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Habitat controls on vent food webs at Eifuku Volcano, Mariana Arc

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Abstract: We studied food web characteristics at three contrasting habitats on the slopes of NW Eifuku volcano in the Northern Mariana Arc, using stable carbon and nitrogen isotopes. The broad and apparently stable plume of hydrothermal flow that was being advected from Champagne site to the Mussel Bed area supported an extensive, high biomass community with a wide trophic base and several levels of consumers. The other two sites, with intense and unstable, or weak hydrothermal regimes, hosted simpler food webs. ^{13}C-enrichment in samples from the vigorously venting environment at Champagne site suggests a strong input of chemosynthesis using Rubisco form II, although this factor alone does not account for the degree of ^{13}C enrichment. In contrast, δ^{13}C depletion, attributable to form I Rubisco or possibly methanotrophy, was observed in the mussels at the other two sites that were receiving advected or weak local hydrothermal flow. At Mussel Bed site, where mussels predominated, this ^{13}C-depleted carbon also contributed to the diet of other species, whereas consumers at Top Tower site did not appear to be relying on carbon produced by the isolated mussels found at this site.

Keywords: Hydrothermal vents • Food web • Stable isotopes • Rubisco • Metanotrophy • Chemosynthesis

Introduction

Vent fluid properties and habitat physical characteristics have a strong influence on community structure at deep-sea hydrothermal vents (Sarrazin et al., 1999). Temperature and sulphide exposure limit the distribution of vent species (Luther et al., 2001), and fluid flow intensities affect primary production by chemoautotrophic bacteria. Habitat conditions can shape vent food webs as well, by controlling faunal composition, and the composition and productivity of the chemoautotrophic microbial community that constitutes the base of the food web. Microbial primary production at vents contributes directly to the diet of symbiotic and grazing organisms (Karl, 1995; Grelon et al., 2006). Links from chemosynthetic bacteria to other trophic levels are not always clear-cut; predation has been identified as an important structuring factor at vents (Micheli et al., 2002) and detrital organic material can support macrofaunal and meiofauna consumers (Levesque et al., 2005 & 2006).

We studied food web characteristics at three contrasting habitats on the slopes of NW Eifuku volcano in the Northern Mariana Arc, using stable carbon and nitrogen isotopes. Stable isotope signatures of organisms and non-living organic matter can reveal food sources and trophic relationships. On average, consumers are similar or slightly enriched (+ 0.4‰) in δ^{13}C values compared to their food source, whereas δ^{15}N displays a stepwise increment of on average 3.4‰ between trophic levels (Post, 2002). We hypothesized that one site, *Champagne*, that was highly unstable and characterized by vigorous vent flows, saturating levels of dissolved CO_2 and abundant free-living chemosynthetic production, would support a very constrained and simple food web, consisting of limited number of tolerant faunal species directly grazing on microbial biomass. At the other two sites, with little or no exposure direct venting, we expected to find a more diverse fauna exploiting a greater variety of food sources.

Materials and Methods

Study sites

NW Eifuku underwater volcano is located in the Northern Mariana Arc at 21°36.0’N, 144°46.5’ E (Fig. 1). Three sites were sampled along the slopes of the volcano, at a depth of around 1550 m. The first site, *Mussel Bed*, was characterized by a weak hydrothermal flow, advecting upslope from intense discharge at the slightly deeper *Champagne* site. There were few visible signs of free-living chemosynthetic microbial growth at *Mussel Bed* site, which was several hundred square metres in area and covered by *Bathymodiolus* mussel colonies (Fig. 2a). Abundant Alvinocarid shrimps were probing the surface of mussels for food (Fig. 2b). Other common macrobenthic species in the mussel bed were galatheid crabs (*Munidopsis* sp.; Fig. 2a) and polynoid polychaetes (scale worms), while harpacticoid copepods, nematodes and small polychaetes were present in sediments within the mussel aggregations.

