

Chemoautotrophic Sulfur Bacteria as a Food Source for Mollusks at Intertidal Hydrothermal Vents: Evidence from Stable Isotopes

by

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Abstract. Stable carbon and nitrogen isotope measurements indicate that limpets (*Lottia limatula*), which normally graze on algae, have diets consisting predominantly of chemoautotrophic sulfide-oxidizing bacteria at southern California intertidal hydrothermal vents. Suspension-feeding mussels (*Mytilus edulis*) at the intertidal vents show only minor inclusion of the chemoautotrophic bacteria in their diet. This is the first evidence from stable isotopes that geothermally driven primary production can be of major importance to intertidal grazers.

INTRODUCTION

The discovery of dense animal and bacterial populations at deep-sea hydrothermal vents, living in the absence of sunlight, suggested that these biological communities were supported locally by chemosynthetic vent bacteria rather than minimal amounts of photosynthetically produced material drifting down from the sunlit zone. Much evidence has since been gathered confirming the hypothesis that chemoautotrophic bacteria are the primary producers at deep-sea vents. These bacteria support a community of invertebrate grazers (*e.g.*, limpets), suspension feeders (*e.g.*, clams, mussels), predators and scavengers (*e.g.*, crabs), and may also supply organic carbon to macroinvertebrates through symbiotic associations (RAU, 1985; HESSLER & SMITHEY, 1984; CAVANAUGH, 1983; FELBECK & SOMERO, 1982; WILLIAMS *et al.*, 1981).

Vent bacteria produce biomass and obtain energy from oxidation of geothermally reduced compounds (*e.g.*, hydrogen sulfide, ammonia, and hydrogen) in vent waters (JANNASCH & WIRSEN, 1979). Sulfide-oxidizing bacteria appear to be among the most conspicuous primary producers at vents (RUBY *et al.*, 1981). Consumption of sulfide-

oxidizing bacteria occurs wherever sulfide levels are high enough to support chemosynthesis. In most of these habitats, however, the sulfide is biologically produced by sulfate-reducing bacteria (SPIES & DESMARAIS, 1983) rather than being geothermally produced as in deep-sea vents. The possibility that geothermally driven chemosynthesis is important for some organisms in habitats other than deep-sea vents has been suggested. STEIN (1984) reported the partial contribution of sulfide-oxidizing bacteria to the diet of black abalone *Haliotis cracherodii* Leach, 1814, at coastal hydrothermal vents. Ciliates, flagellates, and aquatic insect larvae consume sulfide-oxidizing bacteria in terrestrial hot springs (LACKEY *et al.*, 1965). Thus, geothermally driven primary production is not unique to deep-sea vents, and may contribute to community biomass wherever hydrothermal vents occur in the photic zone.

Little is known of the relative nutritional importance of chemosynthesis versus photosynthesis at sunlit vents. STEIN (1984) observed that in addition to black abalone, several species of limpets appeared to graze on benthic mats of bacteria (the most conspicuous species of the bacterial assemblage resembled *Thiothrix* Winogradsky, a known sul-

