuals. Stalked barnacles (*Neolepas* n. sp.) occurred as small clusters of tens of individuals on local prominences in a transition zone between anemones and shrimp. Barnacles (*Neolepas* sp.) are presumed to be suspension feeders on bacteria (Southward and Newman 1998). Video records document the retraction of individual cirri toward the mouth rather than the rhythmic retraction of all cirri familiar in shallow-water barnacle species. Episymbiotic bacteria growing on cirral setae are an alternative diet for Indian Ocean vent barnacles (Southward and Newman 1998). Scaly-footed vetigastropods were collected together with *Alviniconcha* n. sp., but no observations of their behavior were made. Small coiled gastropods (*Desbruyeresia* n. sp.), limpets (*Lepetodrilus* n. sp.), and a small ampharetid polychaete (*Amphisamytha* n. sp.) were not documented by video due to their small sizes and cryptic habitats associated with sulfide deposits in the anemone zone.

Stable-isotope compositions (see Appendix 13 <http://dx.doi.org/10.1007/s00227-002-0865-y>, electronic supplementary material for a spreadsheet of paired carbon and nitrogen isotopic compositions for individuals within species)

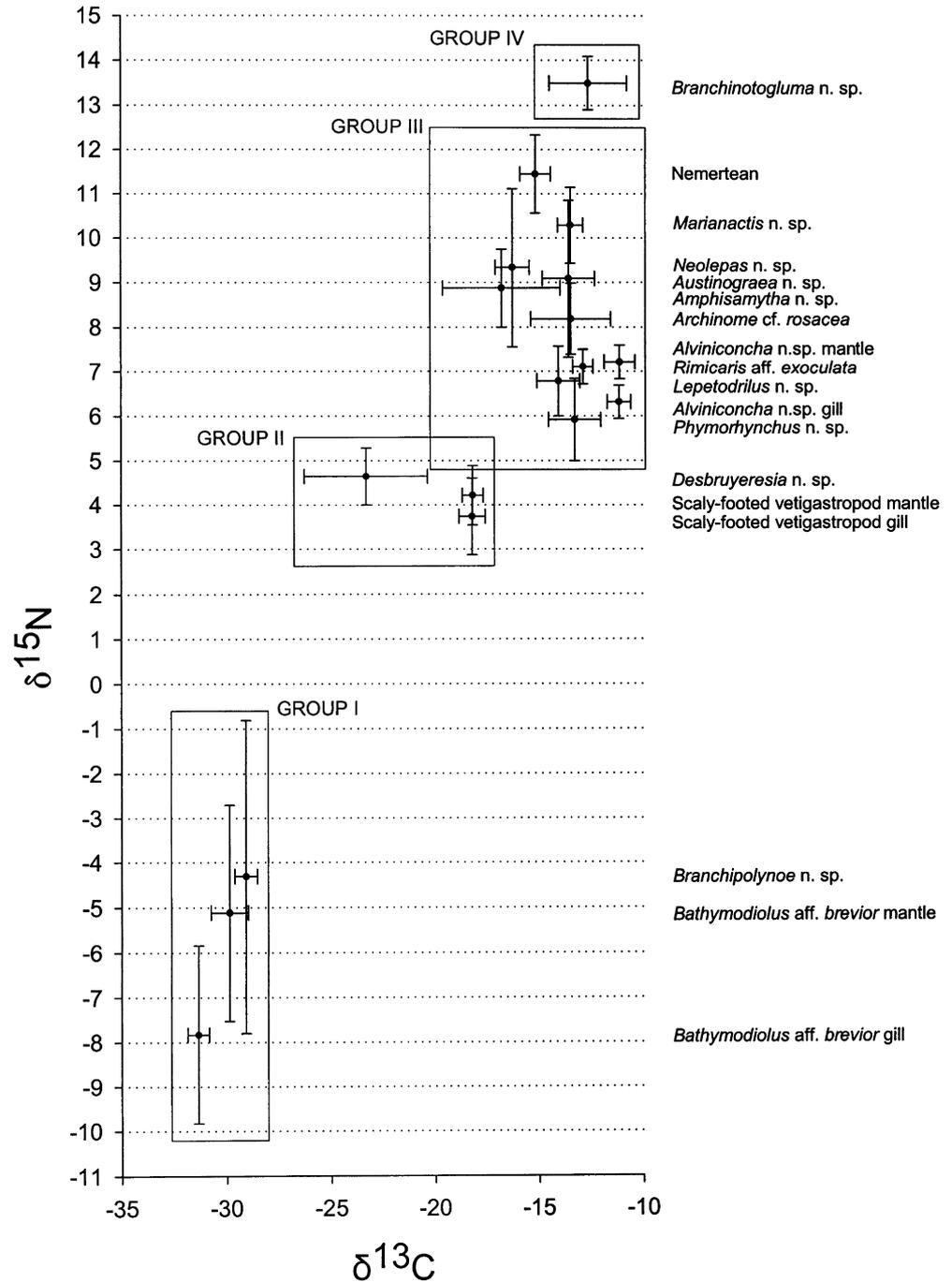
Adult shrimp and anemones (*Marianactis* cf. *bythios*) were the overwhelming biomass dominants at Kairei. Shrimp are likely to be an important dietary item for the anemones, based on visual observations of shrimp capture and ingestion by anemones, gut contents of anemones, similar $\delta^{13}\text{C}$ values of shrimp (-12.8‰) and anemones (-13.5‰), and the 3‰ enrichment in ^{15}N in anemones relative to shrimp. Biomass of the Kairei vent field was thus dominated by organic carbon with $\delta^{13}\text{C}$ values of about -13‰ . Since adult shrimp are likely to derive most of their nutrition from their episymbionts, the episymbiotic bacteria are implicated as the most important primary producers for maintenance of primary consumer biomass in the Kairei food web.