In contrast with *Mussel Bed*, the *Champagne* site (Fig. 2c) was characterized by intense diffuse and focused hydrothermal flow and obvious evidence of free-living chemosynthetic production. Several white smokers and seafloor vents issued fluids (up to 105°C) supersaturated with CO_2. Thick microbial mats covered portions of the seafloor between the white smoker chimneys. Lupton et al. (2006) report CO_2 concentrations of up to 2.2 moles $CO_2.kg^{-1}$ for the *Champagne* vent fluids. This is more than double the theoretical CO_2 solubility for 100°C at 1600 m depth (Lupton et al., 2006). Macrobenthic species were less abundant at this site, meiofauna were absent in samples, and community composition was dominated by the vent shrimp *Opaepele loihi* Williams & Dobbs, 1995, scale worms and occasional galatheid crabs.

The third site, *Top Tower*, a 1-2m high edifice of brecciated talus (Fig. 2d), was weakly discharging hydrothermal fluid. Isolated mussels colonized the edifice surface along with small numbers of galatheid crabs, scale worms and patches of orange microbial mat. As at *Mussel Bed* site, nematodes, harpacticoid copepods and polychaetes were present in particulate debris suctioned from the edifice surface.

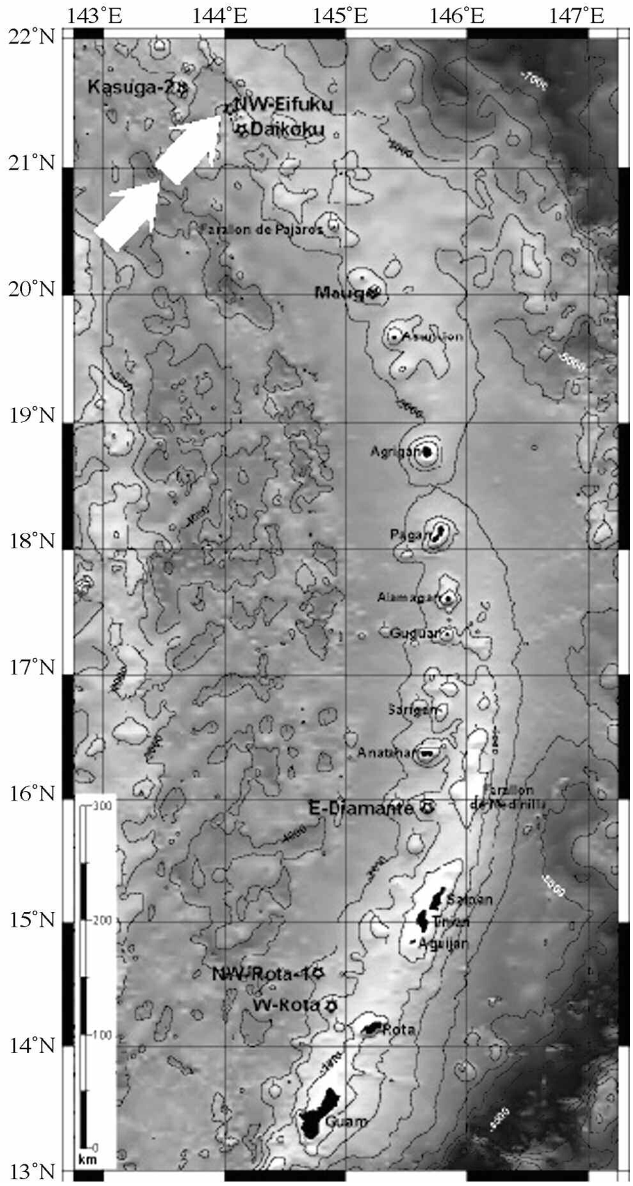

Figure 1. Satellite bathymetry map of Mariana Arc showing location of NW Eifuku Volcano (arrows).

Figure 1. Carte de bathymétrie satellitaire de l’Arc des Mariannes montrant la localisation de NW Eifuku Volcano (flèches).