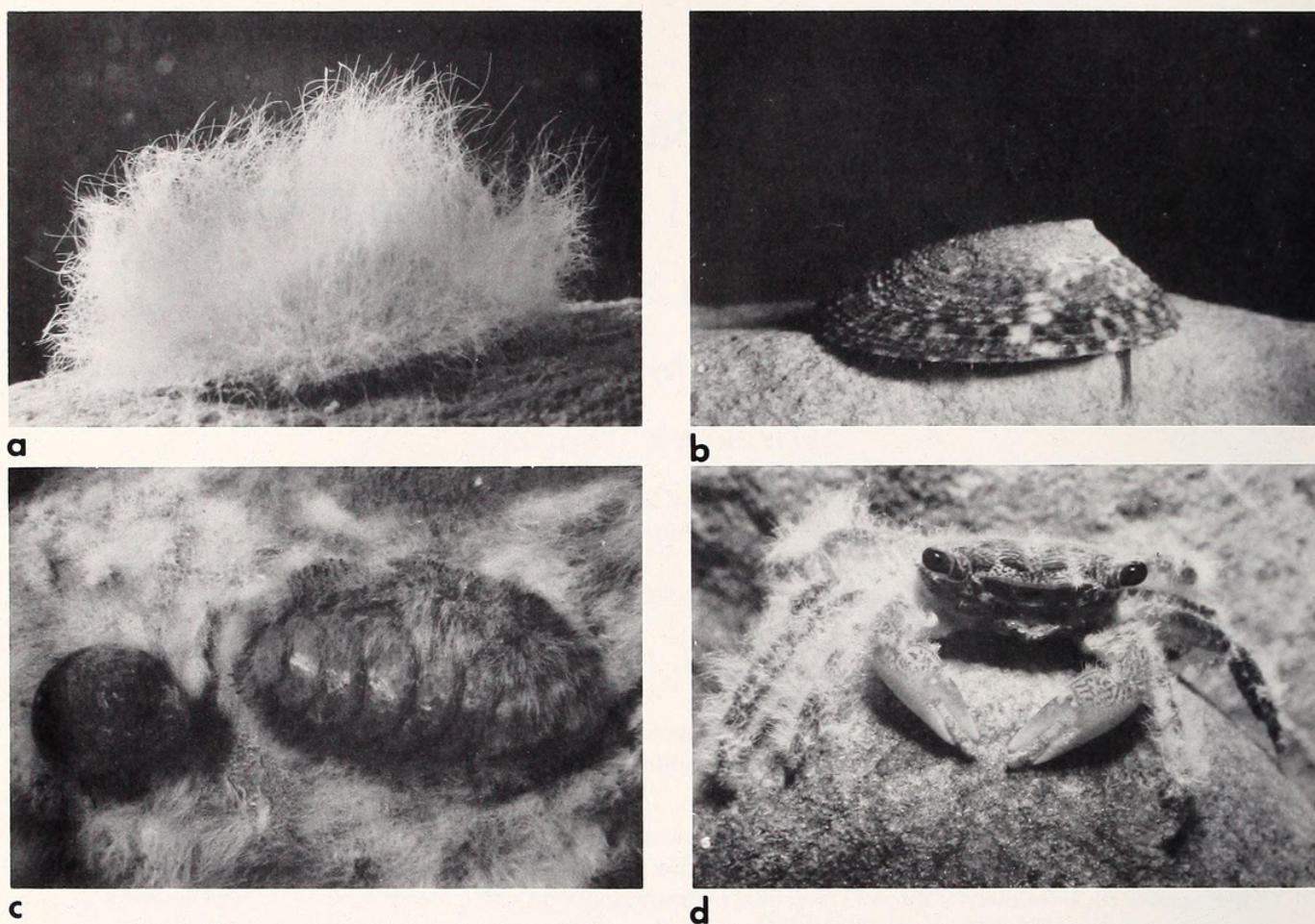


Figure 1

a. File limpet *Lottia limatula* from intertidal vent, with dense growth of filamentous sulfide-oxidizing bacteria on shell. b. File limpet collected 10 m from nearest vent. c. Mossy chiton *Mopalia muscosa* (Gould, 1846) and black turban snail *Tegula funebris* (A. Adams, 1855) on bacteria-covered rock at intertidal vent. d. Shore crab *Pachygrapsus crassipes* from intertidal vent, with bacterial growth on exoskeleton.

fide oxidizer) at intertidal hydrothermal vents located at White Point, southern California. In our study, closer examination of intertidal vents in the same area where STEIN (1984) worked subtidally revealed the presence of numerous common intertidal mollusks and crabs. The hard body surfaces (shell and exoskeleton) of many individuals at these vents were covered with a thick growth of attached filamentous sulfide-oxidizing bacteria (Figure 1). These bacteria grow only in the immediate vicinity of vents where sulfide levels are high enough to support chemosynthetic production of biomass, and indicate that animals covered with bacteria spend a considerable amount of time bathed in the warm, sulfide-rich vent effluent (up to 28°C and 141 $\mu\text{M/L}$; STEIN, 1984).

These observations suggested that vent bacteria might form a substantial portion of the diets of some intertidal macroinvertebrates. We examined this possibility for two species of intertidal mollusks that occur commonly at and away from vents. The file limpet *Lottia limatula* (Carpen-

ter, 1864) is a benthic grazer, while the common mussel *Mytilus edulis* Linnaeus, 1758, was chosen as a representative suspension feeder.

MATERIALS AND METHODS

Possible dietary sources were investigated by measuring the stable carbon and nitrogen isotopic composition ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ ratios) of both animal tissues and their potential food items. This approach was used because, if stable isotope ratios of different food types differ substantially, these isotopic ratios can be used as natural markers to trace dietary sources, as the ratios change little when assimilated into animal tissue (DENIRO & EPSTEIN, 1978, 1981). Use of this method in other studies has indicated a trophic link between deep-sea vent bacteria and deep-vent consumers (RAU, 1985; RAU & HEDGES, 1979).

Samples were taken from intertidal vent (*i.e.*, within 10 cm of a vent opening) and non-vent (at least 10 m from

Table 1

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for vent and non-vent organisms and their potential food sources. Sample size (n) for animals = the number of individuals analyzed. Three samples of vent bacteria and one algal sample were analyzed. Values are given as the mean $\pm$ SE if n is greater than 1.

Organism	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Vent			
Bacteria	3	-31.0 ± 0.3	-4.1 ± 1.3
<i>Lottia limatula</i>	2*	-30.4	-1.3
<i>Mytilus edulis</i>	3	-16.6 ± 0.7	$+6.4 \pm 1.0$
Non-vent			
Benthic algae	1	-15.1	+6.8
<i>Lottia limatula</i>	3*	-15.5	+6.3
<i>Mytilus edulis</i>	1	-15.3	+8.6

* These limpets were analyzed as one sample.

the nearest detectable vent opening) sites at White Point in southern California. Vent openings were easily detected because the rocky substrate surrounding an opening is devoid of algal growth but covered with dense, off-white mats of sulfur bacteria.