Four isotopically distinctive groupings among the 15 Kairei invertebrates are suggested by a plot of carbon- and nitrogen-isotope values (Fig. 1), but only the mussel and its commensal (group I) are significantly different from all other species in terms of both carbon and nitrogen (Tables 2, 3). Isotopic compositions of mussel gill and mantle tissues are significantly different from each other (paired *t*-test; $P < 0.001$). Isotopic ratios of gill tissues in a single *Bathymodiolus brevior* specimen from

the Marianas vents ($\delta^{13}\text{C}_{\text{gill}} = -34.8\text{‰}$; $\delta^{15}\text{N}_{\text{gill}} = -3.0\text{‰}$; Van Dover and Fry 1989) are outside the range of values measured in Kairei mussels. The differences are small, however, implicating a causal agent such as site-specific variation in the isotopic values of the source inorganic carbon and nitrogen rather than a novel nutritional strategy. There are notably large variances associated with nitrogen-isotope compositions in the Kairei mussels, suggesting that the inorganic nitrogen sources used by the symbiotic (or free-living) bacteria on which it feeds have variable isotopic compositions. The commensal polynoid polychaete (*Branchipolynoe* n. sp.) has mean $\delta^{13}\text{C}$ values about 2‰ heavier than the host mussel gill tissue and $\delta^{15}\text{N}$ values enriched in ^{15}N by about 3.5‰. These isotopic values are consistent with, but do not prove, a consumer–diet relationship between the polynoid and its host mussel (Fisher et al. 1994). Kairei group I species are trophically and isotopically isolated from the other groups. Turrid gastropods in the genus *Phymorhynchus* have been implicated as consumers of mussels at vents (Fisher et al. 1994), but, at Kairei, isotopic data indicate that *Phymorhynchus* n. sp. does not belong to this trophic group. Were mussels and their commensals to disappear from the Kairei site, there would likely be no effect on the isotopic relationships or trophic interactions of the other taxa analyzed here.

Species within group II of Fig. 1 are both gastropods. They have intermediate values of carbon-isotope ratios that are statistically different from those of all, or all but one, other species (Table 2). The carbon and nitrogen isotopic compositions of *Desbruyeresia* n. sp. are sufficiently unique to suggest that this small coiled gastropod species may host endosymbiotic bacteria or otherwise have an unusual diet. The behavior and anatomy of the vetigastropod seem to preclude it from deriving its nutrition from a mixed diet of species with isotopically light and heavy carbon. Instead, the sedentary life-style within the diffuse-flow transition zone and anatomical details of its reduced digestive system suggest that the scaly-footed vetigastropod is a candidate host for endosymbionts (Van Dover et al. 2001; A. Waren, personal communication), but tests for enzymes diagnostic of autotrophic metabolism and microscopic examination of gill tissues for symbionts were negative (Van Dover et al. 2001). There is empirical evidence that gill tissues in symbiont-hosting mollusk species have more-negative $\delta^{15}\text{N}$ values than non-gill tissues (Van Dover and Fry 1989; Fisher et al. 1994; present study). Lack of a significant difference ($P > 0.01$) between gill and mantle nitrogen-isotope ratios in the scaly-footed vetigastropod might place the weight of isotopic evidence toward a non-symbiont mode of nutrition, but there is also no significant difference between gill and mantle nitrogen-isotope ratios in *Alviniconcha* n. sp., which is known to have sulfide-oxidizing symbionts in its gills. $\delta^{13}\text{C}$ values of -18‰ are characteristic of particulate organic material of non-vent, deep-sea environments derived from surface photosynthetic primary production (Van Dover and Fry 1989), but the nitrogen isotopic composition of

Fig. 1. Carbon and nitrogen isotopic compositions (‰; mean \pm SD) for hydrothermal vent invertebrates from the Kairei field on the Central Indian Ridge. Boxes defining groups I–IV are used to highlight isotopically similar species groups. *Rimicaris* aff. *exoculata* data are for individuals with carapace length > 13 mm



this detrital organic material when it reaches the seabed is on the order of 18‰. Thus neither of the group II species ($\delta^{15}\text{N}$ of ~ 4 ‰) are likely to be reliant on photosynthetically derived organic material.