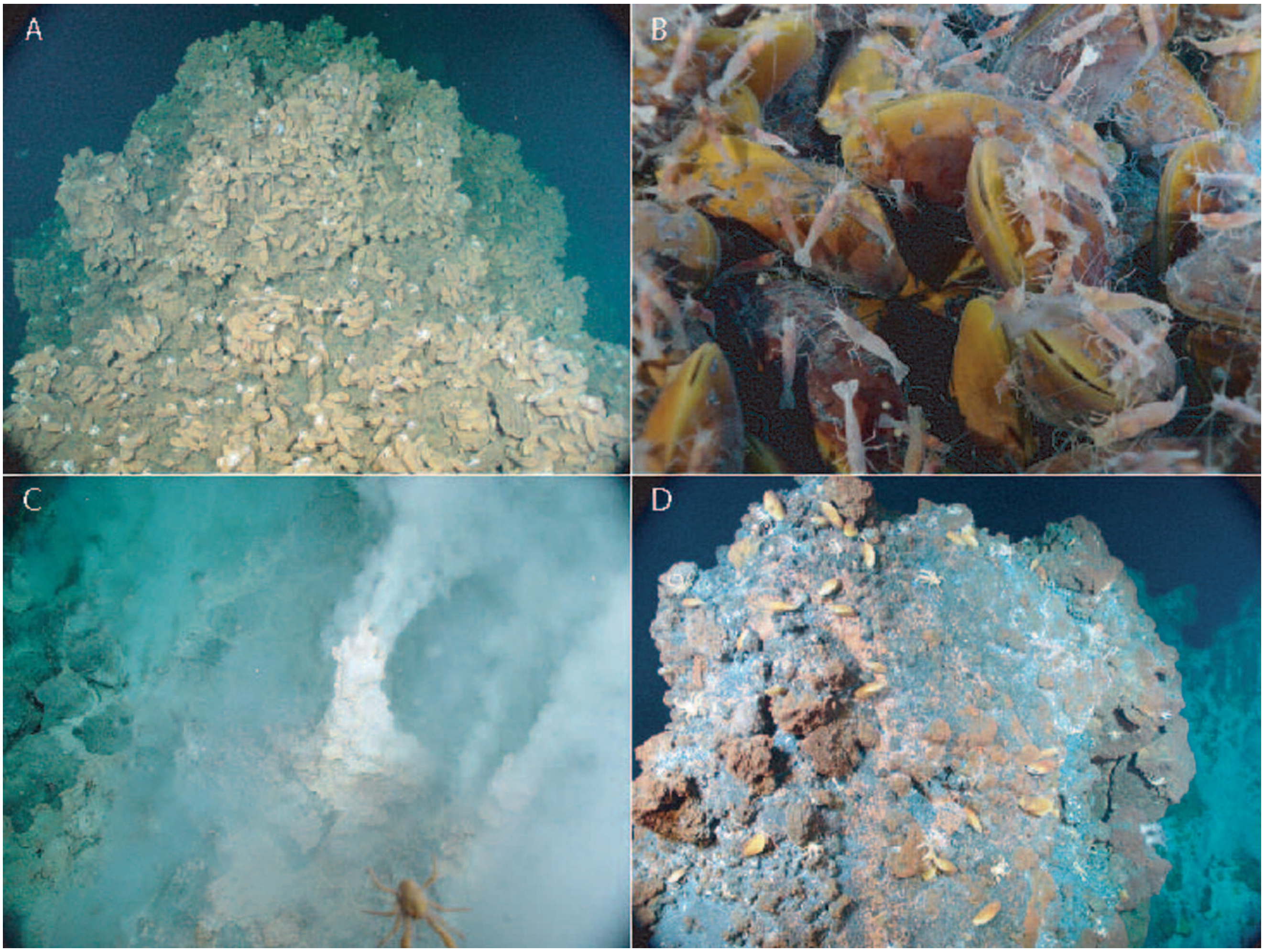

Figure 2. Digital still photos of the three sampled vent sites on NW Eifuku Volcano. **A & B.** Mussel Bed. **C.** Champagne. **D.** Top Tower.

Figure 2. Photos numériques des trios sites hydrothermaux échantillonnés sur NW Eifuku Volcano. **A & B.** Mussel Bed. **C.** Champagne. **D.** Top Tower.

Sampling

Samples were collected by the remotely-operated vehicle ROPOS during a March/April 2004 cruise on the R/V Thomas G. Thompson. Fauna, sediment and particulate organic matter were collected in 2 L acrylic jars with the ROPOS suction sampler. The suction hose was flushed with bottom seawater between samples, to minimize cross contamination. Suspended particulate organic matter was sampled during ROPOS imaging transects above the mussel bed at an altitude of 5 m, using a McClane pump to concentrate particles from 200 L of seawater onto a 142 mm diameter, pre-combusted GF/F filter.