Vent bacterial and non-vent benthic algal samples were scraped from rocks. Limpets were collected and kept live in 25- μm filtered seawater to allow for gut evacuation, so the isotopic composition of the whole body could be determined without contamination from ingested but unassimilated material. Mussels were collected and their foot and mantle tissues were dissected out for analysis, to avoid contamination by gut contents. All samples were lyophilized, ground to powder, treated with 1 N HCl to remove carbonate, and lyophilized again before combustion (NORTHFELT *et al.*, 1981). The resulting CO_2 and N_2 were separated cryogenically and their volumes determined manometrically prior to mass spectrometric analysis. The isotopic compositions are expressed in the usual δ notation where $\delta X = \{[R(\text{sample})/R(\text{standard})] - 1\} \times 1000\text{‰}$ and $X = ^{13}\text{C}$ or ^{15}N , $R = ^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, and the standard = PDB belemnite or atmospheric N_2 , respectively, for carbon and nitrogen (RAU, 1985).

RESULTS AND DISCUSSION

The carbon isotope ratios reported in Table 1 suggest that the diet of intertidal vent limpets consisted predominantly of sulfide-oxidizing bacteria. The $\delta^{13}\text{C}$ values of vent limpets match those of vent bacteria. The $\delta^{13}\text{C}$ values of non-vent limpets, in contrast, were decidedly less negative than those of the vent limpets, and are similar to those of their likely food source, photosynthetic algae encrusting the rocks on which they live.

Carbon isotope ratios of mussels living near and away from the intertidal vents (Table 1) suggest that the vent mussels are using only small amounts of vent bacteria as

a food source. The $\delta^{13}\text{C}$ values for vent mussels are only about 1‰ more negative than those for non-vent mussels. This 1‰ difference contrasts with the 16‰ difference in the $\delta^{13}\text{C}$ values between the vent bacteria and the non-vent algae, which may be taken as the difference between the chemosynthetically and photosynthetically derived food sources in the area. If the photosynthetically derived food used by the mussels had $\delta^{13}\text{C}$ values similar to the benthic algae we analyzed (Table 1), the mussel $\delta^{13}\text{C}$ values suggest that more than 95% of the vent mussel food was not derived from vent bacteria. Water column concentrations of suspended bacteria detached from vent bacterial mats were not measured in this study, but are presumably high near vents because many suspended filaments of mat bacteria are visible to the naked eye over vent openings (especially when wave action detaches parts of the attached mat). Presumably, a suspension feeder, like *Mytilus edulis*, that is situated directly over a vent must entrain some bacteria in its feeding current. Most of these bacteria may subsequently be sorted out and rejected in favor of plankton in non-vent water that mixes with vent effluent.

Nitrogen isotope measurements may also provide information on trophic relationships. For deep-sea vent and other marine organisms, the $\delta^{15}\text{N}$ values increase approximately 2–3‰ with each change in trophic level (RAU, 1985). Our measurements for intertidal vent limpets are in good agreement with this trend. The $\delta^{15}\text{N}$ value for limpets is 2.8‰ higher than that for vent bacteria (Table 1), strengthening the conclusion that the bulk of vent limpet diet consists of vent bacteria. The unusually low $\delta^{15}\text{N}$ value for vent bacteria may be an indication of bacterial N_2 fixation as a vent nitrogen source, rather than a biologically cycled nitrogen source (*e.g.*, nitrate), which would have a more positive $\delta^{15}\text{N}$ value (RAU, 1985). On the other hand, it is also possible that vent water contains an ^{15}N -depleted organic source of nitrogen. For non-vent control limpets, the $\delta^{15}\text{N}$ values are close to that of their algal food source but do not show the slight rise predicted with a change in trophic level. Nevertheless, the values are consistent with a non-vent photosynthetically generated food source (RAU, 1985).

The $\delta^{15}\text{N}$ values for vent and non-vent mussels are consistent with our conclusion that vent mussels use vent bacteria as only a minor food source.

According to STEIN (1984), mollusks are the only macroinvertebrates occurring commonly at subtidal White Point hydrothermal vents. At the littoral zone vents examined in our study, mollusks are the dominant macroinvertebrates, but two arthropods, the striped shore crab *Pachygrapsus crassipes* Randall, 1839 (Figure 1d) and the blue-clawed hermit crab *Pagurus samuelis* (Stimpson, 1857), are also common. It is therefore possible that these and other shallow-water marine macroinvertebrates, in addition to file limpets and black abalone, use vent bacteria as an important food source. Also, the assemblage of tiny organisms associated with mats of coastal vent bacteria (*e.g.*, copepods, flatworms, polychaetes, flagellates), and

the bacteria themselves, have not yet been fully characterized. Because of easy access, there is great potential to investigate further the nature of trophic relationships within these shallow-water hydrothermal vent communities.

Our study is a first account of both carbon and nitrogen stable isotope measurements indicating that geothermally driven production of biomass is of major nutritional importance for certain consumers, even in the sunlit zone where photosynthesis dominates.

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