Group III of Fig. 1 embraces a variety of trophic modes, including thiotrophic endosymbionts in *Alviniconcha* n. sp. (Van Dover et al. 2001), thiotrophic epibionts in *Rimicaris* aff. *exoculata*, grazing and/or suspension-feeding in *Lepetodrilus* n. sp., mucociliary feeding in *Amphisamytha* n. sp., suspension feeding or epibionts in *Neolepas* n. sp., carnivory in *Marianactis* cf. *bythios*, and omnivory in *Phymorhynchus* n. sp.,

Archinome cf. *rosacea*, *Austinograea* n. sp., and the nemertean. Group III species have a relatively large range (4‰) of mean carbon-isotope ratios, with many species (five to seven) that are statistically similar to one another (Table 2). As expected from such a trophically diverse grouping, there is a large range in mean nitrogen-isotope compositions (5.5‰). Host tissues of symbiont-bearing species in group III (shrimp and hairy gastropods) have nitrogen isotopic compositions that could not be statistically distinguished from those of other primary consumers (e.g. *Lepetodrilus* n. sp., *Amphisamytha* n. sp.) or from omnivores (e.g. *Austinograea* n. sp.). The inability to

Table 3. Kairai invertebrate nitrogen-isotope groups formed based on results of Tukey's pairwise comparisons (post hoc with error rate of 0.1). Species showing statistically similar $\delta^{15}\text{N}$ diets are linked by *asterisks*. Mean (SD) $\delta^{15}\text{N}$ (‰) values are given for each species. For symbiont-hosting mollusk species, the isotopic composition of host mantle tissue was used; for shrimp, isotopic values for adults only were used

| | <i>Branchinotoglutina</i> n. sp. | Nemertean | <i>Marianactis</i> cf. <i>bythios</i> | <i>Neolepas</i> n. sp. | <i>Austinograca</i> n. sp. | <i>Amphisamytha</i> n. sp. | <i>Archinome</i> cf. <i>rosacea</i> | <i>Alviniconcha</i> n. sp. | <i>Rimicaris</i> aff. <i>exoculata</i> | <i>Lepetodrilus</i> n. sp. | <i>Phymorhynchus</i> n. sp. | <i>Desbrayeresia</i> n. sp. | Scaly-footed vetigastropod | <i>Branchiopolynoë</i> n. sp. | <i>Bathymodiolus</i> aff. <i>brevior</i> | |
|--|-------------------------------------|-----------|--|---------------------------|-------------------------------|-------------------------------|--|-------------------------------|---|-------------------------------|--------------------------------|--------------------------------|-------------------------------|----------------------------------|---|---|
| Mean $\delta^{15}\text{N}$ | 13.5 | 11.4 | 10.3 | 9.3 | 9.1 | 8.9 | 8.2 | 7.2 | 7.1 | 6.8 | 5.9 | 4.6 | 4.2 | 4.3 | -5.1 | |
| (SD) | (0.6) | (0.9) | (0.8) | (1.8) | (1.8) | (0.8) | (0.8) | (0.4) | (0.4) | (0.8) | (0.9) | (0.6) | (0.7) | (3.5) | (2.4) | |
| <i>Branchinotoglutina</i> n. sp. | * | | | | | | | | | | | | | | | |
| Nemertean | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| <i>Marianactis</i> cf. <i>bythios</i> | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| <i>Neolepas</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Austinograca</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Amphisamytha</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Archinome</i> cf. <i>rosacea</i> | | | | | | | | | | | | | | | | |
| <i>Alviniconcha</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Rimicaris</i> aff. <i>exoculata</i> | | | | | | | | | | | | | | | | |
| <i>Lepetodrilus</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Phymorhynchus</i> n. sp. | | | | | | | | | | | | | | | | |
| <i>Desbrayeresia</i> n. sp. | | | | | | | | | | | | | | | | |
| Scaly-footed vetigastropod | | | | | | | | | | | | | | | | |
| <i>Branchiopolynoë</i> n. sp. | | | | | | | | | | | | | | * | * | * |
| <i>Bathymodiolus</i> aff. <i>brevior</i> | | | | | | | | | | | | | | * | * | * |