Stable isotopes

Stable isotope analysis was performed on individual macrobenthic species. For small invertebrates, such as harpacticoid copepods and nematodes, individuals of the same species were pooled together. Invertebrate samples, particulate matter and GF/F filters were acidified (0.1N HCl) dried and analysed using a Micromass Isoprime isotope ratio mass spectrometer, in line with a Carlo Erba C/N element analyzer. All samples were analysed for both $\delta^{13}C$ and $\delta^{15}N$ isotopes except for those which were very small in mass (harpacticoid copepods, filters with suspended material), for which we report only the carbon isotopic signature. Stable isotopic compositions are reported relative to Vienna Pee-Dee Belemnite and atmospheric nitrogen as:

$$\delta X = [(R_{sample} / R_{standard}) - 1] \times 1000 \ (‰) \qquad (1)$$

where X is ^{13}C or ^{15}N, and R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

Results

Mussel Bed site

Organisms and organic debris at this site showed the widest range of δ^{13}C values, with the limpet, *Lepetodrilus* nr *schrolli* Beck, 1993 being the most enriched (δ^{13}C = -18‰, Fig. 2), and the large scale worm and the mussel, *Bathymodiolus* sp. the most depleted with a δ^{13}C signature of -34‰. Samples from *Mussel Bed* site also showed the widest range in δ^{15}N values, with *Bathymodiolus* sp. the most depleted (δ^{15}N = -3‰) and the shrimp *Alvinocaris* sp. and the galatheid crab *Munidopsis* sp. the most enriched (δ^{15}N = 6‰, Fig. 3). While *Munidopsis* sp. and *Alvinocaris* sp. had similar δ^{15}N signatures, they differed in δ^{13}C; *Munidopsis* sp. was more enriched (δ^{13}C = -22‰) in comparison to *Alvinocaris* sp. (δ^{13}C = -26‰). Stable carbon isotope signatures of potential food sources at this site range from -36‰ for a mucus aggregation sampled with the mussels to -26‰ for suspended particles collected at 5 m altitude above the mussel bed (Fig. 3). None of the potential carbon sources that were sampled corresponded to a potential food source for the species at the ^{13}C-enriched end of the range (*Lepetodrilus* nr *schorolli* and *Alvinocaris* sp. nov.) (Fig. 3).

Top Tower site

While the overall δ^{13}C range at this site was large, tissues of most of the species were within the range of -25 to -21‰, while the mussels were far more depleted in ^{13}C (δ^{13}C = -38‰, Fig. 2). Most fauna ^{15}N signatures fell within a narrow range (6-9‰) as well, except for the Dorvilleid polychaetes, which were more ^{15}N-depleted (δ^{15}N = 1.2‰). The δ^{13}C and δ^{15}N of sampled detritus from this site measured -28‰ and -1‰, respectively (Fig. 3).

Champagne site

The range of δ^{13}C values at this site was much narrower in comparison to the two previous sites. In addition, all species except for one (*Munidopsis* sp.) were very enriched in ^{13}C compared to all other species found at the two previous sites. The most enriched species were the shrimp *Opaepele loihi* and the scale worm, having δ^{13}C signatures of -11 and -10‰, respectively (Fig. 3). Potential food sources at this site were also, in comparison to the previous sites, much more enriched in ^{13}C; the δ^{13}C of the white bacterial mat and detritus measured -13‰ and -17‰, respectively (Fig. 3).