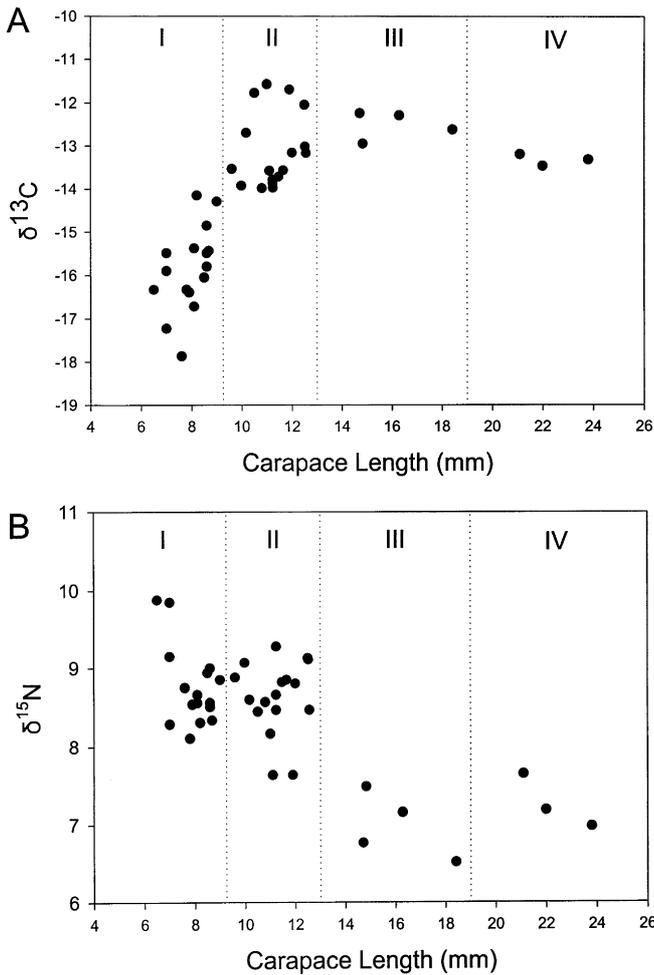


Fig. 2A, B. *Rimicaris aff. exoculata*. **A** $\delta^{13}\text{C}$ (‰) versus carapace length. **B** $\delta^{15}\text{N}$ (‰) vs carapace length. Roman numerals I–IV approximate successive molt stages, but the classification is subjective, based on morphological characteristics. The numerical sequence does not imply that all molt stages are represented in these samples. Individuals classified as adults (as reported in Table 1) are > 13 mm carapace length

distinguish trophic strategies between primary and secondary consumers using nitrogen isotopes in these group III species (Table 3) is most likely due to large variations in nitrogen isotopic compositions (Fig. 1) that result from different feeding histories within omnivorous individuals, but differences in source inorganic nitrogen cannot be eliminated as an alternative explanation. Standard deviations of mean carbon-isotope values of omnivorous species (*Archinome cf. rosacea*, *Austinograea n. sp.*, and *Phymorhynchus n. sp.*) are also relatively large (Fig. 1; Table 2) and, in the case of the crab, are correlated with age/size of the individual. Variation in isotopic composition of inorganic resources used by the shrimp and hairy gastropod symbionts appears to be low, based on standard deviations of both carbon and nitrogen isotopes in these species.

Group IV of Fig. 1 consists of a single species, the white polynoid polychaete (*Branchinotogluma n. sp.*). Its

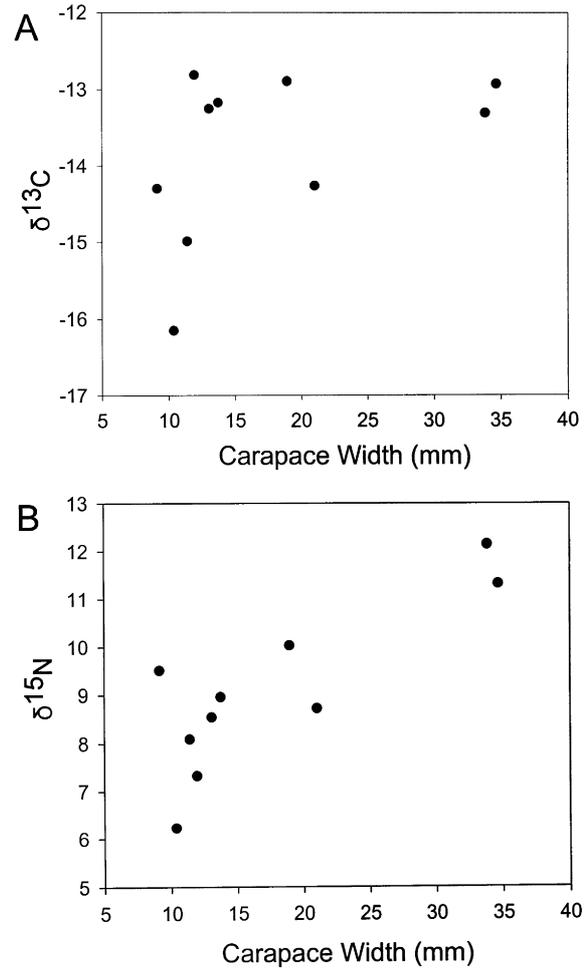


Fig. 3A, B. *Austinograea n. sp.* **A** $\delta^{13}\text{C}$ (‰) versus carapace width. **B** $\delta^{15}\text{N}$ (‰) versus carapace width