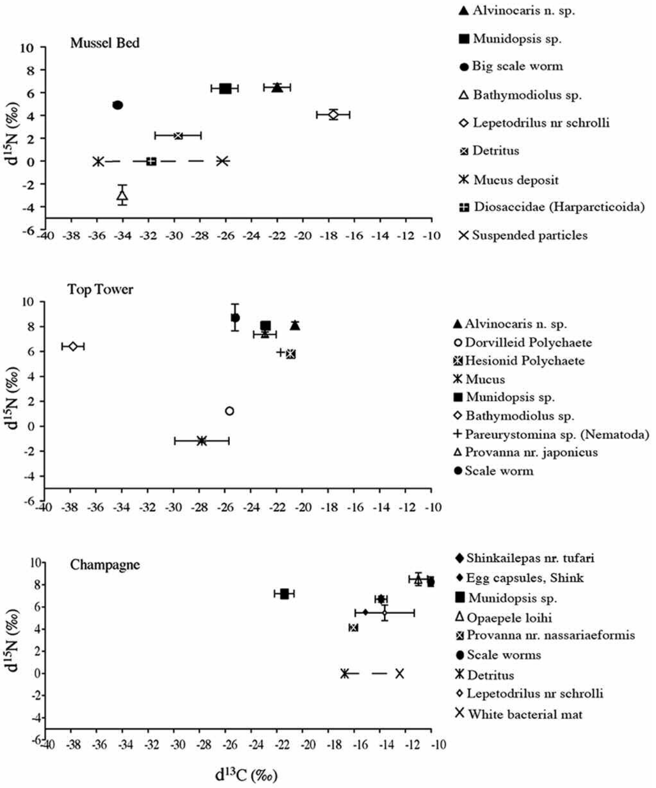

Figure 3. Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopic signatures of inverterbrates and potential food sources at three hydrothermal vent sites on NW Eifuku Volcano. Symbols joined with dashed line at δ^{15}N = 0.0 represent samples for which only δ^{13}C could be obtained. Error bars represent standard error.

Figure 3. Signatures isotopiques stables du carbone (δ^{13}C) et de l'azote (δ^{15}N) des invertébrés et des sources potentielles de nourriture sur trois sites hydrothermaux sur NW Eifuku Volcano. Les symboles joints par une ligne pointillée au niveau δ^{15}N = 0.0 représentent les échantillons pour lesquels seul le δ^{13}C a été obtenu. Les barres d'erreur représentent l'erreur standard.

Discussion

The extensive area and biomass of the *Mussel Bed* site suggest the advected flow from the white smoker chimneys and seafloor vents at nearby *Champagne* site has been abundant and relatively stable over the past years or more. Compared to the other two sites, the community at the *Mussel Bed* site was supported by several sources of primary productivity (broad δ^{13}C range), and two or more consumer trophic levels were present (δ^{15}N range > 9.0). The stable isotope portraits for the other two sites are simpler, in terms of both carbon sources and the number of potential trophic levels. Overall stable isotope trends for the fauna at the three sites are summarized in box plots in Figure 4.