carbon isotopic composition cannot be distinguished from that of seven other species (Table 2), but its nitrogen isotopic composition is statistically different from all species except the nemertean (Table 3). This polychaete appears to be at the top of the food web among the species analyzed. It seems likely that the diet of this polychaete (and perhaps of the nemertean) is more specialized or restricted to prey of higher trophic levels than the diet of the brachyuran crab, for example, which likely feeds across multiple trophic levels (diets of other vent brachyurans include filamentous bacteria, limpets, polychaetes, and small crustaceans; Van Dover, personal observations) and hence would have a more-negative nitrogen isotopic composition. The identity of the putative diet items of *Branchinotogluma n. sp.* remains unknown, due to the small number of individuals collected.

To test the null hypothesis that isotopic composition does not vary with size in vent invertebrates, isotopic compositions of different-sized individuals within selected species were measured and tested for significant correlations between variables. There was a significant positive correlation ($P < 0.01$) between carapace length and $\delta^{13}\text{C}$ in juvenile shrimp (*Rimicaris aff. exoculata*;

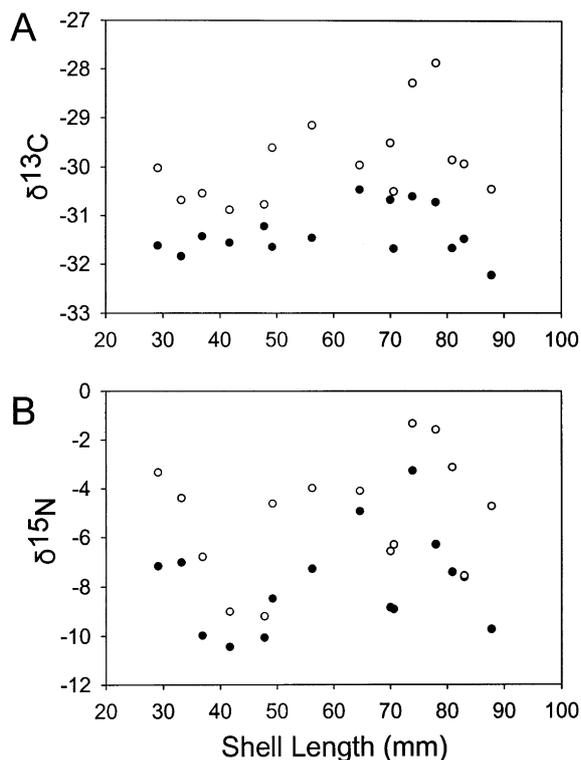


Fig. 4A, B. *Bathymodiolus* aff. *brevior*. **A** $\delta^{13}\text{C}$ (‰) versus shell length. **B** $\delta^{15}\text{N}$ (‰) versus shell length (*open circles* mantle tissue; *closed circles* gill tissue)

6–13 mm carapace length) and a significant negative correlation ($P < 0.01$) between carapace length and $\delta^{15}\text{N}$ for shrimp with carapace lengths of 6–19 mm (Fig. 2). Shifts in diet during post-larval growth have been well-documented using lipid biomarkers for shrimp species (*Rimicaris exoculata*, *Mirocaris fortunata*) at Mid-Atlantic Ridge hydrothermal vents (Pond et al. 1997, 2000). Larval and juvenile stages of the Mid-Atlantic Ridge vent shrimp species acquire lipid reserves from photosynthetically produced organic material, with $\delta^{13}\text{C}$ values of -17‰ to -28‰ . Bulk carbon-isotope values decrease to an average of about -13‰ in adults (Rieley et al. 1999). From the relationship between bulk carbon isotopic data and carapace length, a similar ontogenetic shift in feeding strategy by the Kairei shrimp is inferred. Changes in carbon- and nitrogen-isotope ratios with growth in *Rimicaris* aff. *exoculata* are consistent with a change in diet from recycled phytodetritus to direct feeding on primary producers. Beyond carapace lengths of 14 mm, there is no correlation between body size and isotope ratios of shrimp ($\delta^{13}\text{C}$ vs carapace length: $P = 0.029$; $\delta^{15}\text{N}$ vs carapace length: $P = 0.837$).