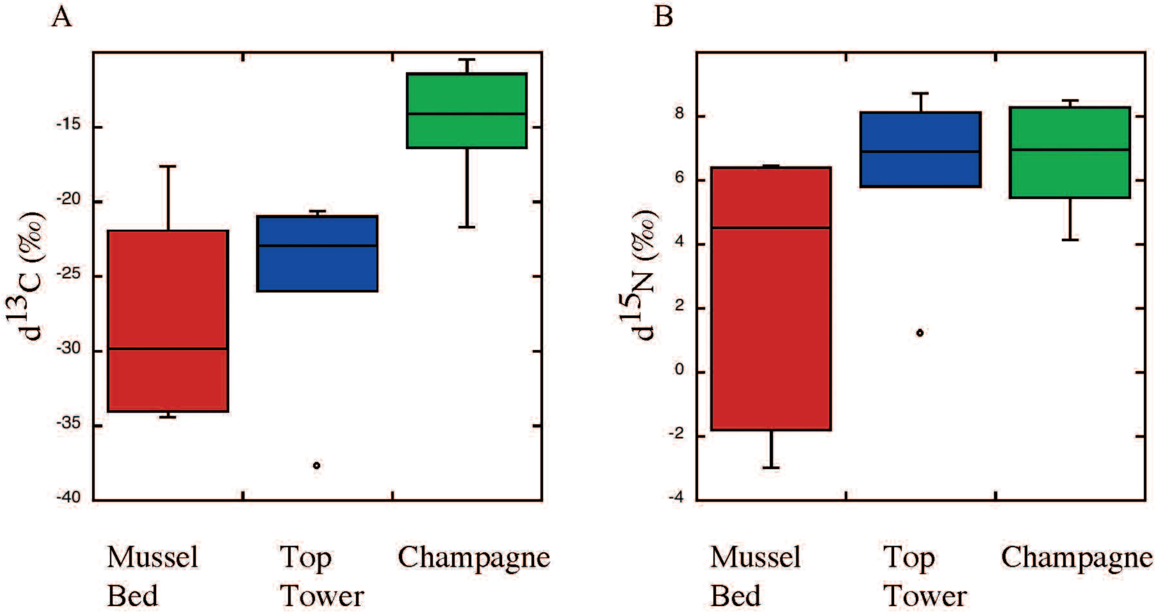

Figure 4. Box plots of fauna only stable isotope ratios from vent sites on NW Eifuku Volcano. Plots derived from mean or single values for 5-7 species sampled at each site. Inter-quartile distances (box length) illustrate width of food web base (A) and number of trophic levels (B). Median values (horizontal line in boxes) permit comparison of carbon (A) and nitrogen (B) sources. Outlier points in Top Tower plots represent the mussel *Bathymodiolus* sp. ($\delta^{13}C$) and the Dorvilleid polychaete ($\delta^{15}N$).

Figure 4. Boites à moustaches des rapports isotopiques de la faune des sites hydrothermaux sur NW Eifuku Volcano. Graphes construits à partir des valeurs uniques ou moyennes des 5 à 7 espèces échantillonnées sur chaque site. La distance interquartile (longueur de la boîte) illustre la diversité des sources (A) et le nombre de niveaux trophiques (B). Les valeurs médianes (trait horizontal) permettent de comparer les sources de carbone (A) et d'azote (B). Les points extérieurs sur le site de Top Tower représentent *Bathymodiolus* sp. ($\delta^{13}C$) et le polychète Dorvilleidé ($\delta^{15}N$).

The narrower food web base at *Champagne* site, and the striking ^{13}C-enrichment that dominated stable carbon signatures at this site, require some particular consideration. A source of primary production with a relatively uniform $\delta^{13}C$ signature is the most likely explanations for the narrow food web base. Less probable would be a situation where several chemoautotrophic primary producers with different stable isotope signatures made the same proportional contribution to the diets of all primary consumers. Levesque et al. (2006) describe a similarly narrow range in fauna $\delta^{13}C$ values (-10 to -12‰) in areas of intense, CO_2-rich fluid flow at vents on Axial Volcano (Juan de Fuca Ridge). The 2.2 moles per kilogram of dissolved CO_2 in the hotter fluids at *Champagne* site is 10x greater than the highest previously reported CO_2 concentrations in vent fluids, and nearly 100x average values for mid-ocean ridge vent fluids (Lupton et al., 2006). Ambient CO_2 concentrations and CO_2:O_2 ratios can affect stable carbon isotope signatures of organic matter fixed by autotrophs by favouring the dominance by one of the two forms of the CO_2 fixing enzyme Ribulose-1-5-biphosphate carboxylase/oxydase (Rubisco). Form I Rubisco is found in higher plants and in most chemoautotrophic bacteria that use the Calvin cycle to fix CO_2 (Tabita, 1988). The occurrence of form II is less well known but it has been confirmed in the symbionts of the vent tubeworm *Riftia pachyptilla* Jones, 1981 (Robinson & Cavanaugh, 1995) and some chemoautotrophs (Tabita, 1988). Form I Rubisco, which has a higher affinity for CO_2, yields organic matter which is more depleted in ^{13}C than does form II Rubisco, which is better adapted to high CO_2:O_2 ratios (i.e. high CO_2 concentrations). A reliance on organic matter produced by one or the other forms of Rubisco has been proposed as a partial explanation for the bimodal distribution of invertebrate $\delta^{13}C$ values observed at some hydrothermal vents (Childress & Fisher, 1992). However, a dominance of CO_2 fixation at *Champagne* site by form II Rubisco cannot fully account for the ^{13}C enrichment in samples from this site. Using the kinetic isotope effect for form II Rubisco of -19.5 determined by Robinson et al. (2003) and the measured $\delta^{13}C$ of -1.75‰ for vent fluid CO_2 at *Champagne* site (Lupton et al., 2006), we obtain a theoretical value of -21.25‰ for autotrophically produced organic matter. This is more depleted in ^{13}C than the -12.8‰ value for the microbial mat sample at *Champagne* site, or the -11.4 to -16.4‰ range for most of the fauna at this site. Factors other than isotope discrimination by Rubisco are therefore contributing to the observed ^{13}C enrichment at *Champagne* site, compared to the other two sites. Robinson et al. (2003) observed a similar discrepancy between theoretical and observed fractionation values for *R. pachyptila* symbionts, and discuss the possible contribution of processes such as anapleurotic carbon fixation, C4 pathways, CO_2 limitation (unlikely in the present case) to further ^{13}C enrichment. They also note that growth rate effects and environmental CO_2 concentrations can influence ^{13}C enrichment. The latter two factors would be particularly interesting to study at *Champagne* site where carbon is being fixed under conditions of high vent fluid flow (= rapid growth?) and extremely high CO_2 concentrations.