No significant change in $\delta^{13}\text{C}$ ($P = 0.206$) was observed with carapace width in the bythograeid crab (Fig. 3A), but there was a significant ($P < 0.01$) positive correlation between carapace width and $\delta^{15}\text{N}$ (Fig. 3B), suggesting that the diet of the crabs changes as they mature. This shift in diet seems likely to be related to a shift in food-handling capabilities toward larger prey

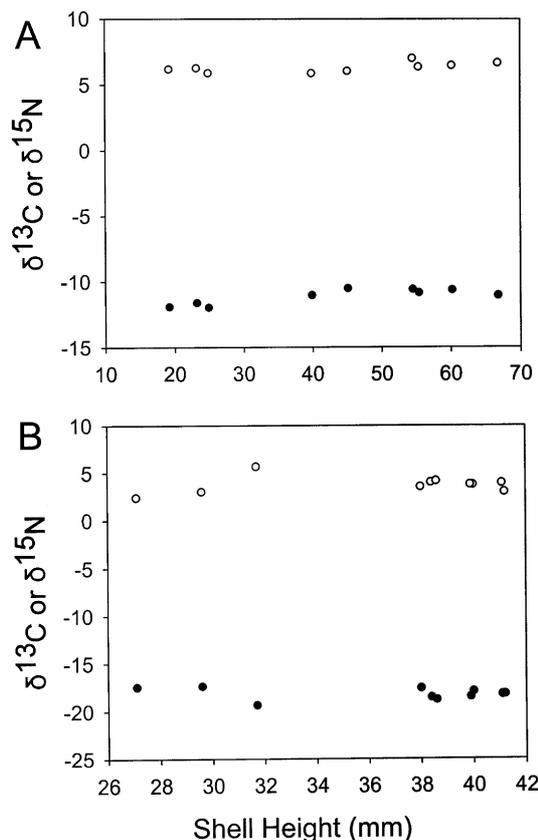


Fig. 5A, B. *Alviniconcha* n. sp. and scaly-footed vetigastropod. Relationship between shell height and isotopic composition (‰) for two gastropod species from Kairei vents. **A** *Alviniconcha* n. sp. **B** Scaly-footed vetigastropod (*open circles* $\delta^{15}\text{N}$; *closed circles* $\delta^{13}\text{C}$)

and higher trophic levels, as has been observed for other aquatic and marine predators (Wyatt 1976).

There was no correlation ($P > 0.1$) between shell length and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in mussel mantle or gill tissues (Fig. 4) or between shell height and either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ in hairy gastropod or scaly-footed vetigastropod gill tissues (Fig. 5). The lack of significant correlations between size and either carbon or nitrogen isotopes in these species indicates that the isotopic composition of their diets and the host-symbiont nutritional relationship are constant for sizes measured here.

Stable-isotope compositions of Indian Ocean vent invertebrate tissues ($\delta^{13}\text{C}$ between -32‰ and -11‰ ; $\delta^{15}\text{N}$ between -8‰ and 14‰ ; Fig. 1) fall within the range of values measured in invertebrates from other deep-sea hydrothermal systems (e.g. Van Dover and Fry 1989; Fisher et al. 1994; Colaço et al. 2002). Carbon and nitrogen isotopic data for vent communities in the eastern Pacific (Galapagos Rift; Fisher et al. 1994), western Pacific (Mariana vents; Van Dover and Fry 1989), Mid-Atlantic Ridge (Lucky Strike; Colaço et al. 2002), and Indian Ocean (present study) are now available (Table 4; Fig. 6).

At the generic level, the most dramatic isotopic discrepancy between species among sites is observed in *Alviniconcha*. Kairei *Alviniconcha* n. sp. are morphologically and genetically distinct from *Alviniconcha*

Table 4. Comparison of stable-isotope compositions at Indian (Kaiei), western Pacific (Mariana Back-Arc), eastern Pacific (Galapagos Rift), and Atlantic (Lucky Strike) Ocean hydrothermal vents. Data are ordered by decreasing $\delta^{13}\text{C}$ values to highlight similarities and differences in carbon resources among sites and species [S symbiont-bearing (epi- or endosymbiont); P primary consumer (suspension feeder or grazer); O omnivore or secondary

consumer]. Isotopic data from Fisher et al. (1994), Van Dover and Fry (1989), and Colaço et al. (2002). For bivalve species with gill symbionts, gill isotopic compositions are reported, except for *Bathymodiolus azoricus* from Lucky Strike, for which gill and muscle tissue were not significantly different and were reported as pooled data by Colaço et al. (2002)

| $\delta^{13}\text{C}$ | Indian Ocean | | Marianas | | Galapagos | | Lucky Strike | |
|-----------------------|--|-----------------------|-------------------------------|-----------------------|-----------------------------------|-----------------------|---------------------------------|-----------------------|
| | Species | $\delta^{15}\text{N}$ | Species | $\delta^{15}\text{N}$ | Species | $\delta^{15}\text{N}$ | Species | $\delta^{15}\text{N}$ |
| -11 | <i>Alviniconcha</i> n. sp. | 6.3 S | <i>Paralvinella</i> sp. | 8.2 P | <i>Riftia pachyptila</i> | 2.0 S | <i>Rimicaris exoculata</i> | 7.9 S |
| -12 | <i>Branchinotogluma</i> sp. | 13.5 O | | | | | | |
| | <i>Rimicaris</i> aff. <i>exoculata</i> | 7.1 S | | | | | | |
| -13 | <i>Phymorhynchus</i> sp. | 5.9 O | Limpet | 8.6 P | | | | |
| | <i>Archinome</i> cf. <i>rosacea</i> | 8.2 O | | | | | | |
| | <i>Marianactis</i> cf. <i>bythios</i> | 10.3 O | | | | | | |
| | <i>Austinograea</i> n. sp. | 9.1 O | | | | | | |
| -14 | <i>Lepetodrilus</i> sp. | 6.8 P | | | | | <i>Chorocaris chacei</i> | 4.9 O |
| -15 | Nemertean | 11.4 O | <i>Austinograea williamsi</i> | 9.3 O | Polynoid | 9.7 O | | |
| | | | <i>Marianactis bythios</i> | 8.6 O | | | | |
| -16 | <i>Neolepas</i> n. sp. | 9.3 P | <i>Chorocaris vandoverae</i> | 8.8 O | | | <i>Mirocaris fortunata</i> | 3.5 P |
| | <i>Amphisamytha</i> n. sp. | 8.9 O | | | | | Anemone | 6.2 O |
| -17 | | | | | <i>Bythograea therydron</i> | 8.8 O | | |
| | | | | | <i>Munidopsis subsquamosa</i> | 7.7 O | | |
| -18 | Scaly-footed vetigastropod | 3.7 P | | | <i>Neomphalus fretterae</i> | 8.6 P | | |
| -19 | | | <i>Neolepas</i> sp. | 7.6 P | <i>Bythites hollisi</i> | 8.0 O | | |
| -20 | | | | | <i>Lepetodrilus elevatus</i> | 6.6 P | | |
| -21 | | | | | <i>Alvinocaris lusca</i> | 5.9 P | | |
| -22 | | | | | | | <i>Bathymodiolus azoricus</i> | -4.6 S |
| -23 | <i>Desbruyeresia</i> sp. | 4.6 S or P | | | | | <i>Amathys lutzi</i> | 0.9 P |
| -24 | | | | | <i>Ventiella sulfuris</i> | 2.3 P | <i>Sericosura</i> sp. | 4.1 P |
| -25 | | | | | | | | |
| -26 | | | | | <i>Eulepetopsis vitrea</i> | 4.2 P | <i>Phymorhynchus</i> sp. | -6.2 O |
| -27 | | | | | | | | |
| -28 | | | <i>Alviniconcha hessleri</i> | 6.1 S | <i>Archinome rosacea</i> | 1.6 O | | |
| -29 | <i>Branchipolynoe</i> n. sp. | -4.3 P | | | | | | |
| -30 | | | | | | | <i>Bathymodiolus azoricus</i> | -9.5 S |
| -31 | <i>Bathymodiolus</i> aff. <i>brevior</i> | -7.8 S | | | <i>Phymorhynchus</i> sp. | 5.5 O | <i>Branchipolynoe seepensis</i> | 6.9 P |
| -32 | | | | | | | | |
| -33 | | | | | <i>Calyptogena magnifica</i> | 1.4 S | | |
| -34 | | | <i>Bathymodiolus brevior</i> | -3.0 S | | | | |
| -35 | | | | | <i>Bathymodiolus thermophilus</i> | 2.7 S | | |

hessleri of the Mariana vents. They are also isotopically distinct with respect to $\delta^{13}\text{C}_{\text{gill}}$ values (Kaiei: -11.1‰ , Mariana: ca. -28‰ ; Van Dover and Fry 1989). This

large disparity in carbon isotopic composition between closely related *Alviniconcha* species and the coincidence of the -11‰ and -28‰ carbon isotopic values with