There were substantial differences in $\delta^{15}N$ signatures between mussels at *Top Tower* site (6‰) and the *Mussel Bed* site (-3‰). Bathymodiolid mussels are generally very depleted in nitrogen isotopes, often showing negative values (Robinson et al., 1998). The unusually enriched nitrogen signature of mussels at *Top Tower* site may be the result of their hosting different microbial symbionts compared to mussels at *Mussel Bed* site. Bathymodiolid mussels can host both thio- and methanotrophic bacteria, separately or as dual symbionts (Fisher et al., 1993).

Thiotrophic mussel symbionts fix CO_2 with form I Rubisco, which has a fractionation factor of around -22 to -30‰ in plants and phytoplankton (Robinson & Cavanaugh, 1995). A slightly greater fractionation factor would be required to explain the $\delta^{13}C$ values of -34 to -38‰ observed in the mussel tissues at *Mussel Bed* site and *Top Tower* site based on thiotrophy alone. Alternatively, methanotrophy could be invoked to explain the more ^{13}C-depleted values in these mussels (Childress & Fisher, 1992). However, Robinson & Cavanaugh (1995) suggest that form I Rubisco in thiotrophic chemoautotrophic symbioses may fractionate against ^{13}C by as much as 40‰.

Munidopsis crabs were present at all three sites. Their notably different carbon isotopic signatures generally reflect between-site trends, suggesting a non-specialized diet exploiting available local resources. In other hydrothermal vent areas, stable isotope studies indicate that *Munidopsis* sp. feed on bacteria and invertebrates associated with bacterial mats (Escobar-Briones et al., 2002). However, at *Champagne* site, the isotopic signature of *Munidopsis* sp. indicates that it was not feeding on any of the sampled bacteria and invertebrates in the area. It was far more depleted in ^{13}C than any of the other species or the bacterial mat. The intense venting activity at *Champagne* site may oblige *Munidopsis* to feed in peripheral areas, away from the sources of primary productivity exploited by the other species.

Conclusion

The different flow regimes clearly influenced food sources and food web structure on the slopes of NW Eifuku. The basic food source, chemolithoautotrophic productivity, varied among sites as indicated by faunal carbon isotopic signatures. The CO_2-rich, vigorously venting environment at *Champagne* site likely favours chemosynthesis using Rubisco form II although this factor alone does not account for the observed ^{13}C enrichment in samples from this site. In contrast, $\delta^{13}C$ depletion, attributable to form I Rubisco or possibly methanotrophy, was observed in the mussels at the other two sites that were receiving advected or weak local hydrothermal flow. At *Mussel Bed* site, where mussels predominated, this ^{13}C-depleted carbon also contributed to the diet of other species, whereas consumers at *Top Tower* site did not appear to be relying on carbon produced by the isolated mussels found at this site. The broad and apparently stable plume of hydrothermal flow that was being advected from *Champagne* site to the *Mussel Bed* site area supported an extensive, high biomass community with a wide trophic base and several levels of consumers. The other two sites, with intense and unstable, or weak hydrothermal regimes, hosted simpler food webs.

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References

Childress J.J. & Fisher C.R. 1992. The biology of hydrothermal vent animals: Physiology, biochemistry and autotrophic symbioses. *Oceanography and Marine Biology Annual Reviews*, **30:** 337-441.