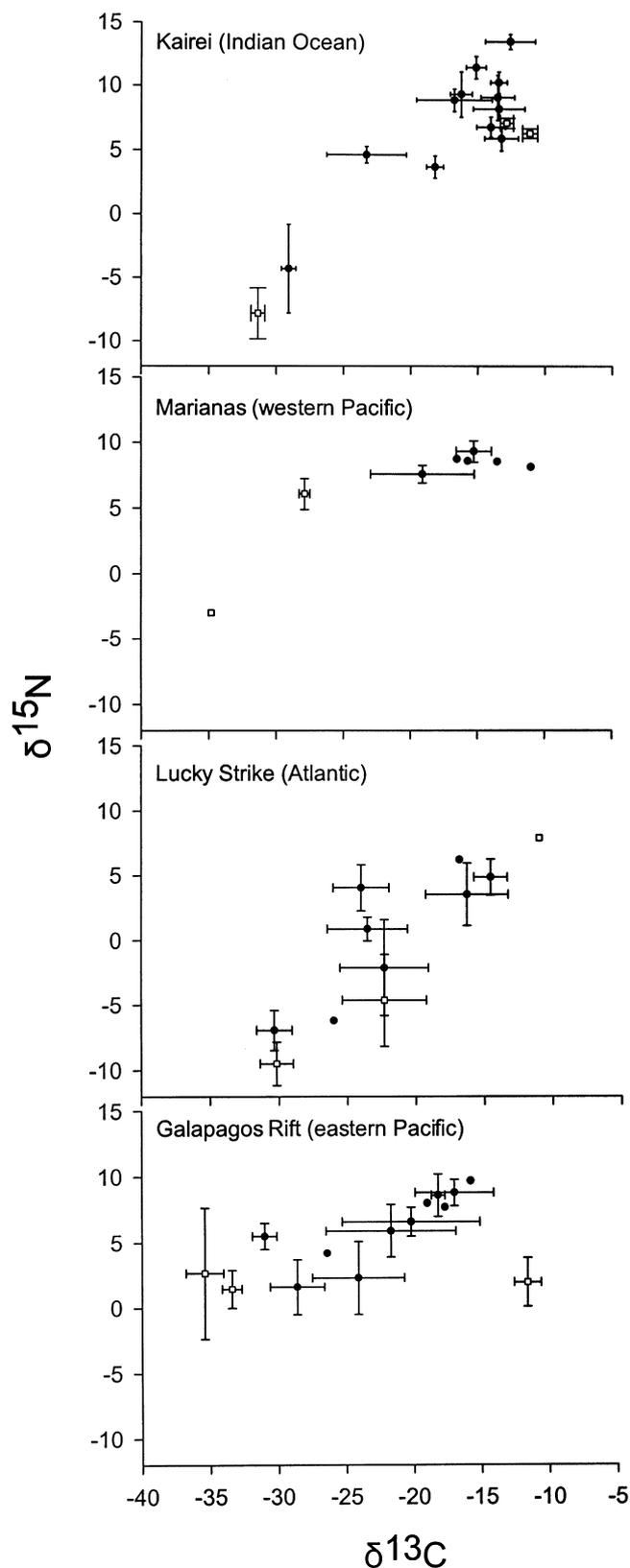


Fig. 6. Comparison of carbon and nitrogen isotopic compositions (‰; mean \pm SD provided where more than two individuals were analyzed) for biogeographically disjunct hydrothermal vent communities. Data from Table 4 (open squares species with endo- or epibionts)

empirically observed fractionation effects of two forms of the CO₂-fixing enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; Robinson and Cavanaugh 1995) suggest that the two *Alviniconcha* species host symbiont species that express distinctive forms of the Rubisco enzyme.

At the community level, there is a consistent pattern of increasing $\delta^{13}\text{C}$ with increasing $\delta^{15}\text{N}$, with the exception of the tubeworm (*Riftia pachyptila*) at Galapagos vents (Fig. 6). Given assumptions of enrichment in ^{15}N at higher trophic levels, fidelity in $\delta^{13}\text{C}$ from one trophic level to another, and the potential for mixed diets, a second, as yet unidentified primary producer with $\delta^{13}\text{C}$ values of ca. -10‰ to -15‰ and $\delta^{15}\text{N}$ values roughly between ca. -1‰ and 5‰ , is implicated in each of the communities (Van Dover and Fry 1989, 1994; Fisher et al. 1994). For Lucky Strike, the one community where the isotopic composition of bacterial mats is available, average $\delta^{13}\text{C}$ values (-24‰ to -35‰) and average $\delta^{15}\text{N}$ values (-10‰ to 4‰) do not meet these isotopic criteria (Colaço et al. 2002). The existence of this primary producer pool does not account for the positive correlation between carbon- and nitrogen-isotope compositions. Fidelity of $\delta^{13}\text{C}$ with diet may be violated at the community level through the additive effects of small trophic shifts toward more positive values of $\delta^{13}\text{C}$. These shifts could be difficult to detect at species level due to mixed diets, but become apparent when the appraisal is executed at the community level. Assuming a 1‰ shift toward more ^{13}C -enriched $\delta^{13}\text{C}$ values with each trophic step (Michener and Schell 1994), this explanation requires a minimum of 13 steps to progress directly from -24‰ to -11‰ and is deemed untenable. The possibility of ^{13}C enrichments per trophic step that are $>1\text{‰}$ should be explored. Resolution of these isotopic and trophic ambiguities requires more comprehensive sampling and analysis of suspended and attached microorganisms than has so far been undertaken.

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