Escobar-Briones E., Morales P., Cienfuegos E. & Gonzales M. 2002. Carbon sources and trophic position of two abyssal species of Anomura, *Munidopsis alvisca* (Galatheidae) and *Neolithodes diomedeae* (Lithodidae). In: *Contributions to the study of East Pacific Crustaceans* (M.E. Hendrickx, ed), pp. 37-43. Unidad Academica Mazatlan, UNAM, Mazatlan, Mexico.

Fisher C.R., Brooks J.M., Vodenicher J.S., Zande J.M., Childress J.J. & Burke Jr R.A. 1993. The co-occurrence of methanotrophic and chemoautotrophic sulfur-oxidizing bacterial symbionts in a deep-sea mussel. *Marine Ecology Progress Series*, **14**: 277-289.

Grelon D., Morineaux M., Desrosiers G. & Juniper S.K. 2006. Feeding and territorial behavior of *Paralvinella sulfincola* a polychaete worm at deep-sea hydrothermal vents of the Northeast Pacific Ocean. *Journal of Experimental Marine Biology and Ecology*, **329**: 174-186.

Karl D.M. 1995. Ecology of free-living , hydrothermal vent microbial communities. In: *The microbiology of deep-sea hydrothermal vents* (D.M. Karl ed), pp. 36-124. CRC Press: Boca Raton.

Levesque C., Limén H. & Juniper S.K. 2005. Origin, composition and nutritional quality of particulate matter at deep-sea hydrothermal vents on Axial Volcano, northeast Pacific. *Marine Ecology Progress Series,* **289**: 43-52.

Levesque C., Juniper S.K. & Limén H. 2006. Spatial organization of food webs along habitat gradients at deep-sea hydrothermal vents on Axial Volcano, Northeast Pacific. *Deep-Sea Research I*, **53**: 726-739.

Lupton, J. et al. 2006. Submarine venting of liquid carbon dioxide on a Mariana Arc volcano. *Geochemisry Geophysics Geosystems,* **7**: Q08007, doi:10.1029/2005GC001152.

Luther G.W. III, Rozan T.F., Taillefert M., Nuzzio D.B., Di Meo C., Shank T.M., Lutz R.A. & Cary S.C. 2001. Chemical speciation drives hydrothermal vent ecology. *Nature,* **410**: 813-816.

Micheli F., Petersen C.H., Mullineaux L.S., Fisher C.R., Mills S.W., Sancho G., Johnson G.A. & Lenihan H.S. 2002. Predation structures communities at deep-sea hydrothermal vents. *Ecological Monographs*, **72**: 365-382.

Paoli G.C. & Tabita F.R. 1998. Aerobic chemolithoautotrophic growth and RubisCO function in *Rhodobacter capsulatus* and a spontaneous gain of function mutant of *Rhodobacter sphaeroides*. *Archives of Microbiology,* **170**: 8-17.

Post D.M. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology,* **83**: 703-718.

Robinson J.J. & Cavanaugh C.M. 1995. Expression of form I and form II ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) in chemoautotrophic symbiosis: Implications for the interpretation of stable carbon isotope ratios. *Limnology and Oceanography,* **40**: 1496-1502.

Robinson J.J., Polz M.F., Fiala-Medioni A. & Cavanaugh C.M. 1998. Physiological and immunological evidence for two distinct C_1-utilizing pathways in *Bathymodiolus puteoserpentis* (Bivalvia: Mytilidae), a dual endosymbitic mussel from the Mid-Atlantic Ridge. *Marine Biology,* **132**: 625-633.

Robinson J.J., Scott K.M., Swanson S.T., O'Leary M.H., Horken K., Tabita F.R. & Cavangh C.M. 2003. Kinetic isotope effect and characterization of form II RubisCO from the chemoautotrophic endosymbionts of the hydrothermal vent tubeworm *Riftia pachyptila*. *Limnology and Oceanography*, **48**: 48-54.

Sarrazin J., Juniper S.K., Massoth G. & Legendre P. 1999. Physical and chemical factors influencing species distributions on hydrothermal sulfide edifices of the Juan de Fuca Ridge, northeast Pacific. *Marine Ecology Progress Series,* **190**: 89-112.

Tabita, F.R. 1988. Molecular and cellular regulation of autotrophic carbon dioxide fixation in microorganisms. *Microbiological Reviews,* **52**: 155-189.